The Impact of Surgical Wound Bacterial Colonization on the Incidence of Surgical Site Infection After Lower Limb Vascular Surgery: A Prospective Observational Study

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WHAT THIS PAPER ADDS
This study investigated the relationship between surgical wound bacterial colonization and the development of surgical site infection after lower limb vascular surgery. The study revealed that a high bacterial load in the surgical wound postoperatively independently increases the risk of the development of surgical site infection after lower limb vascular surgery.

Objective: To study the relationship between surgical wound bacterial colonization and the development of surgical site infection (SSI) after lower limb vascular surgery. SSI is a major problem after lower limb vascular surgery. Most SSIs in vascular surgery are caused by Staphylococcal species that are part of normal skin flora. A prospective observational investigator blind study to examine quantitative and qualitative analysis of surgical wound bacterial colonization and the correlation with the development of SSI has been conducted.

Methods: The study cohort comprised 94 consecutive patients with 100 surgical procedures. Swabs for microbiological analyses were taken from surgical wounds at four different time intervals: before surgery, just before the surgical area had been scrubbed, at the end of surgery, and on the first and second postoperative days. Postoperative complications were recorded.

Results: Three hundred and eighty-seven skin bacterial samples from 100 surgical wounds were analyzed. The most common bacteria isolated were coagulase-negative staphylococci (80%), Corynebacterium species (25%), and Propionibacterium species (15%). In 13 (62%) cases, the same bacterial isolates were found in the perioperative study samples as in the infected wounds. The incidence of SSI was 21%. Multivariate analysis revealed that high bacterial load on the second postoperative day and diabetes independently increased the risk of SSI. Elective redo surgery was protective against the development of SSI.

Conclusions: A high bacterial load in the postoperative surgical wound independently increases the risk of the development of SSI after lower limb vascular surgery.

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Keywords: Peripheral vascular surgery, Bacterial colonization, Surgical site infection

INTRODUCTION
Surgical site infection (SSI) is a major problem particularly after peripheral vascular surgery. SSIs increase the cost of vascular surgery and the risk of major amputations, as well as the mortality.1–3 The incidence of SSI in patients undergoing peripheral vascular surgery procedures ranges from 4 to 27% according to prospective studies.1,4–8

The majority of SSIs in vascular surgery are caused by staphylococcal species that are part of the normal skin flora. Staphylococcus aureus has been reported to be responsible for 30–60%1,3,7–10 and Staphylococcus epidermidis for 17–24%9,10 of these infections.

A few studies have examined the relationship between the bacterial load of the surgical wound and the development of SSIs.11–13 The aim of this study was to measure quantitatively and qualitatively the bacterial colonization of the surgical wound peri- and postoperatively, and to examine whether there was an association between bacterial load and the incidence of SSI in patients undergoing peripheral vascular surgery.

MATERIALS AND METHODS
Study design
The prospective descriptive study was conducted at the Department of Vascular Surgery, North Karelia Central Hospital. The data were collected between January and...
October 2012. Swabs for microbiological analysis were taken from all surgical wounds peri- and postoperatively. Qualitative and quantitative analyses of various bacterial species were performed at Eastern Finland Laboratory Centre, Regional Laboratory of Joensuu. Blinded microbiological analyses were performed by an investigator who remained unaware of the origins of the coded bacterial samples. The study was approved by the ethics committee of Kuopio University Hospital (no. 100/2011, approved 3 November 2011). Written informed consent was obtained for every patient. The study was registered with ClinicalTrials.gov (no NCT01505738).

Patients
Consecutive adult patients undergoing non-emergency lower limb revascularization surgery were included in the study. The exclusion criteria were patients’ refusal to participate, patients’ inability to give informed consent, antibiotic treatment in the 2 weeks prior to surgery, or cephalosporin allergy. Aorto-iliac procedures were not included in the study. Incision drapes were not used in any procedures.

One hundred and nineteen patients underwent lower limb revascularization surgery during the study period. Altogether, 94 patients having 100 surgical procedures were included. Reasons for exclusion from the study were: patient underwent surgery at night or during the weekend (92%); patient was receiving antibiotic treatment (4%); or patient was allergic to cephalosporin (4%).

