Evaluation of hyperspectral technology for assessing the presence and severity of peripheral artery disease

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Background: **Hyperspectral imaging is a novel technology that can noninvasively measure oxyhemoglobin and deoxyhemoglobin concentrations to create an anatomic oxygenation map. It has predicted healing of diabetic foot ulcers; however, its ability to assess peripheral arterial disease (PAD) has not been studied. The aims of this study were to determine if hyperspectral imaging could accurately assess the presence or absence of PAD and accurately predict PAD severity.**

Methods: **This prospective study included consecutive consenting patients presenting to the vascular laboratory at the Jesse Brown VA Medical Center during a 10-week period for a lower extremity arterial study, including ankle-brachial index (ABI) and Doppler waveforms. Patients with lower extremity edema were excluded. Patients underwent hyperspectral imaging at nine angiosomes on each extremity. Additional sites were imaged when tissue loss was present. Medical records of enrolled patients were reviewed for demographic data, active medications, surgical history, and other information pertinent to PAD. Patients were separated into no-PAD and PAD groups. Differences in hyperspectral values between the groups were evaluated using the two-tailed *t* test. Analysis for differences in values over varying severities of PAD, as defined by triphasic, biphasic, or monophasic Doppler waveforms, was conducted using one-way analysis of variance. Hyperspectral values were correlated with the ABI using a Pearson bivariate linear correlation test.**

Results: **The study enrolled 126 patients (252 limbs). After exclusion of 15 patients, 111 patients were left for analysis, including 46 (92 limbs) no-PAD patients and 65 (130 limbs) PAD patients. Groups differed in age, diabetes, coronary artery disease, congestive heart failure, tobacco use, and insulin use. Deoxyhemoglobin values for the plantar metatarsal, arch, and heel angiosomes were significantly different between patients with and without PAD ($P < .005$). Mean deoxyhemoglobin values for the same three angiosomes showed significant differences between patients with monophasic, biphasic, and triphasic waveforms ($P < .05$). In patients with PAD, there was also significant correlation between deoxyhemoglobin values and ABI for the same three angiosomes ($P = .001$). Oxyhemoglobin values did not predict the presence or absence of PAD, did not correlate with PAD severity, and did not correlate with the ABI.**

Conclusions: **These results suggest the ability of hyperspectral imaging to detect the presence of PAD. Hyperspectral measurements can also evaluate different severities of PAD. (J Vasc Surg 2011;54:1679-88.)**

Peripheral artery disease (PAD) is an increasingly prevalent disorder affecting millions of patients across the world, including 8 million Americans. Among these patients, the prevalence of PAD among Americans aged ≥65 years is 12% to 20%.[1] These patients are vulnerable to a variety of sequela such as rest pain, lower extremity ulceration, and even limb amputation. Critical limb ischemia patients comprise 1% of all Americans with PAD, only 50% of whom will remain amputation-free at 1 year.[2]

To combat this disease, effective diagnostic and prognostic technologies are necessary for earlier detection and treatment to avoid unnecessary complications and interventions. Physicians currently use a combination of the ankle-brachial Index (ABI), Doppler waveform analysis, segmental limb pressures, and transcutaneous partial pressure of oxygen for screening, diagnosis, and preprocedural evaluation. These methods have been well validated for screening and diagnosis, but none have been shown to predict with high specificity or sensitivity the healing of tissue loss in patients with PAD.[3]

The ABI and segmental pressures are limited by calcified arteries where measurements are not accurate, as is commonly seen in patients with diabetes.[2] The measurement of transcutaneous partial pressure of oxygen can only be performed on skin adjacent to tissue loss and is limited by surrounding tissue edema and inflammation. Elevated transcutaneous oxygen levels have been associated with successful healing; however, its usefulness has been contested in active infection and swelling.[4]

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Competition of interest: HyperMed Inc supplied the OxyVu imager used in this study. However, HyperMed had no input into the design, execution, data collection, or analysis of the results of this study. None of the authors are affiliated with HyperMed Inc, the makers of the OxyVu technology, and have no vested interest in the clinical success or failure of the hyperspectral imaging system.

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Hyperspectral imaging is a new technology that constructs spatial maps of tissue oxygenation through scanning spectroscopy using wavelengths of visual light.[5] It is noninvasive, nonionizing, noncontact, and automated to reduce user-dependence. It has previously demonstrated an ability to show changes in skin microcirculation in diabetes.[6] Further studies have shown a facility to predict healing of diabetic foot ulcers with sensitivity, specificity, and positive predictive value of up to 86%, 88%, and 96%, respectively.[7] These studies included only small numbers of participants or excluded patients with severe PAD.

Hyperspectral imaging may present a novel, fast, and effective method to diagnose, monitor, and prognosticate PAD and associated wound healing. The aims of the current study were (1) to assess the ability of hyperspectral imaging to evaluate the presence or absence of PAD, and (2) to assess the ability of hyperspectral imaging to grade the severity of PAD in lower extremities. We hypothesized that oxygenation values returned by the imager would be significantly different between patients with and without PAD and that oxygenation values would also be significantly different between patients with varying severities of PAD as classified by standard methods.

METHODS

The protocol for this study was approved by the Northwestern University Institutional Review Board and by the Jesse Brown VA Research and Development Office. All participating patients gave written informed consent before undergoing hyperspectral imaging scans.

Patient cohort. Consecutive patients who presented to the vascular laboratory of Jesse Brown VA Medical Center in Chicago during a 10-week period from June 2009 to August 2009 for a lower extremity arterial flow study were invited to participate in this study. The sole exclusion criterion was lower extremity edema in the evaluated limb.

Each lower extremity of every patient was measured and then analyzed separately in the study. Limbs were initially divided into two groups: those with and without PAD. The presence of PAD was established using the criteria of an ABI $\leq$0.9 or a nontriphasic arterial Doppler waveform in the evaluated limb.[1,8,9] On further analysis for severity of PAD, limbs were classified into three groups by Doppler waveform analysis: (1) limbs with triphasic waveforms, (2) limbs with biphasic waveforms, and (3) limbs with monophasic waveforms. These criteria and classifications are consistent with the Society of Vascular Surgery and American College of Cardiology/American Heart Association guidelines and other published recommendations for the diagnosis of PAD.[10-12]

Procedures. All patients were initially assessed for basic demographics, medical, and surgical history. Standard noninvasive lower extremity arterial flow studies were conducted, including measurement of the ABI; segmental pressures for the upper thigh, lower thigh, calf, dorsalis pedis, posterior tibial, metatarsal, first digit areas, and second to fifth digits if first digit pressures were <50 mm Hg; and arterial Doppler waveforms of the dorsalis pedis and posterior tibial arteries.

