The effect of closed-incision negative pressure wound therapy on clinical and ultrasonographic seroma formation and wound healing following forequarter amputation in large dogs - a randomized pilot trial

Effect van negatiedruktherapie bij gesloten wonden op klinische en echografische seroomvorming en wondheling na voorpootamputatie bij grote honden - een gerandomiseerde pilootstudie

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ABSTRACT

This pilot study aimed at evaluating whether closed-incision negative pressure wound therapy (ciNPWT) has an effect on seroma formation and wound healing following forequarter amputation in dogs above 20 kg. Twelve client-owned dogs weighing more than 20 kg, presented for forequarter amputation, were randomly assigned after surgery into two groups (six ciNPWT and six controls with soft-padded bandage, both bandages applied for three days). A clinical and ultrasonographic control (newly developed scoring system) was performed at bandage removal (three days postoperatively) and ten days, postoperatively. A postoperative seroma was present in 4/6 dogs in the ciNPWT group and in 5/6 dogs in the control group. There were no apparent differences in the ultrasonographic scores or subcutis measurements at three versus ten days, postoperatively. The results of this pilot trial do not support expansion to a larger-scale study evaluating ciNPWT after forequarter amputation in dogs.

SAMENVATTING

Deze pilootstudie had als doel te bepalen of negatiedruktherapie bij een gesloten incisie een effect zou hebben op seroomvorming en wondheling na voorpootamputatie bij honden boven de 20 kg. Twaalf honden van meer dan 20 kg, aangeboden voor voorpootamputatie, werden na de ingreep willekeurig verdeeld in twee groepen (zes honden ondergingen negatiedruktherapie en een controlegroep van zes honden werd behandeld met polsterverband, gedurende drie dagen). Een klinisch onderzoek en een echografische controle (aan de hand van een nieuw ontwikkeld scoresysteem) werden uitgevoerd op het moment van het verwijderen van het verband, i.e. drie dagen postoperatief en tien dagen postoperatief. Een postoperatief seroom was aanwezig bij 4/6 honden in de groep die negatiedruktherapie onderging en bij 5/6 honden in de controlegroep. Er waren geen duidelijke verschillen in de echografische scores noch in de subcutismetingen op drie versus tien dagen postoperatief. De resultaten van deze pilootstudie pleiten niet voor uitbreiding naar een grootschalige studie naar het gebruik van ciNPWT na voorpootamputatie bij honden.
INTRODUCTION

Negative pressure wound therapy (NPWT) is a wound management technique applied in human and veterinary wound care that has gained popularity over the last decades (Go et al., 2017). A foam, applied as a contact layer, is covered by a self-adherent polyurethane plastic drape to provide an airtight seal (Spilbeeen et al., 2013). In the center of the plastic, a hole is created and a suction tube connects the foam with a vacuum device with replaceable reservoir. It has been shown that NPWT enhances granulation tissue formation when applied to chronic open wounds (Daigle et al., 2013) and decreases pain and swelling (Howe, 2015). The mechanism of action of NPWT involves removal of exudate, increased perfusion — secondary to macro- and macro-deformations due to the vacuum — and decreased tension leading to wound contraction (Scherer et al., 2008; Howe, 2015). Additional advantages of NPWT include reducing edema and interstitial fluid (Morykwas et al., 1997; Daigle et al., 2013) and potentially decreasing bacterial colonization (Khashram et al., 2009; Anesater et al., 2011), although the latter is questioned in other articles (Weed et al., 2004; Borgquist et al., 2010). Several veterinary studies showed positive impact of NPWT following skin and reconstructive surgeries, such as skin mesh grafting in dogs (Stanley et al., 2013; Or et al., 2017) or burn wounds (Mullally et al., 2010) amongst others.

