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HEALTHCARE ASSOCIATED INFECTIONS IN VASCULAR SURGERY

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Healthcare Associated Infections in Vascular Surgery

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Ann-Sophie

Difficult to see. Always in motion is the future…
Yoda

Contents

Abstract

Health care associated infections (HCAI) affect roughly four million people in Europe annually, resulting in 16 million extra days of hospital stay, 37 000 attributable deaths and direct costs of approximately € 7 billion. HCAI in vascular surgery are relatively common complications with potentially devastating consequences. The aim of this thesis, based on four papers, was to study the scope of these complications and investigate the viability of methods for large-scale follow-up of patients after discharge and potentially more accurate laboratory diagnostic tools for postoperative infections.

The first paper, which is also the first of the two epidemiological studies included 10 547 patients who had undergone elective, infrainguinal revascularization – open or endovascular – during the period 2005-2010. Data were collected from the Swedish National Registry for Vascular Surgery (Swedvasc), the Swedish National Patient Register (NPR) and the Cause of Death Register. The total incidence of HCAI within 30 days was 9.7% (n = 1 019). The rate of 30-day HCAI for endovascular procedures was 7.8% (490/6 262) and for bypass operations 13.3% (430/3 224). The rate of major amputation at one-year was 11.8% (98/833) for patients with postoperative HCAI and 5.6% (446/7 933) for those without HCAI postoperatively.

The second paper, a prospective, clinical pilot-study, evaluated the effect of surgically induced inflammation on neutrophil CD64 receptors, which have been shown to undergo a rapid up-regulation in response to bacterial infection. This study included 153 elective, vascular patients. The results showed a non-significant effect of surgical trauma on CD64 receptors, as well as, a significant increase in CD64 expression postoperatively, in response to bacterial infection. *The third paper* aimed to investigate some of the cellular responses to surgical trauma and infection by measuring the changes in serum concentration of an array of cytokines in 96 consecutive patients undergoing non-emergent vascular surgery. The results showed a decrease in eight pro-inflammatory cytokines and a positive correlation between perioperative infection and the anti-inflammatory cytokines, interleukin (IL)-10 and IL-13.

The fourth paper, which included a similar cohort to the first paper, collected data on 9 894 patients from Swedvasc, NPR and the Prescribed Drug Register, between 2005 and 2010. The aim of this study was to determine the rate and type of postoperative HCAI, after discharge from hospital, using antibiotic prescription as a surrogate marker. We have shown that 33% of patients received an antibiotic prescription within 30 days of the index operation. In the endovascular group, there was a 52% increase in the rate of prescriptions postoperatively compared to the preoperative period. Prescriptions for urinary tract infections dominated the 30-day postoperative period for patients with claudication.

The rates of postoperative HCAI after lower limb revascularization warrant a more extensive follow-up regimen, including post-discharge, for both open and endovascular procedures. The need for more accurate laboratory tests in the early postoperative period still remains to be fulfilled. Neutrophil CD64 in combination with other markers, such as IL-10 and C-reactive protein have shown potential in this area and merit further investigation.

Populärvetenskaplig sammanfattning

De vanligaste komplikationerna inom sjukvården är vårdrelaterade infektioner (VRI). Enligt Världshälsoorganisationen (WHO), drabbas årligen över fyra miljoner människor i Europa, resulterande i 16 miljoner extra dagar av sjukhusvistelse samt 37 000 relaterade dödsfall och en direkt kostnad på ca € 7 miljarder.

Inom epidemiologisk och klinisk forskning ingår i klassificering av VRI vanligen urinvägsinfektion, luftvägsinfektion, postoperativ sårinfektion och blodburen infektion. Postoperativa VRI inom kärlkirurgi kan få ytterst allvarliga konsekvenser och uppskattad incidens inom en månad efter operation är uppemot 35 %. Dessa infektioner är vanligast efter kärlkirurgiskt ingrepp på benen. Tidig diagnostik och behandling av infektion är avgörande för ett gynnsamt utfall. Kateterbaserade (endovaskulära) ingrepp som numera utgör mer än hälften av alla kärlkirurgiska ingrepp, är ofta utförda på dagkirurgiska enheter, vilket kan försvåra uppföljning och identifiering av eventuella postoperativa VRI inom denna grupp. Den tidiga laboratoriebaserade diagnostiken av en eventuell infektion försvåras av den inflammatoriska effekten av kirurgi. Idag saknas det tester som kan åtskilja mellan inflammation förorsakad av kirurgi och infektion.

Syftet med denna avhandling är att undersöka omfattningen av VRI bland kärlkirurgiska patienter på nationell basis, utforska användbarheten av nya laboratoriemetoder för tidig diagnostik och utvärdera epidemiologiska metoder för uppföljning av patienter, även efter utskrivning från sjukhus.

I de två epidemiologiska studierna (Studie I och IV), har vi kartlagt förekomsten av VRI efter planerade kärlkirurgi på benens pulsådror. Vi samlade in data från det svenska kärlregistret (Swedvasc), Patientregistret, Läkemedelsregistret och Dödsorsaksregistret. Studierna inkluderade 10 547 (Studie I) och 9 894 (Studie IV) patienter under perioden 2005-2010. Data från Läkemedelsregistret har använts för att identifiera postoperativa infektioner efter utskrivning från sjukhus genom att jämföra antibiotika förskrivning före och efter operation. Den totala förekomsten av VRI inom 30 dagar efter kärlkirurgi var 9.7%. För endovaskulära ingrepp var 30-dagars förekomst 7.8% och för bypassoperationer 13.3%. Amputationsfrekvensen inom ett år för de med och utan postoperativ VRI var 12 % respektive 5,6 %. Totalt sett hade 33 % av patienterna fått minst ett recept på antibiotika efter ett planerat kärlkirurgiskt ingrepp. I den endovaskulära gruppen var det en 52% ökning i frekvensen av antibiotikaförskrivning efter operation jämfört med perioden före operationen. Förskrivningen för antibiotika mot urinvägsinfektioner inom 30 dagar efter ingrepp var den största antibiotikagruppen bland patienter med fönstertittarsjuka (claudicatio).

I den andra och tredje studien, båda prospektiva kliniska studier, har vi undersökt den potentiella användbarheten av olika inflammatoriska markörer som laboratorietester för postoperativ infektion. I den andra studien (153 patienter), har vi undersökt effekten av kirurgiskt

trauma samt bakteriell infektion på uppgradering av CD64 receptorer på en subgrupp av vita blodkroppar (neutrofiler). Vi har visat en kliniskt icke-signifikant effekt av kirurgiskt inducerad inflammation på CD64 receptorerna. Resultaten visade också en signifikant upp-gradering av CD64 i respons till bakteriellinfektion. Den tredje studien (96 patienter), fokuserade på effekten av kirurgisktrauma och infektion på cytokiner som fungerar som signalmolekyler mellan cellerna. Resultaten visade en nedgång i postoperativa värden för åtta pro-inflammatoriska cytokiner och en positiv korrelation mellan två anti-inflammatoriska cytokiner, interleukin (IL)-10 och IL-13, och postoperativa infektioner.

Den höga förekomsten av VRI efter kärlkirurgiska ingrepp på benen understryker behovet av ett noggrant uppföljningsprogram under den postoperativa perioden efter utskrivning. CD64 i kombination med andra markörer som IL-10 har visat en viss potential som en förbättrad laboratoriemarkör för postoperativ infektion men mer omfattande klinisk forskning krävs innan detta kan säkerställas.

List of scientific papers

I **Healthcare-associated Infections After Lower Extremity Revascularization**
Daryapeyma A, Östlund O, Wahlgren CM.
Eur J Vasc Endovasc Surg. 2014 Jul;48(1):72-7. Epub 2014 Mar 6.

II **Neutrophil CD64 as a Marker for Postoperative Infection: A Pilot Study**
Daryapeyma A, Pedersen G, Laxdal E, Corbascio M, Johannessen HB, Aune S, Jonung T.
Eur J Vasc Endovasc Surg. 2009 Jul; 38(1): 100-3. Epub 2009 Apr 8.

III **Perioperative Cytokine Response to Infection Associated With Elective Arterial Surgery**
Daryapeyma A, Aarstad HJ, Wahlgren CM, Jonung T.
Vasc Endovascular Surg. 2014 Feb; 48(2): 116-22. Epub 2013 Nov 21.

