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**INDIRIZZO: Scienze Cardiovascolari**

**CICLO XXVI**

**PREDICTIVE VALUE OF NONINVASIVE EVALUATION BY  
EXERCISE ECHOCARDIOGRAPHY AND BNP MEASUREMENT  
FOR EARLY IDENTIFICATION OF PULMONARY ARTERIAL  
HYPERTENSION IN PATIENTS AFFECTED BY SYSTEMIC  
SCLEROSIS: 5 YEARS FOLLOW-UP**

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## RIASSUNTO

Background. La sclerosi sistemica (SSc) è una complessa patologia caratterizzata da una eccessiva produzione di tessuto connettivo, una vasculopatia del microcircolo e disturbi dell'autoimmunità. L'ipertensione arteriosa polmonare (IAP) rappresenta una delle complicanze più severe di questa patologia, gravata da una prognosi piuttosto infausta. La diagnosi, data l'aspecificità dei sintomi, viene solitamente posta in uno stadio avanzato della malattia, quando l'interessamento del letto vascolare polmonare è già severo. Proprio per questo è fondamentale lo sviluppo di adeguate metodiche di screening per individuare la patologia in uno stadio precoce, che potrebbe permettere l'instaurazione di una appropriata terapia e migliorare la prognosi.

Scopo dello studio. Valutare il comportamento della pressione arteriosa sistolica polmonare in un gruppo di pazienti affetti da SSc, asintomatici o paucisintomatici, sottoposti ad ecocardiografia da sforzo. I pazienti sono stati poi seguiti con un follow-up a medio-lungo ripetendo un ecocardiogramma di base per valutare l'eventuale sviluppo di ipertensione polmonare, definendo così il valore predittivo dell'ecocardiografia da sforzo nell'identificare precocemente l'ipertensione polmonare.

Materiali e Metodi. 40 pazienti affetti da SSc (33 femmine e 7 maschi), in classe funzionale NYHA I e II, e con normali valori di pressione arteriosa polmonare sistolica (PAPs) a riposo sono stati sottoposti ad ecocardiogramma da sforzo. Sono stati valutati i valori di PAPs a riposo e da sforzo, mediante la misurazione del jet di rigurgito tricuspide considerando patologica una PAPs da sforzo  $\geq 48$  mmHg. Sono stati analizzati inoltre alcuni parametri emodinamici basali e da sforzo, le caratteristiche cliniche dei pazienti e della patologia reumatologica di base, oltre che i valori di BNP basali e dopo sforzo. È stato eseguito un follow-up a medio-lungo termine ( $5,1 \pm 0,4$  anni) sottoponendo i pazienti

ad una valutazione clinica e ad un ecocardiogramma di base per valutare lo sviluppo di ipertensione polmonare.

Risultati. I valori di PAPs a riposo sono stati di  $26,5 \pm 4,6$  mmHg, raggiungendo valori di  $45,1 \pm 14,2$  mmHg durante esercizio ( $p < 0,00$ ), con un incremento medio di  $18,7 \pm 11,9$  mmHg. In 25 (62,5%) pazienti la PAPs da sforzo è risultata normale (Gruppo A), mentre in 15 pazienti (37,5%), tutti di sesso femminile, abbiamo riscontrato un'alterata vasoreattività polmonare da sforzo (Gruppo B). I due gruppi differivano significativamente sia per i valori di PAPs a riposo ( $p < 0,007$ ) che da sforzo ( $p < 0,00$ ) ma anche per i valori di BNP da sforzo e la variazione del BNP con l'esercizio (rispettivamente  $p < 0,02$  e  $p < 0,001$ ). Dei 40 pazienti iniziali, 2 sono deceduti per cause extracardiache e 2 pazienti sono stati persi; abbiamo pertanto valutato 36 pazienti al follow-up di  $5,1 \pm 0,4$  anni. La PAPs media a riposo è risultata di  $29,67 \pm 7,4$  mmHg. 5 pazienti, tutti appartenenti al gruppo B, hanno presentato valori di PAPs a riposo patologici. Tra questi, in 3 pazienti i valori di PAPs erano suggestivi per la diagnosi di possibile IAP (PAPs  $> 37$  mmHg), in 2 erano invece indicativi di probabile IAP (PAPs  $> 50$  mmHg). Nessuno dei pazienti del gruppo A ha invece presentato nel follow-up valori di PAPs patologici.

Conclusioni. La prevalenza di alterata vasoreattività polmonare indotta dall'esercizio, valutata tramite l'ecocardiografia da sforzo, è elevata nei pazienti affetti da SSc. Questi pazienti hanno un rischio più elevato di sviluppare ipertensione polmonare ad un follow-up a medio-lungo termine. L'ecocardiografia da sforzo, associata al dosaggio del BNP, si è quindi dimostrata utile nell'identificare i pazienti con Ssc, asintomatici o paucisintomatici, che, presentando un'anomalo incremento della PAPs con l'esercizio, possono sviluppare ipertensione polmonare a medio-lungo termine.



## SUMMARY

**Background.** Systemic Sclerosis (SSc) is a chronic multisystem disease characterized by fibrosis of the skin and internal organs, microangiopathy and autoimmune disturbance. Pulmonary arterial hypertension (PAH) is one of the most severe complications of the disease, carrying a poor prognosis. Because of the aspecificity of the symptoms, diagnosis is often made when the disease is in an advanced stage and the pulmonary vascular bed involvement is already severe. For this reason it is essential to develop suitable screening tools to early detect the pathology, providing an appropriate therapy, that could improve the prognosis.

**Aim of the study.** To evaluate the pulmonary arterial systolic pressure (PAsP) during exercise echocardiography in a group of asymptomatic or mildly symptomatic patients affected to SSc. Patients were then submitted to a medium-long term follow-up performing a resting echocardiogram to evaluate the development of pulmonary hypertension, defining the predictive value of the exercise echocardiography to early identify such complication.

**Material and methods.** 40 patients affected by SSc (33 females and 7 males) with NYHA functional class I and II and with normal PAsP at rest, underwent exercise echocardiography. Resting and exercise PAsP values were estimated through measurement of tricuspidal regurgitant jet velocity by Doppler, considering values of exercise PAsP  $\geq 48$  mmHg to be pathological. We also analysed resting and exercise hemodynamic parameters, clinical characteristics of the patients and of the underlying rheumatic disease as well as measurement of resting and exercise BNP values. A medium-long term follow-up was done ( $5,1 \pm 0,4$  years), performing a clinical and resting echocardiographic evaluation to detect the development of pulmonary hypertension.

**Results.** Resting PAsP values were  $26,5 \pm 4,6$  mmHg, reaching values of  $45,1 \pm 14,2$

mmHg during exercise ( $p < 0,00$ ), with a mean increase of  $18,7 \pm 11,9$  mmHg. 25 patients (62,5%) had normal values of exercise PAsP (group A), whereas 15 patients (37,5%), all women, had exercise-induced altered pulmonary vasoreactivity (group B). Comparing group A and B, we found significant differences regarding both resting and exercise PAsP values (respectively  $p < 0,007$  and  $p < 0,00$ ), but also regarding exercise BNP values and the variation between resting and exercise BNP (respectively  $p < 0,02$  and  $p < 0,001$ ). Out of 40 initial patients, 2 patients died because of extracardiac causes, and 2 patients were lost; therefore 36 patients were followed for a mean time of  $5,1 \pm 0,4$  years. Data from follow-up showed mean resting PAsP of  $29,67 \pm 7,4$  mmHg. 5 patients, belonging to group B, presented pathological resting PAsP values: 3 of these patients had PAsP values suggestive for the diagnosis of possible pulmonary arterial hypertension (PAsP  $> 37$  mmHg), 2 patients had PAsP values suggestive for the diagnosis of probable PAH (PAsP  $> 50$  mmHg). None of the group A patients developed pathological PAsP values at follow-up.

Conclusions. Prevalence of exercise-induced altered pulmonary vasoreactivity, detected by exercise echocardiography, is high in patients affected by SSc.

Such patients had a higher risk for developing pulmonary hypertension at medium- to long- term follow-up. Exercise echocardiography, associated with BNP testing, has been demonstrated to be useful to identify SSc patients presenting an abnormal increase of PAsP during exercise that could, far away, develop PAH.

# INTRODUCTION

## PULMONARY ARTERIAL HYPERTENSION

### Definition

The first classification of the Pulmonary Hypertension (PH) come from the first World Conference on this topic, supported by the World health Organization, held in Geneva in the 1973. The initial classification designated only two categories: Primary Pulmonary Hypertension and Secondary Pulmonary Hypertension, depending on the absence or presence of risk factors or identifiable causes. In 1998 a second World Symposium was held in Evian and the previous classification of PH was modified, introducing 5 categories. In 2003, during the third World Symposium, held in Venice, the clinical classification was further modified. Pulmonary hypertension was defined as the presence of a mean pulmonary artery pressure (PAsP)  $\geq 25$  mmHg at rest and/or  $> 30$  mmHg during exercise, assessed by right heart catheterization, with pulmonary vascular resistance  $> 3$  mmHg/L/min (Wood Units), in presence of normal or reduced values of cardiac output. On the basis of the pulmonary capillary wedge pressure (PCWP  $\leq 15$  mmHg o  $\geq 15$  mmHg), two different forms of PH were distinguished: the precapillary and the postcapillary forms. During the fourth Symposium on PH held in Dana Point, in 2008, a new definition of PH was done. Moreover, the importance to distinguish the pulmonary arterial hypertension (PAH) from the other forms of PH was also highlighted [1].

The new definition of PH was based on the presence of a mean PAsP  $\geq 25$  mmHg at rest, no more including the value during exercise, because there were not, certain criteria to identify pathologic values of PAsP during the exercise, due to the extreme variability and influence of these values by the age, weight, cardiac output, type of the exercise [2].

**Table 1.** Haemodynamic definitions of pulmonary hypertension.

Definition	Characteristics	Clinical group(s)
Pulmonary hypertension (PH)	Mean PAP $\geq 25$ mmHg	All
Pre-capillary PH	Mean PAP $\geq 25$ mmHg PWP $\leq 15$ mmHg CO normal or reduced <sup>a</sup>	1. Pulmonary arterial hypertension 3. PH due to lung disease 4. Chronic thromboembolic PH 5. PH with unclear and/or multifactorial mechanisms
Post-capillary PH	Mean PAP $\geq 25$ mmHg PWP $> 15$ mmHg CO normal or reduced <sup>a</sup>	2. PH due to left heart disease
Passive	TPG $\leq 12$ mmHg	
Reactive (out of proportion)	TPG $> 12$ mmHg	

All values measured at rest.

<sup>a</sup>High CO can be present in cases of hyperkinetic conditions such as systemic-to-pulmonary shunts (only in the pulmonary circulation), anaemia, hyperthyroidism, etc.

CO = cardiac output ; PAP = pulmonary wedge pressure; TPG = transpulmonary pressure gradient (mean PAP – mean PWP).

From: Galiè N et al. Guidelines for the diagnosis and treatment of pulmonary arterial hypertension. Eur Heart J 2009;30:2497-2537.

**Table 2.** Important definitions.

- Pulmonary hypertension (PH) is a <i>haemodynamic and pathophysiological condition</i> defined as an increase in mean pulmonary arterial pressure (PAsP) $\geq 25$ mmHg at rest as assessed by right heart catheterization. PH can be found in multiple clinical conditions.
- The definition of PH on exercise as a mean PAsP $> 30$ mmHg as assessed by right heart catheterization is not supported by published data.
- Pulmonary arterial hypertension (PAH, group 1) is a <i>clinical condition</i> characterized by the presence of pre-capillary PH in the absence of other causes of pre-capillary PH such as PH due to lung disease, chronic thromboembolic PH, or other rare diseases. PAH includes different forms that share a similar clinical picture and virtually identical pathological changes of the lung microcirculation

From: Galiè N et al. Guidelines for the diagnosis and treatment of pulmonary arterial hypertension. Eur Heart J 2009;30:2497-2537.

## **Physiology and anatomy of the pulmonary circulation**

From the embryological point of view the common pulmonary artery and the two main branches derived from the primitive cardiac tube, meanwhile the peripheral pulmonary arteries derived from a vascular network around the bronchial buds. From the pulmonary artery trunk towards the capillaries, four types of vessels are present:

- Elastic arteries: the common trunk of the pulmonary artery and the first 5 series of the branches are elastic vessels (although in a lesser extent than the aorta e its main branches).

The following 3 series of branchings are considered transitional types of vessels;

- Muscular arteries: this type represents the majority of the pulmonary vessels, up to a diameter of 150-200 micron; they have a continuous muscular layer (tunica muscularis), that is however thinner than that of the systemic arteries;

- Partially muscularized arterioles: they have an incomplete muscular layer, alternated to a capillary-like wall;

- Non-muscular arterioles: they lack of smooth muscle cells, have a caliber of 30-75 micron; they terminate into a capillary network of the alveolar unit and are involved in the respiratory gas exchanges.

The muscular and partially muscular arterioles represent the resistance vessels system and accompany the respiratory bronchioles and the alveolar ducts. At this level there are pericytes, that are cells able to differentiate into smooth muscle cells in the presence of an hypoxic stimulus, thereby they constitute the site of vascular remodeling involved in the pathogenesis of PH.

The lung has a dual arterial circulation, derived from the bronchial arteries and the pulmonary arteries, and a dual venous drainage system involving the pulmonary veins and the azygos veins. Into the lungs, every pulmonary artery accompanies the branching of the

bronchial tree and divides at the level of the bronchial bifurcations, up to the respiratory bronchioles.

The pulmonary circulation is, in the adult, a low resistance and very high capacitance system as compared with the systemic circulation. All the pulmonary artery tree has a larger vessels section than the systemic district. Moreover it lacks the high resistance muscular arterioles in the peripheral districts and the pulmonary capillaries are numerous and widely anastomized. For these reasons pulmonary circulation has a low pressure gradient. The systolic and diastolic pulmonary artery pressures are about 22 and 10 mmHg, and the mean pulmonary artery pressure is about 15 mmHg. These values are much lesser than those measured in the aorta, because the pulmonary vascular resistance is only 1/10<sup>th</sup> of the systemic one. Pulmonary vascular resistance in the adults is about 1 Wood Units (1 WU =  $67 \pm 23$  dyne-sec/cm<sup>-5</sup>). With exercise the pulmonay blood flow bed increases also five times, without a significant pressure increase. This is due to the activation of two mechanisms:

- Increase and distension of the vessels caliber, especially in the basal regions;
- Recruitment of the hypoperfused vasal districts, especially in the apical regions (the, so-called, pulmonary vascular reserve).

Moreover the pulmonary vascular resistance (PVR) is regulated by some factors that act to the vascular tone. Neurogenic, hormonal and chemical factors are involved to the vascular tone regulation acting to the muscular and fibroelastic elements of the vessels wall: non-adrenergic and non-cholinergic Neuropeptides (Angiotensin II, Atrial Natriuretic Peptide, Intestinal Vasoactive Peptide), the Autacoids (Histamine, Serotonin, Bradykinin), the Prostaglandins with the both vasoconstriction and vasodilation actions, the Nitric Oxide (NO) produced by the pulmonary endothelium, with the vasodilation, antiproliferative and

antithrombotic effects, and the Endothelin with a vasoconstriction effect.

The alveolar hypoxia is a strong stimulus for the pulmonary arteriolar vasoconstriction: this is a regulatory mechanism of the pulmonary circulation that acts to reduce the blood flow through the hypoventilated alveoli, in favour of the normoventilated ones, to reduce the ventilation/perfusion mismatch. The acidosis causes vasoconstriction too, acting together with the hypoxia. A prolonged vasoconstriction leads to structural alterations in a short time: the arteriolar wall undergoes the hypertrophy of tunica media, the appearance of muscular elements more peripherally and the tunica intima hypertrophy. Hence the global cross-sectional area of the pulmonary vascular bed reduces, the arterial stiffness increases, resulting in peripheral pulmonary vascular resistance and pulmonary pressure increase.

### **Clinical classification of Pulmonary Hypertension**

The last classification recommended during the fourth World Symposium on Pulmonary Hypertension maintained the general organization of the previous classification, making some changes in specific points (Table 3).

The five groups are the following:

- Pulmonary arterial hypertension (PAH)
- Pulmonary hypertension secondary to left heart disease
- Pulmonary hypertension due to lung diseases and/or hypoxia
- Chronic thromboembolic pulmonary hypertension (CTPH)
- Pulmonary hypertension with unclear or multifactorial mechanisms

**Table 3.** Updated clinical classification of pulmonary hypertension (Dana Point, 2008).

<p><b>1 Pulmonary arterial hypertension (PAH)</b></p> <p>1.1 Idiopathic</p> <p>1.2 Heritable</p> <p>1.2.1 BMPR2</p> <p>1.2.2 ALK1, endoglin ((with or without hereditary haemorrhagic telangiectasia)</p> <p>1.2.3 Unknown</p> <p>1.3 Drugs and toxins induced</p> <p>1.4 Associated with (APAH)</p> <p>1.4.1 Connective tissue diseases</p> <p>1.4.2 HIV infection</p> <p>1.4.3 Portal hypertension</p> <p>1.4.4 Congenital heart disease</p> <p>1.4.5 Schistosomiasis</p> <p>1.4.6 Chronic haemolytic anaemia</p> <p>1.5 Persistent pulmonary hypertension of the newborn</p>
<p><b>1' Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomas</b></p>
<p><b>2 Pulmonary hypertension due to left heart disease</b></p> <p>2.1 Systolic dysfunction</p> <p>2.2 Diastolic dysfunction</p> <p>2.3 Valvular disease</p>
<p><b>3 Pulmonary hypertension due to lung disease and/or hypoxia</b></p> <p>3.1 Chronic obstructive pulmonary disease</p> <p>3.2 Interstitial lung disease</p> <p>3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern</p> <p>3.4 Sleep-disordered breathing</p> <p>3.5 Alveolar hypoventilation disorders</p> <p>3.6 Chronic exposure to high altitude</p> <p>3.7 Developmental abnormalities</p>
<p><b>4 Chronic thromboembolic pulmonary hypertension</b></p>
<p><b>5 PH with unclear and/or multifactorial mechanisms</b></p> <p>5.1 Haematological disorders: myeloproliferative disorders, splenectomy.</p> <p>5.2 Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangiomyomatosis, neurofibromatosis, vasculitis</p> <p>5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders</p> <p>5.4 Others: tumoural obstruction, fibrosing mediastinitis, chronic renal failure on dialysis</p>

ALK-1 = activin receptor-like kinase 1 gene; APAH = associated pulmonary arterial hypertension;

BMPR2 = bone morphogenetic protein receptor, type 2;

HIV = human immunodeficiency virus; PAH = pulmonary arterial hypertension.

From: Galiè N et al. Guidelines for the diagnosis and treatment of pulmonary arterial hypertension. Eur Heart J 2009;30:2497-2537.

In the group 1 the term familial PAH was replaced by the term heritable PAH, because specific gene mutations have been identified in sporadic cases without a family history.

Therefore the heritable form of PAH includes both the sporadic and the familial cases. In group 1 there are also the idiopathic PAH (IPAH), the drug and toxins induced PAH, the



persistent pulmonary hypertension of the newborn and the form associated with various clinical conditions (APAH): connective tissue diseases, HIV infection, portal hypertension, congenital heart disease, schistosomiasis and chronic haemolytic anaemia (such as sickle cell disease, thalassaemia, hereditary spherocytosis, stomatocytosis, and microangiopathic haemolytic anaemia).

In the group 1' pulmonary veno-occlusive disease and pulmonary capillary haemangiomas remain difficult disorders to classify since they share some characteristics with IPAH, but also demonstrate a number of differences. So the consensus agreement of experts decided that these conditions should be a distinct category, but not completely separated from PAH, and have been designated as clinical group 1'.

Group 2 includes PH due to left heart disease (systolic, diastolic dysfunction or valvular diseases) and group 3 PH due to lung diseases and/or hypoxia (chronic obstructive pulmonary disease, interstitial lung disease, etc.).

Group 4 is chronic thromboembolic PH, secondary to proximal or distal obstructive lesions, caused by thromboemboli (CTEPH).

Group 5 comprises a heterogeneous collection of diseases with uncertain pathogenetic mechanisms leading to PH, including haematological, systemic, metabolic, and other rare disorders.

## **Hystopathology and pathogenesis**

Although the clinical, hemodynamic and prognostic aspects of the different forms of PAH are rather heterogeneous, they share the same hystopathological substrate that is represented by a wide spectrum of proliferative and obstructive lesions of the pulmonary vessels defining the pulmonary hypertensive arteriopathy.

The pathological process mostly involves the peripheral pulmonary arteries, such as the preacinar and intraacinar muscular arterioles (resistance vessels), but it could also involve the venous and capillary district.

The pulmonary hypertensive arteriopathy is characterized by the following structural lesions:

- Hypertrophy of the tunica media
- Thickening of the tunica intima
- Thickening of the tunica adventitia
- Complex obstructive endoluminal lesions with reparative and with reparative/proliferative characteristics.

Tunica media hypertrophy is a diffuse process, common to all type of PAH, due to both the hypertrophy and hyperplasia of the smooth muscle cells, the increase of the elastic fibres and of the connective tissue.

The thickening of the tunica intima is caused by the intimal cells proliferation and is an obliterative diffuse lesion, frequent in all type of PAH. The tickkening can be: concentric laminar, concentric non-laminar or eccentric.

The thickening of the tunica adventitia is caused by the perivascular connective tissue hypertrophy, stimulated by growth factors. It is responsible for the increased stiffness of the vessel wall.

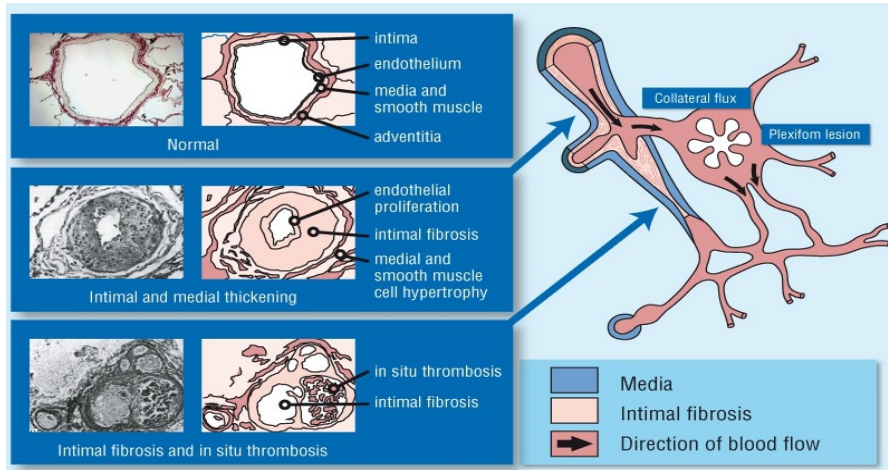
The complex endoluminal lesions are reparative and proliferative alterations and include the plexiform lesions, the angiomatoid lesions and the necrotizing arteritis.

The plexiform lesions are focal alterations, comparable to renal glomeruli, and are constituted by a plexus of capillaries, frequently associated to thrombi. They are characterized by highly proliferative endothelial cells surrounded by myofibroblasts, smooth muscle cells and connective tissue. The angiomatoid lesions are very thin, dilated and tortuous vascular structures often localized distal to the plexiform lesions. The necrotizing arteritis is characterized by segmental fibrinoid necrosis of the muscular arterioles with inflammatory cells infiltration.

