LeucoPatch system for the management of hard-to-heal diabetic foot ulcers in the UK, Denmark, and Sweden: an observer-masked, randomised controlled trial

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Summary

Background The LeucoPatch device uses bedside centrifugation without additional reagents to generate a disc comprising autologous leucocytes, platelets, and fibrin, which is applied to the surface of the wound. We aimed to test the effectiveness of LeucoPatch on the healing of hard-to-heal foot ulcers in people with diabetes.

Methods This was a multicentre, international, observer-masked, randomised controlled trial of people with diabetes and a hard-to-heal foot ulcer done in 32 specialist diabetic foot clinics in three countries (UK, Denmark, and Sweden). After a 4-week run-in period, those with a reduction in ulcer area of less than 50% were randomly allocated (1:1) by computer-generated, web-based randomisation (block sizes of two, four, and six) to either prespecified good standard care alone or care plus weekly application of LeucoPatch. The primary outcome was the proportion of ulcers that healed within 20 weeks assessed in the intention-to-treat population (all participants with post-randomisation data collected), defined as complete epithelialisation (confirmed by an observer who was masked to randomisation group), and remained healed for 4 weeks. This trial is registered with the ISRCTN registry, number 27665670, and ClinicalTrials.gov, number NCT02224742.

Findings Between Aug 30, 2013, and May 3, 2017, 269 participants were randomly allocated to receive treatment (137 to receive standard care and 132 to receive LeucoPatch). The mean age was 61·9 years (SD 11·6), 217 (82%) were men, and 222 (83%) had type 2 diabetes. In the LeucoPatch group, 45 (34%) of 132 ulcers healed within 20 weeks versus 29 (22%) of 134 ulcers in the standard care group (odds ratio 1·58, 96% CI 1·04–2·40; p=0·0235) by intention-to-treat analysis. Time to healing was shorter in the LeucoPatch group (p=0·0246) than in the standard care group. No difference in adverse events was seen between the groups. The most common serious adverse event (SAE) was diabetic foot infection (24 events in the LeucoPatch group [24% of all SAEs] and 20 in the standard care group [27% of all SAEs]. There were no device-related adverse events.

Interpretation The use of LeucoPatch is associated with significant enhancement of healing of hard-to-heal foot ulcers in people with diabetes.

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Introduction

Diabetic foot ulcers are common and are a major source of disability, distress, and cost. Healing is often delayed for many months and amputation is common. The incidence of new ulceration after healing is about 40% at 12 months, thus diabetic foot ulcers can be a financial burden for patients, their families, and health-care services.[1,2] There is an absence of treatments that have been proven to be effective, which relates to the quality of available research, which is mostly of poor design.[3]

Trials that seek to document the effectiveness of treatments for this complex clinical problem should conform to defined criteria for trial design and reporting, which has not been done thus far.[4] To that end, it is necessary that the evaluation of any treatment should be undertaken in a population that responds poorly to good standard care (ie, hard-to-heal ulcers) and should be based on a comparison of the effect of the treatment being tested with contemporaneous controls in an appropriately blinded randomised trial.

One possible treatment option for non-healing ulcers is the use of platelet-rich plasma or platelet-rich fibrin, which might promote healing of hard-to-heal ulcers in people with diabetes, as assessed by the release of cytokines and growth factors involved in tissue repair, angiogenesis, and inflammation.[3,5–8] Although the use of platelet preparations is not new, evidence of their benefits is inconsistent.[3,9,10] However, the recent development of multi-layered patches comprising autologous leucocytes, platelets, and fibrin, which can be made by the bedside and without adding any reagents (Leucopatch, Reapplix ApS, Birkerød, Denmark; appendix), is a possible new option.[11,12] Two pilot studies, of which one included participants with hard-to-heal diabetic foot ulcers only,

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See Online for appendix

Research in context

Evidence before this study

Successive systematic reviews done by the International Working Group of the Diabetic Foot and others have not shown the consistent cost-effective benefit of any topical therapy to accelerate healing of foot ulcers in patients with diabetes. Most published studies on this topic have substantial methodological weaknesses. Nevertheless, a number of studies have suggested the potential benefit of blood-derived products, even though the results have been inconsistent.

Added value of this study

This study is, to our knowledge, the first large randomised, observer-blinded, controlled trial of the use of multi-layered patches comprising autologous leucocytes, platelets, and fibrin generated by the bedside and without the addition of any reagents. The effect of the patches was compared with good standard care in people with diabetic foot ulcers that were not healing despite good standard care. The study was done to a high standard, as recommended by International Working Group of the Diabetic Foot guidelines. In the intention-to-treat analysis, the addition of the autologous multi-layer patch to usual good standard care was associated with a significant increase in the proportion of wounds confirmed healed. The number of adverse events, in particular the number of patients who developed anaemia, did not differ between the two groups.

Implications of all the available evidence

In people with diabetes complicated by foot ulcers that are not healing despite best standard of care, this new bedside treatment has the potential to significantly accelerate wound healing.

have reported beneficial effects on the healing of ulcers, without raising any safety issues.[13,14]

In this multicentre, randomised, controlled trial, we aimed to determine whether the application of LeucoPatch, when used in addition to standard care in a multidisciplinary specialist diabetes foot clinic setting, is superior to standard care alone in healing of hard-to-heal diabetic foot ulcers that were not infected at the time of randomisation.

