

# Continuous intrafemoral artery infusion of urokinase improves diabetic foot ulcers healing and decreases cardiovascular events in a long-term follow-up study

Jiayue Tong,<sup>1,2</sup> Junxia Zhang,<sup>1</sup> Lin Xiang,<sup>1</sup> Shuguang Li,<sup>1</sup> Jinling Xu,<sup>1</sup> Guangping Zhu,<sup>1</sup> Jing Dong,<sup>1</sup> Yangyang Cheng ,<sup>1</sup> Hujun Ren,<sup>1</sup> Min Liu,<sup>1</sup> Ling Yue ,<sup>1</sup> Guangda Xiang <sup>1,2</sup>

**To cite:** Tong J, Zhang J, Xiang L, *et al.* Continuous intrafemoral artery infusion of urokinase improves diabetic foot ulcers healing and decreases cardiovascular events in a long-term follow-up study. *BMJ Open Diab Res Care* 2024;**12**:e003414. doi:10.1136/bmjdr-2023-003414

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/bmjdr-2023-003414>).

Received 22 March 2023  
Accepted 8 December 2023



© Author(s) (or their employer(s)) 2024. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

<sup>1</sup>Department of Endocrinology, General Hospital of Central Theater Command of People's Liberation Army, Wuhan, Hubei, China

<sup>2</sup>Southern Medical University, Guangzhou, Baiyun District Guangdong, China

## Correspondence to

Dr Guangda Xiang;  
Guangda64@hotmail.com

## ABSTRACT

**Introduction** Diabetic foot ulcer (DFU) is a disabling complication of diabetes mellitus. Here, we attempted to assess whether long-term intrafemoral artery infusion of low-dose urokinase therapy improved DFUs and decreased cardiovascular events in patients with DFUs.

**Research design and methods** This trial was a single-center, randomized, parallel study. A total of 195 patients with DFU were randomized to continuous intrafemoral thrombolysis or conventional therapy groups. The continuous intrafemoral thrombolysis group received continuous intrafemoral urokinase injection for 7 days, and conventional therapy just received wound debridement and dressing change. Then, a follow-up of average 6.5 years was performed.

**Results** Compared with conventional therapy, at the first 1 month of intervention stage, the ulcers achieved a significant improvement in continuous intrafemoral thrombolysis group including a complete closure (72.4% vs 17.5%), an improved ulcer (27.6% vs 25.8%), unchanged or impaired ulcer (0% vs 56.7%). During the 6.5-year follow-up, for the primary outcome of ulcer closure rate, continuous intrafemoral thrombolysis therapy obtained a better complete healing rate (HR 3.42 (95% CI 2.35 to 4.98,  $p < 0.0001$ )). For the secondary outcome of cardiovascular disease events, continuous intrafemoral thrombolysis therapy had a lower incidence of cardiovascular events (HR 0.50 (95% CI 0.34 to 0.74,  $p < 0.0001$ )). Importantly, intrafemoral thrombolysis therapy decreased the incidence of cardiovascular death (HR 0.42 (95% CI 0.20 to 0.89,  $p = 0.0241$ )). Additionally, continuous intrafemoral thrombolysis therapy improved local skin oxygenation and peripheral neuropathy as well as glycolipid metabolic profiles when compared with conventional therapy group ( $p < 0.05$ ).

**Conclusions** Continuous intrafemoral thrombolysis therapy has a better therapeutic efficacy to improve DFUs and decrease cardiovascular events.

**Trial registration number** NCT01108120.

## INTRODUCTION

Diabetic foot ulcer (DFU) is a disabling complication of diabetes mellitus (DM).<sup>1</sup> The

## WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Diabetic foot ulcer (DFU) is a disabling complication of diabetes mellitus.
- ⇒ Whether long-term intrafemoral artery infusion of low-dose urokinase therapy improved DFUs in patients with DFUs remains uncertain.

## WHAT THIS STUDY ADDS

- ⇒ In this randomized clinical trial that included 195 patients with DFU, for the primary outcome of ulcer closure rate, compared with conventional therapy, continuous intrafemoral thrombolysis therapy obtained a better complete healing rate (HR 3.42 (95% CI 2.35 to 4.98),  $p < 0.0001$ )).

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ For people with DFUs, continuous intrafemoral thrombolysis therapy can improve their quality of life and prolong life.

foot ulcer development is commonly considered as a cause of leading to non-healing chronic wounds that are difficult to treat.<sup>2</sup> Meanwhile, it is also a significant risk factor for non-traumatic foot amputations of the inferior limbs and mortality in individuals with DM.<sup>2</sup> Approximately 80% of diabetes-related lower-extremity amputation are preceded by foot ulceration,<sup>3</sup> resulting in a heavy burden of medical care and expenditure in patients with DM.<sup>4,5</sup> Furthermore, the risk of cardiovascular events is dramatically increased in patients with diabetic foot.<sup>6,7</sup> Conservative therapies for DFU, such as wound off-loading, wound debridement, and infection control, have inconsistent outcomes and undesired side effects.<sup>8,9</sup> Therefore, approaches, which

are safe and effective treatments for DFU, are actively pursued.

Given that impaired function in large vessels and microcirculation displays a central role in the development of foot ulcers and their subsequent failure to heal.<sup>9,10</sup> Moreover, classic markers of macrovascular and microvascular risk such as hyperglycemia, hyperlipidemia, and longer diabetic duration were associated with lower extremity amputations in patients with type 2 DM.<sup>11</sup>

The tissues of feet in patients with DM are particularly prone to the complications of chronic ischemia.<sup>12</sup> Vascular insufficiency in patients with DM is frequently due to infrapopliteal arterial involvement.<sup>12,13</sup> The microthrombi formation in the small arteries and microcirculation of the forefoot and toes contributes to ischemia and hypoxic environment of local tissues, and consequently resulting in ulceration and gangrene.<sup>14</sup> Thus, we hypothesized that thrombolytic agents may have an important role in promoting the thrombolysis, improving microcirculation and oxygen supply in the extremity, and accelerating healing of the DFUs. Therefore, in this study, we treated DFUs by continuous intrafemoral artery injection of low-dose urokinase to explore effectiveness and safety, and performed a long-term follow-up study.

