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A Placebo-controlled, Double-blind Study of Mesoglycan in the Treatment of Chronic Venous Ulcers

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Objectives: *to assess the effect of treatment with mesoglycan, a sulphated polysaccharide compound, on the healing of venous ulcers.*
Design: *randomised, placebo-controlled, double-blind, multicentre trial.*
Methods: *non-diabetic outpatients with chronic venous insufficiency confirmed by duplex ultrasound, normal ankle/arm pressure index and presence of a leg ulcer were eligible. Patients were randomised to mesoglycan, 30 mg/day intramuscularly for 3 weeks followed by 100 mg/day orally, or matching placebo, as an adjunct to compression therapy and topical wound care. Treatment and observation were continued until complete ulcer healing or for 24 ± 1 weeks. Time to ulcer healing and healing rates were estimated with the Kaplan–Meier method.*
Results: *One hundred and eighty-three patients were randomised and included in the analysis (92 mesoglycan, 91 placebo). Median ulcer area upon inclusion was 3.6 cm^2 in the mesoglycan group and 3.9 cm^2 in the placebo group. The estimated time to heal 75% of the patients was 90 days on mesoglycan versus 136 days on placebo, while the cumulative rate of healing by the end of observation was 97% versus 82%, respectively. The difference in favour of mesoglycan was statistically significant ($p<0.05$, centre-stratified Cox's model). The relative risk of ulcer healing with mesoglycan was 1.48. The rate of adverse events was 7/92 on mesoglycan and 6/91 on placebo.*
Conclusions: *treatment with mesoglycan in addition to established venous ulcer therapy resulted in a significantly faster and more frequent ulcer healing, and did not raise any safety concerns.*

Key Words: Chronic venous insufficiency; Venous ulcers; Mesoglycan; Randomised clinical trial.

Introduction

Chronic venous insufficiency (CVI) and ulceration represents an important medical problem because of its prevalence (0.3% of the adult Western population), poor healing (20% of the ulcers remain open after 2 years while the annual recurrence rate is 6 to 15%), and healthcare costs.[1] Since the underlying CVI cannot be cured, therapy of venous ulcers is mainly aimed at counteracting (by limb compression) or eliminating (by surgery) the transmission of increased venous pressure to the skin. Several systemic drug treatments have been tested for a possible effect on venous ulcer healing, but none has been widely accepted as standard therapy in this setting.[1]

Mesoglycan is a sulphated polysaccharide compound commercially available in some European countries, both in a parenteral and oral form, for the treatment of vascular disease with an associated thrombotic risk. Mesoglycan is extracted from porcine intestinal mucosa and is composed of heparan sulphate (52%), dermatan sulphate (35%), electrophoretically slow-moving heparin (8%) and chondroitin sulphate (5%). Heparan and dermatan sulphate are thrombin inhibitors acting through complementary pathways, i.e. antithrombin III and heparin cofactor II, respectively.[2,3] Mesoglycan and/or its major components have been shown to inhibit neutrophil adhesion and activation,[4] to decrease capillary permeability,[5] to enhance systemic fibrinolysis in humans,[6] and to prevent venous thrombus formation in experimental and clinical settings.[7–9] These properties may be relevant to the proposed pathogenic mechanisms for venous ulceration, involving microcirculatory neutrophil activation, an increase in capillary permeability and the

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formation of pericapillary fibrin cuffs.[10] Oral absorption of mesoglycan and dermatan sulphate fractions has previously been demonstrated.[11,12] Symptomatic benefits of mesoglycan in CVI have been reported in earlier small-scale studies.[13,14]

The present controlled trial was aimed at assessing the effect of adjunct treatment with mesoglycan on the healing of chronic venous ulcers managed with compression therapy and topical wound care. Patients were observed until complete ulcer healing or for 24 weeks.

Patients and Methods

This was a randomised, parallel-group, placebo-controlled, double-blind, multicentre study. Randomisation to mesoglycan or placebo was stratified by centre. The study was conducted at the outpatient clinics of 18 Italian hospital departments of vascular or general surgery ($n=12$), angiology ($n=4$) and dermatology ($n=2$). The study protocol and patient information were approved by the ethics committee of each centre.

