Endothelial function and arterial stiffness assessment as early surrogate markers of vascular risk in patients with systemic lupus erythematosus

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ABSTRACT

Patients with systemic lupus erythematosus (SLE) are at risk of premature atherosclerosis. Conventional prediction risk equations do not adequately predict the cardiovascular risk of these patients because of the complex interaction of traditional and SLE specific risk factors and treatment effects, as well as, the dynamic insult to the vasculature. Non-invasive vascular assessment is able to evaluate the vascular damage accumulated over time. The aim of this review is to examine the role of non-invasive assessment of endothelial function and arterial stiffness as surrogate markers for vascular risk in SLE patients.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease associated with a wide range of clinical manifestations and complications (1-3). More importantly, it demonstrates a bimodal pattern of mortality. Within the first year of the disease, mortality is associated with complications of active disease, such as infection and active nephritis. In contrast, in patients with disease duration exceeding 5 years, mortality is associated with cardiovascular complications and recurrent disease activity (4). The cardiovascular mortality in patients with SLE has not improved over time (5, 6). On the contrary, it may rise as the life expectancy increases because of improved treatment strategies.

The lack of improvement in cardiovascular mortality in patients with SLE reflects the complex and dynamic interaction of disease activity and duration, adverse or beneficial effects of treatment, SLE specific and traditional risk factors (Fig. 1). Consequently, identifying patients at risk for developing cardiovascular disease becomes a challenge. The use of conventional risk stratification tools has poor predictive value in these patients as these tools evaluated only the traditional cardiovascular risk factors (7).

Furthermore, the parameters evaluated in risk equations are static and reflect the metabolic status of the individual at the time of evaluation. For instance, metabolic changes from repeated inflammation and the use of glucocorticoids or immunosuppressants in a patient with multiple flares can result in vascular damage over time (8). However, these metabolic abnormalities may not be present at the time of evaluation and thus, the vascular risk of the individual will be underestimated.

As a result of these limitations, non-invasive vascular assessment is appealing because it evaluates the vascular damage accumulated with time. This is particularly attractive in assessing the vascular risk in populations with complex interaction of risk factors. Furthermore, scans can be repeated in the same individual to monitor progression (or improvement) of disease and the effects of a treatment or an intervention.

The aim of this review is to explore whether non-invasive assessment of endothelial function and arterial stiffness has the potential to be surrogate markers for vascular risk in patients with SLE.

Non-invasive assessment of endothelial function and arterial stiffness

Nitric oxide (NO) produced by the endothelium, plays a pivotal role in maintaining vascular tone and reactivity. During changes in myocardial flow from increased metabolic demands, the conduit coronary arteries respond by NO-mediated vasodilatation (9). However, NO production is impaired in

early atherosclerosis because of oxidative stress and this follows the ischaemic cascade of coronary artery disease (10).

In patients with SLE, flow-mediated dilatation (FMD) of the brachial artery is the most widely used technique to assess endothelial dysfunction in the macrocirculation, circulation involving the conduit coronary arteries (11). In this non-invasive method of assessing endothelial function, NO release from the endothelium in response to hyperemia causes brachial artery dilatation (endothelial-dependent vasodilatation) (12). Exogenous NO either with nitroglycerin spray or sublingual tablet is routinely incorporated in the same technique to determine the maximum obtainable vasodilator response (13). This endothelial-independent vasodilatation response reflects vascular smooth muscle function and arterial compliance.

Arterial stiffness and endothelial dysfunction represent different but related aspects of vascular disease (14). Traditionally, arterial stiffening has been considered an age-related degenerative process. However, some studies have suggested the role of endothelial dysfunction in regulating arterial stiffening. This has been supported by histological examination of intima of stiffened arteries revealing abnormal and disarrayed endothelial cells (15).

In the assessment arterial stiffness, there are many non-invasive techniques and methodologies available (16), and it is beyond the scope of this review to describe them in detail. It is noteworthy to mention of the various techniques, the measurement of the carotid artery and femoral artery pulse wave velocity (carotid-femoral PWV) and augmentation index (derived from central pulse-wave analysis) are the more commonly utilised techniques (Fig. 2).

