

**PREVALENCE AND RISK FACTORS FOR PULMONARY ARTERIAL  
HYPERTENSION IN PATIENTS WITH LUPUS**

by

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## ABSTRACT

**Background:** Pulmonary arterial hypertension (PAH) is a recognised complication of SLE. The risk of developing PAH in SLE may be increased in a subset of patients with antiphospholipid antibodies.

**Aims:** To estimate the point prevalence of PAH, to evaluate screening tests and to identify risk factors for PAH in a large cohort of SLE patients

**Methods:** A prospective cross-sectional study of 288 patients with SLE using resting transthoracic echocardiography to estimate the systolic pulmonary artery pressures (sPAP) and to assess cardiac morphology and function. We assessed potential risk factors such as the presence of lung disease, autoantibodies and anti-phospholipid syndrome (APS). We evaluated screening tests such as pulmonary function tests, six minute walk test and biomarkers.

**Results:** Twelve out of 283 patients had PAH with sPAP >30 mm Hg (range 31-59 mmHg). Only 3 patients had sPAP >40 mm Hg. The only significant risk factor for PAH was lupus anticoagulant ( $p=0.005$ ).

**Conclusion:** The point prevalence of PAH was 4.2% in our cohort of patients with SLE. The significant association of lupus anticoagulant and presence of APS in PAH cases suggests that thrombosis may play an important role in the development of PAH with SLE patients.

This thesis is dedicated to my parents

Athiveeraramapandian Koothappanambalam and Radha Pandian

for encouraging and motivating me all my life

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## **LIST OF ABBREVIATIONS**

<b>ACA</b>	<b>Anticardiolipin antibody</b>
<b>ACR</b>	<b>American college of Rheumatology</b>
<b>ANA</b>	<b>Antinuclear antibody</b>
<b>APLA</b>	<b>Antiphospholipid antibodies</b>
<b>APS</b>	<b>Antiphospholipid syndrome</b>
<b>APS</b>	<b>Antiphospholipid syndrome</b>
<b>ARA</b>	<b>American Rheumatology Association</b>
<b>BDI</b>	<b>Baseline dyspnoea index</b>
<b>BILAG</b>	<b>British Isles lupus assessment group</b>
<b>BNP</b>	<b>Brain natriuretic peptide</b>
<b>CAD</b>	<b>Coronary artery disease</b>
<b>CCB</b>	<b>Calcium channel blockers</b>
<b>CHB</b>	<b>Congenital heart block</b>
<b>CTD</b>	<b>Connective tissue disease</b>
<b>CTD</b>	<b>Connective tissue disease</b>
<b>CTPA</b>	<b>CT pulmonary angiogram</b>

<b>CVD</b>	<b>Cardiovascular disease</b>
<b>DS</b>	<b>Damage score</b>
<b>ECG</b>	<b>Electrocardiogram</b>
<b>ECG</b>	<b>Electrocardiogram</b>
<b>ECHO</b>	<b>Echocardiogram</b>
<b>EF</b>	<b>Ejection fraction</b>
<b>ENA</b>	<b>Extractable nuclear antibody</b>
<b>FEV</b>	<b>Forced expiratory volume</b>
<b>Fkn</b>	<b>Fractalkine</b>
<b>FVC</b>	<b>Forced vital capacity</b>
<b>HRCT</b>	<b>High resolution CT</b>
<b>ILD</b>	<b>Interstitial lung disease</b>
<b>IPAH</b>	<b>Idiopathic PAH</b>
<b>KCO</b>	<b>Diffusion factor coefficient</b>
<b>LAC</b>	<b>Lupus anticoagulant</b>
<b>LVSD</b>	<b>Left ventricular systolic dysfunction</b>
<b>MCTD</b>	<b>Mixed Connective tissue disease</b>

<b>MEP</b>	<b>Maximal expiratory pressure</b>
<b>MIP</b>	<b>Maximal Inspiratory pressure</b>
<b>MRC</b>	<b>Medical research council</b>
<b>NT-proBNP</b>	<b>N-terminal brain natriuretic peptide</b>
<b>NYHA FC</b>	<b>New York Heart Association functional class</b>
<b>OAD</b>	<b>Obstructive airways disease</b>
<b>PAH</b>	<b>Pulmonary arterial hypertension</b>
<b>PCWP</b>	<b>Pulmonary capillary wedge pressure</b>
<b>PFT</b>	<b>Pulmonary function test</b>
<b>PH</b>	<b>Pulmonary hypertension</b>
<b>RAP</b>	<b>Right atrial pressure</b>
<b>RHC</b>	<b>Right heart catheterisation</b>
<b>RLD</b>	<b>Restrictive lung disease</b>
<b>RNP</b>	<b>Ribonuclear protein</b>
<b>RP</b>	<b>Raynaud's phenomenon</b>
<b>RPM</b>	<b>Respiratory pressure meter</b>
<b>RVSP</b>	<b>Right ventricular systolic pressure</b>

<b>SGRQ</b>	<b>St.George’s respiratory questionnaire</b>
<b>SLE</b>	<b>Systemic lupus erythematosus</b>
<b>SLEDAI</b>	<b>SLE disease activiy index</b>
<b>SLICC</b>	<b>Systemic Lupus International Collaboration Committee</b>
<b>SLS</b>	<b>Shrinking lung syndrome</b>
<b>SMR</b>	<b>Standardised mortality rate</b>
<b>SPAP</b>	<b>Systolic pulmonary artery pressure</b>
<b>TLC</b>	<b>Total lung capacity</b>
<b>TLCO</b>	<b>Diffusion factor</b>
<b>TOE</b>	<b>Trans-oesophageal echocardiogram</b>
<b>TTE</b>	<b>Transthoracic echocardiogram</b>
<b>VEGF</b>	<b>Vascular endothelial growth factor</b>
<b>WHO</b>	<b>World Health Organisation</b>
<b>6MWT</b>	<b>Six minute walking test</b>

## **PUBLICATIONS**

The following are the list of publications from this research study

**1. Prevalence and risk factors for pulmonary arterial hypertension in patients with systemic lupus erythematosus (Appendix 8)**

Athiveeraramapandian Prabu, Kiran Patel, John Townend, David Thickett, Peter Nightingale, Veronica Toescu, Chee-Seng Yee, R Situnayake, Caroline Gordon

Ann.Rheum.Dis 2007;66 (Suppl II);477 (abstract)

**2. Prevalence of cardiovascular abnormalities determined by transthoracic echocardiography in patients with systemic lupus erythematosus**

Athiveeraramapandian Prabu, Kiran Patel, John Townend, David Thickett, Peter Nightingale, Veronica Toescu, Chee-Seng Yee, R Situnayake, Caroline Gordon

Ann.Rheum.Dis 2007;66 (Suppl II);478 (abstract)

**3. Prevalence and risk factors for pulmonary arterial hypertension in patients with lupus**

Athiveeraramapandian Prabu, Kiran Patel, Chee-Seng Yee, Peter Nightingale, Rohan D. Situnayake, David R. Thickett, Jonathan N. Townend, and Caroline Gordon

Rheumatology 2009;48;1506-1511

# CHAPTER 1 INTRODUCTION

## 1.1. Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is an autoimmune disease with multiple systemic manifestations causing significant increase in morbidity and mortality(1;2). The overall standardised mortality ratio (SMR) which is the ratio of deaths observed to deaths expected was 2.4 in a large multicenter international lupus cohort of 9,547 patients with an average follow up of 8.1 years. In particular the SMR for SLE patients with renal disease and infections were 7.9 and 5 respectively in this cohort. Though skin and joint involvement are usually the presenting features, other system involvement such as haematological, renal and central nervous system can result in serious organ damage. Cardiovascular and respiratory involvement is not uncommon, and they can occur together resulting in symptom mimicry, delayed diagnosis and rapidly progressive disease.

More than 90% patients who develop SLE are women in 20-50 year age group, but the level of activity in men is broadly similar. Though the actual cause of SLE is unknown, genetic susceptibility, hormonal and environmental factors may play a role in the development of SLE.

### 1.1.1 Epidemiology

The worldwide SLE prevalence range is 3-241/100,000(3-5) and incidence range is 0.37-8.7/100,000(5-7). The study methods, the country and ethnicity studied, sex, and case definitions are among several reasons that explain this wide variability. A community based study on adult women from Birmingham, UK reported a point prevalence of 1 in 2000 and an

incidence of 6.8 per 100,000(5). SLE more often affects women than men, supporting a role for sex hormones in the pathogenesis of SLE. The female to male ratio is 6-18:1, which depends on ethnicity and age of onset of SLE. The sex ratio is less than 10:1 females to males in younger and older patients. SLE affects any age between 18 months to 89 years but more frequently presents in the 20-50 year age group. More than 90% patients who develop SLE include females in the reproductive age group, and this has several implications for the management of SLE around pregnancy. It is well recognised that ethnic groups differ in incidence, prevalence, disease course, and outcomes such as organ damage and mortality in SLE. In a UK study, the Afro-Caribbean and Asian ethnicity had 5-6 times higher incidence and 2-5 times higher prevalence of SLE. In a US study(8), African Americans had two times higher incidence of SLE. The non-Caucasians with SLE are more likely to have severe disease course, renal and neurologic involvement(9), damage accrual and higher mortality rates(10;11).

### **1.1.2 Etiopathogenesis**

Over the last 60 years since the advent of anti-nuclear antibody (ANA) and following the description of LE cell phenomenon, it has been proposed that the etiology of SLE includes autoantibodies produced in response to genetic, hormonal and environmental factors.

#### **1.1.2.1 Etiology**

Several genes have been identified to be acting in additive fashion to confer susceptibility to SLE(12;13). Both genetic and allelic heterogeneity are involved in the initiation of SLE. The familial aggregation of SLE, high sibling recurrence risk ratio (14)and increased SLE concordance rate in monozygotic twins (24-69%) being almost 10 times higher than that seen in dizygotic twins (2-9%) supports the genetic factors involved in SLE(15;16). The genetic linkage studies have identified multiple regions with loci that increase risk of lupus. The



homozygous hereditary complement deficiencies particularly of C1q, C2 and C4 increases the risk of SLE as these complement gene products participate in the rapid clearance of apoptotic debris(17). The risk for developing SLE associated with hereditary C1q, C4 and C2 deficiency are >90%, 75% and 10% respectively(18). The C1q genes are located in chromosome 1, and C4 and C2 genes are located within Major Histocompatibility Antigen (MHC) class III region of chromosome 6. Other alleles of importance in the pathogenesis of SLE are HLA-DR2, HLA-DR3(19) and Fcγ receptor genes(20;21).

The environmental factors that are implicated in SLE risk include drugs, Ultraviolet (UV) rays in sunlight, heavy metals, chemicals, infections and lifestyle including diet.

Procainamide and Hydralazine are among 100 other drugs implicated in lupus risk which modifies epigenetic mechanisms that control gene expression in T cells such as inhibition of deoxyribo-nucleic acid (DNA) methylation and induce autoreactivity(22). DNA methylation is a mechanism by which cells regulate gene transcription and DNA hypomethylation could result in abnormal gene expression implicated in the pathogenesis of SLE. UV rays increases apoptosis of keratinocytes, expression of autoantigens such as Ro, La, Sm and RNP on apoptotic cell surface(23) and impaired clearance of apoptotic cells. Crystalline silica and mercury are among the several metals and chemicals associated with SLE(24;25). The production of autoantibodies and subsequent development of SLE following infections such as Epstein-Barr virus (EBV)(26), Cytomegalovirus (CMV), herpes viruses and parvovirus B19 infections have been shown to be associated with SLE.

The role of oestrogen in SLE disease expression has been considered as SLE is predominant in females and the disease onset often follows puberty. Though oestrogen acts through oestrogen receptors in T and B cells(27), the exact mechanism by which oestrogen exerts its role in pathogenesis is unclear. The use of oestrogen containing contraceptive pill(28) and

postmenopausal oestrogen replacement therapy(29) are associated with increased risk of developing SLE.

### **1.1.2.2 Pathogenesis of SLE**

**T cell abnormalities:** The disruption of immune tolerance in SLE results in the escape of pathogenic autoreactive T cells into the peripheral circulation, where they recognize autoantigens (including DNA, histones and small ribonuclear proteins) presented by antigen presenting cells. The autoreactive T cells assist autoantibody production by autoreactive B cells as well as produce cytokines that promote autoantibody production(30). The SLE T cells display an activated phenotype and aberrant T cell receptor (TCR) are found in large proportion of SLE patients. The pathologic alterations in signal transduction across the TCR play a role in SLE pathogenesis(31).

**B cell abnormalities:** The abnormalities in B cells include alterations in phenotype, life span, function and signal transduction. Phenotypically the naïve B-cells are replaced by activated plasma cells that are long lived. Autoreactive B cells in SLE produce autoantibodies through isotype switching and affinity maturation unlike in normal individuals where the autoantibodies are usually IgM isotype that does not undergo isotype switching or affinity maturation. The autoantibody production by B cells is mediated by both T cell dependent and T-cell independent mechanisms. In SLE, the failure of peripheral tolerance with the presence of autoreactive T cells and follicular dendritic cells presenting autoantigens results in survival and expansion of autoreactive B cells producing autoantibodies(32). The T cell independent mechanisms include Toll like receptors-9 and interleukin-10 (IL-10) and subsequently mediated by B cell receptor (BCR) and B cell activating factor (BAFF)(33). The autoreactive B cells efficiently present the autoantigen to T cells and modulate the activity of T cells to secrete cytokines that contributes to SLE pathogenesis. The intrinsic defects in B cells lead to

aberrant signal transduction across BCR resulting in B cell hyperactivity. The increased production of BAFF by dendritic cells also stimulates B cell survival and maturation. The activated B cells also secrete proinflammatory cytokines such as IL-6 and IL-10.

**Role of cytokines:** Several cytokines have been linked to SLE pathogenesis and the key cytokines among those studied in SLE include Interferon-alpha, IL-6, IL-10 and TNF-alpha(34). The high disease activity in SLE has been associated with increased expression of genes regulated by Interferon-alpha (IFN-alpha) in peripheral blood cells (“IFN signature”)(35). The cytokine IL-6 contributes to SLE pathogenesis through its action on B cells. B-cells abnormally respond to IL-6 by virtue of spontaneous surface expression of IL-6 receptors. High levels of IL-6 correlate with SLE disease severity(36). The increased production of IL-10 has been shown in SLE patients(37), and serum levels of IL-10 are also correlated with disease severity(38). The promotion of B- cell proliferation and differentiation by IL-10 promotes autoantibody production. The association of TNF-alpha gene polymorphism with SLE has been demonstrated (39)but the precise role of TNF-alpha in SLE is not yet clear.

**Defective Apoptosis:** Apoptosis or programmed cell death is an active, tightly regulated physiological process leading to destruction of self-reactive lymphocytes. This is followed by the rapid clearance of apoptotic cells and fragments by mononuclear phagocytic cells. The disruption of either of these processes promotes development of autoimmunity. Apoptosis prevents the exposure and release of intracellular components such as nucleosomes (containing DNA) into the extracellular microenvironment that would lead to an inflammatory response and promote autoimmunity(23).The clearance of apoptotic cells are mediated by complements such as C1q, C3 and C4 as well as C-reactive protein (CRP). In SLE, the altered maturation of phagocytes results in defective clearance of apoptotic cells(40). The

congenital or acquired complement component deficiency seen in SLE patients also promotes defective apoptosis. In SLE, massive apoptotic rates of cells overwhelm the phagocytic cells and thus possibly unmask potential autoantigens. Conversely, lower rates of apoptosis of autoreactive cells in SLE can also augment the autoimmune response. Thus the defective apoptosis with both increased and decreased rates of apoptosis play a role in SLE pathogenesis. In addition, the autoantibodies found in SLE may also bind to apoptotic cell surface and the resulting antibody mediated phagocytosis may trigger secretion of pro-inflammatory cytokines(41).

The variation in its initial presentation in each patient with SLE is as diverse as the etiopathogenesis of SLE. However, some clinical features have been frequently associated with pathogenic autoantibodies in SLE such as anti double stranded DNA (dsDNA), extractable nuclear antigen antibody (ENA) such as anti Ro, anti La, anti Sm and anti RNP. For instance, anti-dsDNA is 97% specific for SLE and strongly associated with lupus nephritis. Similarly, renal, haematologic or vasculitis features are attributed to pathogenic autoantibodies forming immune complex deposits and activating complement.

### **1.1.2.3 Autoantibodies and Immune Complexes**

SLE is an autoimmune disease characterized by autoantibodies and over a hundred autoantibodies have been described in sera of SLE patients(42). Only a few of these autoantibodies are measured in clinical practice. Antinuclear antibody is the commonest autoantibody found in more than 95% of lupus patients. Anti-ssDNA (single stranded DNA), anti- dsDNA (double stranded DNA), anti Ro, anti-poly ADP ribose polymerase, anti-histone/nucleosome antibodies and anti-phospholipid antibodies are found in more than 25% of SLE patients. Anti-dsDNA is found in 50-78% of lupus patients with specificity of 97% for SLE(43). Anti-Ro antibodies are present in 30-40% and approximately 50% of those with

anti-Ro positivity also have anti-La antibodies(44). Anti-Sm antibodies are highly specific and found in 25-40% of Afro-Caribbeans and Asians but only in 10-30% of Caucasian lupus patients. The prevalence of anti-Sm is three times higher in Afro-Caribbeans compared to Caucasians(45). Lupus anticoagulant and anticardiolipin antibodies are the antiphospholipid antibodies that are present in 34% and 44% of lupus patients respectively(46). Anti-RNP (ribonucleoprotein) is present in 20-50% of lupus patients. These antibodies are non-organ specific such as ANA but organ specific antibodies may be also found such as anti-neuronal antibody. Pathogenic antibodies such as anti-dsDNA are a useful marker preceding a flare of lupus nephritis. The majority of these autoantibodies have been detected up to 9.4 years (mean 3.3 years) prior to the diagnosis or onset of lupus disease(47;48).

Immune complexes are formed when these autoantibodies react with target antigens. The clearance of circulating immune complexes (IC) by mononuclear phagocyte system is defective in SLE, resulting in tissue deposition and inflammation. The tissue deposition of IC activates the classical complement system which results in consumption of complement proteins reducing the plasma complement levels. Hypocomplementemia is common in SLE which contributes to defective complement mediated IC clearance.

Anti-Ro and anti-La antibody are associated with neonatal lupus. The serious manifestation in neonates associated with transplacental passage of anti-Ro /La antibody is congenital heart block (CHB) usually detected in the second trimester with absent structural cardiac abnormality. The risk of CHB is 2% in an offspring to primigravida lupus pregnancy and the recurrence rate of CHB in subsequent pregnancies is 19%(49;50). The mortality with CHB is 15-30% (fetal and neonatal) and 67% morbidity requiring permanent pacemaker before adulthood(51). Neonatal lupus rash is another manifestation associated with maternal anti-Ro/La and anti-U1RNP antibodies, and is usually apparent by 8 weeks after birth, and clears

by 6 to 8 months coinciding with clearance of maternal autoantibodies in the infant. Fetal and neonatal lupus is independent of maternal disease and can even occur with asymptomatic mothers(51).

Antiphospholipid antibodies (APLA) which predispose to arterial and venous thrombosis include lupus anticoagulant, anticardiolipin antibodies (ACA) of subtype IgG and IgM and anti-beta<sub>2</sub> GPI antibodies. Lupus anticoagulant is the most pathogenic followed by IgG ACA and then IgM ACA. Antiphospholipid antibodies are present in 30% of lupus patients(46;52) and antiphospholipid syndrome (APS) is present in 10%(53), whereas APLA is present in 2-5% and APS in 2% of the general population(54). The association of APLA with cardiac valvular disease as well as PH in SLE has been reported(55;56). Antiphospholipid antibodies have been found in up to 83% of SLE patients with PH(57-59) and only rarely these patients showed features of thromboembolic pulmonary vascular disease leading to PH. The concept of APLA leading to abnormal coagulation and microthrombi in small pulmonary arteries resulting in PH has been reported. It has also been shown that APLA are present in patients with thromboembolic and non-thromboembolic PH without SLE(60;61).

### **1.1.3 Clinical features**

The 1982 revised American college of Rheumatology (ACR) classification criteria for SLE(62) include 11 clinical, laboratory and pathologic features of lupus [Appendix 1]. The presence of 4 or more of the 11 criteria increases the specificity and the sensitivity of lupus classification. Though not compulsory, a positive ANA is considered by many an essential criterion to improve this specificity, particularly for recruitment to trials and other research studies.

The cardiac manifestations in SLE are often under recognised as they don't always cause an acutely symptomatic flare. Although pericarditis is the commonest cardiovascular manifestation, only 25% of those with pericardial effusion have symptomatic pericarditis during the disease course(63) and pericardial tamponade occurs only in 2.5% of SLE patients(64). Other forms of cardiac lupus including myocarditis, endocarditis, valvular disease and cardiac dysfunction are either rare or subclinical. Cardiac arrhythmias are seen in 10%, with sinus tachycardia most frequent. Systemic hypertension is present in 50% and often associated with lupus nephritis. Pulmonary hypertension is considered rare (see below). Accelerated coronary atherosclerosis is increasingly recognised as cause of death in SLE, irrespective of age. A 10-fold relative risk of non-fatal MI and 17-fold relative risk of death from coronary heart disease even after correcting for Framingham study risk factors was reported by Esdaile et al (65).

The most common respiratory manifestations comprise pleuritis with pleural effusions in 50% of lupus patients(66;67). Though abnormal pulmonary function tests are often recorded, only a few develop interstitial lung disease such as alveolitis and pulmonary fibrosis, or shrinking lung syndrome, pulmonary haemorrhage or thromboembolic disease. Indirect lung involvement such as pneumonia during immunosuppressive therapy is more often seen in SLE patients.

Raynaud's phenomenon is seen in 18-46% of patients with SLE. It is prevalent in patients with subacute cutaneous lupus, discoid lupus, malar rash, arthritis and photosensitivity(68). The presence of high titre ANA, anti-RNP antibody and nucleolar antibodies are predictors of LE-associated RP. Raynaud's phenomenon represents vasculopathy resulting in vasospasm of small blood vessels. RP is present in 74% of SLE patients with PH compared to those with normal pulmonary artery pressures(69). This raises the possibility of vasospasm involving the

pulmonary arteries similar to reports on systemic sclerosis patients(70). Raynaud's phenomenon carries a poor prognosis in patients with PAH associated with SLE(71). This suggests that generalised vasculopathy could be contributing to the pathogenesis of PH in SLE, but the degree to which vasospasm plays a role in PH is unknown.

Thrombosis in SLE is multifactorial and especially associated with nephrotic syndrome and antiphospholipid antibodies (APLA). These antibodies are lupus anticoagulant (LA) and anticardiolipin antibodies (ACA), and increases arterial and venous thrombotic risk six fold and two fold respectively. Antiphospholipid syndrome occurs in the presence of APLA resulting in miscarriages, still births, and/or arterial and venous thrombosis(72). The criteria for diagnosis of antiphospholipid syndrome (APS) requires at least one clinical and one laboratory criterion, which includes detection of one of these antiphospholipid antibodies tested positive at moderate to high titres 12 weeks apart (72)(Table 1-1). The clinical criteria include either arterial or venous thrombosis, or obstetric criteria including three miscarriages before 10 weeks gestation in the absence of an alternate explanation or one miscarriage  $\geq 10$  weeks gestation or premature delivery before 34 weeks due to preeclampsia, eclampsia or intrauterine growth restriction. It is important to note that thrombosis in SLE patients can be due to thrombophilic factors other than antiphospholipid antibodies(73).



**Table 1-1 International consensus statement on revised classification criteria for the antiphospholipid syndrome**

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met\*

Clinical criteria

1. Vascular thrombosis<sup>†</sup>

One or more clinical episodes<sup>‡</sup> of arterial, venous, or small vessel thrombosis<sup>§</sup>, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.

2. Pregnancy morbidity

(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or

(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe pre-eclampsia defined according to standard definitions, or (ii) recognized features of placental insufficiency<sup>¶</sup>, or

(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

In studies of populations of patients who have more than one type of pregnancy morbidity,

investigators are strongly encouraged to stratify groups of subjects according to a, b or c above.

Laboratory criteria\*\*

1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies).
2. Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA
3. Anti- $\beta_2$  glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

\*Classification of APS should be avoided if less than 12 weeks or more than 5 years separate the positive aPL test and the clinical manifestation. †Coexisting inherited or acquired factors for thrombosis are not reasons for excluding patients from APS trials. However, two subgroups of APS patients should be recognized, according to: (a) the presence, and (b) the absence of additional risk factors for thrombosis. Indicative (but not exhaustive) such cases include: age (>55 in men, and >65 in women), and the presence of any of the established risk factors for cardiovascular disease (hypertension, diabetes mellitus, elevated LDL or low HDL cholesterol, cigarette smoking, family history of premature cardiovascular disease, body mass index  $\geq 30 \text{ kg m}^{-2}$ , microalbuminuria, estimated GFR  $< 60 \text{ mL min}^{-1}$ ), inherited thrombophilias, oral contraceptives, nephrotic syndrome, malignancy, immobilization, and

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surgery. Thus, patients who fulfil criteria should be stratified according to contributing causes of thrombosis. <sup>‡</sup>A thrombotic episode in the past could be considered as a clinical criterion, provided that thrombosis is proved by appropriate diagnostic means and that no alternative diagnosis or cause of thrombosis is found. <sup>§</sup>Superficial venous thrombosis is not included in the clinical criteria. <sup>¶</sup>Generally accepted features of placental insufficiency include: (i) abnormal or non-normal or non-reassuring fetal surveillance test(s), e.g. a non-reactive non-stress test, suggestive of fetal hypoxemia, (ii) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, e.g. absent end-diastolic flow in the umbilical artery, (iii) oligohydramnios, e.g. an amniotic fluid index of 5 cm or less, or (iv) a postnatal birth weight less than the 10th percentile for the gestational age. <sup>\*\*</sup>Investigators are strongly advised to classify APS patients in studies into one of the following categories: I, more than one laboratory criteria present (any combination); IIa, LA present alone; IIb, aCL antibody present alone; IIc, anti- $\beta_2$  glycoprotein-I antibody present alone.

#### **1.1.4 Assessment of disease**

The fluctuating nature and unpredictable course of lupus disease has led to monitoring the disease closely as a key management plan to achieve a better outcome. Recurrent activity in these organs can lead to cumulative permanent damage resulting in structural and functional compromise in these vital organs. Thus it is prudent to monitor the SLE disease activity regularly, and promptly control lupus activity with appropriate treatment. Early detection of affected organs is important and depends on the sensitivity and specificity of clinical features assessed as well as the use of appropriate tests. In almost 50% of lupus patients, changes in levels of anti-dsDNA antibodies and complement levels (C3 and C4) can help predict flare as well as monitor the response to therapy. Among several validated disease activity monitoring indices, the British Isles Lupus Assessment Group (BILAG)(74;75) [Appendix 2] and SLE disease activity index (SLEDAI) are commonly used in research setting and increasingly in clinical practice(76). Their use in the clinical setting cannot be underestimated despite their limitations, with benefits to both patients and physicians. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index (SLICC/ACR DI) (77;78) [Appendix 3] is used to record the accumulated damage in 12 organ/systems (ocular, neuropsychiatric, renal, pulmonary, cardiovascular, peripheral vascular, gastrointestinal, musculoskeletal, skin, gonadal failure, diabetes mellitus and malignancy) since the diagnosis of SLE. For example, damage recorded in pulmonary system includes pulmonary hypertension, pulmonary fibrosis, shrinking lung, pleural fibrosis, pulmonary infarction or resection. This damage should be clinically ascertained and present for at least 6 months unless otherwise stated in the index such as radiographic evidence. In addition to the damage due to disease activity, the damage due to comorbidities and therapies is also recorded such as diabetes mellitus, myocardial infarction and malignancy. Stoll et al(79) reported that mean pulmonary damage score (DS) at 1 year significantly predicted death within 10 year of

diagnosis, and mean renal DS at 1 year after diagnosis was a strong predictor of end stage renal failure. Please refer to methods section for more details on BILAG and SLICC/ACR damage index.

### **1.1.5 Treatment**

The principles of treatment in SLE are directed towards controlling disease activity, prevention of damage accumulation and treatment of complications. The prompt and adequate treatment of disease flares in a lupus patient reduces long term complications related to disease and treatment as well as reduces morbidity and mortality from uncontrolled immune-mediated tissue damage to organs. The major determinant of the treatment used in lupus management is the level of disease activity which is broadly classified as mild, moderate and severe. Severe disease activity in SLE is defined as manifestations that are life threatening or cause significant organ dysfunction such as inability to perform daily activities due to inflammatory arthritis, nephrotic syndrome or seizures. By comparison, mild disease activity is defined as manifestations that only lead to some discomfort such as joint pain without interruption to daily activities. Thus, the treatments used in lupus are tailored to each patients needs and the treatment categories include: symptomatic treatment, antimalarials, thalidomide, dapson, retinoids, corticosteroids, immunosuppressives/cytotoxics, intravenous immunoglobulins (IVIG), plasmapheresis, and biologics such as Rituximab and Belimumab.

Severe lupus disease activity requires aggressive treatment with high dose corticosteroids (more than 20mg/day of prednisolone or equivalent) usually combined with immunosuppressive therapy. The efficacy of cyclophosphamide(80), mycophenolate mofetil(81) and intravenous methylprednisolone (with cyclophosphamide combination)(82) has been shown in the treatment of severe lupus nephritis. It is important to note that the clinical trials on lupus patients with severe disease activity predominantly include lupus

nephritis and measure renal outcomes for efficacy. Intravenous immunoglobulins(83;84) and plasmapheresis(85-88) are used only in those with life threatening complications. Rituximab is a chimeric monoclonal antibody against CD20 that causes depletion of B cells is also increasingly used in the treatment of severe disease activity in SLE(89-93). Belimumab is a fully humanised recombinant IgG1 lambda monoclonal antibody that inhibits B-lymphocyte stimulator binding to B cells. Belimumab is the first biologic therapy has been shown to achieve disease control, reduce severity of flares, reduce morbidity and improve quality of life in SLE patients(94). The immunosuppressants such as azathioprine, methotrexate, cyclosporin and mycophenolate mofetil are often the mainstay of maintenance treatment in SLE. Cyclophosphamide and Rituximab are used mostly for renal and neuropsychiatric lupus.

Moderate disease activity is usually treated with lower dose steroids (less than 20mg/day of prednisolone or equivalent) with or without concomitant immunosuppressive therapy.

Corticosteroids in different modes of administration (local, oral, intramuscular and intravenous) are used in lupus treatment. Intra-articular injections of corticosteroids are used to treat inflammatory arthritis while topical corticosteroids are used to treat discoid lupus rash. Antimalarials especially hydroxychloroquine have been shown to be effective in mucocutaneous manifestations(95) and inflammatory arthritis in SLE and are often used in place of immunosuppressive agents. Thalidomide, Dapsone and Retinoids are used for refractory lupus rash. Prasterone is an androgen that has been shown to have steroid sparing effect(96) as well as efficacy in reducing lupus flares(97)

Mild disease activity in SLE requires symptomatic treatment with analgesics and nonsteroidal anti-inflammatory drugs. Topical corticosteroids and antimalarials have also been used in resistant mild mucocutaneous manifestations and inflammatory arthritis.

Other treatments include calcium channel blockers for Raynaud's phenomenon, comorbidity treatment such as anti-hypertensives, cholesterol lowering agents and anti-resorptive drugs, and aspirin, heparin and warfarin for thrombosis prevention.

The difficulty in designing and conducting clinical trials in SLE has led to failure of several drug trials except Belimumab trial(94) in recent years. The non-inferiority randomised controlled trials of newer therapies in combination with steroids and standard therapy in SLE could have failed due to the complex nature of multisystem involvement making disease activity assessment difficult and challenging. The composite SLE Responder Index (SRI) was used in the Belimumab trial as the disease activity measure, and SRI incorporates SELENA (Safety of Estrogens in Lupus Erythematosus National Assessment) version of SLEDAI, BILAG assessment and physician's global assessment score. It is possible that future drug trials in SLE would consider SRI to assess disease activity given the success of Belimumab as the only approved treatment in SLE in the last 25 years.

### **1.1.6 Prognosis**

The 5 year survival rate was less than 55% prior to the introduction of corticosteroids in lupus treatment(98). In recent years, further improvement in treatment approaches of this chronic disease and increased detection of milder disease has led to better 10-year survival rate of over 90%(1) and 20-year survival of 68%(99). Despite this, the death rate is at least three times greater than the general population(2). According to two 24-year studies in Holland(100) and Toronto(101) the improved survival was not due to changing demographics, age at diagnosis, severity of lupus at presentation, major change in disease patterns or new modalities of treatment.

The cause of death varied depending on disease duration, such as active lupus if less than 5 years duration, and vascular events and end organ failure not due to SLE in late deaths(99) (Table 1-2). The increased risk of death in SLE patients is associated with female sex, younger age at diagnosis, shorter disease duration and black/African American race(2) The risk of death from active renal disease and infection have decreased whereas it remains the same for circulatory disease.



**Table 1-2 Unadjusted SMR estimates for all-cause mortality and for death by cause\* in patients with SLE**

Cause of Death		Observed	Expected	SMR** (95% CI)
1. All deaths		1255	526	2.4 (2.3-2.5)
2. Disease of the circulatory system	All disease	313	184.3	1.7 (1.5-1.9)
	Heart disease	126	73.8	1.7 (1.4-2.0)
	Stroke	21	19.3	1.1 (0.7-1.7)
3. Malignancy	All neoplasms	114	138	0.8 (0.6-1.0)
	All haematological cancer	15	7.2	2.1 (1.2-3.4)
	Non-Hodgkin's Lymphoma	8	2.8	2.8 (1.2-5.6)
	Lung cancer	44	19.4	2.3 (1.6-3.0)
4. Infections	Infections	45	9.0	5.0 (3.7-6.7)
	Pneumonia	19	7.2	2.6 (1.6-4.1)
5. Other	Respiratory excluding pneumonia	14	10.4	1.3 (0.8-1.6)
	Renal	34	4.3	7.9 (5.5-11.0)

\*Data shown are for 9,547 patients from 23 participating sites from North America, Europe, Asia and Iceland (76,948 person-years) for calendar period 1958-2001.

\*\*SMR: Standardised mortality ratio is the ratio of observed deaths to expected deaths. This table is adapted from the publication on 'Mortality in SLE' by Bernatsky.S et al(2).

## **1.2. Pulmonary arterial hypertension (PAH)**

Pulmonary arterial hypertension is a rapidly progressive devastating disease with significant morbidity and mortality. PAH is characterised by increased pulmonary vascular resistance leading to impending right heart failure and death, unless treated appropriately and early.

An epidemic of pulmonary hypertension (PH) following the use of anorexigens in mid-60s led to the first World Health Organisation (WHO) conference on PAH in 1973(102). A national registry of primary pulmonary hypertension (PPH) patients in USA in early 80's set the platform for initial understanding of PAH such as natural history of disease, clinical features and lack of effective therapy and poor survival.

Over the last two decades, there has been a great improvement in various aspects of PAH management. This includes awareness of PAH, early recognition by prompt screening of high risk patients, use of right heart catheterisation for accurate measurements of pulmonary artery pressures as well as development of modern treatment regimes to improve patient outcomes such as survival and quality of life.

### **1.2.1 Definition and classification of PH and PAH**

#### **1.2.1.1 Pulmonary Hypertension (PH)**

Pulmonary hypertension (PH) is a haemodynamic and pathophysiological condition which is defined by an increase in mean PAP  $\geq 25$ mm Hg at rest as assessed by RHC(102-104). PH can be found in many clinical conditions and thus the need to separate them into subgroups with PH that share similar pathophysiologic and clinical characteristics. The haemodynamic definition of pulmonary hypertension (PH) includes arterial, capillary and/or venous hypertension in the pulmonary vasculature.

### **1.2.1.2 Pulmonary arterial hypertension (PAH)**

PAH is a clinical condition characterised by the presence of precapillary PH haemodynamically(105). The PAH group excludes other causes of precapillary PH such as PH due to lung diseases, chronic thromboembolic PH and other rare disorders such as glycogen storage diseases. The various causes of PAH also share similar clinical picture and histopathological changes in the lung microcirculation.

The precapillary PH is defined as an increase in mean PAP  $\geq 25$  mm Hg at rest as assessed by RHC, and in addition the presence of normal pulmonary capillary wedge pressure(104) (PCWP)  $\leq 15$  mmHg. This haemodynamic definition of PAH helps exclude PH due to left heart disease.

### **1.2.1.3 Classification of PH**

Since the first classification of PH at WHO International symposium on pulmonary hypertension (PH) in 1973(102), it has undergone modifications in three further symposiums held in Evian in 1998, Venice in 2003(106) and Dana Point in 2008(105). The aim of the PH classification was to group together the different manifestations of the disease that share similar pathophysiologic mechanisms, clinical features and treatment strategies(107).

In 1973, pulmonary hypertension (PH) was classified as primary and secondary depending on the absence or presence of identifiable cause or risk factor. In 1998, the term secondary PH was abandoned. In 2003, the term PPH was replaced by Idiopathic PAH (IPAH) to separate familial PAH with presence of family history or associated PAH if another cause such as connective tissue diseases (CTD) is present. In 2008, the architecture of classification was maintained with minor changes proposed to include schistosomiasis and chronic haemolytic anaemia in the PAH subgroup under associated diseases, to make this subgroup more homogenous.

The clinical classification of PH updated in Venice and Dana Point in 2003 and 2008 are shown in Table 1-3 and Table 1-4 respectively. In these classifications, PH is broadly divided into 5 main groups: 1. Pulmonary arterial hypertension (PAH); 2. PH with left heart disease; 3. PH associated with respiratory diseases and/or hypoxia; 4. PH due to chronic thromboembolic disease; 5. Miscellaneous or PH with unclear multifactorial mechanisms.