## METHODS

### The inclusion and exclusion criteria

The inclusion criteria were: (1) type 2 DM with Wagner grade 1–3 foot ulcers, (2) age 30–80 years, (3) Chinese Han from Hubei province. The exclusion criteria were: (1) patients with Wagner grade 4 or 5 DFUs, (2) bleeding diatheses, (3) malignant hypertension, (4) hepatic or renal failure, (5) mental illness, (6) cancer, (7) current pregnancy, (8) diabetic ketoacidosis or diabetic lactic acidosis, and (9) neuropathic ulcer. Diagnosis criteria: (1) DM was defined in 1999 WHO standard, (2) the DFU was classified by Wagner scale,<sup>15</sup> (3) cardiovascular events included coronary heart disease events (non-fatal myocardial infarction, angina), total stroke, cardiovascular death, coronary and carotid revascularization,<sup>16,17</sup> and (4) cardiovascular death was deemed when a fatal event occurred after myocardial infarction, ventricular fibrillation, sudden death, or heart failure. All other deaths were classified as non-cardiovascular.<sup>18,19</sup> All myocardial infarctions, strokes, and deaths—that is, all cardiovascular disease events and all other deaths were adjudicated by an end point assessment committee unaware of treatment allocation, (5) cigarette smoker was defined as subjects who had smoked at least one cigarette daily for 1 year,<sup>19</sup> (6) malignant hypertension was defined as per the American College of Cardiology/American Heart Association guidelines, (7) albuminuria was defined as per the American Diabetes Association criteria,<sup>20</sup> and (8) major amputation was defined as any above-the-ankle amputation.<sup>18</sup>

### Study subjects and design

A total of 195 patients with DFU were randomly divided into either continuous intrafemoral thrombolysis group (98 patients) or conventional therapy group (97 patients) between May 2010 and December 2015. Characteristics of the patients are shown in [table 1](#).

This trial was registered in ClinicalTrials.gov (NCT01108120), and was a randomized, parallel group trial of investigations with or without continuous intrafemoral thrombolysis. The continuous intrafemoral thrombolysis group received continuous intrafemoral urokinase injection for 7 days, and conventional therapy group just received wound debridement and dressing change. Then, a follow-up of average 6.5 years was performed ([figure 1](#)). The actual time of completion of the study was January 2020.

### Study objective

The primary end point of this study was to compare the percentage of completed ulcers closure between two groups at the first month and the terminal of the follow-up study. The secondary end point was the time of ulcer healing, incidence of ulcer recurrence, incidence of amputation, the cardiovascular event and the cardiovascular death during the follow-up period.

### Study procedures

Eligible patients were numbered according to their admission order. The numbers were arranged in odd or even groups, with the odd number as continuous intrafemoral thrombolysis group and the even number as conventional therapy group. After diabetic dietary advices, all patients received insulin therapy to control blood glucose (BG) within a range of 5–10 mmol/L during first month intervention period as much as possible. Then the patients received conventional care for their ulcers such as wound debridement including remove extensive callus and necrotic tissue, and broad spectrum antibiotics when ulcers showed clinical signs of infection. Adjustments to the treatment are performed when indicated on the basis of microbiologic cultures and sensitivity testing. All participants received a regular dressing change with sterile gauze 2–3 times per week to the end of the study if needed.

Patients were examined weekly after the terminal of the intrafemoral urokinase injection assay. Then the patients were followed monthly for the first year and followed trimonthly to January 2020. At each study visit, ulcers were assessed for area via wound tracing, ulcer closure, or adequate granulation tissue formation. The ulcer area was determined in square millimeters by multiplying the largest width and length of the ulcer. The largest ulcer was considered as the study ulcer when more than one ulcer was present. The measurement was performed after revision of the ulcer.<sup>21</sup> During the follow-up period, the treated target of hemoglobin A1c (HbA1c) was <7.0%, and the therapy medicines and strategy were determined

**Table 1** Baseline characteristics of 195 patients with diabetic foot ulcers who were randomized to treatment with continuous intrafemoral thrombolysis or conventional therapy

	Thrombolysis therapy (n=98)	Conventional therapy (n=97)
General characteristics		
Male	71 (72.5%)	69 (71.1%)
Age (years)	72 (4.8)	72 (4.6)
Diabetes duration (years)	14 (3.0)	14 (3.1)
BMI (kg/m <sup>2</sup> )	27 (3.2)	27 (3.0)
SBP (mm Hg)	139 (11.8)	139 (10.5)
DBP (mm Hg)	82 (8.1)	82 (6.3)
Current or ex-smoker	25 (25.6%)	22 (22.7%)
Clinical history		
Previous non-traumatic amputation or diabetic skin ulcer	2 (2.0%)	2 (2.1%)
Previous cardiovascular disease	66 (67.3%)	63 (64.9%)
Neuropathy	67 (68.4%)	68 (70.1%)
NDS	7.9 (2.1)	7.7 (2.3)
Laboratory data		
TC (mmol/L)	5.2 (1.0)	5.1 (0.9)
LDL-C (mmol/L)	3.0 (0.8)	3.0 (0.6)
HDL-C (mmol/L)	1.2 (0.3)	1.3 (0.3)
TG (mmol/L)	2.1 (0.7)	2.1 (0.7)
HbA1c (%)	8.7 (1.4)	8.5 (1.5)
FBG (mmol/L)	9.5 (1.6)	9.5 (1.7)
2-hour BG (mmol/L)	13.6 (3.1)	14.2 (3.6)
Fibrinogen (g/L)	4.7 (1.0)	4.6 (1.0)
CR (μmol/L)	85.2 (12.1)	88.0 (10.4)
UACR (mg/g)	111 (31.7)	120 (39.1)
AST (U/L)	47 (5.1)	48 (6.1)
ALT (U/L)	46 (4.5)	47 (4.9)
Baseline medication		
Aspirin	71 (72.5%)	75 (77.3%)
ACE inhibitor	48 (50.0%)	51 (52.6%)
Angiotensin-II receptor antagonist	18 (18.4%)	16 (16.55%)
Calcium channel blocker	43 (43.9%)	45 (46.4%)
Metformin	68 (69.4%)	70 (72.2%)
Sulfonylurea	54 (55.1%)	55 (56.8%)
Insulin	52 (53.1%)	51 (52.6%)
Statins	40 (40.8%)	38 (39.2%)
Clopidogrel	15 (15.3%)	13 (13.4%)
Wound conditions, Wagner grade		
1	12 (12.2%)	15 (15.5%)

