ORIGINAL RESEARCH ARTICLE – CLINICAL SCIENCE

A randomized, double-blind, placebo-controlled multicenter trial evaluating topical zinc oxide for acute open wounds following pilonidal disease excision

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ABSTRACT

The purpose of this randomized, double-blind, placebo-controlled multicenter trial was to compare topical zinc oxide with placebo mesh on secondary healing pilonidal wounds. Sixty-four (53 men) consecutive patients, aged 17–60 years, were centrally randomized to either treatment with 3% zinc oxide ($n=33$) or placebo ($n=31$) by concealed allocation. Patients were followed with strict recording of beneficial and harmful effects including masked assessment of time to complete wound closure. Analysis was carried out on an intention-to-treat basis. Median healing times were 54 days (interquartile range 42–71 days) for the zinc and 62 days (55–82 days) for the placebo group ($p=0.32$). Topical zinc oxide increased ($p < 0.001$) wound fluid zinc levels to 1,540 (1,035–2,265) μM and decreased ($p < 0.05$) the occurrence of *Staphylococcus aureus* in wounds. Fewer zinc oxide ($n=3$) than placebo-treated patients ($n=12$) were prescribed postoperative antibiotics ($p=0.005$). Serum-zinc levels increased ($p < 0.001$) postoperatively in both groups but did not differ significantly between the two groups on day 7. Zinc oxide was not associated with increased pain by the visual analog scale, cellular abnormalities by histopathological examination of wound biopsies, or other harmful effects. Larger clinical trials will be required to show definitive effects of topical zinc oxide on wound healing and infection.

Local management of wounds should provide optimal healing conditions and reduce the risk of infections.[1,2] Unambiguous clinical evidence is lacking regarding the use of topical agents for local wound care and their effect on wound healing. The authors of a recent systematic review concluded that the clinical trials performed to support the use of these topical agents in general were very small and of very poor quality.[2]

Pilonidal disease in the sacrococcygeal region is a common disease, occurring predominantly in young males.[3,4] Although the preferred surgical treatment is debated,[3,5–8] retrospective studies indicate that primary closure and open granulation are chosen as the two most frequent treatment options.[9–11] The open wound resulting from excision follows a predictable healing time course and typically heals within 2 months.[5,12–17] The open pilonidal wound is therefore suitable for testing wound-healing modulators.

Zinc is an essential trace element for tissue repair and may accelerate wound healing.[18] Following an initial enthusiastic report on the beneficial effect of oral zinc sulfate on open pilonidal wounds,[12] the results could not be reproduced in an identically designed trial where the median healing times were 56 days for the 10 zinc-treated patients and 59 days for the 10 control patients.[17] However, these trials used unconcealed allocation and were not blinded. It has been suggested that oral zinc is efficacious only in patients with subnormal serum-zinc levels.[19,20] Zinc may also be administered topically.[18,21–24] In a double-blind trial, topical zinc oxide significantly increased the healing rate of leg ulcers compared with placebo treatment.[22] Only one wound became infected in the zinc oxide group compared with six wounds in the placebo group.[22] Antimicrobial, immunomodulatory, and cytoprotective actions of zinc oxide may account for the clinical effects.[25–28]

The aim of the present randomized clinical trial was to compare the effect of topical zinc oxide (3%) meshes with placebo meshes on nonsutured wounds following surgical

IQR interquartile range
VAS visual analog scale

removal of pilonidal disease. Zinc oxide in a mesh provides a depot and sustained release of bioavailable zinc to the wound at noncytotoxic levels.[29–31] Topical zinc in this form was found to be efficacious,[22,32] while readily water-soluble zinc sulfate had no or even deleterious effects on wound healing.[32,33]

MATERIALS AND METHODS

In this randomized, double-blind, placebo-controlled multicenter trial, the patients were centrally randomized to receive either topical zinc oxide meshes or placebo meshes during pilonidal wound healing. The trial was performed in accordance with the Helsinki Declaration II of 2000, approved by the local Ethics Committees (KF 01-162/01, 11-106/01, 11-003/02, and 11-081/02), and registered by The Danish Data Protection Agency (2001-41-1167) and International Standard Randomized Controlled Trial Number (ISRCTN35311675) registry (www.controlled-trials.com). Data were collected and monitored following The International Conference on Harmonization—Good Clinical Practice Guidelines.[34]

Eligibility criteria for patients

Patients, 18 years or older, operated on for the first time for pilonidal abscess or chronic pilonidal disease were recruited after giving their written informed consent. Patients who were hypersensitive to zinc, had dementia, insufficient in Danish, or pregnant or lactating were excluded. The patients' skin phototype,[35] concomitant diseases, medication known to influence wound healing, and tobacco consumption were recorded. Patients were enrolled at two university hospitals in Copenhagen and Aalborg, Denmark.

Surgery, allocation, and interventions

Before surgery, pain intensity due to the pilonidal disease was assessed by the patient on a nongraduated 100 mm visual analog scale (VAS). A preoperative venous blood sample was drawn from the fasting patients into evacuated glass tubes certified free of significant zinc contamination (Vacutainer®, Cat. No. 367737; BD Vacutainer Systems, Plymouth, UK).

The patients were placed prone and received general anesthesia. The skin was shaved and disinfected with 83% ethanol and 0.5% chlorohexidine gluconate. Perioperative prophylactic antibiotics were not given. A charcoal cotton swab was taken from pus of an abscess or the pilonidal sinus and sent in Stuart transport medium for aerobic and anaerobic culturing. An adhesive tape (Tensoplast®; Smith and Nephew, Hull, UK) was used to pull the buttocks apart. The pilonidal cyst was injected with methylene blue and excised with diathermy preserving the presacral fascia.[36] The excised tissue, including the pilonidal disease, was fixed in 10% neutral-buffered formalin. Following hemostasis, the wound volume was measured by filling the sealed cavity with physiological saline from a graduated syringe with a cannula.[37] This method is convenient and rapid but less precise than wound volume estimation with dental molding material.[38,39] The wound margins were marked on a transparent plastic sheet and the wound area was determined.[40]

The patient allocation sequence was computer-generated 1 : 1 in variable block sizes of four or six stratified for center. Allocation concealment was performed using centrally packaged, consecutively numbered, identical packages containing zinc oxide or placebo meshes. The investigators were asked to use the next available number when a new patient entered the trial. Randomization codes were kept confidential until final assessments of the patients, data entry, statistical analyses, and main conclusions were completed.