**Table 1-3 Venice clinical classification of Pulmonary Hypertension (2003)**

1. Pulmonary arterial hypertension (PAH)
1.1. Idiopathic (IPAH)
1.2. Familial (FPAH)
1.3. Associated with (APAH)
1.3.1. Collagen vascular disease
1.3.2. Congenital systemic-to-pulmonary shunts
1.3.3. Portal hypertension
1.3.4. HIV infection
1.3.5. Drugs and toxins
1.3.6. Other (thyroid disorders, glycogen storage disease, Gaucher disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders, splenectomy)
1.4. Associated with significant venous or capillary involvement
1.4.1. Pulmonary veno-occlusive disease (PVOD)
1.4.2. Pulmonary capillary hemangiomatosis (PCH)
1.5. Persistent pulmonary hypertension of the newborn

2. Pulmonary hypertension with left heart disease

2.1. Left-sided atrial or ventricular heart disease

2.2. Left-sided valvular heart disease

3. Pulmonary hypertension associated with lung diseases and/or hypoxemia

3.1. Chronic obstructive pulmonary disease

3.2. Interstitial lung disease

3.3. Sleep-disordered breathing

3.4. Alveolar hypoventilation disorders

3.5. Chronic exposure to high altitude

3.6. Developmental abnormalities

4. Pulmonary hypertension owing to chronic thrombotic and/or embolic disease

4.1. Thromboembolic obstruction of proximal pulmonary arteries

4.2. Thromboembolic obstruction of distal pulmonary arteries

4.3. Nonthrombotic pulmonary embolism (tumour, parasites, foreign material)

5. Miscellaneous

Sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumour, fibrosing mediastinitis)

**Table 1-4 Updated Clinical Classification of Pulmonary Hypertension (Dana Point, 2008)**

1. Pulmonary arterial hypertension (PAH)
  - 1.1. Idiopathic PAH
  - 1.2. Heritable
    - 1.2.1. BMPR2
    - 1.2.2. ALK1, endoglin (with or without hereditary hemorrhagic telangiectasia)
    - 1.2.3. Unknown
  - 1.3. Drug- and toxin-induced
  - 1.4. Associated with
    - 1.4.1. Connective tissue diseases
    - 1.4.2. HIV infection
    - 1.4.3. Portal hypertension
    - 1.4.4. Congenital heart diseases
    - 1.4.5. Schistosomiasis
    - 1.4.6. Chronic hemolytic anemia
  - 1.5 Persistent pulmonary hypertension of the newborn
- 1' Pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis

2. Pulmonary hypertension owing to left heart disease

2.1. Systolic dysfunction

2.2. Diastolic dysfunction

2.3. Valvular disease

3. Pulmonary hypertension owing to lung diseases and/or hypoxia

3.1. Chronic obstructive pulmonary disease

3.2. Interstitial lung disease

3.3. Other pulmonary diseases with mixed restrictive and obstructive pattern

3.4. Sleep-disordered breathing

3.5. Alveolar hypoventilation disorders

3.6. Chronic exposure to high altitude

3.7. Developmental abnormalities

4. Chronic thromboembolic pulmonary hypertension (CTEPH)

5. Pulmonary hypertension with unclear multifactorial mechanisms

5.1. Hematologic disorders: myeloproliferative disorders, splenectomy

5.2. Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, vasculitis



5.3. Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders

5.4. Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

ALK1: activin receptor-like kinase type 1; BMPR2 :bone morphogenetic protein receptor

type2; HIV: human immunodeficiency virus

### **1.2.2 Prevalence; incidence; clinical presentation of PH**

Idiopathic PAH (IPAH) is considered to be rare. The NIH registry estimated an annual incidence of primary PH of 1-2 per million(104) among general population. The French national registry estimated the annual incidence and prevalence of PAH diagnosed by cardiac catheterisation at 2.4 and 15 (range 5-25) cases per million population(108). The possibility of underestimation was considered due to geographical variation of prevalence in France. The prevalence of PAH due to any cause was estimated 30-50 cases per million and the incidence of PAH associated with CTD at 1-2 per million. IPAH is common in the third and fourth decades of life with a female to male ratio of 1.7:1, and has no racial predilection. IPAH accounts for 40% of total PAH cases.

The rarity of the disease makes it difficult to understand the disease process and to perform meaningful experimental and clinical research from small groups of PAH patients. Thus, it becomes imperative to have national registries and specialised PAH centres to achieve progress in understanding the pathogenesis, improving management strategies to have better outcomes and newer treatment modalities.

The classical symptoms of PH include breathlessness, fatigue, poor exercise tolerance, syncope, angina, and palpitations (104). Lupus can manifest with other cardio-pulmonary complications which can also result in few or all of the above symptoms. Due to the non specific nature of these symptoms, the diagnosis of PH is delayed unless other common conditions with similar symptoms are ruled out. Moreover, these symptoms are present at rest only in patients with advanced PAH.

The physical signs of PH include left parasternal lift, accentuation of pulmonary component of second heart sound, pansystolic murmur of tricuspid regurgitation, diastolic murmur of

pulmonary regurgitation and a right ventricular third heart sound. The physical signs are often subtle until the signs of right heart failure develop in advanced stages of PAH. This includes elevated jugular venous pressure (JVP), hepatomegaly, peripheral edema, ascites and cool extremities. It is also important to look for signs of diseases that can cause PH. The presence of calcinosis, sclerodactyly, telangiectasia and skin tightness in systemic sclerosis, malar rash or discoid rash in SLE, swollen hands and arthritis in mixed connective tissue disease, spider naevi and palmar erythema in liver disease may be present. Though lung sounds are often normal, the end inspiratory fine crackles of interstitial lung disease or bi basal crackles of cardiac failure may point to the cause of PH. The presence of digital clubbing raises possibility of congenital heart disease and pulmonary veno-occlusive disease.

It is well known that the diagnosis of PH is often delayed for more than 2 years with a majority of patients diagnosed in advanced stages(108;109). The prolongation of the time to diagnosis of PAH from onset of symptoms has a significant impact on poor survival in these patients. The physician awareness of PAH in at risk groups such as those with a strong family history of PAH, connective tissue diseases, congenital heart diseases, HIV infection, portal hypertension among several others is crucial for earlier diagnosis of PAH. Thus the need to improve physician awareness is of paramount importance to reduce delays in diagnosis of PAH.

The severity of dyspnoea and exertional intolerance is quantitated according to World Health Organization (WHO) classification of functional status(110) in patients with PH. This is the modification adapted from the New York Heart Association (NYHA) functional classification. The role of functional assessment in patients with PH was emphasised in the pulmonary hypertension working group meeting in Evian, France 1998 and led to the WHO functional assessment classification(105) (Table 1-5). The determination of functional class

helps to apply the appropriate management options for patients with PAH at multiple levels. This could be with initial presentation, coexistent clinical signs, screening for PAH, severity of elevated pulmonary artery pressure, treatment choice, quality of life and survival.

The clinical signs of PH are usually subtle until significant right heart failure has developed. It is vital to consider PH in this setting of clinical features, particularly in the absence of other cardiac and respiratory conditions presenting with similar clinical features. ECG, Chest x-ray, pulmonary function testing and Doppler echocardiography are commonly used initial tests in screening for PAH.

The other categories of pulmonary hypertension such as intrinsic lung disease, hypoxia, left heart disease and miscellaneous conditions that can present with similar clinical features have to be excluded by relevant tests before making a diagnosis of PAH.

**Table 1-5 World Health Organisation functional classification for patients with pulmonary hypertension 1998\***

Functional Class	Symptomatic profile
I	Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause dyspnoea or fatigue, chest pain, or near syncope
II	Patients with pulmonary hypertension resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near syncope
III	Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes undue dyspnoea or fatigue, chest pain, or near syncope
IV	Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnoea and/or fatigue may even be present at rest. Discomfort is increased by any physical activity

\*Functional classification of pulmonary hypertension modified after the New York Heart Association functional classification

## Definition of PH

The definition of PH adopted from the 1973 World Health Organisation (WHO) conference on PH is used in the epidemiological studies to evaluate prevalence of PH as well as clinical trials to evaluate PH treatments. PH is defined by RHC measurements of mean PAP  $\geq 25$  mmHg at rest or  $\geq 30$  mmHg at exercise, with pulmonary capillary wedge pressure (PCWP)  $\leq 15$  mmHg and pulmonary vascular resistance (PVR)  $> 3$  Wood units(111). The systolic PAP estimated in the presence of tricuspid regurgitation (TR) by Doppler ECHO use a cutoff of either  $> 30$  mmHg or  $> 35$  mmHg for the definition of PH. As most PH patients have systolic PAP in the range of 30-40 mmHg in the epidemiological studies, these cut off values play a key role in the determination of PH prevalence rates.

The PH diagnosis by ECHO depends on Doppler evaluation of tricuspid regurgitant signal. The tricuspid insufficiency is too weak or insufficient to be measured by Doppler to derive the pressure gradient across the tricuspid valve in 10-25% of patients undergoing PH screening(112;113). There has been good correlation of systolic PAP (sPAP) measured by ECHO and mean PAP measured by RHC. The mean sPAP among 3212 healthy population aged 1-89 years with a detectable TR on transthoracic echocardiogram (TTE) was  $23 \pm 4.7$  mmHg assuming right atrial pressure of 5 mmHg(114). The sPAP increases with age and body mass index(114). Similarly, the mean sPAP in 134 adults aged 20-85 years was 26.6 mmHg and had normal cardiac structure and function detected on transthoracic echocardiography(115). The calculation of sPAP from ECHO parameters are described in detail in the methods section. Mukerjee et al(116) showed that ECHO screening is of value only in screening of advanced PH provided high thresholds of sPAP  $> 45$  mmHg is used to define PH. In another large study on SSc patients using sPAP of  $> 35$  mmHg and  $> 25$  mmHg

in asymptomatic and dyspnoeic patients respectively , the false positive rate of ECHO defined PH was 45% in comparison to RHC measurements(117).

While the non-invasive ECHO has been a pivotal screening test for PH in those suspected to have PH, the gold standard test to confirm PH remains the invasive right heart catheterisation. However, there are limitations to RHC measurements. The RHC procedures are associated with morbidity of 1.1% and mortality of 0.055%. The measurements are susceptible to fluctuation due to respiratory cycle, hyperventilation and Valsalva manoeuvre. The RHC measurements are done at artificial conditions such as rest and overnight fast which might not reflect the true pulmonary artery pressures during active daily lives of patients.

### **1.2.3 Pathogenesis**

Though the exact mechanisms that initiate the pathological changes seen in PH are unknown, a great deal of understanding of pathological and pathophysiological changes as well as pathobiology of PH has led to better monitoring and newer treatment strategies in management of PH.

The complex multifactorial process involved in the development of PAH includes vasoconstriction, proliferation and obstructive remodelling of pulmonary vessel wall, insitu thrombosis and inflammation. Adventitial thickening, medial hypertrophy, intimal proliferation and fibrosis, perivascular inflammatory infiltrates, complex plexiform lesions and thrombotic lesions are pathological characteristics of PAH(118)

In PAH, the pathological lesions particularly affect the distal pulmonary arteries <500micrometer in diameter. Pulmonary veins are unaffected. The vasoconstriction of pulmonary vessels results from endothelial dysfunction as well as potassium channel dysfunction in the smooth muscle cells. Endothelial dysfunction (ED) leads to chronic

impaired production of vasodilators and antiproliferative agents such as nitric oxide, prostacyclin, vasoactive intestinal peptide (VIP) and receptors of bone morphogenetic protein (BMP) pathway. The ED also results in overexpression of vasoconstrictors and proliferative substances such as endothelin-I and Thromboxane A<sub>2</sub>. Endothelial dysfunction promotes proliferation of several cell types including smooth muscle cells, endothelial cells and fibroblasts which leads to vascular remodelling. The increased production of extracellular matrix including collagen, elastin, fibronectin and fibroblast growth factor results in thickening and fibrosis of adventitial layer(119). The medial layer undergoes smooth muscle cell hypertrophy and hyperplasia resulting in lumen reduction. The migration and proliferation of myofibroblasts and fibrosis of both media and intimal layers results in severe lumen reduction of distal pulmonary vessels. The morphological changes affecting all layers of the vessel wall results in severe reduction of vessel lumen and increasing vascular resistance propagating PAH. The severity of each vessel wall layer involvement is variable in different forms of PAH, for example adventitial fibrosis is prominent in PAH secondary to scleroderma.

In addition, the increased production of several growth factors such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), insulin like growth factor-I and epidermal growth factor have been reported in PAH patients. These growth factors also contribute to the vascular remodelling of pulmonary vessels in PH. The hypercoagulable state and platelet dysfunction may also contribute to the pathology of PH. The role of inflammation and thrombosis in the pathogenesis of PAH particularly in SLE is discussed later.



### 1.2.4 Treatment

The treatment of PAH includes supportive treatment with oxygen, anticoagulants, diuretics, digoxin and calcium channel blockers (CCB).

As hypoxia is a potent vasoconstrictor of pulmonary circulation, oxygen therapy is recommended to maintain oxygen saturation above 90%. The role of anticoagulation in supportive therapy of PH has been established with the rationale that thromboembolic phenomena play a significant role in the pathogenesis of PH. The presence of vascular thrombotic lesions in IPAH patients at post-mortem(120), the demonstration of abnormal coagulation(121), fibrinolytic pathways(122) and platelet function in PH(123), and the association of antiphospholipid antibodies with SLE-PH which could possibly increase thromboembolic risk have all led to support the use of anticoagulation in IPAH as well as other forms of severe PAH including SLE associated PH (SLE-PH) and other CTD associated PH. The risk to benefit ratio of anticoagulation in CTD-PAH is not well understood. The evidence for possible benefits such as improved survival with anticoagulation is derived from 1 prospective and 2 retrospective studies(120;124;125) on IPAH, hereditary PAH, and anorexigens induced PAH. The 3 year survival improved from 21% to 49%(120) and 5 year survival from 31% to 62%(124). Anticoagulation should be avoided in patients at high risk of bleeding. Patients with indwelling catheters for prostanoid infusions are also advised to be anticoagulated to prevent catheter related thrombosis. Anticoagulants have not shown to be effective in reducing functional class severity or pulmonary haemodynamics. There is a need for randomised controlled studies to evaluate the benefits of anticoagulation in PAH and SLE-PH. Calcium channel blockers (CCB) such as Nifedipine, Amlodipine and Diltiazem are used in patients with PAH who exhibit an acute vasodilator response (a fall in mean PAP  $\geq$  10 mmHg, to a mean PAP  $\leq$  40mmHg, with an

unchanged or increased cardiac output) to intravenous adenosine or inhaled nitric oxide at right heart catheterisation(126;127). This response is most often seen in IPAH patients, while other forms of PAH especially CTD-PH rarely show long term response to CCB. In patients with high left heart filling pressures and overt right heart failure, CCB is avoided. Rich et al reported on a very select group of IPAH patients to have 94% survival rate at 5 years with high dose CCB treatment(124). Digoxin is sometimes used in IPAH patients with right heart failure, low cardiac output and atrial arrhythmias(128).

The better understanding various drivers of PAH led to the newer therapies including Prostacyclin, Endothelin receptor antagonist (ERA) and phosphodiesterase inhibitors in the last decade to improve the survival and quality of life in PAH patients. The outcome measures commonly comprise of dyspnoea grade, exercise capacity, hemodynamic measures, quality of life and survival. In recent years, the treatment approach in PAH is one of goal oriented therapy rather than escalation of treatment only with signs of deterioration(129). This incorporates using prognostic indicators of survival as treatment targets, such as WHO functional class and various clinical parameters. For example, patients in WHO FC II have better survival than those in WHO III/IV. The escalation of treatment from monotherapy to sequential combination therapy(105;130) is a recommended step for goal oriented therapy of PAH.

Several Prostacyclin analogues used in PAH treatment for patients in WHO-FC III/IV share similar pharmacodynamic properties(131). Prostacyclin induces vasodilatation of all vascular beds, inhibits platelet aggregation, and also has cytoprotective and antiproliferative properties. Iloprost, Epoprostenol(132-134), and Treprostinil(135) are commonly used chemically stable prostacyclin analogues. These are administered by continuous intravenous infusion, subcutaneous infusion and aerosol administration depending on individual drug

properties. The continuous intravenous infusion of Epoprostenol has been shown to improve symptoms, exercise capacity, haemodynamics, survival(133) and have persistent effect in patients with IPAH, CTEPH and CTD-aPAH. Similar efficacy end points were met with subcutaneous Treprostinil infusion in a large randomized controlled trial in IPAH (135). Inhaled and intravenous Iloprost therapy has shown efficacy as monotherapy as well as combination with Bosentan therapy(136) in IPAH.

The role of high plasma levels of Endothelin-1 in the pathogenesis of PAH led to the introduction of ERA in the PAH treatment. Endothelin-1 exerts vasoconstrictor and mitogenic effects through endothelin-A receptor activation in smooth muscle cells, and causes release of nitric oxide (NO) and prostacyclin through activation of endothelin- B receptors in endothelial cells resulting in vasodilatation and antiproliferation effects.

Bosentan is an oral active dual endothelin receptor antagonist(137-140), and Ambrisentan is selective antagonist for endothelin- A receptor(141;142). Both have been shown through RCTs to improve exercise capacity, functional class, haemodynamics and time to clinical worsening in patients with IPAH and CTD-aPAH. The ERA are effective for PAH patients in WHO-FC II as well as those in WHO- FC III/IV.

The phosphodiesterase type-5 inhibitors (PDE-5i) are the latest addition to the treatment armamentarium for PAH, which results in vasodilatation through NO/cGMP pathway in pulmonary vessels and also has antiproliferative effects. Sildenafil(143) and Tadalafil have shown efficacy both as monotherapy and in combination with epoprostenol(144) and Bosentan(145) respectively.

Few RCTs have been published on the use of combination therapy in PAH(140;144;145). These include two or more drugs from prostanoids, ERA and PDE-5i group used either

simultaneously or in stepwise fashion particularly in PAH patients in WHO FC III/IV.

Though the lack of response to monotherapy could be adequate reason to try combination therapy, the optimal timing of using combination therapy is still not clear and is variable in individual patients.

At the time of our study period, most of these advanced therapies were at early stages of use in PAH therapy. These newer target-specific treatments have offered hope to patients with a devastating and progressive disease with poor prognosis by improving symptoms, exercise capacity, quality of life, pulmonary haemodynamics as well as extending survival. Over the recent years since our study period, there has been increasing evidence through randomised controlled trials (RCT) of the advantages of combination therapy over monotherapy of these drugs in several subgroups of PAH. These valid reasons make it worthwhile diagnosing PAH at early stages and monitoring closely to achieve meaningful outcomes.

### **1.2.5 Survival**

The NIH registry from the early 1980's showed the poor median survival of untreated Idiopathic PAH (IPAH) patients to be 2.8 years, with 1, 2, 3 and 5 year survival rates at 68%, 57%, 48% and 34% respectively(103).Historically the prognosis of patients with severe PAH has been very poor until the introduction of advanced therapies including prostanoids, endothelin receptor blockers and phosphodiesterase inhibitors in the treatment algorithm. The exercise capacity and WHO functional class are shown to be closely related to survival in patients with IPAH(146;147). The predictors of poor prognosis includes advanced functional class, reduced exercise capacity as measured by six minute walk test, high right atrial pressure, significant right ventricular (RV) dysfunction, evidence of RV failure, low cardiac index, elevated brain natriuretic peptide (BNP), and underlying diagnosis of scleroderma spectrum of diseases(148).

In mid 90's the introduction of intravenous prostacyclin for IPAH patients with New York Heart Association (NYHA) dyspnoea class III and IV improved these survival rates to 88%, 76% and 63%. A decade later, the introduction of endothelin receptor blocker Bosentan as first line therapy in this group improved the survival rates to 97%, 89% and 86% respectively(149).

Since this present study has been undertaken, several PAH registries around the world particularly from UK, Europe and USA have published the survival rates of patients registered in their cohorts. The 1-year survival observed in the USA registry incident cohort with patients with PH of any cause was 91% and 88.4% in the French registry incident cohort of PH patients. The UK registry(150) reported 1 and 3 year survival rates of 78% and 74% respectively in SLE-PAH compared to 78% and 47% in SSc-PAH (Table 1-6) Among the 86% of SLE patients who were immunosuppressed, 11% were newly immunosuppressed after PAH diagnosis. Seventy five percent underwent advanced therapy for PAH. The REVEAL registry(151) reported 1- year survival rate to be significantly worse in SSc-PAH compared to SLE-PAH, which were 82% and 94% respectively. This reflects the REVEAL registry has predominantly prevalent cases unlike incident cases in UK registry as the risk is initially high. Moreover, only 22% of 110 SLE-PAH patients were on immunosuppressive therapy. It was also shown that hospitalization rates were high in CTD-PAH and similar among all CTD compared to IPAH, and mostly for primary disease flares rather than PAH. In contrast, the 1, 2 and 3 year survival rates in IPAH with advanced therapies were reported at 86%, 70% and 55%(152) compared to 1, 3 and 5 year survival rate of 68%, 48% and 34% respectively in pre- advanced treatment era(103).

The survival in SLE-PAH (median survival 13 months) is poor compared to IPAH (median survival 66 months) but appears to be better than SSc-associated PAH.

**Table 1-6. Survival rates in PAH**

Disease associated with PAH	Source of publication, year	1 year survival	2 year survival	3 year survival	5 year survival
SLE	Asherson et al(57;153) 1986, 1990		>50		50
SLE	Wilmslow et al(154) 1995				86
SLE	Chung et al(155) 2006	50		45	17
SLE	UK PH centres(150) 2009	78		74	
SLE	USA PH centres(151) 2010	94			

SSc	Koh et al(156) 1996	45	35		
CTD  (includes 8 SLE out of 64 CTD patients)	Denton et al(157) 2006	86	73		
SSc	UK PH centres(150) 2009	78		47	
SSc	USA PH centres(151) 2010	82			
Idiopathic PAH	Chung et al(155) 2006	77		73	68

SLE: Systemic lupus erythematosus; SSc: Systemic sclerosis; CTD: Connective tissue disease; PAH: Pulmonary arterial hypertension

### 1.3. PAH and CTD

Pulmonary arterial hypertension is an increasingly recognised complication of several connective diseases, in particular systemic sclerosis (SSc), systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD). PAH associated with CTD contributes to 8% of all cause severe PAH. The national PAH registries from France, Scotland and UK have estimated the prevalence and incidence of PAH associated with CTD to be 2.3 and 0.4, 10 and 2.8, 4.3 and 1.55 cases per million respectively. The prevalence is highly variable depending on the definition of PAH, diagnostic method used, study population number and characteristics. Patients with PAH and CTD are mostly women (4:1), diagnosed at older age (mean 66years), have concomitant disorders such as interstitial lung disease and left heart disease, and have shorter survival rates in comparison to IPAH patients(158).

Systemic sclerosis is the most common CTD associated with PAH and thus most data on clinical features and treatment strategies are derived from SSc patients. The prevalence of PAH in SSc is 10-15%(158) and it is more frequent in limited SSc compared to diffuse SSc. Though isolated PAH is common in SSc, the presence of interstitial lung disease particularly in diffuse SSc could contribute to development of PH. It is recognised that PH associated with hypoxic lung disease have relatively mild elevation of pulmonary pressures without compromise in cardiac output(159). This was also reflected from the UK study(150) that SSc patients with PAH and interstitial lung disease had similar pulmonary haemodynamics to those with isolated PAH and disproportionate to the severity of lung disease. In the 90's it was reported that the median survival of patients with PAH in SSc was 12 months irrespective of the disease type of SSc. The same study on 17 SSc patients with PAH also showed patients with PAH and restrictive lung disease lived longer than isolated PAH (156).



Chang et al(160) also reported no difference in survival between PAH patients with (n=112) and without (n=119) restrictive lung disease. Though this study had a larger cohort, significant limitations were low sPAP threshold of 35 mmHg for PAH diagnosis and the lack of RHC data. In 2009, the UK PH centres reported on a large cohort of 315 unselected incident SSc cases with PAH diagnosed by RHC. The survival rate in SSc patients with PAH and interstitial lung disease(n=56) was found to be significantly worse compared to those with isolated PAH(n=259) (150). A recent study(161) has retrospectively analysed 70 SSc patients with PH and ILD who received advanced targeted therapy including prostanoids, ERA and/or PDE-5i. No benefits of therapy were found and the 1, 2 and 3-year survival rates in this subgroup were 71%, 39% and 21% respectively. The risk of death from PAH related to SSc is three to fourfold higher than for IPAH(162;163).The 1 year survival rate was 55 % in SSc-PH compared to 84% in IPAH patients who were treated with prostanoids and other supportive therapy such as anticoagulation, digoxin and diuretics(162).

Despite the similar histopathological features in IPAH and CTD-associated PAH, the poor prognosis in PAH with CTD suggests that the pathophysiological processes are different, complex and not yet clearly understood. The initiating and propagating pathways are not known though several hypothesis exist involving the vasoconstriction, vascular proliferation and remodelling, and endothelial dysfunction. The drivers of PAH can be different at various stages of this disease. It is vital to target these drivers at the most appropriate time in order to achieve better outcome. Vasospasm plays a role in the early stages of PAH development while proliferation and fibrosis of vessels in later stages of severity.

Though the pulmonary vascular lesions in PAH associated with CTD are similar to IPAH, the prognosis and response to therapy are distinctly poor in comparison to IPAH. The prognosis of PAH patients associated with CTD is very poor with 1, 2 and 3 year survival rates of 45%,

35% and 28% (156). The median survival with CTD associated PAH was only 1 year in comparison to 2.8 years with IPAH prior to introduction of prostacyclin treatment in mid-90s. Though the advent of advanced therapies in PAH have improved survival rates, the long term outcome is still poor in patients with PAH associated with CTD than in IPAH (refer to section 1.2.5)

### **1.3.1 Why PAH in SLE is important**

Both these conditions independently can contribute to poor morbidity and mortality. The association of these conditions together can reduce the quality of life and survival considerably. The cardiac and respiratory functional compromise could be catastrophic if PAH develops in the background of active SLE affecting major organs. Moreover, pathogenesis such as thrombosis and vasculitis are common to both these conditions favouring the association as well as poor survival in this association.

In SLE, the possible reasons for PAH development includes thrombosis, vasculopathy, active lupus disease, valvular heart disease, and interstitial lung disease. Vasospasm is another mechanism that may be important and Raynaud's has been increasingly recognised as an association in SLE patients with PAH. Raynaud's phenomenon is seen in 75% of patients with PAH compared to 25% of patients without PAH in SLE (71;153;164;165) suggesting vasculopathy as a cause. This raises suspicion that antiphospholipid antibodies, Raynaud's phenomenon, vasculitis and autoantibodies, interstitial and thromboembolic lung disease, and left heart disease causing pulmonary venous hypertension could be potential risk factors for development of PAH in SLE.

To date SLE patients have been poorly studied to assess the true prevalence of PAH in non tertiary centre SLE patients. Most studies until now from tertiary centres are likely to

represent patients with severe SLE disease activity and do not necessarily reflect the general SLE population. Depending on the definition of PAH and diagnostic methods used, the prevalence of PAH in SLE patients range from 0.5-43%(69;154;166-170). The highly variable prevalence of PAH in the few epidemiological studies of SLE patients reflects the inconsistent epidemiological data. In addition, the retrospective nature of these studies with fewer SLE patients are also sources of error such as confounding and bias. These studies predominantly used ECHO as the screening tool. Only few patients have severe PAH, both symptomatic and haemodynamic. It is not known who progresses and how soon do they progress. What is known is that prognosis is poor in this group of secondary PAH compared to IPAH (see section 1.2.5)

### **1.3.2 Variable data on prevalence of PAH in SLE**

Pulmonary arterial hypertension is considered to be uncommon in SLE unlike other CTD such as systemic sclerosis and mixed connective tissue disease (MCTD). Overall, the range of prevalence of PH in SSc diagnosed by echocardiogram (ECHO) is 16-59%(171-176) and by right heart catheterisation (RHC) is 7-29%(117;158;172;177-179), with majority of these studies reporting at lower end of the range mentioned above except older studies. In 2003, an UK study on 794 patients with SSc reported the prevalence of PH by ECHO was 18% and RHC 12%(158). Excluding interstitial lung disease related PH, the prevalence of PAH in SSc patients by RHC is 7.85%(117). In a population based approach from UK, the prevalence of PH in SSc was 2.93 per million(150). The prevalence rate of PH in MCTD is 23-53%(180-182). It is also known that a significant number of CTD patients may have undiagnosed PAH (UNCOVER study)(175). Undiagnosed PAH was found in 13.3% in a community cohort of 669 patients with systemic sclerosis or MCTD with an overall prevalence of 26.7%(175)

The lack of robustness in the prevalence studies in PH in SLE stems from studies reported retrospectively or only in a small group of SLE patients. The wide variability depends on the definition of PH, diagnostic methods used, awareness of PH in SLE, study centres, number of study patients and retrospective nature of most studies. The prevalence rates are lower with studies using the gold standard right heart catheterisation (RHC) to diagnose PAH, but these studies are very few with SLE-associated PH (SLE-PH). PAH is also of mild degree in patients with lupus. The inconsistent epidemiological data in PAH associated with SLE influence the prevalence rates to a great degree. The following studies on lupus patients describe the wide variation in prevalence rates of PH in SLE (Table 1-7).

- 1954: Harvey et al(183) reported that none of the 138 SLE patients had PAH.
- 1981: Perez and Kramer(167) found a 9% prevalence of PAH in 43 SLE patients seen over 3 year follow up.
- 1984: Quismorio et al(69) reported a prevalence of 0.5% of PAH in 400 SLE patients followed up over 10 years. They also reported the clinical and haemodynamic characteristics of the 20 PAH cases reported until then, concluding that the disease course is variable but small numbers of patients and short duration of follow up could bias these findings.
- 1985: Badui et al(166) identified a prevalence of 9% for PAH in 100 SLE patients with Doppler ECHO measurements
- 1989: Simonson et al(168) found that 5 out of 36 SLE patients representing 60% of their tertiary care cohort to have PAH. This prevalence rate of 14% was evaluated by Doppler Echocardiography in this case control study. Only

one of the 5 patients had systolic PAP > 40 mm Hg and had past history of PAH prior to this study. In 1995, Winslow et al(154) reported the 5 year follow up data of these patients with a prevalence rate of 43% for PAH in 28 SLE patients. Among the 5 PAH patients from initial study, 2 had died, 2 had normal systolic PAP and only 1 had persistent PAH. The maximum systolic PAP was 38 mm Hg suggesting only mild elevation of PAP in all patients.

- 1990: Asherson et al(57) reported 5% prevalence for PAH in 500 SLE patients over 5 years.
- 1999: Among the 419 SLE patients from Hong Kong, 176 patients underwent Doppler echo in this retrospective analysis over 12 years. This study by Li et al(170) showed a prevalence of 4.3% for PAH in SLE.
- 2000: Pan et al(169) reported PAH prevalence of 5.8% in a retrospective study of 786 SLE patients diagnosed over last 20 years from Singapore. Twenty four of the total 46 PAH patients had a secondary cause for PAH with valvular heart disease, interstitial lung disease, pulmonary embolism or combination at 50%, 8%, 13%, and 29% respectively.
- 2002: Tanaka et al(184) studied 194 SLE patients retrospectively and reported a prevalence of 6.2% for PAH. A third of these PAH patients had overlapping SSc features, predominantly limited SSc.
- 2004: Johnson et al(185) reported a retrospective survey of 117 SLE patients who underwent Echocardiography over previous 7 years, from the cohort of

1100 patients from Canada. The prevalence rate of 5.4% for PAH was calculated, with systolic PAP >40 in a quarter of PAH patients.

Most of these studies were retrospective analyses of SLE cohorts worldwide. These patients underwent ECHO Doppler studies to investigate cardiac and respiratory symptoms, abnormal chest X-ray or ECG, suspicion of valvular heart disease and rarely clinical signs of right heart failure. Thus, ECHO was neither done nor performed early in the asymptomatic or mild PAH patients with subtle clinical features. The possibility of underestimation of PAH is highly likely because the studies were not prospective and only a fraction of the cohort underwent Doppler ECHO. The few prospective studies were of small size which could significantly influence the reported prevalence rates. The tertiary care cohorts are likely to represent patients with severe disease activity and unlikely to reflect the true prevalence in the general SLE population. Except a very few, Doppler ECHO has been the screening method of choice in above studies due to the invasive nature or cost of the right heart catheterization (RHC).

Except for a very few PAH patients, the systolic PAP were of mild and moderate degree less than 45 mmHg. The progress of PAH is variable depending on the presence of any secondary cause for PH in these patients. The advantages of management of severe and symptomatic PAH patient of any cause to improve symptoms, exercise capacity or haemodynamic measures has been clearly shown with newer targeted and combination therapies. The evidence for the benefits of treatment in mild and moderate PAH especially when asymptomatic is not known and needs further evaluation in future. The PH registries from UK, France and USA and few other research groups including Chung et al(155), Condliffe et al(150), Fois et al(186), Cefle et al(55) and Ruiz-Irastorza et al(187) have published on the epidemiological data on PH in CTD since the present study has been undertaken and will be detailed in chapter 3 (section 3.4).

**Table 1-7 Epidemiologic studies of the prevalence of pulmonary hypertension (PH) in SLE**

First author of study (n= No. of patients in cohort)	No. under-gone test	Prevalence rate of PH (%)	PH definition (mmHg)	Country where study was conducted	Type of SLE cohort	PH diagnosed by ECHO or RHC	Durati- on of study (years)	Nature of study (R/P)#	Year of study
Harvey (n=138)	138	0	n/a	USA	n/a	n/a	n/a	n/a	1954
Perez (n=43)	43	9.3	n/a	USA	T	RHC	3.5	R	1981
Quismorio (n=400)	20	0.5	>40	USA	T	RHC	10	R	1984
Badui	100	9	n/a	n/a	n/a	ECHO	n/a	P	1985
Simonson (n=36)	36	14	> 30	USA	T	ECHO	1	P	1989

Asherson (n=500)	n/a	5	n/a	UK	T	n/a	5	R	1990
Ong (n=40)	40	10	>55	Malaysia	T	ECHO	1	P	1992
Winslow (n=28)	28	43	>30	USA	T	ECHO	5	P	1995
Li (n=419)	176	4.3	>30	Hong Kong	T	ECHO	12	R	1999
Shen (n=84)	84	11	>30	China	T	ECHO	1	P	1999
Pan (n=786)	n/a	5.8 (2.8% had PAH)	>30	Singapore	T	ECHO	20	R	2000
Tanaka (n=194)	194	6.2	> 40*	Tokyo	T	ECHO	8	R	2002
Johnson (n=1100)	117	1.3  5.4	≥ 40  > 30	Toronto	T	ECHO	8	R	2004



Chung (N=725)	181	2.8	>45*	South Korea	T	ECHO	9	R	2006
Chung (n=725)	10	1.4	>30	South Korea	T	RHC	9	R	2006
Fois (n=93)	93	13  (9.6 % had PAH)	≥35	France	T	ECHO	11	R	2010

\* Includes right atrial pressure of 10mm Hg; # Nature of study: R- retrospective, P-prospective; T: Tertiary centre; RHC-Right heart catheterisation; ECHO-Echocardiogram; Data unknown from original publications are entered n/a (not available).

### **1.3.3 ECHO as a screening tool for PAH**

The modern Doppler ECHO techniques have been the screening tool of choice for PAH in most studies on SLE patients. Doppler echocardiography is an indirect and observer dependent measure of systolic pulmonary artery pressure using tricuspid gradient (TG) estimation. Only a few retrospective studies reported on the right heart catheterization (RHC) measurements in patients that met PAH criteria on Doppler ECHO. The correlation of mean PAP measured by RHC has shown good correlation with estimated systolic PAP measured by Doppler ECHO(112;188-190). There are other echocardiographic variables suggestive of PH including increased velocity of pulmonary valve regurgitation and a short acceleration time of right ventricular (RV) ejection into pulmonary artery (PA), increased dimensions of right atrium and ventricle, abnormal shape and function of interventricular septum, increased RV wall thickness, and dilated main PA. In 2009, the European Society of Cardiology (ESC) published guidelines to estimate the presence of PH using these ECHO variables in addition to the estimated sPAP(105).

Mukerjee et al(116) prospectively studied 137 patients with SSc to compare Doppler echocardiography and RHC in differentiating patients with and without PAH. It was noted that resting echocardiography performs adequately as a screening tool only in the diagnosis of advanced PAH, provided high thresholds of TG >45mmHg for PAH diagnosis is used. There were several limitations to this study including inter-observer variability and also, echocardiographic parameters studied were useful in advanced rather than early PAH. It was concluded that echocardiography as an adjunct to clinical evaluation was the optimal screening approach to identify patients with PAH.

### **1.3.4 Valvular diseases and other cardiac manifestations in SLE**

Cardiac involvement in SLE can present with anatomical as well as functional abnormalities.

It affects all layers of cardiac wall including endocardium, myocardium and pericardium.

SLE can manifest as pericarditis or pericardial effusion, myocarditis, valvular heart disease, endocarditis, arrhythmias, systemic hypertension and ischemic heart disease. These manifestations can present at any stage of disease and could worsen during active lupus disease.

Initially when cardiovascular disease (CVD) in SLE was increasingly recognised in 70's, autopsy studies in SLE confirmed the presence of anatomical cardiac abnormalities in the range of 15-75% (63;191). Later on, transthoracic echocardiography (TTE) based studies reported the prevalence of similar CVD in SLE was 40-50% (192) and the transoesophageal echocardiography (TOE) studies reported morphological cardiac abnormalities in 50-60% (192;193) of SLE patients. To begin with it was considered that cardiovascular manifestations were predominantly a feature of SLE in later stages resulting in severe life threatening complications. The increasing use of non invasive screening with TTE brought to light the commoner manifestations of pericardial involvement as well as valvular abnormalities.

Moreover, these were present in early stages of SLE onset with less disease duration and rarely caused cardiac functional impairment. This change in pattern recognition of cardiac involvement led researchers to identify risk factors and associations in lupus patients with cardiac valvular abnormalities. In particular antiphospholipid antibodies and antiphospholipid syndrome were significantly associated with cardiac valvular abnormalities.

In 1924, Libman and Sacks (194) reported on verrucous non-bacterial endocarditis predominantly affecting mitral valve and in 1940, Gross (195) associated these lesions with SLE. The Libman-Sacks endocarditis lesions were found in 7 out of 74 SLE patients in a

study by Galve et al(196). Though earlier studies predominantly reported from autopsy findings, the introduction of 2 dimensional and M-mode transthoracic echocardiogram, Doppler studies and transoesophageal echocardiogram led to non-autopsy larger series studies reporting 11-72% of lupus patients with valvular heart disease(197). The valvular heart disease in SLE is predominantly asymptomatic, but if symptomatic can lead to substantial morbidity and mortality(197). The common echocardiographic abnormalities of valves include mild to moderate thickening of valve cusps and valvular vegetations, and these are often accompanied by valvular regurgitation and rarely lead to stenosis. Valvular replacement may be needed in 22% with valvular stenosis and/or regurgitation(196;197). The association of antiphospholipid antibodies such as anticardiolipin antibodies and lupus anticoagulant with cardiac valve abnormalities in SLE has been well documented(198-203), though a few studies did not find this association(204;205). It has also been shown that patients with primary antiphospholipid syndrome have higher prevalence of valvular lesions in 32-60% of patients(206;207). It was found that 50% of lupus patients with a history of cardiovascular and/or cerebrovascular disease had valvular abnormalities(208). The aortic and mitral valve lesions resulting in significant cardiac dysfunction can lead to secondary PH in SLE.

Myocardial involvement is seen in 8-81% of patients with SLE (209-212)and can result in systolic and diastolic cardiac dysfunction. Predominantly myocardial involvement is asymptomatic and noted in autopsy findings. The clinical manifestations due to myocardial involvement are reported less frequently(196;211;213). The increase in left ventricular systolic dimensions and decrease in ejection fraction indicates systolic dysfunction and the increased mitral valve A:E ratio indicates diastolic dysfunction on echocardiographic evaluation.

The cardiovascular disease is considered among the leading causes of increasing mortality and morbidity in SLE. Coronary arterial disease (CAD) is also common in SLE. This is due to accelerated atherosclerosis from increasing number of traditional risk factors in SLE patients, inflammatory risk factors related to lupus as well as treatment such as corticosteroids. It is well known that the number of traditional cardiovascular risk factors is higher in lupus patients in comparison to age and sex matched controls(214-216). Petri et al(217) reported 3 or more known risk factors for CAD in 53% of their cohort. Systemic hypertension, hyperlipidemia and increasing age are predictors of cardiovascular disease in lupus. The accelerated coronary atherosclerosis and systemic hypertension seen in SLE can also contribute to left heart systolic and diastolic dysfunction. It is well known that left heart disease can cause pulmonary hypertension, which is a separate entity in itself in the WHO classification of pulmonary hypertension. In particular, the systolic and diastolic dysfunction, and left heart valvular disease can lead to secondary PH. Echocardiogram and Doppler studies are used to assess cardiac systolic and diastolic function and valvular diseases as part of screening for PH in order to discriminate PAH from secondary PH due to left heart disease in SLE.