Continued

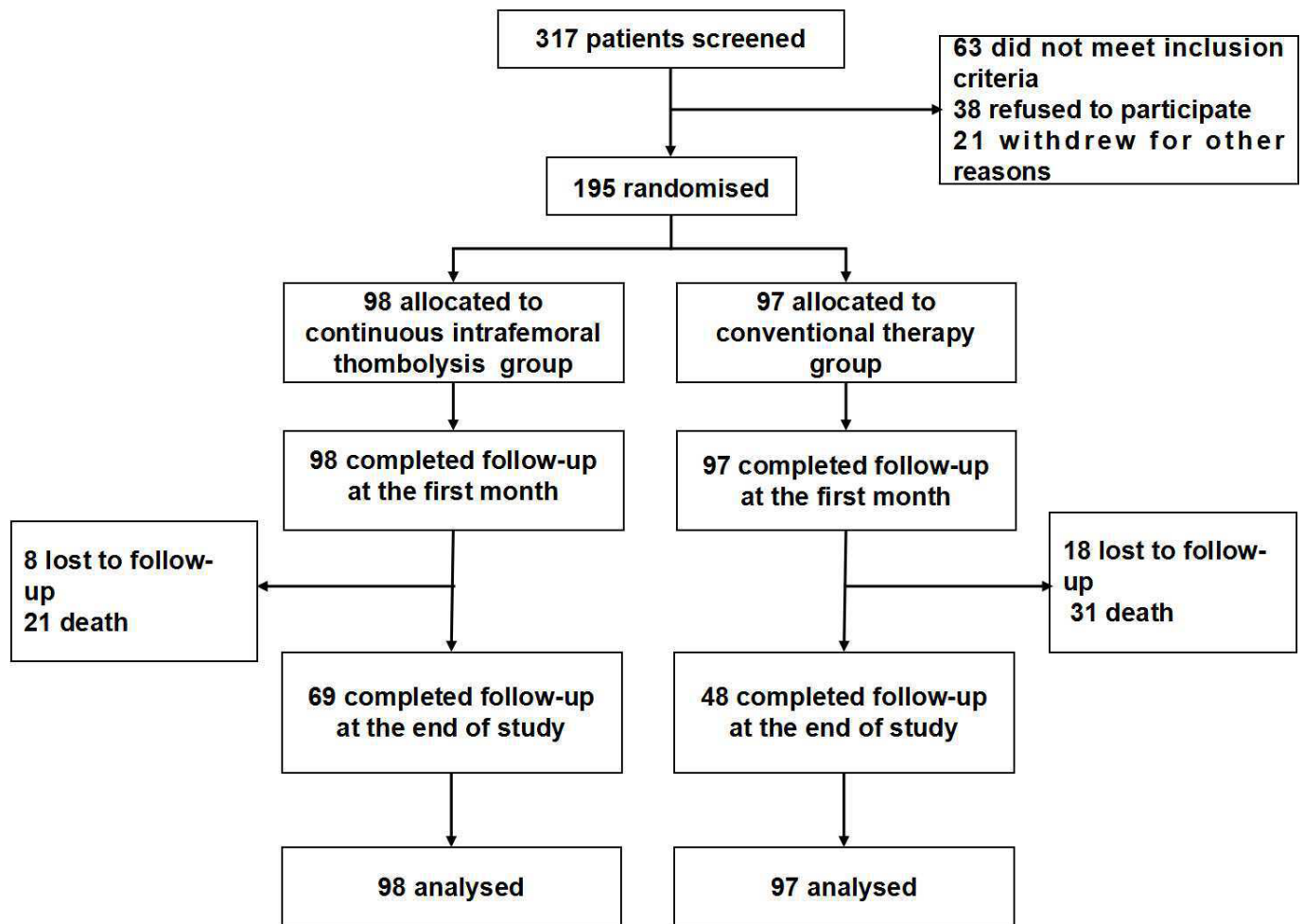
**Table 1** Continued

	Thrombolysis therapy (n=98)	Conventional therapy (n=97)
2	31 (31.6%)	29 (29.9%)
3	55 (56.1%)	53 (54.6%)
Ulcer size (cm <sup>2</sup> )	8.1 (2.0)	7.5 (1.8)
Ulcer duration (month)	7.5 (2.0)	7.4 (2.0)
ABI	1.0 (0.2)	1.0 (0.2)
TBI	0.3 (0.1)	0.3 (0.1)
TcPO2 (mm Hg)	41.9 (9.5)	41.9 (10.5)
Treated for ulcer infection prior to randomization	22 (22.4%)	24 (24.7%)
Only first event for each patient counted in each row. Data are number (%), mean (SD). There were no statistically significant differences between the groups. ABI, ankle-brachial index; ALT, alanine transaminase; AST, aspartate transaminase; BG, blood glucose; BMI, body mass index; CR, creatinine; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; NDS, Neuropathy Deficit Score; SBP, systolic blood pressure; TBI, toe brachial index; TC, total cholesterol; TcPO2, transcutaneous oxygen tension; TG, triacylglycerol; UACR, urinary albumin/creatinine ratio.		

by the responsible physicians based on Chinese Diabetes Society guidelines 2010.

### The continuous intrafemoral urokinase injection approach

Based on our previous report in Chinese in 2007 (Guangda Xiang, Chinese Journal of Diabetes, 2007, 15(11): 649–650), a continuous intrafemoral urokinase injection approach was performed for patients in continuous intrafemoral thrombolysis group. In brief, an ultrasound Doppler examination of vessels including artery and venous of lower limbs was performed. To avoid pulmonary infarction, a filtrator was placed in the inferior vena cava before the thrombolysis process if ultrasound results showed venous thrombosis. Next, a percutaneous artery canal was inserted from femoral at the base of thigh into the distal of popliteal artery in affected lower limb as far as possible from femoral artery. After finishing this process, the outside part of this artery catheter is fixed at thigh (online supplemental figure 1), and the patients must keep in supine position in the bed for 7 days. When the above operation was completed, 200 000–400 000 units urokinase was injected via the catheter to diseased foot. Afterward, the continuous infusion urokinase (100 mL 0.9% sodium chloride+1 000 000 unit urokinase at a rate of 4 mL/hour) was administered with an artery pump via femoral artery for 7 days. Considering that catheter-associated infection risk increased after 7 days,<sup>22</sup> as well as the effective role of the duration of catheterization was 5–9 days in our previous study (Guangda Xiang, Chinese Journal of Diabetes, 2007, 15(11): 649–650), thus, we chose 7 days as the course of treatment. In addition, the



**Figure 1** Trial profile.

safety outcomes such as infections and bleeding or oozing blood for continuous intrafemoral urokinase injection approach were observed.

#### Definition of ulcer outcome

Three professional physicians assessed ulcer healing and they were blinded to the intervention. Ulcer outcome was defined as: (1) complete ulcer closure was defined as skin closure (100% re-epithelialization) without drainage or dressing requirements, (2) improved ulcer was defined as 50% reduction of the ulcer area but not complete ulcer closure, (3) unchanged ulcer was defined as decreased or increased ulcer area <50%, (4) impaired ulcer area was defined as increased ulcer area  $\geq$ 50% or amputation above or below the ankle.