Apart from the NPWT to enhance healing of open wounds, various more specialized types of NPWT have been developed, including closed-incision NPWT (ciNPWT) for application immediately after primary closed sutured or stapled surgical wounds. This system differs from the traditional NPWT in several ways. The foam is soft and already integrated in the adhesive drape, as is the connection to the suction tube (Figure 1). Over the last fifteen years, ciNPWT has been developed in human surgery patients to be used over high-risk incisions in order to decrease the complication rate (Ingargiola et al., 2013; Gillespie et al., 2015; Willy et al., 2017). Positive clinical outcomes have been obtained after sternotomy in obese patients (Grauhan et al., 2013), total hip or knee arthroplasty (Gillespie et al., 2015; Manoharan et al., 2016; Pauser et al., 2016), spinal fracture stabilization (Nordmeyer et al., 2016), and extremity trauma (Stannard et al., 2006; Stannard et al., 2012). In all these human studies, the most consistent outcomes of ciNPWT have been a decreased risk of dehiscence and reduced postoperative incision drainage (Stannard et al., 2006; Stannard et al., 2012; Grauhan et al., 2013; Gillespie et al., 2015; Manoharan et al., 2016; Nordmeyer et al., 2016; Pauser et al., 2016). Some authors claim prevention of surgical site infections by using ciNPWT (Fernandez et al., 2019; Tran et al., 2019). However, data are not consistent regarding this advantage (Masden et al., 2012; Shen et al., 2017; Kuper et al., 2020). Unfortunately, the studies mentioned above did never focus on reporting pain scores. There is one opinion paper voicing that ciNPWT subjectively decreases pain after hindfoot and ankle reconstructions (DeCarbo and Hyer, 2010), as well as one human study claiming decreased pain level when using NPWT following skin flaps (Maruccia et al., 2017). It has been suspected recently that ciNPWT would present similar advantages in veterinary patients with a decreased risk of complication following high-risk procedures. In a preliminary clinical study in six dogs and four cats undergoing distal limb fracture stabilization or arthrodesis, it has also been suggested that the significant decrease in postoperative swelling in the ciNPWT compared to the control group was likely to decrease discomfort (Perry et al., 2015). An experimental study, creating dead space on the dorsum of pigs was used as a model for procedures leading to large postoperative dead space and risk of seroma, and has demonstrated an increase in skin perfusion and a decrease in fluid drainage after cyclic NPWT (Suh et al., 2016). However, a clinical trial in horses failed to demonstrate any beneficial effects on wound healing of primary closed wounds after median laparotomy (Gaus et al., 2017).

Up to date and to the authors’ knowledge, there has been only one clinical study (Perry et al., 2015) and two single case reports (Nolff et al., 2015; Go et al., 2017) describing the use of ciNPWT in dogs. Perry et al. (2015) published the use of ciNPWT following surgery of high-energy fractures of the distal limbs. The technique has also been described in a Rottweiler following surgical management of a large recurrent abscess on the thoracic wall (Nolff et al., 2015). More recently, one of the cases included in the current study has been described (Go et al., 2017).

The aim of the current blinded, randomized pilot trial was to evaluate the effect of a commercially available ciNPWT bandage on postoperative seroma formation and wound healing following forequarter amputation in dogs above 20kg. Forequarter amputation was chosen based on the relatively high risk of postoperative seroma. It was postulated that application of ciNPWT would decrease seroma formation as well as postoperative complications related to wound healing. Evaluation of postoperative seroma in

Figure 1. The Prevena system: dressing and vacuum pump (from: base.euro-pharmat.com)
humans relies on ultrasonographic volume measurement, software not available in veterinary patients. Therefore, an ultrasound (US) scoring system was designed to enable objective evaluation of US images of the subcutaneous tissues.

MATERIALS AND METHODS

The study was conducted after approval by the Ethical Committee of the Faculty of Veterinary Medicine and Bioscience Engineering of Ghent University (EC2016/67). Subsequently, approval of the Deontological Committee of the FPS Public Health, Food Chain Safety and Environment was obtained.

A computer-generated randomization list was created to allocate the dogs to one of the two postoperative treatment groups, cNPWT group (Prevena, GD Medical Belgium BV, Belgium) or control group (soft-padded bandage). The allocation to the group remained blinded to the surgeons until surgery was completed.