### **1.3.5 Inflammation in PAH with SLE**

PAH is considered to have an inflammatory pathogenesis in CTD, HIV and IPAH. Tuder et al (218) identified perivascular inflammatory infiltrates such as T cells, B cells and macrophages suggesting the role of cytokines and growth factors in plexiform lesions found in lungs of patients displaying severe IPAH. Cool et al(219) reported the perivascular mononuclear cells were present only in the plexiform lesions and absent in extravascular and uninvolved vessels of the lungs of scleroderma patients with PAH. Patients with IPAH have detectable levels of ANA(220) and elevated proinflammatory cytokines IL-1 and IL-6(221)

in the absence of systemic autoimmune disease. 40% of IPAH patients had positive ANA without any features of CTD. It is possible that some of these patients had subtle ANA related CTD symptoms which could have been missed at the diagnosis of PAH. Balabanian et al(222) showed patients with severe IPAH have increased plasma levels of various inflammatory markers compared to normal controls.

The presence of IgG and complement fraction immune deposits(223) similar to the renal glomeruli deposits were found in pulmonary vascular endothelium of PAH secondary to CTD. On the other hand, disseminated immune deposits have been found in lungs of lupus patients without PAH(224).

As SLE is an autoimmune inflammatory disease that can cause vasculitis, the concept of pulmonary vasculitis causing PAH is plausible. The inflammatory pathogenesis supports the fact that active lupus disease could predispose to pulmonary vasculitis causing PAH that can respond to immunosuppressive therapy. The response to immunosuppressive therapy and corticosteroids have been shown to improve PAH in CTD patients particularly SLE(225-227). The lack of similar response in other CTDs especially scleroderma, suggests that different mechanisms could play a role in the pathogenesis of PAH in different CTDs. Most of this evidence is derived from small series and case reports, and the lack of placebo controlled studies makes it difficult to assess the true efficiency of immunosuppressant therapy in PAH. Moreover, the frequent use of vasodilators in addition in these patients could also bias the outcome.

Lupus nephritis and Raynaud's phenomenon have been shown to be associated with PAH in SLE. However disease activity of other organ manifestations as well as damage scores in lupus has not been studied and it is not known if they are associated with PAH or not.

A meta-analysis of antiphospholipid antibodies (APLA) including 1000 SLE patients reported an average frequency of 34% for lupus anticoagulant and 44% for anticardiolipin antibodies in lupus patients (46)..A review by Petri et al(52) reported a frequency range of 7-65% for lupus anticoagulant and 17-86% anticardiolipin antibodies in SLE. However, definite secondary APS occurs only in 10% of SLE patients(53). The prevalence of secondary APS in SLE in the same cohort of 667 SLE patients increased to 15% when follow up was extended from 7.5 months to 3 years(228) and further increased to 23% with a follow up of 18 years. APLA have been shown to be associated with PAH in SLE. None of these patients had thromboembolic pulmonary vascular disease leading to PH; however the concept of APLA and microthrombi in small pulmonary vessels resulting in PH is possible.

The presence of inflammatory cells in the PAH lesions, the association of autoantibodies and inflammatory markers and cytokines with PAH, a few lupus manifestations known to have predictive value for PAH, and significant response of PAH to immunosuppressive therapy all do suggest the possibility of inflammatory pathogenesis for PAH in SLE.

### **1.3.6 Assessment of pulmonary involvement in SLE**

Pleurisy, pleural effusion, interstitial lung disease (ILD), pulmonary embolism, shrinking lung syndrome (SLS) are the most important lung manifestations in SLE. The symptoms of lung disease include chest pain, dyspnoea, poor exercise tolerance, and cough. These non specific symptoms are not uncommon in renal or cardiac involvement including PAH in SLE. It is important to be aware of lung diseases that cause secondary PH such as interstitial lung disease, pulmonary embolism and emphysema. The assessment of lung diseases, whether it is serosal, interstitial, airways or vascular disease is vital to ascertain the cause of PH, degree of lung involvement and the impact of cardiorespiratory symptoms in SLE patients. High resolution CT (HRCT) scanning of lungs and CT pulmonary angiography (CTPA) are very

useful imaging investigations in diagnosing interstitial lung disease and pulmonary thromboembolic disease respectively.

### **1.3.6.1 Pulmonary function tests**

The pulmonary function testing includes spirometry, lung volumes and transfer factor measurement. The PFT parameters help to determine the restrictive or obstructive nature of lung disease. The decline in lung function is used as a guide to monitor closely with repeated lung function tests as well as assess the need for imaging and invasive testing modalities for cardiac or lung involvement.

It has been shown that abnormal PFT is common in patients with SLE, particularly low transfer factor (TLCO) and low forced vital capacity (FVC)(229;230) often in the presence of normal chest x ray. Most patients with SLE and abnormal PFT were asymptomatic and did not have a history of lung disease. It is possible that chronic ILD can lead to secondary PH in SLE. The prevalence of clinically significant chronic ILD is 3-13%. The restrictive lung pattern with reduced FVC <80% predicted is seen in patients with chronic ILD. A reduced FVC without evidence of ILD on HRCT lungs is seen in patients with SLS(231) and with imaging evidence in pleural fibrosis. The PFT abnormalities if detected in patients with SLE associated PH would help to ascertain the secondary cause of PH in these patients and guide further investigations necessary such as HRCT scan of chest and CTPA lungs.

It has been shown that reduced TLCO is a valuable screening test for PAH associated with systemic sclerosis (SSc)(116;179) and less often in Idiopathic PAH (IPAH)(232). The UK PH registry reported that TLCO was low in SSc patients with PH especially those with ILD compared to SLE related PAH.



### **1.3.6.2 Respiratory muscle strength**

The respiratory muscle predominantly involved in quiet breathing is the diaphragm. During exercise, the inspiratory muscles include external intercostals, scalene and sternomastoids and the expiratory muscles include abdominal wall muscles and internal intercostals. Respiratory muscle dysfunction involving both inspiratory and expiratory muscles have been reported in patients with SLE(233-235), scleroderma, RA(236) as well as IPAH(237). Shrinking lung syndrome seen in SLE has been attributed to diaphragm malfunction or weakness. The severity of inspiratory and/or expiratory muscle weakness has been different in patients with IPAH compared to those with chronic obstructive airways disease (COPD) and congestive cardiac failure (CCF). There has been good correlation between maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) in patients with IPAH, and the pressure changes are independent of NYHA functional class and haemodynamic parameters such as mean PAP. A similar correlation of MIP and MEP are seen in COPD. In patients with CCF the reduction in MIP is greater than MEP(237;238) and is associated with NYHA functional classes(238). Isolated diaphragm weakness is associated with normal MEP and low MIP. The normal values of MIP and MEP vary widely depending on sex, and the mouthpiece type used for measuring pressures. The normal values vary widely for MIP and MEP and range 70-73 cmH<sub>2</sub>O and 89-94 cmH<sub>2</sub>O in females, and 105-113 cmH<sub>2</sub>O and 140-154 cmH<sub>2</sub>O in males respectively(239-242). In this study, values lower than 40 cmH<sub>2</sub>O for both MIP and MEP were used as evidence for respiratory muscle weakness.

### **1.3.6.3. Respiratory symptom questionnaires**

There are several validated respiratory questionnaires which reliably relate respiratory symptoms and lung function. Their role in determining the risk of PAH development in patients with respiratory symptoms has not been proven. Among several respiratory

questionnaires, Modified medical research council (MRC)dyspnoea scale, Baseline dyspnoea index (BDI) and St.George's respiratory questionnaire (SGRQ) are often used, These questionnaires are easy to complete in a short duration. They are valid, reliable and rates dyspnoea during different tasks and levels of activity.

#### **1.3.6.4. Six minute walking test (6MWT)**

There are several clinical exercise tests to evaluate objective functional exercise capacity.

The most popular exercise tests include stair climbing, 6MWT, shuttle walk test and cardiac stress test (eg. Bruce protocol)(243).6MWT is a practical simple self-paced test to assess submaximal level of functional exercise capacity. As the patients choose their own intensity of walking, and are allowed to stop and rest during the test they do not achieve maximal exercise capacity. The daily activities are most often performed at submaximal levels of exertion and thus, the six minute walking distance reflects the functional exercise level for daily activities better than other exercise tests such as shuttle walk test.

6MWT has been used to measure the response to medical interventions in patients with moderate or severe heart or lung disease. 6MWT also helps predict mortality and morbidity in heart failure, COPD and PAH(244;245). The 6MWT does not measure peak oxygen uptake, diagnose the causes of dyspnoea or exercise limitation. Though 6MWT does not provide all the objective information that a formal cardiopulmonary test provides, a significant correlation between six minute walking distance (6MWD) and peak oxygen flow uptake in end stage lung diseases such as PAH has been reported(245;246). 6MWT also correlates better with quality of life measures, and in contrast questionnaire indices of functional status have larger short term variability.

### **1.3.7 Biomarkers**

Biomarkers can be a useful tool to provide prognostic, diagnostic or therapeutic response value in a disease process. The National Institute of Health (NIH), USA biomarkers definition working group defines 'The biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic response to therapeutic intervention(247). It is essential that these biomarkers are validated in a large longitudinal cohort study.

Several biomarkers have been researched in the area of pulmonary hypertension, particularly in patients with systemic sclerosis. These include brain natriuretic peptide (BNP), vascular endothelial growth factor (VEGF), Fractalkine, endothelin (ET) and soluble CD40ligand.

#### **1.3.7.1 BNP and NT-proBNP**

B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) are released from both cardiac ventricles in response to volume or pressure overload. The increase in pulmonary vascular resistance in PAH leads to right ventricular systolic dysfunction (RVSD) and death. It has been shown that RVSD at presentation can predict early death in PAH patients. The gold standard for detecting RVSD is by cardiac magnetic resonance imaging (MRI) which is expensive and not readily available. The right ventricular ejection fraction (RVEF) can be indirectly estimated by transthoracic echocardiography. This estimation called the Tei Index is detectable easily in the presence of overt right heart failure but otherwise an experienced competent echocardiographer is essential to detect this. B-type natriuretic peptide and NT-proBNP correlates well with RVEF measured by ECHO(248), invasive pulmonary haemodynamics(249) and survival(250-252) in PAH patients. The threshold levels of BNP and NT-proBNP indicating RVSD has also been determined, and thus are an ideal alternative to identify early RVSD by expensive and invasive methods(248).

Both in IPAH and secondary PAH particularly systemic sclerosis, it has been shown that plasma levels of BNP and NT-proBNP correlate well with mean PAP(249). It is important to understand that the levels of BNP and NT-proBNP could be elevated in other conditions such as left ventricular (LV) dysfunction, ischaemic heart disease, renal failure, LV hypertrophy, hypertrophic cardiomyopathy, aortic stenosis, pregnancy induced hypertension, pulmonary embolism, hypoxia and subarachnoid haemorrhage.

NT-proBNP is more stable than BNP both in vivo and in vitro. Thus, it is considered an ideal biomarker to measure cardiac dysfunction in PAH. The baseline levels would identify RVSD at presentation. The follow up levels would help monitor response to therapy or can be an indicator for further haemodynamic testing such as cardiac catheterisation and need for change of therapy for PAH.

Since the completion of my study, Chung et al(253) in 2008 reported that SLE patients have elevated NT-proBNP levels compared to healthy controls. NT-proBNP levels correlated well with disease duration, damage index but not with disease activity or markers of vascular disease such as augmentation index in patients with SLE.

Blyth et al(248) showed a threshold of NT-proBNP levels at 1684 pg/ml specific for detecting RVSD in PAH. Mukerjee et al ascertained that NT-proBNP cut off value of 395 pg/ml had a sensitivity of 69%, specificity of 100% and high negative predictive value of 91% in predicting PAH in systemic sclerosis as determined by mean PAP value >25mmHg by RHC (249).

### **1.3.7.2 Fractalkine (FKN)**

Fractalkine is a chemokine that has been implicated in the pathogenesis of several inflammatory diseases such as rheumatoid arthritis, atherosclerosis, HIV, crescentic

glomerulonephritis and psoriasis. The presence of circulating ANA, inflammatory cell infiltrates in plexiform lesions, elevated levels of proinflammatory cytokines IL-1 and IL-6, and increased expression of chemokines including RANTES and FKN supports the role of inflammation in the pathogenesis of PAH including IPAH and connective tissue disease related PH.

Fractalkine is expressed as membrane bound (mFKN) protein on endothelial cells activated by proinflammatory cytokines such as tumour necrosis factor-alpha and interferon-gamma. The soluble forms (sFKN) are released by proteolytic cleavage of mFKN. Fractalkine receptor CX3CR1 expressed on T cells, mast cells and natural killer cells rapidly and firmly adhere to membrane bound FKN on EC. The upregulation of CX3CR1 in circulating T lymphocytes and elevated sFKN levels has been shown in patients with PAH(222). The lung biopsies from patients with PH have confirmed the presence of mononuclear inflammatory cell infiltrate in the perivascular areas surrounding plexiform lesions in CTD related PH(219) and IPAH(218). The detection of sFkn mRNA in the lung biopsies of PAH and the significantly increased sFkn mRNA expression in PAH patients compared to controls further supports the role of sFkn in PAH(222). Animal studies(222;254) have shown that sFkn is possibly a growth factor for pulmonary artery smooth muscle cells resulting in vascular remodeling in PAH.

Soluble FKN has also been shown to be elevated in active neuropsychiatric SLE(255;256). The sFKN levels in serum and cerebrospinal fluid of SLE patients correlated with SLEDAI, SLICC/ACR Damage index, anti- double stranded DNA and anti-Sm antibody titres and serum complement CH50 levels. The serum and CSF levels of sFKN reduced with treatment for active NPSLE. Fractalkine has been described as a mediator with anti-apoptotic properties supporting the survival of multiple cell types during inflammation(257). SLE being

a disease of defective apoptosis would support the increased expression of chemokine such as Fractalkine. Patients with PAH associated with SLE have shown improvement with immunosuppressive therapy(227) raising the possibility of inflammation playing a role in the pathogenesis of PAH. Fractalkine being associated with PAH as well as SLE activity separately made us explore the possibility of raised sFkn levels in PAH associated with SLE.

### **1.3.7.3 Vascular endothelial growth factor (VEGF)**

VEGF is a selective endothelial cell growth factor, potent enhancer of microvascular permeability and angiogenic peptide which is secreted by a variety of cell types including endothelial cells, macrophages, fibroblasts and smooth muscle cells. VEGF interacts with two specific tyrosine kinase receptors VEGFR-1 and VEGFR-2 found in endothelial cells. Endothelial cell proliferation is associated with several physiological and pathological processes and VEGF has been implicated in these processes. VEGF has been measured in several kinds of biological fluids and cells of the lung parenchyma. The most common origins used for its measurements are blood (serum or plasma), bronchoalveolar lavage (BAL) fluid, sputum, bronchial epithelial cells, alveolar type II cells, alveolar macrophages, neutrophils, endothelial cells of the alveolar capillaries, and airway or vascular smooth muscle cells. Serum VEGF levels are 2 -7 fold higher than plasma due to ex vivo platelet and leucocyte release of VEGF during blood clotting(258).

Elevated serum levels of VEGF have been shown to be associated with RA(259), scleroderma and SLE (260;261). Dysregulated VEGF expression has been shown in primary APS(262), glomerular disease, diabetic retinopathy and in metastases. Basic fibroblast growth factor associated with angiogenesis has been detected in serum of SLE and dermatomyositis patients(263). In SLE, the serum VEGF levels have been shown to be higher than the controls. Serum VEGF levels were found to be higher in active SLE compared to

inactive SLE(264;265), and lupus nephritis was associated with strong expression of VEGF in renal tissues and high serum VEGF levels(266). It has been reported that VEGF plays a significant role in the pathogenesis of PH(267) as the serum VEGF levels were markedly raised in idiopathic and secondary pulmonary hypertension. It has also been shown that continuous prostacyclin infusion increases circulating VEGF levels thus raising an important question as to whether VEGF has deleterious or protective effects. VEGF has been shown to be strongly expressed in the normal pulmonary circulation as well as plexiform lesions of primary pulmonary hypertension(268). Elevated levels of serum VEGF has been reported in SLE patients with pulmonary hypertension (PH) compared to those without PH and controls(269).

### **1.3.8 Pregnancy in SLE**

It is well documented that PAH in pregnancy carries a poor prognosis, with maternal mortality of 56% with secondary PAH compared to 30% with IPAH(270). As SLE commonly affects women in childbearing age group, it is important to identify those with PAH or at risk of developing PAH. This would help plan multidisciplinary care from early pregnancy onwards or prevent pregnancy, and achieve a favourable maternal and fetal outcome. The timing of diagnosis of PAH and admission to hospital with individualised care are predictors of good prognosis.

Antiphospholipid antibodies (APLA) and antiphospholipid syndrome (APS) have also been shown to be associated with PAH in SLE patients. Though antiphospholipid antibodies is present in 30% of SLE patients, only a third have antiphospholipid syndrome(53). However, there is lack of evidence for thromboembolic phenomenon causing PAH associated with APLA. The risk of miscarriages and fetal deaths is high in the presence of APS, and the added burden of PAH in SLE is likely to increase this risk significantly. In the absence of

predictors to point who will deteriorate in pregnancy with SLE, we have to be vigilant of high risk groups such as those with suspected cardiorespiratory symptoms, interstitial lung disease and pulmonary embolism.

#### **1.3.8.1. Pregnancy, PAH and SLE**

Pulmonary arterial hypertension shortens survival and when it coincides with pregnancy, this risk considerably increases during labour and immediate postpartum period. The cardiovascular demands during pregnancy help to adapt to the fetal development and placental circulatory changes. During pregnancy, the increase in heart rate, circulatory blood volume and cardiac output is tolerated with dilatation in the systemic and pulmonary vasculature resulting in only minimal change to PAP. There are further haemodynamic changes that can occur rapidly during labour, delivery and puerperium. However, in the first 72 hours postpartum, the cardiac output further increases secondary to maternal auto transfusion and increased venous return to right heart. These physiological demands during pregnancy can significantly compromise the cardiopulmonary status and result in poor tolerance leading to complications and mortality soon after delivery. In PAH, there is increased pulmonary vascular resistance such that this high cardiac output status results in right heart failure that can cause death.

A systematic overview in pregnancy from 1978 through 1996 showed that among 27 patients with primary and 25 with secondary PH had maternal mortality of 30% and 56%, and neonatal survival of 89% and 88% respectively(270) . The secondary PH patients with SLE and scleroderma had the worst prognosis with 100% maternal mortality. The independent predictive risk factors of maternal mortality were diagnosis of pulmonary vascular disease during or after pregnancy (odds ratio 5.4) and late hospital admissions (odds ratio 1.1 per week of pregnancy).



The rapid deterioration of PAH occurs when pulmonary thromboembolic disease complicates PAH(271). The risk is highest in those with a prothrombotic tendency such as antiphospholipid syndrome during pregnancy.

McMillan et al(271) reported a case series of 3 SLE patients with PAH from our cohort, who declined termination of pregnancy and had high mortality rate of 66%. Both the patients who died were diagnosed with PAH during pregnancy and deteriorated rapidly, while the survivor had mild PAH and diagnosed very early in pregnancy and managed with close monitoring of multidisciplinary team as well as invasive monitoring in intensive care following delivery.

This report by McMillan et al(271) suggests the importance of diagnosing PAH prior to pregnancy planning in high risk groups such as SLE. We can advise appropriately regarding pregnancy prevention and early interruption of pregnancy to improve the survival of lupus patients with PAH. An individually tailored treatment during pregnancy with particular attention to medical care postpartum is vital in patients with early diagnosis and preferably mild PAH. As there are no clear pointers to who will deteriorate and how rapidly they would do so during pregnancy in this subgroup, we are in need of further detailed study to address this. Until then, we have to be vigilant of high risk groups such as those with suspected cardiorespiratory symptoms, interstitial lung disease and pulmonary embolism.

## 1.4. Aims and Objectives of the study

Aims:

To estimate the point prevalence of PAH, identify risk factors and evaluate screening tests for PAH in a large cohort of SLE patients.

Objectives:

1. To estimate the point prevalence of PAH in patients with SLE using transthoracic echocardiogram (TTE).
2. To evaluate risk factors for patients with PAH in SLE
  - a. To estimate the prevalence of left heart structural and functional abnormalities using TTE in patients with PAH and SLE
  - b. To evaluate the role of autoantibodies, BILAG disease activity scores and SLICC/ACR damage scores as predictors for PAH in patients with SLE.
  - c. To evaluate respiratory muscle strength (RMS) abnormalities in SLE patients and compare RMS abnormalities in those with and without PAH.
  - d. To evaluate serum NT-proBNP, plasma sFkn, and plasma VEGF concentration in patients with SLE and compare between patients with and without PAH.
  - e. To assess relationship of serum NT-proBNP, plasma sFkn, and VEGF levels with lupus disease activity measures such as monitoring tests especially anti-dsDNA antibody titre and complement C3 and C4 levels and autoantibodies associated with SLE, BILAG disease activity scores and SLICC/ACR organ damage scores

3. To evaluate the role of other screening tests in patients with PAH in SLE

- a) To evaluate pulmonary function test (PFT) abnormalities in patients with SLE, and assess relationship with lupus related autoantibodies and BILAG disease activity scores
- b) To compare PFT abnormalities in lupus patients with and without PAH, and in patients with and without known restrictive lung diseases
- c) To evaluate dyspnoea in patients with SLE using respiratory symptom questionnaires, and compare dyspnoea scores between patients with and without PAH in SLE.
- d) To determine distance walked in six minutes (6MWD) in SLE patients and compare 6MWD by patients with and without PAH

## CHAPTER 2 METHODS

### 2.1 Patient Recruitment

This prospective cross-sectional study was conducted in the Wellcome Trust Clinical Research Facility (WTCRF) at the Queen Elizabeth Hospital (QEH), Birmingham UK between January 2004 and December 2005. This study was carried out in accordance with Helsinki declaration and received ethical approval from multicentre research ethics committee, UK (MREC 03/9/067). The study protocol synopsis submitted to ethics committee is shown in Appendix 4. Three hundred and ninety-two patients in the cohort with SLE who attend the Lupus UK centre of excellence clinics at QEH and City Hospital (CH), Birmingham were invited to take part in this study. Pregnancy and/or age below 18 years were the exclusion criteria for this study. Two hundred and eighty-eight (73%) patients gave informed written consent to participate. Two hundred and eighty-five (99%) patients fulfilled 4 criteria for SLE diagnosis defined by 1982 revised American college of Rheumatology (ACR) criteria(62) while the remaining patients fulfilled 3 criteria and a clinical diagnosis of SLE by the Consultant Rheumatologist responsible for patient's care (CG or RDS). The patients attended no more than twice at WTCRF for this study.

At the initial visit, a detailed clinical evaluation including history of smoking and Raynaud's phenomenon plus, ECG, assessment of SLE disease activity using BILAG index(74;75) and of accumulated damage using the SLICC/ACR damage index(77;78) was undertaken. Echocardiography and the screening tests and assessments of risk factors for PAH were performed either at the same visit or within 4 weeks of this assessment. Echocardiography was done on a separate day to the other assessment in 19.8% (57/288) of patients.

## 2.2 Demographics

The demographic details of patients were collected and includes their full name, hospital where recruited, hospital identification (ID) number, study ID, age, sex, disease duration, race (Caucasian, Asian, Afro-Caribbean, Oriental and other), the date of onset of 1<sup>st</sup> criteria for SLE and the date when SLE was diagnosed and if fulfilling ACR criteria for SLE (Appendix 1). The presence of Raynaud's phenomenon, history of antiphospholipid syndrome and presence of overlap syndrome features of systemic sclerosis and/or mixed connective disease were also recorded. The written copies of the patient confidential data as well as the study results data were stored at the WTCRF and the electronic copies in an access database at WTCRF.

## 2.3 Electrocardiography (ECG)

During the initial visit, all patients had 12 lead ECG performed. The ECG was analysed to identify heart rate, axis deviation, ventricular hypertrophy and bundle branch block. The following definitions were used to record the ECG abnormalities relevant to this study.

Left ventricular hypertrophy (LVH)(272): 'probable' if the sum of S wave amplitude in V1 and R wave amplitude in V5 or V6 is  $\geq 35\text{mm}$ , and 'definite' if features of left ventricular strain pattern such as T wave inversion or ST segment depression in lateral leads were present in addition.

Right ventricular hypertrophy (RVH): R/S ratio is  $>1$  in V<sub>1</sub> or  $<1$  in V<sub>6</sub>, or sum of R in V1 and S in V5/6 is  $>10.5\text{mm}$ . In addition, the presence of right axis deviation and/or RV strain pattern such as ST segment depression or T wave inversion in anterior or inferior leads makes RVH highly likely.

Right bundle branch: QRS >120ms and terminal R wave in V1 and a slurred S in lead I.

Left bundle branch block: QRS >120m and QS or rS complex in leadV1 and a monophasic R wave in lead I.

Right axis deviation if axis  $\leq -30$  degrees

Left axis deviation if axis  $\geq +90$  degrees

## **2.4 Echocardiography (ECHO)**

All patients had transthoracic echocardiographic examinations performed at rest using a Sonos 7500 echocardiogram (Philips Medical systems) by experienced echocardiographers, who were unaware of the patient's previous clinical characteristics and cardiovascular diagnosis. The scan was performed by a consultant cardiologist (KP) in 75% of patients, and the remainder by 2 senior echo cardiographers. All scan results were reviewed by consultant cardiologists (KP and JT). 2D, M-mode and colour Doppler echocardiography were obtained from parasternal long- axis and short-axis, apical four chamber and subcostal four-chamber views. These were used to evaluate valvular anatomy and function, flow abnormalities, cardiac morphology including left and right sided chamber sizes, and cardiac functional status including ejection fraction (EF). Tricuspid regurgitant flow was identified by colour flow Doppler techniques, and the maximum jet velocity was measured by continuous wave Doppler.

The data recorded were:

1. Right ventricular systolic pressure (RVSP) - This was estimated in all patients in whom tricuspid regurgitation was detected by colour flow Doppler technique. This maximum velocity of the tricuspid valve regurgitant (TR) jet was measured by the continuous wave

Doppler. RVSP was calculated by using the modified Bernoulli equation. This was considered to be equal to the systolic PAP in the absence of right ventricular outflow tract obstruction

2. Systolic PAP – This was estimated by adding RVSP and right atrial pressure (RAP), in the absence of any right ventricular outflow tract obstruction such as pulmonary stenosis. RAP was estimated using standard criteria. Those patients without tricuspid regurgitation had a very low probability of having elevated sPAP and thus, were considered to have ‘normal’ sPAP.

3. Grade of valvular regurgitation and stenosis as mild, moderate and severe in keeping with guidance from British Society of Echocardiography and American College of Echocardiography(273-275)

4. Presence of anterior and/or posterior mitral valve thickness, and to assess for focal valve abnormalities differentiating from age related diffuse nonspecific thickness.

5. Grade of systolic and diastolic dysfunction in left and right ventricle as mild, moderate and severe.

6. Calculation of left ventricular ejection fraction (EF).

7. Right and left ventricular systolic and diastolic measurements.

8. Any other cardiac findings such as septal wall thickness or motion abnormality, and limitations of the study were also recorded.

The World Health Organisation (WHO) defines PAH as a mean PAP>25 mm Hg at rest and >30mmHg with exercise measured by RHC, in the presence of normal pulmonary capillary

wedge pressure. We have defined PAH as systolic pulmonary artery pressure (sPAP) > 30 mm Hg at rest estimated by ECHO, as in other studies using Echo as a screening tool for PAH(169;170;276;277).

## **2.5 Right heart catheterisation (RHC)**

Right heart catheterisation is an invasive test which is performed using femoral approach under local anaesthesia. RHC is the gold standard test to confirm PAH by measuring the mean PAP. The pulmonary capillary wedge pressure (PCWP) is also measured to assess the pulmonary vascular resistance and influence of left heart disease on PAP. If the resting mean PAP was  $\leq 25$  mm Hg, then a 2 minute benchfly exercise is performed to measure post-exercise mean PAP to confirm PAH.

All patients who were found to have severe PAH with sPAP>40mmHg on ECHO, with World Health Organisation (WHO) class III or IV dyspnoea were offered referral to a cardiologist (JT and KP) for consideration of right heart catheterisation. Patients with PAH that did not meet these criteria were not offered this invasive test at the request of the ethics committee as they felt that the risks outweighed the benefits(278) . Patients were explained about RHC during consent at recruitment and if they were found eligible for RHC, additional consent would be taken from patients by the cardiologist (JT or KP).

## **2.6 Assessment of Risk factors for PAH**

### **2.6.1 Autoantibodies**

The autoantibodies were measured as part of routine care using kits from The Binding Site, UK at The Department of Clinical Immunology, University of Birmingham, UK. Anti-nuclear antibody (ANA) was assessed by indirect immunofluorescence on Hep2 cells



(FK001.2), anti- double-stranded DNA (dsDNA) antibody done by indirect immunofluorescence on *Crithidia Luciliae* (FK002.2) and enzyme-linked immunosorbent assay (ELISA) (MK017), and Complements C3 and C4 done by turbidimetry (Roche Diagnostics TinaQuant kits). Antibodies to extractable nuclear antigens (ENA) were assayed using an ELISA screen kit (MK201) and if positive serum was assayed on separate ELISAs for RNP (MK306), Sm (MK305), Ro (MK303), La (MK304), Jo-1 (MK308) and Scl-70 (MK307) antibodies. Anti-IgG and anti-IgM anticardiolipin antibodies (aCL) were done by ELISA (MK027, MK029). Lupus anticoagulant (LAC), as determined by dilute Russel viper venom test (DRVV), was recorded if it was ever positive. Antiphospholipid antibodies were considered positive only if tested positive on 2 occasions at least 3 months apart(72).

ANA, anti dsDNA and complements C3 and C4 were recorded if tested within the previous month of study assessment. ANA titre  $\geq 1:40$ , anti dsDNA levels  $\geq 75$ ku/l, and Complement C3  $<0.75$ g/l and complement C4  $<0.14$ g/l were considered abnormal. Antibodies to ENA and LAC were reported positive if ever done and present. For anticardiolipin antibodies, an aCL IgG titre  $>15$ IU/ml and aCL IgM titre  $>10$ IU/ml were reported as positive if present at these titres at least twice anytime in the past but with a minimum of 3 months between both results.

### **2.6.2 BILAG index and SLICC/ACR damage index**

The BILAG index(74;75) is a valid, reliable and comprehensive clinical measure of lupus disease activity (Appendix 2). This index reports disease activity in eight organ/systems separately and is based on the scores calculated for each system depending on the presence of clinical features and relevant investigations. The eight organ/systems assessed include general features, mucocutaneous, neurological, musculoskeletal, cardiovascular and respiratory, vasculitis, renal and haematology. The scores were determined using the BLIPS (British Lupus Integrated Prospective System) software programme. The scores are graded A

through to E. Grade A refers to the most active score in each organ or system indicating disease activity that is usually treated with high dose steroids or immunosuppressive therapy. Grade B applies to those patients with disease activity usually requiring lower dose steroids, antimalarials or non-steroidal anti-inflammatory drugs (NSAIDs). Grade C refers to patients with mild features only needing symptomatic therapy. Grade D refers to a previously involved system but no current activity and grade E applies to systems that have never been involved. The SLICC/ACR damage index(77;78;279) describes the accumulated damage in 12 organ/systems since the diagnosis of SLE (Appendix 3). The 12 organ/systems assessed include ocular, neuropsychiatric, renal, pulmonary, cardiovascular, peripheral vascular, gastrointestinal, musculoskeletal, skin, premature gonadal failure, diabetes mellitus and malignancy. The damage score ranges from 0 (no damage) to a possible maximum of 47 that accumulates over time.

### **2.6.2 Respiratory muscle strength**

The respiratory muscle strength was assessed by measuring maximum static expiratory and inspiratory mouth pressures, and sniff nasal inspiratory pressure (SNIP). These pressures do vary with the point in lung volume that they are performed at. So, the maximum expiratory mouth pressure (MEP) is assessed at total lung capacity, maximum inspiratory pressure (MIP) at residual volume, and the SNIP at functional residual capacity.

The inspiratory and expiratory muscle strength measurements were performed using a portable, non invasive, handheld respiratory pressure meter (MicroRPM, Micro medical Ltd). The device is fitted with expiratory and inspiratory valve assembly to help perform the manoeuvre without much difficulty as well as controlled leak through the meter during manoeuvres to prevent high false positive values. The flanged mouthpiece attached to the

valve assembly enables the patient to perform the mouth pressure measurements easily. The appropriate sizes of nasal probes were used to avoid incorrect SNIP measurements.

The MIP and MEP were measured using a forceful inspiratory and expiratory manoeuvre respectively with nostrils closed, leading to a sustained maximal effort lasting for at least 2 seconds followed by natural release upon fatigue. The SNIP was measured through one plugged nostril with the nasal probe while the other remained open. A forceful inspiratory sniff manoeuvre performed and the peak pressure value achieved to measure SNIP. There was at least 1 minute recovery between all the respiratory efforts, to avoid any effects from muscle fatigue.

MIP measurement: The flange of mouth piece is positioned over the gums and inside the lips, and the 'bite blocks' between teeth. The patient was instructed to exhale fully to residual volume and then inhale with maximal effort possible for at least 2 seconds with the nostrils closed. The MIP measured was the maximal average inspiratory pressure in 1 second.

MEP measurement: With the flanged mouthpiece in place, the patient was instructed to inhale fully to total lung capacity, and then exhale to maximum effort possible for at least 2 seconds. The MEP measured was the maximal average expiratory pressure over 1 second.

SNIP test: The SNIP test was performed by plugging one nostril with a nasal probe so that a good seal was made. The patient was instructed to exhale normally to functional residual capacity, and then to inhale with maximal effort possible through the open nostril with mouth closed. The SNIP measured was the peak inspiratory nasal pressure.

The normal values of MIP, MEP and SNIP vary widely depending on sex, and the mouthpiece type used for measuring pressures. The normal values vary widely for MIP and

MEP and range 70-73 cmH<sub>2</sub>O, 89-94 cmH<sub>2</sub>O and 82-182 cmH<sub>2</sub>O in females, and 105-113 cmH<sub>2</sub>O, 140-154 cmH<sub>2</sub>O and 112-204 cmH<sub>2</sub>O in males respectively(239-242). The MIP, MEP and SNIP were determined from the best of five consecutive manoeuvres, in units of cmH<sub>2</sub>O gauge pressure. As there is a wide variability in the published reference values(242) for these parameters, we have considered values  $\geq 40$  cmH<sub>2</sub>O to be normal.

### **2.6.3 Pre-existing lung disease**

In patients with SLE, the lung diseases can be a manifestation of lupus activity, a complication (damage) or comorbidity (for example, infection). The non specific nature of respiratory symptoms and the risk of secondary pulmonary hypertension in patients with lung disease are important points to remember during assessment.

The presence of SLE related lung diseases in these patients were obtained from clinical examination and medical records, pulmonary function tests, chest X-ray and high-resolution CT chest. The restrictive lung diseases recorded were pulmonary fibrosis, shrinking lung syndrome, pleural fibrosis, and pulmonary infarction. Patients with shrinking lung syndrome (SLS) have unexplained dyspnoea with restrictive pattern and small lung volumes on lung function testing and absence of parenchymal disease with elevated diaphragm on CT chest (280;281). The obstructive lung diseases were asthma and chronic obstructive pulmonary disease (COPD). The presence of other lung diseases such as TB lung, lung malignancy, and bronchiectasis were obtained also.

Smoking history status was recorded as non smoker, ex-smoker and current smoker. The relevant chest X-ray and HRCT results were obtained from patient records if ever done.

## **2.7 Screening tests for PAH**

### **2.7.1 Six minute walking test (6MWT)**

The six minute walking test was performed according to international guidelines(282). This test is easy to administer with good reproducibility(283;284), well tolerated by patients with respiratory symptoms and also reflects the patient's daily living activities better than shuttle walk test(285).

The 6MWT was performed indoors in a corridor, with a 15m walking course marked with bright coloured tapes at 0 and 15 m as turnaround points and white tape marking at every 5m. There was provision of chairs alongside the corridor for patient to rest if necessary.

The patient was advised to use appropriate clothing and footwear, and walking aids if any. The pulseoximeter and heart rate monitor were attached. The Borg scale(286) (Appendix 4) for scoring dyspnoea and fatigue were explained to patient and baseline scores recorded. The instructions were made clear to the patient such as the objective of the test is to walk as far as possible for 6 minutes, and that they are allowed to slow down, stop and rest if necessary, and resume when able to. Patients who were on bronchodilators were advised to use them as they would have normally used in daily routine. A demonstration lap was performed by the nurse and clarified any queries from the patient. The countdown timer was set to 6 minutes and started with the patient commencing the test. The patient was informed the remaining time at every minute, and 15 seconds before end of test. The completed laps and additional distance measured at final partial lap if any, were entered and calculated on worksheet. The modified Borg Dyspnoea and fatigue scores were recorded post exercise and it was made sure that the patient was comfortable at end of test. The modified Borg scale is a descriptive 10 point scale

and the adjectives used in this scale assist patients to determine intensity of dyspnoea and fatigue.

The data recorded include:

Total distance walked (meters) in 6 minutes, baseline and post-exercise Borg dyspnoea and fatigue scores (Grades 0-10 with 0 being asymptomatic and 10 being severe symptoms) (Appendix 4), heart rate (rate/min) and oxygen saturation (%) were recorded (Pulsox-3i, Konica Minolta, Japan).

### **2.7.2 Respiratory symptom questionnaires**

The respiratory symptom questionnaires used in this study were modified Medical Research Council (MRC) dyspnoea scale(287), Baseline dyspnoea index (BDI)(288;289) and St.George's respiratory symptom questionnaire (SGRQ)(290;291).

Modified MRC dyspnoea [Appendix 5] is an easy to administer 5 point scale. It grades dyspnoea between 0-4 according to different levels of activity, 0 being dyspnoea with strenuous exercise and 4 being dyspnoea with minimal activity.

Baseline dyspnoea index (BDI) (Appendix 7) measures the functional impairment due to dyspnoea, magnitude of task and effort required to produce dyspnoea and they are graded 0-4, 0 being severe impairment and 4 being no impairment. The total score is the sum of all three categories with a maximum score of 12. This scale also includes grade W for amount uncertain, X for unknown and Y for reasons other than dyspnoea causing impairment.

SGRQ is a standardised self-completed questionnaire (Appendix 5) for measuring quality of life in patients with dyspnoea. This contains 76 items divided into three sections: 1. 'Symptoms' – the frequency and severity of respiratory symptoms. 2. 'Activity'- the

activities that cause or are limited by dyspnoea. 3. 'Impact'- the social functioning and psychological disturbances resulting from airways disease. The SGRQ score calculator is a software programme based on the calculation algorithms and missing data imputation (if total number of missing items  $\leq 10$ ). The scores ranging from 0 (no impairment) to 100 (maximum impairment) is calculated for each section and for the overall score. Each item in the questionnaire has an empirically derived weight.

