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Computerized respiratory sounds are a reliable marker in COPD

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Running head: Respiratory sounds are reliable in COPD

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The authors report no conflict of interests.

Contributors

CJ performed data collection and analysis and drafted the manuscript. AM revised it critically for important intellectual content and provided final approval of the version to be published.

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ABSTRACT

Introduction: Computerized respiratory sounds (RS) have shown potential to monitor respiratory status in patients with COPD. However, variability and reliability of this promising marker in COPD are unknown. Therefore, this study assessed the variability and reliability of RS at distinct airflows and standardized anatomic locations in patients with COPD.

Methods: A two-part study was conducted. Part one assessed the intra-subject reliability of RS at spontaneous and target (0.4-0.6L/s and 0.7-1L/s) airflows in 13 outpatients (69.3±8.6yrs; FEV_1 70.9±21.4% predicted). Part two characterized the inter-subject variability and intra-subject reliability of RS at each standardized anatomic location, using the most reliable airflow, in a sample of 63 outpatients (67.3±10.4yrs; FEV_1 75.4±22.9% predicted). RS were recorded simultaneously at seven anatomic locations (trachea, right and left: anterior, lateral and posterior chest). Airflow was recorded with a pneumotachograph. Normal RS intensity, mean number of crackles and wheezes were analyzed with developed algorithms. Inter-subject variability was assessed with the coefficient of variation (CV) and intra-subject reliability with Intraclass Correlation Coefficient (ICC) and Bland and Altman plots.

Results: Relative reliability was moderate to excellent for normal RS intensity and mean number of crackles (ICCs .66-.89) and excellent for mean number of wheezes (ICCs .75-.99) at the three airflows. Absolute reliability was greater at target airflows; especially at 0.4-0.6L/s. Inter-subject variability was high for all RS parameters and across locations (CV .12-2.22). RS parameters had acceptable relative and absolute intra-subject reliability at the different anatomic locations. The only exception was the mean number of crackles at trachea, which relative and absolute reliability was poor.

Conclusions: RS parameters are more reliable at an airflow of 0.4-0.6L/s and overall reliable at all anatomic locations. This should be considered in future studies using computerized auscultation.

Key-words: computerized auscultation; respiratory sounds; normal respiratory sounds; crackles; wheezes; chronic obstructive pulmonary disease; reliability.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is characterized by persistent airflow limitation that is usually progressive.[1] The forced expiratory volume in one second (FEV_1) has been established as the global marker for COPD diagnosis and monitoring.[1] Nevertheless, changes in FEV_1 in response to treatment are small in relation to its repeatability.[2, 3] New clinical markers are therefore needed for evaluating the effectiveness of treatments in COPD.[4] These markers should be simple in terms of measurement, interpretation and resources used, and have acceptable reliability, to ensure that the error involved in measurement is small enough to detect actual changes.[4]

Respiratory sounds (RS) are a simple, objective and non-invasive marker to assess the function of the respiratory system,[5] which do not require special resources beyond those typical of a patient–health professional encounter. However, variation and reliability of this promising marker across and within patients with COPD are still unknown.

It has been shown, using computerized auscultation, that in stable patients with COPD, adventitious RS are mainly characterized by inspiratory crackles and expiratory wheezes.[6-9] More recently, RS were suggested to be useful to diagnose community-acquired pneumonia in this population.[10] These recent studies showed that RS might have potential to monitor the respiratory status of patients with COPD. However, inter-subject variability and intra-subject reliability was not explored, hindering the interpretation of actual changes. In addition, RS have been recorded with no control over patients' airflows, despite the well-known influence of airflow on respiratory acoustic and breathing pattern.[11-13]

Computerized respiratory sound analysis (CORSA) guidelines recommend recordings with an inspiratory and expiratory peak airflow of 1–1.5L/s or 10–15% of the predicted maximum peak expiratory airflow.[14] However, it is unknown if the airflow recommended suit the breathing pattern specificities of patients with COPD. It has been shown that breathing pattern in patients with COPD has reduced complexity compared with healthy subjects,[15] which may affect RS reliability at different airflows. CORSA guidelines also standardized seven anatomic locations (trachea; right and left: anterior, lateral and posterior chest) to record RS.[14] Nevertheless, inter-subject variability and intra-subject reliability of RS at each anatomic location in patients with COPD has never been investigated. To address these relevant research needs, this study

assessed the i) intra-subject reliability of breathing pattern and RS at distinct airflows and ii) inter-subject variability and intra-subject reliability of RS at each standardized anatomic location in patients with COPD.

