Endothelial dysfunction in chronic obstructive pulmonary disease and its impact on gas exchange

Lucilla Piccari

UNIVERSITAT DE BARCELONA

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Doctoral thesis dissertation presented by

Lucilla Piccari

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Directed by

Prof. Joan Albert Barberà Mir
Universitat de Barcelona
Hospital Clínic de Barcelona

Prof. Isabel Blanco Vich
Universitat de Barcelona
Hospital Clínic de Barcelona

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Abbreviations

CI: cardiac index

CO: cardiac output

COPD: chronic obstructive pulmonary disease

COVID-19: coronavirus disease-19

CT: computerised tomography

CTEPH: chronic thromboembolic pulmonary hypertension

eNOS: endothelial nitric oxide synthase

ERS: European Respiratory Society

ESC: European Society of Cardiology

HIF-2: hypoxia-inducible factor 2

FC: functional class

FMD: flow-mediated dilation

ILD: interstitial lung disease

MIGET: multiple inert gas elimination technique

mPAP: mean pulmonary artery pressure

PAH: pulmonary arterial hypertension

PAWP: pulmonary artery wedge pressure

PH: pulmonary hypertension

PVR: pulmonary vascular resistance

PWA: pulse wave analysis

PWV: pulse wave velocity

RHC: right heart catheterization

ROC: receiver-operating curve

SD: standard deviation

V_A/Q: ventilation/perfusion

WHO: World Health Organisation

WSPH: World Symposium on PH

WU: Wood units

Presentation

This doctoral thesis is presented in the format of compendium of publications.

In the introduction, the basic concepts of pulmonary hypertension and pulmonary hypertension associated with chronic obstructive pulmonary disease (COPD) are laid out. There is an exposition of the pathophysiologic mechanisms involved in the development of systemic and pulmonary vascular disease in the context of COPD. These concepts, which have been extensively studied in previous works, are the object of a review article co-authored by the candidate with the title "Pulmonary vasculature in COPD: The silent component" and that is included as supplementary work in the annex of this thesis. The hypotheses of the thesis are stated.

The central part of the thesis consists of two objectives and two articles:

1. **Piccari L**, Del Pozo R, Blanco I, García-Lucio J, Torralba Y, Tura-Ceide O, Moises J, Sitges M, Peinado VI, Barberà JA. Association Between Systemic and Pulmonary Vascular Dysfunction in COPD. Int J Chron Obstruct Pulmon Dis. 2020 Aug 26;15:2037-2047. **Q1 (2020), IF 3.35**
2. **Piccari L**, Blanco I, Torralba Y, Arismendi E, Gistau C, Ramírez A, Gimeno-Santos E, Roca J, Burgos F, Rodríguez-Roisín R, Barberà JA. Mechanisms of hypoxaemia in severe pulmonary hypertension associated with COPD. Eur Respir J. 2023 Jul 20;62(1):2300463. **Q1, IF 24.3**

Thesis summary

Disfunción endotelial en la enfermedad pulmonar obstructiva crónica y su impacto sobre el intercambio de gases

INTRODUCCIÓN: La hipertensión pulmonar (HP) es una complicación frecuente en la enfermedad pulmonar obstructiva crónica (EPOC) y conlleva un importante aumento de morbi-mortalidad. Es conocido que los pacientes con EPOC presentan alteraciones vasculares pulmonares incluso en estadios iniciales de la enfermedad y dichas alteraciones se pueden presentar hasta en sujetos fumadores que no padezcan la EPOC. El consumo de tabaco de hecho es uno de los factores etiológicos principales de la disfunción endotelial y del remodelado arterial pulmonar, siendo ambos mecanismos patogenéticos de la enfermedad vascular pulmonar en la EPOC. Por otro lado, la EPOC también está asociada a riesgo cardiovascular aumentado y a varias enfermedades cardiovasculares, de forma independiente del consumo de tabaco. Sin embargo, no está claro si los pacientes con EPOC y enfermedad vascular pulmonar presenten también disfunción endotelial a nivel de las arterias sistémicas *per se*, de forma independiente de los otros factores cardiovasculares (tabaco, enfermedades cardiovasculares) que se asocian a dicha disfunción. Por otro lado, tampoco es conocido si la disfunción endotelial de arterias pulmonares juega un papel en la regulación del intercambio de gases en formas graves de hipertensión pulmonar que raramente se asocian a EPOC leve-moderada.

HIPÓTESIS: dados los antecedentes expuestos, las hipótesis de este trabajo de tesis son:

1. La entidad de la disfunción endotelial y de la rigidez arterial en arterias sistémicas puede estar relacionada a mayor disfunción endotelial en arterias pulmonares y a desarrollo de enfermedad vascular pulmonar.
2. En pacientes con EPOC e HP grave, la presencia de hipoxemia podría estar relacionada con una combinación de factores intrapulmonares (aumentado desequilibrio de las relaciones ventilación/perfusión, V_A/Q) y extrapulmonares (gasto cardíaco reducido), resultando en una reducida respuesta al estímulo hipóxico debido a mayor disfunción endotelial.

OBJETIVOS: el objetivo general de esta tesis es de valorar la función vascular sistémica y pulmonar de pacientes con EPOC en todo el espectro de la enfermedad vascular pulmonar. Los objetivos específicos de cada estudio se detallan a continuación:

Primer estudio: evaluar la función endotelial y la rigidez arterial en la misma cohorte de pacientes con EPOC, y de relacionarla con la presencia de enfermedad vascular pulmonar.

Segundo estudio: analizar los determinantes de la hipoxemia en pacientes con EPOC y HP grave y averiguar si estos pacientes presentan una respuesta atenuada al estimulo hipóxico.

RESULTADOS PRINCIPALES: En el primer estudio, hemos averiguado que los pacientes con EPOC presentan reducción de la flow-mediated dilation (FMD), que es una forma de valorar la disfunción endotelial de arterias sistémicas, y que esta se reduce de forma constante y progresiva entre sujetos sanos no fumadores, sujetos sanos fumadores, pacientes con EPOC y pacientes con EPOC y enfermedad vascular pulmonar. Además, hemos observado que el sexo, la edad y la presencia de comorbilidades cardiovasculares no influencia el descenso de la FMD. También observamos que la rigidez en arterias sistémicas es mayor en pacientes con EPOC y enfermedad vascular pulmonar respecto a sujetos sanos fumadores y no fumadores, y hay tendencia hacía una diferencia significativa entre la rigidez arterial sistémica de pacientes con EPOC y enfermedad vascular pulmonar frente a los sin patología vascular pulmonar.

En el segundo estudio, hemos confirmado que los pacientes con EPOC e HP grave presentan una más leve alteración del flujo aéreo y una hipoxemia más grave respecto a pacientes con EPOC sin HP o con HP leve-moderada. Asimismo, hemos demostrado que la hipoxemia grave que padecen los pacientes con EPOC y HP grave es debida a un mayor desequilibrio de las relaciones V_A/Q respecto a los otros dos grupos, asociado a un peor gasto cardíaco en estos pacientes. Además, hemos evidenciado como en pacientes con EPOC y HP grave, la vasoconstricción pulmonar hipóxica juega un papel mínimo en el mantenimiento del equilibrio V_A/Q, posiblemente debido a una mayor disfunción endotelial pulmonar en estos pacientes. Por esta razón, el uso de fármacos vasodilatadores pulmonares en esta población de pacientes podría no ser perjudicial sobre el intercambio de gases.

CONCLUSIONES: la disfunción endotelial sistémica está asociada a la enfermedad vascular pulmonar en la EPOC, y dicha asociación es independiente de la presencia de factores de riesgo y enfermedades cardiovasculares. La exposición al tabaco podría ser un común denominador entre disfunción vascular sistémica y pulmonar en la EPOC. La hipoxemia en los pacientes con EPOC e HP grave está determinada por factores intrapulmonares (desequilibrio V_A/Q) y extrapulmonares (reducción del gasto cardíaco), con mínima respuesta de vasoconstricción pulmonar a la hipoxemia. En consecuencia, el uso de fármacos vasodilatadores pulmonares no sería perjudicial para el intercambio de gases en estos pacientes.

Introduction

Pulmonary hypertension

Pulmonary hypertension (PH) is a pathologic increase of pressure in the pulmonary circulation. As such, it is not a disease, but a hemodynamic condition that is common to many different pathologies, and even a normal response to certain physiologic states. The diagnosis of PH requires pulmonary hemodynamic evaluation through right heart catheterization (RHC).

Hemodynamic definition and pathophysiology

Pulmonary hypertension is the haemodynamic state characterised by an abnormal elevation of pressure in the pulmonary vasculature (1). The pulmonary circulation is a system of low pressure and high distensibility, which is the ideal condition to allow it to handle normal cardiac output returning from the systemic veins at rest as well as accommodating higher blood flow from the increased cardiac output necessary for exercise in the healthy subject. These physiologic features ensure that the pressure in the pulmonary circulation never increases too much, even with increasing age and during maximal exercise. Pulmonary arterial pressure measured during RHC varies in healthy subjects with position (supine, semi-recumbent or upright) and age.

The definition of PH was established at the arbitrary threshold of mean pulmonary artery pressure (mPAP) ≥25 mmHg ever since the first expert meeting held in Geneva in 1973 to discuss PH(2) and organised by the World Health Organisation (WHO). The meeting was a response to the growing number of world-wide cases of PH, which had been partly caused by the diffusion of anorexigens drugs with toxic effects on the pulmonary circulation. During the meeting, the threshold for abnormal pulmonary pressure was chosen because “hypertension is definitely present if the pressure exceeds 25 mmHg”; therefore, it was intended as a safe limit that would not lead to over-diagnosis, although even then it was known that the value of mPAP “never exceeds 20 mmHg”. This led to the fact that patients with mPAP between 20 and 24 mmHg were always considered to have an above-normal pulmonary artery pressure, even though this level did not qualify for the diagnosis of PH, effectively placing them in a “grey zone” of a pre-pathological condition.

Indeed, the normal values across all age groups in the supine position are 14 ± 3.3 mmHg of mPAP and 0.92 ± 0.38 mmHg/L/min (Wood units, WU) of pulmonary vascular resistance (PVR)(3). Despite this well-known fact, the threshold of mPAP ≥25 mmHg to define PH has been maintained from 1973 until 2018. During the 6th World Symposium on PH (WSPH) held in Nice in 2018(4), for the first time after 45 years, it was proposed to lower the threshold for the diagnosis of PH to mPAP >20 mmHg. Following this, the latest guidelines on PH issued by the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) in September 2022(1) have established that the threshold for the diagnosis of PH is a value of mPAP of >20 mmHg (2 standard deviations, SD, above the normal average value in healthy subjects) and a value of PVR of >2 WU, as these values are associated with increased mortality(5). The definition of PH during exercise is a slope of the increase in mPAP over that of cardiac output (CO) between rest and exercise >3 mmHg/L/min, being the normal value among all age groups between 1.6 and 3.3 mmHg/L/min in the supine position(6).

Pulmonary hypertension at rest, when associated with parallel elevation of pulmonary vascular resistance above 2 WU and a normal pulmonary artery wedge pressure (PAWP) of ≤15 mmHg, is called pre-capillary PH. In contrast to this, if the PAWP is above 15 mmHg but with normal PVR, PH is termed isolated post-capillary PH; finally, if PAWP is increased above the normal value and PVR is also increased above 2 WU, this condition is termed combined pre- and post-capillary PH. Additionally, the latest guidelines recognise a new hemodynamic entity termed unclassified PH, which is characterised by increase mPAP but normal PVR, and characteristic of high CO states such as hyperdynamic conditions (liver cirrhosis, anaemic states and hyperthyroidism, among others).

Whichever the origin of the increased PAP, whether arterial, capillary or venous, a sustained increase in PVR when left untreated will invariably lead to right ventricular failure and death. The right ventricle, much like the pulmonary circulation, is better suited to accommodate increases in volume rather than in pressure, and especially when the increased pressure intervenes after the first decade of life the right ventricle undergoes a maladaptive remodelling constituted by progressive dilation which ultimately leads to reduced cardiac output, when the right heart is no longer able to counter the increased vascular resistance.

Current classification of pulmonary hypertension

The classification of PH for the 2022 ESC/ERS guidelines (1) (Table 1) recognises several forms of PH which are organised into five groups: Group 1 includes pulmonary arterial hypertension (PAH) in its idiopathic form, as well as hereditary PAH, drugs or toxins-related PAH and PAH associated with other conditions like congenital heart disease, connective tissue disease, portal hypertension, or infectious diseases such as schistosomiasis and human immunodeficiency virus. Following, Group 2 includes PH associated with diseases of the left heart (left valvular disease, heart failure or cardiomyopathy) and it's the only post-capillary form; Group 3 comprises of PH associated with diffuse lung disease such as chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD) and hypoxic states such as hypoventilation syndromes and high-altitude hypoxia; Group 4 is for PH associated with any obstruction of the pulmonary vasculature such as chronic thromboembolic pulmonary hypertension (CTEPH) or vascular tumours; and finally Group 5 is a miscellaneous group of PH with unclear and/or multi-factorial mechanisms, including systemic diseases such as sarcoidosis and pulmonary Langerhans cell histiocytosis or common conditions such as chronic renal failure and haematologic disorders. For the first time, the ESC/ERS 2022 guidelines clearly state that all groups of PH (including Group 5, PH of unclear mechanism) may comprise both pre- and post-capillary components which may contribute to the elevation of mPAP, and that the primary PH diagnosis will depend upon the assessment of the PH specialist(1). In fact, some epidemiological studies in PH have started to adopt this concept of primary and secondary PH diagnosis, demonstrating that a secondary PH diagnosis is very common: in the recent "PVDOMICS" initiative, a secondary PH diagnosis was present in more than a third of patients(7). This situation is very common in PH expert centres, where assigning a PH diagnosis to a patient may be challenging due to significant overlap in clinical features. The most common overlap is seen between patients with PAH and patients with chronic lung disease(7), especially if they suffer from a highly prevalent condition such as COPD. The difficulty in establishing the main PH diagnosis has management consequences since Group 1 PH has a set of established targeted pulmonary vasodilator treatments, whereas in Group 3 PH evidence-based treatment is very scarce and not usually recommended except in certain countries and for certain conditions.

Table 1. Clinical classification of pulmonary hypertension

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 PH with features of venous/capillary (PVOD/PCH) involvement
1.6 Persistent PH of the newborn
Group 2. PH associated with left heart disease
2.1 Heart failure:
2.1.1 with preserved ejection fraction
2.1.2 with reduced ejection fraction
2.2 Valvular heart disease
2.3 Congenital/acquired cardiovascular conditions leading to post-capillary PH
Group 3. PH associated with lung disease 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 mechanism
5.1 Haematological disorders
5.2 Systemic disorders
5.3 Metabolic disorders
5.4 Chronic renal failure with or without haemodialysis
5.5 Pulmonary tumour thrombotic microangiopathy
5.6 Fibrosing mediastinitis
Adapted from the ESC/ERS 2022 PH guidelines (Humbert M et al, Eur Respir J 2023) (1)

For these reasons, the study of the pathophysiological mechanisms that underpin different forms of PH is fundamental to increase our understanding of the different groups and ultimately to deliver appropriate treatment, when available.

This classification (summarised in Table 1) is based not only on the grouping of associated diseases, but also on pathophysiologic evidence of the development of PH in their context.

Pulmonary hypertension in COPD

Prevalence of pulmonary hypertension in COPD

Chronic obstructive pulmonary disease is a heterogeneous, systemic disease defined by chronic and irreversible obstruction to airflow in the lungs, caused by alterations in the airways (chronic bronchitis) or in the alveoli (emphysema) and responsible for chronic respiratory symptoms such as dyspnoea, cough and acute exacerbations(8). Pulmonary hypertension is a common complication of COPD, its prevalence being variable in different studies according to the definition of PH and populations considered (9–11); the prevalence of PH in COPD patients is difficult to determine because they are not systematically screened for PH with echocardiography and because the gold standard test for the diagnosis of PH, RHC, is not routinely performed in COPD patients(1). Therefore, the estimation of the prevalence of PH in COPD mostly rests on data from cohort studies, often undertaken in transplant centres where all patients who are undergoing lung transplant evaluation systematically undergo RHC. This is done because the presence of PH is one of the indications for lung transplant, as well as requiring special care during the peri-operative time. As a result, most cohort studies have been carried out in advanced COPD patients (9,11). Some cohort studies have shed light on the prevalence of PH in a more general COPD population(10), not confined to patients referred for evaluation of lung transplant, and found that PH is more often present in more advanced stages of the disease(10,12). However, this does not mean that the development of PH is always parallel in its severity to the severity of COPD. A recent meta-analysis(13) pooling 38 studies across 16 countries found that the overall prevalence of PH in COPD patients who had been studied for it with either echocardiography or RHC was 39.2%, with increasing incidence along the severity spectrum of COPD (mild, moderate, severe and very severe COPD presented a PH prevalence of 25%, 34%, 39% and 62%, respectively; $p = 0.024$). Interestingly, they found significant differences in regional prevalences, with the highest

prevalence in Africa and the lowest prevalence in Europe (30% vs 60%, respectively; p <0.001); however, the figures from Africa relied on two studies from Egypt which were both conducted with echocardiography as a diagnostic tool for PH, which is known to be unreliable in patients with COPD(14), and one of the two studies was conducted on patients who were experiencing an exacerbation of COPD, which is not recommended in the current PH guidelines(1). In this meta-analysis, the prevalence of PH was found to be decreasing with increasing hemodynamic severity (mild, moderate and severe PH prevalences of 30%, 10% and 7%, respectively; p <0.001), although authors did not mention the hemodynamic criteria assigned to each PH "grade".

There is ample evidence that the majority of patients with COPD who develop PH suffer from mild hemodynamic impairment(9,15), which is often associated with end-stage or pre-transplant disease; however, even when it is present, PH may have different repercussions on morbidity and mortality in COPD patients depending on its level severity. In fact, although in this mild form, PH greatly increases morbidity for COPD patients and indeed even elevations of mPAP below the current threshold for the diagnosis of PH entail a greater risk of hospitalizations due to a COPD exacerbation(16). The presence of PH is a strong independent predictor of increased mortality compared to patients with COPD without PH(9), and each mmHg increase of mPAP in COPD confers increased risk of mortality(17,18).

Definition, prevalence and impact of severe pulmonary hypertension in COPD

A small proportion of patients with COPD may present with severe PH. This was initially evidenced by a wide cohort study(15) finding that only 1.1% of the 998 COPD patients who underwent RHC had severe PH (in that case, defined as mPAP ≥40 mmHg), but later studies using varying thresholds for severe PH found a higher and varied prevalence (1.5-13%) depending on the characteristics of the population considered(19). It has to be acknowledged that many cohort studies suffer from a referral bias. Right heart catheterization, according to the latest guidelines, is currently restricted to patients who may benefit from the information provided by the result of the test for therapeutic decisions (namely surgery such as in lung volume reduction surgery or lung transplant), in the case of enrolment in clinical trials, or if there is a suspicion of severe PH which might indicate referral to an expert centre and decision of optimal management on a case-to-case basis. Thus, patients in which there is a suspicion of severe PH are more often referred for

RHC compared to patients in which the suspicion of mild PH is evidenced (usually through echocardiogram, as there is not a clear indication to perform RHC upon suspicion of mild PH). Furthermore, as mentioned, the definition of severe PH in COPD has been changing significantly in the last two decades, both in cohort studies and in official definitions of consensus statements and guidelines. In some cohort studies, the definitions of severe or "out of proportion" PH rested on varying thresholds of mPAP (≥40 mmHg(9,15,20), mPAP ≥35 mmHg(9), ≥30 mmHg(21)), or on combinations of mPAP, PVR and cardiac index (CI)(22). At the Cologne Conference(23), for the first time, hemodynamic severity of Group 3 PH was established through expert consensus and rested on a complex definition of severe PH in lung disease as being satisfied when at least two out of three elements were present: mPAP ≥35 mmHg, mPAP ≥25 mmHg in the presence of CI <2 L/min/m^2 and/or PVR >480 dyn/s/cm^{-5}. This less than agile definition was then confirmed four years later in a simplified version in the 5th WSPH held in Nice in 2013(24), where severe PH was defined as mPAP ≥35 mmHg or mPAP ≥25 mmHg with CI <2 L/min^2 and subsequently confirmed in later proceedings from PH World Symposia and European PH guidelines with minor changes (ESC/ERS 2015 PH guidelines(25): mPAP ≥35 mmHg or mPAP ≥25 mmHg with CI <2.5 L/min/m^2; 6th WSPH proceedings(26): mPAP ≥35 mmHg or mPAP ≥25 mmHg with CI <2 L/min/m^2).

Recently, the ESC/ERS 2022 guidelines on PH have changed the definition of severe PH in lung disease yet again, defining it simply as the presence of PVR >5 WU. This change was in part the result of a push for simplification of definitions, both within PH groups and among them; in this way, instead of having to consider several parameters at once, PH specialists only had to evaluate the PVR; at the same time, PVR >5 WU was considered as "severe PH" in all PH groups, and not only in Group 3, which was until then the only group with a classification of hemodynamic severity.

But in the case of PH in lung disease, the threshold for severity at PVR >5 WU also rested on two short twin studies published the previous year, which found this limit as significantly discriminating mortality among patients with PH and COPD(27) and PH and ILD(28). The work by Zeder et al(27) is a retrospective cohort study of patients with PH and COPD evaluated in a single centre and whose data was collected in a local registry; patients undergoing lung transplant evaluation were excluded. The authors performed a Cox regression analysis to determine which

hemodynamic parameters would better predict mortality (according to the lowest p value) after adjustment for age, sex and forced expiratory volume in one second (FEV_1) and found that a PVR >5 WU was the best predictor of worse survival (HR 2.59, 95% CI 1.58–4.27; p<0.001), closely followed by values of mPAP ≥44 mmHg (HR 2.44, 95% CI 1.43–4.16; p=0.001) and mPAP ≥33 mmHg (HR 2.26, 95% CI 1.37–3.71; p=0.001). Patients with PVR >5 WU were more frequently males with lower distance walked in the 6-minute walking test and presented lower peak oxygen uptake in the cardio-pulmonary exercise test as well as higher values of N-terminal pro-brain natriuretic peptide. In an analysis combining a level of mPAP ≥33 mmHg and/or PVR >5 WU, the authors found that patients which did not meet any of the two criteria had the best survival and those with mPAP ≥33 mmHg but PVR ≤5 WU had intermediate prognosis, while those with PVR >5 WU had worse prognosis irrespective of whether they associated mPAP <33 mmHg or ≥33 mmHg. Thus, they concluded that PVR >5 WU was the best hemodynamic predictor of prognosis in their cohort.

The study by Olsson et al(28), which used data from patients diagnosed with ILD in a large European registry of PH (the COMPERA registry) where the majority of data (80%) is included by German PH centres, used similar criteria to the ones previously described (they also excluded patients who underwent lung transplant) and analysis (multivariable Cow regression). It found that higher age, male sex, lower total lung capacity (TLC) and higher PVR were all independent predictors of survival, while other hemodynamic parameters were not predictive. When looking at PVR specifically, the cut-off of PVR >5 WU was statistically significant (p =0.03) although higher thresholds such as PVR >8 WU had lower p-values. Patients with PVR >5 WU had lower 6MWD and lower diffusion capacity for carbon monoxide (DL_{CO}). This work was not limited in its design by the single centre cohort like the previous one; however, its cohort was on average very severely affected by PH (mean hemodynamic values for the whole cohort were: mPAP 39 mmHg, CI 2.1 L/min/m^2 and PVR 7.6 WU), likely as a result of selection bias, since these patients had all been referred to an expert PH centre and underwent RHC. Furthermore, all patients enrolled in the COMPERA registry receive pulmonary vasodilator treatment, which could conceivably have an effect, albeit variable, on their prognosis.

