



# Metagenomics Next Generation Sequencing Differentiates Infected Versus Non-Infected Diabetic Foot Ulcers

Brian M. Schmidt, DPM<sup>1</sup>, Yiyuan Huang MS<sup>2</sup>, Crystal M Holmes DPM CWSP<sup>1</sup>, Piyush Ranjan<sup>3</sup>, John Erb-Downward<sup>3</sup>, Mousumi Banerjee PhD<sup>2</sup>, Rodica Pop-Busui MD PhD<sup>1</sup>, and Robert Dickson, MD<sup>3</sup>  
(1) University of Michigan, Ann Arbor, MI, Department of Internal Medicine, Division of Metabolism, Endocrinology & Diabetes, (2) University of Michigan School of Public Health, (3) University of Michigan, Ann Arbor, MI. Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine

## INTRODUCTION

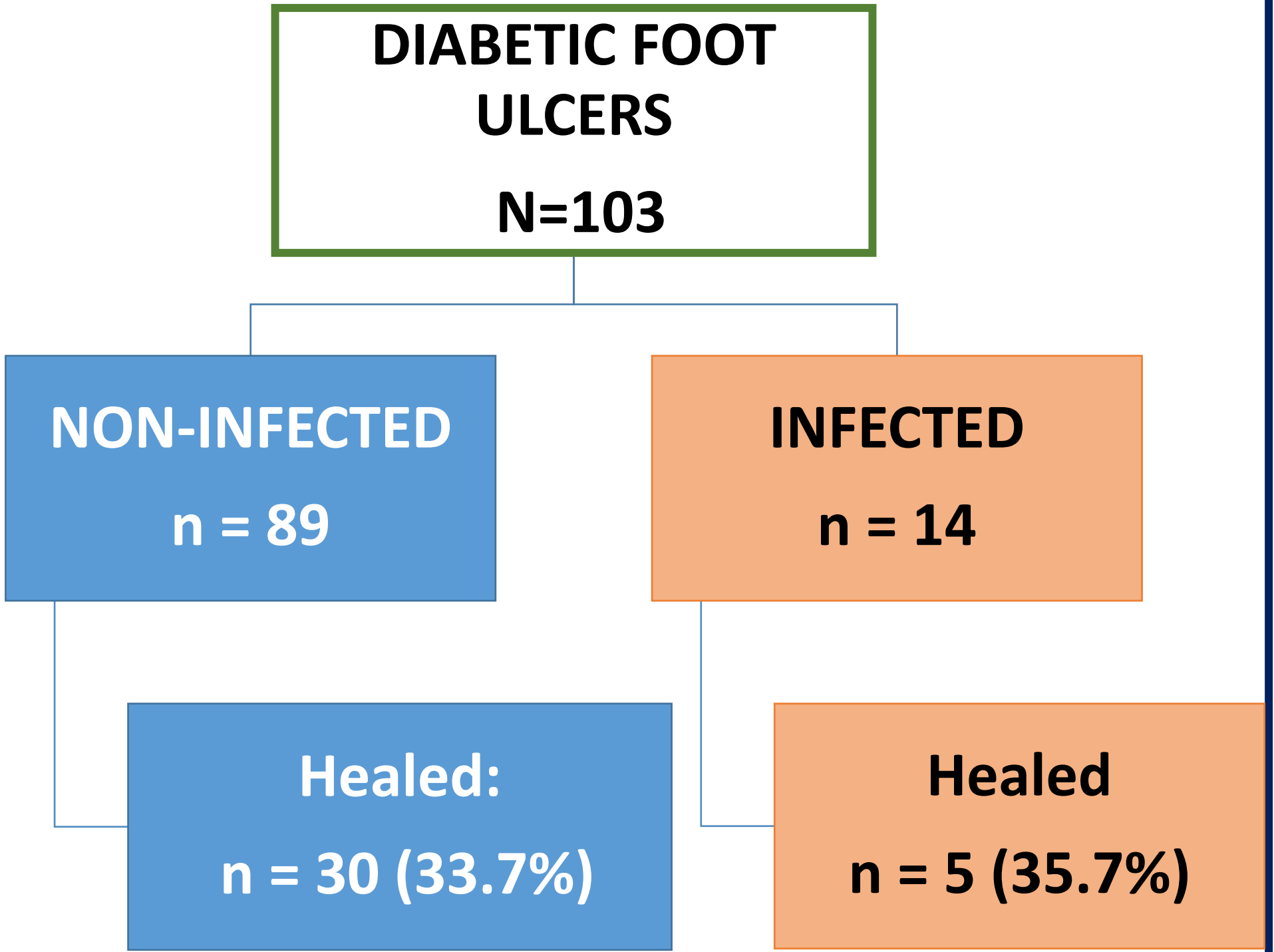
Clinical options to identify diabetic foot ulcer (DFU) infection, which constitute the most frequent diabetes-related cause of hospitalization, remain limited. This study was undertaken to determine if the use of next generation sequencing to quantify microbial presence in DFU infection, is an effective strategy to identify clinical infection.