METHODS

Study design

A two-part study was conducted. Part one assessed the intra-subject reliability of breathing pattern and RS at three distinct airflows, using a small sample of outpatients with COPD. Part two characterized the inter-subject variability and intra-subject reliability of RS at each anatomic location, using the most reliable airflow from part 1 and a larger sample of outpatients with COPD.

Participants

Outpatients with COPD were recruited from two primary care centers. Inclusion criteria were diagnosis of COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (presence of a post-bronchodilator FEV1/FVC<0.70)[1] and clinical stability for 1 month prior to the study (no hospital admissions, exacerbations as defined by the GOLD[1] or changes in medication for the respiratory system). Patients were excluded if they presented co-existing respiratory diseases or had severe neurological, musculoskeletal or psychiatric impairments. Approval for this study was obtained from the ethics committee of the Center Health Regional Administration (2013-05-02) and from the National Data Protection Committee (3292/2013). Eligible patients were identified via clinicians and then contacted by the researchers, who explained the purpose of the study and asked about their willingness to participate. When patients agreed to participate, an appointment with the researchers was scheduled. Written informed consent was obtained prior to data collection.

Data collection

Socio-demographic, anthropometric (height and weight) and clinical (smoking habits, dyspnea, exacerbations in the past 3 months and in the previous year, medication) data were first recorded in the two study parts. Then, airflow and RS were collected. Lung function was assessed with spirometry (MicroLab 3500, CareFusion, Kent, UK) according to standardized guidelines.[16] Patients were classified in 4 groups (A, B, C, D) using the GOLD combined

assessment (symptoms-mMRC, spirometry and risk of exacerbations).[1] All assessments were performed by two physiotherapists and the order was standardized.

Part one

Airflow and RS were acquired simultaneously. Recordings were performed at spontaneous airflow, at a peak of 0.4-0.6L/s (typical tidal airflow range), and at a peak of 0.7-1L/s (modestly increased airflow). Similar target airflows have been used in previous research.[17] After 5-min of quiet sitting, the three distinct airflows were acquired following the standardized order: spontaneous, 0.4-0.6L/s and 0.7-1L/s. Spontaneous breathing was tested first, so it would not be influenced by the target airflows and the order of the two target airflows was selected based on the increased airflow demand. Patients were in a seated-upright position, wearing a nose clip and breathing through a mouthpiece connected to a heated pneumotachograph (3830, Hans Rudolph, Inc., Shawnee, KS, USA). For each airflow, patients performed three trials of 20 seconds each[18], followed by a 2-min recovery period. During spontaneous airflow, patients were instructed to breathe normally and biofeedback of the flow signal was not presented. During target flows, patients had visual biofeedback of the flow signal (RSS 100R Research Pneumotach System, Hans Rudolph, Shawnee, KS, USA) and were instructed to maintain the flow between two horizontal lines. Recording of each target flow was preceded by a training phase of at least 3 breathing cycles.

RS recordings followed CORSA guidelines for short-term acquisitions[14] and were performed simultaneously at seven anatomic locations (trachea; right and left anterior chest; right and left lateral chest; right and left posterior chest)[19] using the LungSounds@UA interface.[20] Seven stethoscopes (Classic II S.E., Littmann®, 3M, St. Paul, MN, USA), with a microphone (frequency response between 20Hz and 19kHz - TOM-1545P-R, Projects Unlimited, Inc.®, Dayton, OH, USA) and preamplifier circuit (Intelligent Sensing Anywhere®, Coimbra, PT) in the main tube, were attached to the patient's skin with adhesive tape (Soft Cloth Surgical Tape, 3M, St. Paul, MN, USA). The analogue sound signals were further amplified and converted to digital by an audio interface (M-Audio® ProFire 2626, Irwindale, CA, USA). The signal was converted with a 24-bit resolution at a sampling rate of 44.1kHz and recorded in .wav format.

Part two

Airflow and RS were acquired simultaneously at the most reliable airflow identified in part one of the study. The same procedures from part one were followed.

