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ORIGINAL RESEARCH

# Increased Expression of miR-155 in Peripheral Blood and Wound Margin Tissue of Type 2 Diabetes Mellitus Patients Associated with Diabetic Foot Ulcer

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**Purpose:** To investigate the correlations of miR-155 expression in the peripheral blood and wound margin tissue of patients with diabetic foot ulcer (DFU) and explore the clinical value of miR-155 as a potential biomarker for the diagnosis and treatment outcomes of DFU.

**Methods:** Sixty newly diagnosed T2DM patients without DFU (T2DM group), 112 T2DM patients with DFU (DFU group), and 60 healthy controls (NC group) were included. MiR-155 levels in the peripheral blood and wound margin tissue were determined by quantitative real-time PCR, while clinical features and risk factors of DFU were explored. Multiple stepwise logistic regression analysis was used to determine whether miR-155 expression was an independent risk factor for DFU. The diagnostic effectiveness of miR-155 level on DFU was evaluated using ROC curve analysis.

**Results:** A significant decrease in the expression level of miR-155 was observed in T2DM group compared with NC group ($P < 0.05$), while a markedly increased miR-155 expression level was noted in DFU group compared with T2DM group ($P < 0.01$). Moreover, there was a negative correlation between the expression levels of miR-155 with healing rate of DFU. Kaplan-Meier survival curve analysis showed that the cumulative rate of unhealed DFU in miR-155 high expression group is higher than that in miR-155 low expression group, both in peripheral blood and wound margin tissue (log rank, $P = 0.004$, $P < 0.001$, respectively). The multivariate logistic regression analysis confirmed that a high expression of miR-155 was an independent risk factor for DFU. The ROC curve analysis indicated that the AUC of miR-155 for the diagnosis of DFU was 0.794, with the optimum sensitivity being 96.82% and the optimum specificity of 95.93%.

**Conclusion:** The increased expression of miR-155 in peripheral blood of T2DM patients is closely related to the occurrence of DFU. MiR-155 is a potentially valuable biomarker for diagnosis and prognosis of DFU.

**Keywords:** miR-155, diabetic foot ulcer, type 2 diabetes mellitus, microRNAs, biomarker

## Introduction

Diabetic foot is a serious chronic complication of diabetes. Diabetic foot ulcer (DFU) is the most common manifestation of the diabetic foot and the most common cause of non-traumatic lower limb amputation.[1] The risk of a patient with diabetes developing a foot ulcer across their lifetime has been estimated to be 19–34%.[2] The pathogenesis of DFU is complex and has not been fully clarified. Early diagnosis, scientific evaluation and timely standardized treatment can effectively improve the prognosis of DFU.[3]

In recent years, an increasing number of studies have shown that abnormal expression of microRNAs (miRNAs) is closely associated with the occurrence and prognosis of DFU.[4] MiRNAs are a class of endogenous non-coding RNAs with a length of approximately 18–25 nucleotides. They can regulate the expression of a target gene by specifically binding to the 3′ untranslated region of downstream target mRNA to guide the silencing complex to degrade mRNA or

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inhibit protein translation.[5,6] MiR-155 is widely involved in many biological processes, such as the development and differentiation of immune cells,[7,8] the inflammatory response,[9] and it has significant effects on the functions of keratinocytes, fibroblasts, dermal mesenchymal stem cells, and other cells involved in the process of wound healing.[10,11] Inhibition of miR-155 expression in local wound tissue can promote skin wound healing in diabetic rats.[12,13] Knockout of microRNA-155 can ameliorate the Th17/Th9 immune response in mice and accelerate wound healing.[14] In addition, recent studies have shown that mesenchymal stem cell-derived exosomes loaded with miR-155 inhibitor can improve diabetic wound healing.[15]

However, the clinical research on the correlation between miR-155 and DFU has not been reported. Accordingly, our study aimed to investigate the changes in the expression level of miR-155 in the peripheral blood and wound tissue of DFU patients and its relationship with the pathogenesis and prognosis of DFU.

# Materials and Methods

## Study Subjects

The participants for this study were selected from the subjects described in a previous study[16] and included 112 patients with type 2 diabetes mellitus (T2DM) having DFU complications (DFU group) who were hospitalized in the Department of Endocrinology at the First Affiliated Hospital of Anhui Medical University from January 2018 to December 2019. In these 112 cases, the duration of foot ulcer was more than four weeks, the ulcer area was 2–20 $cm^2$, the Wagner grade was 2–4, the ankle-brachial index (ABI) was 0.7–1.3. Sixty patients with newly diagnosed T2DM without DFU in the Department of Endocrinology at our hospital in the same period were selected as a diabetic group (T2DM group). These patients had a course of T2DM ranging from one week to five months with no lower extremity atherosclerosis disease, and no diabetic peripheral neuropathy. In addition, sixty healthy subjects who underwent physical examination in the Health Management Center of our hospital in the same period were selected as a normal control group (NC group). All the subjects in the NC group underwent a 75 g oral glucose tolerance test to confirm normal glucose tolerance. All subjects had no severe heart, liver, or renal insufficiency; no autoimmune diseases; no severe sepsis; and no cancerous ulcer wounds. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Anhui Medical University, and we obtained the informed consent of the subjects.

## Study Methods

### The Treatment Process of DFU

All patients with DFU were given routine systemic treatment previously reported,[16] including anti-infection, reducing blood pressure, reducing blood glucose, correcting hypoproteinemia, nourishing nerves, improving blood supply of lower limb wounds, etc. Wound debridement was performed to remove blackened necrotic soft tissue and bone tissue. The full-sickness skin tissue within 0.5 cm of the wound margin was cut by skilled surgeons using tissue scissors according to the sampling protocol and stored in a refrigerator at −80°C. According to the specific conditions of each DFU, decompression or continuous negative pressure wound therapy was given. The course of the DFU was monitored, and the diabetic foot multidisciplinary team decided whether amputation should be performed. All patients with DFU were followed up until the wound healed completely, and the healing time was recorded. Complete wound healing after 8 weeks was defined as spontaneous complete closure, ie, 100% reepithelization,[17] and recorded after eight weeks of treatment.

