Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

SCIENZE CARDIO-NEFRO-TORACIHE

Ciclo XXXV°

Settore Concorsuale:
06/D1 - MALATTIE DELL'APPARATO CARDIOVASCOLARE E MALATTIE DELL'APPARATO RESPIRATORIO

Settore Scientifico Disciplinare:
MED/11 - MALATTIE DELL'APPARATO CARDIOVASCOLARE

Triple Combination Therapy in Pulmonary Arterial Hypertension

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Esame finale anno 2023

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ABSTRACT

Background

Pulmonary arterial hypertension (PAH) is a progressive and severe disease characterized by increasing in pulmonary vascular resistance (PVR) leading to right ventricular failure and premature death. Currently available drugs for treatment of PAH act on three different pathways responsible of the pathogenesis of this disease: the endothelin pathway, the nitric oxide pathway and the prostacyclin pathway.

Focusing on the prostacyclin pathway, the first synthetic agent introduced in clinical practice was Epoprostenol, which demonstrated significant efficacy in the improvement of hemodynamic parameters, exercise capacity and mortality. However, its short half-life requires continuous intravenous infusion, needing central line placement and potentially introducing the risk of central line-associated blood stream infection.

An alternative synthetic agent is Treprostinil, which has a much more stable half-life and can be administrated at much lower infusion rates via a subcutaneous pump. While effective in terms of 6MWD, the frequent occurrence of infusion site pain limits dose escalation and clearance may be affected by renal and hepatic impairment.

For both drugs, dose titration is individualized according to the individual patient and characteristic pattern of adverse effects - hypotension, flushing, diarrhea, and muscle pain – frequently limit dose escalation. Furthermore, the drug delivery must be continuous, as abrupt withdrawal may precipitate to rebound pulmonary hypertension, which can be fatal.

In recent years a new synthetic agent has been introduced. Selexipag is a selective IP prostacyclin receptor agonist that is structurally distinct from prostacyclin but exerts similar effects: it is rapidly hydrolyzed to a long-acting metabolite that binds to IP receptors, resulting in vasodilation, inhibition of platelet aggregation and anti-inflammatory effects. It showed significant clinical and hemodynamic benefits and was able to reduce disease progression at each tolerated dose, in all population subgroups and in the presence of optimal background therapy. Nevertheless, this has not sorted the issue of tolerability seen with prostanoids, though pharmacodynamic data may suggest that more frequent drug dosing could smooth the dose–response curve and lessen side effect burden. Therefore, Selexipag represents a potentially more stable drug, with less complex administration and titration.

Objectives

The purpose of our study was to reassess our experience on the use of drugs that interact on the pathobiological line of prostacyclin, so we compared the efficacy of intravenous Epoprostenol, subcutaneous Treprostinil and oral Selexipag for the treatment of PAH.

Materials and methods

We retrospectively included all patients, referred to our center from February 1995 to December 2021, who received therapy with i.v. Epoprostenol, s.c. Treprostinil or oral Selexipag. Non-invasive and invasive parameters were collected at baseline and at first follow-up (after 3–4 months of treatment).

In total 301 patients were included into the study: 152 were treated with Epoprostenol, 105 with Treprostinil and 44 with Selexipag. Invasive follow-up data, assessed after 3-4 months of therapy, were available in 238 patients: 105 in the Epoprostenol group, 97 in the Treprostinil group 36 in the Selexipag group.

In the second part of our analysis, we aimed at assessing the effects of the same drugs when they were used as third line strategy, therefore we selected 181 patients: 44 in the Selexipag group, 80 patients in the Treprostinil group and 57 patients in the Epoprostenol group. Invasive follow-up data were available in 153 patients: 44 in the Epoprostenol group, 73 in the Treprostinil group 36 in the Selexipag group.

Results and discussion

Firstly, we observed that patients treated with Epoprostenol were significantly more compromised at baseline in terms of symptoms, functional capacity and hemodynamics when compared to the two other groups, while patients treated with Treprostinil appeared to be slightly worse at baseline with respect to those treated with Selexipag. Then, evaluating the effects of the three different drugs between baseline assessment and first follow-up, it emerged that patients treated with Epoprostenol had significantly greater clinical and hemodynamic improvements in respect to those treated with Treprostinil and Selexipag, while patients treated with Treprostinil showed only a trend trough better progresses if compared to patients treated with Selexipag.

There are many confounding factors that could have influenced demographic, clinical and hemodynamic characteristics of patient populations, as well as drug response. Firstly, the different era of drug approval has influenced the treatment strategy, according to the introduction of new compounds targeting the two other pathways (the endothelin and the nitric oxide pathway). That is why initially intravenous and subcutaneous prostacyclin analogs have been used predominantly in

mono therapy and subsequently as second- and third-line compounds when the initial strategy with oral medications was failing. Furthermore, different treatment invasiveness plays an important role in the drug choice, so that less invasive drugs are used in less advanced clinical and hemodynamic conditions.

In the second part of our analysis we aimed at limiting these confounding factors, comparing the effects of the same drugs when they were used as third line strategy.

The baseline characteristics of the three populations were quite similar, even if patients treated with Selexipag were older in comparison with the two other groups: in subjects out of transplantability range we more often prescribe Selexipag, while in youngest patients we tend to be more aggressive. Furthermore, the differences emerged in exercise capacity and baseline hemodynamics reflect the fact that in our clinical practice, we add Epoprostenol as third line therapy in more compromised patients, Treprostinil in intermediate situations and Selexipag in less impaired conditions. Comparing the effects of treatments between baseline and first follow-up we noticed smaller benefits with Selexipag when compared with intravenous and subcutaneous strategies but it's important to weight baseline patient's differences.

Conclusions

Our analysis confirmed clinical and functional benefits for the use of both prostacyclin analogues and prostacyclin receptor agonists in terms of improved functional class, six-minute walking distance and cardiopulmonary hemodynamics.

In particular, the efficacy of Selexipag in triple combination therapy in less advanced patients suggests the possibility to anticipate further its use to achieve as soon as possible triple combination therapy.

1. Introduction: Pulmonary Hypertension

1.1 Definition and classifications

1.1.1 Definition

Pulmonary hypertension (PH) is a pathophysiological disorder that may involve multiple clinical conditions and can complicate many cardiovascular and respiratory diseases.

According to recent guidelines, PH is defined as an increase in mean pulmonary arterial pressure (PAPm) ≥20 mmHg at rest, as assessed by right heart catheterization (RHC)[1]. Furthermore, it is essential to consider other hemodynamic parameters as pulmonary vascular resistance (PVR) and pulmonary arterial wedge pressure (PAWP) in order to discriminate elevated PAP due to pulmonary vascular disease (PVD) from that due to left heart disease (LHD), elevated pulmonary blood flow or increased intrathoracic pressure[1].

The term PAH includes a group of PH patients haemodynamically characterized by the presence of pre-capillary PH, in the absence of other causes of pre-capillary PH, such as CTEPH and PH associated with lung diseases.

1.1.2 Classifications

Haemodynamic classification

According to various combinations of PAP, PAWP and PVR, different haemodynamic definitions have been delineated (shown in *Table 1*).

Pre-capillary PH is hemodynamically defined as mPAP >20 mmHg, PAWP <15 mmHg and PVR >2 Wood Units (WU).

Post-capillary PH is defined as mPAP >20 mmHg and PAWP >15 mmHg and pulmonary vascular resistance is used to distinguish patients with post-capillary PH who have a significant pre-capillary component (PVR >2 WU—combined post- and pre-capillary PH [CpcPH]) and those who do not (PVR ≤2 WU—isolated post-capillary PH [IpcPH]).

In last guidelines exercise PH, defined by an mPAP/cardiac output (CO) slope >3 mmHg/L/min between rest and exercise[2], has been re-introduced. The mPAP/CO slope is age dependent and its upper limit of normal ranges from 1.6–3.3 mmHg/L/min: an mPAP/CO slope >3 mmHg/L/min is not

physiological in subjects aged <60 years and may rarely be present in healthy subjects aged >60 years. A pathological increase in pulmonary pressure during exercise is associated with impaired prognosis in patients with exercise dyspnea[3] and in several cardiovascular conditions[4].

Definition	Haemodynamic characteristics
PH	mPAP >20 mmHg
Pre-capillary PH	mPAP >20 mmHg PWAP ≤15 mmHg PVR >2 WU
Isolated post-capillary PH (Ipc-PH)	mPAP >20 mmHg PAWP >15 mmHg PVR ≤2 WU
Combined post-capillary and pre-capillary PH (Cpc-PH)	mPAP >20 mmHg PAWP >15 mmHg PVR >2 WU
Exercise PH	mPAP/CO slope between rest and exercise >3 mmHg/L/min

Table 1. Haemodynamic definitions of pulmonary hypertension

Clinical classification

The clinical classification of PH aims at categorizing multiple clinical conditions into five groups according to their similar clinical presentation, pathological findings, haemodynamic characteristics and treatment strategy.

Group 1 includes all forms of pulmonary arterial hypertension (PAH): different conditions in which the increasing PAP values is due to microvascular remodeling; group 2 incorporate patients affected by PH due to left heart disease; group 3 encloses cases of PH due to lung diseases and/or hypoxia; group 4 identifies patients affected by pulmonary artery obstructions and finally group 5 incorporates all forms of PH with unclear and/or multifactorial mechanisms.

A comprehensive and up-to-date version of the clinical classification is presented in *Table 2*[1].

GROUP 1. Pulmonary arterial hypertension (PAH)

1.1 Idiopathic

1.1.1 Non-responders at vasoreactivity testing

1.1.2 Acute responders at vasoreactivity testing

1.2 Heritable

1.3 Associated with drugs and toxins

1.4 Associated with:

1.4.1 Connective tissue disease

1.4.2 HIV infection

1.4.3 Portal hypertension

1.4.4 Congenital heart disease

1.4.5 Schistosomiasis

1.5 PAH with features of venous/capillary (PVOD/PCH) involvement

1.6 Persistent PH of the new-born

GROUP 2. PH associated with left heart disease

2.1 Heart Failure

2.1.1 with preserved ejection fraction

2.1.2 with reduced or mildly reduced ejection fraction

2.2 Valvular Heart Disease

2.3 Congenital/acquired cardiovascular conditions leading to postcapillary PH

GROUP 3. PH associated with lung diseases and/or hypoxia

3.1 Obstructive lung disease or emphysema

3.2 Restrictive lung disease

3.3 Lung disease with mixed restrictive/obstructive pattern

3.4 Hypoventilation syndromes

3.5 Hypoxia without lung disease (e.g. high altitude)

3.6 Developmental lung disorders

GROUP 4. PH associated with pulmonary artery obstructions

4.1 Chronic thrombo-embolic PH

4.2 Other pulmonary artery obstructions

GROUP 5. PH with unclear and/or multifactorial mechanisms

5.1 Hematological disorders
5.2 Systemic disorders
5.3 Metabolic disorders
5.4 Chronic renal failure with or without hemodialysis
5.5 Pulmonary tumor thrombotic microangiopathy
5.6 Fibrosing mediastinitis

Table 2. Comprehensive clinical classification of pulmonary hypertension

1.2 Diagnosis

PH diagnosis requires clinical suspicion based on symptoms and physical examination and a comprehensive set of investigations to confirm the diagnosis and to assess the functional and hemodynamical severity of the condition.

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. Furthermore, special awareness should be directed towards patients with associated conditions and/or risk factors for the development of PAH, such as family history, CTD, CHD, HIV infection, portal hypertension or a history of drug or toxin intake known to induce PAH.

A pragmatic approach to diagnosis should be considered, and the diagnostic algorithm proposed by last ESC guidelines is shown in *Figure 1*.

Firstly, patients with PH are often visited by general practitioners for non-specific symptoms. Initial evaluation should include a comprehensive medical history, physical examination, blood test to determine BNP/NT-proBNP, and resting ECG.

The second step include non-invasive lung and cardiac testing. In particular, echocardiography is a fundamental step in the diagnostic algorithm (*Figure 2*) as it assigns the level of probability of PH and could help in identifying other cardiac disorders.

Patients should be referred to a PH center for further evaluation when an intermediate/high probability of PH is established, or in the presence of risk factors for PAH/history of PE. Then, a comprehensive work-up should be performed (including invasive assessment if needed), with the goal of establishing

the differential diagnosis and distinguishing between the various causes of PH according to the current clinical classification[1].

