

The Interplay of Vascular Function, Low Grade Inflammation and Innate Immunity in the Disease Process of Raynaud's Phenomenon and Systemic Sclerosis

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Background

Systemic sclerosis (SSc) is a multi-system disorder, characterised by distinctive pathological vascular abnormalities, typical immunological features and chronic fibrosis of skin / internal organs. Despite considerable disease morbidity and risk of early mortality, the aetiology and pathophysiology of SSc is poorly understood. As a result, disease measures and current therapeutic options are limited.

Aim

Building on preliminary work from our department, we aimed to develop novel, integrated measures of disease activity and severity for use in SSc, whilst elucidating and characterising the interplay between low grade inflammation, innate immune response and vasculopathy in this disease.

Methods

- Patients with SSc, primary Raynaud's phenomenon, undifferentiated connective tissue disease (UCTD) and healthy control subjects were studied.
- Three groups of parameters studied:
 - 1) **NK cell and neutrophil phenotype and function:** in peripheral venous blood.
 - 2) **Inflammatory response and vascular activation:** peripheral venous blood was collected for assay of
 - a) hsCRP and IL-6.
 - b) von Willebrand factor, endothelial cell adhesion molecules, vascular endothelial growth factor and endothelin-1 (ET-1).
 - 3) **Vascular physiological function:**
 - a) Macrovascular function: applanation tonometry (Figure 1).
 - b) Microvascular morphology: digital nailfold video capillary microscopy (Figures 2 & 3).
 - c) Microvascular function: digital iontophoresis of acetylcholine (endothelial-dependent vasodilatation) and nitroprusside (endothelial-independent vasodilatation) measured by laser Doppler imaging (Figure 4).



Figure 1. Applanation tonometry.

Pulse wave analysis was used to assess radial artery stiffness (peripheral arterial function / morphology) and the velocity of the pulse wave has been used as a measure of generalised large vessel function in study participants.