IV **Antibiotic Treatment as a Marker for Healthcare Associated Infections After Lower Extremity Revascularization**
Daryapeyma A, Hammar U, Wahlgren CM
Manuscript submitted

List of abbrevation

AAA-OR	Abdominal aortic aneurysm-open repair
BMI	Body mass index
BSI	Bloodstream infection
C	Celsius
CAS	Carotid artery stenting
CD	Cluster of differentiation
CDC	Centers for disease control and prevention
CEA	Carotid endarterectomy
CLI	Critical limb ischaemia
CRN	Civic registration number
CRP	C-reactive protein
EDTA	Ethylenediaminetetraacetic acid
EPOF	Early postoperative fever
EVAR	Endovascular aortic repair
Fc	Fragment, crystallizable
GEE	Generalized estimating equations
GM-CSF	Granulocyte macrophage-colony stimulating factor
HCAI	Healthcare associated infections
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IP	Interferon-γ induced protein
MCP	Monocyte chemoattractant protein
MFI	Mean fluorescence intensity
MICE	Multiple imputation with chained equations
MIG	Monokine induced by interferon gamma
MIP	Macrophage inflammatory protein
MNP	Mononuclear phagocytes
MRSA	Methicillin-resistant Staphylococcus aureus
NHS	National health system
NK	Natural killer
NPR	National patient register
OR	Odds ratio
PCT	Procalcitonin
PDR	Prescribed drug register
PMN	Polymorphonuclear neutrophils
RANTES	Regulated upon activation normal T-cell expressed and secreted

ROC	Receiver operating characteristics
RTI	Respiratory tract infection
SSI	Surgical site infection
Swedvasc	Swedish national registry for vascular surgery
TEA	Thrombendarterectomy
TNF	Tissue necrosis factor
UTI	Urinary tract infection
WBC	White blood cells
WHO	World health organization

Introduction

Historical perspective

The concept of healthcare related infections, traditionally referred to as nosocomial (Greek: "nosus" = "disease" + "komeion" = to take care of) infections, is as old as the practice of medicine itself. According to a Sanskrit textbook of medicine, *Charaka-Samhita* from c. fourth century B.C., the principles to be followed when constructing a hospital were as follows: [1]

"It should be spacious and roomy. One portion at least should be open to the currents of wind. It should not be exposed to smoke, or dust, or injurious sound or touch or taste or form or scent…After this should be secured a body of attendants of good behaviour, distinguished for purity and cleanliness of habits."

Hippocrates c. 460 – c. 370 B.C. notes, concerning the treatment of wounds that "the surgeon should aim at keeping the wound dry, that condition being a healthier one than when it is wet… Wounds should be permitted to bleed freely and be carefully cleansed." [2]

These ancient teachings seem, however, to have been generally shunned during the Middle Ages as demonstrated by a comment about the 16th century Paris hospital, Hôtel-Dieu: [1]

"A young surgeon who is bred in the Hôtel-Dieu, may learn the various forms of incisions, operations too, and the manner of dressing wounds; but the way of curing wounds he cannot learn. Every patient he takes in hand must die of gangrene."

Figure I. *Hôtel Dieu in Paris, c. AD 1500. The priest on the right is issuing the last sacraments, while a nun administers to the patient on the left. Patients often slept two, three and even four to a bed. Source: Google Images*

These conditions provided, through the force of necessity, a breeding ground for experimentation and new ways of thinking. Ambroise Paré (c. 1510-1590), considered to be the father of French surgery, broke with the tradition of cauterising traumatic wounds with red-hot iron and instead adopted the practice of ligation, débridement and dressing of wounds. [2]

It took approximately another two centuries after Paré for a return to the old principles of hospital hygiene as exemplified by an account of the newly built Edinburgh Royal Infirmary in 1739: [1]

"The beds are designed only to hold one Person… the House is kept clean and sweet; you find nothing in it to offend either your Smell or your Eye; the Patients are used with great tenderness, and the Order established in the House, gives great satisfaction."

One of the noteworthy physicians during this era, as highlighted by Selwyn (1965) was Sir John Pringle, physician to the British Army, who observed that a major cause of morbidity and mortality among soldiers were the hospitals themselves. His major contributions in this field included his insistence on improved ventilation and avoiding overcrowding in wards and indeed, beds. He also played a pioneering role in the epidemiological study of "hospital-fever" and postulated about the causes of infection in his "Experiments on Septic and Antiseptic Substances." Other notable contributions from the same period include, Alexander Gordon's theory on the transmission of "puerperal fever" by the hands and clothing of personnel. This was followed, almost five decades later, by Semmelweis in Vienna, where he used chlorine solution to "deodorize attendants' hands." Parallel to these efforts, Sir James Simpson published his seminal works on "Hospitalism" in which he charted postoperative mortality after amputaions in various healthcare institutions. One of the major conclusions of this work was the observation that the rate of mortality was proportional to the size of the institution and degree of overcrowding. [1]

The culmination of these advances can be seen in the better known and widely acknowledged practices and discoveries of Joseph Lister by desinfection of surgical wounds with carbolic acid to avoid infection and sepsis; Louis Pasteur's establishment of the "germ theory of infection" and Sir Alexander Fleming's discovery of penicillin, which revolutionized modern medicine.

Healthcare associated infections (HCAI) – clinical burden and implications

The most common complications in clinical care are acquired infections resulting from contact with healthcare facilities. The World Health Organization (WHO) defines HCAI as: "An infection occurring in a patient during the process of care in a hospital or other healthcare facility which was not present or incubating at the time of admission. This includes infections acquired in the hospital, but appearing after discharge, and also occupational infections among staff of the facility."

It is estimated that annually, over four million people in Europe are affected, resulting in 16 million extra days of hospital stay, 37 000 attributable deaths and direct costs of approximately € 7 billion. [3]

HCAIs can be broadly divided into two groups based on the source of the infectious agent: an endogenous source, which refers to the bacterial flora on the skin and the bodily cavities, or an exogenous source, which is transmitted to the patient via contact with the healthcare environment. In clinical and epidemiological practice, HCAIs are generally classified as, urinary tract infections (UTI), respiratory tract infections (RTI), surgical site infections (SSI) or, bloodstream infection (BSI). A European report from 2008 found that the most frequent type of HCAI was UTI (27%), followed by RTI (24%), SSI (17%) and BSI (10,5%). [4]

Postoperative HCAI in vascular surgery have an estimated 30-day incidence of up to 35%. [5] The use of prosthetic grafts in aortic surgery and various vascular reconstructions in the limbs implies serious consequences in the event of an infection. [6, 7] Early diagnosis and treatment are therefore of utmost importance. The steady advance of endovascular techniques, often performed on an outpatient basis, poses new challenges to the follow-up of this group of patients.

One of the most challenging aspects of dealing with HCAI, from an epidemiological point of view, is the choice of surveillance method. [8, 9, 10] Although, a prospective method is considered the gold standard, it is also the most demanding in terms of resources. [11, 12] A retrospective design requires complete databases with a high degree of validity. A major drawback is that earlier definitions and criteria cannot be changed. [13] A middle ground is sometimes chosen to get a snapshot picture of the situation by conducting a point prevalence study, as has been done in the WHO study quoted earlier. The limitations of this design are mainly due to the fact that there cannot be any follow-up of the subjects and furthermore, historical comparisons can be difficult to interpret due to shifting trends in diagnosis and treatment. A relatively novel approach for circumventing the problem of post-discharge follow-up is by using a surrogate marker, such as antibiotic prescriptions, to track the postoperative course for each patient. [14, 15,16]

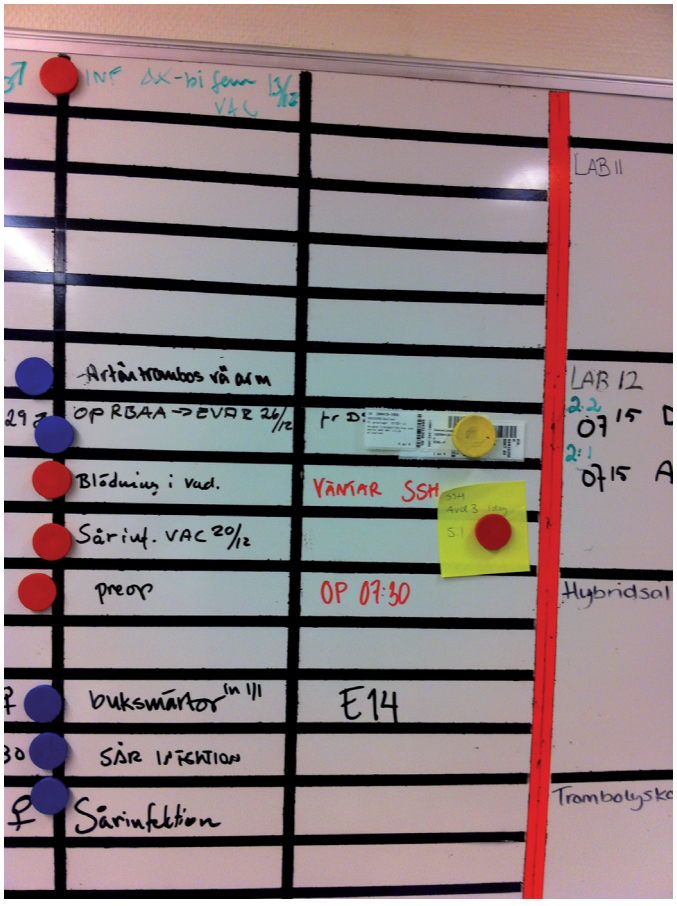

Figure II. *List of patients at the Vascular Surgery ward, Karolinska University Hospital, Christmas 2013. Four of the eight vascular patients were admitted due to surgical site infections*

The main purpose of a surveillance program, irrespective of the method used, is to provide a vision of reality, which can be used to improve the measured outcomes. A study by *Haley et al* from 1985 showed that feedback to healthcare personnel could reduce HCAI rates by 32%.

