Field exercise testing in COPD

Citation for published version (APA):

Andrianopoulos, V. (2017). *Field exercise testing in COPD*. [Doctoral Thesis, Maastricht University]. Datawyse / Universitaire Pers Maastricht. <https://doi.org/10.26481/dis.20170510va>

Document status and date:

Published: 01/01/2017

DOI:

[10.26481/dis.20170510va](https://doi.org/10.26481/dis.20170510va)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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FIELD EXERCISE TESTING IN COPD

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ISBN: 978 94 6159 687 1

Lay out: Datawyse
Cover design: Lina Koulountzou
Production: Datawyse | Universitaire Pers Maastricht

The thesis was financially supported by CIRO+, center of expertise for chronic organ failure, the Netherlands

Printing and distribution of this thesis was financially supported by CIRO+, center of expertise for chronic organ failure, the Netherlands

Field exercise testing in COPD

DISSERTATION

to obtain the degree of Doctor at Maastricht University,
on the authority of the Rector Magnificus, Prof. dr. Rianne M. Letschert
in accordance with the decision of the Board of Deans,
to be defended in public
on Wednesday, 10 May 2017, at 10:00 am

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Chapter 1

General Introduction

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable disease which is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases.¹ It includes two key components: the chronic bronchitis which is partially reversible, and pulmonary emphysema (parenchymal destruction) that is an irreversible component.¹ COPD may be asymptomatic in its early stages but cause severe symptoms such as dyspnea, cough and sputum overproduction in later stages. In the progression of the disease, a decline in forced expiratory volume in 1 second (FEV₁) occurs due to loss of lung elasticity and increase in airways resistance in small and/or large airways. In some patients with advanced disease severity, reduced FEV₁ is accompanied by arterial hypoxemia with or without hypercapnia indicating respiratory insufficiency.² Regarding the prevalence of COPD in Europe, a number of well-designed studies showed prevalence estimates ranging from 4% to 10% among the adults population while new research suggests as many as 66 million people in Europe live with COPD.^{3,4} Statistics for global population have shown that 4.8% of people in the entire world are diagnosed with COPD.⁵ These findings may only partly represent the burden of the disease because a significant proportion of patients is undiagnosed or misdiagnosed.^{3,6,7}

Besides the high prevalence of COPD, the multi-morbidity of disease represents a real public health problem and a major burden on health-care resources.^{8,9} According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) report of 2017, COPD is a leading cause of morbidity and mortality worldwide and social burden is both substantial and increasing.¹ Despite worldwide medical research, health care effort and costs, mortality in COPD reveal a continuing upward trend, in contrast with other major causes of death such as cancer and cardiovascular disease. Globally more than 3 million people died of COPD in 2012 and this was the 6% of all deaths globally.¹⁰ The World Health Organization (WHO) estimates that COPD will be the third most common worldwide cause of death and disability by 2030, from its current fourth ranking.¹¹

The most important risk factor for COPD in developed countries is the tobacco smoke, with 85% of all cases attributable to smoking^{8,12}, while in developing world the exposure to noxious particles and gases (air pollution) often contributes to the prevalence of COPD.^{1,13} Although tobacco smoking is a significant risk factor increasing the odds for COPD development by 5 times¹², fewer than 50% cigarette smokers develop clinically significant COPD during their lifetime.^{14,15} Passive smoking may also contribute to respiratory symptoms and COPD due to inhaled particles and gases.¹⁶ Besides exposures, host factors predispose individuals to develop COPD. These factors include genetic disorders, abnormal lung development and accelerated aging.¹ In some rare cases, which represent the 1-5% of people with COPD, the disease results from a

genetic abnormality that causes low levels of a protein called alpha-1 antitrypsin or from recurrent respiratory infections in childhood.^{17, 18} In symptomatic individuals, the diagnosis of COPD is based on spirometry confirming $FEV_1 < 80\%$ and a ratio of FEV_1 and forced vital capacity (FEV_1/FVC) below 0.70 after bronchodilation.¹⁹ Until recently, COPD classification was largely based on FEV_1 , however, updated GOLD report comprises four quadrants (A-B-C-D) based on symptoms and risk of exacerbations, besides airflow limitation, for classifying patients with COPD.²⁰

IMPACT ON EXERCISE CAPACITY AND PHYSICAL ACTIVITY

Exercise capacity defined as the maximum amount of sustainable physical exertion²¹, and physical activity described as the quantity of body movements produced by skeletal muscles²² are significantly reduced in patients with COPD compared to healthy individuals.^{23, 24} Markedly impaired exercise capacity and physical activity in COPD is usually reduced in proportion to disease severity as assessed by the GOLD staging system.^{25, 26} Patients with COPD often complain for dyspnea that limits the duration and intensity of exercise activities or tasks that can be sustainable. There appears to be an important role for ventilatory limitations which become more prominent as disease progresses and often correlates well with the intensity of dyspnea.²⁷

Exercise capacity in COPD may be negatively affected by several factors related to respiratory system such as ventilatory constraints, hyperinflation, diminished oxygen uptake, alveolar wall destruction with impaired gas exchange, increased respiratory muscle fatigue, and decreased ventilatory reserve.²⁸⁻³² Dynamic ventilatory constraints during exercise deteriorated over time can also significantly contribute to exercise capacity deterioration.³³ Moreover, yet even factors that are not directly related to the lungs may have a negative impact on exercise capacity in COPD including early lactate production³⁴⁻³⁷, muscle dysfunction^{38, 39}, and cardiovascular deconditioning^{40, 41} usually compounded by sedentary lifestyle^{42, 43}. Consequently, all of these factors results in different grades of dyspnea and leg-fatigue that can be occurred even in relatively low levels of physical activity or exercise intensity in patients with COPD. Indeed, a fair correlation of exercise capacity and physical activity has been already reported.⁴⁴ In COPD, a vicious circle may occur as dyspnea is associated with abstention from physical activity that leads to muscle decondition which in turn results in increased ventilatory requirements causing even higher dyspnea and decrements in exercise capacity.⁴⁵⁻⁴⁷ Patients with COPD can be trapped in the vicious cycle of declining physical activity levels and increasing symptoms with exercise (Figure 1).

Figure 1. The vicious circle of inactivity in COPD can result in low exercise capacity and inactivity

EVALUATION OF EXERCISE CAPACITY IN COPD

The evaluation of exercise capacity can be considered as important as the evaluation of pulmonary function in COPD. Evidence has shown that exercise capacity is even superior to single functional measurements obtained at rest (e.g., forced expiratory volume in 1 second) in demonstrating the positive effect of a specific intervention.⁴⁸ It provides useful information for the impact of disease severity in patients' functional status, the effectiveness of therapeutic interventions and it has also prognostic value.⁴⁸

Exercise capacity can be assessed by maximal and submaximal tests, which reflect different pathophysiological properties. Several exercise tests have been developed for measuring exercise capacity and can be categorized in two types: A) the field-based walk tests such as the six-minute walk test and the shuttle walk test, and B) the laboratory tests such as the incremental and cycle endurance ergometer tests.⁴⁹ These tests have different primary outcomes (i.e. walking distance, cycle endurance time, oxygen consumption etc.) that may reflect different physiological parameters. Maximal exercise capacity is commonly assessed by incremental cycle ergometry, whereas the six-minute walk test (6MWT) and cycle endurance test (CET) are responsive to interventions such as drug therapy or exercise training.⁵⁰⁻⁵² Specifically, in the evaluation of exercise capacity, simple improvements in exercise tolerance test

performance, submaximal or maximum aerobic capacity levels, and the functional state between respiratory patients can be observed. The evaluation of exercise capacity can help to demonstrate to patients how they are progressing in relation to their exercise capacity and to detect changes in performance over time or after therapeutic interventions.⁵³

FIELD WALKING TESTS

Measurement of walking distance was first advocated by Balke in 1963 as a means of assessing physical fitness.⁵⁴ Nowadays, field walking tests are widely used and play a key role in the evaluation of functional exercise capacity in COPD.⁵⁵⁻⁵⁷ They usually include the shuttle walk test (SWT), which is implemented as incremental (ISWT) or as endurance shuttle walk test (ESWT), and the six-minute walk test (6MWT).⁵⁵⁻⁵⁸ Field walking tests can be considered as great clinical instruments for assessing prognosis, determining outcome of clinical trials and evaluating response to treatment. Both, SWT and 6MWT employ walking task and do not require expensive and sophisticated technology.^{55, 56}

The available data have indicated that both 6MWT and SWT are valid, reliable and responsive for the assessment of exercise capacity in COPD. The choice of an individual test may depend on the circumstances and purpose of the test.⁵⁵ The importance of field walking test is reflected by the fact that exercise capacity constitutes an important outcome as a major determinant of impaired health status and as a predicting factor of hospitalization and mortality in COPD.⁵⁹ Moreover, exercise capacity has become an integral part of the management of patients with COPD.⁶⁰ There is also a substantial body of evidence that supports the conceptualisation of the 6MWT as a test of functional exercise capacity in patients with COPD while new insights further strengthen its clinical value.

SIX-MINUTE WALK TEST AND ITS CLINICAL VALUE

The six-minute walk test (6MWT) was first described by Guyatt and colleagues as an effective, simple and safe tool for assessing walking capacity in clinical practice.⁶¹ The 6MWT is self-paced test and is considered to be a test of submaximal exercise capacity, even though some patients reach maximal exercise levels.^{62, 63} It is performed by instructing the participant to walk as far as possible in six-minute along a flat corridor and it is well-tolerated as walking is the most common pattern of activity in daily life.⁵⁵ Side-effects of chest pain, dyspnea, or musculoskeletal pain are usually mild to moderate, while serious adverse events have not been described due to the self-paced

nature of the test.^{48, 55, 56, 64} The main outcome of the 6MWT is the six-minute walk distance (6MWD), which is measured by actual meters or feet and expressed as percentage of predicted value. In addition, oxygen saturation (SpO₂) and heart rate (HR) are also recorded during walking by the use of pulse oxymetry. Perceived levels of dyspnea and subjective leg-fatigue are measured at the beginning and at the end of the 6MWT using validated measurement scales, such as the Borg scale. Standardised instructions and encouragement are commonly given during the walking test.^{55, 56}

The 6MWT is widely used to measure functional exercise capacity, assess prognosis and evaluate response to treatment across a wide range of chronic respiratory diseases. It has been proven as a reliable clinical instrument in providing reproducible data which are used to detect and quantify changes in exercise capacity after an intervention in COPD.⁶⁵⁻⁶⁷ Evidence suggests that the minimal clinically important difference (MCID) for the 6MWT is conservatively estimated between 25 and 33 meters (median estimate 30m).⁶⁷ Practically, 6MWT outcomes regarding patients' health status alterations can be used to prompt a change in medical interventions and management of COPD. In some cases, 6MWD can be used to estimate peak oxygen uptake.⁶⁸ Moreover, exercise intensity can be prescribed from the 6MWT using typically the 80% of maximum walking speed throughout the test, however, this is not particularly accurate because of the speed which can be variable during walking. In case that a patient walked 300 meters the maximum walk speed can be estimated by the equation of $[300 \times 10 \div 1000 = 3.0 \text{ km/hour}]$.⁶⁹ Given the ubiquitous clinical use of the 6MWT, clinicians should be familiar with 6MWT procedures, limitations and test utility.^{70, 71}

SIX-MINUTE WALK TEST AND DETECTION OF EXERCISE-INDUCED DESATURATION

Six-minute walk test due to the simplicity of performance and the high responsiveness to arterial oxygen desaturation is the preferred exercise test to screen patients for exercise-induced desaturation (EID) in COPD.^{55, 56} It is documented that 6MWT is more sensitive to detect EID compared to cycle ergometry testing.⁷² There is no uniform definition of EID, however, decline of oxygen saturation (SpO₂nadir) to 88% or lower at exertion is commonly accepted for the discrimination of patients with and without EID.⁷³⁻⁷⁵

EID is highly prevalent among patients with COPD and especially in those with more advance disease severity. In COPD patients with moderate to severe lung disease (mean FEV₁ 43%pred.), the prevalence of exercise-induced desaturation on 6MWT was 62%, where desaturation was defined as a drop of >4% in SpO₂ to <90%.⁷⁶ The presence of EID can be associated with reduced daily physical activity, poor exercise performance, a faster decline in FEV₁, and worse prognosis which support its clinical importance.^{31, 76, 77}

Several determinants of oxygen desaturation can be used to predict which COPD patients will or won't exhibit EID during the 6MWT.⁷⁸ These determinants include FEV₁, diffusion capacity for carbon monoxide (DLCO), baseline standard pulse oxymetry (SpO₂) or a combination of thereof.⁷⁹⁻⁸³ Furthermore, there are evidences that EID is related to emphysema^{84, 85} and to increased pulmonary arterial pressure⁸⁶. EID may also be triggered by depressed ventilatory drive which may be typically accompanied by exercise-induced hypercapnia (pCO₂ >46 mmHg) and low pH.⁸⁷

SIX-MINUTE WALK TEST AND PROGNOSIS

The association of the 6MWD with hospitalization and mortality is well documented.^{55, 77, 88} Lower 6MWD has been associated with increased hospitalization and mortality in patients with COPD. Particularly, patients with 6MWD lower of 357m have increased risk of hospitalization, whereas a 6MWD threshold of 334m is a strong predictor of mortality in COPD.⁸⁹ A 6MWD reduction of 30m or more is associated with increased risk for mortality but not hospitalization due to exacerbation in patients with COPD.⁹⁰ Moreover, 6MWD of 325m or lower has been demonstrated as a threshold for predicting postoperative pulmonary complications with 77% sensitivity and 100% specificity, with similar strong predictive value with FEV₁.⁹¹ Besides 6MWD, walking speed, walk-work, distance-saturation products, and exercise-induced desaturation during 6MWT have demonstrated a long-term prognostic value.⁹²⁻⁹⁵

AIMS OF THE THESIS

Clinical exercise testing is a complex concept regarding to exercise pathophysiology in patients with COPD and the interpretation of exercise outcomes. Therefore, the main objectives of this thesis are:

1. To investigate the characteristics of functional exercise performance and determinants of the CET and 6MWT in a large clinical cohort of COPD patients.
2. To explore the impact of several 6MWD reference equations for adults in patients with chronic obstructive pulmonary disease (COPD) and factors accountable for different 6MWD% predicted values.
3. To study the association between 6MWT-derived variables and mortality as well as hospitalization in COPD patients and to compare it with the BODE index.
4. To investigate the relationship between EID and patient-related outcomes during 6MWT, including pulmonary emphysema in a large well-characterized cohort of COPD subjects.

5. To validate a proposed cut-off of baseline-SpO₂ ≤95% as simple screening procedure to predict EID during 6MWT and study the prevalence and characteristics of patients exhibited EID to SpO₂nadir ≤88%.
6. To study pCO₂ response during the 6MWT and compare clinical characteristics between COPD patients with and without pCO₂ retention and those with and without EIH during the 6MWT.

OUTLINE OF THE THESIS

Chapter 1	provides a background of exercise pathophysiology in COPD and the 6MWT as a widely-used exercise assessment in COPD
Chapter 2	provides more insights in the characteristics and determinants of endurance cycle ergometry and six-minute walk distance in patients with COPD.
Chapter 3	presents impact of several 6MWD reference equations on 6MWD predicted values of patients with COPD and the factors accountable for the discrepancy in reference values.
Chapter 4	evaluates the prognostic value of the 6MWT-derived variables to predict 3-year mortality and hospitalization.
Chapter 5	describes the role of emphysema in EID and the determinants of EID in emphysematous patients during the 6MWT.
Chapter 6	reports for the accuracy of certain cut-off of baseline-SpO ₂ (≤95%) as simple screening procedure to predict EID and demonstrates determinants of EID during the 6MWT.
Chapter 7	describes the exercise-induced pCO ₂ response and demonstrates determinants of pCO ₂ retention and exercise-induced hypercapnia (EIH) during the 6MWT.
Chapter 8	provides a general discussion regarding the findings of this thesis and their clinical implications.

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Chapter 2

Characteristics and determinants of endurance cycle ergometry and six-minute walk distance in patients with COPD

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BMC Pulmonary Medicine 2014; 14:97

ABSTRACT

Background

Exercise tolerance can be assessed by the cycle endurance test (CET) and six-minute walk test (6MWT) in patients with Chronic Obstructive Pulmonary Disease (COPD). We sought to investigate the characteristics of functional exercise performance and determinants of the CET and 6MWT in a large clinical cohort of COPD patients.

Methods

A dataset of 2053 COPD patients (43% female, age: 66.9 ± 9.5 years, FEV_1 : $48.2 \pm 23.2\%$ predicted) was analyzed retrospectively. Patients underwent, amongst others, respiratory function evaluation; medical tests and questionnaires, one maximal incremental cycle test where peak work rate was determined and two functional exercise tests: a CET at 75% of peak work rate and 6MWT. A stepwise multiple linear regression was used to assess determinants.

Results

On average, patients had impaired exercise tolerance (peak work rate: $56 \pm 27\%$ pred., 6MWT: $69 \pm 17\%$ pred.). A total of 2002 patients had CET time of duration ($CET-T_{end}$) less than 20min, while only 51 (2.5%) of the patients achieved 20min of $CET-T_{end}$. In former patients, the percent of predicted peak work rate achieved differed significantly between men ($48 \pm 21\%$ predicted) and women ($67 \pm 31\%$ predicted). In contrast, $CET-T_{end}$ was longer in men (286 ± 174 s vs 250 ± 153 s, $p < 0.001$). Also, six minute walking distance (6MWD) was higher in men compared to women, both in absolute terms as in percent of predicted (443m, 67% pred. vs 431m, 72% predicted, $p < 0.05$). Gender was associated with the $CET-T_{end}$ but BMI, FEV_1 and FRC were related to the 6MWD highlighting the different determinants of exercise performance between CET and 6MWT.

Conclusions

$CET-T_{end}$ is a valuable outcome of CET as it is related to multiple clinical aspects of disease severity in COPD. Gender difference should temper the interpretation of CET.

INTRODUCTION

Constant work rate cycle ergometry, the so-called cycle endurance test (CET), has been put forth as an important assessment of exercise tolerance for patients with chronic obstructive pulmonary disease (COPD).¹⁻³ CET endurance time (CET-T_{end}) can be used in the evaluation of exercise tolerance.⁴ Yet our understanding of CET lags behind that of the commonly used six minute walk test (6MWT).⁵⁻⁸ In fact, the first report of minimal clinically important difference for the CET has only been published in 2008.⁹

Neder and colleagues¹⁰, in a detailed study aimed at plotting power duration relationship from a series CETs, found that the average critical power asymptote (i.e.: the power at which an individual can cycle for 20 minutes) in patients with COPD was 81% of the peak work rate achieved on a maximal incremental test. Anecdotal clinical experience, however, suggests that few patients with COPD can cycle that long, even at 75% of peak work rate, that is the level at which most CETs are performed.¹¹ CET performed at this fixed proportion of peak incremental workload capacity does not cause identical physiological stress among patients.¹² Furthermore, 20 minutes is considered the maximal allowable CET-T_{end} for a CET¹³, and the test is usually stopped at that point.

Constant work rate endurance protocols in CET are based on the maintenance of exercise effort until the exhaustion.¹⁴ Although CET is considered as sub-maximal exercise, it is aimed to assess the maximal aerobic capacity of patients and it is suggested as a highly repeatable test across run-in visits.¹⁵ However, the utility of CETs at 75% peak work rate comes into doubt as more patients would cycle to the obligatory stopping point of 20 minutes.¹⁰ Similarly, the 6MWT even though self-paced, it can be considered as a maximal sustainable exercise in patients with COPD.¹⁶ Both testing modalities for assessing exercise tolerance may provoke different physiological responses and cause different levels of dyspnea and fatigue during the exertion to patients with COPD.^{17, 18} Furthermore, the sex of patients has been related to dyspnea in the 6MWT¹⁹ and may also determine the exertion during the CET. From a physiological point of view, CET and 6MWT may not interchangeable exercise tests and the determinants of exercise performance between the tests can differ.

Herein, we report a retrospective analysis of CET-T_{end} in a large clinical cohort of patients with COPD undergoing initial evaluation for pulmonary rehabilitation (PR).²⁰ We sought to compare the results of CETs in a large clinical cohort to those of a 6MWT and we sought to determine how often a clinical cohort of patients with COPD achieved a maximal CET-T_{end} of 20 minutes during a CET performed at a predetermined 75% of peak work rate. Moreover, we investigated the determinants of CET-T_{end} and 6MWD.

METHODS

Design and participants

We extracted data from the records of 2991 patients with the diagnosis of COPD who were evaluated during the initial assessment of a comprehensive pulmonary rehabilitation program at CIRO+, center of expertise for chronic organ failure in Horn (The Netherlands) between January 1, 2005 and July 1, 2013.²⁰ Of these records, 2053 patients met the following inclusion criteria: all necessary data present, a primary diagnosis of COPD, a FEV₁/FVC ratio of ≤ 0.70 , evidence that they had reached a physiological maximum during the incremental maximal cardiopulmonary exercise test on a stationary cycle ergometer (i.e., ventilation $\geq 85\%$ of maximal voluntary ventilation; heart rate $\geq 80\%$ of the age-predicted heart rate; symptom score for dyspnea $\geq 7/10$ points; and/or symptoms score for fatigue $\geq 7/10$ points), CET done at 75% of peak work rate, and not representing a repeat admission for the same patient. Ethical approval was not indicated because all of the tests were done as part of the routine initial assessment, and analyzed retrospectively. The Board of Directors of CIRO+ approved the use of de-identified patients' record.

As part of routine initial assessment, patients underwent, amongst other tests and questionnaires, a maximal incremental cycle test where peak work rate is determined. Subsequently, on a different day, patients performed a CET at 75% of the determined peak work rate. Patients also performed two 6MWTs²¹ while the test with the longest distance was used for further analysis. All tests were done according to ATS/ERS guidelines.^{1, 8} Routine post-bronchodilator spirometry, physical examination, medical history, and Medical Research Council (MRC) dyspnea scale were obtained. Using dual energy x-ray absorptiometry (DEXA), the fat free mass index (FFMI) for each individual was calculated.

Statistical analysis

All analyses were carried out using SPSS 19, Graphpad Prism 6 and Sigmaplot 12. Data are reported as means with standard deviation ($\pm$ SD). For parametric data, comparison between men and women were made using a two tailed unpaired t-test, and for categorical data Pearson Chi-Square tests were used, as appropriate. Frequency distributions were used to determine performance quartiles for the CET-T_{end} and six-minute walk distance (6MWD) allowing for comparison of the clinical parameters between quartiles. For all comparisons, patients reaching CET-T_{end} of 20 minutes (51 patients, 2.5 %) were excluded from the dataset of 2053 patients as they represented an artificial stopping point, and thereby could artificially reduce standard error. After excluding this group of patients, a dataset of 2002 patients was analyzed for detecting significant differences. For parametric data quartiles were compared using one way

ANOVA and for non-parametric data quartiles were compared using Kruskal-Wallis test. All multiple comparisons were followed by Bonferonni correction. A stepwise multiple linear regression was used to determine clinical parameters that independently influenced test performance. The level of significance was set at $p < 0.05$.

RESULTS

Clinical parameters

On average, patients ($n=2002$; $CET_{end} < 20min$) had moderate to very severe COPD, a normal BMI, normal resting arterial oxygen and carbon dioxide tension, and symptoms of dyspnea. Gender differences were noticed: men were older, and had a significantly higher height, weight, BMI, FFMI, and FEV_1 %predicted compared to women. Then again, women had a significantly worse DLCO. One out of five patients was using long-term oxygen therapy (LTOT) (Table 1).

Table 1. Demographics of COPD patients

Characteristics	All patients ($n=2002$)	Males ($n=1134$) (57%)	Females ($n=868$) (43%)
Age, years, $\pm$ SD	66.9 $\pm$ 9.5	69.0 $\pm$ 9.1	64.3 $\pm$ 9.2*
Weight, kg	71.9 $\pm$ 16.5	77.2 $\pm$ 15.9	65.0 $\pm$ 14.7*
Height, m	1.68 $\pm$ 0.9	1.73 $\pm$ 0.7	1.62 $\pm$ 0.6*
BMI, kg/m ²	25.4 $\pm$ 5.2	25.8 $\pm$ 4.9	25.0 $\pm$ 5.5*
FFMI, kg/m ²	17.0 $\pm$ 2.4	18.1 $\pm$ 2.2	15.5 $\pm$ 1.8*
FEV_1 , %predicted	48.2 $\pm$ 23.2	47.2 $\pm$ 20.7	49.6 $\pm$ 26.1*
FVC, %predicted	95.9 $\pm$ 22.3	93.0 $\pm$ 21.7	99.7 $\pm$ 22.7*
FEV_1/FVC , %	39.8 $\pm$ 11.8	38.9 $\pm$ 11.9	40.9 $\pm$ 11.4*
FRC, %predicted	150.1 $\pm$ 35.9	146.3 $\pm$ 37.5	154.9 $\pm$ 33.0*
DLCO, %predicted	52.9 $\pm$ 18.1	55.3 $\pm$ 30.1	50.9 $\pm$ 25.7*
PO ₂ , kPa	9.8 $\pm$ 2.3	9.8 $\pm$ 2.2	9.7 $\pm$ 2.4
PCO ₂ , kPa	5.3 $\pm$ 1.4	5.2 $\pm$ 1.2	5.4 $\pm$ 1.5
MRC dyspnea grade	3.3 $\pm$ 1.1	3.3 $\pm$ 1.1	3.4 $\pm$ 1.0
GOLD stage I	96 $\pm$ 4.8	48 $\pm$ 4.2	48 $\pm$ 5.5
GOLD stage II	649 $\pm$ 32.4	362 $\pm$ 31.9	287 $\pm$ 33.1
GOLD stage III	730 $\pm$ 36.5	419 $\pm$ 36.9	311 $\pm$ 35.8
GOLD stage IV	527 $\pm$ 26.3	305 $\pm$ 26.9	222 $\pm$ 25.6
Receiving LTOT, n, (%)	408(20.4)	230 $\pm$ 20.3	178 $\pm$ 20.5

Abbreviations: BMI: Body Mass Index, FFMI: Fat-Free Mass Index, FEV_1 : Force Expiratory Volume at 1 sec, FVC: Force Vital Capacity, FRC: Functional Residual Capacity, DLCO: Diffusing capacity of the lung for carbon monoxide; PO₂: Partial pressure of Oxygen, PCO₂: Partial pressure of Carbon-dioxide, MRC: Medical Research Council dyspnea scale, LTOT: Long-term oxygen therapy. Average values are shown for exercise tests and

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clinical parameters. The values are reported for the entire cohort and then for men and women. Data are mean $\pm$ SD unless specified otherwise. Asterisk denotes significant differences ($p < 0.05$).

Maximal incremental cycle tests

On average, patients had a limited maximal exercise tolerance. The percent of predicted peak work rate achieved was significantly different between men ($48 \pm 21\%$ predicted) and women ($67 \pm 31\%$ predicted; $p < 0.001$; Table 2).

Endurance tests

Average CET- T_{end} was 271 ± 166 s. This differed significantly between men (286s) and women (250 s; $p < 0.001$). Average 6MWD was 438 ± 113 m. The 6MWD was higher in men compared to women (443m vs 431m; $p = 0.019$). Then again, 6MWD % predicted differed between men ($67 \pm 17\%$ predicted) and women ($72 \pm 15\%$ predicted; $p < 0.001$; Table 2).

Table 2. Parameters of exercise tests in patients with COPD

Characteristics	All patients (n=2002)	Males (n=1134) (57%)	Females (n=868) (43%)
Maximal incremental cycle test			
Work Rate peak, watts, $\pm$ SD	69 $\pm$ 30	77 $\pm$ 33	60 $\pm$ 23**
Work Rate peak, %predicted	56 $\pm$ 27	48 $\pm$ 21	67 $\pm$ 31**
VE, %Max Voluntary Ventilation	87 $\pm$ 24	86 $\pm$ 23	89 $\pm$ 25
HR max, % age-predicted	81 $\pm$ 12	80 $\pm$ 13	81 $\pm$ 12
Systolic BP post, mm Hg	176 $\pm$ 33	176 $\pm$ 34	176 $\pm$ 32
Diastolic BP post, mm Hg	84 $\pm$ 15	84 $\pm$ 15	84 $\pm$ 14
Dyspnea (Borg) post, points	7.2 $\pm$ 2.0	7.1 $\pm$ 2.0	7.2 $\pm$ 2.0
Leg Fatigue (Borg) post, points	5.9 $\pm$ 2.6	5.8 $\pm$ 2.5	6.0 $\pm$ 2.6
Cycle endurance test (CET)			
Work Rate mean, w	52 $\pm$ 23	58 $\pm$ 25	45 $\pm$ 17**
Endurance cycling time, s	271 $\pm$ 166	286 $\pm$ 174	250 $\pm$ 153**
Dyspnea (Borg) post, points	5.3 $\pm$ 2.3	5.4 $\pm$ 2.3	5.1 $\pm$ 2.3*
Leg Fatigue(Borg) post, points	5.7 $\pm$ 2.2	5.8 $\pm$ 2.2	5.6 $\pm$ 2.1
Six-minute walk test (6MWT)			
6MWD, m	438 $\pm$ 113	443 $\pm$ 120	431 $\pm$ 104*
6MWD, %predicted	69.0 $\pm$ 16.5	66.9 $\pm$ 17.1	71.7 $\pm$ 15.3**
Dyspnea (Borg) post, points	5.2 $\pm$ 4.4	5.2 $\pm$ 4.2	5.1 $\pm$ 4.7
Leg Fatigue(Borg) post, points	4.4 $\pm$ 4.6	4.4 $\pm$ 4.2	4.4 $\pm$ 4.8

Abbreviations: VE: Expired Ventilation, HR: Heart Rate, BP: Blood Pressure, 6MWD: Six-minute walk distance. Average values are shown for exercise tests and clinical parameters. The values are reported for the entire cohort and then for men and women. Data are mean $\pm$ SD unless specified otherwise. Asterisks denotes significant differences (* $p < 0.05$; ** $p < 0.001$).

Distribution of cycle endurance times and six minute walking distances

When separated into 1 minute intervals, the frequency distribution curve for CET-T_{end} shows a leftward kurtosis in men and women (Figure 1A). Only 51, or 2.5%, of patients achieved CET-T_{end} of 20 minutes. When separated into 50m intervals, the frequency distribution curve for 6MWD shows a rightward kurtosis in men and women (Figure 1B).

Associations

The CET-T_{end} and the 6MWD were significantly associated in the whole sample, as well as after stratification for gender presenting a moderate positive correlation (Figure 2).

Examination of performance quartiles

There is clear performance quartile gradation for FEV₁%, FVC%, CET-T_{end}, peak power output of absolute watt and watt %predicted (sole exception 3rd/4th quartile in CET) and the 6MWD with significant differences amongst all quartiles for the exercise performance of CET and the 6MWT (Figure 3). Moreover, there is performance quartile gradation for age with significant differences of age for the 4th and the rest of quartiles for CET and amongst all the quartiles for the 6MWT. Additionally, performance quartile gradation significant differences were detected across the GOLD stages between 1st and 4th, 1st and 3rd, and 2nd and 4th in cycling and amongst all quartiles in 6MWT. The performance quartile gradation for the FRC demonstrated significant differences in all quartiles for CET while a similar pattern was observed for the 6MWT. The performance quartile gradation for the DLCO demonstrated significant differences amongst 1st and 3rd-4th, 2nd and 4th quartiles for the CET and 6MWT respectively. Significant difference in BMI were observed only in CET for 1st and 3rd-4th, 2nd and 3rd-4th, but only between 1st and 4th in 6MWT. Similarly, FFMI performance quartile gradation was significant for almost all quartiles but no significance was detected for the 6MWT (Figure 3). When performance quartiles were determined separately for men and women, men demonstrated more evident differences in FFMI quartiles compared to women. That fact indicates that FFMI may determine the exercise performance more in men than women with COPD (see supplemental material for details, Tables S1-S2, Figures S2-S3).

Stepwise multiple linear regression

Reverse stepwise linear regression revealed that age, gender, FFMI, DLCO%, GOLD stage, Dyspnea and leg fatigue Borg scores at rest independently influence CET-T_{end} (Table 3). Age, BMI, FFMI, FEV₁%, FVC/FEV₁, GOLD stage, FRC, DLCO, and leg fatigue Borg score at rest were retained in the model when 6MWD was the dependent variable (Table 4). The model for CET was able to account for only 15.1% of the variability in

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cycle endurance time, while that for 6MWT accounted for 34.0% of the variability in 6MWD.

Table 3. Stepwise linear regression model of endurance cycle time in seconds

r^2 : 15.1%				
Variable	Coefficient	Standard Error	t value	p value
Age, years	-3.35	0.57	-5.84	<0.001
Sex, m/f	-31.51	12.91	-2.44	0.015
FFMI, kg/m ²	8.49	2.79	3.04	0.002
GOLD stage	-42.09	6.59	-6.39	<0.001
DLCO, %predicted	1.21	0.32	3.83	<0.001
Dyspnea before test, index	-13.38	4.43	-3.02	0.003
Leg fatigue before test, index	-6.96	3.52	-1.98	0.048

Multiple linear regression model for parameters predictive of endurance cycle time. Using isolated linear regression determination a stepwise multiple linear regression model was established with an r^2 of 15.1%.

Table 4. Stepwise linear regression model of Six minute walking distance in meters

r^2 : 34.0%				
Variable	Coefficient	Standard Error	t value	p value
Age, years	-3.97	0.29	-13.98	<0.001
BMI, kg/m ²	-7.48	0.75	-9.87	<0.001
FFMI, kg/m ²	8.77	1.59	5.52	<0.001
FEV ₁ , %predicted	1.28	0.29	4.44	<0.001
FEV ₁ /FVC, %	-1.60	0.42	-3.89	<0.001
GOLD stages	-15.04	5.04	-3.02	0.003
FRC, %predicted	-0.43	0.11	-3.85	<0.001
DLCO, %predicted	1.11	0.16	6.89	<0.001
Leg fatigue before test, index	-15.80	1.64	-9.57	<0.001

Multiple linear regression model for parameters predictive of six minute walk distance. Using isolated linear regression determination a stepwise multiple linear regression model was established with an r^2 of 34.0.

Figure 1. Frequency distribution of CET-Tend and 6MWD in patients with COPD
(A) Frequency distribution of 75% of peak work rate CET-Tend. The number of individuals for each bin of duration time is represented by the height of each column (y-axis). Each bin represents 1 minute as central value (x-axis). Only 51 (2.5%) of the 2053 patients who met the inclusion criteria reached the 20 minute maximal duration. (B) Frequency distribution of six minute walking distance (6MWD). The number of individuals for each bin of walking meters is represented by the height of each column (y-axis). Each bin represents 1 minute as central value (x-axis).

Figure 2. Pearson's correlations between CET-Tend and 6MWD.
A positive moderate correlation was detected in all models between the CET-T_{end} and 6MWD for all patients and stratified by gender.

Figure 3. Performance quartile comparisons

Using the frequency distribution patients were separated into performance quartiles. With the quartiles increasing left to right (CET: quartiles with wave hashing, 6MWT: quartiles with diagonal hashing). Comparisons were then made between the quartiles of CET and between quartiles of 6MWT for all variables. Significant differences are demonstrated by lines above the relevant bars. Dashed line on gender graph represents the point where is an equal number of men and women.

DISCUSSION

In current study, we aimed to investigate the characteristics of functional exercise performance and the determinants which can influence the exercise performance in CET and the 6MWT in a large cohort of patients with moderate to severe COPD (Table 1). The main finding of this study is that CET-T_{end} can be determined by different physiological and clinical characteristics in comparison with the 6MWT. Of particular clinical importance, the sex of patients influenced CET-T_{end} but not in the 6MWT (Table 2-4). Moreover, 6MWT seems to be more determined by lung function compared to CET-T_{end} (Tables 3-4). Also, a number of independent physiological and clinical characteristics demonstrated a different pattern in performance quartile gradation between CET and 6MWT (Figure 3).