Methods

Study design

The trial protocol and rationale has already been published.[15] This was a multinational, observer-masked, randomised controlled trial, undertaken in 32 centres with specialist diabetic foot clinics in the UK, Denmark, and Sweden. Participants had a 4-week run-in period before they were randomly allocated (1:1) to receive either the intervention plus standard care or standard care alone. The intervention period of 20 weeks was followed by a 6-week observation period. The study was done in compliance with the regulatory requirements of the three countries, in accordance with the ethical principles of the Declaration of Helsinki and recommendations for Good Clinical Practice. The study was approved by the National Research Ethics Committee West Midlands–South Birmingham and by the Research and Development departments of the participating UK National Health Service trusts; the ethics committee for Region Midtjylland, Committee 1, Denmark; and by the Regionala Etikprovningsnamnden, Lund, Sweden.

Participants

Participants were people aged 18 years and over who had diabetes (as defined by WHO criteria) complicated by one or more foot ulcers, and a baseline HbA_{1c} of no more than 12% (108 mmol/mol). Ulcers were below the level of the malleoli, but we excluded people with ulcers that were confined solely to the interdigital clefts because of the difficulty in measurement and in placing a patch directly on the wound. All ulcers were hard to heal, meaning that the cross-sectional area decreased by less than 50%, and the cross-sectional area of the index ulcer was 50–1000 mm², at the end of the 4-week run-in period.[16,17] At baseline, the index ulcer was clinically non-infected according to the criteria of the Infectious Diseases Society of America[18] and either the ankle brachial pressure index of the affected limb was 0·50–1·40 or the dorsalis pedis pulse or tibialis posterior pulse was palpable. Participants had to have the capacity to understand study procedures, and to provide written informed consent.

Exclusion criteria were cross-sectional area of the index ulcer had increased by at least 25% or had decreased by less than 50% during the 4-week run-in period, or was either smaller than 50 mm² or larger than 1000 mm² at the end of the 4-week run-in period; clinical signs of infection of the index ulcer or other reason to suspect an infection at randomisation; if a revascularisation procedure in the affected limb was planned or had been undertaken within the 4 weeks before the baseline visit; foot ulcer had been treated with growth factors, stem cells, or an equivalent preparation within the 8 weeks before the baseline visit or there was a need for continued use of negative pressure wound therapy; haemoglobin concentration was less than 105 g/L at screening; diagnosis of sickle-cell anaemia, haemophilia, thrombocytopenia ($<100\times10^9$/L), or any other clinically significant blood dyscrasia; known potential infectivity of blood products, including known HIV and hepatitis; receipt of renal dialysis or an estimated glomerular filtration rate (based on cystatin C or serum creatinine) of less than 20 mL/min per 1·73 m²; current treatment with cytotoxic drugs or with systemically administered glucocorticoids or other immunosuppressants; unlikely to comply with

the need for weekly visits because of other planned activity; involvement in another interventional clinical foot ulcer healing trial within the 4 weeks before the baseline visit; previous randomisation in the current study; or the investigator decided that they did not have the capacity to understand the study procedures or provide written informed consent.

Participants were recruited from and were managed in one of 32 specialist diabetic foot clinics in three countries (UK, Denmark, and Sweden). All participants gave written informed consent.

Randomisation and masking

After the run-in period, eligible participants were randomly assigned to receive either standard care plus intervention or standard care alone. A computer-generated, web-based, randomisation code was used, with permuted blocks of randomly varying size (two, four, and six), as created by the Nottingham Clinical Trials Unit. Trial participants were allocated with equal probability to each treatment group, with stratification by centre and by ulcer area (≤100 mm² *vs* >100 mm²).

Clinical investigators that assessed outcomes were unaware of group assignment throughout the study, as was the study statistician before the clinical database had been cleaned and locked. Participants, caregivers, and site investigators were not masked to treatment allocation. In the event of a disagreement between site investigators and the masked clinical primary outcome assessor, or if a blinded assessment was not done or was delayed beyond the permitted window described in the protocol, a masked adjudication committee reviewed the digital images.

Procedures

Clinical investigators were instructed to manage all eligible ulcers according to the best available standard care (as per International Working Group of the Diabetic Foot guidelines[19]), including offloading, either alone (during the run-in period and in the control group) or in addition to the intervention (during the 20-week active treatment phase in the intervention group).[19] Basic demographic data, medical history, and eligibility criteria were assessed at baseline. Wound characteristics, wound size by acetate tracing (area assessed by a single masked assessor at a later date using Image J software[20]), digital images of the ulcer taken post debridement, active medication including antibiotic prescriptions, type of offloading used (classified into nine types), adverse device effects, and serious adverse events, were recorded at every visit. Participant visits were scheduled every 2 weeks during the run-in period, and weekly during the intervention period. If the index ulcer had healed during the intervention period, participants were seen again at 2 weeks and 4 weeks post healing, with a blinded assessment of healing done at the point of healing and at the 4-week post healing visit. A detailed description of study procedures has already been published.[15]

The active intervention was the application of a LeucoPatch patch directly to the wound, which was done at the bedside in the clinical centres. Each week, a patch was produced by drawing 18 mL of the participant's venous blood into a LeucoPatch device, which was then transferred to a LeucoPatch Centrifuge (Reapplix ApS; Birkerød, Denmark) and spun for 20 min according to an automatic and prespecified programme. The final three-layered LeucoPatch was then removed from the device using aseptic precautions, cut to an appropriate size (if necessary), and put onto the ulcer with the leucocyte side adjacent to the surface of the ulcer. The LeucoPatch was then covered by a low adherent, knitted viscose rayon primary dressing (Tricotex, Smith & Nephew, London, UK) and a protective secondary dressing. Participants with an ulcer area larger than 5 cm² had two patches applied. New patches were made and applied on a weekly basis until healing or the end of the study.