Four registered vascular technicians performed the lower extremity arterial flow studies per standard laboratory protocol. They were blinded to the hyperspectral imaging results because these scans were conducted after the completion of the vascular laboratory studies. One of the authors (J.C.) performed all of the hyperspectral imaging studies and data analysis subsequent to vascular laboratory interpretations and was not blinded to their results.

Hyperspectral imaging evaluation. Hyperspectral imaging data were collected using the OxyVu Hyperspectral Tissue Oxygenation Mapping System (HyperMed, Waltham, Mass). The technology has been previously described with respect to physiologic imaging; but briefly, hyperspectral imaging works on the basis of scanning spectroscopy. Wavelengths of visual light between 500 and 660 nm, which includes the absorption peaks for oxyhemoglobin and deoxyhemoglobin, are collected from each pixel in an image and broken down by a spectral separator to generate a diffuse reflectance spectrum. These spectra from each pixel are compared against standard transmission solutions to determine the concentration of oxyhemoglobin and deoxyhemoglobin present in each visualized pixel.

These wavelengths of light penetrate to 1 to 2 mm below the skin and thus obtain information from the subpapillary plexus.[7] The imager and hemoglobin calculation algorithm are calibrated for different skin pigmentations. The OxyVu system used in this study had a 17-inch focal distance and a resolution of 100 μm. Cutaneous surface temperature values in the imaged area were also obtained and documented by the OxyVu system using a remote infrared temperature sensor on the imaging unit. Scans of approximately 30 seconds were obtained from each imaged area after the imager was calibrated to an OxyVu Check Pad for each new patient.

All patients who received hyperspectral scans were imaged in a standard hospital bed with the head of the bed elevated to approximately 45°. Nine sites on each lower extremity were imaged for each patient and were determined from angiosomes, which are specific vascular beds supplied by major named arteries. These sites were chosen with the idea that tissue oxygenation in watershed angiosomes supplied by end arteries in the foot and ankle could be preferentially affected by PAD.[13] The nine sites chosen on each limb were the anterior calf, lateral calf, posteromedial calf, lateral ankle, medial ankle, dorsal metatarsal, plantar metatarsal, plantar arch, and plantar heel angiosomes (Fig 1).

OxyVu targets were placed in the center of each image. Oxyhemoglobin and deoxyhemoglobin values were calculated automatically by the imaging system using a default region of interest consisting of a 1-cm donut around each central target with an area of 204 mm^2 (Fig 2). Beyond the nine sites imaged for each limb, any additional sites of tissue loss were also imaged.

An OxyVu target was placed immediately adjacent to the tissue loss area with oxyhemoglobin and deoxyhemo-

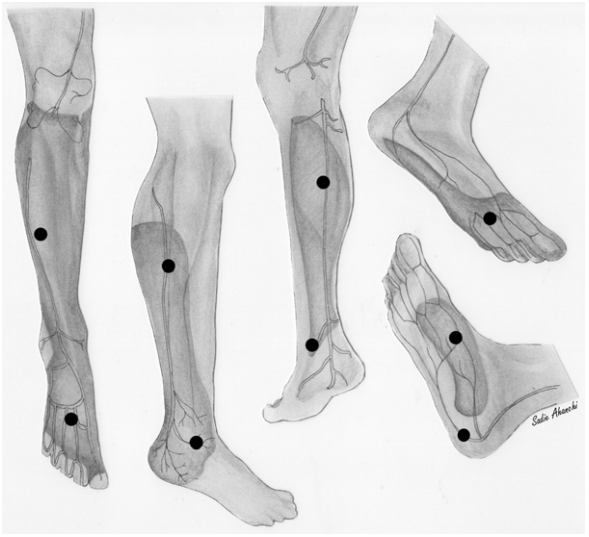

Fig 1. Locations are shown of the angiosomes that were imaged for all patients. The *black circles* represent the approximate position of the OxyVu targets.

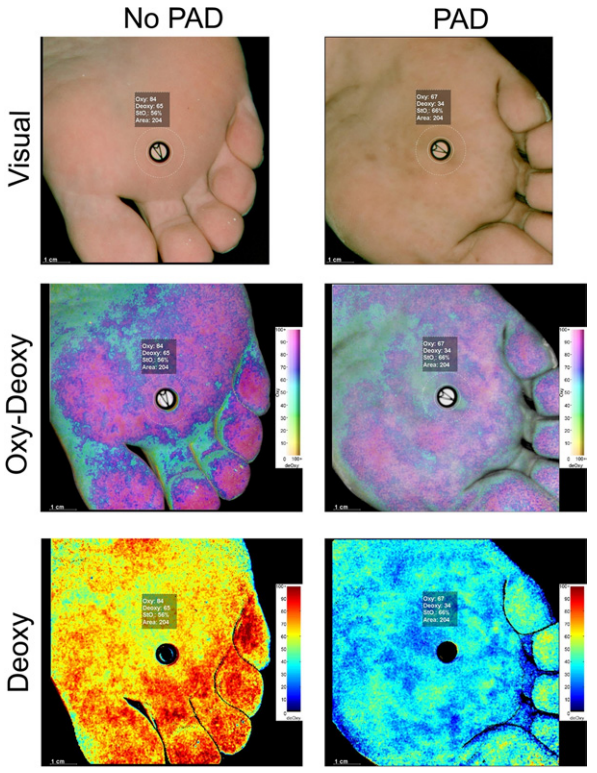

Fig 2. Visual, integrated oxyhemoglobin-deoxyhemoglobin, and deoxyhemoglobin hyperspectral images are shown of the plantar metatarsal angiosome for a **(left)** foot with no peripheral arterial disease (*PAD*) and **(right)** a foot with PAD. The foot with PAD has substantially decreased oxyhemoglobin and deoxyhemoglobin values throughout the angiosome.

globin values calculated using a 1-cm-wide strip traced directly around the area in the image. Oxyhemoglobin, deoxyhemoglobin, and cutaneous surface temperature values were returned by the system for each image. Oxyhemoglobin and deoxyhemoglobin values represent their quantities in analyzed tissue and are influenced by microvascular volume of capillary density and hemoglobin concentration.

