Diabetic Complications Consortium

Application Title: Prognostic Imaging and Blood Markers of Wound Healing Among Patients with Diabetic Foot Ulcers

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1. Project Accomplishments:

The objective of this proposal is to establish and translate novel noninvasive imaging methods and blood markers prognostic of incomplete wound healing in the setting of diabetic foot ulcers. We have completed enrollment and study visits for our study cohort and met projected enrollment numbers: 10 patients with diabetes and foot ulcer (DFU), 5 matched patients with diabetes and no foot ulcer (DP), and 5 matched controls with no diabetes (HC). All study volunteers completed MRI and blood draws. We obtained follow-up visit endpoints of wound size/resolution out to 12 weeks after the initial study visit in all DFU patients.

2. Specific Aims:

Specific Aim 1. Establish MRI measurements to characterize microcirculatory abnormalities in DFU.

We adapted our previous MRI protocols used for perfusion measurements in calf and thigh skeletal muscle studies to this study for imaging in the foot. We piloted both resting perfusion and reactive hyperemia MRI quantification to ensure image quality and minimize motion artifacts. Resting perfusion was measured using a diffusion weighted MRI sequence consisting of multiple low diffusion weighted images to capture molecular motions of microvascular blood flow and of cellular water diffusion within the muscle cells. We applied the bi-exponential intravoxel incoherent motion (IVIM) signal model quantify model (**Figure 1**) parameters of perfusion fraction (fp), pseudodiffusion coefficient (D*), and tissue water diffusion (D). We also applied the fractional Fickian diffusion (FFD) signal model, recently developed in our lab, to estimate the microvascular volume fraction (MVF).

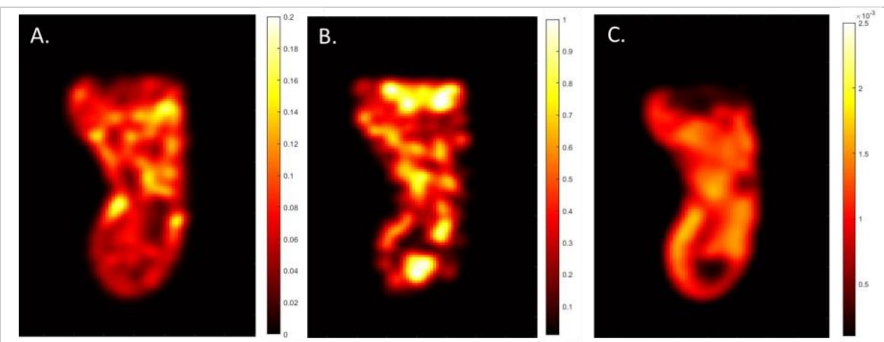

Figure 1. *Representative resting perfusion based on IVIM maps from a slice in the plantar region showing **(A.)** perfusion fraction, fp **(B.)** pseudo-diffusion coefficient, D* and **(C.)** mean diffusivity, D.*

We measured microvascular reserve capacity using a dynamic quantitative T2* MRI procedure, a form of quantitative blood oxygenation level dependent (BOLD) imaging (**Figure 2**). For BOLD imaging, we dynamically collected multi-echo gradient echo images with full coverage of the foot and time resolution of one volume per second for a total of 700 seconds. Dynamic imaging was collected during a reactive hyperemia protocol consisted of 100 seconds at baseline resting perfusion, 300 seconds of ischemia with a pneumatic cuff inflated around the ankle to 50 mmHg above systolic blood pressure, followed by 300 seconds of reperfusion after the pneumatic cuff was depressurized. T2* values, representing the oxygenation of blood in the microvasculature, were mapped using the multi-echo images and quantified over the 700 dynamics.