Outcomes

The primary outcome was the proportion of ulcers in the intention-to-treat population that healed within 20 weeks after randomisation. Healing was assessed after any necessary debridement and was defined as complete epithelialisation without drainage, maintained for 4 weeks. Healing was confirmed both at the start and the end of the 4-week period by a trained observer who was unaware of treatment allocation. The date of healing was defined as that at which the ulcer was first noted by the clinical researcher to have healed and confirmed by an observer who was unaware of treatment allocation.

Secondary ulcer-related outcomes were time to healing in those that healed within the 20-week active intervention period, proportion of healed ulcers at 12 and 26 weeks, change in ulcer area at 4, 12, 16, 20, and 26 weeks (compared with week 0), which was assessed from digital images of acetate tracings using Image J (version 1),[20] incidence of secondary infection, and number of days of systemic antibiotic therapy administered for infection of the foot ulcer during the 20 weeks after randomisation. Secondary patient-related outcomes were incidence of major (above ankle) amputation affecting the target limb by 12, 20, and 26 weeks, incidence of major amputation affecting the contralateral limb by 26 weeks, incidence of minor (below ankle) amputation affecting the target limb by 12, 20, and 26 weeks, incidence of minor amputation affecting the contralateral limb by 26 weeks, incidence of new anaemia, defined as a haemoglobin concentration below 105 g/L (6·5 mmol/L), and a decrease of more than 10% compared with baseline, quality of life measured using Short Form-12 and EuroQol 5-dimensions at baseline, week 12, and week 20, and pain measured by a visual analogue scale.

Statistical analysis

Previous LeucoPatch outcome data suggested a healing rate (in the intention-to-treat population) of 54% (24 of 44)

