Efficacy and safety of autologous whole blood clot in diabetic foot ulcers: a randomised controlled trial

Objective: Diabetic foot ulcers (DFUs) present a significant global health challenge, resulting in high morbidity and economic costs. Current available treatments often fail to achieve satisfactory healing rates, highlighting the need for novel therapies. This study evaluated the safety and efficacy of a novel autologous whole blood clot (AWBC)—a blood-based, biodegradable provisional matrix—in conjunction with standard of care (SoC) when compared to SoC alone in the treatment of hard-to-heal DFUs.

Method: A multicentre, prospective, blinded assessor, randomised controlled trial was conducted at 16 sites across the US, South Africa and Turkey. A cohort of patients with hard-to-heal DFUs was enrolled and randomised to either the AWBC group or the control group. The primary endpoint was complete wound closure at 12 weeks, while secondary endpoints included time to heal and percentage area reduction (PAR) at four and eight weeks. Data were analysed using both intention-to-treat (ITT) and per-protocol (PP) populations.

Results: The cohort included 119 patients. AWBC treatment resulted in a significantly higher healing rate compared to the control in both ITT (41% versus 15%, respectively; p=0.002) and PP populations (51% versus 18%, respectively; p=0.0075). AWBC treatment also resulted in a shorter mean time to heal and higher durability of wound closure. Safety analysis showed a similar incidence of adverse events (AEs) between groups, with no device-related AEs.

Conclusion: The AWBC system, by modulating the wound microenvironment and providing a functional extracellular matrix, offered a promising new approach to treating hard-to-heal DFUs, demonstrating superior healing outcomes compared to SoC alone in this study.

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autologous • blood • cell-based therapy • diabetic • foot ulcer • randomised controlled trial • tissue-based therapy • wound • wound care • wound dressing • wound healing

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Globally, diabetic foot ulcers (DFUs) pose a significant social and economic burden, with Medicare incurring nearly $4 billion USD in DFU-related costs in 2019.[1] Despite many advances made in the management of diabetes, complications related to DFUs remain a global public health issue. The mortality rate among patients with a DFU is two-times greater compared to patients with diabetes who do not present with a DFU.[2] Cases of DFU account for the highest rate of non-traumatic lower extremity amputations, with a five-year mortality rate of 30%.[3,4] Among real-world patients in the US Wound Registry, only 30% of those who presented with a DFU healed at 12 weeks.[5] Despite advances in wound management options for DFUs, safe and effective novel treatment options for hard-to-heal DFUs are urgently needed to improve wound healing outcomes.

Hard-to-heal (chronic) wounds do not follow a linear or predictable pattern of healing, with outcomes that for most remain unpredictable. Impaired and or delayed healing is a key characteristic of DFUs and, to date, treatment has been focused on managing the macroenvironment alone, namely, managing the bacterial and necrotic burden, as well as ensuring exudate control and adequate offloading.[6] However, traditional approaches to the management of the DFU do not always achieve favourable healing outcomes. Delayed healing in DFUs can be attributed to cellular dysfunction within the microenvironment of the wound, resulting in impaired immune responses. Research suggests that the wound environment of the DFU is proinflammatory, which leads to a proteolytic environment resulting in the degradation of the extracellular matrix (ECM), thereby inhibiting the normal matrix–cell interactions.[6,7] As a result, the wound enters a vicious circle of prolonged

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inflammation, degradation of the ECM and attraction of proinflammatory cytokines, with the net effect of delayed and/or impaired healing.[8]

To facilitate DFU healing, treatment should be focused on the management of the wound microenvironment and the associated cellular dysfunction. This can be achieved by modulating the proinflammatory environment, providing a functional ECM to restore matrix–cell interactions, and replenishing growth factors to facilitate wound healing.[6]

In this paper, we present, a novel autologous, blood-based, biodegradable provisional matrix that modulates the proinflammatory wound environment, provides a provisional ECM, and is suggested to deliver topical growth factors to the wound bed, which could overcome the challenges faced in the management of hard-to-heal DFUs.[7,9] The autologous provisional wound matrix is a bedside treatment formed from the patient's own blood. Whole blood is collected into an acid citrate dextrose solution A (ACD-A) vacuum tube containing anticoagulant, and is activated with calcium gluconate and kaolin to control the coagulation process.[9,10] This treatment was found to be safe and effective in healing hard-to-heal wounds, showing 78% (7/9) and 65% (13/20) healing rates in two studies[9,10] in which most patients had advanced comorbidities, and for whom 25% had ulcers that lasted >12 months.

The efficiency and usability of AWBC at the point of care in the management of exuding cutaneous wounds have demonstrated good healing outcomes in several observational studies.[11–14] In a registry study of 22 patients with DFUs, 76% of wounds treated with AWBC achieved a percentage area reduction (PAR) of 50% at four weeks, and 95% complete closure by week 12.[11] In a study of 24 large and hard-to-heal pressure injuries (mean wound area: 21cm^2, mean wound duration: 13 months), 78% of wounds achieved a 50% PAR at week four.[12] By week 12, the mean PAR was 96% and 45% of the wounds were completely closed. Moreover, in small registry studies looking at complex and surgical wounds, AWBC resulted in 80–100% closure rates in three complex wounds with exposed structures,[15] and in 14 patients with complex surgical wounds, showing PAR of a mean of 72% at four weeks, with 78% of wounds closed by 12 weeks.[13]

We present the results of a randomised controlled trial (RCT) that aimed to assess the safety and efficacy of AWBC in conjunction with best practice SoC when compared to best practice SoC alone (control) on the complete closure of hard-to-heal DFUs.

Methods

Ethical approval and patient consent

This study was registered on ClinicalTrials.gov (NCT04185558), where the protocol outline is available. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in Institutional Review Board (IRB) approval. The central IRB, Advarra (No. Pro00033859; Columbia, MD, US) approved the study protocol, as well as the following institutional IRBs: Boston Medical Center (No. H-38929; Boston, MD, US); Olive View UCLA Education & Research Institute (No. 1528465; Los Angeles, US); Temple University School of Podiatric Medicine (No. 27358-002; Philadelphia, US); VA Greater Los Angeles Healthcare System (No. 1641792; Los Angeles, US); University of the Witwatersrand (No. 211015; Johannesburg, South Africa); and Acinadem Altunizade (No. 2023/200; Istanbul, Turkey).

Written informed consent was obtained from all the patients who participated in the study, which included the use of their medical records, wound characteristics, and the use of their wound photographs taken throughout the study.

Study design

This was a multicentre, prospective, blinded assessor, RCT to evaluate the use of AWBC + SoC (AWBC arm) compared to SoC alone (control arm) on hard-to-heal DFUs. Patients were treated at 16 sites in the US, South Africa and Turkey.