Patient selection and preoperative examination

Client-owned dogs weighing more than 20kg presented for forequarter amputation for any reason were eligible to enroll into the study, after written consent of the owner. The dogs were excluded if a neoplasia, a fracture or an open wound was involving the proximal part of the limb, in order to eliminate additional risk of infection or seroma due to severe soft tissue trauma at the location of the surgical incision.

Patient details (age, breed, sex, neuter status, weight, body condition score (BCS), reason for amputation, usual behavior and recent activity level), as well as any medication given prior to inclusion in the study were recorded on dedicated forms (Appendix available on request). Preoperative blood analysis included hematocrit, total serum proteins and serum albumin, amongst any other blood analysis dictated by the primary pathology of the patient. The total serum protein and albumin measurements were made in an attempt to eliminate hypoproteinemia as a factor contributing to seroma formation, although postoperative fluid drainage was not correlated to serum albumin or total proteins concentration in a retrospective study by Shaver et al. (2014).

Anesthesia and surgical procedure

A peripheral venous catheter was placed in the cephalic vein of the unaffected limb and the dogs were premedicated with a combination of dexmedetomidine 2 to 5 μg/kg (Dexdomitor, Vetoquinol, France) and methadone 0.2 mg/kg IV (Comfortan, Eurovet Animal Health BV, the Netherlands). Anesthesia was induced with propofol to effect (Propovet, Abbott Laboratories Ltd., UK) and maintained with isoflurane vaporized in oxygen via endotracheal intuba-

Figure 2. Image of a Prevena bandage in place 24 hours after its application following forequarter amputation in a Rottweiler.
locoregional bloc was performed, a local bloc with bupivacaine was used before transecting the nerves. Muscles were routinely apposed with interrupted sutures of polydioxanone, and the subcutaneous appositional pattern and skin intradermal closure were achieved with poliglecaprone 25 (Monocryl, Ethicon, Johnson & Johnson Medical N.V., Belgium). The incision length, intraoperative details and duration of the surgery were recorded. A small blood sample was drawn immediately following skin closure to reassess hematocrit and total serum proteins.

**Postoperative bandage and care**

A postoperative bandage was placed while the patient was still under general anesthesia. The dogs in the control group had a standard bandage around the thorax consisting of an absorbent American dressing (Zetuvit E, Paul Hartmann AG, Germany), synthetic orthopedic padding (Orthoband, Millpledge, Belgium) and self-adherent wraps (Wrapz, Millpledge, Belgium). The ciNPWT group received a Prevena bandage, applied over the wound following the instructions provided by the company (Prevena Incision Management System, Clinician guide). The skin surrounding the surgical incision was first cleaned from remaining organic materials and subsequently degreased with ether. The center of the dressing was applied over the suture line, whereas the adhesive surrounding sheet was firmly pressed onto the intact skin, ensuring an airtight seal (Figure 2). The system was set at a continuous negative pressure of -125 mmHg and any air leakage was corrected by applying additional adhesive cover and stoma paste.

During hospitalization, the modified Glasgow Pain Score (Murrell et al., 2008) was recorded every four hours. In general, the dogs received methadone 0.2 mg/kg IV every four hours for two days and were then switched to tramadol 3 to 5 mg/kg every eight hours orally for two more days (Tramadol Sandoz 50mg, Sandoz nv/SA, Belgium), and carprofen 2 mg/kg twice daily orally for seven days (Rimadyl, Zoetis, Belgium). Antimicrobials were discontinued postoperatively except in case of therapeutic use. The need for any additional analgesic was recorded.

All dogs remained hospitalized for at least three days postoperatively. They were stimulated to go for short leash walks three to four times a day, with support if ambulation was difficult. The bandages (Prevena or soft padded) were closely monitored for integrity, and any need for modification or re-enforcement was recorded.