Magnetic resonance imaging (MRI) or intra-arterial digital subtraction angiography by experienced angiographers was used for peripheral arterial disease evaluation. Data, including demographic characteristics and peri- and postoperative factors, were collected prospectively. A standardized antibiotic prophylaxis of 3 g of cefuroxime was administered within the hour before incision. A further dose of 1.5 g of cefuroxime was administered if the blood loss was >1,500 mL or if the operation took more than 4 hours.

Blood pressure, pulse, fingertip oxygen saturation, and blood glucose level were measured twice daily, and the number of blood white cells, the hemoglobin value, and the C-reactive protein value once a day for two postoperative days.

Development of postoperative wound infections and other postoperative complications were recorded. Surgical wounds were examined at the 1-month follow-up visit by a vascular surgeon. Patients with postoperative surgical wound infections were followed up until the wound had healed.

Variables
The main outcome in this study was whether a patient developed SSI. A wound complication was considered to be an infection if it met the criteria developed by the Centers for Disease Control and prevention (CDC). The criteria were as follows: bacteria isolated from the wound or areas of localized redness, and heat, swelling and pain around the wound, appearing within 30 days of the operative procedure. The classification of SSI into three categories was based on the CDC criteria: a superficial wound infection involves only skin and subcutaneous tissue, a deep wound infection involves both fascia and muscle layers, and a graft infection is defined as the involvement of an artery or a graft. The general complications, including pneumonia, cardiac complications, strokes, major amputations, and graft thromboses, were considered as secondary outcomes. These complications were defined as follows:

- Pneumonia— infection of lung diagnosed by pulmonologist with correlative changes in chest X-ray film
- Cardiac complications— ischemic changes in electrocardiogram (ECG) with serum troponin-T value >0.5 µg/L or a new Q-wave or atrial fibrillation on ECG, or clinical diagnosis of cardiac insufficiency with correlative radiographic findings
- Stroke— one of the following symptoms: an inability to move limbs on one side of the body, inability to formulate or understand speech, or inability to see one side of the visual field with correlative changes at computed tomography or MRI
- Major amputation— above- or below-knee amputation
- Graft thrombosis— occlusion of revascularized native artery or vein, or prosthetic graft.

Sampling methods
A modified swabbing technique was used in this study. The specimen for bacterial culture was collected with the liquid-based Copan ESwab collection and transport system (Copan Italia, Brescia, Italy) by twirling the pre-wetted (0.9% sterile saline) nylon-flocked swab applicator with a sufficient pressure on the surgical site area of 2 cm × 4 cm for 30 seconds. The swabs were collected from the groin in 96 cases and from distal part of lower limb in four cases. If the revascularization surgery did not include the groin area, the swab was taken from the most proximal incision site. The applicator was placed on the ESwab transport tube containing 1 mL of modified liquid Amies (Copan Italia) according to the manufacturer’s instructions.

Bacterial samples were collected at four different time intervals. Before surgery, the first sample was taken from the operative field just before the surgical area was scrubbed. At the end of surgery, the second sample was taken after suturing the wound and before the dressing was applied. Opsite-Post-Op dressings (Smith & Nephew Medical, Hull, UK) were used to cover the surgical wounds in all patients in the study. The third and fourth samples were taken directly from the wound on the first and the second postoperative days. The first two samples were taken in the operating room and last two in the surgical ward. An additional bacterial sample was taken from those surgical wounds that developed SSI.
**Microbiologic techniques**

The samples were processed within 2 h after collection. Initially the ESwab tube containing the applicator was vortexed for 5 seconds to efficiently elute the bacteria from the nylon swab into the medium and to mix the sample thoroughly. To ensure more reliable counting of heavier bacterial loads a dilution series (1:10 and 1:100) was made as follows: 100 µL of original sample was diluted with 0.9% sterile saline to create a 1:10 dilution, which was further diluted 10-fold to achieve a 1:100 dilution. One hundred microliters of original sample, as well as the 1:10 and 1:100 dilutions, was plated onto duplicate blood agar plates. Additionally, the original sample and the 1:10 dilution were plated onto chocolate agar, cysteine lactose electrolyte-deficient (CLED) agar and anaerobic agar with a 5-µg metronidazole disk. All plates were incubated at 35 °C for 48 hours. Blood agar and chocolate agar plates were incubated in 5% CO₂, CLED agar plates in ambient air, and anaerobic agar in anaerobic atmosphere. Bacterial growth was quantified by counting colony-forming units (CFU). Bacterial identification was conducted according to standard methods and using the automated bacterial identification system Vitek2 (bioMérieux, Marcy l’Ettoile, France). Some isolates were identified with DNA sequencing. Antibiotic susceptibility testing was performed using the EUCAST disk diffusion method [www.eucast.org](http://www.eucast.org) and Vitek2.