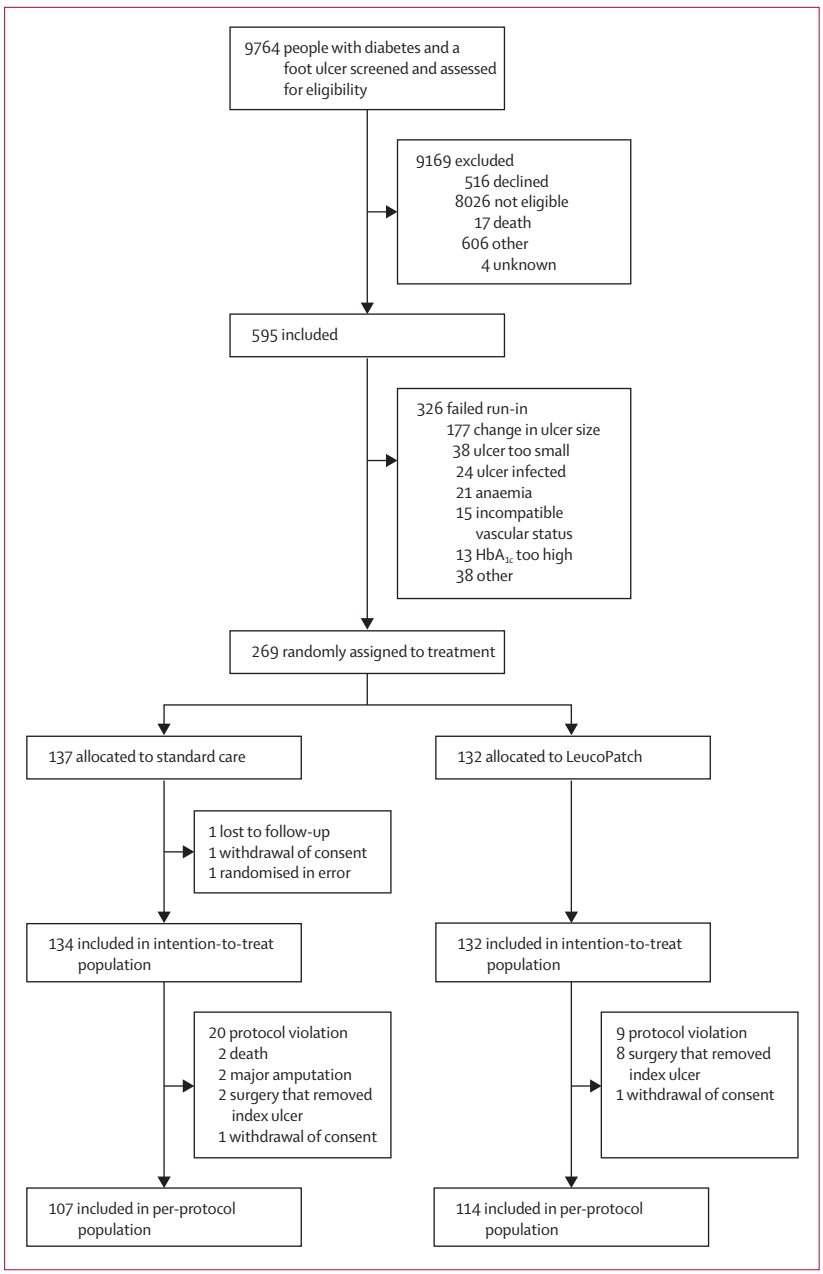

Figure 1: **Trial profile**

during a 20-week follow-up period, in a cohort of patients with a less than 40% ulcer area reduction during the 2-week run-in period.[14] The incidence of healing in a matched control group and in placebo control groups in other diabetic foot studies with inclusion and exclusion criteria similar to those used in the present study was 27–32% at 20-week follow-up, although some authors have reported healing rates below 10%.[3] We calculated that a sample size of 250 evaluable participants would be needed to compare two proportions with Fleiss continuity correction, based on α=0·05 and β=80% with an incidence of healing in the control group of 30% and an improvement of 18% (ie, to 48%) in the treatment group. To allow for 30% dropout, 350 randomised participants were needed. When 140 participants had completed 20-week follow-up, a prespecified interim analysis of the primary outcome was done by a statistician who was not otherwise involved in the study. The purpose was to check for non-futility, establish the dropout rates and, if necessary, recalculate the sample size. The results of the interim analysis were only given to the independent data monitoring committee. On the advice of the data monitoring committee, the trial steering committee suggested that the sample size should be adjusted from 350 participants to 260 participants on the basis of fewer dropouts than anticipated and, because of a slight imbalance in the number of participants randomly assigned to each group, to 269 participants. The interim analysis meant that the level of significance, for the primary outcome, was reduced to 0·04.

We used SAS Enterprise Guide software (version 7.1) for all statistical analyses. Each analysis was done on both the intention-to-treat population and the per-protocol populations. The intention-to-treat population included all randomised participants from whom any post randomisation data were collected. The per-protocol population included all participants in the intention-to-treat population without major protocol deviations who were treated with the allocated dressing for at least 4 weeks; this population was used for a confirmatory sensitivity analysis. We assessed safety outcomes in the safety population, which consisted of all randomised participants. The difference between the intention-to-treat and safety populations was three participants who were randomised but never had a follow-up visit.

We used the χ^2 test to analyse the primary outcome, along with logistic regression analysis with healed or not healed as outcome and treatment and other explanatory variables. We exploratively included pertinent variables, such as wound area at baseline, ulcer depth, duration of wound, ankle brachial pressure index, and country, but only wound size at baseline and duration of wound (<1 year or ≥1 year) were used as covariates.

We used regression analysis of survival data to analyse time (measured in days) to healing, on the basis of the Cox proportional hazards model, with a censor variable indicating whether the ulcer had healed or not. A graphical illustration is presented with survival curves. We used χ^2 tests for all other proportions, except when low numbers required the use of Fisher's exact test (eg, amputations, adverse events, and serious adverse events). We used Student's *t* tests to analyse the total number of days of antibiotic therapy and used repeated measures analysis to analyse reductions in pain.

A p value of less than 0·05 was considered to be statistically significant in all other analyses except the primary outcome analysis.

This trial is registered with the ISRCTN registry, number 27665670, and ClinicalTrials.gov, number NCT02224742.

Role of the funding source
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The statistician (JLJ) had full access to all the data in the study. The chief investigators (FG, WJ, LT, and ML) had full access to all the data after the database was locked and had final responsibility for the decision to submit for publication.

Results
Between Aug 30, 2013, and May 3, 2017, we recruited 595 people with diabetes, of whom 269 were randomly allocated to receive treatment (137 to receive standard care and 132 to receive LeucoPatch). 326 (55%) of 595 participants failed run-in; the main reason for this was a change in ulcer area of more than 50% over 4 weeks (figure 1). 134 participants in the standard care group and 132 in the LeucoPatch group were included in the intention-to-treat population. Participants were recruited from 32 specialist centres; 22 in the UK, three in Sweden, and seven in Denmark. The mean number of participants who consented from each centre was 18·5 (SD 8·5, range 1–78), and the mean number of participants randomised was 8·4 (18·9, 0–31) per centre.

The baseline characteristics were well balanced between treatment groups (table 1). The mean age of participants was 61·9 (SD 11·6) years, 217 (82%) of 266 were men, and 222 (83%) had type 2 diabetes. The median duration of diabetes was 16 years (IQR 10–23) and the median HbA_{1c} was 8·2% (IQR 7·2–9·2; 66 mmol/mol, IQR 55–77). Most index limbs were neuropathic, with 227 (85%) of 266 participants unable to feel a 10-g monofilament on at least two of three prespecified sites on the affected foot. Most ulcers were greater than 100 mm², superficial, and on the forefoot. The two groups were well matched in terms of the types of offloading used throughout the study.