According to these studies, there seems to be therefore little difference between the impact on prognosis of severe PH on COPD and on ILD. However, as is the case for mild PH, severe PH has a very distinct effect on patients with COPD and with ILD, at least in mostly untreated patients who did not undergo lung transplantation. In a study(17) that focused on 317 such patients, and excluded those with combined pulmonary fibrosis and emphysema, when COPD and ILD were considered together there was no difference in survival between patients without PH and with very mild, mild or severe PH. However, when the two cohort were analysed separately, COPD patients presented worse prognosis only when affected by severe PH; while in the case of ILD, any level of PH (even the very mild level) provided worse outcomes. Furthermore, in the same study, the cut-off for severity of PH at PVR >5 WU was statistically significant in terms of survival in COPD patients (thereby confirming the results by Zeder et al.) but not in ILD patients. The discrepancies between the study by Olsson et al and this later study may be explained by the different population considered: predominantly severe versus mild PH patients (median PVR 7.6 WU vs 3.6 WU), the exclusion or inclusion of patients without PH (providing a useful comparison with a control group) and the percentage of patients treated with pulmonary vasodilators (100% vs 9%). Furthermore, other studies which compared patients with idiopathic pulmonary fibrosis with and without PH, thereby also employing analysis of a control group, have similarly found that mortality was increased when mPAP >20 mmHg(29).

Besides the severe clinical consequences of PH in COPD in the form of hospitalizations due to acute exacerbation and mortality, which confer patients with COPD and PH one of the worst 5-year prognoses of the whole 5 groups of PH, the impact of PH on the quality of life and symptoms burden of COPD patients cannot be overstated. Symptoms of PH significantly overlap with those of COPD, making suspicion of PH difficult; the most common symptoms are dyspnoea (initially on exertion, later in the disease present even at rest), weakness and fatigue. Despite the lack of specific studies focusing on symptoms and quality of life of COPD patients with PH, some insights may be gained from data derived from registries. When PH is diagnosed, most patients suffer from severe dyspnoea: functional class according to the World Health Organization classification (FC-WHO) III or IV is found in 28% of COPD patients at diagnosis of PH(30), as opposed to 15% of patients with PAH. This high percentage is mostly driven by patients with severe PH (mPAP ≥35

mmHg or CI <2 L/min/m^2), since when these are considered separately to those with mild-moderate PH, the proportion of those suffering from FC-WHO III-IV increases to 32% in severe PH and decreases to 11% in mild-moderate PH. Data from the French Registry of severe PH in COPD returned an even higher proportion of severe dyspnoea, with 56% of patients experiencing FC-WHO III and 22% FC-WHO IV(31). Patients may also present palpitations, chest pain during exercise, dizziness and occasionally syncope, although these symptoms have not been specifically studied in COPD and PH and they are not systematically collected in registries. The appearance of symptoms and signs of right heart failure is considered a hallmark of advanced PH, although in COPD the presence of suggestive signs such as peripheral oedema may be caused by other mechanisms than right ventricular failure(32), such as the activation of the renin-angiotensin-aldosterone system and through an imbalance of factors promoting the formation of oedemas in the lower extremities and those protecting from it. The French Registry for COPD and severe PH also offers an insight into the frequency of adverse events in these patients: 26% of patients presented at least 1 non-serious adverse event in the previous 12 months of the study, while 43% of patients required at least 1 hospitalization due to a severe adverse event in the previous 12 months. Interestingly, some of the hospitalizations for exacerbation of COPD may in fact have a cardiovascular component(33), at least in part: in a study(34) using an implantable continuous monitoring device of pulmonary artery pressure, allowing for careful management of heart failure medications, patients with COPD experienced a 62% reduction in hospitalizations that had been deemed “respiratory” (i.e. caused by the COPD), while the non-COPD control group did not experience such a reduction in respiratory hospitalizations.

Pulmonary hypertension is recognised to have a clear effect in reducing exercise capacity in COPD patients: compared to patients with COPD without PH, those with PH have lower exercise capacity at maximal (cardiopulmonary exercise test) and submaximal tests (6MWD) as well as lower endurance time(35). A subsequent systematic review and meta-analysis(36) showed that COPD patients with PH had lower maximum workload and peak oxygen consumption as well as lower oxygen pulse at the cardiopulmonary exercise test, compared to those without PH. Interestingly, this difference was driven by patients with less severe COPD who were not candidate for lung

transplant, because when patients who were being evaluated for lung transplant were excluded, the difference between the group with and without PH was even more pronounced.

Given the effects mentioned of PH on exercise tolerance, it is not surprising that even when PH is not manifest during RHC at rest, a high proportion of COPD patients will develop PH during exercise. Cohort studies have shown that an abnormal response to exercise may be evidenced in 58% of COPD patients who do not show PH at rest, and the presence of PH during exertion is a risk factor for developing PH at rest in the future in COPD patients(37). Thus, special consideration must be given to worsening of dyspnoea on exertion and in general to symptoms arising during exercise in COPD patients.

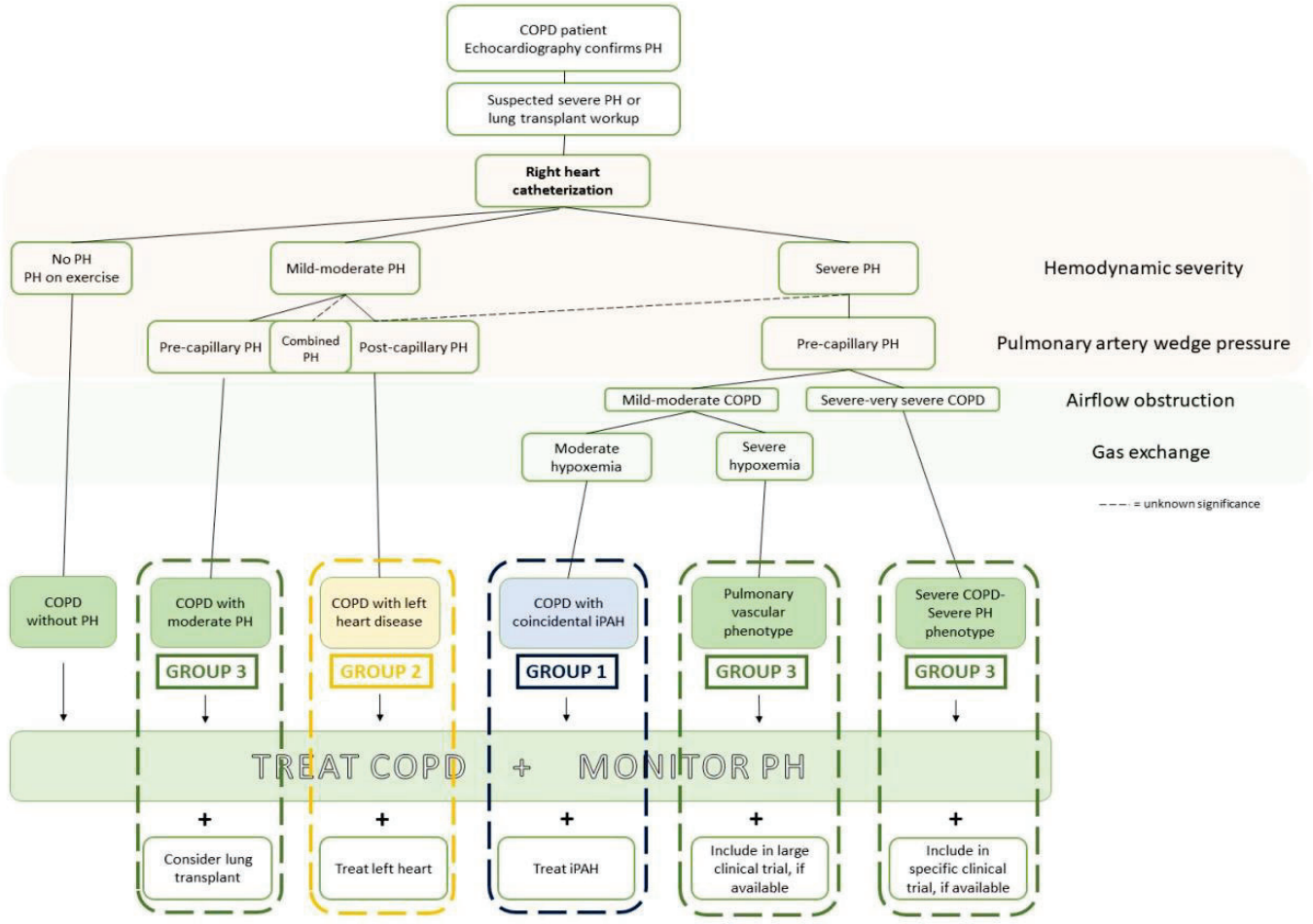

Figure 1. Phenotypes of PH associated with COPD. Adapted from García AR et al, Curr Opin Pulm Med 2022 (34)

Phenotypes of PH in COPD

Pulmonary hypertension may exist in patients with COPD in distinct forms or clinical situations (Figure 1). While historically the development of right heart failure was lumped in one condition called *cor pulmonale* and largely considered as a consequence of the parenchymal lung disease, in recent decades it has been evidenced that although right heart failure may be the common endpoint of PH, there are differences in the way PH manifests itself in COPD patients. With the current knowledge, it is possible to discern at least five different circumstances in which resting PH is associated with COPD: COPD of any severity with mild-moderate pre-capillary PH; COPD with post-capillary pulmonary hypertension (Group 2 PH); the so-called pulmonary vascular phenotype; PAH coinciding with COPD in the same patient (Group 1 PH); and finally, the "severe COPD-severe PH" phenotype(38).

COPD with mild-moderate pre-capillary pulmonary hypertension

This is the most common form of PH in the context of COPD. It is more common in advanced obstructive disease, and its development may be associated with worsening of hypoxemia and of exercise intolerance. It is thus characterised by severe or very severe airflow obstruction, moderate or severe hypoxemia, mild-moderate PH and a limitation to exercise that is typically of ventilatory origin, when assessed through the cardiopulmonary exercise test. It usually progresses slowly, increasing by 0.4 mmHg/year(37) and its treatment is optimal management of the underlying COPD, including correction of respiratory failure if present. The presence of PH is an important indication for lung transplant in eligible patients with COPD, and its diagnosis should prompt consideration of referral to a lung transplant centre.

COPD with post-capillary pulmonary hypertension

Left heart disease is common in COPD(39) and it shares the common aetiologic factor of cigarette smoking. Conversely, patients with systemic arterial hypertension are at higher risk of cardiovascular events if they also suffer from COPD(40) or even if they present reduced lung function without overt COPD(41). Furthermore, in patients with COPD there is a high incidence of diastolic dysfunction, which is an important factor determining their exercise intolerance(42). All these elements may contribute to the development of post-capillary PH, characterised by PAWP >15 mmHg and present in up to half of patients with COPD in retrospective cohorts(14).

Because PAWP requires measurement through RHC to be assessed, and because the latest PH guidelines establish that invasive hemodynamic tests may be foregone when there is a high suspicion of post-capillary PH in a given patient(1), the real prevalence of post-capillary PH in COPD is not known. To assess for the pre-test probability of Group 2 PH, clinicians can use a number of scores, among which the most-commonly employed is the "H_2FPEF" score(43). This tool assigns points to each contributing cardiovascular risk factors such as atrial fibrillation, obesity, age, sex, structural left heart disease, and when the sum of these points is >6, the probability of post-capillary PH is considered high. A further development of the H_2PEF score is the OPTICS score, which aimed to refine the pre-test probability of post-capillary PH by improving the sensitivity and specificity of the assessment, since in patients with newly-diagnosed PH a high H_2PEF is not able to fully exclude pre-capillary PH(44). Both these scores require data derived from the echocardiogram, which is a test prone to pitfalls in COPD patients(14), especially when there is significant emphysema; therefore, the proper assessment of post-capillary PH, which may be particularly relevant during episodes of exacerbation, still requires performance of a RHC in selected cases. There is no specific treatment for COPD with post-capillary PH and the current guidance is to ensure optimal management of the cardiac conditions and of COPD.

The pulmonary vascular phenotype of COPD and its distinction with pulmonary arterial hypertension with COPD: two similar phenotypes

Since the first reports of severe PH in COPD as a rare occurrence(15), it was observed that some patients with severe hemodynamic compromise presented with specific, notable characteristics: milder airflow obstruction manifesting with higher FEV_1 and thus a lower GOLD stage, associated with very low DL_{CO} and arterial oxygen partial pressure (PaO_2), and cardiocirculatory limitation to exercise as opposed to the ventilatory impairment usually observed in COPD patients with mild-moderate PH(15,45). This combination of specific features was called the "pulmonary vascular phenotype"(45). It was proposed that its pathophysiology sat in an intermediate position between mild-moderate PH with COPD on one side and PAH on the other side, where the severity of the hemodynamic impairment was higher than the classical COPD with mild PH phenotype, but lower than that observed in Group 1 PH; conversely, the severity of the lung disease was

proposed to be less extensive in the pulmonary vascular phenotype than in the case of mild-moderate PH with COPD, but more extensive than in PAH.

Of course, it is possible to conceive that patients with COPD (a highly prevalent disease in the general population) might also be diagnosed with a rare disease such as PAH, a situation that would explain the coincidence of an obstructive respiratory pattern of any degree with severe hemodynamic impairment(46). In this case, the patient should be consequently classified as suffering from Group 1 PH, with the implications for vasodilator treatment that this ensues, although with the comorbidity of COPD which may perhaps indicate a simpler therapeutic approach in the first line of treatment. On the other hand, it is possible also to speculate that patients with mild COPD and severe PH could constitute a separate phenotype, with its specific aetiopathogenesis, risk factors, clinical course and prognosis: this would be the proposed pulmonary vascular phenotype. In this latter case, the patient would be classified as having Group 3 PH, and despite the fact that in the presence of severe hemodynamic compromise the latest PH guidelines endorse referral to a PH expert centre for a tailored management approach, the evidence for pulmonary vasodilator treatment is much less clear and at most may consist in a trial treatment with one pulmonary vasodilator for a period of time followed by close monitoring. In recent years, the identification of features or traits that might help distinguish the pulmonary vascular phenotype from the casual concurrence of PAH and COPD has generated much debate, as this is a very frequent clinical conundrum for PH specialists attending patients with COPD and the obvious implications for management. A recent analysis of patients labelled as PAH in the COMPERA and ASPIRE registry(47) (the latter being a single, high-volume PH expert centre based in the United Kingdom) has revealed that those patients with PAH, a low DL_{CO} and a history of smoking (but no discernible lung disease, thus categorised as suffering from Group 1 PH) have outcomes that are virtually non-distinguishable from those of patients with PH associated with lung disease and much worse than those of non-smoker PAH patients with higher DL_{CO}. The association of milder airflow obstruction and more severe gas exchange impairment is a particularly puzzling feature that has defied the study of COPD associated with PH in the last decades. Whichever the cause, it has been proposed that the presence of severe hypoxemia, more than the level of airflow impairment, might be the key feature distinguishing the pulmonary

vascular phenotype compared to the chance co-existence of PAH and COPD(38). Indeed, cohort studies and data from registries show that the level of hypoxemia in patients with the characteristics commonly held as typical of the pulmonary vascular phenotype is seldom seen in patients categorised as suffering from PAH(38). However, this question still needs to be addressed in future, prospective trials.

Severe COPD-severe pulmonary hypertension phenotype

The occurrence of severe PH is not confined to the association with milder airflow obstruction in COPD patients. Indeed, severe hemodynamic compromise may also be found in patients with severe or very severe COPD. Because the presence of severe PH, despite the well-known referral bias in most cohort study, is still a rare finding, these patients can't be lumped together with those with mild-moderate PH and COPD. In fact, a very recent work have found that patients with severe PH and severe or very severe COPD have the worst survival of all combinations of obstruction and PH severity across the spectrum of COPD(48). Despite this, there are few studies specifically focused on this subgroup, and most registry data and retrospective cohorts previously published lump together all patients with COPD and severe PH in one group, mixing the pulmonary vascular phenotype and the "severe COPD-severe PH" phenotype. This "lumping" may be evidenced by the fact that there is no significant difference between airflow obstruction in the mild-moderate PH group versus the severe PH group in registry data studies looking at COPD patients(30), despite the fact that milder airflow obstruction is one of the hallmarks of the pulmonary vascular phenotype. The dire prognosis of the severe COPD-severe PH phenotype suggests that future studies should focus on elucidating the pathogenesis, clinical course and characteristics of these patients specifically, as they might require specific interventions that are not liable to generalization from other phenotypes of PH in COPD.

Aetiopathogenesis of pulmonary hypertension in COPD

The initial insults that cause PH in patients with COPD are not well known. Despite significant advances in understanding the mechanisms that contribute to the development of PH in COPD, there is less evidence about the root causes.

Some of these causes may be genetic, as a striking number of polymorphisms in genes encoding for cytokines and growth factors have been discovered in COPD patients with PH. Perhaps the most studied of these is the polymorphism of the gene encoding for the 5-hydroxytryptamine transporter, one form of which seems to be linked to severe PH in COPD(49) in a proportional fashion to the severity of PH(50). Other polymorphisms in mild-moderate COPD with and without PH have been observed in the genes for the endothelial nitric oxide synthetase (eNOS), where the BB genotype was associated with significantly higher systolic pulmonary artery pressures on echocardiography, compared to patients with the AB and AA genotypes(51); this genotype had been previously associated with common cardiovascular risk factors such as diabetes mellitus and coronary artery disease. Other polymorphisms, such as the one for the angiotensin converting enzyme gene, have been studied in COPD patients in association with electrocardiographic findings(52) and thus are less-robustly linked with the presence or absence of PH; however, the DD variant of the angiotensin converting enzyme gene has been convincingly linked to exercise-induced PH in COPD patients in a small study using RHC to detect the condition(53).

The evidence arising from mutations of the gene for kinase insert domain receptor is more complicated. Carriers of this mutation may exhibit pulmonary emphysema(54) in lung scans and in post-mortems(55), as well as developing PH; this is similar to carriers of a different mutation, of the gene for T-box transcription factor 4, who may exhibit obstructive lung disease and PH(56). However, these two rare genetic disorders are also sometimes associated with interstitial lung disease on imaging and/or with restrictive functional lung pattern, thus the causative link is less clear and the relative frequency of the morphological or functional alterations as well as its relationship with the frequency and severity of PH are not known.

Recently, the hypoxia-inducible factor 2 (HIF-2) has been proposed(57) as a key actor in the development of COPD, operating a switch between progression to a "non-vascular phenotype" (characterised by severe airflow obstruction and emphysema and mild to moderate vascular injury and remodelling) and progression to the pulmonary vascular phenotype (mild to moderate airway obstruction and emphysema and severe vascular injury and remodelling). In the first phenotype, in response to a series of endogenous and exogenous stimuli, there would be a loss of adaptive upregulation of HIF-2 (effectively leading to its downregulation); on the contrary, in

the pulmonary vascular phenotype there would be a maladaptive upregulation leading to greater vascular disease and remodelling. This intriguing hypothesis may give a pathophysiological explanation to the increased pulmonary vascular remodelling seen in COPD with severe PH(58).

Chronic obstructive pulmonary disease is of course mostly considered to be a disease of environmental exposure, although new paradigms are arising concerning different aetiologies of this disease and the definition of "aetiotypes"(8). The most studied causative agent in COPD is cigarette smoking, which is a well-known cause of endothelial dysfunction(59,60), while biomass smoke has been identified as an alternative mechanism to COPD, but it has not been evaluated as an agent causing pulmonary circulatory impairment per se(61). Other environmental exposures such as indoor and outdoor air pollution have been evaluated for COPD(62) and PH(63,64) separately, establishing clear links between the concentration of pollutants and each of the two diseases, but they have never been assessed in the specific case of PH associated with COPD. The intersection between cigarette smoking, endothelial dysfunction and pulmonary and systemic arterial stiffness and remodelling will be exposed in the next paragraph, dealing with the pathophysiology of PH in COPD.

Pathophysiological mechanisms of pulmonary hypertension in COPD

The pathophysiology of PH in COPD is complex and the result of several interplaying factors, which are incompletely understood. Some of these factors have been identified in previous studies: endothelial dysfunction, arterial stiffness and remodelling, parenchymal emphysematous destruction and hypoxic pulmonary vasoconstriction.

The main mechanisms focus of this thesis are endothelial dysfunction and pulmonary vascular remodelling, both playing a prominent role in the pathogenesis of PH in COPD, triggered and sustained by exposure to cigarette smoke. Cigarette smoke also affects systemic arteries and there is ample evidence of a strong association between systemic endothelial dysfunction and cardiovascular risk and disease; thus, the common etiopathogenetic factor of cigarette smoke explains the frequent association between COPD and cardiovascular disease.

Endothelial dysfunction, arterial stiffness and arterial remodelling in COPD

A healthy endothelium plays a fundamental role in maintaining circulatory homeostasis throughout the body(65). Besides its other functions in the regulation of immune responses, vascular permeability and hemostasia, the endothelium reacts to fluctuations in blood pressure by secreting nitric oxide (NO), a small but extremely potent molecule with modulatory effects on inflammation, oxidation and vasodilation. Secretion of NO is modulated through the action of an endothelium-specific enzyme called endothelial nitric oxide synthase enzyme (eNOS). Cardiovascular risk factors such as cigarette smoking, systemic hypertension and diabetes mellitus, however, alter this homeostatic state and reduce the bioavailability of NO by increasing oxygen reactive species and depleting cardioprotective factors such as tetrahydrobiopterin(66). Cardiovascular disease is thus associated with endothelial dysfunction, and the removal of cardiovascular risk factors has restorative effects of the endothelium. Endothelial cells affected by endothelial dysfunction are both those found lining the blood vessels within the distinct organs and tissues as well as circulating progenitor endothelial cells, which participate in vascular proliferation and repair. Several invasive and non-invasive methods have been developed to measure endothelial dysfunction in humans, the first mostly directed at the coronary vascular territory such as coronary epicardial function or coronary micro-vascular function, while the latter assessing peripheral vascular competence as a surrogate measurement. Among these, flow-mediated dilation of the brachial artery (FMD) is the most common technique as it is non-invasive, relatively cheap and highly reproducible if performed by properly trained personnel(66). It consists of assessing the ability of the brachial artery to dilate following the reactive hyperaemia caused by supra-systemic pressure inflation of a sphygmomanometer cuff and subsequent rapid release of the pressure; when the artery dilates poorly, endothelial dysfunction is detected.

Another indicator of cardiovascular risk is arterial stiffness, an increased rigidity of the arterial wall due to the loss of its normal elastic properties(67). The pathogenesis of arterial stiffness is incompletely understood, but it relates to degradation of elastic fibres(68) and an increased deposition or switch in the type of collagen fibres(69). This process may be genetically determined or as a result of a pathologic process in the cardiovascular system, and it results in vascular remodelling of the elastin-collagen components in the arterial wall(69). Arterial stiffness has been

recognised not only as a main physical mechanism for systemic arterial hypertension, but it has also been implicated in other cardiovascular pathologies and as a hallmark of cardiovascular risk factors in general. Arterial stiffness may be measured through non-invasive mechanisms, among which there are pulse wave velocity (PWV) and pulse wave analysis (PWA). Carotid-femoral PWV is the measurement of the delay between the ventricular contraction and the pulse in the carotid and femoral arteries; PWA is performed at the level of the brachial artery and it's the measurement of the reflex-wave produced by the pulse plus the increased component due to the stiffened arterial wall.

Patients with COPD suffer from increased risk of cardiovascular events(39) and death(70) and, unsurprisingly, they also present endothelial dysfunction (71,72) and arterial stiffness(73) in systemic arteries. At least part of this association can be traced back to the common, deleterious effect of cigarette smoke. The loss of vasodilating properties of the endothelium in COPD is proportional to the degree of airflow obstruction and of emphysema; similarly, emphysema correlates positively with arterial stiffness(74). In patients with COPD, higher levels of systemic inflammation are associated with the presence of PH(75) assessed through echocardiography, which may play a role in the higher frequency of acute exacerbations of COPD patients with mPAP >18 mmHg previously observed(16). Cigarette smoking is a known causative agent of endothelial dysfunction (76) and of COPD(77), where it causes endothelial dysfunction(59,78). The mechanisms linking endothelial dysfunction and COPD are complex: in two previous studies by our group(78,79), patients with COPD had lower levels of circulating endothelial progenitor cells compared to non-smoking and smoking controls; smoking controls and COPD patients also showed reduced FMD compared to non-smoking controls, even though these two phenomena appear to be unrelated(78,79). In a subsequent paper from our group(80) studying circulating endothelial microparticles (which are associated with endothelial apoptosis and damage) and progenitor cells (associated with repair), COPD patients showed an imbalance between endothelial damage and repair capacity which suggested a phenotype of loss of endothelial competence.