Surgical site infections (SSI)

The vulnerability of vascular procedures to postoperative infections and specifically, the susceptibility of infrainguinal procedures to SSI warrant a more detailed look at this particular type of HCAI. [17, 18] The Centers for Disease Control and Prevention (CDC) defines SSI as either incisional or organ/space. Incisional SSIs are further divided into Superficial and Deep incisional SSIs occurring within 30 days of the operation. Organ/space SSIs involve other parts of the anatomy, other than the incision, manipulated during the operation. [19]

Table I. Adapted from Centers for Disease Control and Prevention (CDC) Criteria for Surgical Site Infection (SSI), Horan TC et al 1992

Superficial Incisional SSI

Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following:
1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.
3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat and superficial incision is deliberately opened by surgeon, unless incision is culture-negative.
4. Diagnosis of superficial incisional SSI by the surgeon or attending physician.

Do not report the following conditions as SSI:
1. Stitch abscess (minimal inflammation and discharge confined to the points of suture penetration).
2. Incisional SSI that extends into the fascial and muscle layers (see deep incisional SSI).

Deep incisional SSI

Infection occurs within 30 days after the operation if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves deep soft tissues (e.g., fascial and muscle layers) of the incision and at least one of the following:
1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site.
2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38ºC), localized pain, or tenderness, unless site is culture-negative.
3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
4. Diagnosis of a deep incisional SSI by a surgeon or attending physician.

Organ/space SSI

Infection occurs within 30 days after the operation if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves any part of the anatomy (e.g., organs or spaces), other than the incision, which was opened or manipulated during an operation and at least one of the following:
1. Purulent drainage from a drain that is placed through a stab wound into the organ/space.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space.
3. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
4. Diagnosis of an organ/space SSI by a surgeon or attending physician.

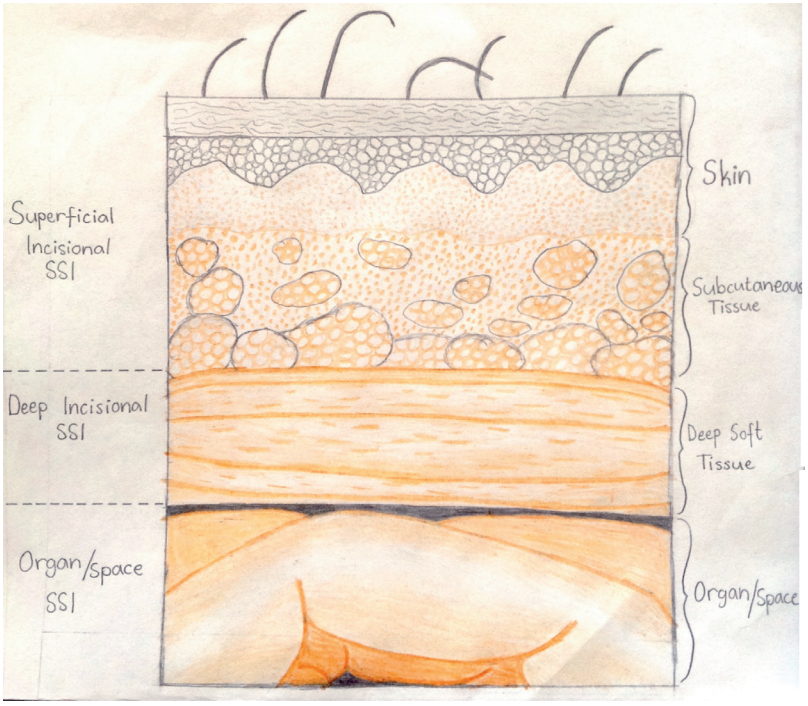

Figure III. *Three categories of SSI, adapted from Centers for Disease Control and Prevention (CDC), illustrated by Hanna Daryapeyma*

Other classification systems, specific to vascular surgery include Szilagyi [20], Samson [21], and Karl-Storck [22] that put more emphasis on the eventual involvement of vascular grafts and anastomoses.

Table II. SSI classification systems in vascular surgery

Class	Szilagyi	Samson	Karl-Storck
1	Infection involves only the dermis	Infection extends no deeper than the dermis	Superficial infection without involvement of the graft
2	Infection extends into the subcutaneous tissue but does not involve the arterial implant	Infections involve subcutaneous tissues but do not come into grossly observable contact with the graft	Partial graft infection without involvement of the anastomosis
3	Infection involves the arterial implant	Infections involve the body of the graft but not at an anastomotic site	Involvement of the anastomosis and the suture line
4		Infection surrounds an exposed anastomosis, but bacteremia or anastomotic bleeding has not occurred	Wound disruption and complete exposure of the graft/patch
5		Infection involves a graft-to-artery anastomosis and is associated with septicemia and/or bleeding at the time of presentation	1-4 with concomitant septic bleeding/ pseudoaneurysm
6			1-4 with graft thrombosis or septic embolization

Pathogenesis of SSI

The development of SSI is contingent on the interplay among three factors: microbial, patient and surgical characteristics. Endogenous contamination from the patient's skin flora is the most common cause of surgical wound contamination. Other microbial related factors include the bacterial load and virulence. [23] Inclusion of prosthetic materials increases the susceptibility of the wound to infection as the number of organisms needed to cause an infection decrease. [24] The virulence of the organisms is dependent on intrinsic factors such as, the ability to bind to tissues and protect themselves from the onslaught of the immune system. The formation of a glycocalyx biofilm is one such defence mechanism. [25, 26] The patient characteristics that can influence the emergence and outcome of SSI include age, comorbidities e.g. diabetes mellitus and obesity and concomitant medications such as

immunosuppressants. [27, 28, 29] The surgical characteristics relevant to SSI are length of surgery, type of wound and the need for blood transfusion. [30, 31, 32]

Microbiology of SSI
The most prevalent bacteria isolated from SSI in vascular surgery patients are Gram-positive bacteria, in particular Staphylococcus aureus, which is reported to account for up to 80% of infections. [17, 33] Chronic graft infections with late clinical presentation are usually caused by S.epidermidis and it is estimated that approximately 20% of vascular SSI are caused by Gram-negative bacteria, which include Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia. The pattern of infections is subject to geographical variations and different practices regarding the use of antibiotics. Several countries in Europe and North America have seen a rise in the number SSI caused by methicillin-resistant Staphylococcus aureus (MRSA). [34]

Laboratory markers of infection

The immune system can be broadly divided into two main branches - the specific and the native or innate. The former possesses specificity and memory but is not initially efficient in eliminating bacteria, unless previously primed. The native immune system includes among other cell types, monocytes and polymorphonuclear neutrophiles (PMN). PMN are mainly involved in phagocytosis and are regulated by cytokines released by other immune competent cells. The neutrophil is the main circulating phagocyte that is delivered to a site of infection. Its function is regulated by changes in the cell membrane that are in turn affected by various receptors. [35]

Cytokines
Cytokines are a group of polypeptides secreted by cells in response to injury and have a central role in mediating the inflammatory response. Their purpose is to organize a concerted response to tissue trauma, which involves recruitment of cells to the site of injury and instigation of a systemic response. [36] Cytokines can generally be classified according to their effects on the inflammatory process e.g. stimulatory or inhibitory. Pattern recognition receptors, such as toll like receptors, recognize bacteria and stimulate the secretion of pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, IL-8, and tissue necrosis factor- α (TNF-α) [37] IL-1 (α/β) and TNF (α/β) are pro-inflammatory cytokines that can induce fever and stimulate the synthesis of IL-6 which in turn sets into motion a cascade of pro-inflammatory and self-regulating anti-inflammatory responses which steer the immune response.

C-reactive protein (CRP)
Tissue damage and acute inflammation, whether caused by surgical trauma or infection, elicits an acute response, which involves the secretion of acute phase proteins, of which CRP is one of the most scrutinized. Its name was derived from its ability to bind the C-polysaccharide of Streptococcus pneumonia and was the first pattern recognition receptor to be identified. [38, 39, 40] CRP production in the liver is induced mainly by IL-6, but also IL-1 and TNF-α. [41] CRP has a high affinity for phosphocholine residues on damaged or dead cells as well certain microorganisms. The complex formed by CRP and the bound ligands

triggers the complement system through recognition by C1q and leads to the stimulation of phagocytosis. Up-regulation of CRP synthesis is rapid (within hours) and its plasma half-life, which remains constant under most conditions, is about 19 hours. [38]

Quantitative measurements of plasma CRP continue to form the corner stone of clinical evaluation of infection, however, due to insufficient sensitivity and specificity, they cannot stand on their own.

Procalcitonin (PCT)

PCT is the prohormone to calcitonin, normally produced by the C-cells of the thyroid in response to hypercalcemia. Its normal circulating levels in plasma are extremely low and it has a half-life of 20-24 hours. In response to inflammatory stimuli such as bacterial endotoxins, procalcitonin is released from non-neuroendocrine parenchymal cells in the liver, lungs and the intestine. [42, 43] Studies have shown that PCT production is rapidly upgraded reaching readily detectable levels within 6-12 hours in response to tissue damage. [44] The use of PCT as a routine laboratory marker for bacterial infections has been hindered by its relative insensitivity to local bacterial infections and a general increase in plasma concentrations in the early postoperative period. [45, 46, 47]

Neutrophil CD64 (FcγRI)

The neutrophil CD64 (cluster of differentiation 64) receptor, also known as FcγRI, is a glycoprotein that binds monomeric immunoglobulin G (IgG)-type antibodies with high affinity. [48] CD64 is a potent cytotoxic trigger expressed exclusively on mononuclear phagocytes and activated polymorphonuclear (PMN) leucocytes. [49] PMN CD64 analysis has been shown to have a high sensitivity and specificity for the early detection of bacterial infection. [50, 51] The up-regulation of CD64 receptors is modulated partly, by the release of interferon- γ (IFN-γ) from natural killer cells (NK-cells) exposed to IL-15 and IL-18. [52]

Aims of the thesis

The general aim of this thesis has been to chart the scope of postoperative infectious complications in vascular surgery, especially after lower extremity procedures, and to evaluate methods for both early diagnosis of infection and extensive follow-up of patients.