Within 20 weeks, 45 (34%) of 132 index ulcers in the LeucoPatch group had healed versus 29 (22%) of 134 in the standard care group, giving an unadjusted odds ratio (OR) of 1·58 (96% CI 1·04–2·40, p=0·0235) for healing in the intention-to-treat population (table 2). On six occasions, the decision that an ulcer had healed was made by the blinded assessment committee on the basis of the digital images. On each occasion, the committee agreed that the ulcer was healed. In the per-protocol population, healing within 20 weeks was achieved in 44 (39%) participants in the intervention group versus 28 (26%) in the standard care group (OR 1·47 [96% CI 0·98–2·23], p=0·0488). Healing incidence at 12 weeks and 26 weeks is shown in table 2. The median time to healing in those who healed was 72 days (IQR 56–103) in the LeucoPatch group and 84 days (IQR 64–98) in the standard care group (table 2; p=0·0343). Time to healing was shorter in the LeucoPatch group (p=0·0246) than in the standard care group (figure 2). The change in ulcer area from baseline is shown in figure 3. The intervention group healed progressively faster up to 12 weeks, although the rates of decline in ulcer area in the two groups were similar thereafter.

	Standard care (n=134)	LeucoPatch plus standard care (n=132)	Total (n=266)
General			
Mean age, years (SD)	62·0 (11·9)	61·9 (11·4)	61·9 (11·6)
Sex, n (%)			
Male	110 (82%)	107 (81%)	217 (82%)
Female	24 (18%)	25 (19%)	49 (18%)
Type 2 diabetes, n (%)	110 (82%)	112 (85%)	222 (83%)
Median duration of diabetes, years (IQR)	15 (8–22)	16 (10–25)	16 (10–23)
Diabetes-related complications, n (%)			
Cerebrovascular	17 (13%)	13 (10%)	30 (11%)
Cardiovascular	57 (43%)	55 (42%)	112 (42%)
Nephropathy	42 (31%)	48 (36%)	90 (34%)
Retinopathy	87 (65%)	87 (66%)	174 (65%)
Median HbA_{1c}, % (IQR)	8·2% (7·1–9·0)*	8·3% (7·2–9·2)†	8·2% (7·2–9·2)‡
Mean baseline haemoglobin, g/L (SD)	131 (15·4)	132 (15·6)	131 (15·5)
Estimated GFR, n (%)			
20–30 mL/min per 1·73 m²	3 (2%)	9 (7%)	12 (5%)
31–45 mL/min per 1·73 m²	15 (11%)	19 (14%)	34 (13%)
46–60 mL/min per 1·73 m²	34 (25%)	35 (27%)	69 (26%)
>60 mL/min per 1·73 m²	82 (61%)	69 (52%)	151 (57%)
Foot ulcer-related complications			
ABPI, n (%)			
0·5–0·79	16 (12%)	14 (11%)	30 (11%)
0·8–0·99	23 (17%)	30 (23%)	53 (20%)
1·0–1·4	73 (55%)	65 (49%)	138(52%)
>1·4	22 (16%)	23 (17%)	45 (17%)
Loss of sensation at two or more sites, n (%)	117 (87%)	110 (83%)	227 (85%)
Area of ulcer, n (%)			
≤100 mm²	34 (25%)	34 (26%)	68 (26%)
>100 mm²	100 (75%)	98 (74%)	198 (74%)
Mean area of ulcer, mm² (SD)	252·7 (226·5)	228·8 (207·43)	240·8 (217·16)
Depth of ulcer, n (%)			
Superficial	120 (90%)	110 (83%)	230 (87%)
Down to tendon	11 (8%)	16 (12%)	27 (10%)
Down to bone	3 (2%)	6 (5%)	9 (3%)
Affected foot position, n (%)§			
Total forefoot	99 (74%)	108 (82%)	207 (78%)
Plantar forefoot	54 (40%)	57 (43%)	111 (42%)
Hind foot	35 (26%)	24 (18%)	59 (22%)
Type of offloading			
Bedbound or immobile	3 (2%)	4 (3%)	7 (3%)
Normal footwear	10 (7%)	14 (11%)	24 (9%)
Normal footwear plus fitted insoles or inserts	6 (4%)	6 (5%)	12 (5%)
Fitted footwear or orthoses	35 (26%)	38 (29%)	73 (27%)
Padded slipper or shoe	27 (20%)	28 (21%)	55 (21%)
Removable cast or device for foot	34 (25%)	31 (23%)	65 (24%)
Removable cast or device for lower leg	25 (19%)	17 (13%)	42 (16%)

(Table 1 continues on next page)

	Standard care (n=134)	LeucoPatch plus standard care (n=132)	Total (n=266)
(Continued from previous page)			
Non-removable cast or device for foot	1 (1%)	3 (2%)	4 (2%)
Non-removable cast or device for lower leg	4 (3%)	6 (5%)	10 (4%)

Data are given as median (IQR) or number of participants (%), unless otherwise stated. Percentages might not sum to 100% because of rounding. GFR=glomerular filtration rate. ABPI=ankle brachial pressure index. *66 mmol/mol (IQR 54–75). †67 mmol/mol (IQR 55–77). ‡66 mmol/mol (IQR 55–77). §Forefoot was defined as distal to and hind foot as proximal to the tarsometatarsal joint.

Table 1: **Baseline clinical characteristics**

The incidence of diabetic foot infection within 20 weeks did not differ between the two groups. No differences in minor or major amputations between groups was seen after 12, 20, or 26 weeks (table 2). Reductions in pain were similar between the groups (table 2).