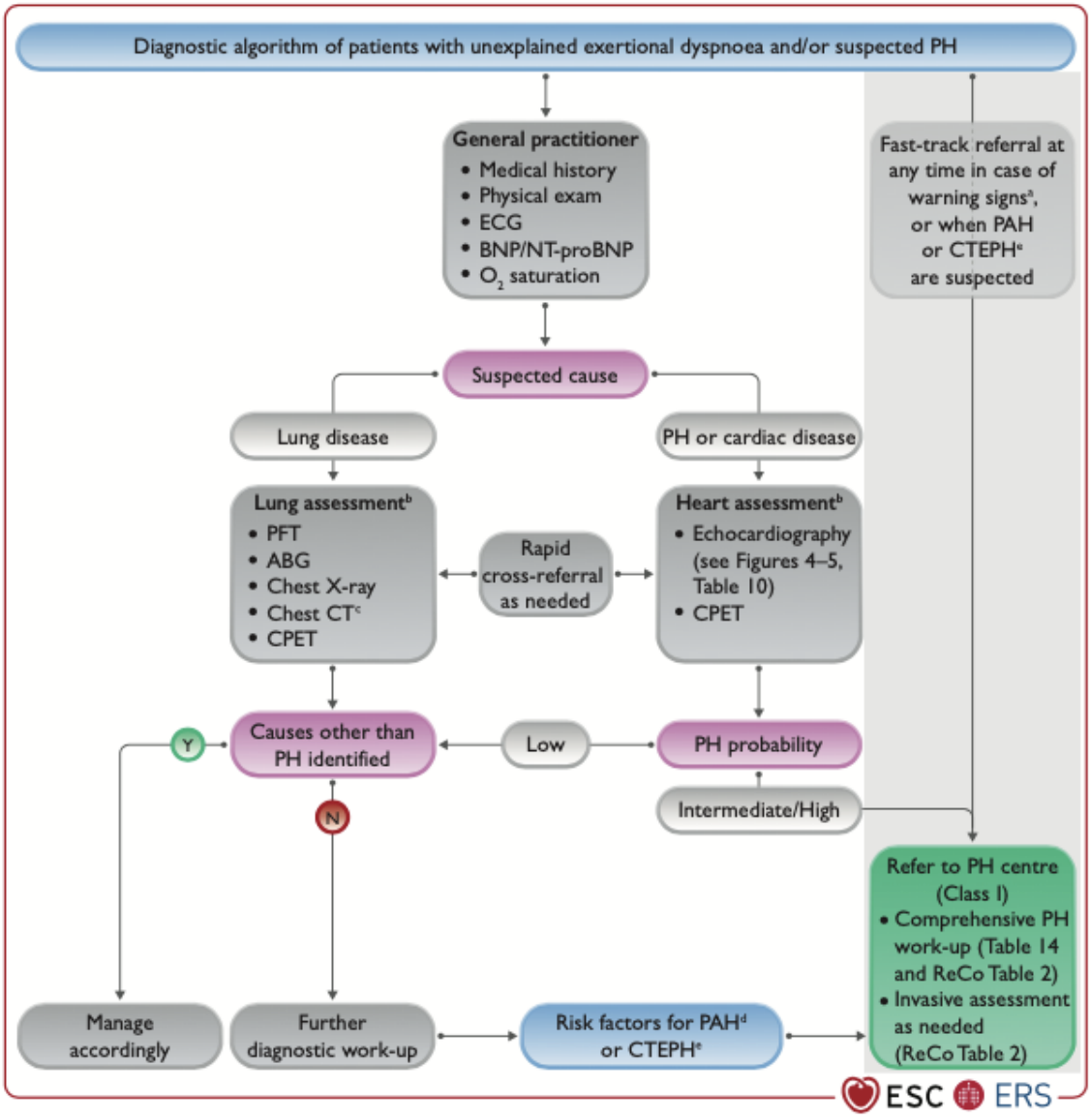

Figure 1. Diagnostic algorithm

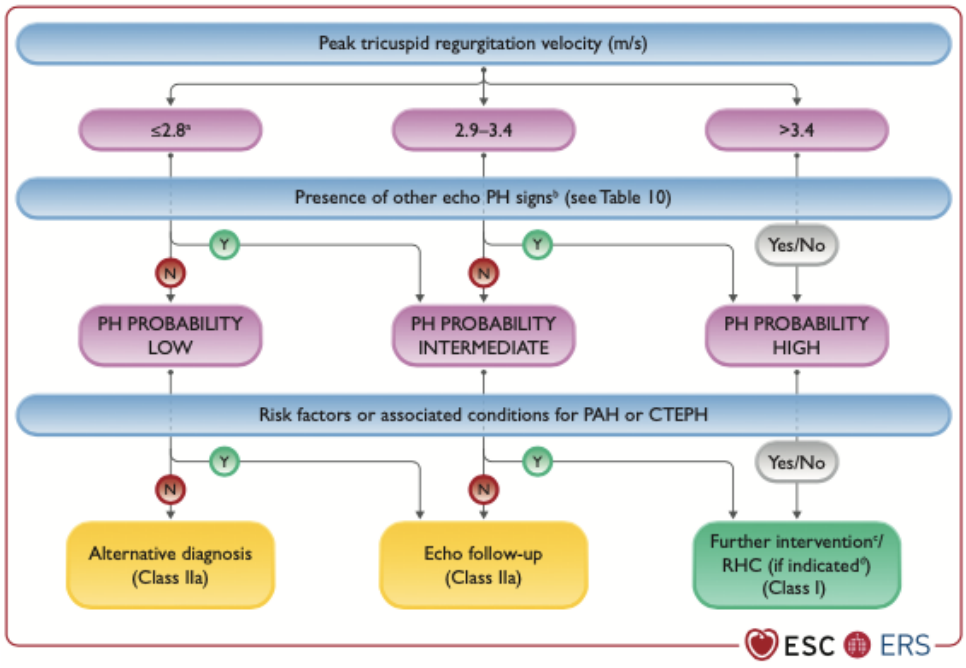

Figure 2. Echocardiographic probability of pulmonary hypertension and recommendations for further assessment

2. Pulmonary arterial hypertension (PAH)

PAH represents the sub-type of PH in which the most important advances in the understanding and treatment have been achieved in the past decades. It is also the group in which PH is the 'core' of the clinical problem and can be treated with specific drugs.

PAH includes a subpopulation of patients with a haemodynamic profile characterized by precapillary PH, with PAWP≤ 15 mmHg and PVR>2 WU, in the absence of other causes of pre-capillary PH such as PH due to lung diseases, CTEPH or other rare diseases.
This definition comprises many apparently heterogeneous conditions that share comparable clinical and haemodynamic pictures and virtually identical pathological changes of the lung microcirculation, the so called "pulmonary hypertensive arterial disease".
These structural changes determine progressive increase in PVR and consequent chronic increase of right ventricle afterload, which results in hypertrophy, dilatation and finally right ventricular failure and death.

PAH it's a rare disease, with an incidence and prevalence respectively of 6 and 48-55 cases/million adults[5] and typically affects relatively young patients (average age of 50 years), mostly females. In registries, IPAH is the most common subtype (around half of PAH patients), followed by PAH associated with connective tissue disease (CTD), CHD and porto-pulmonary hypertension (PoPH)[6].

2.1 Pathogenesis (pathology, pathobiology and pathophysiology)

Even if many pathobiological mechanisms have been identified in cells and tissues of patients with PAH, the exact interactions between them in the initiation and progression of the pathological processes have not been completely understood.
It has been hypothesized that an interaction between genetic predisposition and environmental risk factors may be involved in the initial stages of the disease: it appears that some specific injury on distal pulmonary arteries vessel's wall may initiate, in predisposed individuals, a pathobiological cascade of events which lead to a common vascular obstructive condition (*Figure 3* and *4*).

The main pathological lesions typically affect distal pulmonary arteries (<500 μm). Lesions are characterized by medial hypertrophy, intimal proliferative and fibrotic changes (concentric and/or eccentric), adventitial thickening with moderate peri-vascular inflammatory, complex lesions (plexiform, dilated) and thrombotic lesions [7] (*Figure 3*).

The pathobiology is multifactorial and involves various cell types and many biochemical pathways (*Figure 4*). Excessive vasoconstriction has been related to abnormal function or expression of potassium channels in the smooth muscle cells and to endothelial dysfunction[8]. Endothelial dysfunction leads to chronically impaired production of vasodilator and antiproliferative agents such as nitric oxide and prostacyclin, along with overexpression of vasoconstrictor and proliferative substances such as thromboxane A2 and endothelin[9] [10] [11]. Many of these abnormalities both elevate vascular tone and promote vascular remodelling by proliferative changes that involve several cell types, including endothelial and smooth muscle cells as well as fibroblasts[7]. Also, in the adventitia, there is an increased production of extracellular matrix including collagen, elastin, fibronectin and of matrix-bound smooth muscle cell mitogens, such as basic fibroblast growth factor. Other matrix metalloproteases can stimulate the production of tenascin, a smooth muscle cell mitogenic cofactor. Several additional growth factors including vascular endothelial growth factor, platelet-derived growth factor, insulin-like growth factor-1 and epidermal growth factor have been implicated in the development of remodelling and all have been reported to be increased (the molecule and/or the specific receptors) in the lung and/or in the blood of PAH patients. Reduced plasma levels of other vasodilator and antiproliferative substances such as vasoactive intestinal peptide have also been demonstrated. Angiopoietin-1, an angiogenic factor essential for vascular lung development, seems to be up-regulated in cases of PAH correlating directly with the severity of the disease[12] [13] [14]. Receptors of the bone morphogenetic protein pathway, involved in cellular proliferation and apoptosis, are down regulated and/or malfunctioning in the lung vasculature of both heritable and

acquired PAH. Inflammatory cells, cyto- and chemokines, and platelets (through the serotonin pathway) may also play a significant role in PAH[15]. Pro-thrombotic abnormalities have been demonstrated in PAH patients and thrombi are present in both the small distal pulmonary arteries and in proximal elastic pulmonary arteries[16].

The increase of pulmonary vascular resistance in PAH patients is therefore related to different mechanisms, including vasoconstriction, proliferative and obstructive remodelling of the pulmonary vessel wall, inflammation, and thrombosis. Vasoconstriction is likely prevalent in the small group of patients responding to the acute vasoreactivity test.

The increase in pulmonary vascular resistance leads to right ventricular overload, hypertrophy, and dilatation and eventually to right ventricle failure and death. The importance of the progression of right ventricle failure on symptoms, exercise limitation, and outcome of PAH patients is confirmed by the prognostic impact of right atrial pressure, cardiac index, and pulmonary arterial pressure, the three main haemodynamic factors linked to right ventricle pump function[17]. Echocardiography and cardiac magnetic resonance parameters and brain natriuretic peptide plasma levels can also identify non-invasively the presence and extent of right ventricular dysfunction.

Afterload mismatch remains the leading determinant of right heart failure in patients with PAH[18] because its removal, as follows lung transplantation, leads almost invariably to sustained recovery of right ventricle function. It is therefore conceivable that the drug therapies tested in PAH patients have included compounds which could potentially interfere with the pathobiological mechanisms of the disease trying to achieve a reverse remodelling of obstructive lesions and a reduction of the right ventricular afterload.