Patients

Ambulatory patients with previous history and current clinical diagnosis of CVI were eligible if the following criteria were met: age, 18 to 80 years; duplex ultrasound evidence of CVI; ankle/arm arterial pressure index at rest >0.90 on both limbs; presence of a leg skin ulcer for less than one year, with maximum orthogonal ulcer diameters having a product between 4 and 20 cm^2; ability to frequent the study centre; and written informed consent. In the patients having multiple skin lesions, the largest ulcer meeting the required size was chosen as the target for the purpose of the study.

Exclusion criteria comprised: diabetes mellitus; other concomitant conditions possibly causing ulceration (e.g. blood dyscrasias, neuropathy, vasculitis); active infection of the target ulcer; saphenectomy or sclerotherapy scheduled for the coming 6 months; need for treatment with heparin, oral anticoagulants, prostanoids, vasoactive drugs or long-term aspirin; active bleeding; potential bleeding from organic lesions; prothrombin activity <50%; platelet count $<100 \times 10^3$ μl; renal insufficiency (creatinine >2 mg/dl); known intolerance to sulphated polysaccharides; and pregnancy.

Randomisation and treatments

Prior to study start, a computer-generated randomisation list was prepared for each centre using balanced blocks of 4 patients. Mesoglycan (Prisma, Mediolanum Farmaceutici, Milan, Italy; 30 mg/1 ml ampoules and 50 mg capsules) or placebo (matching saline solution ampoules and excipient capsules) were packaged according to the lists in consecutively numbered containers. At each centre, treatment containers were assigned to patients according to their chronological order of enrolment. Centres were also provided with sealed envelopes to reveal individual treatment in case of an emergency. These were retrieved and checked for integrity at the end of study. Otherwise, all personnel involved in study implementation remained unaware of treatment assignation until the final data analysis.

Treatment with mesoglycan or placebo was initiated intramuscularly with one ampoule once daily for 3 weeks, and continued orally with one capsule twice daily.

Compression therapy and topical wound care were applied to all patients. Permitted materials for compression therapy were short stretch elastic bandages and zinc oxide elastic or non-elastic bandages. Other components of ulcer dressing (gauzes, ointments) were used at the investigator's discretion. Wound care consisted of regular ulcer cleansing with saline and local antiseptics. Lifestyle and postural instructions to relieve venous hypertension were given to all patients. Concomitant treatments with anticoagulant agents, prostanoids, vasoactive drugs or long-term aspirin were prohibited.

Patient observation

Upon inclusion, the contours of the target ulcer were drawn on a standard transparent sheet (OpSite Flexigrid, Smith & Nephew Medical, Hull, U.K.). Patients were requested to assess ulcer-associated pain and to fill in the Medical Outcomes Study Short Form-36 (SF-36), a generic questionnaire for self-assessment of health-related quality of life,[15] and underwent baseline laboratory tests.

After treatment initiation, patients were followed-up by fixed visits every 3 weeks, plus any supplementary visits needed to renew compression and topical therapies. Randomised treatment and patient observation were terminated when complete healing of the target ulcer was ascertained by the investigator, or upon the final visit scheduled at the end of week 24 (± 1 week). Ulcer-associated pain was recorded at each fixed visit.

The SF-36 questionnaire was repeated at the end of observation. Laboratory tests were repeated at week 3 visit and at the end of observation. Adverse events were recorded irrespective of their presumed relationship to study treatment.

Assessments

The primary end-point for efficacy was time to healing of the target ulcer, based upon the actual dates of randomisation and of the visit (be it fixed or supplementary) when complete ulcer epithelialisation was observed. The time-course of ulcer-associated pain was a secondary efficacy variable. Data from SF-36 were used to assess the impact of ulcer healing, irrespective of treatment, on health-related quality of life.

Duplex ultrasound evidence of CVI was defined as presence of (a) valvular incompetence and/or reflux at the sapheno-femoral or sapheno-popliteal junctions; and/or (b) vessel wall or valve morphologic alterations, endoluminal obstruction or reflux in the deep vein system. Concomitant incompetence of perforating veins was recorded when present. Baseline ankle/arm index was determined according to current guidelines.[16] Baseline ulcer areas were calculated from contour sheets at a central location by computerised planimetry, using digital scanning and Analytica-Lite 3.0 software (Immagini & Computer, Bareggio, Italy). Ulcer-associated pain was assessed on a visual-analogue chromatic scale from 0 to 10.[17] SF-36 was used in a validated Italian version.[18] For each of 8 health domains explored, SF-36 produces a score from 0 to 100 (the higher the score, the better the perceived health status). Adverse events that were fatal, life-threatening, or involved hospitalisation or persistent or significant disability were classified as "serious".[19] Laboratory tests included activated partial thromboplastin time (APTT), complete blood count and blood chemistry (fibrinogen, renal and liver function tests).