Accordingly, the work can be divided into two parts:

1. Epidemiological studies to reveal the extent of postoperative HCAI on a nationwide scale and to evaluate the feasibility of post-discharge follow-up using the available national databases.

2. Clinical investigations of possible new laboratory markers for bacterial infections after vascular surgery.

The chronological order of the studies, however, does not follow the abovementioned division into two phases, but rather, reflects the order of my interest and progression in this field. The order of presentation of the studies in this thesis has, therefore, been arranged to follow a more logical thread.

The specific aims of the studies were as follows:

- To evaluate the rate of healthcare associated infections (HCAI) and related risk factors in patients treated for lower extremity arterial disease on a national basis, thereby providing a means of assessing the choice of treatment and likely outcomes for different patient categories (Study I).

- To evaluate the clinical utility of quantitative CD64 measurements to differentiate between inflammation in response to surgical trauma and postoperative bacterial infection (Study II).

- To assess the cytokine response to the acute inflammation caused by vascular surgery and to evaluate the potential for the clinical utility of quantitative measurements of cytokines as markers for infection in conjunction with CRP and CD64 (Study III).

- To investigate the rate and pattern of HCAI and related risk factors in patients treated for lower extremity arterial disease on a national basis using antibiotic prescription as a surrogate marker for postoperative HCAI (Study IV).

Methods and Patients

The two epidemiological studies were retrospective analysis of prospectively collected data from national databases and the two clinical studies were single-centre, prospective cohort studies.

Table III. List of studies

Study	Design	Method	Place & Date
I	Nationwide retrospective cohort	Cross-matching of national databases	Stockholm, 2014
II	Single-centre prospective cohort	Consecutive inclusion of eligible subjects	Bergen, 2009
III	Single-centre prospective cohort	Consecutive inclusion of eligible subjects	Stockholm/Bergen, 2014
IV	Nationwide retrospective cohort	Cross-matching of national databases	Stockholm, 2015

The epidemiological studies (I and IV)

Design
Retrospective analysis of prospectively collected data from the Swedish National Vascular Surgery registry (Swedvasc), National Patient Registry (NPR), Prescribed Drug Register (PDR) and Cause of Death Register.

Population
All patients who underwent non-emergent primary infrainguinal surgery, open or endovascular, for lower extremity arterial disease between January 2005 and December 2010 were included in the study. Patients with secondary procedures, reoperations or combined endovascular and open surgery were excluded (n = 2 763). Only primary surgery/intervention in the time span for each patient was included in the analysis. 10 547 patients were included in Study I and 9 894 in Study II.

Data sources

The Swedish National Registry for Vascular Surgery (Swedvasc), the Swedish National Patient Register (NPR), the Prescribed Drug Register (PDR) and the Cause of Death Register were used as data sources to collect information about patients who have undergone either open or endovascular lower extremity revascularization during the study period. Using the subjects Civic Registration Number (CRN), which is registered at every contact with the health authorities, facilitated the cross- matching of data between different databases. The external validity of Swedvasc has been shown to be over 90%. [53] All contacts with healthcare providers whether hospitals or community care providers are registered at the NPR. According to a recent report, the rate of missing main diagnosis code was 0.9% for hospital admissions and 10% for primary care contacts.[54] In Sweden, antibiotics can only be obtained through a medical prescription at certified pharmacies. Starting 2005, the Prescribed Drug Register (PDR) contains data (type, dose, total amount and time) on all dispensed prescriptions, on an out-patient basis and is linked to the CRN, providing basic demographic data such as age and gender. Drugs prescribed and administered in-hospital are not included in the register. All deaths, including their causes are registered in the national Cause of Death Register.

Data management

Data regarding demographics, presenting symptoms, comorbidities/risk factors, type of procedure and follow-up visits at 30-days and one-year were collected from Swedvasc. These were cross-matched with the NPR and the Cause of Death Register in the first study and NPR and PDR data in the fourth study. The Swedvasc data were stratified according to severity of lower extremity symptoms and types of procedure. NPR data regarding postoperative complications were categorized into skin-and-soft-tissue infections, including SSI, urinary tract infections (UTI), pneumonia and sepsis. PDR data - regarding antibiotic prescription and dispensation - in the fourth study were classified into three categories based on their main therapeutic indications and time of dispensation relative to the index operation (a six-month period leading up to the index operation and a six-month period after the operation for each patient (Fig. II). The postoperative prescription data were further divided into two groups; the first pertaining to the one-month period after the operation and the second to the remaining five months).

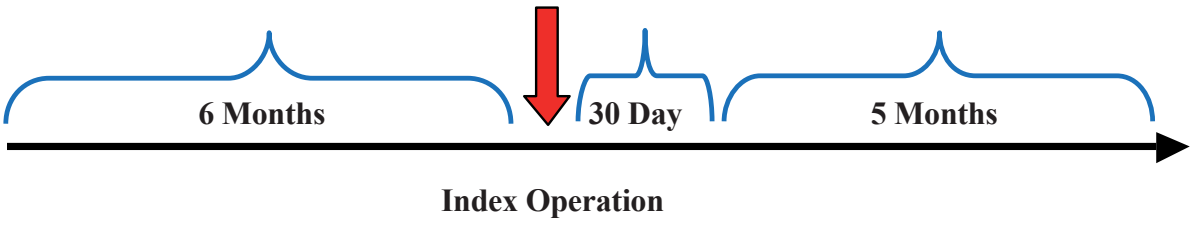

Figure IV. *Time axis for antibiotic prescriptions*

Statistical methods

Study I

Data were presented as the number and proportion of patients for each independent variable both as a total and the proportion with an infection within 30 days.

The impact of the selected variables on all types of infections within 30 days were analysed using logistic regression – with both univariate and multivariate models.

To account for missing data in the variables, all analyses were performed using multiple imputation with chained equations (MICE)**,** [55] under the assumption that data were missing at random, conditional on the selected variables and the infection outcome.

Estimated odds ratios of amputation within one year and death within 30 days were analysed using univariate and multivariate logistical regression analyses for patients with and without 30-d infection and presented with 95% confidence intervals and p-values.

Study IV

To measure frequency of antibiotic purchases we used incidence rates (number of purchases/day). A Poisson model was used for comparisons between groups unless overdispersion was present, in which case we used a negative binomial model. Stratification was used to account for the effect of other variables.

When comparing periods, the groups could not be considered independent since the same persons were followed both before and after operation. A generalized estimating equations (GEE) framework was used to adjust for this dependency.

$P < .05$ was considered to be statistically significant.

Methodological considerations (Study I and IV)

The advantages of a retrospective analysis and cross-matching of national databases with a high degree of validity are in the large numbers provided by the multicentre nature of the data which avoid the type of potential bias posed by regional differences and smaller numbers which can affect sub-group analyses. The major disadvantages are inherent to the retrospective nature of registry-based studies, especially regarding missing data and the type of variables recorded. Plausible alternative methods could include a nested case-control study, representing a more cost-effective method, which would provide us with a ratio of incidence rates instead of the absolute rates of outcome provided by the longitudinal cohort study. A point-prevalence study would also be a tenable alternative providing us with a snapshot of the situation, which could be used as a proxy for the longitudinal data.

The clinical studies (II and III)

Design
Single-centre, prospective cohort studies.

Population
During the period 2006-2010, patients undergoing elective vascular surgery, comprised of carotid endarterectomy (CEA), carotid artery stenting (CAS), open aortic repair (AAA-OR), endovascular aortic repair (EVAR), aortoiliac/aortobifemoral bypass and open infrainguinal procedures, were consecutively included. Study II included 153 patients and Study III 96 patients.

Laboratory methods
Peripheral venous blood samples were taken preoperatively on admission and postoperatively during the first 24 hours. The samples were analysed for C-reactive protein (CRP), total leukocyte counts, serum-procalcitonin and neutrophil CD64 expression. The analyses for CRP (mg/L), leukocyte counts ($x10^9$/L) and s-procalcitonin (μg/L) were performed using well-established laboratory protocols on the Cell Dyn Sapphire Hematology Analyzer®.

CD64 expression was measured using the LK64-H-Assay™ kit produced by Trillium Diagnostics, LLC on the Cell Dyn Sapphire Hematology Analyzer®. The kit contains a mixture of two CD64 specific monoclonal antibodies (clones 22 and 32.2) and one CD163 specific monoclonal antibody (Mac2-158) in addition to a fluorescence bead suspension for standardisation of CD64 quantification. The use of two different antibodies to CD64 and the inclusion of an antibody to CD163, a monocyte specific antigen, increase the sensitivity and specificity of the assay. The analysis required 50μL of EDTA anticoagulated whole blood drawn peripherally and included a 10-minute incubation period. The samples were analysed individually and the data output from the Cell Dyn Sapphire Hematology Analyzer® was then fed into the Leuko64 QuantiCALC™ software which reported the results as a neutrophil CD64 index (PMN-MFI (Mean Fluorescence Intensity) x Bead Calibration Factor / Beads-MFI).

