NIDIA APARECIDA HERNANDES FUJII

AVALIAÇÃO DA CAPACIDADE DE EXERCÍCIO POR MEIO DE TESTES DE CAMPO NA SAÚDE E NA DOENÇA:

VALORES DE REFERÊNCIA PARA O *INCREMENTAL SHUTTLE WALKING TEST* E REPRODUTIBILIDADE DO TESTE DE CAMINHADA DE 6 MINUTOS EM DOENÇA PULMONAR OBSTRUTIVA CRÔNICA

Londrina
2012

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Centro de Ciências da Saúde da Universidade Estadual de Londrina.

Orientador: Prof. Dr. Fabio Pitta

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Londrina, 23 de agosto de 2012.

“Se eu vi mais longe, foi por estar de pé sobre ombros de gigantes.”

Isaac Newton

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FUJII, Nidia A. H. **Avaliação da capacidade de exercício por meio de testes de campo na saúde e na doença: valores de referência para o *incremental shuttle walking test* e reprodutibilidade do teste de caminhada de 6 minutos em doença pulmonar obstrutiva crônica.** 2012. 73 f. Tese de Doutorado (Pós-Graduação em Ciências da Saúde) – Universidade Estadual de Londrina, Londrina, 2012.

RESUMO

Introdução: Testes de campo como o *incremental shuttle walk test* (ISWT) e o teste de caminhada de 6 minutos (TC6) são amplamente utilizados para avaliar a capacidade de exercício de pacientes portadores de doença pulmonar obstrutiva crônica (DPOC). Apesar da vasta literatura existente sobre esses testes, valores de referência do ISWT ainda não foram solidamente estabelecidos, e a reprodutibilidade do TC6 é inconsistente. **Objetivos:** investigar quais variáveis determinam o ISWT em indivíduos saudáveis, bem como estabelecer uma equação de valores de referência do ISWT; e investigar a reprodutibilidade do TC6 em pacientes com DPOC, quantificar o efeito aprendizado existente entre dois TC6 e estudar os fatores determinantes de mudanças no segundo TC6. **Métodos:** No primeiro estudo, 242 indivíduos saudáveis realizaram dois ISWT e tiveram avaliados peso, altura e índice de massa corpórea (IMC). No segundo estudo, 1514 pacientes com DPOC realizaram dois TC6 em dias subsequentes e foram submetidos à avaliação de composição corporal, dispneia e comorbidades (índice Charlson). **Resultados:** Gênero, idade e IMC foram preditores independentes dos valores de referência do ISWT ($ISWT_{pred}$=1449,701–(11,735*idade)+(241,897*gênero)–(5,686*IMC); r^2=0,71; p<0,0001). No segundo estudo, o coeficiente de correlação intraclasse (CCI) entre os dois TC6 foi de 0,93 (p<0,0001), embora os pacientes tenham apresentado distância percorrida significativamente maior no segundo teste (27m (IC95%: -37-107). Os fatores determinantes do aumento no segundo TC6 foram: primeiro TC6<350m, índice Charlson<2 e IMC<30 $kg.m^{-2}$. **Conclusões:** A maior parte da variação do ISWT em indivíduos saudáveis é explicada por idade, gênero e IMC. Baseada nessas variáveis, uma equação para predição de valores de referência do ISWT foi estabelecida. Alto CCI foi observado entre os dois TC6 em pacientes com DPOC, embora um efeito aprendizado significativo tenha ocorrido. Desempenho ruim no primeiro TC6, poucas comorbidades e ausência de obesidade foram associados ao aumento da distância percorrida no segundo teste.

Palavras-chave: Doença pulmonar obstrutiva crônica. Saúde, tolerância ao exercício. Valores de referência. Reprodutibilidade dos testes.

FUJII, Nidia A. H. **Assessment of exercise capacity using field tests in health and illness: reference values for the incremental shuttle walking test and reproducibility of 6-minute walking test in chronic obstructive pulmonary disease.** 2012. 73 p. PhD thesis (Post-Graduation in Health Sciences) – Universidade Estadual de Londrina, Londrina, 2012.

ABSTRACT

Background: Field tests, such as incremental shuttle walk test (ISWT) and 6-minute walking test (6MWT) are widely used to assess exercise capacity in patients with chronic obstructive pulmonary disease (COPD). Despite the vast existing literature about those tests, reference values for the ISWT were not solidly established yet, and the reproducibility of the 6MWT is inconsistent. **Aims:** To investigate which variables determine the variation of the ISWT in healthy subjects, as well as to establish a prediction equation for reference values of the ISWT; and to investigate the reproducibility of the 6MWT in patients with COPD, to quantify the learning effect between two 6MWT and to study the factors which determine improvement in the second 6MWT. **Methods:** For the first study, 242 healthy subjects performed two ISWT and had their weight, height and body mass index (BMI) evaluated. For the second study, 1514 patients with COPD performed two 6MWT in subsequent days and had their body composition, dyspnea and comorbidities (Charlson index) evaluated. **Results:** Gender, age and BMI were independent predictors of the reference values of the ISWT ($ISWT_{pred}$=1449.701–(11.735*age)+(241.897*gender)–(5.686*BMI); r^2=0.71; p<0.0001). In the second study, the intraclass correlation coefficient (ICC) between the two 6MWT was 0.93 (p<0.0001), although patients with COPD had a significantly higher walked distance during the second test (27m (CI95%: -37-107). Determinants of improvement in the second 6MWT were: first 6MWT<350m, Charlson index<2 and BMI<30kg.m^{-2}. **Conclusions:** The major part of the ISWT variation in healthy subjects is explained by age, gender and BMI. Based on those variables, a reference equation for the ISWT was established. High ICC was observed between the two 6MWT in patients with COPD, although there was a significant learning effect. A poor performance in the first 6MWT, few comorbidities and non-obesity were associated to increase in the walked distance of the second test.

Keywords: Pulmonary disease chronic obstructive. Health. Exercise tolerance. Reference values. Reproducibility of results.

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ABREVIATURAS E SIGLAS

6MWT	*6-minute walking test*
BMI	*body mass index*
BODE	*Body-mass index, airway Obstruction, Dyspnea and Exercise capacity*
bpm	*beats per minute*
CCI	Coeficiente de Correlação Intraclasse
CI	*Confidence Interval*
CIRO+	*a centre of excellence for chronic organ failure*
COPD	*Chronic obstructive pulmonary disease*
CPCS	Centro de Pesquisa em Ciências da Saúde
DEXA	*Dual Energy X-ray Absorptiometry*
DPOC	doença pulmonar obstrutiva crônica
EELO	Estudo do Envelhecimento e Longevidade
ERS	*European Respiratory Society*
FEV_1	*forced expiratory volume in the first second*
FFM	*fat-free mass*
FFMI	*fat-free mass index*
FVC	*forced vital capacity*
GOLD	*Global Initiative for Chronic Obstructive Lung Disease*
HR	*heart rate*
IC	intervalo de confiança
ICC	*intraclass correlation coefficient*
IMC	índice de massa corpórea
IQR	*interquartile range*
ISWT	*incremental shuttle walk test*
LFIP	Laboratório de Pesquisa em Fisioterapia Pulmonar
MRC	*Medical Research Council*
MUMC+	*Maastricht University Medical Centre*
MVV	*maximal voluntary ventilation*
OR	*odds ratio*
SD	*standard deviation*
SpO_2	*oxygen saturation measured by oximetry*
TC6	Teste de Caminhada De Seis Minutos

TCPE	Teste Cardiopulmonar de Esforço
UEL	Universidade Estadual de Londrina
UNOPAR	Universidade Norte do Paraná
USA	*United States of America*
VEF_1	Volume Expiratório Forçado no Primeiro Segundo
$VO_{2máx}$	Consumo Máximo de Oxigênio

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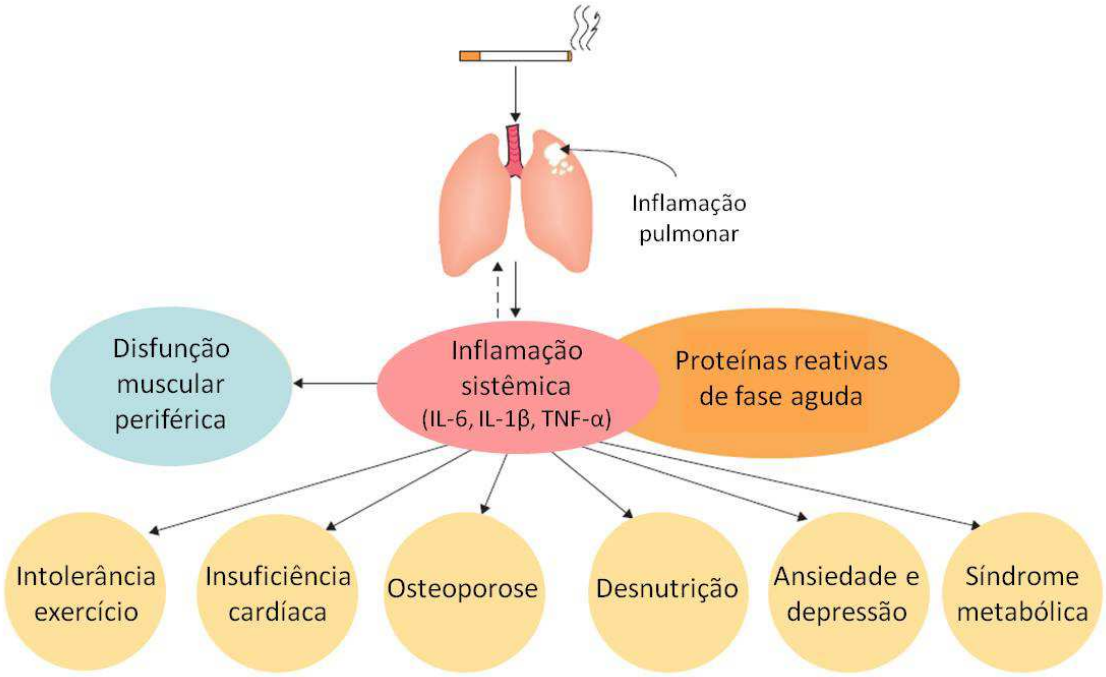
1 INTRODUÇÃO

Segundo o *Global Initiative for Chronic Obstructive Lung Disease* (GOLD), a doença pulmonar obstrutiva crônica (DPOC) é definida como "uma doença prevenível e tratável, caracterizada por obstrução ao fluxo aéreo persistente que é usualmente progressiva e associada a uma resposta inflamatória crônica anormal nas vias aéreas e nos pulmões a partículas nocivas ou gases" [1]. Além do comprometimento pulmonar, a DPOC apresenta manifestações extrapulmonares, como disfunção muscular periférica, intolerância ao exercício e desnutrição, as quais contribuem para a gravidade da doença (Figura 1) [2]. Exacerbações e comorbidades também contribuem para a gravidade geral da doença em pacientes individuais [1].

A intolerância ao exercício é um sinal cardinal da DPOC e está diretamente relacionada a um pior prognóstico [3;4]. Além disso, a limitação da capacidade de exercício de pacientes portadores de DPOC não pode ser predita a partir de variáveis tradicionalmente avaliadas, como idade, gênero, volume expiratório forçado no primeiro segundo (VEF_1) e índice de massa corpórea (IMC) [5]. Por isso, a avaliação da capacidade de exercício desses pacientes é essencial em pesquisas e na prática clínica.

O teste cardiopulmonar de esforço (TCPE) é considerado o método padrão ouro para avaliar a redução na capacidade de exercício, bem como as causas fisiológicas dessa redução. Entretanto, apesar de sua importância, o TCPE nem sempre está disponível na rotina clínica e científica devido ao seu elevado custo e à necessidade de profissionais devidamente treinados para a sua realização [5]. Por essa razão, testes de campo como o *incremental shuttle walking test* (ISWT) e o teste de caminhada de 6 minutos (TC6) foram propostos e vêm sendo amplamente utilizados na avaliação de pacientes portadores de DPOC.

Figura 1 – Manifestações extrapulmonares da DPOC [2].



Adaptado de: Barnes e Celli (2009, p. 1165-1185)

O ISWT foi proposto por Singh e colaboradores (1992) para a avaliação da capacidade máxima de exercício de pacientes portadores de DPOC. Trata-se de um teste simples e de baixo custo que consiste na quantificação da distância percorrida pelo indivíduo em um percurso de 10 metros, seguindo velocidades incrementadas progressivamente ditadas por um sinal sonoro [6]. Além de avaliar a capacidade de exercício, o ISWT tem sido utilizado em DPOC como preditor de mortalidade e do risco de exacerbações agudas da doença [7;8], bem como para avaliar efeitos de intervenções [9;10] e detectar dessaturação de oxigênio durante o esforço [11].

Diversas questões importantes para a correta utilização do ISWT na prática clínica e em pesquisas envolvendo pacientes portadores de DPOC já foram elucidadas em estudos prévios. Primeiramente, demonstrou-se que a distância percorrida durante o ISWT correlaciona-se fortemente com o consumo máximo de oxigênio ($VO_{2máx}$) (r=0,88) obtido durante TCPE [12], comprovando sua característica de teste máximo de exercício. Além disso, sabe-se que a realização de dois ISWT é necessária para avaliar pacientes portadores de DPOC, visto que existe um efeito aprendizado de aproximadamente 20 m (IC 95%: 9-31 m) entre o primeiro e o segundo teste [13]. É conhecido também que um aumento de aproximadamente 48 metros no ISWT é o mínimo necessário para se comprovar benefícios de uma

intervenção [14]. Embora o ISWT tenha se tornado bastante útil para a avaliação de pacientes com DPOC, até há pouco tempo não havia nenhuma equação de referência para predizer a distância percorrida esperada para os indivíduos avaliados. Isso impossibilitava relativizar (ou corrigir) a comparação do desempenho no teste entre indivíduos que apresentam diferentes características de idade, altura, peso e gênero. Recentemente, um grupo de pesquisa desenvolveu uma equação de predição para o ISWT [15]; entretanto, o estudo apresenta algumas limitações que podem comprometer a validade externa da equação, como uma variação limitada de faixa etária da população estudada impossibilitando a predição para indivíduos abaixo de 40 anos de idade, e um baixo coeficiente de determinação para a equação (r^2=0,50) [15]. Considerando a importância de se poder comparar o desempenho no ISWT entre diferentes indivíduos e grupos de indivíduos tanto em pesquisa quanto na prática clínica, torna-se necessário estabelecer uma equação de valores de referência para esse teste de maneira sólida, garantindo a aplicabilidade do teste.