**Statistical methods**

Statistical analyses were made using SPSS version 19.0 for Windows (SPSS, Chicago, IL, USA). In order to find the parameters affecting the infection probability, Pearson’s chi-square test was used for categorical variables, and an independent samples t test for continuous variables in univariate analysis. The non-parametric Mann—Whitney U test was initially used to analyze differences in total bacterial counts (CFU/ml) between SSI- and non-SSI groups at different time intervals owing to their skewed distribution. A natural logarithmic transformation of total bacterial counts was applied before performing t tests. As the parameters correlate with each other and may have some interaction effects, the parameters showing statistically significant association with SSI in univariate analyses were further studied with a binary logistic regression model in order to identify those factors that are independently associated with SSI. A p-value <.05 was considered statistically significant. Odds ratios (OR) and the corresponding 95% confidence intervals (CI) were calculated from the regression models. Receiver—operator characteristic (ROC) analysis was performed to set the ideal cut-off value for total bacterial counts on the second postoperative day. This new dichotomous categorical variable describing total bacterial counts was used in the binary logistic regression.

**RESULTS**

Sixty-six percent of the patients were men with a mean age of 72 years. Half of the patients had coronary artery disease and one-third had diabetes. The indication for surgery was critical ischemia in 47% of cases. Thirty-three percent of the procedures were either femoro-popliteal or femoro-distal bypasses, and 47% were femoral endarterectomies. The other 15% of procedures included thrombectomies, reconstruction of femoral pseudoaneurysm, and axillo-femoral bypass. In one of the thrombectomies the surgical incision was made below knee; in the other cases, patients received a groin incision. A vascular prosthesis or prosthetic patch was used in 18 (18%) patients. Demographic and operative data of the patients are presented in Table 1. Endovascular procedures were not included into the study.

**SSI and other complications**

The incidence of SSI was 21%. Of the 21 SSIs, 76% were superficial and 19% were deep. In this study, there was one complication considered to be a graft infection; a patient with femoral endarterectomy developed a groin infection involving the femoral artery. Infection was located in the groin in 15 cases and in the thigh incision for saphenous vein harvest in six. The infection healed by radical wound revision and cover by muscle flap. No statistically significant differences between SSI and non-SSI groups were found in fasting glucose levels with t test on the first (p = .899) and second (p = .183) postoperative days in our study. Four patients died during the study period; these patients had not developed SSI. Causes of death were myocardial infarction in three patients and cardiac insufficiency in one patient. Two patients needed a major amputation due to critical ischemia. In one patient with SSI due to critical ischemia and graft thrombosis, SSI was the cause of major amputation. Of the 21 patients who developed SSI 20 were healed with treatment by the end of the study period. The other postoperative complications were as follows: graft thrombosis (9%), cardiac complication (3%), stroke (1%), sepsis (1%), and, hemorrhage from the surgical area (3%) (Table 2).

**Microbiological analyses**

The range of total microbial counts in this group of patients was large (range: 0—2,800,000 CFU/ml). The dynamics of wound bacterial colonization at different time intervals is shown as a scatterplot in Fig. 1. Total microbial counts in the SSI group compared with the non-SSI group were statistically significantly higher only in samples taken on the second postoperative day (p = .001, Mann—Whitney U test). Thus this time-point was selected for further analysis. Natural logarithmic transformation was performed in order to normalize the highly skewed distribution of microbial counts. The differences in microbial counts between SSI and non-SSI groups were also studied with a parametric t test after logarithmic transformation. Total microbial counts were statistically significantly higher in the SSI group compared with the non-SSI group only on the second postoperative day sample (Fig. 2).