Zinc oxide (USP) was evenly bound, verified by light microscopy examination, with polyvinylpyrrolidone (Kollidon® 90F; BASF, Ludwigshafen, Germany) to a nonfray 50-cm-long fine-mesh cotton weave (Corman, Lacchiarella, Italy) of 2 (0.5 g)-or 5 (1.25 g)-cm widths. The edges of the mesh were thread-locked and the ends heat-sealed with 30 μm polyethylene to minimize fiber shedding. The median zinc oxide concentration of mesh equaled 33 mg/g (interquartile range [IQR] 33–35 mg/g, $n=12$), determined by atomic absorption spectrophotometry. Placebo consisted of polyvinylpyrrolidone alone bound to the same mesh. The zinc and placebo meshes were manufactured in Class 100,000 facilities, sterile, and indistinguishable in color, texture, and smell.

The dry meshes were applied to the wounds in at least four layers to ascertain that at least the same total zinc oxide dose was delivered as in our previous trial.[22] A hydrofiber absorptive dressing (Aquacel®, ConvaTec, Deeside, UK) was loosely packed into the wound on top of the meshes. The hydrofiber dressing, composed of carboxymethylcellulose, neither influences zinc oxide solubilization nor binds zinc ions chemically. The surrounding skin was protected with a barrier film (Cavilon™, 3M, St. Paul, MN). Finally, the wound was covered with a transparent polyurethane adhesive film dressing (Tegaderm® HP, 3M) to maintain a moist healing milieu.

The time spent by the patient in the operating theater was registered.

Postoperative follow-up

Wounds were treated daily during the first 7 postoperative days with either zinc oxide or placebo meshes and thereafter every second day by community nurses, except at the follow-up visits at the study centers. At the scheduled treatment sessions, fresh zinc oxide or placebo meshes were applied. If wound treatment was required in between the scheduled wound treatments, only secondary dressings were changed. Application of zinc oxide and placebo meshes, and wound closure or not were recorded in the patient's logbook. Upon change of mesh the wounds were cleansed with saline-moistened nonwoven swabs. Exuberant granulations were surgically debrided or treated with 10% silver nitrate.

Patients were reexamined at the surgical centers on postoperative day 7, 30, 60, and 90. On these visits, pain intensity was assessed before and 30 minutes after removal of intervention meshes. Whether or not the meshes adhered to the wound was recorded. The wound was evaluated clinically with respect to complete wound closure by assessors blinded to treatment. The necessity of systemic

antibiotic treatment was judged on clinical signs of wound infection. Duration of sick leave was also recorded. In addition to these procedures, a venous blood sample, accumulated wound fluid in meshes, and a bacterial swab of the wound surface were obtained from fasting patients on postoperative day 7. Also, 6-mm punch biopsies were taken from adjacent uninjured skin, wound edge, and wound center under local anesthetic (1% lidocaine containing epinephrine) on postoperative day 7 and fixed in 10% neutral-buffered formalin. Any serious or nonserious adverse events occurring until wound closure or day 90 were reported to the coordinating investigator. The investigator judged the adverse event as definitely not, improbable, probable, or most probably related to the intervention.

Wound microbiology

The collected bacteriological specimens were cultured aerobically on 5% horse blood agar and Conradi–Drigalski agar, and anaerobically on chocolate agar for 48 hours. All isolates were identified according to general laboratory diagnostic standards. The hemolytic streptococci were grouped A, B, C, D, F, or G using the Oxoid Dryspot Streptococcal Grouping kit (Oxoid, Greve, Denmark).[66]

Histopathology

The formalin-fixed tissues were embedded in paraffin and 5-μm-thick sections were cut from each paraffin block. The sections were stained with hematoxylin and eosin. The presence of hair fragments and/or epithelium, abscess, granulation tissue, fibrosis, and chronic inflammation were evaluated in excised pilonidal disease specimens. Resection margins were evaluated regarding the presence of acute inflammation, chronic inflammation, and/or fibrosis.[41] Day 7 biopsies were examined for foreign body reactions, and acute and chronic inflammatory cell infiltrate. Inflammatory tissue responses were graded as follows: 0, none; 1, slight; 2, moderate; and 3, pronounced. Evaluations were performed by one of the authors (K. K.) without prior knowledge of group affiliation.

Zinc and albumin analyses of sera and wound fluids

The blood samples were left standing at room temperature for 1 hour, centrifuged at $2{,}000\times g$, and the serum fraction was transferred via a cannula to a new Vacutainer® tube to avoid contamination from outside. Wound fluids were squeezed from the meshes in 10-mL plastic syringes, centrifuged at $4{,}000\times g$, and passed through a 0.20 μm syringe filter (Serum Acrodisc®, Pall Gelman, Ann Arbor, MI) to separate ionized from nonsolubilized zinc oxide essentially as described earlier.[30] To validate this assay for zinc levels, 0.5 mL of wound fluid from placebo-treated wounds, either spiked to 1,500 μM zinc or not, was incubated at 37 °C for 1 hour in the presence or absence of 0.1 g of placebo mesh. The incubated wound fluids were analyzed for zinc content both with and without subsequent filtration. Also, wound fluid from zinc oxide-treated wounds was filtered a second time. The sera and wound fluids were maintained at −80 °C until analyzed collectively in one run without prior knowledge of group affiliation. Zinc concentrations were determined colorimetrically with the complexometric reagent 2-(5-bromo-2-pyridylazo)-5-(N-n-propyl-N-3-sulfopropylamino)phenol[42] using an automated chemical analyzer (Hitachi 911; Tokyo, Japan). The precision and accuracy of this method were validated using atomic absorption spectrophotometry as the reference method in our laboratory.[42] Albumin levels were determined by the dry reagent slide technology using a Vitros 950 automated analyzer (Ortho-Clinical Diagnostics, Raritan, NJ).