Cytokines in serum were detected using the Luminex immunobead technology, 25-plex-kit (Invitrogen/Biosource, Carlsbad, CA, USA). In short, antibody-coupled beads were incubated with target analyte after which they were incubated with biotinylated detection antibody before finally being incubated with streptavidin–phycoerythrin. Samples were then read by the Luminex's laser based fluorescent analytical test instrument Luminex® 100™ (Luminex Corporation Austin, TX, USA).

Blood and urine cultures were taken on the suspicion of infection. The diagnosis of infection, whether pre- or postoperative, was based on either a positive culture result or the presence of a clinically manifest wound infection. Fever was defined as body temperature ≥ 38° C.

Staistical methods

Study II

The paired t-test was used for intra-group analyses and the Mann-Whitney U-test was used for inter-group comparisons and ROC curve analysis was used for establishing cut-off points, sensitivity and specificity for CRP, procalcitonin and CD64.

Study III

The Pearson's r, regression analysis, student's t-test, paired samples test, the Kruskal-Wallis, receiver operating characteristics (ROC) analyses and logistical regression were performed as indicated.

Methodological considerations (Study II and III)

The main obstacle in studies attempting to evaluate new diagnostic methods or markers for infection is the confirmation of the presence or absence of infection in a clinical setting. The gold standard is considered to be a positive culture result, which unfortunately can be elusive. A standardized and validated protocol for clinical evaluation can function as an effective substitute. The designs of the two clinical studies could have been further strengthened by the inclusion of a previously defined control group and a more homogenous cohort in regards to exposure or type of operation.

Results and Discussion

Study I

The presenting symptoms based on the degree of lower extremity ischaemia were divided into claudication 27% (n=2 827), critical limb ischaemia without ulceration (CLI) 17% (n=1 835) and gangrene 56% (n=5 885). The treatment modalities consisted of endovascular intervention 59% (n=6 266), thrombendarterectomy (TEA) 10% (n=1 061) and bypass surgery 31% (n=3 224).

The total incidence of postoperative infection (< 30-d) was 9.7% (n=1 019) including:

- skin-and-soft-tissue infection 6.9%, including surgical site infection (SSI
- urinary tract infection (UTI) 1.6%
- pneumonia 1.1%
- sepsis 0.9%.

The incidence of postoperative infections according to the degree of lower extremity ischaemia was: claudication 4.1%; CLI 7.5% and gangrene 13%. The rates of postoperative infection based on type of procedure were: endovascular 7.8%; TEA 9.3% and bypass surgery 13.3%. The incidence of diabetes was 49% in the gangrene group, compared to CLI 28% and claudication 24%.

In both claudicants and patients with CLI, TEA was associated with a higher rate of infection compared to endovascular procedures (OR, 6.7; 95% CI, 3.9 – 11.6, $P < .001$ and OR, 2.0; 95% CI, 1.1 – 3.7, $P < .001$, respectively). The odds increase was even greater when comparing bypass surgery to endovascular for claudicants (OR, 8.4; 95% CI, 5.0 – 14, $P < .001$) and CLI patients (OR, 3.4; 95% CI, 2.3 – 5.1, $P < .001$) (*Table IV*).

Table IV. Multivariate analysis of degrees of lower extremity ischaemia and operative method associated with postoperative healthcare associated infections

Procedure	Characteristics	Multivariate OR (95% C.I.)	P-value
Bypass *vs.* Endovascular	Claudication	8.38 (5.03-13.95)	< .001
	CLI	3.41 (2.29-5.10)	< .001
	Gangrene	1.39 (1.18-1.65)	< .001
TEA *vs.* Endovascular	Claudication	6.71 (3.89-11.55)	< .001
	CLI	2.03 (1.12-3.69)	0.019
	Gangrene	1.04 (0.75-1.46)	0.808

OR, Odds ratio; CI, confidence interval; CLI, critical limb ischaemia; TEA, thrombendarterectomy

The overall one-year major amputation rate of 6.2% was significantly associated with 30-day infection (OR, 2.25; 95% CI, 1.78-2.83, $P < .001$). Patients without postoperative infection had an amputation rate of 5.6%, whereas patients with postoperative infection had an amputation rate of 11.8%. Based on multivariate analysis, there were also significant associations with diabetes (OR, 1.58; 95% CI, 1.3-1.92, $P < .001$) and cerebrovascular disease (OR, 1.41; 95% CI, 1.13-1.76).

The overall 30-d mortality rate (2.7%) was associated with 30-d infection (OR, 1.7; 95% CI, 1.22-2.37, $P = .002$). The degree of severity of lower extremity ischaemia was associated with increased mortality: CLI *versus* claudication (OR, 8.28; 95% CI, 3.54-19.83, $P < .001$); gangrene *versus* claudication (OR, 20.42; 95% CI, 9.07-45.94, $P < .001$). Patients without postoperative infection had a mortality rate of 2.5%, whereas patients with infection had a mortality rate of 4.2%. Using multivariate analysis, the predictors of 30-d mortality were degree of lower extremity ischaemia, renal insufficiency and heart disease (*Table V*).

Table V. Multivariate analysis of risk factors in patients undergoing lower extremity revascularization and association to 30-day mortality

Risk Factors	Multivariable model OR (95% C.I.)	P-value
Diabetes	1.00 (0.87- 1.16)	0.997
Hypertension	1.00 (0.86- 1.17)	0.988
Renal insufficiency	1.27 (1.06- 1.53)	0.009
Cerebrovascular disease	1.11 (0.93- 1.33)	0.252
Heart disease	1.15 (1.00-1.33)	0.049
Lung disease	1.37 (1.14- 1.65)	< .001
Smoking	0.91 (0.77- 1.08)	0.280
Age 60-69 *vs.* <60	0.83 (0.61- 1.12)	0.224
Age 70-79 *vs.* <60	0.86 (0.64- 1.16)	0.320
Age >79 *vs.* <60	0.79 (0.58- 1.07)	0.120
Female gender	1.05 (0.92-1.21)	0.467

OR, Odds ratio; CI, confidence interval

We have shown that both the degree of ischaemia and the presence of preoperative comorbidities have a significant impact on the rate of postoperative HCAI and by extension, on the rates of 30-day mortality and one-year amputation. The choice of operative method is also significantly associated with the risk of developing postoperative infectious complications.

The majority of the HCAI consisted of surgical site infections, however, the combined impact of UTI, pneumonia and sepsis were also quite substantial. Endovascular interventions are generally associated with fewer infectious complications, 7.8% compared to TEA 9.3% and bypass 13.3%. The choice between a bypass operation and endovascular treatment for a claudicant translates into an 8-fold increased risk of developing an HCAI after bypass surgery.

The total incidence of postoperative HCAI in this study (9.7%) is somewhat lower than the rates of SSI after lower extremity revascularisation as reported in other studies.[56, 57] We suspect the main reason for this discrepancy is the lack of a unified definition for SSI and a systematic underreporting of postoperative infections in outpatient care. Compared to studies that have looked at HCAI after open vascular surgery [58], the rate of infection in this study is considerably higher, presumably due to the chosen cohort of infrainguinal procedures and the inclusion of patients with gangrene.

This study has several weaknesses, which are primarily a result of the retrospective design of the study and the registries used for data collection. Certain parameters, such as body mass index (BMI)/obesity, length of operation, and length of hospital stay were not included in the databases. [59]

Study II

The patients were divided into four groups based on the presence or absence of infection pre – and postoperatively (*Figure V*).

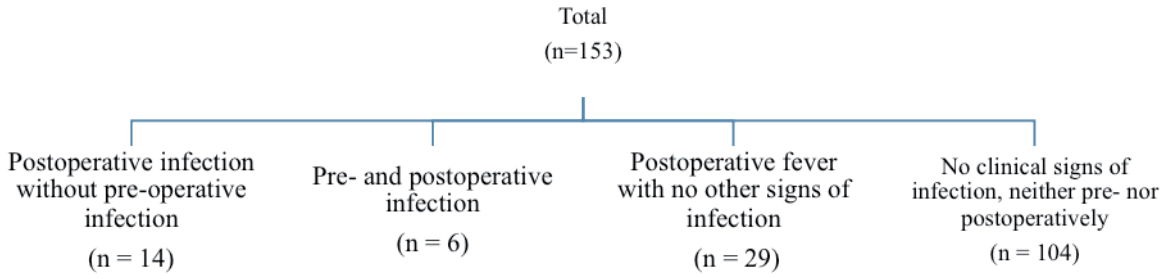

Figure V. *Classification of Patients in Study II*

In the group with postoperative infection (n = 14), the infectious agent was successfully identified in blood and/or urine cultures in five of the 14 cases. For the other nine cases, no infectious agent was isolated, but there was a clinical suspicion of infection and all but one patient responded to antibiotics, the non-responder was diagnosed with ischaemic colitis at laparotomy.
In group 1, post-operative infection only, all four markers were significantly increased in the
24h after surgery: CD64 (P = 0.001); CRP (P = 0.001); WBC (P= 0.002); PCT (P = 0.012). Results for those with post-operative fever only (group 3) were similar. CD64 values were

significantly higher, both pre- and post-operatively than for patients with no pre- or postoperative infection (Table 1). Postoperative values of s-procalcitonin were also higher than those for patients with no infection (Table 1).
In group 2, pre- and postoperative infection, there was no significant difference in the CD64 (P = 0.12, WBC (P = 0.25), and PCT (P = 0.14) values, whereas a marginal significance was shown for CRP (P = 0.046). The postoperative CD64 values were lower than the corresponding
preoperative values in two cases in this group. Post-operative values of CD64 were higher than for patients with no pre- or post-operative infection.
Based on the ROC curve analysis of the postoperative values for CD64, CRP and PCT for the combined group of patients with postoperative infections, CD64 as a diagnostic marker, had a sensitivity of 93% and a specificity of 69% (cut-off: 1.03), compared to CRP which had a sensitivity of 79% and a specificity of 80% (cut-off: 82) and PCT with a sensitivity of 64% and a specificity of 78% (cut-off: 0.26).

