Laser speckle contrast analysis is a reliable measure of digital blood perfusion in Black Africans with systemic sclerosis

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ABSTRACT

Objective. *Laser speckle contrast analysis (LASCA) is evolving as a promising non-invasive tool to assess cutaneous microvascular function in systemic sclerosis (SSc). Reliability studies have mainly focused on Caucasian populations. To determine for the first time the inter-rater reliability of fingertip blood perfusion (BP) using LASCA in Black South African patients with SSc.*

Methods. *Consecutive Black adult patients with SSc were evaluated for peripheral BP using LASCA. Mean BP in defined regions of interest for dorsal fingertips and volar fingertips were measured in two subgroups of 20 SSc patients, each by three independent operators. Two operators were experienced in the use of the LASCA instrument and one was newly trained. Standardised protocols for conditions were followed for all measurements. Inter-rater reliability was tested using the intraclass correlation coefficient (ICC).*

Results. *The majority (87.5%) of the 40 patients included were females and 67.5% had diffuse cutaneous SSc. The mean age (standard deviation) was 48.5 (9.9) years and the median disease duration (interquartile range) was 8.5 (4, 13) years. There was good to excellent agreement, inter-rater ICC (dorsal fingertip range: 0.86-0.97 and volar fingertip range: 0.85-0.96), in both subgroups irrespective of operator skill.*

Conclusion. *LASCA is a credible instrument in patients of Black ethnicity with SSc, and across operator experience.*

Introduction

Systemic sclerosis (SSc) is a connective tissue disease with multisystem organ involvement. Endothelial damage manifesting clinically as peripheral microvascular dysfunction is common in patients with SSc (1). The progressive fibroproliferative vasculopathy often leads to complications such as digital ulcers or critical ischaemia (2, 3). There has been growing recognition for innovative tools to evaluate the microangiopathy in SSc (4).

While nailfold videocapillaroscopy is a well-established technique to assess capillary morphological alterations in SSc, various laser-based imaging tools have become available to evaluate functional aspects of the cutaneous microcirculation (5-9). One such non-invasive technique to dynamically measure blood perfusion (BP) over large tissue areas in real-time is laser speckle contrast analysis (LASCA), which in recent years has shown potential application in the diagnosis and measuring therapeutic response in SSc (10, 11). To date, studies on the clinical utility and reliability of LASCA have been limited mainly to Caucasian patients with predominantly limited cutaneous SSc (LcSSc) and living in colder climates (12, 13).

Whereas diffuse cutaneous disease is the predominant subset accounting for around two-thirds of cases in Black patients, the reverse is true in Caucasian patients in whom the limited subset accounts for most of the cases (14-16). Moreover, a greater burden of digital ulcers has been observed in Black patients compared to whites with SSc (17, 18).

The present study was undertaken to investigate the reliability of LASCA by operators of varying experience in Black South African patients with mainly diffuse cutaneous SSc (DcSSc) living in a subtropical climate.

Materials and methods

Consecutive Black adult patients with SSc, fulfilling the 2013 American College of Rheumatology/European League against Rheumatism classification criteria, were recruited during the summer season, from 11 January to 15 January 2016, at the Rheumatology Clinic of Chris Hani Baragwanath Aca-

demic Hospital, Johannesburg, South Africa (19). The study was approved by the Human Research Ethics Committee (Medical), University of the Witwatersrand (M150514) in compliance with the Helsinki Declaration. All patients provided written informed consent. Demographic and clinical features were documented, including the modified Rodnan skin scores which were performed on the same day as the LASCA measurements. Patients were classified as having DcSSc or LcSSc based on the criteria by LeRoy *et al*. (20, 21). Disease duration was defined as onset from first non-Raynaud's sign/symptom. All patients were taking their regular treatments including aspirin, vasodilator drugs and immunosuppressants.