Sample size calculation and statistical methods

The healing effects of topical zinc oxide in pilonidal wounds have not been reported previously. Thus, we relied on control healing proportion and intervention effect results from a randomized double-blind trial on leg ulcers.[22] These are the only available comparative clinical healing data on topical zinc oxide.[22] The required sample size was estimated using data for the proportion of patients healed at 12 weeks.[22] Owing to the expected faster healing of pilonidal wounds compared with chronic leg ulcers, 80% of the patients in the zinc oxide and 40% in the placebo group were assumed to achieve complete healing within 90 days. Based on these assumptions, it was estimated that 27 patients in each group were required to demonstrate a 40% difference between the two groups with $\alpha=5\%$ and $\beta=20\%$.

Data were double entered into a Lotus Notes database independently by two persons. Any mismatch was discovered by the program and corrected for.

The primary outcome was time in days to wound closure, defined by complete coverage of the wound with visible epithelium. Participants lost to follow-up were censored at the date of last contact to the investigators. Wound closure was analyzed by Kaplan–Meier estimators on an intention-to-treat basis. A log rank test was applied to compare the healing times between the two groups. The Cox proportional hazard regression model was used to adjust for factors (covariates) other than the intervention. The covariates chosen were body mass index, skin type, smoking, preoperative antibiotics, emergency or elective surgery, wound volume, and the serum-zinc/serum-albumin ratio. The ratio between serum-zinc and serum-albumin preoperatively was used because of the correlation between the two variables ($r_S=0.54$, $p<0.001$, $n=61$). Missing data were replaced by the mean value of both groups of respective covariates in the Cox model. Baseline characteristics were not tested for statistical significance due to inappropriateness of such analyses.[43]

Secondary outcomes were postoperative antibiotic treatment, reoperations, pain intensity, and adverse events. Serum-zinc levels and sick leave were considered tertiary outcomes. Student's t test, chi-square test, and general linear model were used for normally distributed data. For nonnormally distributed data, we used the Mann–Whitney's test. Normally distributed data are presented as mean ± standard error of the mean (SEM) and nonnormally distributed data as median (IQR). All statistical analyses were performed using SPSS® 12.0 for Windows (Chicago, IL).

The level of statistical significance was defined as $p<0.05$.

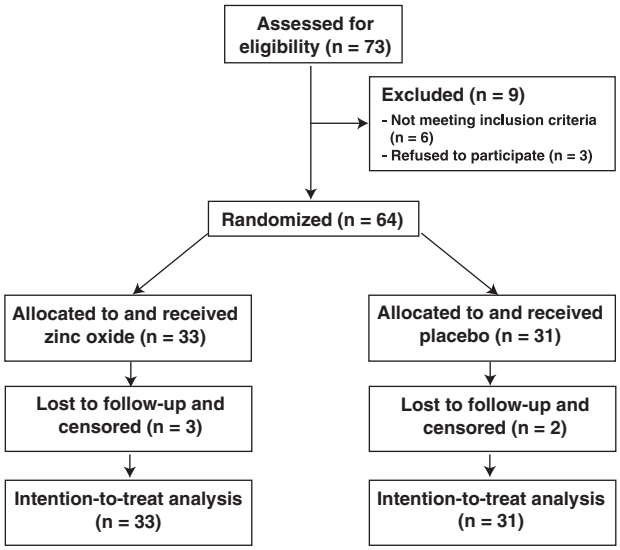

Figure 1. Flow of patients through the trial for the zinc oxide and placebo groups.

RESULTS

Sixty-four patients, five from Aalborg and 59 from Copenhagen, were randomized to zinc oxide or placebo from February 2002 to October 2003. Follow-up continued from randomization to May 2004. A 17-year-old female patient was included by mistake, but has been retained in the trial for analyses. Three patients in the zinc oxide group and two patients in the placebo group withdrew from the study within 2 weeks after randomization for unknown reasons (Figure 1).

The two intervention groups were comparable concerning baseline characteristics, except for more smokers in the zinc group (Table 1). Fourteen patients had self-evaluated skin type II, 27 skin type III, 20 skin type IV, and three skin type V with a similar distribution in the two groups.[44] Neither serum-zinc nor serum-albumin levels differed between the two groups before operation (Figure 2).

Seventy percent of bacteriological cultures from the pilonidal disease obtained before excisions were positive. Of the positive preoperative isolates, 60% were aerobes, 28% anaerobes, and 12% were a mixture of aerobic and anaerobic organisms (Table 2).

Two-thirds of the patients received emergency surgery with no intervention group difference. The duration of surgery, and the size of the wounds following excision were similar in the two groups (Table 1).

Pathological examination verified the features of pilonidal disease with hair fragments and/or epithelialized sinuses present in 90% (47/52) of the specimens. Abscess formation was observed in 83% (43/52) of the excised pilonidal diseases. No resection margin was free of inflammation (Table 3). Malignancies were not observed in any of the specimens examined.

The median healing time was 54 days (IQR 42–71 days) in the zinc oxide group and 62 days (55–82 days) in the placebo group. The proportions of patients with healed wounds in the two groups are shown in Figure 3. Although there was a trend toward earlier wound closure with topical zinc oxide compared with placebo, the log rank test revealed no significant difference ($p=0.32$) between the two groups. Unadjusted hazard ratio of zinc oxide vs. placebo was 1.30 (95% confidence interval 0.77–2.19). Our Cox regression model for prespecified variables revealed that increasing wound volume on the day of surgery was a negative predictor ($p=0.016$) of time to wound closure. Smokers showed faster wound healing ($p=0.011$) than nonsmokers (Table 4). There was a trend ($p=0.053$) of earlier wound healing in patients subjected to emergency compared with those operated electively (Table 4).

With respect to secondary and tertiary outcomes, significantly fewer zinc oxide-treated patients were given systemic antibiotics postoperatively (Figure 4). Wound infections, manifested by a foul smell, were observed in one zinc-treated and in eight placebo-treated patients. The one patient in the zinc group and six of the eight patients in the placebo group were given oral metronidazole (500 mg t.i.d.) for 7–10 days beginning at the first postoperative week. Oral dicloxacillin (500 mg t.i.d.) was administered for 7–10 days on two separate occasions in one placebo-treated patient (counted as one event) and once in another placebo-treated patient.