The first aim of the study was to establish the effect of the inflammatory response due to surgical trauma on the degree the CD64 expression on neutrophils. Analysis of the pre- and postoperative values of CD64 in Group-4 (no infections), showed a significant difference between these values (range: 0.84 – 0.93). However, this increase, presumably due to surgical trauma can be considered of little clinical significance, since the range of values fell short of the cut-off value (1.03). It can therefore be argued, from a clinical point of view, that the effect of surgical trauma on CD64 expression in non-infected patients is negligible.

The second aim of the study was to evaluate the clinical utility of CD64 measurements in diagnosing bacterial infections postoperatively.
In the group with postoperative infection (n = 14) which included five proven cases of infection based on culture results and nine cases based on clinical signs, there was a clear tendency toward higher preoperative CD64 values as represented by the borderline mean value (0.99) for the whole group. There was also, as expected a clear and statistically significant increase in the postoperative values of CD64 correlating with the postoperative infections. In Group –2 (n = 6), the CD64 values indicated the presence of bacterial infection both pre- and postoperatively (*Table VI*). Interestingly, there was no significant increase in the postoperative values, which can be due to the small size of the group or the insignificant effect of trauma in the presence of a clinically significant infection. To show the extent of the effect of trauma on CD64 postoperatively we can highlight the results for two patients in this group with proven preoperative bacterial infection – one with distal gangrene and the other with an infected prosthetic graft. The patients were operated with amputation and excision of infected graft, respectively. The postoperative values of CD64 were lower than the preoperative values. This anecdotal evidence shows not only the insignificant effect of trauma on the CD64 values, but also the correlation between removing the source of infection and the fall in the CD64 index.

Procalcitonin (PCT), which is a pro-hormone to calcitonin is another marker of inflammation shown to be superior to CRP in detecting bacterial infection [60]. The usefulness of PCT measurements postoperatively, for detecting bacterial infections has not been unequivocally established. Studies carried out on patients undergoing thoracic surgery

have shown an unspecific and short-term increase following surgery [61, 62]. In one of these studies the PCT levels returned to normal (<1ng/ml) on the third postoperative day [61], and increased proportionally thereafter in response to varying types of infection ranging from mediastinitis to septic shock. Although, PCT has been shown to be a better marker of infection than CRP, its use in clinical practice has been limited in most part to critically ill patients in intensive care units where its prognostic value is inferred from fluctuations and general trends in the serum concentrations over periods of one week or longer. [60, 61, 63]
As it was our intention to evaluate the usefulness of CD64 measurements in the day to day management of patients after surgery, we chose to use the version of the Leuko64™ assay kit designed specifically for use on Abbott Cell-Dyn 4000® and Sapphire® instruments. By adopting this method of analysis, we were able to streamline the logistics for sample taking and analysis for all four variables (CRP, WBC, PCT and CD64), through running all analyses on the same instrument and thereby minimising the need for extra personnel and laboratory resources. The reliability of this method of analysis has been proven by both the producer of the kits (Trillium Diagnostics, LLC) and a published clinical study comparing the results from flow cytometri with those from Cell-Dyn 4000®. [64]

The shortcomings in this study are due to the heterogeneity of the vascular procedures and patients, the relatively low number of postoperative infections, the lack of a standardised clinical protocol for classification of postoperative SSI and the short postoperative follow-up period.

Table VI. Pre- and postoperative values of each marker expressed as a mean for the designated patient group. Neutrophil CD64 index (CD64); C-reactive protein (CRP); Total leukocyte count (WBC); serum-procalcitonin (PCT).

Infection Marker		**No Pre- or Postop. infection** (n = 104)	**Postop. infection** (n = 14)	**Pre- and Postop. infection** (n = 6)	**Postop. Fever** (n = 29)	**All Patients** (n = 153)
CD64	Preop.	0.84	0.99	1.36	0.92	0.89
	Postop.	0.93	1.42	1.68	1.34	1.07
CRP	Preop.	5.9	12	83.5	5.4	10
	Postop.	49	119	130	93	66
WBC	Preop.	7.2	7.1	14.3	7.3	7.5
	Postop.	8.8	10.4	11.3	9.9	9.2
PCT	Preop.	0.10	0.10	0.25	0.10	0.11
	Postop.	0.44	9.4	1.28	9.4	2.64

Study III

The patients were categorized into four groups as shown in *Figure VI*.

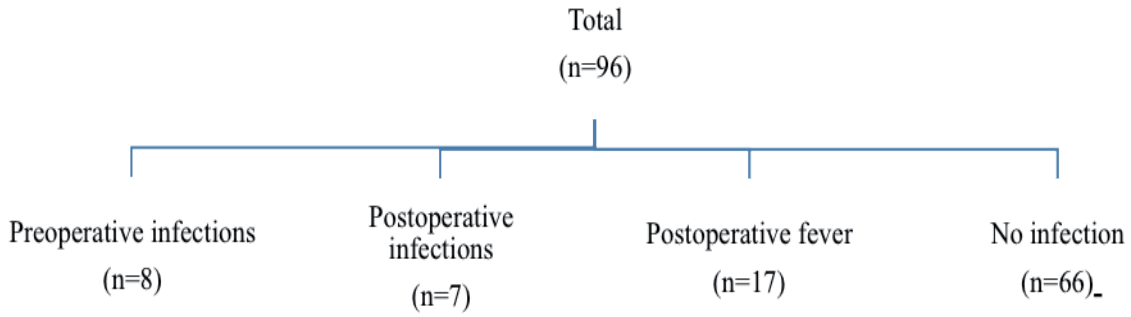

Figure VI. *Classification of patients in Study III*

The cytokines analysed in the study are presented in *Table VII*.

Preoperative versus Postoperative cytokine analyses
Eighty-eight per cent (22/25) of investigated cytokines had lower postoperative mean values compared to preoperative values. IL-6 was the only cytokine that showed a significant increase postoperatively ($P < .05$). CRP ($P < .028$) and CD64 ($P < .001$) also showed significant increases postoperatively.

Patients with postoperative infection
Associations with postoperative infection were shown for pre- and postoperative values of IL-2 and IL-13, as well as, postoperative values of MIP-1α, IP-10 and RANTES ($P < .05$).

Patients with early postoperative fever (EPOF)
Associations with EPOF were shown for pre- and postoperative values of IL-10 and IFN-α in addition to preoperative values of GM-CSF, MIP-1α and postoperative values of IL-6 and Eotaxin.

Cytokine, CRP and CD64 analyses based on postoperative infection or fever
Analysis of the postoperative values of CRP ($P < .001$) and CD64 ($P = .028$) together with IL-6 ($P = .006$) and IL-10 ($P = .02$) using logistical regression showed a significant association with postoperative infection or fever when analysed individually. Combined analysis with CRP and CD64 together with either IL-6 or IL-10 values showed a statistical significance when employing a one-tailed test.

The logistical regression analysis showed a significant predictive value for combining either IL-6 or IL-10 with CRP and CD64, in the diagnosis of postoperative infection. This implies a significant supplementary role for IL-6 and IL-10 in the postoperative diagnosis of infection.

Early postoperative fever (EPOF) described as fever (temp. > 38°C) during the first 48 hours after surgery is quite common in vascular surgery. [71] The aetiology of the fever can

be either infectious as caused by endotoxins or an acute phase response to surgical trauma, both of which are mediated by cytokines. [71, 72] Further complicating the picture, is the finding that postoperative infections do not necessarily present with fever [73], as shown in our series where only two of the postoperative infections (1case of pneumonia and 1case of UTI) were accompanied by fever. Therefore, the patients with fever but no other clinical signs of infection have been assigned to a separate group.

There was a significant change in values for eight of the 22 cytokines that showed a postoperative decrease. All eight are implicated in the pro-inflammatory response, where four (IL-1β, IL-2, IL-12p40 and GM-CSF) induce a pro- inflammatory response through the activation of T cells and mononuclear phagocytes (MNP), and the remaining four (IP 10, MIG, Eotaxin and MCP-1) function as chemoattractants for various immunocompetent cell types. A possible explanation for this, may involve the role of anaesthetic agents and surgical stress in attenuating the cytokine induced pro-inflammatory response. Previous studies have shown impaired neutrophil chemotaxis, MNP phagocytic capacity and a decline in the number of circulating NK-cells postoperatively. [74]

Due to the paucity of postoperative infections in this material and the relatively small number of surgical procedures in each category, the results do not lend themselves to subgroup analyses based on type of infection or surgical procedure and neither can we draw any conclusions about the predictive value of these cytokines. Furthermore, we cannot specify the source of the cytokines, as the samples were not differentiated into various cell type origin.