### **2.7.3 Pulmonary function testing (PFT)**

The results of pulmonary function tests performed in the respective referring hospitals within 12 months of the study were recorded. Spirometric measurements were performed using a wedge bellows spirometer (Model S; Vitalograph, Jaeger Compact system, Viasys Healthcare, UK) and included assessment of vital capacity (VC), forced vital capacity (FVC) and forced expiratory volume in 1 second ( $FEV_1$ ). Functional residual capacity was estimated via the steady-state helium dilution method (Benchmark; Morgan Medical, Gillingham, UK) from which residual volume and total lung capacity (TLC) was calculated. All tests were performed according to national guidelines(292) and the results compared with standard reference values(293). Transfer factor (TLCO) was measured using the single-breath method (Benchmark; Morgan Medical; Jaeger Compact system) according to the Association for Respiratory Technology and Physiology (ARTP)/ British Thoracic Society (BTS) guidelines(292).

The measurements of  $FEV_1$ , FVC,  $FEV_1/FVC$ , TLC, TLCO and transfer coefficient for carbon monoxide (KCO) were expressed as percentages of predicted values and any percentage less than 80% predicted was considered potentially abnormal(231). KCO is derived from DLCO divided by alveolar volume.

## 2.7.4 Biomarkers

### 2.7.4.1 N-terminal pro brain natriuretic peptide (NT-proBNP)

The electrochemiluminescence immunoassay for in vitro quantitative determination of NT-proBNP (Elecsys Systems E170 proBNP assay 03121658122, Roche) in human serum was performed by Joanne Heynes at the Department of Clinical Biochemistry and Immunology, Birmingham Heartlands Hospital, UK (BHH). An aliquot of serum sample collected and stored in gel separator tubes at initial visit was sent to BHH. These samples were collected on the same day as the patient had their echocardiogram and six minute walk test. The test was performed on serum samples (0.2-0.5ml) from 12 patients with PAH, 16 patients with systolic PAP 26-30mmHg, and also on 56 patients with systolic PAP  $\leq 25$  mmHg who were age and sex matched. As the sample of one patient with systolic PAP 26-30 mmHg was lost, the 2 matched patients with systolic PAP  $\leq 25$ mmHg were removed from analysis. Thus, 81 samples in total were included in the analysis. The assay calculated the NT-proBNP concentration of each sample in values of pg/ml on automated analysers.

The analytical range for NT-proBNP assay is 5 pg/ml to 35,000 pg/ml. According to the manufacturer of the assay, NT-proBNP levels above 125pg/ml may indicate cardiac dysfunction. The values below 144pg/ml in women and 93pg/ml in men reliably exclude cardiac failure in symptomatic patients with a negative predictive value (NPV) of 97% (294). The expected NT-proBNP values (95<sup>th</sup> percentile) for women at age <50 was 125pg/ml, 50-59 years were 186 pg/ml,  $\geq 60$  years was 204 pg/ml, for men at age <50 years was 64 pg/ml, 50-59 years were 125 pg/ml and  $\geq 60$  years was 194 pg/ml. These expected normal NT-proBNP values were used to classify our study patients with and without high NT-proBNP concentration for their age and sex.



#### **2.7.4.2 Fractalkine (CX3CL1)**

Soluble CX3CL1 was measured in human plasma using a sandwich ELISA method according to the protocol supplied by R & D systems. The tests were performed by Dr Gemma White at the Greaves Lab in Sir William Dunn School of Pathology at University of Oxford. The 96 well Maxisorp ELISA plates were coated overnight at RT with 100 µl capture antibody diluted to 8 µg/ml in D-PBS. Unbound antibody was removed by washing three times with 200 µl ELISA wash buffer (PBS, 0.05% Tween-20). Non-specific binding was blocked with 200 µl ELISA blocking buffer (1% BSA, 5% sucrose, 0.05% NaN<sub>3</sub>, D-PBS) for 2 hours at RT, before a second wash step. Samples/standards (50-100 µl) were added and incubated for 2 hours at RT. Recombinant chemokine domain CX3CL1 was used as a standard and was diluted in ELISA dilution buffer (1% BSA, D-PBS). Unbound antigen was again removed by washing. Biotinylated detection antibody was diluted in ELISA dilution buffer (250 ng/ml) and 100 µl added to each well before incubation for 2 hours at RT. Plates were washed again before the addition of 100 µl of poly-HRP Streptavidin diluted 1:5000 in endogen buffer for 45 minutes at RT. Unbound reagent was removed by washing for a final time. OPD substrate tablets were reconstituted in water (1 tablet in 3 ml dH<sub>2</sub>O + 1.25 µl hydrogen peroxide 30% solution) and 100 µl added per well. Colour was allowed to develop to give a suitable detection range before the reaction was stopped with 50 µl 3 M H<sub>2</sub>SO<sub>4</sub>. Plates were read at 492 nm using a 96 well spectrophotometer (BioTek) and Gen5 software.

The cut off value was defined for each individual ELISA plate as determined by the mean background optical density (OD) + 3 standard deviations (SD). The OD 490nm values were converted to ng/ml using nonlinear regression. The original assay was performed across 8 plates and the range of cut off values was 0.49 - 0.62 ng/ml, with a mean of 0.57ng/ml. All samples which were below the mean cut off point cannot have a concentration assigned

because they cannot be distinguished from the background absorbance with buffer alone.

Fractalkine assay was performed in all 283 patients in this study.

#### **2.7.4.3 Human Vascular endothelial growth factor (VEGF)**

Human VEGF concentration in plasma was quantitatively measured on undiluted samples of all 81 patients who had undergone NT pro-BNP assay. Plasma VEGF was measured using a sandwich ELISA kit and manufacturer's instructions were followed (R&D systems, Abingdon, UK). This assay measures biologically active VEGF<sub>121</sub> and VEGF<sub>165</sub>. A monoclonal antibody specific for VEGF was coated onto a microplate. Standards and samples were pipetted into the wells and any VEGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme linked polyclonal antibody specific for VEGF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour developed in proportion to the amount of VEGF bound in the initial step. The intensity of the colour was measured spectro-photometrically in a plate reader. The lower limit of detection of VEGF was 5 pg/ml. This in vitro quantitative immunoassay for VEGF was performed by Anne Garfield at the Department of Rheumatology and Immunology, University of Birmingham, Birmingham, UK. According to assay manufacturer, the mean detectable VEGF level was 61 pg/ml and range 0-115 pg/ml for EDTA plasma from 37 healthy volunteers. Plasma VEGF assay was performed in the 81 SLE patients who underwent NT-proBNP assay in this study. Among the 81 patients with SLE, 12 had PAH, 15 had sPAP 26-30mmHg and 54 patients who were age and sex matched had sPAP <25mmHg.

## 2.8 Statistical methods

SPSS 13.0 software was used for statistical analysis. Data were analysed with the SPSS 13.0 statistics package. Variables are summarised as counts and/or percentages or as medians and ranges. Comparisons between groups were made using the Mann Whitney U test, two-sided Fisher's exact test or Pearson's chi-square tests. . Association between variables were assessed with Spearman rank correlation. The p value $<0.05$  was considered significant. The p values quoted have not been adjusted for multiple comparisons as this is an exploratory study aiming to identify possible risk factors. A Bonferroni correction would not be appropriate as the risk factors are not independent of each other.

## CHAPTER 3 PREVALENCE OF PAH

### 3.1 Abstract

#### Objectives:

1. To estimate the point prevalence of PAH in patients with SLE using transthoracic echocardiogram.
2. To evaluate the role of electrocardiogram (ECG) in screening for PAH in patients with SLE
3. To evaluate the role of autoantibodies, BILAG disease activity scores and SLICC/ACR damage scores as predictors for PAH in patients with SLE.

**Methods:** A prospective cross-sectional study of 288 patients with SLE were recruited from lupus clinics in Birmingham, UK. Resting transthoracic echocardiography was performed to estimate the pulmonary artery pressures and to assess cardiac morphology and function. PAH was defined as systolic pulmonary artery pressure (sPAP) >30 mm Hg. We assessed potential risk factors such as autoantibodies, Raynaud's phenomenon, anti-phospholipid syndrome, prior drug therapy, BILAG disease activity scores and SLICC/ACR damage index in predicting PAH in patients with SLE.

**Results:** Of 288 patients who consented for participation, 283 patients were suitable for analysis. 12 patients were found to have PAH with systolic pulmonary artery pressure (sPAP) >30 mm Hg. The range of sPAP in our PAH patients was 31-59 mmHg and 3 patients had sPAP >40 mm Hg. The only significant risk factor for PAH was lupus anticoagulant (Fisher's exact test,  $p=0.005$ ).

**Conclusion:** The point prevalence of PAH was 4.2% in our cohort of patients with SLE.

Most of the PAH cases were found to be of mild severity (<40mm Hg). The significant association of lupus anticoagulant and presence of antiphospholipid syndrome in PAH cases suggests that thrombosis may play an important role in PAH with SLE. This is important, as it is treatable.

## **3.2 Introduction and Methods**

The prevalence of PAH in SLE is widely variable depending on the nature of study. The wide variability depends on the definition of PH, diagnostic methods used, awareness of PH in SLE, study centres, number of study patients and retrospective nature of most studies. Transthoracic echocardiogram has predominantly been the screening test in the studies for estimating PAH in SLE, and a few studies using RHC to screen for PAH in SLE reported a low prevalence rate compared to echo estimated PAH prevalence in SLE. Please refer to methods chapter for full details on methods used to estimate the prevalence and assess risk factors for PAH in SLE.

## **3.3 Results**

### **3.3.1 Demographics**

Two hundred and eighty three patients with SLE were studied, and 266 patients (94%) were females. The median age was 41 years (range 18-82) and median disease duration was 8.7 years (range 0-32). Raynaud's phenomenon occurred in 66.4%, and 63.6% were non-smokers. Antiphospholipid syndrome (APS)(295) was known to be present in 12.4%. The racial distributions were Afro-Caribbean 18.7%, Asian (Indian Subcontinent) 19.8%, Caucasian 56.2%, Oriental 0.7% and others 4.6%.

### **3.3.2 Prevalence of PAH**

Among the 288 patients with SLE that took part in the study, 5 patients were excluded from analysis as their echocardiographic views were very limited and thus the echocardiographer was unable to estimate the pulmonary artery pressure. Of the remaining 283 patients, tricuspid regurgitation (TR) was absent in 114 (40.3%) patients by ECHO assessment. In all

cases with absent TR, there were no other echocardiographic features (right ventricular dilation or hypertrophy) to suggest PAH. There was only one patient each with right ventricular dilatation and hypertrophy in the remainder of cases but their estimated sPAP was normal at 25 and 9 respectively. Of those with absent TR, 85 patients had residual volume (RV) measured in their pulmonary function testing (PFT) and their %predicted RV was more than 120 and 150 in only 4 and 2 patients respectively. The difficulty with TR estimation was not statistically relevant to high residual volume noted in hyperinflated lungs. The reason for inability to estimate TR was poor and/or limited echocardiographic views and patient's body habitus making right heart assessment technically difficult, which was noted in 24 patients with absent TR.

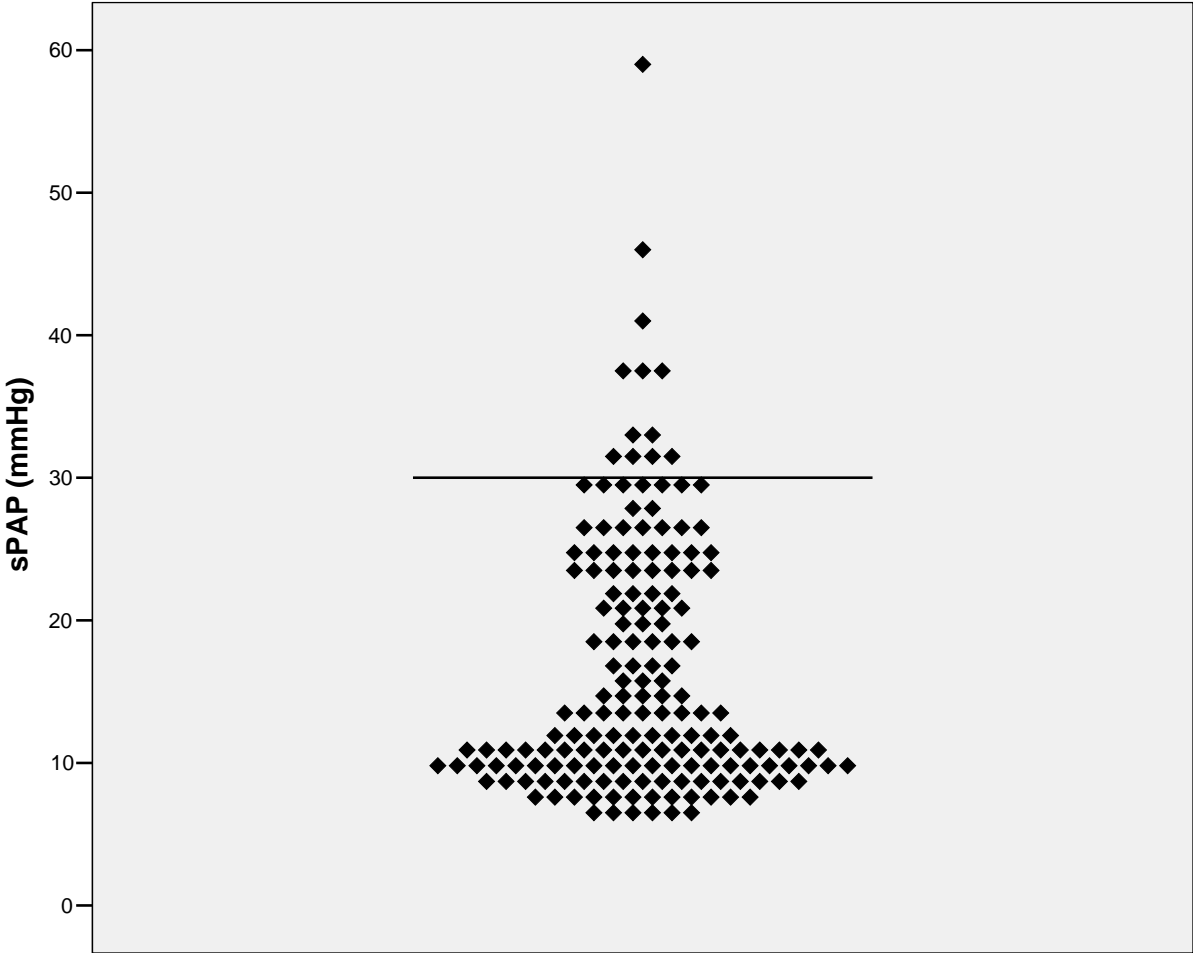
Among the 169 patients in whom TR was present, 12 were estimated to have sPAP>30 mmHg at rest. According to the definition of PAH used in this study which was performed between January 2004 and December 2005, the point prevalence rate of PAH in patients with SLE was 4.2% (CI 2.2%-7.3%). Excluding patients with no TR, the prevalence of PAH in our study group would be 7.1% (CI 3.7%-12.1%).

The distribution of PAP among the 169 patients in the latter group is shown in Figure 3-1. Only 1 patient was previously known to have PH diagnosed by echocardiography and was recorded in their SLICC/ACR damage index scores. There were 4 other patients with a previous history of PH by echocardiogram who did not meet the criteria for PAH by echocardiogram in this study. These 4 patients did not have persistent PH as they all had a secondary treatable cause for PH such as PE, left heart valvular disease and/or borderline PH and thus were not recorded in SLICC/damage scores. The characteristics of these patients with previous PAH diagnosed by echocardiography are shown in Table 3-1. Among the remaining 109 patients in the cohort (104 who refused to participate in this study and 5 whose

echo assessment was not suitable for analysis), three patients (2.7%) had PAH diagnosed by echocardiography in the past.



**Figure 3-1 Distribution of systolic pulmonary artery pressure (sPAP) in SLE cohort**



**Reference line= Upper limit of normal range sPAP (30mmHg)**

### **3.3.3 Risk factors for PAH in SLE**

#### **3.3.3.1. Raynaud's and APS**

The comparison of the demographic variables between lupus patients with and without PAH is shown in Table 3-1 and 3-2. The association of PAH with history of APS in patients with SLE was statistically significant but weak (Fisher's exact test p value 0.043).

#### **3.3.3.2 BILAG index**

At the time of this study, only 29.3% had active SLE in one or more of the eight organ/systems scoring A or B using assessment by BILAG index (Appendix 1). Two-thirds of these patients had evidence of activity either in the musculoskeletal or haematological system. None had scored A, one had scored B and 15 had scored C in cardiorespiratory system. There was no statistical difference in the number of patients with and without active disease in any systems according to BILAG scores between the subgroups of lupus patients with and without PAH (Table 3-3).

#### **3.3.3.3. SLICC/ACR damage index**

Assessment of patients with SLICC/ACR damage index (Appendix 2) revealed that 57.2% did not have any organ damage. It was noted that 5.6% of patients had pulmonary damage and 4.9% of patients had cardiovascular damage secondary to SLE. In this cohort, the highest damage score for both cardiovascular and pulmonary systems was 2, which was noted in 3 (1%) patients for cardiovascular damage and 3 (1%) patients for pulmonary damage. The maximum damage score that can be accumulated for cardiovascular system is 6 and respiratory system is 5. Eleven patients had pleural fibrosis, 2 had pulmonary fibrosis, 2 had shrinking lung syndrome, 1 had pulmonary hypertension and 1 had pulmonary infarction. The cardiovascular damage score included 8 patients with valvular disease, 7 with angina, 1 with cardiomyopathy and 1 with pericarditis. The total score by SLICC/ACR damage index was

significantly different between patients with PAH (median 0, range 0-5) and without PAH (median 0, range 0-8) (Mann Whitney U test, p value 0.03). The number of lupus patients with SLICC/ACR damage score (total) >2 was 7/12 (58.3%) in PAH subgroup and 56/271 (20.6%) in those without PAH, and this difference between groups with and without PAH were statistically significant (Fisher's exact test 2 sided, p value 0.006). Left heart valvular disease, pulmonary embolism interstitial lung disease and/or shrinking lung syndrome were present in all 5 patients with previous history of pulmonary hypertension in the study cohort (Table 3-4). Patients with shrinking lung syndrome (SLS) have unexplained dyspnoea with restrictive pattern and small lung volumes on lung function testing and absence of parenchymal disease with elevated diaphragm on CT chest (280;281).

#### **3.3.3.4. Autoantibodies**

The presence of ANA, dsDNA, ENA, low complements C3/C4, anticardiolipin antibody and lupus anticoagulant were compared between PAH cases and non-PAH patients. Lupus anticoagulant (p=0.005) was significantly associated with PAH while other antibodies did not show any difference between both groups (Table 3-5). Anti-La antibody might be considered borderline (p=0.03). Some results were not available for ENA (6/283) and lupus anticoagulant analysis (67/283) due to either technical reasons (no result from the laboratory) or to patients being on warfarin therapy and not suitable for testing of lupus anticoagulant (Table 5).

#### **3.3.3.5. Prior drug therapy**

The distribution of drug therapy in the study cohort is shown in Table 3-6. The prior use of warfarin (Fisher's exact test 2 sided, p value 0.005) and calcium channel blockers (Fisher's exact test 2 sided, p value 0.031) were found more frequently with PAH in SLE. None of the patients were on Bosentan or Sildenafil prior to this study.

**Table 3-1 Characteristics of SLE patients with and without PAH.**

	SLE	SLE without PAH	SLE-PAH	p value*
No. of SLE patients	283	271	12	-
Age (years)	41 (18-82)	41 (18-82)	45.5 (30-72)	0.22
Sex (M:F)	266:17	255:16	11:1	0.53
Disease duration (years)	8.7 (0-32)	8.6 (0-32)	9.6 (1.9-22.1)	0.95
Afro-Caribbean (%)	18.7	18.8	16.7	1.0
Asian (%)	19.8	20.3	8.3	1.0
Caucasian (%)	56.2	55.7	66.7	1.0
Oriental (%)	0.7	0.4	8.3	1.0
Other race (%)	4.6	4.8	0	-

Variables expressed in median and range and unless specified. \*p value calculated by Mann

Whitney U test for comparison between groups of SLE patients with and without PAH.

**Table 3-2 Frequency of clinical parameters between PAH and non-PAH patients with SLE.**

Risk factors	non-PAH patients  n	PAH patients  n	Positive in non-PAH patients  n (%)	Positive in PAH patients  n (%)	p value**
APS	271	12	30 (11)	4 (33)	0.043*
History of Raynaud's	271	12	180 (66)	8 (67)	1.000
Ever smoked	271	12	97 (36)	6 (50)	0.364
Current smokers	271	12	44 (16)	4 (33)	0.127

APS: antiphospholipid syndrome. \*p<0.05. APS = antiphospholipid syndrome.

\*\*Fisher's exact test 2 sided, p value

**Table 3-3 Comparison of SLE disease activity scores assessed by BILAG index in lupus patients with and without PAH**

Systems involved	SLE without PAH (no. of patients)	SLE with PAH (no. of patients)	p value comparing SLE groups with and without PAH (Mann Whitney U test)
General, active/inactive	2/265	0/12	1.0
Mucocutaneous, active/inactive	13/254	0/12	1.0
Neurological, active/inactive	1/266	0/12	1.0
Musculoskeletal, active/inactive	29/238	1/12	1.0
Cardiorespiratory, active/inactive	1/266	0/12	1.0
Vasculitis, active/inactive	4/263	0/12	1.0
Renal , active/inactive	11/256	2/10	0.102
Haematological, active/inactive	28/239	1/11	1.0

\* p value <0.05. Active SLE- BILAG score A or B, Inactive SLE- BILAG score C, D or E

**Table 3-4 Characteristics of five SLE patients in our study with prior diagnosis of PAH (all causes) by Echocardiography that were reassessed during this study.**

Patient study no.	Year of SLE diagnosis	Year of PAH Diagnosis	Associated conditions	sPAP when diagnosed (mmHg)	Treatment	sPAP in this study (mmHg)
058	2003	2003	Previous PE and ILD	44	Amlodipine, Perindopril, Warfarin	10.7
060	1994	1994	Previous PE and pericarditis	55	Warfarin, Nifedipine	46
064	1995	2000	Mitral regurgitation, MV replacement. Previous PE.	60	Amlodipine, Doxazosin, Perindopril, Warfarin	11.8
065	1986	1997	Aortic and mitral valve disease	44	Amlodipine, Perindopril	TR absent
216	2000	2003	Shrinking lung syndrome	31	Amlodipine, Warfarin	25

PE = pulmonary embolism; ILD = interstitial lung disease; MV = mitral valve; TR =

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tricuspid regurgitation; PAH = pulmonary arterial hypertension; sPAP = systolic pulmonary  
arterial pressure



**Table 3-5 Frequency of serological risk factor variables and results of Fisher's exact test between PAH and non-PAH patients with SLE.**

Risk factors	non-PAH patients n	PAH patients n	Positive in non-PAH group n (%)	Positive in PAH patients n (%)	p value
ANA (Titre $\geq$ 1:40) <sup>††</sup>	271	12	225 (83)	12 (100)	0.226
Anti-dsDNA <sup>††</sup>	271	12	112 (41)	7 (58)	0.371
ENA <sup>†</sup>	265	12	148 (56)	10 (83)	0.076
Anti-Ro <sup>†</sup>	265	12	98 (37)	4 (33)	1.000
Anti-La <sup>†</sup>	265	12	56 (21)	6 (50)	0.030*
Anti-Sm <sup>†</sup>	265	12	29 (11)	1 (8)	1.000
Anti-Jo <sup>†</sup>	265	12	1 (0)	0 (0)	1.000
Anti-scl70 <sup>†</sup>	265	12	6 (2)	0 (0)	1.000
Anti-RNP <sup>†</sup>	265	12	67 (25)	3 (25)	1.000
Low C3 (<0.75) <sup>††</sup>	271	12	29 (11)	0 (0)	0.619
Low C4 (< 0.14) <sup>††</sup>	271	12	64 (24)	5 (42)	0.173
LA <sup>†</sup>	205	11	32 (16)	6 (55)	0.005**

aCL <sup>†</sup>	271	12	63 (23)	5 (42)	0.168
(IgG/IgM) <sup>#</sup>			(44/38)	(3/3)	

<sup>†</sup>Test results if ever done <sup>††</sup>Test results at or within previous month of study assessment

<sup>#</sup>aCL IgG titre >15 IU/ml and aCL IgM titre >10 IU/ml were considered positive according to local laboratory reference criteria \*p<0.05, \*\*p<0.01. aCL = anticardiolipin antibody; LA = lupus anticoagulant.

**Table 3-6 Frequency of prior drug therapy among SLE patients with and without PAH.**

Drug therapy	Prior use in SLE patients without PAH (n=271)		Prior use in SLE patients with PAH (n=12)		p value (Fisher's Exact test- 2 sided)
	(n)	(%)	(n)	(%)	
Warfarin	26	9.6	5	41.7	0.005**
Aspirin	107	39.5	4	33.3	0.770
CCB	58	22.9	6	50	0.031*
ACE inhibitors	61	22.5	1	8.3	0.474

\*p<0.05, \*\*p<0.01. CCB = Calcium channel blockers; ACE inhibitors =angiotensin converting enzyme inhibitors.

### **3.3.4 Screening with ECG for PAH in SLE**

One of the 7 patients with resting tachycardia had PAH. Only one of 14 patients with right axis deviation (RAD), and 2 of 8 patients with left axis deviation (LAD) had PAH. The statistical significance for association of LAD with non-PAH is weak ( $p=0.041$ ). Right ventricular hypertrophy (RVH) was absent in all 278 patients. Left ventricular hypertrophy (LVH) was present in 54 patients in non-PAH group, with 47 probable and 7 definite LVH. Both patients with LVH in PAH group had definite LVH. In the study cohort, RBBB and LBBB were also not associated with PAH. None of the ECG parameters especially right heart parameters such as axis deviation and right ventricular hypertrophy were associated with PAH in patients with SLE (Table 3-7)

**Table 3-7 ECG abnormalities in SLE patients with and without PAH**

	SLE patients without PAH (Group I)	SLE patients with PAH (Group II)	p value comparing Group I and II (Fisher's exact test 2 sided)
Number of patients, n (%)	266 (94)	12 (4)	
Resting HR<60, n (%)	46 (17)	0 (0)	0.228
Resting HR>100, n (%)	6 (2)	1 (8)	0.268
Right axis deviation, n (%)	13 (5)	1 (8)	0.469
Left axis deviation*, n (%)	6 (2)	2(17)	0.041*
RBBB, n (%)	10 (4)	2 (17)	0.089
LBBB, n (%)	3 (1)	0 (0)	1.0
RVH, n (%)	0 (0)	0 (0)	-
LVH, n (%)	54 (20)	2 (17)	1.0

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HR: heart rate, RBBB: right bundle branch block, LBBB: left bundle branch block, RVH: right ventricular hypertrophy, LVH: left ventricular hypertrophy. \*p value <0.05

### **3.3.5 Right heart catheterisation**

Only 1 of 3 patients with sPAP $\geq$ 40 mmHg had WHO grade III dyspnoea and was eligible for right heart catheterisation (RHC) according to study protocol approved by research ethics committee. However, this patient refused to undergo this invasive test. Thus none of our study patients underwent RHC during the study period for estimation of pulmonary artery pressures.

## **3.4 Discussion**

In this study, we found that the prevalence rate of PAH in patients with SLE was 4.2%. If patients with no TR jet on echocardiography were excluded from the analysis, the prevalence rate was 7.1%. These rates are at the lower end of the range of prevalence rates reported from previous studies (69;154;168;210). This prospective cross-sectional cohort study is likely to reflect the prevalence rate better than the previous retrospective and smaller studies. The majority (80.4%) of this cohort have Birmingham postcodes suggesting that the majority are from local community rather than a patient cohort mainly referred from other centres for tertiary care. The SLICC/ACR damage index scores recorded as part of this study were low suggesting that only few patients had severe disease. It has been suggested that studies with predominantly a tertiary care based cohort(168) and/or patients with severe SLE(154) are likely to overestimate the true prevalence of PAH. We believe that our cohort has minimal bias from these factors resulting in a more accurate estimation and lower prevalence rate of PAH in SLE patients. Those patients who refused to participate (n= 104; 27%) could have done so for various reasons including active disease, lack of interest in participating in research studies, inconvenience due to distance required to travel to the centre, and work commitments. We are not aware of any main reason for non-participation.

In this study, TR velocity was measurable in one hundred and sixty nine patients (59.7%). This is in keeping with previously reported frequencies of identifying measurable TR in the range of 39% to 86% of patients (172;296). This frequency increases to almost 100% in patients with signs of right heart failure and in those with sPAP >50mmHg (190). The interobserver variability in the measurement of maximal TR velocity has been found to be less than 3% (112). Arcasoy et al (297) reported that the estimation of sPAP in patients was achieved less frequently in patients with obstructive airways disease compared with interstitial lung disease, and in those with residual volume (RV) exceeding more than 150% of predicted compared with RV <150% predicted. Only 2 patients in our cohort had RV >150% predicted and 27 patients had obstructive airways disease. Neither the presence of obstructive airways disease nor the predicted RV >150% were statistically significant between patients with and without detectable TR (data not shown). Several studies have shown significant correlation between RVSP measured by Doppler echocardiography and mean PAP measured by RHC (172;276;277;298).

Of 12 patients with PAH, 9 had sPAP <40 mmHg and 10 had WHO Grade II dyspnoea or less. This suggests PAH in this SLE cohort was predominantly mild with minimal or no symptoms. In the PAH group, only 1 patient each had restrictive lung disease, known valvular heart disease and previously recorded PAH. This supports the mild nature of cardio-respiratory manifestations and lack of significant dyspnoea in our PAH cases. The role of left heart disease (discussed in Chapter IV in detail) is negligible in our patients with PAH as none of them had significant left ventricular dysfunction with or without valve disease evaluated by ECHO.

The ECG may demonstrate signs of right ventricular hypertrophy and/or right atrial enlargement in pulmonary hypertension. Thus, the ECG findings may include right axis



deviation, P-pulmonale, right bundle branch block, and R/S ratio  $>1$  in lead  $V_1$ . The higher the pulmonary artery pressure, the more sensitive is the ECG. As most patients in our PAH cohort have mild PAH, this could explain the lack of significant ECG findings of PAH in our study. A recent study by Goncalvesova et al(299) showed the ECG signs of RVH/overload may help distinguish PAH from LV dysfunction related secondary PH in SLE patients.

Though the RHC measurement of mean PAP is the gold standard to confirm PAH, only one patient with PAH fulfilled criteria of systolic PAP  $\geq 40$  mmHg with dyspnoea Grade III or more required to undergo RHC according to the protocol approved by the research ethics committee. But this patient refused to undergo RHC measurements due to the invasive nature of intervention. It is thus possible that our ECHO prevalence rate of PAH in SLE might have been an overestimation as they have not been confirmed by RHC mean PAP measurements.

Since our study was completed, several epidemiological studies on the prevalence of PH in SLE have been published including the reports by PH registries from UK, USA and France. In 2006, Chung et al(155) reported a retrospective analysis and found that 181 out of 725 SLE patients who underwent either ECHO or RHC over 9 year period. The prevalence of PAH was 11%, using sPAP  $>45$  mmHg including 10 mmHg as right atrial pressure (RAP). This study reported on 20 patients with PAH and SLE with an average sPAP 65.3mmHg by ECHO, including 10 patients with mean ( $\pm$ S.D) PAP  $50\pm 13.5$ mmHg and RAP  $7\pm 4$ mmHg by RHC. They published the prevalence, as 2.8% (20 out of 725) while in fact it should be 11% (20 out of 181) as the others were not assessed. In 2010, Fois et al(186) reported on 93 lupus patients in a tertiary centre in France over 10 year retrospective analysis and found 13% prevalence using a cut off  $\geq 35$ mmHg by ECHO. In 2011, Cefle et al(55) reported a prevalence of 1.8% from a retrospective analysis of 544 patients with SLE of which 104 underwent echocardiography. In 2012, Ruiz-Irastorza et al(187) prospectively studied 245

patients with lupus and reported 50% of patients had sPAP  $\geq$  30 mmHg and 13.5% had sPAP  $\geq$  40mmHg but only 5% (12 patients) had definite PH with persistent sPAP  $\geq$  40mmHg which was confirmed on a repeat echo 6-12 months later. They also concluded that all lupus patients with PH diagnosis had secondary PH (cardiomyopathy or valvulopathy in 8 patients, severe COPD in 2, SLS in 1 and LV dysfunction in 1 patient) and none had PAH which is considerably different from most previous studies published. The definitions of PH and secondary PH in the study by Ruiz- Irastorza et al if applied to our cohort would result in 5.3% having sPAP  $\geq$  30mmHg, 1% having sPAP  $\geq$  40mmHg and two-thirds of our reported PAH patients would have secondary PH due to valve regurgitation, though all those patients had EF  $\geq$  50% and none had LVSD. But the limitation of these results is that repeat echocardiogram was not performed on all our patients.

The PH registries from UK, Scotland, France and USA have provided vast information on epidemiology of PH in various subgroups which otherwise would have been difficult to ascertain. The trend towards older age at diagnosis is reported from all registries compared to National Institute of Health (NIH) registry. The US and UK registries report a female predominance in SLE -PAH of 94.5-96% compared to 79-82% with IPAH. Table 3-8 and 3-9 represent the recent epidemiology data published from registries worldwide.

**Table 3-8 Prevalence of pulmonary hypertension published by PH registries**

Registry (Year reported)	PAH  (cases/million)	CTD-PAH  (cases/million)	PAH  No. of patients	SLE-PAH  No. of patients
France (2006)	15	2.3	674	15
Scotland (2007)	52	15	n/a	n/a
Scottish PVU* (2007)	26	10	n/a	n/a
UK (2008)	24.9	4.23	484	35
USA-REVEAL** (2011)	12.4	n/a	2967	110

\* PVU: pulmonary vascular unit; n/a: Not available

\*\*REVEAL: Registry to Evaluate Early And Long-term PAH management

**Table 3-9 Incidence of pulmonary hypertension published by PH registries**

Registry (Year reported)	PAH (cases/million)	CTD-PAH (cases/million)	SLE-PAH (cases/million)
France (2006)	2.4	0.4	n/a
Scotland (2007)	7.1	2.1	n/a
Scottish PVU* (2007)	7.6	2.8	n/a
UK (2008)	n/a	1.55	35
USA-REVEAL**	2.3	n/a	n/a

\* PVU: pulmonary vascular unit

\*\*REVEAL: Registry to Evaluate Early And Long-term PAH management

In 2006, the French registry(108) reported on a one-year study commencing in October 2002 including 121 incident and 553 prevalent cases of PAH. The prevalent cases differed from incident cases with higher mean pulmonary artery pressure (mPAP) and female predominance. The CTD-PAH was present in 103 patients (15.3% of all PAH) with 22 incident and 81 prevalent cases. This included 76% systemic sclerosis and 15 % SLE-PAH. Most patients were of NYHA III or IV functional class at diagnosis.

In 2007, the Scottish study(300) investigated Scottish Morbidity Records (SMR) from 1986-2001 of 16-65 year old ever-hospitalised patients with a diagnosis of pulmonary hypertension. Among 374 cases of PH, 111 patients had CTD-PAH with annual incidence of 1 and 2.2 cases per million in males and females respectively. The female incidence was twice that previously reported. The estimated incidence of CTD-PAH for whole adult population was 7 and 2 cases per million in females and males respectively. A comparison with the records from tertiary Scottish Pulmonary vascular unit (SPVU) from 1997-2005 was also reported in this study. An annual incidence of 2.8 cases per million and a prevalence of 10 cases per million in patients with CTD-PAH was reported from SPVU records, compared to 2.1 and 15 cases per million from SMR data respectively.

In 2008, the UK consensus statement from PH centres(301) reported a prevalence of 24.9 patients with PAH treated per million in mid-2005. In 2009, Condliffe et al(150) reported on 484 patients with CTD-PAH collected from unselected incident cases from UK PH centres over a 5.5year period from January 2001 with follow up ranging from 3.3 to 6 years. The PAH diagnosis was made by right heart catheterisation. The incidence of CTD-PAH increased from 0.68 to 1.55 cases per million over the 5 years and the prevalence was 4.23 per million. Among the 35 patients with SLE and PH (8% of 429 CTD-associated PAH diagnosed at rest), 80% had isolated PAH. The SLE-PAH patients were of mean age 42 years and statistically

differed from SSc-PAH with more females (96% vs. 82%), non-Caucasians (30% vs. 4%), and less TLCO% reduction (59.2% vs. 41.5%).

The US registry to evaluate early and long-term PAH disease management (REVEAL) includes 2967 patients from 54 centres(151). Among the 641 with associated CTD, 110 had SLE. Approximately 15% of patients in CTD and SLE group were newly diagnosed with PAH during the 19 month study until September 2007. SLE-PAH patients were younger compared to SSc and CTD associated PAH patients (mean 45 vs 62 and 57 respectively) and had a female to male ratio of 10:1. The REVEAL study characteristically had more female predominance overall at a ratio 4.3:1 compared to 1.7:1 in NIH registry and 1.9:1 from French registry. All above reports confirm the wide variability published on the prevalence of PAH in SLE.

It is well recognized that patients with SLE can develop PH at anytime during the course of their illness, most often in the first 5 years (mean delay  $4.9 \pm 3.7$  years) or as an initial manifestation(302). In the present study, the PAH subgroup as well as the whole SLE cohort had a long disease duration with median more than 8 years. The increased prevalence of Raynaud's phenomenon (62-80%) in lupus patients with PAH(57;69;167) suggests the role of pulmonary arterial vasospasm in pathogenesis of PAH in addition to other factors. However, we did not find the association of RP with PAH in SLE as there was a high prevalence of RP in both groups with and without PAH (66% vs 67%). The under-reporting of Raynaud's phenomenon in lupus patients (16%) recruited in the PH registry cohort in USA has also been noted.

Most of our patients with systemic hypertension or Raynaud's phenomenon were on treatment with vasodilators such as calcium channel blockers (CCB) and angiotensin

converting enzyme (ACE) inhibitors. 31/283 patients with previous thromboembolic disease and/or antiphospholipid syndrome (APS) were on anticoagulant therapy. The significant association of prior use of warfarin and calcium channel blockers (Table 3-6) suggests the possible role of these drugs in either lowering the sPAP or preventing the sPAP from rising to significantly higher values indicative of PAH, as well as supporting the hypothesis that there may be a thrombotic mechanism underlying the pathogenesis of PAH in these SLE patients.