Serum-zinc (Figure 2A) and serum-albumin (Figure 2B) levels increased postoperatively in the zinc group by 18%

Table 1. Preoperative and intraoperative group demographic comparability

Parameter	Zinc oxide mesh	Placebo mesh
n	33	31
Age (years)*	26 (22–31)	25 (21–32)
Gender (males/females)†	27 M/6 F	26 M/5 F
Ethnic background (Nordic/non-Nordic)†	26/7	21/10
Body mass index (kg/m^2)*	25.2 (23.4–28.7)	26.6 (24.0–28.7)
Insulin-dependent diabetes mellitus†	0	3
Occupational position (sitting/standing)†	16/17	18/13
Present smoker†	25	12
Preoperative oral antibiotics†	10	8
Preoperative oral analgesics†	12	12
VAS score (mm)‡	23 ± 4.6	26 ± 5.2
Emergency operation†	21	22
Elective operation†	12	9
Duration of operation (minutes)‡	27 ± 1.2	28 ± 1.6
Wound volume (cm^3)‡	15 ± 1.6	15 ± 2.5
Wound area (cm^2)‡	10 ± 1.2	9 ± 1.6

*Median (IQR).
†Number of patients.
‡Mean ± SEM.

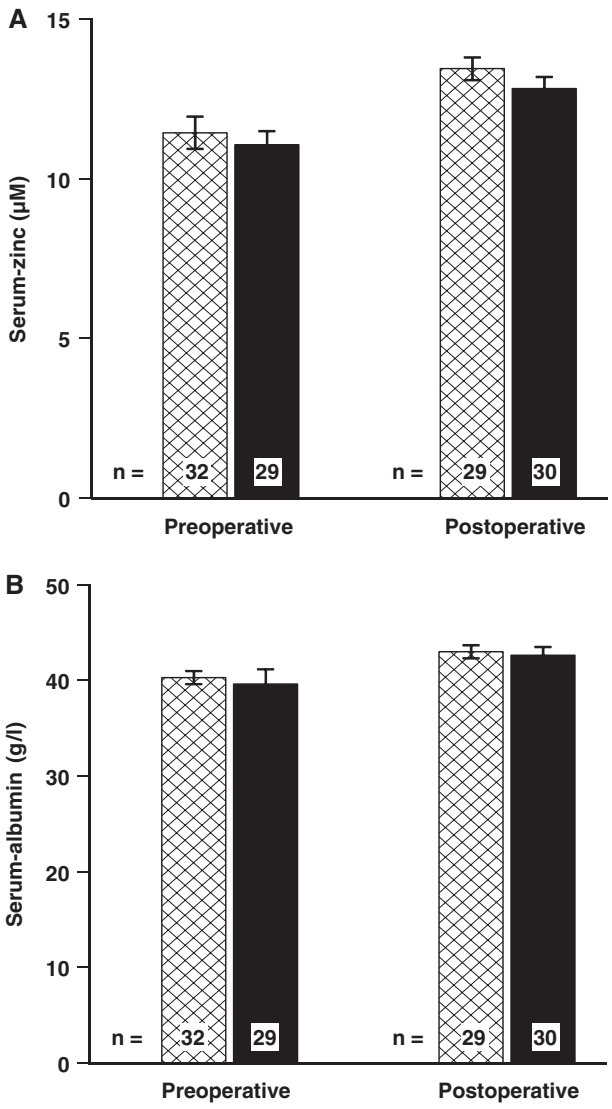

Figure 2. Serum zinc and albumin levels in preoperative and postoperative blood samples. (A) Zinc and (B) albumin concentrations in sera preoperatively and 7 days postoperatively in the zinc oxide (cross-hatched bars) and placebo (solid bars) groups. There were no differences in zinc or albumin levels between zinc and placebo groups either preoperatively or postoperatively. Mean ± SEM. *n*, number of patients.

($p<0.001$) and 7% ($p<0.001$) and by 15% ($p<0.001$) and 8% ($p<0.001$) in the placebo group compared with preoperative levels. No significant differences in serum-zinc or serum-albumin levels were found between the zinc and placebo groups on day 7. The wound fluid zinc concentration was significantly ($p<0.001$) increased in zinc oxide-treated (1,540 [1,035–2,265] µM, $n=17$) compared with placebo-treated patients (0 [0–2.5] µM, $n=15$) on postoperative day 7. Neither the mesh nor the filter material bound zinc ions selectively and all nonsolubilized zinc oxide was eliminated by the single filtration procedure.

Table 2. Microbiological qualitative analyses preoperatively and 7 days postoperatively in the pilonidal wounds

	Preoperative		Postoperative day 7	
Organism	Zinc oxide	Placebo	Zinc oxide	Placebo
n	30	28	30	28
Hemolytic Streptococci (A, B, C, and G)*	1	3	4	6
Nonhemolytic Streptococci*	1	2	0	2
*Staphylococcus aureus**	4	4	6†	13
Coagulase-negative Staphylococci*	3	3	11	6
Corynebacterium sp.*	3	5	2‡	10
Enterococcus sp.*	0	2	6	1
Escherichia coli and other enterobacteria*	0	0	19	13
Bacteroides sp. and mixed anaerobic flora*	5	13	3	4
Candida sp.*	0	0	1	0
Patients with no growth	14	4	1	1
Patients with aerobes only	11	13	26	23
Patients with anaerobes only	4	7	0	0
Patients with aerobes and anaerobes	1	4	3	4

*Number of isolates per group and occasion. In several patients, more than one bacterium was isolated.
†$p=0.038$ compared with placebo day 7.
‡$p=0.007$ compared with placebo day 7.

Wound fluid albumin concentrations were close to those reported earlier[45] but did not differ significantly between the zinc oxide (22.5 [18–25] g/L, $n=17$) and placebo (27.0 [23–32] g/L, $n=15$) groups day 7.

The bacterial flora shifted to a more aerobic composition postoperatively. The Gram-positive organisms *Staphylococcus aureus* and *Corynebacterium species* were cultured less frequently from zinc oxide-treated wounds (Table 2).