These patients were contacted by standardized telephone interview again at 6 and 12 months after imaging for follow-up evaluation of healing.

Statistical analysis. Data were entered into an Excel file (Microsoft Corp, Redmond, Wash), and statistical analysis was performed using SPSS 19.0 software (SPSS Inc, Chicago, Ill). Data were summarized using mean, median, and standard deviation for continuous variables and count and percentage for categoric variables. Differences between the two primary analysis groups of PAD-positive and PAD-negative limbs were calculated with a Pearson χ^2 or Fisher exact test for dichotomous variables and the *t* test for continuous variables. Further analysis for examining differences in oxyhemoglobin and deoxyhemoglobin with varying PAD severity subgroups, as determined by Doppler waveform analysis, was performed using analysis of variance (ANOVA). Hyperspectral parameters were correlated with ABIs using a Pearson bivariate linear correlation analysis. Statistical significance was established at a two-sided $\alpha = 0.05$.

RESULTS

Baseline characteristics. The study recruited 126 patients; however, 15 were excluded due to lower extremity edema, which left 111 patients (222 limbs) for analysis. This total included 65 patients (130 limbs) with PAD and 46 patients (92 limbs) without PAD. In the PAD group, 27 patients (38 limbs) had an ABI >0.9 but abnormal Doppler waveforms; and 4 patients (6 limbs) had an ABI <0.9 but normal Doppler waveforms. The baseline demographic and comorbidity characteristics associated with all analyzed limbs can be found in Table I.

Statistically significant differences were noted between groups of limbs with and without PAD for age, presence of diabetes, history of congestive heart failure (CHF), history of coronary artery disease, use of tobacco ≤8 weeks, use of insulin, and the ABI. The association of these factors was consistent with previously documented risk factors and comorbidities of PAD.[12] The groups were otherwise similar in factors, including body mass index (BMI); hypertension; use of cholesterol-lowering, antihypertensive, adrenergic medications, including inhaled bronchodilators; use of steroids, including inhaled corticosteroids; use of supplemental oxygen; and prior lower extremity revascularization.

One limb in the PAD group and one limb in the non-PAD group had ulcer sites with a history of infection. Although active infection and swelling may affect the reliability of hyperspectral measurements, a review of images from these sites demonstrated no clinically apparent edema or erythema that would presumably compromise the reli-

Table I. Baseline demographic and clinical characteristics for limbs with and without peripheral arterial disease (*PAD*)

Variables[a]	*Without PAD (n = 92)*	*With PAD (n = 130)*	P[b]
Age, years	64.4 ± 10.9	67.4 ± 9.4	.032
Body mass index, kg/m^2	29.4 ± 5.0	28.3 ± 6.0	.123
Male	88 (95.7)	126 (96.9)	.617
Diabetes	35 (38.0)	67 (51.5)	.047
History of CHF	11 (12.0)	29 (22.3)	.048
History of hypertension	75 (81.5)	113 (86.9)	.271
History of CAD	15 (16.3)	43 (33.1)	.005
Tobacco use ≤8 weeks	26 (28.3)	60 (46.2)	.007
Caffeine use ≤12 hours	55 (59.8)	71 (54.6)	.444
Medications			
Statin	57 (62.0)	85 (65.4)	.600
β-blocker	44 (47.8)	68 (52.3)	.511
ACE inhibitor	46 (50.0)	70 (53.8)	.572
Insulin	15 (16.3)	37 (28.5)	.035
Steroid[c]	20 (21.7)	16 (12.3)	.060
Other adrenergic agent[c]	6 (6.5)	18 (13.8)	.083
Other antihypertensive	58 (63.0)	76 (58.5)	.492
Other diabetic agent	26 (28.3)	38 (29.2)	.875
Supplemental oxygen use	1 (1.1)	1 (0.8)	.805
Prior limb vascular surgery	8 (8.7)	20 (15.4)	.139
SBP, mm Hg	134 ± 19.6	136 ± 21.4	.604
Ankle-brachial index	1.2 ± 0.2	0.9 ± 0.4	<.001

ACE, Angiotensin-converting enzyme; *CAD,* coronary artery disease; *CHF,* congestive heart failure; *SBP,* systolic blood pressure.

[a]Continuous data are shown as mean ± standard deviation; categoric data as number (%).

[b]Fisher exact test or χ^2 for categoric variables; *t* test for continuous variables. $P < .05$ is significant.

[c]Includes inhalers.

ability of the oxygenation measurements in the region of interest used to derive oxygenation values.

In addition to the standard nine sites for hyperspectral imaging, 18 patients with 21 further sites of tissue loss were recorded during the course of the study. Four of these patients (four sites) were excluded because of unreliable hyperspectral measurements caused by lower extremity edema. An additional four patients (seven sites) could not be found for follow-up at 6 and 12 months. Of the remaining 10 patients (10 sites), 7 had undergone surgery that would confound follow-up analysis, including amputation, bypass grafting, and stenting.

Evaluation for the presence or absence of PAD. The primary analysis comparing sites with and without PAD showed no significant differences in hyperspectral oxyhemoglobin values for the imaged angiosomes (Table II). In contrast, mean deoxyhemoglobin values in the plantar metatarsal (56.0 ± 17.3 vs 49.7 ± 12.9, $P = .004$), plantar arch (51.4 ± 14.7 vs 45.1 ± 12.6, $P = .001$), and plantar heel (58.6 ± 16.5 vs 50.8 ± 14.6, $P < .001$) angiosomes showed statistically significant differences for non-PAD vs PAD limbs (Table II). In each case, deoxyhemoglobin values were elevated in the images of angiosomes not affected by PAD. An example of OxyVu images acquired at the plantar metatarsal angiosome in limbs with and without PAD can be seen in Fig 2. Although these angiosomes did show these statistically significant differences for deoxyhemoglobin, overlap existed in the ranges of these values between PAD and non-PAD groups.

Mean cutaneous surface temperature measurements (Table II) were similar between groups at all imaged angiosomes. This suggests that external temperature and patient skin temperature played no significant role in regulating the oxygenation of imaged sites in this study.