Blood perfusion was evaluated using the LASCA device (Pericam PSI, Perimed, Jarfalla, Sweden). Patients were requested to refrain from caffeine intake 20 minutes before the examination. Smoking was not permitted on the day of the test. All patients were acclimatised at rest for 20 minutes in the same room at 25 ± 1°C before the assessment. Standardised instrumental parameters included: laser wavelength of 785nm; point density of 1386 x 1036; frame rate of 10 images/s and measurement area of 12.5 x 14/15 cm. Patients were seated; hands were kept flat (working distance of 14.3–14.8 cm from LASCA camera) and no speaking and movement were allowed during measurements. Peripheral BP was measured over the dorsal and volar regions of both hands for 30 seconds. Blood perfusion was assessed independently by three operators (V.S., V.L., and C.I.) and patients were divided into two subgroups of 20 patients. The first subgroup was assessed by the two experienced operators, V.L and V.S., and the second subgroup by V.L and the newly trained operator, C.I. The latter operator received a three hour-long training session in the use of LASCA, prior to commencement of the study. All operators (V.S., V.L, C.I.) were rheumatologists and two (V.S.,V.L) were proficient in the use of LASCA (12). Patients were assessed on the same day under identical conditions to minimise variability. Each observer was blinded to the others' results. Regions of interest, 1cm in diameter, were created at the 2nd–5th fingertips. The mean BP for each patient, reported as perfusion units, was calculated in regions of interest per finger bilaterally for the dorsal fingertips (DF) and volar fingertips (VF). Image analysis was performed using the LASCA software (PIMSoft 15.1, Perimed).

Descriptive statistics for demographic and clinical data were reported as means with standard deviation (SD), or median with interquartile range (IQR), as appropriate for continuous variables. Categorical variables were presented as numbers and percentages. The Wilcoxon signed-rank test was used to compare paired groups of variables. A p-value of <0.05 was considered statistically significant.

Inter-rater reliability was assessed by intraclass correlation coefficient (ICC) with 95% confidence interval (CI) based on a two-way random effects model using STATA v. 13.0. The interpretation of the level of inter-rater reliability (based on the 95% CI of ICC values) was described using the guideline outlined by Cicchetti *et al*. where values less than 0.40, between 0.4 and 0.59, between 0.60 and 0.74, and above 0.75 were indicative of poor, fair, good, and excellent reliability, respectively (22).

Table I. Demographic, clinical features and blood perfusion values for 40 SSc patients.

Parameter	Value
Female:Male	7:1
Age (years), mean (SD)	48.5 (9.9)
Disease duration (years), median (IQR)	8.5 (4, 13)
Disease duration >5 years	23 (57.5)
Ever smoker	4 (10)
Current smoker	2 (5)
Limited cutaneous SSc	13 (32.5)
Diffuse cutaneous SSc	27 (67.5)
mRSS median (IQR)	9.5 (6, 14)
Raynaud's phenomenon	39 (97.5)
History of digital ulcers	11 (27.5)
Pitting scars	24 (60)
Digital calcinosis	6 (15)
Telangiectasia	5 (12.5)
Tendon friction rubs	3 (7.5)
Anti-centromere positive	2 (5)
Anti-topoisomerase I positive	6 (15)
Vasodilator therapy (calcium channel blockers)	39 (97.5)
Dorsal fingertip BP (PU), median (IQR)	37.39 (26.41, 58.42)
Volar fingertip BP (PU), median (IQR)	59.07 (46.30, 88.79)

Values are n (%) unless otherwise specified.
BP: blood perfusion; IQR: interquartile range; mRSS: modified Rodnan skin score; PU: perfusion units; SSc: systemic sclerosis.

Results

Seven of the 47 patients originally recruited had to be excluded because of technical difficulties related to severe hand flexion contractures. The demographic, clinical, and BP data of the remaining 40 patients with SSc are shown in Table I. Most patients were female (87.5%) and 27 (67.5%) had DcSSc with a mean age (SD) of 48.5 (9.9) years and 23 (57.5%) had a disease duration >5 years. A history of ever smoking was documented in 10% and two patients (5%) were current smokers. Eleven patients (27.5%) had a history of digital ulcers, none with active lesions at the time of assessment, six (15%) had digital calcinosis cutis and 24 (60%) had pitting scars. Anti-topoisomerase I antibody was present in six (15%) patients while 5% had a positive anti-centromere antibody. All except one (97.5%) were treated with oral calcium channel blockers as vasodilatory therapy.

The results for the mean BP per operator and fingertip with corresponding ICCs are presented in Table II. The ICC (95% CI) for BP assessments did not differ for the subgroup assessed by two experienced V.S. and V.L. (DF range: ICC=0.88 [0.72–0.95] - 0.95 [0.77–0.98] and VF range: ICC=0.85 [0.61–

Table II. The inter-rater reliability of digital blood perfusion measured by LASCA in black African patients with SSc (n=40).