O TC6 também é considerado um teste de baixo custo e de fácil execução no qual o indivíduo deve caminhar durante seis minutos atingindo a maior distância possível em um corredor de 30 metros. O teste avalia respostas globais e integradas dos diferentes sistemas envolvidos no exercício físico [16;17]. Troosters e colaboradores (2002) demonstraram que, em indivíduos com DPOC, o TC6 induz a respostas metabólicas e cardiopulmonares altas porém constantes a partir do terceiro minuto [17]. Portanto, por se tratar de um teste submáximo de exercício, o TC6 reflete melhor a capacidade funcional dos indivíduos [16]. Apesar de não fornecer informações sobre os mecanismos de limitação da tolerância ao exercício, o desempenho durante o TC6 tem sido utilizado para avaliar resposta a intervenções (farmacológicas e reabilitação pulmonar) [18;19], prescrever carga de treinamento físico [20], detectar dessaturação durante o esforço e indicar uso de oxigenoterapia domiciliar [21], bem como para predizer mortalidade em DPOC [22-24].

Valores de referência para o TC6 já foram propostos na literatura, permitindo uma melhor interpretação de seus resultados [25-32]. Além disso, sabe-se que a mínima diferença importante para o TC6 para avaliar benefícios de intervenções em DPOC é de 35 metros ou 10% de aumento com relação ao valor inicial [18]. Apesar de diversos aspectos práticos sobre o TC6 já terem sido amplamente discutidos na literatura científica, a reprodutibilidade do teste em DPOC ainda é objeto de debate. Embora o teste possa ser considerado reprodutível, alguns

autores sugerem que existe um efeito aprendizagem acentuado, ou seja, pacientes percorrem uma maior distância quando um segundo teste é realizado [33-38]. Entretanto, há uma divergência na literatura sobre a extensão desse efeito aprendizagem o qual varia de 2,6% a 22% [17;33;34;39-41]. Além disso, a maioria dos estudos apresenta alguma limitação que compromete a aplicabilidade de seus resultados, como um tamanho reduzido de amostra e análise estatística inconsistente. Tendo em vista a importância do TC6 para a avaliação de pacientes portadores de DPOC, sua reprodutibilidade e fatores determinantes do aumento durante o segundo teste necessitam ser solidamente estudados.

Considerando-se que valores de referência para ISWT ainda não foram solidamente estabelecidos e a inconsistência de resultados dos estudos relacionados à reprodutibilidade e ao efeito aprendizagem do TC6, dois estudos foram realizados com objetivos voltados a preencher tais lacunas existentes na literatura científica sobre o assunto. Estes dois artigos compõem a presente tese e estão apresentados a seguir seguindo a linha geral de formatação sugerida pelos periódicos nos quais foram publicados, com pequenas adaptações de formato requeridas conforme o modelo dessa tese.

2 OBJETIVOS

2.1 OBJETIVOS GERAIS

- Estabelecer uma equação para predição de valores de referência do ISWT para indivíduos saudáveis com uma ampla faixa etária;
- Investigar a reprodutibilidade do TC6 em uma amostra representativa de pacientes portadores de DPOC.

2.2 OBJETIVOS ESPECÍFICOS

- Determinar quais variáveis antropométricas e demográficas (peso, altura, gênero e idade) explicam a distância percorrida no ISWT de indivíduos saudáveis;
- Quantificar o efeito aprendizagem existente entre dois TC6 realizados em dias subsequentes;
- Estudar os fatores determinantes de aumento na distância percorrida no segundo TC6.

3 ARTIGOS CIENTÍFICOS

Reference Values for the Incremental Shuttle Walking Test

Running title:

Reference values for the ISWT

Vanessa S. Probst*, Nidia A. Hernandes*, Denilson C. Teixeira, Josiane M. Felcar, Rafael B. Mesquita, Cristiane G. Gonçalves, Daniela Hayashi, Sally Singh, Fabio Pitta.

Abstract: Background. Reference values for the incremental shuttle walking test (ISWT) which are applicable to the whole population need to be solidly established. This study aimed to determine which anthropometric and demographic variables influence the walking distance achieved in the ISWT in healthy subjects with a broad age range and to establish a reference equation for predicting ISWT for that population. **Methods.** In a cross-sectional study, 242 healthy subjects (102 male) performed two ISWT and had their weight, height and body mass index (BMI) measured. **Results.** In general, healthy subjects walked 810 [IQR 25-75%: 572 – 1030] m in the ISWT, presenting large variability (range 210–1820 m). The walked distance correlated with age (r = -0.76), height (r = 0.49) and BMI (r = -0.23) ($p<0.001$ for all), but not with weight (r = 0.06, p=0.315). A model of stepwise multiple regression showed that gender, age and BMI were independent contributors to the ISWT in healthy subjects, explaining 71% ($p<0.0001$) of the variability. The derived reference equation was: $ISWT_{pred}$ = 1449.701 – (11.735 x age) + (241.897 x gender) – (5.686 x BMI), where male gender = 1 and female gender = 0. **Conclusion.** In conclusion, the variability of the ISWT is explained largely by gender, age and BMI. The reference values for the ISWT can be adequately predicted using the equation proposed in this study.

Keywords: Incremental shuttle walking test. Reference value. Statistical regression.

* These authors contributed equally to this article.

INTRODUCTION

Exercise tests are commonly used both in clinical practice and in scientific investigations since exercise intolerance is a typical feature in a number of chronic diseases. The objective assessment of exercise capacity through a cardiopulmonary exercise test provides information about the causes of reduced exercise tolerance, which can be eventually improved by specific interventions [1]. Moreover, outcome parameters of exercise tests are used as guide for workload prescription during exercise interventions [2]. As laboratory assessment is not widely available and may be expensive, field tests such as the incremental shuttle walking test (ISWT) have become increasingly popular.

The ISWT is a simple and inexpensive test which evaluates maximal exercise capacity based on the distance walked around a 10 m course according to different speeds dictated by an audio signal [3]. This exercise test has been used as a predictor of mortality[4] and morbidity [5, 6] in patients with chronic respiratory and other diseases, as a predictor of exacerbation in patients with pulmonary disease [7], as well for assessing benefits of interventions [8, 9, 10] and detecting oxygen desaturation during exertion [11].

Although the ISWT has become very useful in clinical and research settings, there were no reference equations to predict the distance walked in the test by healthy subjects. Recently, an equation was developed [12]; however, there are some limitations which can compromise the external validity of that study. Firstly, the sample was composed of subjects aged 40 to 84 years; therefore the resulting equation is not applicable for young people. Moreover, the coefficient of determination of its regression analysis was relatively modest, explaining only 50% of the total variance in the ISWT. Considering the importance of the ISWT in assessing adequately patients of all ages with cardiopulmonary disease and other health conditions, an equation with higher coefficient of determination and applicable for the whole adult population needs to be solidly established.

Hence, the aims of this study were to determine which anthropometric and demographic variables (weight, height, age and gender) influence the walking distance in the ISWT of healthy subjects with a broad age range and to establish an equation for predicting reference values of the ISWT for this population.

METHODS AND MATERIALS

Subjects

In a cross-sectional study, 246 healthy subjects were included in a convenience sample. They were recruited among students and employees of two universities in Londrina (Brazil), as well as their relatives. Part of the sample of subjects older than 60 years old was also composed by individuals participating in a project which investigates the health conditions of the elderly in Londrina, Brazil (EELO Project). All subjects had their exercise capacity, pulmonary function, anthropometric and demographic data evaluated. Data were collected from November 2008 to December 2010. The study was approved (PP000709) by the Research Ethics Committee of the Universidade Norte do Paraná (UNOPAR), Brazil, and all participants gave written informed consent.

Inclusion criteria were: subjects of both genders aged 18 to 83 years and absence of any severe and/or unstable disease which could limit the exercise tolerance. Participants were excluded if they were unable to understand or perform any procedure during the protocol or if they would like to leave the study for any reason.

After obtaining the informed consent, a questionnaire was applied in order to investigate health status, medication, smoking habits and whether subjects were engaged in any regular physical activity (Anexo A). Height (cm) and body weight (Kg) were measured and the body mass index (BMI) was calculated. Spirometry (Pony Cosmed, Italy) was performed to ensure normal lung function. The test was conducted according to international standardization [13] and the lung function parameters obtained were forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1) and the direct measurement of maximal voluntary ventilation (MVV). Reference values adopted were those reported by Pereira and colleagues specifically for the Brazilian population [14].

Incremental shuttle walking test

Two incremental shuttle walking tests were performed with, at least, 30 minutes of resting in between them. The tests were performed in a 10 m course identified by two cones placed 0.5 m from each end point [3]. The best test, that is, the longest walked distance was considered for analysis. Participants should walk (or

run) around the course according to the speed dictated by an audio signal. The initial walking speed was 0.5 m/s and it increased by 0.17 m/s each minute; the speed increment was always indicated by a triple bleep. An adaptation of the modified protocol was used, that is, the audio signals continued until subjects reach their maximal effort, exceeding 12 levels of speed proposed by the modified protocol and even running, if necessary. The authors opted for adapting the protocol in order to avoid a ceiling effect since participants were healthy subjects and could possibly exceed the 12th level in order to ensure their maximal effort. The tests were executed by a physiotherapist or physiotherapy student, all familiarized with the ISWT, and the two tests were conducted by the same evaluator. The initial explanation was standardized and no encouraging phrases were given to the participants during the test. The ISWT was interrupted in case of participants presented one of the following conditions: if the subject could not maintain the required speed due to dyspnea or fatigue; or if the subject failed to complete a shuttle in the time allowed for the second time. Heart rate (HR), arterial blood pressure, perceived dyspnea and leg fatigue (modified Borg scale) (Anexo B) were assessed before and after the tests. Maximal predicted HR was calculated as 220 minus age (in years) [15].

Statistical analysis

The statistical analysis was performed using the statistical packages SPSS 17.0 (SPSS Inc., USA) and GraphPad Prism 5 (GraphPd Software Inc., USA). The normality of data distribution was checked by the Shapiro-Wilk test. Data were described as median [interquartile range 25%-75%]. Wilcoxon test was used to compare outcome parameters of the ISWT. Mann-Whitney test was used to compare subjects' characteristics between male and female genders. Spearman correlation coefficients were calculated and a model of multiple linear regression was applied (ISWT distance as dependent variable; demographic and anthropometric data as independent variables) since its standardized residual presented normal distribution (p=0.785). The categorical variable gender was expressed as 0 for female and 1 for male because in general male subjects reach a higher walked distance in the ISWT. The Bland & Altman plot was used to evaluate agreement between the actual ISWT distance and the predicted value. In order to verify the reliability of the regression equation, it was applied *a posteriori* in a different group of healthy subjects,

composed by 23 individuals selected according to the same inclusion criteria of the present sample. The level of statistical significance was considered as $p<0.05$.

RESULTS

The study sample included 103 male and 143 female apparently healthy subjects. Four subjects (1 man and 3 women) were excluded because they interrupted the test abruptly due to severe joint pain. Therefore, the results of 242 subjects are presented. The subjects' age ranged from 18 to 83 years. They presented normal lung function and, in general, a normal body composition. As expected, men were taller and weighed more than women, as well as presented higher distance walked in the ISWT in absolute values (table 1).

Health status, medication, smoking habits and physical activity level

The comorbidities more prevalent among the study subjects were systemic hypertension (34%), followed by peripheral vascular disease (24%) and arthritis (22%). Other reported comorbidities were: stable cardiac disease (13%), diabetes mellitus (13%), thyroid disorders (8%), osteoporosis (6%) and asthma (6%). One hundred and forty three participants (41% among those were elderly) needed to take some medication continuously. The medications were prescribed by physicians and were related to the specific health condition presented by the individual. Among the medications in use, 45% were drugs known to affect blood pressure and/or heart rate. Approximately 30% of the subjects were current smokers. Regarding the physical activity level, 61% of participants were not engaged in regular physical activity.

Variability of the ISWT

Considering the best of the two ISWT, subjects walked 810 [572–1030] m, presenting large variability (range of 210–1820 m). Twenty-eight percent of subjects exceeded the 12^{th} level of the protocol, presenting a walked distance higher than 1020 m. Subjects walked a median of 770 [530–1010] m in the first ISWT and 790 [550–1030] m in the second ISWT ($p<0.0001$) (table 2). The distance walked during the second ISWT increased by 20 [-10–70] m (increment of 3%); 63% of subjects

had their best ISWT in the second test and 28% in the first test, whereas 9% of the individuals had the exact same distance in the two tests. When comparing genders, male walked farther than female (1010 [755–1200] m vs. 720 [480–910] m, p<0.0001, respectively). In the best test, subjects reached 99 [89–105]% of their maximal predicted HR.

