The influence of vacuum-assisted closure on inflammatory tissue reactions in the postoperative course of ankle fractures

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Summary
Vacuum-assisted closure (vacuum sealing) is a surgical procedure for the local treatment of severe soft-tissue damage. However, systemic consequences to the host are unknown. The aim of this study was to disclose the effects of vacuum sealing on the host’s immune response and to demonstrate the early time course of endotoxin, interleukin-6 (IL-6), C-reactive protein (CRP), haptoglobin, transferrin, orosomucoid, 6-keto-prostaglandin (6KPG), α1-antitrypsin and complement C3 and C4. A total of 35 patients with closed ankle fractures were randomized into two groups and operated on within 6 h after injury. After osteosynthesis, one group was treated by vacuum sealing (VS) and the other by immediate skin closure (IS). Blood was collected immediately after admission and regularly up to 96 h after surgery. Morbidity was checked during the first year after injury. Preoperative endotoxin plasma level was increased compared with that of voluntary individuals (0.06 ± 0.02 EU/ml versus 0.021 ± 0.001 EU/ml) and peaked in patients with immediate skin suture 0.5 h after the surgical procedure at 0.11 ± 0.03 EU/ml. However, in patients with vacuum sealing, this peak was absent (0.07 ± 0.02 EU/ml). Endotoxaemia decreased to almost normal values after 24 h. Plasma IL-6 peaked 12 h postoperatively, decreasing thereafter with no difference between the groups. The plasma level of 6KPG decreased immediately after the surgical procedure in vacuum-sealed patients (before operation, 415 pg/ml; 12 h later, 251 pg/ml), but increased first in patients with immediate skin suture. CRP peaked 48 h after injury (VS, 48 ± 6 mg/l; IS, 38 ± 7 mg/l) with no difference between the groups. Transferrin decreased postoperatively (pre-op: VS, 2.49 ± 0.14 g/l; IS, 2.85 ± 0.19 g/l; 24 h: VS, 2.16 ± 0.08 g/l and IS 2.33 ± 0.11 g/l), whereas haptoglobin (pre-op: VS, 2 ± 0.21 g/l; IS, 1.7 ± 0.18 g/l; 96 h: VS, 3.4 ± 0.25 g/l, IS, 3.2 ± 0.24 g/l) and orosomucoid (pre-op: VS, 0.85 ± 0.05 g/l, IS, 0.83 ± 0.07 g/l; 96 h: VS, 0.85 ± 0.05 g/l, IS 1.14 ± 0.08 g/l) increased until day 4 with no significant difference.
between VS and IS. There was no relevant intergroup difference for complement C3, C4, α1-antitrypsin and morbidity (VS/IS: wound infection, 1/1; metal loosening, 1/1; prolonged healing, 1/0; prolonged pain, 3/2; and motor disturbance, 1/1). Surgery for ankle fractures is associated with temporary endotoxaemia and substantial changes in acute-phase proteins. Vacuum-assisted closure has only limited and no negative systemic immune consequences after surgery for malleolar fractures, is safe and can be used to manage severe soft-tissue damage. However, if feasible, primary skin closure is preferable.

**Keywords:** ankle fractures; vacuum-assisted closure; acute-phase proteins; endotoxin; 6-keto-prostaglandin

### Introduction

Vacuum-assisted closure (vacuum sealing) is a surgical procedure to achieve secure and rapid wound healing in severe soft-tissue damage including open and closed fractures and wound infections [1-5]. An open-cell foam is placed into the wound sealed with an adhesive drape, and a suction device applies a subatmospheric pressure to the wound via perforated tubes that run through the foam [1-6]. Clinical experiences comprise accelerated healing processes, rapid clearing of infected wounds, stimulation of the development of a healthy and tense granulation tissue, prevention of infection, time and cost effectiveness and increased patient comfort [1-6]. Animal experiments demonstrated increased blood and nutrient flow, increased granulation tissue formation (up to 63%) and decreased tissue bacterial counts (increased bacterial clearance) [7]. Several interacting mechanisms were postulated to be responsible: mechanical removal of interstitial fluid decompressing small blood vessels, perturbation of the cytoskeleton causing release of intracellular messengers (e.g. prostaglandins) with subsequent matrix molecule synthesis and cell proliferation [1, 2, 4, 7]. Reduction in bacterial contamination prevents disturbance of wound healing [7, 8]. Tumour necrosis factor (TNF) is a proinflammatory mediator and participates in the early inflammatory response to wounding with high concentrations in wound exudate fluids [9]. Removal of the wound fluid by the vacuum-assisted closure could be speculated to influence the systemic immune response. These mechanisms focus on local processes. However, interactions between the local vacuum-sealing technique and the systemic host response are unknown. Therefore, the intention of this study was to randomize patients with comparable injuries, to treat them with or without the new technique and to elucidate early differences in systemically measurable markers of the inflammatory response. The following markers were selected.