n=20	Mean BP (SD) Operator V.L.	Mean BP (SD) Operator V.S.	ICC (95% CI)	n=20	Mean BP (SD) Operator V.L.	Mean BP (SD) Operator C.I.	ICC (95% CI)
DORSAL				DORSAL			
Left				**Left**			
F2	37.85 (23.47)	39.25 (29.64)	0.93 (0.68 to 0.98)	F2	48.70 (36.88)	61.61 (41.32)	0.91 (0.77 to 0.96)
F3	37.89 (30.77)	38.80 (34.56)	0.94 (0.85 to 0.97)	F3	47.79 (37.15)	54.88 (41.02)	0.91 (0.78 to 0.97)
F4	40.05 (27.87)	43.87 (36.73)	0.94 (0.86 to 0.98)	F4	51.20 (34.94)	57.48 (39.45)	0.86 (0.66 to 0.95)
F5	46.34 (48.67)	45.85 (42.47)	0.88 (0.72 to 0.95)	F5	50.67 (32.00)	59.40 (43.41)	0.91 (0.76 to 0.96)
Right				**Right**			
F2	42.74 (24.34)	38.11 (18.12)	0.94 (0.80 to 0.98)	F2	56.99 (36.61)	66.62 (47.91)	0.88 (0.70 to 0.95)
F3	45.84 (29.41)	47.79 (34.72)	0.94 (0.62 to 0.98)	F3	49.13 (39.30)	62.44 (43.29)	0.97 (0.92 to 0.99)
F4	54.39 (38.08)	52.28 (44.57)	0.94 (0.79 to 0.98)	F4	58.43 (39.77)	68.42 (43.04)	0.95 (0.88 to 0.98)
F5	53.21 (35.40)	55.78 (40.20)	0.95 (0.77 to 0.98)	F5	58.80 (44.08)	71.30 (52.28)	0.93 (0.83 to 0.97)
VOLAR				VOLAR			
Left				**Left**			
F2	71.73 (46.38)	68.44 (52.32)	0.93 (0.81 to 0.97)	F2	86.39 (56.27)	90.53 (55.88)	0.91 (0.77 to 0.96)
F3	60.14 (46.72)	63.43 (54.35)	0.85 (0.62 to 0.94)	F3	78.66 (46.26)	85.23 (50.49)	0.95 (0.86 to 0.98)
F4	68.47 (47.68)	67.59 (47.87)	0.88 (0.69 to 0.95)	F4	75.41 (46.76)	78.33 (45.68)	0.89 (0.72 to 0.96)
F5	72.81 (65.85)	69.67 (64.96)	0.85 (0.61 to 0.94)	F5	73.37 (41.87)	78.77 (48.55)	0.94 (0.85 to 0.98)
Right				**Right**			
F2	77.17 (58.15)	84.77 (71.18)	0.95 (0.86 to 0.98)	F2	105.4 (61.35)	99.43 (63.07)	0.95 (0.89 to 0.98)
F3	75.23 (66.94)	80.79 (71.92)	0.95 (0.87 to 0.98)	F3	85.77 (53.05)	84.18 (56.83)	0.96 (0.90 to 0.98)
F4	68.61 (44.55)	74.81 (58.84)	0.92 (0.78 to 0.97)	F4	90.22 (57.86)	87.37 (56.61)	0.91 (0.77 to 0.96)
F5	73.73 (56.47)	78.28 (60.61)	0.91 (0.77 to 0.97)	F5	97.51 (72.14)	88.32 (69.63)	0.93 (0.83 to 0.97)

BP: blood perfusion; CI: confidence interval; F: fingertip; ICC: intraclass correlation coefficient; LASCA: laser speckle contrast analysis; n: number; SD: standard deviation.