#### The determination of toe brachial index and ankle-brachial index

All subjects were asked to refrain from coffee and smoking for at least 8 hours before the examinations and room temperature was kept between 22°C and 24°C. Participants were kept in a supine position with their feet at heart level after 20 min of acclimatization. The blood pressure was determined sphygmomanometrically in the right arm. Then, the cuff was placed around the ankle

immediately above the malleolus and the ankle pressure was measured with a Doppler signal followed by release sphygmomanometrically on the dorsal right foot.<sup>23</sup> The ankle-brachial index (ABI) was calculated by the ratio of the ankle systolic pressure to the brachial systolic pressure.<sup>24</sup>

The measurement of toe pressure was followed, a 25 mm digital cuff was placed around the proximal phalanx of the hallux and the Doppler signal was measured from the distal pad of the great toe.<sup>25</sup> The cuff was inflated to a maximum of 200 mm Hg and then slowly deflated. The point at which the arterial waveform reappeared was defined as the toe systolic blood pressure. The toe brachial index (TBI) was calculated as the toe systolic pressure divided by the brachial systolic pressure.<sup>24</sup>

#### The determination of peripheral neuropathy

Peripheral neuropathy was based on loss of sensation in peripheral neuropathy including vibration, proprioception, temperature, and pin-prick sensations.<sup>26</sup> All patients were evaluated by vibration sensation using a 128 Hz tuning fork<sup>27</sup> and pin-prick sensation using Neuro tip and temperature sensation using warm and cool rods and Achilles tendon reflex using a tendon hammer. Then, we

used the Neuropathy Deficit Score (NDS) to evaluate peripheral neuropathy. The perceptions were scored 0 if present and normal, and 1 if absent, reduced, or uncertain. On either side, the ankle reflex was scored 0 if present and normal, and 2 if absent. The maximum NDS score was 10. The severity of neuropathy was graded as follows: mild (NDS scores: 3–5), moderate (NDS scores: 6–8), and severe (NDS scores: 9–10). We defined peripheral neuropathy as at least moderate NDS scores with or without symptoms (NDS  $\geq$ 6).

### The measurements of transcutaneous oxygen tension

Local skin oxygenation was evaluated by measurement of transcutaneous oxygen tension (TcPO<sub>2</sub>).<sup>28</sup> TcPO<sub>2</sub> is a non-invasive method reflecting local arterial skin blood flow and oxygenation, and it can be used as a predictor of ulcer heal.<sup>29</sup> TcPO<sub>2</sub> was measured by an electrochemical transducer and the measurements of TcPO<sub>2</sub> were taken at the dorsum of the foot in the first intermetatarsal space (PF5001, Pehimed, Sweden). A calibration period of method was 10–15 min, the TcPO<sub>2</sub> signal was continuously recorded, and has good reproducibility.<sup>30</sup>

### Biochemical measurements

Blood samples were obtained from all patients after a 12-hour fast. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), aspartate transaminase (AST) and alanine transaminase (ALT) were measured enzymatically. HbA<sub>1c</sub> was measured by liquid chromatography (G8-90SL, Tosoh, Japan). Fasting blood glucose (FBG) and postprandial 2-hour glucose (after 75 g glucose loading, 2-hour BG) was measured by a glucose oxidase procedure (SYNCHRON Biochemical Analysis System, Suzhou, China). Creatinine was measured enzymatically. Total plasma fibrinogen was analyzed with a syneresis method,<sup>31</sup> which measures the amount of clottable protein incorporated into the clot, after addition of thrombin to the plasma sample. As our previously described,<sup>32</sup> urinary albumin was examined with nephelometry (N antiserum to human albumin kit; BN prospec, Siemens, Germany) and urinary creatinine (Creatinine Plus V.2 kit; Cobas c701, Roche, Germany) according to the manufacturer's instructions. Coefficients of variation for these assays were 1%–2% (HbA<sub>1c</sub>, TC, HDL-C, creatinine), 2%–3% (BG, TG, LDL-C, UACR), and 3%–6% (fibrinogen, AST, ALT).

### Health-related quality of life

At the end of follow-up study, we randomly selected 20 participants in both groups to complete a validated health-related quality of life (HRQoL) questionnaire that is specific to patients with DFU, the DFS-SF.<sup>33</sup> The DFS-SF tool contained 29 items consisting of 6 subscales that were related to leisure, physical health, dependence or daily life, negative emotions, being worried about ulcers or feet, and being bothered by ulcer care. Each domain had a score ranging from 0 to 100, with higher scores

implying better quality of life. Data generated were used to evaluate the effect of the interventions on HRQoL.

### Sample size calculation and statistical analysis

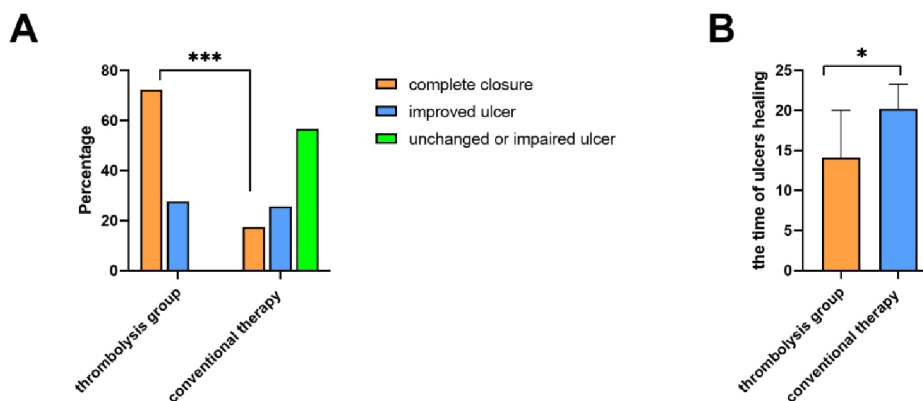
We were conducting a pilot study with no prior data of the effects of continuous intrafemoral thrombolysis on DFU. Based on the previous study, the ulcer healing rate was 45% with a regular dressing change. In this study, we want to achieve a target of 70% ulcer healing rate. Therefore, we performed a power calculation based on the ulcer healing rate. Based on an expected difference in ulcer healing rate of 25%, with a power of 0.90 and an  $\alpha$  of 0.05 (two-sided), we calculated that 80 subjects would be needed. Considering that patients drop out in long-term follow-up, we enrolled 100 participants in each group.

Continuous normally distributed data are reported as mean  $\pm$  SD. The Student's t-test was used to evaluate differences in continuous variables between groups. Contingency tables were analyzed using the  $\chi^2$  test. Kaplan-Meier methodology with log-rank test was used to evaluate the time to complete ulcer closure and cumulative incidence of cardiovascular events and cardiovascular mortality between groups.

