Microcirculatory Hemodynamics and Endothelial Dysfunction in Systemic Lupus Erythematosus

Stephen A. Wright, Fiona M. O’Prey, Derrick J. Rea, Rick D. Plumb, Andrew J. Gamble, William J. Leahey, Adrian B. Devine, R. Canice McGivern, Dennis G. Johnston, Michael B. Finch, Aubrey L. Bell, Gary E. McVeigh

Objective—Impaired flow-mediated dilation (FMD) occurs in disease states associated with atherosclerosis, including SLE. The primary hemodynamic determinant of FMD is wall shear stress, which is critically dependent on the forearm microcirculation. We explored the relationship between FMD, diastolic shear stress (DSS), and the forearm microcirculation in 32 patients with SLE and 19 controls.

Methods and Results—DSS was calculated using (mean diastolic velocity × 8 × blood viscosity)/baseline brachial artery diameter. Doppler velocity envelopes from the first 15 seconds of reactive hyperemia were analyzed for resistive index (RI), and interrogated in the frequency domain to assess forearm microvascular hemodynamics. FMD was significantly impaired in SLE patients (median, 2.4%; range, −2.1% to 10.7% versus median 5.8%; range, 1.9% to 14%; P < 0.001). DSS (dyne/cm²) was significantly reduced in SLE patients (median, 18.5; range, 3.9 to 34.0 versus median 21.8; range, 14.1 to 58.7; P = 0.037). A strong correlation between FMD and DSS, r = 0.65, P = 0.01 was found. Postischemic RI was not significantly different between the 2 groups; however, there were significant differences in the power-frequency spectrums of the Doppler velocity envelopes (P < 0.05).

Conclusions—These data suggest that in SLE, altered structure and function of the forearm microcirculation contributes to impaired FMD through a reduction in shear stress stimulus. (Arterioscler Thromb Vasc Biol. 2006;26:2281-2287.)

Key Words: eigenvector • flow-mediated dilation • microcirculation • shear stress • systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is the archetypal autoimmune disease, with a wide range of clinical manifestations. Among the clinical challenges of SLE, one of the most compelling is the high incidence of atherosclerosis in young women. In 1976, Urowitz et al showed a bimodal mortality pattern in SLE, with late deaths (comprising 45%) attributed to myocardial infarction.1 Women with SLE have a high prevalence of coronary artery disease (CAD)2 and an incidence of myocardial infarction up to 50 times higher than age-matched normals.3 Classical risk factors are similar to those in the general population,3 but the increased risk of atherosclerosis is not exclusively related to traditional Framingham risk factors alone,4 with a recent report highlighting SLE itself as an independent risk.5 Whereas several studies have highlighted the presence of subclinical atherosclerosis in SLE,6,7 the pathogenesis is not fully understood. It has been proposed that autoimmune vascular injury in SLE may predispose to atherosclerotic plaque formation through mechanisms that promote endothelial dysfunction, the earliest precursor for plaque development.8–10

Flow-mediated dilation of the brachial artery (FMD) is used clinically as an indirect bioassay for endothelium-derived nitric oxide (NO) production. The primary hemodynamic determinant of FMD is wall shear stress,11–13 and the degree of FMD has been shown to be proportional to both systolic and diastolic shear stress (DSS) in response to increased flow produced by an ischemic stimulus.14,15 The magnitude of the postischemic flow increase in the brachial artery that determines the shear stress stimulus and thus nitric oxide release is critically dependent on the degree of dilation of the downstream microvasculature.16 Structural and functional changes in the microvasculature that may predate or accompany cardiovascular disease development can limit flow reserve and influence the shear stress stimulus for conduit artery dilation.17 In addition to measuring diameter change in conduit arteries, characterization of ultrasound-based Doppler flow velocity waveforms are also used to determine the status of downstream microvascular networks.18

We hypothesize, that in SLE, an alteration in the forearm microcirculatory hemodynamics contributes to the impairment in FMD through a reduction in the shear stress stimulus. To minimize potential confounding factors on the measure-
ment of endothelial dysfunction, we studied SLE patients with no history of major organ involvement, without excess of conventional risk factors and with no history of cardiovascular or cerebrovascular disease.

**Methods**

Please see http://atvb.ahajournals.org for detailed methodology.