ISWT determinants and reference equation

There were significant correlations between the distance walked in the ISWT with age (r=-0.76), BMI (r=-0.23) and height (r=0.49) (p<0.001 for all), but not with weight (r=0.06, p=0.315). A model of stepwise multiple regression showed that gender, age and BMI explained 71% (p<0.0001) of the variability in the ISWT. Unstandardized coefficients, part correlation and significance are shown in table 3. The reference equation for the distance walked in the ISWT was:

$ISWT_{pred}$ = 1449.701 – (11.735 x age) + (241.897 x gender) – (5.686 x BMI)

(where male gender = 1 and female gender = 0).

The Bland & Altman plot shows the agreement between the actual ISWT and the predicted value obtained from the reference equation (figure 1). A weak but significant correlation was observed between the difference of the ISWT (actual – predicted) and the mean of the ISWT (actual and predicted) (r=0.28, p<0.0001).

Since the mean±standard deviation distance walked in the ISWT was 833±273 m, the lower limit of normal calculated according to the confidence interval was 798 m.

Reliability of the reference equation

The characteristics of the other group composed by 23 healthy subjects (10 male) included in the *a posteriori* analysis were: age 49±20 years, BMI 24±3.8 $Kg.m^{-2}$, FEV_1 93±15 %pred and FVC 91±17 %pred. When the reference equation was applied in this group, there was no difference between the actual and predicted ISWT (839±269 m vs. 838±271 m, respectively; p=0.970; figure 2). Furthermore, there was a strong correlation between the actual and predicted ISWT (r=0.85, p<0.0001) (figure 3).

DISCUSSION

The present study demonstrated that healthy subjects aged from 18 to 83 years present large variability in the ISWT (210–1820 m), and that 71% of this variance is explained by age, gender and body mass index. Moreover, a reference equation was established using anthropometric and demographic variables which can be easily applied in clinical and research settings.

Recently, a Brazilian research group has published a reference equation for the ISWT [12]. However, there were some limitations in their study which could limit the use of the equation. Firstly, the analysis did not include subjects younger than 40 years old, which is a limitation since some cardiopulmonary diseases affect subjects in this age range. Secondly, the regression coefficient was modest (50%), increasing the chance of bias in predicting the walking distance in the ISWT. In fact, this was confirmed when that equation [12] was applied in our sample studied *a posteriori*. The predicted value was greatly underestimated when compared with the actual ISWT (579±173 m vs. 839±269 m, p<0.0001, respectively), which corresponds to a 45% difference. This discrepancy did not happen when the equation from the present

study was used. Finally, those authors considered for the statistical analysis only the second test instead of the best test. This approach could definitely have influenced the results since it was observed in our study that almost one in each three subjects was able to walk farther during the first ISWT.

The present results demonstrated that gender, age and BMI influence the ISWT in apparently healthy subjects. These findings are in agreement with previously published data about walking speed. In a study with 42 male subjects, Pearce et al. [16] affirmed that age alone was associated with walking speed. Himann et al. [17] also found that age was the only significant independent predictor for walking speed when 438 subjects aged 19 to 102 years walked over an 80-m indoor course. Bohannon [18] studied 1923 subjects who had their gait speed evaluated when walking in a corridor, and showed that younger, male and taller individuals walk faster. Recently [19], the same author observed that body composition also influences the walking ability in elderly people, demonstrating that higher BMI was significantly associated with limited walking (r=-0.42). A negative association between adiposity and walking speed in older women was also observed by Ortega-Alonso et al. [20].

Previous studies have shown that patients reach higher peak HR during the ISWT than during the 6-minute walking test, characterizing the ISWT as a maximal exercise test [3, 21]. Furthermore, in our study, the peak HR was even higher than those reported previously [3, 21], since participants achieved 99% of their maximal predicted HR. It can be attributed to the fact that our sample was composed of healthy subjects, who do not present any ventilatory or cardiovascular limitation, differently from the subjects included in those studies. Moreover, in those studies, it was not allowed to exceed the 12th level of the protocol and this could also contribute to the difference in the peak HR found between those results and ours. In contrast,

Jürgensen et al. [12] reported that healthy subjects reached only 78% of their maximal predicted HR when performing the ISWT, which represents 21% less in comparison to the healthy subjects from the present study. This possibly represents a methodological issue in the study of Jürgensen et al., since it might be that those subjects did not perform their maximal effort during the ISWT despite the fact that the authors also adapted the modified protocol of the test, allowing subjects to reach more than 12 levels.

The majority of subjects increased their walking distance during the second test with an average improvement of 20 m. This is in accordance with results previously reported, in which the magnitude of the improvement in the second test ranges from 20 to 40 m [3, 22, 23].

Strengths and limitations

The clinical importance of our findings is evident since the use of absolute values in assessing patients usually induces bias in the results. The use of a reference equation for the ISWT correcting values for gender, age and BMI allows interpreting the results adequately taking into account each subject's characteristics, enabling more reliable intergroup comparisons.

Although a reference equation for the ISWT has been recently published, some points in the present study update those findings. Firstly, our sample included a higher number of subjects (242 vs. 131 subjects) and ages from 18 to 83 years, being more representative of the vast majority of cardiopulmonary patient's population. Secondly, in our analysis, the anthropometric and demographic variables could explain a larger part of the variance in the ISWT (71%). Thirdly, our sample

reached a higher HR in terms of % predicted of the maximal HR, suggesting more effort during the test.

Despite all efforts, some limitations occurred. A few variables which could also explain the variance in the ISWT were not assessed, such as peripheral muscle force and balance. On the other hand, this study aimed at establishing a reference equation using independent variables which are easily available in clinical settings. Another limitation was the use of a convenience sample, although caution was taken concerning the number of participants in each age range, as well as the proportion of male and female subjects. Finally, it can be speculated that the subjects from the present study could have performed even better if a third ISWT was performed instead of two. However, it has been shown that a third ISWT had no benefit in terms of walking distance in patients following artery bypass [24]. Therefore, we hypothesized that this could also be the case for people without cardiac disease. Nevertheless, the necessity of performing a third ISWT still remains to be completely elucidated in healthy individuals.

CONCLUSIONS

In summary, it can be concluded that variability in the performance of the incremental shuttle walking test in healthy people can be explained (71%) by using age, gender and body mass index. A reference equation could be established based on these anthropometric and demographic variables. Standardization of the ISWT is necessary to ensure maximal effort during the test in order to avoid incorrect interpretation of results when considering reference values.

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REFERENCES

1. Palange P, Ward SA, Carlsen KH, Casaburi R, Gallagher CG, Gosselink R et al. Recommendations on the use of exercise testing in clinical practice. Eur Respir J 2007; 29:185-209.

2. Nici L, Donner C, Wouters E, ZuWallack R, Ambrosino N, Bourbeau J et al. American Thoracic Society/European Respiratory Society statement on pulmonary rehabilitation. Am J Respir Crit Care Med 2006; 173:1390-1413.

3. Singh SJ, Morgan MD, Scott S, Walters D, Hardman AE. Development of a shuttle walking test of disability in patients with chronic airways obstruction. Thorax 1992; 47:1019-1024.

4. Ringbaek T, Martinez G, Brondum E, Thogersen J, Morgan M, Lange P. Shuttle walking test as predictor of survival in chronic obstructive pulmonary disease patients enrolled in a rehabilitation program. J Cardiopulm Rehabil Prev 2010; 30:409-414.

5. Geddes D, Davies M, Koyama H, Hansell D, Pastorino U, Pepper J et al. Effect of lung-volume-reduction surgery in patients with severe emphysema. N Engl J Med 2000; 343:239-245.

6. Murray P, Whiting P, Hutchinson SP, Ackroyd R, Stoddard CJ, Billings C. Preoperative shuttle walking testing and outcome after oesophagogastrectomy. Br J Anaesth 2007; 99:809-811.

7. Emtner MI, Arnardottir HR, Hallin R, Lindberg E, Janson C. Walking distance is a predictor of exacerbations in patients with chronic obstructive pulmonary disease. Respir Med 2007; 101:1037-1040.

8. Evans RA, Singh SJ, Collier R, Williams JE, Morgan MD. Pulmonary rehabilitation is successful for COPD irrespective of MRC dyspnoea grade. Respir Med 2009; 103:1070-1075.

9. Murray MP, Turnbull K, Macquarrie S, Hill AT. Assessing response to treatment of exacerbations of bronchiectasis in adults. Eur Respir J 2009; 33:312-318.

10. Hillerdal G, Lofdahl CG, Strom K, Skoogh BE, Jorfeldt L, Nilsson F et al. Comparison of lung volume reduction surgery and physical training on health status and physiologic outcomes: a randomized controlled clinical trial. Chest 2005; 128:3489-3499.

11. Sandland CJ, Morgan MD, Singh SJ. Detecting oxygen desaturation in patients with COPD: incremental versus endurance shuttle walking. Respir Med 2008; 102:1148-1152.

12. Jurgensen SP, Antunes LC, Tanni SE, Banov MC, Lucheta PA, Bucceroni AF et al. The incremental shuttle walk test in older Brazilian adults. Respiration 2011; 81:223-228.

13. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A et al. Standardisation of spirometry. Eur Respir J 2005; 26:319-338.

14. Pereira CAC and Neder JA. Diretrizes para testes de função pulmonar. J Bras Pneumol 2002; 28:S2-S237.

15. Karvonen MJ, Kentala E, Mustala O. The effects of training on heart rate; a longitudinal study. Ann Med Exp Biol Fenn 1957; 35:307-315.

16. Pearce ME, Cunningham DA, Donner AP, Rechnitzer PA, Fullerton GM, Howard JH. Energy cost of treadmill and floor walking at self-selected paces. Eur J Appl Physiol Occup Physiol 1983; 52:115-119.

17. Himann JE, Cunningham DA, Rechnitzer PA, Paterson DH. Age-related changes in speed of walking. Med Sci Sports Exerc 1988; 20:161-166.

18. Bohannon RW. Population representative gait speed and its determinants. J Geriatr Phys Ther 2008; 31:49-52.

19. Bohannon RW. Body mass index and mobility of older home care patients. Physiother Theory Pract. Published online first: 1 November 2010. doi: 10.3109/09593985.2010.523058

20. Ortega-Alonso A, Sipila S, Kujala UM, Kaprio J, Rantanen T. Body fat and mobility are explained by common genetic and environmental influences in older women. Obesity 2008; 16:1616-1621.

21. Green DJ, Watts K, Rankin S, Wong P, O'Driscoll JG. A comparison of the shuttle and 6 minute walking tests with measured peak oxygen consumption in patients with heart failure. J Sci Med Sport 2001; 4:292-300.

22. McKeough ZJ, Leung RW, Alison JA. Shuttle walk tests as outcome measures: Are two incremental shuttle walk tests and two endurance shuttle walk tests necessary? Am J Phys Med Rehabil 2011; 90:35-39.

23. Jolly K, Taylor RS, Lip GY, Singh S. Reproducibility and safety of the incremental shuttle walking test for cardiac rehabilitation. Int J Cardiol 2008; 125:144-145.

24. Fowler SJ, Singh SL, Revill S. Reproducibility and validity of the incremental shuttle walking test in patients following coronary artery bypass. Physiotherapy 2005; 91:22-27.

Table 1 - Characteristics of the study subjects.

	Total group (n=242)	Male (n=102)	Female (n=140)	p-value
Age (yrs)	50 [31-66]	49 [30-69]	51 [33-66]	0.989
Height (m)	1.64 [1.58-1.71]	1.71 [1.66-1.75]	1.59 [1.55-1.64]	<0.0001
Weight (Kg)	69 [60-79]	75 [68-85]	64 [57-72]	<0.0001
BMI ($Kg.m^{-2}$)	26 [23-29]	27 [23-29]	25 [21-29]	0.146
FEV_1/FVC	89 [83-95]	87 [82-93]	89 [85-99]	0.044
FEV_1 %pred	97 [89-106]	96 [89-106]	97 [89-106]	0.793
FVC %pred	94 [85-104]	92 [86-103]	94 [85-104]	0.772
MVV %pred	98 [81-110]	103 [89-113]	93 [77-105]	<0.0001
ISWT (m)	810 [572-1030]	1010 [755-1200]	720 [480-910]	<0.0001

Data are expressed as median [interquartile range 25-75%]. P-value concerns the difference between male and female subjects. BMI: body mass index; FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity; MVV: maximal voluntary ventilation; ISWT: incremental shuttle walking test.

Table 2 - Outcome parameters of the two incremental shuttle walking tests.

	ISWT 1	ISWT 2	p-value
ISWT (m)	770 [530-1010]	790 [550-1030]	<0.0001
% HR max (%)	96 [84-102]	99 [90-105]	<0.0001
HR before (bpm)	84 [75-94]	95 [83-105]	<0.0001
HR end (bpm)	165 [137-184]	173 [146-188]	<0.0001
Δ HR (bpm)	78 [54-93]	73 [56-86]	0.008
% HR max (%)	96 [84-102]	99 [90-105]	<0.0001
SBP before (mmHg)	120 [110-130]	120 [110-130]	0.483
SBP end (mmHg)	160 [150-180]	165 [150-190]	0.959
Δ SBP (mmHg)	40 [20-60]	40 [30-60]	0.613
DBP before (mmHg)	80 [70-80]	80 [70-80]	0.908
DBP end (mmHg)	80 [80-90]	80 [80-90]	0.862
Δ DBP (mmHg)	10 [0-10]	0 [0-10]	0.863
Borg D before (pts)	0 [0-0]	0 [0-0]	0.128
Borg D end (pts)	4 [2-6]	4 [3-6]	0.026
Δ Borg D (pts)	4 [2-5]	4 [2-6]	0.190
Borg F before (pts)	0 [0-0.5]	0 [0-0.5]	0.541
Borg F end (pts)	2 [0-4]	3 [0-4]	0.199
Δ Borg F (pts)	1 [0-4]	2 [0-4]	0.400

Data are expressed as median [interquartile range 25-75%]. ISWT: incremental shuttle walking test; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; Borg D: Borg dyspnea scale; Borg F: Borg fatigue scale.