HRs and 95% CIs were determined by univariate and multivariate Cox proportional hazards regression analyses for end point of ulcer closure and cardiovascular events. Initial univariate analyses identified demographic and clinical variables that predicted ulcer closure and cardiovascular events. Significant variables were entered into subsequent multivariate Cox regression with the variables included in the models using forward stepwise selection, with a probability value of  $<0.05$  required for entry into the model. A probability value of 0.05 or less was considered significant. All statistical analyses were performed using SPSS statistics V.18 software.

## RESULTS

During the course of the study, a total of 317 patients were screened. Of them, 63 patients were excluded owing to inclusion/exclusion criteria, 38 patients refused to participate and 21 patients withdrew for other reasons. Finally, 195 were enrolled and randomized (figure 1). All randomized patients finished the first month intervention stage. During the follow-up period, a total of 117 completed the follow-up study. In the continuous intrafemoral thrombolysis group, 8 patients lost to follow-up, 21 patients dropped out due to death. In the conventional therapy group, 18 patients lost to follow-up, 31 patients dropped out due to death (figure 1). As shown in table 1, the baseline data suggested that no statistically significant demographic differences existed between two groups. The mean age was 72 years and the patients were predominantly male (71.8%). Importantly, the main baseline ulcer clinical characteristics between two groups did not differ, such as the degrees of foot



**Figure 2** The foot ulcers healing at the first month after interventions. (A) Comparison of ulcer outcome between thrombolysis and conventional therapy groups. (B) Comparison of the time of ulcers healing between thrombolysis and conventional therapy groups. Data information: results are shown as mean $\pm$ SD. \*P<0.05, \*\*\*p<0.001 (Student's t-test).

ulcers, glycolipid metabolic profiles, TcPO<sub>2</sub>, TBI, ABI, peripheral neuropathy, etc.

### The intrafemoral thrombolysis therapy improved the foot ulcers at the first month of intervention stage

At the first 1 month of intervention stage, compared with conventional therapy group, the ulcers achieved a significant improvement in continuous intrafemoral thrombolysis group (online supplemental figure 2), including a complete closure (72.4% (71/98 patients) vs 17.5% (17/97 patients), (p<0.001)), an improved ulcer (27.6% (27/98 patients) vs 25.8% (25/97 patients)), unchanged or impaired ulcer (0% vs 56.7% (55/97 patients)) (figure 2A). Importantly, among patients with a complete closure, the time of ulcers healing in continuous intrafemoral thrombolysis group was dramatically shorter than that in conventional therapy group (figure 2B). Of note, as shown in online supplemental figure 3A, in Wagner 1 subgroup, all patients obtained completed ulcers closure in continuous intrafemoral thrombolysis treatment, which is significantly higher than that in conventional therapy group (100% vs 60%). In Wagner 2 subgroup, 80.6% of patients obtained completed ulcers closure in continuous intrafemoral thrombolysis treatment, whereas just 17.2% patients obtained complete closure in conventional therapy group (p<0.05). In Wagner 3 subgroup, 61.8% of patients obtained completed ulcers closure in continuous intrafemoral thrombolysis treatment, whereas no patient obtained complete closure in conventional therapy group (p<0.05). In addition, continuous intrafemoral thrombolysis therapy improved significantly glycolipid profiles, fibrinogen, TcPO<sub>2</sub>, toe systolic pressure, TBI, peripheral neuropathy (p<0.05) (online supplemental figures 3 and 4).

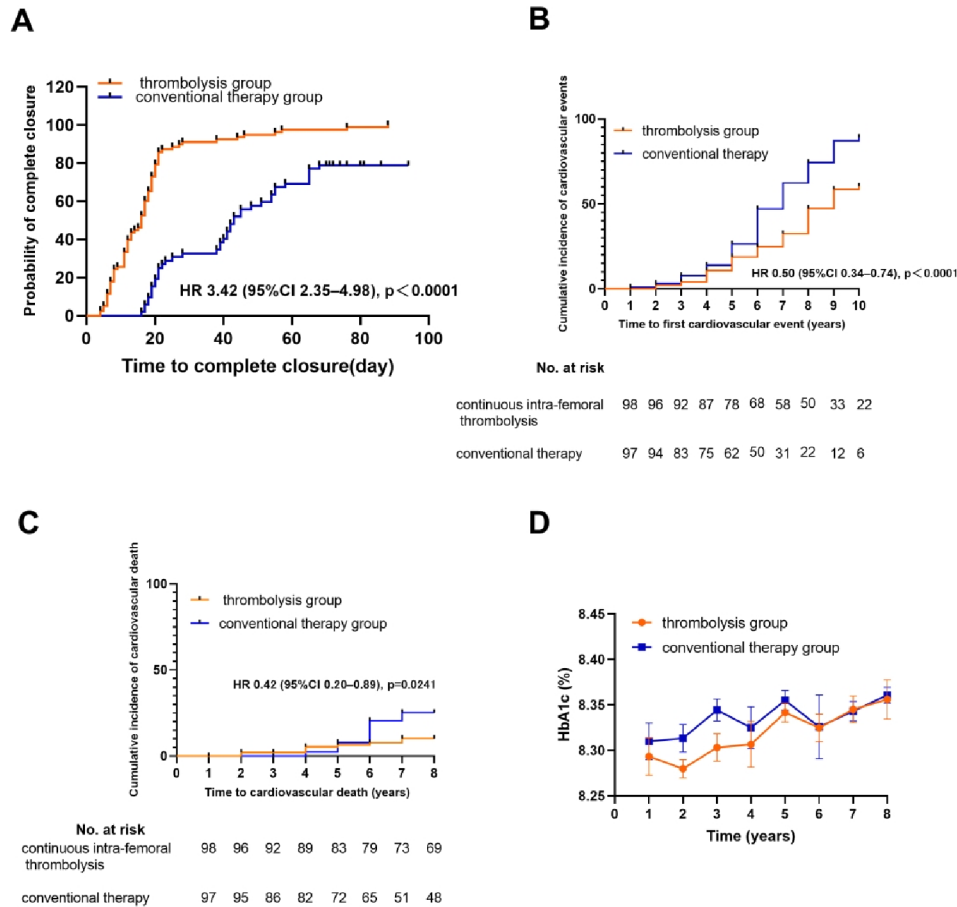
Ulcer duration, ulcer size, and HbA<sub>1c</sub> levels are known to be associated with prognosis of DFUs.<sup>34 35</sup> Therefore, univariate and multivariate Cox proportional hazards regression analyses were conducted on baseline ulcer duration (6 months as a cut-off), baseline ulcer area (6 cm<sup>2</sup> as a cut-off size), and baseline HbA<sub>1c</sub> level (7% as a cut-off). Compared with the conventional therapy group,

the results displayed a significant HR in continuous intrafemoral thrombolysis group (HR 0.21 (95% CI 0.073 to 0.68; p=0.004) for HbA<sub>1c</sub>; HR 0.094 (95% CI 0.026 to 0.34; p<0.001) for ulcer size; HR 0.56 (95% CI 0.35 to 0.88; p=0.012) for ulcer duration) (online supplemental table 1).