Mesh adherence to the wound was recorded in no instance of the total of 67 changes in the zinc group and only once of 70 changes in the placebo group. On postoperative day 7, pain intensity scores neither differed between the groups nor increased significantly 30 minutes after mesh removal (Table 5). Beyond day 7, all VAS scores were below 5 mm.

Table 3. Histopathological assessment of excised pilonidal disease

	Zinc oxide	Placebo
n	27	25
Sinusoidal cavity*		
Hair and/or epithelium	25	22
Abscess	23	20
Granulation tissue	19	15
Chronic inflammation and fibrosis	24	23
Resection margin*		
Normal	0	0
Acute inflammation	3	7
Chronic inflammation	24	18
Fibrosis	0	0

*Number of occurrences. Not all specimens were processed for light microscopy due to technical errors.

Histopathologic examination of 36 wounds on day 7 revealed no significant differences in the composition or grade of the inflammatory cell infiltrate between the two groups (Table 6). In one wound, a few multinucleated giant cells containing birefringent material were observed (Figure 5). No granulomas were observed in this section.

The median sick leave duration was 10 (2–26) days in the zinc oxide and 7 (0–30) days in the placebo group.

One male patient in the placebo group developed a residual midline sinus 4 weeks after wound closure and was reoperated. No other anticipated or unexpected adverse events were recorded.

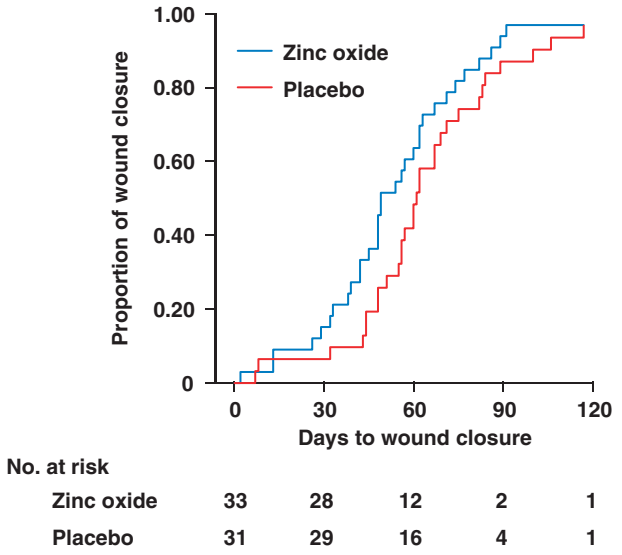

Figure 3. Kaplan–Meier curves depicting proportion of complete healing in the zinc oxide group (n=33) and placebo group (n=31) as a function of time. p=0.32 (log rank test).

Table 4. Cox multiple forward stepwise regression analysis

Variable	Hazard ratio (95% confidence interval)	*p*-value
Emergency vs. elective operation	1.83 (0.99–3.39)	0.053
Smoker vs. nonsmoker	2.40 (1.23–4.69)	0.011
Wound volume	0.97 (0.94–0.99)	0.016
Zinc oxide vs. placebo	0.98 (0.53–1.81)	0.947

DISCUSSION

A recent Cochrane systematic review failed to identify any high-quality randomized clinical trial on topical agents for surgical wounds healing by secondary intent.[2] In the present trial, we took all precautions to avoid bias including a priori sample-size calculation, adequate allocation concealment, placebo-controlled, blinded outcome assessment, a predefined primary outcome measure, and intention-to-treat analyses.[46–48]

Although the time to closure of open pilonidal wounds tended to be shorter with zinc treatment, topical zinc oxide did not significantly accelerate healing, i.e., the number of included patients was insufficient to detect the predicted 40% promotion of healing with zinc oxide. Admittedly, the small sample size limits the impact of our trial as we are unable to exclude beneficial or harmful effects. We identified two factors, which seem to have profound effects on the time to wound closure. The volume of the wound just after excision significantly predicted the healing time. This finding is what one would expect and is in accordance with previous observations.[15,49] Unexpectedly, smoking was associated with significantly faster healing. Although smoking increased dehiscence of small sutured wounds in a prior study,[50] the beneficial effect of smoking on the healing of the nonsutured pilonidal wound has not, to the best of our knowledge, been reported previously. Adjustment of the primary analysis for these prognostic factors did not change the main conclusion. Thus, it is advisable to

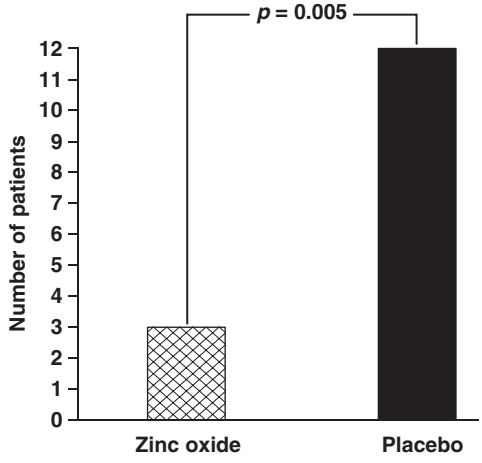

Figure 4. Oral antibiotics prescribed postoperatively (chi-square test).

Table 5. Pain experienced and assessed by the patient on a VAS before and after removal of mesh on postoperative day 7

VAS score (mm)	Zinc oxide	Placebo
n	30	29
Before mesh removal*	27 ± 3.3	18 ± 3.7
After mesh removal*	32 ± 4.8	22 ± 3.5

*Mean ± SEM. No significant differences were found between or within the two groups.

stratify for wound volume and smoking in future trials on this wound type. Moreover, our data tend ($p=0.053$) to support the perceived clinical wisdom that emergency operations for pilonidal disease are superior to elective procedures in terms of time to healing.

Table 6. Histopathological assessment of pilonidal wounds postoperative day 7*

	Zinc oxide	Placebo
n	21	15
Adjacent skin†		
Foreign body reaction	0	0
Acute inflammation	5	3
Chronic inflammation	10	6
Grade of acute/chronic inflammation		
Slight	11	6
Moderate	0	0
Pronounced	0	0
Wound edge†		
Foreign body reaction	0	0
Acute inflammation	17	14
Chronic inflammation	17	13
Grade of acute/chronic inflammation		
Slight	19	11
Moderate	2	1
Pronounced	0	2
Wound center†		
Foreign body reaction	1‡	0
Acute inflammation	19	15
Chronic inflammation	13	11
Grade of acute/chronic inflammation		
Slight	17	9
Moderate	1	2
Pronounced	2	4

*Because some patients did not consent to have biopsies taken, not all wounds were evaluated.
†Number of occurrences.
‡Photomicrograph of this specimen is shown in Figure 5. There were no statistically significant differences between the zinc and placebo groups for any of the variables.