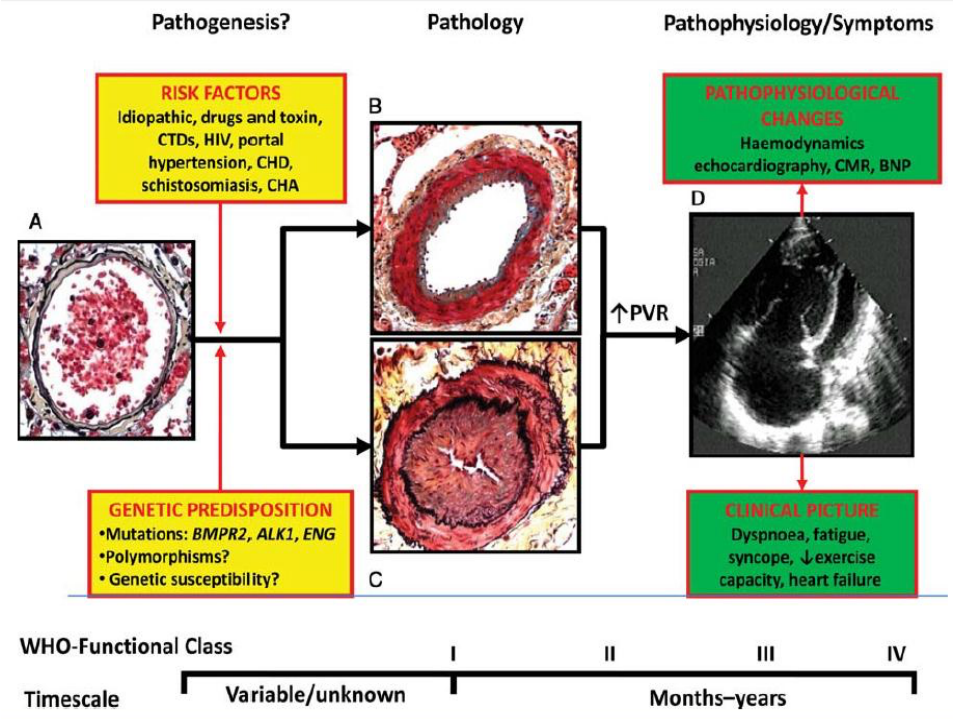

Figure 3. Schematic representation of pathogenesis, pathology, pathophysiology, and symptoms in PAH

(A) Normal small distal pulmonary artery: the thin wall is constituted by a single elastic lamina and a thin layer of smooth muscle cells; a large lumen with red blood cells is also shown. (B) Distal pulmonary artery in pulmonary arterial hypertension: increased thickness of the media due to hypertrophy and hyperplasia of smooth muscle cells and moderate lumen reduction are present. This picture may represent an initial phase of the disease and/or the prevalent changes in patients responding to vasoreactivity tests. (C) Distal pulmonary artery in pulmonary arterial hypertension: increased thickness of the media and also of the intima due to proliferation/migration of myofibroblasts and fibrosis are present. Severe lumen reduction is also shown. This picture may represent an advanced phase of the disease and/or the prevalent changes in patients not responding to vasoreactivity tests. (D) Echocardiographic four-chamber view in pulmonary arterial hypertension: Severe dilatation of the right atrium and ventricle and reduction in size of the left ventricle are shown. ALK1, activin-like kinase-type 1 gene; BNP, brain natriuretic peptide; BMPR2, bone morphogenetic protein receptor type 2 gene; CHA, chronic haemolytic anaemia; CHD, congenital heart disease; CTD, connective tissue diseases; ENG, endoglin gene; HIV, human immunodeficiency virus; CMR, cardiac magnetic resonance; PAH, pulmonary arterial hypertension; PVR, pulmonary vascular resistance; WHO, world health organization.

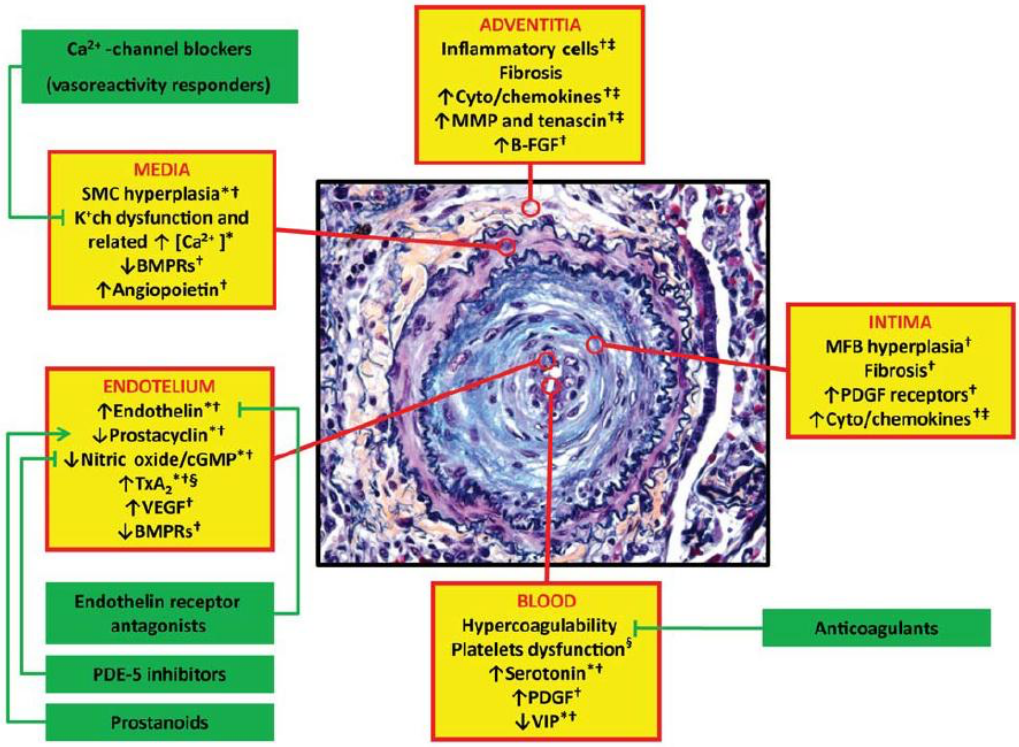

Figure 4. Obstructive remodelling of a small pulmonary artery in pulmonary arterial hypertension (increased thickness of the three vessel layers and severe lumen reduction are shown) and ongoing pathobiological processes in the different layers of the vessel wall (yellow boxes) and in the blood.

Asterisks indicate the potential processes involved. Corrective interactions of the related approved therapeutic interventions are also reported (green boxes). *Vasoconstriction; †Proliferation/migration; ‡Inflammation; §Thrombosis. B-FGF, basic fibroblast growth factor; BMPR, bone morphogenetic protein receptor; [Ca2+], intracellular calcium concentration; K+ch, membrane potassium channels; SMC, smooth muscle cells; MFB, myofibroblasts; MMP, matrix metalloproteases; PDE-5, phosphodiesterase type 5; PDGF, platelet-derived growth factor; TxA2, thromboxane A2; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide.

2.2 Clinical subgroups

PAH includes at least nine clinical subgroups with virtually identical obstructive pathologic changes (*Figure 3* and *4*) in distal pulmonary arteries: idiopathic, heritable, drug and toxin-induced, associated with connective tissue diseases, HIV infection, portal hypertension, congenital heart disease and schistosomiasis.

Idiopathic PAH (IPAH)

Idiopathic PAH describes a sporadic disease with neither a family history of PAH nor an identified risk factor.

Heritable PAH (HPAH)

In 80% of families with multiple cases of pulmonary arterial hypertension (PAH), mutations of the bone morphogenic protein receptor type 2 (BMPR2), a member of the tumour growth factor (TGF)-beta super family, can be identified[19]. In addition, 5% of patients have rare mutations in other genes belonging to the TGF-b super family: activin-like receptor kinase-1 (ALK1)[20], endoglin (ENG)[21], and mothers against decapentaplegic 9 (Smad 9)[22]. Approximately 20% of families have no detectable mutations in currently known disease-associated genes. Recently two new gene mutations have been identified: a mutation in caveolin- 1 (CAV1) which encodes a membrane protein of caveolae, abundant in the endothelial cells of the lung[23] and KCNK3, a gene encoding potassium channel super family K member-3[24]. The identification of these new genes not intimately related to TGF-b signalling may provide new insights into the pathogenesis of PAH.

Drug- and Toxin Induced Pulmonary Hypertension

A number of drugs and toxins are associated with the development of PAH[25 26 27]. The association between exposure to drugs and toxins and PAH is classified as definite or possible, as proposed at the 6th WSPH (*Table 3*). There is a definite association with drugs if data based on outbreaks, epidemiological case-control studies, or large multicenter series are available. A possible association is suggested by multiple case series or cases with drugs with similar mechanisms of action.

Definite	Possible association
Aminorex	Alkylating agents (cyclophosphamide, mitomycin C)
Benfluorex	Amphetamines
Dasatinib	Bosutinib
Dexfenfluramine	Cocaine
Fenfluramine	Diazoxide
Methamphetamines	Direct-acting antiviral agents against hepatitis C virus (sofosbuvir)
Toxic rapeseed oil	Indirubin (Chinese herb Qing-Dai)
	Interferon alpha and beta
	Leflunomide
	L-tryptophan
	Phenylpropanolamine
	Ponatinib
	Selective proteasome inhibitors (carfilzomib)
	Solvents (trichloroethylene)
	St John's Wort

Table 3. Updated risk level of drugs and toxins known to induce PAH

PAH Associated with Connective Tissue Diseases (CTD-PAH)

Pulmonary arterial hypertension may be a pulmonary vascular complication of SSc[28] [29], systemic lupus erythematosus, mixed CTD, and, rarely, dermatomyositis and Sjögren's syndrome.

PAH-CTD is the second most prevalent type of PAH in western countries, after IPAH. Systemic sclerosis, particularly in its limited variant, represents the main cause of PAH-CTD in Europe and the USA[28]. The prevalence of pre-capillary PH in large cohorts of patients with SSc is 5–19%[29].

To notice, in these patients PH may occur in association with ILD and/or HFpEF due to myocardial involvement, so is essential to carefully determine which mechanism is operative in each patient before initiating PAH therapy.

The prognosis for patients with PAH associated with scleroderma remains poor and worse if compared to other PAH subgroups[30]. Recent data suggests that in scleroderma, early diagnosis and early intervention may improve long-term outcome, that is why according to recent guidelines resting echocardiography is recommended as a screening test in asymptomatic patients with SSc. In other

CTDs, PH screening in the absence of suggestive symptoms is not recommended, while echocardiography should be performed in the presence of symptoms.

PAH Associated with HIV Infection (HIV-PAH)

With the availability of highly active antiretroviral therapy (HAART), given in combination with PAH specific therapies[31], the prognosis of PAH-HIV patients has markedly improved while in parallel the incidence of PAH-HIV has declined[32]: this resulted in stable PAH prevalence in HIV during last decades.

Before the era of highly active antiretroviral therapy (HAART) and the development of specific PAH drugs, the prognosis for HIV-PAH was extremely poor, with a mortality rate of 50% at 1 year[33]. The advent of these specific drugs has dramatically improved the prognosis of these patients leading to a better survival than most patients with other forms of PAH[34]. Interestingly, approximately 20% of these patients experience a normalization of hemodynamic parameters after several years of treatment[35].

PAH Associated with Portal Hypertension (POPH)

PAH is found in 2% to 6% of patients with portal hypertension (with or without liver disease) and it's called porto-pulmonary hypertension (POPH)[36 37].
To notice, PoPH is different from hepatopulmonary syndrome (HPS), which is characterised by intrapulmonary vascular dilatation and hypoxaemia, while the two diseases can occur contemporarily/sequentially in patients with portal hypertension[38].
Echocardiography is recommended in patients portal hypertension with signs/symptoms suggestive of PH and as a screening tool in patients evaluated for liver transplantation.

PAH Associated with Congenital Heart Disease in Adults (CHD-PAH)

Nowadays, thanks to progressive improvements in the management of congenital heart diseases (CHD), an increasing number of children affected by CHD survive to adulthood and both the number and the complexity of these patients continue to grow.
It is estimated that approximately 3-7% of adults with CHD will develop PAH, with incidence depending on the underlying lesions and increasing with age and age at defect closure[39], which has an adverse impact on quality of life and outcome[40 41].

There is a well-recognized clinical phenotype of patients with volume and pressure overload (i.e., with large ventricular or arterial shunts) that are at higher risk of developing early PAH than patients with volume overload only (i.e., with atrial shunts). Nevertheless, there are some exceptions, and it can be speculated that a permissive genotype might place some patients with CHD at higher risk of developing PAH.

Last clinical classification of CHD-PAH is shown in *Table 4*.

Other types of PH in association with CHD who do not belong to Group 1 (PAH) are included in different groups of the general clinical classification (i.e., congenital or acquired left heart inflow/outflow obstructive lesions and congenital cardiomyopathies in Group 2).

In addition, some patients with PH associated with CHD are difficult to classify, such as patients with transposition of great arteries and those with PH following atrial redirection surgery or following neonatal arterial switch operation: this strengthens the need to delineate the underlying cardiac anatomy/physiology and severity of PAH/PVR in every single patient.

1. Eisenmenger's syndrome
Includes all large intra- and extra-cardiac defects which begin as systemic-to- pulmonary shunts and progress with time to severe elevation of PVR and to reversal (pulmonary-to-systemic) or bidirectional shunting. Cyanosis, secondary erythrocytosis and multiple organ involvement are usually present. Closing de defect is contraindicated.
2. PAH associated with prevalent systemic-to-pulmonary shunts
- Correctable - Non-correctable Include moderate to large defects. PVR is mildly to moderately increased and systemic-to- pulmonary shunting is still prevalent, whereas cyanosis at rest is not a feature.
3. PAH with small/coincidental heart defects
Marked elevation in PVR in the presence of small cardiac defects (usually ventricular septal defects < 1 cm and atrial septal defects < 2 cm), which themselves do not account for the development of elevated PVR. The clinical picture is very similar to IPAH. Closing the defects in contraindicated.
4. PAH after defect correction
Congenital heart disease is repaired, but PAH either persists immediately after correction or recurs/develops months or years after correction in the absence of significant, postoperative, hemodynamic lesions.