In our SLE study cohort, there were no association between PAH and activity in any system scored by BILAG index. None of the patients in PAH group had BILAG score A or B in the cardiorespiratory system, and 8 out of 12 (75%) patients in the PAH group did not have BILAG score A or B in any system. It has been previously reported that the development of PH and its severity do not correlate with SLE disease activity in non-pulmonary disease or duration of SLE(303). Active SLE in pulmonary or non-pulmonary disease(185) is not associated with PH in a cohort of 129 patients with SLE. There have been several anecdotal reports(184;304) as well as small retrospective series suggesting immunosuppressive therapy (IST) such as intravenous cyclophosphamide (IVCYC) and steroids are an effective treatment in the early stages of PAH in SLE. A randomised controlled study(305) in 36 SLE associated PAH patients comparing IVCYC therapy to oral enalapril reported the benefits of IVCYC in reducing sPAP in mild and moderate PH. This report also reported higher SLE disease activity index (SLEDAI) scores in patients with PH who responded to treatment. Jais et al(306) showed that high SLEDAI scores greater than 3 in PAH associated with SLE was not a predictive factor for response to immunosuppressive therapy and concluded the reason for response in a subset of lupus patients is unclear. Another recent study(307) showed intensive IST in combination with specific vasodilator therapy improved haemodynamics and prognosis in 6 out of 7 SLE patients, especially when used in early phase of PAH. Sanchez et

al(308) showed disease duration from PAH diagnosis in SLE patients does not predict response to IST. Interestingly, the UK PH registry data(150) from unselected incident cases of SLE shows 86% were on immunosuppressive agents, and 11% of those were newly commenced on IST after PH diagnosis. This compared with only 22% in the PH registry (REVEAL) from USA(151). The UK consensus statement(301) recommends immunosuppressive therapy (IST) for active CTD in addition to other advanced vasodilator treatment for PH, and points to lack of published data on combination therapy with IST in PAH associated with CTD. Though this present study did not find any association between disease activity and PAH in SLE, the concept of subclinical disease activity in relation to PH associated with SLE in early stages is possible given the response to IST reported in several publications discussed above.

We have shown that PAH in SLE is strongly associated with SLICC/ACR damage index score >2. This reflects the long disease duration of the study cohort and the PAH subgroup resulting in damage accumulation due to recurrent active disease, comorbidities, drug therapy and overall inflammatory burden. Another recent echo screening study(187) for PH in a series of 245 patients with SLE reported a lack of association of SLICC/ACR damage index score with PAH. Another study(55) on patients with SLE reported that high SLICC/ACR damage scores in patients with PH compared to those without PH reached statistical significance. It is important to be aware of the risk of PH in those lupus patients with high SLICC/ACR damage index scores but low or normal scores should not preclude suspicion of PAH.

In this study, lupus anticoagulant was the only significant risk factor for PAH in SLE. Several researchers have reported the increased frequency of lupus anticoagulant(58) and anticardiolipin antibodies(53;57;59;309) in association with PH in SLE. The presence of



antiphospholipid antibodies in SLE patients with PAH is most often not accompanied with pulmonary thromboembolism(309). The antiphospholipid antibodies are also detected in patients with and without thromboembolic PH without SLE(60;61). The frequency of antiphospholipid antibodies is 30-40% in SLE and usually is present in low titre, while the frequency can be as high as 80% in patients with PH in SLE(55). Some studies have also reported that the anticardiolipin antibodies is neither frequently seen(170) nor associated with PAH(185;310) in patients with SLE. The significant association of lupus anticoagulant in addition to the borderline significance of clinical APS, suggests the role of thrombosis as a mechanism in the pathogenesis of PAH in our SLE patients. However, a recent study by Farzaneh-Far et al on 200 patients from a tertiary care lupus cohort with a PAH prevalence of 17.5% did not find any association with antiphospholipid antibodies, defined as IgG or IgM anticardiolipin antibody titre >40 IU/ml and/or positive lupus anticoagulant. When we increased the sPAP threshold to >35mm Hg for PAH we found a statistically significant association between antiphospholipid antibodies (anticardiolipin antibodies and/or lupus anticoagulant) and PAH (p=0.005, data not shown) though numbers were small. The association of autoantibodies such as anti-RNP antibodies and rheumatoid factor with PAH in SLE has been previously reported but we did not find any similar association in the present study. The weak association of anti-La antibodies to PAH in SLE in the study is unlikely to be of clinical significance. The absence of any association of low complements C3/C4 or dsDNA positivity with PAH in SLE is keeping with the minimal disease activity scores evaluated by BILAG index in the study cohort. We have published the prevalence and risk factors for PAH in SLE study results in a peer reviewed journal (Appendix 8)

### **3.5 Conclusion**

The point prevalence of PAH in SLE is 4.2% in our cohort of SLE patients. Most of the PAH cases (75%) were found to be of mild severity (sPAP <40mmHg) and were asymptomatic and such would not meet criteria for guideline-based treatment(301). It is currently unclear whether establishing the diagnosis of PAH in the pre-symptomatic phase improves outcome in SLE patients. A strong association of PAH in SLE to those with SLICC/ACR damage scores >2 and presence of lupus anticoagulant is evident from this study.

## CHAPTER 4    CARDIOVASCULAR DISEASE IN LUPUS

### 4.1 Abstract

**Background:** The cardiovascular manifestations in systemic lupus erythematosus (SLE) may involve the pericardium, myocardium and endocardium. These include pericarditis, valvular diseases, ischaemic heart disease, impaired cardiac function and conduction defects. Left heart disease leading to secondary PH is a recognised complication in patients with SLE.

**Objectives:** To determine the prevalence of cardiac morphological and functional abnormalities as assessed by electrocardiogram (ECG) and transthoracic echocardiography (TTE) in patients with SLE, and compare between patients with and without PH in SLE.

**Methods:** A prospective cross-sectional study of 283 patients with SLE was recruited from lupus clinics in Birmingham, UK. They underwent a 12-lead ECG and resting transthoracic echocardiography (TTE) to assess cardiac morphology and function. The systolic pulmonary artery pressure was estimated using Doppler echocardiogram. PH was defined as systolic pulmonary artery pressure (sPAP) >30 mm Hg. We assessed the role of autoantibodies, the BILAG disease activity scores and the SLICC/ACR damage index in predicting cardiac abnormalities detected on TTE in patients with SLE.

**Results:** The median age was 41 years (range 18-82) and disease duration was 8.7 years (range 0-32). 94% were female and 98.2% fulfilled 4 of the 1982 ACR classification criteria for SLE. 14/283 (4.9%) were known to have SLICC/ACR

damage index cardiovascular score of 1 (3.8%) or 2 (1.1%), including 8 patients with valvular heart disease, 6 with ischaemic heart disease, 1 with cardiomyopathy and 1 with pericarditis. Mitral valve thickening, valvular regurgitation, left ventricular systolic dysfunction (LVSD) and concentric left ventricular hypertrophy (LVH) were the commonest abnormalities detected on TTE (Table 4-2). 41/283 (14%) had mitral valve thickening, commonly involving the anterior leaflet. Other abnormalities included left ventricular and left atrial dilatation, and segmental wall motion abnormality. We were able to calculate the ejection fraction (EF) in 256/283 patients and 10 patients had an EF <50%. None of the patients had pericardial effusion or thickening at the time of assessment. Mitral regurgitation was significantly associated with the combined positive anticardiolipin IgG titre >40IU/l, positive anticardiolipin IgM titre >40 IU/l and positive lupus anticoagulant. There was no significant difference in the frequency of cardiac valvular and functional abnormalities between lupus patients with and without PAH. None of the patients with PAH had right and/or left ventricular systolic/diastolic dysfunction.

**Conclusion:** Thickening of the anterior mitral valve was the most common cardiac morphological abnormality in our SLE cohort. The majority of valvular abnormalities are mild without compromise in cardiac function. Left heart morphological and functional compromise was not associated with PAH in this cohort. In contrast to previous studies, pericardial disease was absent in our cohort at the time of assessment. A strong association between MR and combined positive LAC and high titre aCL IgG and aCL IgM was present.

## 4.2 Introduction and Methods

Cardiac involvement in systemic lupus erythematosus (SLE) may involve the pericardium, myocardium and endocardium. The cardiovascular (CV) manifestations include pericarditis, myocarditis, endocarditis, valvular diseases, ischaemic heart disease, impaired cardiac function and conduction defects(311). Coronary artery disease (CAD) secondary to accelerated atherosclerosis is due to traditional CV risk factors as well as other factors such as elevated homocysteine level, and combination of oxidative stress, inflammation and presence of antiphospholipid antibodies(312-314). The leading causes of death in SLE patients with disease duration greater than 5 years are CAD, infection and malignancy. Females aged 35-44 years with SLE are 52 times increased risk of myocardial infarction compared to healthy age matched women(315). The evaluation of CAD in SLE patients is not within the remit of this present study. Predominantly the valvular diseases noted in SLE is subclinical being asymptomatic and of mild degree in severity. Though left heart disease causing secondary PH is not often reported in the prevalence studies in SLE, a recent study by Ruiz-Irastorza reported PH in 5% of their lupus cohort and all had secondary PH(187) which is considerably different from most previous studies published. The association of antiphospholipid antibodies with valvular diseases and PAH in SLE is well recognised(56).

Please refer to section 1.3.4 in the introduction chapter for details on CV disease in SLE, and refer to methods section 2.2 for patient demographics, 2.3 for ECG, 2.4 for echocardiogram, 2.6.1 for autoantibodies and 2.6.2 for BILAG and SLICC/ACR damage index.

## **4.3 Results**

### **4.3.1 BILAG disease activity index and SLICC/ACR damage index in SLE patients**

Only 1 patient had activity in cardiorespiratory system with BILAG score B in this cohort. None had severe activity with BILAG score A in cardiorespiratory system (Appendix 1).

Fourteen out of 283 (4.9%) SLE patients were known to have SLICC/ACR damage index (Appendix 2) cardiovascular score of 1 (3.8% of patients) or 2 (1.1% of patients), including 8 patients with valvular heart disease, 7 with ischaemic heart disease, 1 with cardiomyopathy and 1 with chronic pericarditis.

### **4.3.2 ECG abnormalities in patients with SLE**

Twelve lead ECG was performed in 278/283 patients. Five patients did not have ECG due to technical difficulties. The majority of patients (204, 73%) had normal rate, rhythm, and axis.

Forty six (17%) patients had a resting heart rate <60/min and 7 (2.5%) had a resting rate >100 beats/min. Right axis deviation was present in 14 (4.9%) patients and left axis deviation in 8 (2.8%) patients. Three (1.1%) patients had left bundle branch block and 12 (4.3%) patients had right bundle branch block. No other conduction defects were detected. Left ventricular hypertrophy present was present in 56 (20%) patients. None had right ventricular hypertrophy.

None of the SLE patients with tachycardia or bradycardia had activity with BILAG index score A or B for cardiovascular system (CVS). The cardiovascular damage

score (SLICC/ACR DI) was 2 in 1/46 patients and 1 in 3/46 patients with bradycardia. None of the patients with tachycardia had damage score in CVS. There were no statistical association between any ECG abnormality recorded and BILAG disease activity or SLICC/ACR damage index scores (Table 4-1).

**Table 4-1 Disease activity and damage indices in the cardiovascular system in 278 SLE patients with ECG abnormalities**

	SLE active  (BILAG disease activity score A or B in CVS)	SLE inactive  (BILAG disease activity score C,D or E in CVS)	Damage in CVS  (SLICC/ACR DI score 1 or 2 in CVS)	No damage in CVS  (SLICC/ACR DI score 0 in CVS)
Bradycardia  No. of patients (%)	0 (0)	46 (17)	4 (1)	42 (15)
Tachycardia  No. of patients (%)	0 (0)	7 (3)	0 (0)	7 (3)
Right axis deviation  No. of patients (%)	0(0)	14 (5)	0 (0)	14 (5)
Left axis deviation  No. of patients (%)	0(0)	8 (3)	1 (0)	1 (0)
Right bundle branch block  No. of patients (%)	0(0)	11 (4)	1 (0)	10 (4)
Left bundle branch block  No. of patients (%)	0(0)	3 (1)	0 (0)	3 (1)



Right ventricular hypertrophy No. of patients (%)	0(0)	0 (0)	0 (0)	0 (0)
Left ventricular hypertrophy No. of patients (%)	0	56	3 (2 OF 1, AND 1 OF 2)	53

BILAG: British Isles lupus activity group; SLICC/ACR DI: Systemic lupus

international collaboration committee/American College of Rheumatology damage

index

### **4.3.3 Echocardiographic evaluation of cardiac morphology/function**

Transthoracic Echocardiography (TTE) was performed in all 283 patients with lupus. Mitral valve thickening, valvular regurgitation, left ventricular systolic dysfunction (LVSD) and concentric left ventricular hypertrophy (LVH) were the commonest abnormalities detected on TTE (Table 4-2).

The mitral valve (MV) thickening was present in 41(14%) patients. It involved the anterior and posterior MV leaflet in 39 and 8 patients respectively, while 6 patients had both leaflets affected. The valvular regurgitation involved mitral valve in 95(34%) patients, tricuspid valve (TCV) in 128(45%) patients and aortic valve (AV) in 26 (9%) patients. A trace or mild pulmonary incompetence was present in 17 (6%) patients. Seven out of 9 patients who had MV, TCV and AV regurgitation together were only of mild degree.

Three (1%) patients had aortic sclerosis. Three (1%) patients had aortic stenosis (AS), with 2 being mild and one moderate degree AS. None of the patients had mitral stenosis.

Three patients had valve replacements, with 2 patients having mitral valve and 1 having aortic valve replacement. The dilated cardiac chambers were all of mild degree with involvement of left atrium in 7 (2%), left ventricle in 10 (4%), right atrium in 5(2%), and bi-atrial in 2 (1%) patients. The segmental wall motion abnormality and thickening of septal wall were noted in 4 patients each. Mild right ventricular hypertrophy was seen in one patient, who did not have PAH.

Left ventricular systolic dysfunction (LVSD) was mild in 15 (5.4%) and moderate in 6 (2.1%) patients. None had severe LVSD. Left ventricular hypertrophy (LVH) was

mild in 35 out of 38 patients (13.4%). We were able to calculate ejection fraction (EF) in 256/283 (90%) patients and 10 (4%) patients had an EF <50%. The EF% was  $\geq 50\%$  in 246 patients,  $\geq 60\%$  in 126,  $\geq 70\%$  in 24,  $\geq 80\%$  in 3 patients.

No patients had pericardial effusion or pericardial thickening at the time of assessment.

The number of patients with left heart valvular abnormalities, LVSD and LVH were not different between SLE patients with and without PAH (Table 4-3).

**Table 4-2 Frequency of cardiac abnormalities detected by TTE in patients with SLE**

Cardiac abnormality detected on TTE	Mild	Moderate	Severe
	No. of patients (%)	No. of patients (%)	No. of patients (%)
Tricuspid regurgitation	122 (43.1)	4 (1.4)	2 (0.7)
Mitral regurgitation	83 (29.6)	11 (3.9)	1 (0.4)
Aortic regurgitation	24 (8.6)	2 (0.7)	0 (0)
LV systolic dysfunction	15(5.4)	6 (2.1)	0 (0)
LV hypertrophy	35 (12.3)	2 (0.7)	1 (0.4)

TTE: Transthoracic echocardiogram; LV: Left ventricular

**Table 4-3 Left heart abnormalities detected by TTE in patients with and without PAH**

Left heart abnormality detected on TTE	SLE without PAH (n=272) No. of patients (%)	SLE with PAH(n=12) No. of patients (%)	p value comparing SLE patients with and without PAH (Fisher's exact 2 sided test)
AMVLT	36 (13)	3 (25)	0.227
PMVLT	7 (3)	1 (8)	0.303
MR	88 (32)	7 (58)	0.115
AR	24 (9)	2 (17)	0.310
MS	0	0	-
AS	6	0	1.0
LVSD	21 (8)	0	0.609
LVH	35 (13)	3 (25)	0.215

MR: mitral regurgitation; AR: aortic regurgitation; LVSD: left ventricular systolic dysfunction; LVH: left ventricular hypertrophy; AMVLT: anterior mitral valve thickening; PMVLT: posterior mitral valve thickening

#### **4.3.4 Autoantibody associations with cardiac valvular abnormalities**

All the autoantibodies listed in the serological risk factors for PAH study (section 2.6.1, Table 3-5) was compared between patients with and without cardiac valvular abnormalities (regurgitation, stenosis or thickening). The association of positive autoantibodies and presence of cardiac valvular defects did not reach statistical significance. In particular, positive anticardiolipin IgG with a titre >15 IU/ml and >40IU/ml, positive anticardiolipin IgM with a titre >10 IU/ml and >40IU/ml, and positive lupus anticoagulant were not associated with cardiac valve defects in this cohort of SLE patients. 24/70 patients with MR and 25/ 150 patients without MR had combined high titre positivity of anticardiolipin IgG >40IU/ml, anticardiolipin IgM>40 IU/ml and positive lupus anticoagulant. The number of patients with (n=49) and without (n=171) combined high titre positivity of anticardiolipin IgG >40IU/ml, anticardiolipin IgM>40 IU/ml and positive lupus anticoagulant were significantly different between patients with and without MR (p=0.005 Fisher's exact test 2 sided). The history of APS was noted in 34/283 patients. There was no difference in the frequency of valvular thickening, stenosis or regurgitation between patients with and without history of APS.

#### **4.4 Discussion**

The majority (99%) of the SLE cohort had inactive disease in cardiovascular system (CVS) according to BILAG disease activity scores and similarly 95% patients had no damage in cardiorespiratory system measured by SLICC/ACR damage index. Only 8 (3%) patients had valvular disease with audible murmur recorded as cardiovascular damage. This is similar to previous studies reporting that majority of SLE patients have subclinical valvular disease. In another study of 217 SLE patients(316), the

mean SLEDAI score was 4.1 and the mean SLICC score was 1 with only 2.3% patients having CAD..

The ECG was predominantly normal in the study cohort and those who had abnormal rate, rhythm, axis or ventricular hypertrophy abnormalities did not show any association with the disease activity or damage in CV system. This is possibly because of very small number of patients who had active disease and/or damage in CVS (see above). An increasing tendency towards tachycardia in SLE patients was reported previously(317). The ECG abnormalities detected in our cohort could possibly reflect subclinical coronary vascular disease often reported in SLE patients. The objectives of this study did not include ischemic heart disease assessment in SLE and thus unable to confirm the reason for ECG abnormalities in this study cohort.

The main aim was to estimate the prevalence of structural and functional cardiac abnormalities by TTE in this large lupus study cohort. Our study confirmed that thickening of the anterior mitral valve was the most common cardiac morphological abnormality (14%), similar to reports published before and after our study period. Mitral valve thickening was found to be focal thickening consistent with nodules in anterior and posterior leaflets and distinct from the normally observed age related diffuse thickening. It has been postulated that MV thickening could be the hallmark of early valve disease preceding valvular damage and insufficiency. However, the clinical significance of MV thickening has remained uncertain for many years now.

The majority of valvular abnormalities in the cohort were mild regurgitation without compromise in cardiac function. Tricuspid and mitral regurgitation were frequently noted. No patients had mitral stenosis and only 2% had aortic stenosis or sclerosis. The valvular defects seen in our study were similar to previous studies confirming

mild valvular disease and focal thickening of mitral valve leaflets being most frequent in SLE. The anatomical lesions were reported in 40-50% of cases with TTE and 50-60% of cases with TOE evaluation in SLE patients (192). The benefits of TOE are better sensitivity, specificity and negative predictive value than TTE evaluation(193). It is important to consider TOE evaluation in SLE patients with non-diagnostic TTE, particularly in those with cardiac thrombo-embolism and suspected endocarditis. The combined incidence of IE, heart failure, stroke, peripheral embolism and valve replacement was 22% in those with valvular heart disease (VHD) compared to 8% in those without VHD(197). There were no significant associations of left heart anatomical defects with PH in suggesting that secondary PH due to valvular disease is unlikely in this SLE cohort (Table 4-3).

Left ventricular hypertrophy was present in 13% and is likely to be due to systemic hypertension (24%) in SLE patients. The LVSD seen in 7% in this cohort is similar to a previous report using TOE in SLE patients(197) and not associated with PAH. The LVH and LVSD in this present study were predominantly mild and not associated with PH. It is possible that LVSD could be due to subclinical ischemic heart disease but the objectives of this study did not include evaluation of IHD and traditional CV risk factors.

A retrospective study(318) of 275 SLE patients reported on the prevalence of infective endocarditis (IE) associated with valvular disease secondary to SLE. This report from American dental association found 18.5% with clinically detectable cardiac murmur and only 4.4% with clinically significant valvular abnormality potentially required antibiotic prophylaxis before certain dental procedures, but no cases of IE were found related to SLE. Roldan et al(197) reported IE as a



complication in 3/45 (7%) patients with SLE related valvular disease evaluated by repeated transoesophageal echocardiogram with an interval of  $29 \pm 13$  months and followed up for 57 months. The antibiotic prophylaxis for bacteremia producing dental procedures such as dento-gingival manipulations is advisable for SLE patients with clinically significant valvular abnormalities confirmed by TTE or TOE (193;197;318;319).

In contrast to previous studies, acute pericardial disease was absent in our cohort at the time of assessment. None of our patients had pericardial abnormalities recorded. This could be attributed to average disease duration of 9.7 years, the cross sectional nature of study, and minimal cardiovascular damage recorded with only one patient scoring for chronic pericarditis in SLICC/ACR damage index in our cohort.

Pericarditis is common and characteristic of SLE. Though this is a manifestation seen at any stage of SLE, it is often noted at disease onset or during active SLE relapse. It is not uncommon for pericarditis to be recurrent. The echocardiographic diagnosis of pericardial abnormalities was found in 16-54% of patients(320) and the variability depends on symptoms, definitions and diagnostic tools. Often asymptomatic pericardial effusion is only elicited by ECHO, and can be associated with active disease in other organs.

The association of antiphospholipid antibodies(56) and other autoantibodies such as anti-Ro and anti-La(321) with cardiac valvular abnormalities in SLE has been reported by several research groups. The combined positive lupus anticoagulant and high titre anticardiolipin IgG and IgM > 40 IU/ml was strongly associated with mitral regurgitation. However, other valvular defects were not found to be associated with autoantibodies including antiphospholipid antibodies.

## 4.5 Conclusion

The thickening of the anterior mitral valve and the mild valvular regurgitation without compromise in cardiac function were the most common cardiac anatomical abnormality detected by TTE in our SLE cohort. Left heart morphological and functional compromise was not associated with PAH in this cohort. In contrast to previous studies, acute pericardial disease was absent in our cohort at the time of assessment. A strong association between MR and combined positive LA and high titre aCL IgG and aCL IgM was present.

## CHAPTER 5 RESPIRATORY ABNORMALITIES IN SLE AND PAH

### 5.1 Abstract

**Hypothesis:** Pulmonary hypertension associated with SLE can be secondary to lung diseases. Dyspnoea can predict severity of PH in SLE.

**Aims:**

1. To assess respiratory muscle strength abnormalities, pre-existing lung diseases and smoking status as the risk factors for PAH in SLE
2. To assess the role of pulmonary function tests, respiratory symptom questionnaires and six minute walk test as the screening tests for PAH in SLE.

**Objectives:**

1. To determine the presence of underlying lung diseases and history of smoking in our SLE cohort based on history, clinical records and/or SLICC/ACR damage scores, and compare between patients with and without PAH in SLE.
2. To evaluate respiratory muscle strength (RMS) abnormalities in SLE patients and compare RMS abnormalities in those with and without PAH.
3. To evaluate dyspnoea in patients with SLE using respiratory symptom questionnaires, and compare dyspnoea scores between patients with and without PAH in SLE.

4. To evaluate pulmonary function test (PFT) abnormalities in patients with SLE, and assess the relationship with lupus related autoantibodies and BILAG disease activity scores
5. To compare PFT abnormalities in lupus patients with and without PAH, determined by Echocardiogram in this study.
6. To compare PFT abnormalities in lupus patients with and without known restrictive lung diseases
7. To determine distance walked in six minutes (6MWD) in SLE patients and compare 6MWD by patients with and without PAH

### **Methods:**

A detailed clinical history and examination, clinical records evaluation including SLICC/ACR damage index were performed in all 283 patients with lupus in this study. Respiratory muscle strength measurements, pulmonary function testing, and six minute walk test were performed according to international guidelines. The modified MRC scale, baseline dyspnoea index (BDI), and St. George's respiratory questionnaire were also self completed by study patients.

### **Results:**

In this SLE cohort, 15% of patients had known lung disease and 17% were current smokers. The presence of SLE related restrictive lung diseases or smoking status between patients with and without PAH in SLE did not reach statistical significance (Table 5-1). Respiratory muscle weakness was present in 55% with PAH and 31% without PAH with no significant difference between the groups. A strong correlation was found between inspiratory and expiratory muscle pressures in the whole cohort.

The screening tests including all respiratory questionnaires, 6MWT and PFT parameters were not able to distinguish patients with PAH from those without in this cohort.

**Conclusion:**

The prevalence of pre-existing restrictive lung disease in lupus was 5%. Two-third never smoked, and predominantly was in NYHA functional class I (85%). Respiratory muscle weakness was present in one-third and a strong correlation existed between inspiratory and expiratory muscle pressures in SLE. There were no respiratory risk factors identified for PAH in SLE in this study. Pulmonary function tests in patients with SLE revealed low TLCO in 55% and isolated low TLCO in 33%, and significant difference in all parameters noted between patients with and without dyspnoea or pre-existing lung disease. Pulmonary function test parameters, 6MWT distance, and respiratory questionnaires did not have screening potential for identifying PAH in SLE in this cohort.

## 5.2 Introduction and Methods

Pleurisy, pleural effusion, interstitial lung disease (ILD), pulmonary embolism, shrinking lung syndrome (SLS) are the most important lung manifestations in SLE. Patients with shrinking lung syndrome (SLS) have unexplained dyspnoea with restrictive pattern and small lung volumes on lung function testing and absence of parenchymal disease with elevated diaphragm on CT chest(280). The prevalence of SLS is 0.6- 0.9% in large multiethnic lupus cohorts(322) while a higher prevalence of SLS of 6% was reported in patients with refractory SLE requiring haematopoietic stem cell transplantation (323). The assessment of lung diseases, whether it is serosal, interstitial, airways or vascular disease is vital to ascertain the cause of PH, degree of lung involvement and the impact of cardiorespiratory symptoms in SLE patients.

A detailed clinical history and examination, clinical records evaluation including SLICC/ACR damage index were performed in all 283 patients with lupus in this study. The results of pulmonary function tests, chest X-ray and high resolution CT scan of chest were noted only from those patients who underwent these tests previously. Respiratory muscle strength measurements were performed using handheld respiratory pressure meter (MicroRPM, Micro medical Ltd). Pulmonary function testing (PFT) including spirometry, lung volumes and diffusion factor were measured as per standardized guidelines(292). Six minute walk test (6MWT) was performed according to international guidelines(282). The modified MRC scale, baseline dyspnoea index (BDI), and St. George's respiratory questionnaire were also self completed by all study patients. Please refer to section 1.3.6 for introduction and sections 2.6 and 2.7 for methods in detail.

**Statistical analysis:** The distribution of variables was not normally distributed and thus non-parametric tests were used for comparison between groups. The continuous variables were expressed in median and range, or counts and percentages. Mann Whitney U test was used to compare continuous variables between groups. Fisher's exact (2 sided) test and Pearson's test when appropriate were used to analyse categorical variables between groups. Spearman Rank test was used for bivariate correlation analysis between two continuous variables. A p value of <0.05 was considered significant.

## **5.3 Results: Respiratory risk factors for PAH**

### **5.3.1 Presence of underlying lung disease in SLE**

The presence of underlying lung diseases in our lupus patient cohort were obtained from clinical examination and medical records including previous pulmonary function tests, chest X-ray and high-resolution CT chest results. The pulmonary function tests performed in our current study were not used to confirm the presence or absence of the nature of lung diseases already known in our patients.

Among the 283 SLE patients, 14 (5%) had known lupus related restrictive lung diseases (RLD) and 27 (10%) had obstructive airways disease (OAD). In the RLD subgroup, 7 had pulmonary fibrosis, 5 had pleural thickening and 2 had shrinking lung syndrome. Of the 27 with OAD, 25 had asthma and 2 had chronic obstructive airways disease (COPD). Among other lung diseases, two patients with PAH had previous history of pulmonary embolism. In the non-PAH group, 7 had previous history of TB, 1 had pleural effusion and 4 had pulmonary embolism. None in our SLE cohort had history of bronchiectasis or lung malignancy.

Among the 12 patients with PAH, only one had asthma and another had shrinking lung syndrome and pleural fibrosis. In other words, 8% had RLD and 8% had OAD in PAH group compared to 5% with RLD and 9.5% with OAD in non-PAH group. There was no statistical significance in the difference in the number of patients with RLD and/or OAD between patients with and without PAH. (Table 5-1)

### **5.3.2. History of smoking**

In the whole SLE cohort, the number of patients who ever smoked was 103 (36%), who currently smoked were 48 (17%) and who never smoked were 180 (64%). In PAH subgroup, 33% were current smokers and 50% had never smoked. In the non-PAH group, 16% were current smokers and 64% have never smoked (Table 5-1). There were no significant difference between current smokers and the rest of cohort, as well as between ever smoked and never smoked status with the risk of PAH in SLE.



**Table 5-1 Risk of smoking and previous lung disease in SLE patients with and without PAH**

	SLE	SLE without PAH (Group I)	SLE with PAH (Group II)	p value  (Fisher's test, 2 sided)*  ( Group I and II comparison)
No. of patients	283	271	12	
Any lung disease,  No. of patients (%)	41 (14.5)	39 (14)	2 (17)	0.687
Restrictive lung diseases  No. of patients (%)	14 (5)	13 (5)	1 (8)	0.463
Obstructive lung diseases  No. of patients (%)	27 (9.5)	26 (9.5)	1 (8)	1.0
Ever smoked  No. of patients (%)	103 (36)	97 (36)	6 (50)	0.364
Current smokers  No. of patients (%)	48 (17)	44 (16)	4 (33)	0.127

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### 5.3.3 Respiratory muscle strength

The inspiratory and expiratory muscle pressure measurements were performed in 11 patients with PAH and 254 patients without PAH. The median maximal inspiratory pressure (MIP) was 53.5 cmH<sub>2</sub>O and maximal expiratory pressure (MEP) was 62 cmH<sub>2</sub>O in non-PAH group, while these were 42 cmH<sub>2</sub>O and 46 cmH<sub>2</sub>O respectively in PAH group. The difference in MIP and MEP between patients with and without PAH was not significant (Mann Whitney U test).

In the PAH group, 5 patients had inspiratory muscle weakness and 3 had expiratory muscle weakness. In the non-PAH group, 70 patients had inspiratory muscle weakness and 38 had expiratory muscle weakness. Both inspiratory and expiratory muscle weakness were found in 2 patients with PAH and 29 patients without PAH in SLE. The sniff nasal inspiratory pressure was abnormal in 7 out of 11 patients with PAH and 96 out of 240 patients without PAH (Table 5-2).

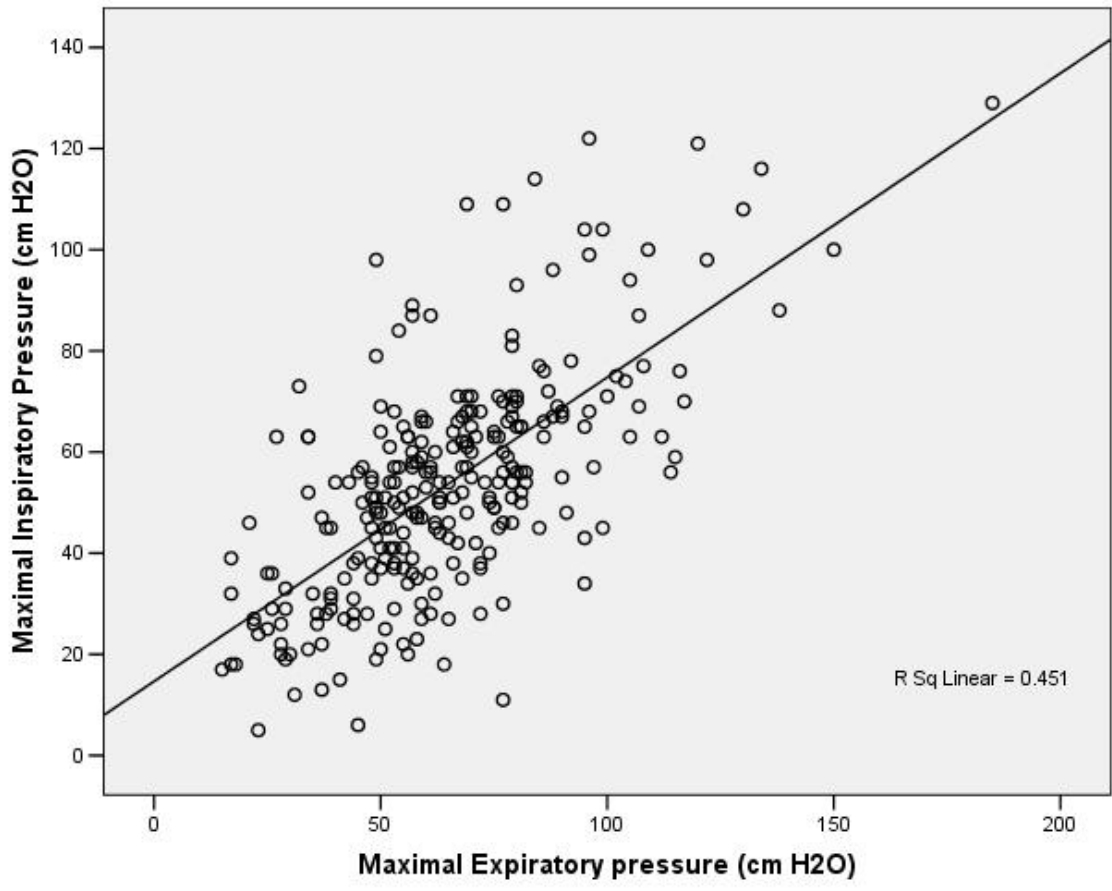
All measurements of respiratory muscle strength including MIP, MEP and SNIP were not statistically significant as a risk factor for PAH in SLE. There was a significant correlation between MIP and MEP (Spearman Rho test p value 0.00) in the whole lupus cohort (Figure 5-1). The correlation between BMI and MIP was strong (Spearman Rho test p=0.005) (Figure 5-2) and that between BMI and MEP was weak correlation (p=0.045).

**Table 5-2 Respiratory pressure measurements (RPM) in SLE patients with and without PAH.**

Respiratory pressure measurements (RPM)	PAH (n=11)	Non PAH (n=254)	Fishers t test (2-sided) p value
MIP $\leq$ 40 cm H <sub>2</sub> O, No. of patients (%)	5 (45)	70 (28)	0.302
MEP $\leq$ 40 cm H <sub>2</sub> O, No. of patients (%)	3 (27)	38 (15)	0.384
MIP and MEP $\leq$ 40 cm H <sub>2</sub> O, No. of patients (%)	2 (18)	29 (11)	0.623
MIP and/or MEP $\leq$ 40 cm H <sub>2</sub> O, No. of patients (%)	6 (55)	79 (31)	0.183

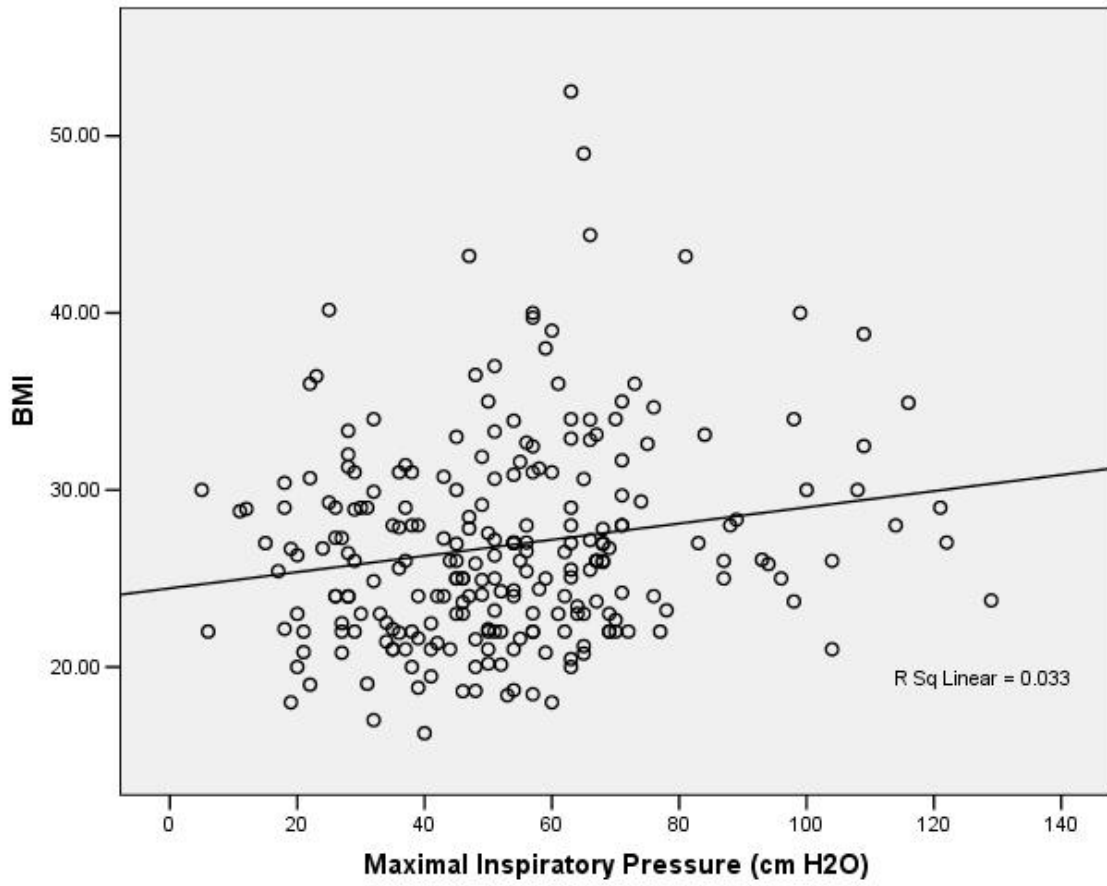
MIP: Maximal inspiratory pressure; MEP: Maximal expiratory pressure

**Figure 5-1 Scatter plot to show the correlation between maximal inspiratory pressure and maximal expiratory pressure.**



Spearman Rho test p value 0.00

**Figure 5-2 Scatter plot to show the correlation between maximal inspiratory pressure and body mass index (BMI).**



Spearman Rho test p value 0.007.

## **5.4 Results: Pulmonary screening tests for PAH in SLE**

### **5.4.1 Respiratory symptom questionnaires**

#### **5.4.1.1. Modified MRC dyspnoea score**

Two hundred and seventy nine patients completed modified MRC questionnaire (Appendix 5). There were 67% patients with dyspnoea score 1-4 in the whole lupus cohort, including 83% in the PAH group and 66% in the non-PAH group (Table 5-3). There was no significant difference in the dyspnoea status between patients with and without PAH in our cohort.

#### **5.4.1.2. Baseline dyspnoea index (BDI)**

Two hundred and fifty eight patients including 11 patients with PAH completed BDI questionnaire (Appendix 6) and there were no significant difference in the dyspnoea scores in all 3 categories between patients with and without PAH (Table 5-4). The BDI total score (range 0-12) is the sum of scores from all three categories in this index. The median BDI total score was 9 for the whole cohort as well as for patients with and without PAH subgroups. This suggests that most patients in this study had mild dyspnoea only, as the maximum total score of 12 is indicative of no dyspnoea with ordinary tasks.

#### **5.4.1.3. St. George's respiratory questionnaire score**

Among the 279 lupus patients who completed the symptom category of SGRQ questionnaire (Appendix 7), 264 completed the activity category and 265 completed the impacts category of the questionnaire. Thus the total score could be calculated in 258 patients. The scores ranges from 0-100 in each of the category, and none of the scoring categories differentiated SLE patients with and without PAH as the statistical significance were not reached (Table 5-5).