These data suggested that continuous intrafemoral thrombolysis therapy accelerated the ulcers closure and improved local skin oxygenation and peripheral neuropathy as well as glycolipid metabolic profiles in patients with diabetic foot. Additionally, oozing blood happened in two individuals in continuous intrafemoral thrombolysis group, and no infections and bleeding were found in study subjects (online supplemental table 2).

### The intrafemoral thrombolysis improved the outcomes of foot ulcers and decreased the cardiovascular events during the long-term follow-up study

For the primary outcome of ulcer closure rate, at the end of follow-up study, patients in the continuous intrafemoral thrombolysis group had a better complete healing rate than those in the conventional therapy group (HR 3.42 (95% CI 2.35 to 4.98), p<0.0001) (figure 3A). The cumulative incidence of complete healing also reflected the continual higher probability in continuous intrafemoral thrombolysis group from day 5 onward. The time to reach median population healing was 16 days in the intrafemoral thrombolysis group, whereas it was 43 days in the conventional therapy group during the follow-up period (figure 3A). The incidence of recurrence in completely healed ulcer during the follow-up phase was 11 of 78 (14.1%) in the continuous intrafemoral thrombolysis group, and 10 of 41 (24.4%) in the conventional therapy group with no statistical significance (p>0.05). On the other hand, comparing between two groups, the incidence of amputation in the continuous intrafemoral thrombolysis group was 10.2% (10/98 patients), which is significantly lower than that in the conventional therapy group (21.7% (21/97 patients), p<0.05) (online supplemental figure 5).



**Figure 3** The outcomes of foot ulcers and cardiovascular events during the long-term follow-up study. (A) Kaplan-Meier curves of time to complete ulcers healing. (B) Cumulative risk curves of time to first cardiovascular events. (C) Cumulative risk curves of time to cardiovascular death. (D) Mean hemoglobin A1c (HbA1c) levels during the long-term follow-up study. Data information: results are shown as mean $\pm$ SD.

For the secondary outcome of cardiovascular disease events, the distribution of cardiovascular events and death registered are shown in online supplemental table 3. In the continuous intrafemoral thrombolysis group, the incidence of cardiovascular events was 48.0% (47/98 patients), which is significantly lower than 59.8% (58/97 patients) in the conventional therapy group (HR 0.50 (95% CI 0.34 to 0.74),  $p < 0.0001$ ) (figure 3B). During follow-up study, 21 (21.4%) all-cause deaths were recorded in patients with the continuous intrafemoral thrombolysis therapy vs 31 (32.0%) patients with the conventional therapy ( $p > 0.05$ ). Notably, in the continuous intrafemoral thrombolysis group, the patients have a lower incidence of cardiovascular death compared with patients in the conventional therapy group (HR 0.42 (95% CI 0.20 to 0.89),  $p = 0.0241$ ) (figure 3C). In addition, in the first 5 years, the HbA1c level was lower in the continuous intrafemoral thrombolysis group than in the conventional therapy group during follow-up period (figure 3D). Moreover, at terminal, glycolipid metabolic profiles and peripheral neuropathy had better improvement in the continuous intrafemoral thrombolysis group when compared with conventional therapy group (table 2) ( $p < 0.05$ ). Compared with baseline, glycolipid

metabolic profiles, the local skin oxygenation and peripheral neuropathy were significantly improved in both thrombolysis group and control group ( $p < 0.05$ ), especially in thrombolysis group ( $p < 0.05$ ). These data illustrate that continuous intrafemoral thrombolysis therapy promoted the healing rate of the DFUs, decreased the incidence of amputation and incidence of cardiovascular events and mortality during the follow-up study.

All participants who were surveyed for life quality returned the questionnaire. The results showed that patients in the continuous intrafemoral thrombolysis group had higher mean scores in every domain with significantly higher scores in physical health (online supplemental table 4,  $p < 0.05$ ), indicating that continuous intrafemoral thrombolysis improves the life quality of patients with DFU.

To explore whether continuous intrafemoral thrombolysis therapy decreased the risk of cardiovascular events, we evaluated univariate and multivariate Cox proportional hazard regression analyses. On univariate analysis, age, diabetes duration, TC, TG, LDL, smoking, HbA1c, and continuous intrafemoral thrombolysis therapy were significantly associated with the end point of cardiovascular events, whereas on multivariate analysis, only age,

**Table 2** Clinical and laboratory data of patients with diabetic foot ulcers who complete the follow-up study at the terminal of long term follow-up study

	Thrombolysis therapy (n=69)	Conventional therapy (n=48)
TC (mmol/L)	4.8 (1.1)	5.1 (0.8)*
LDL-C (mmol/L)	2.5 (0.6)	3.0 (0.8)*
HDL-C (mmol/L)	1.3 (0.3)	1.3 (0.3)
TG (mmol/L)	1.6 (0.5)	2.1 (1.0)*
FBG (mmol/L)	7.8 (1.6)	8.9 (1.0)*
2-hour BG (mmol/L)	9.5 (1.5)	12.7 (2.0)*
Fibrinogen (g/L)	4.7 (0.9)	5.0 (1.1)
TBP	63.4 (16.9)	55.3 (13.2)
TBI	0.4 (0.1)	0.3 (0.1)
ABI	1.2 (0.2)	1.0 (0.3)
TcPO <sub>2</sub> (mm Hg)	44.8 (15.8)	40.1 (14.4)*
Peripheral neuropathy	28 (28.6%)	48 (49.5%)*
NDS	4.9 (3.2)	6.7 (3.5)*