Preoperative serum-zinc levels normalized to albumin, the major zinc-binding protein,[51] did not significantly influence the time to wound closure. In contrast, Zorrilla et al.[20] found that impaired wound healing was significantly related to preoperative low serum-zinc levels in 97 hip fracture patients. Their patients were 80 years of age compared with 25 years in our trial. Remarkably, the serum-zinc levels did not correlate to albumin in their study.[20]

Noteworthy were the low serum-zinc levels before surgery in the otherwise healthy young patients included in our trial. This was most likely due to the local infection because infections in general can cause hypozincemia[52–54] and the serum-zinc levels increased significantly in our study following surgical removal of the abscess beyond the time point of serum-zinc depression due to the surgical trauma.[55]

Metronidazole is often prescribed for malodorous wounds.[56] In the present trial, metronidazole was administered postoperatively due to wound malodor in six of the placebo-treated patients but only in one patient in the zinc group. Marks et al.[49] reported significantly increased healing of nonsutured pilonidal excisions by oral metronidazole administered 7–14 days postoperatively. Thus, this may have favored the healing outcome in the placebo group and confounded our analyses of time to healing in the two groups.

The elevated levels of ionized zinc oxide, exceeding 1,500 μM, in the wound fluid are sufficient to inhibit attachment[57] and growth of *S. aureus* including methicillin-resistant *S. aureus*[26,58,59] and corynebacteria in vitro.[60] In accordance with our bacteriological findings, zinc oxide also inhibited the growth of *S. aureus* in experimental wounds.[25,26] However, the mere colonization by these Gram-positive microorganisms is unlikely to be detrimental to wound healing,[61] and the benefit of topical antimicrobials in wound management is unclear.[62] Zinc oxide applied topically to open pilonidal wounds may reduce the need for systemic antibiotics. Unlike many topical antiseptics such as silver,[63] zinc oxide does not appear to compromise fibroblast functions in vitro.[31]

It is known from previous wound-healing studies that high zinc ion concentrations ($>15{,}000$ μM) are cytotoxic manifested as impaired epithelialization and augmented inflammatory responses.[32,33] We performed histopathological examination of biopsies from treated wounds as part of the safety assessment. No harmful effects such as foreign body reactions or increased inflammatory cell infiltration were attributed to the use of topical zinc oxide.

We covered the wounds with a polyurethane film dressing that prevented wound desiccation and this maneuver may explain why the fine-mesh cotton carrier neither adhered to the wound nor caused increased pain at removal, contrasting previous reports.[64,65] Also, the cotton carrier rarely elicited adverse tissue responses due to fiber shedding.

No systemic zinc absorption was observed from topically applied zinc oxide, evaluated by serum-zinc levels on postoperative day 7. This could be ascribed to the relatively small-sized wounds. In rats with large full-thickness skin wounds, absorption of zinc ions from zinc oxide in the wounds was reflected in increased serum-zinc levels.[30]

The pilonidal excisional wound heals mostly by contraction while formation of granulation tissue and new

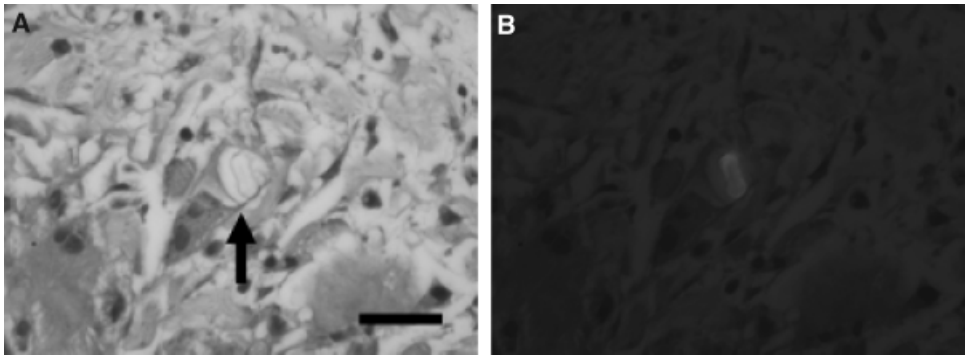

Figure 5. Multinucleated giant cell (arrowed) in the center of a wound containing material (A), which showed birefringence when viewed in polarized light (B); hematoxylin and eosin stain (original magnification × 400). Bar=20 μm.

epithelium contributes <15% to healing, as shown by detailed histomorphometry.[17] Earlier experimental and clinical studies indicate that topical zinc oxide primarily promotes epithelialization during wound healing.[21–24,32] This may explain the less obvious effect of zinc oxide on the healing of the deep open pilonidal wound.

One placebo-treated patient was reoperated due to recurrence. In another study, a 13% recurrence rate of secondary healed pilonidal wounds was reported over a 3-year follow-up period.[5] After the completion of our trial, the standard surgical procedure has been modified and we now drain acute pilonidal abscesses by simple incision and routinely use the Bascom procedure with asymmetric excision and primary closure for elective patients.[8]

In conclusion, topical zinc oxide did not significantly enhance the closure of nonsutured acute wounds healing mostly by contraction. Neither local adverse events nor systemic zinc absorption were observed from zinc oxide in open pilonidal wounds. The potential of topical zinc oxide to promote wound healing and reduce infections would be worthwhile to pursue in larger-scale prospective trials and our present trial may serve as a basis for future sample size calculations.