Table 3 - Multiple linear regression analysis with ISWT as the dependent variable.

	Unstandardized coefficients (B)	95% Confidence interval for B	p-value	Part correlation
Constant	1449.701	1316.884 – 1582.519		
Age (yrs)	-11.735	-12.867 – -10.603	<0.0001	-0.70
Gender #	241.897	198.491 – 285.302	<0.0001	0.38
BMI ($Kg.m^{-2}$)	-5.686	-10.832 – -0.539	0.031	-0.07

Residual standard deviation = 167.8m
The derived equation from the regression analysis was:
$ISWT_{pred}$ = 1449.701 – (11.735 x age) + (241.897 x gender) – (5.686 x BMI)
male gender = 1 and female gender = 0

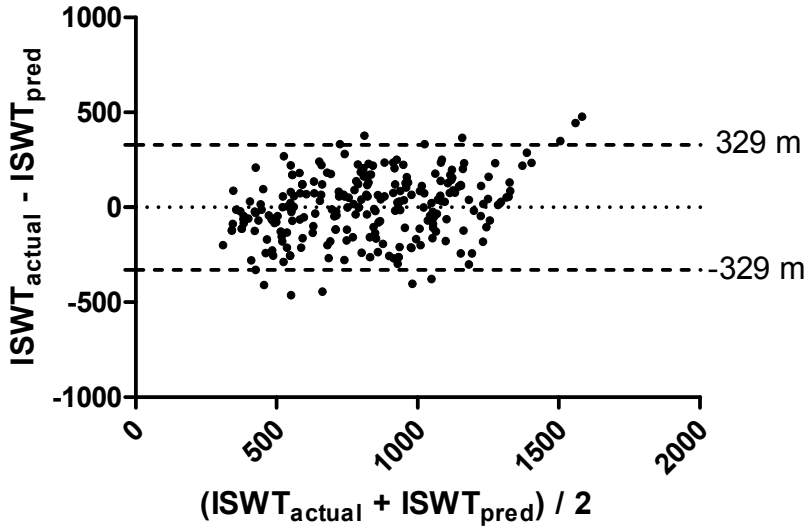

Figure 1 - Bland & Altman plot of the difference between the actual and predicted value of the ISWT plotted against the mean of the actual and the predicted value of the ISWT for the entire subjects group. The central dotted line corresponds to the average difference between the actual and predicted value of the ISWT, whereas the lower and upper dotted lines correspond to lower and upper limits of agreement, respectively.

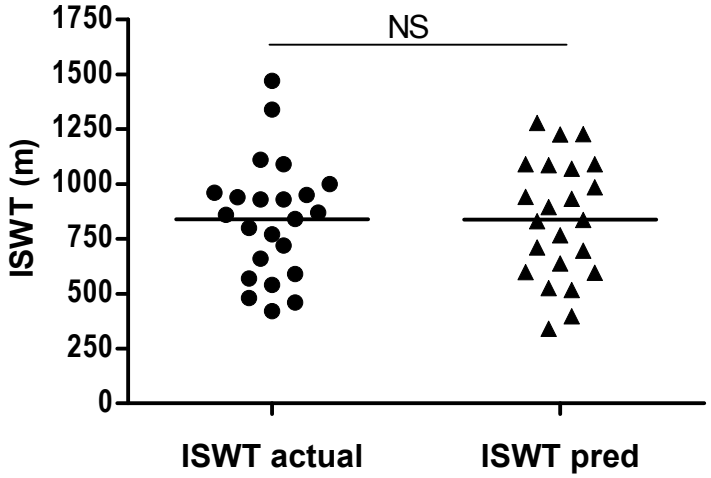

Figure 2 - Comparison between the actual and predicted ISWT in the *a posteriori* analysis.

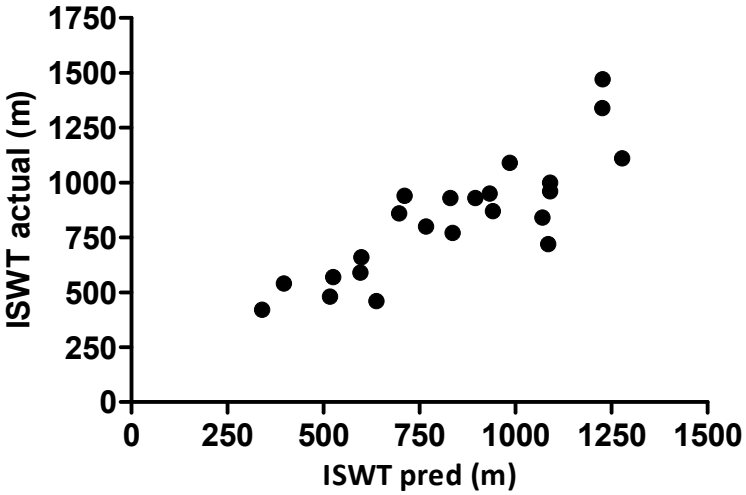

Figure 3 - Correlation between the actual and predicted ISWT in the *a posteriori* analysis ($r = 0.85$; $p<0.0001$).

Artigo 2

REPRODUCIBILITY OF 6-MINUTE WALKING TEST IN PATIENTS WITH COPD

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Abstract: Background: The reproducibility of 6-minute walking test (6MWT) needs to be more solidly studied. **Objectives:** This study aimed to investigate the reproducibility of two 6MWT performed in subsequent days in a large and representative sample of patients with chronic obstructive pulmonary disease (COPD) and to quantify the learning effect between the two tests, as well as its determinants. **Methods:** In a retrospective observational study, 1514 patients with COPD performed two 6MWT in subsequent days. Other measurements included body composition (dual X-ray absorptiometry), dyspnea (Medical Research Council scale) and comorbidity (Charlson index). **Results:** Although the 6MWT was reproducible (ICC=0.93; p<0.0001), patients walked farther in the second test [391m (95%CI 155 to 585m) *vs*. 418m (185 to 605m); p<0.0001]. On average, the second 6MWT increased by 27m (or 7%), and 82% of patients improved in the second test. Determinants of improvement ≥42m in the second test (upper limit of the clinically important change) were: first 6MWT <350m, Charlson index <2 and BMI <30 $kg.m^{-2}$ (OR 2.49, 0.76 and 0.60, respectively). **Conclusions:** The 6MWT was statistically reproducible in a representative sample of patients with COPD. However, the vast majority of patients improved significantly in the second test by an average learning effect of 27m.

Key-words: six-minute walking test; reproducibility of results; pulmonary disease, chronic obstructive

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a systemic disease characterized by progressive airflow limitation, exercise intolerance and physical inactivity [1,2]. Although the degree of airflow obstruction is frequently used as marker of disease severity, it does not adequately reflect extra-pulmonary manifestations of COPD [3,4]. Some modalities of field tests are available to assess these patients' exercise capacity [4], which better reflect the extra-pulmonary features of the disease.

The 6-minute walking test (6MWT) is a simple and inexpensive test which provides a global and integrated response of both physical (pulmonary and non-pulmonary factors) and psychological factors [5,6]. The 6MWT is used to assess functional exercise capacity before and after interventions [7,8] and as a predictor of morbidity and mortality in COPD [9].

In general, the 6MWT is a reliable test in COPD patients, but a learning effect has been suggested [10-15], i.e., patients achieving a considerably higher walked distance when a second test is performed. Indeed, there is controversy about the size of the learning effect, which may range from 2.6% to 22% [10,11, 16-20]. Moreover, the external validity of the previous studies is limited due to the pre-specified inclusion criteria [10,11,13,18]. Moreover, researchers usually used statistical analysis that did not demonstrate trends and agreement between both 6MWTs compromising the internal validity of the results [10]. Furthermore, the determinants of improvement in walking distance remain unknown. Considering the importance of the 6MWT to clinical practice when assessing patients with COPD and to put together exercise training programs [21], its reproducibility and determining factors need to be more solidly studied.

Therefore, the aim of this study was to investigate the reproducibility of the 6MWT in a large and representative sample of patients with COPD and to quantify the learning effect between two 6MWTs performed in subsequent days. Secondly, we aimed to study the determining factors of changes in the second 6MWT.

METHODS

In a retrospective observational study, 1683 patients with COPD were included. Data were collected from patients who were evaluated during the baseline

assessment before entering a pulmonary rehabilitation program at the CIRO+, a center of expertise for chronic organ failure (Horn, the Netherlands) from January 2005 to August 2009. These retrospective analyses are institutional review board exempt due to the use of de-identified, preexisting data.

Inclusion criteria were: diagnosis of COPD according to criteria determined by the *Global Initiative for Chronic Obstructive Lung Disease* (GOLD) [1], clinical stability (absence of exacerbations in the last 3 months), non-participation in rehabilitation programs in the last 2 years, absence of unstable cardiac disease and absence of neurological comorbidities which may limit 6MWT performance. One hundred sixty-nine patients were excluded because they only performed one 6MWT. Therefore, 1514 patients with stable COPD (59% men) completed two 6MWTs and were included in the analyses (table 1).

Six-minute walking test

Two 6MWTs were performed according to the guidelines of the American Thoracic Society [6] in subsequent days in a triangular walking course of 125 meters. The walking tests were executed by a physiotherapist or a biometrist who walked behind the patient. Patients were instructed to walk as fast as they could and the distance walked was registered after 6 minutes. During the test, standardized encouraging phrases were given to patients each minute. Patients who used walking aids in daily life were allowed to use the devices during the 6MWT. Heart rate, SpO_2, perceived dyspnea and leg fatigue (modified Borg scale) were assessed before and after the 6MWTs. Oxygen supplementation was used if required, and oxygen desaturation during the 6MWT was defined as difference between end and beginning SpO_2 value of $\geq$-4% and/or an end SpO_2 of <88% [15]. Reference values for delta of Borg score and HR were those from van Stel et al. [22] and for 6MWT were those from Troosters et al. [23].

Lung function

Spirometry (Masterlab®, Germany) was performed according to the European Respiratory Society [24] and reference values were those from Knudson et al. [25]. Lung function parameters used for analysis were forced expiratory volume in the first second (FEV_1) and forced viral capacity (FVC).

Body composition

A total body scan was performed by whole-body dual energy X-ray absorptiometry (DEXA) using a Lunar Prodigy system (GE Healthcare, USA) as described before [26]. Fat-free mass (FFM) was provided from the sum of lean and mineral bone mass. The fat-free mass index (FFMI) was calculated as FFM (in kilograms) divided by height squared (in metres) [27]. The body mass index (BMI) was calculated as body weight (in kilograms) divided by height squared (in metres).

Functional limitation due to dyspnea

The Medical Research Council (MRC) scale (Anexo C) was used to evaluate the level of functional limitation due to breathlessness in activities of daily living [3].

BODE index

The BODE index (Body-mass index, airway Obstruction, Dyspnea and Exercise capacity) (Anexo D) is a multidimensional grading system used as predictor of risk of death in COPD patients and as an outcome reflecting disease severity. The index was calculated according to Celli et al. [28].

Comorbidities

The presence of comorbidities was evaluated using the Charlson index (Anexo E). It is composed by 19 categories of comorbidities and the total score reflects the cumulative increased likelihood of one-year mortality [29]. Higher score indicates more severe burden of comobidities.

Statistical analysis

Statistical analysis was performed using the statistical packge SPSS 17.0 (SPSS Inc., USA) and GraphPad Prism 5 (GraphPd Software Inc., USA). Data were described as mean ± standard deviation (SD). The intra-class correlation coefficient was used to verify the reproducibility of the 6MWT. The Bland & Altman plot was used to evaluate trend and agreement between first and second tests, as well as the paired t-test was used to compare outcome parameters between the two tests. Unpaired t-test and one-way ANOVA (*post-hoc* Tukey) were used to compare patient characteristics between different groups. Ordinal data were analyzed using

nonparametric tests. Logistic regression assessed determinant factors of a clinically important change in walked distance between the first and second tests (≥ 42 meters, which is the current upper limit of a minimal important difference for the 6MWT [7]). The independent variables included in the logistic regression model were those which have been shown to influence the performance in the 6MWT previously in the literature and those which reflect deconditioning and limitation due to the disease. They were: age, gender, severity of disease (GOLD stages), body composition (BMI and FFMI), use of LTOT, use of walking aids (cane and rollator), comorbidities (Charlson index), limitation due to dyspnea (MRC scale), a poor walking distance in the first test (< 350 m), symptoms (Borg scale for dyspnea and fatigue) and physical deconditining (desaturation and change in HR during the first test). The variables were dichotomized using values indicated in the current literature. The level of statistical significance was considered p≤0.05 for all.

RESULTS

Characteristics

In general, patients presented moderate to very severe COPD. Twenty-four percent of patients used ambulatory oxygen therapy, 32% were rollator users and 1.3% was cane users. The majority of patients (60%) had an abnormal BMI (low, overweight or obese), whereas 30% had an abnormally low FFMI. Moreover, 18% of patients had Charlson index >2 points, and 97% scored grade 2 or higher on the MRC dyspnea scale. Men were older than women, presenting lower FEV_1 and FVC, higher inspiratory capacity, lower MRC dyspnea grade and higher FFMI (table 1).

Reproducibility of the 6MWT

On average, patients walked 391m (95% CI 155; 585m) in the first 6MWT and 418m (185; 605 m) in the second 6MWT. The distance walked in the second test increased on average by 27m (-37; 107m). Thirty-five percent of the patients had a poor first 6MWT (<350m). This proportion of poor walkers decreased in the second 6MWT to 28%.

Eighty-two percent of patients with COPD walked farther during the second 6MWT. In fact, 28% of the subjects increased their walked distance by more than 42m, which is currently the upper limit of change considered as an important

treatment effect [7]. Conversely, 6% of COPD patients decreased their walked distance by more than 42m in the second test.