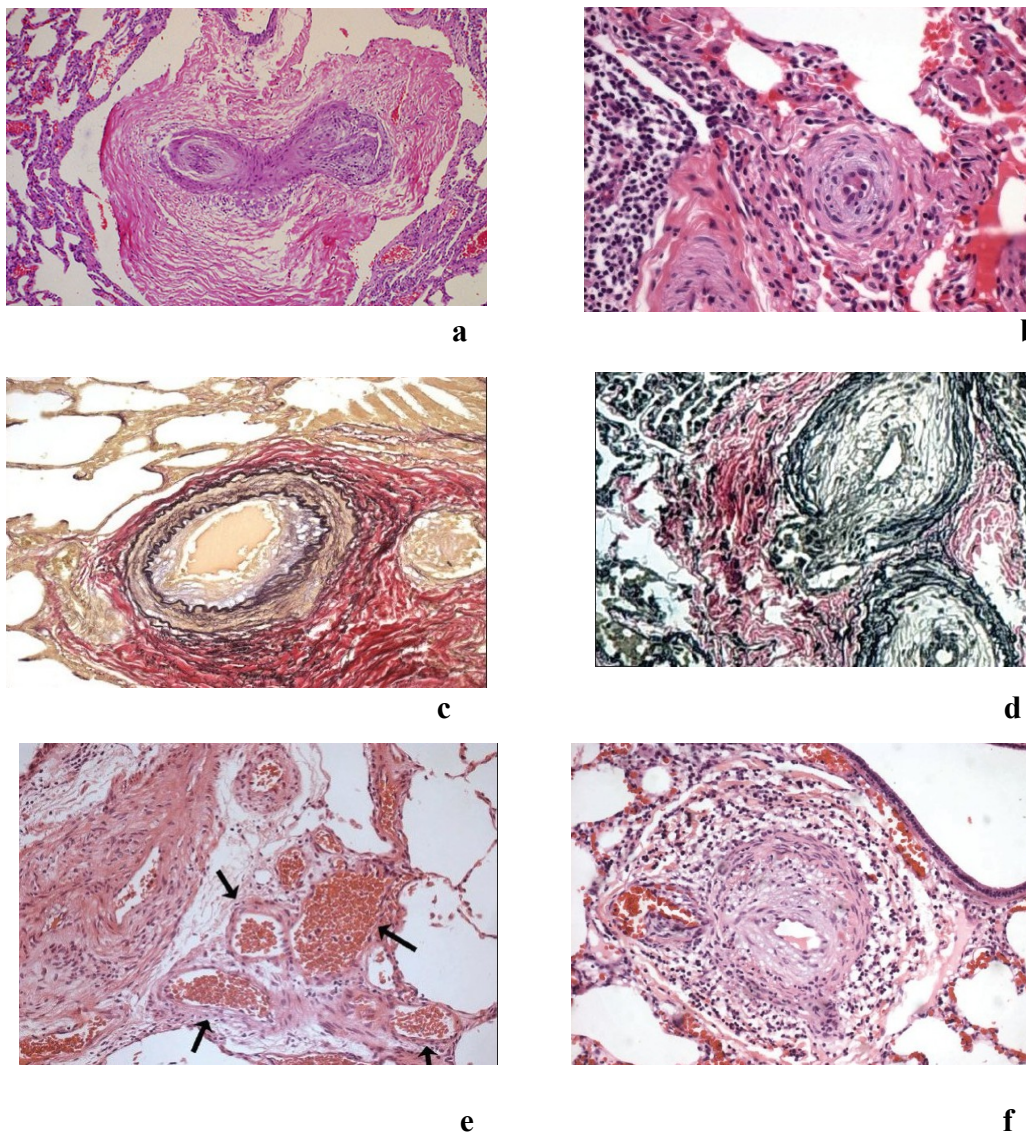
In PAH the histological examination reveals a panvasculopathy, affecting the distal arterioles (with a diameter < 500 micron); it is present intimal fibrosis and hypertrophy, smooth muscle cells hypertrophy, vasoconstriction, adventitial thickening with inflammatory perivascular infiltrates, plexiform lesions and in situ thrombosis. Over time these alterations cause the obstruction of the blood flow within the vessels, together with disorganized angiogenesis and plexiform arteriopathy. The process that causes these pathological changes is not known, however the common opinion is that the etiology of the PAH is multifactorial, with both a substrate of genetic and constitutional predisposition and some environmental factors (toxins exposure, infective or inflammatory processes, etc.).

The processes responsible for the vascular resistance increase in PAH are:

- **Obstructive vascular remodeling**
- **Vasoconstriction**
- **Inflammation**
- **Thrombosis**



**Figure 1.** Histology in pulmonary arterial hypertension.



**Figure 2.** Proliferative lesions of the tunica intima. Plexiform lesion (figure a). Lesions are rich of cells (figure b) with fibrosis and elastic fibres proliferation (figure c); occlusion of the vessel lumen due to fibrosis (figure d); fibrinoid necrosis (arrows, figure e); arteritis (figure f).

The **obstructive vascular remodeling** is considered the key element in the pathogenesis of PAH. The obstructive lesions involve all the vascular layers and various type of cells (endothelial cells, smooth muscle cells and fibroblasts). The histopathological hallmark of vascular remodeling is the anomalous and disorganized cellular proliferation leading to an increased thickness of the vessel wall. Many growth factors are considered involved in the vascular remodeling process: an increased expression of vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), fibroblast growth factor beta (bFGF), insuline-like growth factor 1 (IGF-1), epidermal growth factor (EGF), and angiopoietin-1 (that is an important angiogenetic factor for the development of the pulmonary vascular bed) have been found in the lung tissues of patients affected by PAH.

**Vasoconstriction** mechanisms seem to be related to the abnormal function or expression of potassium channels in the smooth muscle cells and to endothelial dysfunction. The latter causes a reduced production of vasodilator agents such as nitric oxide, prostacyclin and vasoactive intestinal peptide (VIP), and an over-expression of vasoconstriction substances such as thromboxane-A<sub>2</sub>, endothelin-1 and serotonin (5-HT).

The prostacyclin is a potent pulmonary vasodilator agent that inhibits the smooth muscle cells proliferation and reduces the platetel aggregation through cyclic AMP (cAMP) pathway activation. Nitric oxide is a additional potent pulmonary vasodilator and acts through cyclic GMP (cGMP) pathway. Endothelin-1 exerts its action of potent vasoconstriction, and pulmonary vessels smooth muscle cells proliferation interacting with two types of receptors (ET<sub>A</sub> and ET<sub>B</sub>). 5-HT is a substance produced by the intestinal enterochromaffin cells and stored in plateles, and it induces vasoconstriction and smooth muscle cells proliferation. VIP is a neurotransmitter acting through the cGMP and cAMP pathway activation: it induces systemic and pulmonary vasodilation and inhibits smooth muscle cells proliferation and platetel aggregation.

The evidence of **inflammatory processes** in PAH is given by the histological finding of accumulation of perivascular inflammatory cells including macrophages, dendritic cells, T and B lymphocytes. Moreover circulating levels of certain cytokines and chemokines are elevated.

**Thrombosis** and platelet dysfunction are also involved in the pathogenesis of the pulmonary hypertensive vasculopathy. Patients affected to PAH have a thrombophilic state and thrombotic lesions are present in both the small distal pulmonary arteries and the proximal elastic pulmonary arteries. The platelet dysfunction role in PAH is not limited to altered coagulation process. Actually, in response to specific stimuli, platelets are able to produce prothrombotic, vasoactive and mitogen factors as thromboxane A<sub>2</sub>, PDGF, 5-HT, TGF- $\beta$  and VEGF, which contribute to vascular remodeling.

### **Genetics, epidemiology and risk factors**

Currently, there are not reliable epidemiological data about the prevalence of the different groups of PH, but a survey, performed in an echocardiography laboratory, showed a prevalence of PH (defined as PASP > 40 mmHg), among 4579 patients, of 10,5% [3].

In 78,7% of cases, PH was secondary to left heart diseases, in 9,7% secondary to lung diseases or hypoxia, 4,2% had PAH (Group 1), and in 0,6% PH was secondary to thromboembolism. Finally, in 6,8% of cases an aetiology was not ascertained.

In the presence of a familial form of PAH, germline mutation in the bone morphogenetic protein receptor 2 (BMPR2) gene is found in 70% of cases. This protein is involved in the control of vascular cell proliferation processes. Such mutation is also found in 11-40% of sporadic cases, thus resulting in major genetic predisposing factor for PAH.

Data obtained by the European countries registries show a prevalence of PAH of about 15-50 cases per million population in Europe [4]. In the French registry, 39,2% of cases had

IPAH, in 3,9% there was a family history of PAH. Among the forms of PAH associated to other clinical conditions, the 15,3% of cases had a connective tissue disease (CTD), mostly the systemic sclerosis, 11,3% a congenital heart disease (CHD), 10,4% portal hypertension, in 9,5% there was the anorexigen-associated PAH, and 6,2% had HIV infection [5].

<b>Prevalence of PAH in the general population</b> 15–50 cases per million (0.0015–0.0050%)
<b>Prevalence of PAH in at risk populations</b> <ul style="list-style-type: none"><li>•Congenital heart disease: 4–15%</li><li>•Systemic sclerosis: 8–10%</li><li>•Portal hypertension: 0.5–10%</li><li>•HIV: 0.5%</li><li>•Sickle cell disease: 2%</li><li>•BMP2 mutations carriers: 20%</li></ul>

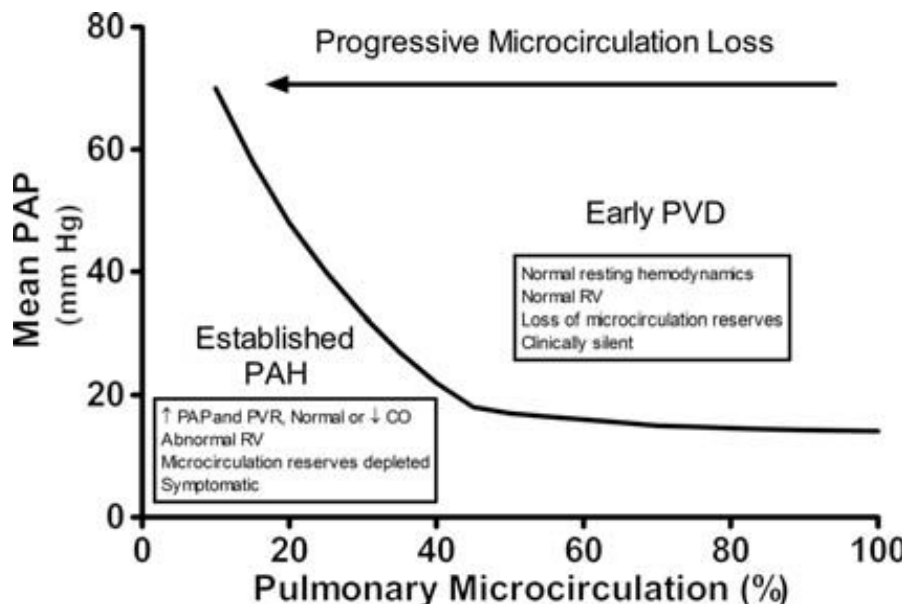
PAH, pulmonary arterial hypertension; CHD, congenital heart disease; HIV, human immunodeficiency virus; BMP2, bone morphogenic protein receptor 2.  
From: Lau E et al. Early detection of pulmonary vascular disease in pulmonary arterial hypertension: time to move forward. Eur Heart J 2011; 32:2489–2498.

### **Clinical presentation and diagnosis**

The evaluation process of a patient with suspected PH requires a series of investigations intended to confirm the diagnosis, clarify the clinical group of PH and the specific aetiology within the PAH group, and evaluate the functional and haemodynamic impairment. Diagnosis of PH is often made very late, because symptoms are non-specific, so an high level of clinical suspicion may be necessary.

Moreover, since PAH, and particularly IPAH, is a diagnosis of exclusion, a diagnostic algorithm may be useful (Fig.11). First of all, the most common causes of PH, such as left heart diseases and lung diseases, should be investigated; thereafter thromboembolic aetiology should be excluded and, at the end, the most rare causes of PH (group 5).

The **symptoms** are non-specific and include breathlessness, fatigue, weakness, angina, syncope. Symptoms at rest are reported only in very advanced cases.



**Figure 3.** Schematic representation of the relationship between pulmonary microcirculation loss and PAP. The high capacitance of the pulmonary circulation implies that early microcirculation loss is not accompanied by a change in resting PAP. Many of the current screening modalities are dependent on detecting a rise in PAP, and thus will fail to detect the early stages of PVD.

PAP: pulmonary artery pressure; PVD: pulmonary vascular disease; RV: right ventricle.

PAH: pulmonary arterial hypertension; PVR: pulmonary vascular resistance; CO: cardiac output;

From: Lau E et al. Early detection of pulmonary vascular disease in pulmonary arterial hypertension: time to move forward. *Eur Heart J* 2011; 32:2489–2498.

Exercise capacity is classified into 4 classes according to the World Health Organization (OMS), redefined in 1998 [6,7].

<b>Class I</b>	Patients with PH without resulting limitation of physical activity. Ordinary physical activity Ordinary physical activity does not cause undue dyspnoea or fatigue, chest pain, or near syncope.
<b>Class II</b>	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.
<b>Class III</b>	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.
<b>Class IV</b>	Patients with pulmonary hypertension and 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.



The **physical signs** include left parasternal lift, an accentuated pulmonary component of second heart sound, a pansystolic murmur of tricuspid regurgitation, a diastolic murmur of pulmonary insufficiency and a right ventricular third sound. Signs of right heart failure (jugular vein distension, hepatomegaly, peripheral oedema and ascites) characterized patients in a more advanced state.

The **electrocardiogram** has a specificity of 55% and a sensitivity of 70% to be a screening tool for detecting PH. In advanced stages may be present signs of right atrial dilatation, right ventricular hypertrophy and right axis deviation. Moreover, supraventricular arrhythmias (atrial flutter and atrial fibrillation) are frequent, whereas ventricular arrhythmias are rare.

**Chest radiograph**, in 90% of patients with IPAH, is abnormal at the time of diagnosis. Findings include central pulmonary arterial dilatation, which contrasts with ‘pruning’ (loss) of the peripheral blood vessels. Right atrium and right ventricular enlargement may be seen in more advanced cases. The chest radiograph allows associated lung diseases or pulmonary venous hypertension due to left heart disease to be reasonably excluded.

**Pulmonary function tests and arterial blood gases.** Pulmonary function tests and arterial blood gases will identify the contribution of underlying airway or parenchymal lung disease. Patients with PAH usually have decreased lung diffusion capacity for carbon monoxide (typically in the range of 40–80% predicted) and mild to moderate reduction of lung volumes. Arterial oxygen (O<sub>2</sub>) tension is normal or only slightly lower than normal at rest and arterial carbon dioxide tension is decreased because of alveolar hyperventilation.

The **exercise capacity** can be assessed by the 6-minute walking test (6MWT) and cardiopulmonary exercise testing. The 6MWT is technically simple, inexpensive, reproducible, and well standardized. In addition to distance walked, dyspnoea on exertion (Borg scale) and finger arterial O<sub>2</sub> saturation are recorded. With cardiopulmonary exercise

testing gas exchange and ventilation are continuously recorded throughout incremental exercise. In PAH, O<sub>2</sub> uptake at the anaerobic threshold and peak exercise are reduced in relation to disease severity, as are peak work rate, peak heart rate, O<sub>2</sub> pulse, and ventilatory efficiency. The 6MWT remains until now the only Food and Drug Administration- and European Agency for the Evaluation of Medicinal Products-accepted exercise endpoint for studies evaluating treatment effects in PAH, because a generally accepted standardization of cardiopulmonary exercise testing is lacking.

**Transthoracic echocardiography** provides several variables which correlate with right heart haemodynamics including PAsP, and should always be performed in the case of suspected PH. The estimation of PAsP is based on the peak velocity of the jet of tricuspid regurgitation. The simplified Bernoulli equation describes the relationship of tricuspid regurgitation velocity and the peak pressure gradient of tricuspid regurgitation: tricuspid regurgitation pressure gradient =  $4 \times (\text{tricuspid regurgitation velocity})^2$ . This equation allows for estimation of PA systolic pressure taking into account right atrial pressure: PA systolic pressure = tricuspid regurgitation pressure gradient + estimated right atrial pressure. Right atrial pressure can be estimated based on the end-expiratory diameter and respiratory variation of the inferior vena cava (measured 0,5-3 cm from its junction to right atrium), although often a fixed value of 5 or 10 mmHg is assumed (Table 1 and 2).

Variable	Normal (0-5 [3] mm Hg)	Intermediate (5-10 [8] mm Hg)		High (15 mm Hg)
IVC diameter	≤2.1 cm	≤2.1 cm	>2.1 cm	>2.1 cm
Collapse with sniff	>50%	<50%	>50%	<50%
Secondary indices of elevated RA pressure				<ul style="list-style-type: none"> <li>• Restrictive filling</li> <li>• Tricuspid E/E' &gt; 6</li> <li>• Diastolic flow predominance in hepatic veins (systolic filling fraction &lt; 55%)</li> </ul>

**Table 1.** Estimation of right atrial pressure.

IVC: inferior vena cava; RA: right atrium; E/E': ratio between E and E' wave of diastolic flow.

**Table 2.** Arbitrary criteria for estimating the presence of PH based on tricuspid regurgitation peak velocity and Doppler-calculated PA systolic pressure at rest (assuming a normal right atrial pressure of 5 mmHg) and on additional echocardiographic variables suggestive of PH.

	<b>Classe</b>	<b>Livello</b>
<b>Echocardiographic diagnosis: PH unlikely</b> Tricuspid regurgitation velocity 2.8 m/s, PA systolic pressure 36 mmHg, and no additional echocardiographic variables suggestive of PH	I	B
<b>Echocardiographic diagnosis: PH possible</b> Tricuspid regurgitation velocity 2.8 m/s, PA systolic pressure 36 mmHg, but presence of additional echocardiographic variables suggestive of PH	IIa	C
Tricuspid regurgitation velocity 2.9–3.4 m/s, PA systolic pressure 37–50 mmHg with/without additional echocardiographic variables suggestive of PH	IIa	C
<b>Echocardiographic diagnosis: PH likely</b> Tricuspid regurgitation velocity .3.4 m/s, PA systolic pressure .50 mmHg, with/without additional echocardiographic variables suggestive of PH	I	B
<b>Exercise Doppler echocardiography</b> is not recommended for screening of PH	III	C

From: Galiè N et al. Guidelines for the diagnosis and treatment of pulmonary arterial hypertension.  
Eur Heart J 2009;30:2497-2537.

However there are some limitations in the Doppler-derived pulmonary pressure estimation: when peak tricuspid regurgitation velocity is difficult to measure (trivial/mild tricuspid regurgitation) the use of contrast echocardiography (e.g. agitated saline) significantly increases the Doppler signal, allowing proper measurement of peak tricuspid regurgitation velocity; moreover this technique is an operator-dependent procedure. Both overestimations or underestimations of systolic PAP can occur. Some studies showed a good correlation between echocardiographic estimation of systolic PAP and its invasive measurement by right heart catheterization (RHC) [8,9], while others have revealed differences between the two methods: 35% of cases, and 45% of cases of

echocardiographic diagnoses of PH were falsely positive [10,11].

These differences can be due to low operator experience, a poor Doppler alignment with the tricuspid regurgitant jet, an incorrect estimation of right atrial pressure, but also to different and variable haemodynamic conditions.

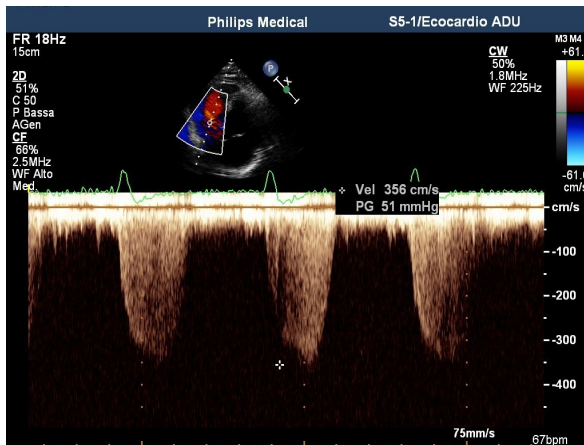


Figure 4. Doppler tricuspid regurgitant jet velocity.

The echocardiographic evaluation also allows the estimate of the mean PAP through the pulmonary valve regurgitant flow analysis, the acceleration time of the pulmonary artery that correlates to mean PAP and PVR, and the estimation of the pulmonary artery end-diastolic pressure using the end-diastolic pulmonary regurgitant jet added to right atrial pressure estimate.

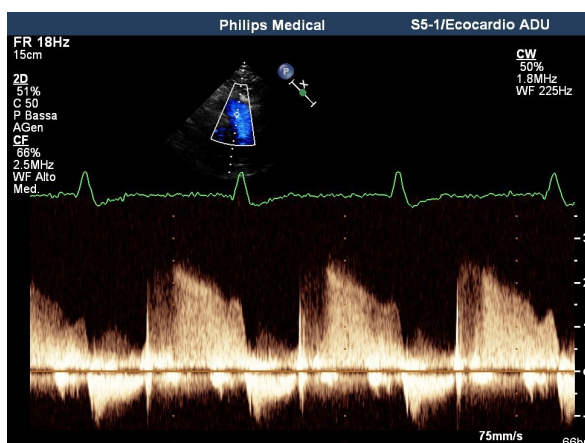


Figure 5. Continuous Doppler pulmonary regurgitant jet velocity.

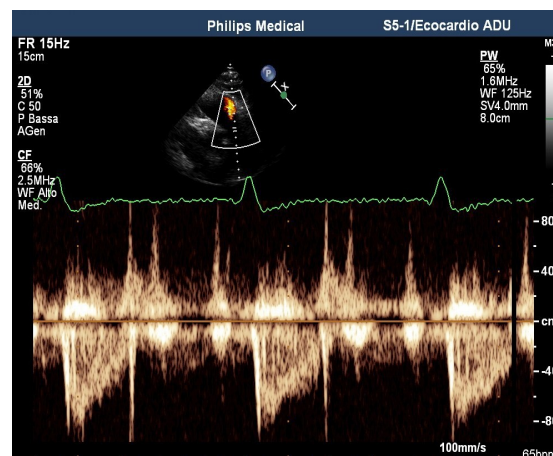
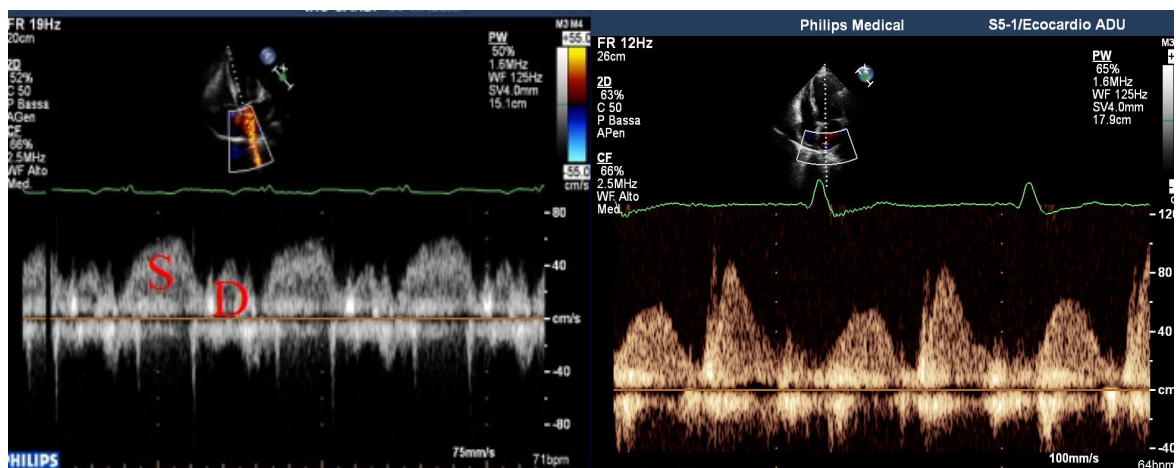


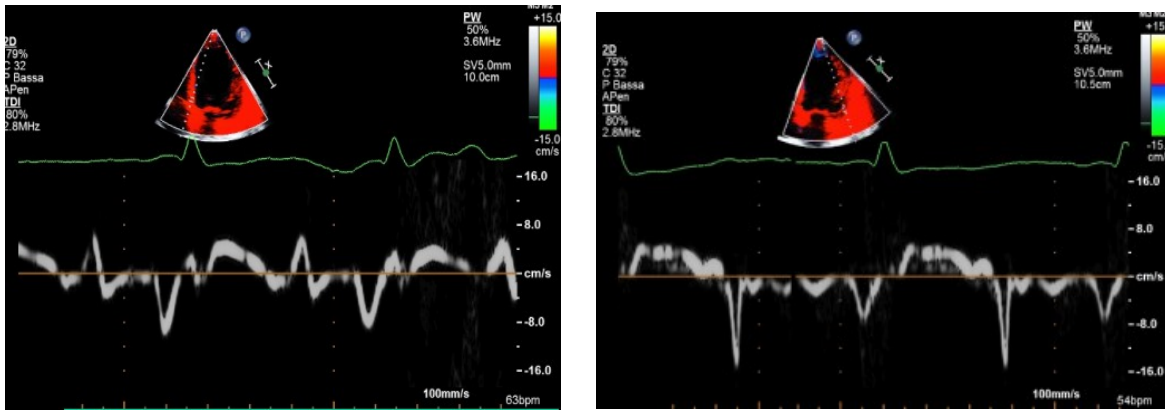
Figure 6. Pulsed Doppler pulmonary artery flow velocity.

Numerous studies are present in literature showing how to estimate left atrial pressure and left ventricular filling pressure using the echocardiography [12-14].

The more useful parameters are: E/E' ratio, pulmonary veins flow analysis using pulsed Doppler. E/E' is a ratio between the left ventricle early diastolic flow velocity, measured by pulsed Doppler at the level of the mitral valve (E wave), and the early diastolic velocity of the mitral annulus measured by tissue Doppler. An E/E' ratio  $> 15$  is suggestive of increased left ventricular diastolic pressures, whereas a E/E' ratio  $< 8$  accurately predicts normal left ventricular filling pressures. E/E' values 8 to 15 represent a grey-zone, where the diagnosis is uncertain. Analysing pulmonary venous flow, peak systolic (S wave) and early diastolic (D wave) flow velocities of the pulmonary flow have a normal pattern when the S/D ratio is between 1,3 and 1,5 ( $\pm 0,3$ ), with a systolic fraction (a ratio of velocity-time integral of the S wave to the sum of velocity-time integrals of the D wave and S wave) of 60-68%; S/D ratio  $< 1$  is suggestive of mean left atrial pressure  $\geq 15$  mmHg, with a sensitivity of 90% and a specificity of 85%.