Table 4. Updated Clinical Classification of Pulmonary Arterial Hypertension Associated with Congenital Heart Disease (updated from Simmoneau et.al[6])

PAH Associated with Schistosomiasis

Schistosomiasis-associated PAH (Sch-PAH) is potentially the most prevalent cause of PAH worldwide. Schistosomiasis affects over 200 million people, of whom 10% develop hepatosplenic schistosomiasis: PAH occurs almost exclusively in this population and 5% of patients with hepatosplenic schistosomiasis may develop PAH[42]. Compared with IPAH patients, patients with schistosomiasis-associated PAH have higher CO, lower PVR and better survival[43]; furthermore, registry data suggest that PAH therapies may beneficial in improving survival in patients with Sch-PAH[44].

2.3 Therapy

The therapy for PAH patients has evolved progressively in the last years, increasing in complexity and in evidence for efficacy[45]. The treatment process of PAH patients should be characterised by a complex strategy that includes the initial evaluation of severity and the subsequent response to treatment.
The treatment approach to PAH patients may be divided into three steps [46]:

- The initial approach includes general measures (physical activity and supervised rehabilitation, pregnancy, birth control and post-menopausal hormonal therapy, elective surgery, infection prevention, psychosocial support, adherence to treatments, genetic counselling and travel), supportive therapy (diuretics, O2), referral to expert centres and acute vasoreactivity testing for the indication of chronic CCB therapy.
- The second step includes initial therapy with high-dose CCB in vasoreactive patients or drugs approved for PAH in non-vasoreactive patients according to the prognostic risk (*Figure 5*) of the patient.
- The third part is related to the response to the initial treatment strategy: in the case of an inadequate response, the association of drugs and lung transplantation are proposed.

Specific drug therapy

Currently available drugs for treatment of PAH act on three different pathways responsible for pathogenesis of the disease: the endothelin pathway, the nitric oxide pathway and the prostacyclin pathway.

- *Calcium channel blockers*: pulmonary vasoreactivity testing for identification of patients suitable for high-dose calcium channel blocker (CCB) treatment is recommended for patients affected by IPAH, HPAH or drug-induced PAH and it should be performed at the time of RHC. In all other forms of PAH and PH the results can be misleading and responders are rare. However, it has been increasingly recognised that only a small number of patients with IPAH who demonstrate a favourable response to acute vasodilator testing at the time of RHC do well with CCBs[47 48], that is why they should be followed closely for safety and efficacy, with a complete reassessment after 3-6 months of therapy. Patients with a satisfactory chronic response present with WHO FC I/II and marked haemodynamic improvement while on CCB therapy, otherwise additional PAH therapy should be added.

  CCBs that have been predominantly used in reported studies are Nifedipine, Diltiazem and Amlodipine, with particular emphasis on Nifedipine and Diltiazem. The choice of CCB is based on patient's heart rate at baseline, with a relative bradycardia favouring Nifedipine and Amlodipine and a relative tachycardia favouring Diltiazem. The daily doses of these drugs that have shown efficacy in IPAH are relatively high (120 – 240 mg for nifedipine, 240–720 mg for diltiazem and up to 20 mg for amlodipine) and they must be reached progressively. Main limiting factors for dose increase are usually systemic hypotension and lower limb peripheral oedema.

- *Endothelin receptor antagonists*: a prominent role for the endothelin system in the pathogenesis of PAH, has been largely demonstrated and activation of the endothelin system has been documented in both plasma and lung tissue of PAH patients[11 49 50].

  Endothelin-1 promotes vasoconstriction and proliferation by binding two different receptor isoforms in the pulmonary vascular smooth muscle cells, endothelin receptors type A and B[51]. Bosentan was the first molecule of its class to be synthetized and it showed improvements in exercise capacity, functional class, haemodynamic, echocardiographic and Doppler variables and time to clinical worsening[52 53]. Increases in hepatic aminotransferases occurred in approximately 10% of patients (found to be dose dependent and reversible after dose

reduction or discontinuation), so that liver function testing should be performed monthly in patients receiving Bosentan[54].

Ambrisentan has demonstrated efficacy on symptoms, exercise capacity, haemodynamics and time to clinical worsening[55] with a significantly lower incidence of abnormal liver function. Finally, Macitentan has been found to increase exercise capacity and reduce a composite endpoint of clinical worsening[56]. While no liver toxicity was shown, reduction in blood haemoglobin ≤8 g/dl was observed in 4.3% of patients receiving 10 mg of Macitentan[56].

- *Phosphodiesterase type 5 inhibitors and guanylate cyclase stimulators*: inhibition of the cyclic guanosine monophosphate (cGMP) degrading enzyme phosphodiesterase type 5 results in vasodilation through the NO/cGMP pathway at sites expressing this enzyme, including the pulmonary vasculature[57]. In addition, PDE-5is exert antiproliferative effects[58 59]. Main drugs approved for PAH treatment are Sildenafil (approved dose 20 mg t.i.d) and Tadalafil (approved dose 40 mg o.d.), both showing favourable results on symptoms, exercise capacity, haemodynamic and time to clinical worsening in RCTs[60 61 62], with a similar safety profile (most side effects of are mild to moderate and related to vasodilation, as headache, flushing, epistaxis).

  While these molecules enhance the NO – cGMP pathway, slowing cGMP degradation, sGC stimulators enhance cGMP production[63] and showed antiproliferative and anti-remodelling properties in various animal models.

  Riociguat has shown favourable results on exercise capacity, haemodynamic, WHO-FC and time to clinical worsening[64]. Important to notice, the combination of Riociguat and PDE-5i is contraindicated due to hypotension and other relevant side effects detected in the open-label phase of an RCT study[65].

- *Prostacyclin analogues and prostacyclin receptor agonists*: dysregulation of the prostacyclin metabolic pathway has been shown in patients with PAH as assessed by a reduction of prostacyclin synthase expression in the pulmonary arteries and of prostacyclin urinary metabolites[66].

  Prostacyclin is manly produced by endothelial cells, is potent vasodilator of all vascular beds, an inhibitor of platelet aggregation and has both cytoprotective and antiproliferative activities[67]. The clinical use of prostacyclin in patients with PAH has been extended by the synthesis of stable analogues having qualitatively similar pharmacodynamic effects. Epoprostenol is a synthetic prostacyclin with short half-life (3 – 5 minutes), stable at room

temperature for only 8 hours, that requires cooling and continuous administration by means of an infusion pump and a permanent tunnelled catheter. A thermos-stable formulation is available to maintain stability up to 48 h[68]. The efficacy of continuous i.v. administration of Epoprostenol has been tested in three unblinded RCTs[69 70 71]: Epoprostenol improves symptoms, exercise capacity and is the only treatment shown to reduce mortality in IPAH in a single RCT study[69]. The meta-analysis for total mortality of the three Epoprostenol RCTs has shown a risk reduction for mortality of about 70% and long-term persistence of efficacy has also been shown[72 73] in IPAH as well as in other APAH conditions[74 33 75]. Treatment with Epoprostenol is initiated at a dose of 2 – 4 ng/kg/min, with doses increasing at a rate limited by side effects (flushing, headache, diarrhoea, leg pain). The optimal dose varies between individual patients, ranging in the majority between 20 and 40 ng/kg/min[72 73]. Serious adverse events related to the delivery system include pump malfunction, local site infection, catheter obstruction and sepsis. Abrupt interruption of the Epoprostenol infusion should be avoided, because in some patients this may lead to a PH rebound with symptomatic deterioration and even death.

Iloprost is a chemically stable prostacyclin analogue available for i.v., oral or aerosol administration. Inhaled Iloprost has been evaluated in one RCT[76] and showed an increase in exercise capacity and improvement in symptoms, PVR and clinical events compared to placebo. Continuous i.v. administration of Iloprost appeared to be as effective as Epoprostenol in a small series of patients with PAH and CTEPH[77], while the effects of oral Iloprost have not been assessed in PAH.

Beraprost is the first chemically active and orally stable prostacyclin analogue. Two RCT[78 79] have shown an improvement in exercise capacity that persists up to 3 – 6 months, without haemodynamic improvements or long-term outcome benefits.

Treprostinil is an Epoprostenol analogue, available for s.c. (by a micro-infusion pump and a small subcutaneous catheter), i.v., inhaled, and oral administration.

The effects of s.c. Treprostinil in PAH were assessed in an RCT and showed improvements in exercise capacity, haemodynamic and symptoms[80]. Treatment with subcutaneous Treprostinil is initiated at a dose of 1 – 2 ng/kg/ min, with doses increasing at a rate limited by side effects (local site pain, flushing, headache). The optimal dose varies between individual patients, ranging in the majority between 20 and 80 ng/kg/min. An RCT with i.v. Treprostinil was performed, but the enrolment was closed because of safety considerations after 45 (36%) of the planned 126 patients had been randomized[81] and data generated from 31 (25%) survivors are not considered reliable.

Inhaled Treprostinil improved the 6MWD, NT-proBNP, and quality of life measures in patients with PAH on background therapy with either Bosentan or Sildenafil[82], but it is not approved in Europe. Similarly, oral Treprostinil has been evaluated in two RCTs of patients with PAH on background therapy with Bosentan and/or Sildenafil and in both trials, the primary endpoint—6MWD—did not reach statistical significance[83 84] and it was not approved in Europe too.

Selexipag is an orally available, selective prostacyclin IP receptor agonist. In a pilot RCT in PAH patients (receiving stable ERA and/or PDE-5i therapy), Selexipag reduced PVR after 17 weeks[85]. An event-driven phase 3 RCT that enrolled 1156[86] patients has shown that Selexipag alone or on top of mono or double therapy with ERAs and/or PDE-5i was able to reduce by 40% a composite morbidity and mortality endpoint. Most common side effects were headache, diarrhoea, nausea and jaw pain.

2.4 Risk assessment and treatment strategies

The initial treatment of patients with PAH should be based on a comprehensive, multiparameter risk assessment, considering disease type and severity and comorbidities.

To notice, the following consideration predominantly apply to non-vasoreactive patients affected by IAPH/HAPAH or PAH-CTD, without cardiopulmonary comorbidities (as they were underrepresented in clinical trials).

Firstly, PAH patients require risk stratification at baseline and regular follow-up including assessment of patient concordance with therapy.

Nowadays we have evidences that patients achieving a low risk status have a much superior long term survival as compared with patients with intermediate or high risk status[87 88 89], so that achieving and maintaining a low risk profile is a key objective in managing patients with PAH.

In the 2015 ESC/ERS Guidelines for diagnosis and treatment of PH, risk assessment was based on a parametric approach using three strata model to classify patients at low, intermediate or high risk of death - respectively with an estimated 1 year mortality of <5%, 5-10% and >10%[90].

The main limitation of this risk assessment tool was that more than half of patients were classified as intermediate risk[88 91 92], so that several attempts was made to sub-stratify patients in the intermediate risk group.

In particular, two recent registry studies evaluated a four strata risk-assessment tool based on refined cut off levels for WHO-FC, 6MWD and NT-proBNP[93 94]. Together, all dedicated studies included more than 4000 patients and showed that a four strata model was more sensitive to changes in risk

from baseline to follow-up and these changes were associated in changes in the long-term mortality risk so it can be considered a useful tool in guiding therapeutic decision making.

According to 2022 ESC/ERS guidelines, the use of three strata model taking into account as many factors as possible is still recommended for initial risk stratification at diagnosis (*Figure 5*), while during follow up the four strata model is suggested as a basic risk stratification tool even if additional variables should be considered as needed, especially right hearth imaging and haemodynamics (*Figure 6*).