**Postoperative follow-up**

In both groups, the bandage was kept in place for 72 hours. Albumin levels were re-assessed at the time of bandage removal. Immediately after removal of the bandage, the visual aspect of the incision, the sensitivity on palpation, and any palpable fluid accumulation were recorded on dedicated forms; five parameters (incision line, scar apposition, surrounding skin, subcutaneous tissue and pain) were scored on a three-to-four-scale (Appendix available on request). An ultrasound (US) was performed by a resident or diplomate in medical imaging, who was blinded to the group assignment. The US machine used was a Philips iU22, with a linear probe L12-5, at 4 cm depth. The gain and focus settings were based on personal preference of the imager. Three movies with the probe moving from dorsal towards ventral, cranially to the incision, on the incision and caudally to the incision were recorded for later interpretation. Additionally, five still images were recorded: one dorsal to, one ventral to, and one centered on the skin incision, as well as one 10 cm cranial and a last one 10 cm caudal to the incision centered on the incision to serve as reference. The radiologist screened in particular for the presence of fluid accumulation and/or subcutaneous edema. In case of any abnormalities or fluid accumulation, additional images or movies were taken. The findings of the US were drawn on paper to help understand the distribution of the lesions at the moment of the review of images and movies. Any fluid accumulation was sampled ultrasound-guided, under strict aseptic conditions, in order to perform microscopical analysis.

All dogs were discharged the day of the US examination, all without bandages. Instructions to limit the dogs’ activity to short lead walks only for two weeks were given to the owners. A control visit was scheduled ten days postoperatively. The clinical examination, assessment of the wound at inspection and palpation together with US were repeated as described above.

**Analysis of the ultrasound images**

All movies and images were blinded and randomized, and retrospectively reviewed (NV, ES) according to a specifically designed US protocol, derived from previous publications (Suehiro et al., 2014; Caggiati, 2016; Oya et al., 2016). It was decided to score each set of images according to three factors. The subcutaneous echogenicity was scored 0 if normal, 1 in case of increased echogenicity with echoic lines still visible, 2 in case of increased echogenicity with echoic lines no longer visible (Suehiro et al., 2014). The second parameter was subcutaneous edema, graded 0 if no edema, 1 if mild edema (thin anechoic lines interspersed in the subcutaneous tissue) and 2 in case of severe edema (cobblestone appearance with large anechoic to hypoechoic pockets) (Oya et al., 2016). The last parameter scored was the presence of subcutaneous seroma, graded from 0 to 3, 0 if no seroma, 1 if seroma less than 1 cm², 2 if seroma between 1 and 5 cm², and 3 if seroma larger than 5 cm². All scores were graded at the dorsal aspect, in the middle and at the ventral aspect of the incision line. All the scores at
the different sites were added to one another in order
to have an echogenicity score, an edema score and a
seroma score. The average of the three scores gave
the ultrasonographic score of each patient. Finally,
the subcutaneous thickness was measured at each of
the five sites mentioned earlier (dorsally, middle, ven-
trally, 10 cm cranial and 10 cm caudal of the incision
line), between the skin and the deeper echoic border
of the muscular fascia (Caggiati, 2016) (Figure 3).

Statistical analyses and data expression

Data were tested for normality by the Shapiro-Wil-
coxon test and expressed as mean +/- standard devia-
tion if normally distributed or as median (range) if not
normally distributed.

Given the fact that this is a pilot trial based on a
limited number of patients, the authors elected to dis-
play all findings as descriptive data rather than per-
forming any further statistical analyses to avoid pre-
mature conclusions being drawn.

RESULTS

Population and preoperative examination

Twelve dogs, six in each group, were included. The
breeds included were mixed breed and German
shepherd (n=2) and one of each following (Presa
Canario, Rottweiler, American Staffordshire terrier,
Labrador retriever, Leonberg, Bouvier des Flandres,
Malinois, White Swiss shepherd). The reason for am-
putation was neoplasia at or distal to the elbow in six
cases (four ciNPWT and two control), trauma in three
dogs in the control group (two highly comminuted
fractures distal to the elbow and one chronic brachial
plexus avulsion), severe osteomyelitis secondary to
open distal fractures in two dogs (2 ciNPWT), and
one necrotizing wound distal to the carpus (control
group). The median age was 78 months (12-120) and
60 months (12-132), the median weight 39 kg (31-48)
and 27 kg (20-64), the sex five males (one castrated),
one female (spayed) and four males (one castrated),
two females (one spayed), and the average preopera-
tive BCS 5 (4-7) and 5 (3-7) for dogs in the ciNPWT
and the control group, respectively. Three dogs were
living outside and three dogs had free access to the
garden in both groups.