### Detection of Clinical Indicators

Venous blood from the elbow was drawn from all subjects into anticoagulation tubes (the anticoagulant was sodium fluoride/EDTA/heparin, selected according to different examination items) or non-anticoagulant tubes at 8:00–8:30 a.m. after fasting for 10 hours. Serum albumin (ALB), blood glucose, blood lipids, glycosylated hemoglobin A1c (HbA1c), white blood cell (WBC) count, hemoglobin (Hb), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and other indicators were measured. Blood glucose, blood lipid, and ALB were measured by an automatic biochemical analyzer (Module P800, Roche, Switzerland). Fasting plasma glucose (FPG) was determined using the glucose oxidase method. Total cholesterol (TCH), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assayed by the oxidase colorimetric method. HbA1c was

detected by high-pressure liquid chromatography, CRP by latex enhanced immunoturbidimetry, and ESR by the Wechsler method. The area of skin ulcers was measured by digital photography combined with ImageJ (Image J-ij133-jdk15, National Institutes of Health, Bethesda, USA) medical image analysis software, ABI was measured by a Doppler blood flow detector (DPL-03, Hangzhou Yuanxiang Medical, China), and transcutaneous oxygen partial pressure (TcPO2) was measured by a transcutaneous oxygen partial pressure detector (TCM400, Ledu, Denmark).

### Detection of miR-155 Expression in Peripheral Blood and Wound Margin Tissue

The expressions of miR-155 in peripheral venous blood (P-miR-155) and the expressions of miR-155 in wound margin tissue (T-miR-155) were measured by real-time quantitative reverse transcription PCR (qRT-PCR). RNA was extracted from 2-mL EDTA anticoagulant blood samples or 50 mg of wound margin tissue according to the instructions of the miRcute miRNA extraction and separation kit (TIANGEN, Beijing, China). cDNA was then synthesized according to the instructions of miRcute miRNA cDNA synthesis kit (TIANGEN, Beijing, China). The primer sequences of miR-155 were as follows: forward primer 5′-CGGCGGTTAATGCTAATTGTGAT-3′, reverse primer 5′-GTGCAGGGTCCGAGGT-3′; The primer sequences of the endogenous control U6 were as follows: forward primer 5'-GCTTCGGCAGCACATATACTAAAA-3', reverse primer 5'-CGCTTCACGAATTTGCCTGTCAT-3'. qRT-PCR was carried out according to the instructions of the miRcute miRNA fluorescence quantitative detection kit (TIANGEN, Beijing, China). The cycling conditions were pre-denaturation at 95°C for 5 min, denaturation at 95°C for 20s, annealing at 58°C for 15s, and extension at 72°C for 10s, for a total of 42 cycles. Using U6 as the internal reference, the relative expression of miR-155 was calculated by the $2^{-\Delta\Delta Ct}$ method. Each sample was repeated three times, and the average value was taken as the result.

### Statistical Processing

SPSS 19.0 software was used for statistical analysis. The measurement data are expressed by the mean ± standard deviation ($\overline{X}\pm S$), whereas the non-normal measurement data are expressed by the median (quartile interval) [M ($P_{25}$, $P_{75}$)]. $X^2$ test or *t*-test was used for comparison between the two groups; analysis of variance test was used for comparison between multiple groups; and LSD-*t* test was used for further pairwise comparison. Spearman correlation analysis was used to evaluate the correlation between the expression of miR-155 in both peripheral blood and wound margin tissue and other clinical variables. Multiple stepwise logistic regression analysis was used to determine whether miR-155 in peripheral blood was an independent risk factor for DFU. Kaplan–Meier survival curve analysis was used to study the correlation between the expression of miR-155 in peripheral blood and wound margin tissue and the wound healing of DFU. ROC curve analysis was used to explore the possibility of miR-155 in peripheral blood as a potential biomarker for the diagnosis of DFU. The best sensitivity and specificity of ROC curve were determined by common methods.[18] All tests were bilateral, $P < 0.05$ was considered to be a statistically significant difference.

## Results

### Comparison of Clinical Parameters Among the Three Groups

There were no significant differences in gender composition, age, TCH, or LDL-C levels among the three groups ($P > 0.05$). The levels of FPG, HbA1c, and TG in the T2DM group and DFU group were higher than those of the NC group, whereas the level of HDL-C was lower than that of the NC group, and the differences were statistically significant ($P < 0.05$). The expression level of miR-155 in the peripheral blood of the T2DM group was lower than that of the NC group, and that of the DFU group was higher than that of the NC group and T2DM group, and the differences were statistically significant ($P < 0.05$). In addition, there were no significant differences in TcPO2, ABI, CRP, ESR, ALB, WBC, and Hb values between the NC group and the T2DM group ($P > 0.05$). Moreover, the duration of diabetes, FPG, HbA1c, CRP, ESR, WBC count, and miR-155 expression levels in peripheral blood in the DFU group were higher than those in the T2DM group, whereas TcPO2, ABI, ALB, and Hb were lower in the DFU group than those in the T2DM group, and the differences were statistically significant ($P < 0.05$). There was no significant difference in TG and HDL-C levels between the two groups ($P > 0.05$) (Table 1).