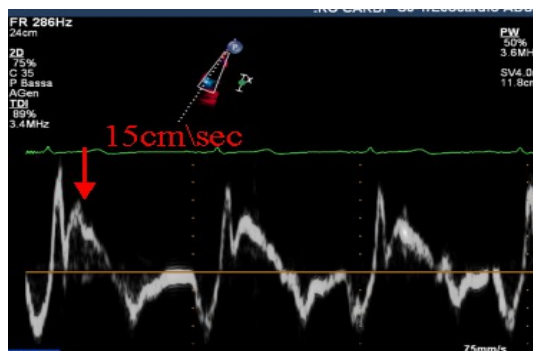
**Figure 7.** Pulsed Doppler pulmonary venous flow (Systolic, S and diastolic, D waves).



**Figure 8 and 9.** Septal and lateral mitral annulus tissue Doppler.

Pluriparametric evaluation of the diastolic function can identify the presence of left heart dysfunction with altered compliance of the left cavities, that is one of the causes of PH (group 2). Echocardiographic parameters useful for the right ventricular systolic function evaluation are: right ventricular fractional area change (RVFAC), TAPSE (Tricuspid annular plane systolic excursion) and TAV (tricuspid annular velocity).

The fractional shortening is the percentage change of the right ventricular area between the end-diastole (EDA) and the end-systole (ESA), expressed with the formula  $(EDA - ESA / EDA)$ . Normal values of RVFAC are  $56 \pm 13\%$ . TAPSE is useful for the assessment of the right ventricular longitudinal function and it is measured by M-mode technique at the lateral tricuspid annulus in apical 4-chamber view. Values  $\geq 16$  mm are generally normal. TAV records the systolic movement of the lateral tricuspid annulus using tissue Doppler: normal values of the systolic peak velocity (S') are  $> 12$  cm/sec.



**Figure 10.** Right ventricle lateral tricuspid annulus tissue Doppler.

The right ventricular diastolic function can be evaluated by the ventricular filling pattern analysis, using pulsed Doppler between the opened tricuspid leaflets in diastole. The flow velocities registered at this level are lower than that of the left heart chambers and there are more respiratory variability.

E/E' ratio of the tricuspid valve and annulus has a good correlation with the mean right atrial pressure. E/A ratio (of the tricuspid diastolic flow), E/E' ratio and right atrial dimension are the more reliable parameters for the study of the right ventricular diastolic function. E/A ratio  $< 0,8$  suggest the presence of diastolic impaired relaxation, E/A ratio between 0,8 and 2,1 associated to E/E' ratio  $> 6$  has a sensitivity of 79% and a specificity of 73% to estimate a right atrial pressure  $> 10$  mmHg. E/A ratio  $> 2,1$ , with a deceleration time of the E wave  $< 120$  msec is a restrictive pattern [15].

**Exercise echocardiography** is a technique not validated for the diagnosis of PH because it lacks a clear cut-off values to make the diagnosis and data have not been validated and compared to right heart cateterization results (class III of recommendations, level of evidence C, according to the latest guidelines) [7].

However many studies are still being performed using this technique, on the basis of the concept that it could be possible to disclose, with the exercise, conditions of impaired pulmonary vascular reserve, that could be a preclinical stage of the pulmonary hypertensive disease [16].

Regarding **blood tests**, it is important to make serological screening for HIV, antibodies assay (Antinuclear, anti-centromere, anti-Scl70, and antiphospholipid antibodies), thrombophilia screening, and liver and thyroid function tests.

Natriuretic peptides (BNP, e NT-proBNP) are useful in the diagnosis and follow-up of patients with heart failure, and are also correlated with prognosis of these patients [17].

**Right heart catheterization** (RHC) is required to confirm the diagnosis of PAH, to assess

the severity of the haemodynamic impairment and to test the vasoreactivity of the pulmonary circulation. When performed at experienced centres, RHC procedures have low morbidity (1,1%) and mortality (0,055%) rates. The following variables must be recorded during RHC: PAP (systolic, diastolic and mean), right atrial pressure, pulmonary wedge pressure, right ventricular pressure, and cardiac output (CO).

Vasoreactivity testing should be performed at the time of diagnostic RHC to identify patients who may benefit from long-term therapy with calcium channel blockers. According to the latest guidelines, vasoreactivity test is indicated with a class of recommendation IC, in patients with idiopathic, heritable or anorexigen use-associated PAH. Currently the agent most used in acute testing is NO; intravenous epoprostenol or adenosine may also be used as an alternative. A positive acute response (positive acute responder) is defined as a reduction of mean PAP  $\geq 10$  mmHg to reach an absolute value of mean PAP  $\leq 40$  mmHg, with an increased or unchanged cardiac output (CO). Positive acute responders are most likely to show a sustained response to long-term treatment with high doses of calcium channels blockers, especially if they are affected by the idiopathic form of PAH.

**High-resolution computed tomography (HRCT)** provides detailed views of the lung parenchyma and facilitates the diagnosis of interstitial lung disease and emphysema.

**Contrast CT angiography and pulmonary angiography** is helpful in determining whether there is evidence of surgically accessible CTEPH (suitability for pulmonary endarterectomy).

**Cardiac magnetic resonance** is not recommended in the diagnostic algorithm of the guidelines, but could provide morphologic and functional informations of the right ventricle.

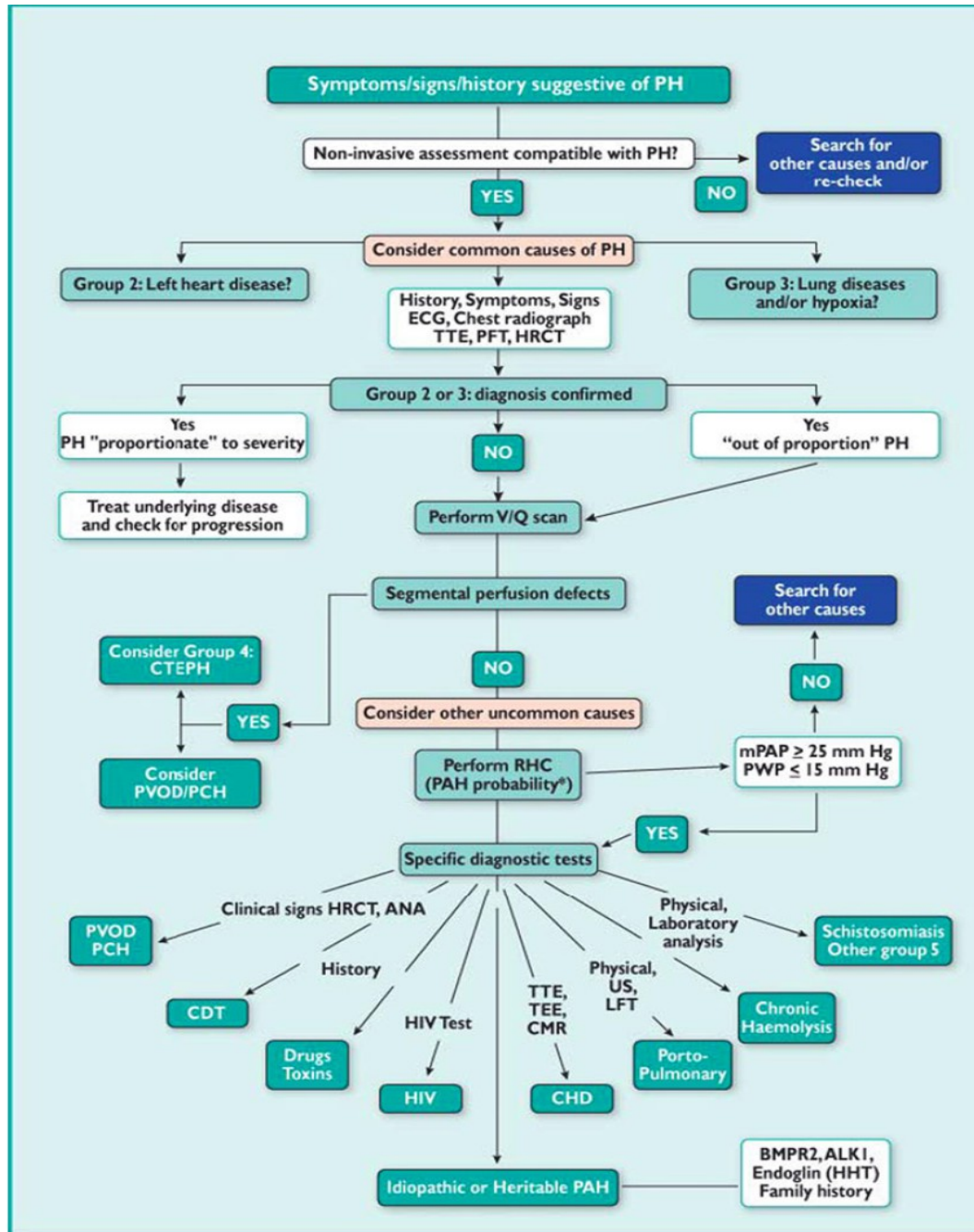
**Ventilation/perfusion lung scan** remains the screening method of choice for CTEPH



because of its high sensitivity.

**Diagnostic algorithm (Figure 11).** Diagnostic process starts with the identification of the more common clinical groups of PH (group 2: left heart disease and group 3: lung diseases), then distinguishes group 4: CTEPH and finally makes the diagnosis and recognizes the different types in group 1 and the rarer conditions in group 5.

PAH should be considered in the differential diagnosis of exertional dyspnoea, syncope, angina, and/or progressive limitation of exercise capacity, particularly in patients without apparent risk factors, symptoms or signs of common cardiovascular and respiratory disorders. Special awareness should be directed towards patients with associated conditions and/or risk factors for development of PAH such as family history, CTD, CHD, HIV infection, portal hypertension, haemolytic anaemia, or a history of intake of drugs and toxins known to induce PAH. In everyday clinical practice such awareness may be low. More often PH is found unexpectedly on transthoracic echocardiography requested for another indication.



**Figure 11. Diagnostic algorithm.** ALK-1 = activin-receptor-like kinase; ANA = anti-nuclear antibodies; BMPR2 = bone morphogenetic protein receptor 2; CHD = congenital heart disease; CMR = cardiac magnetic resonance; CTD = connective tissue disease; Group = clinical group (Table 3); HHT = hereditary haemorrhagic telangiectasia; HIV = human immunodeficiency virus; HRCT = high-resolution computed tomography; LFT = liver function tests; mPAP = mean pulmonary arterial pressure; PAH = pulmonary arterial hypertension; PCH = pulmonary capillary haemangiomas; PFT = pulmonary function test; PH = pulmonary hypertension; PVOD = pulmonary veno-occlusive disease; PWP = pulmonary wedge pressure; RHC = right heart catheterization; TEE = transoesophageal echocardiography; TTE = transthoracic echocardiography; US = ultrasonography; V/Q scan = ventilation/perfusion lung scan.  
 From: Galie N et al. Guidelines for the diagnosis and treatment of pulmonary arterial hypertension. Eur Heart J 2009;30:2497-2537.

If non-invasive assessment is compatible with PH, clinical history, symptoms, signs, ECG, chest radiograph, transthoracic echocardiogram, pulmonary function tests (including nocturnal oximetry, if required), and high resolution CT of the chest are requested to identify the presence of group 2 or group 3. If these are not found or if PH seems 'out of proportion' to their severity, less common causes of PH should be looked for. If a ventilation/perfusion scan shows multiple segmental perfusion defects, a diagnosis of group 4 should be suspected. The final diagnosis of CTEPH (and the assessment of suitability for pulmonary endarterectomy) will require CT pulmonary angiography, RHC, and selective pulmonary angiography. The CT scan may also show signs suggestive of group 1' (PVOD). If a ventilation/perfusion scan is normal or shows only subsegmental 'patchy' perfusion defects, a tentative diagnosis of group 1 or the rarer condition of group 5 is made.

Additional specific diagnostic tools including haematology, immunology, serology, biochemistry and ultrasonography will allow the final diagnosis to be refined.

Open or thoracoscopic lung biopsy entails substantial risk of morbidity and mortality. Because of the low likelihood of altering the diagnosis and treatment, routine biopsy is discouraged in PAH patients.

### **Evaluation of severity and prognostic factors**

Prognosis in PAH depends largely on its etiology. A French registry showed a 1-year survival of these patients of 85,7%, 2-years survival of 69,5% and 3-years survival of 54,9%. The US REVEAL registry confirmed these data and indicated as important prognostic factors the etiology, the functional class, sex, reduced exercise capacity, haemodynamic parameters and right ventricular function.

The evaluation of severity of functional impairment in PAH has a pivotal role to identify

the optimal therapeutic strategy, the response to therapy and the need of possible escalation of therapy during the follow-up.

Based on the clinical, non-invasive and invasive findings the clinical condition of a patient can be defined as:

- **Stable and satisfactory:** patients in this condition should fulfil the majority of the findings listed in the 'better prognosis' column in table 3. In particular, the patient is characterized by absence of clinical signs of right ventricular failure, stable WHO functional class I or II without syncope, a six-minute walking test distance > 500 m, a peak VO<sub>2</sub> > 15ml/min/Kg, normal or near-normal BNP/NT-proBNP plasma levels, no pericardial effusion, TAPSE > 20 mm, right atrial pressure < 8 mmHg, and a cardiac index ≥ 2,5 L/min/m<sup>2</sup>.
- **Stable and not satisfactory:** this is a patient who, although stable, has not achieved the status which patient and treating physician would consider desirable. Some of the limits described for a stable and satisfactory condition and included in the first column of table 3 are not fulfilled. These patients require re-evaluation and consideration for additional or different treatment.
- **Unstable and deteriorating:** Patients in this condition fulfil the majority of the findings listed in the 'worse prognosis' column of table 3. In particular the patient is characterized by evidence of progression of right ventricular failure symptoms and signs, worsening WHO functional class, i.e. from II to III or from III to IV, a 6 min walk distance of < 300 m, a peak VO<sub>2</sub> < 12 mL/min/kg, rising BNP/NT-proBNP plasma levels, evidence of pericardial effusion, TAPSE < 1.5 cm, right atrial pressure > 15 mmHg and a cardiac index that is ≤ 2.0 L/min/m<sup>2</sup>. Clinical warning signs are increasing oedema and/or the need to escalate diuretic therapy, new onset or increasing frequency/severity of angina which can be a sign of deteriorating RV function, and the onset or increasing frequency of

syncope which is often a grim prognostic sign and requires immediate attention as it heralds low output heart failure. Supraventricular arrhythmias may be seen in this condition and contribute to clinical deterioration.

**Table 3.** Parameters with established importance for assessing disease severity, stability and prognosis in PAH (adapted from McLaughlin and McGoon [18]).

Better prognosis	Determinants of prognosis	Worse prognosis
No	Clinical evidence of RV failure	Yes
Slow	Rate of progression of symptoms	Rapid
No	Syncope	Yes
I, II	WHO-FC	IV
Longer (>500 m) <sup>a</sup>	6MWT	Shorter (<300 m)
Peak O <sub>2</sub> consumption >15 mL/min/kg	Cardio-pulmonary exercise testing	Peak O <sub>2</sub> consumption <12 mL/min/kg
Normal or near-normal	BNP/NT-proBNP plasma levels	Very elevated and rising
No pericardial effusion TAPSE <sup>b</sup> >2.0 cm	Echocardiographic findings <sup>b</sup>	Pericardial effusion TAPSE <sup>b</sup> <1.5 cm
RAP <8 mmHg and CI ≥2.5 L/min/m <sup>2</sup>	Haemodynamics	RAP >15 mmHg or CI ≤2.0 L/min/m <sup>2</sup>

<sup>a</sup>Depending on age.

<sup>b</sup>TAPSE and pericardial effusion have been selected because they can be measured in the majority of the patients.

BNP = brain natriuretic peptide; CI = cardiac index; 6MWT = 6-minute walking test;

RAP = right atrial pressure; TAPSE = tricuspid annular plane systolic excursion;

WHO-FC = WHO functional class.



## **PULMONARY ARTERIAL HYPERTENSION ASSOCIATED WITH CONNECTIVE TISSUE DISEASE**

PAH is a well-known complication of connective tissue diseases (CTD) such as systemic sclerosis, systemic lupus erythematosus, mixed CTD, and, to a lesser extent, rheumatoid arthritis, dermatomyositis, and Sjögren's syndrome. PAH associated with CTD (about 15%) is the second most prevalent type of PAH after IPAH (about 53%) in registries [5].

Systemic sclerosis, particularly in its limited variant (CREST syndrome), represents the main CTD associated with PAH. The prevalence of haemodynamically proven PAH in large cohorts of patients with systemic sclerosis is between 7 and 12% [19].

In these patients, PAH may occur in association with interstitial fibrosis or as a result of an isolated pulmonary arteriopathy. For this reason it is imperative to determine which mechanism is operative since this dictates treatment. Histopathological changes in PAH associated with CTD are generally indistinguishable from those of classical IPAH, although there is more frequent involvement of the pulmonary veins [20]. The pathophysiological mechanisms leading to PAH in patients with CTD remain unknown. The presence of antinuclear antibodies, rheumatoid factor, immunoglobulin G, and complement fraction deposits in the pulmonary vessels wall suggests a role of immunological mechanisms.

**Diagnosis.** Compared with IPAH, patients with CTD and PAH are mainly women (women/men ratio 4:1), are older (mean age at diagnosis: 66 years), may present concomitant disorders (pulmonary fibrosis, left heart disease), and have shorter survival [19]. The unadjusted risk of death for systemic sclerosis-associated PAH compared with IPAH is 2.9 [21] and the predictors of outcome are the same as for IPAH (RAP, PAP, and CI). Symptoms and clinical presentation are very similar to those of IPAH and occasional

patients thought to have IPAH can be identified as having an associated CTD by immunological screening tests. High-resolution CT is helpful for evaluating the presence of associated interstitial lung disease. An isolated reduction of diffusion capacity of carbon monoxide is a frequent abnormality in systemic sclerosis associated with PAH.

Echocardiographic screening for the detection of PH has been recommended annually in asymptomatic patients with the scleroderma spectrum of diseases but only in the presence of symptoms in other CTDs. As in other forms of PAH, RHC is recommended in all cases of suspected PAH associated with CTD to confirm the diagnosis, determine severity, and rule out left-sided heart disease. RHC is mandatory if targeted treatments are considered. The proportion of responders in the acute vasodilator test is lower than in IPAH and its clinical usefulness is unclear [22].

**Table 1.** Recommendations for PAH associated with connective tissue disease.

Statement	Class	Level
In patients with PAH associated with CTD the same treatment algorithm as in patients with IPAH is recommended	I	A
Echocardiographic screening for the detection of PH is recommended in symptomatic patients with scleroderma spectrum of diseases	I	B
Echocardiographic screening for the detection of PH is recommended in symptomatic patients with all other CTDs	I	C
RHC is indicated in all cases of suspected PAH associated with CTD, in particular if specific drug therapy is considered	I	C
Oral anticoagulation should be considered on an individual basis	IIa	C
Echocardiographic screening for the detection of PH may be considered in asymptomatic patients with the scleroderma spectrum of disease	IIb	C

From: Galiè N et al. Guidelines for the diagnosis and treatment of pulmonary arterial hypertension. Eur Heart J 2009; 30: 2497-2537.



## SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is a chronic multisystem disease characterized by fibrosis of the skin and internal organs, microangiopathy and autoimmune disturbance. Because of its peculiar histopathological lesions, it is considered a systemic connective tissue disease.

The disease was previously called *Scleroderma*, that is derived from the Greek for 'hard skin' and emphasises the dermatological component of the disease. It was described by Hippocrates.

**Classification.** Two major subsets are recognized, according to the extent of the skin involvement, namely, SSc with limited cutaneous involvement and SSc with diffuse cutaneous involvement [23].

The diffuse cutaneous form has a more rapid onset, the skin changes (thickening and tightening) may spread rapidly within a few months of Raynaud's phenomenon onset, and symmetrically involves all the body surface. Often there is an early internal organ involvement and the prognosis is poor.

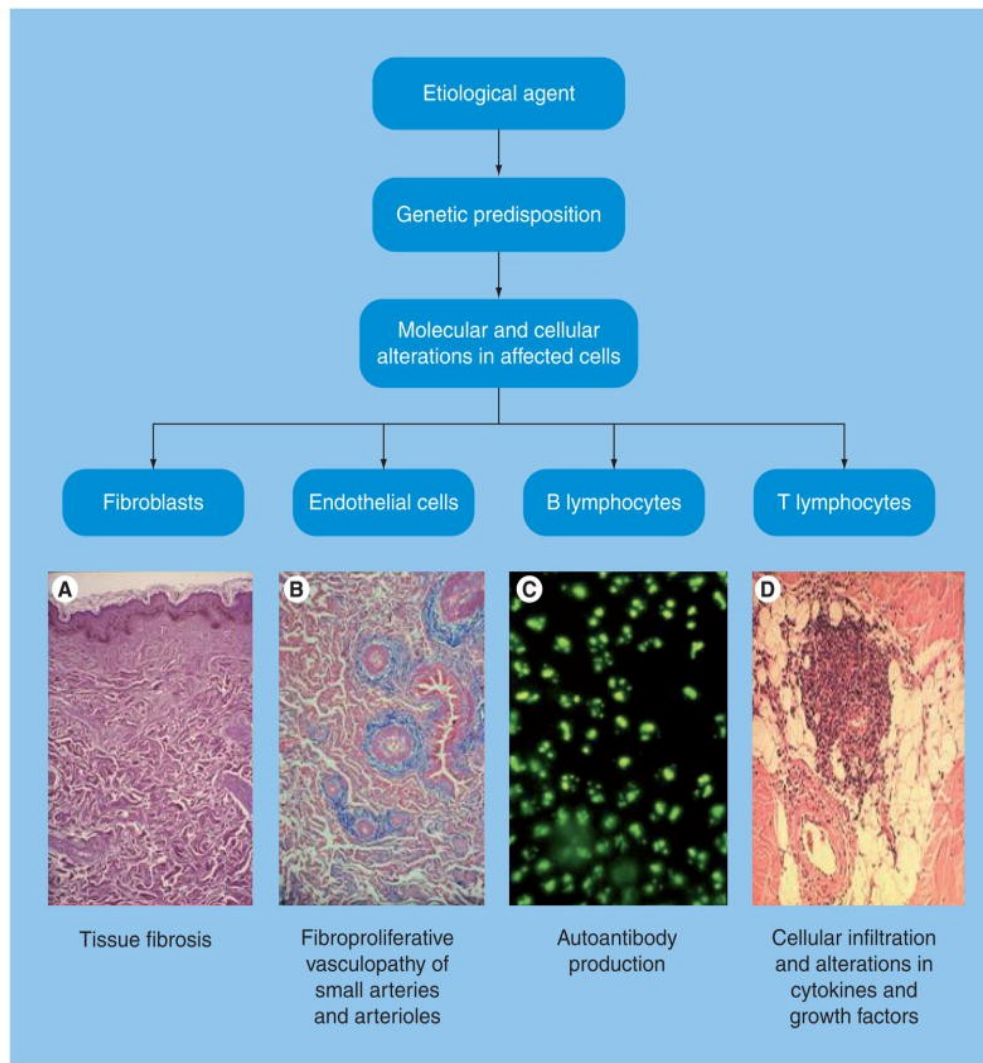
The limited cutaneous form, previously called CREST syndrome (**C**alcinosis, **R**aynaud's disease, **E**sophageal dysmotility, **S**clerodactyly, **T**eleangiectasia) is the more frequent type of SSc (approximately 60% of patients). The onset is characterized by the Raynaud's phenomenon, that usually precedes the skin involvement by some years. Skin changes affects only the face, forearms and lower legs up to the knee. Internal organs are less often affected, and the prognosis is less severe. However an obliterative vasculopathy of the pulmonary arterioles can occur, leading to isolated pulmonary hypertension, without signs of interstitial lung disease.

**Epidemiology.** In Europe the annual incidence of SSc is estimated to be 10 to 20 cases per 1 million persons, whereas the prevalence is 50 cases per 1 million persons [24,25]. In US

the incidence and prevalence are higher [26]; African Americans are at greater risk for diffuse disease. Women are roughly four times more likely than men to develop SSc. The diagnosis is usually made in the third or fourth decade of life.

**Etiology and pathophysiology.** The pathological features of the Ssc are:

- Relevant and often progressive cutaneous and visceral fibrosis.
- Fibroproliferative vasculopathy.
- Humoral and cell mediated immunity disorders (circulating autoantibodies).