Rollator users tended to have a higher change than non-rollator users (30±51 vs. 25±46m, respectively; p=0.058). No difference was observed comparing the changes in walked distance between male and female subjects (26±51 vs. 28±42m, respectively; p>0.05). A similar proportion of men and women increased their walked distance in the second test (81% vs. 83%, respectively). Furthermore, no difference was observed comparing the changes in walked distance among GOLD stages I, II, III and IV (26±38, 24±48, 27±45 and 26±49m, respectively; p>0.05).

The Bland & Altman plot confirms that patients increased the distance walked in the second test (figure 1). Indeed, statistically the 6MWT was reproducible (ICC=0.93, p<0.0001). Then again, the limits of agreement between the two 6MWTs ranged from -67m to 120m. There was no correlation of the mean distance walked in the two 6MWTs on the improvement in the second test (r =-0.01; p = 0.61).

A model of logistic regression showed that a higher odds ratio for a clinically important improvement of ≥42m in the second 6MWT in comparison to the first 6MWT occurred in patients who had a poor first 6MWT (<350m) (OR 2.49 [95% CI 1.80; 3.46], p<0.0001), patients without self-reported co-morbidities other than COPD itself (OR 0.76 [0.58; 0.99], p=0.043) and patients with BMI <30 kg/m^2 (OR 0.60 [0.43; 0.85], p=0.004) (table 2). On the other hand, none of the studied patient characteristics was a determinant for decreasing the second 6MWT.

Reproducibility of oxygen desaturation, heart rate and Borg symptom scores

On average, the change in oxygen saturation during the first and second 6MWT was -5.7% (-15; 0%) and -5.5% (-16; 0%), respectively. The change in oxygen saturation was reproducible (ICC=0.81, p<0.0001). The Bland & Altman plot shows agreement between change in oxygen saturation during both 6MWTs, with limits of agreement ranging from -7% to 8% (figure 2). Moreover, the sensitivity and specificity to detect oxygen desaturation during the second 6MWT based on oxygen desaturation during the first 6MWT were 80% and 77%, respectively.

When comparing the change in HR, patients in GOLD stage IV had a lower change (16±14 bpm) comparing to GOLD I, II and III during the first test (24±15, 24±15 and 22±14 bpm, respectively; p>0.05 for all) and during the second test (18±13 bpm vs. 25±13, 24±14 and 22±14 bpm, respectively; p>0.05 for all).

However, when taking into account the whole group, change in heart rate during the first 6MWT was on average 21 bpm (0; 46 bpm) and 21 bpm (1; 46 bpm) during the second 6MWT. The reproducibility of change in heart rate when two 6MWTs were performed was only modest (ICC=0.62; p<0.0001).

Change in Borg dyspnea score was 2.60 pts (0-6 pts) and 2.52 pts (0-6 pts) during the first and second 6MWT, respectively. In addition, the change in Borg leg fatigue score was 1.98 pts (0-5 pts) during the first test and 2.01 pts (0-6 pts) during the second test. Once again, the reproducibility of change in Borg dyspnea and leg fatigue score when two 6MWTs were performed was only modest (ICC=0.59 and p<0.0001 for both).

DISCUSSION

This study examined the reproducibility of the 6MWT when two tests on subsequent days were performed in daily clinical practice in a large sample of patients with COPD entering pulmonary rehabilitation. Statistically, the 6MWT showed to be reproducible. However, a majority of patients increased the distance walked in the second test (mean change: 27m or 7% of the initial 6MWT), suggesting that there is a considerable learning effect. The Bland & Altman analysis confirmed that the second 6MWT was better than the first, with limits of agreement largely exceeding 42m, which is considered the upper limit of a clinically important change in the 6MWT [7]. Furthermore, we determined that a poor first 6MWT (<350m), Charlson index <2 points or a BMI <30 kg/m^2 are significant determinants of a clinically important changes in the second 6MWT (≥42m) in comparison to the first 6MWT.

Various studies have reported a learning effect when repeated walking tests are performed in COPD. However, the results are controversial regarding the size of this learning effect. For example, Troosters and colleagues found a learning effect of 2.6% in 20 patients with COPD who performed two 6MWTs in the same day [17]. Leach and colleagues found that patients with COPD and interstitial lung disease increased 8.6% the distance walked when two 6MWTs were performed in the same day [11]. Stevens and colleagues demonstrated a learning effect of approximately 10% in patients with advanced lung disease who were participants of a pulmonary rehabilitation program [18]. Spencer et al. found a 7% improvement on the second 6MWT in patients with COPD, which is in accordance with our findings [19]. Sciurba and colleagues [10] also found an increase of 7% when two 6MWTs were performed.

However, they included only patients with severe and very severe COPD. Besides the heterogeneity in the learning effect size, those studies often included patients with COPD and patients with other lung diseases [11,13,18] or only patients with severe disease [10], and this could compromise the external validity of their findings. Moreover, the majority of studies did not show the limits of agreement between the walking distance assessments, compromising the interpretation of the results and their internal validity [10,18-20].

The clinical implication of our findings is evident since the disagreement between two 6MWTs can influence interpretation of the performance in the test. Our results demonstrated that 82% of patients walked further in the second 6MWT, meaning that the vast majority of patients entering in a pulmonary rehabilitation program could have an incorrect conclusion about their functional exercise capacity if only one test was performed. Indeed, we have found that a poor first 6MWT, a score of <2 points on the Charlson index and non-obesity are significant determinants of a change in 6MWT $\geq$42 m. Therefore, incorrect exercise training workloads may be applied during exercise training in case of only 1 baseline 6MWT. Furthermore, this disagreement can be clinically relevant since the 6MWT has been widely used as a predictor of morbidity and mortality in patients with COPD. For example, the proportion of patients who had a poor 6MWT (<350m) decreased when the best of two walked distances was considered for analysis in our sample (35% vs. 28%). It shows that, at the first time, 7% of patients had a false poor walked distance detected. Therefore, it is recommended that, at least, two 6MWT are performed in clinical settings.

In our study, we also found that the change in oxygen saturation during the 6MWT was reproducible. Moreover, its sensitivity and specificity were 0.80 and 0.77, respectively. These findings are interesting for clinical practice since the 6MWT has been used to determine the need for oxygen ambulatory prescription in patients with COPD and results about its reproducibility are controversial. Poulain and colleagues found that oxygen desaturation, defined as a fall of $\geq$4% of resting SpO_2 value during at least 3 min, was reproducible when three 6MWTs were performed in 10 patients with COPD [30]. In contrast, Chatterjee and colleagues demonstrated that the 6MWT oxygen saturation has only modest reproducibility in determining the need for ambulatory oxygen (SpO_2 ≤88% during at least 5s) in stable COPD patients actively

participating in a pulmonary rehabilitation program when three 6MWT were performed (κ statistic=0.62, 72% of agreement between measurements) [15].

Strengths, limitations and future perspectives

Although some authors have already studied the reproducibility of the 6MWT, our study shows several points that strengthen the trustworthiness of our findings. Firstly, the present sample (n=1514) was undoubtedly representative of a COPD population and composed by patients with different levels of disease severity. Secondly, the statistical analysis produced results of clinical importance since not only average data were described, but limits of agreement between assessments as well. Furthermore, some methodological strategies were carefully provided to ensure results reliability: the same walking course was always used, avoiding influence of length and layout track on the walked distance [10]; tests were performed at the same period of the day; encouragement and instructions were standardized and supervisors were familiarized with the 6MWT [6]; and patients who previously participated in rehabilitation programs were not included in the study. However, some limitations occurred. Firstly, some variables which can influence the improvement of the second 6MWT were not studied, such as anxiety, depression and balance [5]. Secondly, the tests were not always executed by the same supervisor; however, as previously mentioned, all supervisors were strongly familiarized with the 6MWT standardization. In addition, the present results are only applicable to 6MWT performed before a PR program and on subsequent days.

CONCLUSION

In summary, the 6MWT was reproducible in a large and representative sample of patients with COPD. However, the vast majority of patients increased the second 6MWT, presenting a learning effect of 27m (or 7%) and with limits of agreement which largely exceed the minimal clinical difference. A poor performance in the first 6MWT, allied to few co-morbidities and non-obesity were the most relevant determinant factors of a change between the first and second tests which exceeds the clinical importance. Standardization of the 6MWT and at least two tests are necessary to avoid incorrect assessment of functional exercise capacity.

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REFERENCES

1. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 2007 Sep 15;176(6):532-55.

2. Pitta F, Troosters T, Spruit MA, Probst VS, Decramer M, Gosselink R. Characteristics of physical activities in daily life in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2005 May 1;171(9):972-7.

3. Spruit MA, Pennings HJ, Janssen PP, Does JD, Scroyen S, Akkermans MA, et al. Extra-pulmonary features in COPD patients entering rehabilitation after stratification for MRC dyspnea grade. Respir Med. 2007 Dec;101(12):2454-63.

4. Palange P, Ward SA, Carlsen KH, Casaburi R, Gallagher CG, Gosselink R, et al. Recommendations on the use of exercise testing in clinical practice. Eur Respir J. 2007 Jan;29(1):185-209.

5. Spruit MA, Watkins ML, Edwards LD, Vestbo J, Calverley PM, Pinto-Plata V, et al. Determinants of poor 6-min walking distance in patients with COPD: the ECLIPSE cohort. Respir Med. 2010 Jun;104(6):849-57.

6. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002 Jul 1;166(1):111-7.

7. Puhan MA, Mador MJ, Held U, Goldstein R, Guyatt GH, Schunemann HJ. Interpretation of treatment changes in 6-minute walk distance in patients with COPD. Eur Respir J. 2008 Sep;32(3):637-43.

8. Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, et al. Outcomes for COPD pharmacological trials: from lung function to biomarkers. Eur Respir J. 2008 Feb;31(2):416-69.

9. Pinto-Plata VM, Cote C, Cabral H, Taylor J, Celli BR. The 6-min walk distance: change over time and value as a predictor of survival in severe COPD. Eur Respir J. 2004 Jan;23(1):28-33.

10. Sciurba F, Criner GJ, Lee SM, Mohsenifar Z, Shade D, Slivka W, et al. Six-minute walk distance in chronic obstructive pulmonary disease: reproducibility and effect of walking course layout and length. Am J Respir Crit Care Med. 2003 Jun 1;167(11):1522-7.

11. Leach RM, Davidson AC, Chinn S, Twort CH, Cameron IR, Bateman NT. Portable liquid oxygen and exercise ability in severe respiratory disability. Thorax. 1992 Oct;47(10):781-9.

12. Iriberri M, Galdiz JB, Gorostiza A, Ansola P, Jaca C. Comparison of the distances covered during 3 and 6 min walking test. Respir Med. 2002 Oct;96(10):812-6.

13. Guyatt GH, Pugsley SO, Sullivan MJ, Thompson PJ, Berman L, Jones NL, et al. Effect of encouragement on walking test performance. Thorax. 1984 Nov;39(11):818-22.

14. Eiser N, Willsher D, Dore CJ. Reliability, repeatability and sensitivity to change of externally and self-paced walking tests in COPD patients. Respir Med. 2003 Apr;97(4):407-14.

15. Chatterjee AB, Rissmiller RW, Meade K, Paladenech C, Conforti J, Adair NE, et al. Reproducibility of the 6-minute walk test for ambulatory oxygen prescription. Respiration. 2010;79(2):121-7.

16. Wu G, Sanderson B, Bittner V. The 6-minute walk test: how important is the learning effect? Am Heart J. 2003 Jul;146(1):129-33.

17. Troosters T, Vilaro J, Rabinovich R, Casas A, Barbera JA, Rodriguez-Roisin R, et al. Physiological responses to the 6-min walk test in patients with chronic obstructive pulmonary disease. Eur Respir J. 2002 Sep;20(3):564-9.

18. Stevens D, Elpern E, Sharma K, Szidon P, Ankin M, Kesten S. Comparison of hallway and treadmill six-minute walk tests. Am J Respir Crit Care Med. 1999 Nov;160(5 Pt 1):1540-3.

19. Spencer LM, Alison JA, McKeough ZJ. Six-minute walk test as an outcome measure: are two six-minute walk tests necessary immediately after pulmonary rehabilitation and at three-month follow-up? Am J Phys Med Rehabil. 2008 Mar;87(3):224-8.

20. Rodrigues SLM, H. F.; Viegas, C. A. A. Six minute walk test: study of the effect of learning in Chronic Obstructive Pulmonary Disease patients. Rev Port Pneumol. 2004;30(2):121-5.

21. Spruit MA, Wouters EF. New modalities of pulmonary rehabilitation in patients with chronic obstructive pulmonary disease. Sports Med. 2007;37(6):501-18.

22. van Stel HF, Bogaard JM, Rijssenbeek-Nouwens LH, Colland VT. Multivariable assessment of the 6-min walking test in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001 Jun;163(7):1567-71.

23. Troosters T, Gosselink R, Decramer M. Six minute walking distance in healthy elderly subjects. Eur Respir J. 1999 Aug;14(2):270-4.

24. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl. 1993 Mar;16:5-40.

25. Knudson RJ, Burrows B, Lebowitz MD. The maximal expiratory flow-volume curve: its use in the detection of ventilatory abnormalities in a population study. Am Rev Respir Dis. 1976 Nov;114(5):871-9.

26. Graat-Verboom L, Spruit MA, van den Borne BE, Smeenk FW, Martens EJ, Lunde R, et al. Correlates of osteoporosis in chronic obstructive pulmonary disease: An underestimated systemic component. Respir Med. 2009 Aug;103(8):1143-51.