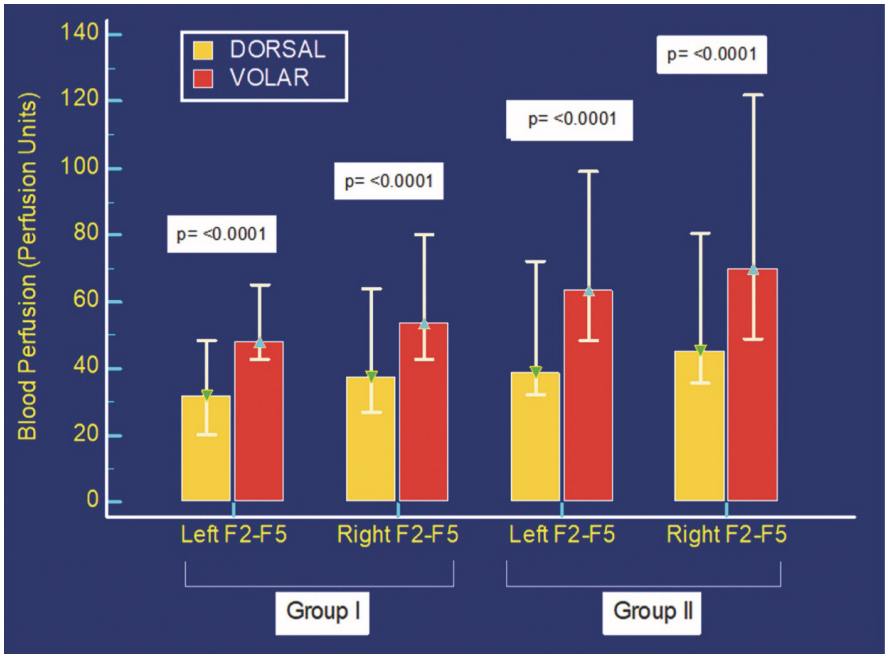

Fig. 1. Comparison between blood perfusion for dorsal and volar fingertips for each group of operators. This figure shows differences in blood perfusion between dorsal and volar fingertips (F) of both hands for Group 1 operators (V.L. and V.S.; n=20 SSc patients) and Group II operators (V.L. and C.I.; n=20 SSc patients).
Bar plots show median and interquartile ranges. *p*-values are according to the Wilcoxon signed rank test.

0.94] - 0.95 [0.86–0.98]) compared to the subgroup assessed by experienced V.L. and newly trained C.I. (DF range: ICC 0.86 [0.66–0.95] - 0.97 [0.92–0.99] and VF range: 0.89 [0.72–0.96] - 0.96 [0.90–0.98]). Moreover, the ICC were similar over a range of BP rates and areas of assessment for both dorsal and volar fingertips. Blood perfusions were significantly higher for VF compared to the corresponding DF of both hands in all 40 SSc patients (p<0.001) (Fig. 1).

Discussion

In the first time ever study of LASCA in Black Africans with SSc there was overall good to excellent inter-rater reliability of dorsal and volar fingertip BP measurements. Most of the patients, living in the warmer subtropical climate of Johannesburg, had diffuse cutaneous disease. Our findings are comparable to the results of two previous studies conducted under the same standardised conditions in Belgian Caucasians with predominantly limited cutaneous disease and living in a relatively cooler climate (12, 13).

In a study by Lambrecht *et al*. of 34 patients with SSc and two independent raters, the ICC (95% CI) ranged from 0.82 (0.67–0.91) to 0.91 (0.83–0.96) for DF, and from 0.74 (0.55–0.86) to 0.86 (0.74–0.93) for VF indicating good to excellent inter-rater reliability (12). In another more recent study of LASCA that included a total of 30 Caucasian SSc patients, using four raters, the ICC

(95% CI) value for inter-rater reliability was 0.97 (0.90–0.99) for average BP measurements (DF and VF combined) in a subgroup of 15 patients. The good to excellent inter-rater agreement was confirmed in an external validation cohort of 15 patients in the same study, with an ICC (95% CI) of 0.87 (0.67–0.96). Additionally, similar acceptable inter-rater reliability results were demonstrated for 30 healthy control subjects (13).

Moreover, we found that the reliability of LASCA was not significantly different and remained good to excellent between assessments made by experienced operators compared to the subgroup assessed by an experienced operator and a recently trainer operator. These results suggest that LASCA is a stable measure for operators with relatively less testing experience.

An interesting observation in both subgroups was the significantly higher digital blood flows in the volar fingertips when compared with the dorsal aspect of fingertips, as demonstrated by LASCA. This has been observed in both SSc patients and healthy subjects in previous reports but the authors did not particularly highlight this finding (23, 24). The differential in BP between the two surfaces might be related to regional differences in anatomical vascularity.

Limitations of the present study include the relatively small sample size and lack of inclusion of control subjects. We did not perform an intra-rater reliability analysis which could have been important to draw additional conclusions, especially for the newly trained operator. This aspect has, however, been attested elsewhere by our group with good to excellent intra-rater reliability demonstrated for LASCA BP measurements, ICC 0.95 and 0.93, in SSc patients and healthy subjects, respectively (13).

Notwithstanding these limitations, our pilot study provides further evidence to the credibility of the LASCA instrument in non-Caucasians, across disease subsets, operator experience, and climates. This marks an important step forward towards the validation of LASCA as a potentially useful tool to quantify microvascular function in SSc. Further studies are needed to explore and better understand inter-ethnic differences with respect to the relationship between LASCA and clinical features in SSc.

Take home messages

- The inter-rater reliability of LASCA was good to excellent in Black Africans with SSc.
- LASCA is a credible tool for application across ethnicities, SSc subsets and operator experience.

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