**Figure 1.** Pathogenetic mechanisms in SSc.

Etiology is unknown, but the complexity of its pathogenesis suggests that one or more environmental agents in genetically susceptible substrate are likely to be responsible for the development of SSc. The pathogenesis of SSc involves endothelium, epithelium, fibroblasts and immunological mediators, with cell-cell, cell-cytokine and cell-matrix interactions [27].

Most hypotheses focus on the interplay between early immunological events and vascular changes, leading to microvascular damage and fibroproliferative vasculopathy. Endothelial activation causes also extravasation of inflammatory cells, like macrofages, T and B lymphocyte, resulting in cytokines and growth factors production that activate a population of fibrogenic fibroblasts generally considered to be the effector cells in the disease. The autonomous activated fibroblasts continue to produce the excessive extracellular matrix that underlies the ultimate fibrotic pathology of SSc.

There is an impairment of endothelium-dependent vascular smooth muscle relaxation demonstrated by the evidence of reduced serum levels of nitric oxide and prostacyclin in SSc. Vascular injury occurs before clinically evident fibrosis. There is an altered functional state of the endothelium characterized by increased permeability, enhanced vasoreactivity, enhanced expression of adhesion molecules, altered balance between hemostatic and fibrinolytic factors, platelet activation, and altered vascular wall growth. Most damage occurs at the level of the cutaneous circulation and in the microvasculature of various internal organs. Small arteries and capillaries constrict, fibroproliferative changes in the vasculature ensue later, eventually leading to obliteration of the vascular lumen, resulting in ischemia.

There are many potent mediators of tissue fibrosis that are believed to play an important role in the pathogenesis of scleroderma. One of the key factors that has received the most attention as a very potent profibrotic factor, indirectly implicated very strongly in the

pathogenesis of SSc, is transforming growth factor (TGF)- $\beta$ 1. A number of studies have shown that TGF- $\beta$ 1 is a potent profibrotic factor in vitro and that its expression is upregulated in the skin and the lungs of Ssc patients. Other important growth factors are connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF) and the beta chemokines monocyte chemoattractant protein (MCP)-1 and MCP-3.

**Clinical manifestations.** Clinical manifestations of SSc are heterogeneous and vary as a results of type of disease (limited or diffuse) and organ involvement (Table 1).

<b>Table 1. Major clinical manifestations of SSc</b>
<p><b>Cutaneous</b></p> <ul style="list-style-type: none"> <li>Diffuse edema of hands and feet (early stages)</li> <li>Progressive skin tightening</li> <li>Sclerodactyly</li> <li>Calcinosis</li> <li>Telangiectasias</li> <li>Digital ulcers and pits</li> <li>Contractures</li> <li>Hyperpigmentation, hypopigmentation, salt and pepper skin</li> <li>Characteristic facies</li> </ul>
<p><b>Vascular</b></p> <ul style="list-style-type: none"> <li>Raynaud's phenomenon</li> <li>Nailfold capillary changes</li> <li>Digital ischemia and ulcers</li> <li>Vasculitic leg ulcers (rare)</li> </ul>
<p><b>Pulmonary</b></p> <ul style="list-style-type: none"> <li>Interstitial lung disease, including alveolitis and interstitial fibrosis</li> <li>Pulmonary hypertension</li> <li>Recurrent aspiration pneumonitis caused by esophageal reflux and dysmotility</li> <li>Chest wall restriction (decreased thoracic compliance)</li> <li>Respiratory muscle weakness</li> </ul>
<p><b>Cardiac</b></p> <ul style="list-style-type: none"> <li>Cardiomyopathy (systolic and diastolic dysfunction): <ul style="list-style-type: none"> <li>Congestive heart failure</li> </ul> </li> <li>Conduction defects: <ul style="list-style-type: none"> <li>Septal infarction pattern</li> <li>Ventricular conduction abnormalities</li> <li>Arrhythmias</li> <li>Heart blocks</li> </ul> </li> <li>Pericarditis or pericardial effusion (impending renal crisis)</li> </ul>
<p><b>Renal</b></p> <ul style="list-style-type: none"> <li>Scleroderma renal crisis (hypertension, renal failure, microangiopathic hemolytic anemia)</li> </ul>
<p><b>Musculoskeletal and Rheumatologic</b></p> <ul style="list-style-type: none"> <li>Arthralgia</li> <li>Tendon friction rubs (relatively specific for diffuse scleroderma)</li> <li>Inflammatory arthritis, erosive arthropathy (rare)</li> <li>Myopathy, myositis</li> </ul>
<p><b>Gastrointestinal</b></p> <ul style="list-style-type: none"> <li>Gastroesophageal reflux</li> <li>Esophageal dysmotility, aperistaltic esophagus</li> <li>Esophageal stricture</li> <li>Adenocarcinoma arising in Barrett's esophagus (occasionally)</li> <li>Watermelon stomach (gastric antral vascular ectasias [GAVE]): Iron-deficiency anemia</li> <li>Decreased peristalsis throughout the GI tract, leading to bloating, early satiety, stasis, and pseudo-obstruction</li> <li>Bacterial overgrowth and malabsorptive diarrhea, alternating diarrhea and constipation</li> </ul>

Megacolon (rare) Colonic wide-mouth diverticuli (usually asymptomatic) Pneumatosis cystoides intestinales Primary biliary cirrhosis Anal incontinence
<b>Endocrine</b> Hypothyroidism
<b>Neurologic</b> Carpal tunnel syndrome Trigeminal neuralgia

**Diagnosis.** Diagnostic criteria have been proposed by the American College of Rheumatology [28].

<b>Table 2. American College of Rheumatology Diagnostic Criteria for Systemic Sclerosis</b>
<b>Major Criterion</b> Proximal sclerodermatous skin changes (proximal to the metacarpophalangeal joints)
<b>Minor Criteria*</b> Sclerodactyly Digital pitting scars of fingertips or loss of substance of the distal finger pads Bibasilar pulmonary fibrosis

\* The patient should fulfill the major criterion or two of the three minor criteria.

A complete blood count, complete metabolic panel, muscle enzymes, thyroid function test, and urinalysis are indicated in all patients. Serologic testing for autoantibodies can be helpful in diagnosing and classifying SSc. However none of the serologic tests is sensitive enough to independently exclude disease. Antinuclear antibodies (antitopoisomerase-I and anticentromere antibodies) are present in the sera of more than 95% of scleroderma patients, with certain clinical phenotypes associated with specific ANAs. Antitopoisomerase-I antibodies (anti-topo-I or anti-Scl-70) are 100% specific for scleroderma and are present in 30% of patients with the diffuse cutaneous form. Anticentromere (ACA) antibodies are highly specific for Ssc and are particularly common in patients with the subset of limited SSc. Antinucleolar antibodies have been reported in 15% to 40% of patients with SSc [24].

**Therapy.** Although no therapy is proved to reverse the vascular and fibrotic damage in patients with SSc, several therapies are available in an effort to slow down disease

progression, improve vascular function, limit mortality and provide supportive symptomatic care. Patients with Raynaud's phenomenon are advised to stop smoking, avoid cold exposure, wear warm clothing and gloves, and avoid vasoconstrictive substances (clonidine, sympathomimetics, cocaine, ergot alkaloids).

Various pharmacologic agents are aimed at reversing digital vasospasm. The dihydropyridine calcium channels blockers (amlodipine and long-acting preparations of nifedipine) are first-line agents for the treatment of scleroderma-associated Raynaud's phenomenon.

The role of antiplatelet and anticoagulant therapy is unclear, although in the absence of contraindications, most experts would recommend low dose aspirin to patients with Raynaud's phenomenon.

Phosphodiesterase-5 (PDE<sub>5</sub>) inhibitors like sildenafil, tadalafil or vardenafil, have been found to be particularly useful in ameliorating refractory digital ischemia and ulceration, presumably because of their vasodilative properties, although they have not yet received regulatory approval for this indication [29].

Endothelin receptors antagonists (such as bosentan) are proved effective for the prevention of ischemic digital ulcers [30,31].

Intravenous prostanoids (e.g. Epoprostenol, alprostadil, or iloprost) have also been shown to ameliorate severe digital ischemia and improve digital ulceration [30].

Cyclophosphamide may be beneficial in patients with interstitial lung disease associated with SSc. Multiple uncontrolled studies had suggested that cyclophosphamide might slow the loss of, or even improve, lung function (specifically FVC) in the setting of early scleroderma with declining FVC and progressive dyspnea [32].

Methotrexate has been shown to improve skin scores, but the effects were modest [33].

Corticosteroids may be useful in the treatment of myositis and alveolitis, but their use is

limited by the observation that high doses can precipitate renal crisis.

Immunoablation combined with autologous stem cell rescue is still considered experimental, but one report documented improved skin scores. However 1-year mortality was high after transplantation [34].

Angiotensin-converting enzyme inhibitors should be used at the first sign of renal crisis (severe hypertension, renal failure, and microangiopathic hemolytic anemia). Their early use is critical in preserving renal function, controlling hypertension, and improving survival during renal crisis. In the hope that control of renin-mediated hypertension can result in renal recovery, therapy with angiotensin-converting enzyme inhibitors should be continued even in the face of renal insufficiency requiring dialysis [35].

Future directions: the central role of TGF- $\beta$  in inducing endothelial damage and fibroblast activation has led investigators to target this molecule as a promising site for future therapies. Indeed, anti-TGF- $\beta$  drugs and others cytokine-based therapies could theoretically provide true disease modification, especially in patients with early disease, before cutaneous and internal fibroses result in significant irreversible damage [36].

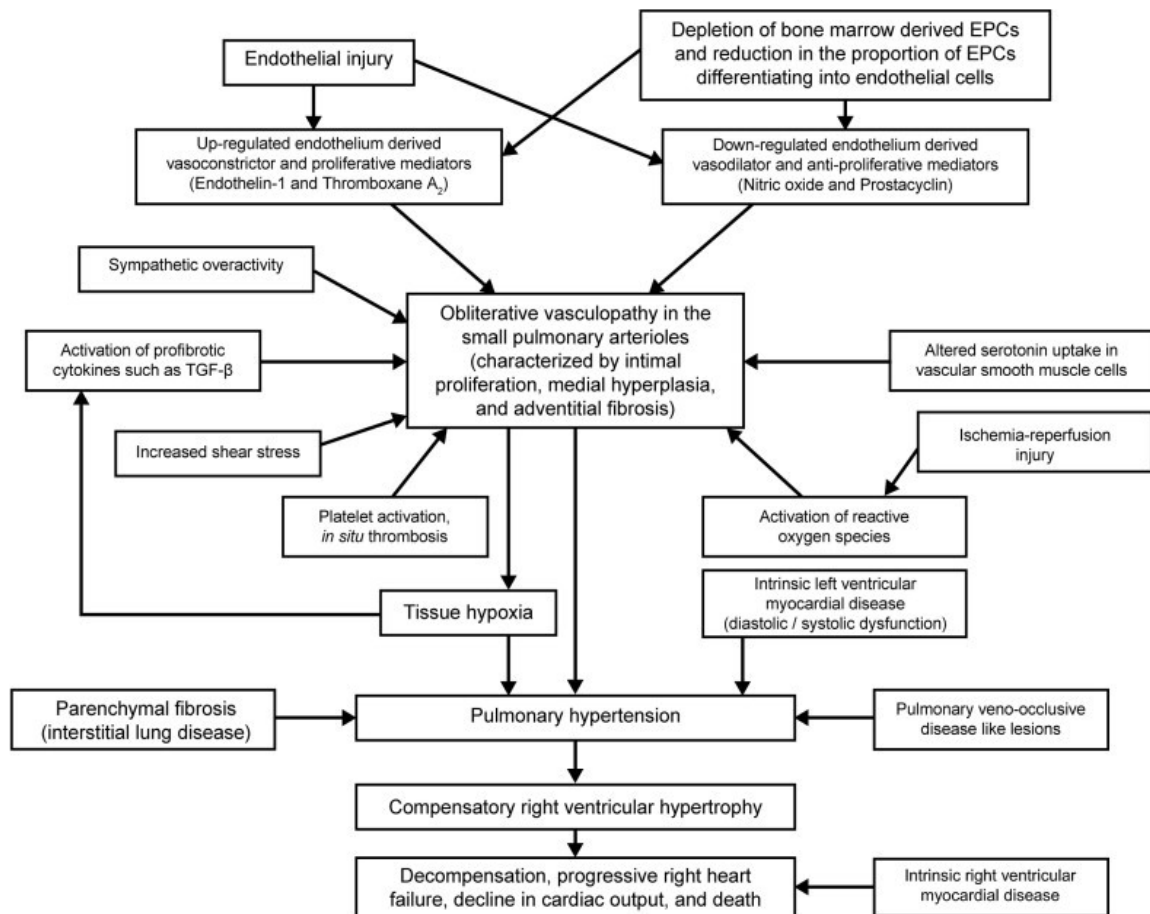




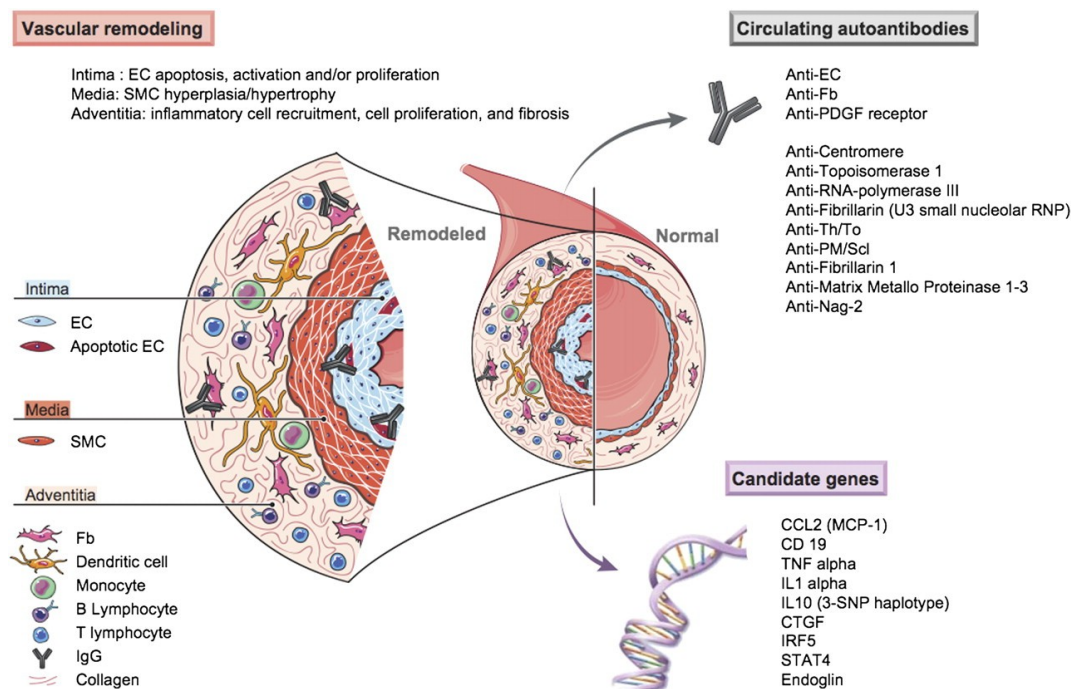
# PULMONARY ARTERIAL HYPERTENSION IN SYSTEMIC SCLEROSIS

## Pathogenesis of Pulmonary Arterial Hypertension in Systemic Sclerosis (SSc-PAH)

The pathogenesis and physiopathology of SSc-PAH look like those of IPAH. They share the same pulmonary artery lesions. This findings suggest similarities in physiopathology, that is the involvement of inflammation and immunity system in both the types of PAH. However it is likely that inflammatory pathways and autoimmunity are more pronounced in SSc-PAH, explaining clinical and prognosis differences between the two forms [37].



**Figure 1.** Pathogenesis of pulmonary hypertension in SSc.  
EPC = endothelial precursor cells.



**Figure 2.** Pulmonary vascular remodeling in SSc-PAH.

From: Le Pavec J et al. Systemic sclerosis-associated pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2010;181:1285-93.

Vascular changes occur at an early stage in SSc and include apoptosis, endothelial cell activation with increased expression of cell adhesion molecules, inflammatory cell recruitment, a procoagulant state, intimal proliferation, and adventitial fibrosis leading to vessel obliteration. Endothelial injury is reflected by increased levels of soluble cell adhesion molecules, disturbances of angiogenesis as reflected by increased levels of circulating vascular endothelial growth factor, and presence of angiostatic factors [38]. Thus, the role of dysregulated angiogenesis in SSc-PAH, whether driven by the inflammatory process or other mechanisms, is a predominant feature of the disease and should be a focus of future studies as a potential target for therapy. The involvement of the inflammatory cells is demonstrated by the finding of macrophages, T and B lymphocytes,

and dendritic cells around plexiform lesions. Levels of macrophage inflammatory protein-1a, IL-1b and IL-6, and P-selectin are increased in severe IPAH. Involvement of leukocytes, macrophages, and lymphocytes, initially described in the complex vascular lesions of IPAH, is also a prominent feature in PAH associated with connective tissue diseases [39]. A role for autoimmunity is suggested by the presence of a number of autoantibodies in the serum of patients with SSc. Antibodies have also been reported in SSc-PAH, including fibrin-bound tissue plasminogen activator in patients with limited cutaneous SSc, and in patients with IPAH with HLA-DQ7 antigen, and anti-topoisomerase II- $\alpha$  antibodies, particularly in association with HLA-B35 antigen. In vitro, autoantibodies from patients with connective tissue diseases (anti-U1-ribonucleoprotein and anti-ds-DNA) can up-regulate adhesion molecules and histocompatibility complex class II molecules on human pulmonary arterial endothelial cells, suggesting that inflammation could lead to pulmonary proliferative vasculopathy [40]. Fibroblasts are found in the remodeled neointimal layer in both SSc-PAH and IPAH. Thus, the detection of anti-fibroblast antibodies in the serum of patients with SSc-PAH and IPAH has significant pathogenic importance because these anti-bodies can activate fibroblasts and induce collagen synthesis, potentially contributing directly to the remodeling process. They induce a proadhesive and proinflammatory response in normal fibroblasts, and have distinct reactivity profiles in IPAH and SSc-PAH, as assessed by immunoblotting. Several fibroblast antigens recognized by serum IgG from patients with IPAH and SSc-PAH have so far been identified, including proteins involved in regulation of cytoskeletal function, cell contraction, cell and oxidative stress, cell energy metabolism, and other key cellular pathways [41].

Despite recent advances in genetics regarding SSc, little is known about genetic involvement in SSc-PAH. Mutations in the gene coding for bone morphogenetic protein

receptor 2 (a member of the transforming growth factor- $\beta$  receptor family) have not been detected in two small cohorts of SSc-PAH [42]. Recently, an association between an endoglin gene polymorphism and SSc-PAH was reported. Endoglin, a homodimeric membrane glycoprotein primarily present on human vascular endothelium, is part of the transforming growth factor- $\beta$  receptor complex. Although endoglin mutations are known causes of hereditary hemorrhagic telangiectasia and have been rarely identified in patients with PAH, the functional significance of endoglin polymorphism in patients with SSc remains to be determined [43].

### **Prevalence and incidence of SSc-PAH**

In prospective studies using right heart catheterization (RHC) for diagnosis, the prevalence of SSc-PAH is between 7.8 and 12% [19,44,45]. With an estimated U.S. prevalence of SSc of about 240 cases per million and a conservative PAH prevalence of 10% among these patients, the estimated overall prevalence of SSc-PAH is around 24 individuals per million, which represents 5 to 10 times the number of patients affected by IPAH [46]. In the French PAH registry, connective tissue disease (mainly represented by SSc) accounts for 15.3% of PAH cases [5]. Probably because of a higher prevalence of SSc in the United States [47], the proportion of SSc-PAH is at least 30% of patients with PAH, as indicated by one single large registry [48]. In a recent prospective study, the estimated incidence of PAH among patients with SSc was 0.61 cases per 100 patient-years [49].

However the histopathological evidence of pulmonary arteriopathy was documented in more than 72% of SSc patients, suggesting some discrepancies between clinical and pathological data [50]. Prevalence of SSc-PAH based on echocardiographic diagnosis is often overestimated; a survey conducted by 59 Societies of Rheumatology, on a population of 909 subjects showed a prevalence of PAH in 27% of patients affected by SSc or other

CTD, defining PAH on the basis of a PAsP value  $> 40$  mmHg [51].

Several clinical markers are associated with an increased risk of developing PAH in the setting of SSc, including limited skin involvement [52-55], disease duration greater than 10 years [53], late age of onset of Ssc [56,57], severity or duration of Raynaud's phenomenon, and reduced nailfold capillary density [53]. Several investigators have emphasized the pivotal role of an isolated reduction in diffusing capacity of carbon monoxide (DLCO) or a progressive decline of DLCO as an independent predictor for subsequent PAH [52,54]. Although the decrease in DLCO is likely the result of progressive pulmonary vascular remodeling over time, it is interesting to note that this alteration is significantly more pronounced in patients with SSc-PAH compared with IPAH patients [37], perhaps suggesting more profound small-vessel remodeling in the former compared with the latter.

### **Diagnosis of Pulmonary Arterial Hypertension in SSc [58]**

As previously mentioned some risk factors are associated to an increased risk for developing PAH in the setting of SSc: limited skin involvement, disease duration greater than 10 years, late age of onset of SSc, severity or duration of Raynaud phenomenon, reduced nailfold capillary density, but also positivity of anticentromere antibodies [59], the presence of aninucleolar antibodies, anti U3 RNP and anti Th/To antibodies [60], increased levels of BNP or NT-proBNP, and altered spirometric parameters that are  $DLCO < 60\%$ , in absence of diffuse interstitial lung disease or  $FVC\%/DLCO\%$  ratio  $> 1,6$ .

Many studies have highlighted the pivotal role of a reduced  $DLCO < 50\%$  of the predicted value or a progressive reduction of DLCO as an independent predictor of PAH development [49], because DLCO could be significantly reduced many years before the diagnosis of PAH [61].

The presence of dyspnea could activate a diagnostic work-up to define the presence of

PAH, especially in those patients with reduced DLCO. In fact the risk of a late diagnosis or a misdiagnosis remains high, because of slowly development of dyspnea, frequently accounted for the presence of musculoskeletal manifestations or because of the reduction of symptoms due to limitation in activity [62].

Fisher and colleagues underlined the need of an yearly echocardiographic screening in patients with SSc, including the asymptomatic ones, to avoid a late diagnosis, usually made after 2 or 3 years from the onset of the disease [60,63].

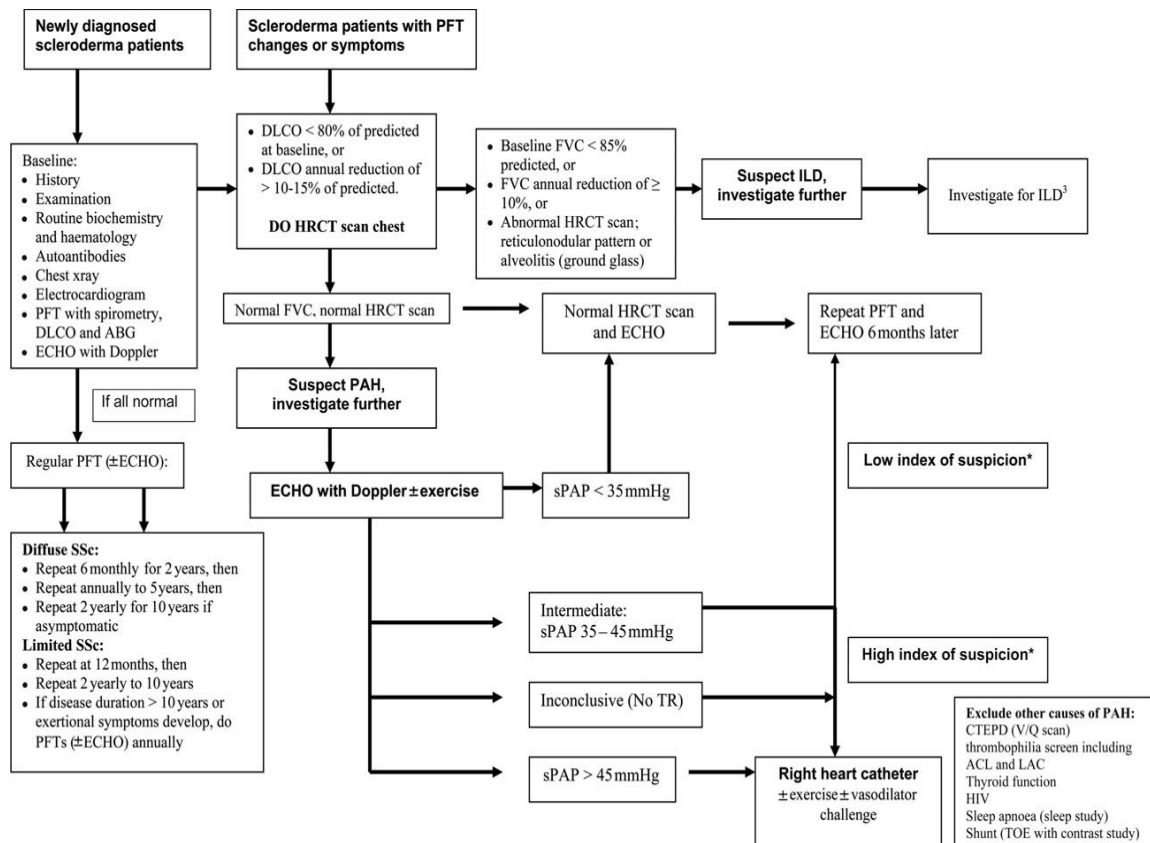
Asymptomatic patients with normal functional respiratory tests (DLCO and FVC), could also undergo echocardiographic study every 2 years.