ROC analysis of total bacterial counts revealed that the area under the curve value was statistically significant between the SSI and non-SSI groups on the second postoperative day (area: 0.746, 95% CI: 0.632—0.861, p = .001).
A CFU/mL value of 155,000 gave the best discriminatory power between SSI and non-SSI groups, and it was used as the cut-off value (area: 0.667, 95% CI: 0.532–0.820, p = .012) to divide the study material into two groups:

- Patients who, on the second postoperative day had bacterial counts > 155,000 CFU/mL (n = 19) and patients who had bacterial counts < 155,000 CFU/mL (n = 76). In these groups, the SSI rate was 52.6% and 14.5%, respectively

### Table 1. Demographics and operative data of 100 surgical procedures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)/mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>100</td>
</tr>
<tr>
<td>Age, y</td>
<td>71.6 (8.8)</td>
</tr>
<tr>
<td>Male gender</td>
<td>66 (66)</td>
</tr>
<tr>
<td>BMI &gt;25 kg/m²</td>
<td>65 (65)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>47 (47)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>35 (35)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>58 (58)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>3 (3)</td>
</tr>
<tr>
<td>COPD</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Asthma</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Use of medication</td>
<td></td>
</tr>
<tr>
<td>Asa</td>
<td>68 (68)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>20 (20)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Statin</td>
<td>73 (73)</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Insulin</td>
<td>18 (18)</td>
</tr>
<tr>
<td>Oral diabetes medication</td>
<td>15 (15)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>39 (39)</td>
</tr>
<tr>
<td>Preoperative blood sample</td>
<td></td>
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<tr>
<td>Hemoglobin value, g/L</td>
<td>132.9 (19.1)</td>
</tr>
<tr>
<td>White blood cells, E9/L</td>
<td>7.8 (2.2)</td>
</tr>
<tr>
<td>C-reactive protein, g/L</td>
<td>11.0 (17.5)</td>
</tr>
<tr>
<td>Creatinine value, μmol/L</td>
<td>82.1 (27.8)</td>
</tr>
<tr>
<td>Indication for surgery</td>
<td></td>
</tr>
<tr>
<td>Claudication</td>
<td>47 (47)</td>
</tr>
<tr>
<td>Ischemic pain</td>
<td>25 (25)</td>
</tr>
<tr>
<td>Ischemic ulcer</td>
<td>22 (22)</td>
</tr>
<tr>
<td>Other</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Type of surgery</td>
<td></td>
</tr>
<tr>
<td>Femoral endarterectomy</td>
<td>47 (47)</td>
</tr>
<tr>
<td>Femoro-femoral bypass</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Femoro-popliteal bypass</td>
<td>20 (20)</td>
</tr>
<tr>
<td>Crural bypass</td>
<td>11 (11)</td>
</tr>
<tr>
<td>Pedal bypass</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Other</td>
<td>15 (15)</td>
</tr>
<tr>
<td>Use of prosthetic material</td>
<td>18 (18)</td>
</tr>
<tr>
<td>Infra-inguinal incision</td>
<td>47 (47)</td>
</tr>
<tr>
<td>Redo surgery</td>
<td>29 (29)</td>
</tr>
<tr>
<td>Operative time, mins</td>
<td>168 (89.9)</td>
</tr>
<tr>
<td>Blood loss, mL</td>
<td>396.0 (473.6)</td>
</tr>
<tr>
<td>Use of drainage</td>
<td>85 (85)</td>
</tr>
<tr>
<td>Blood transfusion perioperatively</td>
<td>12 (12)</td>
</tr>
<tr>
<td>Bacterial colonization on the second postoperative day</td>
<td></td>
</tr>
<tr>
<td>CFU/swab &gt;50 000</td>
<td>25 (25)</td>
</tr>
<tr>
<td>CFU/swab &gt;100 000</td>
<td>20 (20)</td>
</tr>
<tr>
<td>CFU/swab &gt;200 000</td>
<td>16 (16)</td>
</tr>
<tr>
<td>CFU/swab &gt;400 000</td>
<td>8 (8)</td>
</tr>
<tr>
<td>CFU/swab &gt;600 000</td>
<td>6 (6)</td>
</tr>
</tbody>
</table>