Endotoxin is a potent trigger of the mediator cascade of the inflammatory response and was shown to be translocated in malleolar fractures [10-12]. The chief stimulator of the production of most acute-phase proteins is interleukin (IL)-6, and high plasma concentrations were measurable after trauma [13-15]. C-reactive protein (CRP) was determined because it is currently the most widely used acute-phase protein to indicate an inflammatory response and reacts fast [14]. Orosomucoid is a second positive acute-phase reactant that increases early during inflammatory disorders. Haptoglobin may have anti-inflammatory actions, because it is an antioxidant, protects against reactive oxygen species and aids in wound repair by stimulating angiogenesis [14, 16]. Transferrin is a negative acute-phase reactant and was demonstrated to neutralize endotoxin [14, 17, 18]. In previous studies, prostaglandins were used to estimate cell perturbation; therefore we measured 6-keto-prostaglandin F1α (6KPG) [7, 19]. α1-Antitrypsin inhibits several serine proteases that confine inflammatory processes. Tissue degradation products can be eliminated by complement; therefore, we determined C3 and C4. The morbidity was checked 1 year after surgery by clinical examination.
Materials and methods

Patients

Thirty-five patients with closed ankle fractures that had to be managed by surgical intervention were enrolled in the study (13 men and 22 women; mean age 52 years; range 27–74 years). Inclusion criteria: patients who could be operated on within 6 h after injury, age ≥ 18 years. Excluded were patients with any additional injury, who were older than 75 years, were immunosuppressed, had malignant diseases, had infectious diseases during the last 4 weeks or were pregnant. The injury pattern could be summarized as follows: 13 fractures of type Danis/Weber B, 22 fractures of type Danis/Weber C, 33 osteosynthesis by plate and two osteosynthesis by screws only; wires were used additionally in 12 patients. After written consent was obtained, patients were randomized into two groups. First group (n = 18; called 'IS'): the surgical incision (10 cm) was sutured immediately after osteosynthesis. Second group (n = 17; called 'VS'): the wound was covered with an oval piece of an open-cell polyvinylalcohol foam (10 x 2 cm; Vacu-Seal®, Coloplast, Humlebaek, Denmark) and sealed with a semi-permeable transparent polyurethane drape. The foam was sutured to the skin edges of the surgical incision. A multiple perforated drainage ran through the foam distributing a subatmospheric pressure (80 kPa) to the surface of the entire wound. This resulted in a tension-free soft-tissue repair. Physical restraint helped to reduce oedema. After the initial operation (96–120 h), the foam was removed, and the skin could be sutured without tension. Both groups were comparable with regard to classification of fractures and surgical procedures. Blood was collected immediately after admission into endotoxin-free tubes (Greiner, Nürtingen, Germany) and regularly up to 96 h after surgery. Heparin was used for anticoagulation (10 IU/ml) or a combination of ethylenediaminetetraacetic acid with indomethacin (EDTA, 1 mg/ml; indomethacin, 5 μg/ml; determination of 6KPG).

Controls

Ten healthy voluntary individuals with no recent history of infection were selected. Blood was drawn once and analysed for all parameters used.

Endotoxin determination

The endotoxin plasma concentration was determined by the limulus–amoebocyte–lysate test with a chromogenic modification as described and expressed as endotoxin units (EU)/ml [20, 21]. The detection limit of the assay was 0.02 EU/ml, which is regarded as sensitive for small amounts of endotoxin.

IL-6 and 6KPG levels

These were determined with commercially available kits (IL-6, Immunotech, Hamburg, Germany; 6KPG, PerSeptive Diagnostics, Cambridge, MA, USA).