Sample size and statistical analysis

The study sample was set at 90 patients per group to ensure that of at least 70 per group were fully assessable. This number was estimated[20] by assuming that all patients would achieve ulcer healing by the end of week 24 ± 1, and that mean (SD) time to healing would be 84 (42) days in the placebo group and 63 (42) days in the mesoglycan group. The specified significance level was 0.05 (two-tailed) and statistical power was 0.80.

Statistical analysis was performed using SAS System software (SAS Institute, Cary, NC, U.S.A.). Time to healing was estimated from Kaplan–Meier curves for cumulative ulcer healing over time; treatment groups were compared using Cox's model, including baseline ulcer area as a covariate. Relative risk for healing with mesoglycan compared to placebo was derived from Cox's model after checking for proportionality of hazards. As some patients did not actually heal by week 24 ± 1, or were lost to follow-up, the relevant observations were censored at the actual date of the last available visit. Hence all randomised patients were included in the analysis. The effect of treatment on pain scores over time was tested by repeated-measures analysis of variance in the patients completing observation. Data from SF-36 were handled according to published guidelines.[15,18] The influence of ulcer healing on SF-36 scores was tested by analysis of covariance (end-of observation scores corrected for baseline scores) in the patients completing observation. All randomised patients were assessed for adverse events. All analyses were centre-stratified. Significance tests were two-tailed. No interim analysis was planned or executed.

Results

From April to October 1999, 183 patients were included in the study and randomised to mesoglycan ($n = 92$) or placebo ($n = 91$). As shown in Table 1, treatment groups were balanced for baseline characteristics including demographics, clinical history and duplex ultrasound findings relevant to CVI, target ulcer area and duration, concomitant conditions and type of compression therapy adopted upon inclusion. Ulcers of very recent onset (less than one month before inclusion) were only present in 3 patients.

Patients completing observation were 168. Of these, 82 on mesoglycan and 69 on placebo achieved healing of the target ulcer, while 4 on mesoglycan and 13 on placebo had still not healed by the end of week 24 ± 1. The mean duration of observation in study completers was 70 days on mesoglycan and 87 days on placebo. The remaining 15 patients were lost to follow-up before healing was achieved, due to their decision to withdraw from the study (4 patients on mesoglycan, 6 on placebo) or following an adverse event (2 and 3 patients, respectively). The individual treatment code was only opened in one case. Development of ulcer infection at any time during observation was reported in 6 mesoglycan and 4 placebo patients.

Table 1. Baseline patient characteristics.

	Mesoglycan ($n=92$)	Placebo ($n=91$)
Female sex, *n* (%)	67 (73)	61 (67)
Age, years, mean (SD)	62 (12)	62 (11)
Clinical history		
Venous thromboembolism, *n* (%)	22 (24)	27 (30)
Years from onset of CVI symptoms, median (IQR)	4.7 (0.5–17.0)	5.6 (0.8–19.4)
Surgery for CVI*, *n* (%)	13 (14.1)	22 (24.2)
Sclerotherapy, *n* (%)	11 (12.0)	11 (12.1)
Duplex ultrasound findings§		
Superficial vein valvular incompetence, *n*	87	85
Sapheno–femoral or sapheno–popliteal reflux, *n*	72	70
Incompetent perforating veins, *n*	63	70
Deep vein morphologic alterations¶, *n*	24	29
Reflux in deep veins, *n*	33	40
Target ulcer		
Months from ulcer appearance, median (IQR)	3 (2–6)	3 (1—7)
Ulcer area, cm^2, median (IQR)	3.6 (2.5–7.0)	3.9 (2.8–7.8)
Associated pain, 0–10 scale, mean (SD)	4.5 (3.0)	4.3 (2.8)
Concomitant conditions		
Concomitant venous ulcer, *n* (%)	12 (13.0)	7 (7.7)
Arterial hypertension, *n* (%)	28 (30.4)	24 (26.4)
Obesity (body mass index ≥ 30), *n* (%)	27 (29.3)	22 (24.4)
Lower limb functional limitation, *n* (%)	4 (4.3)	4 (4.4)
Current smoking, *n* (%)	11 (12.0)	16 (17.6)
Abnormal blood glucose‡, *n* (%)	2 (2.2)	3 (3.3)
Compression therapy adopted upon inclusion		
Short stretch elastic bandage, *n* (%)	43 (46.7)	36 (39.6)
Zinc oxide non-elastic bandage, *n* (%)	15 (16.3)	22 (24.2)
Combination of the above, *n* (%)	10 (10.9)	10 (11.0)
Zinc oxide elastic bandage, *n* (%)	22 (23.9)	20 (22.0)
Other, *n* (%)	2 (2.2)	3 (3.3)

