

voriconazole for *C. glabrata* complex, which we could not assess as the current Vitek 2 system issues no result for fluconazole or voriconazole for this species complex. Additionally, Posteraro *et al.* did not test *C. krusei* which in our study had poor essential agreement for voriconazole. Categorical agreement between Vitek 2 and Sensititre YeastOne for caspofungin was noted to be suboptimal in our study, congruent with Astvad *et al.* who found 6/31 *fkS* mutant *Candida* isolates were misclassified as susceptible by the Vitek 2 system, while the reference CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) methods misclassified <4% of these isolates.<sup>9</sup>

In our study skewed for resistant *Candida* isolates, essential agreement and categorical agreement between Vitek 2 AST YS08 and Sensititre YeastOne were suboptimal. More validation data with resistant isolates needs to be obtained for the Vitek 2 AST YS08 system.

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## A descriptive account of sequential nailfold capillaroscopy in scleroderma



Sir,

Microvasculopathy is an early and prominent pathological feature of systemic sclerosis (SSc), or scleroderma, and is most easily recognised in the capillaries of the nailfold using the simple technique of nailfold capillaroscopy (NFC).<sup>1</sup> Many studies have confirmed that NFC has proven utility in the early diagnosis of SSc and it has been included in the classification criteria for this disease since 1988.<sup>1</sup> However, to date there is little information concerning sequential NFC in scleroderma and whether documentation of nailfold capillary morphology and density over time may assist in disease management and prognosis.

In this current study we have compared and contrasted sequential nailfold capillary density and morphological characteristics in both healthy subjects and scleroderma patients for periods of up to 12 months.

There were nine patients in the scleroderma study group, three with diffuse cutaneous scleroderma, five with limited cutaneous scleroderma and one with overlap scleroderma. All patients were recruited from the South Australian Scleroderma Register and all patients fulfilled the diagnosis of scleroderma according to the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2013 classification criteria and stratified as limited cutaneous, diffuse cutaneous or overlap scleroderma according to LeRoy's criteria.<sup>2–4</sup> The demographics, clinical and serological features and medications of the scleroderma study group (at entry to the study) are shown in Table 1.

There were four healthy control subjects, three females and one male with an age span of 28–66 years. None were smokers and none had Raynaud's phenomenon. NFC was performed on both patients and controls at regular intervals over the year study period.

NFC was performed on the fourth finger nailfold of each hand using a Capiscope (supplied by KK Technology, United Kingdom). Paraffin oil or KY jelly was applied to the nailbed to reduce the skin/air refractive barrier. The Capiscope technique allows visualisation of the nailfold capillaries at magnifications of 100× and 300× and has the capability of digitalisation of the nailfold images or video capillaroscopy. The procedure was done exactly as described in the Capiscope user's manual enabling the capture of multiple digitalised images.<sup>5</sup> Overlapping images were then aligned and electronically spliced to form a composite mosaic of the nailfold and its capillary arcades. The microvasculature was then assessed from the digitalised images. In particular, we assessed either quantitatively or qualitatively the symmetry or otherwise of the capillary arcades, the morphology and dimensions [normal size dilated or grossly dilated (giant) of the capillary loops, nailfold capillary density, the presence and appearances of capillary microbleeds and the appearance of the cuticle (widened, roughened, discoloured)].

Capillary density was measured from the digitalised nailfold images of each nailfold by the direct observation method

**Table 1** Clinical and laboratory characteristics of patient cohort

Patient no.	Age, years	Gender	Disease subtype	Auto Ab	Disease duration, years	Medication
1	62	M	D/ILD/PAH	Topo 1	11	Bosentan, CCB, O <sub>2</sub> , Sildenafil, ACE
2	69	F	L/DU	ANA(sp)	2	ACE
3	51	F	L	C	11	PPI
4	51	F	D	RNAP	3	MTX, MPO, Pred, CCB
5	70	F	L/PAH	ANA(n)	22	CCB, PPI
6	41	F	D	RNAP	2	MPO, PPI
7	66	F	L	ANA(sp)	9	PPI
8	61	M	L/DU	C	15	CCB
9	26	F	O	ANA(n)	5	CCB

ACE, angiotensin converting enzyme inhibitor; ANA(n), nucleolar pattern; ANA(sp), speckled pattern; C, centromere; CCB, calcium channel blockers; D, diffuse cutaneous; DU, digital ulceration; ILD, interstitial lung disease; L, limited cutaneous; MPO, mycophenolate; MTX, methotrexate; O, overlap; PAH; pulmonary arterial hypertension; PPI, protein pump inhibitor; Pred, prednisolone; RNAP, RNA polymerase 3; Topo 1, topoisomerase 1.

of Hofstee by counting directly the number of capillary loops in the most distal arcade across a transverse 1 mm marker.<sup>6</sup> The capillary density measurement was performed at the midpoint of the nailfold as assessed from the nailfold capillary mosaic.