Gender appears to be a strong determinant of CET-T_{end} in contrast to 6MWT. We found that men on average achieved a higher peak work rate and cycled longer during the CET than women; in spite of the fact the men were older with more impaired lung function (Table 1 and 2). Previous studies have demonstrated that quadriceps muscle strength²² and peak VO₂^{23, 24} have small reductions in COPD patients over a 1 to 5 year period. Moreover, quadriceps muscle strength is positively related to DLCO in patients with COPD²⁵ and the studied male population had a greater diffusing capacity which could be accountable for the better cycle performance.

When we examined the performance quartiles, there was not as much of a gradation in gender proportion except the 4th quartile amongst the 6MWT performance quartiles compared to that was seen in the CET-T_{end} of performance quartiles. Furthermore, there was a significant difference between all the CET performance quartiles in FFMI, which was not seen 6MWT performance quartiles. However, as would be expected, FFMI was on average lower in the female population compared to men (Table 1). Thus, the question remains whether and to what extent gender differences in CET reflected the lower FFMI in women, or if the FFMI differences were simply a reflection of inherent gender differences. To examine this question we stratified the data into performance quartiles for each gender. We found that the difference in FFMI between performance quartiles was no longer apparent in women except for the 1st and 4th CET performance quartiles (Supplementary figures S1-S2), but still apparent in

men between all the quartiles in CET and the 1st and 4th, and the 1st and 3rd quartiles in the 6MWT. There was also less of a CET quartile gradation in FVC% and GOLD stage compared to that seen in 6MWD. To further elucidate the importance of gender and FFMI as determinants of test performance we carried out stepwise multiple regressions. The parameters that were retained when CET-T_{end} was used as the dependent variable were age, gender, FFMI, DLCO%, GOLD stage, Dyspnea and leg fatigue Borg scores at rest (Table 3). When 6MWD was used as the dependent variable, age, BMI, FFMI, FEV₁%, FEV₁/FVC, FRC%, DLCO%, GOLD stage and leg fatigue Borg score at rest were retained, but gender was not retained (Table 4). Performing a stepwise multiple regression after stratification by gender, FFMI was not retained in women but it was retained in men for the CET-T_{end} (Supplementary tables S1-S2). Apparently quartile difference in FFMI (Figure 3) can be likely a reflection of an inherent gender differences beyond fat free mass. Thus, it can be concluded that gender is an important determinant of CET-T_{end}. Interestingly, peak aerobic capacity of patients with COPD was not determined by gender, after correction for isokinetic quadriceps peak torque, DLCO, FEV₁, BMI, age and lower-limb lean muscle mass.²⁶

The model for 6MWD was much better at explaining variability in test performance (r^2 of 34.0%) compared to that for CET (r^2 of 15.1%). Other authors have also found it difficult to build a model that is highly predictive of CET performance.^{12, 27} This perhaps explains why we do not have a predictive equation for CET-T_{end} yet. It does however suggest that there are determinants of CET which we do not understand. This may reflect the complex nature of exercise intolerance in COPD.

CET-T_{end} seems to be influenced in a high extent from the degree of disease severity classified by GOLD stages more than the inter-individual variation of FEV₁ or other specific clinical characteristics compared to 6MWT. It is observed that patients across the GOLD stages are characterized by increased physical deconditioning which leads to a progressive deterioration in lower limb muscle function.²⁸ The impact of the power/duration relationship on the exercise tolerance indicates that endurance is influenced by the intensity of exercise.²⁹ Both exercise tests are sub-maximal, however, the 6MWT is a self-paced while the CET at 75% is a constant load exercise test. The termination of exercise in CET is more associated to the inability to maintain the constant work load of exercise and thus CET-T_{end} is associated to an overall impaired health status, sex of patients and physical deconditioning more than specific lung function characteristics compared to the 6MWT.

In contrast to the findings of Neder and colleagues¹⁰, we found that the vast majority of patients with COPD did not reach the 20 minute time point during a CET at 75% of peak work rate. This discrepancy between two studies is likely the result of differences in methodology and study populations. We retrospectively examined a clinical cohort of patients who were referred for pulmonary rehabilitation, whereas Neder and colleagues¹⁰ studied a proscribed, smaller cohort with the intent of defining

the power duration asymptote. In the present study, CET-T_{end} varied from 2 to 5min for the most of the patients (Figure 1A). This is similar to the findings of Vivodtzev and colleagues¹² where most of the patients sustained CET for 3 to 5 minutes. Although the proportion of patients with CET-T_{end} of 20 minutes was very small (2.5%), we evaluated the possibility of an underestimation in peak work rate that may result in unduly prolonged CET. Nevertheless, peak heart rate, ventilation and dyspnea scores following a cardiopulmonary exercise test indicated a maximum effort for those patients (Supplementary table S3). Moreover, a quartile gradation of ventilation pattern derived by the maximum cardiopulmonary exercise test (CPET) in cycle ergometer indicates the effort to maintain O₂ and CO₂ homeostasis and may partly provide an insight of the ventilatory profile in patients of current study (Supplementary figure S3).

Our findings showed a large variation in the CET-T_{end} values (Figure 1A) which is also observed in the 6MWD, though to a lesser extent (Figure 1B.). Therefore, evaluating patients with a CET at 75% of peak work rate leaves room for demonstrating improvement with an intervention, especially considering that the minimal clinically important difference has been reported to be between 100 and 200 seconds⁹ or ~33% increased from baseline⁴. Although the identical exercise intensity of 75% of peak work rate, the physiological stress may differ amongst patients and thus the space for improvement through PR exercise modalities can vary. Moreover, in daily life the majority of patients have been adapted to walking but not to cycling exercise and that may results in a greater potential of improvement in CET compared to 6MWT after PR. Nevertheless, O'Donnell and colleagues¹⁵ demonstrated that CET at 75% peak work rate was highly repeatable across run-in visits: 7.9+/-4.8 and 8.4+/-5.1min (R = 0.84) in 463 COPD patients indicating the appropriateness of CET in the exercise assessment of patients with COPD.

There are methodological considerations to our study. It is a retrospective cohort study which means there may be unidentified confounders. We have no way of knowing why some of the records were incomplete. Surely some may be incomplete due to a failure of the patients to complete the initial pulmonary rehabilitation assessment, or refusal to perform a particular test. Some were also repeat evaluations. Nonetheless the large sample size helps to mitigate some of these potentially confounding factors. Secondly, this is a population of patients who have been referred for pulmonary rehabilitation which could result in a referral bias towards patients with a greater degree of limitation. Regardless, our findings sufficiently demonstrate that performing CET at 75% of peak work rate is a reasonable effort level for a population of COPD patients being referred for pulmonary rehabilitation. Although a fixed proportion of peak work rate exercise does not provide identical physiological stress amongst patients Furthermore, this study increases our understanding of gender differences in exercise testing and highlights the complex pathophysiology of exercise intolerance in COPD. There have been a number of gender differences reported in COPD.^{27, 30-36} Most

intriguingly, it has previously been shown that women have higher symptom scores on the St. George's Respiratory Questionnaire (SGRQ) compared to men.²⁷ It may therefore be that compared to 6MWT, which simulates a more routine daily activity, cycle ergometry evokes more symptoms in women compared to men resulting in reduced CET-T_{end} for women. This could be a reflection of the fact that most patients with COPD, even in the Netherlands, do not routinely ride a bicycle, and thereby cycling may evoke more dyspnea because of its unfamiliarity. In lieu of predictive equations, this gender difference in CET should be taken into consideration and used to temper the interpretation of CET. In summary, CET at 75% of peak work rate appears to be a useful clinical exercise tool in COPD. There is plenty of room to demonstrate improvement with an intervention and it measures a different aspect of exercise tolerance compared to 6MWT. The fact that the determinants of CET-T_{end} are not reducible to a model built from a handful of clinical parameters also speaks to the importance of CET-T_{end} as an independent measurement before and after pulmonary rehabilitation. Furthermore, CET reflects gender differences in COPD, an aspect of the disease that is gaining increasing recognition.

CONCLUSION

CET and 6MWT are not interchangeable exercise tests. CET-T_{end} is a valuable outcome of CET as it is related to multiple clinical aspects of disease severity in COPD. Gender difference should temper the interpretation of CET. BMI, FEV₁ and FRC influence the walking performance of 6MWT rather than the CET. Although the fixed proportion of workload capacity does not provide the same physiological stress among patients, improvement in CET-T_{end} after pulmonary rehabilitation can reflect a total improvement of clinical status in patients with COPD.

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SUPPLEMENTAL MATERIAL

Table S1. Stepwise linear regression model of CET-T_{end}

r ² : 17.2%				
Variable	Coefficient	Standard Error	t value	p value
MALES				
Age, years	-2.74	0.78	-3.52	<0.001
FFMI, kg/m ²	13.00	3.56	3.65	<0.001
GOLD stage	-44.24	8.91	-4.97	<0.001
DLCO, %predicted	1.25	0.42	2.98	0.003
Dyspnea before test, index	-21.9	4.73	-4.64	<0.001
r ² : 11.0%				
FEMALES				
Age, years	-3.99	0.84	-4.74	<0.001
FEV ₁ , %predicted	1.79	0.44	4.04	<0.001
DLCO, %predicted	1.06	0.47	2.26	0.024
Leg fatigue before test, index	-12.01	4.91	-2.45	0.015

Abbreviations: FFMI: Fat-Free Mass Index, FEV₁: Force Expiratory Volume at 1 sec, DLCO: Diffusing capacity of the lung for carbon monoxide. Multiple linear regression model for parameters predictive of CET-Tend (in seconds) in males and females.

Table S2. Stepwise linear regression model of Six minute walking distance in meters

r ² : 32.5%				
Variable	Coefficient	Standard Error	t value	p value
MALES				
Age, years	-3.79	0.41	-9.22	<0.001
BMI, kg/m ²	-3.46	0.81	-4.27	<0.001
FEV ₁ , %predicted	1.72	0.42	4.11	<0.001
FEV ₁ /FVC, %	-1.25	0.55	-2.26	0.024
GOLD stage	-16.39	7.36	-2.23	0.026
DLCO, %predicted	1.26	0.22	5.77	<0.001
Leg fatigue before test, index	-19.28	2.32	-8.31	<0.001
r ² : 35.9%				
FEMALES				
Age, years	-4.06	0.39	-10.43	<0.001
BMI, kg/m ²	-4.92	0.69	-7.19	<0.001
FEV ₁ , %predicted	0.69	0.30	2.28	0.023
GOLD stage	-16.04	6.61	-2.43	0.016
DLCO, %predicted	1.01	0.22	4.50	<0.001
Leg fatigue before test, index	-12.61	2.27	-5.55	<0.001

Abbreviations: BMI: Body Mass Index, FEV₁: Force Expiratory Volume at 1 sec, FVC: Force Vital Capacity, FRC: Functional Residual Capacity, DLCO: Diffusing capacity of the lung for carbon monoxide. Multiple linear regression model for parameters predictive of 6MWD (in meters) in males and females.

Chapter 2

Table S3. Evidences of maximum CPET in 51 patients with CET ≥ 20 min

HRmax $\geq 80\%$ predicted	VEmax $\geq 85\%$ MVV	Dyspnea and/or leg fatigue ≥ 7
↓	↓	↓
N= 31 (61%) patients	N= 18 (35%) patients	N= 43 (84%) patients
Of rest patients: N= 20 (39%)	Of rest patients: N= 33 (65%)	Of rest patients: N= 8 (16%)
<ul style="list-style-type: none"> ● 1 (2%) had only VEmax $\geq 85\%$ MVV ● 12 (23%) had only dyspnea and/or leg fatigue ≥ 7 by BORG ● 7 (14%) had both VEmax 85% MVV & dyspnea and/or leg fatigue by BORG scale 	<ul style="list-style-type: none"> ● 5 (10%) had only HRmax $\geq 80\%$pred. ● 13 (26%) had only dyspnea and/or leg fatigue ≥ 7 by BORG ● 15 (29%) had both HRmax $\geq 80\%$pred. & dyspnea and/or leg fatigue by BORG scale 	<ul style="list-style-type: none"> ● 1 (2%) had only VEmax $\geq 85\%$ MVV ● 5 (10%) had only HRmax $\geq 80\%$pred. ● 2 (4%) had both VEmax $\geq 85\%$ MVV & HRmax $\geq 80\%$predicted

Abbreviations: HR: Heart Rate, VE: Expired Ventilation, MVV: Maximal Voluntary Ventilation. Percentages of patients who reached a high threshold value in heart rate (HRmax) and/or ventilation (VEmax) and/or Borg scores (Dyspnea/Leg-fatigue) as evidence of an achieved maximum effort during the maximum cardiopulmonary exercise test in cycle ergometer.

Figure S1. Performance quartile comparisons of men

Using the frequency distribution 1134 male patients was categorized into performance quartiles. With the quartiles increasing left to right (CET: quartiles with wave hashing, 6MWT: quartiles with diagonal hashing). Comparisons were then made between the quartiles of CET and between quartiles of 6MWT for all variables. Significant differences are demonstrated by lines above the relevant bars.

Figure S2. Performance quartile comparisons of women
Using the frequency distribution 868 female patients was categorized into performance quartiles. With the quartiles increasing left to right (CET: quartiles with wave hashing, 6MWT: quartiles with diagonal hashing). Comparisons were then made between the quartiles of CET and between quartiles of 6MWT for all variables. Significant differences are demonstrated by lines above the relevant bars.

Figure S3. Performance quartile comparisons of CPET

Using the frequency distribution patients were separated into performance quartiles. Quartiles were increasing from left to right (Quartiles with wave hashing for all samples, males and females). Comparisons were then made between the quartiles of maximum cardiopulmonary exercise test (CPET) in cycle ergometer for the achieved maximum ventilation (VEmax) expressed as absolute values and percentage of predicted. Significant differences are demonstrated by lines above the relevant bars.

Chapter 3

Six-minute walk distance in patients with chronic obstructive pulmonary disease: Which reference equations should we use?

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Chronic Respiratory Disease 2015; 12:111-9

ABSTRACT

Background

The use of different six-minute walk distance (6MWD) reference equations probably results in different predicted 6MWD reference values. We wished to investigate the impact of several 6MWD reference equations for adults in patients with chronic obstructive pulmonary disease (COPD) and factors accountable for different 6MWD %predicted values.

Methods

Twenty-two 6MWD reference equations were applied to a dataset of 2757 patients with COPD. The predicted 6MWD reference value of Troosters and colleagues was used as point of reference.

Results

Four out of 21 remaining equations resulted in comparable 6MWD %predicted; sixteen equations resulted in significantly higher 6MWD %predicted; and one equation resulted in a significantly lower 6MWD %predicted. Similar differences in 6MWD %predicted were observed after stratification by sex. Body mass index and global initiative for chronic obstructive lung disease (GOLD) stages classification demonstrated varying results within and between the groups; 9 out of 21 equations resulted in comparable 6MWD %predicted in underweight patients but only 1 equation demonstrated comparable result in obese. Eight equations in GOLD I, whilst 5 out of 21 equations in GOLD IV resulted in comparable 6MWD %predicted.

Conclusions

Existing 6MWD reference equations will give varying results. The choice of 6MWD reference equation should consider the consistency of 6-min walk test operating procedures and at least be specific for the country/region of origin.

INTRODUCTION

The six-minute walk test (6MWT) can be used to assess functional exercise capacity, treatment efficacy and prognosis in chronic respiratory disease.¹⁻⁴ New insights of COPD affliction, which include cardiac and peripheral muscle wasting effects, provide an additional value in the assessment of functional exercise capacity measured by the 6MWT.⁵ The 6MWT elicit high but submaximal cardiorespiratory responses that mimic day life activities.⁶ In some clinical situations, the six-minute walking distance (6MWD) may be a better index of actual patients' ability to perform daily activities in comparison with the peak oxygen uptake.^{7, 8} The 6MWD has been indicated for the prediction of hospitalization and mortality in COPD⁹ and has been also recognized for its predictive value to postoperative outcome in candidates for volume reduction surgery.¹⁰

Multiple 6MWD reference equations¹¹⁻³⁰ were derived to express the 6MWD as a percentage of these reference values (% predicted) (see supplemental material for details - Table S1). Adjusting the measured 6MWD for physiological variation using the percentage of walk predicted facilitates the interpretation of the walked 6MWD and help to estimate the degree of exercise tolerance in patients.³¹ Erroneous selection of a reference equation may result in misinterpretation of the levels of exercise capacity or improvements of physical performance after participation in pulmonary rehabilitation programs.³⁰ The large number of published 6MWD reference equations and the fact that those equations are derived from healthy individuals with different clinical characteristics and do not consistently employ the same variables makes the choice of 6MWD reference equation often vague.

Several demographic, anthropometric and physiological characteristics can determine the 6MWD in healthy individuals and in patients with COPD.³² However, the strongest determinants that can independently affect the 6MWD in healthy adults seem to be the age, height, weight, sex and race and these characteristics have been proposed for clinical use.^{15, 18, 26, 33} Muscle strength, true leg length, symptoms of depression, health-related quality of life impairment and other factors such as the standardization of the 6MWT can influence the 6MWD.^{11, 34-37} Therefore, it might be anticipated that the use of different 6MWD reference equations derived by several studies would result in different predicted 6MWD reference values.

Herein, we report a retrospective analysis in a large cohort of patients with COPD who were evaluated at CIRO+ between 1 January 2005 and 1 July 2013.³⁸ We assessed the impact of 22 existing 6MWD reference equations in patients with COPD and investigated the factors accountable for different predicted 6MWD reference values using data from 2757 patients with COPD. Furthermore, we wished to identify criteria for the choice of 6MWD reference equation in COPD patients.

METHODS

A dataset of patients with COPD³⁹ including demographic, anthropometric characteristics, lung function evaluation and 6MWT exercise outcomes was analyzed. All patients performed two 6MWTs according to the American Thoracic Society guidelines⁷, including a practice walk.⁴⁰ The 6MWT with the highest 6MWD was used for further analyses.⁴¹ During the 6MWTs, transcutaneous oxyhaemoglobin saturation (SpO₂%) and heart rate (HR) were measured using a pulse oxymeter (Nonin-2500; Nonin Medical Inc., Plymouth, USA). The predicted maximum heart rate (HRmax %) was derived from the formula $HR_{max} = 220 - \text{age in years}$ ⁴² and the Δ values of HR (ΔHR) were calculated by the difference between the HR at the end of the test minus the baseline HR.

The 6MWD % of Troosters and colleagues²⁶ was used as a point of reference. These reference values were used in CIRO+ since the last decade, as these reference values were derived from a Belgian sample of healthy elderly subjects with a mean 6MWD of $622 \pm 75\text{m}$, which is comparable to the mean 6MWD of Dutch healthy elderly subjects.⁴³ All available 6MWD reference equations were applied with the exception of the alternative equation of Dourado and colleagues²⁴ which required handgrip strength measurement not an available in our dataset. All statistical analyses were carried out using Sigmaplot Version 11 and Statistical Package for the Social Sciences (SPSS) Version 19.0. Data are presented as mean ($\pm$ SD) or proportion, as appropriate. For parametric data, comparisons were made using a two tailed unpaired *t*-test. The 6MWD % predicted by Troosters and colleagues²⁶ was compared with the other reference values using Dunnett's multiple comparisons test. A priori, a two-sided level of significance was set at *p* value of <0.05 .

RESULTS

A total of 22 reference equations were applied to a dataset of 2757 patients with COPD (age range: 38-92 years). Reference equations encompassed race/ethnic differences and variety of technical aspects that can determine the 6MWD (Supplementary figure S1 & table S2). The clinical features of patients are summarized in Table 1. In brief, patients generally had moderate to very severe COPD and an impaired lung diffusion capacity (DLCO). Men were slightly older compared with women and had more impaired Tiffeneau index (FEV_1/FVC) and lower total lung capacity (TLC), whilst women had worse DLCO%. On average, the functional exercise capacity was profoundly limited: $422 \pm 124\text{m}$, which equals $68 \pm 18\%$ of reference values of Troosters and colleagues²⁶ (Table 1). Four out of 21 remaining equations resulted in comparable 6MWD %predicted; 16 equations resulted in significantly higher 6MWD %predicted (all $p < 0.05$); and 1 equation resulted in a significantly lower 6MWD % predicted ($p < 0.001$) (Figure 1). Similar

discrepancies in the 6MWD %predicted among the equations were observed in patients stratified by sex (Figure 2).

Table 1. Anthropometric and clinical characteristics of patients with COPD

Characteristics	All patients (n=2757)	Males (n=1547) (56%)	Females (n=1210) (44%)
Age, years, $\pm$ SD	67.4 $\pm$ 9.6	69.4 $\pm$ 9.2	64.8 $\pm$ 9.4*
Height, cm	168 $\pm$ 9	173 $\pm$ 7	161 $\pm$ 6*
Weight, kg	72 $\pm$ 18	77 $\pm$ 17	66 $\pm$ 16*
BMI, kg/m ²	25.5 $\pm$ 5.6	25.8 $\pm$ 5.2	25.2 $\pm$ 6.1
FFMI, kg/m ²	17.0 $\pm$ 2.4	18.2 $\pm$ 2.1	15.6 $\pm$ 1.8*
FEV ₁ , L	1.3 $\pm$ 0.6	1.4 $\pm$ 0.6	1.1 $\pm$ 0.5*
FEV ₁ , %predicted	48.0 $\pm$ 19.7	47.4 $\pm$ 19.7	48.8 $\pm$ 19.7
FVC, %predicted	93.9 $\pm$ 22.8	92.6 $\pm$ 32.2	96.7 $\pm$ 23.5*
Tiffeneau index, %	40.8 $\pm$ 13.2	39.9 $\pm$ 13.5	42.0 $\pm$ 12.8*
TLC, %predicted	117.8 $\pm$ 19.2	113.8 $\pm$ 18.5	123.0 $\pm$ 18.7*
DLCO, %predicted	52.8 $\pm$ 19.4	54.2 $\pm$ 20.1	50.5 $\pm$ 18.1*
SPO ₂ , %	94.4 $\pm$ 3.6	94.2 $\pm$ 4.1	94.6 $\pm$ 2.6
mMRC dyspnea grade	3.4 $\pm$ 1.1	3.3 $\pm$ 1.1	3.5 $\pm$ 1.1*
6MWD, m	422 $\pm$ 124	432 $\pm$ 127	409 $\pm$ 120*
6MWD, %predicted	68 $\pm$ 18	66 $\pm$ 18	70 $\pm$ 17*

Abbreviations: BMI: Body Mass Index, FFMI: Fat-Free Mass Index, FEV₁: Force Expiratory Volume at 1 sec, FVC: Force Vital Capacity, TLC: Total Lung Capacity, DLCO: Diffusing capacity of the lung for carbon monoxide; SpO₂: Saturation of peripheral oxygen, mMRC: modified Medical Research Council dyspnea scale, 6MWD: Six-minute walk distance. Average values are shown for exercise tests and clinical parameters. The values are reported for the entire cohort and then for men and women. Data are mean $\pm$ SD unless specified otherwise. Asterisk denotes significant differences ($p < 0.05$).

Discrepancies between 6MWD expressed as %predicted of the reference values of Troosters and colleagues and the 6MWD expressed as %predicted using the remaining reference equations were also observed after stratification by body mass index (BMI) and the degree of airflow limitation as given by GOLD stages within all groups of patients. Furthermore, differences in the number of 6MWD predicted values with clinically important discrepancies were noticed between the BMI and between the GOLD stages groups. Patients with underweight BMI compared those with obese BMI and patients in GOLD stage I compared those in GOLD stage IV had a smaller number of discrepant 6MWD %predicted values (Figures 3-4).

ALL PATIENTS
N = 2757

Figure 1. Frequency distribution of estimated 6MWD
The frequency distribution of estimated 6MWD applying the existing equation of healthy individuals to patients with COPD, demonstrated large variation. The numbers on the right are mean values per equation.

Figure 2. Frequency distribution of estimated 6MWD stratified by sex
The frequency distribution of estimated 6MWD, applying reference equations of healthy individuals to patients with COPD demonstrated similar impact after stratification by sex. The numbers on the right are mean values per equation.

A. UNDERWEIGHT (BMI 13.3 - 18.4 kg/m²)

N= 200 (7.2%)

B. NORMAL RANGE (BMI 18.5 - 24.9 kg/m²)

N= 1204 (43.7%)

Figure 3. Frequency distribution of estimated 6MWD stratified by BMI

The frequency distribution of estimated 6MWD, applying reference equations of healthy individuals to patients with COPD, demonstrated fewer differences in patients with underweight BMI compared with those with normal range or higher BMI. The numbers on the right are mean values per equation.

A. GOLD Stage I
N= 156 (5.7%)

B. GOLD Stage II
N= 857 (31.1%)

DISCUSSION

Even though a majority of the existing 6MWD reference equations included similar characteristics, such as sex, age, height, weight or BMI, clear differences were found when applying the existing 6MWD reference equations to a dataset of 2757 patients with COPD (Figure 1). The best choice for the predicted 6MWD reference values probably depends on the country/region of origin of the healthy subjects that were used to develop the 6MWD reference equation. For example, the 6MWD reference equation of Poh and colleagues¹⁸ resulted in a mean 6MWD of 81% predicted. This suggests that applying Asian-derived 6MWD %predicted values to Dutch patients with COPD may result in an overestimation of their true functional capacity. As pointed out by authors, one possible explanation for this discrepancy is the differences in body stature between Asians and Caucasian. Herein, it is worth noting that some countries and regions are very ethnically and racial diverse and reference values derived from mixed-race populations could be recommended. When we applied the reference equation of Enright and colleagues¹¹, which is derived from a mixed-race elderly group (aged: 77 ± 4 years), true functional capacity of our patients seems to be overestimated compared to the rest of reference values (Figure 1). We assume that it could be partly attributed both to their younger age and to racial differences.

Next to demographic characteristics, a different operating procedure of 6MWT can also affect walking distance and modify the reference values.⁴ For example, in the study of Troosters and colleagues²⁶ the length of the corridor of used reference equation was 50m which can be considered as long corridor compared to 20m corridor of Gibbons and colleagues.²⁸ This fact can also result in overestimation of 6MWD %predicted relative to a number of other reference equations. Accordingly, our triangular track for the 6MWTs of our patients has the characteristics of a long corridor. Therefore, at CRO+ the predicted reference values of Troosters and colleagues²⁶ are used, who studied healthy Belgians 50-85 years of age.

Methodological differences in the 6MWT, with track lengths ranging from 20m to 50m, the number of test repetitions ranging from 1 to 3, test-retest intervals from 30min to 24h and variable practices regarding the given test instructions (i.e. “walk at your own pace” or “walk as far as you can”) and the encouragement, may partly account for the observed discrepancies in the 6MWD %predicted values. Sciruba and colleagues⁴⁴ demonstrated that the long compared with short corridors can increase the 6MWD as the number of turns is less and thus the physical effort is less.⁴⁴ In the study of Alameri and colleagues¹⁴ healthy individuals reached a low average of HRmax (45%pred.) when instructed to walk at their own pace.¹⁴ Moreover, the impact of a learning effect on the walking distance of 6MWT has been investigated in COPD. Steven and colleagues⁴⁵ performed three 6MWTs in 21 COPD patients on separate days and observed an average increase of 10% in the second test and an additional 3% increase

in walking distance in the third test compared with first 6MWT.⁴⁵ Such factors are known to have an impact on the measured distance (Supplementary Table S2).

The health status of the “healthy subjects” may also have impacts on findings. For example, the study of Enright and colleagues¹¹ included participants with risk factors associated with cardiovascular disease with likely limitations in functional capacity probably resulting in an overestimation of the 6MWD %predicted when applied to individuals with COPD. The physical condition of the volunteers participated at the several studies should be considered. The HR recorded at the end of the 6MWT can vary substantially in moderate and intense levels of effort in different studies.^{15, 25} These facts indicate that differences in characteristics of healthy population, 6MWT protocols and/or different levels of effort during the 6MWT can result in different 6MWD % predicted values.

On the other side, certain clinical characteristics of patients’ populations, where the equations are applied, can also be accountable for the discrepancies in the 6MWD %predicted values. After stratification for BMI and the degree of airflow limitation as given by GOLD stages, the number of discrepant 6MWD values varied between the groups of BMI and GOLD stages, respectively (Figures 3-4). Applying the 6MWD reference equations to underweight or GOLD stage I groups of patients leads to more consistent 6MWD %predicted values. Therefore, the choice of 6MWD reference equation in patients with those characteristics (underweight and/or GOLD stage I) may be of less importance. BMI and/or disease severity as given by GOLD stages are major determinants of exercise performance especially in individuals with higher BMI and/or worse COPD. The exclusion of these characteristics from reference equations could account for the discrepancies in 6MWD %predicted values and that can be more obvious in obese or patients with GOLD stage IV disease. This might explain the larger number of discrepant 6MWD %predicted values among the reference equations applied in the groups of obese and patients with GOLD stage IV disease compared with the groups of underweight patients with GOLD stage I disease (Figures 3-4). Recently, Capodaglio and colleagues³⁰ provided a reference equation specific for obese population and suggested that specific predicted reference values may have the advantage of providing a benchmark for functional capacity assessment and changes monitoring after pulmonary rehabilitation.³⁰

Further studies with large cohorts of volunteers in wide range of age would be necessary in order to create a 6MWD reference equation that will be highly representative and applicable to patients with COPD. Specifically, a population-based sample, a large number of healthy subjects in each decade of age, adequate exclusion criteria for poor health of “healthy subjects” and methodological approach based on American Thoracic Society (ATS) guidelines⁷ should be considered for the creation of 6MWD reference equations. Anthropometric and demographic characteristics of healthy individuals recruited for the development of a reference equation should be

ideally matched to the corresponding patients' characteristics in which the equation is being applied. Additionally, the 6MWT operating procedures of studies formulated reference equations should be in line with those operating procedures followed by the population in which the reference equation is being applied. All of these criteria should be considered for the development and the choice of 6MWD reference equation that can provide an instant practical clinical information and interpretation for the levels of exercise capacity. In summary, existing 6MWD reference equations will give varying results in patients with COPD. The choice of 6MWD reference equations should consider the consistency of 6MWT operating procedures and at least be specific for the country/region of origin. This seems of less importance in underweight patients with mild COPD.

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SUPPLEMENTAL MATERIAL

Table S1. Reference equations of the six-minute walk test in adults

Studies	List of Equations	R ²
Capodaglio et al. ^{20*}	$\text{6MWD}_m = 894.2177 - (2.0700 \times \text{age}_{\text{yrs}}) - (51.4489 \times \text{gender}_{\text{men}=0;\text{women}=1}) - 5.1663 \times \text{BMI}_{\text{kg/m}^2}$ <i>* Reference equation for Obese subjects</i>	0.48
Ramanathan et al. ¹⁹	$\text{6MWD}_m = 561.022_m - (2.507 \times \text{age}_{\text{yrs}}) + (1.505 \times \text{weight}_{\text{kg}}) - (0.055 \times \text{height}_{\text{cm}})$ $\text{6MWD}_m = 30.325_m - (0.809 \times \text{age}_{\text{yrs}}) - (2.074 \times \text{weight}_{\text{kg}}) + (4.235 \times \text{height}_{\text{cm}})$	0.29 0.27
Britto et al. ¹⁸	$\text{6MWD}_m = 890.46_m - (6.11 \times \text{age}_{\text{yrs}}) + ((0.0345 \times (\text{age}_{\text{yrs}})^2) + (48.87 \times \text{gender}_{\text{men}=1;\text{women}=0}) - (4.87 \times \text{BMI}_{\text{kg/m}^2}))$ <i>Alternative:</i> $\text{6MWD}_m = 356.658_m - (2.303 \times \text{age}_{\text{yrs}}) + (36.648 \times \text{gender}_{\text{men}=1;\text{women}=0}) + (1.704 \times \text{height}_{\text{cm}}) + (1.365 \times \Delta\text{HR})$	0.46
Hill et al. ¹⁷	$\text{6MWD}_m = 970.7 + (-5.5 \times \text{age}_{\text{yrs}}) + (56.3 \times \text{gender}_{\text{men}=1;\text{women}=0})$	0.49
Casanova et al. ¹⁶	$\text{6MWD}_m = 361_m - (\text{age}_{\text{yrs}} \times 4) + (\text{height}_{\text{cm}} \times 2) + (\text{HR}_{\text{max}}/\text{HR}_{\text{max}\% \text{pred}} \times 3) - (\text{weight}_{\text{kg}} \times 1.5)$ $\text{6MWD}_m = 361_m - (\text{age}_{\text{yrs}} \times 4) + (\text{height}_{\text{cm}} \times 2) + (\text{HR}_{\text{max}}/\text{HR}_{\text{max}\% \text{pred}} \times 3) - (\text{weight}_{\text{kg}} \times 1.5) - 30$	0.38 0.38
Dourado et al. ¹⁵	$\text{6MWD}_m = 299.296_m - (2.728 \times \text{age}_{\text{yrs}}) - (2.160 \times \text{weight}_{\text{kg}}) + (361.731 \times \text{height}_m) + (56.3860.54 \times \text{gender}_{\text{men}=1;\text{women}=0})$ <i>Alternative:</i> $\text{6MWD}_m = 109.764_m - (1.794 \times \text{age}_{\text{yrs}}) - (2.383 \times \text{weight}_{\text{kg}}) + (423.110 \times \text{height}_m) + (2.422 \times \text{grip strength}_{\text{kg}})$ Factor grip strength: men=1;women=0	
Soares et al. ¹⁴	$\text{6MWD}_m = 511_m + ((\text{height}_{\text{cm}})^2 \times 0.0066) - ((\text{age}_{\text{yrs}})^2 \times 0.030) - ((\text{BMI}_{\text{kg/m}^2})^2 \times 0.068)$	0.55
Osses et al. ¹³	$\text{6MWD}_m = 530_m - (3.31 \times \text{age}_{\text{yrs}}) + (2.36 \times \text{height}_{\text{cm}}) - (1.49 \times \text{weight}_{\text{kg}})$ $\text{6MWD}_m = 457_m - (3.46 \times \text{age}_{\text{yrs}}) + (2.61 \times \text{height}_{\text{cm}}) - (1.57 \times \text{weight}_{\text{kg}})$	0.55 0.63
Alameri et al. ¹²	$\text{6MWD}_m = (2.81 \times \text{height}_{\text{cm}}) + (0.79 \times \text{age}_{\text{yrs}}) - 28.5_m$	0.25
Ben Saad et al. ¹¹	$\text{6MWD}_m = 720.50_m - (160 \times \text{gender}_{\text{men}=0;\text{women}=1}) - (5.14 \times \text{age}_{\text{yrs}}) - (2.23 \times \text{weight}_{\text{kg}}) + (2.72 \times \text{height}_{\text{cm}})$ <i>LLN: Of the predicted 6MWD values a less of -89m can be considered as abnormal</i>	0.77
Iwama et al. ¹⁰	$\text{6MWD}_m = 622.461_m - (1.846 \times \text{age}_{\text{yrs}}) + (61.503 \times \text{gender}_{\text{men}=1;\text{women}=0})$	0.30
Jenkins et al. ⁹	$\text{6MWD}_m = 867 - (5.71 \times \text{age}_{\text{yrs}}) + (1.03 \times \text{height}_{\text{cm}})$ $\text{6MWD}_m = 525_m - (2.86 \times \text{age}_{\text{yrs}}) + (2.71 \times \text{height}_{\text{cm}}) - (6.22 \times \text{BMI}_{\text{kg/m}^2})$	0.40 0.43
Masmoudi et al. ⁸	$\text{6MWD}_m = 299.8_m - (4.43 \times \text{age}_{\text{yrs}}) + (342.6 \times \text{height}_{\text{cm}}) - (1.46 \times \text{weight}_{\text{kg}}) + (62.5 \times \text{gender}_{\text{men}=0;\text{women}=1})$ <i>LLN: Of the predicted 6MWD values a less of -124.5m can be considered as abnormal</i>	0.60
Camarri et al. ⁷	$\text{6MWD}_m = 64.69_m + (3.12 \times \text{height}_{\text{cm}}) + (23.29 \times \text{FEV}_{1\text{L}})$ <i>Alternative:</i> $\text{6MWD}_m = 216.90_m + (4.12 \times \text{height}_{\text{cm}}) - (1.75 \times \text{age}_{\text{yrs}}) - (1.15 \times \text{weight}_{\text{kg}}) - (34.04 \times \text{gender}_{\text{men}=0;\text{women}=1})$	0.36
Chetta et al. ⁶	$\text{6MWD}_m = 518.853_m + (1.25 \times \text{height}_{\text{cm}}) - (2.816 \times \text{age}_{\text{yrs}}) - (39.07 \times \text{gender}_{\text{men}=0;\text{women}=1})$	0.42
Poh et al. ⁵	$\text{6MWD}_m = (5.50 \times \text{HR}_{\text{max}}/\text{HR}_{\text{max}\% \text{pred}}) + (6.94 \times \text{height}_{\text{cm}}) - (4.49 \times \text{age}_{\text{yrs}}) - (3.51 \times \text{weight}_{\text{kg}}) - 473.27_m$	0.78
Enright et al. ⁴	$\text{6MWD}_m = 510_m + (2.2 \times \text{height}_{\text{cm}}) - (0.93 \times \text{weight}_{\text{kg}}) - (5.3 \times \text{age}_{\text{yrs}})$ $\text{6MWD}_m = 493_m + (2.2 \times \text{height}_{\text{cm}}) - (0.93 \times \text{weight}_{\text{kg}}) - (5.3 \times \text{age}_{\text{yrs}})$ <i>LLN: Of the predicted 6MWD values a less of -100m for men & women can be considered as abnormal</i>	0.20

Chapter 3

Studies	List of Equations	R ²
Gibbons et al. ³	♀♂: $6MWD_m = 868.8_m - (age_{yrs} \times 2.99) - (gender_{men=0;women=1} \times 74.7)$	0.41
Troosters et al. ²	♀♂: $6MWD_m = 218_m + (5.14 \times height_{cm} - 5.32 \times age_{yrs}) - (1.80 \times weight_{kg}) + (51.31 \times gender_{men=1;women=0})$ <i>LLN: Of the predicted 6MWD values <82% can be considered as abnormal</i>	0.66
Enright & Sherrill ¹	♂: $6MWD_m = (7.57 \times height_{cm}) - (5.02 \times age_{yrs}) - (1.76 \times weight_{kg}) - 309_m$	0.42
	♀: $6MWD_m = (2.11 \times height_{cm}) - (2.29 \times weight_{kg}) - (5.78 \times age_{yrs}) + 667_m$	0.38
	<u>Alternatives</u> : ♂: $6MWD_m = 1.140_m - (5.61 \times BMI_{kg/m^2}) - (6.94 \times age_{yrs})$	0.42
	♀: $6MWD_m = 1.017_m - (6.24 \times BMI_{kg/m^2}) - (5.83 \times age_{yrs})$ <i>LLN: Of the predicted 6MWD values a less of -153m for men & -139m for women can be considered as abnormal</i>	0.38

List of 6MWT references equations derived from 6MWD reference values of healthy adults.