Preoperative hematocrit was 38% (25-49) and
35% (27-40), total serum proteins 59g/L (47-75) and
66g/L (45-69), and albumin 26g/L (22-33) versus
32g/L (20-33) in the ciNPWT and the control group,
respectively; all were within the normal ranges. The
anesthesia duration was 205 minutes (175-305) and
223 minutes (160-260), respectively. Postoperative
blood analyses (hematocrit and total serum proteins)
were slightly decreased compared to the preoperative
values, but similar for both groups, with a median he-
matocrit of 31% (21-38) and 29% (17-36), and a me-
dian total serum proteins of 51g/L (38-63) and 58g/L
(45-69), respectively.

Postoperative care and clinical follow-up

The Prevena device was easy to apply in all cases,
although there was often a certain degree of pressure
leakage at the first attempt and additional adhesive
sheets had to be applied. In all cases with ciNPWT,
minimal incidents occurred during the three days of
treatment with some short episodes of lost negative
pressure because of air leakage. In one case, it re-
quired additional sheets to cover the defects and in
another case, it required changing of the tubing. All
the other cases were managed without changing any
material. The average modified Glasgow Pain Scale
was 4 (2-6) and 7 (3-9) on day 1; 3 (1-4) and 2 (1-
5) on day 2; 1 (1-4) and 1 (0-2) on day 3 for dogs
in the ciNPWT and the control group, respectively;
no dog required additional pain medication. All cases

![Figure 3. Ultrasound image of a three-day postoperative control after forequarter amputation. The green arrows
indicate the skin, the blue ones indicate the subcutis and the orange ones show the muscular fascia, deeper limit for measuring the thickness.](image)
had minimal (not quantifiable) to complete absence of fluid accumulation in the canister of the vacuum system.

Removal of the cohesive sheets of the Prevena bandage was not well-tolerated and painful in two cases, and a light plane of sedation was required during removal and US (dexmedetomidine 2µg/kg IV). The wound edges were in perfect apposition in 5/6 dogs of the ciNPWT group without any crust or signs of inflammation whereas one dog presented with mild erythema of the surgical incision. In the control group, 3/6 dogs had no postoperative crust nor signs of inflammation whereas two dogs had erythema and the remaining dog had ecchymosis at the level of the incision. Two dogs in each group were sensitive to palpation at the level of the scar after bandage removal, the other ones were not painful. Ten days postoperatively, all incisions were dry and nicely opposed in the ciNPWT group whereas one dog in the control group presented with crusts and irregular apposition. None of the dogs was reacting to palpation of the surgical area. The median total serum proteins (55g/L (46-65) in ciNPWT group versus 61g/L (54-62) in control group) and albumin (25g/L (20-30) versus 26g/L (22-31)) at three days postoperatively were within normal limits.

**Ultrasonographic examination**

On US examination, a subcutaneous fluid pocket was present in 4/6 dogs in the ciNPWT and in 5/6 dogs in the control group at three days postoperatively, and in respectively 4/6 and 3/6 dogs at ten days postoperatively. In case of subcutaneous fluid accumulation, US-guided aspiration was performed, and cytology revealed sero-hemorrhagic fluid, with red blood cells and few degenerative neutrophils with no signs of bacteria, compatible with seroma formation.

The US echogenicity, edema, seroma and global scores are presented in Table 1. The absolute values of the thickness of subcutaneous tissue at the different locations of the skin incision are displayed in Table 2. There was no major difference between the groups in any of the five measured locations. The ratio values (compared to normal skin) were also similar in both groups (Table 3).