**Table 1** Comparisons of Clinical Parameters Among NC Group, T2DM Group, and DFU Group [n (%), ($\overline{X}$±S), M ($P_{25}$, $P_{75}$)]

| Characteristics | NC (n=60) | T2DM (n=60) | DFU (n=112) | *F*/*t*/$x^2$ value | *P* value |
|---|---|---|---|---|---|
| Gender | | | | 1.183 | 0.562 |
| Male | 36 (60.0) | 34 (56.7) | 60 (53.6) | | |
| Female | 24 (40.0) | 26 (43.3) | 52 (46.4) | | |
| Age (y) | 54.7±10.1 | 55.1±11.9 | 54.5±10.6 | 1.327 | 0.315 |
| Diabetes course (y) | – | 0.3±0.2 | 11.3±5.2[d] | 8.946 | <0.001 |
| FPG (mmol/L) | 4.8±0.6 | 9.9±2.5[b] | 11.2±3.8[bd] | 28.357 | <0.001 |
| HbA1c (%) | 5.1±0.4 | 8.3±1.9[b] | 9.1±2.7[bc] | 26.123 | <0.001 |
| TG (mmol/L) | 1.4±0.8 | 1.8±0.8[b] | 1.7±0.9[b] | 9.236 | <0.001 |
| TCH (mmol/L) | 4.3±0.8 | 4.9±0.7 | 4.6±0.7 | 1.868 | 0.127 |
| LDL-C (mmol/L) | 2.4±0.3 | 2.8±0.5 | 2.6±0.5 | 1.981 | 0.092 |
| HDL-C (mmol/L) | 1.3±0.2 | 1.0±0.3[a] | 0.9±0.5[b] | 2.357 | 0.044 |
| TcPO2 (mmHg) | 76.4±7.2 | 70.5±8.9 | 48.5±10.2[bd] | 19.823 | <0.001 |
| ABI | 1.12±0.16 | 1.05±0.21 | 0.84±0.32[bc] | 7.865 | <0.001 |
| CRP (mg/L) | 7.2±1.1 | 9.2±1.2 | 48.2±19.3[bd] | 83.127 | <0.001 |
| ESR (mm/h) | 10.2±2.1 | 12.7±3.1 | 48.2±19.5[bd] | 35.763 | <0.001 |
| ALB (g/L) | 39.9±0.6 | 39.3±0.8 | 33.7±3.5[ac] | 2.964 | 0.023 |
| WBC (×$10^9$) | 4.2±0.8 | 4.7±0.9 | 11.2±4.2[bd] | 30.413 | <0.001 |
| Hb (g/L) | 128.5±2.2 | 126.4±2.9 | 112.3±1.8[ac] | 2.745 | 0.031 |
| P-miR-155 | 1.09 (0.61,1.92) | 0.43 (0.19, 1.37)[b] | 4.38 (1.26,10.13)[bd] | 6.985 | <0.001 |
| T-miR-155 | – | – | 7.41 (2.47,13.86) | – | – |

**Notes**: Data are presented mean±standard deviations or numbers (%) or median with IQR; differences among three groups analyzed using one-way analysis of variance or $x^2$ test, and least-significant difference (LSD) analysis was used for comparison between the two groups. Versus NC group, [a]P< 0.05, [b]P< 0.01; versus T2DM group, [c]P< 0.05, [d]P< 0.01.

**Abbreviations**: NC, normal control group; T2DM, type 2 diabetes group; DFU, diabetic foot ulcer group; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin A1c; TG, triglycerides; TCH, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TcPO2, transcutaneous oxygen partial pressure; ABI, ankle-brachial index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ALB, serum albumin; WBC, white blood cell count; Hb, hemoglobin; P-miR-155, miR-155 expression in peripheral blood; T-miR-155, miR-155 expression in the wound margin tissue; MiR, microRNA.

## Relationship Between miR-155 Expression Levels in Both Peripheral Blood and Wound Margin Tissue and Clinical Characteristics of DFU

The 112 patients with DFU were divided into two subgroups according to the median level of miR-155 expression in peripheral blood and wound margin tissue, respectively. The patients with miR-155 expression lower than the median were classified as the low expression group and those with expression higher than or equal to the median were classified as the high expression group. The clinical characteristics of DFU between the high expression group and the low expression group were compared. As shown in Tables 2 and 3, the expression levels of miR-155 in both peripheral blood and wound margin tissue of patients with DFU were negatively correlated with the healing rate of foot ulcer after eight weeks (P = 0.037, P = 0.035, respectively), and positively correlated with the course of foot ulcer (P = 0.033, P = 0.034, respectively), and Wagner grade of foot ulcer (P = 0.010, P = 0.013, respectively). There was no correlation between miR-155 expression in both the peripheral blood and wound margin tissue and other clinical characteristics of foot ulcers. In order to further explore the effect of the change of miR-155 expression in peripheral blood and wound margin tissue on wound healing, we used Kaplan–Meier survival curve analysis method. The results showed that the estimated time median of wound healing in peripheral blood miR-155 high expression group and low expression group were 10.23 weeks and 9.51 weeks, respectively; Similarly, the estimated time median of wound healing in wound margin tissue miR-155 high expression group and low expression group were 10.12 weeks and 8.67 weeks, respectively. Whether in peripheral blood or wound margin tissue, the cumulative rate of unhealed DFU in miR-155 high expression group is higher than that in miR-155 low expression group (log rank, P = 0.004, P < 0.001, respectively). The wound healing time of high expression group is longer than that of low expression group (P < 0.05) (Figure 1A and B).

**Table 2** Relationship Between miR-155 Expression Levels in Peripheral Blood and Clinical Characteristics of DFU [n (%)]

| Characteristics | High Expression Group (n=72) | Low Expression Group (n=40) | $x^2$ value | *P* value |
|---|---|---|---|---|
| Age (y) | | | 0.003 | 0.955 |
| ≥55 | 40 (55.6) | 22 (55.0) | | |
| <55 | 32 (44.4) | 18 (45.0) | | |
| Gender | | | 0.287 | 0.592 |
| Male | 34 (47.2) | 21 (52.5) | | |
| Female | 38 (52.8) | 19 (47.5) | | |
| Ulcer area ($cm^2$) | | | 3.121 | 0.210 |
| ≤5 | 8 (11.1) | 9 (22.5) | | |
| 5–10 | 48 (66.7) | 21 (52.5) | | |
| >10 | 16 (22.2) | 10 (25.0) | | |
| Ulcer course (w) | | | 6.844 | 0.033 |
| ≤6 | 17 (23.6) | 19 (47.5) | | |
| 6–10 | 44 (61.1) | 16 (40.0) | | |
| >10 | 11 (15.3) | 5 (12.5) | | |
| Wagner grade | | | 9.151 | 0.010 |
| II | 4 (5.6) | 10 (25.0) | | |
| III | 56 (77.8) | 26 (65.0) | | |
| IV | 12 (16.7) | 4 (10.0) | | |
| Amputation rate (%) | | | 2.619 | 0.106 |
| Amputated | 16 (22.2) | 4 (10.0) | | |
| Not amputated | 56 (77.8) | 36 (90.0) | | |
| Ulcer healing rate after 8 weeks (%) | | | 4.352 | 0.037 |
| Healed | 32 (44.4) | 26 (65.0) | | |
| Not healed | 40 (55.6) | 14 (35.0) | | |