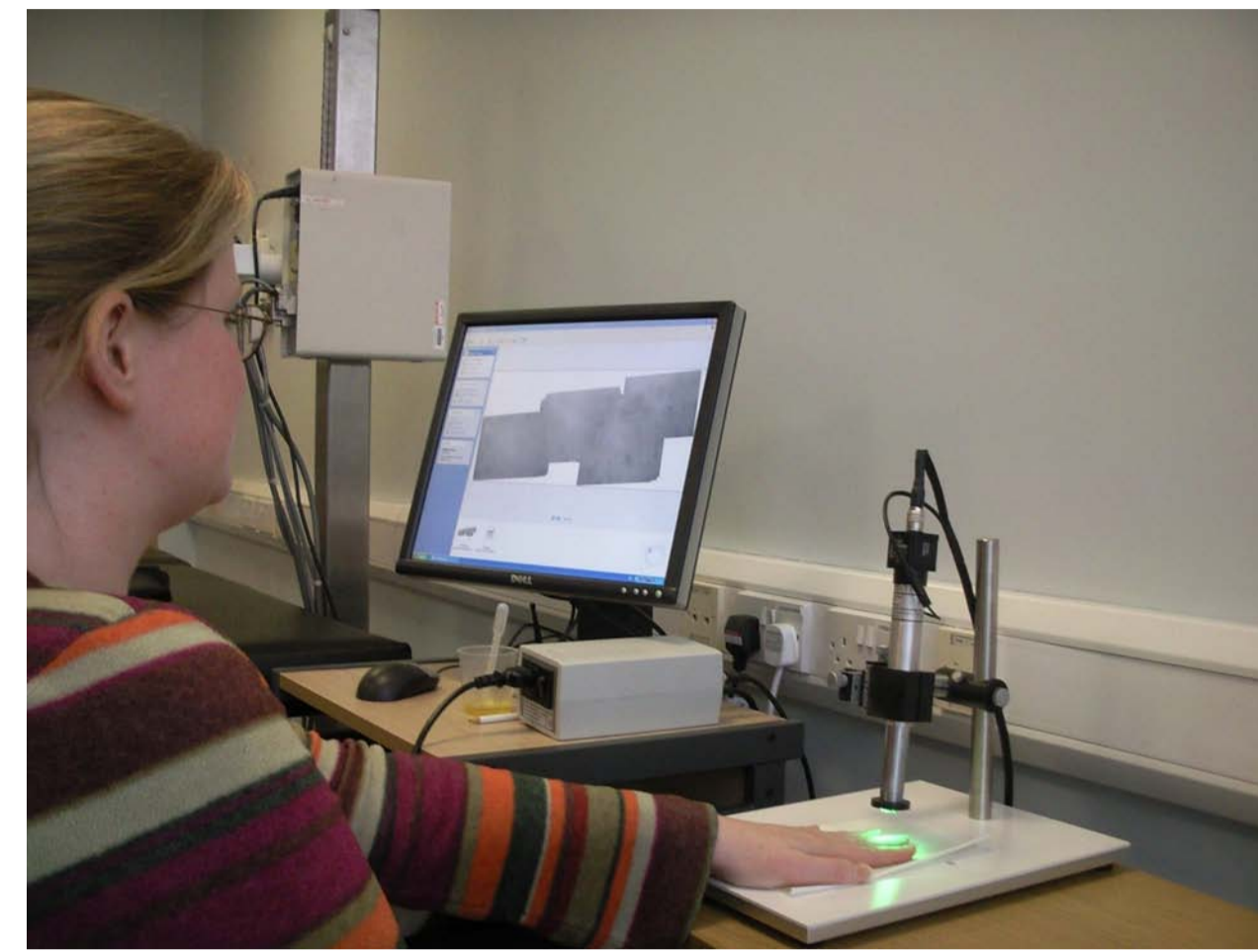


Figure 2. Digital nailfold video capillary microscopy.

A panoramic mosaic of the nailfold capillaries of the ring finger of the non-dominant hand has been recorded, and measures of capillary dimensions and density performed on each study subject.



Figure 3. Digital nailfold video capillary microscopy mosaic of patient with UCTD, demonstrating early capillary enlargement and tortuosity.



Figure 4. Simultaneous iontophoresis of acetylcholine and nitroprusside on adjacent fingers with measurement of blood flow response with concomitant serial rapid laser Doppler scans of the tested digits was conducted on each study participant.

Results

NK cell and neutrophil phenotype and function

NK cells

- Serum cytokine levels: marked inter-individual variability in all groups.
- Th1/Th2 cytokines production (*in vitro* stimulation assay): marked variability within populations. However, IL-6, IFN- γ and IL-2 production distinguishes between groups (Table 1).

Neutrophils: activation and altered function in SSc

Proteomics:

- A number of proteins are expressed in higher concentrations in SSc neutrophils than in control neutrophils. Similar protein changes are found in neutrophils activated by bacterial products.
- Of note, actin is over-expressed. The over-expression of this protein (confirmed in several ways), that is so integral to neutrophil movement, implies that patient neutrophils are activated in the blood of patients SSc.

Apoptosis:

- Neutrophils from SSc patients become apoptotic much earlier than those from healthy controls, but are not prone to necrosis.
- Sera from patients with SSc causes increased apoptosis of healthy neutrophils compared with control sera.

Peripheral venous blood measures of inflammatory response / vascular activation and tests of vascular physiological function

Collection of data is being completed, and results are pending for this section of the study.

	IL-6	IFN- γ	IL-2
Undifferentiated connective tissue disease	high	high	high
Limited cutaneous SSc	normal	normal	normal
Diffuse cutaneous SSc	normal	normal	low

Table 1. NK cell production of IL-6, IFN- γ and IL-2

Conclusions

Preliminary results support the hypothesis that the innate immune system is important in the pathogenesis of UCTD and SSc.

With the imminent completion of measures of vascular physiological function and assays of inflammatory response and vascular activation, we will be able to correlate these findings with results from the studies of the innate immune system.

Our long-term aim is to apply these combined measures in clinical studies of therapeutic agents.

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