The D3/D10 ratio was inferior to 1.0 in 5/6 dogs in the ciNPWT group and 0/6 dogs in the control group dorsally, 4/6 and 1/6 dogs in the middle of the incision, and 3/6 and 1/6 ventrally. Thus, most of the dogs in the ciNPWT group had thicker subcutaneous tissue at ten days compared to three days postoperatively, whereas most of the dogs in the control group had thinner subcutaneous tissue at ten days postoperatively (Table 4).

There was a 100% consensus in the scoring. Yet, the measurements of the subcutaneous thickness were not always identical; the values presented are the average of the two observers for each location.

**DISCUSSION**

This pilot trial in dogs undergoing forequarter amputation was conducted in order to evaluate the effect of ciNPWT on postoperative seroma formation and wound healing. The study design was prospective randomized controlled, the surgeon was blinded to the procedure and so were the US scoring and thickness measurements. The current data did not reveal a decreased risk in postoperative seroma formation after three days of ciNPWT compared to a standard bandage in dogs with forequarter amputation. As already described (Fahie, 2016), seroma formation following limb amputation was a frequent complication, with an overall rate of 8/12 dogs (67%) with a similar incidence in both groups.

In several human clinical studies, a decreased risk of seroma formation has been demonstrated when using postoperative ciNPWT, such as following total hip replacement (Pachowsky et al., 2012) or trauma to the extremities (Stannard et al., 2006). In some studies, measuring the amount of fluid production using Redon drains, the drainage volume was three times (Nordmeyer et al., 2016) to eight times (Pauser et al., 2016) lower in the ciNPWT group than in the control group. This way of quantification was not possible in the present study design without drain placement. Unlike in humans, drains are less routinely used in small animals because of the high risk of infection in clean surgeries leading to very strict asepsis required in the management of the drains (Bristow et al., 2015). There are very few studies in human medicine, in which the use of ciNPWT without simultaneous placement of drains has been described. The few existing ones do not disclose exact volumes of fluid collected in the canister, as it is always minimal to non-existing, as in the current study. In a clinical trial in horses in which ciNPWT was applied after laparotomy, it has been suggested that the collected fluid was condensation water instead of exudate (Gaus et al., 2017).

Studies in human medicine, in which ultrasound has been described to quantify seroma formation usually use high-frequency US machines with a very specific software reconstruction (Pachowsky et al., 2012; Nordmeyer et al., 2016; Pauser et al., 2016) – equipment not available in the Small Animal Veterinary Teaching Hospital of the Faculty of Veterinary Medicine (UGhent) nor in most veterinary institutions. For the current study, all images were stored and retrospectively reviewed after blinding and randomization. In order to objectively evaluate the subcutaneous echogenicity, edema and seroma, a US scoring system, based on previous publications (Suehiro et al., 2014; Oya et al., 2016), was established. The consensus in scoring the US images between the two observers was perfect. Based on the limited experience gained in this pilot trial, it seems worthwhile to further validate this scoring system in a larger cohort of surgical patients. On the other hand, precise measurement of the sub-
cutaneous thickness proved troublesome at various occasions because of the poorly defined edges of subcutaneous tissue, as previously described by Caggiati et al. (2016). Several factors could contribute to the apparent difference in the effect of ciNPWT on the incidence of postoperative seroma formation between canine and human patients. In a recent meta-analysis describing the efficacy of ciNPWT in abdominal wall reconstructions in humans, the difference in overall incidence of postoperative seroma failed to reach statistical significance (Tran et al., 2019). Major contributors could be the relatively uncontrolled postoperative movement in dogs (Amsellem, 2011; Fahie, 2016) but especially the extensive surgical area created by limb amputation versus the relatively small rim of foam provided in a Prevena bandage. Limb amputation in large dogs was chosen as a natural model by the authors, because of the relatively high risk of postoperative seroma formation (Fahie, 2016) to bypass the need of an experimental model. But, in retrospect, the authors believe that one of the reasons why surgical wounds after amputation proved not ideally suited to explore the potential benefits of the Prevena bandage is the limited contact area. In case of forequarter amputation, the skin incision line is not overlying all of the deeper suture lines. Potentially, the application of NPWT using white foam over a much more extensive area, as described for free skin grafts (Stanley et al., 2013; Or et al., 2017) might have potential to be more efficacious in dogs undergoing limb amputation. Yet, skin grafts frequently involve meshing of the skin with multiple perforations whereas the likelihood of drainage through traditionally closed suture lines remains more doubtful (Gaus et al., 2017). In human medicine, a proportional association between the width of foam dressing and the decrease in lateral tensile forces has been suggested (Tran et al., 2019). In that study, only a decrease in infection rate and