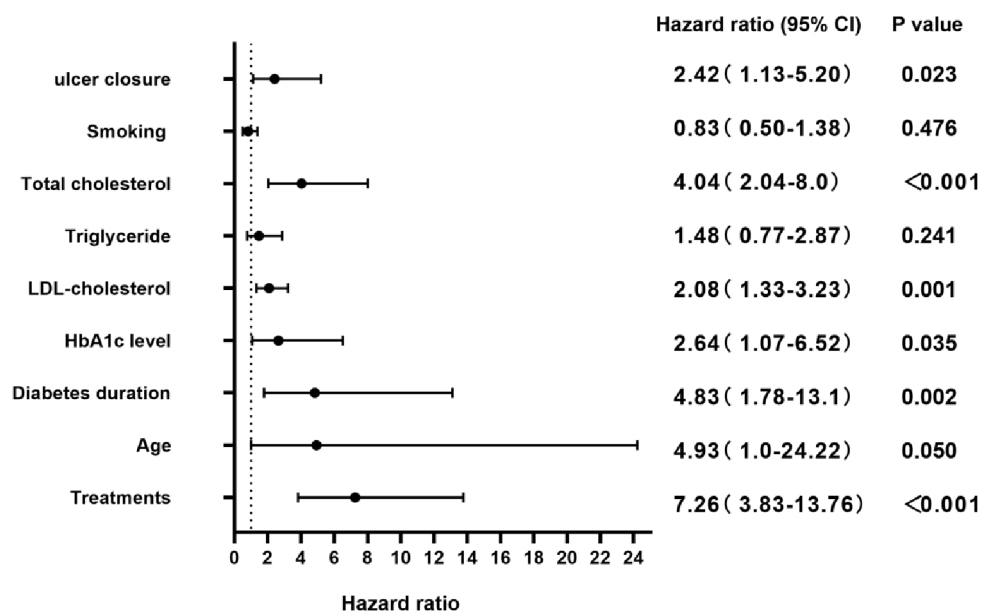
Data are number (%), mean (SD).

All results were from analyses adjusted for baseline.

\*P<0.05.

ABI, ankle-brachial index; BG, blood glucose; FBG, fasting blood glucose; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; NDS, Neuropathy Deficit Score; TBI, toe brachial index; TBP, toe blood pressure; TC, total cholesterol; TcPO<sub>2</sub>, transcutaneous oxygen tension; TG, triacylglycerol.

TC, LDL, HbA1c, and continuous intrafemoral thrombolysis therapy maintained a significant association with cardiovascular events (figure 4).



**Figure 4** Cox regression analysis of demographic and clinical variables associated with cardiovascular events. HbA1c, hemoglobin A1c; LDL, low-density lipoprotein.

## DISCUSSION

In this randomized and parallel study, our main findings were as follows: (1) continuous intrafemoral thrombolysis accelerated the ulcers closure and improved the outcomes of foot ulcers compared with conventional therapy; (2) continuous intrafemoral thrombolysis therapy decreased the incidence of amputation and incidence of cardiovascular events as well as cardiovascular mortality in the long-term follow-up; (3) continuous intrafemoral thrombolysis had a better skin microcirculation response and glycolipid metabolic profiles. As far as we know, this is the first time that our results showed continuous intrafemoral thrombolysis therapy by artery pump is an effectiveness and safety therapy for DFUs.

Given that ischemia, microthrombi formation, and hypoxic environment of local tissues display important roles in the development of foot ulcers, which always resulted in progression of foot lesions to gangrene and eventual amputation.<sup>12</sup> As the development of vascular interventional assays, regional intra-arterial infusion of thrombolytic agents, such as urokinase, has been proposed as a therapeutic alternative to surgical treatment in patients with diabetic lower limb vascular disease.<sup>12</sup> This treatment allows fibrinolytic drug to be continuously infused directly into the occluded artery, consequently avoiding unwanted systemic effects of lytic therapy, and minimizing the risk of hemorrhagic complications. Of note, one single-arm study showed that short-term local infusion of high-dose urokinase reestablished blood flow in the collateral vessels of the foot and leg, and in some patients in the dorsal pedal and plantar arch arteries in a small sample (just eight cases of patients) with rapidly progressive foot lesions.<sup>12</sup> But whether long-term intrafemoral artery infusion of low-dose urokinase



therapy improved DFUs in patients with diabetes has not been reported. Here, our results showed that continuous intrafemoral thrombolysis therapy accelerated the ulcers closure and improved the outcomes of foot ulcers in patients with DM. The improved local skin oxygenation, toe systolic pressure, TBI, and peripheral neuropathy as well as glycolipid metabolic profiles may contribute to the better ulcer closure and to the lower incidence of amputation.

It is established that the risk of cardiovascular events increases in patients with DFU.<sup>6,7</sup> Here, we found that long-term intrafemoral artery infusion of low-dose urokinase therapy decreased the cardiovascular events and cardiovascular death in patients with DFUs. Next, we ought to explore the mechanisms underlying the improvement of cardiovascular events by urokinase. Given that disorders in glycolipid metabolic profiles were associated with cardiovascular events. In consistence with this, the improvement on glycolipid metabolic profiles was obtained by continuous intrafemoral thrombolysis therapy in our follow-up study, which may contribute to the decreased cardiovascular events and cardiovascular mortality. Additionally, due to the improvement of foot ulcers outcomes, consequently the quality of life was improved, which also contributed to the decreased cardiovascular events and improved glycolipid metabolic profiles.

Some limitations of the study should be mentioned here. First, this study was an open-label and single-center study. The study design cannot mask the interventions to patients, which may increase some possible bias. Second, the study sample is relatively small, thus, a long-term follow-up study based on representative large DFU cohorts is needed. Third, predominantly male patients in our study limited generalisability of the findings. Finally, the thrombolysis group had 7 days bed rest, but our control group did not, which may affect healing of the ulcers.

In conclusion, this clinical trial shows that low-dose urokinase continuous intrafemoral thrombolysis therapy exhibited a better therapeutic efficacy for DFUs, including high complete healing rate and short time to complete healing, a lower incidence of amputation and cardiovascular events as well as cardiovascular mortality. The findings of this study suggest that continuous intrafemoral thrombolysis therapy provides a safe and effective treatment for patients with DFUs.

**Contributors** GX performed research design. LY, JZ, LX, and JX conducted the clinical study. SL and GZ were responsible for the daily conduct of the study, acquisition, analysis and interpretation of data. JD, YC, HR, and ML conducted the follow-up study and data analysis. JT and GX drafted the manuscript. The manuscript was read, critically revised and finally approved by all authors. All authors read and approved the final manuscript. GX is the guarantor in the overall content.

**Funding** This work was supported by grants from the National Natural Science Foundation of China (NSFC 81870573, 81370896, 81570730), National Key Research and Development Program of China (2016YFC1305601), and Research Project of Health Commission of Hubei Province (WJ2017H0031).