**Notes**: Data are presented numbers (%); differences between two groups analyzed using $x^2$ test. The cut-off point of miR-155 expression level for grouping was 4.38.

## Correlation Between miR-155 Expression in Both Peripheral Blood and Wound Margin Tissue and Other Clinical Parameters in the Three Groups

In the NC group, no significant correlation was observed between the expression of miR-155 in peripheral blood and other clinical parameters ($P > 0.05$). In the T2DM group, the expression of miR-155 in peripheral blood was negatively correlated with the levels of FPG and HbA1c ($P < 0.05$) and had no significant correlation with other indicators ($P > 0.05$). In the DFU group, the expression of miR-155 in both peripheral blood and wound margin tissue was positively correlated with the course of foot ulcer, Wagner grade, CRP, and WBC count ($P < 0.05$), and had no significant correlation with other indicators ($P > 0.05$). In addition, the expression of miR-155 in peripheral blood was positively correlated with that in wound margin tissue in the DFU group (Tables 4 and 5).

## Analysis of Risk Factors of DFU

In diabetic patients, DFU was used as a dependent variable, and sex, age, and other variables with $P < 0.1$ obtained from univariate logistic regression analysis (including the course of diabetes, FPG, HbA1c, TG, LDL-C, HDL-C, ALB, Hb, TcPO2, ABI, CRP, WBC, ESR, P-miR-155) were used as independent variables for multivariate stepwise logistic regression analysis. The course of diabetes, HbA1c, CRP, low TcPO2, high expression of P-miR-155 were independent risk factors for DFU (Table 6).

**Table 3** Relationship Between miR-155 Expression Levels in Wound Margin Tissue and Clinical Characteristics of DFU [n (%)]

| Characteristics | High Expression Group (n=80) | Low Expression Group (n=32) | $x^2$ value | *P* value |
|---|---|---|---|---|
| Age (y) | | | 0.178 | 0.673 |
| ≥55 | 44 (55.0) | 19 (59.4) | | |
| <55 | 36 (45.0) | 13 (40.6) | | |
| Gender | | | 0.289 | 0.591 |
| Male | 38 (47.5) | 17 (53.1) | | |
| Female | 42 (52.5) | 15 (46.9) | | |
| Ulcer area ($cm^2$) | | | 1.934 | 0.380 |
| ≤5 | 10 (12.5) | 6 (18.8) | | |
| 5–10 | 56 (70.0) | 18 (56.2) | | |
| >10 | 14 (17.5) | 8 (25.0) | | |
| Ulcer course (w) | | | 6.786 | 0.034 |
| ≤6 | 14 (17.5) | 16 (50.0) | | |
| 6–10 | 50 (62.5) | 8 (25.0) | | |
| >10 | 16 (20.0) | 8 (25.0) | | |
| Wagner grade | | | 8.756 | 0.013 |
| II | 5 (6.3) | 7 (21.9) | | |
| III | 61 (76.2) | 16 (50.0) | | |
| IV | 14 (17.5) | 9 (28.1) | | |
| Amputation rate (%) | | | 2.492 | 0.114 |
| Amputated | 21 (26.3) | 4 (12.5) | | |
| Not amputated | 59 (73.7) | 28 (87.5) | | |
| Ulcer healing rate after 8 weeks (%) | | | 4.444 | 0.035 |
| Healed | 30 (37.5) | 19 (59.4) | | |
| Not healed | 50 (62.5) | 13 (40.6) | | |

**Notes**: Data are presented numbers (%); differences between two groups analyzed using $x^2$ test. The cut-off point of miR-155 expression level for grouping was 7.41.

## Marker Verification

To further explore the potential value of miR-155 in the diagnosis of DFU, the expression level of miR-155 in the peripheral blood was assessed on an independent group of 172 peripheral blood samples including 60 cases of T2DM and 112 cases of DFU. The ROC curve was determined to evaluate the sensitivity and specificity of peripheral blood miR-155 in diagnosing DFU. The results showed that the AUC of peripheral blood miR-155 for diagnosing DFU was 0.794 (95% CI, 0.726–0.863, P < 0.001), the best cut-off point of miR-155 was 1.01, the sensitivity was 96.82%, and the specificity was 95.93% (Figure 2).