| Table 1. Ultrasonographic echogenicity, edema, seroma and global US scores at three days (D3) and at ten days (D10) postoperatively (median value with range). |
| Echogenicity | Edema | Seroma | Global US |
| ciNPWT D3 | 4 (2-7) | 6 (3-7) | 3 (0-7) | 4.0 (3.0-5.7) |
| Control D3 | 5 (1-6) | 7 (3-10) | 1 (0-5) | 4.2 (3.0-5.7) |
| ciNPWT D10 | 6 (4-8) | 5 (4-9) | 2 (3-6) | 4.5 (3.0-6.7) |
| Control D10 | 6 (3-7) | 8 (4-10) | 3 (0-9) | 6.0 (3.3-7.7) |

| Table 2. Ultrasonographic measurement of thickness (mm) of subcutaneous tissue at three days (D3) and ten days (D10) postoperatively at different locations on the skin incision (median value with range). |
| Dorsal | Middle | Ventral |
| ciNPWT D3 | 13 (10-16) | 16 (14-19) | 16 (14-19) | 8 (7-9) | 8 (6-13) |
| Control D3 | 13 (9-26) | 17 (15-24) | 17 (9-21) | 10 (6-12) | 10 (6-13) |
| ciNPWT D10 | 14 (13-22) | 18 (15-26) | 15 (9-24) | 7 (7-9) | 9 (6-11) |
| Control D10 | 12 (7-19) | 16 (9-22) | 11 (6-13) | 7 (4-12) | 9 (5-12) |

| Table 3. Three-day (D3) and ten-day (D10) ultrasonographic measurement ratios of subcutaneous tissue thickness compared to normal skin 10 cm away from the incision, at different locations (median value with range). |
| Dorsal | Middle | Ventral |
| ciNPWT D3 | 1.2 (0.9-1.4) | 1.4 (1.3-1.8) | 1.3 (1.2-1.7) |
| Control D3 | 1.2 (0.8-2.4) | 1.5 (1.3-2.2) | 1.5 (0.8-2.0) |
| ciNPWT D10 | 1.3 (1.2-2.0) | 1.7 (1.3-2.4) | 1.3 (0.8-2.2) |
| Control D10 | 1.0 (0.7-1.7) | 1.5 (0.8-2.0) | 1.0 (0.6-1.2) |

| Table 4. Ultrasonographic measurement ratios of subcutaneous tissue thickness compared to normal skin 10 cm away from the incision, at different locations (median value with range). |
| D3/D10 dorsal | D3/D10 middle | D3/D10 ventral |
| ciNPWT | 0.9 (0.6-1.0) | 0.9 (0.6-1.2) | 1.0 (0.6-1.5) |
| Control | 1.2 (1.0-1.8) | 1.1 (0.9-1.9) | 1.6 (0.9-2.3) |
wound dehiscence was found due to ciNPWT; however, no significant effect on postoperative seroma or hematoma formation was noted. Therefore, the Prevena system used may not be ideally suited in case of forequarter amputation.