Six-minute walk distance in patients with COPD: Which reference should we use?

Table S2. Technical and methodological information of studies that formulated 6MWT reference values

Studies	Partic. (n)	Females (%)	Age (Range)	Race	6MWD, m	Methodological notes
Capodaglio et al. ^{20*}	323	194 (60)	37 (20-60)	Caucasian Italians	565±48	*BMI≥30 kg/m ² , Equation specific for obese subjects
Ramanathan et al. ¹⁹	125	67 (54)	46 (25-80)	Indians	495±84	Convenience-based sample of participants
Britto et al. ¹⁸	617	321 (52)	52 (29-67)	Brazilians	586±106	Not randomized sample
Hill et al. ¹⁷	77	40 (52)	65 (45-85)	Canadians	640±99	Participants from 1 specific region
Casanova et al. ¹⁶	444	206 (46)	58 (42-76)	Mixed	571 ±90	Not randomized sample
Dourado et al. ¹⁵	90	50 (56)	60 (40-70)	Brazilians	572 ±83	Convenience-based sample of participants, Inclusion of Grip Strength
Soares et al. ¹⁴	132	66 (50)	(20-80)	Brazilians	All: 552 (Weighted) ♂: 566 ±87 ♀: 538 ±95	Convenience-based sample of participants
Osses et al. ¹³	175	98 (56)	55 (20-80)	Brazilians	All: 605 (Weighted) - ♂: 644 ±84 ♀: 576 ±87	
Alameri et al. ¹²	238	111 (47)	29 (16-50)	Arabs	All: 409 ±51 ♂: 429 ±47 ♀: 386 ±45	"walk at your own pace", HRmax pred. Males: 44% Females: 47%
Ben Saad et al. ¹¹	229	125 (55)	56 (45-59)	Tunisians	All: 624±111 ♂: 711 ±81 ♀: 551 ±75	40m corridor
Iwama et al. ¹⁰	134	73 (55)	33 (13-84)	Brazilians	All: 583 ±83 ♂: 622 ±80 ♀: 551 ±74	Only 5 participants were >65yrs, the 2 nd 6MWD was used and not the best
Jenkins et al. ⁹	109	61 (56)	62 (45-85)	Caucasian Australian	All: 660 (Weighted) ♂: 682 ±73 ♀: 643 ±70	45m corridor
Masmoudi et al. ⁸	155	75 (48)	(40-80)	Tunisians	-	-
Camarri et al. ⁷	70	37 (53)	65 (55-75)	Caucasian Australian	659 ±62	HRmax pred. 81%, 45m corridor
Chetta et al. ⁶	102	54 (53)	34 (20-50)	Caucasian Italian	All: 614 (Weighted) ♂: 638 ±44 ♀: 593 ±57	Relatively young group of participants
Poh et al. ⁵	35	19 (54)	61 (45-85)	Chinese Singapor.	560 ±105	45m corridor
Enright et al. ⁴	2117	1270(60)	77 (≥70)	Mixed	All: 334 ♂: 362 ♀: 332	Participants with risk factors associated with cardiovascular disease
Gibbons et al. ³	79	38 (48)	45 (20-80)	Canadian	698 ±96	20m corridor, Equation based on the maximum of four 6MWT
Troosters et al. ²	51	22 (43)	65 (50-85)	Caucasian Belgian	631 ±93	HRmax pred. 77%
Enright & Sherrill ¹	290	173 (60)	61 (40-80)	Caucasian American	All: 527 (Weighted) ♂: 576 ♀: 494	No practice test, HRmax pred. <65%

Technical and methodological differences of studies that formulated 6MWT reference values derived from healthy adults.

Figure S1. Performance quartile comparisons of CPET
Weighted means of the 6MWD of several healthy populations categorized by race. Asterisks denote significant differences between the first group (*) and between the second group (**) ($p < 0.05$).

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Chapter 4

Prognostic value of variables derived from the six-minute walk test in patients with COPD: Results from the ECLIPSE study

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Respiratory Medicine 2015; 109:1138-46

ABSTRACT

Background

In addition to the six-min walk distance (6MWD), other six-min walk test (6MWT) derived variables, such as mean walk-speed (6MWSpeed), 6-min walk-work (6MWW), distance-saturation product (DSP), exercise-induced oxygen desaturation (EID), and unintended stops may be useful for the prediction of mortality and hospitalization in patients with chronic obstructive pulmonary disease (COPD). We studied the association between 6MWT-derived variables and mortality as well as hospitalization in COPD patients and compared it with the BODE index.

Methods

A three-year prospective study (ECLIPSE) to evaluate the prognostic value of 6MWT-derived variables in 2010 COPD patients. Cox's proportional-hazard regressions were performed to estimate 3-year mortality and hospitalization.

Results

During the follow-up, 193 subjects died and 622 were hospitalized. An adjusted Cox's regression model of hazard ratio [HR] for impaired 6MWT-derived variables was significant referring to: mortality (6MWD ≤ 334 m [2.30], 6MWSpeed ≤ 0.9 m/sec [2.15], 6MWW ≤ 20000 m $\cdot$ kg [2.17], DSP ≤ 290 m% [2.70], EID $\leq 88\%$ [1.75], unintended stops [1.99]; and hospitalization (6MWW ≤ 27000 m $\cdot$ kg [1.23], EID $\leq 88\%$ [1.25], BODE index ≥ 3 points [1.40]; all $p \leq 0.05$).

Conclusions

The 6MWT-derived variables have an additional predictive value of mortality in patients with COPD. The 6MWW, EID and the BODE index refine the prognosis of hospitalization.

INTRODUCTION

The six-minute walk test (6MWT) is a reliable, widely-used test to assess functional exercise performance in patients with chronic obstructive pulmonary disease (COPD).¹⁻³ Its primary readout is the distance (meters) walked in six minutes (6MWD). A 6MWD <350m predicts an increased risk of hospitalization and mortality rates in COPD.^{4,5}

The 6MWT is generally well tolerated, but some patients with COPD are unable to walk at a continuous pace for six minutes and need to stop to rest. Those unintended stops due to exercise-induced symptoms such as dyspnea and leg discomfort may even lead to test discontinuation.^{6,7} Obviously, unintended stops significantly affect 6MWD, and, in turn, may be the underlying reason that a poor 6MWD is associated with mortality in patients with COPD. Indeed, unintended stops during corridor walking are associated with higher rates of mortality in older adults and should be evaluated.⁸⁻¹¹ Therefore, we hypothesized that unintended stop(s) during the 6MWT may provide an additional prognostic information besides the 6MWD of patients with COPD. In addition, other 6MWT-derived outcomes, such as the average walking speed (6MWSpeed, m/sec of actual walking)^{5,9,10}, the 6-min walk work (6MWW, m·kg; defined as the product of 6MWD in meters and body weight in kilograms)^{12,13}, the distance-saturation product (DSP, in meters% defined as the product of the 6MWD in meters and the lowest SpO₂%)¹⁴, and the exercise-induced oxygen desaturation (EID; defined as a nadir SpO₂ ≤88% in the 6MWT)¹⁵ might also possess significant prognostic value in identifying patients at high risk for mortality and hospitalization in COPD.

To date, little is known about the prognostic value of the above mentioned 6MWT-derived variables in patients with COPD. Therefore, we sought to determine the prognostic value of the 6MWT-derived variables exploring further data from the ECLIPSE study.¹⁶

We used the well validated BODE index^{17,18}, calculated from body mass index (B), degree of airflow obstruction as expressed by FEV₁ (O), dyspnea with the modified medical research council (D) and exercise (E) measured with the 6MWD, as a comparator.

METHODS

Design and participants

The ECLIPSE study (ClinicalTrials.gov identifier: NCT00292552; GSK study code SCO104960) was a 3-year non-interventional longitudinal prospective study. The inclusion and exclusion criteria of the ECLIPSE study have been described elsewhere.¹⁶ In brief, individuals (age: 40-75 years) were recruited to the ECLIPSE if they had a smoking

history of ≥ 10 pack-years and a diagnosis of COPD.¹⁹ The ECLIPSE study was carried out in accordance with the Declaration of Helsinki and good clinical practice guidelines, and approved by the ethics committees of participating centers. The Institutional Review Boards of all participating institutions approved the study and all participants signed their informed consent.

Subject characterization

Demographic and physiological characteristics, level of dyspnea (using the modified Medical Research Council (mMRC) dyspnea scale) and measurements of lung function (post-bronchodilator spirometry and lung volumes), amount of emphysema (low-dose volumetric computed tomography [CT scan] 120 kV peak, 40 mA and 1.00 or 1.25-mm slice thickness), and exercise data (6MWT; see below) were used for analyses. Fat mass and fat free mass were determined using bioelectrical impedance (Bodystat 1500). Fat free mass index (FFMI, kg/m^2) was calculated as the mass (kg) divided by the squared height (m^2) and the body mass index (BMI, kg/m^2) was calculated as weight (kg) divided by squared height (m^2).

Six-minute walk test

The 6-min walk test was performed according to the 2002 ATS guidelines.²⁰ Briefly, participants were asked to walk indoors in a flat, straight, 30 m walking course supervised by a well-trained researcher. A practice 6MWT was not undertaken. Subjects were encouraged using standard methodology every minute of the 6MWT.²¹ They were allowed to stop and rest during the test, but were instructed to resume walking as soon as possible. Rest and post-exercise SpO_2 were measured using a pulse oxymeter with a finger probe. A modified Borg scale was used to quantify the level of dyspnea and fatigue perceived by subjects at the beginning and end of the test. The BODE index was calculated using the algorithm developed by Celli and colleagues.¹⁷ We report that 6MWT-data from the ECLIPSE study have been published before. Specifically, the association between baseline 6MWD and outcomes; and between changes in 6MWD and outcomes has been published.^{2, 22, 23} The current study is complementary, as the 6MWT-derived variables and their associations with survival and hospitalization are novel.

Several 6MWT derived variables were used for analyses. The 6MWD was recorded by measuring the actual walking distance in meters during the 6MWT; 6MWSpeed was calculated by dividing the 6MWD by the total walking time (6MWSpeed, $\text{m}/\text{sec} = 6\text{MWD, m}/\text{walking time, sec}$).^{5, 9} For example, in a 6MWD of 400m during which patient have unintended stop(s) of a total duration of 30 seconds, the 6MWSpeed would be 1.2 m/sec (e.g. 400m / 330sec). 6MWW was estimated by the product of body weight and

walking distance (6MWW, $m \cdot kg = 6MWD, m \times \text{body weight, kg}$).^{12, 13} DSP was calculated as the product of the final distance walked in meters and the post-exercise $SpO_2\%$ (DSP, $m\% = 6MWD, m \times SpO_2\%_{\text{post}} / 100$).¹⁴ EID was defined as the post-exercise $SpO_2 \leq 88\%$.¹⁵ Unintended stops were defined as a (temporary) discontinuation of the 6MWT.

Statistical analyses

Subjects were classified as: (1) “Completers” or “Stoppers” according to their ability to complete the 6MWT; (2) “Hospitalized” or “Non-hospitalized” according to ≥ 1 exacerbations requiring hospitalization during follow up (exacerbation-related hospitalization); and (3) “Survivors” or “Non-survivors” according to their vital status at the end of 3-year follow-up.

Results are shown as mean (standard deviation) or proportion (%). The statistical significance of between-group differences was assessed using analysis of variance, paired T-tests and Chi-square test, as appropriate. Multiple comparisons were testing with one-way Anova followed by Bonferroni’s post-hoc tests. Receiver operating characteristics (ROC) curves were used to determine threshold values with the best sensitivity and specificity to predict 3-year survival and hospitalization. The Area Under the curve (AUC) was calculated by the trapezoidal rule and the confidence intervals of the AUC was computed by the method of DeLong.²⁴ Kaplan-Meier curves based on unadjusted and adjusted analyses with Cox’s proportional-hazard regression estimated the 3-year survival probability and the hospitalization-free probability based on patients’ characteristics. Cox’s proportional-hazard regression model was adjusted for age, sex, FEV_1 , FEV_1/FVC ratio (Tiffeneau index), SGRQ, emphysema and smoking. Estimated hazard ratio (HR) and 95% confidence intervals (CI) were calculated. Log-minus-log plots confirmed the validity of the proportionality of hazards assumption over time. A two-sided level of significance was set at $P < 0.05$. Statistical analyses were carried out using MedCalc v.12 and SPSS v.19.0.

RESULTS

Study characteristics

A total of 2010 COPD patients with complete data was included in the study. As shown in Table 1, subjects had normal BMI, moderate to severe airflow limitation (56% GOLD grade III or IV) and mild to severe emphysema on CT (34% had severe to very severe emphysema). The 53% of subjects had dyspnea (mMRC scale ≥ 2), 48% severely impaired health status (SGRQ Total Score ≥ 50 points), and 27% symptoms of depression (CES-D scale ≥ 16 points; Table 1). On average, subjects walked $372 \pm 122m$; 6MWSpeed was 1.0

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$\pm 0.3\text{m/s}$; 6MWW was $28271 \pm 11232\text{m}^2\text{kg}$; and DSP was $341 \pm 117\text{m}\%$. The 6MWD was $<350\text{m}$ in 41% of the cohort, 23% exhibited EID, and 4% had unintended stops during the 6MWT. The BODE index in the entire cohort was 3.1 ± 2.1 points (Table 1).

Table 1. Baseline characteristics of COPD patients categorized by mortality and hospitalization during a 3-year follow-up

Characteristics	All (n=2010)	Survivors (n=1817, 90%)	Non- survivors (n=193, 10%)	P Value	Non- hospitalized (n=1388, 69%)	Hospitalized (n=622, 31%)	P Value
Women, n, (%)	698 (35)	641 (35)	57 (30)		478 (34)	220 (35)	
Age, years	63.4 ± 7.1	63.1 ± 7.1	66.1 ± 6.5	<0.001	63.1 ± 7.2	64.1 ± 6.7	0.002
BMI, kg/m^2	26.5 ± 5.6	26.5 ± 5.5	26.2 ± 6.3	NS	26.7 ± 5.6	26.0 ± 5.5	0.007
<21	295 (15)	254 (14)	41 (21)		185 (13)	110 (18)	
21-30	1273 (63)	1170 (64)	103 (54)		891 (64)	382 (61)	
≥ 30	442 (22)	393 (22)	49 (25)	NS [#]	312 (23)	130 (21)	NS [#]
FFMI, kg/m^2	17.8 ± 3.4	17.8 ± 3.3	17.7 ± 4.1	NS	18.0 ± 3.4	17.3 ± 3.5	<0.001
FEV ₁ , % pred.	48.5 ± 15.5	49.2 ± 15.5	42.3 ± 15.0	<0.001	51.3 ± 15.3	42.4 ± 14.4	<0.001
FVC, % pred.	80.0 ± 19.7	80.6 ± 19.7	73.8 ± 18.4	<0.001	81.8 ± 19.6	75.8 ± 19.3	<0.001
FEV ₁ /FVC, %	44.5 ± 11.4	44.8 ± 11.4	41.7 ± 11.0	<0.001	46.1 ± 11.4	40.9 ± 10.5	<0.001
GOLD Stage							
Stage II	891 (44)	835 (46)	56 (29)		720 (52)	171 (28)	
Stage III	862 (43)	767 (42)	95 (49)		542 (39)	320 (51)	
Stage IV	257 (13)	215 (12)	42 (22)	<0.001 [#]	126 (9)	131 (21)	<0.001 [#]
Emphysema extent [§]							
<5%	461 (25)	430 (26)	31 (18)		372 (29)	89 (16)	
5-25%	397 (22)	368 (22)	29 (17)		289 (23)	108 (19)	
25-50%	351 (19)	316 (19)	35 (21)		238 (19)	113 (20)	
>50%	618 (34)	543 (33)	75 (44)	<0.001 [#]	362 (29)	256 (45)	<0.001 [#]
LAA%, -950 _{HU}	17.5 ± 12.1	17.3 ± 12.2	19.5 ± 11.9	0.032	16.0 ± 11.6	20.9 ± 12.7	<0.001
mMRC Dyspnea	1.7 ± 1.0	1.6 ± 1.0	2.1 ± 1.1	<0.001	1.5 ± 1.0	2.0 ± 1.1	<0.001
≥ 2	1034 (53)	906 (50)	128 (67)	<0.001 [#]	621 (46)	413 (66)	<0.001 [#]
SGRQ-C							
Total score	48.1 ± 18.2	47.4 ± 18.1	53.9 ± 18.2	<0.001	44.9 ± 18.0	55.1 ± 16.5	<0.001
≥ 50 points	927 (48)	810 (45)	117 (62)	<0.001 [#]	549 (41)	378 (61)	<0.001 [#]
CES-D Score	11.5 ± 9.3	11.4 ± 9.4	12.7 ± 8.8	NS	11.0 ± 9.2	12.5 ± 9.5	0.001
≥ 16	526 (27)	463 (26)	63 (33)	0.048 [#]	336 (24)	190 (31)	0.003 [#]
Smokers	728 (36)	658 (36)	70 (36)	NS [#]	516 (37)	212 (34)	NS [#]

Abbreviations: BMI: Body Mass Index, FFMI: Fat-Free Mass Index, FEV₁: Force Expiratory Volume at 1 sec, FVC: Force Vital Capacity, LAA%: Low-attenuation area, mMRC: modified Medical Research Council Dyspnea Scale, SGRQ-C: St. George's Respiratory Questionnaire for COPD (40-item version), CES-D: Center for Epidemiologic Screening Test Questionnaire. Data is represented as means $\pm$ SD and percentages (%). In total 183 patient had undefined extent of emphysema (% valid percent); Hash tag (#) denotes statistical significance detected by Pearson Chi-square analysis. Significance level was set at $P < 0.05$.

Events during follow-up

A total of 193 subjects (10%) died during the 3-year follow-up. Non-survivors were older, had more severe airflow limitation, a higher degree of emphysema, higher dyspnea grades, and more impaired health status than survivors (Table 1). In addition, non-survivors had a lower baseline 6MWD, 6MWSpeed, 6MWW, DSP, a higher proportion of stoppers, a higher proportion of patients with EID, and a higher BODE index (Table 2). Similar differences were found between subjects with and without exacerbation-related hospitalization (Tables 1 and 2). Moreover, significant correlations were found amongst survival days, time to hospitalization and the several 6MWT-derived variables (Supplementary table S1).

Predictive value of 6MWT-derived variables for mortality and hospitalization

Tables 3-4 present the threshold values with the best specificity and sensitivity to predict mortality and hospitalization during follow-up for the 6MWD, 6MWSpeed, 6MWW, DSP, and the BODE index (as a validated reference). The prognostic accuracy of these cut-off points did not vary significantly within different age groups, sex, GOLD stages and the degree of emphysema (Supplementary table S2). Cox's regression analysis unadjusted HR indicated that patients with 6MWD (≤ 334 m), 6MWSpeed (≤ 0.9 m/sec), 6MWW (≤ 20000 m·kg), DSP (≤ 290 m%), BODE index (≥ 4 points), EID (SpO₂ post $\leq 88\%$) or unintended stop(s) have a relative higher risk for mortality (Figure 1). Additionally, the unadjusted HR for the prediction of hospitalization revealed that patients with 6MWD (≤ 357 m), 6MWSpeed (≤ 1.0 m/sec), 6MWW (≤ 27000 m·kg), DSP (≤ 310 m%), BODE index (≥ 3 points), EID (SpO₂ post $\leq 88\%$), or unintended stop(s) were more likely to be hospitalized during the 3-year follow-up (Figure 2). An adjusted Cox's proportional-hazard regression model for age, sex, BMI, FEV₁, FEV₁/FVC, SGRQ, emphysema, and smoking, indicated that the risk for mortality remained high for subjects with impaired 6MWT-derived variables, EID and unintended stop(s) during the 6MWT. Interestingly BODE index did not remain significant after the adjustment (Table 5). Regarding to hospitalization, 6MWW, EID and the BODE index remained significant in the adjusted regression model.

Posterior Cox's regression analyses, which were performed on patients with impaired 6MWT-derived variables according to the proposed cut-off points, incidence of EID and increased BODE index to predict mortality and hospitalization respectively (Tables 3 and 4), demonstrated that unintended stop(s) during the 6MWT retained an additional negative impact on survival (Figure 3). However, this was not observed for hospitalization-free probability (Supplementary figure S1).

Table 2. Exercise outcomes during the 6MWT and categorised by mortality and hospitalization during a 3-year follow-up

Characteristics	All (n=2010)	Survivors (n=1817, 90%)	Non-survivors (n=193, 10%)	P Value	Non-hospitalized (n=1388, 69%)	Hospitalized (n=622, 31%)	P Value
6MWD, m, $\pm$ SD	372 $\pm$ 122	379	304	$\pm$ 127	385	342	$\pm$ 116 <0.001
6MWD, %pred.	64 $\pm$ 21	65.2	54.5	$\pm$ 23.1	66.2	59.5	$\pm$ 19.9 <0.001
<350m, n, (%)	829 (41)	707 (39)	122 (63)	(63)	505 (36)	324 (52)	<0.001 [#]
Walk time, sec	355 $\pm$ 30	357	344	$\pm$ 54	356	354	$\pm$ 30 NS
6MW5speed, m/sec	1.0 $\pm$ 0.3	1.1	0.9	$\pm$ 0.3	1.1	1.0	$\pm$ 0.3 <0.001
6MWW, m/kg	28271 $\pm$ 11232	28873	22611	$\pm$ 11268	29655	25183	$\pm$ 10388 <0.001
DSP, m%	341 $\pm$ 117	349	273	$\pm$ 119	356	309	$\pm$ 109 <0.001
SpO ₂ pre, %	94.5 $\pm$ 2.6	94.6	93.3	$\pm$ 3.3	94.7	93.9	$\pm$ 2.8 <0.001
SpO ₂ post, %	91.5 $\pm$ 5.3	91.8	89.3	$\pm$ 6.2	92.1	90.2	$\pm$ 5.8 <0.001
HR pre, b/m	82 $\pm$ 14	82	84	$\pm$ 15	80	84	$\pm$ 14 <0.001
HR post, b/m	101 $\pm$ 18	101	101	$\pm$ 20	99	103	$\pm$ 19 <0.001
<i>Borg Scale</i>							
Dyspnea pre	1.6 $\pm$ 1.9	1.6	1.8	$\pm$ 1.8	1.5	1.8	$\pm$ 1.9 0.016
Dyspnea post	3.9 $\pm$ 2.4	3.8	4.3	$\pm$ 2.4	3.5	4.7	$\pm$ 2.3 <0.001
Fatigue pre	1.3 $\pm$ 1.8	1.3	1.6	$\pm$ 1.9	1.2	1.4	$\pm$ 1.9 NS
Fatigue post	2.7 $\pm$ 2.5	2.6	3.1	$\pm$ 2.7	2.4	3.1	$\pm$ 2.6 <0.001
BODE index	3.1 $\pm$ 2.1	3.0	4.4	$\pm$ 2.3	2.7	4.1	$\pm$ 2.1 <0.001
EID, n (%)	466 (23)	394 (19)	72 (37)	(37)	262 (19)	204 (33)	<0.001 [#]
Stoppers, n	75 (4)	56 (3)	19 (10)	(10)	41 (3)	34 (6)	0.007 [#]

Abbreviations: 6MWD: Six-min walk distance, 6MWSpeed: Six-min walk speed, 6MWW: six-min walk work, DSP: Distance saturation products, SpO₂: Oxygen saturation, HR: Heart rate, EID: Exercise-induced desaturation (SpO₂ post $\leq$ 88%) Data is represented as means $\pm$ SD and percentages (%). Hash tag (#) denotes statistical significance detected by Pearson Chi-square analysis. Significance level was set at P < 0.05.

Table 3. Cut-off points and the ROC curve parameters for the prediction of long-term mortality

	ROC curve parameters							
Predictive index	Best cut-off	AUC segment	Sensitivity (%)	95% CI Binomial exact	Specificity (%)	95% CI Binomial exact	Likelihood Ratio LR+	LR-
Age, years	≥65	0.63	0.62	0.54-0.69	0.60	0.57-0.62	1.52	0.65
BMI, kg/m ²	≤23	0.53	0.35	0.28-0.42	0.75	0.73-0.77	1.37	0.88
FEV ₁ , %pred.	≤43	0.63	0.61	0.53-0.68	0.62	0.60-0.65	1.61	0.63
FEV ₁ /FVC, %	≤39	0.58	0.50	0.43-0.57	0.65	0.63-0.67	1.42	0.77
SGRQ, score	≥55	0.60	0.53	0.45-0.60	0.65	0.62-0.67	1.49	0.73
6MWD, m	≤334	0.67	0.62	0.54-0.69	0.65	0.63-0.67	1.76	0.59
6MWSpeed, m/sec	≤0.9	0.67	0.57	0.49-0.64	0.69	0.66-0.71	1.80	0.63
6MWW, m/kg	≤20000	0.66	0.46	0.39-0.53	0.79	0.77-0.81	2.17	0.68
DSP, m%	≤290	0.68	0.58	0.51-0.65	0.72	0.70-0.74	2.06	0.59
BODE index	≥4	0.67	0.60	0.53-0.67	0.63	0.61-0.66	1.64	0.63

Abbreviations: Please see legends in Tables 1 and 2.

Table 4. Cut-off points and the ROC curve parameters for the prediction of long-term hospitalization

Predictive index	Best cut-off	ROC curve parameters					Likelihood Ratio	
		AUC segment	Sensitivity (%)	95% CI Binomial exact	Specificity (%)	95% CI Binomial exact	LR+	LR-
Age, years	≥64	0.54	0.53	0.49-0.57	0.55	0.53-0.58	1.20	0.84
BMI, kg/m ²	≤23	0.54	0.32	0.28-0.36	0.75	0.73-0.78	1.29	0.90
FEV ₁ , %pred.	≤44	0.67	0.60	0.56-0.64	0.65	0.63-0.68	1.73	0.61
FEV ₁ /FVC, %	≤45	0.63	0.67	0.63-0.70	0.52	0.49-0.54	1.38	0.64
SGRQ, score	≥45	0.66	0.73	0.69-0.76	0.50	0.47-0.53	1.45	0.55
6MWD, m	≤357	0.60	0.54	0.50-0.58	0.62	0.59-0.65	1.40	0.75
6MWSpeed, m/sec	≤1.0	0.60	0.56	0.52-0.60	0.58	0.56-0.61	1.35	0.75
6MWW, m/kg	≤27000	0.62	0.61	0.58-0.65	0.56	0.53-0.59	1.40	0.69
DSP, m%	≤310	0.62	0.52	0.48-0.56	0.67	0.64-0.69	1.55	0.73
BODE index	≥3	0.69	0.57	0.53-0.61	0.69	0.67-0.72	1.85	0.62

Abbreviations: Please see legends in Tables 1 and 2.

Figure 1. Kaplan-Meier survival curves referring to impaired 6MWT-derived variables
The cut-off values used are indicated within the plots. Unadjusted hazard ratio (HR) and 95% confidence intervals (95%CI) were calculated by Cox's proportional-hazard regression. Significance set on $P < 0.05$.

Figure 2. Kaplan-Meier curves for hospitalization referring to impaired 6MWT-derived variables. The cut-off values used are indicated within the plots. Unadjusted hazard ratio (HR) and 95% confidence intervals (95%CI) were calculated by Cox's proportional-hazard regression. Significance set on $P < 0.05$.

Table 5. Determinants of mortality and hospitalization during a 3-year follow-up of patients with COPD

MORTALITY				HOSPITALIZATION					
Determinants	n	Non-survivors n, (%)	Adjusted Hazard Ratio (95%CI)	P value	Determinants	n	Hospitalized n, (%)	Adjusted Hazard Ratio (95%CI)	P value
6MWD, m					6MWD, m				
>334	1254	74 (6)	1		>357	1148	289 (25)	1	
≤334	756	119 (16)	2.30 (1.62–3.28)	<0.001	≤357	862	333 (39)	1.12 (0.94 – 1.35)	NS
6MWSpeed, m/sec					6MWSpeed, m/sec				
>0.9	1339	84 (6)	1		>1.0	1086	273 (25)	1	
≤0.9	671	109 (16)	2.15 (1.52–3.04)	<0.001	≤1.0	924	349 (38)	1.11 (1.93 – 1.34)	NS
6MWW, m/kg					6MWW, m/kg				
>20000	1535	104 (7)	1		>27000	1015	239 (24)	1	
≤20000	475	89 (19)	2.17 (1.51 – 3.12)	<0.001	≤27000	995	383 (38)	1.23 (1.01 – 1.50)	0.045
DSP, m%					DSP, m%				
>290	1391	82 (6)	1		DSP >310	1224	301 (25)	1	
≤290	619	111 (18)	2.70 (1.89 – 3.86)	<0.001	DSP ≤310	786	321 (41)	1.18 (0.98 – 1.42)	NS
BODE index, points					BODE index, points				
BODE <4	1189	75 (6)	1		BODE <3	838	144 (17)	1	
BODE ≥4	759	113 (15)	1.47 (0.96 – 2.24)	NS	BODE ≥3	1110	457 (41)	1.40 (1.08 – 1.82)	0.012
SpO ₂ % post					SpO ₂ % post				
>88	1544	121 (8)	1		>88	1544	418 (27)	1	
≤88	466	72 (15)	1.75 (1.25 – 2.44)	0.001	≤88	466	204 (44)	1.25 (1.04 – 1.51)	0.020
Unintended Stops					Unintended Stops				
Completers	1935	174 (9)	1		Completers	1935	588 (30)	1	
Stoppers	75	19 (25)	1.99 (1.20 – 3.30)	0.008	Stoppers	75	34 (45)	1.04 (0.73 – 1.50)	NS

Abbreviations: 6MWD: Six-min walk distance, 6MW5speed: Six-min walk speed, 6MWW: six-min walk work, DSP: Distance-saturation products, SpO₂: Oxygen saturation. Hazard ratio for survival was adjusted for age ≥65 years, sex (Female), FEV₁ ≤43%pred., FEV₁/FVC ≤39%, Emphysema (mild to very severe) and smoking. Hazard ratio for hospitalization was adjusted for age≥64 years, sex (Female), FEV₁ ≤44%pred., FEV₁/FVC ≤45%, Emphysema (mild to very severe), and smoking.

DISCUSSION

This study explores the prognostic value of the 6MWT-derived variables including 6MWD, 6MWSpeed, 6MWW, DSP, EID, and unintended stop(s) for the prediction of mortality and hospitalization during a 3-year follow-up in patients with COPD. The 6MWD data of the ECLIPSE has been already investigated^{2, 22, 23}, however the essential novelty of our approach is that we demonstrated the 6MWT-derived variables in association with mortality and hospitalization.

We found that unintended stop(s) during the 6MWT can be a prognostic factor for mortality and hospitalization (Figures 1 and 2). Interestingly, unintended stop(s) together with the rest of 6MWT-derived variables can refine the prognosis to predict mortality (Figure 3). Unintended stop(s), which can also lead to withdrawal from the 6MWT, may reflect the overall impact of poor 6MWT-derived variables and increased BODE index on patients' functional capacity. However, unintended stop(s) combined with impaired 6MWT-derived variables did not lend support in the prediction of hospitalization (Supplementary figure S1). A possible explanation could be that patients with poor 6MWT-derived variables of 6MWD, 6MWSpeed, 6MWW, DSP, incidence of EID and increased BODE index, usually experience the first severe exacerbation related to hospitalization before there is an impact on their ability to keep a continuous pace during the 6MWT. Using an adjusted Cox's proportional-hazard regression model, the prognostic value of unintended stops to predict mortality remained after adjustment for age, sex, BMI, FEV₁, FEV₁/FVC, SGRQ, emphysema, and smoking suggesting that unintended stop(s) can be an important prognostic outcome for survival (Table 5). To the best of our knowledge, this is the first study that demonstrates the prognostic value of unintended stop(s) during the 6MWT in patients with COPD and suggests that more attention should be given to this outcome for the identification of high-risk patients.

Comparison with other studies

The 6MWSpeed has been already shown as a functional capacity indicator in 511 patients with mild to severe COPD.¹⁰ A mean 6MWSpeed <1.0 m/s has been related to disability, hospitalization and decreased survival in older adults.^{5, 10} A mean 6MWSpeed ≤0.9 m/sec increased the risk of death while 6MWSpeed ≤1.0 m/sec increased the risk of hospitalization in our patients with COPD, respectively. The present study is the first one which provides certain cut-off points for the 6MWSpeed with prognostic value on mortality (Figure 1B) and hospitalization (Figure 2B) in COPD patients.

In the study of Chuang and colleagues²⁵, 6MWW was used to assess work and energy expenditure during the 6MWT demonstrating a better correlation with the anaerobic threshold, peak oxygen uptake, DLCO and vital capacity when compared to

6MWD. Carter and colleagues¹² reported that 6MWW is an improved outcome measure with a solid physiologic foundation for the assessment of functional capacity in COPD. DSP variable has also been shown to be a potential indicator of functional status in patients with sarcoidosis²⁶ while DSP <200 m% has been associated with a seven-fold greater risk of 12-month mortality in patients with idiopathic pulmonary fibrosis (IPF).¹⁴ The prognostic value of 6MWW and DSP to predict mortality and hospitalization has also been confirmed by our findings (Figures 1 – 2C & 2D).