Patients were screened and underwent assessment for eligibility criteria. Patients who met the criteria for inclusion (Table 1) were enrolled for a run-in period of 14 days (+2 days). During this run-in phase, patients were treated using the best practice SoC, based on the US guidelines for treating DFUs, which consisted of weekly cleansing, sharp debridement (as required), infection control, an offloading regimen and wound dressing (alginate dressing or hydrogel plus gauze) to facilitate the wound environment, moisture and exudate control.[16] If required, patients received an additional visit/treatment during a one-week period, where the dressings were changed. All patients were provided with a Cam walker boot (for plantar ulcers) or a surgical shoe (for dorsal ulcers) for the purpose of providing standardised offloading. At the end of the run-in phase, patients were assessed again for eligibility. Patients whose wounds had not decreased by ≥30% of the area at the initial screening visit (post debridement) were randomised to a weekly application of AWBC or control treatment for up to 12 weeks.

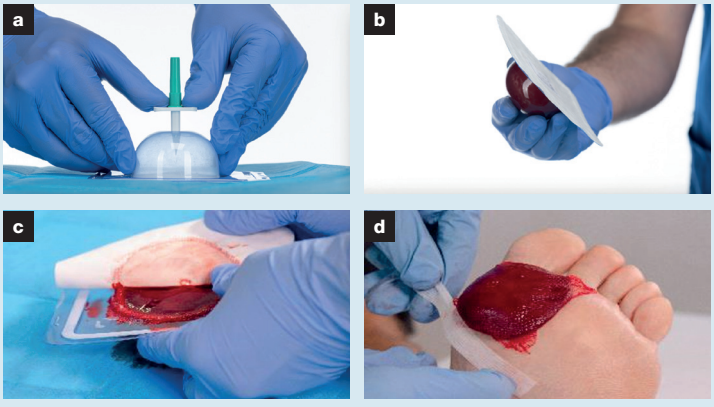
Both the AWBC and the control group were treated weekly, where a thorough wound assessment was performed by the investigator. The wounds were assessed for clinical signs of infection and wound exudate, and debridement and cleansing were performed at the investigator's discretion. Measurement of wound area and depth was conducted using an advanced wound imaging system (eKare inSight; eKare Inc., US). For validation purposes, a blinded, independent assessor reviewed all images for tracing accuracy and healing validation throughout the study, using a central online review process that included images of the ulcers without the allocated treatment, keeping the assessor blinded to the treatment arm. Pre- and post-debridement (if applicable) images were taken by the clinical team at the centre and were uploaded to

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
>18 years of age (≥19 years at one site only*)	Index ulcer has known/suspected malignancy
Has type 1 or type 2 diabetes	Has underlying osteomyelitis
Hard-to-heal DFU (≥30 days), located distal to the malleolus (excluding ulcers between the toes but including those of the heel) and depth ≤0.5cm with no exposed capsule, tendon or bone and no major tunnelling or undermining, or sinus tracts	Received (within the past 30 days) or planning to receive a medication or treatment which would interfere with wound healing (e.g., systemic steroids, immunosuppressive/ autoimmune disease therapy, cytostatic therapy within the previous year, dialysis, radiation therapy to the index foot, planned vascular surgery on the study ulcer limb 90 days post screening, angioplasty or thrombolysis, and/or chemotherapy)
Wound area >1cm^2 but <28cm^2	Sepsis or active infection likely to interfere with trial, such as urinary tract infection
Index ulcer separated from other ulcers by ≥1cm	Index foot has active Charcot foot
No infection on index ulcer/limb	Alcohol/substance misuse within the past 2 months
No necrotic wound tissue post debridement	Coagulation problems, abnormal thrombocytes, or received intravenous heparin†
Adequate circulation to the index limb ($TcPO_2$ ≥30mmHg; ABI >0.7 but <1.2; triphasic or biphasic Doppler arterial waveforms at the ankle of affected leg; TBI >0.6	Treated with wound dressings that included growth factors, engineered tissues, or skin substitutes within 30 days of randomisation, or scheduled to receive them during the study
HbA1c ≤12.0%	Had HBOT within 5 days of screening or scheduled to receive it during the study.
Adequate offloading regimen	Life expectancy <12 months
Willing and able to adhere to the protocol, including having 15ml blood drawn weekly to create the AWBC	Participated in another clinical trial involving a device or a systemically administered study drug or treatment within 30 days of randomisation visit
Female patients capable of conceiving using an acceptable form of contraception (including condoms for male partners)	History of: ESRD, immunosuppression, severe malnutrition, liver disease, scleroderma, HIV or AIDS, active connective tissue disorder, and/or exacerbation of sickle cell anaemia
Able to provide informed consent	Haemoglobin anaemia (<9g/dl) in the previous 3 months
	Wound area decreased by ≥30% during the 2-week screening period (+ 2 days)
	Pregnant or breastfeeding

*This site was located in Alabama, US, where law (Code 26-1-1) defines a minor as <19 years old; †Patients taking coumadin, aspirin, clopidogrel, apixaban or dabigatran were not excluded. ABI—ankle–brachial index; AWBC—autologous whole blood clot; DFU—diabetic foot ulcer; ESRD—end-stage renal disease; HBOT—hyperbaric oxygen therapy; TBI—toe–brachial index; $TcPO_2$—transcutaneous oxygen pressure test

Fig 1. Autologous whole blood clot coagulation mould. The punch tool is used to puncture the coagulation mould at the top centre to allow the insertion of the blood **(a)**. The blood is withdrawn from the tube, using a safety needle, into a 30ml syringe and inserted into the coagulation mould. The blood is mixed with the coagulation component inside the mould for 20 seconds **(b)**. After five minutes, the clot has formed and is gently removed from the coagulation mould **(c)**. The clot is applied to the wound bed and secured with skin closure adhesive strips **(d)**

