Microcirculatory Haemodynamics and Endothelial Dysfunction in Systemic Lupus Erythematosus

Wright: Microvascular Haemodynamics in SLE

Supplemental data to include detailed methodology, tables and figures.
Detailed Methods

Subjects

Patients fulfilling the ACR criteria for the diagnosis of SLE(1) were recruited from the lupus research group at Queen’s University Belfast (QUB). This database contains the details of over 350 patients with SLE from throughout Northern Ireland. A total of 126 of these patients fulfilled the inclusion and exclusion criteria for this study and were then invited by post to participate in the study. The SLE patients were then studied consecutively as each patient consented to be involved. Control subjects were recruited from the secretarial and support staff at QUB and matched to the SLE patients according to age. Patients and controls were excluded if they had any of the following:- diabetes mellitus; hypertension; significant pulmonary, hepatic or renal disease; typical angina or myocardial infarction; cerebrovascular disease or history of transient ischaemic attack; use of antihypertensive, oral hypoglycaemic or lipid lowering agent (in the last 3 months); glucocorticoids equivalent to greater than 10mg prednisolone daily; all pregnant or lactating women. All subjects gave written informed consent to take part in the study, which was approved by the QUB Local Research Ethics Committee and conducted according to the Declaration of Helsinki.

In all subjects, a detailed clinical interview was conducted to ascertain presence of conventional cardiovascular risk factors. An electrocardiogram and full screening blood tests were performed, including plasma homocysteine concentration, fasting lipid profile, serum anti-nuclear antibody levels, anti-dsDNA antibodies, anti-cardiolipin antibodies and complement C3
and C4 levels. The assays used standardised conditions employed in recent SLE studies in Belfast(2;3). Platelet production of 8-epi prostaglandin F$_{2\alpha}$ (8-epi PGF$_{2\alpha}$) was measured as previously described(4). In the SLE patients, disease activity was assessed using SLAM (Systemic Lupus Activity Measure)(5) and organ damage was assessed using the American College of Rheumatology/Systemic Lupus International Collaborating Clinics (ACR/SLICC) score(6).

Flow-mediated dilation
The right brachial artery was assessed using high-resolution B-mode ultrasound (ATL HDI3500 with a 7.5MHz linear-array transducer) following the previously published protocol(7). The ultrasound system was connected to a PC equipped with a frame grabber (National Instruments, Texas) and artificial neural network(8) wall detection software (VIA, MD Medic). The accuracy and reproducibility of FMD measurement using this software in our laboratory is equivalent to previously published work(8;9).

All subjects were studied between 8am and 10am after a 12-hour overnight fast. They were asked not to smoke, drink tea, coffee or alcohol for 12 hours prior to the study. All studies were performed in a quiet, temperature controlled, dedicated vascular research laboratory by the same operator. The brachial artery was scanned longitudinally in the antero-medial plane 2-10cm above the antecubital fossa. Pulsed Doppler was used to record velocity waveforms for 10 seconds at baseline and immediately after cuff deflation for 15 seconds.
A resting scan was taken for 2 minutes. A tourniquet located on the forearm immediately below the antecubital fossa was inflated to 50mmHg above the patients’ systolic blood pressure for 4.5 minutes. Brachial artery diameter measurements were recorded for 2 minutes after cuff release. The subject then rested for at least 10 minutes before measuring endothelium independent dilation (EID), 3 minutes after 500μg sublingual glyceryl trinitrate (GTN).

At the end of the study VIA immediately displayed a graph of diameter against time (figure 1). FMD (using diastolic diameters) was automatically calculated by VIA and expressed as a percentage increase in brachial artery dilation from baseline to provide an estimate of endothelium dependent dilation (EDD). EID was similarly calculated and expressed as the percentage increase in mean diameter 3 minutes after GTN administration.

Hyperemic Diastolic shear stress
Pulsed Doppler velocity waveforms were recorded for 15 seconds immediately after cuff release using a carrier frequency of 6.0MHz, an insonation angle of 70° and a 1.5mm gate range in the centre of the artery. The velocity waveform envelopes were digitised at 100Hz, low passed filtered at 20Hz and stored onto a networked personal computer and analysed off-line using HDI Lab (ATL, Advanced Technologies Laboratory, Bothell, WA). The mean velocity waveforms in cm/s were automatically calculated by HDI Lab using the Doppler frequencies and insonation angle transmitted from the ultrasound machine. The hyperemic mean diastolic velocity (MDV) was then
identified using the ECG ‘R’ wave as a fiducial point and averaged for all of
the velocity envelopes obtained during the 15 seconds.