27. Schols AM, Soeters PB, Dingemans AM, Mostert R, Frantzen PJ, Wouters EF. Prevalence and characteristics of nutritional depletion in patients with stable COPD eligible for pulmonary rehabilitation. Am Rev Respir Dis. 1993 May;147(5):1151-6.

28. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med. 2004 Mar 4;350(10):1005-12.

29. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol. 1994 Nov;47(11):1245-51.

30. Poulain M, Durand F, Palomba B, Ceugniet F, Desplan J, Varray A, et al. 6-minute walk testing is more sensitive than maximal incremental cycle testing for detecting oxygen desaturation in patients with COPD. Chest. 2003 May;123(5):1401-7.

Table 1 - Characteristics of patients with COPD and outcome parameters of the 6MWT.

	COPD patients (n=1514)	Male (n=888)	Female (n=626)
Age (years)	64 ± 10	66 ± 9	62 ± 9*
BMI ($kg.m^{-2}$)	25 ± 5	25 ± 5	25 ± 6
BMI < 21 $kg.m^{-2}$ (%)	16	14	20
BMI 21 - 24 $kg.m^{-2}$ (%)	40	40	42
BMI 25 - 29 $kg.m^{-2}$ (%)	28	31	24
BMI ≥ 30 $kg.m^{-2}$ (%)	16	15	14
FFMI ($kg.m^{-2}$)	16 ± 2	17 ± 2	15 ± 2*
FFMI < 16 $kg.m^{-2}$ (%)	-	24	-
FFMI < 15 $kg.m^{-2}$ (%)	-	-	39
FEV_1 (% pred)	45 ± 18	44 ± 17	47 ± 19*
FVC (%pred)	92 ± 23	90 ± 22	95 ± 24*
Inspiratory Capacity (L)	3.04 ± 0.97	3.45 ± 0.91	2.46 ± 0.73*
GOLD I/ II/ III/ IV (%)	5/ 31/ 44/ 20	4/ 29/ 47/ 20	6/ 35/ 38/ 21
MRC dyspnea scale (grade)	4 ± 1	3 ± 1	4 ± 1*
MRC grade ≥ 2 (%)	97	97	98
BODE index (points)	4 ± 2	4 ± 2	4 ± 2
LTOT (%)	24	22	26
Charlson index 1 /2 / >2 (%)	59/23/18	53/26/22	69/19/12
Rollator (%)	32	28	37
Cane (%)	1.3	1.6	0.8
6MWT T1 (m)	391 ± 127	405 ± 128	372 ± 124*
6MWT T2 (m)	418 ± 127†	430 ± 128†	399 ± 122*†
∆6MWT (m)	27 ± 48	26 ± 51	28 ± 42
Borg D baseline T1 (points)	2.20 ± 1.58	2.26 ± 1.55	2.11 ± 1.61
Borg D baseline T2 (points)	2.17 ± 1.53	2.20 ± 1.56	2.14 ± 1.49
∆Borg D T1 (points)	2.60 ± 1.97	2.55 ± 1.95	2.66 ± 1.99
∆Borg D T2 (points)	2.52 ± 1.95	2.47 ± 1.98	2.60 ± 191
Borg F baseline T1 (points)	1.84 ± 1.65	1.86 ± 1.66	1.79 ± 1.65
Borg F baseline T2 (points)	2.10 ± 1.74†	2.17 ± 1.76†	2.00 ± 1.69
∆Borg F T1 (points)	1.98 ± 1.93	1.93 ± 1.98	2.05 ± 1.84
∆Borg F T2 (points)	2.01 ± 2.02	2.02 ± 2.06	1.99 ± 1.96
SpO_2 baseline T1 (%)	94 ± 2	94 ± 4	95 ± 5
SpO_2 baseline T2 (%)	94 ± 3	94 ± 4	95 ± 3*
ΔSpO_2 T1 (%)	-5.7 ± 5.1	-5.5 ± 5.0	-5.8 ± 5.1
ΔSpO_2 T2 (%)	-5.5 ± 5.0	-5.5 ± 5.0	-5.5 ± 4.9
HR baseline T1 (bpm)	86 ± 16	85 ± 16	87 ± 16*
HR baseline T2 (bpm)	87 ± 14	85 ± 15	89 ± 14*
∆HR T1 (bpm)	21 ± 15	22 ± 15	21 ± 14
∆HR T2 (bpm)	22 ± 14	22 ± 14	21 ± 14

BMI: body mass index, FFMI: fat-free mass index, FEV_1: forced expiratory volume in the first second, FVC: forced vital capacity, GOLD: Global Initiative for Chronic Obstructive Lung Disease, MRC: Medical Research Council, BODE: Body mass-index, airflow Obstruction, Dyspnea and Exercise capacity, LTOT: long-term oxygen therapy, 6MWT: six-minute walking test, T1: first test, T2: second test, Borg D: Borg dyspnea scale, Borg F: Borg fatigue scale, SpO_2: peripheral oxygen saturation, HR: heart rate, bpm: beats per minute. Data are expressed as mean ± SD.

* Male vs. Female, $p<0.0001$. † T1 vs. T2, $p<0.0001$ for all.

Table 2 - Relationship between patient characteristics and change in the walked distance in patients with COPD.

		n	Delta 6MWT ≥ 42 m	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Gender	Male	888	250 (58.4%)	1.0	0.905	1.0	0.130
	Female	626	178 (41.6%)	1.01 (0.81-1.27)		0.82 (0.63-1.06)	
Age	≥65 yrs	761	209 (48.8%)	1.0	0.484	1.0	0.172
	<65 yrs	753	219 (51.2%)	0.92 (0.74-1.15)		0.83 (0.64-1.08)	
GOLD	I/II	555	148 (34.6%)	1.0	0.292	1.0	0.519
	III/IV	959	280 (65.4%)	0.88 (0.70-1.11)		1.10 (0.82-1.47)	
BMI	≥ 30 $kg.m^{-2}$	293	68 (16%)	1.0	0.031*	1.0	0.004*
	< 30 $kg.m^{-2}$	1208	357 (84%)	0.72 (0.53-0.97)		0.60 (0.43-0.85)	
FFMI	≥15 $kg.m^{-2}$ (F)/ ≥16 $kg.m^{-2}$ (M)	1032	283 (68%)	1.0	0.242	1.0	0.885
	<15 $kg.m^{-2}$ (F)/<16 $kg.m^{-2}$ (M)	437	133 (32%)	1.16 (0.91-1.48)		0.98 (0.74-1.29)	
LTOT	No	1150	318 (74.3%)	1.0	0.343	1.0	0.675
	Yes	364	110 (25.7%)	1.13 (0.87-1.47)		0.93 (0.66-1.31)	
Rollator	No	1029	270 (63.1%)	1.0	0.011*	1.0	0.602
	Yes	485	158 (36.9%)	1.36 (1.07-1.72)		0.91 (0.63-1.30)	
Cane	No	1495	423 (98.8%)	1.0	0.849	1.0	0.788
	Yes	19	5 (1.2%)	0.90 (0.32-2.53)		0.86 (0.29-2.58)	
Charlson index	≥ 2pt	610	164 (38.3%)	1.0	0.326	1.0	0.043*
	1 pt	904	264 (61.7%)	0.89 (0.71-1.12)		0.76 (0.58-0.99)	
MRC dyspnea grade	Grade 4/5 pts	830	247 (59.5%)	1.0	0.176	1.0	0.415
	Grade ≤ 3 pts	633	168 (40.5%)	0.85 (0.68-1.07)		1.12 (0.85-1.47)	
6MWT1	≥350m	982	225 (52.6%)	1.0	0.000*	1.0	0.000*
	<350m	532	203 (47.4%)	2.07 (1.65-2.61)		2.49 (1.80-3.46)	
Desaturation during 6MWT1	No	575	179 (42.3%)	1.0	0.04*	1.0	0.061
	Yes	930	244 (57.7%)	1.27 (1.01-1.60)		0.78 (0.60-1.01)	
Borg $D_{baseline}$ stable	Yes	1107	300 (70.8%)	1.0	0.130	1.0	0.818
	No	399	124 (29.2%)	1.21 (0.94-1.56)		1.22 (0.93-1.61)	
Borg $F_{baseline}$ stable	Yes	1022	283 (66.6%)	1.0	0.538	1.0	0.389
	No	486	142 (33.4%)	1.08 (0.85-1.37)		1.12 (0.86-1.46)	
ΔHR 6MWT1	>15bpm	994	252 (60%)	1.0	0.001*	1.0	0.238
	≤15bpm	498	168 (40%)	1.50 (1.19-1.89)		1.17 (0.90-1.52)	

OR: odds ratio, 95% CI: 95% confidence interval, 6MWT: six-minute walking test, GOLD: Global Initiative for Chronic Obstructive Lung Disease, BMI: body mass index, FFMI: fat-free mass index, LTOT: long-term oxygen therapy, MRC: Medical Research Council, 6MWT1: first six-minute walking test, Borg D: Borg scale for dyspnea, Borg F: Borg scale for leg fatigue, HR: heart rate, bpm: beats per minute. * p≤0.05 for all.

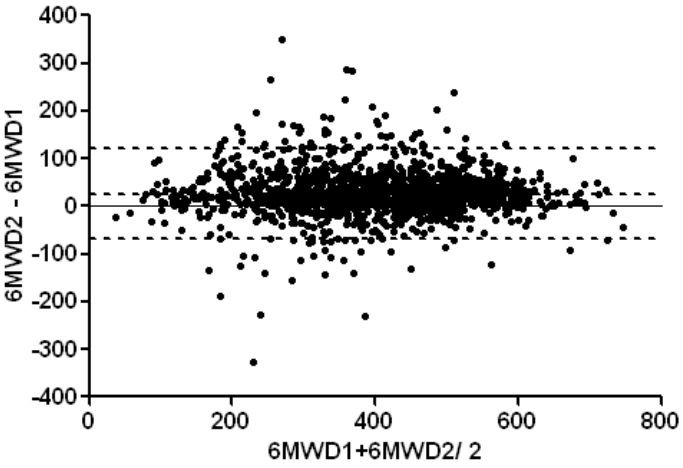

Figure 1 - Bland and Altman plot of the difference between two 6-min walking tests (6MWTs) plotted against the mean value of the first (1) and second (2) 6MWT for the entire group of patients. The central dotted line corresponds to the average difference between two 6MWTs, whereas the lower and upper dotted lines correspond to lower and upper limits of agreement, respectively.

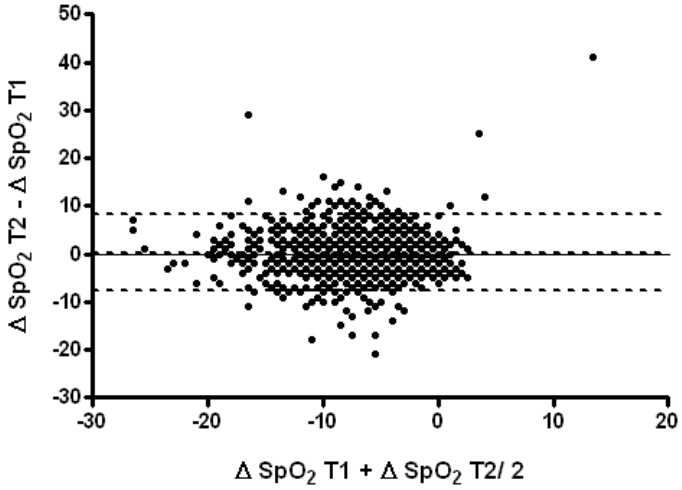

Figure 2 - Bland and Altman plot of the difference between the change of arterial oxygen saturation measured by pulse oximetry (DSp,O2) during two 6-min walking tests (6MWTs) plotted against the mean value of the change of oxygen saturation during the first (1) and second (2) 6MWT for the entire group of patients. The central dotted line corresponds to the average difference between change of oxygen saturation during two 6MWTs, whereas the lower and upper dotted lines correspond to lower and upper limits of agreement, respectively.

4 CONCLUSÔES E CONSIDERAÇÕES FINAIS

4.1 CONCLUSÕES

Primeiramente, com base nas variáveis idade, gênero e índice de massa corpórea, uma equação para predição de valores de referência do ISWT foi estabelecida para indivíduos saudáveis de diferentes faixas etárias. Além disso, pode-se concluir que 71% da variação da distância percorrida no ISWT por indivíduos saudáveis é explicada pela combinação dessas variáveis antropométricas e demográficas.

Por fim, verificou-se que o TC6 foi reprodutível em uma amostra representativa de pacientes portadores de DPOC. Entretanto, a maioria dos pacientes aumentou a distância percorrida quando um segundo teste foi realizado, existindo um efeito aprendizagem de 27 m (ou 7%) com limites de concordância que excederam a diferença mínima clinicamente importante estabelecida para o TC6 em DPOC. Demonstrou-se também que um desempenho ruim no primeiro TC6 associado à presença de poucas comorbidades e à ausência de obesidade foram fatores associados a um aumento clinicamente importante na distância percorrida no segundo teste.

4.2 CONSIDERAÇÕES FINAIS

O presente trabalho estabeleceu uma equação para predição de valores de referência do ISWT com base em variáveis antropométricas e demográficas facilmente obtidas em pesquisa e na prática clínica. Isso possibilitará a comparação do desempenho no teste entre indivíduos e grupos de indivíduos com diferentes características antropométricas e demográficas, além de permitir a quantificação do grau de limitação da capacidade de exercício dos indivíduos avaliados. Dessa maneira, torna-se possível uma melhor interpretação clínica dos resultados obtidos no ISWT.