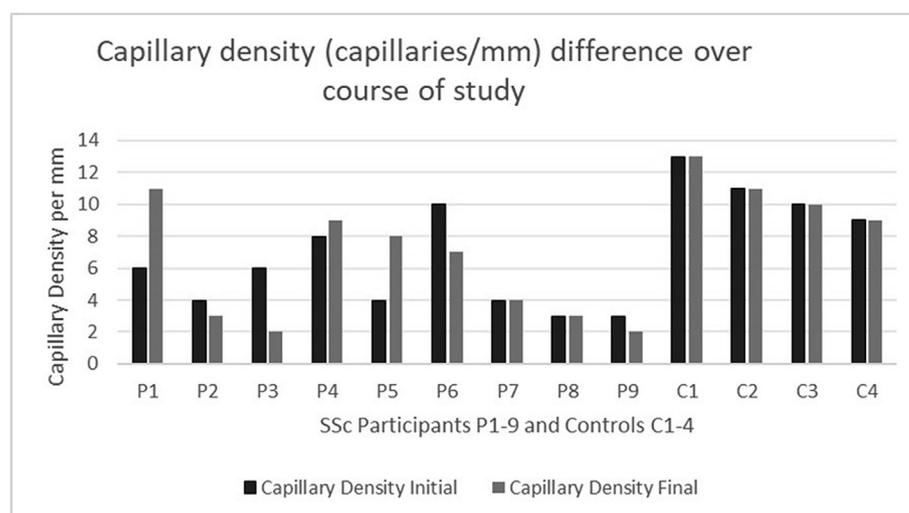
In health the nailfold appeared very stable (Supplementary Fig. 1, Appendix A) with no change in capillary density over the study period (Fig. 1), findings consistent with previous reports.<sup>7,8</sup> In contrast, in the scleroderma cohort we observed that the mean capillary density decreased in four patients, increased in two patients and remained unchanged in three patients over the study period (Fig. 1). Furthermore, we observed progressive disruption and distortion of the capillary arcades to varying degrees in different patients. Notably, we observed examples of capillary dilatation, repeated capillary bleeding with the formation of 'beads and waves' of extravasated blood deposits migrating distally into the cuticle, capillary destruction leaving avascular areas, and finally, the emergence of new capillaries moving into these bare areas (Fig. 2; Supplementary Fig. 2–4, Appendix A). The wave pattern of bleeding/extravasation, evident particularly in the cuticle, was consistent with synchronous bleeding occurring from several sites within the nailfold. The cuticle was frequently 'ragged' and had a slight brownish discolouration. There was no apparent relationship between episodes of

Raynaud's (often several times per day) and the appearance of microhaemorrhages in individual patients. Morphological changes were apparent in some patients with scleroderma in a period as short as 6 weeks.

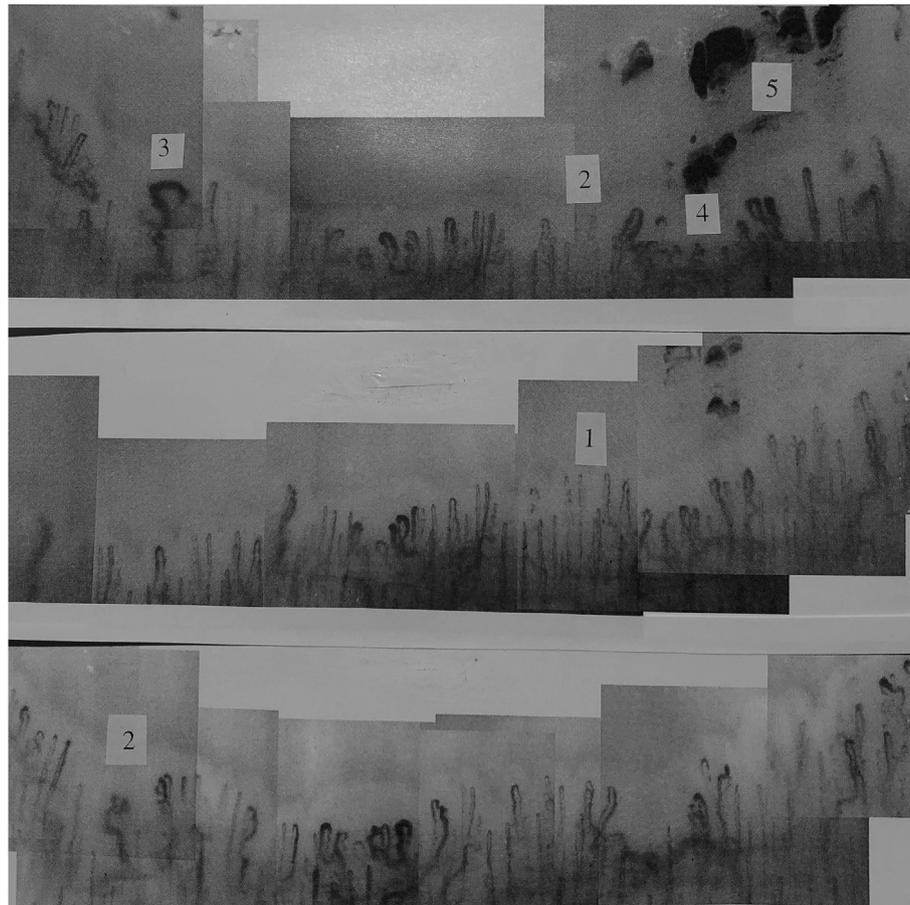
We conclude that in scleroderma the nailfold vascular tissue is in a state of constant flux with capillary dilation and repeated capillary bleeding associated with capillary loss and regeneration.