Other studies have demonstrated that the levels of oxygen saturation and incidence of EID during the 6MWT could improve the predictive ability of the 6MWD in COPD patients.^{27, 28} In this study, the occurrence of EID was also an indicator for increased mortality and hospitalization. Beside the prognostic value of worse 6MWT-derived variables, we also observed that older patients with lower FEV₁ and increased scores of SGRQ have higher risk for mortality and hospitalization (Supplementary figure S2). Regarding to the BODE index, a study of Ong and colleagues¹⁸ in 127 COPD patients who were followed up for 16 months demonstrated the BODE index as a better predictor of hospitalizations for COPD than the FEV₁ alone. Previously, BODE score of 3 points or higher has been associated to mortality and exacerbation rates in patients with COPD.²⁹ Our findings suggested that increased BODE score was an independent predictor of mortality and hospitalization in our large cohort of patients with COPD (Figures 1 –2E & 2F).

A Cox's regression analysis with proportional hazard model for the prediction of 3-year mortality and hospitalization adjusted for age, sex, BMI, FEV₁, FEV₁/FVC, SGRQ, emphysema, and smoking indicated that patients with impaired 6MWT-derived variables have increased risk of death (Table 5). After this adjustment, the cut-off point used for DSP (≤ 290 m%) was the best predictor of mortality (Hazard Ratio: 2.70) while, surprisingly, the BODE index did not remain significant. We assume that the inclusion of SGRQ and BMI in the adjustment of regression model reduced the BODE index significance as a result of variables' interrelation. In terms of hospitalization, patients with worse 6MWW, BODE index and EID had higher risk of exacerbation-related hospitalization (Table 5). The cut-off point of the BODE index (≥ 3 points) appeared to be the strongest predictor (Hazard Ratio: 1.40). Our adjusted proportional hazard models demonstrated that DSP is the stronger predictor of mortality while the BODE index is the most sensitive tool for the prediction of hospitalization compared to the 6MWT-derived variables.

Figure 3. Kaplan-Meier survival curves referring to unintended stop(s) during 6 MWT of patients with impaired 6 MWT-derived variables

Unintended stops (Completers/Stoppers) during the 6 MWT were used for the estimation of survival probability (%). Unadjusted Hazard ratio (HR) and 95% confidence intervals (95%CI) were calculated by Cox's proportional-hazard regression. Significance level was set at $P < 0.05$.

Strengths and weaknesses

There are certain limitations to our study. First, the 6MWT screening was performed only once at baseline, while the 2014 ERS/ATS Technical Standard for field walking tests suggest the conduct of two 6MWT.³⁰ At the time of designating the ECLIPSE study, the

2002 ATS statement on 6MWT was used, which did not recommend a practice walk. The 6MWT-derived variables, such as oxygen saturation, are reproducible.³¹ Obviously, 6MWW and DSP may have been a little bit higher as a second 6MWT would increase the 6MWD in about 80% of the patients with COPD.³¹ Second, the number of the unintended stops during the 6MWT is not available; nevertheless, the total walking time indicated whether or not patients stopped to rest during the 6MWT. Third, the EID was defined based on the SpO₂ at the end of the test (SpO₂ post) and not the SpO₂ nadir which can lead to an overestimation of the DSP in patients who exhibit EID during the 6MWT. Even though SpO₂ nadir and the SpO₂ post for most patients with chronic respiratory disease are relatively similar during the 6MWT with differences ranging from 1% to 10%³², the use of SpO₂ nadir would be optimal absent the biases. Concerning the diagnostic accuracy, the area under the ROC curve quantifies the overall ability of the tested variables to discriminate between patients who have a good or poor prognosis. Even though the diagnostic accuracy of the 6MWT-derived variables can be considered as sufficient based on the AUC (≥ 0.60)³³, a range of values is rather low (< 0.60). In turn, omissions errors and commission errors may occur.³⁴

Despite these limitations, this study contributes with important information about the 6MWT-derived variables and the BODE index in respect of their relationship to mortality and hospitalization in patients with COPD. Our findings may help clinicians to better predict the survival and hospitalization in patients with COPD. Additionally, unintended stop(s) during the 6MWT may represent an advanced stage of disease severity in which enrolment in a pulmonary rehabilitation program could be urgently recommended.

CONCLUSIONS

The 6MWT-derived variables of 6MWD, 6MWSpeed, 6MWW, DSP, incidence of EID, unintended stop(s) and the BODE index are important predictors of long-term mortality and hospitalization in patients with COPD. New insights into the 6MWT-derived variables indicate the DSP as the strongest predictor of mortality and the BODE index as the most sensitive tool for assessing the risk of hospitalization. Besides the poor 6MWT-derived variables and the BODE index, novel evidences suggesting that unintended stop(s) during the 6MWT can refine the prognosis of mortality in COPD. Obviously, these findings need to be reproduced by others and reliability needs to be studied.

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SUPPLEMENTAL MATERIAL

Table S1. Correlation matrix amongst survival days, time to hospitalization and several 6MWT derived outcomes

	Survival days	Time to hospitalization	6MWD, m	6MW-Speed, m/sec	6MWW, m/kg	DSP, m%	BODE, score	SpO ₂ % post	Unintended Stops
Survival days	1	.134 **	.149 **	.141 **	.141 **	.158 **	-.150 **	.132 **	-.103 **
Time to hospitalization	.134 **	1	.142 **	.148 **	.161 **	.154 **	-.192 **	.111 **	-.062 *
6MWD, m	.149 **	.142 **	1	.981 **	.809 **	.987 **	-.679 **	.191 **	-.285 **
6MWSpeed, m/sec	.141 **	.148 **	.981 **	1	.793 **	.969 **	-.661 **	.183 **	-.202 **
6MWW, m/kg	.141 **	.161 **	.809 **	.793 **	1	.800 **	-.621 **	.156 **	.267 **
DSP, m%	.158 **	.154 **	.987 **	.969 **	.800 **	1	-.697 **	.340 **	-.286 **
BODE, score	-.150 **	-.192 **	-.679 **	-.661 **	-.621 **	-.697 **	1	-.321 **	-.258 **
SpO ₂ % post	.132 **	.111 **	.191 **	.183 **	.156 **	.340 **	-.321 **	1	-.072 **
Unintended Stops	-.103 **	-.062 *	-.285 **	-.202 **	-.267 **	-.286 **	.258 **	-.072 **	1

Abbreviations: 6MWD: Six-min walk distance, 6MWSpeed: Six-min walk speed, 6MWW: six-min walk work, DSP: Distance saturation products, SpO₂: Oxygen saturation.

Chapter 4

Table S2. ROC curves for the prediction of mortality and hospitalization

PREDICTORS OF MORTALITY	AREA UNDER THE CURVE (AUC)										
	Age, years			Sex		GOLD Stages			Emphysema extend		
	<55	55-65	>65	M	F	II	III	IV	<5%	5-50%	>50%
6MWD ≤334 m	0.60	0.63	0.62	0.62	0.67	0.60	0.62	0.59	0.65	0.62	0.65
6MWSpeed ≤0.9 m/sec	0.61	0.61	0.63	0.61	0.67	0.60	0.62	0.59	0.62	0.62	0.64
6MWW ≤20000 m kg	0.60	0.64	0.60	0.60	0.69	0.55	0.63	0.62	0.61	0.57	0.66
DSP ≤290 m%	0.62	0.63	0.64	0.64	0.68	0.60	0.63	0.61	0.60	0.66	0.66
BODE index ≥4 score	0.60	0.63	0.61	0.59	0.68	0.54	0.60	0.52	0.61	0.56	0.64
EID (SpO ₂ post ≤88%)	0.53	0.55	0.59	0.58	0.58	0.53	0.56	0.57	0.53	0.60	0.56
Unintended stops	0.53	0.56	0.52	0.52	0.56	0.50	0.52	0.59	0.56	0.52	0.54
HOSPITALIZATION											
6MWD ≤357 m	0.63	0.57	0.57	0.57	0.59	0.55	0.56	0.51	0.55	0.55	0.59
6MWSpeed ≤1.0 m/sec	0.63	0.57	0.56	0.56	0.59	0.56	0.55	0.52	0.55	0.54	0.59
6MWW ≤27000 m kg	0.57	0.60	0.58	0.58	0.60	0.59	0.55	0.53	0.54	0.58	0.57
DSP ≤310 m%	0.63	0.59	0.58	0.58	0.61	0.58	0.55	0.51	0.55	0.57	0.59
BODE index ≥3 score	0.67	0.64	0.63	0.64	0.63	0.59	0.56	0.50	0.60	0.63	0.59
EID (SpO ₂ post ≤88%)	0.56	0.57	0.57	0.59	0.54	0.55	0.54	0.51	0.54	0.55	0.55
Unintended stops	0.50	0.53	0.51	0.51	0.51	0.51	0.50	0.50	0.51	0.51	0.52

Abbreviations: 6MWD: Six-min walk distance, 6MWSpeed: Six-min walk speed, 6MWW: six-min walk work, DSP: Distance saturation products, SpO₂: Oxygen saturation, EID: Exercise-induced desaturation.

Figure S1. Kaplan-Meier curves for hospitalization referring to unintended stops of patients with impaired 6MWT-derived variables

Unintended stops (Completers/Stoppers) during the 6MWT were used for the estimation of hospitalization-free probability (%). Unadjusted hazard ratio (HR) and 95% confidence intervals (95%CI) were calculated by Cox's proportional-hazard regression. Significance level was set at $P < 0.05$.

Figure S2. Kaplan-Meier curves for mortality and hospitalization according to clinical characteristics of patients with COPD

Certain thresholds of age, FEV₁, SGRQ and sex (m/f) were used for the estimation of survival and hospitalization-free probability (%). Unadjusted hazard ratio (HR) and 95% confidence intervals (95%CI) were calculated by Cox's proportional-hazard regression. Significance level was set at $P < 0.05$.

Chapter 5

Determinants of exercise-induced oxygen desaturation including pulmonary emphysema in COPD: Results from the ECLIPSE study

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Respiratory Medicine 2016; 119:87-95

ABSTRACT

Background

Exercise-induced oxygen desaturation (EID) is related to mortality in patients with chronic obstructive pulmonary disease (COPD). We investigated: (1) the prevalence of EID; (2) the relative-weight of several physiological determinants of EID including pulmonary emphysema, and (3) the relationship of EID with certain patients' clinical characteristics.

Methods

Data from 2050 COPD patients (age: 63.3 ± 7.1 years; FEV_1 : $48.7 \pm 15.7\%$ pred.) were analyzed. The occurrence of EID (SpO_2 post $\leq 88\%$) at the six-minute walking test (6MWT) was investigated in association with emphysema quantified by computed-tomography (QCT), and several clinical characteristics.

Results

435 patients (21%) exhibited EID. Subjects with EID had more QCT-emphysema, lower exercise capacity and worse health-status (BODE, ADO indexes) compared to non-EID. Determinant of EID were obesity ($BMI \geq 30 \text{ kg/m}^2$), impaired FEV_1 (44% pred.), moderate or worse emphysema, and low SpO_2 at rest (93%). Linear regression indicated that each 1-point increase on the ADO-score independently elevates odds ratio (1.5fold) for EID.

Conclusions

About one in five COPD patients in the ECLIPSE cohort present EID. Advanced emphysema is associated with EID. In addition, obesity, severe airflow limitation, and low resting oxygen saturation increase the risk for EID. Patients with EID in GOLD stage II have higher odds to have moderate or worse emphysema compared those with EID in GOLD stage III-IV. Emphysematous patients with high ADO-score should be monitored for EID.

INTRODUCTION

Exercise-induced oxygen desaturation (EID), as defined by fall in oxygen saturation to 88% or lower during exercise^{1, 2}, can occur in patients with Chronic Obstructive Pulmonary Disease (COPD), even in those without resting hypoxemia.³⁻⁵ Yet, their physiological determinants and clinical consequences are still subject of debate. In general, patients with EID are characterized by severe airflow limitation, low diffusing carbon monoxide capacity (DLCO), reduced resting arterial oxygenation⁶⁻⁹ and the presence of emphysema¹⁰⁻¹². In addition, EID is also related to numerous variables that include the rate of lung function decline and health status¹³, physical activity¹⁴, and the outcomes of hospitalization as well as mortality.^{15, 16} The determinants of EID, however, have not been validated in a large cohort of COPD patients followed up prospectively. The design and size of the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) study offers a unique opportunity to overcome these limitations.¹⁷

Herein, we investigated in the ECLIPSE: (1) the prevalence of EID in COPD; (2) the relative weight of several physiological determinants of EID in COPD, particularly the severity of airflow limitation and the presence of emphysema as defined by quantitative computed tomography (QCT) analysis¹⁸⁻²⁰ and, (3) the relationship of EID with certain patients' clinical characteristics.

METHODS

Design and participants

The ECLIPSE study (Clinicaltrials.gov identifier NCT00292552; GSK study code SCO104960) was a 3-year non-interventional longitudinal prospective study, as described elsewhere.¹⁷ In brief, individuals (age: 40-75 years) were recruited to ECLIPSE if they had a smoking history of ≥ 10 pack-years and a diagnosis of COPD.²¹ We excluded subjects with resting hypoxemia (as defined by $\text{SpO}_2\%$ pre-walk $\leq 88\%$) from this analysis. The ECLIPSE study was carried out in accordance with the Declaration of Helsinki and good clinical practice guidelines, and was approved by the ethics committees of participating centers. The Institutional Review Boards of all participating institutions approved the study and all participants provided written informed consent. Data from the ECLIPSE study have been published before^{16, 22-24} but the current analysis, which is mostly focus on EID in association with the severity of emphysema, is novel and, therefore, complements previous reports.

Subject characterization

Demographic and physiological characteristics, level of dyspnea (using the modified Medical Research Council (mMRC) dyspnea scale)²⁵, measurements of lung function (post-bronchodilator spirometry and lung volumes), QCT-emphysema degrees, health status (using the St. George's Respiratory Questionnaire (SGRQ))²⁶, and data from 6MWT were used in this analysis. Fat mass and fat free mass were determined using bioelectrical impedance (Bodystat 1500). The fat free mass index (FFMI, kg/m²) was calculated as the mass (kg) divided by the squared height (m²) and the body mass index (BMI, kg/m²) was calculated as weight (kg) divided by squared height (m²). Furthermore, the following multidimensional COPD indices were calculated for the whole cohort; (1) the ADO index²⁷, which includes age, dyspnea by the MRC, and FEV₁%, and (2) the BODE index²⁸, which incorporates dyspnea by the MRC, BMI, FEV₁%, and exercise capacity as measured by six-minute walking distance (6MWD).

CT imaging

All subjects underwent a low-dose volumetric computed tomography (CT scan) at full inspiration. Scans were obtained using multi-detector row CT scanners (120 kVp, 40 mAs) and were reconstructed using contiguous 1.00 mm or 1.25 mm slice thickness and an intermediate spatial frequency reconstruction algorithm. The radiation dose was estimated to be 1.67 mSv per CT study or 5 mSv for the entire ECLIPSE protocol. All CT scans were sent for evaluation at the central imaging unit at the University of British Columbia (Vancouver, BC, Canada).²⁹ All CT scans were analyzed by the use of Pulmonary Workstation 2.0 software (VIDA Diagnostics, Coralville, IA, USA) and the severity of emphysema was quantified in CT scans (QCT) by two radiologists using the % of lung pixels that were low attenuation areas (%LAA) defined as being below 950 Hounsfield Units (density mask technique).^{18, 20, 30} The degree of QCT-emphysema was classified according to a 5-scale score as absent-trivial: <5% (scores 0-1), mild: 5-25% (score 2), moderate: 25-50% (score 3), or severe to very severe: >50% (scores 4-5).²⁹

Six-minute walk test (6MWT)

The 6MWT was performed according to international recommendations.³¹ Briefly, participants were asked to walk indoors in a flat, straight, 30-m walking course supervised by a well-trained researcher. A practice 6MWT was not undertaken. Patients were encouraged using standardized encouragement every minute of the 6MWT.¹ Resting (pre-walk) and post SpO₂ were assessed using a pulse oxymeter with a finger probe. A modified Borg scale was used to quantify the levels of dyspnea and fatigue perceived by patients at the beginning and end of the test. All subjects included in the

analysis were non-hypoxemic at rest (SpO_2 pre-walk $\geq 89\%$). EID was defined as a fall of oxygen saturation to $\text{SpO}_2 \leq 88\%$ at the end of the test.^{1,2}

Statistical analysis

Results are reported as mean (standard deviation) or proportion (%). The statistical significance of differences between groups was assessed by analysis of variance, paired T-tests and Chi-square, as appropriate. Effect sizes (Cohen's d) using absolute standardized differences among the comparisons between the non-EID and EID groups were calculated. Pearson's correlation coefficients were used to assess the bivariate relationship amongst patients' characteristics and the variables of $\text{SpO}_2\%$ post-walk, $\Delta\text{SpO}_2\%$ and QCT-defined emphysema. Linear regression and univariate and multivariate binary logistical regression models were used to assess individual predictors of EID. Receiver operating characteristics (ROC) curves were used to determine threshold values to predict EID. The Area Under the curve (AUC) was calculated by the trapezoidal rule and the confidence intervals of the AUC was computed by the method of DeLong.³² Two-sided level of significance was set at $P < 0.05$. Statistical analyses were carried out using MedCalc v.12, Sigmaplot v.12 and SPSS v.19.0.

RESULTS

Subject characteristics

A total of 2050 subjects with COPD were included in the study. As shown in Table 1, subjects generally had severe airflow limitation, low oxygen saturation (SpO_2 pre-walk), and were normal-weight to overweight. Of these, 1124 subjects (55%) were assigned into GOLD III and IV while 622 subjects (33%) were diagnosed with severe to very severe emphysema. Fifty-three percent (53%) of the subjects complained of severe dyspnea (mMRC scale ≥ 2), and 47% had severely impaired health status (SGRQ Total Score ≥ 50). Thirty-six percent (36%) were current smokers (Table 1).

Determinants of EID

Four hundred thirty-five subjects ($n=435$, 21%) exhibited EID while the prevalence of EID was higher across GOLD stages and the degrees of emphysema severity (Figure 1). Subjects with EID were older with more severe airflow limitation, lower FVC, and worse dyspnea (mMRC) as well as SGRQ total scores. Also, they were more commonly ex-smokers compared those without EID (non-EID; Table 1). Subjects with EID had a shorter walk distance (6MWD), lower pre-walk SpO_2 and higher pre/post-walk heart

rate. In addition, EID subjects had greater increase of heart rate from baseline (Δ HR) and more intense dyspnea at exertion compared to non-EID (Table 2). Moreover, those with EID had higher scores in the ADO²⁷ and the BODE²⁸ indices compared to non-EID subjects (Table 2).

The effect of QCT-emphysema and the severity of airflow limitation on the lowest values of oxygen saturation levels, which defined the EID (SpO_2 post $\leq 88\%$) in the 6MWT, varied widely across the disease severity (Figure 2). Both, severity of airflow limitation and the extent of emphysema had a negative impact on the SpO_2 levels at the end of 6MWT. Severe to very severe emphysema in advanced airflow limitation is highly related to EID. On the other hand, the odds for patients with EID to have moderate or worse emphysema were greater in patients with GOLD II compared to GOLD III-IV (ORs 5.22, 2.11 and 1.48, respectively; Figures 1 and 2).

Despite the large variability in the proportion of patients who presented EID across the disease severity, the overall change in oxygen saturation (ΔSpO_2) associated with the severity of emphysema was relatively consistent (Figure 3).

Figure 1. Prevalence of EID in COPD according to GOLD stages and pulmonary emphysema
EID progressively increased across the GOLD stages and the degrees of pulmonary emphysema in COPD. A total of 435 subjects (21%) in the ECLIPSE cohort exhibited EID.

Table 1. Baseline characteristics of COPD patients categorized by the prevalence of EID

Characteristics	All Subjects (n=2050)	Non-EID (n=1615, 79%)	EID (n=435, 21%)	P Value	Cohen's d
Women, #, (%)	710(35)	544(34)	166(38)		
Age, years	63.3±7.1	63.1±7.2	64.1±6.8	0.012	0.143
BMI, kg/m ²	26.5±5.6	26.5±5.5	26.7±5.8	NS	0.035
<i>Underweight</i>	98(5)	81(5)	17(4)		
<i>Normal range</i>	751(37)	591(36)	160(37)		
<i>Overweight</i>	743(36)	594(37)	149(34)		
<i>Obese</i>	458(22)	349(22)	109(25)	NS [#]	0.928
FFMI, kg/m ²	17.8±3.4	17.8±3.5	17.6±3.4	NS	0.058
FEV ₁ , % predicted	48.7±15.7	51.2±15.2	39.5±13.9	<0.001	0.803
FVC, % predicted	80.0±19.8	81.2±19.2	75.4±21.0	<0.001	0.288
FEV ₁ /FVC, %	44.7±11.4	46.2±11.0	39.1 11.1	<0.001	0.643
<i>GOLD Classification[§]</i>					
Stage II	925(45)	836(52)	89(20)		
Stage III	861(42)	639(39)	222(51)		
Stage IV	263(13)	139(9)	124(29)	<0.001 [#]	0.114
<i>Emphysema extent^{§§}</i>					
<5% [absent-trivial]	478(26)	431(30)	47(11)		
5-25% [Mild]	406(22)	356(24)	50(12)		
25-50% [Moderate]	361(19)	293(20)	68(17)		
>50% [Severe-very severe]	622(33)	375(26)	247(60)	<0.001 [#]	0.403
mMRC, Dyspnea grade	1.7±1.0	1.6±1.0	2.0±1.0	<0.001	0.400
≥2	1062(53)	775(48)	287(66)	<0.001 [#]	0.835
SGRQ-C Total Score	47.8±18.1	46.5±18.5	52.6±15.6	<0.001	0.356
≥50 points, n (%)	934(47)	700(43)	234(54)	<0.001 [#]	0.893
Current Smokers, n (%)	742(36)	639(40)	103(24)	<0.001 [#]	1.171

Abbreviations: BMI: Body Mass Index, FFMI: Fat-Free Mass Index, FEV₁: Force Expiratory Volume at 1 sec, FVC: Force Vital Capacity, mMRC: modified Medical Research Council Dyspnea Scale, SGRQ-C: St. George's Respiratory Questionnaire for COPD (40-item version). Values expressed as mean ±SD. [§]One patients had a confirmed diagnosis for COPD but could not be assigned to a GOLD stage. ^{§§}In total 183 patient had undefined extent of emphysema (% valid percent). Hash tag (#) denotes statistical significance detected by Pearson Chi-square analysis Significance level was set at P <0.05.

Table 2. Six-Minute Walk Test characteristics and multi-dimensional indices categorized by the prevalence of EID

Characteristics	All Subjects (n=2050)	Non-EID (n=1615, 79%)	EID (n=435, 21%)	P Value	Cohen's d
6MWD, m, $\pm$ SD	372 $\pm$ 121	382 $\pm$ 119	334 $\pm$ 121	<0.001	0.400
6MWD, % predicted	64 $\pm$ 21	66 $\pm$ 20	58 $\pm$ 21	<0.001	0.390
SpO ₂ , % pre	94.7 $\pm$ 2.2	95.1 $\pm$ 2.0	93.1 $\pm$ 2.3	<0.001	0.928
SpO ₂ , % post	91.8 $\pm$ 5.0	93.9 $\pm$ 2.6	84.1 $\pm$ 4.3	<0.001	2.758
Δ SpO ₂ , %	-2.9 $\pm$ 4.3	-1.2 $\pm$ 2.4	-9.0 $\pm$ 4.8	<0.001	2.055
HR pre, b/m	82 $\pm$ 14	81 $\pm$ 14	85 $\pm$ 14	<0.001	0.286
HR post, b/m	100 $\pm$ 18	98 $\pm$ 17	108 $\pm$ 19	<0.001	0.555
Δ HR, b/m	18 $\pm$ 14	17 $\pm$ 13	23 $\pm$ 16	<0.001	0.412
<i>Borg Scale</i>					
Dyspnea, pre	1.6 $\pm$ 1.8	1.5 $\pm$ 1.8	1.7 $\pm$ 1.9	NS	0.108
Dyspnea, post	3.9 $\pm$ 2.4	3.6 $\pm$ 2.3	4.7 $\pm$ 2.3	<0.001	0.478
Δ Dyspnea, [Δ]	2.3 $\pm$ 2.0	2.1 $\pm$ 1.9	3.0 $\pm$ 2.2	<0.001	0.438
Fatigue, pre	1.3 $\pm$ 1.8	1.3 $\pm$ 1.8	1.3 $\pm$ 1.8	NS	0.000
Fatigue, post	2.7 $\pm$ 2.5	2.6 $\pm$ 2.5	2.7 $\pm$ 2.5	NS	0.040
Δ Fatigue, [Δ]	1.4 $\pm$ 1.9	1.4 $\pm$ 1.8	1.4 $\pm$ 1.9	NS	0.000
ADO index, score	3.7 $\pm$ 1.5	3.5 $\pm$ 1.5	4.4 $\pm$ 1.4	<0.001	0.620
≥ 6 points, n, (%)	245 (12)	148 (9)	97 (22)	<0.001 [#]	0.408
BODE index, score	3.1 $\pm$ 2.1	2.8 $\pm$ 2.0	4.2 $\pm$ 2.1	<0.001	0.683
≥ 6 points, n, (%)	456 (22)	286 (18)	170 (39)	<0.001 [#]	0.493

Abbreviations: BMI: Body Mass Index, FFMI: Fat-Free Mass Index, FEV₁: Force Expiratory Volume at 1 sec, FVC: Force Vital Capacity, 6MWD: Six-min walk distance, SpO₂: Oxygen saturation, HR: Heart rate, ADO index: "Age, Dyspnea, and airflow Obstruction" – index, BODE index: "Body-mass index, airflow Obstruction, Dyspnea, and Exercise" – index, EID: Exercise-induced oxygen desaturation (SpO₂ post 88%). Values expressed as mean $\pm$ SD unless specified otherwise. Hash tag (#) denotes statistical significance detected by Pearson Chi-square analysis. Significance level was set at P < 0.05.

Multivariate correlates of EID

Using receiver operating characteristics (ROC) curves, the threshold values with the best specificity and sensitivity to predict EID were determined for age (≥ 60 years), sex (female), BMI (≥ 30 kg/m²), FEV₁ (≤ 44 %pred.), QCT-emphysema (moderate to very severe), resting oxygen saturation (SpO₂ pre-walk $\leq 93\%$) and ADO index (ADO score ≥ 6 points; Supplementary table S1). Univariate logistical regression analysis revealed that older age and impaired levels of FEV₁, QCT-emphysema, and SpO₂ pre-walk were independent predictors of EID. Multivariate logistical regression model showed that only impaired levels of FEV₁, QCT-emphysema, and SpO₂ pre-walk remained significant (p ≤ 0.016). Interestingly, obesity (BMI ≥ 30 kg/m²) reached the statistical significance

($p < 0.003$) for contributing EID only within this multivariate-adjusted model and not as independent determinant (Table 3).

A combination of certain clinical characteristics including obesity, moderate to very severe emphysema, severe airflow limitation ($FEV_1 \leq 44\%$ pred.), and resting $SpO_2 \leq 93\%$ revealed that the 81% of the subjects with all of these characteristics exhibited EID (Figure 4).

Relationship of ADO score with EID

The presence of EID was related to higher ADO index²⁷ scores in EID subjects with GOLD II-III and across all the degrees of emphysema compared to those with non-EID (Supplementary figure 1). For each 1-point increase on the ADO score, the odds ratio for EID was independently increased by 1.5 fold while 40% of subjects with $ADO \geq 6$ points had EID (Supplementary table S2).

Table 3. Logistic regression: Determinants of EID in COPD patients

Determinants	Subjects n	EID n, (%)	Crude OR (95%CI)	P value	Adjust OR (95%CI)	P value
Age						
<60 years	594	106 (18)	1		1	
≥ 60 years	1456	329 (23)	1.34 (1.05-1.71)	0.017	1.21 (0.91-1.61)	NS
Sex						
Male	1340	269 (20)	1		1	
Female	710	166 (23)	1.22 (0.98-1.51)	NS	1.30 (1.00-1.68)	NS
BMI						
$< 30 \text{ kg/m}^2$	1592	326 (20)	1		1	
$\geq 30 \text{ kg/m}^2$	458	109 (24)	1.21 (0.95-1.55)	NS	1.57 (1.15-2.14)	0.005
FEV_1						
$> 44\%$ predicted	1198	141 (12)	1		1	
$\leq 44\%$ predicted	851	294 (35)	3.96 (3.16-4.96)	< 0.001	3.14 (2.42-4.07)	< 0.001
Emphysema						
Absent to mild	884	97 (11)	1		1	
Moderate to very severe	983	315 (32)	3.83 (2.98-4.91)	< 0.001	3.37 (2.52-4.51)	< 0.001
$SpO_2\%$ pre-walk						
$> 93\%$	1506	194 (13)	1		1	
$\leq 93\%$	544	241 (44)	5.38 (4.29-6.75)	< 0.001	4.75 (3.69-6.13)	< 0.001

Abbreviations: BMI: Body Mass Index, FEV_1 : Force Expiratory Volume at 1 sec, SpO_2 : Oxygen saturation. Multivariate-adjusted model including all the determinants of EID. A multivariate-adjusted regression analysis; all the determinants of EID (age, sex, BMI, FEV_1 , emphysema, and SpO_2 pre-walk) were included in the multivariate model. Significance level was set at $P < 0.05$.

Figure 2. Frequency distribution of post-walk SpO₂ and EID (88%) stratified by the GOLD stages and the degrees of QCT-emphysema

The dot line marks the threshold value for the presence of EID (SpO₂ post-walk 88%).

Figure 3. Frequency distribution of the changes in oxygen saturation (ΔSpO_2), as occurred from baseline to post-walk values, stratified by the GOLD stages and the degrees of QCT emphysema. The interquartile range (IQR) and the median changes in oxygen saturation (ΔSpO_2 [M]) were calculated.

Figure 4. Combination of certain lung function characteristics and resting oxygen saturation applied for the prediction of EID in patients with COPD

A combination of certain lung function characteristics and resting oxygen saturation for the prediction of EID revealed that the highest proportion of patients (81%) who exhibit EID are characterized by moderate to very severe emphysema (QCT score 3-5), obesity, impaired FEV₁ and low resting SpO₂ pre-walk values.

DISCUSSION

This study provides three major observations: (1) about 20% of COPD patients included in the ECLIPSE cohort exhibited EID; (2) emphysema, severity of airflow limitation, arterial oxygen saturation at rest and obesity are associated with EID; and (3) a high ADO index is predictive of EID.

It has been previously suggested that the presence of emphysema may determine EID in COPD.¹¹ However; this is the first time that the role of emphysema was investigated in a large well-characterized COPD population (ECLIPSE cohort). In this study, emphysema in association with other determinants, such as obesity (BMI ≥30kg/m²), have a prognostic value in the prediction of EID. Moreover, the relationship of the ADO score with EID was a novel investigation.

Interpretation of results

In current study, the prevalence of EID was increased across the GOLD stages (Figure 1). The severity of airflow limitation seems to contribute to the occurrence of EID along with than the grades of QCT-emphysema (Figure 2). In addition, the occurrence of EID in subjects with GOLD II may indicate the presence of emphysema according to the findings of this study (Figure 2). However, this was not true in GOLD III and IV as both airflow limitation and emphysema likely contribute to EID.

Impaired oxygen transport and utilization is a common consequence in both airway limitation and pulmonary emphysema, and can lead to ventilation/perfusion (V/Q) mismatch resulting in EID. Airflow limitation often leads to air-trapping and dynamic hyperinflation, which result in higher work of breathing due to high inspiratory threshold load and is associated to increased oxygen consumption.³³⁻³⁵ On the other hand, emphysema is linked to decreased alveolar surface area and loss of elastic recoil of the lungs.^{36, 37} In addition, the presence of large airway disease (airway thickening) may also contribute to V/Q inequality during exercise and to EID.^{38, 39} In current study, the negative influence of very low FEV₁ (GOLD III-IV)^{5, 39} and severe emphysema on oxygen saturation levels was exclusively taken into account showing that very low FEV₁ might be overlapping with the contribution of severe emphysema on the occurrence of EID. Nevertheless, correlation analysis of this study indicated that emphysema as well as airflow limitation similarly correlated to the post-walk SpO₂ ($r = -0.35$ and $r = 0.38$, respectively, $p < 0.001$; Supplementary table S3) showing that severity of emphysema can be also a major determinant of EID, especially in moderate COPD. Specifically, severe to very severe emphysema seems to have a significant association with EID rather than the lower degrees of emphysema in patients with COPD.

In this study, an important finding is that obesity was associated with the presence of EID in COPD. Previously, the association of lower SpO₂ with obesity has been reported for 871 emergency department patients and hospital workers.⁴⁰ Kapur and colleagues have also demonstrated that obesity is associated with a lower resting oxygen saturation examining a large cohort of 2252 elderly subjects from the Cardiovascular Health Study.⁴¹ In COPD, one study confirms a correlation between COPD severity with BMI and oxygen saturation using measurements of SpO₂ values at rest.⁴² To the best of our knowledge, our findings revealed for the first time a potential relationship between obesity and EID during the 6MWT in subjects with COPD. However, the effect of obesity on the occurrence of EID is likely depended from the degree of emphysema. The direct physiological mechanism underlying the effect of obesity on EID is complex; however, obesity and EID could be related to low baseline SpO₂, which can be considered as one of the major determinant of EID.^{5, 8}

In this study, we also investigated the relationship of the ADO index²⁷ and EID. Previously, the prognostic value of the ADO index regarding the risk of exacerbation and

death has been reported, however, ADO prognostic value of EID has never been investigated.^{43, 44} Multidimensional indices for the prognosis of oxygen desaturation have been proposed by Cutaia and colleagues⁴⁵ who suggested that patients with an increased BODE index²⁸ (≥ 7 points) should be evaluated for oxygen desaturation during daily activities.⁴⁵ In our analysis, the selection of the ADO index²⁷ for screening EID was based on the fact that exercise or a 6MWT is not required in order to record a score for this clinical index. Patients with high score of ADO index²⁷ (≥ 6 points) presented a greater occurrence of EID (40% vs 19%; $p < 0.001$; Table 2). This can be attributed to severe airflow limitation and/or extended emphysema which was common in those patients with high ADO scores. Subjects with EID in GOLD II-III had higher ADO scores compared to non-EID, but this was not true in GOLD IV, while ADO scores were also greater in subjects with EID across the emphysema severity (Supplementary figure S1). However, we did not include the ADO index in our multivariate logistical model as age and FEV₁, which are the main elements of the ADO index, were already included as independent determinants in this statistical analysis.

In our multivariate logistical regression model, obese subjects with an impaired FEV₁ ($\leq 44\%$), moderate to very severe emphysema, and low pre-walk SpO₂ ($\leq 93\%$) have higher odds for EID (Table 3 and Figure 4). As it was expected, the role of advanced emphysema in EID was critical and therefore emphysematous patients should be screened for EID, especially those with high ADO scores.

Strengths and weaknesses

There are certain limitations to our study. First, the 6MWT screening was performed only once at baseline, while the 2014 ERS/ATS technical standard for field walking tests suggests the conduct of two 6MWT.¹ Second, EID was defined based on the SpO₂ at the end of the test (SpO₂ post) and not the SpO₂ nadir which can lead to an underestimation of the patients who exhibit EID during the 6MWT. Even though SpO₂ post and the SpO₂ nadir for most patients with chronic respiratory disease are relatively similar during the 6MWT⁴⁶, the use of SpO₂ nadir would be optimal absent the biases. Moreover, the definition of EID did not include the magnitude of SpO₂ decrease, however, only 33 of the patients with EID (7.5%) had a SpO₂ decline less than 4%. Third, blood gas analysis and diffusion capacity (DLCO) were not available in our dataset, which could provide further physiological evidence for the presence of EID. Fourth, additional factors such as quantified air-trapping and airway wall thickness³⁹ did not evaluate for potential contribution on EID. Despite these limitations, this study contributes with important information about emphysema, airflow limitation and obesity as determinants of EID and the use ADO index²⁷ as an additional clinical tool for screening EID in patients with COPD. Our findings may help clinicians to better understand the relationship of emphysema with EID and predict more accurately the EID.