On the other hand, Campo et al. suggested the diagnostic work-up for PAH immediately after the appearance of one of the symptoms referable to the underlying disease (except for the Raynaud's phenomenon) [64].

**Table 1.** Findings associated with PAH in SSc.

Symptoms	Recent onset of dyspnea on exertion
Physical examination	Signs of right heart failure
Echocardiographic findings	TR jet > 3.0 m/s (PAsP > 40 mmHg) Right ventricular dilatation/hypokinesis Pericardial effusion
PFT	DLCO% < 60% without significant pulmonary lung disease FVC%/DLCO% >1.6
Other parameters	Increased levels of BNP or pro-NT-BNP Reduced oxygen saturation during eexercise

PFT: pulmonary functional tests.



**Figure 3.** Algorithm for the diagnosis of lung disease in SSc.

ABG, arterial blood gas; ACL, anticardiolipin Ab; CTEPD, chronic thromboembolic pulmonary disease; LAC, lupus anticoagulant; IAP, pulmonary arterial hypertension; PFT, pulmonary function tests; RHC, right heart catheter; SPAP, systolic pulmonary artery pressure; TOE, transoesophageal echocardiogram; V/Q scan, ventilation/perfusion scan.

From: Proudman SM et al. Pulmonary arterial hypertension in systemic sclerosis: the need for early detection and treatment. Intern Med J 2007;37(7):485-94.

### Biohumoral markers and evaluation of disease severity

Over the past few years, the Brain Natriuretic Peptide (BNP) and its precursor, NT-proBNP, have been studied in various forms of PAH and found to be useful as prognostic indicators in SSc-PAH and to assess the efficacy of the therapies [65].

BNP is released by the ventricular myocardium in response to volume or pressure overload

and represents a marker of ventricular stretching; it was demonstrated that levels of BNP correlate with functional capacity, mortality and right ventricular systolic dysfunction. Recent studies have also demonstrated that BNP and NT-proBNP could be useful in the early diagnosis of PAH in patients at risk, such as those affected by SSc [66,67].

A NT-proBNP cut-off value of 365 pg/ml has been proven to have a sensitivity of 56-69% and a specificity of 95-100% to identify the presence of PAH in SSc patients [65]. Mathai et al. showed that SSc-PAH patients had significantly higher levels of NT-proBNP compared to IPAH patients, despite similar hemodynamic conditions. In SSc-PAH patients, in addition to cardiac wall stretching, another trigger mechanism for the production of natriuretic peptides seems to be a more pronounced neuro-hormonal activation [68].

Combining the elevated levels of NT-proBNP with the reduced values of DLCO it could be possible to obtain useful informations for the diagnosis and the follow-up of patients with SSc-PAH [69].

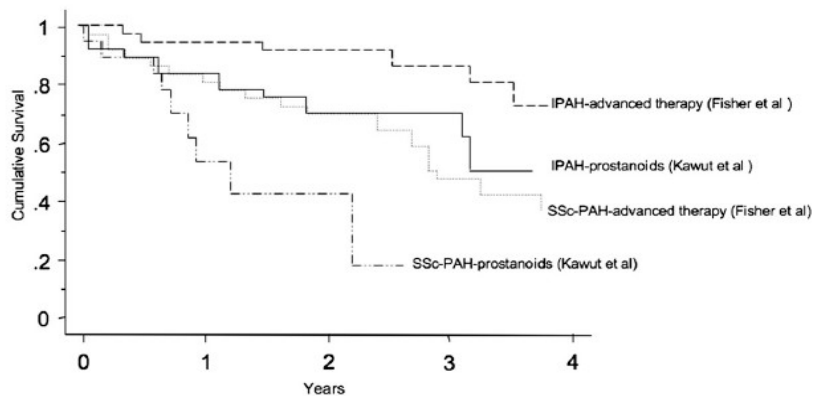
A novel biomarker that could identify patients affected by IPAH or SSc-PAH is the Growth differentiation factor 15 (GDF-15), that is a protein belonging to the transforming growth factor beta superfamily that has a role in regulating cellular growth, differentiation, cell-to-cell signaling and apoptotic pathways. Probably it is also involved in the pathogenesis of PAH. Recent data showed that SSc-PAH patients had increased levels of GDF-15 [70]; these levels directly correlate with pulmonary artery pressure and with FVC/DLCO ratio, and are associated with a poor prognosis.

## **Survival**

SSc-PAH has emerged as a leading cause of mortality [71]. Before the introduction of the new therapies, 5-year survival in patients with SSc-PAH was 10%, differently from the 80% survival of SSc patients without PAH [60].



Compared with IPAH patients, those with SSc-PAH are almost four times more likely to die from their disease [37,72]. Moreover, outcomes in SSc-PAH remain worse than those in PAH associated with other CTD [73]. At a time of broader treatment availability, and despite substantial improvements in other PAH categories, 3-year survival remains less



**Figure 4**

Kaplan-Meier analysis showing survival in patients with systemic sclerosis-related pulmonary arterial hypertension (SSc-PAH) and idiopathic pulmonary arterial hypertension (IPAH) treated with prostanoids or advanced therapy. ERA = endothelin receptor antagonists; mPAP = mean pulmonary arterial pressure; NYHA = New York Heart Association; PDE5 = phosphodiesterase 5; PVR = pulmonary vascular resistance.

Studies	Kawut et al (21)		Fisher et al (37)	
Period of time	1997-2001		2000-2005	
Patients	IPAH n = 33	SSc-PAH n = 22	IPAH n = 41	SSc-PAH n = 50
First line PAH therapy	Prostanoids		ERA, PDE5 inhibitors, or prostanoids according to guidelines	
NYHA FC III-IV, %	-		77.5	64
mPAP, mmHg	52 ± 12	48 ± 10	54 ± 1.9	47 ± 1.5
CI, L.min <sup>-1</sup> .m <sup>-2</sup>	2.1 ± 0.62	2.2 ± 0.54	2.1 ± 0.1	2.2 ± 0.1
PVR, dyn.s.cm <sup>-5</sup>	918 ± 433	755 ± 238	941 ± 88	855 ± 83

From: Le Pavec et al. Systemic sclerosis-associated pulmonary arterial hypertension. Am J Respir Crit Care Med 2010;181:1285-93

than 60% (Figure 4) [73,74,37,49,19,75].

Markers of worse prognosis include male sex, late age at diagnosis, pericardial effusion, functional severity based on New York Heart Association functional class, right heart dysfunction, and hyponatremia [19,37,73,76].

Moreover patients with SSc-PAH have a more severe right ventricular dysfunction than patients affected by IPAH, although it is unclear if this datum can explain the increased mortality rate of SSc patients [77].

## **Pulmonary involvement in Systemic Sclerosis**

Involvement of the lungs is present in about 60-70% of SSc patients, represents the leading cause of mortality and morbidity [50] and consists most often of interstitial fibrosis or interstitial lung disease (ILD) and pulmonary vascular disease leading to PAH. Autoptic examinations showed lung fibrosis in the majority of SSc patients [71].

There are also patients that develop both PAH and ILD, as two distinct and independent processes. Patients with limited SSc will typically develop isolated PAH 10 to 15 years after the onset of their disease, whereas patients with diffuse SSc are at greater risk for ILD (occurring in the 75% of cases), usually within the first 5 years after diagnosis. In these patients fibrosis can cause pulmonary hypertension (PH-ILD). PH is generally modest (mean pulmonary arterial pressure: 25-35 mmHg), but in some patients, particularly those with only moderate pulmonary function impairment, PAP elevations can be more substantial (mean PAP: 35-50 mmHg). In this case PH is considered out of proportion to the degree of lung impairment. However, although SSc patients with ILD alone have a mean survival of 5 to 8 years, development of PH will significantly shorten survival.

Recent studies have concluded that PH-ILD was associated with a fivefold increased risk of death compared with SSc-PAH [73]. When dyspnea occurs in SSc patients it is necessary to make the differential diagnosis between ILD, PAH, or other causes.

Respiratory functional tests, particularly DLCO, as already asserted, represent an early marker of ILD and PAH and correlate with the severity of the underlying disease in both cases [78]. An isolated reduction of DLCO is observed in 20% of SSc patients [79]. Pulmonary fibrosis causes more specifically the reduction of respiratory volumes, and so the total pulmonary capacity and the forced vital capacity (FVC). It has been proposed that pulmonary disease severity in SSc was defined as mild, moderate or severe when values of FVC and DLCO were 70-79, 50-69 and < 50% of predicted, respectively [80].

High resolution computed tomography (HRCT) permits to establish the severity grades of ILD, considering the extent, the localization of the fibrosis, the extent of the reticular pattern, and of the ground-glass opacities. HRCT has also a key role in the prognosis of SSc patients [81]. A study enrolling 215 subjects, patients with more extensive pulmonary fibrosis seen at HRCT (abnormalities involving > 20% of the pulmonary volume) have a greater mortality and more progression of the lung disease than patients with pulmonary involvement < 20%. HRCT has a high sensitivity to detect zones of fibrosis that have been found in 55-65% of SSc patients, up to 96% of patients with abnormal functional respiratory tests [82].

The risk of lung cancer is increased in patients with both limited or diffuse cutaneous SSc. The incidence rate of malignant lung neoplasms is approximately fivefold higher than that for an age and gender matched subset of the general population.

### **Cardiac involvement**

Heart involvement in SSc patients is either primary, related to myocardial fibrosis, or secondary, as may occur in cases complicated by PAH or systemic hypertension in those with renal crisis. Primary involvement can exhibit as myocardial dysfunction, conduction defects, arrhythmias and pericardial disease.

Clinically evident cardiac involvement is present in less than 25% of the subjects, although 80% of SSc patients have cardiac abnormalities seen at autopsy [83]. The responsible mechanisms are: microvascular injury, interstitial fibrosis, pulmonary hypertension.

Myocardial fibrosis is thought to result from recurrent vasospasm of small vessels and it is often associated with contraction band necrosis, that is a histological lesion indicative of myocardial ischemia followed by reperfusion. The degree of myocardial fibrosis may be increased in SSc patients with a long history of Raynaud's phenomenon.

Diastolic and systolic dysfunctions of both the right and left ventricles are common in SSc patients [84]. Echocardiographic evaluation using tissue Doppler has a high sensitivity to detect myocardial abnormalities, especially the diastolic dysfunction [85].

Cardiac RMN allows to identify the subclinical myocardial involvement in asymptomatic SSc patients, showing reduced end-diastolic and end-systolic ventricular volumes compared to healthy subjects [86,87].

Cardiac RMN seems to have higher sensitivity than echocardiography (75% versus 48%), because its ability to directly visualize the presence of fibrosis or scar using late gadolinium enhancement, signal intensity, and myocardial wall thinning [87].

The prevalence of atherosclerotic coronary artery disease is not higher in SSc patients than the general population; they often have coronary microvascular dysfunction rather than epicardial coronaries involvement. Moreover the spasm of small coronary arteries can occur, termed myocardial Raynaud's phenomenon.

Conduction system disease and arrhythmias are common. They are likely to result from fibrosis of the myocardium and conduction system. Many death among SSc patients are sudden, and some may result from ventricular arrhythmias.

Symptomatic pericarditis occurs in 7 to 20% of patients with SSc, but pathological evidence of pericardial involvement is observed in 70 to 80% at autopsy. Pericardial effusions may be small or large and can develop rapidly.

### **The role of echocardiography in Systemic Sclerosis**

Echocardiography allows the evaluation of many cardiac complications of SSc. The main role is the study of systolic and diastolic left ventricular function, the right ventricular systolic function evaluation, the assessment of pericardial involvement and the estimate of systolic pulmonary arterial pressure.

*Left ventricular diastolic function.* Diastolic function is often impaired in SSc patients, and can present different grades of severity, coming from the impaired relaxation up to the restrictive pattern; the study of diastolic function consists in a pluriparametric evaluation as already previously described.

*Left ventricular systolic function.* It is less often impaired than diastolic function. It could be the manifestation of a primary cardiac damage or secondary to arterial hypertension or renal crisis. Systolic dysfunction can be classically evaluated by the measurement of the ejection fraction of the left ventricle, but recently other techniques are available to detect subclinical and early stages of systolic impairment.

TDI: Doppler technique permits the evaluation of myocardial tissue velocities; the measurement of longitudinal systolic velocity S' of the left ventricular lateral wall, is an index of the longitudinal contractile function of the left ventricle, that is a parameter of early systolic dysfunction [84].

Speckle-tracking: this technique is used for the measurement of myocardial strain that is the percentage change in length of a myocardial segment respect to its initial length. It is an index of myocardial wall deformation that was recently introduced in clinical practice [88], and in SSc patients has been demonstrated to be a valid technique to detect subclinical myocardial involvement [89].

*Right ventricular systolic function.* SSc patients can have right ventricular systolic dysfunction either primary or secondary to pulmonary hypertension. The routine evaluation of the systolic function of the right ventricle provides the measurement of TAPSE, TAV and right ventricle fractional area change, as already illustrated in a previous chapter [90].

*Pericardial diseases.* Echocardiographic evaluation of the pericardial layers help in the diagnosis of pericardial complications that are: acute pericarditis, pericardial effusion,

pericardial tamponade or constrictive pericarditis.

*Pulmonary hypertension.* Echocardiography is the more used non-invasive tool for initial diagnosis of PAH, for the screening of high risk population, for the evaluation during follow-up, and for the assessment of prognosis and the efficacy of therapies.

The echocardiographic estimate of systolic arterial pulmonary pressure and the other useful parameters that integrate the study are already previously described.

The PAsP values, assessed by ecocardiography, that indicate the diagnosis of PAH and recommend the execution of RHC, are still not clear. Moreover PAsP estimate is never the only parameter used to establish the need to further evaluations. In both the IPAH and the SSc-PAH cardiac function, particularly the right ventricle function, represents one of the most important predictors of survival, hence deserving a careful assessment, using also new techniques such as speckle tracking and strain measurement.

### **Exercise echocardiography in Systemic Sclerosis**

Pulmonary hypertension induced by exercise (mean PAP  $\geq$  30 mmHg) is no more considered a diagnostic definition, because it is not supported by published data, and it lacks standardised parameters concerning the type and level of exercise, that could affect the value of the mean PAP. Moreover, in contrast to the PAP at rest, PAP during exercise is largely age-dependent; younger healthy subjects aged < 50 years can reach values > 30 mmHg during submaximal or maximal exercise; after a mild exercise, 50% of subject over fifty years of age can reach values > 30 mmHg [91,92].

In healthy subjects a moderate effort induces an increase of cardiac output and pulmonary blood flow with a reduction of vascular resistance and only mild increase of pulmonary pressures [91]. Nevertheless in well-trained athletes, high levels of work are accompanied by remarkable increase of cardiac output and increase of left ventricular and left atrial

pressures, causing higher pulmonary pressures levels [93]. Thus, with the previous definition many individuals were incorrectly labelled as pulmonary hypertensive, that in fact had simply a physiological exercise response. Taking into account all these variables, it seems impossible to come up with a solid definition of PH during exercise. So in the current guidelines it was decided to abandon the exercise criterion. Further research is ongoing in this field to generate the data that are needed to move forward in this important area of pulmonary vascular disease. In the last years many works have focused on the use of exercise echocardiography to study the pulmonary artery pressure during exercise. The first symptom in PAH patients is often dyspnea on exertion, so the change in PAP with exercise could provide a possible tool to detect a subclinical phase of pulmonary vascular disease, that is an intermediate stage between resting pulmonary arterial hypertension and normal [16].

Many studies have shown that exercise echocardiography could be a useful tool to make an early diagnosis of pulmonary vascular disease in patients with SSc, to start appropriate therapies in an early stage of the disease that could be more efficacious, although long-term results and prognostic value of such test remains unknown [94].

Data about the prevalence of exercise pulmonary arterial hypertension are limited: Grunig et al. in 2000 have published studies involving patients carrying genes for familial pulmonary hypertension: in 48% of these subjects they found PAP values on exercise > 40 mmHg [95]. Collins et al. have studied 51 patients affected by autoimmune diseases and have found exercise pulmonary arterial hypertension in 59% of subjects, considering a PAsP cut-off of 35 mmHg [96].

Reichemberger and Callejas-Rubio using a PAsP cut-off value of 40 mmHg have found exercise PAH in 45-50% of SSc patients [97,98]. Recent data have showed prevalence of exercise PAH in 40% of SSc patients (defining the PAsP cut-off  $\geq$  50 mmHg) [99].

Steen et al. studied 54 patients affected by SSc at high risk to develop PAH because presenting dyspnea on exertion or abnormalities on functional pulmonary tests or on echocardiogram (PAsP > 35 mmHg). These subjects underwent stress echocardiography and the result of the test was considered positive when mean PAP increased at least 20 mmHg. Patients with positive result at stress echocardiography were submitted to right heart catheterization that confirmed the presence of PAH in 81% of cases [100].

D'Alto and coworkers have compared the values of PAsP during exercise in 172 SSc patients with a control group and demonstrated that the first group had PAsP values higher than the second group, especially in the presence of interstitial lung disease or diastolic dysfunction. Moreover the distribution of exercise PAsP was bimodal and 13% of SSc patients had exercise PAsP values  $\geq 48$  mmHg [101].

Assuming that PAH induced by exercise could be an intermediate stage between normal condition and rest PAH, the follow-up of such patients could be important, but in literature there are few data.

Condliffe and his group described 42 SSc patients with PAH induced by exercise and found the progression toward rest PAH, after a mean period of 2,3 years, in 8 patients (19% of cases), performing right heart catheterization; 4 out of these 8 patients (9,5%) died as a consequence of pulmonary vascular disease within 3 years after the diagnosis [73].

More recently Codullo and coworkers studied a group of patients with altered pulmonary response to exercise diagnosed by stress echocardiography (PAsP  $\geq 48$  mmHg) and confirmed by RHC; patients were followed for about 3,5 years and 3,5 % of cases developed resting PAH [102]. In conclusion exercise echocardiography has still a role in the research field because it is a complex tool that need a significant expertise and has not yet an appropriate standardization, regarding the methods of execution and interpretation of the test. However the potentialities of such tool, in a selected group of patients, could be



enormous and should have necessarily still investigated.

Right heart catheterization (RHC) is mandatory to make a definitive diagnosis of PAH; SSc patients with dyspnea of unknown origin and the finding of a PAsP > 40 mmHg or signs of right ventricular dysfunction should undergo RHC [60].

RHC should be considered also in patients with risk factors for the development of PAH (limited cutaneous form of Ssc, Raynaud's phenomenon lasting > 8 years, positivity of anti-centromere antibodies, isolated positivity of anti-nucleolar antibodies, diffuse teleangectasia), patients with low DLCO (< 60%), or FVC%/DLCO% ratio > 1,6, with dyspnea of unknown origin and echocardiographic signs of right ventricular dysfunction. During RHC it is possible to perform the pulmonary vasoreactivity tests, although its utility in SSc patients is uncertain. On the other hand only 1% of SSc patients have positive results at pulmonary vasoreactivity tests, and high doses of calcium channels blockers could be dangerous in SSc-PAH patients because their negative inotropic and hypotensive effects [103].

Table 2. Decision algorithm for screening and recommendation for RHC in SSc.

Parameters	Low risk	Intermediate risk	Moderate risk	High risk
<ul style="list-style-type: none"> <li>• Dyspnea or</li> <li>• Raynaud's phenomenon lasting &gt; 8 years</li> <li>• Anti centromere or isolated anti nucleolar antibodies-ANA</li> </ul>		yes	yes	yes
DLco (without emphysema or interstitial lung disease)	> 70%	> 70%	< 70%	< 60%
FVC%/DLco%	<1.6	<1.6	>1.6	> 1.6
PAsP	< 35 mmHg	< 35 mmHg	> 35 mmHg	> 40 mmHg
<b>Recommendations</b>	Repeat PFT every year, and echo cardiogram every 2-3 years	Repeat PFT every year, and echo cardiogram every year	Repeat echo cardiogram after 3-6 months or perform right heart catheterisation	Perform right heart catheterisation

PFT: pulmonary functional test.



## **THERAPEUTIC OPTIONS IN PULMONARY ARTERIAL HYPERTENSION**

Before the approval of the more recent drugs, the therapeutic options in PAH consisted of the use of supplemental oxygen, diuretics, digoxin, high doses of calcium channels blockers and anticoagulation, in addition to an adequate training program to improve functional capacity. Although the use of supplemental oxygen and anticoagulation have contributed to improve survival, prognosis remains severe, except for the minority of patients with IPAH that respond to high doses calcium channels blockers therapy.

Currently there are three main approaches to the pharmacological management of PAH, which targets its underlying pathophysiology via the prostacyclin-, endothelin- and NO-mediated pathways. So the new available main therapeutic modalities are: prostacyclin analogs (epoprostenol, treprostinil, iloprost), endothelin receptor antagonists (bosentan, ambrisentan and sitaxsentan) and phosphodiesterase inhibitors (sildenafil, tadalafil) [104].

**Prostacyclin analogs.** Epoprostenol, a synthetic prostacyclin, is the first agent of this class approved in 1995 for the treatment of PAH. It is a potent nonselective vasodilator and antiproliferative agent and has a short half-life so it is given via continuous intravenous infusion through a peripheral or central line. Epoprostenol have demonstrated its efficacy in improving survival in IPAH [105], but data regarding patients with SSc-PAH are insufficient. Menon et al. studied 7 patients with SSc-PAH demonstrating that treatment with epoprostenol significantly reduced both mean PAP and pulmonary vascular resistance, with simultaneous increase of cardiac output [106]. The works of Humbert, Badesch e Klings also confirmed the positive data regarding the use of epoprostenol in SSc-PAH patients, but all the studies were performed in small group of patients [107-109].

Treprostinil offers the option of subcutaneous administration and improves symptoms,

exercise capacity and hemodynamics in connective tissue disease associated with PAH [110].

Inhaled iloprost, has been approved in 2004 for the treatment of NYHA class III and IV PAH patients and its action seems to be selective for pulmonary circulation [111].

Beraprost, an orally administered prostacyclin, has been demonstrated efficacious in improving DLCO in patients with SSc-PAH [112].

**Endothelin-receptors antagonists.** PAH is associated with excess production of ET-1, therefore blocking the effects of ET-1 via antagonism of the ET<sub>A</sub> and ET<sub>B</sub> receptors, which mediate its deleterious vasoconstrictive and mitogenic effects, is an important therapeutic strategy. In 2002 bosentan, a dual receptor antagonist has been introduced in the treatment of PAH. In two placebo-controlled trials, bosentan, orally administered, demonstrated its efficacy in IPAH and SSc-PAH, improving functional capacity, hemodynamics, and time to clinical worsening [113,114]. Generally this drug is well tolerated, but the typical side effect is an increase of liver function tests, that occurs in about 10% of patients; these hepatic effects could be managed with simple measures (treatment interruption or dose reduction).

Ambrisentan e Sitaxsentan, selective antagonists of ET<sub>A</sub> receptor has been introduced later, but there are still not clear the advantages of such selective inhibition [115].

**Phosphodiesterase inhibitors.** Inhibition of phosphodiesterase type-5 (PDE-5), an enzyme that rapidly degrades cGMP, limiting the NO-mediated pulmonary vasodilatation, causes vasodilation and antiproliferative actions, especially in the pulmonary vascular bed where it is well represented. Sildenafil, tadalafil and vardenafil, has been approved for use in PAH, including SSc-PAH) [116].

**Combination therapy.** Given that currently available therapies target different pathways implicated in the pathophysiology of PAH, there is a strong rationale for combining

different therapies with the aim of optimizing treatment response. This can be achieved by either the simultaneous administration of two or more targeted treatments or by the sequential addition of one or more agents to ongoing therapy. A metanalysis conducted by Galiè and coworkers in 2009, including all the randomized studies performed from 1990 to 2008, regarding the treatment with the new therapies, demonstrated an improvement in survival in these patients [117]. Nowadays combination therapies have become common practice and many multicenter studies have evaluated the efficacy of various combination of oral, endovenous or inhaled agents [118].