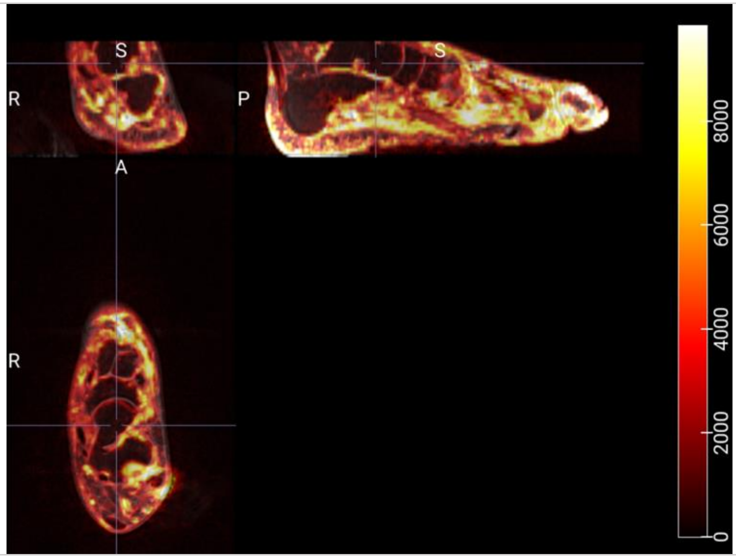

Figure 2. *Representative volumetric reconstruction of BOLD MRI image contrast from a single time point (dynamic) in a DFU patient.*

We developed a procedure for segmenting the foot into respective plantar regions based on the foot angiosomes, for analysis of IVIM, FFD, and BOLD perfusion images. We wrote an in-house MATLAB code that allows for interactive user-selection of anatomical landmarks of the foot to guide segmentation into regions of interest. These masked regions of interest are directly applied to quantitative perfusion maps produced by IVIM and T2* analysis. Ongoing work is focused on improving the pre-processing of these MRI data. Motion artifacts are inevitable particularly with the long scan times for dynamic T2* imaging. There are also distortion artifacts that can degrade data quality. Current work is focused on developing workflows that robustly identify artifacts and mitigate them to improve data quality.

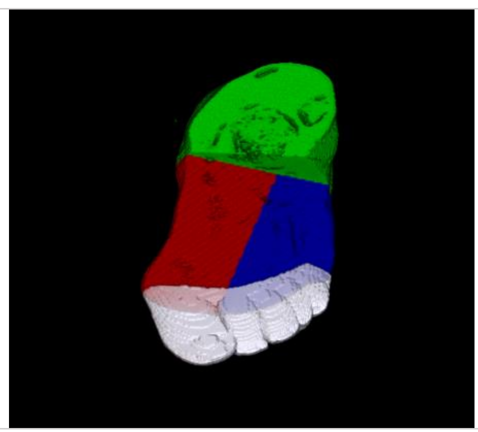
Figure 3. *Rendered plantar view of angiosome segmentation showing forefoot regions of interest used for diffusion-weighted analysis: heel region (green), toes region (white), medial plantar (blue) and lateral plantar (red). Representative IVIM maps from a slice in the plantar region showing*

We have processed resting perfusion data from a subset of DFU (n=5) and HC (n=5) subjects that exhibited sufficient data quality without more extensive pre-processing and artifact mitigation steps. Subject characteristics are displayed in Table 1, showing these subjects to be well-matched by age and BMI.

Table 1. *Study participant characteristics for preliminary resting perfusion analysis.*

	Diabetic Foot Ulcer (n = 5)	Healthy Control (n = 5)	p-value
Age, yrs	59.2 (7.5)	52.8 (7.5)	0.23
Height, cm	176.3 (11.4)	172.7 (10.9)	0.63
Weight, kg	99.5 (18.7)	85.1 (20.4)	0.28
Body mass index, kg/m^2	31.7 (2.0)	28.2 (4.5)	0.17

Data are expressed as mean (standard deviation). Differences between DFU and HC were assessed using Student's t*-tests.*

DFU patients tended to show elevated tissue diffusion and perfusion indices compared with healthy controls. Effect sizes observed in this initial analysis (**Table 2**) support further investigation into the use of diffusion-weighted MRI to forecast likelihood of wound healing in patients with diabetic foot ulcers. Ongoing work is focused on analysing data from the remaining study cohort. Additionally, we will compare resting diffusion and perfusion metrics to longitudinal measures of wound healing and blood markers of inflammation. **Table 2** shows that the largest mean differences between groups occurs in the toes with effect sizes f_p=1, D*=1.29, D=2.79, and MVF=2.28. The medial plantar forefoot also showed large effect sizes with D*=1.52, D=2.26, and MVF=1.15.