Determinants of prognosis (estimated 1-year mortality)	Low risk (<5%)	Intermediate risk (5–20%)	High risk (>20%)
Clinical observations and modifiable variables			
Signs of right HF	Absent	Absent	Present
Progression of symptoms and clinical manifestations	No	Slow	Rapid
Syncope	No	Occasional syncope[a]	Repeated syncope[b]
WHO-FC	I, II	III	IV
6MWD[c]	>440 m	165–440 m	<165 m
CPET	Peak VO_2 >15 mL/min/kg (>65% pred.) VE/VCO_2 slope <36	Peak VO_2 11–15 mL/min/kg (35–65% pred.) VE/VCO_2 slope 36–44	Peak VO_2 <11 mL/min/kg (<35% pred.) VE/VCO_2 slope >44
Biomarkers: BNP or NT-proBNP[d]	BNP <50 ng/L NT-proBNP <300 ng/L	BNP 50–800 ng/L NT-proBNP 300–1100 ng/L	BNP >800 ng/L NT-proBNP >1100 ng/L
Echocardiography	RA area <18 cm^2 TAPSE/sPAP >0.32 mm/mmHg No pericardial effusion	RA area 18–26 cm^2 TAPSE/sPAP 0.19–0.32 mm/mmHg Minimal pericardial effusion	RA area >26 cm^2 TAPSE/sPAP <0.19 mm/mmHg Moderate or large pericardial effusion
cMRI[e]	RVEF >54% SVI >40 mL/m^2 RVESVI <42 mL/m^2	RVEF 37–54% SVI 26–40 mL/m^2 RVESVI 42–54 mL/m^2	RVEF <37% SVI <26 mL/m^2 RVESVI >54 mL/m^2
Haemodynamics	RAP <8 mmHg CI ≥2.5 L/min/m^2 SVI >38 mL/m^2 SvO_2 >65%	RAP 8–14 mmHg CI 2.0–2.4 L/min/m^2 SVI 31–38 mL/m^2 SvO_2 60–65%	RAP >14 mmHg CI <2.0 L/min/m^2 SVI <31 mL/m^2 SvO_2 <60%

6MWD, 6-minute walking distance; BNP, brain natriuretic peptide; CI, cardiac index; cMRI, cardiac magnetic resonance imaging; CPET, cardiopulmonary exercise testing; HF, heart failure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAH, pulmonary arterial hypertension; pred., predicted; RA, right atrium; RAP, right atrial pressure; sPAP, systolic pulmonary arterial pressure; SvO_2, mixed venous oxygen saturation; RVESVI, right ventricular end-systolic volume index; RVEF, right ventricular ejection fraction; SVI, stroke volume index; TAPSE, tricuspid annular plane systolic excursion; VE/VCO_2, ventilatory equivalents for carbon dioxide; VO_2, oxygen uptake; WHO-FC, World Health Organization functional class.

[a]Occasional syncope during heavy exercise or occasional orthostatic syncope in a stable patient.

[b]Repeated episodes of syncope even with little or regular physical activity.

[c]Observe that 6MWD is dependent upon age, height, and burden of comorbidities.

[d]To harmonize with the four-strata model shown in *Table 18*, the BNP and NT-proBNP cut-off levels have been updated from the 2015 version based on data from the REVEAL registry, acknowledging that the European validation studies have used the original cut-off levels.[274,292,293,295,296,302]

[e]cMRI parameters adapted from *Section 6.2.2.2*.

Figure 5. Comprehensive risk assessment in pulmonary arterial hypertension (three strata model)

Determinants of prognosis	Low risk	Intermediate–low risk	Intermediate–high risk	High risk
Points assigned	1	2	3	4
WHO-FC	I or II[a]	-	III	IV
6MWD, m	>440	320–440	165–319	<165
BNP or NT-proBNP,[a] ng/L	<50 <300	50–199 300–649	200–800 650–1100	>800 >1100

6MWD, 6-minute walking distance; BNP, brain natriuretic peptide; NT-proBNP, N-terminal pro-brain natriuretic peptide; WHO-FC, World Health Organization functional class.
Risk is calculated by dividing the sum of all grades by the number of variables and rounding to the next integer.
[a]WHO-FC I and II are assigned 1 point as both are associated with good long-term survival.

Figure 6. Variables used to calculate the simplified four-strata risk-assessment tool

For patients presenting at low or intermediate risk, initial combination therapy with ERA and PDEi5 is recommended. This approach was assessed in the AMBITION trial, which included patients with IPAH/HAPAH/DPAH or PAH-CTD, who were randomised to Ambrisentan plus Tadalafil or to initial monotherapy with either drugs[95]. The primary endpoint - time to first clinical failure event – was significant reduced in the combination therapy group, as there were significant improvements in 6MWD and NT-proBNP.

Then, in the TRITON study, treatment naïve PAH patients (predominantly IAPH/HAPAH/DPSH or PAH-CTD patients) were assigned to receive initial dual combination therapy with Macitentan and Tadalafil or initial triple combination therapy with Macitentan, Tadalafil and Selexipag[96]. The study failed to demonstrate a benefit of triple vs. oral double combination therapy in terms of reduction of PVR at week 26 (primary endpoint) but confirmed substantial improvements in haemodynamics and exercise capacity with initial ERA/PDE5i combination therapy.

Based on the available evidence, initial dual combination therapy with ERA and PDE5i is recommended for newly diagnosed patients presenting at low or intermediate risk, while initial oral triple combination therapy is not recommended given the current lack of evidence. In patients at high risk and in patients at intermediate risk presenting with severe haemodynamic impairment, initial triple combination therapy including an i.v./s.c. prostacyclin analogue should be considered[97 98]. Evidence for this approach is limited to case series but there is consensus that this strategy has the highest likelihood of success, especially thanks to registry data from France showing that initial triple combination therapy including an i.v./s.c. prostacyclin analogue was associated with better long term survival than dual combination therapy[99].

3. Clinical study

3.1 Background

Pulmonary arterial hypertension (PAH) is characterised by pulmonary vascular changes leading to elevated pulmonary artery pressure, right heart failure - dyspnoea, reduction in exercise tolerance, and ultimately death.
Goals of therapy are relieving of symptoms, improving exercise capacity and quality of life, arresting disease progression and reducing mortality. People affected by PAH often respond to disease specific modifying therapies, including calcium channel blockers, endothelin receptor antagonists, phosphodiesterase-5 inhibitors and prostacyclin analogues.

Prostacyclin is endogenously synthesised by endothelial cells using the cyclo-oxygenase arachidonic pathway and exerts vasodilatory, antithrombotic and antiproliferative effects that are essential for endothelial function[99]. The principal target of prostacyclin is the IP G protein coupled receptor in the smooth muscle of arterioles: its activation triggers intracellular cyclic adenosine monophosphate formation, activating protein kinase A, which mediates vasodilation of the pulmonary arteries, inhibition of platelet aggregation and relaxation of the smooth muscles[100]. Disequilibrium between vasodilating mediators, such as a reduction in the normal release of prostacyclin and increased release of vasoconstricting mediators, such as thromboxane A2, plays a causative role in PAH[101,102].

Currently there are three prostacyclin analogues available for PAH therapy: Epoprostenol, Iloprost and Treprostinil. The key attributes of synthetic prostacyclin agents are prostacyclin's short half-life at room temperature (minutes) and that they mainly only exert local effects[99].
The first synthetic agent (Epoprostenol) demonstrated significant efficacy as a therapeutic agent in the improvement of haemodynamic parameters, exercise capacity, and mortality[75]. However, it is not without drawbacks: its short half-life requires continuous intravenous infusion, via a central venous catheter and continuous pump. This entails the need of central line placement which potentially introduces the risk of central line-associated blood stream infection[103]. Initial preparations were required to be refrigerated or kept on ice, however newer preparations have a more stable half-life of 24 hours[84].
Iloprost is a prostacyclin analogue that is most frequently used via inhalation. It has a slightly longer half-life of 20 to 30 minutes but still requires 5 to 10 inhalation doses throughout the day. Iloprost has been proved effective as monotherapy reducing clinical event and PVR and improving exercise capacity and symptoms[85].

Treprostinil has a much more stable half-life (four hours) and can be administrated at much lower infusion rates via a subcutaneous or intravenous pump[104]. While effective in terms of 6MWD, the doses achieved in the pivotal trial were low because of the frequent occurrence of infusion site pain[105]. As centres have become experienced, escalation to more effective doses has been shown to be feasible. However, the side effect burden remains high, and it can take an average of 6 months to achieve a stable effective dose[106]. Furthermore, Treprostinil is metabolised by liver cytochrome P450 (CYP) and its metabolites are renally excreted. Hence, clearance may be affected by hepatic and renal impairment. In addition, cumulative effects of Treprostinil can occur if used with antihypertensives or anticoagulants[102].

For all prostacyclin agents, dose titration is individualised according to the individual patient. A characteristic pattern of adverse effects - hypotension, flushing, diarrhoea, and muscle pains[76,102] - may limit dose escalation. Indeed, the dose is often up titrated until side effects are evident making patient and investigator concealment (blinding) somewhat problematic in clinical trials. Both delivery method and the drug itself are expensive. Furthermore, therapy must be continuous, as abrupt withdrawal may precipitate rebound pulmonary hypertension, which can be fatal.

Selexipag is a selective IP prostacyclin receptor agonist that is structurally distinct from prostacyclin but exerts similar effects: it is rapidly hydrolysed to a long-acting metabolite that binds to IP receptors, resulting in vasodilation, inhibition of platelet aggregation, and anti-inflammatory effects[106].

In a study published by Simmoneau and colleagues[91] Selexipag showed significant hemodynamic benefit at 17 weeks and an event-driven, phase 3 RCT[86] showed that Selexipag alone or on top of mono or double therapy with an ERA and/or a PDE5i reduced the relative risk of composite morbidity/mortality events by 40%. The most common side effects were headache, diarrhea, nausea, and jaw pain but this has not sorted the issue of tolerability seen with prostanoids, though the pharmacodynamic data may suggest that more frequent drug dosing could help smooth the dose–response curve and lessen side effect burden. Therefore, Selexipag offers a potentially more stable drug, with less complex administration and titration[86].

A recent review demonstrated clinical and statistical benefit for the use of intravenous prostacyclin compared to control in terms of improved functional class, six-minute walk distance (6MWD), mortality, symptoms scores, and cardiopulmonary haemodynamics, but at a cost of increased risk of adverse events. Furthermore, in clinical trials, significant mortality benefits have been demonstrated using intravenous preparations and not in subcutaneous, oral, or inhaled preparations. Finally, Selexipag when compared to placebo in large, long-term trials was associated with less clinical worsening but increased adverse events, while the effect on other clinical outcomes is less certain[100].

3.2 Objectives

The purpose of this study was to reassess our experience on the use of drugs that interact on the pathobiological line of prostacyclin, so we compared the efficacy of intravenous Epoprostenol, subcutaneous Treprostinil and oral Selexipag for the treatment of PAH.
Particularly, we evaluated their effects on symptoms, exercise capacity, haemodynamic response and survival.

3.3 Materials and methods

Patients

This study was conducted in the centre dedicated to study and treatment of pulmonary arterial hypertension of the Bologna Sant.Orsola University Hospital.
We retrospectively included all patients, referred to our centre from February 1995 to December 2021, who received therapy with i.v. Epoprostenol, s.c. Treprostinil or oral Selexipag.

Assessment

Non-invasive and invasive parameters were collected at baseline and at first follow-up, including the determination of WHO functional class, 6MWD, right atrial pressure (RAP), mean pulmonary arterial pressure (mPAP), pulmonary wedge pressure (PAWP), cardiac index (CI), pulmonary vascular resistance (PVR) and venous oxygen saturation (SvO2).
Baseline was defined as the time of RHC preceding prostanoid therapy starting. First follow-up data were collected after 3–4 months of prostanoid treatment. All patients were treated according to 2015 ESC/ERS guidelines for pulmonary arterial hypertension.

Statistical analysis

6MWD and haemodynamic parameters absolute changes and percentage changes after 3-4 months of therapy were expressed as median and interquartile ranges.
Changes in NYHA functional class were assessed using the McNemar test, while changes in 6MWD and haemodynamic parameters were analysed using the Wilcoxon and Mann-Whitney test.
Comparison of absolute changes and percentage changes of 6MWD and haemodynamic parameters after treatment with the three different drugs were analysed using the Dunn test with Bonferroni correction.

3.4 Results

Firstly, a total 321 patients were considered suitable for the study. Among them, 20 patients were excluded because of lack of hemodynamic parameters at the basal evaluation (15 patients started therapy in another centre and for 5 patients we chose a non-invasive follow-up strategy), so that a total of 301 patients were included into the study: 152 of them were treated with Epoprostenol, 105 with Treprostinil and 44 with Selexipag.