The incidence of new anaemia (not classified as serious) or any adverse events did not significantly differ between groups (table 2, appendix). The most common serious adverse event (SAE) was diabetic foot infection; there were 24 events in the LeucoPatch group (24% of all SAEs) and 20 events in the standard care group (27% of all SAEs; table 2, appendix). Of these diabetic foot infections, 16 infections (67%) in the LeucoPatch group (16% of all SAEs) and 12 infections (60%) in the standard care group (16% of all SAEs) were attributed to the index ulcer. There were no device-related adverse events. The country of recruitment did not significantly affect the results (data not shown).

Discussion

This multicentre, observer-masked, randomised controlled trial found a significantly higher incidence of healing within 20 weeks (unadjusted OR 1·58) in those receiving LeucoPatch applications for hard-to-heal diabetic foot ulcers when compared with good quality standard care. Ulcer area also significantly decreased in the intervention group compared with the standard care group, as did time to healing in those with ulcers that healed. The incidence of major or minor amputation and of any adverse events or serious adverse events did not differ between the two groups. In particular, there was no increased incidence of anaemia in the intervention group—even in those with reduced glomerular filtration rate—despite the need for weekly venesection. The incidence of either the number of episodes of clinical infection or of antibiotic use also did not differ between the two groups, even though a difference might have been expected because of the leucocytes contained within the application.

The main strength of the study was that the design and conduct of this study fulfilled the exacting requirements specified for work in this field.[4] The study population was designed to focus on those with hard-to-heal ulcers—the group for which new treatments are most needed. All investigators managed participants according to the principles of good standard care using prespecified criteria and this procedure was reinforced at regular investigator meetings. The groups were well matched. The target number of participants were recruited and retention was high, with few dropouts.

The main weakness of our study design and conduct was that it was not possible to mask either the participant or the clinical researcher to treatment allocation. The use of sham venepuncture was rejected as being unethical, but assessment of the primary outcome was undertaken by an independent and masked observer and backed up with digital imaging. The inclusion and exclusion criteria ensured that the recruited population was representative of a hard-to-heal population; this assumption is reflected in the low overall incidence of healing in the non-intervention group. Nevertheless, an element of selection is evident in that the mean age was slightly less than anticipated (62 years as opposed to the expected 67 years), which might reflect the need for participants to attend each week for up to 5 months. We recruited a high proportion of males (82% instead of the expected 67%), but this finding is now recognised as a typical feature of large trials in this field. The overall incidence of healing was lower than anticipated, and lower than that observed in the pilot studies, but probably reflects the more rigid selection of a defined hard-to-heal population. It is also possible that the low healing rate reflected poor standard care, which might have been different between the two groups. We believe that this hypothesis is unlikely because the low healing rate in those already preselected as having hard-to-heal ulcers after the 4-week run-in period was similar to that reported by Coerper and colleagues.[17] Additionally the two groups were well matched in terms of their baseline characteristics and offloading strategies, and similar numbers were revascularised during the 26-week follow-up. Therefore, it is unlikely that a differential standard of care explains the additional benefit seen on healing in the intervention group.

Notably, the OR for healing in the intervention group (1·58) was similar to that which we used to calculate the sample size (48% *vs* 30%; OR 1·60). We acknowledge that the number of participants who we screened and were not eligible for inclusion might suggest that the patient population was not representative of patients seen in a specialist diabetic foot clinic. We felt that for this protocol, although we were including hard-to-heal ulcers, we had to exclude those with little chance of healing within the 20 weeks of the study (eg, very large ulcers, those with severe ischaemia, and those with severe renal disease) as their data had little chance of contributing to the final results. The median number of ulcers included per centre was, however, similar to a published randomised controlled trial of another product designed to accelerate wound healing in patients with neuroischaemia.[21]