CONCLUSIONS

In summary, EID occurs in about 20% of COPD patients. Several physiological determinants including presence of advanced emphysema, severe airflow limitation, low oxygen saturation at rest, and obesity, contribute to EID which seems to be captured by high ADO index (≥ 6 points) in GOLD stage II-III. Also, patients with EID in GOLD stage II have higher odds of having moderate or worse emphysema compared those with EID in GOLD stage III-IV. Emphysematous patients with high ADO score should be monitored for EID upon embarking on a clinical exercise training program.

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SUPPLEMENTAL MATERIAL

Table S1. Cut-off points and the ROC curve parameters for the prediction of EID

Predictive index	Best cut-off	ROC curve parameters					Likelihood Ratio	
		AUC segment	Sensitivity (%)	95% CI Binomial exact	Specificity (%)	95% CI Binomial exact	LR+	LR-
Age, years	≥60	0.54	0.68	0.68-0.76	0.35	0.32-0.37	1.09	0.82
BMI, kg/m ²	≥30	0.51	0.25	0.21-0.29	0.78	0.76-0.80	1.16	0.96
FEV ₁ , % pred.	≤44	0.72	0.68	0.63-0.72	0.66	0.63-0.68	1.96	0.49
QCT-Emphysema	≥3	0.71	0.72	0.55-0.65	0.74	0.72-0.77	2.33	0.54
SpO ₂ % pre-walk	≤93	0.75	0.55	0.51-0.60	0.81	0.79-0.83	2.95	0.55
ADO index, score	≥6	0.66	0.23	0.19-0.27	0.91	0.89-0.92	2.42	0.85

The best detected threshold values and the ROC parameters for the prediction of EID in the studied COPD population. The area under the curve (AUC) represents the expected performance of the best cut-off points for the predictive indexes. *Abbreviations:* BMI: Body Mass Index; FEV₁%; Force expiratory volume in 1 sec; QCT-emphysema: Quantified by Computed-Tomography emphysema; SpO₂: Saturation of peripheral oxygen; ADO: “Age, Dyspnea, airflow Obstruction” – index.

Table S2. Logistical regressions for ADO index and EID as dependent variable

Linear logistic regression	Coefficient	Std. Error	Odds ratio (OR)	P value
ADO index	0.405	0.0394	1.50 (1.39-1.62)	<0.001
Binary logistic regression				
Univariate	Subjects	EID, n (%)	CRUDE OR	P value
ADO index <6 points	1751	328 (19)	1	<0.001
ADO index ≥6 points	245	97 (40)	2.84 (2.14-3.78)	

Linear regression (method: Enter) and binary logistic regression (Univariate) for ADO index and EID as dependent variable. *Abbreviation:* ADO: “Age, Dyspnea, airflow Obstruction” – index, EID: Exercise-induced desaturation.

Table S3. Pearson's correlations between patients' characteristics, SpO₂% and QCT-emphysema

Characteristics	SpO ₂ % post-walk		ΔSpO ₂ % [Delta values]		QCT-emphysema [degrees]	
	r (95%CI)	P value	r (95%CI)	P value	r (95%CI)	P value
Age, years	-0.07 (-0.12, 0.03)	0.002	-0.04 (-0.08, 0.01)	0.109	0.06 (0.02, 0.11)	0.008
BMI, kg/m ²	-0.04 (-0.08, 0.01)	0.098	0.02 (-0.02, 0.06)	0.020	-0.36 (-0.40,-0.32)	<0.001
FFMI, kg/m ²	0.01 (-0.03, 0.06)	0.517	0.07 (0.02, 0.11)	0.066	-0.35 (-0.38, -0.30)	<0.001
FEV ₁ , % pred.	0.38 (0.34, 0.42)	<0.001	0.32 (0.28, 0.35)	<0.001	-0.41 (-0.45,-0.38)	<0.001
FVC, % pred.	0.14 (0.10, 0.19)	<0.001	0.07 (0.03, 0.12)	0.001	-0.03 (-0.01, 0.08)	0.173
SpO ₂ pre, %	0.50 (0.47, 0.53)	<0.001	0.09 (0.04, 0.13)	0.001	-0.06 (-0.11,-0.02)	0.005
SpO ₂ post, %	1 -	-	0.89 (0.88, 0.89)	<0.001	-0.35 (-0.39,-0.31)	<0.001
ΔSpO ₂ , %	0.89 (0.88, 0.89)	<0.001	1 -	-	-0.38 (-0.41, -0.34)	<0.001
QCT-emphysema	-0.35 (-0.39,-0.31)	<0.001	-0.38 (-0.41,-0.34)	<0.001	1 -	-
6MWD, m	0.17 (0.12, 0.21)	<0.001	0.08 (0.04, 0.13)	0.001	-0.16 (-0.20,-0.11)	<0.001
6MWD, %pred.	0.15 (0.11, 0.19)	<0.001	0.08 (0.04, 0.13)	0.001	-0.20 (-0.24,0.15)	<0.001
ADO index	-0.28 (-0.33, -0.24)	<0.001	-0.20 (-0.25, -0.16)	<0.001	0.28 (0.23,0.32)	<0.001
BODE index	-0.30 (-0.34, -0.26)	<0.001	-0.22 (-0.26, -0.18)	<0.001	0.36 (0.31, 0.39)	<0.001

Abbreviations: BMI: Body Mass Index; FFMI: Fat free mass index; FEV₁%, Force expiratory volume in 1 sec; FVC: Force vital capacity; SpO₂: Saturation of peripheral oxygen; QCT-emphysema: Quantified by Computed-Tomography emphysema; 6MWD: Six-minute walk distance; ADO: "Age, Dyspnea, and airflow Obstruction" – index; BODE: "Body mass index, airflow Obstruction, Dyspnea and Exercise capacity" – index.

Figure S1. Comparisons of the ADO index between EID and non-EID across the GOLD stages and the degrees of QCT-emphysema

Abbreviations: ADO: "Age, Dyspnea, and airflow Obstruction" – index; EID: Exercise-induced desaturation. Asterisks denote significant difference (* $p < 0.05$; ** $p < 0.001$).

Chapter 6

Exercise-induced oxygen desaturation in COPD patients without resting hypoxemia

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Respiratory Physiology & Neurobiology 2014; 190:40–6

ABSTRACT

Background

Exercise-induced oxygen desaturation (EID) is associated with increased risk of mortality in chronic obstructive pulmonary disease (COPD). Several screening tests have been proposed to predict EID, including FEV₁, DLCO and baseline-SpO₂. We aimed to validate a proposed cut-off of baseline-SpO₂ ≤95% as simple screening procedure to predict EID during six-minute walk test (6MWT). In addition, we studied the prevalence and characteristics of patients exhibited EID to SpO₂nadir ≤88%.

Methods

402 non-hypoxemic COPD patients performed 6MWT. Sensitivity and specificity of baseline SpO₂ ≤95% as a cut-off to predict EID and determinants of EID were investigated.

Results

158 patients (39%) exhibited EID. The sensitivity of baseline-SpO₂ ≤95% to predict EID was 81.0%, specificity 49.2%, positive and negative predictive values were 50.8% and 80.0%, respectively. In a multivariate model, DLCO <50%, FEV₁ <45%, PaO₂ <10 kPa, baseline-SpO₂ <95%, and female sex were the strongest determinants of EID.

Conclusions

Baseline oxygen saturation solely is inaccurate to predict EID. A combination of clinical characteristics (DLCO, FEV₁, PaO₂, baseline-SpO₂, sex) increases the odds for EID in COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a highly prevalent chronic respiratory disease, characterized by partially irreversible airflow limitation.¹ Patients with COPD frequently experience a significant fall of oxygen saturation during exercise that is attributed to the imbalance between oxygen delivery and demand during exercise.² Exercise-induced oxygen desaturation (EID) has been related to reduced exercise performance, a faster decline in forced expiratory volume in the first second (FEV₁), and increased mortality in patients with COPD.^{3, 4} Moreover, ambulatory oxygen supplementation reduces exercise-induced breathlessness and oxygen desaturation; and improves exercise performance in patients with COPD, irrespective of resting arterial oxygen pressure.^{5, 6} In fact, oxygen saturation $\leq 88\%$ with physical activity is currently an indication for reimbursement of continuous oxygen use by Centres for Medicare & Medicaid Services in COPD patients with resting non-hypoxemia.⁷ Therefore, identifying non-hypoxemic COPD patients with EID seems clinically relevant, but has not been studied previously.

A 6-minute walk test (6MWT) has been suggested as preferred measure to identify COPD patients with EID.⁸ Indeed, EID occurs frequently during 6MWT in patients with COPD.⁹ Moreover, the 6MWT is more sensitive for identifying EID in patients with COPD compared to cardiopulmonary exercise test.¹⁰

Even though the 6MWT is a relatively simple test¹¹, several screening tests have been proposed to predict which COPD patients will (not) have EID, including FEV₁, diffusion capacity for carbon monoxide (DLCO) and/or baseline standard pulse oxymetry (SpO₂).^{8, 12, 13} Indeed, Knower and colleagues suggested that a resting SpO₂ of 95% or less is a good screening test for the detection of EID during a 6MWT (sensitivity: 73%; negative predictive value: 84%), especially in patients with a DLCO $>36\%$ of the predicted value (sensitivity: 100%; negative predictive value: 100%).⁸ To date, this readily available office screening procedure has not been validated in a large cohort of non-hypoxemic patients with COPD.

Herein, we report a prospective analysis in a large clinical cohort of patients with COPD undergoing baseline evaluation for pulmonary rehabilitation.¹⁴ The aim of the study was two-fold: 1) to validate the cut off of resting SpO₂ of 95% or less as a simple screening procedure to predict EID during a 6MWT in patients with COPD as proposed by Knower and colleagues⁸; and 2) to study the characteristics of non-hypoxemic patients with COPD who have a nadir SpO₂ $\leq 88\%$ during the 6MWT. A priori, resting SpO₂ was expected not to be accurate enough to identify non-hypoxemic patients with EID.

METHODS

Design and participants

Patients with COPD¹⁵ referred for a comprehensive pulmonary rehabilitation program at CIRO+ or its pulmonary rehabilitation network were recruited prospectively.¹⁴ Age between 45 to 85 years was set as inclusion criterion. The use of long-term oxygen therapy and/or treatment or hospitalisation for an acute exacerbation in the last 4 weeks before the study and resting hypoxemia were pre-defined exclusion criteria. The medical ethical committee of the Maastricht University Medical Centre (METC 11-4-042) approved this study and all subjects provided written informed consent for the use of data that were obtained during the routine pre-rehabilitation assessment. Initially, a power calculation was performed, suggesting to include 402 patients (power: 90%; level of significance: 0.01; please see supplemental material for all details). A completed dataset of 402 patients was derived from a total number of 438 eligible patients according to the study criteria after the exclusion of those who were unable reliably to perform spirometric maneuvers of FEV₁, DLCO or the 6MWT.

Assessments

As part of a 3-day pre-rehabilitation assessment¹⁴, patients underwent a physical examination by a chest physician (including assessment of body weight, height and medical history), post-bronchodilator spirometry, DLCO, resting arterial blood gas analysis and 6MWTs according to international recommendations.¹⁶⁻¹⁹ Body mass index (BMI, kg/m²) was calculated as weight divided by squared height. Overweight was defined as a BMI of 25.0–29.9 kg/m² and obesity as a BMI of 30.0 kg/m² or more. In addition, fat mass (FM) and fat-free mass index (FFMI) (using dual-energy X-ray absorptiometry (DEXA))²⁰ and symptoms of dyspnea (using the Medical Research Council (MRC) dyspnea scale) were assessed.

Six-minute walk test

Two 6MWTs were performed according to the American Thoracic Society guidelines¹⁹, including a practice walk.²¹ Participants were asked to walk in a 125m triangular track during 6MWTs while SpO₂ and heart rate were continuously recorded by the use of pulse oxymeter (Nonin-2500). Both 6MWTs were conducted by healthcare professionals who instructed and supervised the patients while pulse oxymetry during walking tests was monitored for possible artefacts. Patients were encouraged every minute of the 6MWTs using two phrases “You are doing well” or “Keep up the good work”. Symptom scores for breathlessness and perceived exertion were recorded using Borg scales.²² The 6MWT with the longest walk distance was used for further analyses.

Acquisition of baseline SpO₂ was obtained in sitting position while patients were silent and relaxed. EID was defined as a nadir SpO₂ ≤88%. A nadir SpO₂ ≤88% is current indications for reimbursement for continuous oxygen use by the Centres of Medicare and Medicaid Services.⁷

Statistical analyses

All statistical analyses were carried out using Sigmaplot 11, GraphPad Prism 4.03 and SPSS 19.0. Data are presented as mean and standard deviation or proportion, as appropriate. For aim 1, patients were stratified by baseline SpO₂ ≤95% (yes/no) and the presence of EID (yes/no). Differences between patients with and without EID were compared using an unpaired T-test. In addition, specificity, sensitivity, the positive predictive value and the negative predictive value for baseline SpO₂ ≤95% were calculated with standard methods. For aim 2, patients with and without EID were compared using an unpaired T-test. Receiver operating characteristic (ROC) curves were used to determine the threshold values with the best sensitivity and specificity to predict EID, best being defined as the point on the ROC curve with the shortest distance from the upper left corner (0,1). Area under the curve (AUC) was calculated by the trapezoidal rule. In addition, univariate and multivariate logistic regression analyses were used to estimate odds ratios for EID. *A priori*, a two-sided level of significance was set at $p \leq 0.05$.

RESULTS

Patient characteristics

A total of 402 patients (age range: 45–85; mean: 64.3 years) with COPD (174 women) were included in this study. Patients generally had moderate to very severe COPD, an impaired lung diffusion capacity, a normal BMI, normal resting arterial oxygen and carbon dioxide tension, severe symptoms of dyspnea at rest, and a poor functional exercise performance. 158 patients (59%) had EID. Desaturators had a lower BMI, a higher degree of airflow limitation, worse DLCO, more static lung hyperinflation, and worse resting arterial blood gases compared to non-desaturators (Table 1).

Validation of algorithm of Knower et al. (Aim 1)

252 patients (63%) had a baseline SpO₂ of 95% or lower, of which 128 patients (51%) had EID. In the group of patients with a baseline SpO₂ of 96% or higher, 30 patients (20%) had EID. Desaturators had a higher degree of airflow limitation, worse DLCO, and more static lung hyperinflation compared to non-desaturators, irrespective of the baseline SpO₂

(Table 2). Sensitivity of the baseline SpO₂ of 95% or lower to predict EID was 81.0%; specificity was 49.2%; positive predictive value was 50.8%; and negative predictive value was 80.0%. These numbers hardly changed after excluding all COPD patients with a DLCO of 36% of predicted or less, as was done by Knower and colleagues⁸ (Table 3).

Table 1. Demographics of COPD patients and the prevalence of EID during 6MWT

Characteristics	All Patients (n=402)	Non-Desaturators (n=244) (61%)	Desaturators (n=158) (39%)
Women, #, (%)	174(43)	95(39)	79(50)
Age, years	64.3±8.0	64.1±8.3	64.6±7.6
BMI, kg/m ²	25.6±5.5	26.2±5.7	24.8±5.2*
FM, kg	23.3±10.5	23.8±11.0	22.5±9.8
FFMI, kg/m ²	17.2±2.4	17.5±2.3	16.6±2.4*
FEV ₁ , L	1.4±0.6	1.6±0.6	1.1±0.4*
FEV ₁ , %pred.	51.5±18.8	58.1±18.3	41.5±14.5*
FEV ₁ /FVC, %	40.2±12.1	44.5±11.7	33.6±9.5*
FVC, %pred.	99.3±21.6	101.2±21.5	96.3±21.5*
FRC, %pred.	143.8±34.1	134.7±31.6	157.9±33.0*
TLC, %pred.	115.8±17.1	112.8±16.4	120.4±17.2*
DLCO, %pred.	53.9±19.7	60.4±19.9	43.7±14.4*
PaO ₂ , kPa	10.1±1.6	10.5±1.5	9.4±1.4*
PaCO ₂ , kPa	5.0±0.6	4.9±0.5	5.2±0.7*
SaO ₂ , %	95.5±2.0	96.1±1.6	94.7±2.2
SpO ₂ , %	94.6±2.4	95.4±1.9	93.3±2.5*
6MWD, m	453.5±103.2	458.8±104.9	445.3±100.1
6MWD, %pred.	70.8±16.4	71.8±15.7	69.3±17.2
MRC, index	3.2±0.9	3.2±0.9	3.3±0.9

Abbreviations: BMI: Body Mass Index, FM: Fat Mass, FFMI: Fat-Free Mass Index, FEV₁: Force Expiratory Volume at 1 sec, FVC: Force Vital Capacity, FRC: Functional Residual Capacity, TLC: Total Lung Capacity, DLCO: Diffusing capacity for carbon monoxide, PO₂: Partial pressure of Oxygen, PCO₂: Partial pressure of Carbon-dioxide, SaO₂: Arterial oxygen saturation, SpO₂: Saturation of peripheral Oxygen, 6MWD: Six-minute walk distance, MRC: Medical Research Council dyspnea scale. Data is represented as means ±SD and percentages (%). Data are mean ±SD unless specified otherwise. Asterisk denotes significant differences for patients exhibited EID (Desaturators) (P ≤ 0.05).

Table 2. Characteristics of COPD patients stratified by baseline saturation and grouped based on the presence of EID during 6MWT

Characteristics	Baseline SpO ₂ ≥96% (n=150)		Baseline SpO ₂ ≤95% (n=252)	
	Non-Desaturators (n=120) (80%)	Desaturators (n=30) (20%)	Non-Desaturators (n=124) (49%)	Desaturators (n=128) (51%)
Women, #, (%)	50(42)	22(73)	45(36)	57(45)
Age, years	62.8±8.6	61.8±8.1	65.3±7.8	65.3±7.4
BMI, kg/m ²	25.4±5.5	23.0±4.3*	27.0±5.7	25.2±5.4*
FM, kg	21.9±10.1	20.6±8.9	25.5±11.5	22.9±9.9
FFMI, kg/m ²	17.3±2.3	15.7±2.2*	17.8±2.3	16.8±2.4*
FEV ₁ , L	1.7±0.6	1.1±0.4	1.5±0.6	1.1±0.4*
FEV ₁ , %pred.	60.1±18.9	41.9±14.6*	56.1±17.6	41.4±14.6*
FEV ₁ /FVC, %	45.5±11.8	34.6±9.9*	43.5±11.6	33.4±9.4*
FVC, %pred.	102.9±20.7	95.2±18.3	99.7±22.3	96.6±22.2
FRC, %pred.	133.4±31.0	163.1±27.9*	135.8±32.3	156.7±34.1*
TLC, %pred.	113.1±16.1	125.0±15.9*	112.6±16.8	119.3±17.4*
DLCO, %pred.	60.8±22.2	42.2±15.5*	60.2±17.5	44.0±14.2*
PaO ₂ , kPa	10.9±1.4	10.1±1.4*	10.1±1.6	9.2±1.4*
PaCO ₂ , kPa	4.8±0.5	5.0±0.6	5.0±0.5	5.2±0.7*
SpO ₂ , %	97.0±0.8	96.4±0.7*	93.8±1.3	92.6±2.1*
6MWD, m	468.5±110.3	488.9±84.5	449.4±99.0	435.1±101.1
6MWD, %pred.	72.6±16.9	76.1±13.1	71.0±14.5	67.6±17.7
MRC, index	3.1±1.0	2.9±0.9	3.2±0.9	3.4±0.9

Abbreviations: Please see legend Table 1. The studied COPD patients categorized by baseline saturation of SpO₂ ≥96% and SpO₂ ≤95% and grouped based on the presence of EID to nadir-SpO₂ ≤88%. Data are mean ±SD unless specified otherwise. Asterisk denotes significant differences for patients exhibited EID (Desaturators) (P ≤ 0.05).

Table 3. Sensitivity and specificity of the parameters tested as predictors of EID in COPD

Predictive index	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Baseline Saturation 95% (Total Sample)	81.0	49.2	50.8	80.0
Baseline Saturation 95% (Excluding DLCO ≤36%)	84.5	48.0	42.4	87.2

A cut-off of baseline saturation of 95% presented low sensitivity to predict EID to nadir-SpO₂ ≤88% in the total sample of patients with COPD and after the exclusion those with DLCO ≤36%.

Exercise-induced nadir SpO₂ ≤88% (Aim 2)

Using ROC curves, the threshold values with the best specificity and sensitivity to predict EID were determined for age, BMI, FEV₁, FRC, DLCO, PaO₂, PaCO₂, and baseline SpO₂ (Table 4). These threshold values were used in an univariate logistical regression model, which revealed that patients aged 60 years or older; with an overweight or

obese BMI; with FEV₁ <45% predicted; with FRC >135 %predicted; with a DLCO <50 %predicted; or with a PaO₂ <10.0 kPa; PaCO₂ ≥5.2 kPa and baseline SpO₂ of 95% or lower have higher odds for EID (Table 5). A multivariate logistical regression model, however, showed that only FEV₁, DLCO, PaO₂ and SpO₂ remained significant. A combination of these critical characteristics revealed that the 95% of patients with FEV₁ <45 %predicted and DLCO <50 %predicted with resting PaO₂ <10 kPa and baseline SpO₂ <95 %predicted, exhibited EID (Figure 1). Moreover, in the multivariate logistical regression model the gender of patients was revealed as an important factor for the prevalence of EID indicating a higher proportion of women who exhibited EID (Table 5 and Figure 1).

Table 4. Cut-off points and the ROC curve parameters for the prediction of EID

ROC curve parameters						
Predictive index	Best cut-off	AUC segment	Sensitivity (%)	Specificity (%)	Likelihood Ratio LR+	LR-
Age, years	59.5	0.52	0.76	0.35	1.17	0.69
BMI, kg/m ²	25.8	0.58	0.68	0.50	1.37	0.63
Baseline SpO ₂ , %	94.5	0.74	0.65	0.70	2.13	0.51
PaO ₂ , kPa	9.95	0.74	0.73	0.65	2.11	0.41
PaCO ₂ , kPa	5.25	0.61	0.42	0.78	1.92	0.74
DLCO, %pred.	50.5	0.78	0.75	0.67	2.30	0.37
FEV ₁ , %pred.	44.5	0.76	0.67	0.76	2.77	0.43
FRC, %pred.	135.5	0.69	0.75	0.54	1.63	0.46

Abbreviations: BMI: Body Mass Index, SpO₂: Saturation of peripheral Oxygen, PO₂: Partial pressure of Oxygen, PCO₂: Partial pressure of Carbon-dioxide, DLCO: Diffusing capacity for carbon monoxide, FEV₁: Force Expiratory Volume at 1 sec, FRC: Functional Residual Capacity. The best detected cut-off points and the ROC parameters for the prediction of EID in the studied COPD population. The area under the curve (AUC) represents the expected performance of the best cut-off points for the predictive indexes.

Table 5. Logistic regression: determinants of EID in COPD patients

Determinants	Subjects n	EID n, (%)	Crude OR (95%CI)	P value	Adjust OR (95%CI)	P value
Age						
<60 years	123	38 (31)	1		1	
≥60 years	279	120 (43)	1.69 (1.08-2.65)	0.02	1.67 (0.91-3.06)	0.10
Sex						
Male	228	79 (35)	1		1	
Female	174	70 (45)	1.57 (1.05-2.35)	0.03	2.26 (1.30-3.92)	0.004
BMI						
<25 kg/m ²	205	95 (46)	1		1	
≥25 kg/m ²	197	63 (32)	0.54 (0.36-0.82)	0.003	1.13 (0.62-2.09)	0.69
FEV ₁						
≥45 %pred.	237	52 (22)	1		1	
<45 %pred.	165	106 (64)	6.39 (4.11-9.95)	<0.001	4.92 (2.58-9.41)	<0.001
FRC						
≤135 %pred.	170	39 (23)	1		1	
>135 %pred.	232	119 (51)	3.54 (2.28-5.50)	<0.001	1.05 (0.54-2.07)	0.88
DLCO						
≥50 %pred.	211	43 (20)	1		1	
<50 %pred.	191	115 (60)	5.91 (3.80-9.21)	<0.001	5.45 (2.96-10.01)	<0.001
PaO ₂						
≥10.0 kPa	201	42 (21)	1		1	
<10.0 kPa	201	116 (58)	5.17 (3.33-8.03)	<0.001	4.89 (2.75-8.69)	<0.001
PaCO ₂						
<5.2 kPa	251	82 (33)	1		1	
≥5.2 kPa	151	76 (50)	2.09 (1.38-3.16)	<0.001	1.49 (0.84-2.63)	0.17
SpO ₂						
≥96%	150	30 (20)	1		1	
<95%	252	128 (51)	4.13 (2.58-6.61)	<0.001	2.25 (1.20-4.23)	0.01

Abbreviations: Please see legend of Table 4. Logistic regression analysis of the determinants for EID: adjusted odds ratios for EID according to gender and physiological characteristics.

A posteriori analyses

Characteristics of desaturators and non-desaturators were studied after stratification for gender (Supplementary table S1). This *posteriori* analysis shows again that desaturators had a lower BMI, a higher degree of airflow limitation, worse DLCO, more static lung hyperinflation, and worse resting arterial blood gases compared to non-desaturators, irrespective of sex.

Figure 1. A combination of certain lung function and baseline oxygen saturation characteristics for the prediction of EID

(A) A combination of certain lung function and baseline oxygen saturation characteristics for the prediction of EID revealed that the highest proportion of patients (95%) who exhibit EID (nadir-SpO₂ ≤88%) are characterized by DLCO <50 %predicted and FEV₁ <45 %predicted with resting values of PaO₂ <10 kPa and SpO₂ <95%.

(B) A combination of certain lung function and baseline oxygen saturation characteristics for the prediction of EID in males. **(C)** A combination of certain lung function and baseline oxygen saturation characteristics for the prediction of EID in females.

DISCUSSION

The current study has two main findings: 1) Baseline oxygen saturation threshold of 95% or lower measured by standard pulse oxymetry fails to identify accurately EID in 402 non-hypoxemic patients with COPD; and 2) Nadir $\text{SpO}_2 \leq 88\%$ occurs frequently during a 6MWT in non-hypoxemic patients with COPD, in particular in those patients with a $\text{FEV}_1 < 45\%$ predicted, $\text{DLCO} < 50\%$ predicted, resting $\text{PaO}_2 < 10\text{kPa}$, baseline $\text{SpO}_2 < 95\%$, and in women.

In 2001, Knower and colleagues reported that baseline oxygen saturation of 95% or lower is a valid and readily available office screening procedure to identify COPD patients without EID during 6MWT, especially in patients with DLCO greater than 36 %predicted.⁸ The analyses using a dataset of 402 non-hypoxemic patients with COPD did not corroborate the study of Knower and colleagues. Based on the power calculation (see supplemental materials for all details), a type II error can be ruled out, and, in turn, it seems fair to state that a baseline pulse oximetry threshold of 95% or lower cannot be used solely in daily clinical practice to identify accurately patients with COPD without EID.

Patients with COPD exhibit EID due to the imbalance between oxygen supply and demand during exercise, which can be attributed to respiratory, hemodynamic and peripheral muscle abnormalities or a combination thereof.²³ Impaired lung mechanical characteristics and gas exchange inefficiency are prominent in COPD and contribute to increased work of breathing. Impaired hemodynamics, lower-limb muscle dysfunction and weakness^{24, 25} in the presence of premature lactic acidosis may negatively impact the dynamic matching of oxygen delivery and utilization.²⁶ Arterial hypoxemia and excessive respiratory muscle work are related to insufficient oxygen transport in the working muscles enhancing EID.²⁷

In the present study, the six-minute walk test (6MWT) due to the simplicity of performance and the high responsiveness to arterial oxygen desaturation is the preferred exercise test to investigate the EID. It has been shown that 6MWT is more sensitive to arterial oxygen desaturation than the cardiopulmonary exercise test on cycle ergometer. For example, Poulain and colleagues showed that ~28% of COPD patients with broad range of FEV_1 (29 – 81 %predicted) desaturate on 6MWT while this was not shown on cardiopulmonary exercise test.¹⁰ However, authors of this study and following relevant studies did not provide an explanation of this phenomenon^{2, 28, 29}, nor did direct arterial blood gas measurements.³⁰ On the other hand, Cockcroft and colleagues showed that a significantly lower PaO_2 was present with matching metabolic rates on self-paced walking exercise on treadmill and on the cycle ergometer, and that this difference was accountable for a lower lactate threshold in cycling exercise.³¹ Later, Hsia and colleagues confirmed that the higher minute ventilation found during cycle exercise at the same equivalent work rate (and oxygen uptake) was in close association

with less EID.³² These observations provided a reasonable pathophysiological mechanism for EID.

In the current study, we also focussed on spirometry, lung diffusion capacity, pulse oximetry, and arterial blood gases, which may be available for many patients with COPD from outpatient routine follow ups. As expected, a lower FEV₁, DLCO, PaO₂ and baseline SpO₂ increased the odds of having EID (Table 4, and Figure 1). Previously, FEV₁ and DLCO have already been suggested as possible strong predictors of EID in patients with COPD.^{8, 12, 13} For example, Owens and colleagues¹³ reported that DLCO of 55% or higher was 100% specific in identifying patients with COPD without EID, as compared with an 82% specificity for an FEV₁ above 55% of predicted. The sensitivity of the diffusing capacity with this cutoff point was 68%, as compared with 46% for the FEV₁. Kelly and colleagues¹² showed that patients with COPD or interstitial lung disease with DLCO of 60% of predicted or higher never experienced EID.

Recently, Van Gestel and colleagues³³ found that a FEV₁ of 50 %predicted or lower was the optimal cut-off value to identify COPD patients with EID. Moreover, in a recent study of Crisafulli and colleagues³⁴ a multivariate regression between anthropometric and functional variables and SpO₂ nadir as the dependent variable indicated that resting saturation of arterial oxygen - SO₂ ($r = 0.65$), PaO₂ ($r = 0.50$) and FEV₁ %predicted ($r = 0.41$) significantly predict EID in COPD. Indeed, a walking desaturation score (WDS) derived from these clinical variables classified and accurately predicted the risk of EID in patients with COPD. In accordance with these findings, the present study indicated FEV₁ <45 %predicted in association with resting SpO₂ <95% and PaO₂ <10.0 kPa as determinants of EID. However, the application of WDS in the current study sample classified 77.5% of the non-desaturating patients as “low risk”, 13.9% as “high risk” and 8.6% as “very high risk” for EID. Thirty-eight percent of the patients with EID were classified as “low risk”, 27.2% as “high risk”, and 34.8% as “very high risk” for EID. In present study the influence of a low DLCO <50 %predicted in the prevalence of EID was crucial and the fact that DLCO is not included in the WDS variables may be possibly attributed for the small percentage of high risk group classification of the desaturators of this study.

Interestingly, EID appears also to be influenced by gender. The multivariate logistic regression model of this study revealed that women have higher odds to exhibit EID compared to men (Table 5 and Figure 1). The current analyses cannot explain these findings, which seems rather hypothesis-generating than definitive.

There is no uniform definition of EID in patients with COPD or standardized exercise protocol to elicit decreases in oxygen levels in individuals with COPD.^{8, 13, 29, 33} Nadir SpO₂ ≤88% was used in the current study, as the clinical relevance of a decrease of 4% or more, highly depends on the baseline SpO₂. A 6MWT was used to induce oxygen desaturation, as this has been shown to be able to identify EID in patients with COPD.¹⁰ Oxygen saturation was monitored continuously during the 6MWT for the detection of

nadir $\text{SpO}_2 \leq 88\%$, as SpO_2 at the end of the 6MWT did not accurately identify desaturation in 21% of patients with COPD.³⁵

CONCLUSIONS

In summary, EID cannot be predicted accurately by just using resting SpO_2 . Moreover, the current analysis revealed that 39% of the 402 non-hypoxemic patients with COPD had nadir $\text{SpO}_2 \leq 88\%$, which may be suitable candidates for ambulatory oxygen therapy. Obviously, future trials have to corroborate the current findings, in particular the observed influence of gender.

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SUPPLEMENTAL MATERIAL

Table S1. Characteristics of COPD patients stratified by gender and grouped based on the presence of EID

Characteristics	MALES (n=228)				FEMALES (n=174)			
	Non-Desaturators (n=149) (65%)		Desaturators (n=79) (35%)		Non-Desaturators (n=95) (55%)		Desaturators (n=79) (45%)	
Age, years	65.1	±8.0	66.1	±7.8	62.5	±8.5	63.1	±7.3
BMI, kg/m ²	26.1	±5.3	25.3	±5.6	26.3	±6.2	24.3	±4.8*
FM, kg	22.1	±10.2	20.9	±9.9	26.4	±11.6	24.0	±9.4
FFMI, kg/m ²	18.5	±1.9	17.9	±2.2	16.0	±2.0	15.3	±1.7
FEV ₁ , L	1.7	±0.7	1.3	±0.5*	1.4	±0.4	0.9	±0.3*
FEV ₁ , %pred.	55.9	±19.0	40.9	±14.7*	61.5	±16.8	42.0	±14.5*
FEV ₁ /FVC, %	42.7	±12.4	33.0	±10.2*	47.1	±10.1	34.3	±8.7*
FVC, %pred.	97.8	±20.8	93.2	±22.0	106.6	±21.7	99.4	±20.6
FRC, % pred.	134.5	±33.9	153.2	±33.0*	134.8	±27.9	162.6	±32.5*
TLC, % pred.	111.2	±16.3	115.6	±16.3*	115.5	±16.3	125.2	±16.9*
DLCO, % pred.	61.3	±22.0	45.0	±15.4*	59.2	±16.2	42.4	±13.4*
PaO ₂ , kPa	10.6	±1.6	9.2	±1.3*	10.4	±1.5	9.5	±1.6*
PaCO ₂ , kPa	4.9	±0.5	5.1	±0.7	4.9	±0.5	5.3	±0.7*
SpO ₂ , %	95.2	±1.9	92.8	±2.3*	95.6	±1.9	93.7	±2.5*
6MWD, m	464.1	±108.7	454.2	±104.7	450.5	±98.7	436.4	±95.2
6MWD, %pred.	69.0	±15.6	67.9	±16.3	76.1	±15.0	70.5	±18.1
MRC, index	3.2	±0.9	3.3	±1.0	3.2	±0.9	3.3	±0.9

Total population of non-hypoxemic patients with COPD categorized by gender and grouped based on the presence of EID to nadir-SpO₂ ≤88%. Data are mean ±SD unless specified otherwise. Asterisk denotes significant differences ($P \leq 0.05$) between groups of patients with and without EID. *Abbreviations:* BMI: Body Mass Index, FM: Fat Mass, FFMI: Fat-Free Mass Index, FEV₁: Force Expiratory Volume at 1 sec, FVC: Force Vital Capacity, FRC: Functional Residual Capacity, TLC: Total Lung Capacity, DLCO: Diffusing capacity for carbon monoxide, PaO₂: Partial pressure of arterial oxygen, PaCO₂: Partial pressure of carbon-dioxide, SpO₂: Saturation of peripheral Oxygen, 6MWD: Six-minute walk distance, MRC: Medical Research Council dyspnea scale.

Power Analysis Calculation

Sample size calculation

Based on the paper of Knowler and colleagues (Knowler et al. 2001) with a power of 90% and a level of significance of 0.01, we estimated the following sample size:

$$N = [Z_{1-\beta} \sqrt{(p_1(1-p_1) + p_2(1-p_2))} + Z_{1-\alpha/2} \sqrt{p_{gem}(1-p_{gem})}]^2 / d^2$$

N = sample size per Group

p_1 = proportion group 1

p_2 = proportion group 2

p_{gem} = mean of both groups

d = difference in group proportions

$Z_{1-\alpha/2}$ = standard normal deviate for two tailed test based on alpha level

$Z_{1-\beta}$ = standard normal deviate for one tailed test based on beta level

$$N = [1.282 \sqrt{0.15(1-0.15) + 0.25(1-0.25)} + 2.326 \sqrt{0.10(1-0.10)}]^2 / (0.25-0.15)^2$$

N = 201 patients per group; a total sample size of N= 402

Chapter 7

Transcutaneous carbon-dioxide partial pressure trends during six-minute walk test in patients with very severe COPD

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Respiratory Physiology & Neurobiology 2016; 233:52–59

ABSTRACT

Background

Transcutaneous carbon-dioxide partial-pressure ($T_c\text{PCO}_2$) can be reliably measured and may be of clinical relevance in COPD. Changes in $T_c\text{PCO}_2$ and exercise-induced hypercapnia (EIH) during six minute walk test (6MWT) need further investigation. We aimed (1) to define patterns of $T_c\text{PCO}_2$ trends during 6MWT and (2) to study determinants of CO_2 -retention and EIH.