Table 2. *Diffusion and perfusion indices derived from diffusion-weighted-MRI analysis. Intravoxel incoherent motion (IVIM) parameters consisted of perfusion fraction (fp), pseudo-diffusion coefficient (D*), and mean diffusion coefficient (D). Fractional Fickian diffusion (FFD) parameters were used to derive microvascular volume fraction (MVF).*

		IVIM			**FFD**
	Foot Region	**f_p (%)**	**$D^* \times 10^2$ (mm^2/s)**	**$D \times 10^3$ (mm^2/s)**	**MVF (%)**
DFU (N=5)	Toes	14.1 (2.5)	2.66 (0.55)	1.14 (0.15)	2.32 (0.56)
	FF Med Plant	13.7 (2.9)	2.82 (0.74)	1.41 (0.17)	2.54 (0.63)
	FF Lat Plant	11.2 (3.9)	2.34 (0.55)	1.23 (0.32)	1.73 (0.95)
HC (N=5)	Toes	10.3 (4.9)	1.75 (0.83)	0.59 (0.24)	0.98 (0.61)
	FF Med Plant	13.3 (2.9)	1.95 (0.32)	1.03 (0.17)	1.87 (0.52)
	FF Lat Plant	10.3 (4.5)	1.78 (0.84)	1.01 (0.09)	1.19 (0.65)
Cohen's d Effect Size	Toes	***1.0***	***1.29***	***2.79***	***2.28***
	FF Med Plant	0.15	***1.52***	***2.26***	***1.15***
	FF Lat Plant	0.23	***0.80***	***0.91***	0.66

Cohen's d effect size is reported instead of significance level; these effect sizes compare mean differences between DFU and HC groups by foot region and MRI parameter, with values in bold signifying a large effect size.

We have processed BOLD MRI perfusion reserve data from a subset of DFU (n=5) and HC (n=5) subjects that exhibited sufficient data quality without more extensive pre-processing and artifact mitigation steps. Subject characteristics are displayed in **Table 3**, showing these subjects to be well-matched by age and BMI.

Table 3. *Study participant characteristics for preliminary reactive hyperemia analysis (HC N=5, DFU N=5)*

	HC	DFU	p-value
Age, yrs	52.80 (8.07)	53.40 (13.67)	0.935
Height, cm	172.72 (10.92)	174.18 (13.23)	0.854
Weight, kg	85.10 (20.40)	86.555 (16.69)	0.905
BMI	28.20 (4.53)	28.46 (3.52)	0.922
Systolic BP, mmHg	121.00 (9.30)	136 (15.85)	0.099
Diastolic BP, mmHg	75.40 (7.99)	86.20 (20.61)	0.323
CO pressure, mmHg	171.00 (9.30)	186 (15.85)	0.099

Values represent mean(std)

DFU patients and healthy controls showed distinctly different BOLD responses to the cuff occlusion protocol (**Figure 4**). DFU patients show a greater decrease in T2* during ischemia and a blunted reperfusion response, compared with healthy controls. **Table 4** shows the T2* in the DFU group dropped significantly lower in the medial plantar regions. There was no difference in the time it took to reach the minimal ischemic value in either region, some subjects reached a minimal value and plateaued while other subjects never plateaued. In both the lateral and medial plantar regions, the DFU patients had a significantly lower maximal reperfusion value and longer time-to-peak than the healthy controls. Additionally, recovery of T2* in the DFU group barely reached baseline values during reperfusion while the healthy controls showed the expected increase above baseline consistent with hyperemic reperfusion of oxygenated blood. Our results indicate blunted microvascular reserve in the patients with DFUs. Ongoing analysis is focused on processing the remaining patient data and improving analysis pipeline and comparison of imaging markers with clinical outcomes of wound healing.