**Subjects**

Patients fulfilling the American College of Rheumatology criteria for the diagnosis of SLE were recruited from the lupus research group at Queen’s University Belfast (QUB). 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 ischemic attack; use of antihypertensive, oral hypoglycemic or lipid-lowering agent (in the past 3 months); glucocorticoids equivalent to >10 mg prednisolone daily; and 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 ECG 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 standardized conditions used in recent SLE studies in Belfast. Platelet production of 8-epi prostaglandin (PG) F$_{2alpha}$ (8-epi PGF$_{2alpha}$) was measured as previously described. In the SLE patients, disease activity was assessed using SLAM (Systemic Lupus Activity Measure) and organ damage was assessed using the American College of Rheumatology/Systemic Lupus International Collaborating Clinics (ACR/SLICC) score.

**Flow-Mediated Dilation**

The right brachial artery was assessed using high-resolution B-mode ultrasound (ATL HDI3500 with a 7.5-MHz linear-array transducer) after the previously published protocol.

**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 1.5-mm gate range in the center of the artery. The velocity waveform envelopes were digitized at 100 Hz, low-passed-filtered at 20 Hz, and stored onto a networked personal computer and analyzed off-line using HDI Laboratory (ATL; Advanced Technologies Laboratory, Bothell, Wash). Hyperemic diastolic shear stress (DSS) was obtained from the following equation: DSS=$8 \times \mu \times (MDV/DBL)^{1.5}$ where $\mu$=blood viscosity, MDV=mean diastolic velocity, and DBL=brachial artery baseline diameter.

**Waveform Analysis**

The velocity waveforms at baseline and during reactive hyperemia were obtained by pulsed Doppler as described. The peak velocity waveform envelopes were extracted using HDI Laboratory and stored for off-line analysis. The resistive index (RI) (peak systolic velocity minus end-diastolic velocity over peak systolic velocity: PSV−EDV/PSV$^{26}$) was calculated from the waveforms using HDI laboratory. 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 hyperemic flow (Figure 1). The first velocity waveform after cuff release was not used because of the potential for the cuff to be released at different times during the cardiac cycle. Percentage change in the power of the first 4 frequency components from baseline was then calculated for each subject using:

$$\%\text{change}(f_i) = \frac{RHP(f_i) - BLP(f_i)}{BLP(f_i) \times 100}$$

where, $(f_i)$=frequency components 1 to 4 (Hz); RHP=reactive hyperemia power (cm/s$^2$); and BLP=Baseline Power (cm/s$^2$).

**Statistical Analysis**

All statistical analysis was performed using SPSS version 12.1. Descriptive variables are presented as mean value±standard devia-
The coefficients of variation for resting diameter, FMD and DSS variability in measurements in 10 subjects on 2 separate occasions. Statistical significance was set at *P* < 0.05. Reproducibility of our technique was assessed by looking at the variability in measurements in 10 subjects on 2 separate occasions. The coefficients of variation for resting diameter, FMD and DSS were 2.3%, 3.8%, and 2.7%, respectively.

**Results**

**Subject Characteristics**

We studied 32 patients with SLE and 19 controls; 28 patients were female, 6 were smokers and 2 had a family history of atherosclerosis. The mean (±SD) age of the SLE patients was 45±8 years, with mean disease duration 15±5 years. The BMI indicates body mass index; CRP, BP, blood pressure; C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

<table>
<thead>
<tr>
<th>TABLE 1. Study Sample Characteristics</th>
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<tbody>
<tr>
<td><strong>SLE</strong> (n=32)</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Female, n (%)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
</tr>
<tr>
<td>Family history,* n (%)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
</tr>
<tr>
<td>CRP, mg/L</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
</tr>
<tr>
<td>8-epi PGF₂α, pg/mg protein</td>
</tr>
</tbody>
</table>

Mean±SD unless stated. *Family history of atherosclerosis as defined by a first-degree relative having confirmed cardiovascular or cerebrovascular disease before the age of 60 years.*

**Vascular Reactivity Studies**

There was no difference in the resting diameter of the brachial artery between groups (Table 2). The SLE patients had a significantly reduced %FMD (median, 2.4%; range, −2.1% to 10.7% versus median 5.8%; range, 1.9% to 14%; *P* < 0.001) but the glyceryl trinitrate (GTN) response between the groups was not different.

