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● *Original Contribution*

PHOTOACOUSTIC OXYGENATION QUANTIFICATION IN PATIENTS WITH RAYNAUD'S: FIRST-IN-HUMAN RESULTS

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Abstract—The purpose of this study was to investigate the use of photoacoustic imaging for quantifying fingertip oxygenation as an approach to diagnosing and monitoring Raynaud's phenomenon. After 30 min of acclimation to room temperature, 22 patients (7 patients with secondary Raynaud's associated to Scleroderma and 15 healthy controls) provided informed consent to undergo fingertip Doppler imaging and high-frequency photoacoustic imaging before and 5, 15 and 30 min after cold stimulus (submerged hand in a 15 °C water bath for 1 min). High-frequency ultrasound and photoacoustic imaging was performed on the nail bed of each patient's second through fifth finger on their dominant hand, using a Vevo 2100 LAZR system with an LZ-250 probe (Fujifilm VisualSonics, Toronto, ON, Canada) in oxy-hemoglobin quantification mode. During each exam, volumetric data across a 3-mm span of data was acquired to produce a volumetric image of percent oxygenation and hemoglobin concentration. Changes in fingertip oxygenation between Raynaud's patients and healthy volunteers were compared, using receiver operator characteristic (ROC) analysis. Photoacoustic signal was detected in both the nail bed and nailfold in all study participants. Doppler ultrasound resulted in poor differentiation of Raynaud's patients from healthy volunteers, with an area under the ROC curve (A_z) of 0.51. Photoacoustic imaging demonstrated improved accuracy at baseline ($A_z = 0.72$), which improved when quantifying normalized changes after cold stimulus ($A_z = 0.89$ 5-min post stimulus, $A_z = 0.91$ 15-min post stimulus, and $A_z = 0.85$ after stimulus). Oxygenation levels derived using photoacoustic imaging are able to identify patients with Raynaud's and safely evaluate their response to a cold stimulus over time. (E-mail: John.Eisenbrey@jefferson.edu)

Key Words: **Photoacoustic imaging, Raynaud's, Tissue oxygenation.**

INTRODUCTION

Raynaud's phenomenon (RP) is a debilitating disorder that can lead to finger and toe amputations in severe cases. It is caused by an exaggerated peripheral vasospasm in response to cold or emotional stress. The subsequent peripheral blood flow reduction is clinically characterized by a triphasic color change (blanching, cyanosis, and erythema) and, although RP is generally inconsequential for patients with no additional pathologies (primary RP), it a disabling disorder for patients with connective tissue disease, such as scleroderma (Maricq et al. 1996). RP is almost a universal finding in patients with scleroderma and is driven by endothelial dysfunction, intimal fibroproliferation and vessel lumen occlusion (Maricq et al. 1996). It is present in more than 90% of patients with scleroderma (Herrick 2008; Maricq et al. 1996). RP can be seriously disabling, leading to digital ulcers, finger necrosis and amputation or associated complications, such as infection and osteomyelitis. Digital ulcerations can be present in up to 30% of the patients with systemic sclerosis-RP and is an expression of its clinical severity (Herrick 2008; Maricq et al. 1996).

Unfortunately, there are very few direct methods to detect and quantify peripheral microangiopathy in RP (Andrade et al. 1990; Korn et al. 2004; Merkel et al. 2002). Widefield nailfold capillaroscopy (NFC) and video capillaroscopy (Andrade et al. 1990) are the most commonly utilized methods. In healthy individuals and those with primary Raynaud's, the NFC pattern is characterized by capillary loops that are similar in size and shape and demonstrate uniform distribution (Cutolo et al. 2003). On the other hand, patients with active NFC

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abnormalities present with abnormally dilated capillary loops (wire loops) that progress to late vasculopathy with disorganized and missing capillary loops and varying degrees of microhemorrhages (Maricq 1981). However, NFC and video capillaroscopy as used in the clinical setting do not provide quantitative measurements, are intrinsically observer dependent and only measure structural capillary abnormalities of the subjacent vasculopathy, but not the severity of the blood flow impairment (Herrick 2008; Maricq 1981). Activity and severity of RP in clinical trials have been measured by the Raynaud's Condition Score and other patient-reported outcomes, such as severity, duration and frequency of attacks. However, the intrinsic subjectivity of these measurements, based in patient-reported outcomes, stress the need for an objective, noninvasive measure of digital blood flow and tissue oxygenation.