Although the carotid-femoral PWV is considered the "gold standard", other arterial sites are also used to evaluate PWV: the upper limb (carotid-brachial), lower limb (femoral-dorsalis pedis) and limb to limb (brachial-ankle).

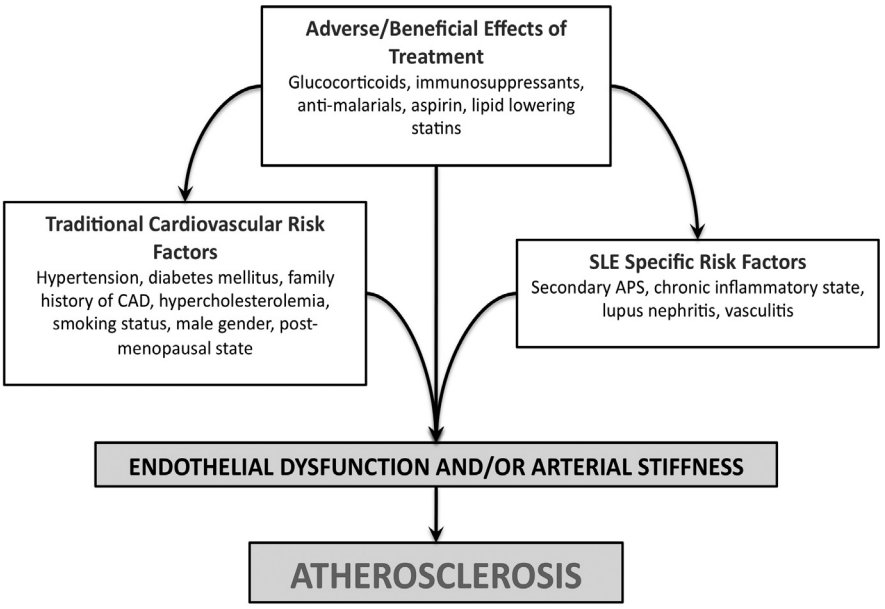

Fig. 1. Complex interaction of various mediators involved in the pathogenesis of atherosclerosis. SLE treatment affects endothelial function and arterial stiffness either directly or indirectly through modifying SLE specific or traditional cardiovascular risk factors (APS antiphospholipid syndrome; CAD coronary artery disease).

Methods

A comprehensive literature review using the Pubmed database (1966 to December 2011) was used to identify all relevant studies on non-invasive assessment of endothelial function and arterial stiffness in patients with SLE. The following search terms were used: "non-invasive assessment", "flow-mediated dilatation", "endothelial-dependent vasodilatation", "endothelial-independent vasodilatation", "brachial artery reactivity testing", "arterial compliance", "arterial stiffness", "pulse wave velocity", "pulse waveform analysis", "subclinical atherosclerosis", "lupus", and "systemic lupus erythematosus". Bibliographies from relevant publications and review articles were also reviewed manually for relevant articles.

The search was limited to the English language with abstracts and we included all study designs, including reviews and meta-analyses. Studies are excluded if they (1) comprised of the paediatric population because SLE is more complex and serious in children and results may not be applicable to the adults, or (2) evaluate biochemical markers of endothelial dysfunction or arterial stiffness because they are not the focus of this review.

Results

A total of 36 relevant articles were identified with the search methodology. There was one meta-analysis on brachial artery FMD and two review articles. Of the remaining 33 original articles, three studies were excluded because they comprised of the pediatric population (n=2) or they evaluated biochemical markers of endothelial function (n=1). No additional articles were identified from bibliographies in review articles and meta-analysis.

Clinical applications of endothelial function assessment in SLE

Evidence of endothelial dysfunction and association with risk factors

There is strong evidence to suggest patients with SLE had impaired endothelial-dependent, but not endothelial-independent, vasodilatation compared to healthy controls (17-29). However, in SLE patients with secondary antiphospholipid syndrome (APS), endothelial-independent vasodilatation of the brachial artery was reduced and this corresponded with an increase in inflammatory markers (18, 28). This interesting observation of secondary APS preferentially affecting the vascular smooth muscle and not the endothelium warrants further investigation.