C-reactive protein, transferrin, orosomucoid (= α1-acid glycoprotein), α1-antitrypsin, haptoglobin, complement C3 and C4

These molecules were determined nephelometrically as recommended by the manufacturer (Nephelometer 100 Analyser, Behringwerke, Marburg, Germany).

Statistical evaluation

The mean value and standard error of each parameter was calculated. The Mann–Whitney U-test was used for the evaluation of statistical differences between individual time points and controls. ANOVA was used to assess differences within and between groups. Significance was assumed when P < 0.05. The protocol was approved by the ethical review committee of the university, and informed consent for participation was obtained from all patients.

Results

Endotoxin (Table 1)

The mean endotoxin plasma levels of 35 patients with ankle fractures are listed. At admission, the concentration was already higher than in healthy voluntary individuals (0.06 ± 0.02 EU/ml versus 0.021 ± 0.001 EU/ml, P = 0.08). The endotoxin concentration peaked 0.5 h after the operation at 0.11 ± 0.03 EU/ml (P < 0.05 versus control) in patients with immediate skin suture (IS). However, the patients whose wounds were treated with the vacuum sealing procedure (VS) had no postoperative
Table 1
Plasma concentrations of endotoxin (EU/ml), transferrin (g/l), α1-antitrypsin (g/l) and complement C3 (g/l)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Before</th>
<th>0.5 h</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endotoxin</td>
<td>VS</td>
<td>0.06 ± 0.02</td>
<td>0.07 ± 0.02</td>
<td>0.045 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>0.05 ± 0.01</td>
<td>0.11 ± 0.03*</td>
<td>0.06 ± 0.01</td>
<td>0.035 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Transferrin</td>
<td>VS</td>
<td>2.49 ± 0.14</td>
<td>2.23 ± 0.1</td>
<td>2.33 ± 0.15</td>
<td>2.16 ± 0.08†</td>
<td>2.21 ± 0.08</td>
<td>2.12 ± 0.09†</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>2.85 ± 0.19</td>
<td>2.38 ± 0.12†</td>
<td>2.44 ± 0.09†</td>
<td>2.33 ± 0.11†</td>
<td>2.38 ± 0.12†</td>
<td>2.41 ± 0.11†</td>
</tr>
<tr>
<td>α1-Antitrypsin</td>
<td>VS</td>
<td>2.23 ± 0.19</td>
<td>1.95 ± 0.18</td>
<td>2.19 ± 0.15</td>
<td>2.34 ± 0.06</td>
<td>2.64 ± 0.06†</td>
<td>2.74 ± 0.08†</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>2.02 ± 0.12</td>
<td>1.75 ± 0.08</td>
<td>2.05 ± 0.12</td>
<td>2.39 ± 0.14</td>
<td>2.76 ± 0.2</td>
<td>2.95 ± 0.2</td>
</tr>
<tr>
<td>C3</td>
<td>VS</td>
<td>0.93 ± 0.06</td>
<td>0.76 ± 0.05</td>
<td>0.83 ± 0.05</td>
<td>0.85 ± 0.04</td>
<td>0.92 ± 0.04</td>
<td>1.04 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>0.92 ± 0.08</td>
<td>0.75 ± 0.04</td>
<td>0.81 ± 0.04</td>
<td>0.85 ± 0.03</td>
<td>0.94 ± 0.05</td>
<td>1.06 ± 0.06†</td>
</tr>
</tbody>
</table>

Mean values and SEM; VS, vacuum-sealed group; IS, immediate suture group.
*P < 0.05 versus control; †P < 0.05 versus admission.

peak (the endotoxin concentration 0.5 h after the procedure was 0.07 ± 0.02 EU/ml; P = 0.24 versus control). After 24 h, the endotoxin concentrations of both groups were almost in the normal range.

IL-6 (Figure 1)

IL-6 was already increased at admission (IS, 80 ± 17 pg/ml; VS, 101 ± 13 pg/ml) and peaked 12 h after the operation (IS, 100 ± 15 pg/ml; VS, 120 ± 16 pg/ml; P < 0.05, when compared with controls). However, there was no difference between IS and VS throughout the observation period.

