Association between the duration of diabetes and gram-negative bacterial infection in diabetic foot infections: a case-control study

Siyong Chen¹, Xianpei Tan², Xia Li¹ and Tongfeng Zhao¹

¹Department of Endocrinology, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, 510655, China
²Department of Neurology, Jingzhou First People’s Hospital, The First Affiliated Hospital of Yangtze University, Jingzhou, 434000, China

Abstract. This retrospective case-control study was designed to explore the association between the duration of diabetes and gram-negative bacterial infection in diabetic foot infections (DFIs). All DFI patients hospitalized in the Department of Endocrinology in the Sixth Affiliated Hospital of Sun Yat-sen University between 2013 and 2019 with positive microbial culture results were included. Cases were defined as DFI patients whose microbial cultures grew gram-negative bacteria (including polymicrobial flora). Controls were defined as DFI patients whose positive microbial cultures did not grow gram-negative bacteria. Clinical data were extracted from the hospital information system. Stabilized inverse probability weighting was used to balance between-group differences at baseline. Confounders were selected using a directed acyclic graph. Missing data were imputed with the multiple imputation of chained equations method. Odds ratios (ORs) with 95% confidence intervals (CIs) and \( P_{\text{trend}} \) for associations between the duration of diabetes and gram-negative bacterial infection were obtained using binomial logistic regression models. The weighted OR of gram-negative bacterial infection for DFI patients with a moderate duration of diabetes (8~19 years) compared with those with a short duration (0~7 years) was 3.87 (95% CI: 1.15 to 13.07), and the OR for those with a longer duration (20~30 + years) was 7.70 (95% CI: 1.45 to 41.00), and there was a dose-response trend with increasing duration of diabetes (weighted \( P_{\text{trend}} = 0.007 \)). The results demonstrated that a long duration of diabetes might be associated with an increased risk of gram-negative bacterial infection in type 2 diabetes patients with DFI.

Key words: Diabetic foot infection, Duration of diabetes, Gram-negative bacteria

DIABETIC FOOT has now become a heavy burden all over the world, and people with diabetes have an approximately 25% chance of developing a foot ulcer in their lifetime [1, 2]. Diabetic foot infection (DFI) can complicate diabetic foot ulcers (DFUs) and increase the risk of lower extremity amputation by 50% [3, 4]. However, before the microbial culture results are obtained, clinicians can only carry out empirical antimicrobial therapy to manage DFIs. Evaluating the distribution of microbes in DFIs is a key step to initiate an effective antimicrobial therapy. Previous studies showed that an assessment of the distribution of pathogens in DFIs should include data on prior use of antibiotics in the past month, the depth and duration of the ulcer, and the severity of the infection [5, 6]. In addition to the factors above, we found that in our clinical work, DFI patients with long duration of diabetes seemed to be more likely to have gram-negative bacterial infections. However, we did not find any studies that have addressed this issue.

For this reason, we retrospectively analyzed the data of DFI patients hospitalized in the Department of Endocrinology, the Sixth Affiliated Hospital of Sun Yat-sen University between 2013 and 2019 to explore the association between the duration of diabetes and gram-negative bacterial infections in DFIs.

Materials and Methods

Study design and population

This retrospective case-control study was performed at the Sixth Affiliated Hospital of Sun Yat-sen University in 2019. The study was approved by the Ethics Committee of the Sixth Affiliated Hospital of Sun Yat-sen University. The ethical committee’s reference number is E201906. All DFI patients hospitalized in the Department of Endocrinology between 2013 and 2019 with positive...
microbial culture results before receiving antimicrobial therapy administered in the hospital were included in this study. Patients with venous stasis ulcers, non-type 2 diabetes or non-Han ethnicity were excluded. Cases were defined as DFI patients whose microbial cultures grew gram-negative bacteria (including polymicrobial flora). Controls were defined as DFI patients whose positive microbial cultures did not grow gram-negative bacteria.