**Immunosuppressive therapy.** Recently, investigators have been evaluating the ability of antineoplastic agents to slow aberrant proliferation of vascular smooth muscle and endothelium in PAH. Imatinib mesylate is a tyrosine kinase inhibitor used in chronic myelogenous leukemia. Initial reports have shown improvement in PVR and cardiac output in PAH patients [119]. Rituximab, an anti-CD20 medication that targets B-cell populations and may lower platelet-derived growth factor-specific antibodies, is also being studied within a SSc-PAH population as a result of case reports of improved outcomes in patients with advanced stage [120].

**Surgical therapy.** Atrial septostomy may provide temporary symptomatic relief in patients who have not responded to other therapies. By creating a right-to-left intraatrial shunt, the intention of atrial septostomy is to decrease right ventricular filling pressures to improve cardiac output and overall systemic oxygen delivery. The long-term benefits of atrial septostomy remain unclear because these data often are skewed by the fact that the procedure is usually used as a bridge to transplant or as a palliative measure [7].

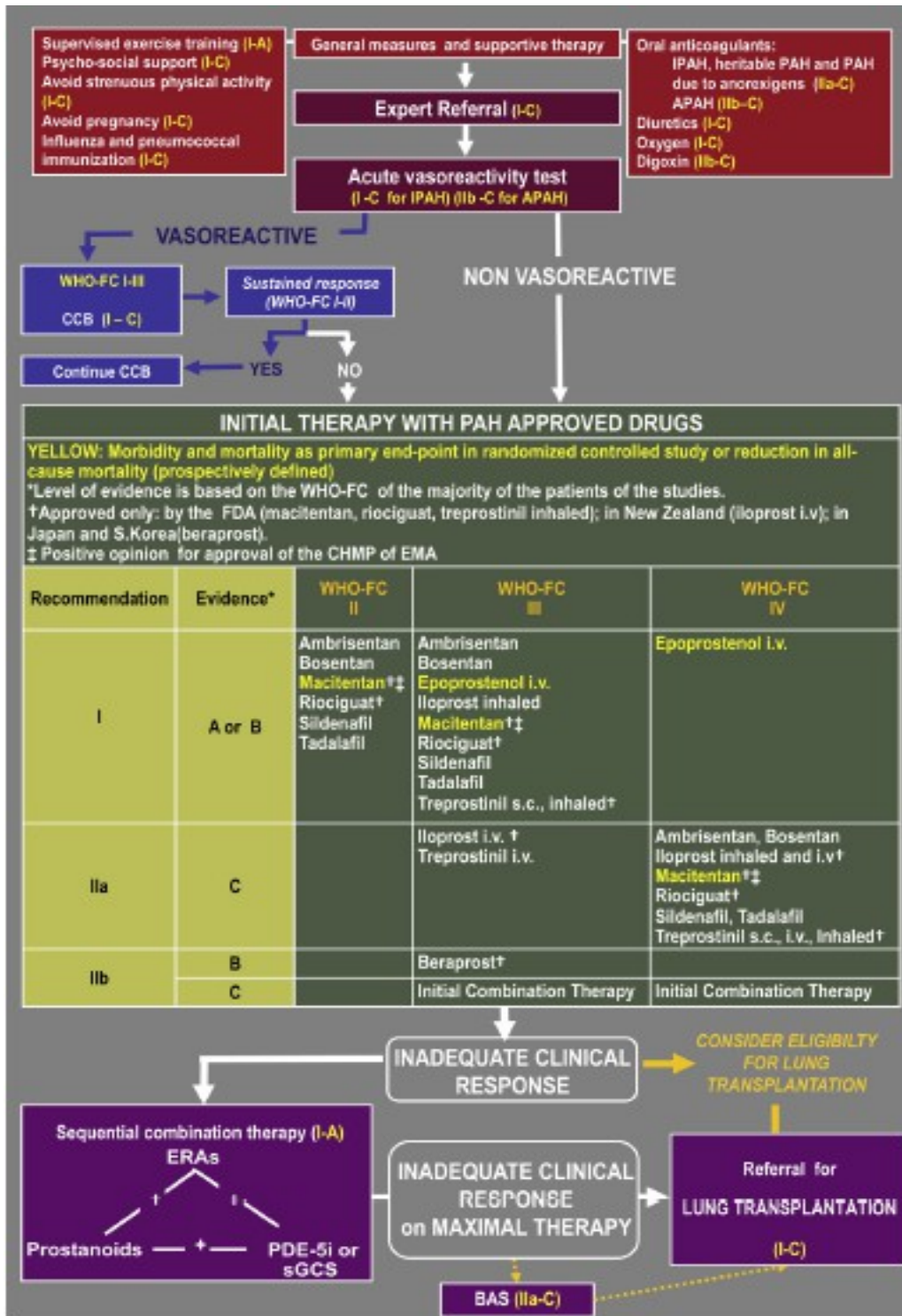
Lung transplantation is a therapy of last resort for many patients. Unfortunately patients with SSc are generally considered poor candidates for lung transplantation because of multi-organ disease or esophageal dysmotility, which may increase the risk of aspiration.

Nonetheless, carefully selected patients may tolerate transplant well [121].

**Treatment algorithm.** The suggested initial approach after the diagnosis of PAH is the adoption of the general measures, the initiation of the supportive therapy and referral to an expert center. Acute vasoreactivity test remains mandatory in all patients with PAH (group 1), although patients with idiopathic PAH, heritable PAH, and PAH associated with anorexigen use are the most likely to exhibit an acute positive response and to benefit from long-term therapy with calcium channels blockers (CCB). CCB should be started at maximal tolerated doses and an adequate response should be confirmed after 3 to 4 months of treatment. Patients with a negative vasoreactive test and with NYHA functional class II should be treated with an endothelin-receptor antagonist or a PDE-5 inhibitor.

NYHA functional class III patients should be treated with an endothelin-receptor antagonist or a PDE-5 inhibitor, or a prostanoid.

As head-to-head comparisons among different agents are not available, there is no evidence-based first-line treatment for either NYHA functional class II or III patients. Intravenous administration of epoprostenol is recommended as first-line therapy for NYHA class IV patients; in case of inadequate clinical response sequential combination therapy should be considered: association of an endothelin-receptor antagonist and/or a PDE-5 inhibitor or a prostanoid (double or triple therapy).



**Figure 6.** Evidence-based treatment algorithm.

APAH: associated pulmonary arterial hypertension; BAS: balloon atrial septostomy; CCB: calcium channel blockers; ERA: endothelin receptor antagonist; sGCS: soluble guanylate cyclase stimulators; IPAH: idiopathic pulmonary arterial hypertension; i.v.: intravenous; PDE-5i: phosphodiesterase type 5 inhibitor; s.c.: subcutaneous; WHO-FC: World Health Organization functional class.

From: Galiè N et al. Guidelines for the diagnosis and treatment of pulmonary arterial hypertension. Eur Heart J 2009;30:2497-2537.





## **AIM OF THE STUDY**

The aim of our study was to evaluate, using exercise echocardiography, the prevalence of exercise-induced abnormal pulmonary vascular reactivity, in asymptomatic or mildly symptomatic patients affected by systemic sclerosis, with normal pulmonary artery pressure at rest. We also measured values of BNP both at rest and during exercise test. During exercise echocardiography we estimated pulmonary artery systolic pressure, and we correlated these values with clinical, echocardiographic and functional parameters. Moreover the results of BNP measurement, at rest and during exercise, were correlated with exercise PAsP values. After a medium-long term follow-up (mean observation period of  $5,1 \pm 0,4$  years) we evaluated patients with a clinical assessment and we performed a rest echocardiography to detect the development of resting PAH, assessing the predictive value of exercise echocardiography for early identify patients at risk to develop PAH.



## **MATERIALS AND METHODS**

### **Study population**

We enrolled 40 consecutive patients affected by Systemic Sclerosis referred to our Echocardiography laboratory (Cardiology Unit of Camposampiero Hospital, Padua) between January 2009 and December 2009, coming from the Rheumatology Unit of the University of Padua. Diagnosis of SSc was based on the American College of Rheumatology criteria [28].

Exclusion criteria were the following: 1) patients older than 70 years, 2) atrial fibrillation/flutter, 3) post-capillary diastolic dysfunction, 4) PAH at rest, 5) deep vein thrombosis history or vascular doppler echo positivity of the lower limbs, 6) inability to perform an exercise stress test because of comorbidity or poor compliance.

The protocol of this study was approved by the ethic committee of the Hospital, and the participants gave written informed consent.

Patients were evaluated on the basis of the age, gender, time to diagnosis, type of SSc (diffuse cutaneous or limited cutaneous form), autoantibody subset (anti-centromere, antiScl-70 and nonspecific ANA antibodies), WHO/NYHA functional class, associated diseases, cardiovascular risk factors, family history of autoimmune diseases, diagnostic test as spirometry with DLCO, and high resolution computed tomography (HRCT), and ongoing treatment.

At the time of the study many patients were treated with oral vasodilators (25) and with anti-ulcer agents; moreover 6 patients received immunosuppressive therapy for SSc (3 with azathioprine, 1 with methorexate, 1 with cyclosporin and 1 with mofetil mycophenolate).

Spirometry test consisted of measurement of TLCO/VA [diffusing capacity for carbon monoxide corrected for alveolar volume in maximal inspiration (ml/min/mmHg/L)] and

FVC (forced vital capacity) assuming that values  $> 100\%$  in males and  $> 85\%$  in females exclude restrictive lung disease [122]. Matching the values of TLCO/VA and FVC and findings of the HRCT we established a grading of the interstitial lung disease to identify the effect of the lung disease on the parameters evaluated in our study:

- grade 0: absence of interstitial lung disease
- grade 1: mild interstitial lung disease
- grade 2: moderate interstitial lung disease
- grade 3: severe interstitial lung disease

During follow-up many clinical parameters were considered: symptoms, functional class, treatment changes, new onset of cardiac or extracardiac diseases or new cardiovascular risk factors.

### **Resting echocardiographic study**

All patients previously underwent a comprehensive rest transthoracic echocardiogram using IE33 ultrasound machine (Philips medical Systems, Andover, MA), equipped with 2.5- to 3.5-MHz phased-array transducers, second harmonic technology, and coupled with tissue Doppler imaging (TDI). The following parameters were assessed: walls thickness and diameters of the cardiac chambers measured in parasternal long axis view using B-mode technique. Volumes of the left ventricle and the ejection fraction were measured from apical 4-chambers view using Simpson method. Diastolic function was assessed through the following parameters: Doppler velocities of the E and A waves of transmitral flow and E/A ratio, the deceleration time (DT) of the E wave, measurement of E' and A' diastolic velocities of the lateral mitral annulus using tissue Doppler, and Doppler velocities of the systolic (S) and diastolic (D) pulmonary venous flow from apical 4-chambers view. Right ventricular function was detected by the measurement of TAPSE (tricuspid annular plane systolic excursion) and TAV (tricuspid annular velocity) using

respectively M-mode and TDI technique. End-diastolic, end-systolic areas and the fractional area change (FAC) of the right ventricle have also been assessed. The systolic pulmonary arterial pressure (PAsP) was derived from the maximal velocity of tricuspid regurgitant jet according to the simplified Bernoulli equation and adding right atrial pressure, estimated from the dimension and collapsibility of the inferior vena cava. The end-diastolic pulmonary pressure (dPAP) was obtained using Bernoulli formula, from the velocity of pulmonary regurgitant jet and adding right atrial pressure; such echocardiographic estimate of dPAP is significantly correlated to dPAP measured by RHC [123]. We also measured the acceleration time (AT) of the pulmonary artery flow, that is inversely correlated to PAsP and to mean PAP [124,125].

During the echocardiographic study systemic arterial pressure, heart rate, oxygen saturation and measurement of BNP blood levels at rest were also assessed.

At follow-up we performed echocardiographic study evaluating the parameters previously described, and in particular we estimated the pulmonary artery systolic pressure to recognize the development of PAH.

### **Exercise Echocardiographic study**

Exercise stress echo was conducted using a Ergoline graded semisupine bicycle ergometer with 25-W incremental loading every 3 minutes. A 12-lead electrocardiogram and blood pressure and oxygen saturation measurement were performed at baseline and every minute thereafter. Transthoracic 2-dimensional echocardiographic monitoring was performed throughout and up to 5 minutes after the end of stress, mostly using the apical 4-chamber view. Every step we measured volumes, and ejection fraction of the left ventricle, diastolic function using pulmonary venous flow and E/e' ratio and pulmonary systolic arterial pressure. Moreover measurement of BNP blood levels was performed at peak exercise.

Resting pulmonary artery hypertension was defined by PAsP values > 40 mmHg (referring

to tricuspid regurgitant velocity  $> 3\text{m/sec}$  plus right atrial pressure of 5 mmHg) [101]. A cutoff value of PAsP  $\geq 48$  mmHg at peak exercise was considered a significant exercise-induced increase in PAsP [99].

Analysis of the diastolic filling of the left ventricle allows to distinguish between pre-capillary forms of PH (with normal left ventricle filling pressure) and post-capillary form (with increased left ventricle filling pressure). During exercise echocardiography it is more reliable to detect pulmonary venous flow and E/e' ratio as indices of diastolic function; actually E and A waves analysis, E/A ratio and deceleration time of the E wave could be difficult to estimate during effort for the frequent fusion of the doppler waves due to the high heart rate and for the presence of artifacts due to the polypnea.

### **BNP**

All patients had two blood samples collected for BNP measurement from the antecubital vein at the time of the clinical and echocardiographic assessment: the first withdrawal was made in the supine position after a rest of at least 20 minutes, the second at peak of the exercise. The blood was stored in a 10 ml test tube containing ethylenediamine tetraacetic acid and no later than 10 minutes we measured BNP levels by Triage BNP test (Biosite, USA), that is a rapid immunofluorometric assay allowing BNP determination ranging from 5 pg/ml to 5,000 pg/ml, with a known cut-off value for heart failure of 100 pg /ml.

## STATISTICAL ANALYSIS

The results are expressed as mean  $\pm$  standard deviation for quantitative variables and as absolute and relative frequencies for qualitative variables. The comparison between SSc patients who developed exercise PAH and those who did not was performed using Student's *t* test. In the event that the applicable conditions were not met, we performed the Mann-Whitney test. The qualitative variables between the two groups were compared using the Chi-square or Fisher's test. Pearson's linear correlation coefficient (*r*) and simple linear regression were used for correlations between exercise PAH and the other variables. Concerning data obtained at follow-up, clinical and echocardiographic parameters of the group of patients who developed resting PAH were compared to the remaining patients population and also to the patients group with exercise altered pulmonary artery vasoreactivity, using Wilcoxon test.

All analyses were performed using a value of  $P < 0,05$  as significant and using the Stata software version 11.0.





## RESULTS

40 patients with SSc were recruited in the study (7 males and 33 females, mean age 52.2 years  $\pm$  9.8), 21 out of them with limited sclerosis (52.5%) and 19 with diffuse sclerosis (47.5%). The clinical characteristics of the patients are presented in Table 1. The echocardiographic study showed that the average PAsP at rest was 26.5 $\pm$ 4.6 mmHg and reached a value of 45.1 $\pm$ 14.2 mmHg with exercise with an average increase of 18.7 $\pm$ 11.9 mmHg. Assuming the PAsP cut-off value  $\geq$  48 mmHg to define the exercise-induced pulmonary hypertension, we divided the population into two groups: group A (25 patients, 62,5%) = normal pulmonary artery vasoreactivity (with PAsP < 48 mmHg) and group B (15 patients, 37,5%) = altered pulmonary artery vasoreactivity (with PAsP  $\geq$  48 mmHg). The two groups were compared in terms of clinical, echocardiographic, hemodynamic and functional data as shown in table 1, 2 and 3.

Regarding the clinical data the patients enrolled in our study were predominantly females (82,5%), and all patients belonging to group B were females, resulting a significant difference in terms of sex between the two groups (p 0,03). In addition, group B patients were older (56  $\pm$  10,3 years) than group A patients (50  $\pm$  9,1 years), but the difference was not statistically significant. In addition, female patients were aged more than 10 years than male patients (p 0,0319). We didn't find any statistically significant difference between the two groups regarding the subsets of disease (diffuse cutaneous and limited cutaneous forms), the autoantibodies subset, and the disease duration.

Regarding pulmonary functional tests we didn't find any significant difference of TLCO/VA, FVC and grade of interstitial lung disease, although group B patients had lower TLCO/VA values (62,9  $\pm$  21,4) than group A patients (72,9  $\pm$  21%). Similarly there was no difference between the two groups regarding NYHA functional class, but 60% of patients

with NYHA functional class I belonged to group A and 46% of patients with NYHA functional class II belonged to group B. All patients affected to systemic arterial hypertension (n = 3) were in group A. With regard to drug therapy, the datum did not significantly correlate to the development of altered pulmonary vascular responsiveness.

**Table 1.** Clinical parameters of the study population, divided according to the pulmonary vascular response to stress.

Clinical parameters	Patients	Subgroup with impaired vascular reactivity	Subgroup with normal vascular reactivity	P value
Number (%)	N= 40 (100%)	N= 25 (62,5%)	N= 15 (37,5%)	
Males (n, %)	7 (17,5%)	7 (28%)	0 (0%)	0,03
Females (n, %)	33 (82,5%)	18 (72%)	15 (100%)	
Age (years)	52,2 ± 9,8	50 ± 9,1	56 ± 0,3	n.s.
Age (males, years)	45,9 ± 7,1	45,9 ± 7,1	-	n.s.
Age (females, years)	53,6 ± 9,9	51,6 ± 9,4	56 ± 10,3	n.s.
Limited sclerosis (n, %)	21 (52,5%)	12 ( 48%)	9 (60%)	n.s.
Diffuse sclerosis (n, %)	19 (47,5%)	13 ( 52%)	6 (40%)	
Length of the disease (years)	8,75 ± 5,87	9,04 ± 5,96	8,27 ± 5,89	n.s.
Length of the disease (Limited form, years)	8,76 ± 5,92	8,67 ± 5,28	8,89 ± 7,01	n.s.
Length of the disease (Diffuse form, years)	8, 74 ± 5,98	9,38 ± 6,73	7,33 ± 4,08	n.s.
Antibody subset:				
Scl-70 (n, %)	17 (42,5%)	12 (48%)	5 (33,3%)	n.s.
ACA (n, %)	16 (40%)	10 (40%)	6 (40%)	
Nonspecific pattern (n, %)	7 (17,5%)	3 (12%)	4 (26,7%)	
Functional class:				
NYHA I (n, %)	23 ( 57,5%)	15 (60%)	8 (53,3%)	n.s.
NYHA II (n, %)	17 (42,5%)	10 (40%)	7 (46,7%)	n.s.
Systemic hypertension (n, %)	3 (7,5%)	3 (12%)	0 (0%)	n.s.
BSA (m <sup>2</sup> )	1,69 ± 0,16	1,73 ± 0,17	1,63 ± 0,11	n.s.
<i>Pulmonary functional test:</i>				
TLCO/VA (%)	69,1±21,4	72,9 ± 21	62,9 ± 21,4	n.s.
FVC (%)	98,7± 19,6	98,7±17,6	98,7±21	n.s.
<i>Interstitial lung disease</i>				
Grade 0 (n, %)	23 (57,5%)	15 (60%)	8 (53,4%)	n.s.
Grade 1 (n, %)	12 (30%)	8 (32%)	4 (26,6%)	
Grade 2 (n, %)	5 (12,5%)	2 (8%)	3 (20%)	
Grade 3 (n, %)	0 (0%)	0 (0%)	0 (0%)	

Regarding the resting echocardiographic measurements (Table 2), thickening, volumes and diastolic and systolic parameters of the left ventricle were normal, as data about the right ventricle. Exercise echocardiography showed a reduction of left ventricular volumes and a slight increase of the LV ejection fraction. Regarding diastolic function, during effort, E/e' ratio and S/D ratio remained within the normal range. Comparing the two groups, we found a significant difference regarding the PAsP at rest (p 0.0077), the exercise PasP (p 0.000), and the increase of the PAsP with the exercise (p 0.000). The other echocardiographic parameters didn't show significant differences between the two groups.

**Table 2.** Echocardiographic parameters of the study population, divided according to the pulmonary vascular response to stress.

Echocardiographic parameters	Patients (N=40)		Subgroup with normal vascular reactivity (N=25)		Subgroup with impaired vascular reactivity (N=15)		P value
	mean	sd	mean	sd	mean	sd	
Left atrium (AP diameter, mm)	31,88	4,76	32,16	4,62	31,40	5,10	n.s.
Right atrium volume (ml)	34,2	10,2	33,2	7,9	34,8	12,7	n.s.
Septum thickness of LV (mm)	9,33	1,62	9,48	1,83	9,07	1,22	n.s.
Posterior wall thickness (mm)	8,78	1,14	8,84	1,21	8,67	1,05	n.s.
LV end-diastolic diameter (mm)	43,28	4,65	43,00	4,43	43,73	5,12	n.s.
Pulmonary AT (msec)	137,70	28,36	137,7	27,21	137,67	31,16	n.s.
TAPSE (mm)	22,80	4,12	22,92	4,37	22,60	3,81	n.s.
TAV (cm/s)	13,03	2,13	13,12	2,23	12,88	2,03	n.s.
RV end-diastolic area (cm <sup>2</sup> )	14,2	2,3	14,3	2,1	14,1	2,9	n.s.
RV end-systolic area (cm <sup>2</sup> )	7,7	2,1	7,4	8,6	7,7	2,1	n.s.
RV FS (%)	46,3	8,3	51,2	7,3	46,3	4,9	n.s.
Right atrial pressure (mmHg)	5,25	0,81	5,36	0,95	5,07	0,46	n.s.
Resting PAsP (mmHg)	26,48	4,64	25,00	3,85	28,93	4,92	0,0077
Exercise PAsP (mmHg)	45,13	14,17	36,88	8,77	58,87	10,20	0,00
Δ PAsP (mmHg)	18,65	11,91	11,88	7,71	29,93	8,65	0,00
Resting LV ESV (ml/mq)	13,76	3,55	14,05	3,24	13,28	4,09	n.s.
Resting LV EF (%)	67,33	5,82	66,76	5,75	68,27	6,01	n.s.
Exercise LV EF (%)	71,00	4,68	70,68	4,33	71,53	5,33	n.s.
Resting E/E' ratio	7,37	1,90	7,00	1,66	7,98	2,17	n.s.
Exercise E/E' ratio	7,73	2,02	7,54	1,93	8,05	2,20	n.s.
Resting VTI s wave PVF	17,14	3,47	16,33	2,89	18,48	4,02	n.s.

Exercise VTI s wave PVF	15,69	3,57	15,32	2,98	16,31	4,43	n.s
Resting VTI d wave PVF	10,98	2,61	11,28	2,67	10,46	2,52	n.s
Exercise VTI d wave PVF	10,56	3,23	10,72	3,39	10,30	3,06	n.s

AP: antero-posterior; LV: left ventricle; RV: right ventricle; VTI: velocity-time integral;  
FS: fractional shortening; PVF: pulmonary venous flow.

Among the functional data, we observed a statistically significant difference in resting systemic blood pressure (p 0.00318), and variation of oxygen saturation with exercise between the two groups (p 0.0481). No other functional variables showed difference between group A and B (Table 3).

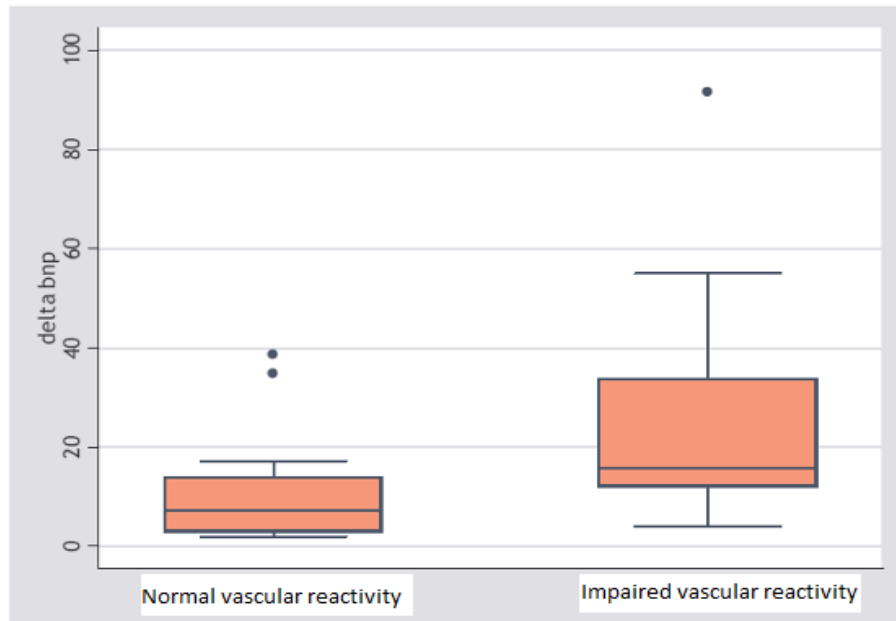
**Table 3.** Functional parameters and BNP measurement of the study population, divided according to the pulmonary vascular response to stress.