CRP (Figure 1)

CRP increased significantly in both groups (P < 0.001) and peaked 48 h after the operation.

Figure 1
Early time course of IL-6 (circles, dots) and CRP (squares) after surgery for isolated ankle fractures. Mean values and SEM. A, admission of patients. Open symbols, immediate skin closure group; closed symbols, vacuum-sealed patients.

Orosomucoid (α₁-acid glycoprotein), haptoglobin (Figure 2)

Both proteins increased significantly and typically for positive acute-phase proteins until day 4 (96 h). The values were comparable, ran parallel, and there was no significant difference between the groups.

Transferrin (Table 1)

The plasma concentration decreased after osteosynthesis in both groups and remained on this level for the rest of the observation period (IS, \(P < 0.05\), admission versus all other time points; VS, \(P < 0.05\), admission versus 24 h + 96 h). All postoperative values were also significantly below control levels. Again, there was no difference between the groups.

\(\alpha_1\)-Antitrypsin, complement C3 and C4 (Table 1)

The kinetics were very similar to those of orosomucoid and haptoglobin. The concentrations increased significantly during the time course, but there were no differences between patients with or without vacuum sealing. Values for C3 and C4 were similar (therefore, only values for C3 are listed).

6KPG (Figure 3)

Patients treated by vacuum sealing had lower levels of 6KPG immediately after surgery, whereas the concentration increased first in patients with IS. However, there was no significant difference between IS and VS.

Discussion

Ankle fractures are associated with soft-tissue damage with subsequent swelling and haematoma, causing circular tension in the skin, pressure to
adjacent tissue and reduced blood flow. To prevent excessive tension after osteosynthesis, vacuum sealing can be used. This technique is an additional surgical procedure and has been shown to be effective in the treatment of various severe soft-tissue damage [1-6]. An open-cell foam is placed into the wound, and a suction device applies a subatmospheric pressure to the adjacent tissue. Clinicians report, among others, accelerated wound healing, stimulation of the development of granulation tissue, reduction in bacterial contamination, prevention of infection, cost effectiveness and increased patient comfort [1-6]. Animal experiments investigating vacuum sealing point to increased blood and nutrient flow, increased granulation tissue formation and increased bacterial clearance [7]. However, all these mechanisms focus on local processes. The aim of this study was to explore whether there is an influence of vacuum sealing on the systemic inflammatory response by disclosing the early time course of the acute-phase reaction. Patients with isolated ankle fractures were selected and operated on within 6 h after injury. The study protocol required that the local conditions of all patients recruited had to be suitable to apply immediate skin suture and vacuum sealing to prevent bias. This prerequisite is different from routine surgery, where primary skin closure is the preferred method if possible. The patients were allocated randomly into two groups (with or without vacuum sealing). The plasma levels of endotoxin, IL-6, 6KPG, several acute-phase proteins and complement C3 and C4 were determined in order to elucidate a different immune response caused by the different procedures. The malleolar fractures were shown to cause endotoxaemia shortly after injury, with a significant increase after an additional surgical procedure [12]. In this study, patients with immediate skin closure had a further increase (to 0.11 ± 0.03 EU/ml) postoperatively, but patients with vacuum sealing did not (0.07 ± 0.02 EU/ml). Except for the ankle fracture, the patients were healthy. However, ankle fractures are minor injuries, and translocation is only slight compared with major injury such as polytrauma [20]. Endotoxaemia was also described in patients with gut disorders,
pancreatitis, burn, minor trauma such as goitre surgery or even colonoscopy and can be considered as a frequent phenomenon [23, 24].