A reprodutibilidade do TC6, bem como o tamanho do efeito aprendizagem existente entre dois testes puderam ser solidamente estudados, obtendo-se resultados com evidente relevância clínica. Com base nos resultados,

demonstrou-se que a padronização da aplicação do TC6 é necessária para evitar erros de interpretação dos resultados obtidos com o teste. Tendo em vista a magnitude do efeito aprendizagem existente (27m ou 7%), recomenda-se que dois testes sejam realizados. Caso contrário, existe grande risco de interpretação incorreta da capacidade funcional de exercício de pacientes com DPOC, bem como do efeito de intervenções nessa população. A não realização de dois TC6 também pode implicar na prescrição incorreta de cargas de treinamento físico e na avaliação prognóstica incorreta desses pacientes.

É válido ressaltar que os resultados obtidos no presente estudo são aplicáveis a situações de avaliação pré-reabilitação pulmonar, sendo necessários mais estudos para investigar a reprodutibilidade do TC6 após a reabilitação. Pesquisas futuras também são necessárias para se estudar o efeito aprendizagem quando três ou mais ISWT são realizados a fim de padronizar o número de testes que devem ser aplicados ao avaliar indivíduos de diferentes faixas etárias ou diferentes graus de comprometimento da capacidade de exercício.

5. REFERÊNCIAS

1. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2011.

2. Barnes PJ, Celli BR. Systemics manifestations and comorbidities of COPD. Eur Respir J 2009; 33: 1165-1185.

3. Celli BR, Cote CG, Marin JM, Casanova C, Montes O, Mendez RA, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med 2004; 350(10):1005-1012.

4. Oga T, Nishimura K, Tsukino M, Sato S, Hajiro T. Analysis of the factors related to mortality in chronic obstructive pulmonary disease: role of exercise capacity and health status. Am J Respir Crit Care Med 2003; 167(4):544-549.

5. Palange P, Ward SA, Carlsen KH, Casaburi R, Gallagher CG, Gosselink R et al. Recommendations on the use of exercise testing in clinical practice. Eur Respir J 2007; 29(1):185-209.

6. Singh SJ, Morgan MD, Scott S, Walters D, Hardman AE. Development of a shuttle walking test of disability in patients with chronic airways obstruction. Thorax 1992; 47(12):1019-1024.

7. Ringbaek T, Martinez G, Brondum E, Thogersen J, Morgan M, Lange P. Shuttle walking test as predictor of survival in chronic obstructive pulmonary disease patients enrolled in a rehabilitation program. J Cardiopulm Rehabil Prev 2010; 30(6):409-414.

8. Emtner MI, Arnardottir HR, Hallin R, Lindberg E, Janson C. Walking distance is a predictor of exacerbations in patients with chronic obstructive pulmonary disease. Respir Med 2007; 101(5):1037-1040.

9. Evans RA, Singh SJ, Collier R, Williams JE, Morgan MD. Pulmonary rehabilitation is successful for COPD irrespective of MRC dyspnoea grade. Respir Med 2009; 103(7):1070-1075.

10. Singh S, Harrison S, Houchen L, Wagg K. Exercise assessment and training in pulmonary rehabilitation for patients with COPD. Eur J Phys Rehabil Med 2011; 47(3):483-497.

11. Sandland CJ, Morgan MD, Singh SJ. Detecting oxygen desaturation in patients with COPD: incremental versus endurance shuttle walking. Respir Med 2008; 102(8):1148-1152.

12. Singh SJ, Morgan MD, Hardman AE, Rowe C, Bardsley PA. Comparison of oxygen uptake during a conventional treadmill test and the shuttle walking test in chronic airflow limitation. Eur Respir J 1994; 7(11):2016-2020.

13. McKeough ZJ, Leung RW, Alison JA. Shuttle walk tests as outcome measures: Are two incremental shuttle walk tests and two endurance shuttle walk tests necessary? Am J Phys Med Rehabil 2011; 90(1):35-39.

14. Singh SJ, Jones PW, Evans R, Morgan MD. Minimum clinically important improvement for the incremental shuttle walking test. Thorax 2008; 63(9):775-777.

15. Jurgensen SP, Antunes LC, Tanni SE, Banov MC, Lucheta PA, Bucceroni AF, et al. The incremental shuttle walk test in older brazilian adults. Respiration 2011; 81(3):223-228.

16. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002; 166(1):111-117.

17. Troosters T, Vilaro J, Rabinovich R, Casas A, Barbera JA, Rodriguez-Roisin R, et al. Physiological responses to the 6-min walk test in patients with chronic obstructive pulmonary disease. Eur Respir J 2002; 20(3):564-569.

18. Puhan MA, Mador MJ, Held U, Goldstein R, Guyatt GH, Schunemann HJ. Interpretation of treatment changes in 6-minute walk distance in patients with COPD. Eur Respir J 2008; 32(3):637-643.

19. Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, et al. Outcomes for COPD pharmacological trials: from lung function to biomarkers. Eur Respir J 2008; 31(2):416-469.

20. Jenkins S, Hill K, Cecins NM. State of the art: How to set up a pulmonary rehabilitation program. Respirology 2010; 15: 1157-1173.

21. Poulain M, Durand F, Palomba B, Ceugniet F, Desplan J, Varray A, et al. 6-minute walk testing is more sensitive than maximal incremental cycle testing for detecting oxygen desaturation in patients with COPD. Chest 2003; 123(5):1401-7.

22. Pinto-Plata VM, Cote C, Cabral H, Taylor J, Celli BR. The 6-min walk distance: change over time and value as a predictor of survival in severe COPD. Eur Respir J 2004; 23(1):28-33.

23. Cote CG, Pinto-Plata V, Kasprzyk K, Dordelly LJ, Celli BR. The 6-min walk distance, peak oxygen uptake, and mortality in COPD. Chest 2007; 132(6):1778-1785.

24. Cote CG, Pinto-Plata VM, Marin JM, Nekach H, Dordelly LJ, Celli BR. The modified BODE index: validation with mortality in COPD. Eur Respir J 2008; 32(5):1269-1274.

25. Troosters T, Gosselink R, Decramer M. Six minute walking distance in healthy elderly subjects. Eur Respir J 1999; 14(2):270-274.

26. Enright PL, Sherrill DL. Reference equations for the six-minute walk in healthy adults. Am J Respir Crit Care Med 1998; 158:1384-1387.

27. Gibbons WJ, Fruchter N, Sloan S, Levy RD. Reference values for a multiple repetition 6-minute walk test in healthy adults older than 20 years. J Cardiopulm Rehabil 2001; 21(2):87-93.

28. Camarri B, Eastwood PR, Cecins NM, Thompson PJ, Jenkins S. Six minute walk distance in healthy subjects aged 55-75 years. Respir Med 2006; 100(4):658-665.

29. Chetta A, Zanini A, Pisi G, Aiello M, Tzani P, Neri M, et al. Reference values for the 6-min walk test in healthy subjects 20-50 years old. Respir Med 2006; 100(9):1573-1578.

30. Iwama AM, Andrade GN, Shima P, Tanni SE, Godoy I, Dourado VZ. The six-minute walk test and body weight-walk distance product in healthy Brazilian subjects. Braz J Med Biol Res 2009; 42(11):1080-1085.

31. Soaresa MR, Pereira CA. Six-minute walk test: reference values for healthy adults in Brazil. J Bras Pneumol 2011; 37(5):576-583.

32. Dourado VZ, Vidotto MC, Guerra RL. Reference equations for the performance of healthy adults on field walking tests. J Bras Pneumol 2011; 37(5):607-614.

33. Sciurba F, Criner GJ, Lee SM, Mohsenifar Z, Shade D, Slivka W, et al. Six-minute walk distance in chronic obstructive pulmonary disease: reproducibility and effect of walking course layout and length. Am J Respir Crit Care Med 2003; 167(11):1522-1527.

34. Leach RM, Davidson AC, Chinn S, Twort CH, Cameron IR, Bateman NT. Portable liquid oxygen and exercise ability in severe respiratory disability. Thorax 1992; 47(10):781-789.

35. Iriberri M, Galdiz JB, Gorostiza A, Ansola P, Jaca C. Comparison of the distances covered during 3 and 6 min walking test. Respir Med 2002; 96(10):812-816.

36. Guyatt GH, Pugsley SO, Sullivan MJ, Thompson PJ, Berman L, Jones NL, et al. Effect of encouragement on walking test performance. Thorax 1984; 39(11):818-822.

37. Eiser N, Willsher D, Dore CJ. Reliability, repeatability and sensitivity to change of externally and self-paced walking tests in COPD patients. Respir Med 2003; 97(4):407-414.

38. Chatterjee AB, Rissmiller RW, Meade K, Paladenech C, Conforti J, Adair NE, et al. Reproducibility of the 6-minute walk test for ambulatory oxygen prescription. Respiration 2010; 79(2):121-127.

39. Spencer LM, Alison JA, McKeough ZJ. Six-minute walk test as an outcome measure: are two six-minute walk tests necessary immediately after pulmonary rehabilitation and at three-month follow-up? Am J Phys Med Rehabil 2008; 87(3):224-228.

40. Stevens D, Elpern E, Sharma K, Szidon P, Ankin M, Kesten S. Comparison of hallway and treadmill six-minute walk tests. Am J Respir Crit Care Med 1999; 160(5 Pt 1):1540-1543.

41. Wu G, Sanderson B, Bittner V. The 6-minute walk test: how important is the learning effect? Am Heart J 2003; 146(1):129-133.

ANEXOS

ANEXO A

Questionário de Comorbidades

1) O Sr./Sra. teve alguma doença grave no passado? □ Sim □ Não
Se sim, qual ?……..
Com qual idade ?…….

2) O Sr./Sra. tem:

Asma ou outra doença pulmonar	□ Sim □ Não	
Artrose / Artrite	□ Sim □ Não	
Doença do coração	□ Sim □ Não	
Pressão alta	□ Sim □ Não	
Diabetes	□ Sim □ Não	
Osteoporose	□ Sim □ Não	
Problema de tireóide (qual ?)	□ Sim □ Não	……
Problema vascular (qual ?)	□ Sim □ Não	……
Alergia (a quê ?)	□ Sim □ Não	……
Doença cardíaca na família (qual ?)	□ Sim □ Não	……

3) O Sr./Sra. toma alguma medicação no momento? □ Sim □ Não
Se sim, qual o nome e a dose do medicamento ?
…….……
……

4) O Sr./Sra tomou algum medicamento nos últimos 12 meses que não esteja mais tomando no momento ? □ Sim □ Não
Se sim, qual o nome e a dose do medicamento ?
……
……

5) O Sr./Sra já foi hospitalizado(a) por um período maior do que um dia ?
□ Sim □ Não
Se sim, quando (aproximadamente) ?…….....
Por qual razão ?…….....

6) O Sr./Sra fuma ou já fumou ? □ Sim □ Não
Se sim, quantos cigarros/maços por dia e desde quando / até quando ?
……....

7) O Sr. Sra teve algum problema ortopédico que gerou alguma limitação importante nas suas atividades da vida diária ? (por exemplo, problemas sérios nas costas ou joelho) □ Sim □ Não
Se sim, qual era o problema e desde quando /até quando ?

……...

8) O Sr./Sra ainda trabalha (profissionalmente) ? □ Sim □ Não
Se sim, por quantas horas e quantos dias por semana ?
……...

9) Qual é a atividade mais cansativa que o Sr./Sra realiza toda semana ?
……...

10) O Sr./Sra participa de competições esportivas ? □ Sim □ Não
Se sim, qual esporte, com qual freqüência e por há quanto tempo?
……...

11) Qual é o seu peso hoje (aproximadamente) ? ……Kg
Qual era o seu peso há um ano (aproximadamente) ?……..Kg

ANEXO B

Escala de Borg modificada

0	Nenhuma
0,5	Muito, muito leve
1	Muito leve
2	Leve
3	Moderada
4	Pouco intensa
5	Intensa
6	
7	Muito intensa
8	
9	
10	Muito, muito intensa
#	Máxima

A escala de Borg modificada é utilizada para a avaliação da sensação de esforço percebido para dispneia e fadiga. Sua pontuação varia de 0 a 10 pontos, sendo que uma maior pontuação indica maior sensação de dispneia ou fadiga.

Referência:

Wilson RC, Jones PW. A comparison of the visual analogue scale and modified Borg scale for the measurement of dyspnoea during exercise. Clin Sci 1989; 76(3):277-82.

ANEXO C

Escala MRC

1. Só sofre de falta de ar durante exercícios intensos
2. Sofre de falta de ar quando andando apressadamente ou subindo uma rampa leve
3. Anda mais devagar do que pessoas da mesma idade por causa de falta de ar ou tem que parar para respirar mesmo quando andando devagar
4. Pára para respirar depois de andar menos de 100 metros ou após alguns minutos
5. Sente tanta falta de ar que não sai mais de casa, ou quando está se vestindo

A escala MRC avalia o grau de limitação nas atividades de vida diária devido à sensação de dispneia de pacientes portadores de DPOC e já foi validada para o idioma português (referência abaixo). A escala é composta por cinco itens e uma maior pontuação indica maior limitação devido à dispneia.

Referência:

Kovelis D, Segretti NO, Probst VS, Lareau SC, Brunetto AF, Pitta F. Validação do Modified Pulmonary Functional Status and Dyspnea Questionnaire e da escala do Medical Research Council para o uso em pacientes com doença pulmonar obstrutiva crônica no Brasil. J Bras Pneumol 2008; 34(12): 1008-1018.