There was no difference between groups in the baseline systolic velocity, hyperemic systolic velocity, or change in velocity after cuff release, but diastolic shear stress (dyne/cm²) was significantly reduced in the SLE patients (median, 18.5; range, 3.9 to 34.0 versus median, 21.8; range, 14.1 to 58.7; *P* = 0.037) (Table 2).

A strong correlation between FMD and DSS (*r*=0.65, *P*=0.01) was found. There was a significant negative correlation between disease activity (as measured by SLAM-R) and FMD (*r*=−0.67, *P*=0.01) and also a weaker negative association with CRP levels and FMD (*r*=−0.41, *P*=0.05) and 8-epi PGF₂α and FMD (*r*=−0.32, *P*=0.03). There was no correlation between the range of systolic and diastolic blood pressures and FMD (systolic BP and FMD *r*=−0.32, *P*=0.97; diastolic BP and FMD *r*=−0.30, *P*=0.75) and no correlation between the use of aspirin, NSAID or COX-2 inhibitors and the vascular reactivity parameters.

**TABLE 2. Brachial Artery FMD Results and Time-Domain Doppler Velocity Results**

<table>
<thead>
<tr>
<th>Velocity Results</th>
<th>SLE (n=32)</th>
<th>Control Subjects (n=19)</th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting diameter, cm</td>
<td>0.35 (0.27–0.51)</td>
<td>0.36 (0.32–0.45)</td>
<td>NS</td>
</tr>
<tr>
<td>Absolute FMD, cm</td>
<td>0.01 (−0.01–0.03)</td>
<td>0.02 (0.01−0.05)</td>
<td>0.001</td>
</tr>
<tr>
<td>FMD %</td>
<td>2.4 (−2.1–10.7)</td>
<td>5.8 (1.9–14.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GTN %</td>
<td>16.2 (7.0–31.4)</td>
<td>15.4 (5.9–23.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline systolic velocity, cm/s</td>
<td>74.5 (68.6–148.5)</td>
<td>86.9 (64.5–157.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperemia systolic velocity, cm/s</td>
<td>188.5 (139.8–270.8)</td>
<td>169.6 (132.3–304.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Difference in systolic velocity, cm/s</td>
<td>92.3 (45.1–179.7)</td>
<td>86.2 (48.5–156.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic shear stress, dyne/cm²</td>
<td>18.5 (3.9–34.0)</td>
<td>21.8 (14.1–58.7)</td>
<td>0.037</td>
</tr>
<tr>
<td>Resistive index</td>
<td>0.6 (0.5–0.8)</td>
<td>0.6 (0.5–0.8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Median (range).
inhibitor and FMD ($r_c = -0.25, P=0.08$). For correlation plots of FMD with DSS, SLAMR, CRP, mean arterial pressure, and use of aspirin/NSAID/Cox-2, please see http://atvb.ahajournals.org.

Waveform Analysis
Immediately after release of the cuff, the forearm is converted from a high-resistance to a low-resistance circuit as demonstrated by the change in the pulsed Doppler velocity waveform (Figure 2). Taking the second velocity waveform immediately after release of the cuff for analysis, we found no significant difference in the resistive index formula between groups (Table 2). However, Figure 3 shows the average of the second velocity waveform for SLE patients and the control group revealing clear differences in the waveform morphology. Using eigenvector analysis, we found no difference in the power-frequency spectrums of the baseline resting velocity waveforms. However, there was a significant difference between groups in the percentage power increase from baseline in frequency component 3 (SLE, median, 17.3%; range, 0.05 to 37.3%; versus controls, median, 5.7%; range, 0.0 to 5.7%; $P=0.041$) and frequency component 4 (SLE, median, −37.3%; range, −88.72% to 25.82%; versus controls, median, −33.6% to 513.7%; $P=0.041$) (Figure 3). There was no significant correlation between blood pressure and the difference in frequency components: systolic BP, $r_c = −0.097$, $−0.075$, $−0.21$, and $−0.13$ for frequency components 1, 2, 3, and 4 respectively; $P>0.05$ and diastolic BP ($r_c = 0.12$, $0.19$, $0.28$, and $0.22$ for frequency components 1, 2, 3, and 4 respectively; $P>0.05$).

In the subgroup (3 controls and 3 SLE patients) in which the right brachial artery was insonated during cuff inflation on the left forearm, there was no significant difference in the heart rate or in the power of the frequency components of the velocity waveform.