Evaluation of the severity of PAD. Further investigation comparing subgroups of limbs with varying PAD severity as classified by dorsalis pedis Doppler waveform analysis demonstrated significant differences in the plantar metatarsal ($P = .015$), plantar arch ($P = .038$), and plantar heel ($P = .006$) angiosomes for mean deoxyhemoglobin values (Table III). Each of these sites showed downward trends in deoxyhemoglobin content moving from triphasic to biphasic to monophasic waveforms. This follows with decreased mean deoxyhemoglobin in the initial PAD group and may indicate a restriction of deoxyhemoglobin flow in increasingly severe PAD. Post hoc analysis illustrated that the triphasic group had significantly higher deoxyhemoglobin content compared with monophasic and biphasic groups (Fig 3). There was no difference in deoxyhemoglobin content between monophasic and biphasic groups for all of these angiosomes.

In further analysis to isolate maximally ischemic limbs, we used three groups: (1) limbs with posterior tibial and dorsalis pedis monophasic waveforms, (2) all other limbs with PAD, and (3) limbs with no PAD. In contrast to the above results of decreased deoxyhemoglobin with more severe waveforms, limbs with both monophasic waveforms did not have significantly different hyperspectral-derived oxygenation values from other limbs with PAD, except at the dorsal metatarsal angiosome, where limbs with both monophasic waveforms actually had higher mean values of deoxyhemoglobin (45.8 ± 16.7 vs 38.2 ± 11.6, $P = .011$).

Differences in mean oxyhemoglobin values were detected at the dorsal metatarsal ($P = .041$) and plantar metatarsal ($P = .031$) angiosomes for severity classified by dorsalis pedis Doppler waveforms (Table III). Further differences were noted in mean deoxyhemoglobin values at the dorsal metatarsal ($P = .042$) angiosome when severity was classified by posterior tibial Doppler waveforms (Table IV). These comparisons of values across the varying severities of disease did not show any apparent trend.

A bivariate linear correlation analysis of limbs with PAD showed statistically significant relationships between limb ABI and the hyperspectral-derived values for deoxyhemoglobin at the lateral ankle ($R = -0.266$, $P = .005$), dorsal metatarsal ($R = -0.257$, 0.006), plantar metatarsal ($R = -0.254$, $P = .004$), plantar arch ($R = -0.302$, $P = .001$), and plantar heel ($R = -0.192$, $P = .033$). Although these bivariate correlations are statistically significant, the clinical significance is not affirmative given the very low correlation coefficient value. Furthermore, this trend is different from that seen with the relationship between mean deoxyhemoglobin values and Doppler waveforms. Whereas Doppler

Table II. Comparison of hyperspectral-derived mean variables among sites with and without peripheral arterial disease (*PAD*)

	Sites without PAD		*Sites with PAD*		
Variable	*No.*	*Mean ± SD*	*No.*	*Mean ± SD*	P[a]
Oxyhemoglobin					
Anterior calf	91	29.0 ± 14.6	126	27.8 ± 13.9	.537
Lateral calf	90	30.5 ± 15.0	121	27.7 ± 11.2	.128
Lateral ankle	89	29.0 ± 14.8	109	27.8 ± 11.7	.524
Medial ankle	89	32.3 ± 14.0	115	32.3 ± 13.3	.986
Posteromedial calf	92	26.4 ± 11.3	120	25.9 ± 11.9	.754
Dorsal metatarsal	88	33.5 ± 17.5	113	34.8 ± 14.9	.561
Plantar metatarsal	92	73.3 ± 21.0	126	71.5 ± 21.4	.518
Plantar arch	90	52.0 ± 22.4	126	50.6 ± 18.9	.632
Plantar heel	90	71.5 ± 20.9	127	72.7 ± 24.5	.704
Deoxyhemoglobin					
Anterior calf	91	35.2 ± 9.6	126	34.9 ± 10.0	.809
Lateral calf	90	39.9 ± 10.2	121	38.9 ± 11.3	.525
Lateral ankle	89	43.5 ± 12.0	109	41.0 ± 12.1	.155
Medial ankle	89	42.8 ± 11.0	115	41.3 ± 12.2	.374
Posteromedial calf	92	37.2 ± 10.1	120	36.8 ± 10.1	.815
Dorsal metatarsal	88	41.4 ± 12.0	113	39.9 ± 13.2	.398
Plantar metatarsal	92	56.0 ± 17.3	126	49.7 ± 12.9	.004
Plantar arch	90	51.4 ± 14.7	126	45.1 ± 12.6	.001
Plantar heel	90	58.6 ± 16.5	127	50.8 ± 14.6	<.001
Temperature					
Anterior calf	91	31.4 ± 1.3	126	31.1 ± 1.2	.072
Lateral calf	90	30.9 ± 1.5	121	30.7 ± 1.8	.469
Lateral ankle	91	30.3 ± 1.9	120	30.1 ± 2.1	.473
Medial ankle	90	30.7 ± 1.9	121	30.4 ± 1.9	.230
Posteromedial calf	92	31.0 ± 1.4	123	30.7 ± 1.4	.296
Dorsal metatarsal	89	29.4 ± 3.1	124	29.2 ± 2.9	.678
Plantar metatarsal	92	29.2 ± 3.4	127	29.5 ± 3.0	.542
Plantar arch	90	29.7 ± 2.5	128	29.7 ± 2.4	.956
Plantar heel	91	27.7 ± 3.1	129	28.2 ± 3.0	.289

SD, Standard deviation.
[a]*t* test; values of $P < .05$ are significant.

waveforms showed decreased deoxyhemoglobin with monophasic waveforms (more severe disease), low ABIs (more severe disease) showed increased deoxyhemoglobin values. A scatter of the relationship between plantar arch deoxyhemoglobin and ABI values is shown in Fig 4. ABI was inversely predictive of plantar arch deoxyhemoglobin ($P = .001$): for every 1-unit increase in ABI, there will be approximately a 10-unit decrease in plantar arch deoxyhemoglobin. No sites showed any significant relationships between oxyhemoglobin values and ABIs. Falsely elevated ABIs were not excluded from this analysis; thus, hyperspectral oxygenation values were also correlated with toe-brachial index, posterior tibial ABI, and dorsalis pedis ABI, which may adjust for these falsely elevated ABIs. These results are tabulated in Table V and showed similarly low correlation coefficients.