Methods

Sixty-two COPD patients (age: 63 ± 8 years, FEV_1 : 33 ± 10 %predicted) were recruited and $T_c\text{PCO}_2$ was recorded by SenTec digital-monitoring-system during 6MWT.

Results

Half of patients (50%) exhibited CO_2 -retention ($T_c\text{PCO}_2$ [Δ] >4 mmHg); 26% preserved and 24% reduced $T_c\text{PCO}_2$. Nineteen (31%) patients presented EIH ($T_c\text{PCO}_2 >45$ mmHg). EIH was associated to higher baseline- $P_c\text{CO}_2$, worse FEV_1 , lower inspiratory-pressures, underweight/normal BMI, and pre-walk dyspnea. Stronger determinants of CO_2 -retention were FEV_1 and pre-walk dyspnea, whereas baseline- $P_c\text{CO}_2$ and pre-walk dyspnea better predict EIH.

Conclusions

PCO_2 response to 6MWT is highly heterogeneous; however, very low FEV_1 and elevated baseline- $P_c\text{CO}_2$ together with pre-walk dyspnea increase the risk for CO_2 -retention and EIH. Overweight BMI seems to carry a protective effect against EIH in very severe COPD.

INTRODUCTION

Six-minute walk test (6MWT) is widely used to assess functional exercise performance and prognosis in patients with chronic obstructive pulmonary disease (COPD).¹⁻³ Moreover, the 6MWT is used as field test to assess exercise-induced oxygen desaturation^{4, 5} which has an additional prognostic value besides the 6-min walk distance (6MWD).^{6, 7} Also, transcutaneous carbon-dioxide pressure (T_cPCO₂) can be measured reliably as an indicator of ventilatory adequacy and may be of clinical relevance in patients with COPD.⁸⁻¹¹

Resting PCO₂ is highly variable amongst patients with COPD.¹² Respiratory mechanics impairments, reduced ventilation (hypoventilation), hyperinflation, and reduced gas exchange capabilities (ventilation / perfusion mismatch) may account for CO₂ retention and often lead to hypercapnia.¹³⁻¹⁶ During exercise, a substantial CO₂ retention (T_cPCO₂ [Δ] >4mmHg from baseline) may also occur frequently in patients with COPD and can result in exercise-induced hypercapnia (EIH; defined by an elevation of PCO₂ levels greater than 45 mmHg / 6.0 kpa).^{15, 16}

Indeed, exercise-induced changes in PCO₂ are highly dependent upon several pathophysiological mechanisms in COPD.^{16, 17} Even though many COPD patients are normocapnic at rest, some patients may exhibit substantial CO₂ retention during exercise, which often results in EIH and it is associated with a greater tendency toward respiratory failure.^{18, 19} In contrast, other patients with COPD may significantly reduce PCO₂ on exertion even to hypocapnic levels and be at risk of acute respiratory alkalosis due to hyperventilation.²⁰ Indeed, levels of PCO₂ have been reported as independent prognostic factor with a U-shaped association with mortality in COPD.²¹

A decline of PCO₂ levels is less common than CO₂ retention and the occurrence of EIH in very severe COPD. Although, EIH has been proposed as an indication for the progress of disease severity regarding resting hypercapnia¹⁸ and also determines a condition of acute respiratory failure, which is related to high risk for death in patients with advanced COPD²², the current literature provides only scarce information on CO₂ responses at cycle ergometer test^{16, 23, 24} or 6MWT.²⁵ To date, the determinants of CO₂ retention and EIH have never been investigated using continuous recording of T_cPCO₂ during the 6MWT in patients with very severe COPD.

Herein, we report an analysis of CO₂ response to exercise during the 6MWT in patients with very severe COPD. We aimed to (1) to define patterns of T_cPCO₂ trends during the 6MWT and (2) to study the determinants of CO₂ retention and the occurrence of EIH during walking in very severe COPD.

METHODS

Design and participants

Patients aged 30 to 75 years with very severe COPD (GOLD IV), with and without the presence of chronic respiratory failure, were recruited prospectively. Exclusion criteria were resting capillary $P_c\text{CO}_2 > 55$ mmHg, use of non-invasive ventilation, and/or acute exacerbation in the last 4 weeks. The Bavarian ethical committee approved this study and all subjects provided written informed consent (Germany: Ethic committee approval number: 12007). Sixty-two ($n=62$) out of 75 patients with very severe COPD, participating in an inpatient Pulmonary Rehabilitation at Schoen Klinik Berchtesgadener Land (Schoenau am Koenigssee, Germany) were used for data analyses. Data from 13 patients were incomplete, and, in turn, not used for further analyses.

Assessments

All patients underwent physical examination, anthropometric and lung function measurements. Capillary blood gases (CBG) at rest were obtained under normal ambient condition and a complete medical history was recorded. Lung function was assessed by post-bronchodilator spirometry, plethysmographic lung volumes at rest (i.e. tidal volume [Vt], maximal inspiratory mouth pressure [Pi max], expired ventilation [VE]), measures of carbon monoxide diffusing capacity (DLCO), and CBG analysis ($P_c\text{O}_2$, $P_c\text{CO}_2$). Body mass index (BMI, kg/m^2) was defined as weight divided by squared height, and fat mass index (FMI, kg/m^2) was calculated as the fat mass (kg) divided by the squared height (m^2). Fat mass and fat-free mass were measured using bioelectrical impedance (NutriGuard MS, Poecking, Germany). Patients completed the self-administered and validated version of the Hospital Anxiety and Depression Scale (HADS)²⁶ and the Chronic Respiratory Questionnaire (CRQ)²⁷.

Two 6MWTs were performed one week apart according to the international guidelines.^{1, 2} The test with the longest walking distance was used for further analysis. Participants were asked to walk indoors in a flat, straight, 30m walking course supervised by a well-trained health-care professional. Patients were encouraged using standard methodology every minute of the 6MWT.¹ They were allowed to stop and rest during the test, but were instructed to resume walking as soon as possible. Oxygen saturation (SpO_2), heart rate (HR) and transcutaneous CO_2 levels ($T_c\text{PCO}_2$) as surrogate for arterial carbon-dioxide partial pressure (PaCO_2)²⁸ were measured continuously using the SenTec digital-monitoring system (Sentec AG, Therwill, Switzerland) carried by a well-trained health-care professional.⁸ Perceived dyspnea and leg fatigue of patients at the 6MWT were quantified by a 10-scale Schoen Klinik index (modified Borg scale) before and at the end of the exertion (see supplemental materials – Table S1). Exercise-

induced desaturation (EID) was defined as SpO₂ nadir $\leq 88\%$. Major changes in T_cPCO₂ (T_cPCO₂, $[\Delta]$ $>4\text{mmHg}$) during the 6MWT defined patients as “non-isocapnic” including those who presented either a decreasing CO₂ pattern (decline of T_cPCO₂ $>4\text{mmHg}$) or an increasing CO₂ pattern (increase of T_cPCO₂ $>4\text{mmHg}$) at exertion. Moreover, the non-isocapnic patients who substantially increased T_cPCO₂ (T_cPCO₂, $[\Delta]$ $>4\text{mmHg}$ from baseline) were characterized as “CO₂ retainers”. Exercise-induced hypercapnia (EIH) was considered as the peak of T_cPCO₂ $>45\text{mmHg}$ during the 6MWT.

Statistical analysis

Results are expressed as mean (standard deviation) or proportion (%). The statistical significance of differences between groups was assessed by analysis of variance, paired T-tests and Chi-square, as appropriate. Pearson’s correlation coefficients were used to assess bivariate relationship between changes of carbon dioxide pressure (T_cPCO₂, $[\Delta]$) during the 6MWT, and physiological characteristics. Spearman’s rank correlation was used to determine the repeatability of the T_cPCO₂ trends (Patterns: Reducing, Isocapnic, Increasing) at 6MWT. Binary logistical regression assessed individual predictors of CO₂ retention and EIH. An adjusted logistic regression model was used to estimate odds ratio for CO₂ retention and EIH. Receiver operating characteristics (ROC) curves were used to determine threshold values to predict EIH. The Area Under the curve (AUC) was calculated by the trapezoidal rule and the confidence intervals of the AUC was computed by the method of DeLong.²⁹ Two-sided level of significance was set at $P < 0.05$. Statistical analyses were carried out using the software of MedCalc v.12 and SPSS v.19.0.

RESULTS

Patient characteristics

A total of 62 patients with very severe COPD (31% women) was included in the study. In general, patients had a normal BMI, very severe airflow limitation, static hyperinflation (defined as ratio of residual volume to total lung capacity (RV/TLC $>60\%$) and very severely impaired lung diffusion capacity (DLCO $\leq 30\%$). They were characterized by impaired respiratory pattern at rest (i.e. small V_t and/or high VE/min) and respiratory muscle weakness (determined by P_i max $<60\%$), while had low P_cCO₂ levels but normal range in P_cCO₂ as obtained by CBG analyses. Patients also had impaired health and mood status (rated by CRQ and HADS, respectively) and the vast majority (81%) was receiving long-term oxygen therapy (LTOT; Table 1).

On the 6MWT, patients demonstrated poor exercise capacity (mean 6MWD <360m) with a substantial exercise-induced SpO₂ decline (SpO₂% [Δ] = -8 points), mild elevation in heart rate (HR[Δ] = 26 b/min) and a total small increase in T_cPCO₂ (T_cPCO₂, [Δ] = 1.8 mmHg). Patients also reported moderate post-walk dyspnea and mild leg-fatigue at the end of test. Thirty-eight patients (61%) exhibit EID, 19 patients (31%) presented EIH, and 13 patients (21%) had both (Table 1).

Table 1. Baseline and exercise characteristics

Baseline Characteristics	All Patients (n=62)	Exercise Characteristics	All Patients (n=62)
Women, #, (%)	19(31)	6MWD, m	358±97
Age, years	63.0±8.1	6MWD, %predicted	53.3±14.4
BMI, kg/m ²	24.2±4.6	SpO ₂ pre, %	95.9±2.7
FMI, kg	5.6±2.7	SpO ₂ post, %	88.0±6.8
FFMI, kg/m ²	18.6±2.8	SpO ₂ [Δ], %	-7.9±5.5
FEV ₁ , %pred.	33.2±10.1	HR pre, b/min	85±14
RV/TLC, %	69.1±8.8	HR post, b/min	112±17
DLCO, %pred.	30.1±16.8	HR [Δ], b/min	26±10
Vt, L	0.96±0.2	T _c PCO ₂ pre, mmHg	40.9±6.0
Bf, br/min	21.0±6.5	T _c PCO ₂ post, mmHg	42.7±7.5
VE, L/min	19.4±5.5	T _c PCO ₂ [Δ], mmHg	1.8±6.0
Pi max, %pred.	59.4±18.9	Dyspnea pre, index	1.7±1.9
P _c O ₂ , mmHg	59.4±8.2	Dyspnea post, index	5.5±2.6
P _c CO ₂ , mmHg	40.8±5.1	Dyspnea [Δ], index	3.8±2.4
CRQ Dyspnea, score	3.3±1.5	Fatigue pre, index	1.4±1.9
CRQ Fatigue, score	4.4±1.4	Fatigue post, index	3.7±2.6
CRQ Emotion F., score	4.3±1.5	Fatigue [Δ], index	2.3±2.2
CRQ Mastery, score	4.1±1.5		
CRQ Total score	4.0±1.3	EID, n, (%)	38(61)
HADS Anxiety, score	6.0±3.9	EIH	19(31)
HADS Depression, score	6.3±4.1	EID & EIH	13(21)
LTOT, n, (%)	50(81)	Oxygen users	49(79)

Abbreviations: BMI: Body Mass Index, FMI: Fat Mass Index, FFMI: Fat-free Mass Index, FEV₁: Force Expiratory Volume at 1sec, RV/TLC: Residual Volume / Total Lung Capacity ratio, DLCO: Diffusing capacity for carbon monoxide, Vt: tidal volume, Bf: Breathing frequency, VE: Expired ventilation, Pi max: Maximal inspiratory mouth pressure; P_cO₂: Capillary oxygen partial pressure, P_cCO₂: Capillary carbon-dioxide partial pressure, CRQ: Chronic Respiratory Disease Questionnaire, HADS: Hospital Anxiety and Depression Scale, LTOT: Long-Term Oxygen Therapy, 6MWD: Six-minute walk distance, SpO₂: Saturation of peripheral Oxygen, HR: Heart Rate, T_cPCO₂: Transcutaneous carbon-dioxide partial pressure, EID: Exercise-induced desaturation, EIH: Exercise-induced hypercapnia. Predicted values of pulmonary measurements were estimated according to European Community for Coal and Steel (ECCS) report 1993 as adopted by Quanjer PhH et al. *ERJ* 1993; 16:5-40. Predicted values of 6MWD% were calculated according to Troosters T. et al. *ERJ* 1999; 14:270-4.

Exercise-induced carbon dioxide retention during 6MWT

The T_cPCO₂ response to exercise was highly heterogeneous during the 6MWT. Half of patients (50%) increased substantially T_cPCO₂ levels (T_cPCO₂ [Δ] >4mmHg; “CO₂ retainers”), while the rest either maintained or even decreased CO₂ at exertion (“CO₂ non-retainers”). From all patients, CO₂ non-retainers consisted of 16 patients (26%) who preserved the CO₂ levels (T_cPCO₂ [Δ] ranged from -4 to 4 mmHg; “Isocapnic T_cPCO₂-pattern”); and 15 patients (24%) who reduced the CO₂ levels (T_cPCO₂ [Δ] decline >4 mmHg; “decreasing T_cPCO₂-pattern”) during the 6MWT (Supplementary Figures S1-S2 and Table 2).

Table 2. Transcutaneous measurements during the 6MWT by SenTec digital-monitoring system

Variables	Total Population (n=62)	CO ₂ non-retainers		CO ₂ retainers
		Decreasing CO ₂ pattern (n=15, 24%)	Isocapnic CO ₂ pattern (n=16, 26%)	Increasing CO ₂ pattern (n=31, 50%)
T _c PCO ₂ pre, mmHg	40.9±6.0	42.6±5.3	40.5±5.8	40.2±6.4
T _c PCO ₂ post, mmHg	42.7±7.5	36.7±5.9	40.8±5.6	46.6±6.9
T _c PCO ₂ [Δ], mmHg	1.8±6.0	-6.0±3.0	0.2±1.5	6.4±3.9
T _c PCO ₂ nadir, mmHg	38.5±6.4	35.5±5.9	39.4±5.7	39.4±6.7
T _c PCO ₂ peak, mmHg	44.8±6.8	42.9±5.4	41.7±5.6	47.4±7.1
T _c PCO ₂ [Δ], mmHg	2.3±7.2	-7.4±3.1	0.5±2.5	7.9±3.9
SpO ₂ %, pre	95.9±2.7	95.8±2.8	96.0±3.1	96.0±2.5
SpO ₂ %, nadir	86.1±7.1	88.3±6.2	85.4±8.4	85.4±6.8
SpO ₂ [Δ], %	-9.8±5.8	-7.5±4.4	-10.6±7.3	-10.6±5.3
HR pre, b/min	85.9±14.4	85.9±15.3	82.8±12.5	87.5±15.0
HR peak, b/min	115.1±17.7	113.3±15.3	115.1±18.1	115.9±19.1
HR [Δ], b/min	29.2±11.0	27.4±5.3	32.3±13.1	28.5±11.8
EID, n, (%)	38(61)	8(53)	9(56)	21(68)
EIH	19(31)	0(0)	0(0)	19(61)
EID & EIH	13(21)	0(0)	0(0)	13(42)

Abbreviations: T_cPCO₂: Transcutaneous carbon-dioxide partial pressure; SpO₂: Saturation of peripheral oxygen, HR: heart rate; EID: exercise-induced oxygen desaturation, EIH: exercise-induced hypercapnia. The CO₂ patterns were defined according to T_cPCO₂ trends ([Δ] >4mmHg) during 6MWT. Predicted values of pulmonary measurements were estimated according to European Community for Coal and Steel (ECCS) report 1993 as adopted by Quanjer PhH et al. *Eur Respir J Suppl* 1993, suppl. 6. Predicted values of 6MWD% were calculated according to Troosters et al. *Eur Respir J* 1999; 14:270-4.

Age, BMI, the degree of airflow limitation (FEV₁), CO₂ transfer factor as defined by DLCO, respiratory muscle weakness as determined by Pi max, baseline P_cO₂, and the proportion of LTOT users were similar between patients with and without CO₂ retention during the 6MWT (Table 3). The CO₂ retainers had a better health status as well as a better mood status (Table 3).

Nineteen (31%) out of 62 patients presented EIH ($T_c\text{PCO}_2 > 45\text{mmHg}$). Patients with EIH had lower BMI, lower FEV_1 , smaller tidal volume resulting in lower ventilation, greater respiratory muscle weakness (as defined by Pi max) and higher baseline P_cCO_2 compared to non-EIH patients (Table 3). CO_2 retention or EIH during the 6MWT did not affect the exercise capacity (6MWD) in patients with very severe COPD, even though patient with EIH had higher pre-walk dyspnea compared to non-EIH (Table 4). Moreover, lowest oxygen saturation (SpO_2 nadir) and peak heart rate do not seem to be related with $T_c\text{PCO}_2$ responses during the 6MWT (Table 4 and Figure 1).

Table 3. Baseline characteristics and categorization according to CO_2 retention and EIH

Baseline Characteristics	CO_2 non-retainers (n=31, 50%)	CO_2 retainers (n=31, 50%)	Non-EIH (n=43, 69%)	EIH (n=19, 31%)
Women, n, (%)	10(32)	9(29)	13(30)	6(32)
Age, years, $\pm\text{SD}$	62.9 $\pm$ 8.6	63.0 $\pm$ 7.6	62.6 $\pm$ 8.4	63.8 $\pm$ 7.4
BMI, kg/m^2	25.2 $\pm$ 4.9	23.2 $\pm$ 4.2	25.0 $\pm$ 4.4	22.3 $\pm$ 4.9*
FMI, kg/m^2	6.2 $\pm$ 3.1	5.1 $\pm$ 2.3	6.0 $\pm$ 2.9	4.8 $\pm$ 2.3
FFMI, kg/m^2	19.0 $\pm$ 3.0	18.2 $\pm$ 2.6	19.0 $\pm$ 2.6	17.7 $\pm$ 3.0
FEV_1 , %pred.	35.0 $\pm$ 11.2	31.3 $\pm$ 8.6	34.8 $\pm$ 10.8	29.5 $\pm$ 7.4*
RV/TLC, %	68.5 $\pm$ 9.8	69.8 $\pm$ 7.8	68.1 $\pm$ 8.9	71.4 $\pm$ 8.4
DLCO, % pred.	28.4 $\pm$ 18.6	32.0 $\pm$ 14.6	30.3 $\pm$ 18.0	29.9 $\pm$ 13.9
Vt , L	0.97 $\pm$ 0.2	0.94 $\pm$ 0.3	1.01 $\pm$ 0.2	0.81 $\pm$ 0.3*
Bf, br/min	21.7 $\pm$ 7.6	20.4 $\pm$ 5.1	20.8 $\pm$ 6.8	21.6 $\pm$ 5.8
VE, L/min	20.4 $\pm$ 5.8	18.5 $\pm$ 5.2	20.5 $\pm$ 5.4	17.0 $\pm$ 5.3*
Pi max , %pred.	58.8 $\pm$ 19.6	60.0 $\pm$ 18.4	62.3 $\pm$ 20.0	52.2 $\pm$ 13.7*
P_cO_2 , mmHg	58.9 $\pm$ 9.0	59.8 $\pm$ 7.4	59.7 $\pm$ 8.2	58.7 $\pm$ 8.3
P_cCO_2 , mmHg	40.3 $\pm$ 4.8	41.2 $\pm$ 5.4	39.6 $\pm$ 4.6	43.5 $\pm$ 5.3**
CRQ Dyspnea, score	3.0 $\pm$ 1.3	3.6 $\pm$ 1.6	3.3 $\pm$ 1.5	3.3 $\pm$ 1.4
CRQ Fatigue, score	3.9 $\pm$ 1.3	4.9 $\pm$ 1.4*	4.3 $\pm$ 1.4	4.5 $\pm$ 1.5
CRQ Emotional function score	3.6 $\pm$ 1.6	4.8 $\pm$ 1.2**	4.2 $\pm$ 1.7	4.5 $\pm$ 1.2
CRQ Mastery, score	3.5 $\pm$ 1.7	4.6 $\pm$ 1.2*	4.0 $\pm$ 1.7	4.3 $\pm$ 1.1
CRQ Total score	3.5 $\pm$ 1.3	4.5 $\pm$ 1.1**	3.9 $\pm$ 1.4	4.1 $\pm$ 1.0
HADS Anxiety, score	7.5 $\pm$ 3.6	4.6 $\pm$ 3.6**	6.4 $\pm$ 4.0	5.1 $\pm$ 3.5
HADS Depression, score	8.1 $\pm$ 4.5	4.8 $\pm$ 3.0**	6.7 $\pm$ 4.5	5.5 $\pm$ 3.0
LTOT, n	27(87)	23(74)	34(79)	16(84)

Abbreviations: Please see legend Table 1. Baseline characteristics of patients categorized according to CO_2 retention and the occurrence of EIH. Asterisks denote significant difference: (*) $p < 0.05$, (**) $p < 0.01$.

Table 4. Six-min walk test characteristics and categorization by CO₂ retention and EIH

6MWT Characteristics	CO ₂ non-retainers (n=31, 50%)	CO ₂ retainers (n=31, 50%)	Non-EIH (n=43, 69%)	EIH (n=19, 31%)
6MWD, m	343±99	373±94	369±98	334±92
6MWD, %predicted	51.3±14.5	55.3±14.0	54.6±14.2	50.3±14.4
SpO ₂ %, pre	95.9±2.9	96.0±2.5	96.0±2.7	95.8±2.8
SpO ₂ %, nadir	86.8±7.5	85.4±6.8	86.4±7.4	85.4±6.7
HR pre, b/m	91±15	94±13	92±15	94±9
HR peak, b/m	114±17	115±19	115±19	114±13
T _c PCO ₂ pre, mmHg	41.5±5.6	40.2±6.4	39.6±5.9	43.8±5.2*
T _c PCO ₂ post, mmHg	38.5±6.0	47.4±7.1**	38.9±5.3	52.0±4.8**
Dyspnea, pre	1.5±2.0	2.0±1.8	1.4±1.8	2.5±2.0*
Dyspnea, post	5.1±2.6	6.0±2.5	5.4±2.8	5.8±2.0
Leg Fatigue, pre	1.2±1.8	1.5±1.9	1.2±1.8	1.7±2.2
Leg Fatigue, post	3.5±2.5	3.9±2.6	3.3±2.5	4.6±2.6
EID, n, (%)	17(55)	21(68)	25(58)	13(68)

Abbreviations: 6MWD: six-minute walk distance; SpO₂: Saturation of peripheral oxygen; HR: heart rate; T_cPCO₂: Transcutaneous carbon-dioxide partial-pressure, EID: exercise-induced oxygen desaturation, EIH: exercise-induced hypercapnia. Six-minute walk test characteristics and categorization performed according to CO₂ retention and the occurrence of EIH. Asterisks denote significant difference: (*) p< 0.05, (**) p< 0.01.

Repeatability of exercise-induced carbon dioxide patterns during 6MWT

High repeatability of the T_cPCO₂ patterns during exercise was observed between the longest 6MWT and the shortest 6MWT for each patient. Specifically, the 73% of the patients demonstrated an identical T_cPCO₂ pattern in both 6MWT (Spearman's rho=0.60, p <0.001). In addition, the mean values of the changes in T_cPCO₂ as well as SpO₂ and heart rate during both performed 6MWT were similar (Supplementary figure S3).

Table 5. Logistic regression: Determinants of CO₂ retention and EIH in patients with very severe COPD

Determinants	n	CO ₂ retention	ADJUST OR (95%CI)	P value	Determinants	n	EIH	ADJUST OR (95%CI)	P value
FEV ₁					FEV ₁				
>36 %pred.	21	6 (29)	1		>36 %pred.	21	3 (14)	1	
≤36 %pred.	41	25 (61)	4.95 (1.41-17.44)	0.013	≤36 %pred.	41	16 (39) (0.96-16.81)	4.02 (0.96-16.81)	0.057
BMI					BMI				
>25 kg/m ²	21	7 (33)	1		>25 kg/m ²	21	3 (14)	1	
≤25 kg/m ²	41	24 (59)	2.77 (0.87-8.89)	0.086	≤25 kg/m ²	41	16 (38) (1.05-18.27)	4.38 (1.05-18.27)	0.043
P _c O ₂ pre-walk (BGA)					P _c O ₂ pre-walk (BGA)				
> 63 mmHg	19	9 (47)	1		> 63 mmHg	19	3 (16)	1	
≤63 mmHg	43	22 (51)	1.29 (0.42-3.93)	0.655	≤63 mmHg	43	16 (37) (0.77-12.39)	3.09 (0.77-12.39)	0.111
P _c CO ₂ pre-walk (BGA)					P _c CO ₂ pre-walk (BGA)				
< 41 mmHg	35	16 (46)	1		< 41 mmHg	35	6 (17)	1	
≥41 mmHg	27	15 (56)	1.73 (0.60-5.00)	0.311	≥41 mmHg	27	13 (48) (1.37-14.79)	4.50 (1.37-14.79)	0.013
Pi max					Pi max				
>64 %pred.	31	13 (42)	1		>60 %pred.	34	7 (21)	1	
≤64 %pred.	31	18 (58)	3.36 (1.00-11.34)	0.051	≤60 %pred.	28	12 (43) (1.00-12.47)	3.53 (1.00-12.47)	0.050
Dyspnea pre-walk					Dyspnea pre-walk				
<2 points	34	13 (38)	1		<3 points	42	9 (21)	1	
≥2 points	28	18 (64)	3.38 (1.11-10.26)	0.032	≥3 points	20	10 (50) (1.16-12.61)	3.82 (1.16-12.61)	0.028

Abbreviations: Please see legend Table 1. A Logistic regression demonstrated the determinants of CO₂ retention and EIH during the 6MWT in 62 patients with very severe COPD. Each determinant was adjusted for age (≥60 years), sex (females), and Long-Term Oxygen Therapy (LTOT). CO₂ retention and EIH were defined as T_cPCO₂ [Δ] >4mmHg and T_cPCO₂ >45mmHg, respectively. Significance was set on p <0.05.

Correlates of T_cPCO₂ during the 6MWT

Post-walk T_cPCO₂ was correlated with the levels of capillary P_cCO₂ (r=0.53, p <0.001) at rest and had an inverse correlation with FEV₁% predicted (r=-0.32, p<0.05). The delta values (T_cPCO₂, [Δ]) were associated only with the FEV₁% (r=-0.26, p<0.005; Supplementary table S2.)

Determinants of T_cPCO₂ increase and EIH during the 6MWT

Using receiver operating characteristics (ROC) curves, the threshold values with the best sensitivity and specificity to predict T_cPCO₂ retention and EIH were determined for BMI

(≤ 24 kg/m²), FEV₁ (≤ 36 %pred.), baseline P_cO₂ (≤ 63 mmHg), baseline P_cCO₂ (≥ 41 mmHg), Pi max (≤ 64 %pred. - CO₂ retainers/ ≤ 60 %pred. - EIH) and pre-walk dyspnea (Schoen Klinik scale: ≥ 2 points - CO₂ retainers/ ≥ 3 points - EIH; Supplementary tables S3-S4).

Using logistic regression of these ROC curve identified cut-offs, showed that FEV₁ ≤ 36 %pred. and higher pre-walk dyspnea (≥ 2 points) were determinants of CO₂ retention while P_cCO₂ ≥ 41 mmHg and higher pre-walk dyspnea (≥ 3 points) were determinants of EIH after adjustment for age (≥ 60 years), sex (females), and LTOT (Table 5). Interestingly, a higher BMI (>25 kg/m²) seems to carry a protective effect against the occurrence of EIH in advanced COPD.

Figure 1. Changes in the variables of T_cPCO₂, SpO₂ and Heart Rate in each minute (means/min) of the 6MWT according to the CO₂ retention

The changes of SpO₂ and Heart Rate in each minute of the 6MWT were similar for all of the three patterns of T_cPCO₂ response to exercise. Error bars represent the Standard Error of Mean (SEM).

DISCUSSION

This study provides four major observations: (1) PCO₂ response to exercise is highly heterogeneous in patients with advanced COPD; (2) half of the patients (50%) demonstrated CO₂ retention, whereas a 31% of total population exhibited EIH during

the 6MWT; (3) exercise-induced CO₂ retention is mostly determined by worse airflow limitation while EIH is mostly associated with higher baseline P_cCO₂ levels; and (4) overweight BMI may prevent the occurrence of EIH in very severe COPD. To the best of our knowledge, this is the first study to demonstrate the patterns of T_cPCO₂ trends in the 6MWT using continuous recording of CO₂ and to reveal the determinants of CO₂ retention and EIH during walking in GOLD stage IV.

The physiological basis of CO₂ retention and EIH is multifactorial as it reflects mechanical constraints on ventilation resulting in ventilatory abnormalities¹⁶, reduced respiratory drive³⁰ and impaired physiological characteristics^{18, 31} such as increased resting PCO₂ levels and indices of airflow limitation. It is also well-documented that physiological dead space is increased in patients with advanced COPD due to underlying V/Q mismatch, which requires a higher ventilation to keep alveolar ventilation (and hence PCO₂) constant.^{32, 33} At increased respiratory rates usually during exercise, an inversely proportional decrease in expiratory time often occurs in patients with COPD contributing to the development of dynamic hyperinflation.³³ Montes de Oca and Celli indicated that EIH is related to lungs' inability to increase ventilation because of factors such as the presence of dynamic hyperinflation.³⁴ O'Donnell and colleagues have also reported that the tendency to develop CO₂ retention during exercise could be predicted by the degree of dynamic hyperinflation.¹⁶

In the current study, dynamic hyperinflation may contribute to CO₂ retention and EIH. Although there were no differences in static hyperinflation (RV/TLC) between CO₂ retainers and non-retainors, a higher airflow limitation may result in greater CO₂ retention and EIH potentially due to higher degree of dynamic hyperinflation at exertion. After categorization based on CO₂ retention, the patients of current study tended to have worse airflow limitation compared to non-retainors and this is more distinct and reaches significance when patients are stratified by EIH (Table 3). Indeed, impaired FEV₁ can result in air-trapping causing hyperinflation which, in turns, leads to CO₂ retention.³⁵ However, a number of patients with severely impaired FEV₁ did not reached the hypercapnic levels (T_cPCO₂ >45mmHg) during 6MWT. Other factors such as respiratory muscle strength could potentially compensate the negative impact of low FEV₁ for the occurrence of dynamic hyperinflation preventing EIH as it has been also reported in previous studies.³⁶⁻³⁸

In this study, resting tidal volume and ventilation has been shown to be comparable between CO₂ retainers and non-retainors; however, patients with EIH had more shallow breaths and an overall lower ventilation compared to non-EIH (Table 3). It has been postulated that CO₂ retention during exercise is attributed to a "behavioral" adoption of shallow breathing pattern, which serves to minimize intrathoracic pressure perturbations, reduce respiratory discomfort, and mitigate the development of respiratory muscle fatigue ("wise fighter" hypothesis).³⁹ Sorli et al. demonstrated that increase in PCO₂ levels was associated with a decreased tidal volume and reduced

alveolar ventilation.⁴⁰ Our findings suggested that shallow breathing and low ventilation are characteristics of patients with EIH rather than those who retain CO₂ but do not reach the hypercapnic levels during the 6MWT (Table 3). Also, health and mood status (CRQ / HADS scores) did not correlate with T_cPCO₂ (Pearson's $r=0.12$ to 0.19 , $p > 0.17$) during the 6MWT, which may imply that several factors can impact on health and mood status besides the lung function impairment as represented by T_cPCO₂. This again illustrates the complex intertwinement of what we measure, expect and the symptoms patients suffer of.

Interestingly, clear differences were found in BMI of EIH and non-EIH patients (Table 3). Although it is well established that obesity causes decreases in resting lung volumes⁴¹, the ventilatory responsiveness to hypercapnia can be higher in patients with increased weight compared those with weight loss.^{42, 43} Indeed, chemoreflex responses to hypercapnia have been shown to be potentiated in eucapnic obese subjects.⁴⁴ Moreover, obesity, compared to normal range BMI, is associated to lower resting and dynamic hyperinflation, lower mechanical loading and thus can result in lower CO₂ retention.⁴⁵⁻⁴⁸ Evidence also has shown that COPD patients with low BMI had 20 times higher risk of having CO₂ retention during an acute exacerbation than those with normal BMI.⁴⁹ The findings of the current study indicate that overweight BMI reduces the risk of EIH in very severe COPD (Tables 3 and 5), which can be attributed to a potential higher ventilatory responsiveness, less dynamic hyperinflation and lower mechanical loading during the 6MWT.

According to the findings, EIH is highly dependent on PCO₂ baseline values (Table 3 and 4). When EIH is defined as reaching above a certain threshold, this is very much defined by the baseline levels. Indeed, patients who exhibited EIH had increased baseline-P_cCO₂ and pre-walk T_cPCO₂ levels compared to non-EIH. However, high baseline PCO₂ levels are not always predictive of the increase in PCO₂ and EIH.⁵⁰ A minority of patients (23%) who did not exhibit EIH presented high baseline T_cPCO₂ levels (T_cPCO₂ ≥ 42 mmHg). Furthermore, increased resting P_cCO₂ levels seem to modify dyspnea sensation at rest. Nevertheless, post-walk dyspnea was comparable in both groups stratified on the presence of EIH as well as the exercise capacity and SpO₂ nadir (Table 4).

A logistical regression model for the prediction of CO₂ retention and EIH (adjusted for age, sex, and LTOT) showed that FEV₁ and pre-walk dyspnea determine CO₂ retention, whereas baseline-P_cCO₂ levels and pre-walk dyspnea had a strong influence on the prevalence of EIH in patients with very severe COPD (Table 5). These findings may imply that airflow limitation is more important factor for CO₂ retention; however, baseline-P_cCO₂ levels are more crucial for reaching hypercapnic CO₂ values during the 6MWT. In addition, a higher BMI reduces the risk of EIH in patients with very severe COPD (Table 5).

Strengths and weaknesses

There are certain limitations to our study. The SenTec electrode has been shown to be accurate enough under resting conditions and also transcutaneous PCO_2 -measurements have been already validated at CPET (TOSCA 500 monitor).⁹ Then again, the accuracy of SenTec recordings has not been proved yet during the 6MWT. Pilot evaluation (n=10 patients with COPD) for the agreement of PCO_2 recordings between capillary BGA and SenTec device, however, showed a very strong intraclass correlation coefficient (ICC) absolute agreement for pre-walk (ICC= 0.98, 95%CI: 0.93 -0.99) and post-walk (ICC = 0.98, 95%CI: 0.73-0.99) values of the 6MWT (Supplementary table S5 and figure S4). Another limitation of the study is that respiratory measurements such as ventilation, tidal flow-volume loops, end-tidal PCO_2 and the detection of dynamic hyperinflation were not performed during walking. There was no detailed assessment of dynamic lung mechanics, which would have provided us with important information for patients' respiratory profile during the 6MWT. Also, a power analysis was not performed and our sample size may be relatively small for this kind of analysis and also included only very severe COPD patients. Despite these limitations, this study offers a novel analysis of PCO_2 response to exercise and the determinants of CO_2 retention during the 6MWT. The detection and interpretation of CO_2 retention and EIH during exercise could be an enhanced diagnostic tool for the clinical management of COPD. Furthermore, it may be useful information for the design of tailored pulmonary rehabilitation programs for patients with elevated PCO_2 levels and EIH. Future studies need to reproduce these findings at isoload exercise time and evaluate the impact of PCO_2 retention and EIH during the 6MWT in respect to prognosis and respiratory failure.