ANEXO D

Índice BODE

Variável	Pontos no índice BODE			
	0	**1**	**2**	**3**
VEF_1 (% predito)	≥ 65	50 - 64	36 - 49	≤ 35
TC6 (m)	≥ 350	250 - 349	150 - 249	≤ 149
Escala MRC modificada	0 - 1	2	3	4
IMC (Kg/ m^2)	> 21	≤ 21		

O índice BODE (*Body-mass index, airflow Obstruction, Dyspnea and Exercise capacity*) é um sistema multigraduado utilizado como preditor de mortalidade em pacientes portadores de DPOC. O índice é calculado com base em quatro variáveis com as seguintes pontuações: uma medida de avaliação da composição corporal (IMC) de 0 a 1 ponto; uma medida de grau de obstrução das vias aéreas (VEF_1 em % do predito após bronco-dilatador) de 0 a 3 pontos; uma medida de sensação subjetiva de dispneia (escala MRC modificada) de 0 a 3 pontos; e uma medida de capacidade de exercício (distância percorrida no TC6) de 0 a 3 pontos. A pontuação final do índice BODE varia de 0 a 10 pontos, sendo que quanto maior a pontuação do paciente, maior é o risco de mortalidade precoce.

Referência:

Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med 2004; 350(10):1005-12.

ANEXO E

Índice Charlson

Comorbidade	Score
Infarto agudo do miocárdio	1
Insuficiência cardíaca congestiva	1
Doença vascular periférica	1
Acidente vascular encefálico	1
Demência	1
Doença pulmonar crônica	1
Doença do tecido conjuntivo	1
Diabetes mellitus sem complicação	1
Úlcera	1
Hemiplegia	2
Insuf. renal crônica moderada a grave	2
Hepatite A	2
Diabetes mellitus com complicação	2
Tumor	2
Leucemia	2
Linfoma	2
Hepatite B ou C	3
AIDS	6
Tumor maligno com metástase	6

O índice Charlson é composto por 19 comorbidades as quais recebem pontuação conforme a sua gravidade. O escore total reflete o risco de mortalidade em um ano. Quanto maior o escore, pior é o prognóstico do paciente.

Referência:

Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol. 1994 Nov;47(11):1245-51.

ANEXO F

Normas para publicação - *Respiratory Medicine*

Manuscript submission checklist

Authors should ensure they have uploaded the following as separate items in order for the editorial office to process their submission. Failure to provide any of the mandatory items below will result in the manuscript being returned to the author.

- Cover letter (mandatory)
- Abstract (including clinical trial registration number where appropriate) (mandatory)
- Conflict of Interest Statement (mandatory) Manuscript including ethics statement as appropriate (mandatory)
- Artwork (optional)
- Supplementary files eg. datasets, video files (optional)
- Permissions letters (As necessary, see below)
- Consolidated Standards of Reporting Trials (CONSORT) flow chart as appropriate

Format and Structure

Most text formats can be accommodated, but Microsoft Word is preferable. In general, articles should conform to the conventional structure of Summary, Introduction, Methods, Results, Discussion and References.

Title page

Your title page, should give the title in capital letters (not exceeding 100 letters), a running title (not exceeding 50 letters) and the authors' names (as they are

to appear), affiliations and complete addresses, including postal (zip) codes. The author and address to whom correspondence should be sent must be clearly indicated. Please supply telephone, fax and e-mail numbers for the corresponding author.

Abstract

An abstract of your manuscript summarizing the content, at a maximum of 250 words, should be provided as a separate submission item.

Reference Format

Manuscripts should use the 'Embellished Vancouver' style for references, as follows:

Text: Indicate references by superscript numbers in the text. The actual authors can be referred to, but the reference number(s) must always be given.
List: Number the references in the list in the order in which they appear in the text.

Examples:

Reference to a journal publication:

1. Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. J Sci Commun 2000; 163:51–9.

Reference to a book:

2. Strunk Jr W, White EB. The elements of style. 3rd ed. New York: Macmillan; 1979.
Reference to a chapter in an edited book:

3. Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age. New York: E-Publishing Inc; 1999, p. 281–304.
Note shortened form for last page number. e.g., 51–9, and that for more than 6 authors the first 6 should be listed followed by 'et al.' For further details you are

referred to "Uniform Requirements for Manuscripts submitted to Biomedical Journals" (*J Am Med Assoc* 1997;277:927–34), see also:

http://www.nlm.nih.gov/tsd/serials/terms_cond.html

Figures

Figures of good quality should be submitted online as a separate file. For detailed instructions on the preparation of electronic artwork, consult: http://www.elsevier.com/authors. Permission to reproduce illustrations should always be obtained before submission and details included with the captions.

Tables

Tables should be submitted online as a separate file, bear a short descriptive title, and be numbered in Arabic numbers. Tables should be cited in the text.

Keywords

A list of three to six keywords should be supplied: full instructions are provided when submitting the article online.

Units and Abbreviations

These should be given in SI units with the traditional equivalent in parentheses where appropriate. Conventions for abbreviations should be those detailed in Units, Symbols, and Abbreviations, available from the Royal Society of Medicine.

Language Editing

Papers will only be accepted when they are written in an acceptable standard of English.

ANEXO G

Normas para publicação - *European Respiratory Journal*

General

- The manuscript file you submit must be saved as .rtf (rich text format) or .doc (MS Word document).
- Original articles should not exceed 3,000 words, not including abstract, references, tables and legends. However, if manuscripts do exceed this limit, please state the final word count and explicit reasons for exceeding the limit in your covering letter.
- Figures and/or tables should be limited to no more than eight altogether. Large figures with a high number of parts should be avoided owing to space limitations. Large figures can be presented in the online depository.
- Abbreviations or unusual terms should be described at the first time of use.
- Symbols as defined by the ad hoc working group of the Commission of the European Communities (see *Eur Respir J* 1993; 6: Suppl. 16) are recommended.
- Système International (SI) units are recommended.
- Equations should be created as normal text.

Title page

- Please provide a concise and informative title, limited to 90 characters, including spaces between words.
- Include a list of all contributing authors and all of their affiliations, with a clear indication of who is associated with each institution.
- Supply the full correspondence details for the corresponding author, including e-mail address. Please note that only one corresponding author per manuscript should be provided.

Abstract

- Please provide an abstract of 200 words or fewer, which is easily understood without reference to the text (see *Ann Intern Med* 1987; 106: 598–604).
- The abstract must have four separate paragraphs, which correspond to the question of the study, materials/patients and methods, results, and the answer to the question. One or two sentences of background information can be included before the question if necessary. The question and answer should be the same as those in the text.
- Include only a few important values.
- Avoid using abbreviations.

Keywords

- Please provide a list of 6 keywords or fewer.
- The keywords should be listed alphabetically.
- The keywords should be listed in full without abbreviations.

Introduction

- State the question you asked (or hypothesis to be tested) and your considerations leading to the formulation of the question.
- Give only pertinent references.

Material and methods

Study subjects or animals

- Clearly describe how the subjects or experimental animals were identified, including the control subjects when used. For animals, see *Laboratory Animals* 1985; 19: 106–108.
- Clearly state the eligibility criteria for cases and controls in observational studies, or for subjects in clinical trials.

- All work involving studies on human subjects is expected to have received approval from local Ethical Committees and the regulatory authority (when appropriate, for example, drug trials).
- Animal experimentation must be performed according to the Helsinki convention for the use and care of animals.
- Provide details of the species and/or strain and number of animals involved in the study.
- The Editors reserve the right to refuse work which does not conform to acceptable ethical criteria.

Study design

- Clearly state the main study objective(s).
- Provide an overview of the main tests or experiments.
- Consider sample size and whether you have enough subjects to reliably address the research question.
- Papers on clinical trials should include details of the sample size calculation (*i.e.* the expected effect size, power, level of statistical significance and one- or two-sided test). The sample size should be reproduced independently.

Methods

- Describe the methods and apparatus in sufficient detail to allow other workers to evaluate or reproduce the tests/experiments.
- For methods that have been published before, provide only a reference or a reference and a brief description.
- Identify drugs and chemicals, including generic name, dosage and route of administration.

- Please provide manufacturer and manufacturer's address for equipment, drugs and chemicals, as necessary, but not in a separate section.
- For systematic reviews, make sure that the keywords used to search electronic medical databases cover different terminology (for example, tumour or cancer) and spelling (for example, randomised or randomized).

Analysis

- Clearly state and define the main outcome measure(s).
- Briefly state the statistical methods used during the analysis if they are standard. New methods should be described with justification.
- In the case of single- or multicentre trials with blinded intervention, the code must have been broken at the end of the study in the presence of the responsible investigator of each centre. The code and the data will then be available to each participating centre. The first author should make provisions so that if needed, the data are available to the *ERJ* for independent statistical analysis.
- See the Statistical Notes below for further information on analysis, presentation and interpretation

Results

- Keep the Results section brief.
- Describe the baseline characteristics or condition of patients or animals.
- Focus on the important results, *i.e.* those that help address the research question.
- Present most data in figures or tables, not in the text. In the text, emphasise or summarise the most important observations.

Discussion

- At the beginning of the Discussion, summarise the main results, and show how they have addressed the research question.
- Make sure that the conclusions are consistent with the results and are pertinent to the research question.
- Describe the limitations of the study and/or analysis, and discuss the possible implications on the conclusions.
- Emphasise the new and important aspects of the study.
- Try to explain contradictory or unexpected results, or discrepancies with previous findings.

Acknowledgements

- All acknowledgements should be grouped into one paragraph placed after the Discussion.
- Only acknowledge persons who have made substantial contributions to the study.
- Provide the names and affiliation details of members of collaborating bodies

References

- Number references consecutively in the order in which they first appear in the text, using full size Arabic numerals in square brackets.
- All authors must be included in the reference list.
- For original articles, the number of references should be limited to 30.
- References should conform to the style used in *Index Medicus* (Vancouver Style) as shown in the following examples:

1. Bannerjee D, Khair OA, Honeybourne D. Impact of sputum bacteria on airway inflammation and health status in clinical stable COPD. *Eur Respir J* 2004; 23: 685–692.

2. Bourbon J, Henrion-Caude A, Gaultier C. Molecular basis of lung development. *In*: Gibson GJ, Geddes DM, Costable U, Sterk PJ, Corrin B, eds. Respiratory Medicine. 3rd Edn. Elsevier Science, Edinburgh/Philadelphia, 2002; pp. 64–81.

- Websites should be listed in the reference list, not in the text, and only used when an original citation is unavailable; citations should be listed as follows:

WHO. Severe Acute Respiratory Syndrome (SARS). www.who.int/csr/sars/en/index.html. Date last updated: June 1 2004. Date last accessed: June 1 2004.

- Work which has not yet been accepted for publication and personal communications should not appear in the reference list.
- One copy of papers cited as “in press” should be uploaded onto manuscript central as supplementary material.

Tables

- Tables should be created and inserted into the text document using the “Table”, “Insert Table” function in your word processing package; DO NOT supply tables in a separate file.
- Tables should be numbered consecutively with Arabic numerals.
- Limit decimals to a sensible number.
- Large tables should be avoided due to space restrictions, and if used may be split.
- Please provide a clear footnote for all tables, making sure ALL abbreviations and symbols used are defined.
- Any references in tables should run on in numerical order from the text where the table is cited.
- The *ERJ* discourages the use of previously published tables unless absolutely essential. If such tables must be used, you must obtain permission from the copyright owner, which is usually the publisher and not the original author. Use of

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Figures

- Figures constitute a key element of manuscripts submitted to the *ERJ*. However, figures should be limited to those required to show the essential features described in the manuscript. Large figures with a high number of parts should also be avoided. Redundant or excessive figures will not be published due to space restrictions. Figures such as these can be presented in the online depository.
- All submitted figures must be clearly named and numbered, giving the figure number both as part of the filename and on the figure itself. If labelling is necessary, see a recent issue of the *ERJ* for the degree of labelling required.
- There is a charge for colour figures; however, a discounted rate is available to Members. Please contact the Publications Office for further details.
- Please attempt to keep each figure under 1 megabyte (MB). If it is over 1 MB, the upload time will be considerably increased; likewise, the download time could be significantly increased.
- Whether for images, drawings, or graphs, we recommend that no more than four components be used for a given figure (*e.g.* a, b, c, d).
- Images embedded in the .doc or .rtf file WILL NOT be accepted.
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copyright owner, which is usually the publisher and not the original author. Use of the figure may involve a fee payable to the original publisher. Please note that some publishers will not provide permission for publication, which precludes these figures from being displayed in both the printed and the online version of the *ERJ*. Once your manuscript has been accepted for publication, the *ERJ* publications team will contact you requesting that all written confirmation details are forwarded to them for their records.

Photographic images

- All photographic images should be provided with a minimum of 300 dots per inch (dpi).
- Figures should preferably be supplied in .jpeg, .tif or .eps format.
- The image mode for colour photographic images should be cyan-magenta-yellow-black (CMYK). For black-and-white photographic images, it should be grey scale.
- An image-editing program such as Adobe Photoshop© is recommended for saving images.
- Due to space restrictions, it is essential that each submitted figure show only the areas of interest with enough surrounding area for orientation purposes.
- Radiographic images should be of high quality and combined into one array, such as posteroanterior and lateral views. They should also be sized the same to facilitate reproduction.
- Image size should be submitted as close as possible to print size; one column width is 88 mm, two-column width is 182 mm.
- When several images of a given type are being shown, please reproduce each specific type at the same magnification.

- Photomicrographs must have internal scale markers (linear scale), since the size and magnification may be altered by the publisher.
- Images should correspond in appearance to the tonal relations of the original radiograph (*i.e.* showing the bones white on a dark background, with the patient's right to the observer's left. CT scans and MR images should employ the internationally-accepted "view from below".
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- If an image has been enhanced electronically, please explain the alterations that have been made and send in the original image along with the enhanced one. Moreover, keep an electronic set of original images, since our Reviewers might ask you to modify their content and the display modus.
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Legends

- Please provide a clear legend for each figure.
- Legends should be brief and nonrepetitive of information given in the text.
- They should include the imaging technique used, the body part imaged and any noteworthy details.
- ALL abbreviations should be expanded.
- Use of internal scales should always be mentioned in the legend.