Hemodynamics and functional parameters	Patients (N=40)		Subgroup with normal vascular reactivity (N=25)		Subgroup with impaired vascular reactivity (N=15)		P value
	mean	sd	mean	sd	mean	sd	
Resting systolic BP (mmHg)	117,95	23,71	111,68	20,86	128,40	25,15	0,00318
Exercise systolic BP (mmHg)	165,63	25,15	162,12	27,39	171,47	20,42	n.s.
Resting diastolic BP (mmHg)	75,08	12,43	71,84	11,07	80,46	13,08	n.s.
Exercise diastolic BP (mmHg)	82,93	11,33	81,32	10,20	85,60	12,93	n.s.
Δ systolic BP (mmHg)	47,68	20,99	50,44	17,91	43,07	25,32	n.s.
Δ diastolic BP (mmHg)	7,85	10,44	9,48	11,21	5,13	8,71	n.s.
Resting heart rate (bpm)	75,08	10,09	74,08	9,69	78,67	10,43	n.s.
Exercise heart rate (bpm)	132,58	20,24	131,48	21,39	134,40	18,74	n.s.
Δ heart rate (bpm)	56,78	22,26	57,40	22,83	55,73	22,01	n.s.
Δ oxygen saturation (%)	1,83	1,77	1,40	1,44	2,53	2,07	0.0481
Maximal heart rate (bpm)	167,78	9,84	170,04	9,02	164,00	10,30	n.s.
% maximal heart rate (%)	79,13%	11,96	77,4	12,47	82,02	10,85	n.s.
Effort duration (min)	7,8	2,5	7,8	2,4	7,3	2,5	n.s.
Resting BNP value (pg/ml)	28,38	27,27	25,27	28,07	33,51	26,05	n.s.
Exercise BNP value (pg/ml)	48,11	40,94	37,72	36,34	64,74	43,58	0,01
Δ BNP value	19,74	19,47	12,55	13,15	31,23	22,7	0,0006

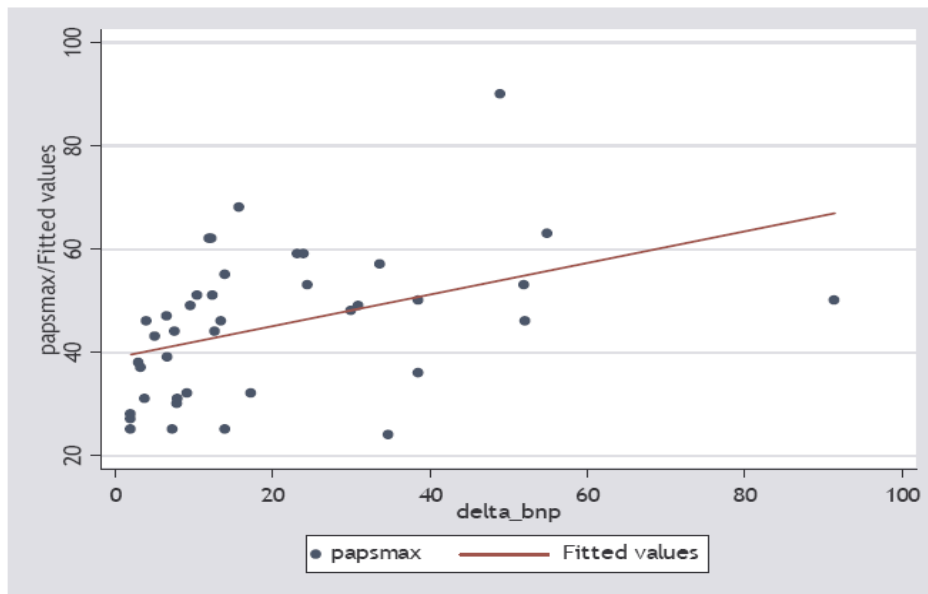
BP: blood pressure.

Finally the BNP dosage showed a wide dispersion of the values with an average concentration of  $28.38 \pm 27.27$  pg/ml at rest, and  $48.11 \pm 40.94$  pg/ml at peak exercise. Moreover subjects of group B showed higher exercise BNP levels than those of group A

( $64,74 \pm 43,58$  pg/ml versus  $37,72 \pm 36,34$  pg/ml), with no differences regarding resting BNP values. Variation of BNP values with exercise ( $\Delta$ BNP) resulted higher in group B patients than group A and there was also a significant linear correlation between  $\Delta$ BNP values and the development of altered pulmonary vascular responsiveness (figure 1 and 2).



**Figure 1.** Comparison of  $\Delta$ BNP during exercise between the two patients groups.

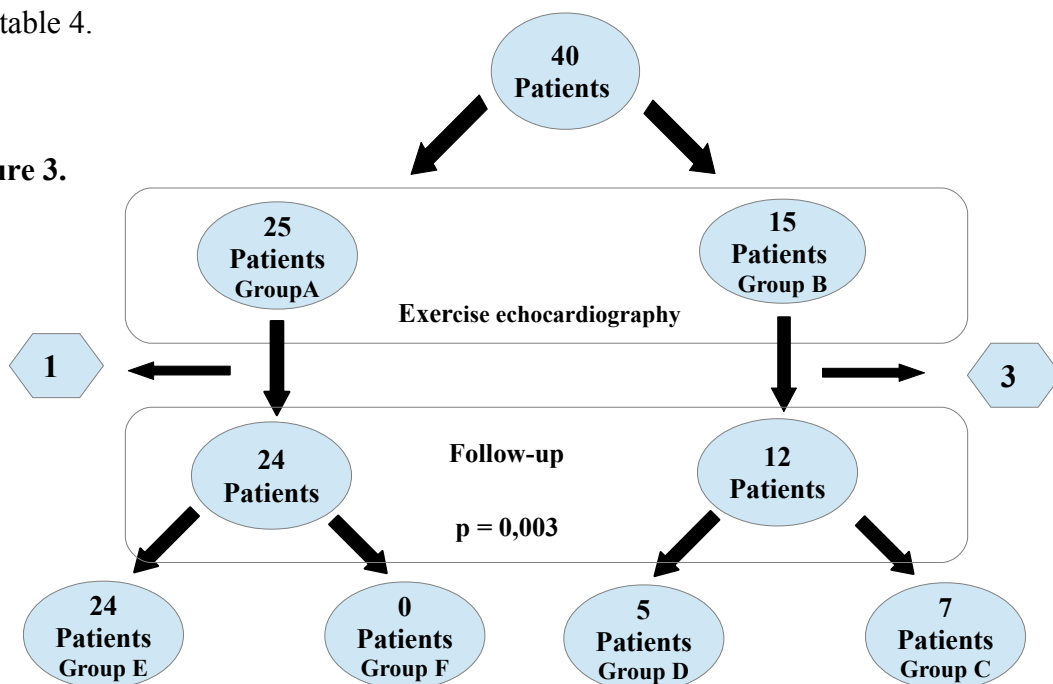


**Figure 2.** Linear correlation between delta BNP and the development of impaired pulmonary vasoreactivity induced by exercise.  $r = 0,4171$ .

## Follow-up

Our patient population were re-evaluated after about five years ( $5,1 \pm 0,4$  years); during this period, 2 patients died for acute leukemia and lung cancer, and 2 patients were lost. One patient belonged to group A, the other 3 patients (included patients that died) belonged to group B. So we performed a clinical and echocardiographic assessment in 36 patients. We found that within group B, 5 patients (41,7%), all female subjects, had echocardiographic diagnosis of PAH (group D); according to the latest ESC guidelines, 3 out of these 5 patients had a diagnosis of possible PAH (presenting a tricuspid regurgitant velocity between 2.9 and 3.4 m/sec, corresponding to a PAsP between 37 and 50 mmHg) and 2 out of 5 patients had a diagnosis of probable PAH (with a tricuspid regurgitant velocity  $> 3.4$  m/sec, corresponding to a PAsP  $> 50$  mmHg). Only one patient underwent right heart catheterization, that confirmed the diagnosis of pre-capillary PAH; the other patient refused the test and for the other 3 patients, with a mild increase of the PAsP, and absence of symptoms, we decided to performed a close clinical and echocardiographic follow-up. None of the patients of group A presented, at follow-up increased values of PAsP at rest. At follow-up we newly defined three groups of patients as shown in figure 3 and table 4.

Figure 3.



**Table 4.** distribution of the groups at follow-up, on the basis of PAsP value at rest.

		Follow-up		
		Normal (PAsP <37 mmHg)	Resting PAH (PAsP >37 mmHg)	Total
<b>Baseline</b>	Normal response (Exercise PAsP < 48 mmHg)	24 (100%) (group E)	0 (0%) (group F)	24
	Impaired response (Exercise PAsP ≥ 48 mmHg)	7 (58,3%) (group C)	5 (41,7%) (group D)	12
	Total	31 (86,1%)	5 (13,9%)	36

**P=0,003**

We made comparison between group C and group D (all belonging to group B, defined as group with altered pulmonary vascular reactivity at exercise echocardiography), and also comparison between group D (who developed at follow-up PAH at rest) and the rest of population (group C and group E), regarding clinical parameters (table 5 and 6).

**Table 5.** Comparison regarding clinical parameters between group D and the other groups.

Clinical parameters	Patients	Group D	Others groups (C+E)	P value
Number (%)	n= 36 (100%)	n=5 (13,9%)	n=31 (86,1%)	
Males (n, %)	6 (21,6%)	0 (0%)	6 (19,3%)	n.s.
Females (n, %)	30 (83,3%)	5 (100%)	25 (80,7%)	
Age (years)	56,7±10,1	68,8± 6,7	54,7±9,2	0,025
Limited sclerosis (n, %)	20 (55,6%)	3 (60%)	17 (54,8%)	n.s.
Diffuse sclerosis (n, %)	16 (44,4%)	2 (40%)	14 (45,2%)	
Length of disease (years)	13,7±5,3	16±6,9	13,5±5,8	n.s.
Antibody subset:				n.s.
Scl-70 (n, %)	14 (38,9%)	2 (40%)	12 (38,7%)	
ACA (n, %)	16 (44,4%)	2 (40%)	14 (45,2%)	
Nonspecific pattern (n, %)	6 (16,7%)	1 (20%)	5 (16,1%)	
Functional class:				n.s.
NYHA I (n, %)	25 (69,4%)	2 (40%)	23 (74,2%)	
NYHA II (n, %)	9 (25%)	3 (60%)	6 (19,4%)	
NYHA III (n, %)	2 (5,6%)	0	2 (6,4%)	n.s.
BSA (m <sup>2</sup> )	1,70±0,2	1,65±0,13	1,7±0,2	n.s.
<i>Pulmonary functional test:</i>				
TLCO/VA (%)	69,7±0,2	72,8±32	69,2±17,3	n.s.
FVC (%)	99,1±0,2	96,6±19,1	99,5±18,7	n.s.
<i>Interstitial lung disease</i>				
	n=34	n=4	n=30	n.s.
Grade 0 (n, %)	19 (55,9%)	0	19 (63,3%)	
Grade 1 (n, %)	11 (32,3%)	2 (50%)	9 (30%)	
Grade 2 (n, %)	4 (11,8%)	2 (50%)	2 (6,7%)	
Grade 3 (n, %)	0	0	0	

**Table 6.** Comparison regarding clinical parameters between group D and C.

Clinical parameters	Patients	Group D	Group C	P value
Number (%)	n= 12 (100%)	n= 5 (41,7%)	n= 7 (58,3%)	
Females (n, %)	12 (100%)	5 (100%)	7 (100%)	-
Age (years)	60,75±11,1	68,8± 6,7	55± 10,2	0,025
Limited sclerosis (n, %)	8 (66,7%)	3 (60%)	5 (71,4%)	n.s.
Diffuse sclerosis (n, %)	4 (33,3%)	2 (40%)	2 (28,6%)	
Length of disease (years)	13,8±5,87	16±6,9	12,2±4,9	n.s.
Antibody subset:				n.s.
Scl-70 (n, %)	8 (66,7%)	2 (40%)	6 (85,7%)	
ACA (n, %)	3 (25%)	2 (40%)	1 (14,3%)	
Nonspecific pattern (n, %)	1 (8,3%)	1 (20%)	0 (0%)	
Functional class:				n.s.
NYHA I (n, %)	8 (66,7%)	2 (40%)	6 (85,7%)	
NYHA II (n, %)	4 (33,3%)	3 (60%)	1 (14,3%)	
BSA (m <sup>2</sup> )	1,64±0,1	1,65±0,13	1,6±0,8	n.s.
<i>Pulmonary functional test:</i>				
TLCO/VA (%)	72,2±22,2	72,8±32	71,7±14,8	n.s.
FVC (%)	100,1±13,6	96,6±19,1	102,6±9,03	n.s.
<i>Interstitial lung disease</i>	n=11	n=4	n=7	n.s.
Grade 0 (n, %)	5 (45,4%)	0	5 (71,4%)	
Grade 1 (n, %)	4 (36,4%)	2 (50%)	2 (28,6%)	
Grade 2 (n, %)	2 (18,2%)	2 (50%)	0	
Grade 3 (n, %)	0	0	0	

We observed significant differences only for the age between group D and group C and also between group D and the rest of population (group C + E), resulting more advanced in group B than the other groups. The other clinical characteristics didn't show differences. Regarding pulmonary functional data, patients belonging to group B had a more advanced grade of interstitial lung disease than group C and the rest of population (group C + group E). Regarding values of BNP at peak of exercise and the difference between BNP values at resting and at peak exercise (during evaluation performed in 2009) we didn't find difference between the groups.



## DISCUSSION

Pulmonary arterial hypertension is one of the leading causes of death in patients with SSc. The growing availability of new efficacious therapies gives a pivotal role to the research of tools able to early identify patients at higher risk for developing this severe complication. Early diagnosis could be the better way to improve the survival. The reduced vascular reserve in response to stress is an important feature of the pulmonary arterial hypertension and, for this mechanism, the exercise, in such patients, induces a marked increase of the PAsP values. Conversely the normal pulmonary circulation is unique in its ability to accommodate the entire cardiac output at low arterial pressure even during condition of maximal exercise. This is accomplished by its high-capacitance and low-resistance circuit, with large microcirculatory reserve which are 'unrecruited' at rest. During exercise, the pulmonary microcirculation is progressively recruited, resulting in the maintenance of relatively low arterial pressure despite increasing flow. The recruitment of the microcirculation also serves to increase the capillary surface area available for gas exchange during exercise. The high capacitance of the pulmonary circulation means that early pulmonary vascular disease can be well compensated for. In fact, > 50% of the pulmonary circulation must be obstructed before a rise in resting PAP is detected. Thus a rise in resting PAP is a late marker of the obliterative and remodeling processes occurring in the distal pulmonary arteries [126]. Exercise could be a useful 'stress' to elicit an abnormal response to facilitate disease detection, and for this reason exercise echocardiography could be a diagnostic tool to early identify pulmonary vascular disease when resting hemodynamics do not permit to detect the initial phase of the disease [16]. In systemic sclerosis abnormalities of the pulmonary physiology recognize the primum movens as the endothelial dysfunction of the arterioles and capillaries leading to platelet

aggregation, muscular layer proliferation, thickening of the tunica intima and adventitia, and lastly reduced vascular compliance and vessel occlusion. These processes lead to reduction of pulmonary vessel bed that exhibits a rise in pulmonary arterial pressure during efforts, and absence of symptom and normal pulmonary arterial pressure at rest.

Pulmonary vascular involvement is present in two-thirds of patients with SSc [127], but this initial phase of the disease is clinically silent and detectable pathophysiological changes in the pulmonary circulation usually appear when the pathological lesions are fully developed. The length of this pre-clinical phase of the disease is currently unknown [128], but if we consider that late symptoms reporting and delayed diagnosis are common in PAH, it is clear how distant the early stage of the disease may be from the initiation of effective therapies. Therefore it is mandatory to reduce the time between the beginning of the pathobiological processes leading to PAH and the initiation of effective medical therapy. The first studies made in the nineties, considered small groups of patients, affected by LES or SSc, that showed a significative increase of the pulmonary pressure respect to healthy subjects [129,130]. From 2001 departments of Rheumatology, Pneumology and Cardiology of the University of Connecticut became to perform annual stress echocardiographic studies in patients with SSc, starting with such experimental tool. Therafter the studies conducted by Steen [61], Tolle [16], Alkobot [131], Collins [96] and D'Alto [101], confirmed the high prevalence of altered pulmonary vasoreactivity induced by exercise in SSc patients, although the cut-off values for its definition are rather not well-defined. More recently study groups of Pavia and Naples made a follow-up of patients with pulmonary altered vasoreactivity induced by exercise revealing in this group a worse prognosis than patients without pulmonary altered vasoreactivity [102].

Our study was conducted only with asymptomatic or midly symptomatic patients, that are not considered at high risk for PAH development (as patients with clear symptoms, altered

pulmonary functional tests or with echocardiographic findings), but they need a close clinical follow-up because represent the majority of patients affected by SSc, and in whom diagnosis of PAH is always made very late.

Exercise echocardiography was able to identify two SSc patients groups presenting a different vascular response to exercise. Assuming a PAsP cut-off value of 48 mmHg, 37,5% of our patients showed an altered pulmonary vasoreactivity induced by exercise, that is more or less the prevalence as already described in literature in various works [61,97-99,131,132]. Actually prevalence of PAH in SSc is much lesser (from 7% to 12%) than the prevalence of altered pulmonary vasoreactivity and the reason of this difference is that the reduced pulmonary vessels compliance and vasodilation response is a peculiar feature of SSc patients. Subjects with exercise pulmonary abnormal vasoreactivity (group B) had both higher resting and exercise PAsP values than the other group, as also confirmed in other studies [101]. Also the change between resting and exercise PAsP (delta,  $\Delta$ PAsP) was found higher in group B than group A; such parameter in a study was also assumed as the index to define altered pulmonary vasoreactivity induced by exercise, instead of the absolute value of PAsP reached during exercise [61]. Regarding the analysis of the functional capacity of SSc patients, group B patients performed less effort than the other group regarding both the duration of the exercise and the submaximal heart rate reached, but the data were not statistically significant. We did not observe a correlation between the exercise PAsP values and the duration of the effort, in agreement to Alkotob et al [131]. In SSc the presence of reduced functional capacity caused by various physiopathological mechanisms, other than the presence of PAH: pleuropulmonary alterations, muscular inflammations, arthritis, steroidal drugs use, deconditioning, but especially the presence of interstitial lung disease and pulmonary fibrosis [16].

Patients of our study had normal systo-diastolic echocardiographic parameters, that were

also confirmed during exercise echocardiography. In particular the estimate of diastolic filling pressures resulted normal during exercise, assuming that the increase of PAsP could have a precapillary origin rather than due to diastolic dysfunction. Recently D'Alto et al. found that diastolic dysfunction and the presence of interstitial lung disease can often explain the increase in PAsP during exercise echocardiography, rather than a primary pulmonary vascular obstructive disease [101]. Another important datum is represented by the difference between group A and B regarding the change in oxygen saturation during exercise (Delta O<sub>2</sub> saturation). Oxygen saturation depends on the respiratory gas exchange diffusion capacity, so alterations of the capillary vessels walls, even in the initial phase of the disease, limit the oxygen and carbon dioxide passage across the alveolo-capillary membrane, causing a slight desaturation after the effort [133].

BNP measurement showed no significant difference between the two group (A and B) regarding resting values, but values at peak exercise and the change between resting and exercise values (DeltaBNP) are higher in group B than group A. So we found a correlation between the deltaBNP value and the development of altered pulmonary vasoreactivity induced by exercise. There are not available studies evaluating BNP values during exercise, in this kind of patients. Studies of patients affected by ischemic heart disease, cardiomyopathies and valvular heart disease have evaluated BNP values during stress echocardiography. It is also important to point out that the physical activity could per se increase BNP or NT-proBNP levels in healthy athletes, especially the endurance exercise, but also in non-athletes subjects [134]. Our study demonstrated that patients at higher risk for development of PAH, had higher BNP levels during exercise; this phenomenon could be explained with the higher pressure overload conditions and wall stretching of the right ventricle in the group of patients with altered pulmonary vasoreactivity. In conclusion we can affirm that variations of BNP values with the exercise could represent a biomarker of

pulmonary vascular involvement in SSc patients and, together with exercise echocardiography, could help in diagnosing the altered pulmonary vasoreactivity. Baseline measurement of BNP values did not show any usefulness in early diagnosis of PAH.

Our follow-up could be defined a medium- to long-term evaluation. We found that a high percentage of patients with altered pulmonary vasoreactivity at exercise echocardiography, developed, after about 5 years PAH (41,7%). These datum is different from that reported by Codullo et al. revealing a 3,5% of patients developing PAH, in a shorter follow-up (3,5 ± 0,2 years) [102]. Condliffe et al. showed in their study that 19% of SSc patients with exercise PAH diagnosed by right heart catheterization developed resting PAH after a 2,3 years follow-up [73]. Alkotob et al. after a 2 years follow-up didn't show development of resting PAH in patients perviously submitted to exercise echocardiography who developed altered pulmonary vasoreactivity. However it is to underline that the cut-off value of PAsP to define exercise PAH in this study was considered 40 mmHg (a very low value) and was measured at the end of the exercise, not at peak exercise [131].

Data in literature about follow-up of this kind of patients are certainly lacking, because there are very few studies, with small patient population and with short-term evaluation.

Other interesting data in our follow-up regard the significant difference in terms of the age between the 5 patients who developed resting PAH and the rest of the population; the first group resulted older than the other. This could be explained by the fact that PAH, as a complication of SSc, is more frequent in patients with advanced age, so much that some authors consider the age a risk factor for developing PAH [135]. Finally our 5 patients with resting PAH at follow-up showed a more advanced stage of interstitial lung disease than the other patients, so it is not possible to exclude a role of the lung disease in favoring the development of altered pulmonary vasoreactivity and ultimately resting PAH.



## STUDY LIMITATIONS

Our study lacks a patient control group and is a single-center study with a limited number of patients enrolled. The right heart catheterization would have certainly provided more accurate informations about the pulmonary artery and right atrial pressures, but it would have been difficult to perform such an invasive measurement at baseline evaluation and during stress test to reach reliable data. In the current guidelines of the European Society of Cardiology the exercise echocardiography is not recommendend (class III, level of evidence C) in patients with suspected PAH [7]. The causes are: the value of a normal PAsP achieved during the exercise is not well established, the technical difficulty to perform the exam, the lack of a sufficient number of prospective studies. The main limitations of this kind of study lie in the limits of the echocardiography itself (the image quality, the difficulty of tricuspid regurgitation jet detection). In our study, echocardiographic investigations were performed by a single operator and the technology applied has allowed a limited variability and the ability to measure the pulmonary pressure in 100% of the cases. Another limitation in our study concerns the parameters of the right ventricular function (TAPSE and TAV) that were assessed only at rest, as well as the right atrial pressure, derived by measuring the size and respiratory excursions of the inferior vena cava at rest and assumed as a constant value during all the phases of the effort.

At follow-up the study limitations consisted in performinng right heart catheterization in only one of the 5 patients who developed echocardiographic signs suggestive of PAH. In this patients RHC confirmed the diagnosis of PAH.





## CONCLUSIONS

Exercise echocardiography is a noninvasive, low cost and low risks technique that could be a useful screening tool for early identify a subclinical stage of PAH in a selected patient population such as SSc patients.

Early detection of PAH is an important strategic objective in such kind of disease carrying a very poor prognosis. The future paradigm of early disease detection in high-risk patients should ideally be aimed at detecting disease prior to a rise in resting PAP.

Exercise echocardiography uses a physiological 'stress' that is the effort, to study the hemodynamic response of the pulmonary circulation to detect the altered pulmonary vasoreactivity induced by exercise. It is hypothesized that such atypical response to exercise is a sign of early PAH disease.

Following this kind of patients could provide an early diagnosis of pulmonary hypertension to early start an effective treatment, improving the prognosis.

BNP measurement during exercise could also support the diagnosis of altered pulmonary vasoreactivity made by exercise echocardiography.

Finally the medium- to long-term follow-up of our patients showed that subjects with an abnormal vasoreactivity response to exercise will progress to resting PAH over time, validating the predictive value of this method to early identify the disease.



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**SUMMARY OF SCIENTIFIC ACTIVITIES  
DURING PhD COURSE**



## **PhD course (2010-2014)**

### **I YEAR (2010/2011)**

**- Attendance to the following lessons, congresses and scientific activities expected from the didactic programme of the first year of PhD course:**

1. Seminary "Anatomy for electrophysiologist: conductive tissue and cardiac pacing". Veneto AIAC Regional course. Padua, 18 February 2011.
2. Seminary "Update on myocarditis". Padua, 11 March 2011.
3. XXX Update course of Pediatric Cardiology. Padua, 19-20 April 2011.
4. Update course "The adult with congenital heart disease". Padua, 13 May 2011.
5. Symposium "Arrhythmogenic cardiomyopathy: from genes to therapy". Padua, 20-21 May 2011.
6. Monday Meeting called "Clinical-pathological conferences". Padua, Division of Anatomy Pathology.
7. Course "The anatomic basis of transcatheter implantation of prosthetic aortic valve. Padua, 21 October 2011.
8. "Morgagnane Lectures" on Wednesday.
9. "Summer School" Week on Basic Sciences. Padua, 26-30 September 2011.
10. I congress of Pediatric Arrhythmias. Abano Terme, 17-18 November 2011.