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REFERENCES

1. Ågren MS, Ostenfeld U, Crawford ME, Kiss K, Gong Y, Gluud C, Friis-Møller A, Kallehave F, Raffn K, Jorgensen LN. Topical zinc oxide for open pilonidal wounds. *Wound Rep Reg* 2005; 13: A4.
2. Vermeulen H, Ubbink DT, Goossens A, de Vos R, Legemate DA. Systematic review of dressings and topical agents for surgical wounds healing by secondary intention. *Br J Surg* 2005; 92: 665–72.
3. Hull TL, Wu J. Pilonidal disease. *Surg Clin N Am* 2002; 82: 1169–85.
4. Chintapatla S, Safarani N, Kumar S, Haboubi N. Sacrococcygeal pilonidal sinus: historical review, pathological insight and surgical options. *Tech Coloproctol* 2003; 7: 3–8.
5. Kronborg O, Christensen K, Zimmermann-Nielsen C. Chronic pilonidal disease: a randomized trial with a complete 3-year follow-up. *Br J Surg* 1985; 72: 303–4.
6. Jensen SL, Harling H. Prognosis after simple incision and drainage for a first-episode acute pilonidal abscess. *Br J Surg* 1988; 75: 60–1.
7. Banerjee D. The aetiology and management of pilonidal sinus. *J Wound Care* 1999; 8: 309–10.
8. Bascom J, Bascom T. Failed pilonidal surgery: new paradigm and new operation leading to cures. *Arch Surg* 2002; 137: 1146–50.
9. Spivak H, Brooks VL, Nussbaum M, Friedman I. Treatment of chronic pilonidal disease. *Dis Colon Rectum* 1996; 39: 1136–9.
10. Baier PK, Baumgartner U, Furtwangler A, Holzinger F, Schoffel U. Therapy of the pilonidal sinus—primary wound closure or open wound after excision. *Zentralbl Chir* 2002; 127: 310–4.
11. Perruchoud C, Vuilleumier H, Givel JC. Pilonidal sinus: how to choose between excision and open granulation versus excision and primary closure? Study of a series of 141 patients operated on from 1991 to 1995. *Swiss Surg* 2002; 8: 255–8.
12. Pories WJ, Henzel JH, Rob CG, Strain WH. Acceleration of wound healing in man with zinc sulphate given by mouth. *Lancet* 1967; 1: 121–4.
13. Barcia PJ. Lack of acceleration of healing with zinc sulfate. *Ann Surg* 1970; 172: 1048–50.
14. Weinstein MA, Rubin RJ, Salvati EP. The dilemma of pilonidal disease: pilonidal cystotomy, reappraisal of an old technique. *Dis Colon Rectum* 1977; 20: 287–9.
15. Marks J, Hughes LE, Harding KG, Campbell H, Ribeiro CD. Prediction of healing time as an aid to the management of open granulating wounds. *World J Surg* 1983; 7: 641–5.
16. Holzer B, Grüßner U, Brückner B, Houf M, Kiffner E, Schildberg FW, et al. Efficacy and tolerance of a new gentamicin collagen fleece (Septocoll) after surgical treatment of a pilonidal sinus. *Colorectal Dis* 2003; 5: 222–7.
17. Berry DP, Harding KG, Stanton MR, Jasani B, Ehrlich HP. Human wound contraction: collagen organization, fibroblasts, and myofibroblasts. *Plast Reconstr Surg* 1998; 102: 124–31.