Hyperemic Diastolic shear stress (DSS) was obtained from the following
equation: \[ DSS = 8 \times \mu \times (MDV/DBL), \] where \( \mu \) = blood viscosity, MDV= mean diastolic velocity and DBL = brachial artery baseline diameter. This is
derived from Poiseuille’s Law governing shear rate and applies to a
Newtonian fluid(11). Blood is a non-Newtonian fluid and hence the viscosity
varies at different shear rates. We assumed a blood viscosity of 0.035
dyne·s/cm², which corresponds with the observed range of shear rates in the
brachial artery(12).

Waveform Analysis
The velocity waveforms at baseline and during reactive hyperemia were
obtained by pulsed Doppler as described above. The peak velocity waveform
envelopes were extracted using HDI Lab and stored for off-line analysis. The
resistive index (RI) (peak systolic velocity minus end-diastolic velocity over
peak systolic velocity: PSV-EDV/PSV)(13) was calculated from the waveforms
using HDI lab. A modified version of the Root-MUSIC algorithm that permits
beat-to-beat analysis of recorded waveforms was applied to each of the
velocity waveform envelopes, using Matlab version 7.0.1 (MathWorks, Inc), to
give representative power-frequency spectrums. These power-frequency
spectrums were then averaged to give a single power-frequency spectrum for
each patient's baseline signal. The modified root-MUSIC algorithm was also
applied to the second complete peak velocity waveform for each patient
during cuff release to represent maximum reactive hyperaemic flow (figure 2).
(The first velocity waveform after cuff release was not used due to the potential for the cuff to be released at different times during the cardiac cycle).

Percentage change in the power of the first four frequency components from baseline was then calculated for each subject using:

\[
\%\text{Change}(f_x) = \frac{\text{RHP}(f_x) - \text{BLP}(f_x)}{\text{BLP}(f_x)} \times 100
\]

where, \( f_x \) = frequency components 1 to 4 (Hz)

\[
\text{RHP} = \text{Reactive Hyperemia Power (cm/s)}^2
\]

\[
\text{BLP} = \text{Baseline Power (cm/s)}^2
\]

In a subgroup of patients, the cuff was placed onto the left forearm and inflated to 50mmHg above systolic pressure for 4.5 minutes. The right brachial artery was insonated and the Doppler velocity waveforms were recorded and stored, at baseline and after cuff release, as described above. This allowed confirmation that the change in velocity waveform morphology was not influenced by central haemodynamic factors during cuff deflation.
Reference List


(6) Gladman DD, Urowitz MB, Goldsmith CH, Fortin P, Ginzler E, Gordon C. The reliability of the systemic luus international collaborating


(9) Sidhu JS, Newey VR, Nassiri DK, Kaski J-C. A rapid and reproducible on line automated technique to determine endothelial function. Heart 2000; 88:289-292.


Table I: Heart rate and brachial artery velocity waveform frequency domain changes after release of cuff on left forearm while insonating the right brachial artery in 6 subjects (3 controls and 3 SLE patients). Results expressed as mean±SD.
Figure I (a)
Figure I (b)
Figure II

Flow Mediated Dilation (%) vs. Diastolic Shear Stress (dyne/cm²)

Patient Group
- ▲ Control
- ○ SLE

$r=0.65; p=0.01$
Figure III

r = -0.67; p = 0.01
Figure IV
Figure V

- $r = -0.71; \ p = 0.63$

Mean Arterial Pressure mmHg

Flow Mediated Dilation %

Patient Group

- Control
- SLE
Flow Mediated Dilation %

p=0.23

If on NSAID, aspirin or COX-2 inhibitor

Figure VI
Figure Legends

Figure I
VIA output showing graph of diastolic diameter change with time for (a) control subject and (b) patient with SLE. FMD calculated by comparing baseline mean diameter before cuff inflation and diameter between ‘peak start’ and ‘peak end’ cursors.

Figure II
Correlation plot of flow mediated dilation (FMD) against diastolic shear stress (DSS) for patients with SLE and control group

Figure III
Correlation plot of flow mediated dilation (FMD) against disease activity in patients with SLE as measured by SLAM-R

Figure IV
Correlation plot of flow mediated dilation (FMD) against C-reactive protein (CRP) for patients with SLE and control group

Figure V
Correlation plot of flow mediated dilation (FMD) against mean arterial pressure (MAP) in patients with SLE and control group
Figure VI

Boxplots to show flow mediated dilation in those subjects taking and not taking aspirin/NSAID/cox-2 inhibitors. Median, interquartile range and extreme values are shown.