Many of the mechanisms here highlighted in systemic arteries of COPD patients are also at play in the lung vessels, illustrating a continuum of vascular injury throughout the body. Our group

found an increased recruitment of endothelial progenitor cells in the pulmonary vessel wall of COPD patients(79,81), likely as a response to injury. However, in vivo studies have shown that these cells present impaired proliferation, differentiation, migration and physiologic senescence in animals exposed to tobacco(82). These defective progenitor cells are able to migrate from the pulmonary vasculature into the intimal layer and differentiate into smooth muscle cells, contributing to vascular remodelling(83). At the level of the pulmonary tissue, endothelial dysfunction has been observed in pulmonary arteries of COPD patients(79) even with mild airflow obstruction, and its severity is directly correlated with that of the obstructive impairment(84). Pulmonary arteries of COPD patients(85) and even those of smokers with normal lung function(86) display reduced expression of eNOS; furthermore, small pulmonary arteries and lung microvasculature from patients with COPD with and without PH show reduced number of endothelial cells(87). It has previously been shown that patients with COPD and pulmonary vascular impairment present endothelial dysfunction in systemic arteries, showing lower FMD compared to COPD patients without derangement of the pulmonary vasculature(78). Inflammatory cells, especially CD8+ T lymphocytes, are also prominent in pulmonary arteries of COPD patients compared to those of non-smokers and smokers with normal lung function(88), in a proportional manner to the level of endothelial dysfunction shown in the same arteries. Arterial stiffness of the pulmonary arteries has been previously evidenced through intra-vascular ultrasound(89) in COPD patients with and without PH.

Endothelial dysfunction and arterial stiffness represent two manifestations of the same vascular damage, which is present in COPD both in the pulmonary and in the systemic circulation. In this sense, endothelial dysfunction and arterial stiffness of systemic arteries might be seen as manifestations of the systemic effects of COPD(90), where these effects see at their centre the vascular endothelium.

The link between endothelial dysfunction and arterial stiffness in COPD patients with and without pulmonary vascular disease has not been studied previously. It is conceivable that, given the common risk factors and aetiopathogenesis of these two phenomena, they might be observed in conjunction in COPD patients with pulmonary vascular disease; however, this has not been prospectively assessed in the same cohort of patients.

Arterial remodelling has been amply documented in COPD, irrespective of the presence of PH(87), and manifests itself as abnormal medial and intimal thickening of pulmonary arteries, with net increase in vessel wall size. This increase is mostly driven by proliferation of smooth muscle cells and muscularization of pulmonary arterioles(91), which is especially prominent in severe PH(58); on the other hand, the presence of a hyperproliferative endothelium with occlusive features such as plexiform lesions is more controversial in COPD and thus far only observed sporadically in association with severe PH(58,92). Muscularization may also extend in the post-capillary territory, where extracellular matrix is more highly represented than in the arterial compartment(93). Cigarette smoking is an important cause of arterial wall remodelling, causing intimal thickening even in smokers with normal lung function(84); however, smoking cessation may partially reverse these effects even in patients with established COPD(94).

Another mechanism of increased pulmonary vascular resistance is loss of lung vascular bed, which is present in small arteries of the COPD lung even in the absence of PH(95), but it's more prominent in severe PH, both microscopically and macroscopically. Loss of capillary density was observed recently in COPD with severe PH in post-mortems(58) as well as in advanced CT-imaging analysis(96). The loss of pulmonary vessels and general parenchymal destruction that exists in the emphysematous lung have long been considered main causes of increased PVR, as an equal amount of blood flow needs to be accommodated in a smaller vascular network, thereby increasing pulmonary pressure and, in the long run, vascular resistance.

Hypoxic vasoconstriction and gas exchange impairment in COPD with and without pulmonary hypertension

Hypoxia is the main mechanism through which, traditionally, the presence of PH is explained in the context of lung disease in general, and COPD specifically(2). Hypoxic vasoconstriction is the mechanism allowing pulmonary vessels to reduce blood flow to hypoxic areas of the lung, in an attempt to reduce ventilation/perfusion (V_A/Q) imbalance. Hypoxia can produce the transient effect of increasing PVR in COPD patients during episodes of acute exacerbation, exercise or during sleep. However, when V_A/Q mismatch is sustained over time, hypoxia is also a potent stimulant of arterial cell proliferation(93). Furthermore, chronic hypoxia can decrease the distensibility of pulmonary arteries even in healthy individuals(97); Arterial remodelling is seen for instance in healthy high-altitude natives who suffer from sustained hypoxia(98); this

remodelling is reversible upon returning to sea-level for a sufficient amount of time(99). The influence of hypoxia in COPD has recently been proposed as a pivotal factor into the expression of a more “parenchymal” versus “vascular” phenotype of COPD, through the action of the hypoxia-inducible factor-2 as a “switch” between the two conditions through its hyperexpression(57).

However, arterial remodelling exists even in mild COPD patients who are not hypoxemic(84); furthermore, oxygen supplementation reduces the progression but does not reverse PH in COPD patients(21), highlighting that other factors besides hypoxia are necessary to sustain the vascular injury in COPD. Certainly, the concurrence of cigarette smoke and chronic hypoxia may have a synergic effect in decreasing distensibility and inducing thickening of the vessel wall ultimately leading to an increase in PVR in COPD patients.

One of the most striking clinical features of PH in patients with COPD is hypoxemia. Causes of hypoxemia have been classically divided into hypoventilation, diffusion impairment, shunt and

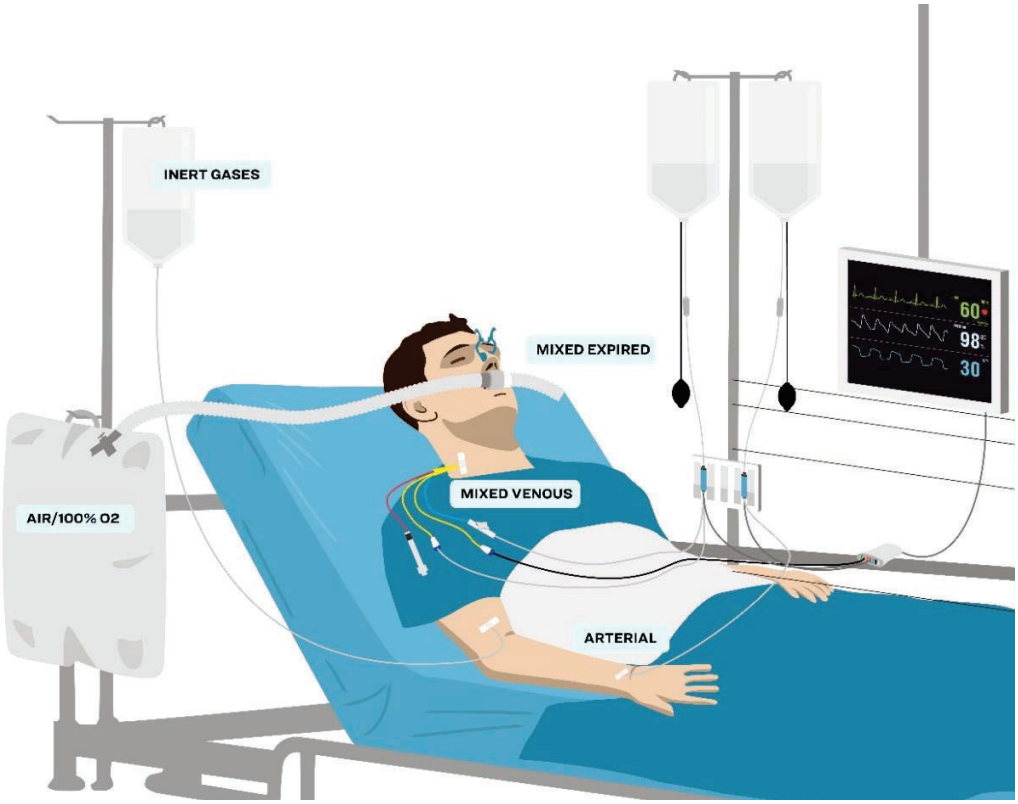

Figure 2. Multiple Inert Gases Elimination Technique. Original figure.

ventilation-perfusion inequality; the reduction of inspired oxygen pressure (e.g. in subjects living at high altitude) is considered a fifth, somewhat special circumstance. With increased knowledge of the main pathophysiologic mechanisms in lung health and disease, as well as the development of techniques able to explore these mechanisms, the understanding of the different causes of hypoxemia in pulmonary pathology has increased significantly and great efforts have been taken in the last decades of the 20th century to elucidate the different components causing hypoxemia in parenchymal lung disease. Hypoxemia may be present in COPD, especially in advanced stages, and its presence is in fact considered a clinical sign of advanced disease, especially when chronic respiratory failure appears. In the 1970s, some studies using the multiple inert gases elimination technique (MIGET) explored the pathophysiology of COPD to elucidate the causes of hypoxemia in COPD patients. The MIGET (Figure 2)(100) is a technique where 6 inert gases, with a measured blood solubility, are injected through a peripheral vein. The patient is placed in a semi-recumbent position and arterial and venous peripheral access are obtained, as well as contemporaneous right heart access through a Swan-Ganz catheter. Exhaled air can be extracted via a heated metal mixing chamber connected to the patient through a mouthpiece fitted with a two-way, non-rebreathing valve and while the patient wears a nose-clip; the patient breathing conditions are reported (commonly either room air conditions or 100% oxygen through a Douglas bag). The gases, mixed into a solvent such as 5% dextrose or saline solution, and injected in the continuous perfusion, in order of increasing solubility, are: sulphur hexafluoride (SF-6), ethane, cyclopropane, halothane, diethyl ether and acetone. Mixed expired oxygen and carbon dioxide are measured with a mass spectrometer and minute ventilation and breathing frequency are recorded minute by minute using a calibrated Wright spirometer. Once the gases have been injected with a continuous pump for at least 20 minutes and during the continuation of the infusion, with the patient in a respiratory and cardio-circulatory steady state (assessed through less than 5% change in heart and respiratory rate and minute ventilation), pulmonary hemodynamic measurements are obtained like in a normal RHC; then, at exactly the same time, a sample of arterial and venous blood as well as exhaled air are obtained. The samples are analysed through a gas chromatographer to establish the exact concentrations of the inert gases. Some other variables such as oxygen and carbon dioxide pressures in arterial and venous blood corrected by body

temperature are calculated, as well as arterial and venous oxygen content, oxygen delivery and consumption and alveolo-arterial gradient of oxygen. From the concentrations of the inert gases, the values of retention and excretion of each of them is determined: the retention of the least soluble gases is sensitive to the presence of shunt and low V_A/Q; the excretion of the most soluble gases is instead sesnsitive to the presence of high V_A/Q and deadspace.(101). Graphically, the results are represented in two curves: the retention curve is the plot of the ratio of arterial to mixed venous concentration against solubility of the gas; the excretion curve is the plot of the ratio of mixed expired concentration to mixed venous concentration against solubility(102). This data is then elaborated by an algorithm that fits the ventilation and perfusion distributions in the patients' lungs that would best explain the retention and excretion of the 6 inert gases. With this, the algorithm builds a model of the amount of blood flow and of ventilation in a lung of 50 compartments with different VA/Q ratios, ranging from 0 (shunt) to >100 (dead space) (Figure 3). From the distributions of blood flow and ventilation in this multicompartimental lung, several key measurements may be derived: the dispersion of perfusion (LogSD Q) and ventilation (LogSD V) distributions computed on a logarithmic scale constitute the extent of V_A/Q imbalance (the upper normal limit being 0.60 for LogSD Q and 0.65 for LogSD V); the retentions and excretions of each of the 6 inert gases, corrected for dead space (DISP R–E*, normal value 3), is considered as a general descriptor of V_A/Q mismatch(103). The relative percentages of blood perfusing poorly ventilated areas (Low V_A/Q) or areas with a negligible amount of ventilation (shunt), as well as the percentages of blood flow perfusing areas with little perfusion (High V_A/Q) or with negligible amount of perfusion (dead space) are also evaluated. The PaO_2 measured during the sampling subtracted to the PaO_2 predicted by the model built by MIGET is assessed as an indirect measurement of the diffusion capacity across the alveolo-capillary interface(104).

The MIGET technique has been used extensively since the 1970s to evaluate the pathophysiology of many respiratory conditions (asthma, COPD, pulmonary fibrosis) and pulmonary vascular diseases (PAH, pulmonary embolism, CTEPH). In the study of COPD, some of the causes that had previously been evoked to explain hypoxemia were alveolo-arterial difference in oxygen, venous admixture and physiologic deadspace; but significant doubts initially remained as to whether impairment of oxygen diffusion across the alveolo-capillary interface could be contributing to

hypoxemia, whether deadspace was a significant factor, and how did they combine with the alterations in pulmonary ventilation which are the hallmark of COPD(105). A landmark study by Wagner et al established in 1972 that gas exchange impairment in COPD was due almost entirely to V_A/Q mismatch(105) with virtually no diffusion impairment. The authors identified three patterns of V_A/Q mismatch which they called H (for high, with high V_A/Q pattern), L (for low, exhibiting a low V_A/Q pattern) and HL (for mixed pattern, high and low) and they correlated these findings with the clinical picture of the 23 patients examined. They were able to establish that the H pattern was more common among patients with predominant emphysema (type A), whereas patients with mostly chronic bronchitis (type B) were equally distributed among the H, L and HL patterns. Therefore, the patterns of V_A/Q mismatch seemed to only partially correspond to the clinical features of COPD commonly employed to describe patients' characteristics. Subsequent studies have shown that patients with mild COPD already present with significantly altered V_A/Q

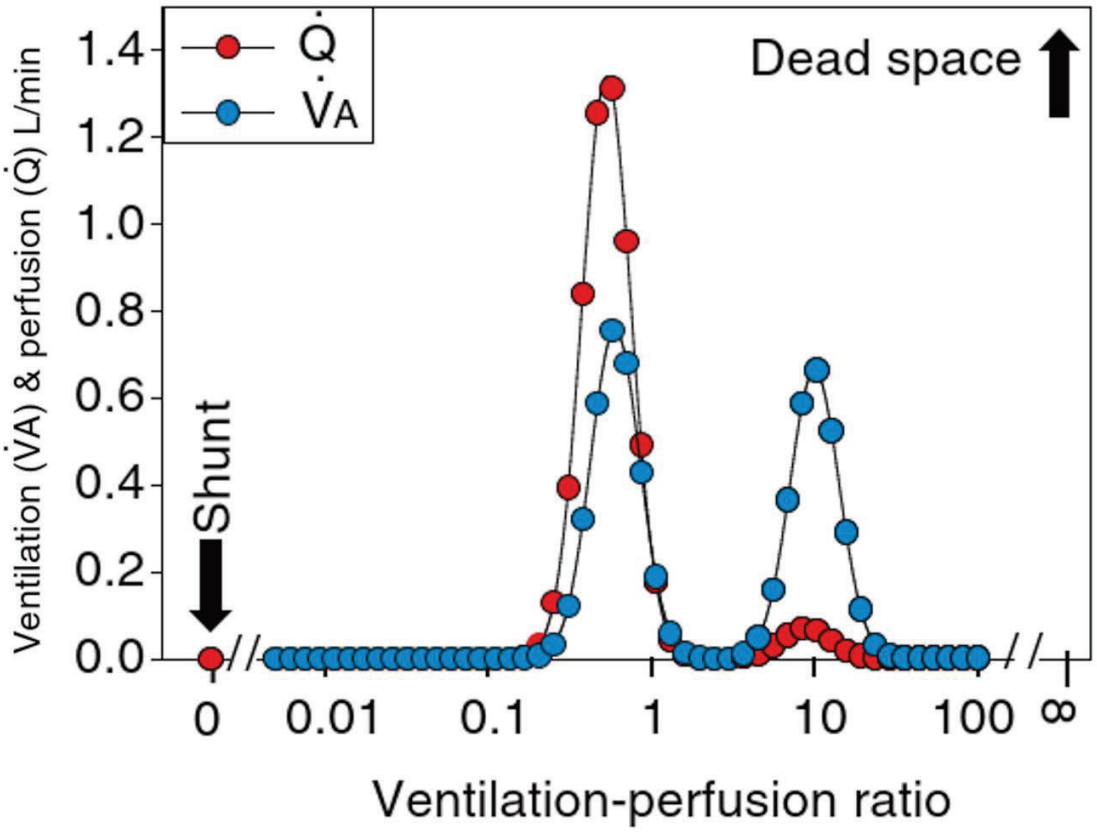

Figure 3. Ventilation/perfusion distribution in a seated young healthy subject. Adapted from Wagner PD, BRN Reviews 2019 (102).

inequality(106,107), pointing out to the fact that V_A/Q mismatch is a phenomenon occurring early in COPD natural history. Despite this, there is a certain proportionality between the level of airflow impairment and V_A/Q mismatch, since an increase in DISP R/E* is correlated with declining post-bronchodilator FEV_1(108); furthermore, the imbalance is worsened during episodes of acute exacerbation and partially reverses to previous levels after acute respiratory failure has subsided(109).

When patients with COPD are exposed to 100% oxygen, V_A/Q mismatch worsens significantly due to the release of hypoxic vasoconstriction and increased perfusion of less ventilated areas(110,111). For the same reason, administering a pulmonary vasodilator in oral (nifedipine, sildenafil) or inhaled form (nitric oxide) to patients with COPD increases overall V_A/Q mismatch and blood flow shifts to areas of lower ventilation, the variation in perfusion increases and gas exchange worsens though an increase in alveolo-arterial oxygen gradient and fall in PaO_2 (106,111,112). These phenomena are all consistent with the release of hypoxic vasoconstriction. The ability of the lung to counter the effects of V_A/Q mismatch through hypoxic vasoconstriction has been found to be inversely correlated with the degree of pulmonary arterial remodelling(113). Thus, pulmonary arteries of COPD patients lose the ability to adapt to hypoxemia as the disease progresses.

The MIGET has also been used to elucidate the characteristics of V_A/Q relationships in pulmonary vascular disease, whether isolated, secondary to chronic embolic disease or in association with COPD. Dantzker et al. studied patients with idiopathic PAH and CTEPH, finding that hypoxemia was present only in half of the patients, but an increased alveolo-arterial gradient of oxygen was present in all of them. This phenomenon was explained by a combination of increased V_A/Q mismatch, intrapulmonary shunt and the presence of a low mixed venous partial pressure of oxygen (PvO_2) as a result of low cardiac output, with no significant diffusion impairment. The authors explained their findings of increased perfusion to areas of low V_A/Q as the result of overperfusion of normal lung units caused by the obliteration of some of the pulmonary vascular bed; they also suggested that the increased blood flow in areas of low V_A/Q and shunt may partly be the consequence of interstitial pulmonary oedema caused by the increased intravascular pressure. As for the low PvO_2, this was explained by the lower cardiac output that some of the

patients presented; in fact, the authors observed that when either V_A/Q inequality or low PvO_2 did not co-exist, the patient was not hypoxemic, since one of the two factors was not sufficient to lower PaO_2 below the normal values.

In studies exploring COPD patients with predominantly mild-moderate PH through the MIGET(114), the V_A/Q mismatch observed was similar to that previously observed in COPD patients without PH(108): the mild PH observed in this patients, therefore, does not seem to affect V_A/Q relationships significantly. However, when patients with COPD and mild PH are administered a pulmonary vasodilating agent, V_A/Q mismatch, perfusion dispersion and arterial oxygenation are worsened(114). For these reasons, even in patients with COPD who present mild or moderate PH, the administration of pulmonary vasodilators is contraindicated: the reduction in PVR is obtained at the cost of worsening gas exchange.

It is known that there is a direct correlation between the increase of mPAP and the decrease of PaO_2 in PH patients with COPD(37). In data collected from the COMPERA Registry, the mean PaO_2 in COPD patients with PH was 55 mmHg (SD ±10 mmHg)(30) and in the French Registry for severe PH in COPD, the median PaO_2 was 50 mmHg (percentiles 25 and 75: 45-62 mmHg) and 82% of patients was on chronic oxygen therapy. Nonetheless, the relationship between the increase in pulmonary artery pressure and the decrease in arterial oxygen is variable across the spectrum of COPD and pulmonary vascular disease. Interestingly, COPD patients with an especially severe form of PH, those affected by the pulmonary vascular phenotype, show a degree of hypoxemia that does not seem to correlate with the ventilatory impairment. The specific clinical picture of patients with COPD and the pulmonary vascular phenotype, where milder airflow obstruction is associated with strongly altered gas exchange manifesting with severe hypoxemia paired with severe pulmonary vascular disease, had never been studied before specifically and poses fundamental questions on the relative role of ventilation impairment versus perfusion impairment in these patients. To what degree is the hypoxemia a cause or a consequence of the pulmonary vascular derangement in COPD patients with severe PH is one of the leading questions of this work.

Considering the accumulated evidence, it is still unclear whether patients with COPD and pulmonary vascular disease present increased endothelial dysfunction and arterial stiffness in systemic arteries, compared with patients with COPD without pulmonary vascular disease; also, the impact of tobacco products and cardiovascular risk or comorbidities on this possible phenomenon have not been clearly elucidated. Furthermore, given the disconnect between mild ventilatory impairment on one side and severe PH and gas exchange impairment on the other, and in the hypothesis that there is indeed an effect of pulmonary vascular disease on endothelial dysfunction in COPD patients, it is unclear whether this increased endothelial dysfunction may lead to loss of pulmonary vascular regulation of gas exchange, resulting in the severe hypoxemia seen in the pulmonary vascular phenotype.

Pulmonary vasculature in COPD: The silent component

Isabel Blanco[1,2,3], Lucilla Piccari[1], Joan Albert Barberà[1,2,3]

[1]Department of Pulmonary Medicine, Hospital Clínic

[2]August Pi i Sunyer Biomedical Research Institute (IDIBAPS), University of Barcelona

[3]Biomedical Research Networking Center in Respiratory Diseases (CIBERES), Madrid, Spain A

Correspondence: Dr Joan A. Barberà, Servei de Pneumologia, Hospital Clínic. Villarroel, 170, Barcelona 08036, Spain. Email: jbarbera@clinic.ub.es

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Abstract

Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction that results from an inflammatory process affecting the airways and lung parenchyma. Despite major abnormalities taking place in bronchial and alveolar structures, changes in pulmonary vessels also represent an important component of the disease. Alterations in vessel structure are highly prevalent and abnormalities in their function impair gas exchange and may result in pulmonary hypertension (PH), an important complication of the disease associated with reduced survival and worse clinical course. The prevalence of PH is high in COPD, particularly in advanced stages, although it remains of mild to moderate severity in the majority of cases. Endothelial dysfunction, with imbalance between vasodilator/vasoconstrictive mediators, is a key determinant of changes taking place in pulmonary vasculature in COPD. Cigarette smoke products may perturb endothelial cells and play a critical role in initiating vascular changes. The concurrence of inflammation, hypoxia and emphysema further contributes to vascular damage and to the development of PH. The use of drugs that target endothelium-dependent signalling pathways, currently employed in pulmonary arterial hypertension, is discouraged in COPD due to the lack of efficacy observed in randomized clinical trials and because there is compelling evidence indicating that these drugs may worsen pulmonary gas exchange. The subgroup of patients with severe PH should be ideally managed in centres with expertise in both PH and chronic lung diseases because alterations of pulmonary vasculature might resemble those observed in pulmonary arterial hypertension. Because this condition entails poor prognosis, it warrants specialist treatment.

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Pulmonary vasculature in COPD: The silent component

ISABEL BLANCO, Lucilla PICCARI AND Joan Albert BARBERÀ

Department of Pulmonary Medicine, Hospital Clínic and August Pi i Sunyer Biomedical Research Institute (IDIBAPS); University of Barcelona and Biomedical Research Networking Center in Respiratory Diseases (CIBERES), Madrid, Spain

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction that results from an inflammatory process affecting the airways and lung parenchyma. Despite major abnormalities taking place in bronchial and alveolar structures, changes in pulmonary vessels also represent an important component of the disease. Alterations in vessel structure are highly prevalent and abnormalities in their function impair gas exchange and may result in pulmonary hypertension (PH), an important complication of the disease associated with reduced survival and worse clinical course. The prevalence of PH is high in COPD, particularly in advanced stages, although it remains of mild to moderate severity in the majority of cases.