# Discussion

Based on the above research, it is easy to find that the miR-155 expression level in peripheral blood of newly diagnosed T2DM patients is significantly lower than that of subjects with normal glucose tolerance. Conversely, the expression level of miR-155 in DFU patients was dramatically higher than that of patients without DFU. Multiple logistic regression analysis showed that high expression of peripheral blood miR-155 was an independent risk factor for DFU. Further analysis revealed that the expression levels of miR-155 in the peripheral blood and wound margin tissue of patients with DFU were closely related to the Wagner grade, the healing rate of foot ulcers. The patients with high expression of miR-155 in the peripheral blood and wound margin tissue had more serious DFU status, lower healing rate, and longer healing time, indicating that the high expression of miR-155 is not only a strong risk factor for the pathogenesis of DFU but also a potential biomarker for the evaluation, treatment, and prognosis of DFU. To our knowledge, this is the first study to

**Table 4** Correlations Between miR-155 Expression in Peripheral Blood and Other Clinical Parameters in NC Group, T2DM Group, and DFU Group (r)

| Variables | NC (n=60) | | T2DM (n=60) | | DFU (n=112) | |
|---|---|---|---|---|---|---|
| | r | *P* value | r | *P* value | r | *P* value |
| Age | 0.041 | 0.825 | 0.052 | 0.812 | 0.048 | 0.785 |
| Gender | 0.102 | 0.346 | 0.039 | 0.857 | 0.049 | 0.722 |
| Course of diabetes | – | – | 0.101 | 0.426 | 0.142 | 0.302 |
| Course of foot ulcer | – | – | – | – | 0.295 | 0.041 |
| Foot ulcer area | – | – | – | – | 0.112 | 0.319 |
| Wagner grade | – | – | – | – | 0.309 | 0.025 |
| FPG | −0.083 | 0.311 | −0.298 | 0.039 | 0.181 | 0.098 |
| HbA1c | −0.078 | 0.335 | −0.287 | 0.046 | 0.173 | 0.102 |
| TG | −0.015 | 0.888 | −0.088 | 0.301 | 0.017 | 0.841 |
| TCH | 0.014 | 0.897 | 0.029 | 0.885 | 0.068 | 0.719 |
| LDL-C | 0.038 | 0.826 | −0.011 | 0.903 | 0.055 | 0.768 |
| HDL-C | 0.067 | 0.553 | 0.046 | 0.652 | −0.094 | 0.458 |
| ALB | 0.012 | 0.902 | 0.008 | 0.925 | −0.019 | 0.826 |
| TcPO2 | −0.009 | 0.926 | −0.068 | 0.474 | −0.075 | 0.453 |
| ABI | 0.095 | 0.344 | 0.086 | 0.798 | −0.103 | 0.382 |
| CRP | 0.085 | 0.501 | 0.123 | 0.295 | 0.315 | 0.021 |
| ESR | −0.069 | 0.552 | 0.106 | 0.304 | 0.203 | 0.069 |
| WBC | −0.011 | 0.828 | 0.178 | 0.224 | 0.296 | 0.042 |
| Hb | 0.093 | 0.415 | −0.085 | 0.302 | −0.081 | 0.336 |

**Abbreviations**: NC, normal control group; T2DM, type 2 diabetes group; DFU, diabetic foot ulcer group; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin A1c; TG, triglycerides; TCH, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALB, serum albumin; TcPO2, transcutaneous oxygen partial pressure; ABI, ankle-brachial index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count; Hb, hemoglobin; MiR, microRNA.

investigate the relationship between changes in miR-155 expression and the onset and treatment outcome of DFU in patients with T2DM.

In the present study, peripheral blood miR-155 levels were notably decreased in patients with T2DM compared with control individuals with normal glucose tolerance. Correlation analysis revealed that in patients with T2DM, the expression of miR-155 was negatively correlated with FPG and HbA1c. Animal studies find that overexpression of miR-155 transgenes can improve glucose tolerance and insulin sensitivity in mice, leading to hypoglycemia; in contrast, miR-155 deficiency in mice can impair islet function and lead to hyperglycemia, impaired glucose tolerance, and insulin resistance in the liver, muscle, and adipocytes.[19,20] Clinical studies show that the expression level of miR-155 in the peripheral plasma and monocytes of T2DM patients is significantly lower than that of sex-age-matched normal glucose tolerance subjects.[21,22] These findings support the results of the present study. Notably, according to the results of this study, we cannot explain the reason for the down-regulation of miR-155 expression in T2DM patients. However, a previous study demonstrated that high blood sugar potentially downregulates the expression of miR-24 by inducing the activation of c-Myc.[23] As for whether hyperglycemia directly downregulates the expression of miR-155, it is still uncertain. Therefore, more studies are needed to clarify the mechanism of miR-155 expression change in high glucose environment. However, in the DFU group, there was no significant correlation between the expression level of miR-155 in peripheral blood and wound margin tissue and FPG or HbA1c. We speculate that the reason is that other factors in DFU patients may have more influence on the expression of miR-155 than FPG and HbA1c.

In addition, our study also demonstrated that in DFU group, both the expression level of miR-155 in peripheral blood and wound margin tissue was positively correlated with the index of inflammatory state, including CRP, ESR, and WBC count, suggesting that the high expression of miR-155 in the peripheral blood and wound margin tissue of patients with DFU may be related to the state of infectious inflammation. Liu et al found that sepsis patients exhibited a significantly elevated miR-155 level, which was positively related to greater severity of sepsis.[24] Chen et al revealed that serum miR-