CONCLUSIONS

The response of PCO_2 to 6MWT is highly heterogeneous in patients with very severe COPD. However, CO_2 retention is related to worse airflow limitation and pre-walk dyspnea, whereas the occurrence of EIH is associated to higher baseline P_cCO_2 levels and pre-walk dyspnea. Reduced FEV_1 and elevated baseline- P_cCO_2 together with pre-walk dyspnea increase the risk for CO_2 -retention and EIH during the 6MWT. In contrast, overweight BMI seems to carry a protective effect against EIH in very severe COPD. Monitoring of T_cPCO_2 during 6MWT might help to identify patients who need special attention regarding to EIH.

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SUPPLEMENTAL MATERIAL

Table S1. Borg’s modified scale for rating dyspnea and/or leg fatigue

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The modified “Borg-scale” for rating dyspnea and/or leg fatigue, as used at Schoen Klinik Berchtesgadener Land* in Germany (*here it is presented translated from German to English and in grayscale).

Table S2. Pearson’s correlations between $T_c\text{PCO}_2$ response to 6MWT and patients’ characteristics

	PcO_2 rest	PcCO_2 rest	FEV_1 %pred	DLCO %pred	Pi max %pred	6MWD %pred	SpO_2 nadir	Dyspnea pre	Dyspnea post
$T_c\text{PCO}_2$, pre-walk	0.01	0.56**	-0.13	-0.10	-0.27*	-0.22	0.05	0.03	-0.01
$T_c\text{PCO}_2$, post-walk (peak values)	0.13	0.53**	-0.32*	-0.08	-0.20	-0.11	-0.09	0.23	0.20
$T_c\text{PCO}_2$ [Δ] (nadir-peak)	0.13	0.13	-0.26*	-0.03	0.08	0.12	0.17	0.20	0.24

Abbreviations: $T_c\text{PCO}_2$: Transcutaneous carbon-dioxide partial-pressure; FEV_1 : forced expiratory volume in 1 sec; DLCO : carbon monoxide diffusing capacity; Pi max : Maximal inspiratory mouth pressure; SpO_2 : Saturation of peripheral oxygen; 6MWD: Six-minute walk distance.

Table S3. Cut-off points and the ROC curve parameters for the prediction of CO₂ retention

		ROC curve parameters						
Predictive index	Best cut-off	AUC segment	Sensitivity (%)	95% CI Binomial exact	Specificity (%)	95% CI Binomial exact	Likelihood Ratio	
							LR+	LR-
Age, years	≥60	0.52	0.68	0.49-0.83	0.39	0.22-0.58	0.90	1.20
BMI, kg/m ²	≤25	0.61	0.74	0.55-0.88	0.45	0.27-0.64	1.35	0.57
FEV ₁ , %pred.	≤36	0.60	0.81	0.63-0.93	0.48	0.30-0.67	1.56	0.40
P _c O ₂ , mmHg	≤63	0.54	0.67	0.49-0.83	0.29	0.14-0.48	1.05	0.90
P _c CO ₂ , mmHg	≥41	0.53	0.42	0.25-0.61	0.65	0.45-0.81	1.18	0.90
Pi max, %pred.	≤64	0.50	0.64	0.44-0.81	0.53	0.34-0.72	1.38	0.67
Dyspnea pre-walk	≥2	0.58	0.58	0.39-0.76	0.67	0.47-0.83	1.74	0.63

Abbreviations: BMI: Body mass index; FEV₁: forced expiratory volume in 1 sec; P_cO₂: Capillary oxygen partial pressure; P_cCO₂: Capillary carbon-dioxide partial pressure; Pi max: Maximal inspiratory mouth pressure; AUC: Area under the curve.

Table S4. Cut-off points and the ROC curve parameters for the prediction of CO₂ retention

		ROC curve parameters						
Predictive index	Best cut-off	AUC segment	Sensitivity (%)	95% CI Binomial exact	Specificity (%)	95% CI Binomial exact	Likelihood Ratio	
							LR+	LR-
Age, years	≥60	0.52	0.63	0.38-0.84	0.35	0.21-0.51	0.97	1.06
BMI, kg/m ²	≤25	0.68	0.84	0.60-0.97	0.44	0.29-0.60	1.51	0.36
FEV ₁ , %pred.	≤36	0.64	0.84	0.60-0.97	0.42	0.27-0.58	1.45	0.38
P _c O ₂ , mmHg	≤63	0.54	0.84	0.60-0.97	0.37	0.23-0.53	1.34	0.42
P _c CO ₂ , mmHg	≥41	0.71	0.63	0.38-0.84	0.67	0.52-0.81	1.94	0.55
Pi max, %pred.	≤60	0.68	0.76	0.50-0.93	0.61	0.45-0.76	1.96	0.39
Dyspnea pre-walk	≥3	0.66	0.53	0.30-0.76	0.76	0.61-0.88	2.21	0.62

Abbreviations: BMI: Body mass index; FEV₁: forced expiratory volume in 1 sec; P_cO₂: Capillary oxygen partial pressure; P_cCO₂: Capillary carbon-dioxide partial pressure; Pi max: Maximal inspiratory mouth pressure; AUC: Area under the curve.

Table S5. Agreement between CBG analysis and SenTec recordings of PCO₂ during the 6MWT

ID	Sex	Age	GOLD Stage	PCO ₂ pre-walk, mmHg			PCO ₂ post-walk, mmHg			O ₂ Liters
				CBG	SenTec	Difference	CBG	SenTec	Difference	
1	M	55	4	60.2	60.1	-0.1	63.5	60.8	-2.7	2.0
2	M	62	4	40.0	39.0	-1.0	40.3	39.1	-1.2	3.0
3	M	84	2	33.0	32.2	-0.8	31.7	30.1	-1.6	-
4	M	74	3	30.0	30.9	+0.9	30.6	30.4	-0.2	-
5	M	64	4	49.5	51.2	+1.7	47.6	44.8	-2.8	3.0
6	F	59	4	38.9	36.6	-2.3	43.8	43.1	-0.7	2.0
7	F	44	4	32.9	33.9	+1.0	33.1	33.5	+0.4	-
8	F	69	4	35.8	36.2	+0.4	38.8	36.4	-2.4	2.0
9	F	63	4	35.0	34.4	-0.6	38.3	35.4	-2.9	3.0
10	F	71	4	46.4	50.9	+4.5	53.7	53.3	-0.4	2.5

Abbreviations: CBG: Capillary Blood Gas values; SenTec: Sentec monitoring-system measures of transcutaneous carbon-dioxide partial-pressure (T_cPCO₂).

Figure S1. Individual changes in T_cPCO_2 during the 6MWT of CO₂ “non-retainers”
Individual changes in T_cPCO_2 were observed during the 6MWT of CO₂ “non-retainers” including patients with exercise-induced isocapnic or decreasing T_cPCO_2 pattern.

Figure S2. Individual changes in $T_c\text{PCO}_2$ during the 6MWT of CO_2 "retainers"

Individual changes in $T_c\text{PCO}_2$ were observed during the 6MWT of CO_2 "retainers" including patients with exercise-induced increasing $T_c\text{PCO}_2$ pattern.

Figure S3. Repeatability of the changes in T_cPCO₂, SpO₂ and Heart Rate in 6MWTs
The repeatability of the changes in T_cPCO₂, SpO₂ and Heart Rate (mean value/minute) was measured in 6MWTs (longest vs shortest 6MWT). Error bars represent the Standard Error of Mean (SEM).

Figure S4. Bland-Altman plots of the means of CBG analysis and SenTec recordings during 6MWT
Bland-Altman plots of the means of both methods (CBG analysis and SenTec recordings) during the 6MWT in patients with COPD. Regression lines of differences and 95% Confidential Intervals are presented. Intraclass Correlation Coefficient (ICC) of absolute agreement and 95%CI were calculated.

Chapter 8

General Discussion

The European Respiratory Society (ERS) and American Thoracic Society (ATS) have recognized the six-minute walk test (6MWT) as a valid and reliable measure of exercise capacity for patients with chronic lung disease.^{1, 2} To date, the six-minute walk test (6MWT) is the most frequently employed “self-paced” test of walking capacity in patients with COPD, expressed as the distance walked in six minutes (6MWD).^{1, 2} The 6MWD is widely-used as clinical outcome measure providing important information about the global and integrated responses of pulmonary and cardiovascular system during exertion, and it has significant prognostic value.^{3, 4} From an evidence-based perspective, 6MWD with associated pathophysiological responses such as dyspnea and leg fatigue as well as oxygen desaturation and carbon-dioxide retention at exertion, is of considerable importance in the multidimensional evaluation in COPD regarding intervention effects and prognosis. The 6MWD appears to be responsive to clinical treatments while it also presents a significant association with mortality and hospitalization in patients with COPD.^{1, 2} In addition, a number of indices derived from the 6MWT may improve the predictive value of information gained from the 6MWD. However, the interpretation of the 6MWT usually remains restricted to 6MWD as the only parameter used in clinical practice. Other 6MWT-derived variables, such as walking speed (6MWSpeed), six-minute walk-work (6MWW), distance saturation products (DSP), exercise-induced desaturation (EID), and unintended stop(s) during walking can provide healthcare professionals, patients and scientists with important additional and prognostic clinical information.⁵

This thesis aimed to increase the current knowledge for the 6MWT and to strengthen its utility by providing novel insights on the determinants and the interpretation of walking performance as well as demonstrating determinants of exercise-induced oxygen desaturation (EID), CO₂ retention and exercise-induced hypercapnia (EIH), and assessing and extending its prognostic value in patients with COPD.

SIX-MINUTE WALK TEST IN COMPARISON WITH CYCLE ENDURANCE TEST (CET)

In **chapter 2** of this thesis, certain characteristics of functional exercise capacity and different factors that determine exercise performance in cycle endurance test (CET) and 6MWT were investigated. In general, the 6MWT is characterized by a self-paced walking involving all major muscular groups, whereas CET utilizes primarily the lower body muscles to sustain a constant work-load.⁶⁻⁸ The results of chapter 2 demonstrated that the variability of endurance time in CET (CET-T_{end}), even though poorly explained (r^2 : 15.1%), included the characteristics of age, sex, fat-free mass index (FFMI), GOLD stage, diffusion lung capacity for carbon dioxide (DLCO), dyspnea and leg-fatigue before test. On the other hand, the variance in 6MWD was better explained (r^2 : 34.0%) by including the

following characteristics of age, BMI, FFMI, disease severity by GOLD stage, FEV_1 , FEV_1/FVC , functional residual capacity (FRC), and DLCO, as well as leg-fatigue before test.⁹

Chapter 2 showed that age, FFMI, GOLD stage, DLCO and leg-fatigue are common determinants of $CET-T_{end}$ and the 6MWD. In healthy elderly, aging is related to an important decrease of muscle power¹⁰ as well as to a less efficient cardiovascular system¹¹ and mainly these factors result in lower exercise capacity affecting both cycling and walking performance.¹² Age-related decrements can also be explained by a general muscle deconditioning as physical activity usually declines with age, while this process in patients with COPD is also being intensified by the progress of disease severity over time.¹³ Another factor which seems to affect both CET and 6MWT in COPD, is the FFMI. In line with the results of chapter 2, a positive correlation has been reported between fat-free mass and the performance in CET ¹⁴ and 6MWT.¹⁵ However, an evaluation of high number of potential confounders such as disease severity, health status, cardiovascular disease, exacerbation rate and oxygen use, might eliminate the relationship between fat-free mass and 6MWT.¹⁶ Furthermore, DLCO has been reported as the strongest predictor in exercise intolerance in COPD¹⁷ reflecting the high impact of DLCO on cycling and walking performance, while very low DLCO levels are particularly related to the need of oxygen supplementation during exercise.¹⁸ In agreement with chapter 2, leg-fatigue has been also shown as an important factor limiting exercise in COPD.¹⁹ The observation of those aforementioned clinical characteristics which are common determinants for $CET-T_{end}$ and 6MWD, suggest that both exercise test can be used to discriminate patients according to underlying disease severity and loss of functional capacity. Indeed, CET and 6MWT seems to be associated with systemic impairment in COPD that is manifested at exertion.

Chapter 2 also demonstrated the different determinants of exercise capacity between CET and the 6MWT. Baseline dyspnea and sex were related to $CET-T_{end}$, whereas BMI and lung-function indices of FEV_1 , FEV_1/FVC , and FRC were related to 6MWD. Previous studies have shown that dyspnea predicts general health status to a greater extent than physiologic measurements in patients with COPD.^{20, 21} Therefore, it may be reasonable that $CET-T_{end}$, as determined by rates of dyspnea, mostly reflects an overall impaired health status in COPD rather than lung function impairment which can be better reflected by the 6MWD.²² On the other hand, BMI has great impact on leg-movement during walking but this is not the case for CET which is a non-weight-bearing test as patient's body weight is supported by the seat.²³⁻²⁵ Evidence has also shown that an increase in FEV_1 is beneficial for improving walking but not cycling endurance in patients with COPD, which confirms that FEV_1 may determine 6MWD but not $CET T_{end}$.²⁶ It seems that 6MWD may reflect improvements in lung function independently of the deconditioned state of the peripheral muscles in COPD. Indeed, an increase in 6MWD due to less dynamic hyperinflation, as a result of better lung function, might be better

represented by the 6MWT which usually causes less quadriceps fatigue compared to CET that imposes a constant work load to the legs.²⁷

Interestingly, sex appears to be a strong determinant of CET-T_{end} in contrast to 6MWD. The male population of studied patients achieved a higher peak work rate (WR_{peak}) and cycling performance at 75% WR-peak during CET compared to the female group. This trend was also observed by Laviolette and colleagues who demonstrated that women with COPD are disadvantaged during exercise as they reached a critical inspiratory reserve volume earlier, leading to a steep increase in dyspnea and to exercise termination.²⁸ In addition, a number of sex-related differences in the clinical expression of COPD have been reported including higher scores on the St. George's Respiratory Questionnaire (SGRQ)²⁹ in women that indicates more impaired health status.³⁰ Therefore, a general worse experience of health status in women may result in more intensive perceived symptoms limiting the CET-T_{end}. This could be also a reflection of the fact that cycling can evoke more dyspnea because of its unfamiliarity compare to walking which simulates a more routine daily activity.

To the best of our knowledge, chapter 2 was the first report demonstrating the performance quartiles between CET and the 6MWT. The recruitment of different muscle groups between CET and 6MWT and the "external-paced" nature of CET compared to "self-paced" 6MWT, contributes to different ventilatory responses resulting in different levels of perceived dyspnea, leg-fatigue and different physiological responses influencing performance.³¹ The relative weight of several clinical characteristics on CET-T_{end} and 6MWD also varies within performance quartiles of these tests highlighting the complex pathophysiology that influence exercise capacity in COPD. Consequently, clinicians and health professionals should consider the choice of CET or 6MWT as critically important according to the aims of the assessment procedure. Moreover, they need to be aware of patients' clinical characteristics which determine exercise performance in CET and 6MWT, when interpreting or comparing results of both test procedures.

Vivodtzev and colleagues have reported that physiological stress caused by CET is not identical among patients even though cycling is performed at fixed proportion (75%) of peak incremental workload capacity.³² In chapter 2, CET-T_{end} had a large variation among patients, even though the work load was predefined. In fact, CET at 75% is influenced by the degree of peripheral muscle deconditioning as the load imposed is fixed and not self-chosen as during the 6MWT. Indeed, this variation may indicate a large heterogeneity in muscle function including different levels of impaired oxidative capacity, muscle atrophy, and physical deconditioning of patients who can additionally present different degrees of exercise-induced desaturation or hypercapnia at exertion. Therefore, it can result in different physiological demands during constant work-load which may lead to different physiological stress during CET that may better reflect the overall state of functional capacity.³³⁻³⁸ However, it seems that performing

CET at 75% of peak work rate is a feasible level of effort for COPD patients being referred for a pulmonary rehabilitation (PR) program according to the findings of chapter 2.

As suggested before, the 6MWT and CET are not interchangeable; ideally, both tests should be performed in clinical settings for the exercise assessment. The fact that the determinants of CET-T_{end} cannot be reproducible from a model, which includes a small number of clinical parameters, underscores the importance of CET-T_{end} as an independent measurement before and after a clinical intervention. In addition, improvements in exercise outcomes after PR program should be evaluated using both 6MWT and CET as patients response outcomes can differ in 6MWT and CET.³⁹ Practical limitations or time and personnel restrictions can impose the use of one test, either 6MWT or CET. For instance, a 6MWT cannot be performed in daily clinical practice in case of a short hall-way (<30m in length), while CET needs a cardiopulmonary exercise test (CPET) to determine the peak cycling load, which requires equipment and technical support that is not always easily available. Chapter 2 expands the knowledge for the determinants of exercise performance in 6MWT and CET and demonstrates different characteristics of those tests as well as factors that influence cycling and walking emphasizing that in the latter peripheral muscle deconditioning is less of importance.

THE IMPORTANCE OF THE CHOICE OF REFERENCE EQUATION IN 6MWT

Recent guidelines on the diagnosis and treatment of COPD indicate that assessment of disease severity is more comprehensive by using additional functional criteria such as exercise capacity.⁴⁰⁻⁴³ The percentage of predicted 6MWD (%), which represents the exercise capacity in the 6MWT, may be different as calculated by several reference equations which have been formulated under different 6MWT operational settings and for different populations.⁴⁴⁻⁴⁶

Chapter 3 indicated that discrepancies among reference values are depending on factors used for the formulation of reference equations including (A) demographic and anthropometric characteristics, (B) methodological differences, and (C) health condition of the healthy population. Recommendation for clinicians and health professionals is to choose a 6MWD reference equation which is derived from 6MWTs performed under same operating procedures and derived from same country/region populations.⁴⁷

The 6MWD has shown a considerable variability in several studies.^{44, 48-54} Regarding the important influence of demographic and anthropometric characteristics in 6MWD, the variables of age, height, weight, BMI and sex can determine walking performance and explain the 25% to 66% of the total variability in 6MWD.^{44, 48-52, 54-66} More specifically, ageing can contribute to impaired walking performance by muscle loss and weakness with an average loss of muscle strength of 20-40% in individuals older than 70

years old resulting in lower gait speed and walking ability.^{67, 68} Evidence has also shown that differences in patients' height, which are attributed to genetics, sex and race, are strongly associated to length of stride, which is a component of walking speed, and determines the 6MWD.^{44, 51, 53, 55} Moreover, differences in patients' weight, as expressed better in BMI, are associated to different energy cost during walking⁶⁹ and may indicate different levels of physical capacity⁷⁰, which determine the 6MWD. For instance, obesity is related to physical inactivity and deconditioning⁷¹ which result in reduced cardiopulmonary capacity and increased metabolic load for a given amount of exercise with a negative effect on walking distance.⁵⁵ Furthermore, increased prevalence of comorbid conditions and obesity-specific disability, which are frequently observed in obese populations, might interfere with walking.⁷² Also, a number of volunteers, often termed as "healthy", may not correspond to criteria for being completely healthy and this can further contribute to the discrepancies in the 6MWD reference values.

Chapter 3 also demonstrated that besides those clinical characteristics, technical or methodological characteristics including different operational procedures, 6MWT instructions and encouragement can influence walking performance. It is reported that there is less walking effort in long corridors because of the smaller number of turns compared to short corridors⁷³ while lack of standardization in given instructions⁷⁴ or encouragement⁷⁵ can also affect 6MWD. Indeed, the standardization of the 6MWT is important for data interpretation as several factors may influence the outcome. In case that these factors are standardized and well controlled, the 6MWT can provide concrete valuable information. Moreover, other factors such as the regional habitual walking⁷⁶, psychological status⁷⁶, and altered walking pattern⁷⁷ as well as balance impairment⁷⁸ can partially determine 6MWD, while the relative weight of these determinants can differ.⁷⁶ Clinicians should consider all factors that potentially influence the 6MWT for the right interpretation of the 6MWD reference values.

Herein, it should be also noted that the choice of right predicted equations is not 6MWT-specific. Indeed, several reference equations exist in other clinical tests (i.e. CPET, muscle function test, lung function, etc.). The findings of chapter 3 regarding to factors used for the formulation and application of reference equations may find a common ground with studies that deal with reference equations in other clinical tests. For instance, ERS/ATS interpretative strategies for lung function tests⁷⁹ recommended taking the characteristics of age, sex, height, health status, ethnicity, equipment and general population characteristics into account when developing and updating reference equations.⁷⁹ BMI was also reported by another study as a factor that could influence lung function.⁸⁰

A practical recommendation for clinicians and health professionals is to choose a reference equation that was formulated under same operating procedures and derived from a representative large population with a comparable range of age. It is noteworthy

that an erroneous selection of reference equation may result in wrong estimation of actual exercise capacity and thus potentially misleading data into clinical decision-making process. Moreover, an underestimation or overestimation of actual exercise capacity of patients may have a negative impact on prescribed exercise protocols (i.e. misestimated exercise workloads) resulting in less efficient exercise interventions in COPD.

THE PROGNOSTIC VALUE OF VARIABLES DERIVED FROM SIX-MINUTE WALK TEST

A significant relationship between 6MWD and mortality or hospitalization has been demonstrated in literature.⁸¹⁻⁸³ Several thresholds for 6MWD have been proposed for the prediction of mortality^{4, 81, 84-94} with a pooled mean of 317 meters indicating an increased risk for mortality in COPD. The range of disparity in the 6MWD may be attributed to the differences in studied populations, sources of recruitment, and different methodological and statistical approaches as well as the heterogeneity of disease which all may influence 6MWD (Figure 1).

Figure 1. Thresholds of 6MWD for the prediction of mortality in COPD. Vertical lines represent weighted mean thresholds for 6MWD across studies for predicting mortality.¹ Reproduced with permission of the European Respiratory Society ©. European Respiratory Journal Dec 2014, 44 (6) 1447-1478; DOI: 10.1183/09031936.00150414.

Chapter 4 expanded the prognostic value of 6MWT beyond the previous studies of the ECLIPSE cohort^{16, 81, 95} and establishes thresholds of 6MWT derived-variables for the prediction of mortality and hospitalization. In specific, 6MWT-derived variables including 6MWD, walking speed (6MWSpeed), six-minute walk-work (6MWW), distance saturation products (DSP), exercise-induced desaturation (EID), and unintended stop(s) demonstrated to be important predictors of mortality and hospitalization in patients with COPD.⁹⁶ An adjusted logistical model indicated the variable of DSP as the strongest predictor of mortality and the BODE index as the most sensitive tool for assessing hospitalization in COPD. Moreover, unintended stop(s) during the 6MWT showed a significant prognostic value regarding the long-term prediction of mortality.⁹⁶

Besides the 6MWD, the use and evaluation of several 6MWT-derived variables increases the total prognostic value of the 6MWT and may facilitate the clinical decision-making process. Specifically, several 6MWT-derived variables could be taken into account and assist clinicians in selecting patients who could most benefit from a clinical intervention and thus substantially increase life expectancy. Indeed, the appropriate selection of patients plays a key role in the success of a therapeutic intervention and the 6MWT-derived variables could be evaluated in this direction. It is known that patients with reduced exercise capacity who experience less ventilatory limitation to exercise and more reduced respiratory and peripheral muscle strength could be more likely to improve with exercise training.⁹⁷ Subsequently, it may be reasonable that among patients with similarly low 6MWD, those with higher DSP, 6MWW and without unintended stop(s) are more likely to benefit from an exercise intervention and, therefore, may have better prognosis. Nevertheless, future research is needed to investigate the role of 6MWT-derived variables in the evaluation of the effect of a clinical intervention (such as a PR program, pharmacotherapy, chest surgery etc.) in terms of prognosis in COPD.

In accordance with previous studies^{81, 98-100}, the variables of 6MWD, 6MWSpeed, 6MWW, DSP, the BODE index¹⁰¹, and the presence of EID demonstrated an independent prognostic value on overall mortality and hospitalization. Next to the 6MWD, the supplementary use of DSP and BODE index could be strongly advised to clinicians and health professionals as independent variables to estimate prognosis. Specifically, the 6MWW and DPS can be considered as multidimensional prognostic variables of the 6MWT combining walking distance with the effect of patients' body weight and oxygen desaturation during walking, respectively.^{4, 99} Moreover, a statistical adjustment of those variables with a number of certain physiological and clinical characteristics including age, sex, BMI, FEV₁, FEV₁/FVC, SGRQ, emphysema, and smoking, revealed also that DSP and BODE index remained significant and the strongest predictors of mortality and hospitalization, respectively.

Intriguingly, even though the BODE index was shown as the strongest predictor of hospitalization, it did not remain significant in the prediction of overall mortality after

the aforementioned logistical adjustment. The crude association of the BODE index with mortality might likely fail to reflect a causal association due to confounding by the variable of SGRQ.^{102, 103} This information is novel as previous studies did not included the variable of SGRQ in the adjustment of the BODE index.

Chapter 4 was also the first study demonstrating that the presence of unintended stop(s) during the 6MWT is an independent predictor of overall mortality and hospitalization. Previously, Wong and colleagues have reported that one in 5 individuals with chronic lung disease rest during the 6MWT.¹⁰⁴ Several factors can intercept the constant walking of patients during the 6MWT.¹⁰⁵ Most common factor is the increased perception of dyspnea while a number of patients also report leg-fatigue as a limiting factor of walking capacity during the 6MWT.¹⁰⁶ Interestingly, unintended stop(s), as an independent variable, remained significantly predictive of mortality into patients' groups assigned by impaired 6MWT-derived variables (6MWD $\leq$ 334m, 6MWSpeed $\leq$ 0.9m/s, 6MWW $\leq$ 20000 m/kg, DSP $\leq$ 290m%, EID) and by increased score of the BODE index ($\geq$ 4points). Clinicians should be aware that unintended stop(s) during the 6MWT may reflect an overall impaired health status beyond the FEV₁ or other respiratory indices and may be clinically relevant in medical decision making in regards to prognosis in COPD patients.

Herein, it is important to remark that findings of chapter 4 could have a clinical implication not only to patients with COPD but also to elderly with underlying comorbidities or to other clinical populations. Especially in the elderly characterized by an increased prevalence of comorbidities (most common cardiovascular, metabolic, rheumatologic or combination of thereof) significant differences can be found in functional exercise capacity according to health status¹⁰⁷, that can be reflected in 6MWD, 6MWSpeed, 6MWW and unintended stop(s). Moreover, taking into consideration that both 6MWD and EID are valuable indicators of exercise capacity and mortality in various pulmonary and non-pulmonary diseases¹⁰⁸⁻¹¹¹, it is reasonable to hypothesise that 6MWT-derived variables as proposed in chapter 4, could be also evaluated in other clinical populations in which exercise capacity is associated with clinical manifestations. Indeed, the findings of chapter 4 could be used as a model to further explore the utility of 6MWT in patients or individuals that can be stratified based on physiological responses during walking.

DETERMINANTS OF EXERCISE-INDUCED OXYGEN DESATURATION IN 6MWT AND THE ROLE OF PULMONARY EMPHYSEMA

Exercise induced oxygen desaturation (EID) is frequently observed in patients with advanced COPD and is predictive of poorer clinical outcome. Several pathophysiological mechanisms and characteristics have been postulated to explain the occurrence of

EID.¹¹²⁻¹¹⁷ Patients who exhibit EID usually present a variety of clinical findings including lower levels of FEV₁ and DLCO^{114, 115, 117-120} with predominant emphysema phenotype¹²¹, worse blood gases (PaO₂)^{120, 122, 123} and higher dyspnea (MRC)¹²⁴ at rest compared to those without EID. A limited number of studies in COPD have shown a relationship between EID and pulmonary emphysema which is characterized by impairment or destruction of alveoli and lung tissue involved in gas exchange.^{125, 126} Fewer alveoli, impaired lung tissue and damage to the pulmonary vasculature are associated with lower levels of DLCO and, therefore, less oxygen availability into the bloodstream which can result in ventilation/perfusion (V'/Q') mismatch.¹²⁷⁻¹³¹ The inequality of V'/Q' due to pulmonary emphysema and small airways disease, can be detected even in patients with mild COPD¹³², but appears to increase with the progress of disease severity.¹³³

Chapter 5 showed that about 20% of subjects with clinically stable COPD, who were included in the ECLIPSE cohort, exhibited EID while occurrence of EID was associated with the degree of lung-density defined emphysema, severity of airflow limitation, baseline arterial oxygen saturation, and obesity. Almost half of the patients in GOLD stage IV and one out of 3 patients with severe to very severe pulmonary emphysema exhibited EID. Moreover, patients with EID in GOLD stage II had higher odds to have moderate or worse emphysema compared those with EID in GOLD stage III-IV.¹³⁴

Patients with pulmonary emphysema usually present severe lung function impairment^{135, 136} and particularly a decreased DLCO, which is attributed to the widespread loss of the pulmonary capillary bed.^{137, 138} Previously, a relationship between DLCO and the degree of emphysema on lung CT scans has been reported^{139, 140} and a fair inverse correlation ($r = -0.63$, $p < 0.001$) between DLCO and the volume fraction of the lung with a density <950 Hounsfield units (i.e. percentage of lung affected by emphysema) has been demonstrated.¹⁴¹ The alveolar tissue destruction as well as V'/Q' inequality can contribute to the occurrence of EID.^{133, 142} In chapter 5, the percentages of EID were progressively increased across the degrees of emphysema. However, pulmonary emphysema as a part of pathophysiology in COPD is confounded by the airflow limitation which also contributes to EID. The degrees of emphysema along with the severity of airflow limitation as defined by FEV₁ or GOLD stages can provide clinicians and health professionals with information about the risks of EID in patients with COPD.

Interestingly, obesity was also identified as an additional determinant of EID in COPD. In order to appreciate the impact of obesity on lung function and pulse oxymetry in COPD, it is important to understand the impact of excessive fat mass on these outcomes *per se*. Isolated obesity is associated with reduced static lung volumes and normal to supraphysiological diffusion capacity.^{143, 144} Reduced ventilatory capacity may result in alveolar ventilation/perfusion inequalities and decreased resting oxygen saturation in isolated obesity.¹⁴³ In patients with co-occurring obesity and COPD, the degree of static hyperinflation is less pronounced than in normal weight COPD patients

with comparable FEV₁, age and same sex¹⁴⁵, while there is no relevant impact of increasing BMI on resting oxygen saturation.¹⁴⁶ Maatman and colleagues using several well-implemented exercise testing protocols in COPD, observed a significantly more pronounced EID during walking in obese female COPD patients, compared to non-obese female COPD patients.²⁵ No difference was observed in male patients or in the same patients when a cycling test, as weight-supported exercise, was performed. The results of the present study partially confirm this finding, suggesting that obesity is a risk factor for EID during weight-bearing exercise in COPD. The observed difference according to sex of patients warrants further investigation, but might be sex-related differences in excessive fat mass distribution.¹⁴³

Chapter 5, also investigated for first time the role of the ADO index⁹¹ in predicting EID in COPD. The ADO index is a multidimensional index including age, dyspnea rate by MRC and FEV₁ (%). According to the findings of Chapter 5, the ADO index may be recommended as an additional indicative index for the risk of EID during 6MWT. Especially, emphysematous patients with high ADO index should be monitored for oxygen desaturation.¹³⁴ In addition; chapter 5 demonstrated that a combination of clinical characteristics could be used to prioritize the assessment for the detection of EID in those patients who are at high risk of EID prior to the participation on PR program. Indeed, a combination of certain lung function characteristics and resting oxygen saturation for the prediction of EID revealed that the highest proportion of patients (81%) who exhibit EID are characterized by moderate to very severe pulmonary emphysema, obesity, impaired FEV₁ and low resting SpO₂ pre-walk values. The use of statistical models including several patient characteristics, as has been already suggested¹²³, can be a useful approach for obtaining fast information along the clinical decision-making process in patients with COPD.

EXERCISE-INDUCED OXYGEN DESATURATION IN NON-HYPOXEMIC PATIENTS

Patients without hypoxemia at rest (non-hypoxemic) are characterized by baseline oxygen saturation more than 88%. Oxygen saturation can be measured by pulse oxymetry (SpO₂), which is an indirect, non-invasive method for assessing arterial oxygen tension (PaO₂) and can detect EID.^{147, 148} EID means that some non-hypoxemic patients with COPD are exposed intermittently to hypoxemia (SpO₂ ≤88%) when performing exercise. Besides the increase of 6MWD, improvements in COPD can be manifested by attenuation of exercise-induced decline of SpO₂ preventing thus the occurrence of EID.¹⁴⁹

Chapter 6 revealed that baseline SpO₂ cannot predict accurately the EID. A threshold of 95% at baseline SpO₂ is not adequate to detect a potential EID during the 6MWT in

patients with COPD, as was suggested by Knower and colleagues.¹⁵⁰ However, the inclusion of additional patients' characteristics (such as sex, FEV₁, DLCO, and baseline PaO₂) found to refine the prediction of EID. It was demonstrated that 39% of non-hypoxemic patients with COPD with GOLD stages II-IV, who were referred for Pulmonary Rehabilitation (PR), exhibited EID during the 6MWT.¹⁵¹

The detection of EID is useful for prognosis but can also provide clinicians information on the SpO₂ trend during daily life of patients with COPD. Evidence has shown that patients who exhibit EID during the first minute of the 6MWT, the "early desaturators", have increased risk to desaturate during activities of daily living. In contrast, those who desaturate after the 3.5 minutes in the 6MWT, the "late desaturators", have lower risk to manifest desaturation in their daily life activities.¹⁵² Moreover, non-hypoxemic patients at rest but with substantial and more rapid EID during the 6MWT will require Long-Term Oxygen Therapy (LTOT) sooner than those with a more late-onset EID. Nowadays, a decline of SpO₂ ≤88% (often followed by a reduction of ≥4% from the baseline) during physical activity is considered as an indication for prescription and reimbursement of continuous oxygen use by Centres for Medicare & Medicaid Services in patients with COPD who are non-hypoxemic at rest. Therefore, the 39% of patients in chapter 6, who exhibited EID, may be suitable candidates for LTOT.¹⁵³ This higher prevalence of EID compared to the ECLIPSE cohort (39% vs 21%) may be explained by the fact that those patients were referred for PR program, which indicates that they had more evident symptoms of COPD.

The correction of EID during exercise or physical activity by LTOT can result in alleviation of dyspnea, increase of physical condition and might contribute to more effective PR programs by allowing more intensive exercise training.^{154, 155} However, the question of whether or not clinicians should administer oxygen to patients at home cannot be easily answered from a 6MWT solely. For instance, a patient with EID during the 6MWT may not reach desaturation at home in case of low intensity physical activity. Indeed, a number of patients may not present EID outside the hospital even though they were detected with EID during the 6MWT.¹⁵⁶ In these cases, SpO₂ measurement during laboratory or field exercise tests is not representative of the SpO₂ in the home setting. However, only the possibility of functional and clinical improvements (such as exercise tolerance, quality of life, survival and hospitalization rate) can provide an important incentive for the use of LTOT in patients with COPD.¹⁵⁷ Considering also that evidence of "harm" induced by relatively modest fraction of inspired oxygen (FiO₂) is absent, the LTOT remains recommended.¹⁵⁸

The detection of EID in clinical settings remains important because it is associated with higher mortality rates^{4, 98, 99, 159} and impaired daily physical activity.¹⁶⁰ The suggested methodological approach for obtaining rapid information about the occurrence of EID after considering an alarm SpO₂ threshold of 95% in pulse oxymetry at baseline¹⁵⁰ is not accurate according to the findings of chapter 6. In fact, measuring

oxygen saturation by pulse oxymetry is subject to important limitations.¹⁶¹ For instance, values of pulse oxymetry generally have a margin of error ± 2 points of the actual SpO_2 levels and very low blood pressure can affect the pulse oxymetry readings.¹⁶²⁻¹⁶⁴ Furthermore, oxygen saturation may be overestimated in heavy smokers because of high carboxyhaemoglobin levels.¹⁶⁵ In addition, baseline levels of oxygen saturation seems to be independent of the degree of airways obstruction, as quantified by the FEV_1 .¹⁶⁶ On the other hand, FEV_1 is associated with dynamic hyperinflation¹⁶⁷ which, in turns, is related to EID.¹⁶⁸ Low FEV_1 (<45%) seems also to be associated with the magnitude of SpO_2 decline during exercise according to the findings of chapter 6. Therefore, clinicians should not solely rely on baseline SpO_2 values of pulse oxymeter but they should also consider other clinical characteristics, such as FEV_1 , DLCO, baseline PaO_2 in respect to EID as suggested in chapter 6.