Data sources and definitions
In this study, clinical variables including age, sex, antibiotic use in the past month, duration of ulcer, HbA1c, smoking, alcoholism, hypertension, respiratory failure, duration of diabetes, CKD (chronic kidney disease), dialysis, statin use, antihyperglycemic agent use and ASCVD (atherosclerotic cardiovascular disease) were extracted from the hospital information system of the Sixth Affiliated Hospital of Sun Yat-sen University.

DFI was defined by the presence of at least two of the following indicators: redness, warmth, pain or tenderness, induration or purulent secretions [7, 8].
The duration of diabetes was defined as known years of diabetes. As some durations of diabetes were recorded as 10+ years or 20+ years, we classified them into groups defined by years of duration as follows: 0–7 (40%), 8–19 (40%) and 20–30 + (20%).

Hypertension was defined as any type of hypertension except those secondary to diabetic nephropathy to estimate the total effect of the duration of diabetes.

Chronic ulcer was defined as an ulcer with a duration >4 weeks [3].

Microbiologic tests
According to the standard operating procedure of our department, the wounds were debrided and cleansed by a specially trained nurse practitioner, and then all the specimens were collected with sterile cotton swab, kept in a sterile tube, and sent to the microbiology laboratory. All isolates underwent phenotypic identification using the VITEK® 2 microbial identification system (bioMérieux, Marcy l’Etoile, France) according to the manufacturer’s instructions.

Statistical analysis
Stabilized inverse probability weighting based on the probability of gram-negative bacterial infection (excluding duration of diabetes) was used to balance between-group differences at baseline [9]. The probability was estimated using multivariable logistic regression that adjusted for baseline characteristics including demographics, known exposures that affect microbial distribution (antibiotic use in the past month and duration of ulcer) and some potential confounders due to their potential effect on metabolism (smoking, alcoholism, HbA1c, hypertension and respiratory failure). To estimate the total effect of duration of diabetes on risk of gram-negative bacterial infection, intermediate factors (statin use, antihyperglycemic agent use, CKD, dialysis and ASCVD) were excluded. Confounder selection is illustrated in a directed acyclic graph (Supplementary Fig. 1). Between-group differences in baseline characteristics were compared using standardized mean differences (SMDs), and differences <10% were considered small differences [10]. Odds ratios (ORs) with 95% confidence intervals (CIs) and P values for associations between duration of diabetes and gram-negative bacterial infection in type 2 diabetes patients with DFI were obtained using binomial logistic regression models.

All variables in this study were complete except for ASCVD (3.5% missing, not included in multivariable logistic regression models) and HbA1c (10.5% missing, missing data were imputed with the multiple imputation of chained equations method using the baseline characteristics) [11].

Four sensitivity analyses were conducted to assess the robustness of the main results: (1) an analysis in which duration of diabetes (in years) was reclassified which duration of diabetes (in years) was reclassified into groups as follows: 0–9 (50%), 10–19 (30%) and 20–30 + (20%); (2~4) three analyses using multivariable logistic regression models including confounders that changed the adjusted OR by more than 10% (HbA1c and chronic ulcer were each excluded from the model separately and then both were excluded. They may be considered as intermediate factors since a long duration of diabetes could result in worsening beta cell function and nonhealing ulcers. HbA1c depended more on the patient’s treatment, while duration of ulcer depended more on admission time).

All statistical tests were 2-sided, and p < 0.05 was considered statistically significant. Statistical analyses were conducted using SPSS Statistics software for mac version 26.0 (IBM, Armonk, NY, USA).