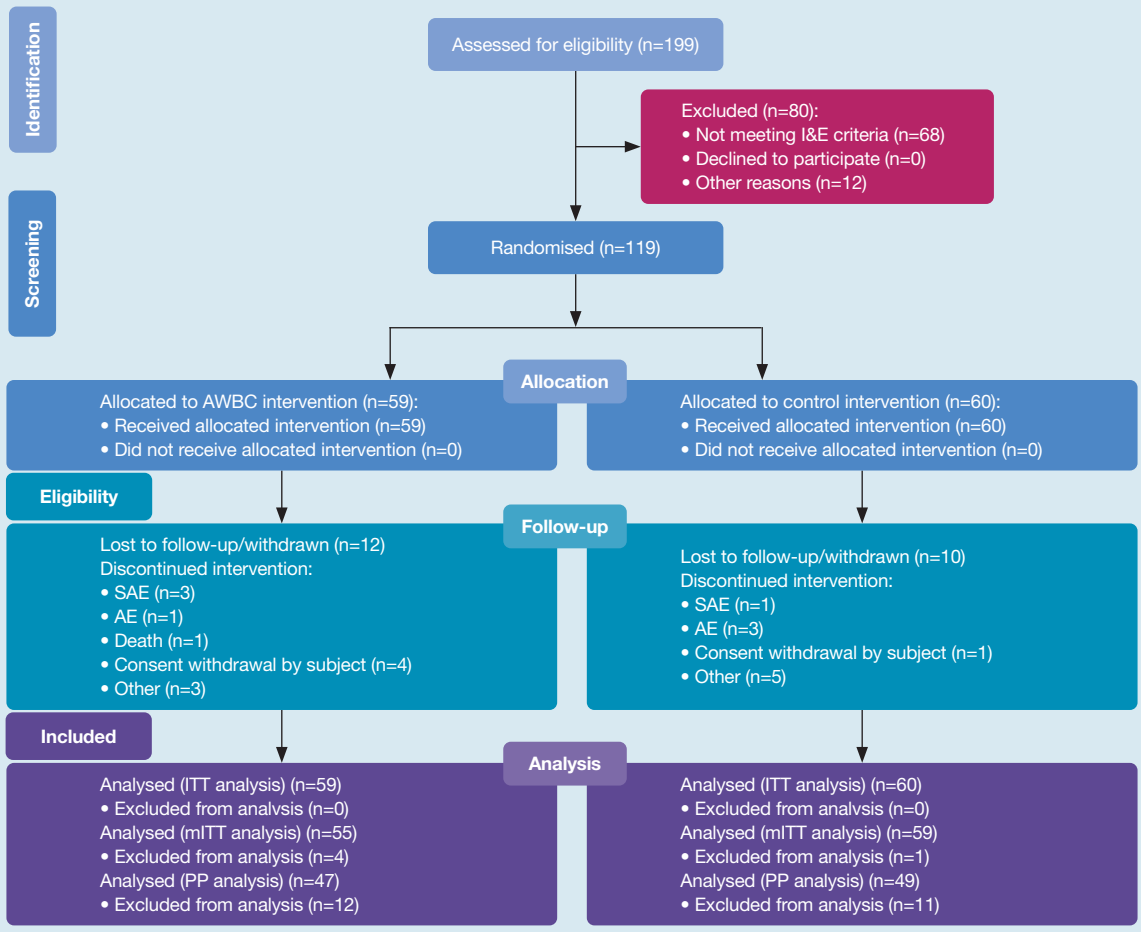


the image capture system, for the blinded assessor's review. The images only contained the patient's number and wound location without any identifications. Patients were instructed to wear the offloading device at all times, including sleeping and bathing (to avoid water from entering the device, patients were instructed to use a cast protector while showering).

Both study arms were permitted a second visit between the weekly treatment visits, where necessary, to change their secondary dressings. For the AWBC arm, this was the outer foam without touching the non-adherent dressing, while patients in the control group had their entire dressing changed.

Complete wound closure was defined as 100% re-epithelialisation of the wound as evidenced by no wound drainage and no requirement for further dressings. Definitive wound closure was confirmed in person by the investigator two weeks later. Regardless of which treatment arm the patient had been randomised to, if the wound reopened at the two-week healing confirmatory visit, the patient resumed the weekly

Fig 2. Patient flow chart. AE—adverse event; AWBC–autologous whole blood clot; I&E—inclusion and exclusion; ITT—intention-to-treat; PP—per-protocol; SAE—serious adverse event



allocated treatment, as long as the patient had not exceeded the 12 weeks of treatment (as defined in the protocol). Patients who had confirmation of complete healing entered a 12-week follow-up phase, during which they were evaluated every two weeks in the first month and then every four weeks for two additional visits, as long as the wound remained healed.

Patients discontinued the study if they missed two consecutive weekly visits, were non-adherent with the study protocol, or had an adverse event (AE) that interfered with treatment or jeopardised their health. AEs included: an infection that could not be controlled; lack of venous access (in the AWBC group); ulcer deterioration ≥50% in area from baseline; and any acute health deterioration requiring hospitalisation or which was likely to negatively interfere with treatment. Patients had the option to withdraw consent at any time, in which case, their data was used up to the point of their withdrawn consent.

Study endpoints

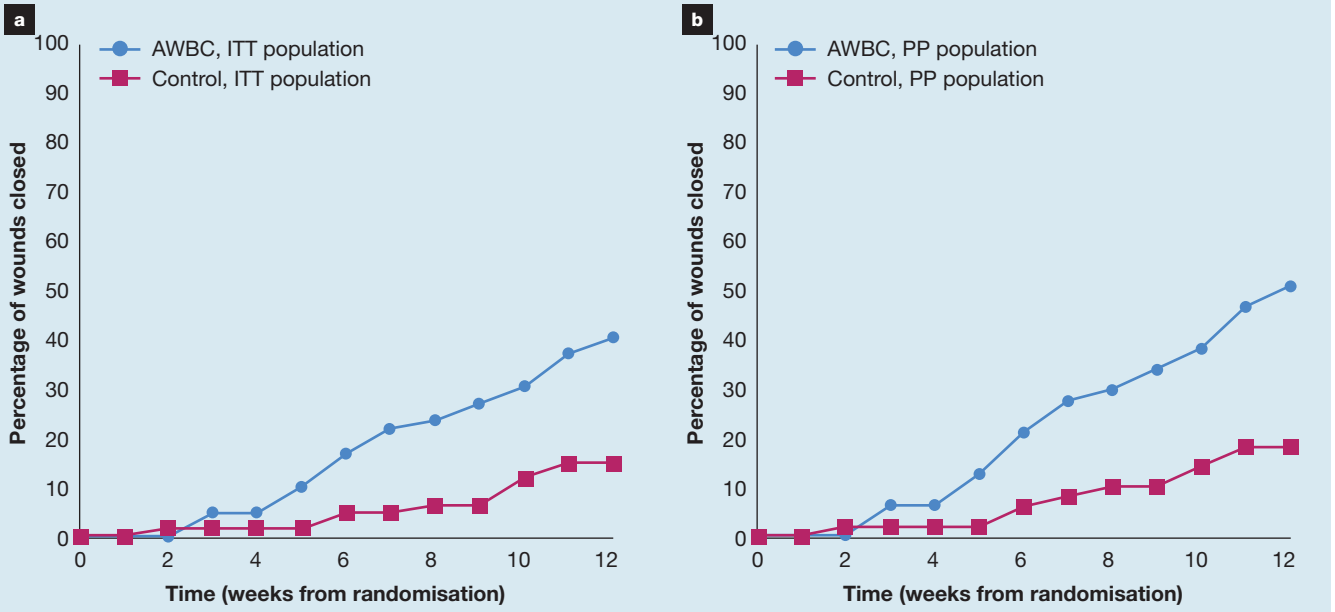
The primary study endpoint was the proportion of wounds closed at 12 weeks, and the secondary endpoints were time to heal within 12 weeks, and mean PAR at four and eight weeks.

Durability of wound closure within 12 weeks of follow-up time was defined as the number of wounds that remained healed after 12 additional weeks of follow-up time.

Patient population

Patients were enrolled between January 2020 and November 2023. The major inclusion criteria were adult patients (≥19 years old) with hard-to-heal (≥30 days) Wagner 1 or 2 DFUs, with a baseline wound area between 1–28cm^2 post debridement, demonstrating adequate circulation of the affected limb (criteria are detailed in Table 1). For patients with multiple DFUs, the largest eligible ulcer was selected. Exclusion criteria included, among others, the presence of underlying osteomyelitis, active Charcot foot and known coagulopathies (patients taking anticoagulants were not excluded). Table 1 describes the complete inclusion/exclusion criteria.