Endothelial dysfunction, with imbalance between vasodilator/vasoconstrictive mediators, is a key determinant of changes taking place in pulmonary vasculature in COPD. Cigarette smoke products may perturb endothelial cells and play a critical role in initiating vascular changes. The concurrence of inflammation, hypoxia and emphysema further contributes to vascular damage and to the development of PH.

The use of drugs that target endothelium-dependent signalling pathways, currently employed in pulmonary arterial hypertension, is discouraged in COPD due to the lack of efficacy observed in randomized clinical trials and because there is compelling evidence indicating that these drugs may worsen pulmonary gas exchange. The subgroup of patients with severe PH should be ideally managed in centres with expertise in both PH and chronic lung diseases because alterations of pulmonary vasculature might resemble those observed in pulmonary arterial hypertension. Because this condition entails poor prognosis, it warrants specialist treatment.

Key words: hypoxia, obstructive disease, pulmonary circulation, pulmonary remodelling, targeted pulmonary arterial hypertension drugs.

Abbreviations: ABG, arterial blood gas; AMP, adenosine monophosphate; bid, twice a day; cGMP, cyclic guanosine monophosphate; CI, cardiac index; COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity of the lung for carbon monoxide; EMP, endothelial microparticles; eNOS, endothelial NO synthase; EP, end point; ET-1, endothelin-1; FC, functional class; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HPV, hypoxic pulmonary vasoconstriction; LTOT, long-term oxygen therapy; LVRS, lung volume resection surgery; mPAP, mean pulmonary artery pressure assessed by right heart catheterization; NO, nitric oxide; PaO_2, partial arterial oxygen concentration; PAP, pulmonary artery pressure; PDE-5, phosphodiesterase-5; Peak VO2, oxygen consumption at peak exercise; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; qd, one a day; RCT, randomized clinical trial; RV, right ventricle; SMC, smoothmuscle cells; sPAP, systolic pulmonary artery pressure estimated by echocardiography; tid, three times a day; VA/Q, ventilation–perfusion; VEGF, vascular endothelial growth factor; 6MWD, distance covered in the 6-min walk test.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction that results from an inflammatory process affecting the airways and lung parenchyma. Despite major abnormalities taking place in bronchial and alveolar structures, changes in pulmonary vessels also represent an important component of the disease. Alterations in vessel structure and abnormalities in their function impair gas exchange and may result in pulmonary hypertension (PH), which is a relevant complication in the natural history of the disease, as it is highly prevalent and associated with reduced survival and worse clinical course.

The etiologic and pathogenic mechanisms responsible for pulmonary vascular abnormalities in COPD have been extensively investigated in recent years. Studies in human cohorts and in experimental models have revealed a crucial role for endothelial damage in the pathogenesis of PH in COPD. Cigarette smoke products have been identified as major factors in the initiation of pulmonary vascular abnormalities. These observations have coincided with the development of drugs that target the vasodilator/vasoconstrictor

Correspondence: Dr Joan A. Barberà, Servei de Pneumologia, Hospital Clínic. Villarroel, 170, Barcelona 08036, Spain. Email: jbarbera@clinic.ub.es

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imbalance between endothelium-derived mediators and that have shown to be of great efficacy in patients with pulmonary arterial hypertension.

In this review, we recap the current knowledge on the clinical impact of pulmonary vascular abnormalities in COPD; we examine their potential mechanisms and causative agents and discuss the results of recent studies with endothelium modulating agents in this condition.

WHAT IS THE CLINICAL IMPACT OF PULMONARY HYPERTENSION IN COPD?

Prevalence

Pulmonary hypertension is defined by the abnormal increase of pulmonary artery pressure (PAP). Despite the upper normal limit of mean PAP being 20 mm Hg,[1,2] current guidelines define PH by a mean PAP ≥25 mm Hg.[3] Assessment of PAP requires right heart catheterization. In COPD, the majority of hemodynamic studies involving a large number of subjects have been performed in patients with advanced disease (stage IV of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification),[4–6] candidates to either lung transplantation or lung volume reduction surgery. In this group, the prevalence of PH is very high, affecting more than one third of patients (Figure 1).

Two recent studies have reported the prevalence of PH in patients with less severe disease. In a study conducted in 98 patients, Hilde *et al.*[7] reported a prevalence of PH of 5%, 27% and 53% in COPD GOLD stages II, III and IV, respectively. Portillo *et al*,[8] in a study conducted in 139 COPD patients, reported a prevalence of PH of 7%, 25% and 22% GOLD stages II, III and IV defined by spirometry, respectively (Figure 1).

In most cases, PH is of mild-to-moderate severity, with mean PAP <35 mm Hg. Severe PH is rarely seen in COPD. In a retrospective study conducted in 998 COPD patients, Chaouat *et al.*[9] identified only 27 patients with PAP >40 mm Hg. Whereas 16 of them had another disease, which could have been causing PH, in 11 (1.1% of the whole group), COPD was the only cause. The latter group of patients had moderate airway obstruction (forced expiratory volume in 1 s 50% predicted), severe hypoxemia, hypocapnia and very low carbon monoxide diffusing capacity. Similar results were obtained by Thabut *et al.*[6] who identified, in a cluster analysis, a subgroup of COPD patients characterized by moderate impairment of airway function and high levels of PAP, along with severe arterial hypoxemia. These findings indicate that there is a reduced subset of COPD patients with severe PH that share some clinical features with idiopathic PAH.[10] The prevalence of severe PH in COPD varies between series, depending on the severity of the disease and the cut-off PAP used to define it, but is generally less than 5% of cases.

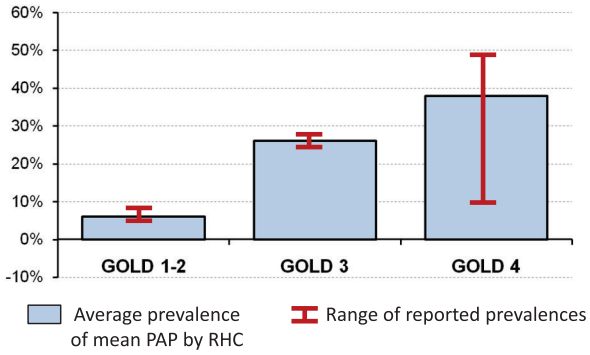

Figure 1 Reported prevalence of pulmonary hypertension, defined by a mean pulmonary artery pressure >25 mm Hg assessed by right heart catheterization, according to the spirometric Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage in chronic obstructive pulmonary disease patients (data obtained from References[4–8]). PAP, pulmonary artery pressure.

Current guidelines classify PH in COPD into three groups: COPD without PH, COPD with PH and COPD with severe PH (Table 1).[3] The latter group is defined by a mean PAP ≥35 mm Hg and/or a cardiac index <2 L/min/m^2, on the basis of the reduced prevalence of patients with these characteristics among COPD patients.

In patients with mild-to-moderate disease, the prevalence of PH is low.[7,8] However, in these patients, PH might not be present at rest but develop during exercise. Hilde *et al.*[7] showed that the majority of COPD patients present abnormal physiological hemodynamic responses during exercise, with an increase of PAP of similar magnitude in patients with and without PH at rest. In their study, 58% of patients without PH at rest developed abnormal increases of PAP, as assessed by the relationship increase of PAP/increase of cardiac output.[7] Portillo *et al.*[8] showed an abnormal increase of PAP during exercise in 71% of patients. Patients with exercise-induced PH are more prone to develop resting PH in the subsequent years.[11] Therefore, particular attention should be paid to these patients as they may suffer greater vascular impairment in their lungs.

Prognosis

The presence of PH is a strong predictor of mortality in COPD, survival being inversely related to the severity of PH.[9] Furthermore, echocardiographic signs of right ventricular dysfunction and electrocardiographic signs of right ventricular hypertrophy[12] or right atrial overload[13] are also predictive of survival.

In addition to the prognostic significance in relation to survival, the presence of PH in COPD is associated with poor clinical evolution and more frequent use of healthcare resources. A mean PAP >18 mm Hg is one of the best predictors of increased risk of hospitalization for COPD exacerbation.[14] Furthermore, Wells *et al.*[15]

Table 1 Classification of pulmonary hypertension in COPD

COPD without PH	PAP <25 mm Hg
COPD with PH	PAP ≥25 mm Hg (with supplemental O_2 if needed)
COPD with severe PH	PAP ≥35 mm Hg (with supplemental O_2 if needed) or PAP ≥25 mm Hg with low CI (<2 L/min/m^2)

CI, cardiac index; COPD, chronic obstructive pulmonary disease; PAP, pulmonary arterial pressure; PH, pulmonary hypertension.

showed that the enlargement of the pulmonary artery diameter, as assessed by a pulmonary artery/aorta (PA:A) ratio >1 in the computed tomography scan, was associated with future exacerbation episodes requiring hospitalization and that this ratio outperformed many established risk factors for exacerbation. Accordingly, COPD patients with an abnormal pulmonary vascular bed might have lesser functional reserve to overcome the changes that occur during exacerbation episodes and are at greater risk for hospitalization.

Pulmonary hypertension in COPD progresses over time, and its severity correlates with the degree of airflow obstruction and the impairment of pulmonary gas exchange. The rate of progression of PH is slow, with an average PAP increase of 0.6 mm Hg/year.[10] Because PAP is only moderately elevated and its rate of progression is slow, the right ventricle (RV) has time to adapt to such a modest increase in afterload. When PAP is chronically elevated, the RV dilates, and both end-diastolic and end-systolic volumes increase. The stroke volume of the RV is usually maintained, whereas its ejection fraction decreases. Yet, in clinically stable patients, RV contractility lies within normal limits irrespective of the PAP value. Decreased RV contractility in COPD has been observed only during exacerbation episodes in patients presenting marked peripheral edema.

In clinically stable COPD, peripheral edema is not a sign of RV failure because it might be present in patients without evidence of reduced cardiac output.[10] In COPD, peripheral edema results from a complex interaction between the hemodynamic changes and the balance between edema-promoting and edema-protective mechanisms. In patients with PH associated with chronic respiratory failure, both hypoxemia and hypercapnia aggravate venous congestion by further activating the sympathetic nervous system, already stimulated by right atrial distension. Sympathetic activation decreases renal plasma flow, stimulates the renin–angiotensin–aldosterone system and promotes tubular absorption of bicarbonate, sodium and water. Vasopressin also contributes to edema formation. It is released when patients become hyponatremic and its plasma levels rise in patients with hypoxemia and hypercapnia.

WHAT ARE THE MECHANISMS OF PULMONARY HYPERTENSION IN COPD?

Potential underlying mechanisms of PH in COPD include (i) increased vascular tone, (ii) arterial narrowing due to the remodelling of the vessel wall, (iii) reduced pulmonary vascular cross-sectional area, (iv) thrombosis and (v) blood hyperviscosity. These different mechanisms may concur in the same patient and determine the severity of PH. Underlying these mechanisms are a number of biological, structural and functional changes that take place in the pulmonary vasculature of COPD patients.

Endothelial dysfunction

Endothelial cells play a crucial role in regulating vascular homeostasis.[16] In pulmonary vessels, endothelial cells contribute to the reduced vascular tone,[17] regulate the adaptation of the vessels to increased flow[18] and modulate hypoxic vasoconstriction.[19,20] As in other pulmonary hypertensive states, endothelial dysfunction of pulmonary arteries plays a crucial role in the pathogenesis of PH in COPD. Endothelial dysfunction of pulmonary arteries has been shown at different degrees of COPD severity, in both patients with end-stage COPD who underwent lung transplantation[21] and patients with mild-to-moderate disease who underwent surgical lobectomy.[22] The impairment of endothelial function is associated with or results from changes in the expression or balanced release of vasoactive mediators with vasodilator properties, such as nitric oxide (NO) or prostacyclin and mediators with vasoconstrictive properties, such as endothelin-1 (ET-1) or angiotensin.

The expression of endothelial NO synthase (eNOS) in pulmonary arteries, which is diminished in patients with idiopathic PAH,[23] is also reduced in patients with COPD,[24] and in smokers without airflow obstruction[25] (Figure 2). Further, Nana-Sinkam *et al.*[26] have shown diminished expression of prostacyclin synthase in pulmonary arteries in patients with severe emphysema. Similarly, Tuder *et al.*[27] have demonstrated loss of expression of prostacyclin synthase in endothelial cells of pulmonary arteries in patients with associated forms of PH. Giaid *et al.*[28] showed that the expression of ET-1 in pulmonary arteries was increased in both primary and secondary forms of PH. However, studies in patients with mild-to-moderate COPD or severe emphysema have failed to show differences in ET-1 expression in pulmonary arteries when compared with nonsmokers.[24,25] Although, the latter finding could be due to the fact that patients evaluated in these series did not have PH. Furthermore, the expression of some growth factors (vascular endothelial growth factor, VEGF)[29] or their receptors (transforming growth factor-β type II receptor)[30] is up-regulated in pulmonary arteries of COPD patients (Figure 2).

Taken together, there is compelling evidence showing that COPD is associated with perturbed endothelium, not only in the pulmonary vasculature but also in systemic vessels. Such a state of altered endothelial function might predispose COPD to cardiovascular events,[31] which are a common cause of death in this disease.[32]

In addition to changes in vasoactive mediators and growth factors, COPD patients present structural damage in pulmonary endothelium. Areas of cell denudation and detachment between endothelial cells have been shown in pulmonary arteries of COPD patients.[33] Furthermore, patients with COPD show increased numbers of circulating endothelial microparticles (EMP),[34,35] which are small membrane-bound vesicles (0.1–1 μm diameter) released by endothelial cells after activation or apoptosis.[36] Such an increase of circulating EMP may reflect endothelial damage in COPD. Indeed, the number of circulating EMP further increases during exacerbation episodes and correlates with the severity of airflow obstruction.[37] Along this line, COPD patients also show reduced numbers of bone marrow-derived circulating progenitor cells.[38–40] These cells play a key role in the repair process of damaged endothelium and are closely related to endothelial function.[41]

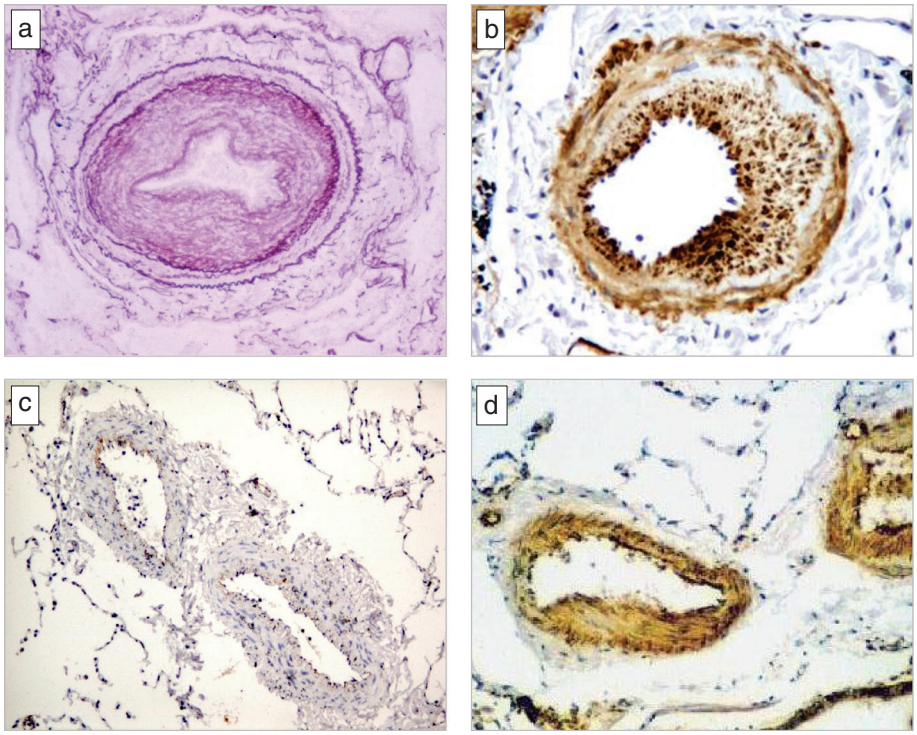

Figure 2 Pulmonary vascular changes in chronic obstructive pulmonary disease. (a) Pulmonary muscular artery stained with orcein stain showing prominent intimal hyperplasia and narrowing of the lumen caused, in part, by abundant elastin deposition. (b) Immunostaining for -smooth muscle actin revealing intimal proliferation of smooth muscle cells. (c) Immunostaining with monoclonal antibody against CD8 revealing CD8 T-lymphocyte infiltrate in the arterial adventitia. (d) Staining for vascular endothelial growth factor, which is increased in the arterial wall.

Endothelial dysfunction in COPD is not restricted to pulmonary vasculature as it has also been shown in systemic arteries.[39,42,43]

Vascular remodelling

Remodelling of pulmonary vessels is a major determinant of PH in COPD. The most prominent feature of pulmonary vascular remodelling in COPD is the enlargement of the intimal layer in muscular arteries, which is more pronounced in small arteries with a diameter of less than 500 μm[22,44,45] (Figure 2). Intimal hyperplasia is produced by the proliferation of cells that express smooth muscle α actin and other mesenchymal markers like vimentin[46] (Figure 2). In addition, there is deposition of elastic and collagen fibres.[46] Pulmonary vascular remodelling is apparent in patients with different degrees of COPD severity, and the presence of PH does not seem to be associated with greater derangement of the vessel structure.[47] Changes in the tunica media are less conspicuous, and the majority of studies have failed to show striking differences in the thickness of the muscular layer in COPD patients, as compared with controls.[22,44,45,47]

The extent of pulmonary vascular lesions correlates with the severity of PH. Whereas in patients with mild-to-moderate PH, vascular lesions are characterized by the intimal reaction with fibrous vascular occlusion, patients with severe PH may show complex vascular lesions including plexiform and angiomatoid lesions similar to those observed in PAH.[48]

Remodelling of pulmonary arteries is not restricted to patients with an established diagnosis of COPD. Indeed, intimal hyperplasia[46] and muscularization of small pulmonary arteries,[49] of similar magnitude than in COPD, also occur in heavy smokers with normal lung function. In fact, studies in experimental models of COPD indicate that pulmonary vascular changes antecede the development of emphysema.[49,50]

Emphysema

Derangement of the pulmonary vascular bed as a consequence of emphysema may contribute to the development of PH. A recent study by Peinado *et al.*[51] in explanted lungs of patients with severe COPD who underwent lung transplantation showed that those who had PH (of mild-to-moderate severity) show more severe morphologic signs of pulmonary emphysema, as compared with patients without PH. Interestingly, the degree of vascular remodelling was similar. These observations suggest that the concurrence of pulmonary emphysema with vessel remodelling might explain the development of severe PH in some COPD patients. In fact, Adir *et al.*[52] described a subgroup of patients with severe emphysema and normal airflow rates presenting with severe PH.

WHAT ARE THE CAUSATIVE AGENTS OF PULMONARY HYPERTENSION IN COPD?

Hypoxia has been classically considered the main pathogenic mechanism of PH in COPD. However, its role has been reconsidered because pulmonary vascular

remodelling and endothelial dysfunction can be observed in patients with mild COPD who do not have hypoxemia and in smokers with normal lung function[53] and because long-term oxygen therapy does not fully reverse PH or the pulmonary vascular lesions.[48] Furthermore, the relationship between arterial PO_2 and PAP is very weak, and arterial oxygen tension only accounts for 8% of the PAP.[4] In addition, the characteristics of pulmonary vascular remodelling in COPD differ substantially from those seen in individuals living at high altitude.[54]

We strongly believe that cigarette smoke exposure may explain the vascular changes observed in smokers and the majority of COPD patients,[53] although it might be insufficient to produce PH or severe PH, which occur only in a proportion of patients. Evidence supporting this contention derives from observations made in 'healthy' smokers and from experimental studies in animal models. Studies conducted in smokers without airflow obstruction have shown that these individuals present remodelling in pulmonary arteries,[22] impairment of endothelial function,[22] reduced expression of eNOS,[25] increased VEGF expression,[29] inflammatory cell infiltrate[55,56] and gene expression of cytokines and angiogenic mediators,[57] which are indistinguishable from those seen in patients with mild-to-moderate COPD and clearly differ from nonsmokers. In addition, experimental animal models chronically exposed to cigarette smoke develop pulmonary vascular changes similar to those seen in COPD patients; endothelial dysfunction,[58] vessel remodelling, vascular inflammation[59] and PH.[58] In these animal models, cigarette smoke exposure induces changes in gene expression of VEGF, VEGF receptor-1, ET-1 and inducible NOS,[49] mediators that regulate vascular cell growth and vessel contraction and are likely involved in the pathogenesis of pulmonary vascular changes of COPD. Interestingly, changes induced by cigarette smoke exposure in pulmonary vessels antecede the development of pulmonary emphysema.[49] Furthermore, exposure of pulmonary artery endothelial cells to cigarette smoke extract causes an irreversible inhibition of eNOS activity, which is due to diminished protein content and mRNA.[60] Cigarette smoke contains a number of products that have the potential to produce endothelial injury, among those the aldehyde acrolein seems to play a prominent role.[26]

Interestingly, individuals exposed to biomass smoke show structural changes in pulmonary arteries similar to those observed in smokers,[61] suggesting similar mechanisms of pulmonary vascular impairment. It is of note that PH appears to be more prevalent in subjects exposed to biomass smoke than in cigarette smokers[62] and that exposed subjects present endothelial dysfunction in systemic vessels.[63]

With this underlying disturbed endothelial function, hypoxia may act as a contributing factor that may further promote the development of PH. Indeed, in guinea pigs, the concomitant exposure to cigarette smoke and hypoxia resulted in a greater increase of PAP and right ventricle hypertrophy than either stimulus alone.[64] Hypoxia may induce the proliferation of smooth muscle cells (SMC) and fibroblasts in the vessel wall,[65] although the proliferative effects of hypoxia on SMCs are enhanced in the presence of mitogenic factors released by endothelial cells or fibroblasts.[65] Accordingly, endothelial damage induced by cigarette smoke and the subsequent imbalance among vasoactive and angioproliferative mediators may conform the substrate where hypoxic stimulus may further induce proliferative responses in SMCs and promote the development of PH.

Damaged pulmonary endothelium not only may predispose to the development of PH but also to the progression of emphysema. Indeed, in recent years, the concept of impaired endothelial cell survival has emerged as a critical factor in the pathogenesis of emphysema.[35,66,67] Thomashow *et al.*[35] have recently shown that the number of circulating EMP of an apoptotic origin was significantly associated with percent emphysema in COPD patients. Whether emphysema represents the lung expression of a generalized endothelial disorder or whether increased levels of EMP resulting from endothelial apoptosis in patients with emphysema may act as signalling elements capable of producing endothelial damage remains a matter of debate.[31] Interestingly, a recent study in rodents chronically exposed to cigarette smoke has shown that soluble guanylate cyclase stimulation, which is the target enzyme for NO in SMCs, not only prevents pulmonary vascular remodelling and the development of pulmonary hypertension, but also the progression of pulmonary emphysema,[68] thereby suggesting a close relationship between vascular changes and the formation of emphysema by alterations in the NO–cyclic guanosine monophosphate pathway axis.

Patients with COPD and also smokers without airflow obstruction show an increased number of inflammatory cells infiltrating the adventitia of pulmonary muscular arteries, largely constituted by activated T lymphocytes with a predominance of the CD8+ T cell subset.[55,69] The number of neutrophils, macrophages and B lymphocytes is minimal and does not differ from control nonsmokers. The role of inflammation on pulmonary vascular changes in COPD remains to be established, but recent evidences in PAH suggest that inflammation may play a significant role in the pathogenesis of PH.[70]

Accordingly, the initial event in the natural history of PH in COPD appears to be the injury of pulmonary endothelium by cigarette-smoke products or inflammatory cytokines. Endothelial damage results in the abnormal synthesis and release of vasoactive mediators that impair endothelium-dependent vasodilation and favours the action of factors that promote the proliferation of SMC and extracellular matrix deposition.[53] All these changes may contribute to intimal hyperplasia in pulmonary vessels resulting in the reduction of the arterial lumen and the increase of pulmonary vascular resistance (PVR). Arteries with endothelial dysfunction are more susceptible to the action of additional factors. Among those, sustained arterial hypoxemia and alveolar hypoxia in poorly ventilated lung units play a crucial role, because they may induce further endothelial impairment and vessel remodelling, either directly or through VEGF-dependent mechanisms, thus amplifying the initial effects of cigarette smoke products.[71] Finally, the loss of the pulmonary capillary bed in patients with severe emphysema may

further contribute to increase PVR and eventually result in severe PH.