In a recent meta-analysis consisting of 13 case-control studies (n=580 patients, 381 matched controls), an increasing

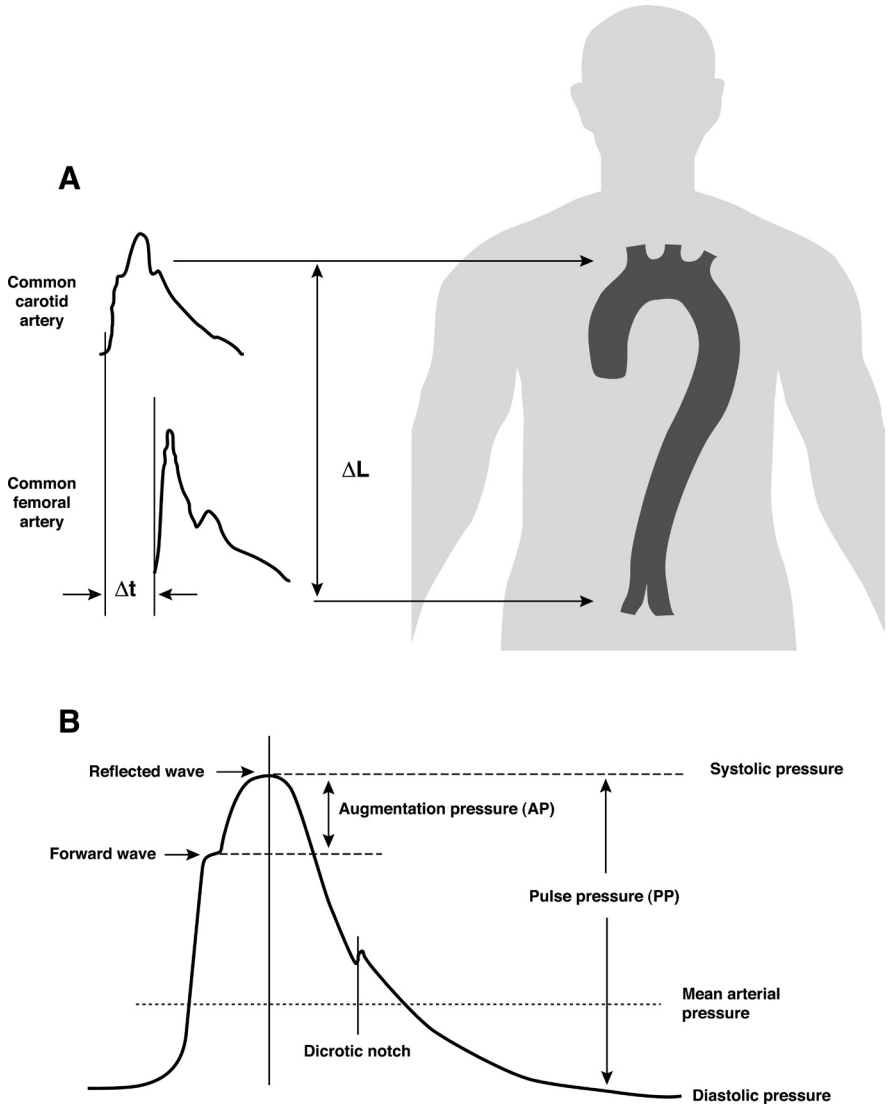

Fig. 2. A. Carotid-femoral pulse wave velocity (PWV) method. Transit time (Δt) is measured between the feet of the 2 waveforms and the distance (ΔL) is measured over the body surface between the 2 recording sites. Pulse wave velocity is then calculated as ΔL/Δt. **B**. Central pulse waveform analysis. Augmentation pressure (AP) is defined as the difference in height between the forward wave and reflected wave, and the ratio of AP to pulse pressure (PP) defines the augmentation index (in percent). Other parameters in the analysis include central systolic pressure and central pulse pressure.

age and a longer duration of SLE were associated with a small difference in brachial artery endothelial-dependent vasodilatation between patients with SLE and controls. Other cardiovascular risk factors evaluated (gender, smoking status, menopausal state, diabetes mellitus, body mass index, blood pressure, fasting lipid profile, C-reactive protein and prednisolone use) were not significant predictors for impaired endothelial function (28).