Fig 3. Weekly percentage of wounds closed, intention-to-treat (ITT) population **(a)**. Weekly percentage of wounds closed, per-protocol (PP) population **(b)**. AWBC—autologous whole blood clot



Randomisation

A computer-generated, site-specific randomisation code was used to generate the automatic random allocation sequence. Randomisation was performed using blocks of four and stratification was carried out between and within sites (RedCap, US). None of the study investigators or site providers had access to the assigned allocation prior to randomisation. At each site, only the study coordinator or the principal investigator had system permission to randomise patients. The system automatically blocked the option to randomise if not all the criteria were met.

AWBC preparation, application, and removal procedures

AWBC is a point-of-care treatment using an AWBC kit (ActiGraft Pro; RedDress Medical, US) which contains three components:

Fig 4. Heat map of proportions of wounds healed, intention-to-treat population. Chi-squared test was used to obtain p-values, which are shown with different combinations of the right-censored wounds healed in the study groups. The nonsignificant areas are highlighted in shades of orange-brown. AWBC—autologous whole blood clot; control—standard of care

AWBC group healed												
	0	.001	.002	.005	.009	.017	.029	.048	.077	>0.1	>0.1	>0.1
	1	<.001	.001	.003	.005	.010	.018	.031	.050	.078	>0.1	>0.1
	2	<.001	<.001	.001	.002	.006	.011	.019	.032	.051	.079	>0.1
	3	<.001	<.001	<.001	.002	.003	.006	.011	.019	.032	.052	.080
	4	<.001	<.001	<.001	<.001	.002	.003	.006	.012	.020	.033	.052
	5	<.001	<.001	<.001	<.001	<.001	.002	.004	.007	.012	.020	.033
	6	<.001	<.001	<.001	<.001	<.001	<.001	.002	.004	.007	.012	.021
	7	<.001	<.001	<.001	<.001	<.001	<.001	.001	.002	.004	.007	.012
	8	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.001	.002	.004	.007
	9	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.001	.002	.004
		0	1	2	3	4	5	6	7	8	9	10
	Control group healed											

1. A phlebotomy tray including a sterile blood draw set (21G winged with 7-inch tube with holder), a vacuum tube containing the citrate-based anticoagulant (for up to 15ml of blood), disposable gloves, tourniquet, and alcohol and gauze pads
2. An AWBC preparation kit, including the coagulation mould/clotting tray containing 85mg calcium gluconate powder and 28mg pharmaceutical grade kaolin powder, a 30ml sterile syringe, coagulation mould punch tool, tube injector, medical-grade cotton gauze, sterile clot extraction ring and a face mask
3. A kit with a sterile round sticker and dressing materials including a drape, gauze, nonadherent dressing, hydrophilic foam dressing and skin closure adhesive strips.

The preparation of the clot was performed according to the steps described below:

1. Blood from the patient (15ml) was withdrawn into an ACD-A vacuum tube containing citrate-based anticoagulant
2. The coagulation mould was punctured once at the top centre (Fig 1a) using the punch tool to allow the insertion of the blood into the mould
3. A safety needle was attached to the 30ml syringe and used to withdraw the blood from the tube and insert it into the coagulation mould through the puncture opening
4. The pierced hub was cleaned using gauze and the sterile round sticker was stuck over the pierced hub to prevent leakage
5. The blood was mixed with the kaolin and calcium gluconate, which accelerate the clot formation, by agitating and turning the coagulation blister for 20 seconds
6. The blood was allowed to clot inside the mould for five minutes until coagulation was complete (Fig 1c)
7. To release the AWBC from the coagulation mould, the backing was removed by securing the gauze stuck to the AWBC and the extraction ring was used to release the AWBC from the mould
8. The AWBC was attached to the wound using skin closure adhesive strips, and a non-adherent dressing was placed on the AWBC and covered by a hydrophilic, secondary dressing foam (Fig 1d).

The treatment phase consisted of a weekly AWBC application for up to 12 weeks or until complete closure occurred. Patients were also allowed to have a second weekly visit to change the secondary dressing, as required.

Data analysis

Categorical variables were analysed using frequencies and percentages; continuous variables were analysed using means±standard deviation (for non-normal distributions, medians and interquartile ranges (IQRs) were given). Baseline variables between groups were tested using t-tests, Mann–Whitney, Chi-squared and Fisher exact tests (as appropriate). No adjustment for multiplicity testing occurred. All statistical testing was two-sided and performed using a significance (alpha)

Table 2. Patient characteristics

Variable	AWBC group	Control group
	n=59	n=60
Age, years, mean±SD	58.3±8.8	56.2±10.9
Race, n (%)		
White	48 (81)	47 (78)
African American	6 (10)	8 (13)
Asian	0 (0)	4 (7)
Other	5 (9)	1 (2)
Hispanic ethnicity, n (%)	16 (27)	18 (30)
Sex at birth, n (%)		
Male	45 (76)	49 (82)
Female	14 (24)	11 (18)
BMI, mean±SD (range)	32.9±8.9 (21.5–74.6)	34.2±9.1 (21.3–81.3)
Smoker, n (%)		
Current	6 (10)	10 (17)
Former	7 (12)	8 (13)
Never smoked	46 (78)	42 (70)
Ambulation status, n (%)		
Full without assistance	37 (62)	37 (62)
Full with assistance	10 (17)	14 (23)
Limited without assistance	5 (9)	3 (5)
Limited with assistance	5 (9)	3 (5)
Wheelchair-bound	2 (3)	3 (5)
Abnormal nutrition status, mean±SD	2±3	2±3
HbA1c (%) (out of range),* mean±SD	8.3±1.62	8.2±1.56
Diabetes duration (where available), years, n (%)		
0–5	17 (33)	11 (21)
5.01–10	7 (14)	8 (15)
10.01–15	5 (10)	10 (19)
15.01–20	9 (18)	10 (19)
>20	13 (25)	14 (26)
Minor amputation, n (%)	5 (8)	12 (20)†
Comorbidity count, mean±SD; median (IQR)	3.9±2.78; 3 (3)	3.8±2.5; 3 (3)
Key comorbidities, n (%)		
Chronic kidney disease	2 (3)	3 (5)
Hypertension	32 (54)	34 (57)
PAD/PVD	6 (10)	4 (7)
Congestive heart failure	7 (12)	3 (5)
Venous disease	2 (3)	2 (3)
Peripheral neuropathy	19 (32)	14 (23)
Anxiety	0 (0)	1 (2)
Depression	2 (3)	5 (8)

*n=50 for AWBC group and 47 for control group; †the control group had a significantly higher number of minor amputations than the AWBC group (p=0.034). AWBC—autologous whole blood clot; BMI—body mass index; IQR—interquartile range; PAD—peripheral arterial disease; PVD—peripheral vascular disease; SD—standard deviation