ASSESSMENT OF PULMONARY HYPERTENSION

Recognition of PH in COPD is difficult, especially in its mildest form, because symptoms due to PH, such as dyspnea or fatigue, are difficult to differentiate from the clinical picture of COPD. Furthermore, the identification of some clinical signs may be obscured by chest hyperinflation or the large swings in intrathoracic pressure. Usually, the main suspicion is based on the presence of peripheral edema, but, as discussed above, in COPD this may not be a sign of right ventricular failure. Cardiac sounds may be disturbed by the presence of bronchial rales or overinflated lungs and the typical auscultatory findings of PH are uncommon in COPD patients.

Severe PH should be suspected in COPD patients presenting dyspnea and exercise intolerance disproportionate to the severity of airflow impairment, especially if these symptoms are accompanied by severe hypoxemia, hypocapnia and markedly reduced carbon monoxide diffusing capacity[9] (Figure 3).

Echocardiography is the most useful non-invasive diagnostic tool for the initial assessment of patients with suspected PH. Indications for echocardiography in COPD patients include the clinical suspicion of significant PH and to rule out concomitant left heart disease, which is frequently associated with COPD and may also produce PH. Nevertheless, the diagnostic accuracy of echocardiography for detecting PH in COPD is low.[72,73]

As recently reported, a PA:A ratio >1 on computed tomography scan outperforms echocardiography for diagnosing resting PH in patients with severe COPD.[74]

Pulmonary hypertension is confirmed by right heart catheterization, but it is not routinely recommended in the assessment of patients with COPD. Confirmatory right heart catheterization is indicated in candidates being put forward for surgical treatments. These include patients undergoing lung volume reduction surgery and bullectomy, those with suspected severe PH potentially amenable to targeted therapy and, in general, in those conditions where the results of the hemodynamic assessment will determine treatment options (Figure 3).

IMPLICATIONS FOR TREATMENT

In patients with associated PH, COPD should be optimally treated according to existing guidelines.[75] Treatments addressed to ameliorate PH in COPD include long-term oxygen therapy (LTOT) or vasodilator therapy.

Long-term oxygen therapy

It has been convincingly shown that LTOT prevents the progressive increase of PAP in patients with COPD and that when administered more than 18 h/day, it decreases progressively PAP.[76] LTOT also improves survival, although this effect is unrelated to the amelioration of pulmonary hemodynamics. Survival benefit seems to be greater in patients presenting a significant decrease in PAP during the acute oxygen administration (acute responders).[77] Despite such beneficial effects of LTOT, PAP rarely returns to normal values, and the structural abnormalities of pulmonary vessels persist.[48]

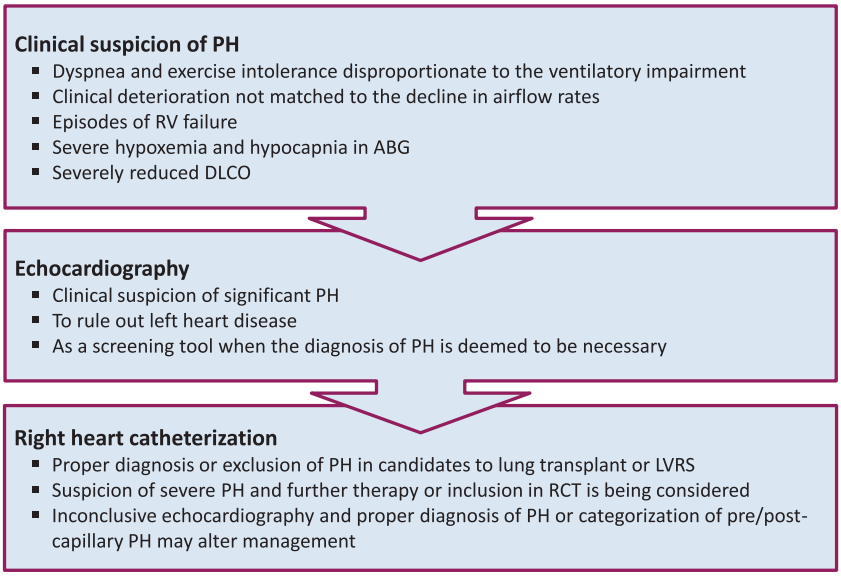

Figure 3 Diagnostic approach to pulmonary hypertension in chronic obstructive pulmonary disease. Definition of abbreviations: RV, right ventricle; ABG, arterial blood gas; DLCO, diffusing capacity of the lung for carbon monoxide; PH, pulmonary hypertension; LVRS, lung volume resection surgery; RCT, randomized clinical trial.

Conventional vasodilators

Calcium channel blockers have been extensively evaluated for the treatment of PH in COPD. The acute administration of nifedipine has been shown to reduce PAP and increase cardiac output in COPD patients studied both at rest and during exercise.[78] However, nifedipine inhibits hypoxic pulmonary vasoconstriction (HPV), a physiologic mechanism that reduces perfusion in poorly ventilated or nonventilated lung units and diverts it to better ventilated units, thereby restoring ventilation–perfusion (V_A/Q) equilibrium and increasing arterial PO_2. Because HPV plays an important role in regulating gas exchange in COPD, the administration of nifedipine to these patients results in worsening of V_A/Q relationships and decrease of partial arterial oxygen concentration (PaO_2).[78] Furthermore, clinical results of long-term treatment with calcium channel blockers in COPD have been disappointing.[71]

In a recent randomized, double-blind, placebo-controlled study, 1-year treatment with the angiotensin-II antagonist losartan also failed to provide significant benefit on systolic PAP, exercise capacity or symptoms in patients with COPD-associated PH.[79]

Selective pulmonary vasodilators such as inhaled nitric oxide (NO) also worsen V_A/Q distributions and decrease PaO_2 due to the inhibition of HPV.[19] The combined administration of low NO doses and oxygen during 3 months was compared with oxygen alone in a randomized study. Combined NO and oxygen caused a greater decrease in PAP and PVR than oxygen alone, without decreasing arterial oxygenation.[80] Yet, due to the complexity of NO delivery, no further studies have extended these initial findings.

Endothelium modulating agents

Current therapy of PAH is based on drugs that target signalling pathways between the endothelial cells and smooth muscle cells. Three signalling pathways are current targets for PAH treatment: the NO–cyclic guanosine monophosphate pathway, the prostacyclin–cyclic adenosine monophosphate pathway and the endothelin-1 pathway (Table 2). The use of these agents (phosphodiesterase-5 (PDE-5) inhibitors, guanylate cyclase stimulators, prostanoids and ET-1 receptor antagonists,) has been shown to improve symptoms, exercise performance, pulmonary hemodynamics and survival in some forms of PAH. Considering that the pathogenesis of PH in COPD shares some common pathways with that of PAH, it is conceivable that drugs that may correct the endothelial vasoconstrictor–dilator imbalance could be of clinical benefit in COPD.

Acute effects

The acute administration of intravenous epoprostenol to patients with a COPD exacerbation reduced PVR, but it also worsened arterial oxygenation.[81] Two studies have assessed the acute effects of inhaled iloprost in COPD. In one uncontrolled study conducted in patients with an estimated systolic PAP of 41 mm Hg, the administration of 5-μg iloprost increased 6-min walk distance and improved arterial oxygenation.[82] In contrast, in a placebo-controlled study, the administration of 10-μg iloprost reduced oxygen consumption in a cardiopulmonary exercise test, and 20-μg iloprost worsened gas exchange at rest.[83]

Table 2 Endothelium modulating agents used for the treatment of pulmonary arterial hypertension

Signalling pathways with vasodilator and antiproliferative effects
a) Nitric oxide–cyclic GMP pathway
a.1.) Phosphodiesterase-5 inhibitors
—Sildenafil
—Tadalafil
a.2.) Soluble guanylate cyclase stimulators
—Riociguat
b) Prostacyclin–cyclic AMP pathway
Prostanoids
—Epoprostenol
—Iloprost
—Treprostinil
—Selexipag
Signalling pathways with vasoconstrictor and proliferative effects
Endothelin-1 pathway
Endothelin-1 receptor antagonists
—Ambrisentan
—Bosentan
—Macitentan

AMP, adenosine monophosphate; GMP, guanosine monophosphate.

The acute effects of 20 and 40-mg sildenafil were assessed in COPD patients with PH, both at rest and during exercise.[84] Both sildenafil doses improved pulmonary hemodynamics at rest and during exercise, although PaO_2 decreased at rest due to increased V_A/Q mismatching due to the inhibition of HPV.[84]

In summary, studies of acute effects of prostanoids and PDE-5 inhibitors indicate that these drugs worsen gas exchange in COPD.

Long-term effects

A number of uncontrolled studies, conducted in reduced number of subjects, have evaluated the long-term effects of PAH targeted therapy in patients with COPD-associated PH.[71] The number of randomized, controlled studies in this condition is limited (Table 3).

The effects of the dual-ET-1 receptor antagonist bosentan were evaluated in a group of COPD patients with systolic PAP estimated by echocardiography ranging between 20 and 42 mm Hg.[85] At the end of 3-month treatment, there were no changes in exercise tolerance or systolic PAP, whereas arterial oxygenation significantly worsened in the group treated with bosentan[85] (Table 3). Valerio *et al.*[86] evaluated the effects of adding bosentan to conventional COPD treatment in 32 patients with COPD-associated PH using a randomized, unblinded design. At the end of an 18-month period, patients who received bosentan showed improved hemodynamics and exercise tolerance, compared with

Table 3 Targeted therapy for pulmonary arterial hypertension in COPD in randomized clinical trials

Author (year)	*n*	Drug, dose	Follow-up	PH, status	Primary EP	Achieved?
Rao (2011)	33	Sildenafil, 20 mg tid	12 weeks	Yes, sPAP 53 mm Hg	6MWD	Yes
Lederer (2012)	10	Sildenafil, 20 mg tid	4 weeks	Excluded	6MWD, Peak VO_2	No
Blanco (2013)	60	Sildenafil[†], 20 mg tid	3 months	Yes, mPAP 31 mm Hg	Endurance time	No
Goudie (2014)	120	Tadalafil, 10 mg qd	12 weeks	Yes, mPAP 30 mm Hg	Δ6MWD	No
Stolz (2008)	30	Bosentan, 125 mg bid	12 weeks	No, sPAP 35 mm Hg	Δ6MWD	No
Valerio (2009)	32	Bosentan[‡], 125 mg bid	18 months	Yes, mPAP 37 mm Hg	PAP, FC, 6MWD	Yes

[†]Added to pulmonary rehabilitation.
[‡]Unblinded.
PH, pulmonary hypertension; EP, end point; sPAP, systolic pulmonary artery pressure estimated by echocardiography;mPAP,mean pulmonary artery pressure assessed by right heart catheterization;6MWD, distance covered in the 6-min walk test; Peak VO_2, oxygen consumption at peak exercise; PAP, pulmonary artery pressure; FC, functional class; tid: three times a day; qd: one a day; bid: twice a day.

control subjects, without deterioration of gas exchange.[86]

The effects of PDE-5 inhibitors in COPD-associated PH have been recently evaluated in four randomized clinical trials (Table 3). Rao *et al.*[87] evaluated the effects of sildenafil (20 mg tid) in 33 patients with COPD and an estimated systolic PAP of 50 mm Hg. After 12-week treatment, patients receiving sildenafil showed a substantial increase in 6-min walk distance (+191 m) and a significant decrease of systolic PAP.[87] In contrast, Lederer *et al.*[88] did not find any significant effect on exercise tolerance in 10 COPD patients without PH treated with sildenafil (20 mg tid) during 1 month, using a cross-over design. At the end of the treatment period, patients receiving sildenafil showed worsened arterial oxygenation and quality of life.[88] In a randomized, placebo-controlled study, Blanco *et al.*[89] evaluated the effects of 3-month treatment with sildenafil (20 mg tid), added to pulmonary rehabilitation, in 60 patients with severe COPD and mild-to-moderate PH. Sildenafil treatment did not improve the results obtained with rehabilitation alone on exercise tolerance and quality of life.[89] In a recent randomized, placebo-controlled clinical trial, conducted in 120 COPD patients with moderate PH (estimated mPAP 30 mm Hg), 12-week treatment with tadalafil (10 mg qd) did not produce any significant effect on 6-min walk distance.[90]

In summary, the treatment of choice in patients with PH associated with COPD who are hypoxemic is LTOT. The use of conventional vasodilators or PAH targeted therapy in patients with COPD and moderate PH is currently discouraged based on the lack of efficacy observed in the majority of randomized clinical trials conducted so far, and because there is compelling evidence indicating that these drugs may worsen pulmonary gas exchange. The subgroup of patients with severe PH should be ideally managed in centres with expertise in both PH and chronic lung diseases and included in randomized, controlled trials if available. Targeted PAH therapy might be considered in patients with severe PH in a compassionate basis due to the poor prognosis of this condition, with careful monitoring of gas exchange and inclusion in prospective registries.

'TAKE HOME' MESSAGES FOR THE CLINICIANS

1. Pulmonary hypertension is a common complication of COPD, particularly in advanced disease, associated with greater mortality and worse clinical course. It is usually of mild-to-moderate severity and progresses slowly, with preserved right ventricular function. Only a reduced subgroup of patients (3–5%) may present severe PH, with mean PAP >35 mm Hg and/or low cardiac index.
2. The distinction between Group 3 PH or the presence of PAH (Group 1) in a patient with a prevalent disease such COPD might be difficult in patients who present severe PH, particularly if airflow obstruction is mild or moderate.
3. Patients presenting symptoms that exceed those expected by the airflow impairment should be further evaluated by echocardiography, although its diagnostic performance is lower than in other forms of PH. Confirmatory right heart catheterization is indicated in candidates to surgical treatments (lung volume reduction surgery, bullectomy or lung transplant), suspected severe PH potentially amenable to targeted therapy and, in general, in those conditions where the result of the hemodynamic assessment will determine treatment options.
4. The treatment of choice for patients with COPD and associated PH who are hypoxemic is long-term oxygen therapy.
5. Drugs approved for PAH or conventional vasodilators are not recommended in COPD patients with mild-to-moderate PH because they may impair gas exchange and because of their lack of efficacy shown in randomized controlled trials.
6. COPD patients with severe PH should be referred to a centre with expertise in PH and lung diseases and ideally included in randomized controlled trials.

Disclosure statement

Dr Blanco reports personal fees from Bayer and Actelion in the last 5 years outside the present work. Dr Joan Albert Barberà has received fees as advisory board member and speaker from Actelion, Bayer, GlaxoSmithKline and Pfizer and has received grant support through his institution from Actelion, Bayer, Ferrer, Pfizer and GlaxoSmithKline.

The Authors

Dr Isabel Blanco graduated in Medicine and Surgery at the University of Barcelona and holds a PhD in medicine, also from University of Barcelona. She was a two-year post-doctoral fellow at the University of Johns Hopkins in Baltimore, USA. Currently, she is a Pulmonologist at Hospital Clinic of Barcelona and a biomedical researcher at the Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS). Dr Blanco has numerous publications in national and international journals, has participated in numerous research projects both locally and internationally and has extensive experience in the management of pulmonary hypertension, exercise test and pulmonary physiology. Dr Lucilla Piccari graduated in Medicine and Surgery at the University of Bologna and studied her residency in Pulmonary Medicine also at University of Bologna. She is a Fellow at the Pulmonary Hypertension National Reference Center directed by Dr Barberà in Barcelona, Spain. Dr Joan Albert Barberà is the Head of the Pulmonary Hypertension Unit at the Hospital Clínic in Barcelona, Spain, and Professor of Medicine at the University of Barcelona, Spain. His research interest involves the physiopathology and biopathology of pulmonary vascular diseases, with particular focus on pulmonary vascular abnormalities associated with chronic respiratory diseases.

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Hypothesis

This thesis work originates from two main hypotheses:

1. Given the common pathogenetic mechanisms and multiple molecular and cellular pathways involved, in COPD, the magnitude of endothelial dysfunction and arterial stiffness in systemic arteries may be associated to greater endothelial dysfunction in pulmonary arteries and the development of pulmonary vascular disease.
2. In patients with COPD and severe PH the presence of severe hypoxemia might be due to the combination of intrapulmonary (greater VA/Q mismatch) and extrapulmonary (decreased cardiac output) factors and, eventually, a reduced response to hypoxic stimulus as a result of impaired endothelial function.

Objectives

According to the background knowledge laud out in the introduction of this thesis, the body of work of the PhD thesis has been directed at assessing the systemic and pulmonary vascular function across the spectrum of pulmonary vascular disease in COPD patients.

For this, two research studies were carried out, with the specific objectives here exposed:

First study:

To assess systemic endothelial function and arterial compliance in the same cohort of patients with COPD, and to analyse their potential relationship with pulmonary vascular disease.

Second study:

To analyse the determinants of hypoxaemia in severe PH associated with COPD and to investigate whether these patients have an attenuated response to hypoxic stimulus.

Association Between Systemic and Pulmonary Vascular Dysfunction in COPD

Lucilla Piccari[1], Roberto Del Pozo[1], Isabel Blanco[1,2], Jessica García-Lucio[1], Yolanda Torralba[1,2], Olga Tura-Ceide[1,2], Jorge Moises[1,2], Marta Sitges[3,4], Víctor Ivo Peinado[1,2], Joan Albert Barberà[1,2]

[1]Department of Pulmonary Medicine, Hospital Clínic, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain;

[2]Biomedical Research Networking Centre on Respiratory Diseases (CIBERES), Madrid, Spain;

[3]Department of Cardiology, Hospital Clínic, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Barcelona, Spain;

[4]Biomedical Research Networking Centre on Cardiovascular Diseases (CIBERCV), Madrid, Spain

Correspondence to: Joan Albert Barberà Department of Pulmonary Medicine, Hospital Clínic, Institute of Biomedical Research August Pi I Sunyer (IDIBAPS), University of Barcelona, Villarroel 170, Barcelona 08036, Spain Tel +34 932275779. Email jbarbera@clinic.cat

Keywords: COPD, pulmonary circulation and pulmonary hypertension, emphysema, cardiovascular diseases

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Abstract

Introduction: In chronic obstructive pulmonary disease (COPD), endothelial dysfunction and stiffness of systemic arteries may contribute to increased cardiovascular risk. Pulmonary vascular disease (PVD) is frequent in COPD. The association between PVD and systemic vascular dysfunction has not been thoroughly evaluated in COPD.

Methods: A total of 108 subjects were allocated into four groups (non-smoking controls, smoking controls, COPD without PVD and COPD with PVD). In systemic arteries, endothelial dysfunction was assessed by flow-mediated dilation (FMD) and arterial stiffness by pulse wave analysis (PWA) and pulse wave velocity (PWV). PVD was defined by a mean pulmonary artery pressure (PAP) ≥25 mmHg at right heart catheterization or by a tricuspid regurgitation velocity >2.8 m/s at doppler echocardiography. Biomarkers of inflammation and endothelial damage were assessed in peripheral blood.

Results: FMD was lower in COPD patients, with or without PVD, compared to nonsmoking controls; and in patients with COPD and PVD compared to smoking controls. PWV was higher in COPD with PVD patients compared to both non-smoking and smoking controls in a model adjusted by age and the Framingham score; PWV was also higher in patients with COPD and PVD compared to COPD without PVD patients in the non-adjusted analysis. FMD and PWV correlated significantly with forced expiratory volume in the first second (FEV1), diffusing capacity for carbon monoxide (DLCO) and systolic PAP. FMD and PWV were correlated in all subjects.

Discussion: We conclude that endothelial dysfunction of systemic arteries is common in COPD, irrespective if they have PVD or not. COPD patients with PVD show increased stiffness and greater impairment of endothelial function in systemic arteries. These findings suggest the association of vascular impairment in both pulmonary and systemic territories in a subset of COPD patients.

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ORIGINAL RESEARCH

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Lucilla Piccari[1]
Roberto Del Pozo[1]
Isabel Blanco[1,2]
Jessica García-Lucio[1]
Yolanda Torralba[1,2]
Olga Tura-Ceide[1,2]
Jorge Moises[1,2]
Marta Sitges[3,4]
Víctor Ivo Peinado[1,2]
Joan Albert Barberà[1,2]

[1]Department of Pulmonary Medicine, Hospital Clínic, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain; [2]Biomedical Research Networking Centre on Respiratory Diseases (CIBERES), Madrid, Spain; [3]Department of Cardiology, Hospital Clínic, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Barcelona, Spain; [4]Biomedical Research Networking Centre on Cardiovascular Diseases (CIBERCV), Madrid, Spain

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Discussion: We conclude that endothelial dysfunction of systemic arteries is common in COPD, irrespective if they have PVD or not. COPD patients with PVD show increased stiffness and greater impairment of endothelial function in systemic arteries. These findings suggest the association of vascular impairment in both pulmonary and systemic territories in a subset of COPD patients.

Keywords: COPD, pulmonary circulation and pulmonary hypertension, emphysema, cardiovascular diseases

Plain Language Summary

Patients who suffer from an obstruction to the flow of air in their airways (a condition that is called chronic obstructive pulmonary disease, or COPD) present with stiffness of the peripheral blood vessels and an impairment of normal dilation of the arteries in response to changes in systemic arterial pressure, as compared to smokers as well as to healthy subjects. Furthermore, patients who suffer from disease of the pulmonary vessels, leading to a higher pressure in the pulmonary circulation, also present the increased stiffness and reduced dilation of peripheral arteries to a greater degree: this finding may suggest that the dysfunction in pulmonary and systemic vascular territories might be associated within this patient subgroup. For this reason, the non-invasive evaluation of dilation and stiffness in the

Correspondence: Joan Albert Barberà
Department of Pulmonary Medicine, Hospital Clínic, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), University of Barcelona, Villarroel 170, Barcelona 08036, Spain
Tel +34 932275779
Email jbarbera@clinic.cat

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peripheral arteries could potentially lead to a suspicion of elevated pressure in the pulmonary circulation of patients with COPD, a condition that usually requires an invasive procedure for its diagnosis.

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disorder with systemic effects that has been associated with increased cardiovascular risk.[1] Indeed, cardiovascular disease is a major cause of death in COPD patients, accounting for 25% of the overall mortality.[2] Previous studies have shown that COPD is associated with changes in systemic vascular function[3,4] and circulating biomarkers of vascular competence[5] thereby suggesting the presence of associated peripheral artery disease.[6] The severity of endothelial dysfunction in systemic arteries, as assessed by flow-mediated dilation (FMD),[7] correlates with the severity of airflow obstruction and the extent of emphysema in COPD.[3] Furthermore, COPD patients exhibit vascular stiffness in systemic arteries,[8,9] which is correlated with the severity of emphysema.[10]

Pulmonary vascular disease (PVD), a term[11] that encompasses abnormal pulmonary hemodynamics and changes suggestive of pulmonary hypertension[12] at echocardiography, is a frequent complication of COPD, especially in advanced disease stages.[13] Pulmonary hypertension is a strong and independent prognostic factor for mortality in COPD patients[14] and the presence of PVD has been associated with more frequent exacerbation episodes and greater use of healthcare resources.[15,16]

The relationship between PVD and systemic peripheral disease has not been well established. Conceivably, given that cigarette smoke exposure is a common risk factor for alterations in both vascular territories, patients with PVD might be more prone to develop peripheral vascular disease. In this respect, in a previous study, we showed that COPD patients with PVD had reduced FMD compared with patients without PVD.[17]

The association of COPD with markers of vascular function in systemic vessels, namely stiffness and endothelial function, has been assessed in separated cohorts. Furthermore, the association of PVD and systemic vascular function in COPD has not been thoroughly evaluated. Accordingly, the current study aimed to assess systemic endothelial function and arterial compliance in the same cohort of patients with COPD, as well as to analyze their potential relationship with PVD.