* Consisting of varicose vein surgery in all cases.
§ On the limb affected by target ulcer. All patients had multiple abnormalities.
¶ Vessel wall or valve alterations or endoluminal obstruction.
‡ 140 mg/dL upon inclusion, confirmed in one further determination.
n: number of patients; CVI: chronic venous insufficiency; IQR: interquartile range.

Kaplan–Meier plots for cumulative target ulcer healing over time are shown in Figure 1. Estimated time to heal 25%, 50% (median) and 75% of the patients was 36, 64 and 90 days with mesoglycan versus 42, 70 and 136 days with placebo, while the estimated rate of healing by the end of observation was 97% versus 82%, respectively. The difference in favour of mesoglycan was statistically significant ($p<0.05$) and the corresponding relative risk for healing was 1.48 (95% confidence interval [CI], 1.05 to 2.09). After adjusting for baseline ulcer area, the difference was still significant ($p<0.05$) and the relative risk for healing with mesoglycan was 1.43 (CI, 1.01 to 2.04). No significant deviation from proportionality was detected in the relative risk for healing over time, indicating a relatively constant effect of mesoglycan over the observation period. Treatment by centre interaction was not significant.

An alternative exploratory analysis was also performed, in which ulcer healing events were attributed to the fixed visit dates as scheduled in the protocol rather than to the actual dates when healing was observed. Following this approach, the difference between treatments was again found to be significant ($p=0.02$; $p=0.03$ after adjusting for baseline ulcer area) and not time-dependent.

Out of 19 patients having a concomitant venous ulcer upon inclusion (Table 1), 15 completed observation (10 mesoglycan, 5 placebo). Among these, 7 patients on mesoglycan and 1 on placebo achieved complete healing of the concomitant ulcer by the end of observation.

The overall time-course for ulcer-associated pain scores did not differ significantly according to treatment ($p=0.27$). The initial decrease in pain tended to be greater on mesoglycan (from a mean [SD] baseline

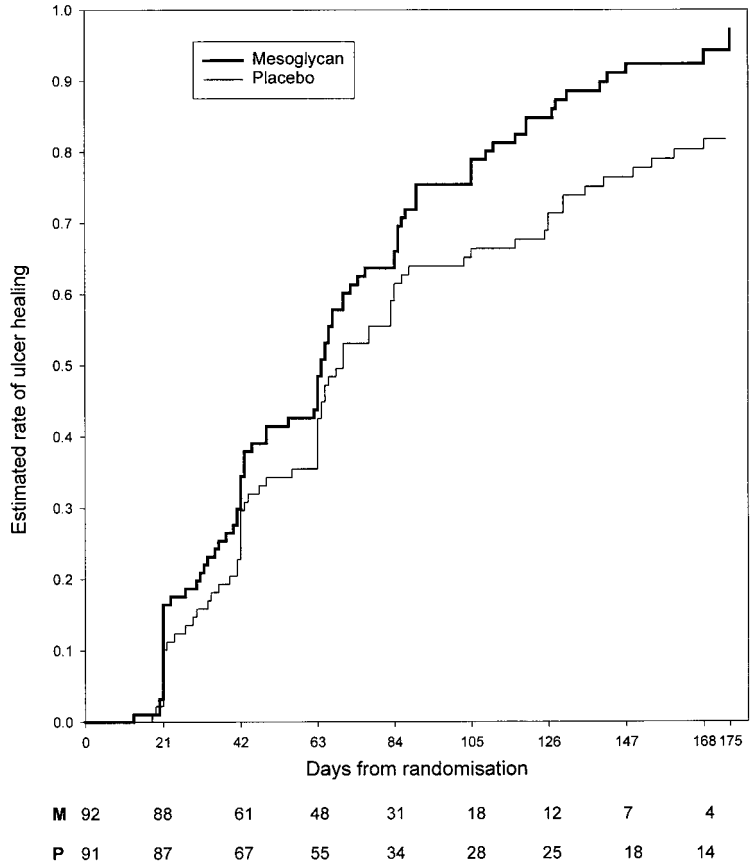

Fig. 1. Kaplan–Meier curves for cumulative ulcer healing over time, including all randomised patients. Numbers of patients that were on observation at each time-point are shown at the bottom of the graph (M: mesoglycan; P: placebo).

score of 4.4 [3.0] to 1.7 [2.4] at the end of Week 3) than on placebo (from 4.2 [2.8] to 2.1 [2.4], $p<0.1$.