**Table 5-3 Modified Medical Research Council (MRC) dyspnoea score in SLE patients**

MRC dyspnoea score	SLE, no. of patients (%)	
	PAH absent (n=267)  No. of patients (%)	PAH present (n=12)  No. of patients (%)
0	91 (34)	2 (17)
1	103 (38)	7 (59)
2	58 (22)	1 (8)
3	10 (4)	2 (16)
4	5 (2)	0 (0)
n: Total number of patients in each group; Score 0: No dyspnoea; Score 4 : severe dyspnoea		

**Table 5-4 Baseline dyspnoea index (BDI) in patients with and with PAH in SLE.**

		BDI dyspnoea scores				
SLE patients	BDI dyspnoea categories	0	1	2	3	4
PAH absent (n=247)  No. of patients (%)	Functional impairment	6 (2)	13 (5)	29 (12)	76 (31)	119 (48)
	Magnitude of task	4 (1)	19 (8)	78 (32)	72 (29)	74 (30)
	Magnitude of effort	2 (1)	24 (10)	85 (34)	66 (27)	67 (27)
PAH present (n=11)  No. of patients (%)	Functional impairment	0 (0)	0 (0)	1 (9)	4 (36)	4 (36)
	Magnitude of task	0 (0)	1 (9)	1 (9)	6 (55)	3 (27)
	Magnitude of effort	0 (0)	0 (0)	5 (45)	2 (18)	3 (27)

Score 0: very severe impairment; 1: severe impairment; 2: moderate impairment; 3:

Mild impairment; 4: No impairment. n: Total number of patients in each group



**Table 5-5 St. George’s respiratory questionnaire scores in patients with and without PAH.**

	SLE	SLE without PAH	SLE with PAH (n=12)	p value (Mann Whitney test)*
Total score (n=258)	19 (0-82)	19 (0-82)	24 (3-78)	0.50
Symptoms score (n=279)	17 (0-100)	17 (0-100)	19 (7-82)	0.32
Activity score (n=264)	35 (0-100)	35 (0-100)	38 (6-100)	0.83
Impacts score (n=265)	9 (0-87)	9 (0-87)	11 (0-64)	0.54

Variables are expressed in median and range in brackets. n= Number of SLE patients.

\*Mann Whitney U test p value comparing median scores between SLE patients with and without PAH. n: Number of patients in each group

### **5.4.2. Pulmonary function tests (PFT)**

Pulmonary function tests were performed in 222 patients with lupus in this cohort, and 207 had all the parameters including spirometry, lung volumes and diffusion factor measured. The remaining 15 patients were unable to complete the tests due to respiratory symptoms limiting their performance of the test. The spirometry parameters included forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC), lung volumes included total lung capacity (TLC), and diffusion capacity included total diffusion capacity adjusted for haemoglobin (TLCO) and TLCO adjusted for alveolar volume (KCO).

Pulmonary function test parameters less than 80% predicted values were considered low. Restrictive pulmonary defect was defined by low predicted FVC <80% and low predicted TLC <80%. Obstructive defect was defined by decreased FEV1 predicted <80% and ratio of FEV1/FVC less than 0.8(231).

#### **5.4.2.1. PFT in SLE cohort**

The median age of the patients who underwent PFT was 41.5 years (range 18-82 years) and the median disease duration was 8.7 years (range 0-32 years). The only median PFT parameter that was below 80% predicted value was TLCO (median 74%, range 16-127%) and low TLCO was found in 130 (59%) patients in this SLE cohort. An isolated reduction of TLCO <80% predicted was found in 73 (33%) patients with SLE (Table 5-8). Reduction of TLCO and FVC less than 80% was present in 30 (13%) patients. The obstructive defect was found in 5 (2%) patients and restrictive defect was found in 24 patients (11%). All PFT parameters were normal in 70 patients (32%). The majority (85%) had no dyspnoea on performing ordinary tasks. Patients with low TLCO were dyspnoeic (19%) more than those with normal TLCO (9%) but the difference was not significant (Fisher's exact test  $p=0.053$ ). The only association

of low TLCO with BILAG disease activity score was found for haematologic activity (Pearson chi square  $p=0.001$ ). The weak association of low TLCO with anti-Sm antibody was also evident (Fishers exact test  $p=0.047$ )

**Association with dyspnoea:** All SLE patients in this study were graded by NYHA functional class according to their symptoms limiting physical activity. We compared patients with dyspnoea (classes II, III, IV) and those without dyspnoea (Class I) in this cohort and found that all parameters except KCO were significantly lower in those with dyspnoea. However, TLCO was the only parameter that was below 80% in both groups, with 63% in those with dyspnoea compared to 77% in those without dyspnoea (Table 5-6).

**Association with lung disease:** All PFT parameter except KCO predicted were significantly lower in patients with SLE related lung diseases compared to those without lung diseases (Table 5-7).

**Table 5-6 Pulmonary function test parameters in SLE patients with and without dyspnoea.**

	SLE	SLE without dyspnoea (Group I)	SLE with dyspnoea (Group II)*	p value  (Mann Whitney test comparing Group I and II)
No. of patients who had PFT	222	189	33	
FEV1, % predicted	96 (21-143)	98 (30-143)	83 (21-127)	0.00
FVC, % predicted	103 (20-157)	104.5 (45-157)	89 (20-129)	0.001
TLC, % predicted	94 (36-156)	96 (52-156)	88 (36-127)	0.005
TLCO, % predicted	74 (16-127)	77 (32-127)	63 (16-96)	0.00
KCO, % predicted	85.5 (43-130)	86 (46-130)	82 (43-128)	0.319

FEV1: Forced expiratory volume in 1 second. FVC: Forced vital capacity. TLC: Total lung capacity. TLCO: Total diffusion capacity. KCO: Transfer coefficient.

\*Group II had NYHA dyspnoea classes' II-IV.

Values are expressed as median and range in brackets, unless otherwise specified.

**Table 5-7 Pulmonary function test parameters in SLE patients with and without related lung diseases\***

	SLE without lung disease  (Group I)	SLE with related lung diseases  (Group II)	p value  (Mann Whitney test comparing Group I and II)
No. of patients who had PFT	210	12	
FEV1, % predicted	97 (31-143)	66 (21-116)	0.00
FVC, % predicted	104 (47-157)	66.5 (20-111)	0.00
TLC, % predicted	95 (56-156)	87 (36-98)	0.001
TLCO, % predicted	76(35-127)	47 (16-72)	0.00
KCO, % predicted	86 (46-130)	75 (43-117)	0.207

FEV1: Forced expiratory volume in 1 second. FVC: Forced vital capacity. TLC: Total lung capacity. TLCO: Total diffusion capacity. KCO: Transfer coefficient.

Values are expressed as median and range in brackets.

\*SLE related lung diseases such as pleural disease including effusion, fibrosis and thickening, interstitial lung disease and shrinking lung syndrome.

#### **5.4.2.2. PFT in SLE patients with and without PAH**

All twelve patients with PAH and 210 patients without PAH had undergone lung function testing. None of the parameters were significantly different between patients with and without PAH. The median values of PFT parameters are shown in Table 5-8. The box plot analysis of PFT parameters for patients with and without PAH is shown in Figure 5-3. Though the median predicted TLCO % was below 80% in both groups, the low TLCO values between patients with and without PAH were not statistically significant.

The numbers of patients with isolated reduction of predicted TLCO less than 80% was found in 73 (35%) patients without PAH and 3 (25%) patients with PAH in SLE (Table 5-9). All PFT parameters were normal in 66 (31%) patients in the non-PAH group and 4 (33%) patients in the PAH group. The restrictive defect was present in 23 patients in non-PAH group and 1 patient in PAH group. The obstructive defect was found only in non-PAH group in 5 patients. TLCO <55% predicted was found in 2/12 and 23/206 patients with and without PAH respectively (Fisher's exact p=0.633). The FVC/TLCO ratio >1.4 in 6/12(50%) patients with PAH and 88/201(44%) patients without PAH and the difference was not significant between groups (Fisher's exact 2-sided test, p value 0.768)

**Association with dyspnoea:** There were 3 out of 12 (25%) patients with dyspnoea in PAH group and 30 out of 210 (14%) patients with dyspnoea in the non-PAH group.

The median TLCO predicted was 66% in SLE patients with PAH. Patients with symptomatic PAH had median TLCO predicted value of 64% compared to 62% in those with asymptomatic PAH and there were no significant difference between them.

The difference in the PFT parameters between patients with and without PAH in relation to presence or absence of dyspnoea did not reach statistical significance.

**Association with lung disease:** There was one patient (8%) in the PAH group and 11 patients (5%) in the non-PAH group with pre-existing lung disease who underwent PFT. Again none of the parameters showed any significant difference between patients with and without PAH in relation to presence or absence of lung disease.

**Correlation of TLCO and sPAP:** There was no correlation of TLCO predicted % with systolic pulmonary artery pressure (sPAP) in patients with SLE, including those with and without PAH in SLE.

**Table 5-8 Pulmonary function test parameters in SLE patients with and without PAH.**

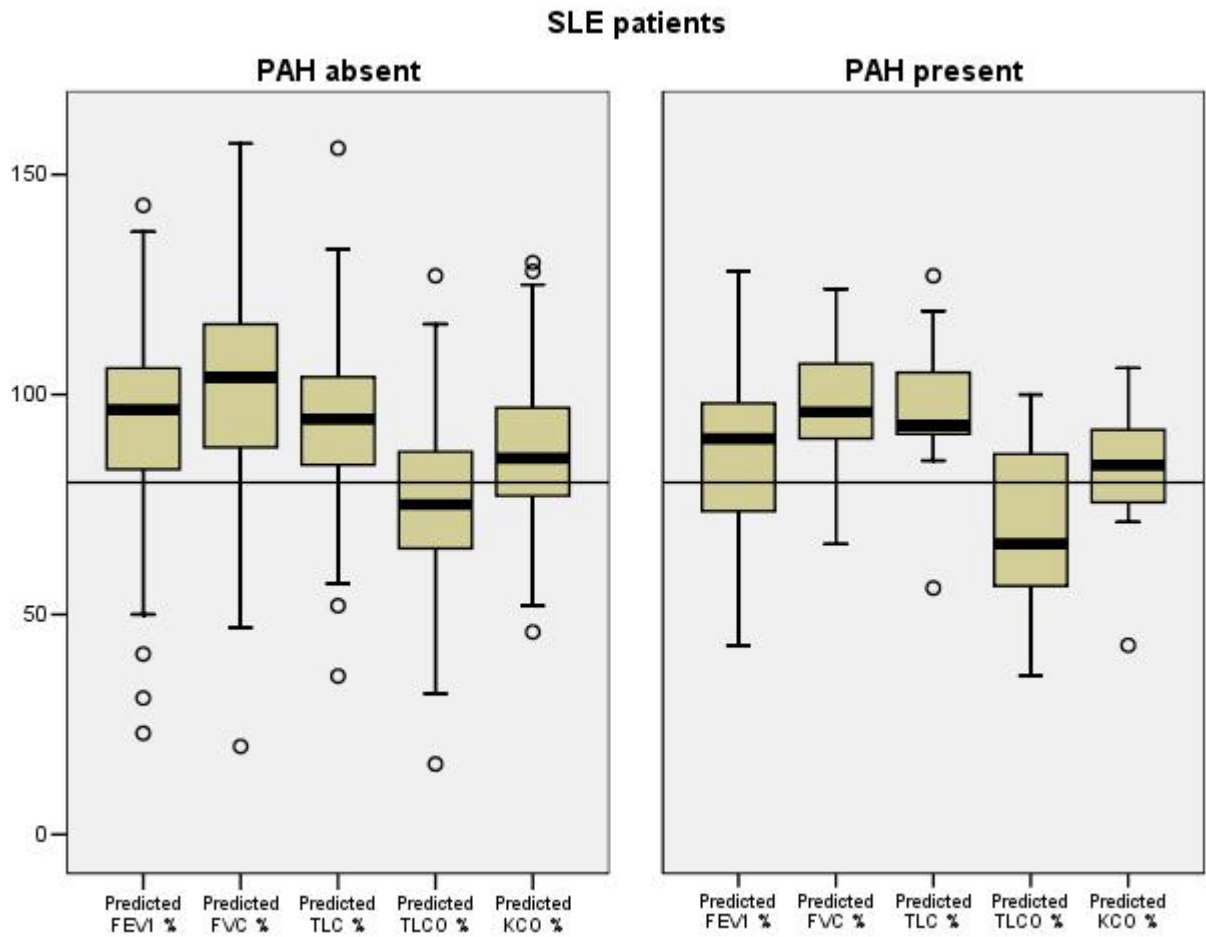
	SLE	SLE without PAH (Group I)	SLE with PAH (Group II)	p value (Mann Whitney test comparing Group I and II)
No.of patients who had PFT	222	210	12	
FEV1, % predicted	96 (21-143)	96 (21-143)	90 (43-128)	0.101
FVC, % predicted	103 (20-157)	103 (20-157)	96 (66-124)	0.435
TLC, % predicted	94 (36-156)	94 (36-156)	93 (56-127)	0.624
TLCO, % predicted	74 (16-127)	75 (16-127)	66 (36-100)	0.263
KCO, % predicted	85.5 (43-130)	85.5 (46-130)	84 (43-106)	0.492

FEV1: Forced expiratory volume in 1 second. FVC: Forced vital capacity. TLC: Total lung capacity. TLCO: Total diffusion capacity. KCO: Transfer coefficient.

Values are expressed as median and range in brackets.



**Figure 5-3 Box plot analysis of pulmonary function tests in SLE patients with and without PAH.**



Reference line drawn at 80%; FEV1: Forced expiratory volume in 1 second. FVC: Forced vital capacity. TLC: Total lung capacity. TLCO: Total diffusion capacity. KCO: Transfer coefficient.

**Table 5-9 TLCO predicted % in association with normal TLC, FVC and FEV1 predicted% values.**

	TLCO predicted %					
	SLE without PAH			SLE with PAH		
	<80	<70	<60	<80	<70	<60
No. of patients (%)	122 (58)	73 (35)	33 (16)	8 (67)	7 (58)	4 (33)
With FVC predicted >80%, No. of patients (%)	90 (43)	46 (22)	13 (6)	7 (58)	6 (50)	3 (25)
With TLC predicted >80%, No. of patients (%)	89 (42)	46 (22)	15 (7)	7 (58)	6 (50)	3 (25)
With TLC and FVC predicted >80%, No. of patients (%)	80 (38)	40 (19)	11 (5)	7 (58)	6 (50)	3 (25)
With TLC, FVC and FEV1 predicted > 80%, No. of patients (%)	73 (35)	35 (17)	10 (5)	3 (25)	2 (17)	1 (8)

FEV1: Forced expiratory volume in 1 second. FVC: Forced vital capacity. TLC: Total lung capacity. TLCO: Total diffusion capacity. KCO: Transfer coefficient.

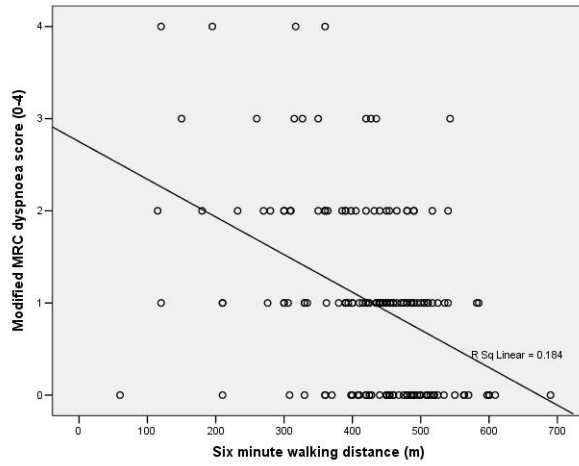
### **5.4.3. Six minute walking test**

This test was performed by 11 patients in the PAH group and 266 patients in the non-PAH group. The median distance walked in 6 minutes (6MWD) in PAH group was 435 m (range 150-600 m) and in non-PAH group was 440.5m (range 60-729). Six patients were unable to perform this test due to walking difficulties, fatigue or refusal to perform it. There was no significant correlation between 6MWD and sPAP in either group.

The pre-exercise fatigue score on modified Borg scale [Appendix 5] was 0 (no fatigue) in 71 (25%) patients, score 0.5-2 (mild fatigue) in 119 (43%), score 3 (moderate fatigue) in 54 (19%) and score  $\geq 4$  (severe fatigue) in 34 (12%) patients with SLE. The fatigue and dyspnoea scores pre- and post-exercise were not significantly different between patients in the PAH and non-PAH group.

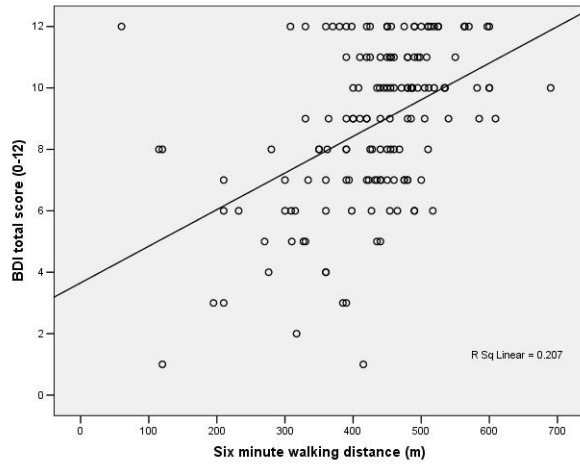
The 6MWD correlated significantly with the total scores from all three respiratory questionnaires (modified MRC, BDI and SGRQ) (Figure 5-4, 5-5, 5-6) with spearman rank test p value of 0.0 for each questionnaire. Similar significance was also reached between 6MWD and NYHA functional classes.

**Figure 5-4 Scatter plot of correlation between six minute walking distance and modified MRC dyspnoea scale.**



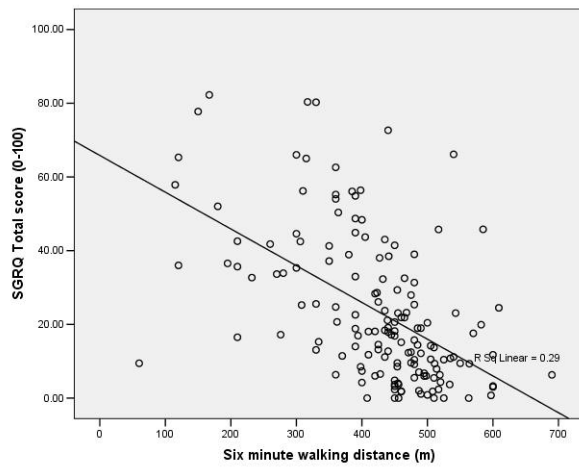
MRC score 4 refers to sever dyspnoea and 0 refers to being asymptomatic. Spearman Rank test p value 0.0

**Figure 5-5 Correlation of six minute walking distance and Baseline Dyspnoea Index (BDI) total score.**



BDI score 0 refers to severe dyspnoea and 12 refers to no dyspnoea with ordinary activities. Spearman Rank test p value 0.0

**Figure 5-6 Scatter plot of correlation between six minute walking distance and St. George's respiratory questionnaire (SGRQ) total score.**



SGRQ score 100 refers to severe dyspnoea and 0 refers to being asymptomatic.  
Spearman Rank test p value 0.0

## **5.5. Discussion**

### **5.5.1 Pre-existing lung disease**

In this study, the presence of pre-existing lung diseases had no predictive value for PAH in SLE. The lung diseases such as interstitial lung disease, pulmonary embolism, and pulmonary veno-occlusive disease can result in secondary PH in SLE. The low prevalence of these lung diseases (ILD in 2.4%, PE in 2.1% and PVOD in 0%) in this lupus cohort could have resulted in the lack of significant association of prior lung diseases and PAH in SLE. In a multiethnic US cohort(322). 7.3% had pulmonary damage after mean disease duration of 5.3 years based on SLICC/ACR damage scores recorded in 626 lupus patients. The pulmonary damage included pulmonary fibrosis in 3.9%, pleural fibrosis in 1.2%, pulmonary infarction and shrinking lung syndrome in 0.6% patients each and PH in 1.9% patients. Pan et al(169) reported the prevalence of secondary PH in SLE due to ILD and/or PE is 1.5% from a retrospective analysis on 786 SLE patients and also concluded both PAH and secondary PH in SLE is equally common with a prevalence of 3% each. 5/24 patients in that study had PH secondary to ILD. But in systemic sclerosis, the prevalence of PH (sPAP>40 mmHg) secondary to ILD was 18% and PH without ILD was 22% in a cohort of 197 patients(324). There was no significant difference in the prevalence of PH between limited and diffuse type of systemic sclerosis. There is a lower prevalence of pre-existing lung diseases in SLE compared to SSc that can predispose to secondary PH, and this is reflected in the low PH frequency both in this present study as well as US registry report.

### **5.5.2 Smoking**

Two thirds of this cohort has never smoked, and there was no significant difference in the smoking status between patients with and without PAH.

### 5.5.3 Respiratory muscle strength

The respiratory muscle weakness was present in 32% in this study cohort which is the largest SLE cohort where RMS has been assessed. This finding is of potential interest in SLE patients as it is the first time that respiratory muscle weakness has been quantified in a large cohort of SLE patients. The subgroup analysis showed 55% of patients with PAH and 31% without PAH in SLE had respiratory muscle weakness but the difference in MIP or MEP between the groups did not reach statistical significance. This is possibly due to small numbers and predominantly mild rise in sPAP in the PAH subgroup. Scano et al(325) reported mild reduction in respiratory muscle strength in 9 SLE patients, with median MIP 61 cmH<sub>2</sub>O (range 43-80 cmH<sub>2</sub>O) and median MEP 90 cmH<sub>2</sub>O (46-139 cmH<sub>2</sub>O). Jacobelli et al(233) described reduced MIP in more than 50% of their study patients In this present study, the corresponding values were much lower with median MIP 53 cmH<sub>2</sub>O (range 5-129 cmH<sub>2</sub>O) and median MEP 61 cmH<sub>2</sub>O (15-185 cmH<sub>2</sub>O). Though the median MIP and MEP values in this study did not reach the values we defined for respiratory muscle weakness (<40 cmH<sub>2</sub>O), they were much lower than the normal values published in studies using mouth flange type pressure meter(242) and relationship to age and sex (326). This would suggest our lupus cohort has moderate respiratory muscle weakness. The good correlation of BMI with respiratory muscle strength in this study suggests the association of low BMI with respiratory muscle weakness previously reported(327). Polymyositis, steroid myopathy, myasthenia gravis or myasthenia like condition, and peripheral neuropathy are other reasons considered in the respiratory muscle weakness in SLE(325). The concept of systemic fatigue and physical deconditioning leading to respiratory muscle fatigue can be considered in SLE patients with respiratory muscle weakness. In this study, the systemic fatigue score that was evaluated by modified Borg scale prior to 6MWT showed moderate or severe fatigue



in 32%, mild fatigue in 43% and no fatigue in 25%. The subanalysis (data not shown) showed that the correlation between fatigue scores and MIP (Spearman Rho test  $p=0.276$ ) or MEP (Spearman Rho test  $p=0.215$ ) were not significant. The difference in the fatigue scores between those SLE patients with and without inspiratory muscle weakness (Mann Whitney U test  $p=0.758$ ) or expiratory muscle weakness (Mann Whitney U test  $p=0.290$ ) was also not significant. These results in our SLE cohort shows there was no association between fatigue and respiratory muscle weakness.

In our study, the strong correlation between MIP and MEP is irrespective of sPAP, exercise capacity or NYHA functional classes. Meyer et al(237) reported that IPAH related respiratory muscle weakness was associated with low MIP and low MEP and were independent of haemodynamic parameters, exercise capacity and NYHA functional class. It was also proposed in IPAH that respiratory muscle weakness could either be a cause or consequence of IPAH due to chronic overload of respiratory musculature. Congestive cardiac failure has been associated with reduced inspiratory muscle strength(328;329). Inspiratory muscle weakness is considered to be a prognostic predictor in CCF(238). Our cohort had only 2 patients with LVEF <50% and none had RV dysfunction. Overall, the mild nature of PAH in this study could have resulted in the lack of association of respiratory muscle weakness with PAH in SLE.

#### **5.5.4 Screening tests for PH**

An ideal screening test for PH in SLE would be a non-invasive test that could be done periodically in predisposed patients so that aggressive surveillance to diagnose or detect progression of PH is possible at the earliest possible stage. The lung function tests, 6MWT and respiratory questionnaires are the screening tests for PH in SLE patients discussed below.

#### **5.5.4.1. Pulmonary function tests in SLE patients**

Similar to previous studies(229;330-332), this study also showed an increased prevalence of abnormal PFT in SLE patients. Reduction of TLCO was the most frequent PFT abnormality noted in 130/222 patients (59%) as found in previous studies (discussed below). Seventy-six (34%) patients had isolated TLCO reduction, 30 (14%) patients also had FVC reduction and 21 (9%) patients also had restrictive pattern with both FVC and TLC reduced. Overall in this lupus cohort, only 3/33 patients with reduced FVC, 5/36 patients with reduced TLC, and 1/24 patients with both reduced FVC and TLC had normal TLCO. Any PFT abnormalities without reduced TLCO were seen in very few patients in this study. The median %predicted TLCO was 74% in this lupus cohort, 63% in those with dyspnoea and surprisingly 47% in those with pre-existing lupus related lung disease. Other lung function test parameters including reduced FEV1, FVC and TLC reached statistical significance in those with dyspnoea or prior lung disease in lupus patients compared to those without these features.

Since the early 70s, abnormal PFTs in SLE patients have been reported by several investigators. Reduced FVC in up to 60%, mostly accompanied with reduced TLCO was observed in earlier studies of smaller lupus cohorts(330;332). But larger cohorts and recent studies have reported reduced TLCO in 46-47% and reduced FVC in 8-32% in unselected SLE cohorts(229;230). Paran et al(333) reported a low KCO of <70% was found in 41% of lupus patients who were asymptomatic. Chick et al(334) reported from a lupus cohort of 24 SLE patients that 90% had pulmonary dysfunction in the presence of lung disease and 71% in the absence of dyspnoea or history of lung disease. Grennan et al (330) reported 14/22 lupus patients had abnormal PFT and only 29% of those were symptomatic. Groen et al(304) showed that 40% had normal PFT,

10% had isolated TLCO reduction and 40% had restrictive defect in a cohort of 48 SLE patients. Another study (332) on 43 SLE patients reported reduced TLCO in 72% and reduced lung volumes in 49%. The possibility of subclinical respiratory dysfunction associated with reduced TLCO in a non-smoking cohort of SLE has also been reported previously(331). In this present study, the median TLCO predicted% was 74 (range 16-116) among 139 SLE patients who were non-smokers.

In 2012, Allen et al reported on 110 SLE patients with abnormal lung function in 66%, respiratory symptoms in 63% and shrinking lung syndrome in 10%. Previously in 2002, Nakano et al(229) reported on a Japanese cohort of 110 lupus patients with low TLCO in 47% and restrictive pattern with FVC <80% predicted in 8%. The prevalence of pulmonary fibrosis was 13% determined by clinical and/or chest x-ray or high resolution CT chest. Over 80% had abnormal PFT in the absence of clinical pulmonary disorder. They also noted that impaired TLCO was more prevalent in the presence of pulmonary fibrosis (PF) or with restrictive pattern PFT, and isolated TLCO reduction was seen in 39% in the absence of PF or restrictive defect. Our study results are very similar to that reported by the Nakano et al. The majority of patients in this present study were not dyspnoeic (85%) and were non-smokers (64%) and very few had prior restrictive lung disease (5%). This raises an important question if this subclinical impairment of pulmonary function, particularly in asymptomatic patients, has any prognostic value in predicting overt ILD.

Sant et al(335) found increased prevalence of HRCT abnormalities (72%) in a lupus cohort of 29 SLE patients, and they were mostly ILD (38%) who often were asymptomatic (31%). Of those who were asymptomatic with a normal CXR and normal PFT, 26% were found to have HRCT evidence of ILD. Nakano et al(229) performed HRCT in addition to CXR in 110 SLE patients with ILD prevalence of

13%. Traynor et al(323) showed 33% prevalence of HRCT proven ILD and 81% of these patients were asymptomatic. Only 5 patients in our PFT study group had undergone HRCT making it difficult to draw any conclusion in this study. The possibility of subclinical ILD in SLE was thus considered highly prevalent in SLE which would partly explain the high prevalence of abnormal PFT seen in SLE. It is possible that abnormal lung function may contribute to hypoxic muscle fatigue in SLE patients.

Disease activity and PFT in SLE patients: Both in adult(336) and paediatric lupus patients(337), disease activity was found to be inversely related to TLCO. In a cohort of 34 severe refractory SLE patients(323), improvement of PFT with stem cell transplantation was evident. Few other studies neither found this correlation(229;332) nor found an improvement with pulmonary function following control of disease activity. These variations of low TLCO with SLE disease activity could be due to the measure of SLE disease activity index used and the number of patients included in these studies. In this study, we have shown a strong association of BILAG disease activity scores for haematologic involvement in patients with low predicted TLCO compared to those with normal predicted TLCO.

Association of TLCO reduction to anti-RNP antibody(229;304) and Raynaud's phenomenon(229) was not found in this study. However, a weak association of anti-Sm antibody in patients with low TLCO found in this study needs further evaluation in a large cohort to confirm this association.

#### **5.5.4.2. PFT in SLE patients with PAH**

There was no significant reduction of TLCO in those with PAH (median predicted% TLCO was 66%) compared to those without PAH. None of the PFT parameters were

significantly different between patients with and without PAH in relation to dyspnoea or prior lung disease. In this cohort, we have been unable to show the role of PFT as a screening test for PH in SLE, but this could just reflect that the PAH cohort is small, and the prevalence of dyspnoea and prior lung disease are also minimal.

Reduction in TLCO has been considered as a screening test for PAH in systemic sclerosis(116;117;338) and less often in IPAH(232). Ungerer et al(179) reported TLCO <40-55% in association with 87 SSc patients, and Steen et al(339) reported reduction in TLCO is a common finding in SSc and only 7% of those developed PH over a follow up of 5.4 years. Mukerjee et al(116) found positive predictive accuracy of TLCO <55% predicted to be adequate to diagnose advanced PAH but cannot be relied to exclude PH where the pre-test probability is high. The same study showed cutoff value of TLCO at 60% had sensitivity, specificity, positive predictive value and negative predictive value of 74%, 45%, 70% and 50% respectively. Chang et al(340) showed TLCO <50% predicted was associated with severe PH in SSc. The consensus from UK PH centres includes DLCO evaluation in the annual screening for PAH in SSc and MCTD, and right heart catheterisation should be performed if DLCO <50% in absence of ILD.

An isolated TLCO reduction in patients with SLE has been reported by several investigators. The UK PH centres registry reported that the reduction in TLCO is much worse in ILD related PH in SSc (mean 29%) and SSc related PAH (mean 41%) than SLE-PAH (mean 59%) from a cohort of 56, 259 and 28 patients in each disease group respectively. The same registry data showed that 86/484 (18%) patients with PH secondary to CTD were due to respiratory disease related and 2/3rds were SSc related ILD. In the US PAH registry, the reduction in TLCO was significant in CTD-PAH compared to IPAH (mean 45% vs 64%), as well as SSc-PAH compared to SLE-

PAH (mean 41% vs 53%). We have found that TLCO reduction in SLE does not differ between patients with and without PAH. This finding supports the previous study reports of low TLCO noted in SLE patients but the underlying cause of this and the clinical significance remains uncertain.

Steen et al(338) reported that TLCO %predicted <55 or a ratio of FVC/TLCO (% predicted) >1.4 is strongly associated with PH in SSc. They also found serial measurements over 15 years prior to diagnosis of PH in limited SSc showed mean TLCO %predicted reduced from 80% to 35%. In this present study, we did not find any predictive value for TLCO<55% predicted, FVC/TLCO ratio >1.4 or FVC/TLCO >2.0 when assessing PAH in this SLE cohort but this may have been due to the relatively mild PAH and small numbers of patients with significant PAH.

#### **5.5.5. Six minute walking test**

Six minute walk test is a useful measure in the evaluation of exercise limitation in pulmonary hypertension which might predict the severity of PAH. In this study we did not find any correlation between distance walked in 6MWT and sPAP in patients with SLE as well as in subgroups with and without PAH. The difference between the median distances walked in both subgroups was also not significant. A strong association between 6MWD and dyspnoea scores and NYHA functional class was found in this SLE cohort. In this study, 85% were asymptomatic in the whole cohort and 75% in the PAH subgroup. This partly explains why patients with and without PAH do not have significant difference with the distance walked in 6MWT. Another plausible reason is the predominance of mild PAH with sPAP <40mmHg in 75% of the PAH subgroup. 6MWT is a well standardized and reproducible test. The prognostic significance of distance walked and oxygen desaturation in 6MWT has been shown in IPAH(341). Walking distance < 332 m(342) or <250 m(147), and

oxygen desaturation >10% (343) are considered poor prognostic indicators in PH that increases the mortality risk 2-3 times higher. 6MWD is also used to assess response to treatment in PH.

#### **5.5.6. Respiratory symptom questionnaires**

In this study cohort, 85.5% of patients were graded NYHA functional class I as they were asymptomatic and had no limitations of activity for ordinary tasks. Among those with NYHA functional class I, the median modified MRC questionnaire was 1, median total BDI score was 10, and median SGRQ total score was 15.1 (median symptom score of 13.3, activity score of 29.5 and impact score of 5.95). These results suggest that few patients in NYHA functional class I category may have dyspnoea and that these respiratory questionnaires are very sensitive in identifying SLE patients with respiratory symptoms and/or limitations with activity. All the respiratory symptom questionnaires tested in this study did not reach statistical significance in differentiating patients with and without PAH. This result is not surprising given the low prevalence of PAH and predominantly mild PAH in our study cohort. This was reflected in the scores calculated by modified MRC scale, BDI and SGRQ questionnaires and confirmed that only a minority had significant dyspnoea of severe grades. Only 3(25%) patients were dyspnoeic in the PAH group with one in NYHA class I and two in NYHA Class II. Modified MRC scale and BDI have been validated in COPD, with a change in scale by  $\geq 1$  unit being representative of clinically significant change. The SGRQ scores have also shown good correlation with spirometry, 6MWD and MRC scale in patients with airways disease(290;291). These observations supported our objective to study the screening potential of these questionnaires to predict PAH in SLE. In this present study, only few patients were dyspnoeic and predominantly had mild degree dyspnoea and patients with PAH were

infrequent and had relatively mild PAH. So, it is not possible to evaluate these questionnaires as screening tools for PAH in this SLE cohort.

## **5.6. Conclusion**

The prevalence of pre-existing restrictive lung disease in lupus was 5%. Two-third never smoked, and the patients were predominantly in NYHA functional class I (85%). Respiratory muscle weakness was present in one-third and a strong correlation existed between inspiratory and expiratory muscle pressures in SLE. There were no respiratory risk factors identified for PAH in SLE in this study. Pulmonary function tests in patients with SLE revealed low TLCO in 55% and isolated low TLCO in 33%, and significant difference in all parameters noted between patients with and without dyspnoea or pre-existing disease. Pulmonary function test parameters, 6MWT distance, and respiratory questionnaires were not useful as screening tests for PAH in SLE in this cohort, with mild and infrequent PAH.



## CHAPTER 6 BIOMARKERS IN SLE ASSOCIATED PAH

### 6.1 Assessment of NT-proBNP concentration in SLE and PAH

#### 6.1.1 Abstract

**Hypothesis:** High concentrations of serum NT-proBNP are associated with cardiac dysfunction due to pulmonary hypertension in SLE.

**Objective:**

1. To evaluate serum NT-proBNP levels in SLE cohort, and assess characteristics in those with high NT-proBNP levels
2. To evaluate the role of NT-pro BNP as a screening test for PAH in SLE.
2. To correlate serum NT-proBNP levels with parameters used in assessment of PAH severity in SLE including systolic pulmonary artery pressure, six minute walking test (6MWT) distance, pulmonary function tests and NYHA functional classes.

**Methods:** We performed NT-proBNP assay on 81 lupus patients from our cohort including all 12 patients with PAH, 15 patients with systolic pulmonary artery pressure (sPAP) 26-30 mmHg, and 54 patients with sPAP  $\leq$ 25 mmHg who were age and sex matched.

**Results:** The median NT-proBNP concentrations in our lupus cohort were 88.3 (range 5-1413) pg/ml. Five out of 25 patients with high NT-proBNP values had

echocardiographic diagnosis of PAH. High NT-proBNP levels were strongly associated with SLICC /ACR damage scores  $>2$  ( $p=0.008$ ) and disease duration ( $p=0.037$ ). There was no statistical difference between the NT-proBNP concentrations in lupus patients with and without PAH. The subgroup analysis on patients with systolic PAP 26-30mmHg showed higher serum NT-proBNP concentrations in comparison to those with sPAP  $\leq 25$ mmHg, but was also statistically insignificant. Among the PAH subgroup, the correlation between serum NT-proBNP concentration and (i) sPAP (ii) 6MWT distance and (iii) pulmonary function test parameters were not statistically significant.

**Conclusion:** Serum NT-proBNP concentration in lupus patients is higher than in general population. SLE patients with high NT-proBNP levels are associated with longer disease duration and higher cumulative damage scores. Elevated serum NT-proBNP concentration is neither a significant predictor of PAH in our cohort, nor help in monitoring severity of PAH.

### **6.1.2 Introduction and Methods**

N-terminal pro-BNP has been shown to be a useful diagnostic marker of early PAH in patients with SSc(344). NT-proBNP is also a valuable prognostic marker in PAH and raised levels of NT-proBNP are directly related to severity of PAH in SSc(250) and idiopathic PAH(252). According to the manufacturer of the NT-proBNP assay, the normal reference range of NT-proBNP in adults is 64.7-278 pg/ml depending on age and sex. Please refer to section 1.3.7.1 for detailed introduction and section 2.7.4.1 for methods.

Statistical methods: The variables were not normally distributed and thus non parametric tests have been used for statistical analysis. The variables are expressed as median and range, or mean and standard deviation (SD). The medians of the groups were compared using Mann Whitney U test (MWU test), as the distribution of variables were not of normal distribution. The correlation of continuous variables was tested with Spearman rank correlation coefficient test. The categorical variables were compared using two-sided Fisher's exact test and Pearson's chi square test. The statistical significance was set at  $p < 0.05$ .