Discussion
In this cohort of SLE patients with mild disease (mean SLAM-R 9.20) and no major organ involvement, without excess of cardiovascular risk factors, and no history of cardiovascular or cerebrovascular disease, we found a significant reduction in FMD and DSS compared with control subjects. A significant positive correlation between DSS and FMD was evident; with DSS contributing ≈42% to the observed measured FMD. Analysis of the pulsed Doppler flow velocity waveform during reactive hyperemia identified morphological differences in the velocity envelope waveform between patients and controls produced by structural or functional alteration in the forearm microcirculation.

Cuff position and duration of occlusion have been shown to influence not only the magnitude of brachial artery dilation but also the underlying mechanisms determining the vasodilator response. In our experimental set-up (distal cuff occlusion and duration of occlusion <5 minutes), the brachial artery vasodilatory response is recognized as primarily NO-dependent. Mechanical shear stress plays a pivotal role in determining NO-mediated vasodilation and strongly correlates to FMD in the study participants, with a significant reduction in calculated DSS in the SLE patients. Although strongly correlated, a change in DSS can only account for 42% of the observed FMD ($R^2=0.42$), indicating that shear and shear stress stimulus for NO production cannot solely account for the observed difference in FMD. Furthermore, the reduced FMD in SLE is unlikely to be insensitivity of smooth muscle to NO or a structural problem in the brachial artery limiting dilation, because the endothelium-independent dilation (EID) in response to the NO donor GTN was not different between groups. Previous studies have described increased sympathetic outflow and increased endothelin-1 in SLE, both of which are constricting factors and could influence FMD. Another contributor to the difference in FMD may be a reduced bioavailability of NO in response to shear stress. This may be as a consequence of reduced production of NO or alternatively increased destruction.

Increased oxidative stress may account for the endothelial dysfunction in SLE. Oxidative stress produced by interaction between superoxide and NO may be of particular importance in relation to vascular functions of NO. Superoxide ($O_2^·−$) combines almost instantaneously with NO to form peroxynitrite that has a dual effect of decreasing NO bioactivity while promoting protein and lipid oxidation and both $O_2^·−$ and peroxynitrite have been shown to be increased in SLE. 8-epi PGF$_{2α}$, a marker of free radical damage and oxidative stress has been shown to be elevated in SLE. Using the platelet as a surrogate marker for vascular cells, we found no difference between groups in platelet production of 8-epi PGF$_{2α}$, although there was a weak negative correlation with FMD. The lack of difference between groups may reflect the low disease activity in our SLE patients in comparison to the study by Ames et al. Uncoupling of endothelial nitric oxide synthase (eNOS) is another potential mechanism leading to endothelial dysfunction and may contribute to the accelerated atherosclerosis observed in other disease states. We have previously shown the functional consequences of eNOS uncoupling in congestive cardiac failure with patients exhibiting enzyme uncoupling demonstrating significantly impaired endothelium-mediated vasodilator responses. Further potential mech-
anisms for impaired NO bioavailability in SLE include an increase in asymmetrical dimethylarginine,39 an endogenous nitric oxide inhibitor, and an imbalance in inducible nitric oxide synthase and eNOS activity.40

Because SLE-related disease activity increased (SLAM-R score), greater impairment in FMD was apparent. In previous studies, the relationship between activity in SLE and FMD may have been caused by a greater use of corticosteroids or immunosuppressive therapies employed with increasing disease severity.8,9 Due to the strict exclusion criteria our study group had relatively low disease activity, with only 8 patients on low-dose corticosteroids at time of study. A potential confounding factor may be the relatively high number of SLE patients taking hydroxychloroquine and NSAIDs/Coxibs. Antimalarials may have potential benefits in regard to the risk of atherosclerosis in SLE. As well as lowering cholesterol,41 antimalarials are thought to have anti-thrombotic effects42 and can also lower fasting glucose levels.43 There is current controversy surrounding the use of NSAIDs and Coxibs and the risk of accelerated atherosclerosis,44 although both classes have been shown to have beneficial effects on endothelial function.45–47 This effect is offset, however, by their effects on blood pressure and platelet aggregation.48 In our study, there was a trend toward a reduced FMD in those patients treated with aspirin, NSAIDs, or Coxib, which may reflect inhibition of endothelial PGI2.49 Similar to previous reports studying FMD in SLE,8,9 blood pressure was higher in the patient group although within the “normal” range as defined by an expert panel.50 As there was no correlation between the range of systolic and diastolic blood pressures and FMD or frequency domain analysis of the velocity waveform the effect of the BP difference influencing the results is negligible.