Modification of endothelial function with therapies

Some therapies have been shown to improve endothelial function in patients with SLE (Table I). The effects of statins on endothelial function assessed by brachial artery FMD were studied in a non-randomised interventional study of 88 patients with SLE. Sixty-four patients received atorvastatin 20mg daily for 8 weeks while the remaining 24 patients did not. After 8 weeks, the use of atorvastatin was associated with an increase in brachial artery diameter at baseline and a 3.1% absolute improvement in endothelial-dependent vasodilatation. This improvement in endothelial function was seen in SLE patients with or without conventional cardiovascular risk factors (hypertension, dyslipidaemia and/or obesity) (30).

Omega-3 polyunsaturated fatty acids (Ω-3 PUFA) reduce non-fatal and fatal myocardial infarction, and sudden cardiac deaths in patients with coronary artery disease (31), and improve endothelial function in patients with diabetes mellitus, dyslipidaemia and heart failure

Table I. Interventional studies with endothelial function assessment (SLE systemic lupus erythematosus; CADRF cardiovascular disease risk factors; EDV endothelium-dependent vasodilation; EDIV endothelium-independent vasodilation).

First author, Year (reference)	Study population	Study design	Intervention	Results
Wright, 2008(37)	60 SLE patients	Randomised, placebo controlled trial	3g Omega-3 Fish Oil *vs*. Placebo for 24 weeks	EDV improves from 3.0% (baseline) to 8.9% (24 weeks) in intervention arm
Ferreira, 2007 (30)	64 SLE patients (33 with CADRFs); 24 healthy controls	Non-randomised, non-placebo, controlled interventional study	Atorvastatin 20mg for 8 weeks in patients	EDV improves from 3.8% (baseline) to 6.9% (8 weeks) in patients; EDV unchanged in controls. EIDV unaffected in both patients and controls.
Tam, 2005 (43)	39 SLE patients	Randomised, placebo controlled trial	Antioxidants (500mg Vitamin C and 800 IU Vitamin E) *vs*. Placebo for 12 weeks	EDV not changed after 12 weeks in intervention arm

Table II. Assessment of arterial stiffness in patients with SLE.

First author, Year (Reference)	Study population	Study design	Stiffness Assessment Methodology	Major findings
Tso, 2005 (52)	83 SLE patients	Cross sectional	Brachial-ankle PWV	Analysis stratified by brachial-ankle PWV. Age and SBP independently associated with brachial-ankle PWV
Sabio, 2009 (53)	128 SLE patients	Cross sectional	Carotid-femoral PWV	Analysis stratified by the presence of MetS. SLE patients with MetS have higher PWV. PWV was associated with age, male gender, presence of MetS, duration of disease and CRP levels
Selzer, 2001 (55)	220 SLE patients (124 premenopausal, 96 postmenopausal)	Case control	Carotid-femoral PWV	PWV associated with traditional factors in postmenopausal women. In premenopausal women, PWV associated with both SLE-related and traditional risk factors
Cypiene, 2009 (45)	30 SLE patients; 66 healthy controls	Case control	Carotid-radial PWV, AIx	PWV and AIx higher in SLE patients compared to controls. PWV not associated with any parameters studied; increasing AIx associated with higher SLICC organ damage index and increasing age.
Cacciapaglia, 2009 (46)	33 SLE patients; 33 controls	Case control	Arterial stiffness parameters from carotid artery (vascular strain, distensibility, pressure-strain elastic modulus)	SLE patients higher "stiffness" indices compared to controls
Shang, 2008 (47)	32 SLE patients; 32 controls	Case control	Brachial-ankle PWV, carotid-ankle PWV, carotid AIx	SLE patients had increased brachial-ankle PWV, carotid-ankle PWV, and carotid AIx compared to controls. Carotid AIx was an independent predictor of active disease with SLEDAI ≥ 3. Carotid-ankle PWV was an independent predictor of organ damage with SLICC ≥1
Roman, 2005 (48)	101 SLE patients; 80 RA patients; 105 controls	Case control	Arterial stiffness parameters from carotid artery (vascular strain) and carotid-radial tonometry (arterial stiffness index, Young's modulus, Peterson's elastic modulus)	Arterial stiffness increased in SLE and RA and associated with disease duration, cholesterol levels and inflammatory biochemical markers
Bjarnegrad, 2006 (49)	27 SLE patients; 27 controls	Case control	Carotid-femoral PWV, Carotid-radial PWV, AIx	Carotid-femoral PWV was higher in SLE patients compared to controls, and it was associated with increasing CRP and C3 levels
Yildiz, 2008 (50)	24 premenopausal SLE patients; 24 controls	Case control	Carotid-femoral PWV	Carotid-femoral PWV higher in premenopausal SLE patients compared to controls. Increasing age, BMI, WHR, and higher HR and BP were associated with PWV
Cypiene, 2010 (51)	31 SLE patients; 63 RA patients; 72 controls	Case control	Carotid-radial PWV, AIx	Carotid-radial PWV, AIx higher in SLE patients compared to controls. AIx associated with organ damage index, age, and mean blood pressure
Tso, 2006 (54)	58 SLE patients; 32 controls	Case control	Brachial-ankle PWV	Homocysteine levels higher in SLE patients compared to controls; and homocysteine levels in SLE patients was positively correlated with brachial-ankle PWV
Kudaravalli, 2011 (58)	32 SLE patients; 10 controls	Case control	Stiffness index, reflection index using digital photoplethysmography	SLE patients had increased arterial stiffness compared to controls. Arterial stiffness improved with N-acetylcysteine and atorvastatin treatment (see text)