### **6.1.3 Results**

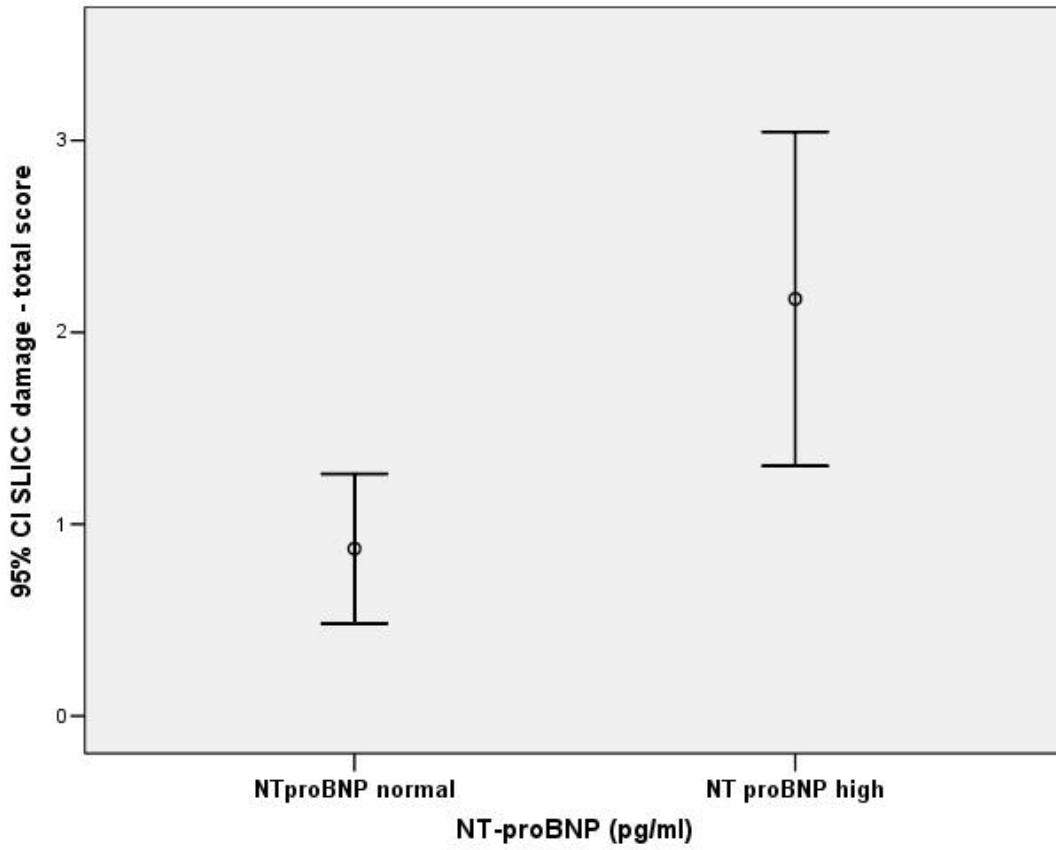
The median serum NT-proBNP concentration in our selected 81 lupus patients was 88.3pg/ml (range 5-1413pg/ml). The median age of these 81 patients was 44 years (range 30-82) and 89% were females. Raynaud's was ever present in 70%. The racial distribution was 59% Caucasian, 22% Asian and 17% Afro-Caribbean. The median disease duration was 9.9 years (range 0.4-26.7, mean 10.2 years).

### **6.1.3.1. Comparison of SLE patients with and without high NT-proBNP levels**

Twenty five patients with SLE had high concentrations of NT-proBNP for their age and sex (median 334.9pg/ml, range 93.1-1413pg/ml). Their median age was 59 years (range 30-82) and 92% were females. The difference in disease duration between patients with normal and high NT-proBNP levels reached statistical significance (median 9 years vs 11.6 years,  $p=0.037$ ). The median SLICC/ACR damage score in those with high NT-proBNP was 2, range 0-6 (mean  $\pm$  SD:  $02.17 \pm 2.01$ ) compared to median 0, range 0-7 (mean  $\pm$  SD  $0.87 \pm 1.33$ ) in those with normal NT-proBNP levels (Mann Whitney U test  $p$  value 0.008) (Figure 6-1). The median NT-proBNP levels was 118.9 pg/ml (range 12-1413 pg/ml) in those with SLICC score  $>2$  compared to median 63.1 pg/ml (range 5-1387 pg/ml) in those with SLICC scores  $\leq 2$  (Mann Whitney U test  $p$  value 0.036). The SLICC/ACR damage score  $>2$  was significantly associated with high NT-proBNP levels in our cohort (Fisher's exact test  $p$  value 0.037). There was a significant difference in the SLICC/ACR damage scores for cardiovascular system (MWU test  $p=0.045$ ), respiratory system (MWU test  $p=0.045$ ) and premature gonadal failure (MWU test  $p=0.042$ ) between patients with and without high NT-proBNP levels. There were no association between NT-proBNP levels and BILAG scores for individual systems including cardiorespiratory and renal systems.

NT-proBNP levels correlated significantly with six minute walk distance ( $p=0.023$ ), but there was weak association with sPAP ( $p=0.049$ ) and no association with NYHA functional classes. In PFT's, predicted TLCO% ( $p=0.014$ ) and predicted TLC% ( $p=0.039$ ) were statistically significant with high NT-proBNP levels (data not shown).

**Figure 6-1 Error bar of SLICC damage score in SLE patients with and without high NT-proBNP levels\***

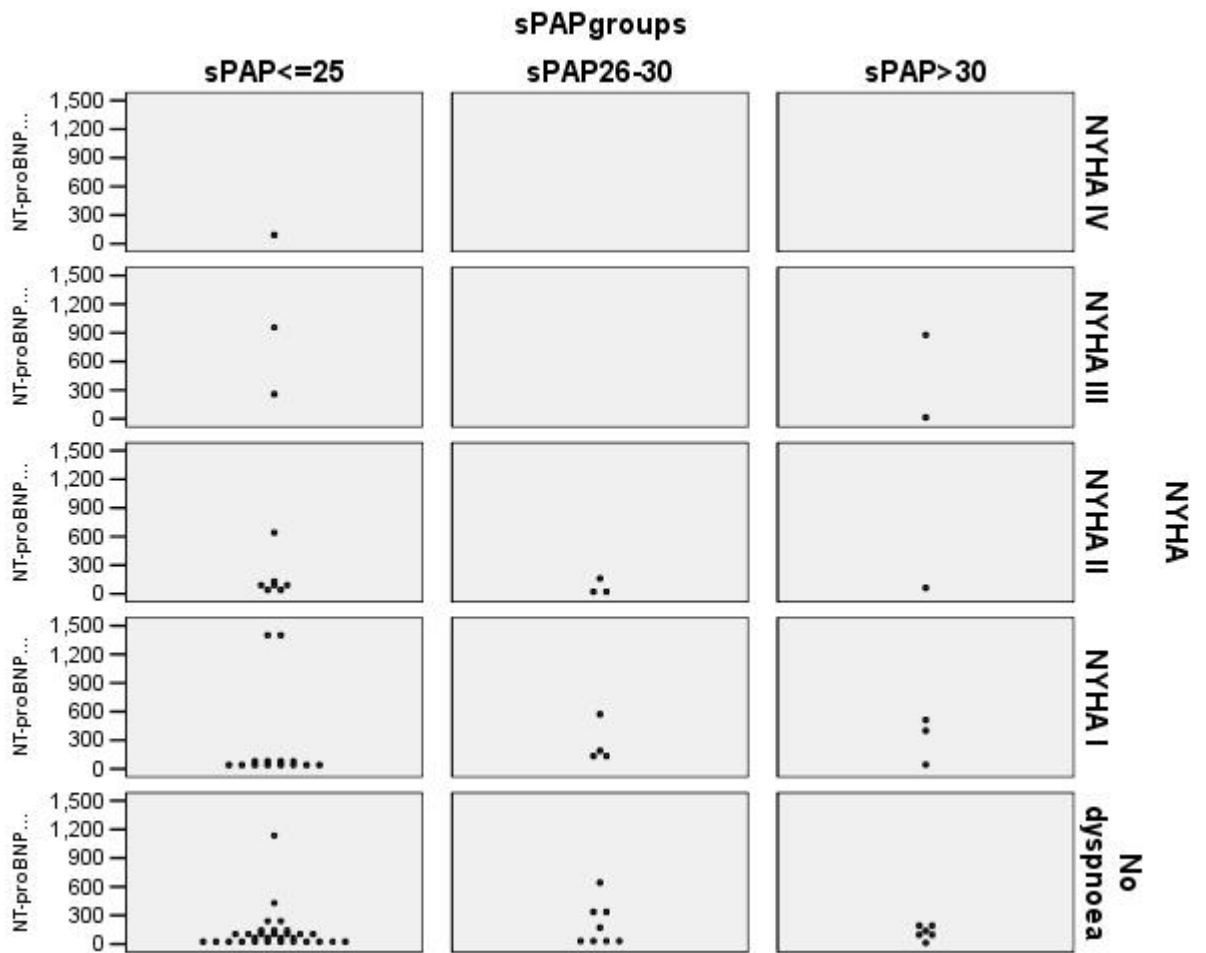


\*NT-proBNP levels (mean and 95% CI) shown in SLE patients with and without high NT-proBNP levels

### **6.1.3.2. Comparison of NT-proBNP concentration in SLE patients with and without PAH**

The characteristics of SLE patients with and without PAH are shown in Table 6-1. The variables of PAH severity including all lung function test parameters, NYHA functional classes, six minute walk test distance, SLICC/ACR damage index scores (total and individual systems) and BILAG disease activity scores were not statistically different between groups. The scatter plots of NT-proBNP concentration according to NYHA subclasses in lupus patients with sPAP < 25mmHg, 26-30mmHg and >30mmHg shows most patients had either no dyspnoea or were in NYHA classes I and II (Figure 6-2).

**Figure 6-2 NT-proBNP concentration (pg/ml) according to NYHA symptomatic subgroups and systolic pulmonary artery pressure (sPAP) subgroups**



**Table 6-1 Characteristics of SLE patients with and without PAH who underwent NT-proBNP assay**

Category	SLE patients with PAH (n=12)	SLE patients without PAH (n=69)
Age (years)	45.5 (30-72)	42 (30-82)
Sex (F:M) (% of no. of patients)	92:8	88:12
Disease duration (median, range in years)	9.6 years (1.9-22.1)	9.9 years (0.4-26.7)
Systolic PAP (median, range in mmHg)	35 (31-59)	10 (5-30)
NT-proBNP ( median, range in pg/ml)	121.35 (12-878)	83.7 (5-1413)
NYHA class I (No. of patients)	9	56
NYHA class II (No. of patients)	1	10
NYHA class III (No. of patients)	2	2
NYHA class IV (No. of patients)	0	1
6MWT ( median, range in meters) (n= number of patients)	435 (150-600) (n=11)	450 (60-609) (n=67)
FVC % predicted (n=67)	96 (66-124)	102 (23-143)
TLCO %predicted (n=68)	66 (36-100)	73 (44-112)



KCO %predicted (n=68)	84 (43-106)	84 (52-122)
SLICC damage score (median, range)	1 (0-7)	1.5 (0-5)
SLICC-CVS Score (no. of patients scoring 0/1/2)	11/0/1	64/4/1
SLICC-RS Score (no. of patients scoring 0/1/2)	10/1/1	63/5/1
BILAG cardiorespiratory (no. of patients in Group A/B/C/D/E )	0/0/4/47/17	0/0/2/8/2
No. of patients with NT-proBNP>125pg/ml (%)	6 (50%)	22 (31%)
No. of patients with NT-proBNP>150pg/ml (%)	5 (41%)	16 (23%)
No. of patients with NT-proBNP>395pg/ml (%)	3 (25%)	8 (12%)

None of the variables were statistically different between groups except the sPAP as expected. NYHA- New York Heart association functional class. 6MWT- six minute walk test. SLICC- Systemic Lupus International Collaboration clinics damage score. BILAG- British Isles Lupus Assessment Group. n: Number of patients. Variables are expressed as counts, or median and range.

The distribution of NT-proBNP concentrations in patients with and without PAH is shown in Figure 6-3. There was no statistical difference between NT-proBNP levels in SLE patients with and without PAH (median 121.3pg/ml vs 83.7pg/ml respectively). The number of patients with high NT-proBNP levels in each age and sex category was also not significant between both groups (Table 6-2). Interestingly, the subgroup analysis of those with sPAP 26-30mmHg (n=15) showed higher NT-proBNP values (median 137.6 pg/ml, range 15.8-642.3 pg/ml) compared to those with sPAP <25mmHg (n=54, median 70.75 pg/ml, range 5-1413pg/ml). Patients with high NT-proBNP levels were strongly associated with systolic PAP 26-30mmHg compared to those with sPAP <=25mmHg (p=0.027).

PAH was present in 11/72 females and 1/9 males in the cohort for NT-proBNP analysis. The difference in NT-proBNP levels in men and women with SLE also did not reach statistical significance (Table 6-3).



**Table 6-2 Analysis of relationship of PAH and NT-proBNP in different sex and age groups.**

	BNP normal for age (no. of patients)		BNP high for age (no. of patients)		P value
	PAH absent	PAH present	PAH absent	PAH present	
Age<50 female (n=50)	35	5	8	2	0.62
Age <50 male (n=4)	2	0	1	1	1.0
Age50-59 female (n=4)	3	0	0	1	0.25
Age50-59 female (n=3)	3	0	0	0	NS
Age>60 female (n=18)	4	2	11	1	0.24
Age>60 male (n=2)	2	0	0	0	NS

**Table 6-3 NT-proBNP concentrations in male and female patients with SLE.**

	NT-proBNP levels in females (n=72), Median (range) in pg/ml	NT proBNP levels in males (n=9), Median (range) in pg/ml
SLE without PAH (n=69)	89.1 (5-1413)	48.7 (11.3-118.9)
SLE with PAH (n=12)	114.4 (12-1878)	197.6

Number of patients in each subgroup. Mann Whitney U test p value- 0.12 in males, and 0.57 in females.

### **6.1.3.3 Correlation of NT-proBNP levels with parameters of PAH severity in SLE patients with and without PAH**

The NT-proBNP levels do not correlate with sPAP and ejection fraction calculated by echocardiogram, 6MWT distance, NYHA functional class and pulmonary function test parameters including FEV1, FVC, TLCO, KCO and TLC (percentage predicted values) in both subgroups of lupus patients, with and without PAH (Table 6-1).

### **6.1.4 Discussion**

Our study revealed that patients with SLE have higher concentrations of serum NT-proBNP, similar to findings in other lupus cohorts(253;345) which were published after our study period. In comparison to the median NT-proBNP concentration of 89pg/ml in our SLE cohort, the expected median for age and sex matched healthy population is 50pg/ml. Also the median NT-proBNP concentration of the lupus patients with high NT-proBNP subgroup was considerably higher at 335pg/ml. In comparison, the expected median NT-proBNP level in similar aged females (median 50-59 years) is 66pg/ml according to assay manufacturer's evaluation of NT-proBNP level in blood donors (Elecsys Systems E170 proBNP assay 03121658122, Roche). The limitation of this study is the lack of control group to compare the NT-proBNP levels in SLE patients.

It is well established that plasma NT-proBNP concentration is a diagnostic, monitoring and prognostic biomarker for heart failure(346;347). NT-proBNP is also shown to be a useful biomarker to stratify cardiovascular risk in general population even in those without known cardiac disease(348) or overt clinical heart failure. This finding has also been shown in patients with rheumatoid arthritis (349) and SLE (253;345;350). In our study there were no significant association between NT-proBNP levels and echo parameters of cardiac failure such as left ventricular ejection

fraction and RV dysfunction (data not shown). With regards to individual system scores for cardiac and respiratory system, NT-proBNP level had a weak association with SLICC/ACR damage scores ( $p=0.045$ ) but no association with BILAG disease activity scores for these systems. This raises the possibility if lupus patients with high NT-proBNP level have in fact undiagnosed cardiac disease or increased susceptibility to cardiac disease. Goldenberg et al have recently published the role of NT-proBNP as a marker of cardiac disease and not vascular disease in SLE patients (350).

Chambers et al(351) followed up SLE patients for >10 years and reported SLICC/ACR damage accrual is associated with increased risk of mortality. In particular, pulmonary and renal damage scores accrual have been shown to predict poor outcomes (79). This study has shown a strong association of SLICC/ACR damage scores (overall) greater than 2 with high NT-proBNP levels. It is possible that NT-proBNP could predict damage accrual and indirectly morbidity and mortality in SLE patients but further studies are needed to confirm this finding. Plasma BNP and NT-proBNP have potent independent association with mortality in patients with IPAH(252) and systemic sclerosis associated PAH(250).

This study was performed in 2007 and was the first to assess the screening potential of NT-proBNP for PAH in SLE, but failed to show any benefit with screening for PAH. This could be the effect of smaller group of SLE-PAH patients. Little is known about the potential use of BNP and NT-proBNP in lupus, and the literature predominantly reports on association of NT-proBNP with cardiac atherosclerotic disease in lupus. The significance of NT-proBNP as a diagnostic marker of early PAH has been established in systemic sclerosis and IPAH but not for SLE patients. The PAH registry from USA compared BNP values (pg/ml) in a cohort of 59 patients

with SLE (263.8+/-338.8) and 179 SSc patients with SSc (552.2+/-977.8pg/ml) and the difference was statistically significant (p=0.0004). The markedly elevated levels of BNP were specifically seen in SSc associated PAH, unlike other CTD associated PAH such as SLE and MCTD as well as IPAH. The high prevalence of myocardial fibrosis and renal insufficiency were speculated as reasons for this marked levels of BNP in SSC-PAH though not studied specifically in the US registry.

The correlation of NT-proBNP to pulmonary pressures in IPAH and SSc associated PAH has led to the use of NT-proBNP in monitoring progression of PAH. The non-invasive, inexpensive and convenient testing of NT-proBNP helps to avoid unnecessary repeated invasive right heart catheter studies in monitoring PAH. In our study, the very weak association in the correlation of NT-proBNP level and sPAP for the whole SLE cohort (p=0.049) did not reach statistical significance in the SLE-PAH subgroup (data not shown). This could be a reflection of most patients in PAH group had mild PAH, with 75% of patients having sPAP 30-40mmHg. Similarly, the correlation was significant between NT-proBNP and distance walked in six minute test for the whole SLE cohort (p=0.023) but was not significant in subgroup analysis of SLE patients with and without PAH. NYHA functional classes help identify patients with significant limitation of function secondary to cardio-respiratory symptoms. However this limitation in function in SLE could relate not just to cardiac and respiratory diseases but also due to musculoskeletal manifestation, fatigue, and other systemic involvement. I did not find any significant correlation between NT-proBNP level and NYHA classes. This could be due to patient numbers in NYHA III and IV classes were very few or none. Overall, none of the PAH severity parameters including sPAP, 6MWT distance, NYHA functional classes and lung function test parameters do not correlate with NT-proBNP level in our SLE-PAH subgroup



analysis. This may reflect the low numbers of PAH patients and the relatively low pulmonary artery pressures they had.

SLE patients are at high risk of atherosclerotic disease despite absence of other cardiovascular risk factors. The good correlation of longer disease duration ( $p=0.037$ ) and higher SLICC scores ( $p=0.017$ ) with high NT-proBNP levels in our whole SLE cohort could well be a reflection of longer exposure to inflammatory disease with resultant damage accrual and increasing the susceptibility for ischemic myocardial disease. It is important to note the absence of BILAG disease activity 'A' and 'B' scores as well as minimal cardiorespiratory SLICC/ACR damage scores recorded in our cohort does not exclude subclinical atherosclerotic disease. The prevalence of high NT-proBNP level is considerably high around 40% both in SLE patients with and without PAH in our study. The interesting and potential question to answer in future prospective studies in SLE patients would be to understand the reasons for elevated NT-proBNP concentration and its value in identifying cardiac disease earlier in SLE. In 2008, Chung et al(253) reported on 113 SLE patients comparing with 80 controls and found high concentrations of NT-proBNP in SLE patient but did not find any association with atherosclerotic burden, augmentation index or inflammatory state in SLE. In 2012 another report on 124 SLE patients concluded high NT-proBNP levels in SLE (median 82.5pg/ml) similar to our study result (median 88.3pg/ml) and reported NT-proBNP to be a marker of ventricular dysfunction but not atherosclerosis, however in our study the number of patients with ventricular dysfunction was very low (see Chapter IV, table 4-2,4-3).

I have shown that patients with sPAP<25mmHg are different from those with sPAP 26-30mmHg with regards to normal and high NT-proBNP level. Though patients in both subgroups do not fulfil diagnostic criteria for PAH by echocardiogram, the

significantly increased number of patients with high NT-proBNP level in the latter group ( $p=0.027$ ) suggests the need for evaluating NT-proBNP in stratifying risk for PAH in this borderline group. Thakkar et al(352) have reported recently on patients with systemic sclerosis who are at high risk for PAH and have proposed screening algorithm for PAH combining NT-proBNP  $> 209.8\text{pg/ml}$ , TLCO $<70\%$  and FVC/TLCO $\geq 1.82$  as cut-off for high risk for PAH.

The association of TLCO predicted % and TLC predicted % in patients with high NT-proBNP levels, especially with only 5 patients in the cohort with SLICC/ACR pulmonary damage scores  $>0$  (mean 1.2) is intriguing. Further evaluation of lung function test parameters either alone or in combination with NT-proBNP level in a larger SLE cohort with PAH or with high risk for PAH would help identify their screening potential for PAH. Allanore et al (353) have reported from a prospective cohort of SSc patients that NT-proBNP level and pulmonary function test parameter KCO are independent predictors of PAH.

### **6.1.5 Conclusion**

Our study confirms high levels of serum NT-proBNP are seen in SLE patients without overt cardiac disease or cardiac failure. High NT-proBNP level in SLE have a strong association with SLICC damage scores  $> 2$  and longer disease duration. I have shown that high levels of NT-proBNP neither have a screening nor monitoring potential in mild PAH associated with SLE but this needs further studies in larger cohorts of SLE-PAH with more severe PAH disease.

## 6.2. Fractalkine in SLE

### 6.2.1 Abstract

**Hypothesis:** Inflammation plays a role in the pathogenesis of PAH associated with SLE.

**Objective:**

1. To determine soluble Fractalkine(sFkn) concentration in patients with SLE
2. To compare sFkn concentration in SLE patients with and without PAH
3. To assess relationship of sFkn levels with BILAG disease activity scores and SLICC/ACR organ damage scores
4. To assess relationship of sFkn concentration with lupus disease activity monitoring tests such as dsDNA titre and complement C3 and C4 levels and other autoantibodies associated with SLE.

**Methods:** We performed sFkn assay by sandwich ELISA method in all our 283 patients with SLE. The mean cut-off point for detection of plasma sFkn was 0.57ng/ml, and those below this sFkn concentration were recorded undetectable by this assay.

**Results:** Forty five patients (15.9%) with SLE had detectable levels of sFkn with median concentration of 1.653ng/ml (range 0.559-18.114). The sFkn levels were detected in 25% patients with PAH and 15.5% patients without PAH. There was no significant difference between sFkn concentration in lupus patients with and without PAH. Though the association of sFkn with BILAG disease activity scores for vasculitis ( $p= 0.036$ ) reached statistical significance, it is unlikely to be of clinical

significance because none scored BILAG “A” and 2 patients in each group scored BILAG ‘B’. The association of sFkn with anti-Ro antibody ( $p=0.042$ ) reached statistical significance.

**Conclusion:** Soluble Fractalkine concentration is increased in patients with SLE and maybe a serological biomarker for active vasculitis in SLE but more patients with active disease are needed to confirm this finding. sFkn is not associated with PAH in SLE.

### 6.2.2 Introduction and Methods

Please refer to section 1.3.7.2 for detailed introduction and section 2.7.4.2 for methods. The reference value of soluble Fractalkine in healthy controls is  $0.003 \pm 0.003$  ng/ml (mean  $\pm$  standard error of mean) (256).

#### Statistical methods

The continuous variables were expressed as median and ranges, and/or mean  $\pm$  standard deviation (S.D). Non-parametric tests were used as the variables were not normally distributed. The comparison between groups was performed using Mann Whitney U test, and the correlation between variables were performed using Spearman Rank correlation test. The categorical variables were compared between groups using Fisher's exact (2 sided) or Pearson's chi-square tests. The p value  $< 0.05$  was considered significant.

### 6.2.3. Results

Among the 283 lupus patients in our study, 91% were females, median age 41 years (range 22-75) and median disease duration of 10.2 years (range 0.1-27.6 years)

Forty-five out of 283 patients with SLE (15.9%) had detectable levels of sFkn. The sFkn levels were raised in 42 (25%) and 3 (15.5%) patients with and without PAH respectively (p=0.4). The median plasma sFkn concentration for the whole cohort was 0 ng/ml (range 0-18.1 ng/ml) and mean  $\pm$ S.D was  $0.54 \pm 0.12$  ng/ml. Though the median sFkn concentration in lupus patients with and without PAH was 0 ng/ml, the mean sFkn concentration in lupus patients were higher in those with PAH (mean  $\pm$ SD,  $0.79 \pm 0.65$  ng/ml) than without PAH (mean  $\pm$ SD,  $0.53 \pm 0.12$  ng/ml; p=0.413). The difference in median sFkn concentration between patients with and without PAH was not significant (Table 6-4).

The detectable sFkn levels were associated with BILAG disease activity scores only for vasculitis in SLE ( $p=0.036$ ), but all other organ system scores did not reach statistical significance with sFkn. The overall SLICC/ACR damage score and individual organ damage score analysis showed no relationship with sFkn levels.

The correlation of sFkn levels with dsDNA titre, complement C3 and C4 were also not significant. Among the autoantibody associations, anti-Ro antibody was the only autoantibody that reached statistical significance with sFkn levels ( $p=0.042$ ).

**Table 6-4 Characteristics of SLE patients with and without PAH who underwent plasma sFkn assay.**

	SLE	SLE without PAH	SLE-PAH	p value (Mann Whitney U test unless specified)
No. of SLE patients underwent sFkn assay , no. of patients	283	271	12	-
sFkn detected, no. of patients(%)	45 (15.9%)	42 (15.5%)	3 (25%)	0.413 (Fisher's)
Age (years)	41 (18-82)	41 (18-82)	45.5 (30-72)	0.22
Sex (M:F)	266:17	255:16	11:1	-
Disease duration (years)	8.7 (0-32)	8.6 (0-32)	9.6 (1.9-22.1)	0.95
sFkn concentration ,median and range (ng/ml) [mean±SD in ng/ml)	0 (0-18.114) [0.54±0.12]	0 (0-18.114) [0.53±1.2]	0 (0-7.867) [0.79±2.25]	0.433
Systolic PAP, mmHg	9 (5-46)	8.9 (5-29)	37 (32-46)	0.00

C3 (g/l)	1.11 (0.16-2.68)	1.11 (0.16-1.68)	0.97 (0.77-1.27)	0.071
C4 (g/l)	0.2 (0.04-0.68)	0.2 (0.04-0.68)	0.17 (0.11-0.40)	0.499
dsDNA titre (kU/l)	20 (7-3887)	20 (7-3887)	109.5 (12-446)	0.083

Variables expressed in median and range and unless specified. sFkn- serum Fractalkine

p value- comparison between groups of SLE patients with and without PAH.



**Table 6-5 Characteristics of SLE patients with and without raised plasma sFkn levels.**

	Plasma SFkn elevated	Plasma sFkn undetectable	p value (Mann Whitney U test unless specified)
No. of SLE patients (n)	45	238	
Age (years)	41.5 (18-82)	41 (22-75)	
Sex (Male: Female)	41/4	225/13	
Disease duration (years)	10.2 (0.1-27.6)	8.35 (0-32)	NS
Plasma sFkn levels , median and range (ng/ml) mean $\pm$ S.D	1.653 (0.559-18.114) (0.54 $\pm$ 1.94)	-	
dsDNA titres	20 (12-245)	20 (7-3887)	NS
Complement C3 (g/l)	1.15 (0.6-1.71)	1.1 (0.16-2.68)	NS
Complement C4 (g/l)	0.23 (0.05-0.48)	0.2 (0.04-0.68)	NS
BILAG neurologic disease (score A/B/C/D/E)	0/0/1/30/14 (n=45)	0/1/10/150/73 (n=234)	NS

BILAG vasculitis (score A/B/C/D/E)	0/2/1/29/13	0/2/32/150/50	0.036 (Pearson Chi-square)
Anti-Ro antibody, no.of patients positive	23 (51%)	79 (33%)	0.042 (Fisher's exact test)

Variables expressed as counts, median and ranges; BILAG: British Isles lupus  
assessment group; NS- Not significant

#### 6.2.4. Discussion

In this study, I have shown that raised sFkn levels are not associated with PAH in SLE. Thus, sFkn levels do not have a significant role in the screening for PAH risk in SLE. As the absolute concentration of sFkn is higher in the PAH group compared to the non-PAH group in SLE, I performed sub analysis only on patients with detectable sFkn and found that the mean ( $\pm$ SD) levels were 3.15 ( $\pm$ 4.1) ng/ml and 3.4( $\pm$ 3.8) ng/ml in both groups respectively with no significant difference.

Fractalkine is considered to play a role in the pulmonary vascular remodelling in PAH. This was evidenced in laboratory research by increased expression of Fractalkine in the inflammatory cells surrounding pulmonary arterial lesions and smooth muscle proliferation(254). A subset of IPAH patients have been shown to have circulating autoantibodies as well as elevated levels of circulating proinflammatory cytokines and chemokines including sFkn(354). In particular, it is thought that inflammation plays a significant role in subsets of PAH associated with SLE and human immunodeficiency virus (HIV). Thus I aimed to test the hypothesis that inflammatory chemokine Fractalkine is associated with PAH in SLE. But my study has not shown a significant association between Fracktalkine and PAH in SLE, which could be due to very few patients in the PAH subgroup who predominantly had mild PAH.

Yajima et al(256) reported that serum sFkn levels were higher in SLE compared to rheumatoid arthritis or healthy controls, and the sFkn levels among 67 SLE patients were  $452\pm 118$  pg/ml (mean  $\pm$  standard error of mean, SEM). Similarly, Sato et al(255) reported increased sFkn levels in SLE compared to RA and primary Sjogren's syndrome. In comparison, the mean  $\pm$  SEM in our study with 283 SLE patients were  $540\pm 120$  pg/ml confirming similar raised levels of sFkn in SLE.

There are only a few studies which have assessed the relationship of sFkn in SLE (255;256;355). They have all reported on the significant correlation of sFkn levels in cerebrospinal fluid (CSF) but not serum sFkn levels with neuropsychiatric involvement in SLE (NPSLE). This follows the evidence that Fractalkine is found in inflammatory brain disease(356). Sato et al also reported serum sFkn levels were higher in those with NPSLE than those without NPSLE. The role of sFkn as a serologic marker of NPSLE disease activity and organ damage in patients with SLE as well as the reduction of sFkn levels with treatment of NPSLE are notable(256). In this study, I did not find any significant correlation of plasma sFkn level with neurologic disease activity scores (BILAG) or SLICC/ACR damage scores for NPSLE. This could be due to minimal number of patients having active NPSLE. There was only one patient with BILAG “C” score and none in “A” or “B” score in neuropsychiatric system in those with raised sFkn (Table 6-5). There were approximately 15% who had SLICC/ACR damage scores>0 with NPSLE in patients with and without raised sFkn levels (6 and 39 patients with mean score 1.33 vs 1.39 in each group respectively). Interestingly, I found a significant association between sFkn levels and BILAG disease activity scores for lupus vasculitis. This fits in with active and inflammatory disease in association with sFkn. It has previously been shown that active SLE patients have higher sFkn levels compared to inactive SLE patients. The BILAG disease activity scores for vasculitis included none with ‘A’ score and only 2 patients with “B” score in each group, which would suggest the statistical significance noted in our study would need confirmation in a future study including more lupus patients with active vasculitis.

I also found that anti-Ro antibody is significantly associated with raised sFkn levels. There has been no previous report of such an association with sFkn. Sato et al showed

sFkn levels in serum positively correlated with dsDNA titre, anti-Sm antibody, immune complex C1q levels and negatively correlated with CH50((256). I did not find any correlation of sFkn with dsDNA titres or complement C3 and C4 levels.

### **6.2.5. Conclusion**

Plasma Fractalkine levels were found to be raised in 15.9% of SLE patients. The elevated sFkn level was similar to previous reports on plasma sFkn level in SLE which were higher than RA. There was lack of association for plasma sFkn with PAH in SLE. The weak statistical association of active lupus vasculitis and anti-Ro antibody with sFkn needs confirmation with a larger cohort of active vasculitis patients in future.

## 6.3. Vascular Endothelial growth factor (VEGF) in SLE and PAH

### 6.3.1 Abstract

**Hypothesis:** Inflammation plays a role in the pathogenesis of PAH associated with SLE.

**Objective:**

1. To evaluate plasma VEGF concentration in patients with SLE
2. To compare plasma VEGF concentration in SLE patients with and without PAH
3. To assess relationship of VEGF level with BILAG disease activity scores and SLICC/ACR organ damage scores
4. To assess relationship of plasma VEGF concentration with lupus disease activity monitoring tests such as anti-dsDNA antibody titre and complement C3 and C4 levels, and other autoantibodies associated with SLE.

**Methods:** We performed plasma VEGF immunoassay by ELISA method on 81 lupus patients from our cohort including all 12 patients with PAH, 15 patients with systolic pulmonary artery pressure (sPAP) 26-30 mmHg, and 54 patients with sPAP  $\leq$ 25 mmHg who were age and sex matched.

**Results:** The median plasma VEGF concentration in our lupus cohort was 103 pg/ml (range 48-610 pg/ml). The median (range) plasma VEGF concentration in patients without and with PAH was 103 pg/ml (64-513) and 109 pg/ml (48-610) respectively (p=0.26). There was no correlation of VEGF concentration with systolic pulmonary artery pressure, anti-dsDNA antibody titres, complement C3 and C4 levels, and SLICC/ACR damage scores. The difference between plasma VEGF level in lupus

patients with active and inactive disease by BILAG scores was borderline significant for musculoskeletal system activity ( $p=0.033$ ).

**Conclusion:** Plasma VEGF concentration in lupus patients is higher than in general population. Patients with and without PAH in SLE do not show difference in VEGF concentration.

### **6.3.2 Introduction and Methods**

According to the manufacturer of VEGF assay used in this study, the reference range of VEGF levels I EDTA plasma is 0-115 pg/ml. Please refer to section 1.3.7.3 for detailed introduction and section 2.7.4.3 for methods.

#### **Statistical Methods:**

The variables tested were not normally distributed. The continuous variables were expressed as median and ranges. Thus comparison of continuous variables between groups was performed using Mann Whitney U test, and the correlation between variables were performed using Spearman Rank correlation test. The categorical variables were compared between groups using Fisher's exact (2 sided) or Pearson's chi-square tests. The p value < 0.05 was considered significant.

### **6.3.3. Results**

There were detectable levels of plasma VEGF in all lupus patients in this study. The median plasma VEGF concentration in our selected 81 lupus patients was 103 pg/ml (48-610 pg/ml) and their mean  $\pm$  S.D was  $133.9 \pm 92$  pg/ml. The median age of these 81 patients was 44 years (range 30-82), median disease duration was 9.9 years (range 0.4-26.7 years) and 89% were females.

The median plasma VEGF concentration in lupus patients with PAH was 109 pg/ml (range 48-610 pg/ml) and in patients without PAH was 103 pg/ml (range 64-513 pg/ml) (Figure 6-4). There was no statistical difference between the plasma VEGF level between groups with and without PAH in SLE (p=0.264). The distribution of plasma VEGF concentration and sPAP in SLE patients is shown in Figure 6-5.

The correlation of plasma VEGF level with BILAG disease activity scores and SLICC/ACR damage scores in our cohort did not reach statistical significance. In



lupus patients with PAH, plasma VEGF level had significant negative correlation with BILAG disease activity scores for cardiorespiratory system (Spearman rank test  $p=0.010$ ). In patients without PAH, plasma VEGF has a weak negative correlation with BILAG scores for musculoskeletal system ( $p=0.045$ ). None of the patients scored BILAG 'A' or 'B' in the cardiorespiratory system. One patient scored BILAG 'A' and 8 patients scored BILAG 'B' in the musculoskeletal system of which only one patient with score 'B' had PAH. To verify these correlation tests between VEGF level and BILAG score, I also performed an analysis comparing lupus patients grouped together as active (BILAG scores A or B) and inactive (BILAG scores C, D or E), and performed sub-analysis in patients with and without PAH. The only significant difference in the median plasma VEGF level between active and inactive SLE groups was for the musculoskeletal system in the BILAG disease activity scores (Table 6-6). However, this significance was weak (Mann Whitney U test  $p=0.036$ ) and the median plasma VEGF level were higher in the inactive group (76 pg/ml) compared to the active group (107.19 pg/ml). In the sub-analysis for patients with and without PAH, similar weak association was present only for musculoskeletal system activity in the non-PAH subgroup (Mann Whitney U test  $p=0.033$ )

The plasma VEGF level did not correlate with systolic PAP (Figure 6-5), anti-dsDNA antibody and complement C3 and C4 levels. There was no association of plasma VEGF level with autoantibodies related to SLE.