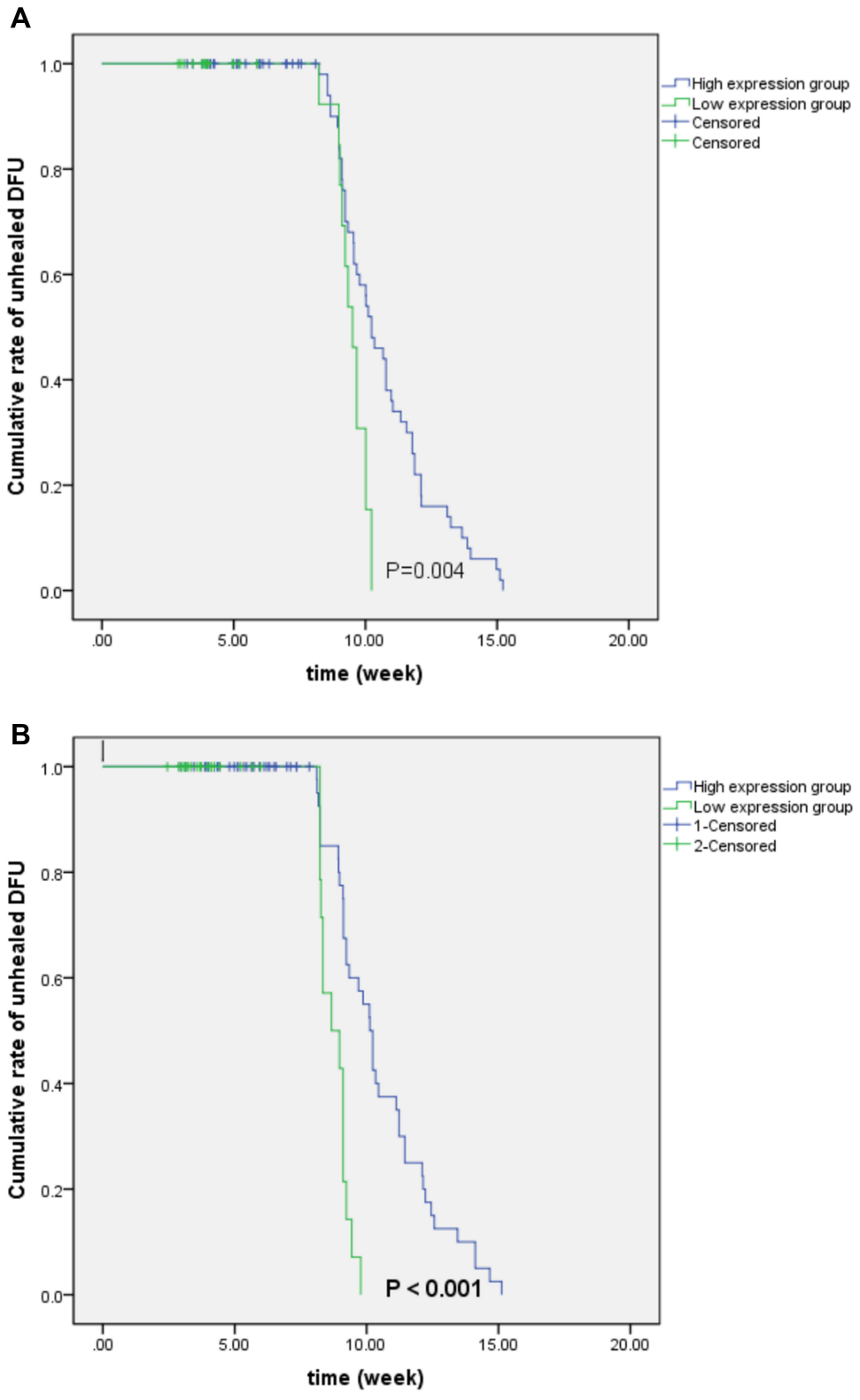


**Figure 1** Curve of wound complete healing rate of DFU between miR-155 high expression group and low expression group by Kaplan-Meier survival curve analysis. (**A**) The cumulative rate of unhealed DFU in the high expression group of peripheral blood miR-155 was higher than that in the low expression group of peripheral blood miR-155 (log rank, P = 0.004). The estimated time median of wound complete healing in peripheral blood miR-155 high expression group and low expression group were 10.23 weeks and 9.51 weeks, respectively (P < 0.05). (**B**) The cumulative rate of unhealed DFU in the high expression group of wound margin tissue miR-155 was higher than that in the low expression group of wound margin tissue miR-155 (log rank, P < 0.001). The estimated time median of wound complete healing in wound margin tissue miR-155 high expression group and low expression group were 10.12 weeks and 8.67 weeks, respectively (P < 0.05).

**Table 5** Correlation Between miR-155 Expression in Wound Margin Tissue and Other Clinical Parameters in the DFU Group (r)

| Variables | DFU (n=112) | |
|---|---|---|
| | r | *P* value |
| Age | 0.031 | 0.802 |
| Gender | 0.113 | 0.305 |
| Course of diabetes | 0.138 | 0.279 |
| Course of foot ulcer | 0.304 | 0.012 |
| Foot ulcer area | 0.179 | 0.114 |
| Wagner grade | 0.357 | 0.009 |
| FPG | 0.193 | 0.067 |
| HbA1c | 0.184 | 0.095 |
| TG | 0.011 | 0.893 |
| TCH | 0.052 | 0.746 |
| LDL-C | 0.101 | 0.493 |
| HDL-C | −0.048 | 0.682 |
| ALB | −0.081 | 0.795 |
| TcPO2 | −0.108 | 0.327 |
| ABI | −0.115 | 0.286 |
| CRP | 0.298 | 0.025 |
| ESR | 0.201 | 0.064 |
| WBC | 0.275 | 0.043 |
| Hb | −0.107 | 0.291 |
| P-miR-155 | 0.418 | < 0.001 |

**Abbreviations**: DFU, diabetic foot ulcer group; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin A1c; TG, triglycerides; TCH, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALB, serum albumin; TcPO2, transcutaneous oxygen partial pressure; ABI, ankle-brachial index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count; Hb, hemoglobin; P-miR-155, miR-155 expression in peripheral blood; MiR, microRNA.

**Table 6** The Multivariate Stepwise Logistic Regression Analysis of Risk Factors for Diabetic Foot Ulcer

| Variable | β | SE | Wald | OR | 95% CI | *P* value |
|---|---|---|---|---|---|---|
| Course of diabetes (y) | 0.73 | 0.52 | 5.51 | 4.69 | 1.98~6.22 | <0.001 |
| HbA1c (%) | 0.57 | 0.33 | 3.86 | 1.83 | 1.17~7.51 | 0.031 |
| TcPO2 (mmHg) | 0.39 | 0.25 | 3.01 | 1.14 | 1.09~8.17 | 0.038 |
| CRP (mg/L) | 0.42 | 0.29 | 2.96 | 1.12 | 1.06~6.75 | 0.046 |
| P-miR-155 | 0.81 | 0.43 | 7.12 | 3.98 | 1.25~8.62 | 0.003 |

**Notes**: Multivariate unconditional logistic regression analysis adjusted for sex, age, the course of diabetes, FPG, HbA1c, TG, LDL-C, HDL-C, Hb, ALB, TcPO2, ABI, CRP, WBC, ESR, P-miR-155.