Standardized differences in quantitative data were calculated by
\[
d = \frac{|\bar{Z}_{\text{case}} - \bar{Z}_{\text{control}}|}{\sqrt{s_{\text{case}}^2 + s_{\text{control}}^2}}
\]
and in qualitative data, they were calculated by
\[
d = \frac{|p_{\text{case}} - p_{\text{control}}|}{\sqrt{(p_{\text{case}}(1-p_{\text{case}}))(1-p_{\text{control}})}}
\]
Pooled standard deviations were calculated by
\[
S = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2 + \cdots + (n_k - 1)S_k^2}{n_1 + n_2 + \cdots + n_k - 1}}
\]
Results

The study included 30 cases and 27 controls, all from Guangzhou and the cities nearby, aged from 40 to 92 years, with the duration of diabetes ranging from 1 to 30+ years. Table 1 shows the baseline characteristics of the study population. Cases were more likely to require dialysis and insulin therapy, have a history of antibiotic use in the past month and have worse glycemic control in the past three months.

Table 2 shows that the weighted OR of gram-negative bacterial infection for DFI patients with a moderate duration of diabetes (8-19 years) compared with those with a short duration (0-7 years) was 3.87 (95% CI: 1.15 to 13.07), and the OR for those with a longer duration (20-30+ years) was 7.70 (95% CI: 1.45 to 41.00), and there was a dose-response trend with increasing duration of diabetes (weighted $P_{\text{trend}} = 0.007$).

The sensitivity analyses (Supplementary Tables 1 and 2) also found that a long duration of diabetes was associated with increased risk of gram-negative bacterial infection in type 2 diabetes patients with DFI, and there was still a dose-response trend.

Discussion

In this study, we observed that a long duration of diabetes was associated with an increased risk of gram-negative bacterial infection in type 2 diabetes patients with DFI, and there was a dose-response trend. If replicated in other populations, the duration of diabetes could be used to evaluate the distribution of microbes in DFI. Our prediction of pathogenic bacteria might be more accurate if assessed in conjunction with the duration of diabetes and known risk factors for gram-negative bacterial infections, such as antibiotic use in the past month and ulcer depth, and we might be able to further improve the effectiveness of empirical anti-infective therapy in DFI.

Table 1  Baseline characteristics

| Characteristics          | Observed data | IPW data |
|--------------------------|---------------|----------|
|                          | Cases ($n = 30$) | Controls ($n = 27$) | SMD (%) | Cases ($n = 30$) | Controls ($n = 27$) | SMD (%) |
| Male                     | 16 (0.53)     | 13 (0.48)  | 10.4  | 16.4 (0.54)     | 14 (0.51)    | 5.7     |
| Age, mean (SD), y        | 71.53 (11.72) | 72.44 (13.29) | 7.3   | 71.06 (10.88)   | 69.86 (14.00) | 9.6     |
| Antibiotics use          | 23 (0.77)     | 15 (0.56)  | 45.8  | 20 (0.67)       | 19 (0.68)    | 2.5     |
| Chronic ulcer            | 10 (0.33)     | 10 (0.37)  | 7.8   | 10.4 (0.34)     | 9.8 (0.36)   | 2.7     |
| Smoking                  |               |           |       |               |             |         |
| Yes                      | 4 (0.13)      | 5 (0.19)  | 14.2  | 6.2 (0.21)      | 4.6 (0.17)   | 9.6     |
| Past                     | 2 (0.07)      | 2 (0.07)  | 3.0   | 2 (0.07)       | 2 (0.07)    | 2.7     |
| No                       | 24 (0.80)     | 20 (0.74) | 14.1  | 22 (0.73)      | 20.8 (0.76)  | 7.0     |
| Alcoholism               | 2 (0.07)      | 1 (0.04)  | 13.4  | 1.2 (0.04)      | 1 (0.04)    | 1.7     |
| HbA1c, mean (SD), %      | 9.10 (3.05)   | 8.58 (2.57) | 18.4  | 8.66 (2.94)    | 8.62 (2.41)  | 1.5     |
| Hypertension             | 19 (0.63)     | 20 (0.74) | 23.3  | 20.8 (0.68)    | 17.2 (0.64)  | 8.1     |
| Respiratory failure      | 0 (0)         | 0 (0)     | 0     | 0 (0)          | 0 (0)       | 0       |
| CKD                      | 18 (0.60)     | 21 (0.78) | 39.1  |               |             |         |
| Dialysis                 | 7 (0.23)      | 2 (0.07)  | 45.3  |               |             |         |
| Statin use               | 11 (0.37)     | 8 (0.30)  | 15.0  |               |             |         |
| Antihyperglycemic agent use |           |           |       |               |             |         |
| None                     | 5 (0.17)      | 5 (0/19)  | 4.9   |               |             |         |
| Oral                     | 11 (0.37)     | 12 (0.44) | 15.9  |               |             |         |
| Oral + insulin           | 1 (0.03)      | 3 (0.11)  | 30.4  |               |             |         |
| Insulin                  | 13 (0.43)     | 7 (0.26)  | 37.2  |               |             |         |
| ASCVD                    | 18 (0.64)     | 19 (0.70) | 13.0  |               |             |         |
| unknown                  | 2 (0.07)      | 0 (0)    |       |               |             |         |