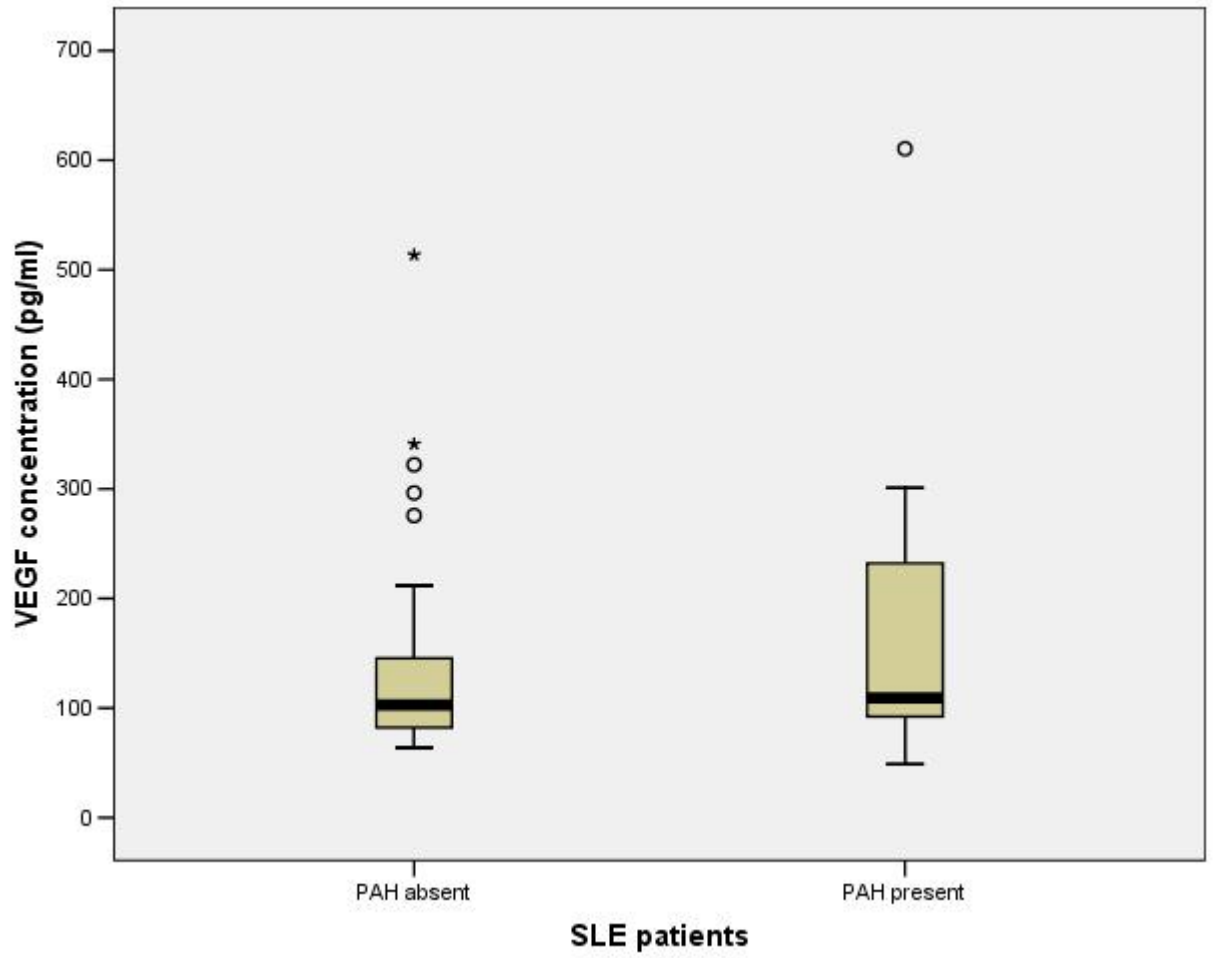
**Table 6-6 Plasma VEGF level in lupus patients with active and inactive disease by BILAG scores.**

Systems involved	Inactive SLE (BILAG score C,D or E)	Active SLE (BILAG score A or B)	p value comparing active and inactive SLE groups (Mann Whitney U test)
General, median pg/ml (range)  [No .of patients]	103.96 (49-610)  [80]	-  [0]	
Mucocutaneous, median pg/ml (range)  [No .of patients]	103.82 (49-610)  [75]	104.09 (67-174)  [5]	0.796
Neurological, median pg/ml (range)  [No .of patients]	103.96 (49-610)  [80]	-  [0]	
Musculoskeletal, median pg/ml (range)  [No .of patients]	107.19 (49-610)  [71]	76.34 (64-146)  [9]	0.036*

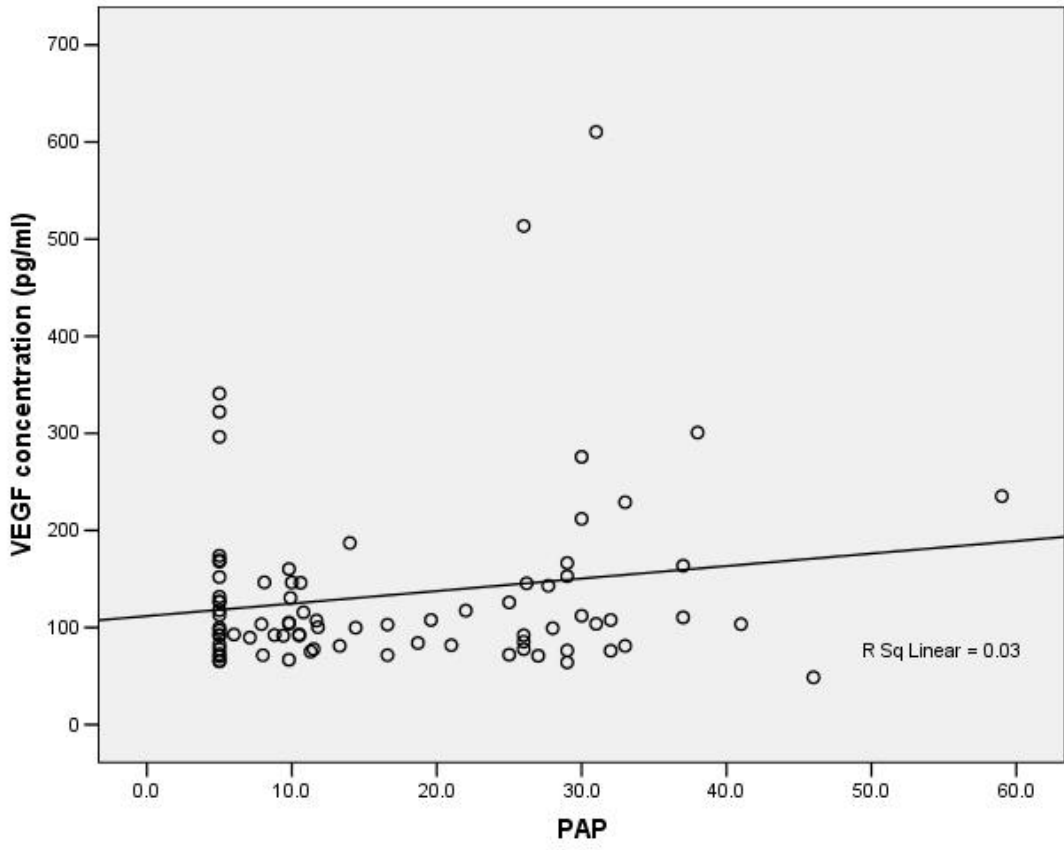
Cardiorespiratory, median pg/ml (range)  [No .of patients]	103.96 (49-610)  [80]	-  [0]	
Vasculitis, median pg/ml (range)  [No .of patients]	103.82 (49-610)  [79]	-  [1]	0.398
Renal , median pg/ml (range)  [No .of patients]	103.96 (49-610)  [74]	108.18 (92-301)  [6]	0.381
Haematological, median pg/ml (range)  [No .of patients]	106.4 (49-610)  [66]	96 (70-322)  [14]	0.352

\* p value <0.05

**Figure 6-4** Box plot graph of plasma VEGF concentration (pg/ml) in SLE patients with and without PAH.



**Figure 6-5 Scatter plot graph of plasma VEGF concentration (pg/ml) and systolic pulmonary artery pressure (mmHg) in SLE patients.**



#### 6.3.4. Discussion

I have shown that plasma VEGF level in SLE (median 103 pg/ml, range 48-610 pg/ml) are higher than in the general population as described by the VEGF assay manufacturer ((R&D systems, Abingdon, UK). The mean (S.D) serum VEGF level in 10 healthy controls was 330 (84) pg/ml in another study on 15 patients with SLE patients with PH(269). In an oncology study with a cohort of 300 patients, the median serum VEGF level in 145 healthy individuals were 294 pg/ml (range 30-1752 pg/ml)(357). I measured plasma VEGF as it has been considered that serum VEGF level could be spuriously high due to platelet release of VEGF during blood clotting. It has been reported previously that SLE patients have higher level of plasma VEGF and serum VEGF compared to controls. Navarro et al (266) showed that the median plasma VEGF level in 28 SLE patients was 70 pg/ml (range 5-341 pg/ml) compared to 23 pg/ml in 24 healthy controls. Robak et al(260) reported elevated plasma VEGF level in a cohort of 52 SLE with a median of 152.5 pg/ml (range 5-916 pg/ml). In another study of children and adolescents with SLE(358), the mean serum VEGF level in SLE was 579 pg/ml compared with 113 pg/ml in healthy controls. These studies on lupus patients confirm the VEGF levels are higher in serum compared to plasma. Our study suggests the high plasma VEGF level seen in SLE patients and is in fact higher in our cohort than the levels previously reported by Navarro et al. The limitation of this study is the lack of control group to compare the VEGF level seen in our SLE cohort.

However, I have not been able to show any difference in the concentration of VEGF levels between lupus patients with and without PAH. It has been debated if VEGF is protective or deleterious in PH, given that VEGF levels have been present both in normal pulmonary circulation and pathogenic lesions of PAH. Moreover, elevated

VEGF has been reported both in patients with PH as well as those receiving continuous prostacyclin infusions. In our study, the small numbers of patients in the PAH subgroup and that most patients had mild PAH could have been the reason for the lack of difference in the median between lupus patients with and without PAH.

Our study cohort did not have any significant difference in the plasma VEGF concentration between active (BILAG score A or B) and inactive (BILAG score C, D or E) disease subgroups (Table 6-6). The difference in the plasma VEGF level between active and inactive disease in the musculoskeletal system suggested that higher VEGF levels were associated with inactive disease. Though this association was statistically significant, it is unlikely to be of clinical significance given that elevated VEGF level being a marker of inflammation were found to be associated with inactive disease. The BILAG scores for the remaining 7 different systems did not show any association with plasma VEGF level. Robak et al have shown that serum VEGF levels are significantly higher in active (median 238 pg/ml) compared to inactive SLE (median 118 pg/ml) using systemic lupus activity measure (SLAM) to define disease activity as >15 points. Another study on lupus patients revealed that plasma VEGF levels were higher with lupus nephritis compared to those with absent renal disease. In this study, I did not find any such associations of plasma VEGF levels with lupus disease activity in any system. This could be possible as very few patients had BILAG scores A or B in any system. Two patients had BILAG 'A' score in our cohort with one each in mucocutaneous and musculoskeletal system. There were 3,8,1,6 and 14 patients scoring BILAG score 'B' in mucocutaneous, musculoskeletal, vasculitis, renal and haematologic systems respectively. None of the patients scored BILAG A or B in cardiorespiratory system. However, the comparison of patients with and without PAH showed that plasma VEGF level negatively

correlated with cardiorespiratory scores in the PAH subgroup and plasma VEGF level also negatively correlated with musculoskeletal scores in the non-PAH subgroup (Spearman Rank correlation test). The scatter plot analysis shows 2 patients in the PAH group to have BILAG disease score 'C' for cardiovascular system with median VEGF concentration of 90pg/ml compared to 137 pg/ml in the rest of PAH group with BILAG score D or E. Thus, the BILAG score associations with plasma VEGF level in cardiorespiratory and musculoskeletal systems in subgroups relate to PAH are both weak and clinically insignificant.

The lack of any correlation of plasma VEGF level with lupus disease activity monitoring tests such as anti-dsDNA antibody titres, complement C3 and C4 levels could also be a reflection of very few patients with active disease in our cohort at the time of assessment. None of the lupus associated autoantibodies showed any relation to plasma VEGF levels in our analysis. There have been no previous reports of any autoantibody association with VEGF in SLE.

### **6.3.5 Conclusion**

Patients with SLE may have higher concentrations of plasma VEGF. In a cohort with low disease activity and few patients with significant PAH, I did not show any significant associations with disease activity or level of PAH. Prospective longitudinal studies may be of interest however to address associations reported by others more definitively.



## CHAPTER 7 CONCLUSIONS

### 7.1 Summary of prevalence of PAH in SLE study

I have shown that the point prevalence of PAH was 4.2% (12 out of 283 patients) in our cohort of patients with SLE studied with TTE. This prevalence rate increases to 7.1% if only cases with detectable TR jet are included (60% of patients). Only 1/12 patients with PAH was known to have PAH prior to the study. Four other patients previously diagnosed with secondary PH did not meet criteria for PAH in this study, and 3/109 patients of the cohort who did not participate in the study were diagnosed with PAH previously based on TTE.

As most of the PAH cases (75%) were found to be of mild severity (<40mm Hg) and asymptomatic, they would not meet criteria for guidelines-based treatment. Only 1/3 patients with PAH fulfilled criteria for RHC (sPAP>40mmHg and WHO grade III/IV FC) according to study protocol, but refused to undergo RHC.

There was a weak association of PAH with history of APS but no such association was found between PAH and history of Raynaud's phenomenon or smoking status. The BILAG disease activity scores for any system evaluated were not significantly different but the total SLICC/ACR DI scores were different between SLE patients with and without PAH. Those SLE patients with total SLICC/ACR DI >2 were strongly associated with PAH. This suggests the possibility of previous disease activity contributing to higher damage scores and PAH.

The autoantibody that showed a strong significant association with PAH in SLE was presence of lupus anticoagulant, and a weak association was found with anti-La

antibody. Patients with PAH and SLE were found to be frequently treated with warfarin and calcium channel blockers prior to this study. The significant association of lupus anticoagulant and presence of antiphospholipid syndrome in PAH cases suggests that thrombosis may play an important role in PAH with SLE. This is important, as it is treatable.

It is currently unclear whether establishing the diagnosis of PAH in the pre-symptomatic phase improves outcome in SLE patients but is likely to be important in those contemplating pregnancy due to high risk of maternal mortality in pregnancy with PAH.

## **7.2 Summary of cardiovascular disease in SLE study**

Thickening of the anterior mitral valve was the most common cardiac morphological abnormality in our SLE cohort. Valvular regurgitation (tricuspid, mitral and/or aortic valve), left ventricular systolic dysfunction (LVSD) and concentric left ventricular hypertrophy (LVH) were the other common abnormalities detected on TTE. The majority of valvular abnormalities are mild without compromise in cardiac function. The benefits of infective endocarditis prophylaxis in patients with mild valvular disease who undergo dental surgery are not yet apparent.

A strong association between MR and combined positive LA and high titre aCL IgG and aCL IgM was present similar to previous reports suggesting association of valvular disease with antiphospholipid antibodies. In contrast to previous studies, acute or chronic pericardial disease was absent in our cohort at the time of assessment. Only 1 patients had active disease in cardiorespiratory system with

BILAG index score 'B', and 4.9% of patients had SLICC/ACR DI cardiovascular score of 1 or 2.

The majority of SLE patients had normal rate, rhythm and axis on ECG and the few abnormalities detected on ECG were not associated with BILAG index or SLICC/ACR DI.

The valvular abnormalities are predominantly of mild degree similar to previous reports. However, those patients particularly at high risk of infective endocarditis should be offered prophylaxis when necessary for gingivo-dental manipulation.

Left heart morphological and functional compromise was not associated with PAH in this cohort. There was no secondary cardiac cause for PH such as left heart dysfunction or severe valvular disease with compromised cardiac function detected on TTE in this SLE study cohort.

### **7.3 Summary of respiratory abnormalities in SLE study**

The prevalence of pre-existing restrictive lung disease related to lupus was 5% and history of PE was recorded in 6 patients (2 had PAH) in this study. Two-third never smoked, and predominantly was in NYHA functional class I (85%). Respiratory muscle weakness was present in one-third in SLE patients. A strong correlation existed between inspiratory and expiratory muscle pressures as well as BMI and MIP in SLE patients. Pulmonary function tests in patients with SLE revealed low TLCO in 55% and isolated low TLCO in 33%, and significant difference in all parameters noted between patients with and without dyspnoea as well as patients with and without pre-existing lung disease.

The respiratory questionnaires (modified MRC, BDI and SGRQ) showed that a third of the patients did not have dyspnoea and less than 10% with severe dyspnoea in the whole cohort. In the whole SLE cohort, the median 6MWD was 440m (range 60-729m), and 6MWD showed significant correlation with total scores from all respiratory questionnaires (modified MRC, BDI and SGRQ) suggesting the limitation of exercise capacity in those with severe respiratory symptoms.

The pulmonary risk factors including pre-existing RLD, history of smoking and respiratory muscle weakness were not associated with PAH in SLE patients. In patients with PAH, the median 6MWD was 435m (range 150-600m), the median % predicted TLCO was 66% (range 36-100) and only 17% had severe impairment recorded on respiratory questionnaires. Pulmonary function test parameters, 6MWT distance, and respiratory questionnaires may not have screening potential for PAH in SLE patients.

The respiratory risk factor/screening test assessment in SLE patients has either highlighted a new finding such as respiratory muscle weakness or confirmed the presence of findings noted previously in other studies such as reduction of TLCO predicted%. There was no evidence of secondary pulmonary cause for PH such as PE, ILD or PVOD but the limitation of this study is that patients were not fully investigated for these conditions with HRCT or pulmonary vascular imaging at the time of study. Though PFT and 6MWT have been recognised as complimentary screening and/or monitoring tests for PH in SSc and IPAH patients, it is highly possible that these tests did not establish their screening potential role in SLE related PAH due to the fact that PAH was infrequent and mostly mild degree disease in this cohort. The minimal number of SLE patients with pulmonary damage also led to

absent secondary PH from pulmonary interstitial and thromboembolic disease in this cohort.

## **7.4 Summary of Biomarkers in SLE associated PAH study**

### **NT-proBNP**

Our study confirms high levels of serum NT-proBNP are seen in SLE patients without overt cardiac disease or cardiac failure. The high NT-proBNP levels in SLE have a strong association with SLICC damage scores > 2 and longer disease duration. This may suggest the possibility of longer exposure to recurrent inflammatory disease with resultant damage accrual and increasing the susceptibility to ischemic heart disease. I have shown that high levels of NT-proBNP neither have a screening nor monitoring potential in mild PAH associated with SLE but this needs further studies in larger cohorts of SLE-PAH with more severe PAH disease.

### **Soluble Fractalkine**

Soluble Fractalkine concentration was shown to be increased in patients with SLE. The elevated sFkn levels were similar to previous reports on plasma sFkn levels in SLE which were higher than RA. Soluble Fkn may be a serological biomarker for active vasculitis in SLE. I did not find an association between sFkn levels and PAH in SLE patients. The association of anti-Ro antibody with sFkn was a new finding of interest. The lack of association of sFkn with active disease markers in lupus reflects the minimal activity recorded in these patients at the time of study and/or it is possible that the patients chose to attend the study visit only when they felt well and had minimal or absent activity of lupus disease.

## **VEGF**

Patients with SLE have higher concentrations of plasma VEGF. In a cohort with low disease activity and few patients with significant PAH, I did not show any significant associations with disease activity or level of PAH. Prospective longitudinal studies may be of interest however to address associations reported by others more definitively.

In summary, the role of NT-proBNP, sFkn and VEGF in predicting PAH in this SLE cohort is not evident but this is most likely to reflect the mild PAH seen in the few PAH cases diagnosed in this cohort.

## **7.5 Limitations of studies**

1. This is an uncontrolled study.
2. The patients studied might have been biased in only attending the study for echocardiogram and assessments while they were not suffering an acute flare. This could have reduced the point prevalence rate of PAH estimated in our cohort of SLE patients as well as reducing the level of disease activity measured. Certainly some patients rebooked their appointments on one or two occasions.
3. The infrequent and predominantly mild PAH identified in this cohort is potentially a major determinant of lack of association with various risk factors and screening tests assessed in PAH related to SLE.
4. The power calculation were performed to detect a significant difference in risk factor assessment between the sample sizes in this lupus cohort of 12 PAH cases and

271 non-PAH cases. The population value for the prevalence of a specific risk factor in lupus patients with and without PAH was assumed in order to perform the power calculation. If the prevalence value of a risk factor is 1%, 5%, 10% and 20% in SLE patients without PAH and the prevalence value of the same risk factor is at least 23%, 34%, 43% and 58% respectively for SLE patients with PAH, then there is 80% or more power to detect a significant difference at the 5% level between our study groups. The statement above holds true whatever the observed prevalence values for the risk factor studied. For example, in the general population the reported prevalence value for antiphospholipid antibodies in SLE is 30-40% in those without PAH and upto 80% in those with PAH. However, such wide differences were not present for all the risk factors assessed in this study.

### **7.3 Future research**

1. A prospective longitudinal long term controlled follow-up study of SLE patients with and without diagnosed PAH confirmed by RHC would help recruit a larger cohort of moderate and severe PAH associated with SLE. This would enable to assess risk factors and screening tests for PAH in patients with SLE in a meaningful and detailed manner.
2. The screening potential of 6MWT in SLE-PAH needs further evaluation in a larger cohort of SLE patients with PAH. This could be done either retrospectively comparing symptomatic SLE patients with and without PAH or prospectively screening symptomatic SLE patients with 6MWT along with periodic PFT and Doppler echo evaluation of sPAP and/or RHC evaluation of mPAP.

3. It would be useful to conduct a prospective longitudinal study in a large unselected SLE cohort to assess the relationship of TLCO to SLE disease activity clinically and on blood tests and to determine if low TLCO has any prognostic significance.
4. A prospective long term follow up study in a large cohort of SLE patients comparing PFTs and HRCT in relation to respiratory symptoms.
5. It would be useful to evaluate the screening potential of biomarkers such as NT-proBNP in combination with TLCO in a prospective controlled study of SLE patients, and also assess their prognostic significance in SLE patients with and without symptoms of PH.



## APPENDIX 1 1997 UPDATE OF THE 1982 ACR REVISED CRITERIA FOR CLASSIFICATION OF SLE

Criterion	Definition
1. Malar Rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Nonerosive Arthritis	Involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Pleuritis or Pericarditis	1. Pleuritis--convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion <i>OR</i> 2. Pericarditis--documented by electrocardiogram or rub or evidence of pericardial effusion
7. Renal Disorder	1. Persistent proteinuria > 0.5 grams per day or > than 3+ if quantitation not performed <i>OR</i> 2. Cellular casts--may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic Disorder	1. Seizures--in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance <i>OR</i>

	<p>2. Psychosis--in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance</p>
<p>9. Hematologic Disorder</p>	<p>1. Hemolytic anemia--with reticulocytosis OR</p> <p>2. Leukopenia--&lt; 4,000/mm<sup>3</sup> on <math>\geq</math> 2 occasionsOR</p> <p>3. Lymphopenia--&lt; 1,500/ mm<sup>3</sup> on <math>\geq</math> 2 occasions OR</p> <p>4. Thrombocytopenia--&lt;100,000/ mm<sup>3</sup> in the absence of offending drugs</p>
<p>10. Immunologic Disorder</p>	<p>1. Anti-DNA: antibody to native DNA in abnormal titer <i>OR</i></p> <p>2. Anti-Sm: presence of antibody to Sm nuclear antigen <i>OR</i></p> <p>3. Positive finding of antiphospholipid antibodies on:</p> <p>1. an abnormal serum level of IgG or IgM anticardiolipin antibodies,</p> <p>2. a positive test result for lupus anticoagulant using a standard method, or</p> <p>3. a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test</p>
<p>11. Positive Antinuclear Antibody</p>	<p>An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs</p>

## APPENDIX 2 CLASSIC BILAG INDEX

All features must be attributable to SLE and refer to last 4 weeks compared with prior disease activity

Indicate features which are present:

1) *Improving*

2) *same*

3) *worse*

4) *new*

*or Y/N or value*

*(where indicated)*

*(default is 0 =*

*not present)*

### GENERAL

- |                                     |     |
|-------------------------------------|-----|
| 1. Pyrexia (documented)             | ( ) |
| 2. Weight loss - unintentional > 5% | ( ) |
| 3. Lymphadenopathy/splenomegaly     | ( ) |
| 4. Fatigue/malaise/lethargy         | ( ) |
| 5. Anorexia/nausea/vomiting         | ( ) |

### MUCOCUTANEOUS

- |  |     |
|--|-----|
| 6. Maculopapular eruption - severe, active<br>(or discoid/bullous) | ( ) |
| 7. Maculopapular eruption - mild                                   | ( ) |
| 8. Active discoid lesions – generalised or<br>extensive            | ( ) |
| 9. Active discoid lesions - localised                              |     |

- |                                  |     |     |
|----------------------------------|-----|-----|
| Including lupus profundus        |     | ( ) |
| 10. Alopecia (severe, active)    |     | ( ) |
| 11. Alopecia (mild)              |     | ( ) |
| 12. Panniculitis (severe)        |     | ( ) |
| 13. Angio-oedema                 |     | ( ) |
| 14. Extensive mucosal ulceration |     | ( ) |
| 15. Small mucosal ulcers         |     | ( ) |
| 16. Malar erythema               |     | ( ) |
| 17. Subcutaneous nodules         |     | ( ) |
| 18. Perniote skin lesions        |     | ( ) |
| 19. Peri-ungual erythema         |     | ( ) |
| 20. Swollen fingers              | Y/N | ( ) |
| 21. Sclerodactyly                | Y/N | ( ) |
| 22. Calcinosis                   | Y/N | ( ) |
| 23. Telangiectasia               | Y/N | ( ) |

**NEUROLOGICAL**

- |   |  |     |
|---|--|-----|
| 24. Deteriorating level of consciousness                |  | ( ) |
| 25. Acute psychosis or delirium or<br>confusional state |  | ( ) |
| 26. Seizures  |  | ( ) |
| 27. Stroke or stroke syndrome                           |  | ( ) |
| 28. Aseptic meningitis                                  |  | ( ) |
| 29. Mononeuritis multiplex                              |  | ( ) |
| 30. Ascending or transverse myelitis                    |  | ( ) |
| 31. Peripheral or cranial neuropathy                    |  | ( ) |

32. Disc swelling/cytoid bodies ( )
33. Chorea ( )
34. Cerebellar ataxia ( )
35. Headache severe, unremitting ( )
36. Organic depressive illness ( )
37. Organic brain syndrome including  
pseudotumor cerebri ( )
38. Episodic migrainous headaches ( )

### **MUSCULOSKELETAL**

39. Definite myositis (Bohan & Peter) ( )
40. Severe polyarthritis - with loss of  
function ( )
41. Arthritis ( )
42. Tendonitis ( )
43. Mild chronic myositis ( )
44. Arthralgia ( )
45. Myalgia ( )
46. Tendon contractures and  
fixed deformity **Y/N** ( )
47. Aseptic necrosis **Y/N** ( )

### **CARDIOVASCULAR & RESPIRATORY**

48. Pleuropericardial pain ( )
49. Dyspnoea ( )
50. Cardiac failure ( )

51. Friction rub ( )
52. Effusion (pericardial or pleural) ( )
53. Mild or intermittent chest pain ( )
54. Progressive cxr changes-lung fields Y/N ( )
55. Progressive cxr changes-heart size Y/N ( )
56. ECG evidence of pericarditis or  
myocarditis Y/N ( )
57. Cardiac arrhythmias including tachycardia  
> 100 in absence of fever Y/N ( )
58. Pulmonary function fall by > 20% Y/N
59. Cytohistological evidence of  
inflammatory lung disease Y/N ( )

#### VASCULITIS

60. Major cutaneous vasculitis incl.ulcers ( )
61. Major abdominal crisis due to vasculitis ( )
62. Recurrent thromboembolism (excluding  
strokes) ( )
63. Raynaud's ( )
64. Livido reticularis ( )
65. Superficial phlebitis ( )
66. Minor cutaneous vasculitis  
(nailfold vasculitis, digital vasculitis,  
purpura, urticaria) ( )
67. Thromboembolism (excl. stroke)  
- 1st episode Y/N ( )

## RENAL

68. Systolic blood pressure (mm Hg) **value** ( )
69. Diastolic blood pressure (mm Hg) **value** ( )
70. Accelerated hypertension **Y/N** ( )
71. Urine dipstick protein (+ = 1, ++ = 2, +++ = 3) **value** ( )
72. 24 hour urinary protein (g) **value** ( )
73. Newly documented proteinuria > 1g / 24hrs **Y/N** ( )
74. Nephrotic syndrome **Y/N** ( )
75. Creatinine (plasma/serum) **value** ( )
76. Creatinine clearance/GFR ml/min **value** ( )
77. Active urinary sediment **Y/N** ( )
78. Histological evidence of active nephritis  
- within 3 months **Y/N** ( )

## HAEMATOLOGY

79. Haemoglobin g/dl **value** ( )
80. Total white cell count x 10<sup>9</sup>/l **value** ( )
81. Neutrophils x 10<sup>9</sup>/L **value** ( )
82. Lymphocytes x 10<sup>9</sup>/L **value** ( )
83. Platelets x 10<sup>9</sup>/L **value** ( )
84. Evidence of active haemolysis **Y/N** ( )
85. Coomb's test positive **Y/N** ( )
86. Evidence of circulating anticoagulant **Y/N** ( )

## APPENDIX 3 SLICC/ACR DAMAGE INDEX FOR SLE

Damage (non-reversible change, not related to active inflammation) occurring since diagnosis of lupus, ascertained by clinical assessment and present for at least 6 months unless otherwise stated. Repeat episodes must occur at least 6 months apart to score 2. The same lesion cannot be scored twice.

<b>OCULAR</b>	<u>Score</u>
Any cataract ever (documented by ophthalmoscopy)	<b>1</b>
Retinal change <b>OR</b> Optic atrophy (documented by ophthalmoscopy)	<b>1</b>
<b>NEUROPSYCHIATRIC</b>	
Cognitive impairment (eg memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance level) <b>OR</b> Major psychosis	
Seizures requiring therapy for 6 months	<b>1</b>
Cerebrovascular accident or surgical resection (for non-malignant causes) ( <i>score 2 if &gt;1</i> )	<b>1 2</b>
Cranial or peripheral neuropathy (excluding optic)	
Transverse myelitis	<b>1</b>
	<b>1</b>
<b>RENAL</b>	
Estimated/Measured GFR < 50%	<b>1</b>
Proteinuria $\geq$ 3.5g/24 hours	<b>1</b>
<b>OR</b>	
End-stage renal failure (regardless of dialysis or transplantation)	<b>3</b>



<b>PULMONARY</b>	
Pulmonary hypertension (right ventricular prominence or loud P2)	1
Pulmonary fibrosis (physical & radiograph)	1
Shrinking lung (radiograph)	1
Pleural fibrosis (radiograph)	1
Pulmonary infarction (radiograph) or resection (for non-malignant causes)	1
<b>CARDIOVASCULAR</b>	
Angina <b>OR</b> Coronary artery bypass	1
Myocardial infarction (score 2 if > 1)	1 2
Cardiomyopathy (ventricular dysfunction)	1
Valvular disease (diastolic, murmur, or systolic murmur > 3/6)	1
Pericarditis for 6 months <b>OR</b> Pericardiectomy	1
<b>PERIPHERAL VASCULAR</b>	
Claudication for 6 months	1
Minor tissue loss (pulp space)	1
Significant tissue loss (eg loss of digit or limb) (score 2 if > 1)	1 2
Venous thrombosis with swelling ulceration <b>OR</b> Venous stasis (clinical)	1
<b>GASTROINTESTINAL</b>	
Infarction or resection of bowel below duodenum, spleen, liver or gallbladder for any cause (score 2 if > 1)	1 2
Mesenteric insufficiency	1
Chronic peritonitis	1
Stricture <b>OR</b> Upper gastrointestinal surgery	1
	1

Pancreatic insufficiency requiring enzyme replacement	
<b>MUSCULOSKELETAL</b>	
Muscle atrophy or weakness	1
Deforming or erosive arthritis (including reversible deformities, excluding avascular necrosis)	1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	1
Avascular necrosis (imaging) <i>(score 2 if &gt; 1)</i>	1 2
Osteomyelitis (supported by culture evidence)	1
Tendon rupture	1
<b>SKIN</b>	
Scarring chronic alopecia	1
Extensive scarring or panniculum other than scalp and pulp space	1
Skin ulceration for > 6 months (excluding thrombosis)	1
<b>PREMATURE GONADAL FAILURE</b> (secondary amenorrhoea before age 40)	1
<b>DIABETES MELLITUS (regardless of treatment)</b>	1
<b>MALIGNANCY (exclude dysplasia site)</b> <i>(score 2 if &gt; 1)</i>	1 2

## APPENDIX 4 PAH IN SLE- STUDY PROTOCOL SYNOPSIS

Protocol synopsis for the study on PAH in SLE submitted to multi-centre research ethics committee, UK

TITLE	Determination of the prevalence and risk factors for PAH in patients with systemic lupus (SLE)).
OBJECTIVES	<p>To evaluate the prevalence of patients with symptomatic Pulmonary Arterial Hypertension (PAH) diagnosed by 3D ECHO Doppler / right heart cardiopulmonary catheterisation in a group of patients with systemic lupus erythematosus.</p> <p>To assess the prevalence of PAH in the different subtypes of SLE defined by autoantibodies (including overlap syndrome such as mixed connective tissue disease).</p> <p>To determine risk factors for PAH in the total population and the different sub groups of patients with SLE.</p> <p>To evaluate the influence of DLCO on the sensitivity and the specificity of the PAH screening algorithm in patients with SLE</p> <p>To assess the frequency of cardiac valve abnormalities and right and left ventricular dysfunction in patients with SLE and their risk factors</p> <p>To assess the correlation between the sPAP determined by Doppler echocardiogram and the resting mPAP measured by</p>

	right cardiac catheterisation, where medically indicated, such as in patients with moderate/severe PAH (>40 mm Hg).
INDICATION	SLE
METHODOLOGY	Prospective observational study
NUMBER OF PATIENTS	400 patients
POPULATION STUDIED	<p><b>Inclusion criteria:</b></p> <p>Male or female <math>\geq</math> 18 years</p> <p>SLE or mixed connective tissue disease</p> <p>Informed consent.</p> <p><b>Exclusion criteria:</b></p> <p>Pregnancy</p>
DURATION OF STUDY	<p>January 2004 to December 2005: cross-sectional study</p> <p>Follow up of patients with confirmed pulmonary hypertension for 5 years total (yearly assessment)</p>
OUTLINE OF STUDY	<p>The following data will be recorded:</p> <p>Clinical data (defined clinical criteria for disease activity and damage, SF36, and serology)</p> <p>Full lung function tests including DLCO (base hospital)</p>

	<p>Electrocardiogram</p> <p>Doppler Echocardiogram (Wellcome Trust CRF)</p> <p>Echocardiographic assessment of cardiac function to determine:</p> <p>Left ventricular systolic function</p> <p>Left ventricular diastolic function</p> <p>Left atrial and aortic measurements</p> <p>Aortic – valve, sinuses, sinotubula, ascending aorta</p> <p>Right ventricular systolic function</p> <p>Right ventricular diastolic function</p> <p>Valvular morphometry and dynamics</p> <p>Pulmonary valve – PA root dimension, PA – pressures</p> <p>Tricuspid valve</p> <p>Aortic valve</p> <p>Mitral valve</p> <p>Cardiac catheterisation (when clinically indicated). (Wellcome Trust CRF)</p> <p>Walking test (6 minutes)</p> <p>Respiratory muscle strength measurement</p> <p>Questionnaire assessing shortness of breath on exertion on a</p>
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	<p>scale of 1-10 (Borg scale) and transient dyspnoea index and/or a respiratory QOL score such as SGRQ.</p> <p>Detailed symptom questionnaire and history re smoking history will be included.</p>
TREATMENT	<p>NA – Non therapeutic study (but therapy taken by the patients will be recorded, in particular, calcium channel blockers, ACE inhibitors and related drugs, aspirin and warfarin)</p>
DEFINITIONS	<p>PAH: resting mPAP <math>\geq</math> 25 mm Hg (echocardiogram)</p>
STATISTICAL ANALYSIS	<p>Prevalence of PAH amongst the patients screened</p> <p>Prevalence of PAH for SLE (autoantibody subtypes)</p> <p>Correlation between ECHO Doppler and PAH confirmed on right heart catheter</p>

## APPENDIX 5 MODIFIED BORG DYSPNOEA SCALE

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight
3	Moderate
4	Somewhat severe
5	Severe
6	
7	Very severe
8	
9	Very, very severe (almost maximal)
10	Maximal

### Patient Instructions for Borg Dyspnoea Scale

“This is a scale that asks you to rate the difficulty of your breathing. It starts at number 0 where your breathing is causing you no difficulty at all and progresses through to number 10 where your breathing difficulty is maximal. How much difficulty is your breathing causing you right now?”

**Modified Borg Fatigue scale:** The same scale is used to score Fatigue level with appropriate instructions.

## APPENDIX 6 MODIFIED MRC DYSPNOEA SCORE

### Grade

- 0 “I only get breathless with strenuous exercise”
  
- 1 “I get short of breath when hurrying on the level or walking up a slight hill”
  
- 2 “I walk slower than people of the same age on the level because of breathlessness or have to stop for breath when walking at my own pace on the level”
  
- 3 “I stop for breath after walking about 100 yards or after a few minutes on the level”
  
- 4 “I am too breathless to leave the house” or “I am breathless when dressing”

NB: This is the modified MRC (Medical Research Council) scale that uses the same descriptors as the original MRC scale in which the descriptors are numbered 1-5.



# **APPENDIX 7 St.GEORGE'S RESPIRATORY QUESTIONNAIRE**

# APPENDIX 8 PREVALENCE OF PAH IN SLE (PUBLICATION)

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## Prevalence and risk factors for pulmonary arterial hypertension in patients with lupus

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**Objectives.** Pulmonary arterial hypertension (PAH) is associated with rapid deterioration and poor prognosis in SLE, especially during pregnancy. The prevalence of PAH in SLE in non-tertiary centres is uncertain. This study aims to estimate the point prevalence of PAH and identify risk factors for PAH in a large cohort of SLE patients.

**Methods.** A prospective cross-sectional study of 288 patients with SLE were recruited from lupus clinics in Birmingham, UK. Resting transthoracic echocardiography was performed to estimate the pulmonary artery pressures and to assess cardiac morphology and function. PAH was defined as systolic pulmonary artery pressure (sPAP) >30 mmHg. We assessed potential risk factors such as the presence of lung disease, respiratory muscle weakness, autoantibodies, smoking, RP and APS.

**Results.** Of 288 patients who consented for participation, 283 patients were suitable for analysis. Twelve patients were found to have PAH with sPAP >30 mmHg. The range of sPAP in our PAH patients was 31–59 mmHg and three patients had sPAP >40 mmHg. The only significant risk factor for PAH was LAC ( $P = 0.005$ ).

**Conclusions.** The point prevalence of PAH was 4.2% in our cohort of patients with SLE. Most of the PAH cases were found to be of mild severity (<40 mmHg). The significant association of LAC and presence of APS in PAH cases suggests that thrombosis may play an important role in PAH with SLE. This is important, as it is treatable.

**KEY WORDS:** Systemic lupus erythematosus, Pulmonary arterial hypertension, Prevalence, Echocardiography, Lupus anti coagulant, Screening, Risk factors, Anti-phospholipid antibodies.

### Introduction

SLE is a well recognized autoimmune multisystem disorder with frequent respiratory and cardiac manifestations that can lead to significant morbidity and mortality. Pulmonary hypertension is a serious complication that is associated with a significant risk of death especially in pregnancy in the early post-partum period [1–3]. In the last few years, new treatment modalities have been developed to improve the symptoms and prognosis of pulmonary hypertension [4, 5]. Most of these studies have been performed in idiopathic pulmonary arterial hypertension (PAH) patients and in connective tissue disease patients with SSc.

Estimates of the prevalence of PAH in SLE vary from 0.5 to 43% [6–9]. These results are from retrospective studies of large groups of patients over a period of 5–10 years or cross-sectional studies involving small numbers of SLE patients. The wide variability in the reported prevalence rates reflects the varying definitions of PAH used, differences in diagnostic methods, population groups studied and number of patients involved. There have been no attempts at diagnosing PAH in large cohorts of SLE.

The non-specific nature of symptoms such as dyspnoea, palpitations, fatigue and syncope associated with PAH could lead to a delay in the diagnosis of PAH in patients with SLE. This suggests a need for appropriate screening methods to identify

PAH. Although the gold standard test to diagnose PAH is right heart catheterization (RHC), this is an invasive and expensive test, which makes it unsuitable for use as a screening tool. Transthoracic Doppler Echocardiography has, however, been shown to be a safe, sensitive and specific tool to screen for PAH as well as to assess the severity of PAH in patients with SSc [10, 11]. Using this technique, we set out to prospectively determine the prevalence of PAH in our SLE cohort and to assess risk factors that may play a role in the development of PAH in SLE patients. We also assessed the value of pulmonary function tests and dyspnoea screening questionnaires as screening tests for PAH.

### Methods

Detailed methods are available as supplementary data at *Rheumatology* Online.

### Patient recruitment

This prospective cross-sectional study was conducted in the Wellcome Trust Clinical Research Facility (WTCRF) at the Queen Elizabeth Hospital (QEH), Birmingham, UK, between January 2004 and December 2005. This study was carried out in accordance with Helsinki declaration and received ethical approval from multicentre research ethics committee, UK (MREC 03/9/067). Three hundred and ninety-two patients with SLE were invited to take part in this study. Two hundred and eighty-eight (73%) patients gave informed written consent to participate. Two hundred and eighty-five (99%) patients fulfilled four criteria for SLE diagnosis defined by 1982 revised ACR criteria [12], whereas the remaining patients fulfilled three criteria and a clinical diagnosis of SLE by the Consultant Rheumatologist responsible for patient's care (C.G. or R.D.S.).

At the initial visit, a detailed clinical evaluation including history of smoking and RP plus, ECG, assessment of SLE disease activity using BILAG index [13, 14] and the SLICC/ACR damage index [15, 16] scores was undertaken. Echocardiography (ECHO) and the screening tests and assessments of risk factors for

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