Figure 2 shows the mean changes in SF-36 scores that occurred at the end of week 24 ± 1 compared to baseline, according to the healing status of target ulcer. Healing was associated with improvements in each of the 8 health domains explored by the questionnaire, whereas non-healed patients showed smaller improvements or no change. Differences between healed and non-healed patients were statistically significant ($p<0.05$) in 6 domains, i.e. bodily pain, general health, vitality, social functioning, role limitation due to emotional problems and mental health.

Reported adverse events are detailed in Table 2. Total adverse event incidence was 7/92 on mesoglycan (8%) and 6/91 on placebo (7%). Only 2 events per group were of serious nature (none being fatal), while those resulting in permanent treatment discontinuation were 2 on mesoglycan and 4 on placebo. Most of the events were considered unrelated to study treatment. Their organ-system distribution (Table 2) did not reveal clear treatment-related differences in

Table 2. Adverse events (AE) reported during the observation period, ordered by system-organ class.

	Mesoglycan ($n=92$)	Placebo ($n=91$)
Skin	Skin rash	Skin rash*
Musculoskeletal	*Road accident trauma**	
CNS	Headache, pruritus	Drowsiness
Gastrointestinal		Epigastric pain
Cardiovascular	*Congestive heart failure**	*Reversible cerebral ischaemia**
	Palpitations, orthostatic hypotension	*Cerebral stroke**
	Episodes of fainting	
	Superficial thrombophlebitis	
Respiratory	Bronchopneumonia	
Haemorrhage		Rectal bleeding*
General		Influenza
Total *n* of AE	8	7
Patients with AE	7 (8%)	6 (7%)

* AE followed by permanent treatment discontinuation.
Serious AE are shown in *italics*.

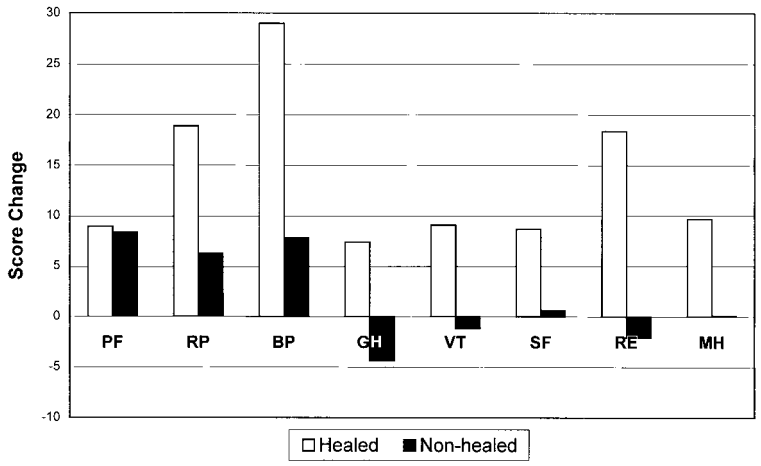

Fig. 2. Self-assessment of health status by SF-36, according to the end-of-observation healing status of the target ulcer. Mean (SD) score differences (end-of-observation – baseline) are shown for the 8 domains explored by the questionnaire. A positive difference means an improvement in perceived health status. PF: Physical functioning; RP: Role-physical limitation; BP: Bodily pain; GH: General health; VT: Vitality; SF: Social functioning; RE: Role-emotional limitation; MH: Mental health.

any event category. No injection site or systemic bleeding was observed in the mesoglycan group.

APTT was unaffected by treatment with mesoglycan. No treatment-related difference was detected for any of the other laboratory tests. An on-treatment platelet count of less than 100×103 µl was detected in one patient per group; the patient on mesoglycan had concomitant liver disease reported upon inclusion.