Table 3. Wound characteristics of diabetic foot ulcers

Variable	AWBC group	Control group
	n=59	n=60
Wound area, cm^2,* mean±SD; median (IQR)	5.3±5.6; 3.2 (5.1)	4.6±4.8; 2.3 (4.4)
Wound depth, cm, mean (SD)	0.23 (0.46)	0.33 (0.46)
Wound age, weeks* mean (SD); median (IQR)	78.8 (191.5); 24 (39)	45.3 (65.1); 22 (42)
Anatomic location, n (%)		
Plantar	45 (76)	48 (80)
Toe	8 (13)	12 (20)
Forefoot	12 (20)	15 (25)
Midfoot	29 (49)	28 (46)
Hindfoot	2 (3)	0 (0)
Heel	7 (12)	4 (7)
Ankle	1 (2)	1 (2)
Offloading type, n (%)		
Boot	32 (54)	31 (52)
Shoe	26 (44)	26 (43)
Wheelchair	1 (2)	3 (5)

AWBC—autologous whole blood clot; IQR—interquartile range; SD—standard deviation

level of 0.05. Missing data within 12 weeks from the start of the study were incorporated in the intention-to-treat (ITT) population using the last observation carried forward principle for the area measurement. For time to heal only, the last visit for which data were available constituted the start of right censoring.

The populations analysed included ITT, per-protocol (PP) and safety populations. The ITT population included all randomised patients, while the PP population excluded patients who withdrew consent and patients who did not complete the study for any reason. The safety population included the ITT population.

For wounds that closed during the treatment phase, the days to heal were calculated as the number of days after the date of randomisation that the ulcer was first closed. For right-censored patients (i.e., patients who did not have an event (did not heal)), time to heal was set to 84 days (12 weeks) in Kaplan–Meier survival analysis. The date of the early termination for those patients who withdrew or terminated early from the study was used as the failure date in the time to heal analysis.

Primary endpoint analysis (complete wound healing at 12 weeks) was calculated for both ITT and PP populations in each treatment group using the two-sample continuity-corrected z-test (healed=success). If significant results were found, a generalised linear model (logit link function) was created to adjust for differences between treatment groups based on patient and wound-related variables that had marginal statistical significance ($p \leq 0.1$). Generalised linear models with additional variables were built using stepwise addition of variables, starting with the treatment group. Model parsimony was checked using stepwise deletion of all available variables. In pair correlation analysis, if $r \geq 0.7$, only one variable of the pair was chosen. If >5% of outcomes were missing, a tipping point analysis, presented using a heat-map plot, was conducted to account for uncertainty of imputation of right-censored outcomes.

Fig 5. Kaplan–Meier plot, intention-to-treat (ITT) population **(a)**. Kaplan–Meier plot, per-protocol (PP) population **(b)**. Lines are shown for treatment (AWBC) and standard of care (control) after adjusting for contribution of other significant variables

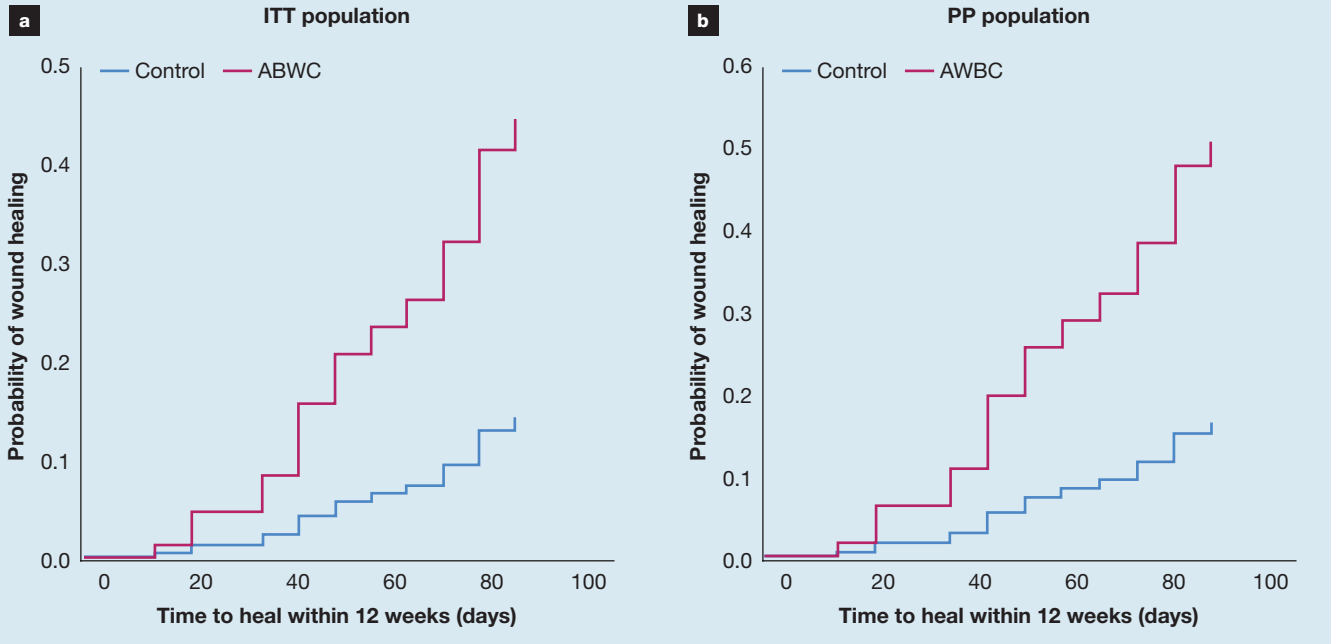


Table 4. Generalised linear model (logit link function) results

Variable	B		p-value		Odds ratio		95% CI	
	ITT	PP	ITT	PP	ITT	PP	ITT	PP
Treatment (AWBC)*	1.63	1.81	0.00084	0.0065	5.1	6.1	2.0–13.2	2.2–17.2
Comorbidity count	–0.38	–0.36	0.00087	0.0009	0.7	0.7	0.5–0.9	0.6–0.9
Full ambulation with assistance or limited†	1.48	1.58	0.006	0.006	4.4	4.9	21.5-12.6	1.6-15.1

*Reference group: standard of care; †reference group: full ambulation without assistance. AWBC—autologous whole blood clot; CI—confidence interval; ITT—intention-to-treat analysis; PP—per-protocol analysis

Secondary endpoints were analysed for the ITT and PP populations. Time to heal was calculated using Cox regression in conjunction with the log-rank test based on patient and wound-related variables that had marginal statistical significance ($p \leq 0.1$). Mean time to heal with 95% confidence intervals (CIs) was calculated with a summary of all variables included in the model and the assumptions met or not met in the final model. A one-minus-survival plot (probability of wound healing) was also made. The PAR was calculated using the following formula:

$$PAR = ((A1–A2)/A1)*100$$

where: A1 is the baseline area (at randomisation) and A2 is the area at the specified timepoint.