In the “response-to-injury” hypothesis of atherosclerosis,51 endothelial dysfunction may result from numerous sources including immune complexes, complement activation and homocysteine, all of which are relevant to SLE.52 We found no relationship between complement levels, homocysteine or anticardiolipin antibodies and FMD in our patients. CRP levels were higher in SLE patients and exhibited a negative correlation with FMD, a finding previously described in association with other disease states.53

**Figure 3.** Mean velocity waveform (A) of second velocity waveform after cuff release for SLE and control subjects showing obvious visible differences in waveform morphology. There was no significant difference in the measured resistive index but analysis of the waveform in the frequency domain (B) revealed significant difference in frequency components 3 and 4.
waveform. Time domain characterization of the Doppler velocity signal has traditionally relied on quantitative measurements such as the resistive index (RI). While these measurements can mirror changes in resistance of downstream vascular beds, it is apparent that changes in resistance and flow pulsatility indices are not closely related in all circumstances. R-I emphasizes isolated points on the waveform that identify the systolic and diastolic excursions of pulsatile flow during the cardiac cycle. Immediately on release of the cuff, the forearm microvascular network is maximally dilated due to the release of ischemic stimulators. Inspection of the average velocity waveform morphology of the second velocity waveform after release of the cuff revealed clear differences in waveform morphology without a significant difference in the RI. The most obvious morphological change occurred in the late systolic and early diastolic interval, produced as a result of wave-reflection from the downstream microvasculature.

These data suggest altered velocity waveform morphology marks the presence of downstream microvascular abnormalities in SLE that predominately influences pulsatile (compliance and distensibility) rather than steady-state (flow resistance) properties of the forearm microcirculation. Previous experimental and modeling work suggests RI should be more appropriately termed “impedance index” to incorporate the importance of the pulsatile phenomena in opposing the total opposition to flow.

While differences in the velocity waveform are visually obvious, to quantify the changes requires frequency domain analysis. Eigen-decomposition of the velocity waveforms revealed significant differences in the power-frequency spectrum between the groups after cuff occlusion that were not apparent on comparing baseline waveforms. Cuff inflation applied to the forearm opposite the site of ultrasound recording did not alter baseline Doppler waveform morphology or heart rate, confirming changes we observed in the power-frequency spectrum identify abnormalities in the arterial properties of the forearm microvascular network. This is the first study in patients with a disease associated with accelerated atherosclerosis to show that alteration in the microcirculatory hemodynamics of the forearm may impair FMD through a reduction in the shear stress stimulus. Thus, in SLE patients the impairment in FMD may be caused by “microvascular dysfunction” in the forearm and hence a reduction in stimulus for dilation rather than solely local brachial artery endothelial dysfunction.

Structural and functional changes in different microvascular beds have been shown to provide predictive information both in terms of disease development and microvascular and macrovascular complications in other disease states. Microcirculatory involvement is well recognized in SLE and this technique may provide information useful in assessing disease activity and response to treatment and ultimately in predicting future vascular complications. This contention requires testing in longitudinal long-term studies.

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Disclosures
None.

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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$_2$α (8-epi PGF$_{2α}$) 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: \( DSS = 8 \times \mu \times \frac{MDV}{DBL} \), 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)

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

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

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>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After cuff release</th>
<th>( p )</th>
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<tr>
<td>Heart rate, bpm</td>
<td>79±8</td>
<td>81±7</td>
<td>NS</td>
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<tr>
<td>Power of frequency component (cm/s)²</td>
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<tr>
<td>1</td>
<td>115±81</td>
<td>119±79</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>121±96</td>
<td>132±83</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>115±97</td>
<td>128±74</td>
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<tr>
<td>4</td>
<td>64±31</td>
<td>71±73</td>
<td>NS</td>
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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 \( mean \pm SD \).
Figure I (a)
Figure I (b)
Figure II

Diastolic Shear Stress (dyne/cm²)

Flow Mediated Dilation (%)

Patient Group
- ▲ Control
- ○ SLE

$r=0.65; p=0.01$
Flow Mediated Dilation %

Figure III

\( r = -0.67; p = 0.01 \)
Figure IV
Figure V
If on NSAID, aspirin or COX-2 inhibitor

Flow Mediated Dilation %

p=0.23

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.