**Abbreviations**: FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin A1c; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Hb, hemoglobin; ALB, serum albumin; TcPO2, transcutaneous oxygen partial pressure; ABI, ankle-brachial index; CRP, C-reactive protein; WBC, white blood cell count; ESR, erythrocyte sedimentation rate; P-miR-155, miR-155 expression in peripheral in blood; MiR, microRNA.

155 level was up-regulated in community-acquired pneumonia patients, and lipopolysaccharides could induce the up-regulation of miR-155 in RAW264.7 cells in vitro.[25] These results strengthened our findings. Moreover, although there was a significant difference in the course of diabetes between the DFU group and the T2DM group, further analysis found that there was no correlation between miR155 and the course of diabetes in either the DFU group or the T2DM

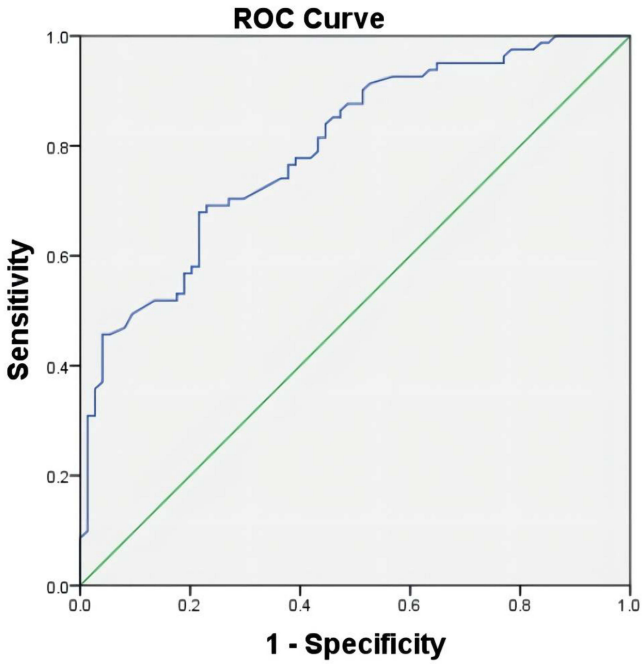


**Figure 2** The biomarker potential of circulating miR-155 for DFU and control. ROC analysis was used to evaluate the ability of circulating miR-155 to distinguish between two groups. MiR-155 distinguished DFU patients from controls with area under curve (AUC) of 0.794 (95% CI, 0.726–0.863, P < 0.001), the best cut-off point of miR-155 was 1.01, the sensitivity was 96.82%, and the specificity was 95.93%. DFU: diabetic foot ulcer.

group. Therefore, the significant difference in the course of disease between the DFU group and the T2DM group might have little effect on the difference of miR-155 expression between the two groups.

In this study, the course of foot ulcers in the DFU group was at least four weeks, which can be classified as a chronic refractory wound.[26] The clinical features of the DFU group are a long course of diabetes, poor control of blood glucose for a long time, abnormal lipid metabolism, peripheral angiopathy, and infectious inflammation. Multivariate regression analysis showed that the course of diabetes, HbA1c, TcPO2, and CRP were independent factors affecting the occurrence of foot ulcers, which was consistent with the results of previous studies.[27,28] Further analysis showed that the levels of miR-155 in the peripheral blood and wound margin tissue of the DFU group were significantly higher than that of the T2DM group; the expression level of miR-155 in both peripheral blood and wound margin tissue of the DFU group was positively correlated with the course of foot ulcer and Wagner grade, and negatively correlated with the healing rate of foot ulcer after 8 weeks, the higher the expression of miR-155, the more difficult and longer the time required for complete healing of DFU wound. Multiple regression analysis showed that the high expression of miR-155 in both peripheral blood and wound margin tissue was an independent risk factor for foot ulcer. These results suggested that miR-155 may be involved in the occurrence of diabetic foot ulcers and can be used as a biomarker for the severity and the prognosis of DFU.

At present, the mechanism of miR-155 involved in wound healing has not been fully clarified. It is widely believed that the persistent and excessive inflammatory state in the wound and the functional impairment of a variety of cells in the epidermis involved in wound healing are important factors in the difficulty of DFU healing.[3,29] Studies[12] show that the expression of miR-155 is increased in skin wounds of diabetic rats. The up regulation of miR-155 not only plays a role in promoting inflammation but also can affect the migration and proliferation of keratinocytes and damage the re-epithelialization of wounds by down-regulating the expression of fibroblast growth factor-7. However, after the knockout of miR-155, M1-like macrophages in wound tissue of mice decrease, M2-like macrophages and type I collagen deposition significantly increase, wound inflammation is alleviated, and repairability is enhanced.[30] Another study also found that knocking down miR-155 in mice can also decrease the expression of inflammatory cytokines Th17/Th9 in wound tissue and promote wound recovery.[14] In addition, in the skin wound model of diabetic rats, after local injection of