Our observations and conclusions are very similar to those of Wong and colleagues who examined capillary appearances under 40× magnification monthly for 7 months in four scleroderma patients and one patient each with dermatomyositis, mixed connective disease (MCTD) and limited connective tissue disease.<sup>9</sup> However, all their published illustrations of capillary injury involved the patients with dermatomyositis and MCTD, these investigators did not formally measure capillary density and made no mention of capillary renewal. These New Zealand authors concluded that their findings were consistent with the hypothesis that the observed capillary dilatation, bleeding and capillary loss was due to recurrent vascular injury (e.g., endothelial cell cytotoxins, immune complexes or exogenous events such as exposure to cold).

Other published studies of sequential NFC are sparse despite NFC being one of the ACR/EULAR diagnostic



**Fig. 1** Capillary density (caps/mm) at mid-part of fourth nailfold for patients P1–P9 and controls C1–4 over the 12 month period study period.



**Fig. 2** Progression of capillary morphological changes over a 12 week period in Patient 1 demonstrating capillary ghosting (1), capillary loss (2), capillary dilatation (3), irregularity of capillary arcades (4) and microhaemorrhages (5). The lower image is the initial image followed by the middle image 6 weeks later and the final image (top) 12 weeks later.

criteria for scleroderma and being in regular clinical use in rheumatological practice.<sup>1</sup>

A detailed sequential NFC study has been recently reported by Avouac and his European colleagues.<sup>10</sup> In their study they examined visual images of a linear 1 mm zone of the nailfold from all eight fingers obtained with NFC on 140 scleroderma patients with mean disease duration of 9 years. NFC examination was performed on four occasions over a 3 year study period. At study entry abnormalities in nailfold capillary morphology  $\pm$  micro bleeds were found in 90% of patients. Of particular interest at entry, 126 patients (90%) showed a low capillary density [ $x = 5.1 \pm 2.3/\text{mm}$  ( $m \pm \text{SD}$ ) compared with a normal value  $>9/\text{mm}$ ]. Over the next 3 year period significant NFC changes were detected in 72 patients (51%) with 20 of these patients showing a progressive capillary loss. Indeed, the progressive capillary loss predicted overall disease progressions, occurrence of new digital ulcers, lung vascular progression in four patients, progression of skin fibrosis and worsening of the Medsger severity score.

Another recent study from The Netherlands examined NFC characteristics annually in 138 scleroderma patients undergoing intensive therapy with the majority of their patients having two or three NFC examinations.<sup>11</sup> NFC morphology was reported stable in 53% of these patients, but importantly 26% showed 'reverse transition' or improvement, particularly those patients who had had intense immunosuppression followed by autologous haemopoietic stem cell transplantation (HSCT). This is

consistent with the finding of Miniati and colleagues in Italy who also reported improvements of nailfold video capillaroscopy features in those patients on immunosuppressive drugs.<sup>12</sup>

In conclusion, we have demonstrated that in health the nailfold capillary morphology appears stable for periods up to one year whilst in nine patients with scleroderma we observed multiple and evolving changes in capillary calibre, capillary bleeding, capillary destruction and loss and angiogenesis with the appearance of new capillaries. The nature of these capillary morphological changes is not known but our observations favour recurrent endothelial injury as a possible causal factor.

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#### APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pathol.2019.05.009>.

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