Tissue damage and endotoxin are capable of inducing the release of proinflammatory mediators such as TNF [14, 25]. TNF is present in significant quantities at the site of healing and can suppress many macrophage and lymphocyte activities [26, 27]. Concentrations of ~4000 pg/ml in cell-free wound exudate were measured after wounding [9]. Additionally, the wound fluid was shown to have immunosuppressive properties [28]. Vacuum sealing mechanically removes interstitial fluid from an extensive wound surface, decompressing small blood vessels and reducing the tension in the skin, which is commonly associated with ankle fractures [7]. It could be hypothesized that the aspiration of wound fluid via the foam is associated with clearing tissue degradation products and mediators that could influence the immune response. The systemic inflammatory responses comprise changes in acute-phase proteins. As already mentioned in the Introduction, we therefore determined the kinetics of CRP, haptoglobin, orosomucoid, transferrin and α1-antitrypsin. A major function of CRP is its ability to bind phosphocholine and thus recognize some foreign pathogens as well as phospholipid constituents of damaged cells [29]. CRP increased after a proper time lag, peaked 48 h after injury, but there was no difference between VS and IS. The time course was typical for an early and fast-reacting positive acute-phase protein [14]. Haptoglobin may have anti-inflammatory actions and aids in wound repair by stimulating angiogenesis [14, 16]. Like CRP, haptoglobin, orosomucoid, and α1-antitrypsin are positive acute-phase proteins that increase during inflammatory disorders by at least 25% [14]. Initially (time 0.5 h), the concentration of these parameters dropped slightly. Thereafter, the concentration increased continuously until the end of the observation period (P < 0.05, all parameters). The profile of the haptoglobin curve was similar to that of orosomucoid, but the relative increase was somewhat larger (approximately a factor of 1.8 versus 1.3). The observation period was limited to 96 h after osteosynthesis because the foam and drainage were removed thereafter. There was no difference between the groups concerning the acute-phase proteins, suggesting a clinical influence of the vacuum sealing of minor importance in the patients selected. However, to minimize bias, the study protocol required wound conditions that were suitable for direct suture in all patients, but vacuum sealing is most advantageous for wounds that are not advised for immediate suture. Complement C3 and C4 fluctuate typically during inflammation and can also be classified as acute-phase parameters [14]. In this study, there was no unusual or conspicuous feature concerning the concentration and kinetics of C3 and C4. Several other acute-phase parameters are known. In this study, we also quantified IgM, IgG, IgA, albumin and neopterin. However, analogous to C3 and C4, there was nothing unusual. Therefore, the data are not shown. Transferrin is a negative acute-phase reactant and has been demonstrated to neutralize endotoxin [14, 17, 18]. As expected, the concentrations decreased during the early period after the operation. Again, there was no difference between the groups. All in all, the time courses of CRP, orosomucoid, haptoglobin, α1-antitrypsin and complements revealed substantial changes in their concentrations, suggesting a moderate inflammatory response.

The vacuum sealing was thought to perturb the cytoskeleton, affecting the release of intracellular messengers such as prostaglandins, possibly responsible for the positive clinical experience [7]. The measurement of 6KPG in the plasma showed an earlier decrease in patients with vacuum sealing than in IS. However, there is a considerable variation of the 6KPG level, and this should be suggested as indicative.

Morbidity was checked during the first year after injury (no deaths). There was no difference in the frequency of undesirable events between the groups.

It was well recognized by the investigators and ethics committee that additional burden and potential risk existed for patients with vacuum-assisted closure by delayed skin suture because of the protocol’s requirements to prevent bias. In routine surgery, primary skin closure is preferable if feasible. Vacuum-assisted closure was developed in our own department, and clinical experience has existed since 1987. Thus, it was a safe method in the hands of our experienced surgeons. Moreover, publications have stated that patients benefit from a pronounced
anti-oedematous effect, which lowers the risk of complications of wound healing [2]. Vacuum-assisted closure is an accepted means for the treatment of serious soft-tissue damage and infection.

In summary, it should be concluded that endotoxin can be measured early and temporarily in the plasma in patients with ankle fractures. The injury and surgical procedure cause substantial changes in the plasma concentrations of acute-phase reactants. Vacuum sealing after surgery for malleolar fractures has limited systemic effects and is a safe procedure in the treatment of soft-tissue damage of ankle fractures. However, the foams used in this study had relatively small dimensions (2 x 10 cm) compared with extensive soft-tissue injuries requiring several plates of the foam (one plate is 10 x 15 cm). Hence, it seems reasonable to hypothesize that the systemic consequences are more intensive the larger the vacuum-sealed area is. Again, it should be mentioned that vacuum sealing should not be used as a routine procedure in the surgical treatment of malleolar fractures, but it offers an alternative in severe soft-tissue damage. New developments use the technique of vacuum sealing to apply antimicrobial drugs several times a day to infected wounds with the objective of controlling infection and enhancing detoxification [30].

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