DISCUSSION

This prospective 10-week study of consecutive patients from our vascular laboratory showed that hyperspectral imaging might be useful in detecting differences in oxygenation levels in the lower extremities of patients with and without PAD. Oxyhemoglobin content in the skin of PAD and non-PAD limbs did not show any differences; however, deoxyhemoglobin content was reduced in the plantar angiosomes of patients with PAD. Furthermore, these same angiosomes also showed decreasing deoxyhemoglobin content with increasing severity of disease. In contrast to previous studies of hyperspectral imaging technology, which predominately studied patients with diabetes and excluded patients with PAD, our study included patients with a spectrum of PAD, including critical limb ischemia. Thus, our data suggest that hyperspectral imaging may be a useful tool for the diagnosis and evaluation of patients with PAD.

Our results are seemingly in contrast to a recent study by Jafari-Saraf and Gordon,[14] which demonstrated no correlation between the ABI and hyperspectral oxygenation parameters in patients being evaluated for PAD, excluding those patients with calcified tibial vessels. They showed that oxyhemoglobin values and hemoglobin oxygen saturation values did not exhibit significant differences across ABI groups of <0.45, 0.45 to 0.9, and >0.9. However, these authors only studied values at the dorsum of the foot and ankle, in contrast to our study, which included values at nine angiosome sites. Thus, our data are consistent with the

Table III. Comparison of hyperspectral-derived variables among sites of varying peripheral arterial disease severity by dorsalis pedis artery Doppler waveform analysis

	Monophasic		*Biphasic*		*Triphasic*		
Variable	*No.*	*Mean ± SD*	*No.*	*Mean ± SD*	*No.*	*Mean ± SD*	P[a]
Oxyhemoglobin							
Anterior calf	34	27.2 ± 16.3	73	28.0 ± 14.1	108	28.8 ± 13.7	.825
Lateral calf	32	27.9 ± 12.2	71	27.7 ± 11.6	106	29.9 ± 14.1	.494
Lateral ankle	29	29.0 ± 14.9	65	27.5 ± 10.5	104	28.8 ± 14.3	.793
Medial ankle	29	34.4 ± 15.8	69	31.6 ± 12.4	105	32.4 ± 13.7	.636
Posteromedial calf	31	24.2 ± 10.9	72	26.6 ± 13.1	108	26.1 ± 10.7	.630
Dorsal metatarsal	32	40.5 ± 19.0	68	31.9 ± 12.1	100	33.9 ± 17.1	.041
Plantar metatarsal	34	63.6 ± 24.0	74	74.8 ± 19.3	109	73.3 ± 21.2	.031
Plantar arch	35	48.8 ± 24.9	73	51.4 ± 16.4	107	52.0 ± 21.4	.722
Plantar heel	35	66.1 ± 26.1	73	74.0 ± 23.6	107	73.1 ± 21.6	.218
Deoxyhemoglobin							
Anterior calf	34	35.2 ± 9.2	73	35.2 ± 10.9	108	35.0 ± 9.2	.985
Lateral calf	32	40.4 ± 13.6	71	38.9 ± 11.0	106	39.6 ± 9.8	.801
Lateral ankle	29	41.0 ± 12.9	65	41.3 ± 12.2	104	42.9 ± 11.8	.613
Medial ankle	29	41.7 ± 10.7	69	40.9 ± 13.1	105	43.0 ± 10.8	.498
Posteromedial calf	31	37.1 ± 9.2	72	36.9 ± 11.2	108	37.1 ± 9.7	.983
Dorsal metatarsal	32	43.8 ± 16.4	68	38.2 ± 11.3	100	41.3 ± 12.0	.084
Plantar metatarsal	34	49.0 ± 16.4	74	49.5 ± 11.9	109	55.3 ± 16.4	.015
Plantar arch	35	44.5 ± 16.1	73	45.8 ± 10.9	107	50.2 ± 14.6	.038
Plantar heel	35	50.7 ± 19.9	73	50.7 ± 12.7	107	57.6 ± 15.9	.006
Temperature							
Anterior calf	34	30.8 ± 1.4	73	31.2 ± 1.2	108	31.4 ± 1.3	.051
Lateral calf	32	30.6 ± 1.7	71	30.6 ± 1.9	106	30.9 ± 1.5	.338
Lateral ankle	33	29.6 ± 2.3	72	30.3 ± 2.0	106	30.4 ± 1.9	.168
Medial ankle	33	30.0 ± 2.0	70	30.5 ± 1.9	107	30.7 ± 1.8	.191
Posteromedial calf	33	30.7 ± 1.5	72	30.8 ± 1.4	109	30.9 ± 1.4	.602
Dorsal metatarsal	34	28.7 ± 2.9	72	29.3 ± 3.0	105	29.4 ± 3.1	.510
Plantar metatarsal	34	28.9 ± 3.1	75	29.7 ± 3.1	109	29.3 ± 3.3	.460
Plantar arch	35	29.4 ± 2.6	75	29.9 ± 2.4	107	29.7 ± 2.4	.592
Plantar heel	35	28.6 ± 2.9	75	27.9 ± 3.1	108	27.9 ± 3.0	.428

SD, Standard deviation.
[a]Analysis of variance test; $P < .05$ is significant.

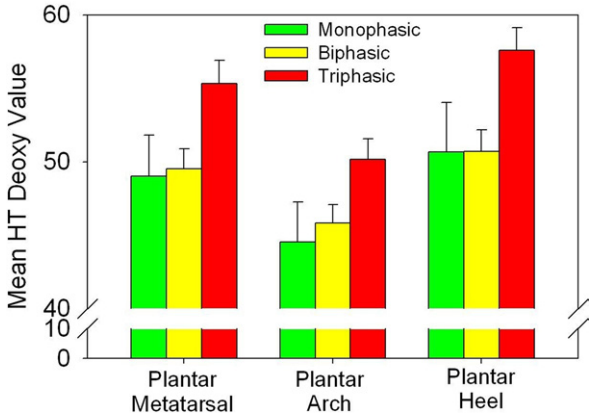

Fig 3. Mean hyperspectral technology (*HT*) deoxyhemoglobin (*Deoxy*) values are shown with the standard deviation (*error bars*) for the plantar metatarsal, plantar arch, and plantar heel angiosomes in individuals with monophasic, biphasic, and triphasic dorsalis pedis waveforms. Individuals with monophasic and biphasic waveforms have lower mean deoxyhemoglobin values than those with triphasic waveforms.

study of Jafari-Saraf and Gordon. One explanation for why we found a difference in the values for the plantar angiosomes is that they are covered by glabrous skin, which is rich in arteriovenous anastomoses, have generally higher oxygenation levels, and are generally more reactive compared with skin over other body parts.[5]