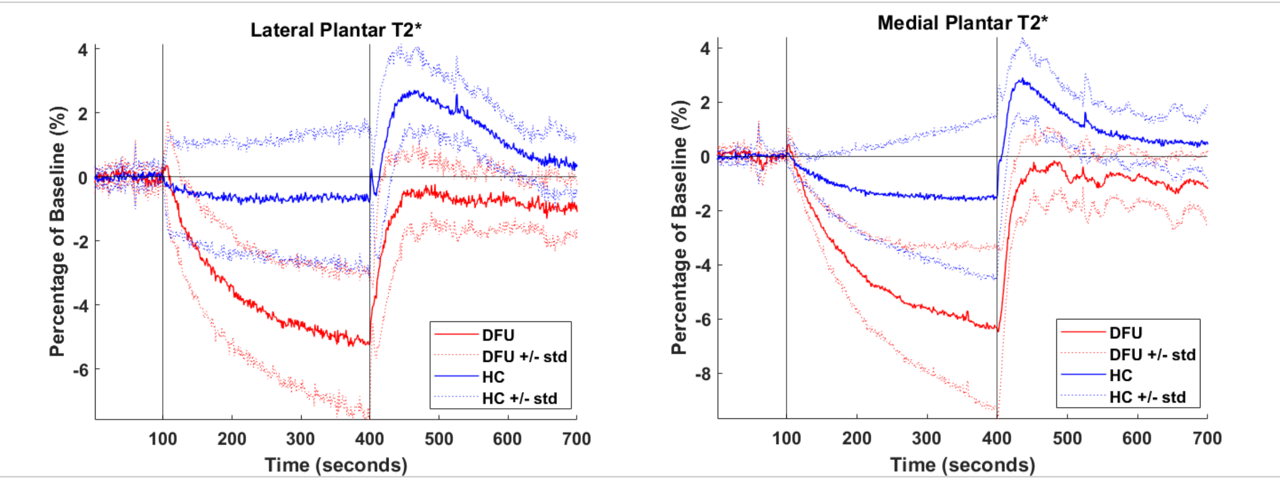

Figure 4. Average traces of the DFU and HC in the (left panel) lateral and (right panel) medial plantar region. Cuff occlusion occurred at t = 100s and released at t = 400s. The thick traces represent group average normalized T2* values and dotted traces represent average +/- standard deviation..

Table 4. *Quantitative BOLD MRI results during ischemia and reperfusion*

	Medial Plantar VOI		Lateral Plantar VOI	
	HC	DFU	HC	DFU
Baseline T2* signal (ms)	25.32 (0.87)	25.78 (2.35)	18.52 (1.14)	18.80 (2.39)
Maximum Perfusion Value (%)	2.82 (1.07)	-0.01 (1.18)*	2.68 (1.51)	-0.81 (1.54)*
Time to Maximum Perfusion (s)	434.8 (5.8)	458.6 (14.1)*	459.4 (9.8)	515.2 (38.7)*
Minimum Ischemic Value (%)	-1.63 (0.82)	-6.46 (2.75)*	-0.86 (0.10)	-4.21 (3.13)
Time to Minimum Ischemic Value (s)	332.4 (63.2)	349.8 (73.7)	327.2 (87.3)	343 (70.3)

Values represent mean (std)
*Significantly different from HC, $p < 0.05$

Specific Aim 2. Test for reduced monocyte reserve respiratory capacity in patients with DFU.

We have collected blood samples from all subjects in our cohort and performed respirometry on isolated monocytes. Data analysis is underway. No data are available to date.

3. Publications:

We focus a substantial effort on developing and refining perfusion MRI methodologies for detection of perfusion abnormalities in unresolved foot ulcers. Given this, and in due to slow-down of clinical research during the first year of the pandemic, we published a review paper on the topic of IVIM in skeletal muscle that examines methodologies used by our group in this research project:

1. Englund E.K., Reiter D.A., Shahidi B., Sigmund E.E., Intravoxel Incoherent Motion MRI in Skeletal Muscle: Review and Future Directions. *Journal of Magnetic Resonance Imaging, August 14, 2021.* https://doi.org/10.1002/jmri.27875

We have two abstracts accepted for presentation reporting our research findings in wound healing of patients with diabetes at the International Society of Magnetic Resonance in Medicine scientific meeting in May of 2022:

1 Reiter D.A., Yao J., Edwards S.J., Schechter M.C., Fayfman M., Santamarina G., Nesbeth P., Giacalone V., Blanco G., Feiweier T., Tirouvanziam R., Alvarez J., Risk B., Rajani R. Elevated Foot Diffusion and Resting Perfusion in Patients with Diabetic Foot Ulcer: A Pilot and Feasibility Study. *International Society of Magnetic Resonance in Medicine 2022 (talk).*

2 Edwards S.J., Yao J., Schechter M.C., Fayfman M., Santamarina G., Nesbeth P., Giacalone V., Blanco G., Tirouvanziam R., Alvarez J., Risk B., Rajani R., Reiter D.A. Dynamic BOLD MRI Shows Greater Foot Ischemia and Blunted Reperfusion Following Cuff-Occlusion Challenge in Diabetic Patients with Foot Ulcers. *International Society of Magnetic Resonance in Medicine 2022 (e-poster).*