Materials and Methods

Study Population

Sixty-one patients with COPD, with and without PVD, and 47 control subjects with normal pulmonary function (20 of them current smokers) were prospectively evaluated. COPD was defined by smoking habit, a compatible clinical history, and evidence of chronic airflow obstruction on forced spirometry (post-bronchodilator forced expiratory volume in the first second/forced vital capacity ratio, FEV_1/FVC, <70%).[18] Patients were clinically stable at the time of the study without exacerbation episodes or oral steroid treatment during the previous 4 months. All COPD patients were on regular bronchodilator treatment and most were also receiving inhaled corticosteroids; PVD was considered to be present when at right heart catheterization mean pulmonary artery pressure (mPAP) was ≥25mmHg, fulfilling the definition of pulmonary hypertension when the study was initiated,[12] or when tricuspid regurgitation velocity was >2.8 m/s at Doppler echocardiography. Patients with left ventricle ejection fraction <50% at echocardiography were excluded. Healthy subjects were allocated into two groups according to their smoking status (non-smokers and active smokers).

A complete clinical history, physical examination, laboratory tests, electrocardiogram, pulmonary function tests and echocardiogram were performed in all subjects. In patients with COPD, arterial blood gas analysis was additionally performed. The following inflammatory and endothelial biomarkers were also analyzed: brain natriuretic peptide (BNP), high-sensitivity C reactive protein (hs-CRP), fibrinogen, vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), interleukin-6 (IL-6), hepatocyte growth factor (HGF), angiopoietin-2 (ANG-2), cyclic guanosine monophosphate (cGMP), soluble tumor necrosis factor receptor type I (sTNF-αRI), soluble intercellular adhesion molecule-1 (sICAM-1), leptin, adiponectin, and soluble tyrosine kinase receptor Axl (sAXL).

The study was approved by the Ethics Committee of Hospital Clínic of Barcelona (20095026) and conducted in accordance with the Declaration of Helsinki on ethical principles for medical research involving human subjects. All subjects gave written informed consent before being included in the study.

All the measurements of vascular distensibility and endothelial function were performed in a quiet, temperature-controlled room (22±2ºC). Subjects fasted and

avoided physical exercise, caffeine, alcohol, drugs and stimulants for at least 6 hours and rested supine for 15 minutes before starting the measurements, maintaining this position throughout the measurements. All studies were performed at the same time of day (afternoon) with the same ultrasound machine and by the same operator. FMD testing was performed 15–30 minutes after the completion of the arterial stiffness tests.

Vascular Stiffness

Arterial stiffness was assessed according to current guidelines.[19] Both pulse wave analysis (PWA) and pulse wave velocity (PWV) measurements were performed by applanation tonometry (SphygmoCor System, AtCor Medical, Sydney, Australia). Blood pressure was measured twice using an automated, non-invasive oscillometric sphygmomanometer.

Pulse wave analysis was obtained on the radial artery of the right wrist and measured by specifically trained staff. The machine derived an aortic pulse pressure waveform from the radial artery wave and calculated the component of pulse pressure related to the reflection wave due to the increased stiffness of the arterial wall by means of the pressure difference between the reflection wave peak and the incident wave peak, expressed as percentage of the central pulse pressure (augmentation index, AI).[20] Since the timing of the reflection wave is obviously affected by heart rate, the AI was also normalized at a standard heart rate of 75 beats per minute (AI75),[21] in order to avoid the potential bias of different heart rates across the study groups.

Pulse wave velocity was measured by specifically trained staff. We used the R-wave of a simultaneously recorded ECG to identify the onset of the pressure wave, and applanation tonometry at the carotid and femoral arteries to record the pressure waveform, establishing the time delay between the R-wave and the beginning of the pulse pressure for each of the two central pulses. This distance (in meters) divided by the time delay of the pressure waveform (seconds) equaled carotid-femoral PWV.[22] A measurement was accepted when it was reproducible at least twice with minimal variation. The final result was the mean of the measurements performed.

Endothelial Function

Endothelial function was assessed by FMD on the brachial artery, according to current guidelines[23,24] as previously described.[17] In brief, a blood pressure cuff was placed around the forearm. Brachial artery diameter was measured longitudinally 5–10 cm above the elbow by ultrasound, using a linear vascular transducer 7.5/5.5 MHz connected to an echocardiogram (Sonos 5500, Philips Medical Systems), and a three-lead electrocardiography was connected to the ultrasound machine. A 60-second baseline period was recorded prior to the cuff inflation. After this, the blood pressure cuff was inflated to 250 mmHg for 5 minutes to achieve total brachial arterial occlusion, and then rapidly deflated. Recording recommenced immediately after deflation and continued for 2 minutes. The brachial artery recovered for 15 minutes, after which another baseline scan was recorded for 15 seconds.

All the recorded images were transferred to a computer for measurement by the automated edge detection software (Brachial artery analyzer, MIA-LLC, IA, USA). The FMD response was calculated as the percentage of change from baseline to peak diameter of the brachial artery after cuff deflation.

Statistical Analysis

Data are expressed as mean ±SD when variable distribution was normal and as median and percentile 25–75 for non-normal distributions. The four groups were compared with a non-adjusted analysis using ANOVA with post-hoc tests (Tukey for continuous variables and Bonferroni for categorical variables). Since comorbidities were not equally represented within the four groups, and in order to correct for their possible confounding effect over endothelial dysfunction and arterial stiffness, a linear regression model was created comprising all the conditions and discarding them one by one using Akaike information criterion (AICc), in order to find out which variables (such as age, systemic arterial hypertension, diabetes or Framingham score) had an effect on the overall differences of FMD, PWA and PWV between groups and should thus be included in the final model for each study variable, while maintaining the group category as the variable of interest. With the linear model thus created, we performed Sidak and Tukey multiple comparison tests in order to carry out pairwise comparisons. Correlations among variables were analyzed using Pearson's correlation tests. $P<0.05$ was considered significant in all cases.

Results

Anthropometric, clinical and functional characteristics of the subjects are shown in Table 1. In the COPD without PVD group, 3 patients were in GOLD 1 stage, 16 patients in GOLD 2, 13 patients in GOLD 3 and 14 patients in

Table 1 Characteristics of the Study Subjects

	Non-Smoking Controls (n=27)	Smoking Controls (n=20)	COPD without PVD (n=46)	COPD with PVD (n=15)
Age, years	56 ± 8	54 ± 8	62 ± 7 [ab]	64 ± 6 [ab]
Male gender, n (%)	12 (44%)	9 (45%)	38 (83%) [ab]	13 (87%) [ab]
Smoking status				
Current smokers, n (%)	0 (0%)	20 (100%)	14 (30%)	2 (13%)
Ex-smokers, n (%)	9 (33%)	0 (0%)	32 (70%)	13 (87%)
Pack-years smoking	4 ± 8	30 ± 24	62 ± 29 [ab]	69 ± 29 [ab]
GOLD stage 1-2-3-4, n	NA	NA	3–16-13-14	0-1-5-9
FEV_1, % ref	107 ± 12	103 ± 10	48 ± 20 [ab]	30 ± 10 [abc]
FVC, % ref	106 ± 11	104 ± 12	84 ± 19 [ab]	65 ± 13 [abc]
FEV_1/FVC, %	79 ± 5	77 ± 5	43 ± 14	34 ± 9
TLC, % ref	106 ± 8	106 ± 9	116 ± 19 [a]	113 ± 21
RV, % ref	108 ± 18	110 ± 23	181 ± 57 [ab]	197 ± 62 [ab]
DL_{CO}, % ref	92 ± 15	85 ± 9	57 ± 20 [ab]	39 ± 12 [abc]
PaO_2, mmHg	NA	NA	73 ± 9	63 ± 10[c]
Systemic arterial hypertension, n (%)	5 (19%)	3 (15%)	21 (46%) [ab]	10 (67%) [abc]
Dyslipidemia, n (%)	7 (26%)	7 (35%)	14 (30%)	6 (40%)
Diabetes mellitus, n (%)	1 (4%)	0 (0%)	2 (4%)	5 (33%) [abc]
Framingham score, points	4.9 ± 5.1	7.00 ± 6.0	10.2 ± 5.8 [a]	12.1 ± 6.8 [ab]
Systolic pulmonary artery pressure, mmHg	27 ± 4	27 ± 3	31 ± 3	43 ± 10 [abc]
Mean pulmonary artery pressure, mmHg *	NA	NA	21.2 ± 1.9	29.9 ± 5.9 [abc]
Cardiac index, L/min/m^2 *	NA	NA	2.24 ± 0.89	2.77 ± 0.47 [abc]
Pulmonary artery wedge pressure, mmHg *	NA	NA	8.3 ± 3.1	9.30 ± 4.1
Pulmonary vascular resistance, dyn·s·cm^{-5} *	NA	NA	247 ± 40	328 ± 113
Left ventricular ejection fraction, %	64 ± 3	64 ± 3	62 ± 5	60 ± 6
Systolic blood pressure, mmHg	124 ± 18	123 ± 16	130 ± 20	135 ± 19

Notes: Results are expressed in mean±standard deviation. [a] $p<0.05$ compared with non-smokers; [b] $p<0.05$ compared with smokers; [c] $p<0.05$ compared with COPD without PVD; * Right heart catheterization was performed in 5 patients of the COPD without PVD group and in 10 patients of the COPD with PVD group.

Abbreviations: COPD, chronic obstructive pulmonary disease; PVD, pulmonary vascular disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; FEV_1, forced expiratory volume in 1st second; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; DL_{CO}, diffusing capacity for carbon monoxide; PaO2, partial pressure of arterial oxygen; NA, not available.

GOLD 4; in the COPD with PVD group no patient was in stage 1, one patient was in GOLD 2, 5 patients in GOLD 3 and 9 patients in GOLD 4. Fifteen patients overall (one patient in the smoking group, 4 patients in the COPD without PVD group and 10 in the COPD with PVD group) underwent right heart catheterization, while the other patients were evaluated by means of echocardiography. Fifteen patients with COPD fulfilled the criteria for PVD. Age, male gender proportion and the number of packs-year were higher in COPD patients. COPD patients with PVD had greater airflow obstruction, lower DL_{CO} and lower PaO_2 than COPD patients without PVD. Systemic arterial hypertension and diabetes mellitus were more prevalent in COPD patients with PVD.

Endothelial Function of Systemic Arteries

In the non-adjusted analysis, FMD was lower in both COPD groups, with and without PVD, as compared with non-smoking controls; and lower in the COPD with PVD group compared with smoking controls (Table 2, Figure 1A). In consideration of the different baseline vessel diameters, we formulated the ratio between flow-mediated dilation and baseline brachial artery diameter, which is an index of endothelial dysfunction independent of vessel

Table 2 Vascular Function in Systemic Arteries

	Non-Smoking Controls (n = 26)	Smoking Controls (n = 20)	COPD without PVD (n = 45)	COPD with PVD (n = 15)
Flow-mediated dilation, % change from baseline diameter	10.10 (6.15–14.30)	6.60 (4.58–9.50)	5.40 (3.13–7.30) [a]	2.70 (0.78–4.70) [ab]
Flow-mediated dilation/Baseline brachial artery diameter, %/mm	2.50 (1.48–3.82)	1.34 (0.80–2.04)	1.16 (0.49–1.65) [a]	0.49 (0.18–1.02) [ab]
Augmentation index, %	25.0 (19.3–35.3)	33.0 (20.0–43.0)	25.0 (17.3–30.0)	27.0 (17.3–34.0)
Augmentation index 75, %	23.5 (17.0–29.3)	26.0 (20.0–40.0)	27.0 (16.8–31.0)	26.0 (19.5–31.8)
Pulse wave velocity, m/s	8.3 (6.6–9.7)	7.6 (6.6–8.9)	9.8 (8.4–11.8) [ab]	11.4 (10.4–13.2) [abc]

Notes: Results are expressed as median (percentile 25-percentile 75). Non-adjusted comparisons: [a]p<0.05 compared with non-smokers; [b] p<0.05 compared with smokers; [c] p<0.05 compared with COPD without PVD.

Abbreviations: COPD, chronic obstructive pulmonary disease; PVD, pulmonary vascular disease.

diameter; this parameter yielded the same results. None of the individual conditions (age, gender, BMI) and comorbidities (systemic arterial hypertension, diabetes, hypercholesterolemia, renal disease, chronic ischemic disease, cardiac failure, Framingham score) included in the linear regression model had an effect on the differences between groups, so the adjusted analysis was not performed.

Vascular Stiffness of Systemic Arteries

In the non-adjusted analysis, PWV was higher in both COPD groups compared with control subjects (smokers and non-smokers). It was also higher in the COPD with PVD group compared with the COPD without PVD group (p =0.037). In the linear model adjusted for age and the Framingham score (selected by the Akaike information criterion method), PWV was higher in the COPD with PVD group compared to non-smoking and smoking control subjects (Table 2, Figure 1B). There was also a trend towards higher PWV in the COPD with PVD group compared to the COPD without PVD group (p=0.06) in the adjusted model. There were no differences in PWV between non-smokers and smokers control groups.

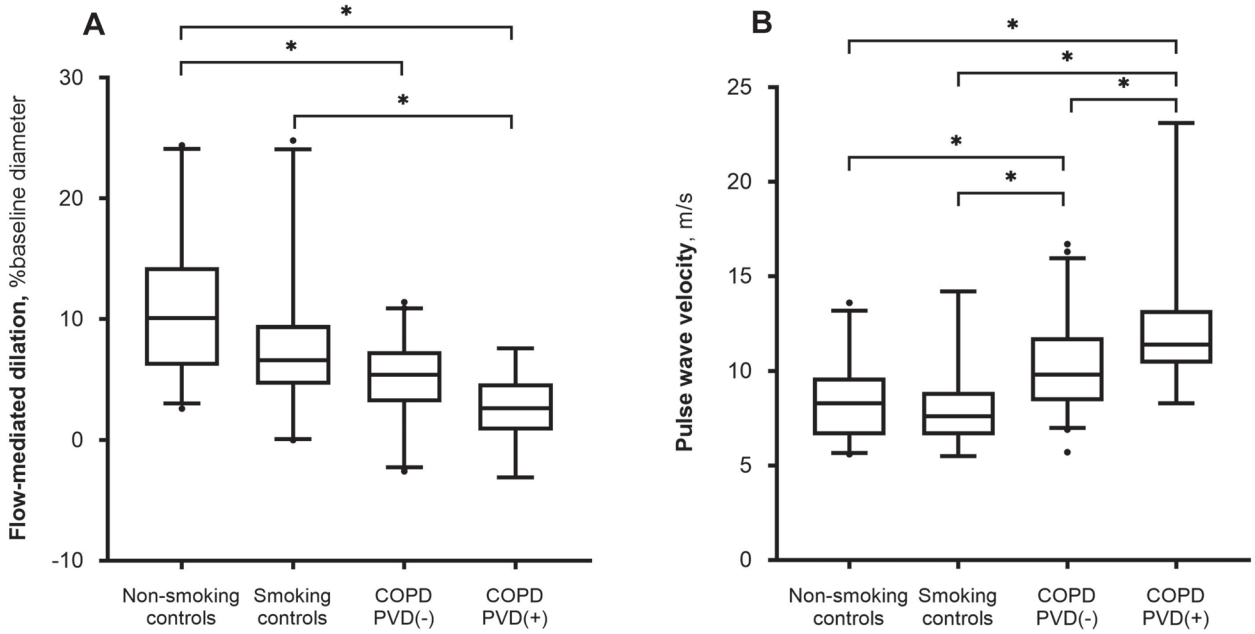

Figure 1 Results of (**A**) endothelial function, assessed with flow-mediated dilation, and (**B**) arterial stiffness, assessed with pulse wave velocity, in non-smoking controls, smoking controls, chronic obstructive pulmonary disease (COPD) without pulmonary vascular disease (PVD) [COPD PVD(-)] and COPD with PVD [COPD PVD(+)]. Boxplots show median and 25–75 percentiles, whiskers show 5 and 95 percentiles, points show values out of this range. Between-group differences were analyzed with unadjusted ANOVA using Tukey as post-hoc test; *p<0.05.

Measurements of PWA, expressed as AI and AI75, did not differ between groups, neither in the non-adjusted nor in the adjusted models (Table 2).

Relationship Between Systemic Vascular Function and Pulmonary Function

Both FMD and PWV correlated with FEV_1, DL_{CO} and systolic PAP values (Figure 2); also, the number of pack/years correlated with FMD ($r = 0.393$, $p < 0.001$) and PWV ($r = 0.357$, $p < 0.001$). FMD and PWV were inversely correlated ($r = -0.3$; $p = 0.04$). Since the group of non-smoking healthy subjects might lead to a correlation bias, we repeated the analysis excluding this group. Correlations between lung function and sPAP and PWV persisted statistically significant, whereas correlations with FMD did not. We tested the performance of FMD and PWV in predicting the presence of PVD using ROC analysis. Both tests had good diagnostic performance in identifying PVD, with areas under the curve (AUC) of 0.815 and 0.803, FMD and PWV, respectively (Figure S1, supplementary material). A FMD <4.5% had a sensitivity of 77% and a specificity of 75% in identifying PVD. A PWV >10.4 m/s had a sensitivity of 87%, and specificity of 72% in identifying PVD.

Inflammatory and Vascular Markers

The plasma levels of the soluble receptor of tumor necrosis factor-alpha (sTNF-αRI) were higher in COPD patients with PVD, compared with both non-smoking and smoking control groups (Table 3). Fibrinogen was increased in COPD patients without PVD, compared with both the non-smoking and smoking control groups.

Discussion

The results of the present study show that in patients with COPD, the presence of PVD is associated with systemic arterial dysfunction, characterized by increased vascular stiffness and worse endothelial function in systemic arteries. This association is irrespective of the presence of cardiovascular risk factors, which are more prevalent in COPD patients with PVD.

Systemic arterial stiffness, as assessed by PWV, was increased in COPD patients compared with control subjects, being patients with COPD and PVD those who presented the highest PWV values, in the non-adjusted analysis. Nevertheless, since in our cohort COPD patients presented more frequently cardiovascular risk factors, we analyzed the potential effect of associated comorbidities in an adjusted linear regression model. In the adjusted model, differences between COPD patients with PVD and both non-smoking and smoking controls persisted after adjusting for potential confounding factors. Furthermore, there was a trend to greater PWV in COPD patients with PVD compared with those without PVD. Accordingly, greater systemic arterial stiffness in the COPD with PVD group cannot be attributed to the effect of existing conditions or comorbidities.

Higher PWV in COPD patients has been previously shown,[10] but to our knowledge, this is the first time that this observation is adjusted for potential confounding comorbidities, namely cardiovascular risk factors, and related to the presence of PVD. Taken together our current findings and previous observations suggest that greater systemic arterial stiffness in COPD might be the result of increased cardiovascular risk factors in this population. However, a subgroup of COPD patients with greater stiffness in systemic arteries might be more prone to develop PVD and eventually pulmonary hypertension, potentially as a result of greater stiffness also in pulmonary vessels.[25]

Several studies have previously reported endothelial dysfunction of systemic arteries in COPD patients,[3,26] and our own group[17] has already demonstrated the presence of reduced FMD in COPD patients with PVD compared to patients without pulmonary vascular impairment. In the current study, we extend these previous observations and demonstrate that endothelial dysfunction of systemic arteries in COPD is independent of the presence of cardiovascular risk factors and that both endothelial dysfunction and arterial stiffness are present in the same cohort of patients, being inversely related among them. Furthermore, COPD patients with PVD consistently show the greatest impairment in systemic vascular function, as shown in both unadjusted and adjusted linear models.

The reasons why COPD patients with PVD show greater systemic vascular disease are not clear. Airway inflammation has been previously linked with reduced vascular nitric oxide production,[27] which could possibly explain a mechanism for endothelial dysfunction. However, the hypothesis of a "spill-over" of inflammatory mediators from the lung to the systemic circulation is not supported by the current findings, since there were no consistent differences among groups in the inflammatory mediators that were tested. Cigarette smoking is a common etiologic factor for both systemic[28] and pulmonary vascular disease.[29] Endothelial dysfunction might predispose to greater vascular damage and pulmonary vascular remodeling which in turn may lead to pulmonary artery stiffness. In fact, the number of pack/years correlated with both FMD and PWV in our cohort. Pulmonary artery

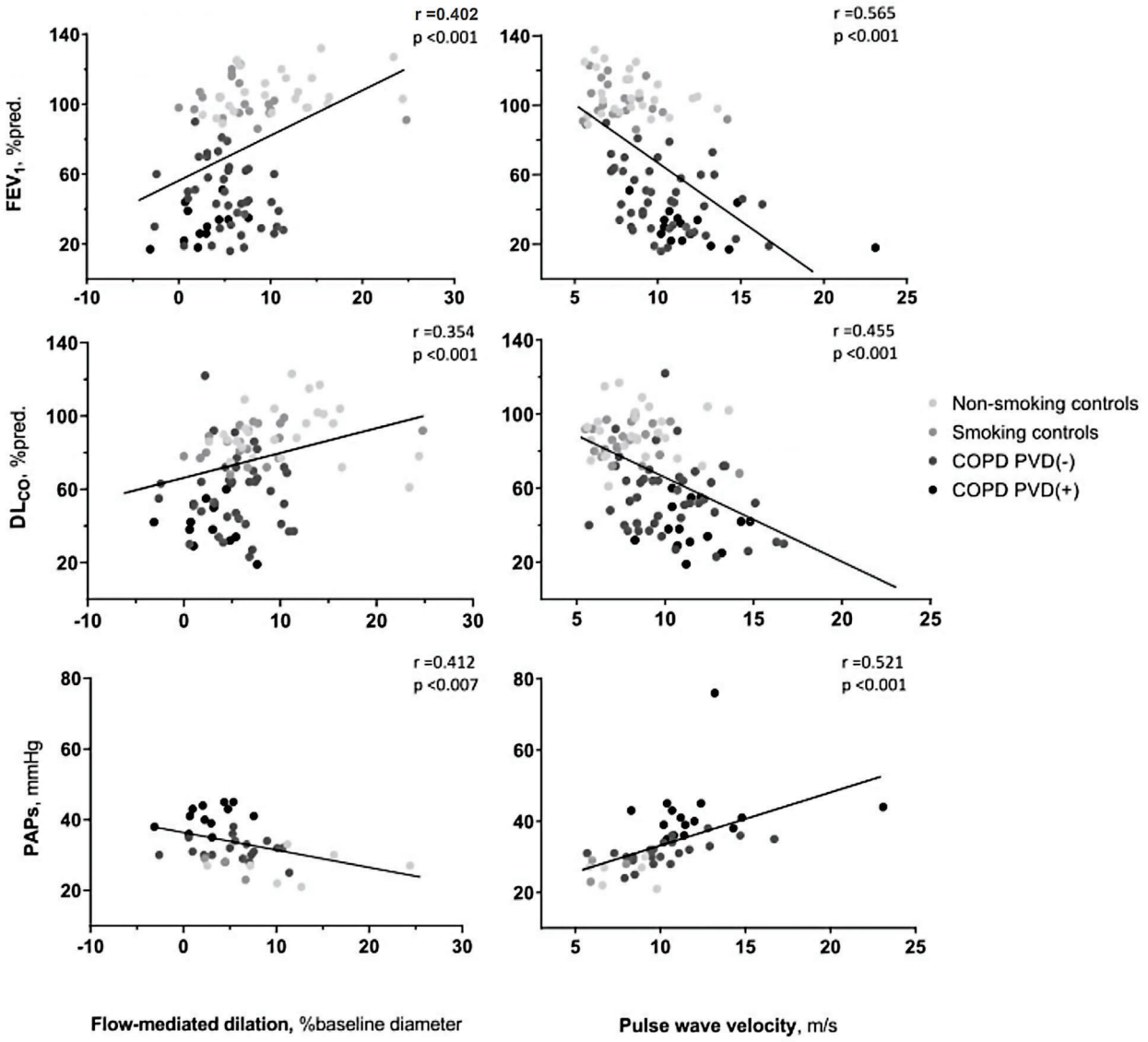

Figure 2 Correlation between flow-mediated dilation (left column) and pulse wave velocity (right column) with forced expiratory volume during the first second (FEV_1), diffusing capacity for carbon monoxide (DLCO) and systolic pulmonary artery pressure (PAPs; estimated by doppler echocardiography in patients with tricuspid regurgitation and thus a measurable PAPs or measured at right heart catheterization). Patients are grouped as non-smoking controls, smoking controls, chronic obstructive pulmonary disease (COPD) without pulmonary vascular disease (PVD) [COPD PVD(-)] and COPD with PVD [COPD PVD(+)].

stiffness has been extensively reported in COPD patients,[25,30] and this finding, as measured by magnetic resonance pulse wave velocity, is able to identify pulmonary hypertension in COPD and is predictive of major cardiovascular events.[31] In our study, arterial stiffness was not associated with smoking habit in patients without COPD, as no differences were observed between control smokers and non-smokers. Conceivably, vessel stiffness might be a contributing factor for the development of PVD. Similar to what has been shown in other forms of pulmonary hypertension,[32] factors involved in vessel stiffening, such as metalloproteases,[33] collagen and extra-cellular matrix gene expression and transcription,[34,35] which have been shown in pulmonary arteries of COPD patients,[36] might contribute to the development of PVD in COPD. Arterial stiffness could also be induced as a result of greater sympathetic nervous activity, through an increased vascular tone enhanced by associated sleep apnea[37] or elevated carotid chemoreceptor activity.[38] However, this seems unlikely in our patients since all of them were clinically screened to rule out sleep apnea.