That we found significant results in these plantar sites as opposed to areas more proximal in the leg is also consistent with the concept of angiosomes described by Galiano[13] explaining that lower extremity angiosomes reach a watershed status in the foot. Also of note, the Jafari-Saraf and Gordon study excluded limbs with noncompressible tibial vessels. Our study did not use this exclusion criteria because our definition of PAD included limbs with an unreliable ABI by taking into account nontriphasic Doppler waveforms, as is currently recommended.[10,11] Despite the decreased mean value of deoxyhemoglobin we found in PAD-affected angiosomes, the distribution of values in these groups does show substantial spread and overlap (Fig 3). A factor contributing to the statistical significance of our

Table IV. Comparison of hyperspectral-derived variables among sites of varying peripheral arterial disease (PAD) severity by posterior tibial artery Doppler waveform analysis

	Monophasic		*Biphasic*		*Triphasic*		
Variable	*No.*	*Mean ± SD*	*No.*	*Mean ± SD*	*No.*	*Mean ± SD*	P[a]
Oxyhemoglobin							
Anterior calf	31	26.6 ± 16.4	79	28.2 ± 13.6	104	28.8 ± 14.1	.893
Lateral calf	29	27.9 ± 11.9	77	27.7 ± 11.3	103	30 ± 14.4	.473
Lateral ankle	24	28.5 ± 15.5	73	28.4 ± 10.6	101	28.3 ± 14.4	.990
Medial ankle	25	32.7 ± 16.5	77	32.6 ± 12.6	101	32.1 ± 13.6	.967
Posteromedial calf	28	24.3 ± 13.3	78	26.3 ± 11.4	105	26.3 ± 11.3	.713
Dorsal metatarsal	29	38.9 ± 20.6	73	33.8 ± 12.4	98	33.2 ± 16.9	.238
Plantar metatarsal	31	64.2 ± 24.1	80	75.1 ± 20.2	105	72.3 ± 20.8	.078
Plantar arch	31	49 ± 25.6	80	51.2 ± 15.7	103	51.9 ± 22.1	.888
Plantar heel	32	67.5 ± 23.6	79	76.2 ± 25.4	103	70.6 ± 20.8	.218
Deoxyhemoglobin							
Anterior calf	31	36.0 ± 10.8	79	34.7 ± 9.4	104	35.2 ± 9.8	.900
Lateral calf	29	39.2 ± 13.8	77	39.2 ± 10.9	103	39.8 ± 9.9	.900
Lateral ankle	24	44.1 ± 14.0	73	40.1 ± 11.5	101	43.1 ± 11.9	.185
Medial ankle	25	43.5 ± 13.6	77	40.7 ± 12.0	101	42.9 ± 10.7	.375
Posteromedial calf	28	37.9 ± 9.5	78	36.9 ± 10.4	105	36.9 ± 10.2	.900
Dorsal metatarsal	29	44.7 ± 16.5	73	38.1 ± 11.7	98	41.3 ± 11.8	.042
Plantar metatarsal	31	50.3 ± 16.0	80	50.1 ± 11.7	105	54.6 ± 17.1	.164
Plantar arch	31	47.3 ± 16.5	80	44.8 ± 11.2	103	50.3 ± 14.5	.051
Plantar heel	32	53.3 ± 18.5	79	50.7 ± 13.5	103	56.9 ± 16.6	.069
Temperature							
Anterior calf	31	31.0 ± 1.3	79	31.2 ± 1.3	104	31.4 ± 1.2	.389
Lateral calf	29	30.8 ± 1.8	77	30.6 ± 1.9	103	30.9 ± 1.4	.548
Lateral ankle	30	29.8 ± 2.4	77	30.2 ± 2.0	104	30.3 ± 1.9	.437
Medial ankle	30	30.0 ± 2.1	77	30.5 ± 1.9	102	30.7 ± 1.8	.374
Posteromedial calf	30	30.8 ± 1.5	78	30.8 ± 1.5	105	30.9 ± 1.4	.898
Dorsal metatarsal	32	28.7 ± 3.0	76	29.3 ± 3.0	102	29.4 ± 3.1	.669
Plantar metatarsal	31	28.8 ± 3.2	81	29.7 ± 3.0	105	29.2 ± 3.3	.426
Plantar arch	32	29.3 ± 2.7	81	29.9 ± 2.4	103	29.7 ± 2.4	.669
Plantar heel	32	28.4 ± 3.0	81	28.1 ± 3.0	104	27.8 ± 3.1	.640

SD, Standard deviation.
[a]Analysis of variance test. $P < .05$ is significant.

results is the larger number of participants and limbs recruited in our study compared with previous trials.[5,7,14]

If hyperspectral imaging is to be clinically useful in the future, a much more thorough evaluation will need to be undertaken to determine the valid ranges and benchmarks for determining diseased vs nondiseased limbs. Once these benchmarks are determined and a model for predicting wound healing in PAD is created, a prospective study can be conducted to determine the sensitivity and specificity of this technology.[5,7]

The differences we found in deoxyhemoglobin and not in oxyhemoglobin—except for oxyhemoglobin in two angiosomes compared for severity by dorsalis pedis Doppler waveforms—are further notable because no previous study, to our knowledge, has documented differences in deoxyhemoglobin without changes in oxyhemoglobin as well. Indeed, previous studies of diabetes and ischemic models have all shown oxyhemoglobin to decrease in diseased and ischemic states.[5,6,15,16] A study of hyperspectral technology by Nagaoka et al[16] actually shows oxyhemoglobin decreasing and deoxyhemoglobin increasing in the middle finger of an individual whose brachial artery was compressed for 1 minute. This is thought to indicate increased tissue consumption of oxygenated hemoglobin with an increase in deoxygenated hemoglobin secondary to blockage in blood flow.

A possible reason for the difference found in our data is the physiology of PAD, which is a chronic process affecting blood flow in both macroperipheral and microperipheral arteries. The peripheral sympathetic postural autonomic vasoregulation mechanism, the venoarteriolar response, is impaired in PAD. This has been demonstrated by increased skin blood flow in PAD limbs in the dependent position (which was the case in our study) due to decreased precapillary resistance.[17] As the hyperspectral imager gathers data from the subpapillary plexus, it can be thought to examine this increased dependent skin blood flow, which could manifest as unaffected oxyhemoglobin levels with decreased deoxyhemoglobin levels due to less than maximal oxygen extraction in more perfused skin.