Abbreviations: IPW, inverse probability weighted; SD, standard deviation; SMD, standardized mean difference; CKD, chronic kidney disease; ASCVD, atherosclerotic cardiovascular disease, including acute coronary syndromes, myocardial infarction, stable angina, coronary/other arterial revascularization, stroke, transient ischemic attack, and peripheral arterial disease of atherosclerotic origin.
To our knowledge, this study is the first to assess the association between duration of diabetes and the risk of gram-negative bacterial infection in DFI. The finding observed in our study is supported by some associations shown in prior studies; for example, chronic kidney disease and peripheral arterial disease, which could be long duration associated complications of diabetes, were associated with a higher incidence of healing failure [12-14], and in deep and nonhealing ulcers, infections were more frequently polymicrobial and the causative pathogens often included gram-negative bacteria [7, 15]. However, the mechanism behind the association is still unclear, and further research is needed.

The study has several strengths. First, confounders in this study were cautiously selected using a directed acyclic graph which ensured that intermediate factors were excluded and helped to better estimate the total effect of the duration of diabetes. Second, several sensitivity analyses were conducted in this study to further support the robustness of our findings.

The study has a few limitations. First, it is a single-center, small sample study. Patients with less severe infections that did not require hospitalization may be less likely to be included in this study. The bacteriology of DFI in other areas may be different from that in Guangzhou [16, 17]. Therefore, further studies are needed to confirm our observations in other areas and populations. Second, due to the limitation of laboratory conditions, our study lacked the results of anaerobic culture. However, studies have shown that an antibiotic regimen aimed at anaerobes is required only when there is strong clinical evidence of anaerobic infection, i.e., the classic ‘fetid foot’ [18, 19]. To treat most of these potential pathogens, complete debridement is enough. Third, swab cultures that we used in the study are considered not as reliable as tissue biopsy, although they are of value, cleansing and debridement may increase their accuracy [20, 21]. Therefore, further studies using tissue biopsy are still needed to support our observation. Fourth, recall bias and the latency of the onset of type 2 diabetes made the duration of diabetes prone to be misclassified. However, we do not have evidence to support that misclassification occurred differently for cases and controls. If the misclassification was nondifferential by case-control status, a long duration of diabetes may actually have a more significant effect increasing risk of gram-negative bacterial infection in DFI patients than that currently observed in this study.

In summary, this study provides evidence that a long duration of diabetes might be associated with an increased risk of gram-negative bacterial infection in type 2 diabetes patients with DFI. This finding should be replicated in other populations to confirm our observation and further establish the potential value in evaluating the distribution of microbes in DFI.

### Disclosure Statement

The authors declare that they have no conflicts of interest.

### Disclaimers

The views expressed in the article are our own and not an official position of our hospital or university.