#### **Active attendance to congresses (as speaker):**

1. Abstract presentation (in oral session) at European Congress of Echocardiography (EuroEcho) held in Copenhagen (Denmark), 8-11 December 2010.

Title of the Abstract: Exercise non-invasive evaluation of the hemodynamic parameters and brain natriuretic peptide in the setting of pulmonary arterial hypertension in patients with scleroderma. Authors: V. Scarabeo, M.G. Leone, F. De Conti, V. Degani, F.

D'Ambrosio, P Piovesana.

2. Abstract presentation (in poster session) at National Congress of Italian Society of Echocardiography (SIEC) held in Naples , 14-16 April 2011.

Title of abstract: Studio dell'ipertensione polmonare nei pazienti sclerodermici mediante l'ecocardiogramma da sforzo: valutazione delle variabili emodinamiche e del dosaggio del BNP. Authors: V. Scarabeo, M.G. Leone, F De Conti, V. Degani, F. D'Ambrosio, P Piovesana.

**Teaching activity:**

- Teaching commitment for training courses on Basic Echocardiography and Pediatric Echocardiography organized by the Italian Society of Echocardiography (SIEC).

- Teaching commitment for the following theoretical and practical courses for Cardiologists (with ECM accreditation) held in the Department of Cardiology of Camposampiero hospital:

1. Pediatric Cardiology Course (15-16 November 2010).

2. Pediatric Cardiology Course (18-19 April 2011).

3. Mitral valve disease: update on diagnostic and therapeutic approaches (6 May 2011).

4. Course "Thromboembolic risk and atrial fibrillation: the echocardiographic diagnostic tool and new therapies on anticoagulant drugs"(8 April 2011).

5. Inflammatory heart diseases (24 June 2011).



## **II YEAR (2011/1012)**

**- Attendance to the following lessons, congresses and scientific activities expected from the didactic programme of the second year of PhD course:**

- “Challenges” in aortic valvulopathy. SIC Meeting of Triveneto. Padua, 26 November 2011.
- Anatomy for the electrophysiologist: ablation of the AV junction and Brugada syndrome. Regional Course of Veneto AIAC. Padua, 10 February 2012.
- Advances in Medicine. Scientific Symposium. Padua, 23-24 March 2012.
- XXXI Update course in Pediatric Cardiology. Padua, 3-4 April 2012.
- Monday Meeting called “Clinical-pathological conferences”. Padua, Division of Anatomy Pathology.
- “Morgagnane Lectures” on Wednesday. Padua, Division of Anatomy Pathology.
- Forum of Electrophysiology. Clinical-pathological Conference. Padua, 25 May 2012.

### **Teaching activity:**

- Teaching commitment for training courses on Basic Echocardiography and Pediatric Echocardiography organized by the Italian Society of Echocardiography (SIEC).
- Teaching commitment for the following theoretical and practical courses for Cardiologists (with ECM accreditation) held in the Department of Cardiology of Camposampiero hospital:
  1. Course “Therapies for Acute Coronary Syndrome and future developments for prosthetic cardiac valves” (7 December 2011).
  2. Pediatric Cardiology Course (10-11 May 2012).
  3. Echocardiography in heart failure (20 April 2012).

4. Thromboembolic risk in atrial fibrillation: new developments on oral anticoagulant therapy in preventing ischemic stroke and echocardiographic imaging techniques" (30 March 2012).
5. Aortic valve stenosis and arterial hypertension (15 June 2012).
6. Diagnostic and therapeutic approaches in bioprosthetic cardiac valves (22 June 2012).

### **III YEAR (2012-2013)**

**Attendance to the following lessons, congresses and scientific activities expected from the didactic programme of the third year of PhD course:**

- Monday Meeting called "Clinical-pathological conferences". Padua, Division of Anatomy Pathology.
- Congress "Anatomy for the electrophysiologist: atrial fibrillation". Padua 2 February 2013.
- Update Course "Pediatric Cardiology, XXXII Course". Padua, 27-28 March 2013.
- Spring School. Bressanone, 12-13 April 2013.
- Biannual Course "The adult patient with congenital heart disease". Padua, 17 May 2013.
- Meeting "Heart transplantation". Padua, 7 June 2013.

#### **Teaching activity:**

- Teaching commitment for training courses on Basic Echocardiography and Pediatric Echocardiography organized by the Italian Society of Echocardiography (SIEC).
- Teaching commitment for the following theoretical and practical courses for Cardiologists (with ECM accreditation) held in the Department of Cardiology of Camposampiero hospital:
  1. Heart failure and hypertensive heart disease: integration between echocardiographic imaging technique and biohumoral parameters (19 April 2013).
  2. Thromboembolic risk in atrial fibrillation: new developments on oral anticoagulant therapy in preventing ischemic stroke and echocardiographic imaging techniques" (3 May 2013).

## **IV YEAR (2013-2014)**

**Attendance to the following lessons, congresses and scientific activities expected from the didactic programme of the fourth year of PhD course:**

- Monday Meeting called "Clinical-pathological conferences". Padua, Division of Anatomy Pathology.
- Congress "Anatomy for the electrophysiologist: the arrhythmic risk in mitral valve prolapse". Padua 1 February 2014.
- International Symposium "Update in Arrhythmogenic cardiomyopathy and sudden cardiac death in the young: novel technologies in cardiology, genetics and pathology. Padua, 20-21 March 2014.
- XXXIII Update Course in Pediatric Cardiology: sudden infant death syndrome, complete transposition of the great vessels. Padua, 16-17 April 2014.
- Spring School-Bressanone, 31 May-1 June 2014.
- Forum of Electrophysiology "Monitoring of arrhythmias: present and future". Padua, 6 June 2014.

### **Teaching activity:**

- Teaching commitment for training courses on Basic Echocardiography and Pediatric Echocardiography organized by the Italian Society of Echocardiography (SIEC).
- Teaching commitment for the following theoretical and practical courses for Cardiologists (with ECM accreditation) held in the Department of Cardiology of Camposampiero hospital:
  1. Hypertensive heart disease and aortic valve stenosis: echocardiographic approach and new surgical perspectives (23 May 2014).
  2. Thromboembolic risk in atrial fibrillation: new developments on oral anticoagulant

therapy and echocardiographic imaging techniques" (9 May 2014).

3. Heart failure and hypertensive heart disease (3 October 2014).

### **Published scientific works:**

- Migliore F, **Scarabeo V**, Corfini A, Piovesana P. Microbubbles contrast inside the cyst: a pathognomonic sign of mitral valve blood cyst. J Cardiovasc Med 2010; 11(4):294-296.

### **Abstracts**

- V. Scarabeo, M.G. Leone, F De Conti, V. Degani, F. D'Ambrosio, P Piovesana. Exercise non-invasive evaluation of the hemodynamic parameters and brain natriuretic peptide in the setting of pulmonary arterial hypertension in patients with scleroderma. European Journal of Echocardiography 2010; 11, Suppl 2 (Abstr): 653.

- V. Scarabeo, M.G. Leone, F De Conti, V. Degani, F. D'Ambrosio, P Piovesana. Studio dell'ipertensione polmonare nei pazienti sclerodermici mediante l'ecocardiogramma da sforzo: valutazione delle variabili emodinamiche e del dosaggio del BNP. Abstract. XV Congresso Nazionale della Società italiana di Ecografia cardiovascolare. Napoli, 14-16 Aprile 2011.

- P.Turrini, M.S. Baccillieri, V. Scarabeo, F. De Conti, A. Stendardo, R. Verlato. Incremento improvviso della soglia ventricolare destra e dell'impedenza di shock cavale in paziente portatore di Sprint Fidelis seguito con CareLink; una causa inattesa. Giornale Italiano di Aritmologia e Cardioritmologia 2012; 15, Suppl 1 (Abstr): 98.

- M.S. Baccillieri, M Busa, P.Turrini, A. Gloria, A. Berton, A. Di Marco, F. De Conti, V. Scarabeo, A. Stendardo, R. Verlato. Inefficacia del dronedarone prescritto secondo le indicazioni dell'AIFA per prevenire la fibrillazione atriale in soggetti con precedente sospensione di amiodarone per qualunque ragione. Giornale Italiano di Aritmologia e Cardioritmologia 2012; 15, Suppl 1 (Abstr): 12

- P. Turrini, M.S. Baccillieri, F. Campisi, A. Di Marco, F. De Conti, A. Stendardo, V. Scarabeo, M. Busa, A. Gloria, A. Berton, G. Gerosa, R. Verlato. Effetto sinergico della CRT con VAD Jarvik: descrizione di un caso. Giornale Italiano di Aritmologia e Cardioritmologia 2012; 15, Suppl 1 (Abstr): 129.

- M. Busa, V.Scarabeo, M. Noni, A. Berton, M. Mion, P. Piovesana. Cardiotossicità da Antracicline: ruolo dell'ecocardiografia parametrica nell'identificazione del danno precoce. Giornale Italiano di Cardiologia 2012; 13, Suppl 2 (Abstr): 164.

- P. Piovesana, P. Offelli, F. D'Ambrosio, F. De Conti, V. Scarabeo, M. Panfili, F. Antonini-Canterin. A new Stress Echo protocol to increase sensitività: dipiridamole follone by exercise. *Giornale Italiano di Cardiologia* 2012; 13, Suppl 2 (Abstr): 499.
- A. Berton, V. Scarabeo, M. Busa, M. Rizzo, F. Cozzi, P Piovesana. Valore predittivo della valutazione non invasiva dei parametri emodinamici e del BNP durante ecocardiografia da sforzo nell'identificazione precoce dell'ipertensione polmonare nei pazienti sclerodermici; follow-up a due anni. *Giornale Italiano di Cardiologia* 2012; 13, Suppl 2 (Abstr): 533.
- M. Beraldo, M. Panfili, F. De Conti, V. Scarabeo, P Piovesana. Ecostress dipiridamolo pù sforzo: quale significato assume un risultato negativo? *Giornale Italiano di Cardiologia* 2013; Vol. 14, Suppl 1 (Abstr): P 451.
- M. Beraldo, M. Panfili, F. De Conti, V. Scarabeo, P Piovesana. Valore diagnostico di una nuova metodologia di induzione di ischemia utilizzando l'associazione di dipiridamolo e sforzo fisico. *Giornale Italiano di Cardiologia* 2013; Vol. 14, Suppl 1 (Abstr) : P452.
- M. Busa, M. Nonj, V. Scarabeo, A. Berton, M. Mion, P. Piovesana. Ruolo dell'ecocardiografia parametrica nella definizione precoce della tossicità da antraci cline. *Giornale Italiano di Cardiologia* 2013; Vol. 14, Suppl 1 (Abstr) : C80.

# Curriculum Vitae

## PERSONAL INFORMATION

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POSITION **Medical doctor**

## WORK EXPERIENCE

- 01/03/2005–Present **Full-time employment with open-ended contract as medical doctor cardiologist at Department of Cardiology of Camposampiero Hospital (ULSS 15 Alta Padovana, Veneto)**  
Camposampiero (Italy)  
Medical activities in Cardiology ward, Intensive Coronary Care Unit, Ambulatory Division and Laboratory of Echocardiography.
- 22/03/2004–28/02/2005 **Full-time employment with open-ended contract, as medical doctor cardiologist at Department of Cardiology of Portogruaro Hospital (ULSS 10 "Veneto Orientale")**  
Portogruaro (Italy)
- 07/04/2003–21/03/2004 **Full-time employment with fixed-term contract as medical doctor cardiologist at Department of Cardiology of Pieve di Cadore hospital (ULSS n.1, Belluno)**  
Pieve di Cadore (Italy)

## EDUCATION AND TRAINING

- 2010–Present **Doctoral (PhD) course in Clinical and Experimental Sciences. Curriculum: Cardiovascular Sciences.**  
University of Padua, Padova (Italy)
- 01/01/2010–16/12/2010 **Second-level short specialisation degree in Cardiovascular Pathology**  
University of Padua, Padova (Italy)
- 01/01/2008–19/01/2009 **Second-level short specialisation degree in Pediatrics and its sub-specialty areas: Cardiology.**  
University of Padua, Padova (Italy)
- 01/01/1998–16/12/2002 **School Specialisation degree in Cardiology**  
University of Padua (II School of Cardiology), Padova (Italy)

10/1992–20/10/1998 **Academic degree in Medicine and Surgery**  
Catholic University of the Sacred Heart in Rome, Roma (Italy)

1987–07/1992 **Secondary School Diploma in Classical Studies**  
Liceo-Ginnasio "A. Giordano", Venafro (IS) (Italy)

#### PERSONAL SKILLS

Mother tongue(s) Italian

Other language(s)	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	B2	B2	B1	B2	B2

Levels: A1/A2: Basic user - B1/B2: Independent user - C1/C2: Proficient user  
[Common European Framework of Reference for Languages](#)

Computer skills Knowledge and application of Microsoft Office programmes

Driving licence A, B

#### ADDITIONAL INFORMATION

From 2012 teaching activity in Basic and Pediatric Echocardiography Courses organized by the Italian Society of Echocardiography (SIEC).

From 2011 teaching activity in theoretical-practical courses for cardiologists held in the Department of Cardiology of Camposampiero hospital.



## Publications and abstracts

1. **Scarabeo V**, Cernetti R, Zorzi C, De Conti F, Bini RM, Pantaleoni A, Piovesana P. Interatrial septal aneurysm and genetic prothrombotic disorders: possible interaction in the pathogenesis of pediatric stroke. *Ital Heart J* 2001;2 (11):854-857.
2. Paschetto G, Reimers B, Napodano M, Saccà S, Cernetti C, Span S, **Scarabeo V**, Pellizzari N, Pascotto P. Prevention of distal embolization during coronary angioplasty in patients with acute coronary syndromes. *Ital Heart J* 2002;3, Suppl 2 (Abstr): C194.
3. **Scarabeo V**, Di Marco A, Pantaleoni A, Contessotto F, Mantovani E, Piovesana P. Dissection of the ascending aorta mimicking an acute coronary syndrome: usefulness of transthoracic echocardiography for the differential diagnosis. *Ital Heart J* 2002;3 (8):490-494.
4. Bertaglia E, **Scarabeo V**, Bonso A, Proclemer A, Verlato R, Coro' L, Mantovan R, D'Este D, Zoppo F, Pascotto P. Variabili differenti predicano le recidive di fibrillazione atriale dopo ablazione dell'istmo cavo-tricuspidalico nei pazienti con e senza storia pregressa di fibrillazione atriale. *Ital Heart J* 2002;3, Suppl 7 (Abstr): C106.
5. **Scarabeo V**, Bertaglia E, Zoppo F, Zerio C, Zerbo F, D'Este D, Pascotto P. Encircling anatomico delle vene polmonari mediante ablazione transcateretere con radiofrequenza: variabili predittive di successo clinico. *Ital Heart J* 2002;3, Suppl 7 (Abstr): C110.
6. Bertaglia E, Zoppo F, Zerio C, **Scarabeo V**, Pellizzari N, Zerbo F, D'Este D, Pascotto P. Variabili predittive di successo clinico dell'encircling anatomico delle vene polmonari per la cura della fibrillazione atriale. *Ital Heart J* 2003; 4, Suppl 1 (Abstr): P172.
7. Napodano M, Paschetto G, Saccà S, Cernetti C, **Scarabeo V**, Pascotto P, Reimers B. Intracoronary thrombectomy improves myocardial reperfusion in patients undergoing direct angioplasty for acute myocardial infarction. *J Am Coll Cardiol* 2003; 42(8): 1395-1402.
8. Bertaglia E, **Scarabeo V**, Zoppo F, Zerio C, Pellizzari N, Zerbo F, D'Este D, Pascotto P. Isolamento circonferenziale delle vene polmonari mediante ablazione transcateretere con radiofrequenza per il trattamento della fibrillazione atriale. *Ital Heart J Suppl.* 2003; 4 (10): 825-832.
9. Dalle Mule J, Perelli R, Mazzella M, Martinelli F, Proclemer A, **Scarabeo V**, Di Sipio ML. Impact of myocardial viability assessment on the beneficial effect of cardiac resynchronization therapy in patients with ischemic cardiomyopathy. *JACC* 2004; 43(5) Suppl 1 (Abstr):P1068-121.
10. Toffanin G, **Scarabeo V**, Verlato R, De Conti F, Zampiero AA, Piovesana P. Transesophageal echocardiographic evaluation of pulmonary vein anatomy in patients

undergoing ostial radiofrequency catheter ablation for atrial fibrillation: a comparison with magnetic resonance angiography. *J Cardiovasc Med* 2006; 7(10):748-752.

11. D'Este D, Zoppo F, Bertaglia E, Zerbo F, Picciolo A, **Scarabeo V**, Pascotto A, Pascotto P. Long-term outcome of patients with atrioventricular node reentrant tachycardia. *Int J Cardiol* 2007; 115(3): 350-3.

12. **Scarabeo V**, Baccillieri MS, Di Marco A, De Conti F, Contessotto F, Piovesana P. Sine-wave pattern on the electrocardiogram and hyperkalemia. *J Cardiovasc Med* 2007; 8(9):729-731.

13. **Scarabeo V**, De Simone G, De Conti F, Turrini P, Verlato R, Lafisca N, Zampiero A, Piovesana P. Pulmonary veins remodeling and transcatheter ablation of atrial fibrillation. *Eur Heart J* 2008; 29 Suppl 1(Abstr): P1918.

14. **Scarabeo V**, De Simone G, De Conti F, Verlato R, Contessotto F, Di marco A, Zampiero A, Piovesana P. Prevalence of variant pulmonary veins anatomy in patients undergoing radiofrequency transcatheter ablation of atrial fibrillation. *Eur Heart J* 2008; 29 Suppl 1 (Abstr): P1206.

15. Stendardo A, **Scarabeo V**, De Conti F, Pengo V, Piovesana P. L'ecocardiografia come metodica incruenta nella diagnosi differenziale tra ipertensione polmonare pre e postcapillare. *Ital Heart J* 2009; 10(6) Suppl 2 (Abstr): P362.

16. Stendardo A, **Scarabeo V**, De Conti F, Pengo V, Piovesana P. Prevalenza di ipertensione polmonare da sforzo in pazienti con malattie del connettivo. *Ital Heart J* 2009; 10(6) Suppl 2 (Abstr): P208.

17. Stendardo A, **Scarabeo V**, De Conti F, Pengo V, Piovesana P. Ruolo dell'ecocardiografia da sforzo nello studio dell'ipertensione polmonare in pazienti con malattie del connettivo. *Ital Heart J* 2009; 10(6) Suppl 2 (Abstr): P203.

18. Milanesi O, **Scarabeo V**, Maschietto N, Biffanti R, Patuelli G. Treatment of severe complications of Fontan circulation with endothelin1 dual (A and B) receptor antagonist Bosentan. *Eur Heart J* 2009; 30, Suppl 1(Abstr): P4799.

19. **Scarabeo V**, Biffanti R, Cerutti A, Maschietto N, Reffo E, Padalino M, Vida V, Stellin G, Milanesi O. Treatment of severe complications of Fontan circulation with Endothelin 1 dual (A and B) receptor antagonist bosentan. *Atti del XXXIX Congresso Nazionale SICP* 2009; (Abstr): P97.

20. Migliore F, **Scarabeo V**, Corfini A, Piovesana P. Microbubbles contrast inside the cyst: a pathognomonic sign of mitral valve blood cyst. *J Cardiovasc Med* 2010; 11(4):294-296.

21. **V. Scarabeo**, M.G. Leone, F De Conti, V. Degani, F. D'Ambrosio, P Piovesana. Exercise non-invasive evaluation of the hemodynamic parameters and brain natriuretic peptide in the setting of pulmonary arterial hypertension in patients with scleroderma. *European Journal of Echocardiography* 2010; 11, Suppl 2 (Abstr): 653.
22. F. D'Ambrosio, **V. Scarabeo**, F. De Conti, M. Panfili, M.G. Leone, P. Piovesana. Un nuovo protocollo per la diagnosi di ischemia inducibile con l'ecostress: dipiridamolo + sforzo. *Giornale Italiano di cardiologia* 2010; 11, Suppl 1 (abstr): P211.
23. M.G. Leone, **V. Scarabeo**, F. De Conti, V. Degani, F. D'Ambrosio, P Piovesana. Valutazione non invasiva delle variabili emodinamiche e della variazione del BNP da sforzo nello studio dell'ipertensione arteriosa polmonare nei pazienti affetti da sclerodermia. *Giornale Italiano di Cardiologia* 2010; 11, Suppl 1 (abstr): P281.
24. **V. Scarabeo**, M.G. Leone, F De Conti, V. Degani, F. D'Ambrosio, P Piovesana. Studio dell'ipertensione polmonare nei pazienti sclerodermici mediante l'ecocardiogramma da sforzo: valutazione delle variabili emodinamiche e del dosaggio del BNP. Abstract. XV Congresso Nazionale della Società italiana di Ecografia cardiovascolare. Napoli, 14-16 Aprile 2011.
- P. Offelli, **V. Scarabeo**, F. De Conti, P. Piovesana. Complicanza inattesa di legatura dell'auricola sinistra in intervento di plastica mitralica. *Giornale Italiano di Cardiologia* 2011; 12, Suppl 1 (Abstr): P420.
25. P.Turrini, M.S. Baccillieri, **V. Scarabeo**, F. De Conti, A. Stendardo, R. Verlato. Incremento improvviso della soglia ventricolare destra e dell'impedenza di shock cavale in paziente portatore di Sprint Fidelis seguito con CareLink; una causa inattesa. *Giornale Italiano di Aritmologia e Cardioritmo* 2012; 15, Suppl 1 (Abstr): 98.
26. M.S. Baccillieri, M Busa, P.Turrini, A. Gloria, A. Berton, A. Di Marco, F. De Conti, **V. Scarabeo**, A. Stendardo, R. Verlato. Inefficacia del dronedarone prescritto secondo le indicazioni dell'AIFA per prevenire la fibrillazione atriale in soggetti con precedente sospensione di amiodarone per qualunque ragione. *Giornale Italiano di Aritmologia e Cardioritmo* 2012; 15, Suppl 1 (Abstr): 12.
27. P. Turrini, M.S. Baccillieri, F. Campisi, A. Di Marco, F. De Conti, A. Stendardo, **V. Scarabeo**, M. Busa, A. Gloria, A. Berton, G. Gerosa, R. Verlato. Effetto sinergico della CRT con VAD Jarvik: descrizione di un caso. *Giornale Italiano di Aritmologia e Cardioritmo* 2012; 15, Suppl 1 (Abstr): 129.
28. M. Busa, **V. Scarabeo**, M. Noni, A. Berton, M. Mion, P. Piovesana. Cardiotossicità da Antracicline: ruolo dell'ecocardiografia parametrica nell'identificazione del danno precoce. *Giornale Italiano di Cardiologia* 2012; 13, Suppl 2 (Abstr): 164.
29. P. Piovesana, P. Offelli, F. D'Ambrosio, F. De Conti, **V. Scarabeo**, M. Panfili, F. Antonini-Canterin. A new Stress Echo protocol to increase sensitività: dipiridamole follone by exercise. *Giornale Italiano di Cardiologia* 2012; 13, Suppl 2 (Abstr): 499.

30. A. Berton, **V. Scarabeo**, M. Busa, M. Rizzo, F. Cozzi, P Piovesana. Valore predittivo della valutazione non invasiva dei parametri emodinamici e del BNP durante ecocardiografia da sforzo nell'identificazione precoce dell'ipertensione polmonare nei pazienti sclerodermici; follow-up a due anni. *Giornale Italiano di Cardiologia* 2012; 13, Suppl 2 (Abstr): 533.

31. M. Beraldo, M. Panfilì, F. De Conti, **V. Scarabeo**, P Piovesana. Ecostress dipiridamolo pù sforzo: quale significato assume un risultato negativo? *Giornale Italiano di Cardiologia* 2013; Vol. 14, Suppl 1 (Abstr): P 451.

32. M. Beraldo, M. Panfilì, F. De Conti, **V. Scarabeo**, P Piovesana. Valore diagnostico di una nuova metodologia di induzione di ischemia utilizzando l'associazione di dipiridamolo e sforzo fisico. *Giornale Italiano di Cardiologia* 2013; Vol. 14, Suppl 1 (Abstr) : P452.

33. M. Busa, M. Nonj, **V. Scarabeo**, A. Berton, M. Mion, P. Piovesana. Ruolo dell'ecocardiografia parametrica nella definizione precoce della tossicità da antraci cline. *Giornale Italiano di Cardiologia* 2013; Vol. 14, Suppl 1 (Abstr) : C80.