PAR analysis at four and eight weeks was carried out using linear mixed modelling (repeated measures) with the area at randomisation as an additional factor to the treatment group. A summary of assumptions met or not met in the final model was included, as well as covariate structure details. Durability of wound closure was performed by calculating the proportions of closed wounds that remained healed for an additional 12 weeks, in each treatment group, using the two-sample z-test (remained healed=success). Adjustment for multiplicity of statistical testing (gatekeeping for testing of secondary endpoints) used hierarchical serial testing as follows:

Time to heal > PAR > durability of wound closure

Exploratory endpoints were analysed without statistical testing. The mean number of sharp debridements performed on each patient for each group and the mean number of AWBC applications per patient were calculated.

For the safety analysis, the counts of AEs and serious AEs (SAEs) per treatment group per patient were analysed. The US National Cancer Institute Common Terminology Criteria for Adverse Events 5.0 scale[17] was used to grade AEs and SAEs. Grades 3–5 of this scale were used to define SAEs, which were any adverse changes in

Fig 6. Weekly percentage area reduction (PAR), intention-to-treat (ITT) population **(a)**. Weekly PAR, per-protocol (PP) population **(b)**. AWBC—autologous whole blood clot

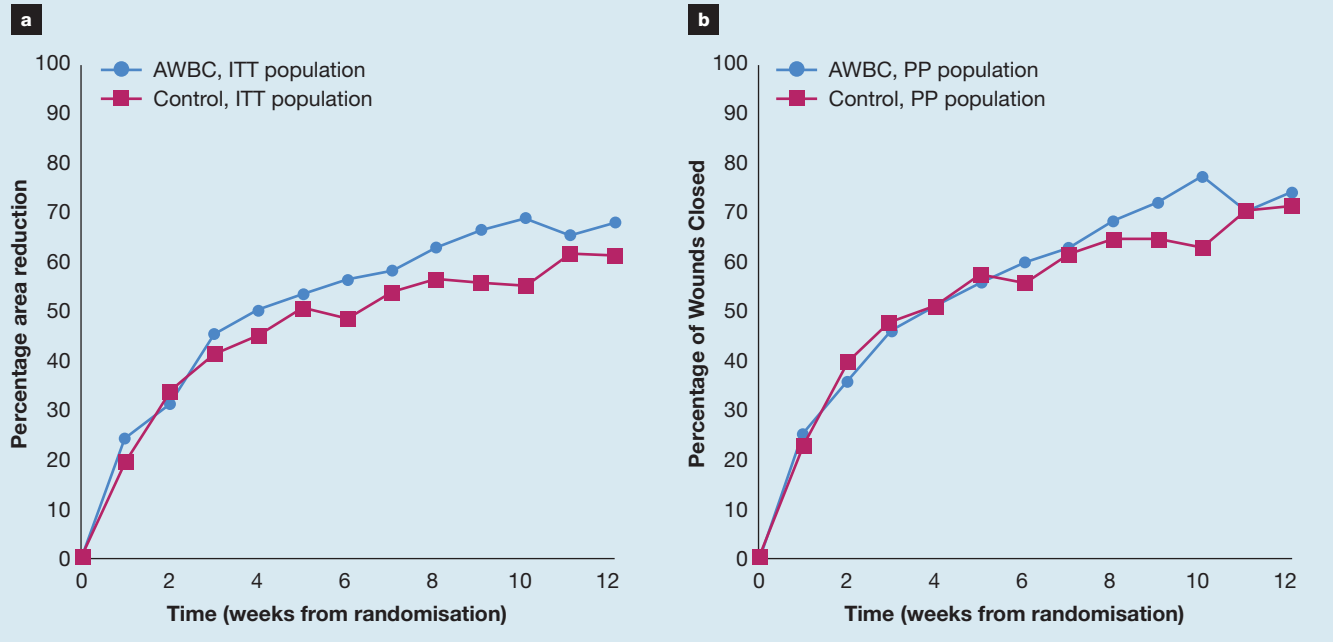


Table 5. Cox regression results

Variable	B		p-value		HR		95% CI	
	ITT	PP	ITT	PP	ITT	PP	ITT	PP
Treatment (AWBC)*	1.35	1.17	0.00081	0.003	3.9	3.2	1.8–8.5	1.5-7
Full ambulation with assistance or limited†	0.78	−0.30	0.031	0.004	2.2	0.8	1.1–4.4	0.6-0.9
Wound age (weeks)‡								
13–40	−0.88	−0.46	0.033	0.22	0.4	0.6	0.2–0.9	0.3–1.3
>40	−0.86	−1.59	0.071	0.034	0.4	0.2	0.2–1.1	0.05–0.9
Comorbidity count time	−0.003	0.022	0.04	0.001	1	1	0.99–1.0	1–1

*Reference group: standard of care; †reference group: full ambulation without assistance; ‡reference group: <13 weeks. AWBC—autologous whole blood clot; CI—confidence interval; HR—hazard ratio; ITT—intention-to-treat analysis; PP—per-protocol analysis

Fig 7. A 59-year-old male patient with type 2 diabetes, hypertension, peripheral vascular disease, hyperlipidaemia and lymphoedema presented with a hard-to-heal diabetic foot ulcer of three months' duration and with a baseline wound area of 9.4cm^2, depth 0.4cm. His wound had previously failed treatment with gauze, foam and antimicrobial dressings, and the index foot had previously undergone a 5th ray partial amputation. The study ulcer formed near the amputation site **(a)**. The wound closed seven weeks after autologous whole blood clot weekly application **(b)**

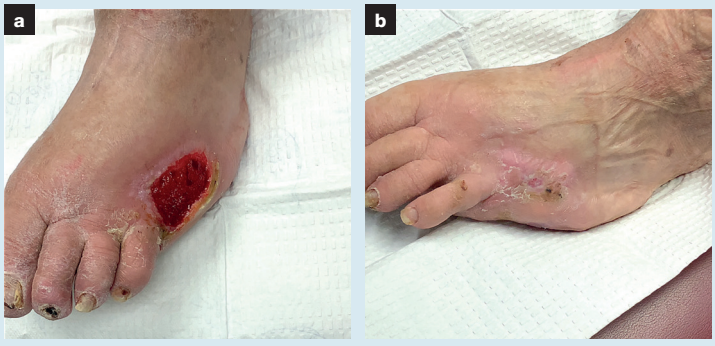
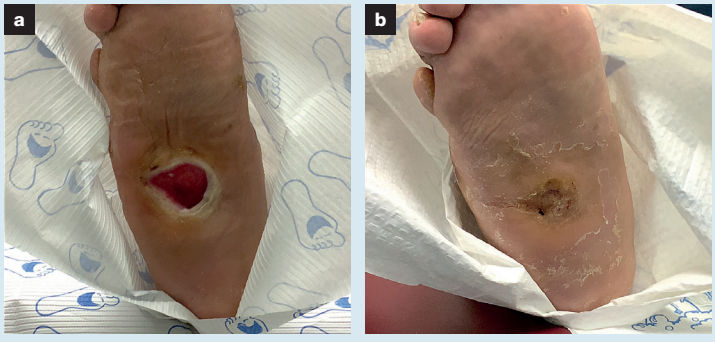


Fig 8. A 72-year-old male patient with type 2 diabetes, congestive heart failure, gastric reflux, osteoarthritis, neuropathy, hypercholesterolaemia and onychomycosis presented with a hard-to-heal diabetic foot ulcer of 14 months' duration and with a baseline wound area of 6.4cm^2, depth 0.3cm. His wound had previously failed treatment with gauze, foam and antimicrobial dressings, a topical antiseptic and germicide, and negative pressure wound therapy **(a)**. The wound closed 11 weeks after autologous whole blood clot weekly application **(b)**



the patient's health state that resulted in death, was life-threatening, required inpatient hospitalisation or prolonged existing hospitalisation, and/or resulted in persistent or significant disability/incapacity. The counts of AEs by severity and relatedness to intervention/product were also analysed. Any lack of venous access during the AWBC preparation procedure was documented and reported among safety analysis data.