### Table 2. Univariate analysis of 100 surgical procedures.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No infection n = 79</th>
<th>Infection n = 21</th>
<th>Univariate p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender, n (%)</td>
<td>48 (61)</td>
<td>18 (86)</td>
<td>.03</td>
</tr>
<tr>
<td>Age</td>
<td>71.8 (8.8)</td>
<td>70.9 (8.2)</td>
<td>.66</td>
</tr>
<tr>
<td>Ischemic ulcer</td>
<td>16 (20%)</td>
<td>6 (29%)</td>
<td>.41</td>
</tr>
<tr>
<td>Claudication</td>
<td>36 (46%)</td>
<td>11 (52%)</td>
<td>.59</td>
</tr>
<tr>
<td>BMI &gt;25 kg/m²</td>
<td>48 (61%)</td>
<td>17 (81%)</td>
<td>.09</td>
</tr>
<tr>
<td>Preoperative hemoglobin, g/L</td>
<td>131.5 (18.8)</td>
<td>138.2 (19.8)</td>
<td>.17</td>
</tr>
<tr>
<td>Preoperative white blood cell count, E9/L</td>
<td>7.9 (2.3)</td>
<td>7.7 (1.6)</td>
<td>.81</td>
</tr>
<tr>
<td>Preoperative CRP g/L</td>
<td>11.8 (19.2)</td>
<td>8.2 (8.2)</td>
<td>.21</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>37 (47%)</td>
<td>10 (48%)</td>
<td>.95</td>
</tr>
<tr>
<td>Hypertension</td>
<td>43 (54%)</td>
<td>15 (71%)</td>
<td>.16</td>
</tr>
<tr>
<td>Diabetes</td>
<td>22 (28%)</td>
<td>13 (62%)</td>
<td>.09</td>
</tr>
<tr>
<td>Use of Asa</td>
<td>53 (67%)</td>
<td>14 (78%)</td>
<td>.04</td>
</tr>
<tr>
<td>Use of Clopidogrel</td>
<td>7 (9%)</td>
<td>3 (14%)</td>
<td>.46</td>
</tr>
<tr>
<td>Use of Statin</td>
<td>59 (75%)</td>
<td>14 (67%)</td>
<td>.46</td>
</tr>
<tr>
<td>Use of prosthetic material</td>
<td>18 (23%)</td>
<td>0 (0%)</td>
<td>.016</td>
</tr>
<tr>
<td>Elective redo surgery</td>
<td>25 (32%)</td>
<td>4 (19%)</td>
<td>.26</td>
</tr>
<tr>
<td>Operation time (mins)</td>
<td>172.4 (91.3)</td>
<td>154.2 (85.0)</td>
<td>.40</td>
</tr>
<tr>
<td>Blood loss during surgery (mL)</td>
<td>403.6 (453.5)</td>
<td>367.6 (554.3)</td>
<td>.79</td>
</tr>
<tr>
<td>Use of drainage</td>
<td>66 (84%)</td>
<td>19 (91%)</td>
<td>.43</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>11 (14%)</td>
<td>1 (4.8%)</td>
<td>.25</td>
</tr>
<tr>
<td>CFU/swab &gt;155 000</td>
<td>9 (11%)</td>
<td>10 (48%)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Note. Categorical variables are presented as frequencies and percentages. Continuous variables are presented as means and standard deviations. BMI = body mass index; CRP = C-reactive protein; Asa = acetylsalicylic acid; CFU = colony-forming units.

patients who, on the second postoperative day had, bacterial counts > 155,000 CFU/mL (n = 19) and patients who had bacterial counts < 155,000 CFU/mL (n = 76). In these groups, the SSI rate was 52.6% and 14.5%, respectively
isolates were found in the perioperative study samples and infected wounds. In 13 (62%) cases, the same bacterial protective against SSI, and infra-inguinal surgery has been because, in an earlier study, redo surgery was found to be surgery parameters were also added into the presented in Table 2. Binary logistic regression analysis was used to determine whether these factors were indepen-
dently associated with SSI. Infra-inguinal surgery and redo surgery were also included in the model (Table 3).

The incidence of SSI after lower limb vascular surgery was 21% in our study, which is in accordance with our previous prospective studies. The incidence of SSI has been reported to range between 4% and 25% in other prospective studies. A possible explanation for the differences in the reported incidence of SSI is the definition of SSI used. Some studies have defined wound complication as infection only if there was a positive culture from the wound. In this study a wound complication was classified as an infection if there were signs of infection, even if the cultures were negative. In this study, 5% of infected surgical wounds had a negative culture.

The main result of this study is that perioperative bacterial load is a significant risk factor for the development of SSI.

Similar results have been observed in two earlier studies, which found peri- and postoperative bacterial load to increase the risk of wound complications after dermatologic and plastic surgery. The difference between those studies and this one is that instead of wound complication, wound infection was used as the outcome.