18. Lansdown ABG. Zinc in the healing wound. *Lancet* 1996; 347: 706–7.
19. Wilkinson EA, Hawke CI. Does oral zinc aid the healing of chronic leg ulcers? A systematic literature review. *Arch Dermatol* 1998; 134: 1556–60.
20. Zorrilla P, Salido JA, Lopez-Alonso A, Silva A. Serum zinc as a prognostic tool for wound healing in hip hemiarthroplasty. *Clin Orthop* 2004; 304–8.
21. Tronnier H. Experimental study on epithelization. *Z Hautkr* 1975; 50: 925–9.
22. Strömberg HE, Ågren MS. Topical zinc oxide treatment improves arterial and venous leg ulcers. *Br J Dermatol* 1984; 111: 461–8.
23. Weingart D, Stoll P. The epithelialization of split skin graft donor sites—a test model for the efficacy of topical wound therapeutic agents. *Eur J Plast Surg* 1993; 16: 22–5.
24. Ågren MS. Zinc in wound repair. *Arch Dermatol* 1999; 135: 1273–4.
25. Sunzel B, Lasek J, Söderberg T, Elmros T, Hallmans G, Holm S. The effect of zinc oxide on *Staphylococcus aureus* and polymorphonuclear cells in a tissue cage model. *Scand J Plast Reconstr Surg Hand Surg* 1990; 24: 31–5.
26. Ågren MS, Söderberg TA, Reuterving CO, Hallmans G, Tengrup I. Effect of topical zinc oxide on bacterial growth and inflammation in full-thickness skin wounds in normal and diabetic rats. *Eur J Surg* 1991; 157: 97–101.
27. Roselli M, Finamore A, Garaguso I, Britti MS, Mengheri E. Zinc oxide protects cultured enterocytes from the damage induced by *Escherichia Coli*. *J Nutr* 2003; 133: 4077–82.
28. Lim Y, Levy M, Bray TM. Dietary zinc alters early inflammatory responses during cutaneous wound healing in weanling CD-1 mice. *J Nutr* 2004; 134: 811–6.
29. Sunzel B, Söderberg TA, Reuterving CO, Hallmans G, Holm SE, Hänström L. Neutralizing effect of zinc oxide on dehydroabietic acid-induced toxicity on human polymorphonuclear leukocytes. *Biol Trace Elem Res* 1991; 31: 33–42.
30. Ågren MS, Krusell M, Franzén L. Release and absorption of zinc from zinc oxide and zinc sulfate in open wounds. *Acta Derm Venereol* 1991; 71: 330–3.
31. Ågren MS, Mirastschijski U. The release of zinc ions from and cytocompatibility of two zinc oxide dressings. *J Wound Care* 2004; 13: 367–9.
32. Ågren MS, Chvapil M, Franzén L. Enhancement of re-epithelialization with topical zinc oxide in porcine partial-thickness wounds. *J Surg Res* 1991; 50: 101–5.
33. Hallmans G. Local absorption of zinc from wounds treated with different concentrations of zinc sulphate. *Acta Derm Venereol* 1978; 58: 413–9.
34. International Conference on Harmonisation Expert Working Group. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH harmonised tripartite guideline. Guideline for good clinical practice. In: *1997 CFR & ICH Guidelines*. USA: Barnett International/PAREXEL, 1997: 1–71.
35. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 1988; 124: 869–71.
36. Duxbury MS, Blake SM, Dashfield A, Lambert AW. A randomised trial of knife versus diathermy in pilonidal disease. *Ann R Coll Surg Engl* 2003; 85: 405–7.
37. Berg W, Traneroth C, Gunnarsson A, Lossing C. A method for measuring pressure sores. *Lancet* 1990; 335: 1445–6.
38. Resch CS, Kerner E, Robson MC, Heggers JP, Scherer M, Boertman JA, Schileru R. Pressure sore volume measurement. A technique to document and record wound healing. *J Am Geriatr Soc* 1988; 36: 444–6.
39. Plassmann P, Melhuish JM, Harding KG. Methods of measuring wound size: a comparative study. *Ostomy Wound Manage* 1994; 40: 50–2, 4, 6–60.
40. Mirastschijski U, Konrad D, Lundberg E, Lyngstadaas SP, Jorgensen LN, Ågren MS. Effects of a topical enamel matrix derivative on skin wound healing. *Wound Rep Reg* 2004; 12: 100–8.
41. Søndenaa K, Pollard ML. Histology of chronic pilonidal sinus. *APMIS* 1995; 103: 267–72.
42. Makino T, Saito M, Horiguchi D, Kina K. A highly sensitive colorimetric determination of serum zinc using water-soluble pyridylazo dye. *Clin Chim Acta* 1982; 120: 127–35.
43. Assmann SF, Pocock SJ, Enos LE, Kasten LE. Subgroup analysis and other (mis) uses of baseline data in clinical trials. *Lancet* 2000; 355: 1064–9.
44. Lock-Andersen J, Wulf HC, Knudstorp ND. Interdependence of eye and hair colour, skin type and skin pigmentation in a Caucasian population. *Acta Derm Venereol* 1998; 78: 214–9.
45. James TJ, Hughes MA, Cherry GW, Taylor RP. Simple biochemical markers to assess chronic wounds. *Wound Rep Reg* 2000; 8: 264–9.
46. Kjaergard LL, Villumsen J, Gluud C. Reported methodologic quality and discrepancies between large and small randomized trials in meta-analyses. *Ann Intern Med* 2001; 135: 982–9.
47. Moher D, Schulz KF, Altman D. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *JAMA* 2001; 285: 1987–91.
48. Ioannidis JP, Evans SJ, Gøtzsche PC, O'Neill RT, Altman DG, Schulz K, Moher D, CONSORT Group. Better reporting of harms in randomized trials: an extension of the CONSORT statement. *Ann Intern Med* 2004; 141: 781–8.
49. Marks J, Harding KG, Hughes LE, Ribeiro CD. Pilonidal sinus excision—healing by open granulation. *Br J Surg* 1985; 72: 637–40.
50. Sorensen LT, Karlsmark T, Gottrup F. Abstinence from smoking reduces incisional wound infection: a randomized controlled trial. *Ann Surg* 2003; 238: 1–5.
51. Homsher R, Zak B. Spectrophotometric investigation of sensitive complexing agents for the determination of zinc in serum. *Clin Chem* 1985; 31: 1310–3.
52. Wannemacher RW Jr., Pekarek RS, Klainer AS, Bartelloni PJ, Dupont HL, Hornick RB, Beisel WR. Detection of a leukocytic endogenous mediator-like mediator of serum amino acid and zinc depression during various infectious illnesses. *Infect Immun* 1975; 11: 873–5.
53. Alarcón OM, Reinosa J, Rangel O, Silva T, Chacón A. Effect of isofluorane anesthesia on serum levels of Zn, Cu, Fe, Mn and Co in humans. *J Trace Elem Med Biol* 1996; 10: 180–4.
54. Gaetke LM, McClain CJ, Talwalkar RT, Shedlofsky SI. Effects of endotoxin on zinc metabolism in human volunteers. *Am J Physiol* 1997; 272: E952–6.
55. Tengrup I, Samuelsson H. Changes in serum zinc during and after surgical procedures. *Acta Chir Scand* 1977; 143: 195–9.
56. Ashford R, Plant G, Maher J, Teare L. Double-blind trial of metronidazole in malodorous ulcerating tumours. *Lancet* 1984; 1: 1232–3.

57. Akiyama H, Yamasaki O, Kanzaki H, Tada J, Arata J. Effects of zinc oxide on the attachment of *Staphylococcus aureus* strains. *J Dermatol Sci* 1998; 17: 67–74.
58. Söderberg TA, Sunzel B, Holm S, Elmros T, Hallmans G, Sjöberg S. Antibacterial effect of zinc oxide in vitro. *Scand J Plast Reconstr Surg Hand Surg* 1990; 24: 193–7.
59. Ugur A, Ceylan O. Occurrence of resistance to antibiotics, metals, and plasmids in clinical strains of *Staphylococcus* spp. *Arch Med Res* 2003; 34: 130–6.
60. Groman N, Judge K. Effect of metal ions on diphtheria toxin production. *Infect Immun* 1979; 26: 1065–70.
61. Edwards R, Harding KG. Bacteria and wound healing. *Curr Opin Infect Dis* 2004; 17: 91–6.
62. O'Meara SM, Cullum NA, Majid M, Sheldon TA. Systematic review of antimicrobial agents used for chronic wounds. *Br J Surg* 2001; 88: 4–21.
63. Poon VK, Burd A. In vitro cytotoxity of silver: implication for clinical wound care. *Burns* 2004; 30: 140–7.
64. Viciano V, Castera JE, Medrano J, Aguilo J, Torro J, Botella MG, Toldra N. Effect of hydrocolloid dressings on healing by second intention after excision of pilonidal sinus. *Eur J Surg* 2000; 166: 229–32.
65. Bethell E. Why gauze dressings should not be the first choice to manage most acute surgical cavity wounds. *J Wound Care* 2003; 12: 237–9.
66. Petts DN. Evaluation of the oxoid dryspot streptococcal grouping kit for grouping beta-hemolytic streptococci. *J Clin Microbiol* 1999; 37: 255–7.