Ultrasonography with Doppler evaluation of peripheral blood flow is commonly used for diagnostic evaluation in patients with a wide variety of connective tissue disorders. Unfortunately, relatively low resolution (~0.2 mm) of current clinical scanners limits its use in studying circulation in small body parts, such as fingers. In addition, quantification of these findings is often difficult and user dependent, resulting in similar drawbacks to those associated with NFC. Photoacoustic (PA) imaging is an emerging imaging modality and PA systems are now commercially available for preclinical research (Becker et al. 2018; Eisenbrey et al. 2015; Needles et al. 2013). Using an optical laser, light (generally in the 650−970 nm wavelength range) is directed through the tissue from a tunable laser. As this light is absorbed, tissue chromophores undergo localized thermal expansion, which in turn generate acoustic signatures that can be detected by an ultrasound transducer. One such measurement is the oxygenation of blood by comparing the ratio of absorption at multiple wavelengths (the absorption spectra of hemoglobin alters based on its oxygenation state). Such optical absorption measurements form the basis of clinically used pulsed-ox measurements (Yoshiya et al. 1980). However, PA benefits from the same specificity of optical imaging, along with the resolution and increased penetration depths of ultrasound, while providing tissue oxygenation information throughout the imaging plane. This modality has been applied to a variety of preclinical models (Eisenbrey et al. 2015; Mallidi et al. 2011; Needles et al. 2013) and has more recently shown some promise in early human trials of cancer detection and characterization (Mallidi et al. 2011). However, PA has yet to be applied as a tool for imaging and quantification of vascular dysfunction of connective tissue diseases in humans.

Quantification of microperfusion and oxygenation remains an important goal in systemic sclerosis-RP. Magnetic resonance angiography has been used to successfully quantify peripheral circulation in RP, but this technique requires an injection of contrast dye and extensive magnetic resonance imaging (MRI) workup, which may limit its applicability for screening (Park et al. 2014; Zhang et al. 2011). Other optical methods such as those using laser-speckle tracking have also been proposed to quantify microperfusion, but they do not quantify tissue oxygenation levels or provide anatomic registration (Leahy et al. 2007; Zhang et al. 2017). Hence, the purpose of this pilot study was to explore the feasibility of PA imaging in RP patients and to compare oxygen quantification from this patient population with healthy volunteers.

MATERIALS AND METHODS

Patient enrollment

All participants (both RP patients and healthy volunteers) provided informed consent to participate in this institutional review board-approved and HIPPA-compliant study. Inclusion criteria for both groups consisted of the subject being 18 y of age or older and being able to provide informed consent. Within the RP group, patients were required to have a documented history of severe scleroderma-related (secondary) RP history of finger ulceration. High-resolution video capillaroscopy was performed by an expert (F.A.M.-B.) in each RP patient (as part of their clinical standard of care) before enrolling to confirm severe scleroderma-related RP. Study exclusion criteria for all patients consisted of nail polish (other than clear), active smoking, occupational exposure to a cold environment, occupational exposure to strong mechanical vibrations, uncontrolled systemic arterial hypertension or diabetes mellitus and clinical evidence of proximal arterial disease. Healthy volunteers from Thomas Jefferson University Hospital (Philadelphia, PA, USA) were approached between November and December 2016. Patients with RP were consecutively approached at The Jefferson Scleroderma Center by Dr. Mendoza-Ballesteros between December 2016 and April 2017. Before participation, all patients were asked to rest for 30 min in the scanning room to ensure temperature acclimation (21°C).

A total of 15 healthy volunteers and 7 RP patients (out of 13 approached patients, 6 declined to participate) were enrolled and successfully completed the study protocol. The sex breakdown of the healthy volunteer group consisted of 67% women (10/15). A total of 80% were Caucasian (12/15), 13% Asian (2/15), and 7% (1/15) African American. The average age in this group was 53.4 ± 5.6 y (range = 41−62 y). The RP group consisted of 86% women (6/7). A total of 71% (5/7) were Caucasian and the remaining 29% (2/7) were African American. The average age in this group was 47.2 ± 13.1 y (range = 32−63 y).