Invasive follow-up data, assessed after 3-4 months of therapy, were available in 238 patients: 105 in the Epoprostenol group, 97 in the Treprostinil group 36 in the Selexipag group; in particular, in the Epoprostenol group 29 patients died, 3 underwent bi-pulmonary transplantation, 1 was lost at follow-up, 1 patient stopped therapy because of adverse effects, in 12 patients we decided not to repeat RHC due to severe comorbidities and in 1 patient it was planned after the end of our study; in the Treprostinil group, 5 patients died, 1 patient underwent bi-pulmonary transplantation, 1 patient was lost at follow-up, and in 1 patient RHC was not repeated due to severe comorbidities; in the Selexipag group, 1 patient died, 3 were lost at follow-up, 1 stopped taking the drug because of adverse reactions, in 1 patient RHC was not repeated due to severe comorbidities and 2 patients were not been seen in follow-up consultation yet when the study was concluded.

Baseline demographic, functional and haemodynamic characteristics of patients are described in *Table 5* and *6*.

	Epoprostenol	**Treprostinil**	**Selexipag**	**p-value**
n°	152	105	44	
Age	45 (32÷56) ∬	44 (37÷52) *	51 (36÷66) *∬	**0.022**
Male gender, n (%)	60 (39.5)+	26 (25)+	13 (30)	**0.042**
NYHA III/IV, n (%)	121 (80)∬+	71 (68)+	20 (45.5)∬	**0.001**
6MWT (m)	300 (216÷400)∬+	452 (331÷507)+	487 (375÷570)∬	**< 0.001**

Aetiology, n (%) **I/H/D** **CTD** **CHD** **Po-HIV**	ǂ 72 (47) 40 (26) 19 (12.5) 23 (8)	ǂ 65 (62) 14 (13) 25 (24) 1 (1)	 27 (58) 8 (18) 8 (18) 1 (2)	 **0.001**
Dose after 3-4 months	17 (14÷22)	45 (28÷54)	2800 (2000÷3200)	**/**
Dose at the end of the study	21 (16÷30)	56 (47÷67)	2800 (2000÷3200)	**/**
Low/intermediate/high risk n (%)	10 (7)/80 (53)/62 (41)ʃʃǂ	14 (13)/ 81 (77)/ 10 (9.5)ǂ	14 (32)/ 28 (64)/ 2 (5)ʃʃ	**0.003**

*ʃʃǂ = $p<0.05$ between respective pair

Table 5. Baseline demographic clinical and functional characteristics

Patients treated with Selexipag were older than patient on Epoprostenol and Treprostinil therapy, while there were no significant differences between the latter two.

Furthermore, we observed a minority of male gender in all groups (according with the epidemiology of PAH), particularly in patient treated with Selexipag and Treprostinil.

Considering the percentages of patients in NYHA functional class III or IV, it emerged that patients treated with Epoprostenol were more compromised at baseline, with a statistically significant difference in respect to those treated with Selexipag and, to a lesser extent, to those treated with Treprostinil.

Similarly, patients treated with Epoprostenol had significantly lower exercise capacity at baseline 6MWT as compared to patients treated with Selexipag and Treprostinil.

Comparing exercise capacity and NYHA functional class, patients treated with Treprostinil appeared to be slightly more compromised at baseline than those treated with Selexipag even though statistical significance was not reached.

Concerning aetiology of PAH, main significant differences were found between patients treated with Epoprostenol and those treated with Treprostinil, with higher percentage of patients affected by CTD and Po-HIV in the Epoprostenol group (considering that in scleroderma patients the use of subcutaneous infusion would be difficult and that Po-HIV patients could benefit from strong treatment before transplantation) and lower percentage of I/H/D and CHD (in this latter group the use of transvenous catheter would increase cardioembolic risk). No significant difference was found between patients treated with Treprostinil and Selexipag.

Concerning posology, for all prostacyclin agents dose titration is needed during follow-up to reach the maximum tolerated dose. At the end of our study, most patients treated with Selexipag received

the maximum approved dose; for Treprostinil and Epoprostenol a dosage like those reports in literature was reached, respectively of 40 and 20 ng/kg/min.

Finally, we observed that most patients treated with Selexipag had a low risk profile, most patients treated with Treprostinil had an intermediate risk profile and patients treated with Epoprostenol an high-risk profile.

To notice, between patients treated with Selexipag, 2 had a high-risk profile - the first patient was included in the TRITON study while for the second Epoprostenol would have been difficult to manage. Conversely, in Treprostinil and Epoprostenol groups, respectively 14 and 10 patients with low risk profile were treated with parenteral prostacyclin analogues: 8 patients in the Epoprostenol group because at that time there were no therapeutic alternatives and 2 patients because of hemodynamic deterioration; in the Treprostinil group, therapy was started in 3 patients when there were no alternative drugs available and for 11 patients Treprostinil was added because of hemodynamic worsening.

	Epoprostenol	**Treprostinil**	**Selexipag**	**p-value**
n°	152	105	44	
RAP (mmHg)	12 (9 ÷ 15) ∬+	9 (7 ÷ 11) *+	7 (5 ÷ 9) *∬	**<0.001**
mPAP (mmHg)	60 (53 ÷ 73) ∬	62 (53 ÷ 72) *	52.5 (44.5 ÷ 64) *∬	**0.001**
PAWP (mmHg)	8 (6 ÷ 11) +	10 (8 ÷ 12) +	9 (8 ÷ 11)	**0.005**
mBP (mmHg)	82 (74.5 ÷ 89.5)	83 (78 ÷ 89)	85 (75 ÷ 95)	0.398
CI (l/min/m2)	2 (1.5 ÷ 2.5) ∬+	2.3 (2 ÷ 2.6) +	2.5 (2.1 ÷ 2.75) ∬	**<0.001**
PVR (W.U.)	18 (13 ÷ 23.5)+	13 (11 ÷ 16) *+∬	10 (8 ÷ 13) *∬	**<0.001**
SVR (W.U.)	22 (17 ÷ 28) ∬+	19 (16 ÷ 22) +	19 (16 ÷ 23)∬	**0.001**
Syst O2 Sat (%)	94 (90 ÷ 96) ∬	94 (91 ÷ 97) *	96 (94 ÷ 97) *∬	**<0.001**
SvO2 (%)	54 (47 ÷ 62) ∬+	64 (58 ÷ 69) +	65 (60 ÷ 70) ∬	**<0.001**

0*∬+ = p<0.05 between respective pair

Table 6. Baseline hemodynamic characteristics

At baseline patients treated with Epoprostenol were significantly more compromised in comparison to patients in the two other groups: RAP and PVR were significantly higher than in patients treated with Selexipag and Treprostinil, while CI and SvO2 were significantly lower.

The comparison between patients treated with Selexipag and Treprostinil showed that the latter were slightly more compromised, presenting higher RAP, mPAP and PVR and lower IC and SvO2.

Subsequently, we compared functional and hemodynamic parameters at baseline and after 3-4 months of therapy (*Table 7, 8, 9*).

Epoprostenol, n 105	Baseline	After 3-4 months of therapy	p-value
Low/intermediate/high risk, n (%)	10 (7)/ 80 (53)/ 62 (41)	30 (29)/ 58 (55)/ 17 (16)	**<0.001**
NYHA III/IV, n (%)	121 (80)	48 (44.5)	**<0.001**
6MWT (m)	311 (216 ÷ 417)	411 (357 ÷ 477)	**<0.001**
RAP (mmHg)	11 (9 ÷ 14)	10 (6 ÷ 13)	**0.021**
mPAP (mmHg)	60 (53 ÷ 72)	55 (49 ÷ 63)	**<0.001**
PAWP (mmHg)	8 (7 ÷ 10)	9 (7 ÷ 11)	**0.004**
mBP (mmHg)	83 (76 ÷ 89)	80 (73 ÷ 85)	**<0.001**
CI (l/min/m2)	2 (1.6 ÷ 2.5)	2.7 (2.3 ÷ 3.5)	**<0.001**
PVR (W.U.)	17 (13 ÷ 22.5)	11 (8 ÷ 15)	**<0.001**
SVR (W.U.)	22 (17 ÷ 28)	15 (12 ÷ 19)	**<0.001**
Syst O2 Sat (%)	94 (92 ÷ 96)	94 (92 ÷ 96)	0.135
SvO2 (%)	56 (49 ÷ 62)	64 (57 ÷ 70)	**<0.001**

Table 7. Comparison between clinical, functional and hemodynamic characteristics at baseline and after 3-4 months of treatment with Epoprostenol

Patients treated with Epoprostenol, after 3-4 months of therapy showed relevant clinical and functional improvements, with statistically significant reduction in risk class, NYHA functional class and improvements in exercise capacity at 6MWT.

Furthermore, all mean hemodynamic parameters were improved too: we observed significant increase in CI and SvO2 and significant reduction of mPAP, mBP, PVR, SVR and RAP.

Treprostinil, n 97	Baseline	After 3-4 months of therapy	p-value
Low/intermediate/high risk, n (%)	14 (13)/ 81 (77)/ 10 (9.5)	35 (36)/ 56 (58) / 6 (6)	**<0.001**
NYHA III/IV, n (%)	71 (68)	44 (45)	**<0.001**
6MWT (m)	425 (331 ÷ 508)	447 (384 ÷ 549)	**<0.001**
RAP (mmHg)	9 (7 ÷ 11)	7 (5 ÷ 10)	**0.005**
mPAP (mmHg)	62 (53 ÷ 72)	57 (50 ÷ 68)	**<0.001**

PAWP (mmHg)	10 (8 ÷ 11)	10 (8 ÷ 11)	0.817
mBP (mmHg)	83 (78 ÷ 89)	79 (73 ÷ 85)	**<0.001**
CI (l/min/m2)	2.3 (2 ÷ 2.6)	2.8 (2.4 ÷ 3.2)	**<0.001**
PVR (W.U.)	13 (11 ÷ 16)	10 (8 ÷ 13)	**<0.001**
SVR (W.U.)	19 (17 ÷ 22)	16 (13 ÷ 18)	**<0.001**
Syst O2 Sat (%)	94 (91 ÷ 97)	94 (90 ÷ 96)	0.408
SvO2 (%)	64 (57 ÷ 69)	68 (63 ÷ 73)	**<0.001**

Table 8. Comparison between clinical, functional and hemodynamic characteristics at baseline and after 3-4 months of treatment with Treprostinil.

Similarly, after 3-4 months of therapy, patients treated with Treprostinil showed relevant functional improvement with statistically significant reduction of patients in intermediate and high class of risk and increment of patient in low-risk class (although many patients remain in at intermediate risk). In the same way, we observed progresses in NYHA functional class (with statistically significant reduction of patients in NYHA functional class III/IV) and improved exercise capacity at 6MWT. All mean hemodynamic parameters ameliorated too, with significant reduction in RAP, mPAP, mBP, PVR and SVR and significant increase in CI and SvO2.

Selexipag, n 36	**Baseline**	**After 3-4 months of therapy**	**p-value**
Low/intermediate/high risk, n (%)	14 (32)/ 28 (64)/ 2 (5)	22 (61)/ 14 (39)/ 0 (0)	**0.001**
NYHA III/IV, n (%)	20 (45.5)	12 (32)	0.059
6MWT (m)	487 (375 ÷ 570)	540 (440 ÷ 608)	**0.020**
RAP (mmHg)	7 (5 ÷ 9)	5.5 (4 ÷ 9)	0.294
mPAP (mmHg)	52.5 (44.5 ÷ 64)	46 (40 ÷ 52)	**<0.001**
PAWP (mmHg)	10 (8 ÷ 11.5)	9 (7.5 ÷ 10.5)	0.143
mBP (mmHg)	84.5 (74 ÷ 92)	78.5 (71 ÷ 87.5)	**0.006**
CI (l/min/m2)	2.4 (2.1 ÷ 2.7)	2.9 (2.5 ÷ 3.6)	**<0.001**
PVR (W.U.)	10.5 (8 ÷ 13)	7 (5 ÷ 10)	**<0.001**
SVR (W.U.)	18.5 (16 ÷ 23)	15 (11 ÷ 20)	**<0.001**
Syst O2 Sat (%)	96 (94 ÷ 98)	95 (93 ÷ 96)	**0.015**
SvO2 (%)	65 (56 ÷ 70)	68 (62.5 ÷ 73.5)	**0.027**

Table 9. Comparison between clinical, functional and hemodynamic characteristics at baseline and after 3-4 months of treatment with Selexipag

Lastly, for patients treated with Selexipag, the risk stratification after 3-4 months of therapy showed a reduction of patient at higher/intermediate risk (with no patient in high-risk category) with an increased number of patients at lower risk.

In patient treated with Selexipag we found no statistical differences between NYHA functional class before and after therapy (even if we can observe a trend toward reduction of patients in NYHA III/IV and a small increase in walking distance), while we observed statistically significant improvements of functional capacity at 6MWT.