	Cohort	Standard care	LeucoPatch plus standard care	Significance
Primary outcome				
Blinded assessment of healing within 20 weeks, n (%)	Intention to treat	29 (22%)	45 (34%)	Unadjusted: OR 1·58 (96% CI 1·04–2·40), p=0·0235; adjusted*: OR 1·89 (96% CI 1·07–3·40), p=0·0237
Blinded assessment of healing within 20 weeks, n (%)	Per protocol	28 (26%)	44 (39%)	Unadjusted: OR 1·47 (96% CI 0·98–2·23), p=0·0488; adjusted*: OR 1·795 (96% CI 0·98–3·32), p=0·0480
Secondary outcomes				
Blinded assessment of healing within 12 weeks, n (%)	Intention to treat	17 (13%)	27 (20%)	p=0·0882
Blinded assessment of healing within 26 weeks, n (%)	Intention to treat	29 (22%)	45 (34%)	Unadjusted OR 1·89 (95% CI 1·09–3·28), p=0·0237
Median days to healing in those who healed within 20 weeks, n (IQR)	Intention to treat	84 (64–98); n=29	72 (56–103); n=45	p=0·0343
Number of patients who developed new infection within 20 weeks, n (%)	Intention to treat	63 (47%)	51 (39%)	OR 0·8350 (95% CI 0·63–1·11), p=0·2080
Percentage of visits at which infection was reported as a proportion of total visits	Intention to treat	10·1%	8·6%	OR 0·8417 (95% CI 0·70–1·02), p=0·0728
Healing by ABPI subcategory, N (% of those in that subcategory)				
0·5–0·79	Intention to treat	2/16 (12·5)	5/14 (35·7)	..
0·8–0·99	Intention to treat	6/23 (26·0)	8/30 (26·7)	..
1·0–1·4	Intention to treat	14/73 (19·2)	25/65 (38·4)	..
>1·4	Intention to treat	7/22 (31·8)	7/23 (30·4)	..
Total number of days of antibiotic therapy	Intention to treat	2822	2662	OR 0·92 (95% CI –9·14 to 7·35), p=0·8314
New minor amputations of index limb				
12 weeks	Intention to treat	2	5	OR 2·49 (95% CI 0·48–12·80), p=0·4526
20 weeks	Intention to treat	5	8	OR 1·63 (95% CI 0·53-4·96), p=0·4196
26 weeks	Intention to treat	9	8	OR 0·90 (95% CI 0·35–2·34), p=1·000
New major amputations of index limb				
12 weeks	Intention to treat	0	0	..
20 weeks	Intention to treat	2	2	OR 1·02 (95% CI 0·14–7·21), p=1·000
26 weeks	Intention to treat	2	2	OR 1·02 (95% CI 0·14–7·21), p=1·000
New minor amputations of contralateral limb				
12 weeks	Intention to treat	1	4	OR 3·98 (95% CI 0·44–35·57), p=0·3746
20 weeks	Intention to treat	2	7	OR 3·56 (95% CI 0·74–17·11), p=0·1062
26 weeks	Intention to treat	3	7	OR 2·37 (95% CI 0·61–9·15), p=0·2226
New major amputations of contralateral limb				
12 weeks	Intention to treat	0	0	..
20 weeks	Intention to treat	1	1	OR 1·02 (95% CI 0·06–6·24), p=1·000
26 weeks	Intention to treat	1	1	OR 1·02 (95% CI 0·06–16·23), p=1·000
Reduction in pain† (% change VAS)	Intention to treat	–45·5%; n=85	–54·5%; n=71	OR 1·20 95% CI –1·22 to 10·54, p=0·1194
Revascularisation of the index limb by 26 weeks, n (%)	Intention to treat	6 (5%)	3 (2%)	OR 0·44 (95% CI 0·08–3·31), p=0·49
Death by 26 weeks, n (%)	Intention to treat	5 (4%)	3 (2%)	OR 0·60 (95% CI 0·14–2·56), p=0·7221
Safety‡				
Incidence of new anaemia, n (%)§	Safety	11 (8%)	13 (10%)	OR 1·20 (95% CI 0·56–2·58), p=0·6408
Any adverse event	..	..	..	OR 0·93 (95% CI 0·78 – 1·12), p=0·4607
Number of participants, n (%)	Safety	90 (66%) of 137	81 (61%) of 132	..
Number of reports	Safety	240	274	..
Device-related adverse events	Safety	0	0	..
Any serious adverse event	..	..	..	OR 1·26 (95% CI 0·91 – 1·76), p=0·1689
Number of participants, n (%)	Safety	42 (31%)	51 (39%)	..
Number of reports	Safety	74	98	..

Data are given for primary and important secondary outcomes for patients allocated to intention-to-treat population in people randomised to standard care alone (n=134) or Leucopatch plus standard care (n=132). For the per-protocol analyses, the population was standard care alone (n=107) and Leucopatch plus standard care (n=114). ABPI=ankle brachial pressure index. VAS=visual analogue scale. *Adjusted for baseline wound size (≤100 mm² *vs* >100 mm²). †In those that had pain at baseline. ‡Full list of adverse events and serious adverse events can be found in the appendix. §Not classified as serious.

Table 2: **Primary, secondary, and safety study outcomes**

This study has shown the apparent effectiveness of this new intervention in the management of people with hard-to-heal diabetic foot ulcers. It adds to the increasing number of studies that have reported benefit from the use of platelets and platelet-derived application to the surface of the chronic wound. Such benefit could be mediated through many mechanisms relating to the process of inflammation and tissue repair. However, in addition to delivering living autologous platelets to the wound surface, LeucoPatch also delivers living autologous neutrophils and macrophages and could, therefore, confer additional advantages. Nevertheless, no difference was seen between the two groups in the apparent incidence of episodes of wound infection.