### Source(s) of Support

We did not receive any grants, equipment, drugs, or other support that facilitated conduct of the work described in the article or the writing of the article.
References

1. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, et al. (2006) Risk factors for foot infections in individuals with diabetes. *Diabetes Care* 29: 1288–1293.
2. Singh N, Armstrong DG, Lipsky BA (2005) Preventing foot ulcers in patients with diabetes. *JAMA* 293: 217–228.
3. Adam KM, Mahmoud SM, Mahadi SI, Widatalla AH, Shawer MA, et al. (2011) Extended leg infection of diabetic foot ulcers: risk factors and outcome. *J Wound Care* 20: 440–444.
4. van Battum P, Schaper N, Prompers L, Apelqvist J, Jude E, et al. (2011) Differences in minor amputation rate in diabetic foot disease throughout Europe are in part explained by differences in disease severity at presentation. *Diabet Med* 28: 199–205.
5. Wheat LJ, Allen SD, Henry M, Kernek CB, Siders JA, et al. (1986) Diabetic foot infections. Bacteriologic analysis. *Arch Intern Med* 146: 1935–1940.
6. Lipsky BA, Pecoraro RE, Wheat LJ (1990) The diabetic foot. Soft tissue and bone infection. *Infect Dis Clin North Am* 4: 409–432.
7. Lipsky BA, Berent AR, Cornia PB, Pile JC, Peters EJ, et al. (2012) 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 54: e132–e173.
8. Schaper NC (2004) Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. *Diabetes Metab Res Rev* 20 Suppl 1: S90–S95.
9. Brookhart MA, Wyss R, Layton JB, Sturmer T (2013) Propensity score methods for confounding control in non-experimental research. *Circ Cardiovasc Qual Outcomes* 6: 604–611.
10. Austin PC, Stuart EA (2015) Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. *Stat Med* 34: 3661–3679.
11. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, et al. (2009) Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ* 338: b2393.
12. Mills JL Sr, Conte MS, Armstrong DG, Pommerselli FB, Schanzer A, et al. (2014) The Society for Vascular Surgery Lower Extremity Threatened Limb Classification System: risk stratification based on wound, ischemia, and foot infection (WIF). *J Vasc Surg* 59: 220–234 e1–2.
13. He Y, Qian H, Xu L, Zhang S, Gu X, et al. (2017) Association between estimated glomerular filtration rate and outcomes in patients with diabetic foot ulcers: a 3-year follow-up study. *Eur J Endocrinol* 177: 41–50.
14. Prompers L, Schaper N, Apelqvist J, Edmonds M, Jude E, et al. (2008) Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. *Diabetologia* 51: 747–755.
15. Uckay I, Gariani K, Pataky Z, Lipsky BA (2014) Diabetic foot infections: state-of-the-art. *Diabetes Obes Metab* 16: 305–316.
16. Bansal E, Garg A, Bhatia S, Attri AK, Chander J (2008) Spectrum of microbial flora in diabetic foot ulcers. *Indian J Pathol Microbiol* 51: 204–208.
17. Yoga R, Khairul A, Sunita K, Suresh C (2006) Bacteriology of diabetic foot lesions. *Med J Malaysia* 61 Suppl A: 14–16.
18. Fierer J, Daniel D, Davis C (1979) The fetid foot: lower-extremity infections in patients with diabetes mellitus. *Rev Infect Dis* 1: 210–217.
19. Lipsky BA (2007) Empirical therapy for diabetic foot infections: are there clinical clues to guide antibiotic selection? *Clin Microbiol Infect* 13: 351–353.
20. Huang Y, Cao Y, Zou M, Luo X, Jiang Y, et al. (2016) A comparison of tissue versus swab culturing of infected diabetic foot wounds. *Int J Endocrinol* 2016: 8198714.
21. Xie X, Bao Y, Ni L, Liu D, Niu S, et al. (2017) Bacterial profile and antibiotic resistance in patients with diabetic foot ulcer in Guangzhou, Southern China: focus on the differences among different Wagner’s grades, IDSA/IWGDF grades, and ulcer types. *Int J Endocrinol* 2017: 8694903.