Results

There were 199 patients assessed for study eligibility; 80 (40%) patients did not pass screening. The main reasons for screening failure included reduction by ≥30% of the ulcer area (post debridement) (n=25, 31%); ineligible ulcer size (n=15, 19%); HbA1c ≥12.0% (n=11, 14%); and haemoglobin anaemia <9g/dl (n=4, 5%).

There were 119 patients enrolled at 16 sites; 59 patients were randomised to the AWBC group and 60 to the control group (Fig 2). Table 2 summarises patient characteristics, which were well balanced between groups, except for minor amputations at the wound site, which were significantly greater in the control group (n=12, 20%) (p=0.034) than in the AWBC group (n=5, 8%). Some 21 (18%) patients had limited ambulation and the average number of comorbidities per patient was approximately four, with the most common comorbidities being hypertension, peripheral neuropathy and congestive heart failure.

Table 3 summarises the wound characteristics, which were well balanced between groups. Compared to other RCTs, there were notably larger wounds included in both groups, with a slightly larger mean wound area of 5.3±5.6cm^2 in the AWBC group versus 4.6±4.8cm^2 in the control group. Older wounds were also included, with longer mean wound duration of 78.8±191.5 weeks in the AWBC group versus 45.3±65.1 weeks in the control group. Some 32 wounds (27%) had a baseline duration of >1 year and 32% of DFUs (n=38) were recurring ulcers.

Some 22 (18%) patients discontinued the study. In the AWBC group, 12 patients discontinued: four withdrew consent; three had an SAE; one had an AE; one was lost to follow-up (LTFU); two were non-adherent

with the study protocol; and one died. In the control group 10 patients discontinued: three were non-adherent with the study protocol; one was LTFU; three had an AE; one had an SAE; one was discontinued at the investigator's discretion; and one withdrew consent.

Primary healing endpoint

AWBC treatment resulted in a 41% healing rate in the ITT population (24/59) compared to only 15% in the control group (9/60). The AWBC treatment showed an odds ratio of 2.73 in healing DFUs compared to control. The group difference was statistically significant (unadjusted p=0.002; adjusted p=0.00084). In the PP population, 51% of DFUs healed (24/47) in the AWBC group and 18% in the control group (9/49). The group difference was also statistically significant (unadjusted p=0.0075; adjusted p=0.0065). Fig 3a,b shows the weekly percentage of wounds that reached complete closure in both treatment groups for the ITT and PP populations. Table 4 summarises the best compromise results for the generalised linear model (logit link function) for the ITT and PP analyses.

Fig 4 represents a heat map of each possible combination of healed wounds within each censored group, with an associated p-value for each study group. For example, if no censored wounds had healed in both groups, this would be represented by the cell 0 (y axis) and 0 (x axis) with a value of 0.001 (top left hand corner). If censored AWBC-treated wounds had healed at a rate of approximately 40%, then the censored SoC–treated wounds would need to have healed at a rate of at least 100% for the result to become nonsignificant. There was no heat map analysis for the PP population because there were no right-censored patients in this group.

Secondary healing endpoints

Analysis was performed to determine the time to heal within 12 weeks for both the ITT and PP populations. In the ITT population, the mean time to heal for the AWBC treatment was 70.6 days (95% CI: 65.3–75.9 days) and 79.2 days (95% CI: 75.7–82.7 days) for the control group (Fig 5a). For the PP population, the mean time to heal was 68.4 days (95% CI: 62.2–74.5 days) for the AWBC group and 78.7 days (95% CI: 74.8–82.6 days) for the control group (Fig 5b). The final Cox regression model included treatment, ambulation, wound age, wound area, and a time-dependent covariate, comorbidity count time, because there was a small but significant lack of proportional hazards for comorbidity count (this variable alone was not significant in the final model for both ITT and PP populations) (Table 5).

When looking at the weekly PAR for both populations, the mixed model PAR analysis did not show a statistically significant result between treatments (Fig 6a,b).

The study also looked at exploratory endpoints, concluding that the number of sharp debridements performed in the AWCB was significantly lower (7.1±4.2) compared to control (8.8±4.3) (p=0.017). It is worth noting that debridement was performed only when necessary as part of the wound bed preparation prior to wound dressing. The number of applications in the AWBC treatment group was 7.4±2.8, and the median (IQR) was 7 (3, 12) in both the ITT and PP populations. Interestingly, among the patients who had previously undergone minor amputations, AWBC demonstrated a better healing outcome, with a 60% healing rate in wounds at the amputation site compared to a 25% healing rate in the control group.

Figs 7–9 provide case examples of three DFUs that reached complete healing following treatment with AWBC.

Table 6. Counts (%) of adverse events by severity and relatedness to study product or standard of care

Adverse event category	AWBC group	Control group
	n=59	n=60
Severity, n (%)		
Mild	29 (60)	21 (66)
Moderate	11 (23)	8 (25)
Severe	6 (13)	3 (9)
Life-threatening	1 (2)	0 (0)
Fatal	1 (2)	0 (0)
Relatedness		
Not related	37 (77)	30 (94)
Unlikely to be related	10 (21)	2 (6)
Possibly related	1 (2)	0 (0)
Probably related	0 (0)	0 (0)
Definitely related	0 (0)	0 (0)

AWBC—autologous whole blood clot

Treatment durability

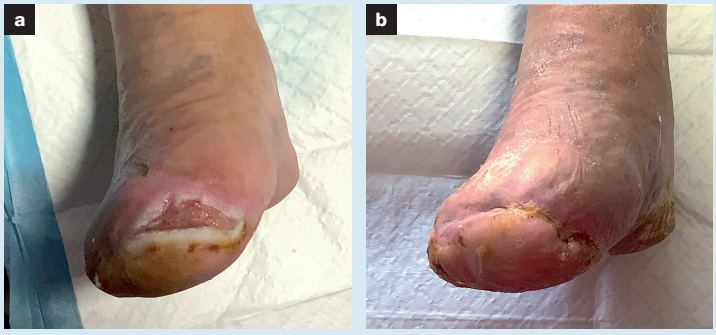
Wounds that had healed were re-evaluated for complete healing during a confirmatory visit two weeks after they were initially observed as being healed. During this visit, two wounds in the AWBC group and one wound in the control group had reopened. Of the reopened wounds in the AWBC group, one was still within the treatment window, allowing for an additional five weeks of treatment as per protocol. This wound subsequently healed and remained closed at the second healing confirmatory visit.