SLE: systemic lupus erythematosus; MetS: metabolic syndrome; BMI: body mass index; WHR: waist-hip ratio; PWV: pulse wave velocity; AIx: augmentation index; RA: rheumatoid arthritis; SLICC: Systemic Lupus International collaborative Clinics; SLEDAI: SLE disease activity index).

(32-36). The benefits of Ω-3 PUFA on endothelial function in patients with SLE was demonstrated in a randomised placebo controlled trial. Patients with SLE who received 3g of Ω-3 PUFA experienced an improvement in brachial artery endothelial-dependent vasodilatation from 3.0% at baseline to 8.9% after 24 weeks (37).

However, the evidence of anti-malarials and anti-oxidants on endothelial function in patients with SLE is less clear. Current evidence suggests the cardiovascular benefits of anti-malarials in patients with SLE (38, 39) are derived from the lipid lowering, anti-inflammatory, anti-coagulant and glucose lowering effects (40-42). But these anti-

Table III. Clinical applicability of non-invasive assessment of endothelial function and arterial stiffness in patients with SLE.

	Techniques to assess are heterogeneous	Ease of use	Evidence of association with risk factors*	Evidence showing modification with treatment	Evidence of prognostic value
Endothelial function assessment	+	+	++	++	±
Arterial stiffness assessment	+++	++	++	+	NA ∏

*Includes traditional cardiovascular and SLE specific risk factors.
∏ No studies performed to date.

atherogenic properties of anti-malarials did not appear to improve endothelial function in patients with SLE.

Increasing use of hydroxychloroquine was associated with lesser differences in brachial artery endothelial-dependent vasodilatation in patients with SLE compared to controls as demonstrated in the recent meta-analysis (31). Of note, there is significant heterogeneity in the studies and the findings may be confounded by a large number of patients who had secondary APS also receiving hydroxychloroquine.

The effect of anti-oxidants on endothelial function in patients with SLE was studied in a randomised placebo-controlled trial of 39 patients with SLE, randomised to either vitamins (vitamin C 500mg and vitamin E 800IU) or placebo for 12 weeks (43). Although plasma concentrations of vitamin C and E were detected after 3 weeks of treatment, most oxidative stress markers and endothelial-dependent vasodilatation assessed by brachial artery FMD remained unchanged in both groups.

Prognostic value of endothelial function assessment

The question of whether an improvement in endothelial function translates into a better prognosis was addressed in a prospective study. The presence of impaired endothelial-dependent vasodilatation assessed at the brachial artery did not predict development of end-organ damage (including the cardiovascular system) over 5 years of follow-up in patients with SLE (n=36) (44). But this study did not have sufficient power to detect a real effect due to the small number of patients, again highlighting the need for larger prospective and well-designed studies.