In addition to the pro-inflammatory cytokines and chemokines that have shown significant variations in this study, there was also a group of anti-inflammatory cytokines represented by IL-10 and IL-13. The role of IL-10 in bacterial disease has not been fully elucidated, but it is thought that through its inhibition of pro-inflammatory cytokines, it provides a protective function against the detrimental effects of systemic inflammation. IL-13 manifests its anti-inflammatory effects through the inhibition of IL-1β, IL-6, IL-8 and TNF-α [65]. To our knowledge, this is the first report linking IL-10 plasma levels to perioperative infection.

Table VII. Summary of the cytokines and their major functions and effects.
(IL: interleukin; PGE_2: prostaglandin E_2; GM-CSF: granulocyte macrophage-colony stimulating factor; TNF-α: tissue necrosis factor-α; IFN-γ: interferon-γ; IFN-α: interferon- α; MIP-1α/β: macrophage inflammatory protein-1α/β; IP 10: interferon-γ induced protein; MIG: monokine induced by interferon gamma; RANTES: regulated upon activation normal T-cell expressed and secreted; MCP-1: monocyte chemoattractant protein-1)

Cytokines	Function	Ref.
Pro-inflammatory		
IL-1β	Stimulates T-cell proliferation; enhances PGE_2 synthesis – induces fever	65
IL-2	Growth factor/activator for T cells, NK cells and B cells	65
IL-2R	Receptor for IL-2; enhances lymphocyte proliferation	65
IL-4	B cell proliferation; IgE production; *inhibition* of IL-1, IL-6 and IL-8	65
IL-5	Eosinophil differentiation; enhances T cell toxicity	65
IL-6	Stimulation of acute phase protein synthesis in liver; growth factor for B cells	66
IL-7	Growth factor for B cells and T cells	65
IL-12p40	Differentiation of TH1 cells. Chemoattractant for macrophages	67
IL-15	Proliferation of T lymphocyte and NK cells	65
IL-17	Stimulates T cell proliferation	65
GM-CSF	Stimulates neutrophils, eosinophils and mononuclear phagocytes	65
TNF-α	Stimulates PGE_2 synthesis; induces production of acute phase reactant proteins by the liver	65
Anti-inflammatory		
IL-1Ra	IL-1 receptor antagonist	65
IL-10	Inhibition of IFN-γ production by T cells and NK cells	65
IL-13	Inhibits the production of IL-1β, TNF-α, IL-8 and IL-6	65
Interferons		
IFN-α	Anti-viral and anti-proliferative activity	68
IFN-γ	Activation of macrophages; regulation of PMN CD64 expression	69
Chemokines		
IL-8	Mediates recruitment and activation of neutrophils	65
MIP-1α	Induce monocyte and T lymphocyte migration	65
MIP-1β	Induce monocyte and T lymphocyte migration	65
IP 10	Chemoattractant for MNP, T cells, NK cells and dendritic cells	65
MIG	T cell chemoattractant	65
Eotaxin	Eosinophil chemoattractant	70
RANTES	Chemoattractant for monocytes, eosinophils, basophils and mast cell	70
MCP-1	Chemoattractant for neutrophils, monocytes and lymphocytes	65

Study IV

The indications for intervention were divided into claudication 27% (n= 2 659), CLI 17% (n=1 681) and gangrene 56% (5 552). The treatment modalities were comprised of endovascular intervention 59% (n=5 865), TEA 13.5% (n=1 332) and bypass surgery 27% (n=2 697).

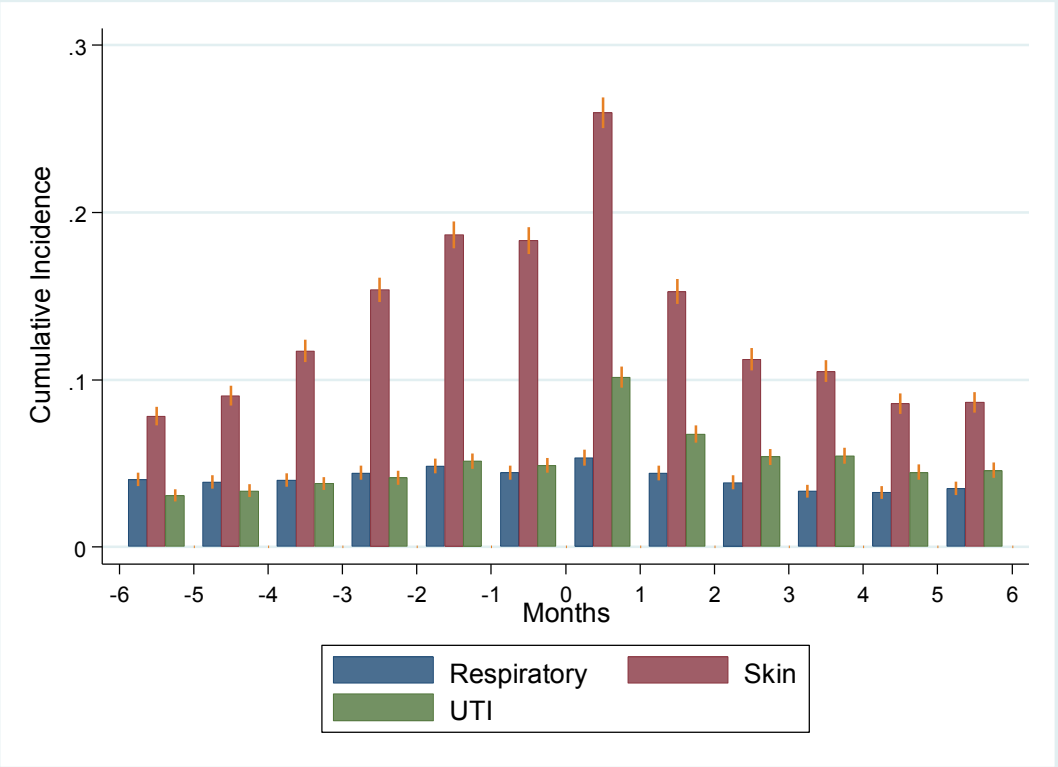

Figure VII. *Total antibiotic prescriptions*

There was a 92% increase in incidence rate for the 1-month postoperative period compared to the preoperative period (P < .001) followed by a 9% decrease in the incidence rate for the extended (1-6 months) postoperative period (P < .001).

Patients undergoing endovascular interventions had a 52% increase in the rate of dispensed antibiotic prescriptions postoperatively, compared to the preoperative period and a 15% decrease during the extended postoperative period.

The overall incidence of 30-day postoperative antibiotic prescription per patient was 33%. These were comprised of antibiotics for skin-and-soft-tissue infections 67%, UTI 21%, and respiratory tract infections (RTI) 12%. During the month leading to the index operation, 32% of patients with postoperative antibiotics had received an antibiotic prescription.

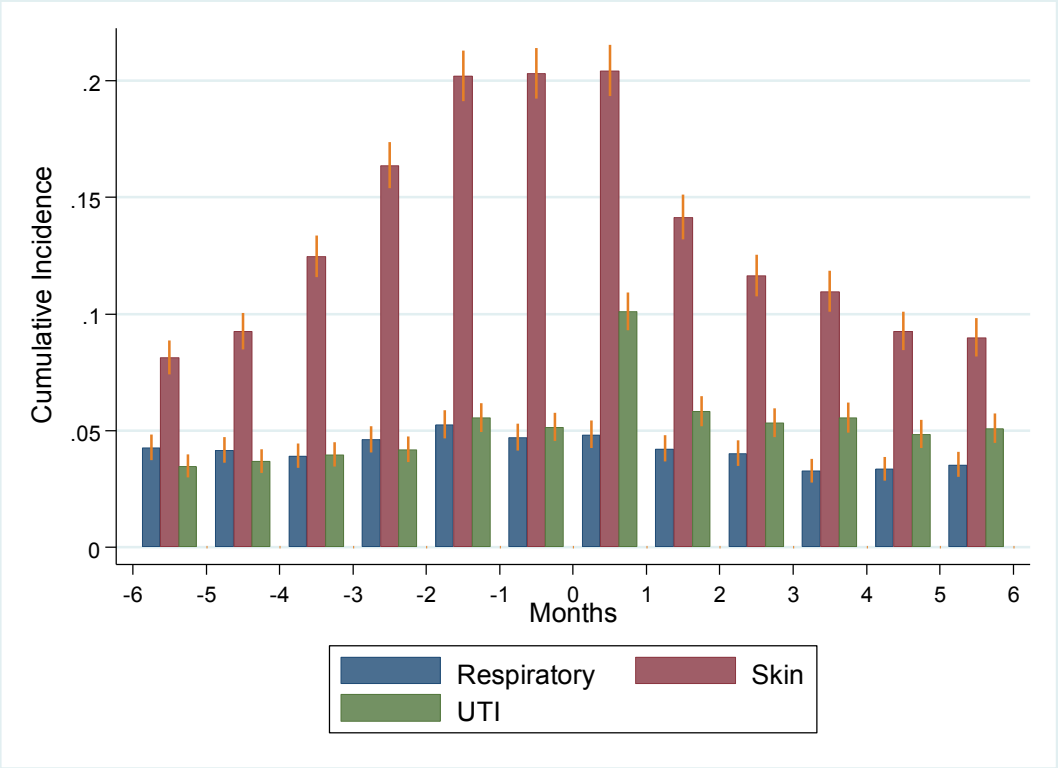

Figure VIII. *Antibiotic prescriptions for the endovascular group.*

In the endovascular group, there was an increase in the incidence of UTI-antibiotics prescriptions during the 30-day postoperative period compared to the preoperative period ($P < .001$). In the subgroup analysis, UTI-antibiotics dominated the postoperative 30-day period for claudicants ($P < .001$), as opposed to skin-and-soft-tissue antibiotics, which were prominent in the ulceration/gangrene group ($P < .001$), during the same period. Patients with CLI without ulceration showed an increase in both skin-and-soft-tissue ($P = .016$), and UTI-antibiotics ($P < .001$) postoperatively, compared to the preoperative period.