Wounds that were confirmed as healed at the healing confirmatory visit by the investigator were followed up for an additional 12 weeks to assess durability of the wound closure. In the AWBC treatment group, 17/24 (71%) wounds remained healed compared to 5/9 (55%) wounds in the control group.

Safety analysis

There were 80 AEs reported among 47 patients. The AWBC group had 48 AEs occurring in 28 patients, while the control arm had 32 AEs occurring in 19 patients (47% versus 31%, respectively). There were 12 SAEs in nine patients (15%) in the AWBC group and four SAEs in three patients (5%) in the control group.

Fig 9. A 64-year-old female patient with type 1 diabetes and a hard-to-heal diabetic foot ulcer that formed three months previously on the amputation site of all metatarsal heads. The baseline wound area was 3.4cm^2, depth 0.3cm. The wound previously failed treatment with calcium alginate **(a)**. The wound closed 11 weeks after autologous whole blood clot weekly application **(b)**



There were 21 wound-related AEs in 19 patients in the AWBC group and 23 in 15 patients in the control group. In the AWBC group, seven (6%) patients had a wound infection, three (3%) patients had cellulitis, and one (1%) patient had osteomyelitis. In the control group, three (3%) patients had a wound infection, six (5%) patients had cellulitis, two (2%) patients had wound inflammation and one (1%) patient had osteomyelitis. There were no treatment-related AEs (Table 6).

Discussion

The results of this RCT compare the efficacy and safety of AWBC to SoC in the treatment of hard-to-heal DFUs. AWBC treatment was found to be superior to control in achieving wound closure, in both the ITT population (41% versus 15%, respectively) and the PP population (51% versus 18%, respectively). AWBC showed significant superiority over SoC, with 2.73-times greater odds of reaching complete wound closure by week 12 in the AWBC group compared with the control. Furthermore, the eligible wounds for randomisation were those that failed to reduce in size by >30%, prior to randomisation, indicating that only hard-to-heal DFUs were randomised and included in the study. The efficacy results were confirmed by a third-party, blinded wound care expert, further removing potential bias and increasing the reliability of the results. Healing outcomes in the trial were further validated by applying the US Food and Drug Administration (FDA) definition for wound closure and inclusion of a healing confirmation visit.[18] Moreover, the wound measurement system used in this study is a registered FDA advanced wound imaging system, that captured the wound area and depth with high accuracy (≤5% error up to ±1mm), which allowed consistency between sites in following the progression of each wound.

Lower healing rates than expected in both arms could be attributed to the patient and wound characteristics being more severe. Patients had an average of four comorbidities each, while >25% of the wounds were more than a year old (Tables 2 and 3). Both groups had large wounds with a mean baseline area of approximately 5cm^2 (Table 3). Interestingly, peripheral neuropathy was the second most prevalent comorbidity in this study, affecting 28% of patients (n=33). It is worth noting that in both the ITT and PP populations, while increased comorbidity count might be expected to cause a delay in wound healing, the increase in odds of healing for patients with limited ambulation or requiring assistance to achieve full ambulation could be due to better offloading or other reasons that are unknown (Table 4). In the heat map analysis (Fig 4), out of 90 combinations of healed wounds, 76 (84%) had statistically significant group differences in favour of AWBC, demonstrating the robustness of the censored healing outcomes. Another factor that may have limited AWBC healing rates is that the number of weekly applications was limited to 12. The data suggest that the unhealed wounds would have had higher complete healing rates if the treatment phase had been longer than 12 weeks and permitted more applications. Some seven wounds that did not reach complete wound closure at week 12 in the AWBC group, had a good healing trajectory and a PAR ranging between 82–98% at week 12. For these wounds, additional applications would likely result in complete closure by the twentieth application.

An important trial limitation that likely impacted healing rates was that this trial was conducted over the entire course of the COVID-19 pandemic, starting only two months before the global pandemic declaration. Nearly 80% of clinical trials not related to COVID-19 stopped early or were interrupted after the onset of the pandemic, and most ongoing trials experienced major delays in recruitment and enrolment.[19,20] The present study was anticipated to be completed in only two years but took twice as long due to recruitment issues. Patients' adherence was likely affected during the pandemic, with patients returning less frequently than anticipated for their secondary dressing changes, which could account for lower healing rates in both groups and could explain why the controls' healing rate was <20%. Similar healing rates for SoC groups were reported in other DFU trials that were conducted during the pandemic.[21]

Our study shows that AWBC treatment results in a shorter healing time than control, supporting the suggested mechanism of action of AWBC by initiating and accelerating the healing process in hard-to-heal wounds.[7] Moreover, AWBC treatment had a better outcome in patients who had a medical history of minor amputation at the wound site, compared to the control arm (60% versus 25%, respectively). The impact of the faster healing rates was especially noticeable by the significant group difference in the debridement data, with the AWBC group requiring an average of seven sharp debridements versus nine in the control group (p=0.017) during the treatment phase. Additionally, AWBC durability showed much better outcomes, with 71% of the healed wounds remaining

closed three months after the initial healing, compared with only 55% in the control group. All of these outcomes were achieved with a high safety profile for the AWBC, as we identified no device-related AEs; therefore, supporting the safety and effectiveness of AWBC use in the management of hard-to-heal DFUs. It is worth noting that a comprehensive economic analysis detailing the financial benefits of AWBC treatment will be detailed in a future paper by our group.

Despite the challenges created by the COVID-19 pandemic, the statistically significant healing outcomes of the AWBC group support the effectiveness of AWBC as a novel, biologically active treatment to facilitate definitive wound closure in hard-to-heal DFUs. The AWBC was suggested to modulate the proinflammatory wound environment, provide a provisional ECM that restores the dynamic reciprocity between the matrix and cells, and provide topical growth factors to the wound bed.[9,10,22,23] The AWBC would appear therefore to allow the wound to transition from chronicity to an acute healing state.

Limitations

The trial commenced shortly before the global declaration of the COVID-19 pandemic, leading to unforeseen challenges. Recruitment was prolonged, and patient adherence may have been adversely affected due to pandemic-related restrictions and concerns. These factors could have influenced both the healing rates and the overall study outcomes.

Reflective questions

- Taking into consideration the economic burden of diabetic foot ulcers, what could be the strategy of the healthcare system to reduce the cost and improve patients' outcomes by using autologous whole blood clot (AWBC) treatment?
- What might be the main factors contributing to the success or failure of the AWBC treatment?
- What other wound types might benefit from AWBC treatment?

Conclusion

In conclusion, this RCT demonstrated the safety and efficacy of AWBC in achieving wound closure in hard-to-heal DFUs when compared to best practice SoC. AWBC had statistically significant healing outcomes when compared to control, presenting a promising and innovative treatment for hard-to-heal DFUs, offering a significant improvement over traditional care. This novel approach addresses the underlying challenges in the wound microenvironment, suggesting a paradigm shift in the management of hard-to-heal DFUs and emphasising the potential advantages and benefits of this innovative treatment. JWC

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