In addition to all the changes noted between purely PAD and non-PAD groups, the trend of decreasing hyperspectral deoxyhemoglobin when evaluating severity of PAD by Doppler waveform should not be understated. This would support the idea that decreased flow of blood and hemoglobin to the extremity would decrease absolute

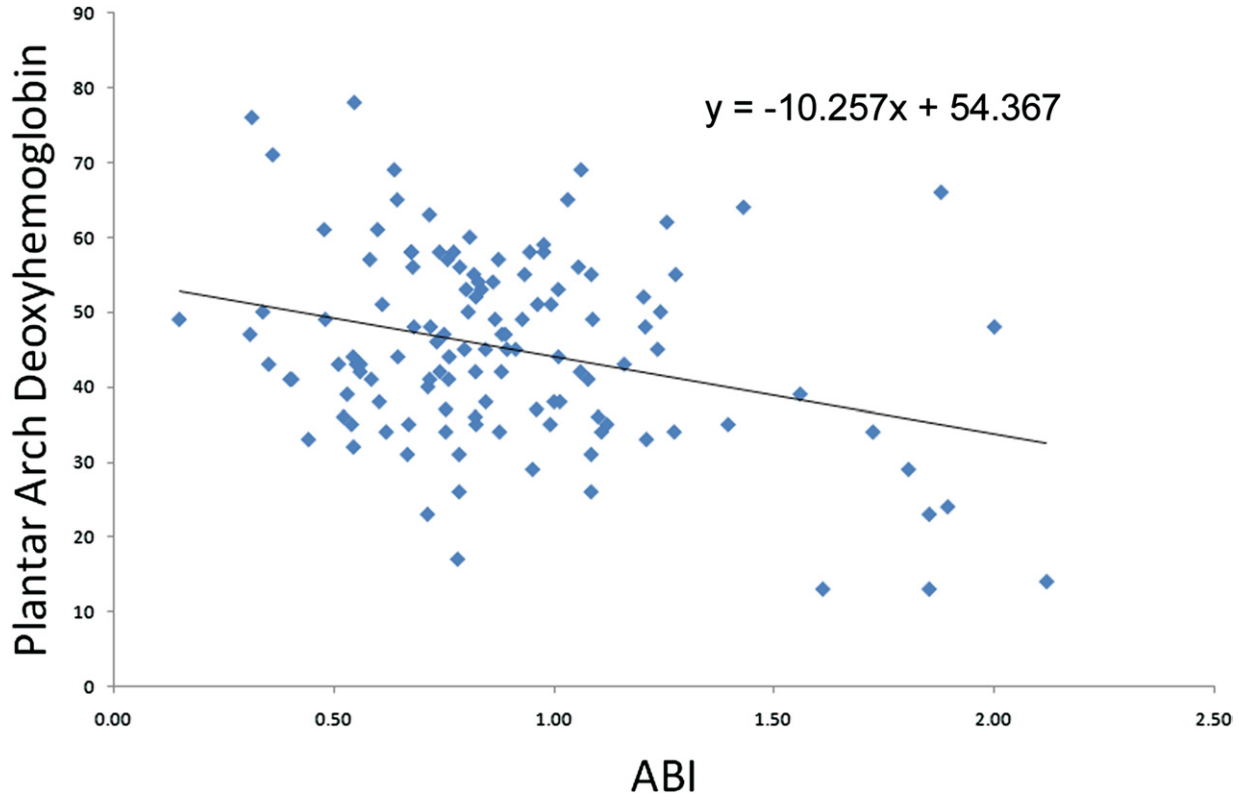

Fig 4. Linear regression of plantar arch deoxyhemoglobin values is shown for the ankle-brachial index (*ABI*) in limbs with peripheral arterial disease. The data show a statistically significant inverse relationship between deoxyhemoglobin and ABI for this angiosome ($P = .001$).

deoxyhemoglobin content in the image. The downward trend in deoxyhemoglobin with increasing severity of PAD can be clearly seen in Fig 3. Although we did note insignificant changes in hyperspectral values between limbs with monophasic and biphasic Doppler waveforms, this may be due to the smaller number of limbs for analysis in both of these groups compared with the triphasic group. A greater number of more severely diseased limbs may have shown a stronger relationship at this level of PAD. Future investigations of this subject will need to address adequate recruitment of all severities of PAD.

Our study is not without limitations. One potential limitation is the baseline disparities between the PAD and non-PAD cohorts. Significantly more limbs in the PAD group were associated with diabetes, congestive heart failure, coronary artery disease, and recent tobacco use. Although this is not completely unexpected, given that these are well-known risk factors and comorbidities for PAD, they may have adversely affected the results of the analysis. This is most suspect for diabetes, given the previous studies of hyperspectral technology examining diabetic microcirculation and foot ulcer healing. However, the results of our study do not mirror the oxygenation patterns found in the previous diabetic studies that excluded patients with PAD.[5-7] Use of other vasoactive substances, such as caffeine, adrenergic agents, and antihypertensive agents, which may have had a more direct effect on the circulatory system and tissue perfusion, was well-matched between groups.

Respiratory diseases, such as chronic obstructive pulmonary disease (COPD), could also play a direct role in the detected hyperspectral oxygenation values that may not be in proportion to mechanical indices like ABI and Doppler waveforms. The technical outline for the imaging system specifies that no oxygen measurement ranges have been established for patients on oxygen assistance. Although we did not exclude COPD patients from the study or interview participants about its presence, we did record several markers, including use of adrenergic agents, including inhaled bronchodilators; steroids, including inhaled corticosteroids; and supplemental oxygen that make us confident it did not significantly confound results. Further trials will need control for these factors.