Clinical applications of arterial stiffness assessment in SLE

Evidence of arterial stiffness and association with risk factors

Patients with SLE had a greater extent of arterial stiffness compared to controls (45-51, 56). Increasing arterial stiffness was associated with traditional cardiovascular risk factors such as increasing age (45, 50-52), blood pressure (50-52), the presence of metabolic syndrome (50, 53) and cholesterol levels (48). SLE related risk factors such as higher organ damage and activity indices (45, 47, 51), longer duration of disease (48, 53) and raised inflammatory biochemical markers (including homocysteine levels) (48, 49, 53, 54) also predicted increasing arterial stiffness.

The impact of SLE related risk factors on increasing arterial stiffness appears to be related to the menopausal state of the patients. In post-menopausal women, traditional risk factors were primarily associated with increased arterial stiffness measured by PWV. On the other hand, SLE related factors (elevated C3 levels, lower leukocyte count, the non use of hydroxychloroquine and the presence of ds-DNA antibodies) predict increased stiffness in pre-menopausal women (55). This observation partially explains the higher incidence of cardiovascular events seen in young patients with SLE.

Despite promising findings, these studies have been retrospective in design. More importantly, the methodologies to assess arterial stiffness are heterogeneous (Table II), making interpretation of results in various studies difficult.

Modification of arterial stiffness with therapies

There is evidence of improvement in arterial stiffness with treatment in patients with SLE. Thirty-two patients with SLE were given N-acetylcysteine 600mg 3 times daily and atorvastatin 10mg daily for 2 weeks. At the end of two weeks, arterial stiffness indices decreased, along with inflammatory and oxidative biochemical markers (56).

The postulated cardiovascular benefits from N-acetylcysteine included enhancing the bioavailability of NO by removing oxygen radicals from plasma and endothelial cells, and increasing the half-life of NO by forming a NO adduct that allows NO to be transported easily to vascular smooth muscle cells and platelets (57, 58).

Currently, no prospective studies have been conducted to investigate the prognostic value of arterial stiffness in patients with SLE.

Early surrogate markers of vascular risk or are we too early

There are (and will be) many non-invasive techniques and new methodologies developed to improve characterisation of endothelial dysfunction and arterial stiffness. Furthermore, there is an emerging enthusiasm to validate these techniques in patients with rheumatologic conditions such as SLE. These patients have an increased propensity for premature atherosclerosis and traditional risk equations do not adequately predict cardiovascular risk.

It is important to distinguish the roles of these vascular parameters as risk factors or surrogate markers. Unlike surrogate markers, risk factors relate to the probability of developing a disease without any causal links to the underlying pathophysiology of the disease. Modifying risk factors with intervention or therapies may have no impact on disease progression whereas surrogate markers can replace clinical endpoints as a guide to therapy. Most importantly, surrogate markers predict future events independently of other risk factors after they have been validated prospectively

(59-61). There are other factors to consider before clinical application and these include the reproducibility of the method, the sensitivity, specificity and predictive value of the test, and whether the test is readily available.

Endothelial dysfunction and arterial stiffness are accepted vascular consequences of sub-clinical atherosclerosis. In patients with SLE, these vascular parameters have been shown to be associated with traditional and SLE specific risk factors. There is also encouraging evidence from current studies showing improvement in endothelial function and arterial stiffness with therapies. However, these studies are limited by small sample sizes and a lack of well-designed prospective studies investigating the predictive value and prognostic roles of these vascular parameters. In order to facilitate such studies, the techniques and methodologies to assess these parameters should be standardised. This will also allow interpretation and comparison of results in the various studies.

Unfortunately, it is unclear whether endothelial function and arterial stiffness assessment are suitable surrogate markers for sub-clinical atherosclerosis in SLE at this time (Table III). Despite these limitations, we remain optimistic that such assessment is important, and they have potential roles in the risk stratification in both primary and secondary prevention of cardiovascular disease in patients with SLE.

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