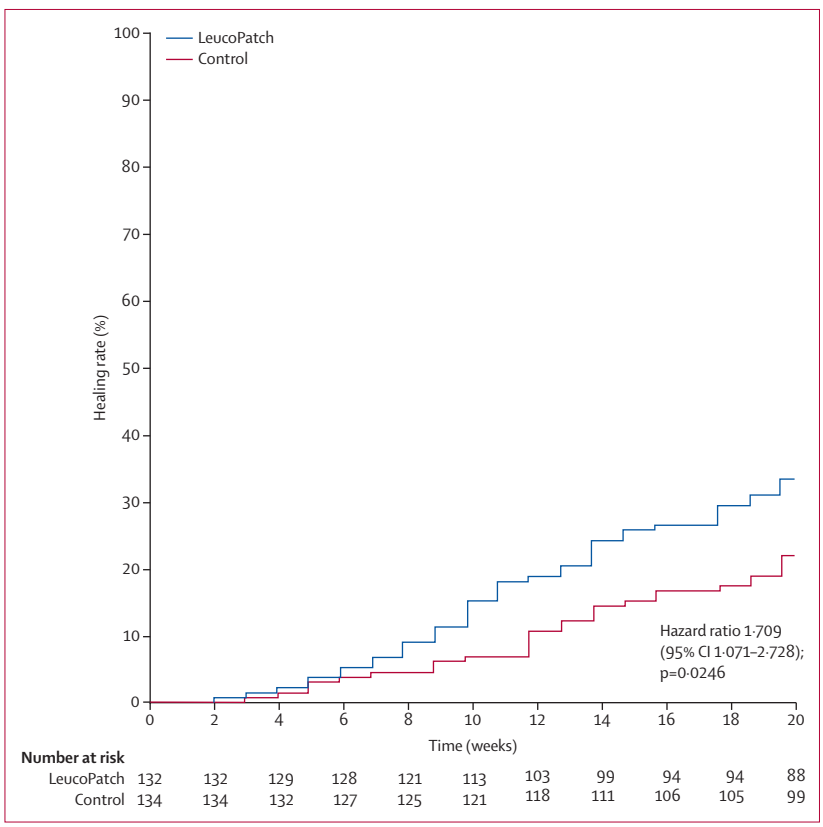

Figure 2: **Time to healing**
Survival curve showing proportion of ulcers healed, with healing defined as complete epithelialisation without any drainage sustained for at least 4 weeks, in the intention-to-treat population randomly assigned to standard care alone (n=134) or standard care and LeucoPatch (n=132).

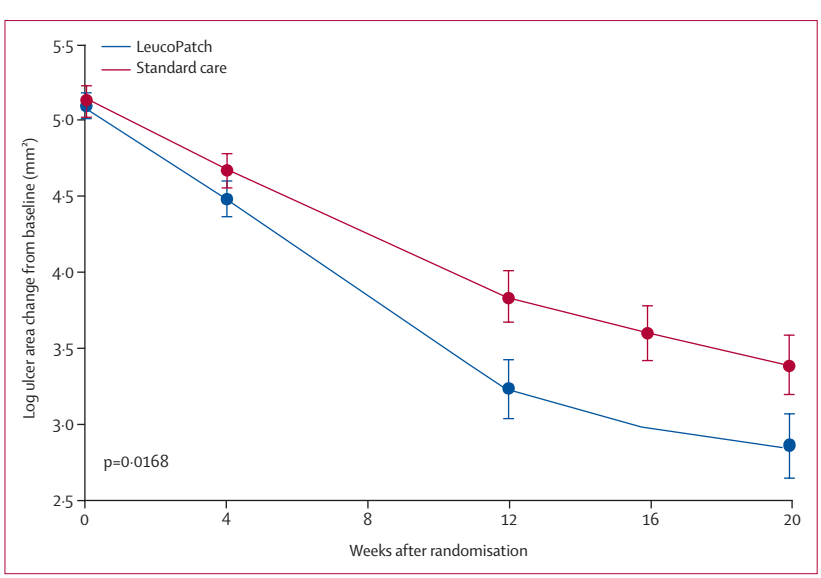

Figure 3: **Reduction in ulcer area**
Data are mean of log, with 95% CI error bars.

The production of LeucoPatch patches is simple and quick, and the patch is easy to apply during the course of routine clinical practice. Weekly application was used for this definitive study. Notably, the analysis of the per-protocol population (ie, those without major protocol deviations and who were treated with LeuocPatch for at least 4 weeks) showed a similar improvement in healing at 20 weeks. It is possible, therefore, that the treatment might not need to be continued until full healing is seen and could be discontinued earlier. This possibility has not been tested with this protocol.

Although acceptability was not directly tested in this study, the low number of dropouts from the study protocol suggests that patients find this an acceptable treatment strategy.

Although reviews of the published literature on wound care products for diabetic foot ulcers have repeatedly emphasised the urgent need for trials of better quality, to our knowledge, this trial is only the third relatively robust randomised controlled trial to be reported in the last 12 months. The other two studies investigated a dressing product that acts on the activity of matrix metalloproteinases (sucrose octasulphate dressing)[21] and a dressing which releases intradermal nitrix oxide (ProNOx1).[22] Although these three approaches represent contrasting modes of action, and include different types of ulcer with different durations and different healing criteria, all three trials reported an almost identical figure for unadjusted OR: about 1·5–1·6.

In summary, this trial shows a clinically and statistically significant benefit associated with the weekly application of autologous immune cell, fibrin, and platelet patches (LeucoPatch) in a population of people with hard-to-heal diabetic foot ulcers. The treatment was without apparent adverse events, specifically without evidence of new onset anaemia. It is possible that this treatment might also be of benefit in other types of diabetic foot ulcer but this theory has not yet been studied.

Contributors
FG, WJ, LT, JLJ, DF, and ML wrote and approved the study protocol. FG, WJ, LT, and ML were the national chief investigators of the study. DJW, EFH, and SJE coordinated the running of the trial and data collection. JLJ did the statistical analysis. FG, WJ, LT, ML, and JLJ interpreted the data. FG, WJ, LT, JLJ, and ML wrote the manuscript. All authors approved the final version.

Declaration of interests
LT and ML have received research support from Reapplix ApS. All other authors declare no competing interests.

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