In contrast, a study of 609 neurosurgical patients did not find any correlation between intraoperative bacterial load and postoperative infection. In that study, samples were taken only during surgery and not postoperatively. However, in this study, it was particularly found that high bacterial load on the second postoperative day increased the risk of SSI. In addition, there was no significant relationship found between high bacterial colonization perioperatively and the development of SSI.

Perioperative bacterial load independently increases the risk of SSI; however, the same bacterial isolates from the perioperative or postoperative study samples and from the

![Figure 2. Total bacterial counts (colony-forming units [CFU]/mL) in patients with and without surgical site infection. Bars represent CFU logarithmic means with 95% confidence interval. P-Values obtained with student t test. Note. SSI = surgical site infection.](image)

(\( p < .001 \)). There were five cases in which the second postoperative day sample was not taken or the sample was missing.

Coagulase-negative staphylococci were the most common bacteria growing in 80% of the cultures. Other commonly isolated bacteria were Corynebacterium spp. (25%), Propionibacterium spp. (15%), Enterococcus faecalis (4%), Micrococcus spp. (3%), and S. aureus (3%).

Positive cultures were obtained from infected wounds in 20 (95%) of 21 patients. The most commonly identified species were S. aureus (52%), E. faecalis (23%), Peptostreptococcus spp. (29%), Escherichia coli (24%), and Corynebacterium species (19%). No methicillin-resistant S. aureus, vancomycin-resistant enterococci, extended-spectrum beta-lactamase producing E. coli or Klebsiella species, or multidrug-resistant (MDR) Pseudomonas aeruginosa or other MDR gram-negative rods were present in these samples. Polymicrobial flora were found in 71% of the infected wounds. In 13 (62%) cases, the same bacterial isolates were found in the perioperative study samples and infected wounds. Staphylococcus aureus was the causative agent in five (71%) of the seven SSI cases with non-matching bacterial samples.

**Statistical analyses**

The univariate analysis found the following factors to increase the risk of SSI: male gender, diabetes, and a high bacterial colonization of the surgical site on the second postoperative day when using the cut-off point of 155,000 CFU/mL. The result of the univariate analysis is presented in Table 2. Binary logistic regression analysis was used to determine whether these factors were independently associated with SSI. Infra-inguinal surgery and redo surgery parameters were also added into the final model because, in an earlier study, redo surgery was found to be protective against SSI, and infra-inguinal surgery has been shown to be associated with SSI. The results of logistic regression analysis are shown in Table 3. High bacterial colonization of the surgical site on the second postoperative day was found to be an independent risk factor for SSI with an OR of 5.97. The other factor that increased the risk of SSI independently was diabetes (OR = 4.77). Redo surgery was protective against SSI, but only when infra-inguinal surgery was included in the model (Table 3).

**DISCUSSION**

The incidence of SSI after lower limb vascular surgery was 21% in our study, which is in accordance with our previous prospective studies. The incidence of SSI has been reported to range between 4% and 25% in other prospective studies. A possible explanation for the differences in the reported incidence of SSI is the definition of SSI used. Some studies have defined wound complication as infection only if there was a positive culture from the wound. In this study a wound complication was classified as an infection if there were signs of infection, even if the cultures were negative. In this study, 5% of infected surgical wounds had a negative culture.

The main result of this study is that perioperative bacterial load is a significant risk factor for the development of SSI.

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Perioperative bacterial load independently increases the risk of SSI; however, the same bacterial isolates from the perioperative or postoperative study samples and from the

**Table 3. Binary logistic regression analysis\(^a\) of factors associated with surgical wound infection after lower limb vascular surgery.**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU/mL &gt; 155,000 on 2nd postoperative day</td>
<td>5.97</td>
<td>1.67–21.42</td>
<td>.006</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4.77</td>
<td>1.46–15.55</td>
<td>.010</td>
</tr>
<tr>
<td>Elective redo surgery</td>
<td>0.16</td>
<td>0.03–0.88</td>
<td>.035</td>
</tr>
<tr>
<td>Infra-inguinal surgery</td>
<td>4.11</td>
<td>0.95–17.84</td>
<td>.059</td>
</tr>
<tr>
<td>Male gender</td>
<td>4.41</td>
<td>0.90–21.71</td>
<td>.068</td>
</tr>
</tbody>
</table>