## METHODS

- Descriptive Cohort Analysis**
- Who:** Individuals enrolled into NIDDK Diabetic Foot Consortium's Biomarkers for Active Diabetic Foot Ulcer protocol (Clinicaltrials.gov Identifier: NCT06104969) with clinically non-infected vs. clinically infected DFU
- Follow-up period:** Up to one year from enrollment
- Aims:**
  - Describe patient characteristics in DFU infection
  - Describe microbial community characteristics in infected and non-infected DFU using nanopore next generation sequencing techniques

## RESULTS

### BREAKDOWN OF PATIENTS



## RESULTS

### CLINICAL CHARACTERISTICS OF 103 OUTPATIENTS WITH DIABETIC FOOT ULCER +/- INFECTION

*Case mix and severity of illness were similar between the infected and non-infected groups*

	Non-Infected (n=89)	Infected (n=14)	p-value
Demographics and Select Patient Characteristics			
Age (years)	57.8 ± 12.5	57.0 ± 19.5	0.85
Male sex, n (%)	69 (78)	11 (79)	0.93
Race			
White, n (%)	64 (72)	12 (86)	0.65
T1DM , n (%)	10 (11.2)	3 (21.4)	0.29
BMI (mean, SD)	36.9 ±10.4	32.8 ± 9.4	0.16
HbA1c (mean, SD)	8.1 ± 1.8	8.3 ± 2.6	0.57
Affected Foot ABI	1.05 (0.24)	1.08 (0.09)	0.70
CKD, n (%)	41 (46)	7 (50)	0.78
CAD, n (%)	20 (22.7)	2 (14.3)	0.48
HTN, n (%)	82 (92.1)	12 (85.7)	0.43
MNSI, Total Score	5.9 (1.7)	5.6 (2.5)	0.46
Wound Characteristics			
Length (in cm)	1.9 ± 1.9	2.5 ± 2.9	0.37
Width (in cm)	1.7 ± 1.2	1.7 ± 1.8	0.97
Depth (in cm)	0.5 ± 0.4	0.9 ± 0.9	<0.01

HbA1c, Hemoglobin A1c; BMI, body mass index; CKD, chronic kidney disease; ABI, Ankle Brachial Index; MNSI, Michigan Neuropathy Screening Instrument; HTN, Hypertension

## CONCLUSION

The infected participants had **higher Gram-positive bacterial and fungi relative abundances** at baseline, and this was distinct from individuals without infection. This method supports the use of next generation sequencing as a feasible tool to quantify and identify infection in DFU.

## RESULTS

### NEXT GEN SEQUENCING CHARACTERISTICS

	Non-infected (n=89)	Infected (n=14)	Total (n=103)	p-value
Gram stain				0.231
0 (Gram positive)	60 (89.6%)	6 (75.0%)	66 (88.0%)	
1 (Gram negative)	7 (10.4%)	2 (25.0%)	9 (12.0%)	
NGS Identified Bacteria, n (% yes)				0.31
1	83 (93.3%)	14 (100.0%)	97 (94.2%)	
NGS Bacterial Species Counts				0.82
Mean (SD)	5.136 (3.350)	4.929 (3.174)	5.108 (3.312)	
Relative Abundance for first most abundant organism, %				0.10
Mean (SD)	52.398 (29.195)	70.463 (20.804)	55.231 (28.646)	
Fungi Present				< 0.001
1	0 (0.0%)	2 (15.4%)	2 (2.2%)	
Gram Positive Abundance, %				0.03
Mean (SD)	56.717 (33.460)	76.600 (27.669)	59.419 (33.322)	
Gram Negative Abundance, %				0.78
Mean (SD)	21.785 (26.183)	19.807 (21.830)	21.517 (25.547)	
Aerobic Abundance, %				0.77
Mean (SD)	20.112 (25.708)	22.321 (29.143)	20.413 (26.058)	
Anaerobic Abundance, %				0.16
Mean (SD)	10.794 (14.921)	17.514 (26.611)	11.708 (16.961)	
Facultative Anaerobic Abundance, %				0.24
Mean (SD)	60.552 (32.774)	71.521 (31.250)	62.043 (32.641)	
Obligate Anaerobe Abundance, %				0.64
Mean (SD)	2.355 (5.909)	1.571 (5.080)	2.249 (5.786)	
Gram Positive Count				0.76
Mean (SD)	3.730 (2.687)	3.500 (2.210)	3.699 (2.619)	
Gram Negative Count				0.93
Mean (SD)	1.393 (1.542)	1.357 (1.151)	1.388 (1.490)	
Aerobic Count				0.81
Mean (SD)	0.989 (0.923)	0.929 (0.829)	0.981 (0.907)	
Anaerobic Count				0.44
Mean (SD)	1.348 (1.631)	1.714 (1.939)	1.398 (1.671)	
Facultative Anaerobic Count				0.23
Mean (SD)	3.011 (1.748)	2.429 (1.158)	2.932 (1.688)	
Obligate Anaerobe Count				0.44
Mean (SD)	0.360 (0.661)	0.214 (0.579)	0.340 (0.650)	

This study was supported by **K23DK131261 (BMS) & U01DK119083 (BMS, CHM, RPB)** from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).