An additional question that arises is whether clinicians should use the lowest or the end value of SpO_2 during the 6MWT for the detection of EID. It is demonstrated that the end value of SpO_2 may lead to an underestimation of the number of patients with EID. Some patients, who have marginally reached $\text{SpO}_2 \leq 88\%$ during 6MWT, may perform unintended stop(s) during the 6MWT or potentially reduce the effort at the end of the exertion allowing a small increase of SpO_2 levels above the threshold of 88% which defines the EID. Even though these cases are not prevalent, the lowest value of SpO_2 should be ideally used for the detection of all patients with potential EID. Therefore, the continuous recording of SpO_2 during the 6MWT should be recommended.

In chapter 6, a predictive logistical model of EID including variables of FEV_1 , DLCO, PaO_2 and baseline SpO_2 was used to detect high risk patients for EID. Interestingly, sex had also an influence on the occurrence of EID: female gender was associated with more prevalent EID during the 6MWT. According to physiological characteristics of women compared to men, women have smaller lungs, reduced lung diffusion (DLCO), and lower maximal oxygen consumption (VO_2max) compared to age- and height-matched men.¹⁶⁹ Furthermore, a study determining reference values for arterial blood gases has demonstrated higher values of PaO_2 in elderly men than in elderly women.¹⁷⁰ This could only partly explain the influence of sex on EID. However, further studies are needed to reproduce and explain these sex-related differences in EID.

CARBON-DIOXIDE PARTIAL PRESSURE IN 6MWT AND THE EXERCISE-INDUCED HYPERCAPNIA

Transcutaneous carbon dioxide partial pressure (TcPCO_2) can be reliably measured providing indirect information on alveolar ventilation performance.¹⁷¹⁻¹⁷⁴ Patients with COPD may exhibit CO_2 retention during exercise to hypercapnic CO_2 levels ($\text{PtCO}_2 > 46\text{mmHg}$). Exercise-induced hypercapnia (EIH) can reflect integrated alterations in

minute ventilation (VE), physiologic dead space (VD/VT), and carbon dioxide output as determined by alveolar ventilation.¹²²

Chapter 7 demonstrated a high heterogeneity of $T_c\text{PCO}_2$ levels in response to 6MWT. Patients who exhibited a substantial CO_2 retention ($T_c\text{PCO}_2 [\Delta] >4$ mmHg from baseline) during exercise had higher pre-walk dyspnea and worse airflow limitation (CO_2 retainers). Patients who reached the hypercapnic CO_2 levels during exercise (EIH: $T_c\text{PCO}_2 >46$ mmHg) were characterized by higher pre-walk dyspnea and increased baseline levels of capillary carbon-dioxide pressure (PcCO_2). In addition, it seems that overweight-BMI has a protective effect against EIH in very severe COPD.¹⁷⁵

In chapter 7, a high variability in the development of $T_c\text{PCO}_2$ during the transition from rest to exercise was detected in agreement with previous study.¹²² O'Donnell and colleagues reported that that retention of CO_2 often occurs during exercise in advanced COPD, even when is not present at rest, and it can be a sign of inadequate ventilation.¹²² CO_2 retention and/or EIH during the 6MWT could also imply a number of certain pathophysiological conditions such as dynamic hyperinflation, reduced gas exchange capabilities (ventilation/perfusion inequality), ventilatory abnormalities such as increased VD/VT, and more rarely the opening of right to left shunt.^{122, 176} Regardless the factors that trigger CO_2 retention, the increase of PCO_2 during exercise amplifies dyspnea by increased activation of central chemoreceptors. The ability to deal with increasing CO_2 during exercise is usually dependent on the degree of FEV_1 , ventilation rate and the inherent sensitivity to carbon dioxide.^{177, 178}

In chapter 7, half of the patients (50%) with very severe COPD demonstrated CO_2 retention, whereas 31% of total population exhibited EIH during the 6MWT. Exercise-induced CO_2 retention was found to be determined by very severe airflow limitation ($\text{FEV}_1 \leq 36\%$) and increased baseline dyspnea. Indeed, very impaired FEV_1 can cause air-trapping generating dynamic hyperinflation which, in turns, is related with the occurrence of CO_2 retention during exercise in COPD.¹⁷⁹ At increased respiratory rates during exercise, an inversely proportional shortening in expiratory time may be occurred and lead to the development of dynamic hyperinflation in COPD.¹⁸⁰ Dynamic hyperinflation can cause lung mechanic dysfunctions resulting in CO_2 retention, while it has also been implicated as major determinant of dyspnea.¹⁸¹ Nevertheless, some patients with severely impaired FEV_1 did not exhibit EIH. In chapter 7, the occurrence of EIH found to be much dependent on baseline PCO_2 levels which are mainly related to impaired gas exchange and/or reduce drive to breathe at rest (low sensitivity to CO_2).^{182, 183} Indeed, patients with increased baseline PCO_2 levels (≥ 41 mmHg) had higher odds to exhibit EIH according to the findings of chapter 7.

Other factors such as inspiratory muscle weakness are also associated with CO_2 retention which can lead to the occurrence of EIH in COPD.^{122, 184} In chapter 7, patients with EIH presented lower peak inspiratory mouth-pressure (Pi max) indicating greater inspiratory muscle weakness compared to non-EIH. Also, the evaluation of Pi max as

determinant of CO₂ retention and EIH showed a strong tendency towards statistical significance ($p=0.051$ and $p=0.050$, respectively). It seems that CO₂ retention due to muscle loading occurs at very low values of $P_{i\max}$. Otherwise, inspiratory muscle weakness is associated with alveolar hypoventilation contributing to CO₂ retention and EIH when the inspiratory loads are high due to static or dynamic hyperinflation.¹⁸⁵ Particularly, the unbalanced inspiratory muscle loading-to-strength ratio at exertion likely due to impaired breathing pattern and/or the occurrence of dynamic hyperinflation can trigger the signal for the integrated response that leads to rapid and shallow breathing contributing to EIH in patients with COPD.¹⁸⁵⁻¹⁸⁷ Shallow breathing presumably represents a key protective mechanism to prevent respiratory muscle fatigue ("wise fighter" hypothesis), however at the price of an insufficient alveolar ventilation.^{188, 189} Mechanical factors may substantially constrain the ventilatory response to the metabolically generated CO₂ in such a way that PCO₂ increases (i.e., compromised respiratory compensation).¹⁹⁰ Indeed, severe mechanical restriction secondary to dynamic hyperinflation and increased respiratory muscle work has been recognized as a contributory factor to EIH in COPD.^{122, 142}

In consistency with previous findings^{145, 191-194}, chapter 7 also demonstrated a protective role of overweight-BMI against the occurrence of EIH in patients with advanced COPD. Although the higher body surface area in overweight or obese patients can result in higher CO₂ production as a result of large metabolically active muscle mass¹⁹⁵, it can also lead to lower static and dynamic hyperinflation and to lower mechanical loading during exercise.^{192, 193} Moreover, the inspiratory capacity as related to total lung capacity (IC/TLC ratio) is higher in overweight or obese compared to patients with a normal range BMI.^{192, 193} These mechanical effects in higher BMI reduce operating lung volumes at rest and throughout exercise¹⁴⁵ and may have favorable influence on PCO₂ levels, especially during effort. In addition, altered metabolic loading at exertion in patients with higher BMI can stimulate an increase in minute ventilation during exercise compared to those with normal-ranged BMI and thus may prevent the occurrence of EIH.^{196, 197}

Chapter 7 did not detect a relationship between CO₂ retention/EIH and EID; however, it should be reported that the majority (79%) of studied patients used oxygen during the 6MWT and this may have diminished the extent of SpO₂ decline contributing to the lack of association between EIH and EID. Evidence has shown that changes in T_cPCO₂ have a fair negative correlation with the magnitude of SpO₂ decline.¹²² Changes in T_cPCO₂ and SpO₂ are dependent on global gas exchange, cardiac output, oxygen content and are also influenced by regional blood flow and oxygen content.¹⁹⁸ Nevertheless, different shapes of the oxygen and carbon dioxide dissociation curves determine the peak and lowest values of T_cPCO₂ and SpO₂.¹⁹⁹ Also, the use of supplemental oxygen in the vast majority of studied patients may diminish the extent of SpO₂ decline and thus the presence of EID and this may attributed for the lack of

association between CO₂ retention, EIH and the occurrence of EID. Chapter 7 also indicated that CO₂ retention and EIH do not have significant impact on the 6MWD. This is in consistence with the observation of Montes de Oca and Celli¹⁷⁸ who reported that exercise capacity is similarly reduced in eucapnic and hypercapnic patients with severe stable COPD, even though hypercapnic patients achieve lower ventilation during exercise.¹⁷⁸

Interpreting the findings of Chapter 7 from a clinical perspective, the occurrence of CO₂ retention and EIH during the 6MWT may represent a marker of disease severity that is beyond the walking capacity. In other words, the case of a patient who did not improve significantly the 6MWD but prevented the high accumulation of CO₂ and/or the occurrence of EIH in 6MWT should be considered as beneficial. Less increase in PCO₂ levels during 6MWT may reflect improvement in respiratory capability at exertion, which, in turns, can be related to a better prognosis, irrespectively from the distance walked. Therefore, monitoring of T_cPCO₂ during walking, especially in severe COPD could be recommended as it may provide clinicians with additional clinical information. Moreover, monitoring of T_cPCO₂ during walking could also be useful to evaluate the impact of inspiratory muscle training (IMT) and/or the effect of non-invasive ventilation (NIV) to counteract respiratory muscle weakness and EIH. Furthermore, a future perspective on exercise-induced PCO₂ changes from baseline levels during the 6MWT or other exercise test in respect to mortality, hospitalization, and other deteriorations such as cognitive deficits during the progress of disease severity, can be suggested for COPD patients.

METHODOLOGICAL CONSIDERATIONS

The present thesis has some important limitations that should be considered in interpreting the results. As general drawback, this thesis includes some retrospective cohort studies (Chapters 2, 3, 4, 5) which were constructed from databases of healthcare records that have been collected in the past. There were a few consequences attributed to the retrospective collection of the data. Some of the records were incomplete without knowing the exact reason of lacking data, which may be either due to the failure of the patient to complete the initial PR assessment or due to the refusal to perform a particular test. Some were also repeat evaluations. Moreover, many different healthcare professionals would have been involved in data recording and therefore the measurement of risk factors and outcomes would probably be not as highly accurate as in a prospective research design. Furthermore, the retrospective analyses in this thesis are subject to unidentified confounders associated with particular tests and/or evaluations. Also, selection bias could occurred if the selected cohorts were not representative of all possible patients with COPD.²⁰⁰

However, the large size of the cohorts analysed retrospectively in this thesis reduces the confounding effects.

There are also certain limitations regarding to the specific aims of each chapter included in this thesis. In chapter 2, it should be noted that the inclusion of patients' population referred for PR program could result in a referral bias towards patients with a greater degree of limitation in clinical care. Nevertheless, the fixed proportion of WR peak in exercise assessment does not provide identical physiological stress among patients and this would be also similar to the patients with more severe COPD. In chapters 4 and 5, a critical criticism could focus on the fact that patients performed only one 6MWT instead of two as recommended by ERS/ATS technical standards.^{1,2} This fact may have resulted in a slightly lower 6MWD, as usually the second 6MWT is longer in about 80% of patients with COPD¹, and consequently in lower 6MWT-derived outcomes (6MWW, DSP) and changes in SpO₂ during walking. Furthermore, the EID was defined based on the SpO₂ at the end of the 6MWT and not the SpO₂ nadir for both chapters 4 and 5, which can lead to an underestimation of the number of patients who exhibit EID. However, this bias is believed that is relatively low as SpO₂ at the end of the 6MWT is similar with the nadir values with differences ranging from 1% to 10%.²⁰¹ Chapter 6 included patients consisted of a convenience sample, while in chapter 7 there was no possibility to acquire respiratory measurements such as ventilation, tidal flow-volume loops, end-tidal PCO₂ and thus the detection of dynamic hyperinflation were not performed during walking but only assumed. Moreover, there was no detailed assessment of dynamic lung mechanics, which would have provided us with important information for patients' respiratory profile during the 6MWT.

Despite these methodological limitations, this thesis contributes with important clinical information about the 6MWT and the 6MWT-derived variables. Due to the large size populations, which are included in analyses, the findings of each chapter could lead to concrete conclusion that enhance the use of the 6MWT in daily clinical routine.

CONCLUSIONS, FUTURE PERSPECTIVES AND RECOMMENDATIONS FOR RESEARCH

In summary, this thesis has shown that 6MWT is a useful clinical tool providing important clinical information well beyond the measure of 6MWD. It is sufficient to provide concrete exercise outcomes based on multidimensional 6MWT-derived variables and to detect the occurrence of oxygen desaturation better than CET. Additionally, the 6MWT has a considerable prognostic value which is enhanced using not only the 6MWD but also other 6MWT-derived variables. Clinicians and health professionals can have a robust prediction in regards to mortality and hospitalization evaluating the DSP and BODE index, respectively. Moreover, the understanding of the

determinant of EID during the 6MWT and the role of pulmonary emphysema in oxygen desaturation can increase the interpretation of the occurrence of EID during 6MWT. The pathophysiological mechanism of EID can be caused by low FEV_1 , $DLCO$, and baseline PaO_2 when the metabolic demands are increasing during walking. On the other hand, EID in patients with mild COPD could imply the existence of pulmonary emphysema. Clinically significant information about the respiratory impairment can also be derived from the monitoring of PCO_2 during 6MWT and the detection of PCO_2 trends as an additional marker of disease severity. Indeed, the utilization of all the clinical information that 6MWT is possible to provide is crucial for the clinical decision-making process in COPD.

Future research is necessary to be able to acquire all the possible clinical information from a 6MWT. Indeed, this thesis increased the utility of the 6MWT by expanding the knowledge for several 6MWT-derived variables. Some of them were already considered in previous research studies but some also were newly analyzed and proposed by this thesis. A step forward has been done; however, more future research is needed especially regarding variable such as the levels of PCO_2 during the 6MWT. The changes in PCO_2 during the 6MWT constitute a variable that is usually neglected in clinical settings. The constant monitoring of PCO_2 during the 6MWT is rarely performed even though it could provide clinicians with important information about the respiratory sufficiency and detect patients at high risk for EIH. Moreover, PCO_2 is the fundamental regulator of cerebral blood flow and, therefore, disturbance of its levels during the 6MWT could be also related to the increased rates of cognitive dysfunction in COPD. Indeed, PCO_2 trends or changes in several multidimensional variables derived from a 6MWT may be able to determine not only the risks for mortality or hospitalization but also the risk for the development of certain comorbidities.

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Chapter 8

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Summary

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by persistent airflow limitation and impaired exercise capacity. Chronic bronchitis and pulmonary emphysema are the two main conditions included in COPD. Patients with COPD demonstrate a generally impaired health status and have increase rates of morbidity and mortality. Dyspnea is the cardinal symptom in COPD, which results in impaired exercise capacity and has a negative impact on daily life activities and quality of life. The perception of dyspnea is exaggerated in those patients during physical activity or exercise due to reduced breathing efficiency that results from the deteriorating ventilatory mechanics and the increased ventilatory requirement. Moreover, pathophysiological effects of cardiovascular and peripheral muscle abnormalities can further explain and intensify dyspnea as well as increased muscle fatigue in COPD. As a consequence, exercise capacity in those patients is symptom-limited and determined by a complex interaction of several pathophysiological factors in COPD. Therefore, exercise capacity can reflect the impact of disease severity on patients with COPD and can provide clinicians and health professionals with important clinical information.

Clinical exercise testing has become an important clinical tool to assess and evaluate exercise capacity and predict outcomes in patients with COPD. Therefore, this thesis focused on field exercise testing and specifically on the six-minute walk test (6MWT) aiming at extending the clinical information derived from the 6MWT and at increasing test utilization in clinical practice. After describing the overall burden of COPD disease and its negative impact on exercise capacity in patients, **Chapter 1** introduced the field tests and demonstrated the clinical and prognostic value of the 6MWT as reliable clinical instrument according to the recent evidences in literature. Indeed, 6MWT has been shown to provide useful information for the impact of disease severity in patients' functional status, the effectiveness of therapeutic interventions and can predict mortality and hospitalization. The main outcome of the 6MWT is the six-minute walk distance (6MWD) which represents exercise capacity and can change after therapeutic intervention or across the progress of disease severity influencing the clinical decision-making process.

Several determinants of exercise performance can affect 6MWD. **Chapter 2** aimed at investigating the characteristics of functional exercise performance and determinants of the cycle endurance test (CET) and 6MWT in a large clinical cohort of COPD patients. A retrospective analysis in a large dataset of 2050 patients demonstrated different determinants of exercise performance between CET and 6MWT. The main findings were that the overall perception of dyspnea and sex-related differences determine the CET- T_{end} but BMI, FEV₁ and FRC influence the 6MWD. Chapter 2 suggested that CET and 6MWT are not interchangeable exercise tests; gender difference should temper the interpretation of CET and that even though the fixed proportion of workload capacity does not provide the same physiological stress among patients, improvement in CET-

T_{end} after a therapeutic intervention such as a pulmonary rehabilitation (PR) program are responsive of improvements in clinical status of patients with COPD.

Several reference equations for the 6MWT have been formulated to facilitate the interpretation of the walked 6MWD and help to estimate the degree of exercise tolerance in patients. However, applying different reference equations could result in different 6MWD values of predicted leading to misinterpretation of the levels of exercise capacity or improvements of physical performance after therapeutic interventions. Therefore, **Chapter 3** investigated the impact of several 6MWD reference equations and factors accountable for different 6MWD %predicted values in a large dataset of 2757 patients with COPD. Chapter 3 reported that existing 6MWD reference equations will give varying results and highlighted the importance of the selection of an appropriate reference equation. It suggested that the choice of 6MWD reference equation should consider the consistency 6MWT operating procedures and at least be specific for the country/region of origin.

Besides the interpretation of 6MWD, the estimation of prognosis according to walking performance is clinically important. In addition to the 6MWD, other 6MWT derived variables, such as mean walk-speed (6MWSpeed), 6-min walk-work (6MWW), distance-saturation product (DSP), exercise-induced oxygen desaturation (EID), and unintended stops seems to be useful for the prediction of mortality and hospitalization in COPD. **Chapter 4**, explored the association between 6MWT-derived variables and mortality as well as hospitalization and compared it with the BODE index using the ECLIPSE cohorts in a three-year follow-up. Cox's proportional-hazard regressions were performed to estimate 3-year mortality and hospitalization and demonstrated that the 6MWT-derived variables have an additional predictive value of mortality in patients with COPD. Chapter 4, indicated the DSP as the strongest predictor of mortality and the BODE index as the most sensitive tool for assessing the risk of hospitalization while suggested that unintended stop(s) during the 6MWT can refine the prognosis of mortality in COPD.

Impaired gas exchange can produce varying degrees of V'/Q' inequalities, diffusion impairment, and hypoxemia during exercise. **Chapter 5**, aimed at investigating the prevalence of exercise-induced desaturation (EID), the relative-weight of several physiological determinants of EID including pulmonary emphysema, and the relationship of EID with certain patients' clinical characteristics using a dataset of 2050 COPD patients from the ECLIPSE cohort. Chapter 5 demonstrated that 21% of patients exhibited EID in the ECLIPSE cohort and showed that advanced emphysema, obesity, severe airflow limitation, and low resting oxygen saturation are associated with EID. Chapter 5 also suggested that emphysematous patients with high ADO-score should be monitored for EID.

In clinical practice, several screening tests have been proposed to predict EID, including FEV₁, DLCO and baseline-SpO₂. **Chapter 6** aimed to validate a proposed cut-off

of baseline-SpO₂ ≤95% as simple screening procedure to predict EID during 6MWT and investigated the prevalence and characteristics of patients exhibited EID to SpO₂nadir ≤88% using 402 non-hypoxemic COPD patients referred for pulmonary rehabilitation (PR). Chapter 6 reported that 39% of those patients, who were referred for PR, exhibited EID and that those patients may be suitable candidates for ambulatory oxygen therapy. Chapter 6 did not corroborate baseline SpO₂ ≤88% as highly accurate in predicting EID, and thus concluded that baseline SpO₂ solely is not adequate to predict EID. In contrast, a combination of several clinical characteristics including DLCO, FEV₁, PaO₂, baseline-SpO₂, and sex, increases substantially the odds for EID and can facilitate the prediction of EID in COPD.

Besides the EID, the variable of carbon-dioxide partial pressure (PCO₂) has been also reported as prognostic factor in COPD. **Chapter 7**, aimed to define patterns of transcutaneous carbon-dioxide partial-pressure (T_cPCO₂) trends during 6MWT and to study determinants of CO₂-retention and exercise-induced hypercapnia (EIH) using a group of 62 patients with very severe COPD. Chapter 7 demonstrated that 50% of the patients with COPD exhibited CO₂-retention, 26% preserved and 24% reduced T_cPCO₂ levels while in all group 31% patients presented EIH during the 6MWT. Chapter 7 also showed that even though PCO₂ response to 6MWT is highly heterogeneous, a very low FEV₁ and increased baseline-P_cCO₂ together with pre-walk dyspnea increase the risk for CO₂-retention and EIH. Moreover, chapter 7 revealed that an overweight BMI seems to carry a protective effect against EIH in very severe COPD.

Valorization

Valorization is the process by which scientific knowledge can shape and influence the world and have practical application. Knowledge valorization specifically refers to “getting the maximum value and usefulness out of education programs and managed projects, by generalizing what has been learnt from the specialist experiences to other, related fields”.

Based on this definition, this chapter will discuss the current findings of this thesis in the light of the social and economic impact as well as innovative concepts for future health care.

RELEVANCE AND INNOVATION

COPD is highly prevalent disease and a leading cause of morbidity and mortality worldwide. Besides the impairment of pulmonary function, several other factors influence the impact and the evolution of COPD. These factors include the occurrence of hypoxemia or hypercapnia at exertion, limiting functional dyspnea and diminished exercise capacity that are associated with higher risk of mortality and hospitalization. Indeed, the degree of exercise tolerance and pathophysiological response to exercise facilitate the prognosis and can constitute an essential dimension in the evaluation of COPD patients. Clear exercise outcomes and their appropriate interpretation can play a superior role in COPD management and treatment in clinical practice.

This thesis provided new evidence in this field of expertise:

In chapter 2, a comparison between two popular tests of exercise assessment (CET and the 6MWT) in clinical practice revealed different determinants of exercise capacity between CET and the 6MWT. According to this, CET and 6MWT are not interchangeable. Patients’ clinical characteristics should be considered in the interpretation of exercise outcomes derived from CET or 6MWT. Indeed, the new knowledge reported in this thesis facilitates the choice of exercise test and the interpretation of the exercise outcomes which have become an integral part of the evaluation and response to treatment in COPD.

Chapter 3 addresses an important concern about the choice of the appropriate 6MWT reference equation. The right interpretation of walking performance according to the estimation and inference for normative values is great of importance for both health professionals and patients. For health professionals, the estimation of walking capacity using reference values can represent the severity of patients’ exercise intolerance and thus influence on the clinical decision. In addition, reference values are critical to tailor appropriately timed exercise interventions indicating the high importance of choosing the right reference equation. For the patients, walking capacity

expressed as percentage of predicted, can be used to increase the awareness about the impact of disease severity on their exercise capacity.

Chapter 4 investigated the prognostic value of existing and new variables derived from the six-minute walk test in patients with COPD. This knowledge can increase the utilization of the 6MWT in praxis and provide health professionals with insight for the prognostic value of each 6MWT-derived variable. Moreover, the demonstrated prognostic value of the 6MWT-derived variable can also be considered in the clinical decision-making process.

In chapter 5, the relationship of EID with certain patients' clinical characteristics including pulmonary emphysema and the relative-weight of these determinants on the occurrence of EID was demonstrated. This knowledge provides health professionals with a better understanding about the pathophysiological interaction of several determinants of EID and the risk factors for the occurrence of EID in COPD patients with different degrees of pulmonary emphysema. Practically, health professionals could partly estimate the risks for the occurrence of EID during the 6MWT based on patients' two basic characteristics which are the degree of pulmonary emphysema and lung obstruction severity according to the data presented in this chapter.

Chapter 6 reported that baseline oxygen saturation levels solely are inaccurate to predict EID, while it demonstrated a combination of clinical characteristics that increases the odds for EID in COPD. Indeed, this is practical knowledge for health professionals who could expect based on certain patients' characteristics the occurrence of EID prior to exercise testing. Also, it can provide health professionals with rapid clinical information about the risk of EID in cases that the application of a 6MWT is not instantly possible.

Chapter 7 demonstrated the high heterogeneity of PCO_2 responses during the 6MWT and the determinants of CO_2 retention and EIH in COPD patients. Indeed, PCO_2 response during the 6MWT may represent a marker of disease severity that is beyond the walking capacity. Practically, health professionals could assess PCO_2 trends during the 6MWT and specifically the degree of CO_2 retention during walking for the evaluation of therapeutic interventions such as inspiratory muscle training (MIT) or consider the efficiency of non-invasive ventilation in COPD. Moreover, the findings of this chapter could suggest the transcutaneous monitoring of CO_2 during 6MWT for the identification of patients who need special attention regarding to EIH.

TARGET GROUPS

Health care professionals

The main target group of this thesis is health-care professionals including mostly physicians, exercise physiologists, physiotherapists, and nurses. The findings of this thesis provided further insight of the widely-used field test of the 6MWT and the COPD pathophysiological responses to exercise. These findings emphasize the importance of exercise assessment in clinical practice and help to increase the utilization of the 6MWT and the interpretation as well as the prognostic value of the 6MWT-derived variables. Health care professionals are encouraged to assess exercise capacity of the patients and consider exercise outcomes in the clinical decision-making process. Exercise outcomes increase the clinical information of patients' health status, support timely treatment and facilitate the prognosis.

Patients with COPD

It is a consensus that patient is the most important member of the interdisciplinary health care team. Patients can benefit of the output of this thesis increasing their awareness of the disease, understanding the significance and the role of field exercise testing in clinical practice, and realizing the clinical importance of assessing exercise capacity. Indeed, patients who are aware about their disease and their clinical condition are more likely to have better outcomes. In contrast, patients with low levels of awareness are likely to have poor motivation and being not adequately cooperative in therapeutic plans and intervention. The consequences can include low therapeutic efficacy and added medical expenses. Therefore, the knowledge including in this thesis can be used by COPD patients for increasing their awareness about the disease and utilizing actions plans more effectively, which can result in obtaining the most benefits from the therapeutic procedure.

ACTIVITIES AND PRODUCTS

The findings of the studies in this thesis have resulted in numerous scientific abstracts on international congresses all over the world. All the studies have been published in recognized scientific journals in the respiratory field and they have received numerous citations. Data from chapter 2 have been published in the official European Respiratory Society (ERS) statement of the use of exercise testing in the evaluation of interventional efficacy (Puentes-Maestu L. et al. *Eur Respir J* 2016). Moreover, some abstracts of the studies included in this thesis were awarded with travel grants in order to be presented in international congresses in Europe and USA and be the topic of discussions amongst

health-professionals. Considering this publicity, the findings of this study have been widely distributed and may constitute an inspiration for future research questions. Indeed, relative future research is essential as exercise testing can be the cornerstone of chronic patients' clinical evaluation.

Acknowledgements

The ancient Greek philosopher Aristotle had told “All human actions have one or more of these seven causes: chance, nature, compulsion, habit, reason, passion and desire”. Writing this note of thanks as the finishing touch of my thesis, I wish to thank all the people who were behind on my personal causes that making me successfully complete this PhD thesis.

First of all, I would like to express my sincere gratitude to my supervisors Professor Wouters and Professor Spruit (or Martijn, as he always liked to be addressed by his first name). Professor Wouters thank you for being an inspiration for me and for giving me motivation throughout the intense period of my PhD. Your immense knowledge and your guidance were more than valuable. Honestly, I have great respect for you and I really appreciate your support and kindness. Dear Martijn, I want to thank you for all the opportunities I was given to conduct my research during the PhD and for your important advices and instruction for writing good scientific articles and completing my thesis. I consider myself lucky that I learned how to write scientific articles from one of the best on it. Also, I appreciate the fact that you helped me to increase my scientific network introducing me to the best scientists on the respiratory field. Dank u wel!

Dear Professor Vogiatzis, the words are really not enough to express my gratitude to you. My whole research career started from you who gave me the opportunity and trust me to be a member of your research team in Athens. I learned how to do research from you who are one of the most capable and recognizable professors on conducting clinical experiments with patients. Undoubtedly, you are my greatest mentor in research. Your positive influence and your support as co-supervisor provided me with the attributes to complete my thesis. Ευχαριστώ!

Dear Professor Klaus Kenn, thank you for the cooperation regarding one of the studies included in my thesis and your kindness. I am also deeply thankful for the post-PhD chance you gave me agreeing to apply together for the Marie Skłodowska-Curie fellowship and your warm welcome at the Schön klinik in Berchtesgaden! I want also to thank my German colleagues Rainer, Inga, and Tessa for their assistance (especially in the German language!) and teamwork. Danke Schön!

I want also to acknowledge my degree- and assessment- committee members: Prof. G.J. Wesseling, Prof. H.P. Brunner-La Rocca, Prof. Y.F. Heijdra, Prof. J.P. Kooman, Prof. E. Derom, Prof. K. Kenn and Prof. N.G. Koulouris who graciously agreed to serve as members on my PhD committee. I appreciate it! Thank you very much!

I need also to express my sincere appreciation to Ingrid Augustin for her valuable assistance during my PhD period. I am also very thankful to Gerrie Janssen for her help and guidance in organizational issues related to my PhD. Many thanks also go to the great physicians of CIRO+, Dr. Frits Franssen, Dr. Lowie Vanfleteren and Dr. Janssen Daisy for their valuable medical opinion and advices regarding findings of this thesis.

I also appreciate the help and advices of Groenen Miriam in statistics and I also want to say a thank you to Dr. Maurice Sillen for his kindness.

I would also like to thank my CIRO+ colleagues with whom I shared the same room during my PhD for their cooperation and friendliness. Dear Rafael, Sarah, Dionne B., Dionne S., Jeannet, Fionna, Carmen, Nienke, Wai-Yan, Anouk, Esther, Yvonne, Cindy and Coby thank you very much for your support and the nice and friendly atmosphere!

I owe my thanks to all of my co-authors in the studies of this thesis, who helped me to improve the manuscripts with their useful and constructive comments and suggestions. In regards to the ECLIPSE studies included in thesis, special thanks go to Professors Alvar Agusti, Jørgen Vestbo, Bartolome Celli, Peter Calverley, Victor Pinto-Plata, Per Bakke, Stephen Rennard, William MacNee, and to Julie Yates and Ruth Tal-Singer for the critical comments and suggestions.

I would like also to acknowledge the European Respiratory Society (ERS) for the tremendous positive impact on my research career. In 2012, I received an ERS Long-Term Research Fellowship (LTRF 63-2012) that offered me the chance to move from THORAX research center in Athens to CIRO in the Netherlands. This LTRF created the great opportunity to continue at CIRO+ for a PhD. Recently (2016) I was the recipient of an ERS-EU RESPIRE2 Marie Skłodowska-Curie Postdoctoral Research Fellowship – Number MCF (8465)-2015 that allowed me to move from CIRO+ in the Netherlands to the Schön klinik Berchtesgadener Land in Germany for a post-PhD fellowship.

I want to thank Professor Robert Bals for all the given support in the beginning of my ERS LTRF when I moved at CIRO+ center where, afterwards, I continued with the PhD course. Also, I truly appreciate his involvement in Marie Skłodowska-Curie Fellowship as my external mentor. Dear Robert, thank you very much!

I am also deeply thankful to the Director of the THORAX Research Foundation of Intensive and emergency Medicine Professor Charalambos Roussos and to Captain Vassilis Konstantakopoulos (Captain Vassilis & Carmen Foundation) for their valuable support to the first steps of my career in research. Although they were not directly involved in my PhD, they laid the foundation stone for starting and developing my career in research. I really thank you!

A special word of thank I would like to address to Kerstin Fritz for her invaluable support to my life abroad throughout my PhD period. Kerstin, I thank you very much for your motivation, your help and all the precious moments. You are really behind of many personal accomplishments and achievements I did these years. Herzlichen Dank!

Many thanks also go to my friend and colleague Zafeiris and Erica, my Paranympths in PhD defense. Zafeiris, we started doing our first steps in research having a very good cooperation and creating a good friendship! Πολλά ευχαριστώ σε σένα και την Έρρικα!

Also, I need to thank my close Greek friends Alexandros and Spiros as well as my Brazilian friend Rodolfo for their support and friendliness during my PhD period.

Last but not least, my family deserves special thanks for supporting me over the years and for encouraging me to pursue an advance degree. Without my parents Eleni, Dimitris and my sister Marianna, I may never have gotten to where I am today. Ευχαριστώ πολύ!

I would also like to acknowledge my home city Athens. Short trips to Athens during the intensive period of PhD course always helped me to rest and relax so that I could feel energetic again

Curriculum vitae

Vasileios Andrianopoulos (VA) was born on 28th January 1980 in Athens (Greece). In 2003, VA obtained his Bachelor Degree in “Physical Education and Sports Science” from the University of Thessaly, Greece. During his bachelor study, VA was selected by Professor Ioannis Koutentakis to join the University’s research team; thereby he had the opportunity to write a bachelor thesis entitled “Smoking and risk factors for coronary disease in a Greek population over 50 years old”. In 2010, VA completed a master degree with excellent mark in “Exercise and Health” at the University of Thessaly. His thesis was entitled “The effect of expiratory flow limitation on oxygen kinetic of peripheral muscles” and the research measurements were performed at Thorax Foundation in Athens (Greece) under the supervision of Professor Ioannis Vogiatzis.

VA worked for almost 5 years as young researcher at Thorax foundation in Athens, a recognized research center of Intensive and emergency Medicine directed by Professor Dr. Charalambos Roussos. Within the frames of his work at Thorax foundation, he also worked for the large European Research project “PROACTIVE”-items of PROactive tools for measuring physical activity in COPD. Additionally, VA offered his services in the Pulmonary Rehabilitation of the 1st University clinic at “Sotiria” hospital in Athens and he implemented numerous ergospirometric measurements for COPD and CHF patients referred to Pulmonary Rehabilitation. In 2012, VA obtained a European Respiratory Society (ERS) Long Term Research Fellowship (LTRF 63-2012) and moved from the Thorax foundation in Athens (Greece) to CIRO+ Center of expertise for chronic organ failure in Horn (the Netherlands). VA completed his LTRF performing research analyses on large databases of patients with COPD and afterwards continued at CIRO+ center for a PhD course. In 2016, VA was the recipient of an ERS-EU RESPIRE2 Marie Skłodowska-Curie Post-doctoral Research Fellowship and moved from CIRO+ to Schön Klinik in Berchtesgaden Land (Germany).

VA is an active member of the “ERS College of Experts” and has participated in the ATS/ERS Task Force for the development of Health Technology Assessment for patients’ field testing. He has expertise in the operation of several clinical devices (NIRO [Hamamatsu], PORTALITE [Artinis], Physioflow [Manatec], SenTec [Sentec AG], OptoElectronic Plethysmography – O.E.P. [BTS]) and at designing and interpreting experiments with patients. VA has participated in many international congresses all over the world as presenter and/or chairman in congresses’ sessions as well as he has written many articles in scientific journals and books.

List of publications

ORIGINAL RESEARCH ARTICLES AND REVIEWS

Athanasopoulos D., Louvaris Z., Cherouveim E., **Andrianopoulos V.**, Roussos Ch, Zakynthinos S., Vogiatzis I. Expiratory muscle loading increases intercostal muscle blood flow during leg exercise in healthy humans. *Journal of Applied Physiology*, 2010; 109:388-395.

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