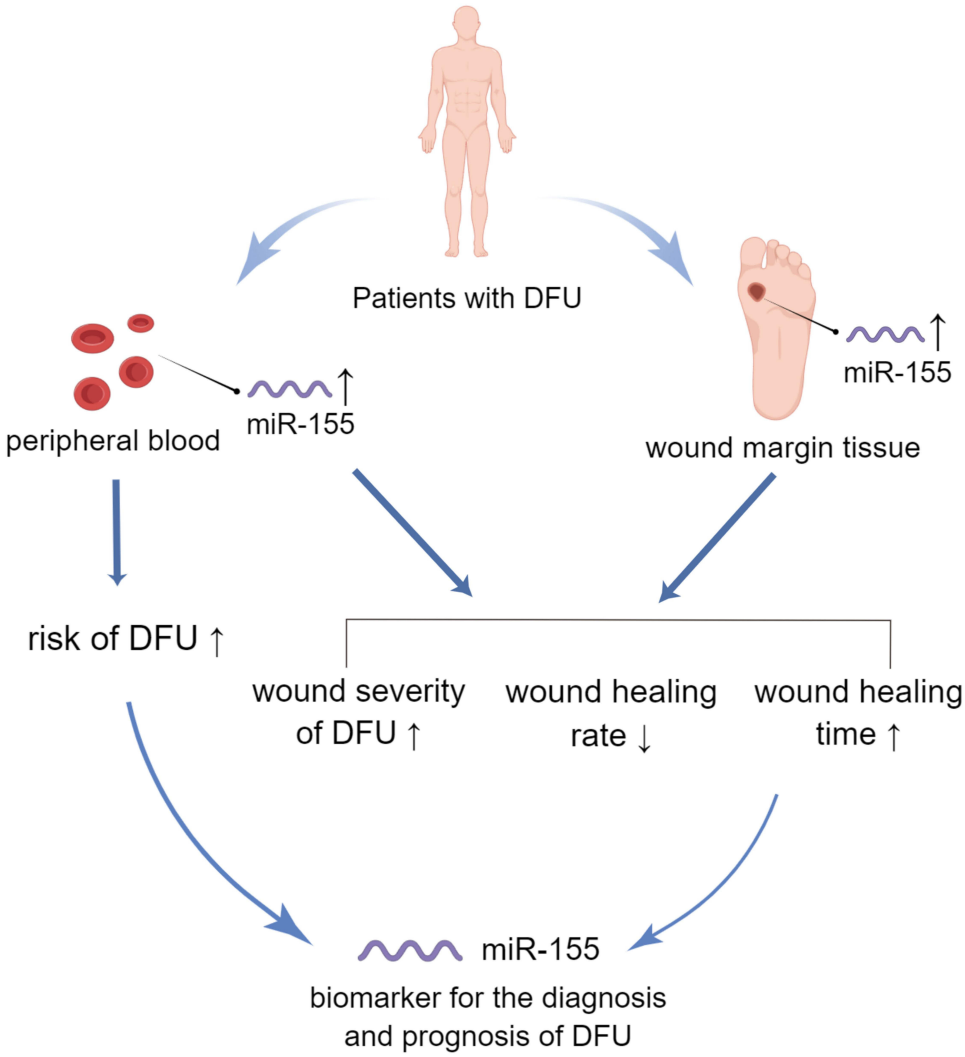


**Figure 3** MiR-155 is a potentially valuable biomarker for diagnosis and prognosis of DFU. ↑: increase; ↓: decrease.

miR-155 inhibitor interferes with miR-155 expression, the number of inflammatory cells, such as T lymphocytes, neutrophils, and macrophages in wound tissue decrease, the levels of interleukin-1β and tumor necrosis factor-α decrease, the inflammatory reaction is alleviated, neovascularization significantly increases, the collagen content in granulation tissue increases and is arranged more regularly, and wound healing is accelerated.[12,13] Furthermore, angiogenesis in wound tissue plays an important role in DFU wound healing and endothelial progenitor cells (EPC) migrate to the peripheral circulation and differentiate into mature cells to participate in angiogenesis.[31] Studies show that miR-155 in M1-like macrophage-derived exosomes can reduce the angiogenesis of endothelial cells,[32] and target PTCH1 to mediate EPC dysfunction caused by high glucose.[33]

It turned out that the expression level of exosomal miR-155 in peripheral blood could be used as a non-invasive biomarker for the diagnosis and progression of hepatic fibrosis[34] as well as a potential biomarker for the detection of lung cancer.[35] In the present study, according to the results of ROC curve analysis, we also found that the expression level of miR-155 in peripheral blood of T2DM patients could serve as a potential biomarker for the prediction DFU. Moreover, we also discovered that the expression level of miR-155 in both peripheral blood and wound margin tissue was positively correlated with the course of DFU, and negatively correlated with the healing rate of DFU after eight weeks and the complete healing time. Therefore, the abovementioned results suggested the functionality of the expression level of miR-155 in both peripheral blood and wound margin tissue for the diagnosis and prognosis of DFU. Nonetheless, further

studies are needed to identify the reasons for the increased expression of miR-155 in both peripheral blood and wound margin tissue of patients with DFU. Significantly, in the present study, miR-155 in peripheral blood and in wound margin tissue exhibited a good consistency in terms of their expression and predictive value for wound healing. Sampling of peripheral blood carries a small risk of trauma, and the determination of miR-155 in peripheral blood is relatively simple and convenient. Therefore, based on our findings and previous studies,[36] our research group recommends that the treatment outcome of DFU can be predicted by detecting the expression of miR-155 in peripheral blood.

## Conclusion

This study finds that the increased level of miR-155 expression in peripheral blood of type 2 diabetes patients is closely related to the occurrence of DFU, and can be a biomarker for diagnosis of DFU (Figure 3). In addition, the increased level of miR-155 expression in peripheral blood and wound margin tissue is closely related to the poor prognosis of DFU. The shortcomings of this study include that it is a single-center study, the sample size is relatively small, and selection bias may exist. Therefore, further studies are needed to confirm these findings. In addition, this study also cannot clarify the causal relationship between miR-155 and DFU. In the future, we need to further explore the mechanism of action of miR-155 and evaluate whether miR-155 can become a new therapeutic target for DFU.

## Abbreviations

T2DM, type 2 diabetes mellitus; DFU, diabetic foot ulcer; AUC, area under the curves; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin A1c; TCH, total cholesterol; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALB, serum albumin; Hb, hemoglobin; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TcPO2, transcutaneous oxygen pressure; ABI, ankle brachial index; qRT-PCR, quantitative real-time PCR assays.

## Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Inquiries for data access may be sent to the following e-mail address: chmw1@163.com.

## Ethical Approval

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Anhui Medical University, and we obtained the informed consent of the subjects. All procedures were performed in studies involving human participants in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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