Discussion

In this randomised, double-blind study, patients with leg ulcers caused by CVI were treated with mesoglycan or placebo as an adjunct to established therapeutic measures (limb compression, topical wound care). Care was taken to enrol patients with "pure" venous ulcers – all had a diagnosis of CVI confirmed by duplex ultrasound scanning and normal ankle/arm arterial pressure index, while those with diabetes or other alternative causes of ulceration were excluded. The duration of observation, up until complete healing of the target ulcer or for 24 ± 1 weeks, was long enough to account for ulcer outcome rather than initial healing.

Treatment with mesoglycan resulted in faster and more frequent ulcer healing. The estimated time to heal 75% of the patients was 90 days on mesoglycan versus 136 on placebo, while the estimated proportion of patients healed by the end of observation was 97.1% on mesoglycan versus 81.8% on placebo. The difference between treatments was statistically significant by intent-to-treat analysis, and remained significant after adjusting for baseline ulcer area. The relative risk of healing with mesoglycan was 1.48, or 1.43 in the adjusted analysis.

The healing rate achieved in the placebo group, 75% after 136 days, compared well with those recently reported for venous ulcers managed with compression at reference wound care centres, 75% after 112 days[21] or 68% after 168 days.[22] This suggests that compression and topical therapy was well implemented.

Healing of the target ulcer resulted in significant improvements of patient-perceived health status, as reflected by SF-36 scores. This confirmed the findings of an earlier study using the Nottingham Health Profile, in which the healing status of chronic venous ulcers was found to be a major determinant of health-related quality of life in the affected patients.[23]

The mechanism by which ulcer healing was promoted by treatment with mesoglycan remains to be verified. Mesoglycan may counteract putative mechanisms for venous ulceration[10] through inhibition of neutrophil adhesion and activation,[4] preservation of endothelial barrier function,[5] prevention of fibrin formation[2,3] and enhancement of fibrinolysis.[6] An alternative or additional explanation is suggested by recent reports highlighting the role of physiological dermatan and heparan sulphate in wound healing processes,[24] including cutaneous wound repair.[25]

The intramuscular followed by oral regimen adopted for the study reflected the use of mesoglycan in current clinical practice. Since the ulcer healing advantage of mesoglycan relative to placebo was not found to vary over the observation period, this would imply that the oral treatment contributed to the overall effect of mesoglycan. Previous reports have shown

that fractions of sulphated polysaccharides are absorbed after oral dosing[11,12,26–28] and can produce *in vivo* antithrombotic effects while generating barely detectable anticoagulant activities.[27,28]

The safety profile of mesoglycan did not show important differences as compared with placebo, confirming a long established clinical experience in the European countries where this agent is available. Virtually all of the patients were able to tolerate mesoglycan treatment, the two cases of discontinuation being due to intercurrent clinical events.

A significant effect of sulphated polysaccharide treatment on venous ulcer healing was previously reported with sulodexide, a compound similar to mesoglycan in composition. In a 2-month, open-label study the healing rate was 58% with intramuscular followed by oral sulodexide and compression therapy, versus 36% with compression alone.[29]

Other systemic drug treatments investigated in chronic venous ulcers include pentoxifylline and a micronised purified flavonoid fraction (MPFF). The relative risk of ulcer healing with pentoxifylline and compression, compared to placebo and compression, was estimated to be 1.30 (CI, 1.10–1.54) in a recent meta-analysis.[30] This was based on a total of 447 patients, most of whom were included in trials having the same duration of observation as in the present study. Significant improvements in ulcer healing were reported with MPFF as an adjunct to compression therapy in two studies.[31,32] The relevant results were difficult to compare with those presently reported, because of the low control healing rates achieved with compression alone in both studies – 13% after 2 months[31] and 28% after 24 weeks.[32]

Assessing the cost-effectiveness of mesoglycan was beyond the scope of this study. However, it can be noted that mesoglycan is a rather inexpensive treatment, with a daily cost of 1.29 EUR (intramuscular regimen) or 1.51 EUR (oral regimen) at Italian prices.

In conclusion, even when systematically applied for several months by experienced operators, compression therapy and local wound care fail to achieve healing of chronic venous ulcers in a significant proportion of patients. Ulcer healing is an important positive outcome for health-related quality of life in the patients affected by CVI and active ulceration. Adjunct treatment with mesoglycan results in a significantly faster and more frequent healing of venous ulcers, and compares favourably with other systemic therapies investigated in this setting. The treatment is well tolerated and does not raise any safety concerns. In terms of benefit/risk ratio, therefore, mesoglycan represents a useful addition to the limited therapeutic options available for managing chronic venous ulceration.

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Appendix

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