Another notable consideration for the data analysis is the possibility of type 1 error. The multiple comparisons that were made increased the probability of returning a false-positive difference. In the primary analysis, 18 values were compared for oxygenation (9 angiosomes each for oxyhemoglobin and deoxyhemoglobin), determined by the presence or absence of PAD, and in the subgroup

Table V. Pearson correlation coefficient for linear relationships of hyperspectral-derived variables with pressure indexes for limbs with peripheral arterial disease (PAD)

	ABI			*TBI*			*ABI*					
							Posterior tibial			*Dorsalis pedis*		
Variable	*No.*	R	P	*No.*	R	P	*No.*	R	P	*No.*	R	P
Oxyhemoglobin												
Anterior calf	123	−0.016	.863	126	0.057	.525	121	0.041	.654	123	−0.03	.744
Lateral calf	118	0.009	.919	121	0.015	.867	117	0.019	.843	118	−0.029	.752
Lateral ankle	108	−0.029	.767	109	0.007	.940	107	0.016	.866	108	0.027	.783
Medial ankle	113	0.044	.646	115	0.022	.812	112	0.046	.634	113	0.123	.194
Posteromedial calf	118	0.026	.777	120	0.058	.526	117	0.012	.900	118	0.052	.576
Dorsal metatarsal	111	0.044	.647	113	−0.042	.656	110	0.031	.751	111	0.060	.532
Plantar metatarsal	124	−0.049	.588	126	0.306	<.001	122	0.013	.886	124	−0.059	.515
Plantar arch	124	−0.104	.252	126	0.134	.134	122	−0.071	.437	124	−0.073	.420
Plantar heal	124	0.049	.589	127	0.176	.048	122	0.104	.257	124	0.027	.767
Deoxyhemoglobin												
Anterior calf	123	−0.101	.267	126	0.001	.990	121	−0.031	.74	123	−0.109	.232
Lateral calf	118	−0.133	.152	121	−0.021	.821	117	−0.085	.362	118	−0.073	.433
Lateral ankle	108	−0.266	.005	109	−0.16	.097	107	−0.315	.001	108	−0.180	.062
Medial ankle	113	−0.136	.151	115	−0.125	.184	112	−0.168	.077	113	−0.046	.627
Posteromedial calf	118	0.018	.848	120	0.047	.612	117	−0.001	.994	118	0.088	.343
Dorsal metatarsal	111	−0.257	.006	113	−0.128	.176	110	−0.298	.002	111	−0.202	.033
Plantar metatarsal	124	−0.254	.004	126	−0.025	.783	122	−0.213	.018	124	−0.201	.025
Plantar arch	124	−0.302	.001	126	−0.008	.928	122	−0.329	<.001	124	−0.212	.018
Plantar heel	124	−0.192	.033	130	−0.082	.359	122	−0.165	.069	124	−0.123	.175
Temperature												
Anterior calf	123	0.248	.006	126	0.164	.067	121	0.244	.007	123	0.275	.002
Lateral calf	118	0.239	.009	121	0.055	.552	117	0.263	.004	118	0.184	.046
Lateral ankle	119	0.298	.001	120	0.179	.050	118	0.298	.001	119	0.284	.002
Medial ankle	119	0.327	.000	121	0.219	.016	117	0.392	<.001	119	0.291	.001
Posteromedial calf	121	0.218	.016	123	0.09	.324	119	0.265	.004	121	0.211	.020
Dorsal metatarsal	121	0.267	.003	124	0.131	.148	119	0.297	.001	121	0.255	.005
Plantar metatarsal	125	0.270	.002	127	0.173	.052	123	0.301	.001	125	0.260	.003
Plantar arch	126	0.271	.002	128	0.152	.086	124	0.305	.001	126	0.235	.008
Plantar heel	126	0.287	.001	129	0.020	.822	124	0.323	<.001	126	0.194	.029

ABI, Ankle-brachial index; *TBI,* toe-brachial index.

analyses of severity by dorsalis pedis and posterior tibial waveforms. However, high levels of significance were achieved in the primary analysis, with $P = .004$ for the plantar metatarsal, $P = .001$ for the plantar arch, and $P < .001$ for the plantar heel. Furthermore, oxygenation values in adjacent angisomes tend to correlate with one another; for example, calf angiosome mean values were all lower than plantar angiosome mean values. The test results in these regions of adjacent angiosomes are likely to be at least partially dependent on one another. Thus, we are confident the differences detected in these results are significant.

One aspect of the hyperspectral images that this study did not directly address is its anatomic mapping of oxygenation throughout the scanned area. The data presented in this study were limited to the relatively small regions of interest around the OxyVu targets in each image. One of the possibly powerful capabilities of hyperspectral imaging is to supply oxygenation information beyond these point measurements in an anatomic view that could be amenable to simple color and pattern recognition by technicians after further study and development. For instances where the automatically sampled region of interest may coincidentally fall in an oxygenation area not representative of the rest of the body part, the rapidly interpretable color mapping in returned images could be invaluable.

Another facet of hyperspectral imaging that our data could not address is the reproducibility of hyperspectral-derived oxygenation values in our participants over time. Although one individual that was initially recruited had repeat studies, this person had to be excluded from the analysis due to lower extremity edema.

Although no large-scale studies have been undertaken for the reliability of measurements in subjects with PAD, hyperspectral imaging has been tested for reliability and establishment of normative oxygenation values in asymptomatic patients with subgroups undergoing repeat same-site, same-subject testing after 8 hours and after transient cuff-induced ischemia with reproducible hyperspectral-derived results.[18] On the basis of these past results, it is reasonable to believe the same technology would perform similarly in our study.

CONCLUSIONS

Hyperspectral imaging presents an interesting new development for the diagnostic imaging and evaluation of PAD. Although our study does not provide an immediate

breakthrough use for this technology at the bedside to replace existing technologies, our analysis has demonstrated its utility in distinguishing ischemia from normal flow states. With further study and understanding of how this technology works, it may be a valuable tool for the prediction of wound healing in severely ischemic patients. Other possible uses for hyperspectral imaging include monitoring and follow-up of revascularization interventions. Future work concentrating on the recruitment of severe PAD and critical limb ischemia patients should yield more immediately applicable results to the surgical management of tissue loss in those patients. A robust study concentrating on the recruitment of severe PAD and critical limb ischemia patients and analyzing its use in predicting the healing of PAD-associated tissue loss and need for revascularization or amputation should yield more immediately applicable results in the surgical management of tissue loss. The clinical applicability of hyperspectral imaging with regards to PAD is clearly still in its adolescence and requires much more validation before it can be used safely and effectively. However, this and other studies suggest an intriguing new opportunity for the physician and vascular laboratory.

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AUTHOR CONTRIBUTIONS

Conception and design: MK
Analysis and interpretation: JC, EW, MK
Data collection: JC
Writing the article: JC, EW, MK
Critical revision of the article: JC, EW, MK
Final approval of the article: JC, EW, MK
Statistical analysis: JC, EW
Obtained funding: Not applicable
Overall responsibility: MK

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