Regarding hemodynamic data, we observed significant increases in CI, SvO2 and reduction in PVR, mPAP, mBP and SVR after 3-6 months of therapy; RAP was reduced too without however reaching statistical significance.

Afterwards, we compared the mean functional and hemodynamic variations, between baseline and the first re-evaluation after 3-4 months of therapy, between the three groups (*Table 10*).

	Epoprostenol	**Treprostinil**	**Selexipag**	**p-value**
n	105	97	36	
Abs. Delta 6MWT (m)	91 (34 ÷ 160)* +	19 (-7 ÷ 55) +	12 (-10 ÷ 57)*	**<0.001**
Abs. Delta RAP (mmHg)	-1 (-5 ÷ 2)	-1 (-3 ÷ 1)	0 (-4 ÷ 2)	0.752
Abs. Delta mPAP (mmHg)	- 6 (- 12 ÷ -2)	-4 (-10 ÷ 0)	-6.5 (-14 ÷ 0)	0.281
Abs. Delta CI (l/min/m2)	0.7 (0.4 ÷ 1.1)* +	0.5 (0.1 ÷ 0.8) +	0.3 (0.1 ÷ 1)*	**0.001**
Abs. Delta PVR (WU)	-6 (-10 ÷ -2)* +	-3 (-5 ÷ -1) +	-2 (-4 ÷ -1)*	**<0.001**
Abs. Delta SvO2 (%)	6 (1 ÷ 14.3)	3 (-2÷ 7)	2 (-2 ÷ 7.5)	**0.003**
Rel. Delta 6MWT (%)	32 (10 ÷ 66) *+	4 (2 ÷ 13) +	2 (-2 ÷ 12)*	**<0.001**
Rel. Delta RAP (%)	-14 (-40 ÷ 22.5)	-14 (-34.5 ÷ 111)	0 (-40 ÷ 31)	0.779
Rel. Delta mPAP (%)	- 10 (-18.5 ÷ -3)	-7 (-14 ÷ 0)	-10 (-18.5 ÷ -3)	0.198
Rel. Delta CI (%)	35 (18 ÷ 60)* +	20.5 (5.5 ÷ 33) +	17 (2 ÷ 39)*	**<0.001**
Rel. Delta PVR (%)	-35 (-49 ÷ -19.5) * +	-24 (-34 ÷ -10) +	-24 (-42 ÷ -8) *	**<0.001**
Rel. Delta SvO2 (%)	10 (-2 ÷ 26)* +	4 (-3.5 ÷ 11) +	4 (-3 ÷ 11)*	**0.001**

*∬+ = p< 0.05 between respective pair

Table 10. Comparison of mean changes (both relative and absolute values) between baseline and first follow-up (after 3-4 months of therapy)

Firstly, significant improvements in exercise capacity were observed in patients treated with Epoprostenol as compared to patients treated with Treprostinil and Selexipag, both in absolute and

percentage values. Concerning hemodynamic parameters, we registered better improvements in IC and SvO2 and greater reduction in PVR in patients treated with Epoprostenol than in patients in the two other groups, both considering absolute and percentage values.
Between patients treated with Selexipag and patient treated with Treprostinil, no statistically significant differences was found, nor in absolute values neither in percentage changes even if patients treated with Treprostinil showed a trend trough better clinical and hemodynamic improvements.

In the second part of our analysis, we aimed at assessing the effects of the same drugs, on clinic and hemodynamic profile, when they were used as third line strategy.

Therefore, we selected 181 patients (120 patients were excluded, in particular 95 in Epoprostenol group and 25 in Treprostinil group): 44 in the Selexipag group (100% of the total), 80 patients in the Treprostinil group and 57 patients in the Epoprostenol group.

Invasive follow-up data, assessed after 3-4 months of therapy, were available in 153 patients: 44 in the Epoprostenol group, 73 in the Treprostinil group 36 in the Selexipag group.
In particular, in the Epoprostenol group 7 patients died, 1 patient underwent transplantation, 1 was lost at follow-up, in 1 patient RHC was not repeated before the end of the study and in 3 patient we decided for a conservative strategy because of severe comorbidities.
In the Treprostinil group, 5 patients died, 1 patient underwent by-pulmonary transplantation and in 1 patient RHC was not repeated because of severe comorbidities.
In the Selexipag group, 2 patients died, 1 patient stopped the drug because of adverse reactions, 2 patients were lost at follow-up, 2 patients had not been seen in follow-up consultation yet when this study was concluded and in 1 patient we choose a conservative strategy.

Baseline demographic, functional and haemodynamic characteristics of this sub-populations are described in *Table 11 and 12*.

	Epoprostenol	**Treprostinil**	**Selexipag**	**p-value**
n°	57	80	44	
Age	42 (32 ÷ 60) *	43 (37 ÷ 52) ∬	51 (36 ÷ 66) *∬	**0.035**
Male gender, n (%)	20 (35)	21 (26)	13 (30)	0.537
NYHA III/IV, n (%)	46 (81)	56 (79)	20 (45.5)	0.101

6MWT (m)	366 (288 ÷ 417) *+	435 (338 ÷ 525) +	487 (375 ÷ 570) *	**<0.001**
Aethiology, n (%) **I/H/D** **CTD** **CHD** **PoHIV**	+ 28 (49) 22 (37) 7 (12) 0	+ 49 (61) 11 (14) 20 (25) 0	 27 (61) 8 (18) 8 (18) 1 (2)	 **0.011**
Dose after 3-4 months	18 (16 ÷ 21)	47 (34 ÷ 54)	2800 (2000 ÷ 3200)	/
Dose at the end of the study	22 (19 ÷ 30)	56.5 (47 ÷ 67.5)	2800 (2000 ÷ 3200)	/
Low/intermediate/high risk n (%)	2 (3.5)/ 39 (68)/ 16 (28)	11 (14)/ 63 (79)/ 6 (7.5)	14 (32)/ 28 (64)/ 2 (5)	0.068

*∫∫+ = p<0.05 between respective pair

Table 11. Baseline demographic and functional characteristics

Patients treated with Selexipag were older in comparison to patient treated with Epoprostenol or Treprostinil, while there were no significant differences between the latter two. Then, we observed a minority of male gender in all groups, particularly in patient treated with Selexipag and Treprostinil.

Patients in the Epoprostenol group where more often in NYHA functional class III or IV at baseline, even if no significant differences between groups were found.

Similarly, patients treated with Epoprostenol had significantly lower exercise capacity at baseline 6MWT as compared with patients treated with Selexipag and Treprostinil.

Considering exercise capacity and NYHA functional class, patients treated with Treprostinil appeared to be slightly more compromised at baseline than those treated with Selexipag even though statistical significance was not completely reached.

Concerning aetiology of PAH, significant differences were found between patients treated with Epoprostenol and those treated with Treprostinil, with higher percentage of patients affected by CTD in the Epoprostenol group and lower percentage of I/H/D and CHD. No significant difference was found between patients in Treprostinil and in Selexipag group.

Concerning posology, for all prostacyclin agents dose titration is needed during follow-up to reach the maximum tolerated dose. At the end of our study, most patients treated with Selexipag received

the maximum approved dose while for Treprostinil and Epoprostenol a dosage like those reported in literature was reached.

Finally, we observed that most patients treated with Selexipag were at low risk, patients treated with Treprostinil were at intermediate risk and patients treated with Epoprostenol were at high-risk. To notice, 2 patients treated with Selexipag had a high-risk profile: the first patient was included in the TRITON study, for the second patient Epoprostenol would be difficult to manage. Conversely, in Treprostinil and Epoprostenol groups, respectively 11 and 2 patients at low risk were treated with parenteral prostacyclin analogues because of hemodynamic deterioration.

	Epoprostenol	Treprostinil	Selexipag	p-value
n°	57	80	44	
RAP (mmHg)	12 (8 ÷ 14) ∬+	9 (7 ÷ 11.5) *+	8 (5 ÷ 9) *∬	**<0.001**
mPAP (mmHg)	64 (53 ÷ 75) ∬	62 (53 ÷ 72) *	52 (44.5 ÷ 64) *∬	**<0.001**
PAWP (mmHg)	9 (8 ÷ 11)	10 (8 ÷ 12)	9 (8÷ 11)	0.083
mBP (mmHg)	79 (72 ÷ 86) ∬	82 (77 ÷ 88.5)	85 (75 ÷ 95) ∬	**0.029**
CI (l/min/m2)	2.1 (1.8 ÷ 2.5) ∬ +	2.4 (2.1 ÷ 2.7) +	2.5 (2.1 ÷ 2.8) ∬	**0.026**
PVR (W.U.)	16 (12.5 ÷ 22) ∬+	13 (10 ÷ 16) *+	10 (8 ÷ 13) *∬	**<0.001**
SVR (W.U.)	20 (16 ÷ 23)	18 (15.5 ÷ 22)	19 (16 ÷ 23)	0.223
Syst O2 Sat (%)	94 (92 ÷ 96) ∬	94 (91.5 ÷ 97) *	96 (94 ÷ 97) *∬	**0.010**
SvO2 (%)	58 (50.5 ÷ 62) ∬+	65 (60 ÷ 70) +	65 (50 ÷ 70) ∬	**<0.001**

*∬+ = p< 0.05 between respective pair

Table 12. Baseline hemodynamic characteristics

At baseline patients treated with Epoprostenol were haemodynamically more compromised in comparison to patients in the two other group: RAP and PVR where significantly higher while SvO2 were significantly lower than in patients treated with Selexipag and Treprostinil.

The comparison between patient in Selexipag and Treprostinil groups showed that the latter are slightly more compromised, even if statistical significance was reached only for RAP and PVR while no differences were found in CI values between groups.

In addition, we compared functional and hemodynamic parameters at baseline and after 3-4 months of therapy (*Table 13, 14, 15*).

Epoprostenol, n 44	Baseline	After 3-4 months of therapy	p-value
Low/intermediate/high risk n (%)	2 (3.5)/ 39 (68)/ 16 (28)	13 (30)/ 24 (55)/ 7 (16)	**0.002**
NYHA III/IV, n (%)	46 (81)	22 (50)	**0.003**
6MWT	385 (300 ÷ 446)	452 (377 ÷ 513)	**0.004**
RAP (mmHg)	12 (8.5 ÷ 14)	10 (7 ÷ 14)	0.367
mPAP (mmHg)	60.5 (53 ÷ 76)	57 (50 ÷ 65.5)	**<0.001**
PAWP (mmHg)	9 (8 ÷ 10)	11 (9 ÷ 12)	**0.011**
mBP (mmHg)	82 (73.5 ÷ 88)	74 (71 ÷ 82)	**<0.001**
CI (l/min/m2)	2 (1.9 ÷ 2.5)	2.8 (2.5 ÷ 3.5)	**<0.001**
PVR (W.U.)	15 (12 ÷ 19)	10 (8 ÷ 13)	**<0.001**
SVR (W.U.)	19 (16 ÷ 22)	13.5 (12 ÷16)	**<0.001**
Syst O2 Sat (%)	94 (92 ÷ 96)	94 (91 ÷ 95)	0.981
SvO2 (%)	59 (54 ÷ 62)	65.5 (59 ÷ 71)	**<0.001**

Table 13. Comparison between clinical, functional and hemodynamic characteristics at baseline and after 3-4 months of treatment with Epoprostenol as third line therapy

Patients treated with Epoprostenol as third line therapy, showed relevant improvements after 3-4 months, with significant reduction of patients at intermediate/high risk and increase in patient at low risk. Furthermore, we observed statistically significant reduction in NYHA functional class and improved exercise capacity at 6MWT.

Finally, all mean hemodynamic parameters were improved too: there was a significant increase in CI, SvO2 and PAWP and a significant decrease of mPAP, mBP, PVR and SVR; we also observed reduction in RAP even if statistically significance was not reached.