Interestingly, sTNF-αRI was significantly elevated in COPD patients with PVD. sTNF-αRI has been shown to

Table 3 Vascular and Systemic Inflammatory Markers

	Non-Smoking Controls (n=27)	Smoking Controls (n=20)	COPD without PVD (n=46)	COPD with PVD (n=15)
hsCRP, mg/dL	0.15 (0.05–0.24)	0.09 (0.05–0.33)	0.40 (0.12–0.87)	0.27 (0.20–0.80)
BNP, pg/mL	13.7 (4.1–23.4)	12.0 (7.6–28.1)	13.5 (7.7–24.9)	21.2 (8.4–40.5)
Fibrinogen, g/L	3.40 (3.00–3.70)	3.30 (2.90–3.78)	3.80 (3.10–5.05)[ab]	4.00 (3.60–4.50)
Glycemia, mg/dL	92 (85–99)	92 (83–99)	96 (87–109)	112 (94–138)
VEGF, detectable %	12 (44.4)	12 (60)	22 (47.8)	7 (46.7)
VEGF, pg/mL	47.0 (20.8–87.3)	13.3 (8.9–40.1)	9.5 (4.6–39.5)	20.7 (16.8–30.0)
TGF-β, ng/mL	2.31 (1.42–3.43)	2.08 (1.39–5.70)	3.00 (2.06–3.65)	2.18 (1.83–3.29)
IL-6, pg/mL	1.74 (0.96–5.23)	2.55 (0.88–8.00)	1.84 (0.87–3.26)	1.12 (0.54–2.07)
HGF, pg/mL	274 (220–437)	257 (218–365)	336 (309–407)	338 (309–448)
Leptin. ng/mL	11.0 (8.8–16.9)	13.9 (6.6–17.4)	11.5 (8.2–22.5)	16.6 (9.5–20.5)
Angiopoietin-2, pg/mL	776 (593–939)	942 (599–1319)	923 (746–1172)	1244 (844–1551)
cGMP, nmol/mL	2.52 (2.02–3.18)	2.19 (1.82–3.00)	2.23 (1.81–3.10)	2.58 (2.20–3.09)
sTNF-αRI, pg/mL	1039 (706–1348)	897 (691–1159)	1050 (878–1359)	1303 (1207–1659) [ab]
sICAM-1, ng/mL	77.1 (59.0–94.5)	85.8 (67.0–144.8)	96.0 (80.7–190.5)	92.6 (82.8–212.4)
Adiponectin, ng/mL	1046 (795–1494)	1118 (859–1628)	927 (691–1223)	815 (717–996)
sAxl, pg/mL	38.3 (15.8–70.9)	42.4 (20.8–69.3)	38.8 (23.0–57.7)	31.9 (20.3–72.7)

Notes: Results are expressed in median (percentile 25-percentile 75). [a] p<0.05 compared with non-smokers; [b] p<0.05 compared with smokers.
Abbreviations: hsCRP, high sensitivity C-reactive protein; BNP, brain natriuretic peptide; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor; IL-6, Interleukin-6; HGF, hepatocyte growth factor; cGMP, cyclic guanosine monophosphate; sTNF-αRI, soluble receptor of tumor necrosis factor-alpha; sICAM-1, soluble intercellular adhesion molecule-1; sAxl, adiponectin, soluble tyrosine kinase receptor Axl. COPD, chronic obstructive pulmonary disease; PVD, pulmonary vascular disease.

be elevated in patients with COPD and systemic hypertension,[39] or coronary artery disease.[40] TNFα was also elevated in COPD patients with pulmonary hypertension.[41]

Taken together, the interplay between pulmonary and systemic vascular disease in COPD patients could be more complex than previously considered, although they share physiopathological mechanisms[42,43] and similar therapeutic strategies.[44] It is therefore conceivable that COPD patients, through the effect of cigarette smoking, develop endothelial dysfunction and that, as the disease progresses, this leads to remodeling of both pulmonary and systemic vascular beds producing stiffness of the vessel wall. Indeed, endothelial damage has recently been suggested as a possible pathogenetic mechanism in the development of COPD, alongside other more traditionally studied pathophysiological pathways.[45,46] Our observations could support this hypothesis, although further studies are needed to better define the role of endothelial dysfunction in this disease.

Results of the present study indicate that pulmonary and systemic vascular impairments are interrelated in COPD. These findings concur with the previous observations of altered markers of vascular competence (defined as an imbalance between injury and repair capacity of the endothelium) in COPD patients with PVD,[5] suggesting common pathways in the pathobiology of alterations in both vascular territories.

Our study has some limitations. First, even though the overall cohort comprises more than one hundred patients, when broken down into groups, the sample size might not be large enough to discern some differences between groups. Second, right heart catheterization was not available in all subjects. Therefore, we cannot ascertain that patients with echocardiographic findings suggestive of pulmonary hypertension certainly had it. For this reason, we used the term PVD to define this subgroup of subjects. The proceedings from the 6th World Symposium on Pulmonary Hypertension (Nice 2018) suggested to lower the mPAP threshold for the diagnosis of pulmonary hypertension;[47] however, we chose to follow the European guidelines[12] that were in effect when the study was started. Nevertheless, the recent proposal to lower the cut-off mPAP value to define pulmonary hypertension[47,48] and the observation in COPD that mPAP values lower than 25mmHg are associated with adverse clinical outcomes[15] are in favor that patients we classified as having PVD certainly had it. Third, we cannot rule out the possibility that some of the patients in the PVD group on the basis of echocardiographic findings had left ventricular dysfunction. We excluded patients with reduced ejection fraction

at echocardiography and those with intermediate or high probability of heart failure with preserved ejection fraction based on clinical criteria.[49] Nevertheless, we cannot completely exclude the latter possibility.

Conclusion

The results of the present study show an association between systemic and pulmonary vascular impairment that suggests common pathophysiological mechanisms. The concurrence of endothelial dysfunction, which might be triggered by cigarette smoke products, and vascular stiffness appears as a potential common mechanism, not fully explained by conventional cardiovascular risk factors. Our results suggest that exploring factors associated with systemic vascular impairment in COPD may also shed light on the understanding of the development of pulmonary hypertension and contribute to reduce the impact of vascular comorbidities in this disease.

Abbreviations

AICc, Akaike information criterion; AI, augmentation index; AI75, augmentation index normalized at 75 bpm; ANG-2, angiopoietin-2; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; COPD, chronic obstructive pulmonary disease; hs-CRP, high-sensitivity C reactive protein; FEV_1, post-bronchodilator forced expiratory volume in the first second; FMD, flow-mediated dilation; FVC, forced vital capacity; HGF, hepatocyte growth factor; IL-6, interleukin-6; PH, pulmonary hypertension; PVD, pulmonary vascular disease; PWA, pulse wave analysis; PWV, pulse wave velocity; sAXL, soluble tyrosine kinase receptor; sICAM-1, soluble intercellular adhesion molecule-1; sTNF-αRI, soluble tumor necrosis factor receptor type I; TNFα, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

Ethical Approvement Statement

The study was approved by the Ethics Committee of Hospital Clínic of Barcelona (20095026) and all subjects gave written informed consent before being included in the study.

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Disclosure

Dr García-Lucio reports grants from Instituto de Salud Carlos III, outside of the current study. The authors report no other potential conflicts of interest for this work.

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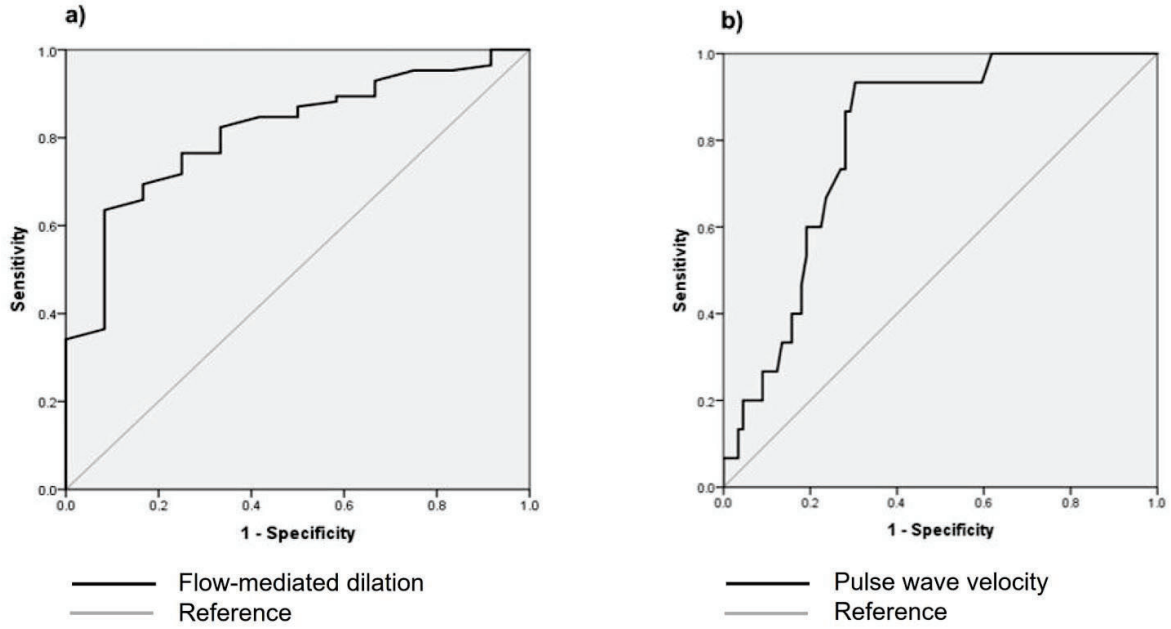
Supplementary Figure S1. Receiver operating curve of flow-mediated dilation (FMD) and pulse wave velocity (PWV) to detect the presence of pulmonary vascular disease (PVD) in the subject cohort. a) FMD <4.5% of baseline diameter was identified as the best predictor of PVD (sensitivity 77%, specificity 75%; area under the curve, AUC, 0.815); b) PWV >10.35 m/s was identified as the best predictor of PVD (sensitivity 87%, specificity 72%; AUC 0.803).



Mechanisms of hypoxaemia in severe pulmonary hypertension associated with COPD

Lucilla Piccari[1], Isabel Blanco[1,2,3], Yolanda Torralba[1,2,3], Ebymar Arismendi[1,2,3], Concepción Gistau[1], Ana Ramírez[1], Elena Gimeno-Santos[1,2], Josep Roca[1,2,3], Felip Burgos[1,2,3], Roberto Rodríguez-Roisín[1,2,3] and Joan Albert Barberà[1,2,3]

[1]Department of Pulmonary Medicine, Hospital Clínic, University of Barcelona, Barcelona, Spain.

[2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

[3]Biomedical Research Networking Centre on Respiratory Diseases (CIBERES), Madrid, Spain.

Corresponding author: Isabel Blanco, Department of Pulmonary Medicine, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Carrer Villarroel 170, 08036 Barcelona, Spain. Ph: +34 932273040. Email: iblanco2@clinic.cat)

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Abstract

Rationale: Patients with chronic obstructive pulmonary disease (COPD) with associated severe pulmonary hypertension (PH) may present with mild airflow obstruction and severe hypoxaemia. The mechanisms of hypoxaemia in this condition are poorly understood.

Objectives: Investigate the determinants of hypoxaemia in severe PH associated with COPD by assessing ventilation-perfusion (V_A/Q) relationships using the multiple inert gas elimination technique (MIGET).

Methods: 88 COPD patients (43 without PH [no-PH], 34 with moderate PH [mPH] and 11 with severe PH [sPH]) underwent simultaneous diagnostic right heart catheterization and MIGET while breathing room air; 51 patients were also assessed while breathing 100% oxygen.

Results: Patients with sPH had more severe hypoxaemia (PaO_2, median [P25-P75], 48 [41-55] mmHg, compared with 68 [58-76] and 60 [55-69] mmHg in no PH and mPH, respectively; p<0.001). The group with sPH had greater V_A/Q mismatching (LogSD Q, 1.24 [0.90-1.35] vs. 0.89 [0.77-1.00] and 0.89 [0.78-1.03] in no-PH and mPH, respectively; p=0.013), with more prominent shunt (6.65 [1.65-9.70] % vs. 2.10 [0.75-3.60] and 2.83 [0.48-3.71] % in no-PH and mPH, respectively; p=0.010), and numerically lower mixed-venous PO_2 (31 [23-35] mmHg vs. 34 [32-36] and 34 [33-36] mmHg in no-PH and mPH respectively; p=0.061), which was the major determinant of hypoxaemia. The contribution of hypoxic vasoconstriction to VA/Q matching was lower in sPH than in the other groups.

Conclusions: COPD patients with severe PH characteristically present with very severe hypoxaemia, which results from the combined effect of reduced mixed-venous PO_2, increased

intrapulmonary shunt and greater V_A/Q mismatch, with a negligible role of hypoxic vasoconstriction in preserving V_A/Q matching.

Mechanisms of hypoxaemia in severe pulmonary hypertension associated with COPD

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To the Editor:

Pulmonary hypertension (PH) is a frequent complication of COPD, with a poor prognosis, especially in its severe form [1]. Accordingly, current guidelines distinguish patients with severe PH from those with moderate PH [2]. Patients with COPD and severe PH often present with worse hypoxaemia than those with moderate PH, despite having milder airflow obstruction [3–5]. The mechanisms underlying severe hypoxaemia in these patients have not been elucidated. This study aimed to analyse the determinants of hypoxaemia in severe PH associated with COPD by assessing ventilation/perfusion (V_A/Q) relationships with the multiple inert gas elimination technique (MIGET).

We retrospectively analysed 88 COPD patients who underwent simultaneous assessments of pulmonary haemodynamics and V_A/Q distributions with MIGET in our laboratory. Patients were grouped as: without PH (mean pulmonary artery pressure (mPAP) ⩽20 mmHg or pulmonary vascular resistance (PVR) ⩽2 Wood units (WU)); moderate PH (mPAP >20 mmHg, PVR 2.5–5.0 WU and pulmonary artery wedge pressure (PAWP) ⩽15 mmHg); and severe PH (mPAP >20 mmHg, PVR >5 WU and PAWP ⩽15 mmHg). Studies were performed as previously described [6]. In a subgroup of 51 patients, measurements were repeated while breathing 100% oxygen to assess the effects of inhibiting hypoxic pulmonary vasoconstriction (HPV).

The dispersion of perfusion (logSD Q) and ventilation (logSD V) distributions were used as indices of V_A/Q mismatch (normal: logSD Q <0.60, logSD V <0.65). The difference between retention and excretion (R−E*) for each inert gas was computed and the whole R−E* of all gases (DISP R−E*, normal <3) was used as an overall descriptor of V_A/Q inequality. The amount of blood flow in units with low V_A/Q ratio (0.005–0.1) or non-ventilated areas (shunt), and the amount of ventilation in units with high V_A/Q ratio (10–100) or non-perfused areas (dead space) were computed. The difference between measured arterial oxygen partial pressure (P_{aO_2}) and that predicted by the MIGET (Pr-Ms P_{aO_2}) was used to assess alveolar-to-capillary diffusion limitation to oxygen.

Groups were compared with an independent samples Kruskal–Wallis one-way analysis of variance for continuous variables, *post hoc* Dunn's test for pairwise comparisons, and chi-square test for categorical variables. Comparisons between room air and oxygen breathing were performed with a paired-samples t-test. A p-value <0.05 was considered significant. The study was approved by the hospital ethics committee.

The groups had similar age and sex distribution. Patients with severe PH had less airflow obstruction than those with moderate or without PH (figure 1a), and lower carbon monoxide diffusing capacity (median (interquartile range) 29 (23–37), 44 (28–58) and 46 (28–61) % predicted for severe, moderate and without PH, respectively; p<0.05). Pulmonary haemodynamic profiles are shown in figure 1a.

Patients with severe PH had significantly lower P_{aO_2} and numerically lower mixed-venous partial pressure of oxygen (P_{vO_2}) than the other groups (figure 1a). They also had greater impairment of V_A/Q relationships, with the highest DISP R−E* (17.3 (13.7–24.4)) and logSD Q values, and an increased proportion of shunt (figure 1a and b). No differences in logSD V, high V_A/Q units or dead space were observed. The P_{aO_2} was fully explained by the severity of V_A/Q mismatching, without differences in Pr-Ms P_{aO_2}.

Shareable abstract (@ERSpublications)
Severe hypoxaemia, characteristic of COPD with severe pulmonary hypertension, is due to a combination of greater ventilation–perfusion mismatch, increased intrapulmonary shunt and reduced P_{vO_2}, with negligible hypoxic pulmonary vasoconstriction regulation https://bit.ly/3Wnzpik

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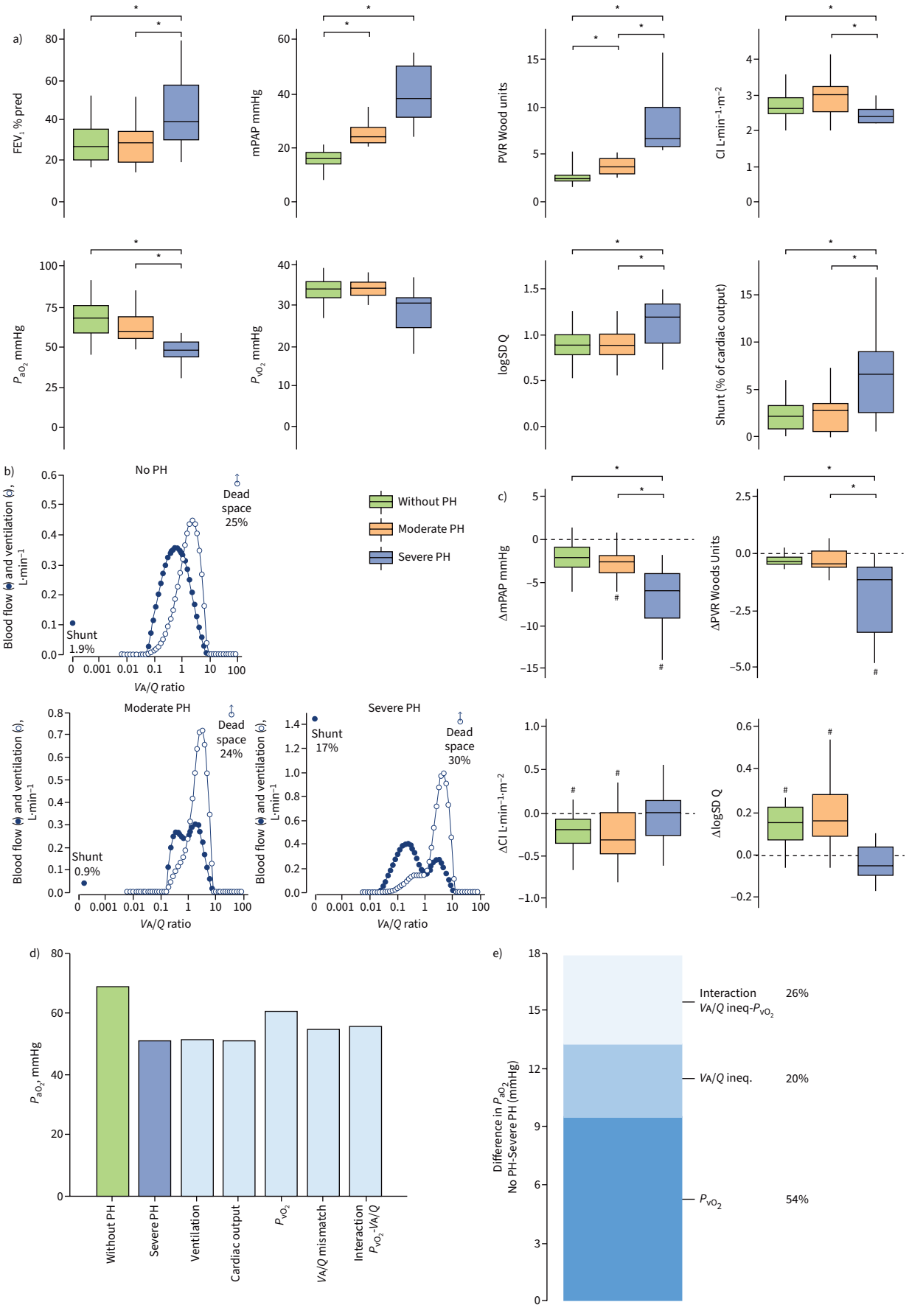
a)
FEV1 % pred
mPAP mmHg
PVR Wood units
CI L·min−1·m−2
PaO2 mmHg
PvO2 mmHg
logSD Q
Shunt (% of cardiac output)
b)
No PH
Dead space 25%
Shunt 1.9%
Blood flow (●) and ventilation (○), L·min−1
VA/Q ratio
Without PH
Moderate PH
Severe PH
Moderate PH
Dead space 24%
Shunt 0.9%
Severe PH
Dead space 30%
Shunt 17%
c)
ΔmPAP mmHg
ΔPVR Woods Units
ΔCI L·min−1·m−2
ΔlogSD Q
d)
PaO2, mmHg
Without PH
Severe PH
Ventilation
Cardiac output
PvO2
VA/Q mismatch
Interaction PvO2-VA/Q
e)
Difference in PaO2 No PH-Severe PH (mmHg)
Interaction VA/Q ineq-PvO2 26%
VA/Q ineq. 20%
PvO2 54%

FIGURE 1 a) Airflow obstruction, pulmonary haemodynamics and gas exchange measurements breathing room air in patients with COPD without pulmonary hypertension (PH), with moderate PH and with severe PH. b) Representative plots of the distribution of blood flow and ventilation as a function of the ventilation/perfusion (V_A/Q) ratio on a logarithmic scale in COPD patients without PH, with moderate PH and with severe PH. Closed symbols indicate blood flow and open symbols ventilation. The amount of shunt and dead space are expressed as percentage of cardiac output and minute ventilation, respectively. c) Changes in pulmonary haemodynamics and gas exchange measurements breathing 100% oxygen in COPD patients without PH, with moderate PH and with severe PH. d) Quantitative contribution of determinants of hypoxemia in COPD patients with severe PH. Green bar shows the average arterial oxygen partial pressure (P_{aO_2}) predicted by the multiple inert gas elimination technique (MIGET) algorithm in patients without PH and the dark blue bar that of patients with severe PH. Light blue bars show the P_{aO_2} predicted by the MIGET in patients with severe PH if their ventilation, cardiac output, mixed-venous partial pressure of oxygen (P_{vO_2}) and V_A/Q distribution data had been those of patients without PH. The last light blue bar reflects the effect of the concurrence of higher P_{vO_2} with more homogenous V_A/Q distributions, as seen in patients without PH. e) Schematic representation of the relative contribution of different factors on the difference in P_{aO_2} between patients with severe PH and those without PH. Boxplots (a, c) show the median and the interquartile range, whiskers mark the 5th and 95th percentiles. *: $p<0.05$ compared with the other group; #: $p<0.05$ compared with the value breathing room air in the same group. FEV_1: forced expiratory volume in the first second; mPAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; CI: cardiac index; logSD Q: dispersion of the distribution of blood flow (normal <0.6) that reflects the severity of V_A/Q mismatch; shunt: perfusion of unventilated lung units. Δ denotes the change between measurements at room air and those breathing 100% oxygen (c).