Analysis of the bypass group showed a 30-day postoperative increase in the skin-and-soft-tissue and UTI-antibiotics prescriptions relative to the preoperative period for all three categories of lower limb ischaemia ($P < .001$).

By cross-matching the national vascular and the prescribed drug registers we have sought to use antibiotic prescription and dispensation as a surrogate marker for identifying postoperative infections. One-third of the patients in our cohort received an antibiotic prescription during the first month after surgery. Patients undergoing endovascular revascularization have a substantial increase in the burden of infection postoperatively, partly explained by UTI-antibiotics. This is best demonstrated by the results for claudication patients, which showed a 62% increase in the cumulative incidence rate of total antibiotic prescriptions during the 30-day postoperative period compared to the preoperative period. This highlights the importance of post-discharge surveillance for endovascular patients.

Disregarding the third of the patients with preoperative antibiotics, there still remains a group of patients, comprising more than a fifth of the whole cohort, who received antibiotics after discharge.

The extrapolation from dispensed antibiotic prescription to an actual HCAI-event and by extension the categorization of a particular infectious complication is admittedly, contentious. As we have already mentioned, the use of antibiotic prescriptions, can at best be considered a surrogate for clinical diagnosis. The defining criterion for diagnosis of an infectious complication is the treating physician's clinical judgment, which can, in certain circumstances lead to a risk of antibiotic over-prescription.

In addition to the limitations posed by the retrospective analysis of registry data, a further point of concern is the classification of the different types of postoperative HCAI based on the type of antibiotics. This is based on the assumption that most physicians follow the common practice of treating uncomplicated cases of RTI, UTI and SSI with specific groups of antibiotics best suited for the particular infection. The resulting classification is far from exact due to an inevitable overlapping of indications for certain types of antibiotics.

General Discussion and Future Perspectives

The somatic, psychological, and socioeconomic burden of healthcare associated infections in vascular surgery can be debilitating, for both the healthcare system and the individual. Patients with postoperative SSI often require prolonged periods of in-hospital care or readmission resulting in loss of productivity and income, increased costs and varying degrees of psychological impairment. More serious cases of infection involving prosthetic aortic grafts can lead to major reoperations and/or life-long treatment with antibiotics or antifungal medications.

The topics discussed in this thesis touch upon two of the main challenges in tackling this subject. The issue of surveillance and follow-up of patients, as discussed in studies I and IV are of importance to the healthcare system and form the backbone of a process in which data collected and analyzed are disseminated to the institutions, leading to decisions concerning corrective and preventive measures, which can then be evaluated by the surveillance systems.

Surveillance strategies can vary depending on the setting and the available resources. [75, 76] There are, however, certain requirements that need to be met in order for a surveillance method to be of value. These requirements, listed in a 2002 WHO report on the *"Prevention of Hospital-acquired infections – a practical guide"* are as follows:

- *simplicity* – aiming to minimize costs and efforts to promote participation
- *flexibility* – to allow adaptive changes when needed
- *acceptability* – judged by the quality of data
- *consistency* – by using standardized definitions and protocols
- *sensitivity* – requiring consistency and representativeness of the collected data
- *specificity* – requiring standardized and precise definitions and trained personnel

Active surveillance methods can be broadly divided into prevalence and incidence studies. Prevalence studies are usually carried out in single healthcare institutions and provide a snapshot of the current status. They are relatively cheap, fast and less labour intensive than longitudinal studies. Incidence or continuous studies are generally more cumbersome but provide a range of information such as rates of infection and associated risk factors that are necessary in detecting patterns and implementing preventive measures.

The results from Study I, which showed a 30-day incidence rate of 9.7% for postoperative infections, were suspected of underestimating the actual rate. In study IV, using the same cohort and national registries (NPR/Swedvasc), we used antibiotic prescription as a surrogate for post-discharge surveillance for HCAI. Our findings from Study IV showed a 30-day incidence rate of HCAI more than double the previous results. The observed difference in the rates of 30-day HCAI may to some extent be explained by differences in coding and classification in the registries, but cannot fully account for the substantial discrepancy between the rate of antibiotic prescriptions and the recorded diagnoses of infection.

The retrospective register based methods used in this work have the advantage of reducing the cost and workload in identifying the rates and patterns of infection and associated risk factors. Using antibiotic prescription and dispensation for post-discharge follow-up can be an extremely cost-effective method for both identifying the scope of the problem and evaluating the effects of procedural improvements. As mentioned earlier, the applicability of the results and conclusions from these studies are directly related to the quality of data in the registries. As such, the Swedish national registries provide an invaluable source of data for quality control and follow-up of vascular patients.

The other main challenge, considered in this work, is the accurate diagnosis, preferably as early as possible, of postoperative bacterial infections. Early diagnosis and treatment of these infections in vascular patients are of paramount importance, if serious consequences are to be avoided. The presence of acute inflammation caused by surgical trauma is a major confounding factor when using the traditional laboratory markers such as total leucocyte counts and CRP. The pursuit of more sensitive and specific markers is on-going and will continue. In studies II and III, we have looked at some possible candidates that upon initial evaluation seem promising. Neutrophil CD64 expression, procalcitonin, and the cytokines IL-6 and IL-10 have all shown potential as useful laboratory markers, however it is unlikely that any one of these will become a gold standard on its own. It is more likely that a battery of tests will be needed to distinguish between the acute inflammatory response to infection and surgical trauma.

The topic of preventive measures has not been discussed in this thesis as it represents another vast field of research ranging from experimental techniques such as carbon dioxide insufflation of the surgical wound during surgery [77], the use of antimicrobial sutures [78], negative pressure wound closure devices [79, 80] and the eventual benefits of supplemental oxygen inhalation [81] to the tentative benefits of preoperative procedures such as showering, hair removal and various antiseptic skin preparations. [82, 83]

A relevant area for future research is the presumed link between the inflammatory response to infection and the increased risk of thrombosis as alluded to by the results in the first study which showed a higher rate of amputation among patients with postoperative infection compared to those without infection and previous research highlighting a possible connection. [84]

The way forward should ideally involve a three-pronged approach consisting of further clinical evaluation of new laboratory markers, further refinement of perioperative techniques

and procedures for reducing postoperative infections and improved prospective surveillance of patients both in hospital and post-discharge with the help of standardized, validated protocols and personnel with the relevant competence.

Conclusions

The conclusions from this thesis are the identification of healthcare associated infections (HCAI) as a major group of complications in lower extremity revascularization, particularly, in endovascular procedures, which have not been formerly associated with a substantial degree of infections and the potential viability of neutrophil CD64 in combination with PCT, CRP, IL-6 and IL-10 as a battery of laboratory tests for early detection of postoperative HCAI.

More specifically:

- We have determined the rate of infection within 30 days, both in-hospital and after discharge, for patients undergoing infrainguinal, open or endovascular revascularizations in a large population based cohort. We have also identified the significant risk factors for the development of postoperative infection, 30-day mortality and one-year amputation for this cohort. Furthermore, we have has shown the effect of the interaction between different classes of lower extremity ischaemia and choice of operative method on postoperative HCAI.

- We have demonstrated the minimal effect of surgically induced inflammation on the up-regulation of neutrophil CD64 receptors. CD64 as a marker for postoperative infection in vascular surgery has shown to have a higher sensitivity than CRP and PCT and may represent an improved diagnostic method.

- We have shown a positive correlation between perioperative infection and both pro- and anti-inflammatory cytokines, represented by IL-6, and IL-10 and IL-13, respectively. To our knowledge this is the first report linking IL-10 plasma levels to perioperative infection.

- Antibiotic treatment can be a useful marker for postoperative infections in vascular surgery. Our results emphasize the appreciable burden of disease and illustrate the pattern of antibiotic treatment of postoperative HCAI in patients undergoing treatment for severe lower extremity arterial disease. Based on these results, we suspect a previous underestimation of the total incidence of postoperative HCAI in patients treated with infrainguinal revascularization. This underscores the need for comprehensive follow-up regimens for this vulnerable category of vascular patients.

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