**Disclaimer** The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

**Competing interests** None declared.

**Patient consent for publication** Not applicable.

**Ethics approval** This study was approved by the ethics commission of General Hospital of Central Theater Command (approval ID: [2009]004-10). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available on reasonable request.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

#### ORCID iDs

Yangyang Cheng <http://orcid.org/0000-0001-9199-8846>

Ling Yue <http://orcid.org/0000-0003-4812-6748>

Guangda Xiang <http://orcid.org/0000-0002-6044-2308>

#### REFERENCES

- Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *JAMA* 2005;293:217–28.
- Blume PA, Walters J, Payne W, *et al*. Comparison of negative pressure wound therapy using vacuum-assisted closure with advanced moist wound therapy in the treatment of diabetic foot ulcers: a multicenter randomized controlled trial. *Diabetes Care* 2008;31:631–6.
- Hingorani A, LaMuraglia GM, Henke P, *et al*. The management of diabetic foot: a clinical practice guideline by the society for vascular surgery in collaboration with the American Podiatric Medical Association and the society for vascular medicine. *J Vasc Surg* 2016;63:3S–21S.
- Perez-Favila A, Martinez-Fierro ML, Rodriguez-Lazalde JG, *et al*. Current therapeutic strategies in diabetic foot ulcers. *Medicina (Kaunas)* 2019;55:714.
- Ramsey SD, Newton K, Blough D, *et al*. Incidence, outcomes, and cost of foot ulcers in patients with diabetes. *Diabetes Care* 1999;22:382–7.
- Pinto A, Tuttolomondo A, Di Raimondo D, *et al*. Cardiovascular risk profile and morbidity in subjects affected by type 2 diabetes mellitus with and without diabetic foot. *Metabolism* 2008;57:676–82.
- Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *JAMA* 1979;241:2035–8.
- Everett E, Mathioudakis N. Update on management of diabetic foot ulcers. *Ann N Y Acad Sci* 2018;1411:153–65.
- Guan Y, Niu H, Liu Z, *et al*. Sustained oxygenation accelerates diabetic wound healing by promoting epithelialization and angiogenesis and decreasing inflammation. *Sci Adv* 2021;7.
- Greenman RL, Panasyuk S, Wang X, *et al*. Early changes in the skin microcirculation and muscle metabolism of the diabetic foot. *Lancet* 2005;366:1711–7.
- Rajamani K, Colman PG, Li LP, *et al*. Effect of fenofibrate on amputation events in people with type 2 diabetes mellitus. *Lancet* 2009;373:1780–8. doi: 10.1016/S0140-6736(09)60698-X.
- Vannini P, Ciavarella A, Mustacchio A, *et al*. Intra-arterial Urokinase infusion in diabetic patients with rapidly progressive ischemic foot lesions. *Diabetes Care* 1991;14:925–7.
- Weck M, Rietzsch H, Lawall H, *et al*. Intermittent intravenous Urokinase for critical limb ischemia in diabetic foot ulceration. *Thromb Haemost* 2008;100:475–82.
- Almér LO, Sundkvist G, Lilja B. Fibrinolytic activity, autonomic neuropathy, and circulation in diabetes mellitus. *Diabetes* 1983;32 Suppl 2:4–7.

- 15 Wagner FW. The dysvascular foot: a system for diagnosis and treatment. *Foot Ankle* 1981;2:64–122.
- 16 Keech A, Simes RJ, Barter P. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus. *Lancet* 2005;366:1849–61.
- 17 Investigators FS. The need for a large-scale trial of fibrate therapy in diabetes: the rationale and design of the fenofibrate intervention and event lowering in diabetes (FIELD) study [ISRCTN64783481]. *Cardiovasc Diabetol* 2004;3:9. doi: 10.1186/1475-2840-3-9.
- 18 Spinetti G, Specchia C, Fortunato O, et al. Migratory activity of circulating mononuclear cells is associated with cardiovascular mortality in type 2 diabetic patients with critical limb ischemia. *Diabetes Care* 2014;37:1410–7.
- 19 Meng B, Li Y, Ding Y, et al. Myeloid-derived growth factor inhibits inflammation and alleviates endothelial injury and atherosclerosis in mice. *Sci Adv* 2021;7:eabe6903.
- 20 American Diabetes A. 10. Microvascular complications and foot care: standards of medical care in diabetes-2018. *Diabetes Care* 2018;41:S105–18.
- 21 Kalani M, Apelqvist J, Blombäck M, et al. Effect of dalteparin on healing of chronic foot ulcers in diabetic patients with peripheral arterial occlusive disease: a prospective, randomized, double-blind, placebo-controlled study. *Diabetes Care* 2003;26:2575–80.
- 22 Thomas F, Burke JP, Parker J, et al. The risk of infection related to radial vs femoral sites for arterial catheterization. *Crit Care Med* 1983;11:807–12.
- 23 Yao ST, Hobbs JT, Irvine WT. Ankle systolic pressure measurements in arterial disease affecting the lower extremities. *Br J Surg* 1969;56:676–9.
- 24 Brooks B, Dean R, Patel S, et al. TBI or not TBI: that is the question. is it better to measure toe pressure than ankle pressure in diabetic patients. *Diabet Med* 2001;18:528–32.
- 25 Wahlberg E, Gush R. A new automated toe blood pressure monitor for assessment of limb ischemia. *Eur J Vasc Endovasc Surg* 2002;24:304–8.
- 26 Azhary H, Farooq MU, Bhanushali M, et al. Peripheral neuropathy: differential diagnosis and management. *Am Fam Physician* 2010;81:887–92.
- 27 Rith-Najarian SJ, Stolusky T, Gohdes DM. Identifying diabetic patients at high risk for lower-extremity amputation in a primary health care setting. A prospective evaluation of simple screening criteria. *Diabetes Care* 1992;15:1386–9.
- 28 Bacharach JM, Rooke TW, Osmundson PJ, et al. Predictive value of transcutaneous oxygen pressure and amputation success by use of supine and elevation measurements. *J Vasc Surg* 1992;15:558–63.
- 29 Howd A, Proud G, Chamberlain J. Transcutaneous oxygen monitoring as an indication of prognosis in critical ischaemia of the lower limb. *Eur J Vasc Surg* 1988;2:27–30.
- 30 Kalani M, Brismar K, Fagrell B, et al. Transcutaneous oxygen tension and toe blood pressure as predictors for outcome of diabetic foot ulcers. *Diabetes Care* 1999;22:147–51.
- 31 Blombäck B, Blombäck M, Paul K-G, et al. Purification of human and bovine fibrinogen. *Acta Chem Scand* 1956;10:147.
- 32 He M, Li Y, Wang L, et al. MYDGF attenuates Podocyte injury and proteinuria by activating AKT/BAD signal pathway in mice with diabetic kidney disease. *Diabetologia* 2020;63:1916–31.
- 33 Bann CM, Fehnel SE, Gagnon DD. Development and validation of the diabetic foot ulcer scale-short form (DFS-SF). *Pharmacoeconomics* 2003;21:1277–90.
- 34 Jalilian M, Ahmadi Sarbarzeh P, Oubari S. Factors related to severity of diabetic foot ulcer: a systematic review. *Diabetes Metab Syndr Obes* 2020;13:1835–42.
- 35 Monteiro-Soares M, Boyko EJ, Ribeiro J, et al. Predictive factors for diabetic foot ulceration: a systematic review. *Diabetes Metab Res Rev* 2012;28:574–600.