Breathing 100% O_2, mPAP and PVR decreased to a greater extent in the severe PH group (figure 1c). The increase in P_{aO_2} was significantly less in patients with severe PH (348 (228–485) mmHg) than in patients with moderate or without PH (498 (443–512) and 505 (474–532) mmHg, respectively; $p<0.05$). V_A/Q relationships worsened (increased logSD Q) in patients without PH and with moderate PH, but not in those with severe PH (figure 1c). The amount of shunt remained unchanged in all groups.

Our study shows that, in COPD patients with severe PH, the marked impairment of gas exchange is explained by the concurrence of severe V_A/Q mismatch, with a prominent amount of blood diverted to areas with low V_A/Q ratio and shunt, lower P_{vO_2} and a poor contribution of HPV to V_A/Q matching. This profile differs from that usually seen in COPD [7, 8], which we corroborated in patients without PH, and with moderate PH. Since the severe PH group had less airflow limitation, further worsening of V_A/Q relationships is likely due to changes in pulmonary vasculature. In fact, pulmonary vascular remodelling is associated with the severity of V_A/Q mismatching in COPD [9] and some features observed in the severe PH group (areas of very low V_A/Q and shunt) concur with those shown in idiopathic pulmonary arterial hypertension [10]. We hypothesise that in COPD patients with severe PH, pulmonary vascular abnormalities add to changes in small airways and lung parenchyma, further increasing the V_A/Q imbalance due to airflow obstruction. Of note, patients with severe PH had a significant amount of shunt, the origin of which is not apparent since they did not have obvious areas of alveolar occupation or collapse. Potential explanations include: a patent *foramen ovale*, although this was ruled out with contrast echocardiography in the majority of patients with severe PH; loss of peripheral vascular bed [11] that could divert blood to poorly ventilated units, further decreasing their V_A/Q ratio; or the hypothetical presence of anastomoses between pulmonary and bronchial vessels bypassing the pulmonary capillaries, similar to those described in pulmonary arterial hypertension [12].

We explored the quantitative contribution of factors determining P_{aO_2}, using the MIGET algorithm, in patients with severe PH, as compared to those without PH [13] (figure 1d). The principal factor, accounting for 54% of the difference in P_{aO_2}, was reduced P_{vO_2}; the remaining 46% was explained by greater V_A/Q mismatch and shunt, which also determine lower capacity to counterbalance the effect of a diminished P_{vO_2} (figure 1e). This analysis highlights the important role of P_{vO_2}, determined by cardiac output, in modulating P_{aO_2} in PH [14].

V_A/Q relationships (logSD Q) significantly worsened on 100% oxygen in patients without PH or with moderate PH, but not in those with severe PH, who instead presented a significant decrease in mPAP and PVR. This suggests that HPV did not contribute substantially to maintaining V_A/Q balance in COPD with severe PH. Presumably, greater endothelial dysfunction in severe PH may reduce the ability to finetune the matching of perfusion to alveolar ventilation [9]. The lack of deterioration of V_A/Q relationships when releasing HPV with oxygen in COPD patients with severe PH may suggest that pulmonary vasodilators might not adversely affect gas exchange in this group. Indeed, controlled trials of pulmonary vasodilators in COPD patients with severe PH have not reported oxygenation worsening [15].

This study is limited by the relatively small sample size of the severe PH group, which is a rare condition, although it was sufficiently robust to detect significant differences in gas exchange determinants, which

was its main goal. Another limitation is the absence of imaging in many patients, which could have informed on the parenchymal derangement, due to the retrospective nature of the study.

In conclusion, our study shows that patients with severe PH associated with COPD present distinctive features in pulmonary gas exchange that may contribute to characterising this clinical phenotype. In these patients, severe hypoxaemia is caused by the combination of greater V_A/Q mismatch, increased intrapulmonary shunt and reduced P_{vO_2}, along with a negligible role of HPV in preserving V_A/Q matching. From a practical point of view, these results suggest that pulmonary vasodilators might not be detrimental to gas exchange in this population; theoretically, they could even improve it by increasing cardiac output.

Lucilla Piccari[1], Isabel Blanco[1,2,3], Yolanda Torralba[1,2,3], Ebymar Arismendi[1,2,3], Concepción Gistau[1], Ana Ramírez[1], Elena Gimeno-Santos[1,2], Josep Roca[1,2,3], Felip Burgos[1,2,3], Roberto Rodríguez-Roisín[1,2,3] and Joan Albert Barberà[1,2,3]

[1]Department of Pulmonary Medicine, Hospital Clínic, University of Barcelona, Barcelona, Spain. [2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain. [3]Biomedical Research Networking Centre on Respiratory Diseases (CIBERES), Madrid, Spain.

Corresponding author: Isabel Blanco (iblanco2@clinic.cat)

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Discussion

Airway disease, emphysema and pulmonary vasculopathy coexist in a complex interaction in COPD. In the last few decades, following a stronger understanding of its pathophysiology, new research has progressively unravelled the systemic ramifications of COPD and the existence of multiple etiopathogenetic mechanisms(90). Cigarette smoking, one of the biggest modifiable causes of COPD, has wide-ranging damaging consequences on very different organs and tissues, and its effect intertwine with those of obstruction of the airways and destruction of the lung parenchyma, causing such diverse effects as systemic inflammation, low body mass index and sarcopenia. Therefore, what was initially considered primarily a lung disease is nowadays being reclassified as a systemic one(115). For these reasons, understanding the connection between systemic and pulmonary vascular alterations is key to understanding the pathophysiology of COPD.

Systemic and pulmonary vascular function have often been explored separately in COPD. However, similar alterations are found in the vascular endothelium of systemic and pulmonary arteries. Even though not all patients with COPD develop PH, there is also ample evidence that some degree of pulmonary vasculopathy, with arterial remodelling, arterial stiffness, endothelial dysfunction and inflammatory infiltrate in the lung vessels is found in patients with COPD without PH and some of these have even been found in smokers without airflow obstruction(78–80,82–84,88). The prevalence of these pulmonary vascular alterations in COPD patients is not known because it has not been systematically explored, nor are there simple, non-invasive tests that can be used to detect them. However, they most likely have an impact on the clinical course of those affected, and possibly may have a role in COPD phenotype, progression, exacerbations and outcomes. The full pathogenetic course of the pulmonary and systemic endothelium from health to disease still needs to be discovered, and this thesis aims to add some pieces to the puzzling path through damage and repair of vasculature in COPD.

Vascular function in systemic arteries in COPD

In the study “Association Between Systemic and Pulmonary Vascular Dysfunction in COPD”, we demonstrated that patients with COPD present systemic arterial dysfunction as assessed through FMD and PWV, and that this is associated with the presence of pulmonary vascular disease.

In the first study object of this doctoral thesis, we confirmed that endothelial dysfunction of systemic arteries is associated with COPD, and that FMD decreases in a continuous fashion through the range from non-smoking subjects, smoking subjects, patients with COPD without pulmonary vascular disease and patients with COPD and pulmonary vascular disease (Figure 1 in the article). Interestingly, the same progressive decrease of FMD among groups was observed even when we corrected the measurements of FMD for the diameter of the brachial artery, which may vary upon individuals and thus introduce a bias in the evaluation of the percentage of change from baseline to dilated vessel during FMD. The fact that lower dilation of the brachial artery in COPD patients existed even when the percentage values were corrected for the individual vessel size indicates that there is a true difference among groups.

In a previous study from our group(78), it was demonstrated that patients with COPD and pulmonary vascular disease had worse FMD compared to COPD patients without it. In this study, we have expanded on this association by demonstrating that this relationship is independent of the presence of comorbid conditions, and therefore it cannot be ascribed to the fact that COPD patients with pulmonary vascular dysfunction had a higher percentage of cardiovascular disease compared to COPD patients without pulmonary vascular disease in our cohort. The fact that the values of FMD for COPD patients with and without pulmonary vascular disease are not statistically different may be due to the relatively small size of the latter group, despite the numerical value of FMD in the COPD with pulmonary vascular disease group being half of that of COPD patients without pulmonary vascular disease. This proportion is observed even when this variable is corrected for brachial artery size in each individual, the dilation of the brachial artery for COPD patients with pulmonary vascular disease being consistently half of that achieved by COPD patients without pulmonary vascular impairment. Alternatively, it could be argued that endothelial dysfunction per se is not enough to cause pulmonary vascular disease and it is necessary the concurrence of other factors, namely reduced vascular compliance.

Arterial stiffness, as measured by PWV, was significantly increased in COPD patients and was even higher in those with pulmonary vascular disease. In the non-adjusted analysis, both COPD groups had increased arterial stiffness compared to both control groups, and COPD patients with pulmonary vascular disease had increased stiffness compared to COPD patients without it.

However, age and Framingham score (a score indicating the 10-year cardiovascular risk of an individual) were retained in the final adjusted model, indicating that these variables had an effect on the results: in the final adjusted analysis, having taken into account the effects of age and the Framingham score, COPD patients with pulmonary vascular disease had increased arterial stiffness compared with both non-smoking and smoking controls. Therefore, even after having taken into account the effect of cardiovascular comorbidities and age, systemic arteries of COPD patients with pulmonary vascular disease were stiffer than those of smoking and non-smoking controls; stiffness was also higher in the COPD group with pulmonary vascular disease compared to the one without, although this difference did not reach statistical significance ($p = 0.06$). Interestingly, there is no influence of the singular cardiovascular disease separately; instead, only the Framingham score significantly influenced the result. Thus, it seems that it is the combination of the different cardiovascular diseases to be significantly affecting arterial stiffness. It is possible to explain these results by considering the ffect of cigarette smoke on the systemic and pulmonary vasculature, which is known to cause endothelial dysfunction in both compartments(78,79) and might predispose to the progression to pulmonary vascular disease. Previous studies have indeed evidenced through different techniques the presence of pulmonary artery stiffness in COPD patients with PH(89,116,117). There was no difference in either endothelial dysfunction or arterial stiffness between non-smoking and smoking subjects with normal lung function in our study, at variance with previous findings from our group(78). This difference could be due to the fact that in the previous study we excluded patients with cardiovascular risk factors other than cigarette smoking, whereas in the current one we included patients with other cardiovascular risk factors, Accordingly, in the presence of other cardiovascular risk factors, cigarette smoking may not be sufficient to significantly increase the severity of endothelial dysfunction. Furthermore, since the number of pack/years was significantly higher in both COPD groups compared to both control groups, it may be that in the latter the degree of exposition had not yet reached the level required to produce vascular damage. Indeed, in the present study cigarette smoke exposure was 27% lower in the control smokers group than in the previous one(78).

Both FMD and PWV correlated with the level of airflow obstruction and gas exchange impairment as expressed by the DLCO. This finding is interesting as it reinforces the relationship between the

pathogenetic mechanisms of COPD and the existence of systemic arterial dysfunction. Furthermore, the direct correlation between PWV and systolic pulmonary artery pressure, as well as the inverse correlation between FMD and the systolic pulmonary artery pressure, illustrate an association between the systemic and pulmonary vascular impairments which may indicate that these two tests could be useful in detecting PH in COPD patients. In fact, as the ROC curves in our work demonstrate, both FMD and PWV are discretely sensitive and specific for the presence of pulmonary vascular disease in COPD patients.

In the analysis of inflammatory mediators, we only detected two differences among groups. We found an elevation of the soluble receptor of tumour necrosis factor-alpha in the COPD group with pulmonary vascular disease: this receptor is elevated in other cardiovascular diseases, which were more represented in this group of patients. Fibrinogen was also elevated in COPD patients without pulmonary vascular disease. Other inflammatory markers were not significantly different among groups, which means that our findings can't be attributed to a higher inflammatory state of COPD groups compared to the control groups. We did not find differences among groups in the augmentation index or in the augmentation index normalised at 75 beats per minute.

Gas exchange and pulmonary arterial function in patients with COPD across the spectrum of pulmonary vascular disease

In the second work of this doctoral thesis entitled "Mechanisms of hypoxaemia in severe pulmonary hypertension associated with COPD", we confirmed that patients with COPD and severe PH presented with milder airflow obstruction but at the same time worse gas exchange, as manifest in the lower PaO_2, compared to patients with COPD without PH and with mild-moderate PH, which conforms the so-called pulmonary vascular phenotype(45).

Severe gas exchange impairment in COPD patients with severe PH was the result of worse V_A/Q mismatch and increased proportion of blood flow to poorly perfused areas and to areas of shunt. In patients with COPD, worse V_A/Q mismatch is associated with more pronounced vascular remodelling(113). This could be a potential explanation as to why the severe PH group, despite showing a functional profile of milder airflow obstruction compared to the other two

groups, presented worse V_A/Q relationships. We therefore posit that severe vascular abnormalities, which have been previously demonstrated histologically in COPD patients with severe PH(58,92), are added to the ventilatory impairment and emphysematous destruction, thereby causing a state of more severe disarray of gas exchange. The increased heterogeneity in the distribution of blood flow, caused by the severe vascular disease, is added to the heterogeneity of ventilation caused by alveolar and airway disease typical of COPD and results in the further increased V_A/Q mismatch.

One of the most interesting features of gas exchange in severe PH patients was the increased percentage of blood flow directed to areas of very low V_A/Q and shunt, which is not common in COPD patients assessed using MIGET(105,108,113). An increased amount of shunt was also found with MIGET in patients with PAH and with CTEPH in the seminal article by Dantzker et al.(118) Calculating from the reported values for each patient, the median shunt was 1.1% of (percentiles 25-75: 0.3%-6.1%), and the median low V_A/Q was 7.1% (percentiles 25-75: 5.8-17.0)(118). In our study, we found an even higher value for both parameters in COPD patients with severe PH: median shunt 6.7% (percentiles 25-75: 1.8%-9.7%), and median low V_A/Q 9.6% (percentiles 25-75: 1.8%-24.4%)(119).

The cause of increased blood flow directed at areas of very low V_A/Q and shunt in COPD patients with severe PH is not clear. The obvious hypothesis of areas of alveolar occupation from infection or oedema was excluded by computerised tomography thoracic scan in all severe PH patients, while the presence of intracardiac shunting of blood flow through a patent foramen ovale was ruled out in the majority of them. It's possible that through the loss of pulmonary vessels, caused by the morphological disarray brought about by emphysema and vessel remodelling found in patients with COPD and severe PH(58,96), blood flow could be redistributed away from areas of restricted perfusion thereby leading to eventual overperfusion of poorly ventilated areas. In fact, this explanation was already evoked in the study of PAH and CTEPH with MIGET by Dantzker et al(118). In that article, the authors also argued that interstitial and alveolar oedema in regions at the base of the lungs, caused by high pulmonary artery pressure and leakage, might explain the distribution of blood flow to areas of shunt; however, in our study all patients with severe PH were explored with high resolution CT scans and showed no interstitial or alveolar oedema.

Another possible explanation is that the increased pulmonary vascular pressure may lead to the development of pulmonary-bronchial vascular anastomoses that would effectively bypass the gas exchange unit. Such structures have been identified in patients with PAH(120,121), CTEPH(122), COVID-19(123) and asthma (124). It is known that one of the hallmark histological signs of PAH is the presence of plexiform lesions(125) which are aberrant vascular formations not in contact with air spaces and arising from the connection between the bronchial and pulmonary circulation; lesions undistinguishable from these have been observed in a previous study comparing the histology of lungs from COPD patients with severe PH and from PAH(92).

In addition to the increase V_A/Q mismatch and proportion of shunt and low V_A/Q, patients with COPD and severe PH presented with reduced PvO_2, which highlights the importance of the interdependency of PvO_2 and PaO_2. Reduced PvO_2 is the product of a decreased cardiac output which in turn is expression of right ventricular systolic dysfunction in patients with severe PH (in the absence of significant left heart dysfunction, as proven by normal PAWP). It is important to highlight that PaO_2 and PvO_2 are interdependent, as the Fick principle establishes. In fact, the reduced PvO_2 is the expression of reduced oxygen delivery, which is in turn caused both by reduced cardiac output as well as reduced oxygen content; the relationship is linear, and the slope is determined by the degree of V_A/Q mismatch. Therefore, in COPD patients with severe PH, who have increased V_A/Q mismatch compared with the other two groups, the same PvO_2 will determine a much lower PaO_2, due to the lower ability of the lung to counterbalance such decrease in PvO_2, as shown by a lower slope of the PaO2/PvO2 curve. This also explains the influence of the interaction between PvO_2 and V_A/Q mismatch that we computed in the model analysis.

To analyse the determinants of impaired gas exchange in COPD patients with severe PH we used the MIGET algorithm to model the individual contributions of minute ventilation, cardiac output, PvO_2 and V_A/Q mismatch on the resulting PaO_2 (Figure 4). The model allowed to identify as major causes of the severe hypoxemia presented by COPD patients with severe PH PvO_2 (54%), closely followed by the V_A/Q mismatch and its interaction with the reduced PvO_2.

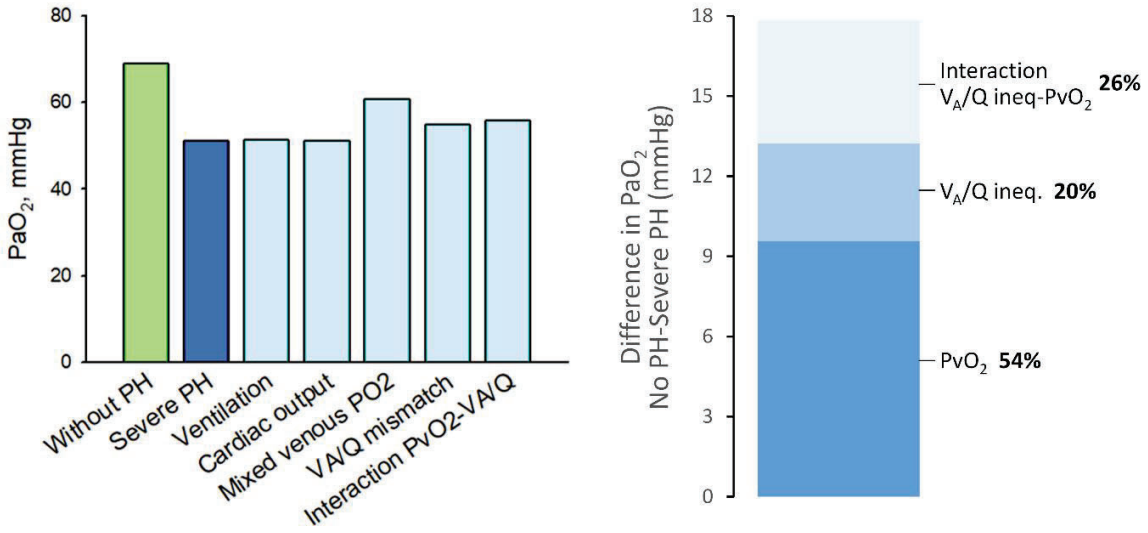

Figure 4. Individual contributions of hemodynamic and V_A/Q mismatch values to gas exchange. Adapted from Piccari L et al, Eur Respir J 2023 (119).

In previous works, similar models had been applied to unravel the gas exchange determinants in patients with pulmonary embolism(126) and in liver cirrhosis(127). In a previous study from our group, the same technique had been used to ascertain the causes of hypoxemia in COPD patients who were experiencing an exacerbation(109), finding that cardiac output and oxygen consumption played similar but antithetic roles in the resulting PaO_2. Strikingly, in the cited study the interaction between V_A/Q mismatch and reduction in PvO_2, the first being amplified by the other as previously explained, accounted for 26% of the reduction of PaO_2 in the model, which is the exact same result we found in our work.

In our study, we showed that, upon administration of 100% oxygen, pulmonary hemodynamics improved in the COPD with severe PH but V_A/Q mismatch did not worsen, contrary to what happened in patients with COPD and mild-moderate PH and COPD without PH, which means that, in the group with severe PH, hypoxic vasoconstriction plays a minimal role in the maintenance of gas exchange in severe PH patients with COPD.

In patients with COPD and severe PH, the administration of 100% oxygen produced a vasodilating effect (reduction in mPAP and PVR) but it did not increase V_A/Q mismatch compared to the measurements obtained at room air. This is different from what we observed in COPD patients

without PH and with moderate PH, where LogSD Q did increase significantly compared to room air measurements. In COPD patients with moderate PH, the mPAP did decrease significantly but PVR did not, while in the group without PH neither mPAP nor PVR were significantly decreased. These findings suggest that hypoxic pulmonary vasoconstriction does not contribute significantly to the maintenance of VA/Q matching in COPD patients with severe PH.

We consider that an increased endothelial dysfunction in the pulmonary vasculature may lead to the loss of fine-tuning abilities of the endothelium to vasoconstrict in response to the hypoxic stimulus(128). In fact, previous studies from our group have established that worse V_A/Q mismatch was associated with more prominent vascular remodelling in COPD(113).

Systemic and pulmonary vascular function in COPD

COPD is a complex disease, where likely all the components of the lung parenchyma present alterations and contribute to the pathophysiology and clinical course. It has been observed in recent years that the pulmonary endothelium may play a bigger role than previously thought(32). Endothelial injury has not only been found in the pulmonary circulation, but also in other distant organs with very different functions, such as the kidneys and the systemic vessels(129). It is possible then that common risk factors such as cigarette smoke, as well as genetic conditions and developmental events (such as reduced lung growth or dysynaptic development of the lung parenchyma) may all play a role in the progressive evolution of endothelial dysfunction, which in turn may predispose to arterial stiffness. In fact, we know that even patients who do not present hemodynamic compromise meeting the definition of PH, may develop some degree of pulmonary vasculopathy. However, PH is not present in all COPD patients and the reason why in some patients the hemodynamic deterioration is more pronounced than in others is not known. It is possible that the coexistence of several factors in the same patient (e.g., cigarette smoking on a favourable genetic terrain, expression of specific molecules such as HIF-2 or stunted lung growth) might result in greater vasculopathy and ultimately PH, and that this is not the case when these factors are not present at the same time. It is also possible that individual characteristics such as inflammatory response to injury and reparative processes may favour one outcome over the other and determine the appearance of a specific phenotype. It may finally be that the recognised

phenotypes of PH in COPD are only a part of those existing and that these should also take into account the different etiotypes of COPD(8) and peripheral vascular dysfunction, so that if we were to consider all of these different manifestation we would observe the full picture of COPD-associated vascular injury in the pulmonary and systemic circulation.

Endothelial dysfunction is clearly at the centre of the pathophysiology of COPD and pulmonary vascular disease, and the ramifications of its molecular effects are still to be fully uncovered.

Conclusions

From the results of the studies conducted in this doctoral thesis, it is concluded:

1. In chronic obstructive pulmonary disease, there is an association between systemic and pulmonary vascular impairment that suggests common pathophysiological mechanisms.

2. Increasing degrees of endothelial dysfunction and of arterial stiffness are associated with the development of chronic obstructive pulmonary disease and especially in chronic obstructive pulmonary disease associated with pulmonary vascular disease.

3. The concurrence of endothelial dysfunction, which might be triggered by cigarette smoke products, and vascular stiffness in patients with chronic obstructive pulmonary disease and its association with pulmonary vascular disease suggest that in these patients both pulmonary and systemic vascular territories might be impaired.

4. Patients with severe pulmonary hypertension associated with chronic obstructive pulmonary disease present distinctive features in pulmonary gas exchange that may contribute to characterising this clinical phenotype.

5. In these patients, severe hypoxaemia is caused by the combination of greater ventilation/perfusion mismatch, increased intrapulmonary shunt and reduced mixed-venous oxygen pressure.

6. In patients with severe pulmonary hypertension and chronic obstructive pulmonary disease there is a negligible role of hypoxic pulmonary vasoconstriction in preserving ventilation/perfusion matching.

7. These results suggest that pulmonary vasodilators might not be detrimental to gas exchange in these patients; instead, they might improve it by increasing cardiac output.

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