Treprostinil, n 73	Baseline	After 3-4 months of therapy	p-value
Low/intermediate/high risk n (%)	11 (14)/ 63 (79)/ 6 (7.5)	32 (44)/ 36 (49)/ 5 (7)	**<0.001**
NYHA III/IV, n (%)	56 (70)	32 (43)	**<0.001**
6MWT (m)	435 (338 ÷ 572)	485 (404 ÷ 565)	**<0.001**
RAP (mmHg)	9 (7 ÷ 11)	8 (6 ÷ 10)	**0.050**
mPAP (mmHg)	63 (53 ÷ 74)	57 (51 ÷ 68)	**<0.001**
PAWP (mmHg)	10 (8 ÷ 12)	10 (8 ÷ 12)	0.864
mBP (mmHg)	83 (77 ÷ 89)	78 (73 ÷ 83)	**<0.001**
CI (l/min/m2)	2.4 (2.1 ÷ 2.7)	2.9 (2.6 ÷ 3.2)	**<0.001**
PVR (W.U.)	13 (11 ÷ 16)	9 (7.5 ÷ 12.5)	**<0.001**
SVR (W.U.)	18 (16 ÷ 22)	15 (12.5 ÷ 17)	**<0.001**
Syst O2 Sat (%)	94 (92 ÷ 97)	94 (90 ÷ 96)	0.083
SvO2 (%)	65 (60 ÷ 70)	69 (63 ÷ 73)	**0.004**

Table 14. Comparison between clinical, functional and hemodynamic characteristics at baseline and after 3-4 months of treatment with Treprostinil as third line therapy

Similarly, after 3-4 months of therapy, patients treated with Treprostinil showed relevant improvement with statistically significant reduction of patients at intermediate/high risk and increase of patients at low risk. Also, we found a statistically significant reduction in NYHA functional class and improved exercise capacity at 6MWT.

All mean hemodynamic parameters were improved too, with a significant reduction in RAP, mPAP, mBP, PVR and SVR and a significant increase in CI and SvO2.

Selexipag, n 36	Baseline	After 3-4 months of therapy	p-value
Low/intermediate/high risk n (%)	14 (32)/ 28 (64)/ 2 (5)	22 (61)/ 14 (39)/ 0 (0)	**0.001**
NYHA III/IV, n (%)	20 (45)	12 (32)	0.058
6MWT (m)	487 (375 ÷ 575)	540 (440 ÷ 608)	**0.020**
RAP (mmHg)	7 (5 ÷ 9)	5.5 (4 ÷ 9)	0.294
mPAP (mmHg)	52.5 (45.5 ÷ 64)	46 (40.5 ÷ 52)	**<0.001**
PAWP (mmHg)	10 (8 ÷ 11.5)	9 (7.5 ÷ 10.5)	0.143
mBP (mmHg)	84.5 (74 ÷ 92)	78.5 (71 ÷ 87.5)	**0.006**
CI (l/min/m2)	2.4 (2.1 ÷ 2.7)	2.9 (2.5 ÷ 3.6)	**<0.001**
PVR (W.U.)	10.5 (8 ÷ 13)	7 (5 ÷ 10)	**<0.001**
SVR (W.U.)	18.5 (16 ÷ 23)	15 (11 ÷ 20)	**<0.001**
Syst O2 Sat (%)	96 (94 ÷ 98)	95 (93 ÷ 96)	**0.015**
SvO2 (%)	65 (60 ÷ 70)	68 (62.5 ÷ 73.5)	**0.027**

Table 15. Comparison between clinical, functional and hemodynamic characteristics at baseline and after 3-4 months of treatment with Selexipag as third line therapy

For patients treated with Selexipag as third line therapy, after 3-4 months of therapy we observed a reduction of patients at worst prognosis: in fact, patients in low and intermediate rick category increased with no patient at high risk.

Concerning NYHA functional class, no statistical differences were found in patient treated with Selexipag even if there is a trend toward reduction of patient in NYHA III/IV.

We observed a statistically significant increase in functional capacity with increasing of meters at 6MWT before and after therapy.

Regarding hemodynamic data, a significant increase in CI and SvO2, with significant reduction in PVR, SVR, mPAP and mBP was observed after 3-4 months of therapy (while reduction in RAP was not statistically significant).

Finally, we compared the mean functional and hemodynamic variations, between baseline and the first re-evaluation after 3-4 months of therapy, between the three groups (*Table 16*).

	Epoprostenol	Treprostinil	Selexipag	p-value
n°	44	73	36	
Abs. Delta 6MWT (m)	65 (25 ÷ 132) ∬+	18 (-8 ÷ 63) +	12 (-10 ÷ 57) ∬	**0.044**
Abs. Delta RAP (mmHg)	-1 (-4 ÷ 2.5)	-1 (-3 ÷ 2)	0 (-4 ÷ 2)	0.953
Abs. Delta mPAP (mmHg)	- 5 (-9 ÷ -1)	-5 (-11 ÷ -1)	-6.5 (-14 ÷ 0)	0.670
Abs. Delta CI (l/min/m2)	0.9 (0.4 ÷ 1.1) ∬+	0.5 (0.2 ÷ 0.8) +	0.3 (0.1 ÷ 1) ∬	**0.022**
Abs. Delta PVR (WU)	-4.5 (-8 ÷ -2) ∬+	-3 (-5 ÷ -2) +	-2 (-4 ÷ -1) ∬	**0.021**
Abs. Delta SvO2 (%)	5 (-2 ÷ 13) ∬+	3 (2 ÷ 6) +	2 (-2 ÷ 7.5) ∬	**0.036**
Rel. Delta 6MWT (%)	16 (5 ÷ 43) ∬+	3 (-2 ÷ 13.5) +	2 (-2 ÷ 12) ∬	**0.031**
Rel. Delta RAP (%)	-10 (-26 ÷ 25)	-14 (-33 ÷ 14)	0 (-40 ÷ 31)	0.768
Rel. Delta mPAP (%)	- 8 (-14 ÷ -2)	-8 (-15 ÷ -2)	-12 (-24 ÷ 0)	0.459
Rel. Delta CI (%)	38 (18.5 ÷ 59) ∬+	21 (7 ÷ 35) +	17 (2 ÷ 39) ∬	**0.010**
Rel. Delta PVR (%)	-35 (-46 ÷ -17)	-26 (-35 ÷ -13)	-25 (-42 ÷ -8)	0.106
Rel. Delta SvO2 (%)	8 (2.5 ÷ 23) ∬+	4 (3.5 ÷ 10.5) +	4 (-3 ÷ 11) ∬	**0.024**

*∬+ = p< 0.05 between respective pair

Table 16. Comparison of mean changes (both relative and absolute values) between baseline and first follow-up (after 3-4 months of therapy)

Comparing the mean variations from baseline and the first re-evaluation, we found better improvements in exercise capacity in patients treated with Epoprostenol compared to patients treated with Treprostinil and Selexipag, both considering absolute and percentage values (even if statistical differences were found only between Epoprostenol-Selexipag and Epoprostenol-Treprostinil groups). Concerning changes in hemodynamic parameters, we observed that patients treated with Epoprostenol had higher improvements in CI compared to patients in the two other groups, both considering absolute and percentage values (with statistically significant differences between Epoprostenol-Selexipag and Epoprostenol-Treprostinil groups).

As observed in the first part of our analysis, no statistically significant differences were found between patients treated with Selexipag and patient treated with Treprostinil (even if patients treated with Treprostinil showed a trend trough better clinical and hemodynamic improvements).

4. Discussion

Dysregulation of the prostacyclin metabolic pathway has been shown in patients with PAH as assessed by a reduction of prostacyclin synthase expression in the pulmonary arteries and of prostacyclin urinary metabolites[66].

The clinical use of prostacyclin in patients with PAH has been extended by the synthesis of stable analogues having qualitatively similar pharmacodynamic effects, however their use carry several difficulties associated with their administration and AEs[101].

Epoprostenol, the first approved PAH therapeutic agent, has shown improvements both in exercise capacity, quality of life and mortality in PAH patients. However, due to his short half-life the only possible way of administration is intravenous infusion by a pump, possibly causing AEs such as local pain, infection and thrombotic complications[102].

In more recent years, research has been undertaken to develop a safer and more convenient preparation of prostacyclin agents including the inhaled, subcutaneous, and oral routes. Treprostinil was first studied as a subcutaneous continuous infusion therapy thus avoiding the risk of sepsis. While effective in terms of 6MWD, the frequent occurrence of infusion site pain limits dose escalation, therefore the issue for the easy administration of prostanoids remains[103].

Selexipag is a selective IP prostacyclin receptor agonist that works similarly to prostacyclin, offering a more stable drug with oral administration and potentially similar efficacy. When compared to placebo in large, long-term trials, Selexipag was associated with fewer clinical worsening but increased adverse events, while the effect on other clinical outcomes is less certain.

The purpose of this study was to reassess our experience on the use of drugs that interact on the pathobiological line of prostacyclin, so we compared the efficacy of intravenous Epoprostenol, subcutaneous Treprostinil and oral Selexipag for the treatment of PAH.

Our analysis confirmed clinical and functional benefit for the use of intravenous and subcutaneous prostacyclin in terms of improved functional class, six-minute walking distance and cardiopulmonary hemodynamics. Concerning the oral prostacyclin receptor agonist, a trend through clinical and hemodynamical benefits emerged, even if improvements were apparently weaker when compared to those observed with intravenous Epoprostenol or subcutaneous Treprostinil.

More in detail, we observed that patients treated with Epoprostenol were significantly more compromised at baseline in terms of symptoms, functional capacity and hemodynamics when compared to patients in the two other groups, while patients treated with Treprostinil appeared to be slightly worse at baseline with respect to those treated with Selexipag.

Then, evaluating the effects of the three different drugs between baseline assessment and first follow-up (after 3-4 months of therapy), it emerged that patients treated with Epoprostenol had significantly greater clinical and hemodynamic improvements in respect to those treated with Treprostinil and Selexipag, while patients treated with Treprostinil showed only a trend trough better progresses if compared to patients treated with Selexipag.

There are definitely many confounding factors that could have influenced demographic, clinical and hemodynamic characteristics of patient populations, as well as drug response.

Firstly, is important to notice the different era of drug approval: 1995 for Epoprostenol, 1999 for Treprostinil and 2018 for Selexipag, so that the use of these drugs has progressively changed over time according to the introduction of new compounds interacting with the other two pathways (the endothelin and the nitric oxide pathway). That is why initially intravenous and subcutaneous prostacyclin analogs have been used predominantly in mono therapy and subsequently as second- and third-line compounds when the initial strategy with oral medications was failing. Nowadays an increasing number of patients is treated with double upfront combination therapy, and therapy with prostacyclin analogues and prostacyclin receptor agonist is used as subsequent strategy in case of inadequate clinical response. Furthermore, different treatment invasiveness play an important role in the drug choice, so that less invasive drugs are used in less advanced clinical and hemodynamic conditions.

Therefore, both baseline characteristics of our patients, treatment clinical and hemodynamical effects at first follow-up and survival rate, were influenced by the temporal sequence of drug introduction in clinical practice and of consequence on their way and timing of administration.

In the second part of our analysis, we aimed at limiting these confounding factors, so we compared the effects of the same drugs on clinic and hemodynamic profile, when they were used as third line strategy.

The baseline characteristics of the three populations were quite similar, even if patients treated with Selexipag were older in comparison with the two other groups: in subjects out of transplantability range we more often prescribe Selexipag, while in youngest patients we tend to be more aggressive. Furthermore, the differences emerged in exercise capacity and baseline hemodynamics data reflect the fact that in our clinical practice, we add Epoprostenol as third line therapy in more compromised patients, Treprostinil in intermediate situations and Selexipag in less impaired conditions. Comparing the effects of treatments between baseline and first follow-up we noticed smaller benefits with Selexipag when compared with intravenous and subcutaneous strategies but it’s important to weight baseline patient’s differences (greater improvements in more severe patients).

The efficacy of Selexipag in triple combination therapy in less advanced patients suggests the possibility to anticipate further its use in order to achieve as soon as possible triple combination therapy. Benefits of this approach emerged from the TRITON study too, in particular exploratory analyses suggested a possible signal for improved long-term outcomes with initial triple versus initial double oral therapy – but we certainly need further study to confirm these results[96].

5. Conclusions

Drugs interacting with the prostacyclin pathway have been the first to be utilized in PAH patients and their use has been changing in last years, according to the introduction of new compounds addressing the two other pathogenetic pathways (endothelin and nitric oxide pathways).

Initially, intravenous and subcutaneous prostacyclin analogs have been used predominantly in mono therapy and subsequently as second- and third-line compounds when the initial strategy with oral medications was failing. The prostacyclin receptor agonist Selexipag has been recently introduced and is used predominantly as third line compound in triple combination therapy.

The different strategy of using parenteral prostacyclin analogs and the new prostacyclin receptor agonist impacts baseline clinical and hemodynamic profile of treated patients, influencing results on drug efficacy.

Our analysis confirmed clinical and functional benefit for the use of both prostacyclin analogues and prostacyclin receptor agonist in terms of improved functional class, six-minute walking distance and cardiopulmonary hemodynamics.

In particular, the efficacy of Selexipag in triple combination therapy in less advanced patients suggests the possibility to anticipate further its use in order to achieve as soon as possible the beneficial effects of triple combination therapy and in particular the reduced risk for disease progression.

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