\(^a\) Regression model with CFU/mL > 155,000 on the second postoperative day, diabetes, elective redo surgery, infra-inguinal surgery, and sex as covariates.
infected wounds were only found in 13 (62%) cases. In the wound healing process, bacteria and debris are phagocy-
tosed and removed by neutrophils during the inflammatory
phase, which begins within 24 hours of injury. The prolif-erative phase follows and overlaps the inflammatory
phase, and is characterized by the migration of epithelial
cells across the new tissue to form a barrier between the
wound and the environment. Until complete epithelial-
ization has created the bacterial barrier, a wound may
become colonized by bacteria. It is suggested that if there is
a large amount of any bacteria in a surgical wound, the
phagocytosis by neutrophils is not effective in preventing
SSI during the period of wound healing when complete re-
epithelialization has not yet occurred. In other words, dur-
ing the inflammatory and proliferative phase of wound
healing the number of bacteria, rather than the bacterial
species, seems to be important in the development of SSI.

Suture materials have a non-shedding surface to which
bacteria can adhere, form biofilms, and potentiate SSI. The
biofilm bacteria are difficult to treat because, shielded
within the matrix, they are less susceptible to antibiotics
and antiseptics. The surgical suture can become contam-
inated by bacteria from the patient’s own skin. It is possible
that contamination of sutures can occur until the complete
re-epithelialization has occurred.

High bacterial growth in a surgical wound predicts the
development of SSI. Quantitative measurement of bacterial
growth will not, however, be used as a screening method
for infections because by the time the bacterial sample
analysis is performed the wound infection already exists.
Instead, in future, more prospective studies are warranted
to test hypotheses about methods of decreasing the bac-
terial load of the wound. One such method could be
postoperative wound-washing with antiseptic agents.

Diabetes was the other independent risk factor for the
development of SSI, and a similar trend was also found for
infra-inguinal surgery, though the p-value was not statistically
significant in this study. Diabetes and infra-inguinal surgery
were other independent risk factors for the development of
SSI. The predictive values of diabetes and infra-inguinal sur-
gery have also been demonstrated in other studies.
Likewise in a previous study, redo surgery was found to be
protective against SSI. The explanation for this surprising
result might be that a vascular prosthesis was used more
often as a graft at redo surgery, meaning that there were no
long vein harvesting incisions. In this study, prosthetic graft
was used in 18 (62%) of 29 redo procedures. The incidence
of SSI was 27% in patients with ischemic ulcers and 19% in pa-
patients without ulcers; the difference was not statistically
significant (p = .41). Ischemic ulcers have also not been a risk
factor for SSI in our previous studies with 190–280 vascular
surgery patients.

In agreement with earlier studies coagulase-negative
staphylococci were the predominant bacteria isolated from
the study samples followed by Corynebacterium spp. and Propionibacterium spp. The leading causative
agent for SSI was S. aureus, found in 52% of the infected
wounds.

The limitation of this study is that it was not possible to
draw any conclusions about infections of vascular prosth-
theses. The study had only 1 month of follow-up time, although
infections of prostheses generally occur a few months, and
sometimes years, after the initial surgery. Furthermore, the
incidence of prosthesis infection in vascular surgery is 1–5%.
There were 18 procedures with prosthetic material in
the study, which means that no more than one vascular
prosthesis infection is expected to occur.

A high bacterial load in the surgical wound on the second
postoperative day increases the risk of SSI after lower limb
vascular surgery. Further research will be required to find
methods to decrease the wound bacterial load post-
operatively. For example, there is only one double-blind
study, which was published almost 30 years ago, with 121
patients comparing the effect of duration of antibiotic
prophylaxis on wound infection after peripheral artery
surgery. The study did not find that continuing prophylactic
antibiotics for more than 24 hours added any benefit
compared with 24-hour antibiotic prophylaxis. A ran-
donized controlled trial with larger sample size should be
conducted to study the effect of the duration of antibiotic
prophylaxis on the incidence of SSI in vascular surgery.
Moreover, the different postoperative wound care
methods, for example daily wound antisepsis, should be
studied.

It is concluded that high bacterial load of surgical wound
on the second postoperative day increases the risk of SSI
after lower limb vascular surgery.

CONFLICT OF INTEREST
None.

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