In human surgery patients, the reported positive effect of ciNPWT is not limited to an effect on postoperative seroma formation, but also on wound care (Pachowsky et al., 2012; Nordmeyer et al., 2016), dehiscence (Willy et al., 2017) and surgical site infection (Stannard et al., 2012; Grauhan et al., 2013; Gillespie et al., 2015; Willy et al., 2017). In experimental studies, the effect of ciNPWT on the healing of surgical incisions has been investigated (Horch, 2014; Loveluck et al., 2016; Suh et al., 2016). A first effect of the negative pressure exerted on the suture line is the increase of perfusion and oxygen delivery to the surgical wound, leading to decreased inflammation and faster healing, as shown on the back of experimental pigs (Suh et al., 2016), or the abdominal skin of healthy human patients using a white light spectroscopy and laser Doppler flowmetry (Horch, 2014). In a biomechanical study, the forces applied to the skin were modelled and it was concluded that there was a decreased tension across the incision line when ciNPWT was applied (Loveluck et al., 2016). The subjective observation of very thin scars in all but one dog in the ciNPWT group of the present study might have been the result of such tension-free skin apposition. None of the surgical incisions in the ciNPWT group of the current study, including the only case with minimal crust formation, presented any visible signs of dehiscence, inflammation or ecchymosis. In the current preliminary study, one dog in the control group developed extensive surgical site infection and could not complete the study, as the subcutaneous measurements would have been at the location of the dehiscence. No surgical site infection occurred in any of the other dogs. Nevertheless, it was not possible to determine objectively if the Prevena system indeed enhanced wound healing. A case-controlled clinical trial in fifty horses failed to demonstrate any beneficial effect of ciNPWT on the healing and complication rate of laparotomy wounds (Gaus et al., 2017).

At the time of the current study, the cumulative number of reported canine patients treated with ciNPWT was seven (Nolff et al., 2015; Perry et al., 2015). Despite the fact that in the present study this number was almost doubled, the total sample size remains extremely small. The clinical application of ciNPWT following surgical repair of high-energy fractures in the distal limbs in six dogs and four cats is up to date the only case series that has been described in the veterinary literature (Perry et al., 2015). In that study, control and ciNPWT patients received a modified Robert-Jones bandage, that was removed after 24 hours and replaced until 72 hours. The treatment group received ciNPWT under the Robert-Jones during the first 24 hours. Despite disparity between the groups and types of fractures, the authors reported a significant decrease in the postoperative limb circumference and wound discharge after ciNPWT application. It should be noted that the length of the applied ciNPWT therapy was extremely short (24 hours). In the current study, the Prevena bandage was left in place for three days, which was retrospectively conceived by the authors as potentially too short to result in any difference between the groups. In a recent experimental study in pigs, incisional wounds with extensive dead space were created on the dorsum to study the effect of ciNPWT applied for seven days concurrently with aspiration drains, and the authors described a significant decrease in the seroma drainage (through the drains) in the treatment group compared to the control group (Suh et al., 2016). From another perspective, in the current human literature, an extensive variety in the duration to apply ciNPWT treatment has been reported, ranging from a few days till more than ten days with most performed four-day protocols (Li et al., 2017; O’Leary et al., 2017; Shen et al., 2017; Kuper et al., 2020). Furthermore, longer treatments also involve longer hospitalization time and higher costs, which may be a limitation to the extended use of ciNPWT in veterinary patients (Gaus et al., 2017). This pilot trial suffered from various limitations. Specific to the study design was the small number of subjects. Furthermore, the evaluation of wounds in general and surgical wounds in particular, is primarily subjective unless based on histologic evaluation, which is not applicable in a clinical setting. To counteract this shortcoming, postoperative US was performed, and a scoring system was developed to evaluate images in a standardized way. An additional limitation to this study was the variation in the pathologies of the cases included; some oncological or infectious cases might have potentially influenced the risk of postoperative complications. The authors tried to limit this bias by excluding dogs that had disease extending proximal to the elbow.

**CONCLUSION**

In this study, the Prevena device was easy to use and the ciNPWT was well-tolerated by the dogs although slight sedation was needed to remove the adhesive sheet in one third of the patients. Despite successful experience with ciNPWT in human patients prone to develop seroma and wound complications, in this preliminary study, an appreciable decrease in either local inflammation or seroma formation was not detected. To further explore the potential benefits of ciNPWT, further investigations in other models are warranted.
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