Signal processing

All files were processed using algorithms written in Matlab®R2009a (Mathworks, Natick, MA, USA). Breathing phases were automatically detected using the positive and negative airflow signals. Mean inspiratory and expiratory time were then calculated. The mean airflows and tidal volumes were calculated per breathing phase using flow and volume raw signals. To combine the detected breathing phases with sound signals, the flow signals were timed synchronized with tracheal sound signals. Due to the simultaneous acquisition of RS at the seven locations, the breathing phases detected on tracheal sounds were applied to the other six locations.

Crackles were detected using a multi-algorithm technique based on established algorithms.[21-23] This multi-algorithm technique showed a 7% performance improvement over the best individual algorithm.[24] Wheezes were detected using an algorithm based on time-frequency analysis.[25] The mean number of crackles and wheezes per breathing phase was extracted. After excluding these adventitious sounds, normal respiratory sounds (NRS) were analyzed based on the methodology proposed by Pasterkamp[26] and the mean intensity was determined within a frequency band of 100 to 2000Hz.[26, 27]

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY, USA). The level of significance was set at 0.05.

Part one

Descriptive statistics were used to characterize the sample. Inspiratory and expiratory mean airflow, tidal volume and time were determined by computing the mean of the three recordings at each airflow. The mean NRS intensity, mean number of crackles and mean number of wheezes per breathing phase were determined by computing the mean of the three recordings at all anatomic locations. One-way repeated measures ANOVA was used to analyze differences in the breathing pattern and RS across airflows. When a statistically significant difference was found, Bonferroni post hoc tests were performed. Statistical analysis was completed with the estimation of effect sizes. The effect size was computed via Partial eta-squared as it is the index

more commonly reported for analysis of variance with repeated measures.[28] Partial eta-squared was interpreted as small ($\eta^2 \geq 0.01$), medium ($\eta^2 \geq 0.06$) or large ($\eta^2 \geq 0.14$) effect.[29]

As recommended for intra-subject reliability,[30] both relative, with Intraclass Correlation Coefficient (ICC), and absolute reliability, with Bland and Altman method, were used. The ICC equation (1, K) was used, where k=3 since three recordings were performed for each airflow. ICC was interpreted as excellent (>0.75), moderate to good (0.4-0.75) or poor (<0.4).[31] Bland and Altman method assesses the agreement between two sets of measures.[32] Thus, random numbers were generated in Matlab to delete one recording. Bland and Altman plots were created to analyze the distribution of results (GraphPad Prism version 5.01, GraphPad Software, Inc., La Jolla, CA, USA).[32]

Sample size was determined as described by Bonett.[33] A sample size of 13 subjects was required to estimate an ICC of 0.9 with a 95% confidence interval width of 0.2 (α=0.05 and k=3).[33]

Part two

Descriptive statistics were used to characterize the sample. The mean NRS intensity, mean number of crackles and mean number of wheezes per breathing phase were determined by computing the mean of the three recordings for each anatomic location (trachea, anterior right and left, lateral right and left, posterior right and left). The inter-subject variability in RS parameters was measured with the coefficient of variation (CV), as it is useful for analyzing the variability of measures, independently of the magnitude of the data.[34] It is defined as the standard deviation divided by the mean.[35] The relative and absolute intra-subject reliability of RS parameters were computed, as described above, per anatomic location.

Sample size for the CV was estimated using the approach of Kelley.[36] Using data from part one, it was found that the CV of NRS intensity was between 0.17 and 0.25. It was determined that a minimum of 59 individuals was needed for a CV of 0.25 with a 95% confidence interval width of 0.1 (α=0.05).[36]

RESULTS

Part one

Thirteen participants (10 male) were enrolled. Four participants had mild, six moderate and three severe-to-very-severe airflow limitation. All patients used long-acting bronchodilators. Table 1 provides participants' characteristics.

(Table 1)

Respiratory sounds

Intensity of NRS during inspiration and expiration was higher at an airflow of 0.7-1L/s (post hocs $p<0.001$) (Table 2). No significant differences were seen in the mean number of crackles (inspiratory $p=0.451$; expiratory $p=0.066$) and wheezes (inspiratory $p=0.296$; expiratory $p=0.121$). Relative reliability of NRS intensity was moderate to excellent at the three airflows (Table 2). Bland and Altman plots indicated greater agreement for NRS intensity at an airflow of 0.4-0.6L/s (Figure 1b and 2b). Relative reliability of the mean number of inspiratory and expiratory crackles was found to be moderate to excellent for the three airflows (Table 2). However, a higher level of agreement existed at an airflow of 0.4-0.6L/s, with narrower limits of agreement (Figure 1e and 2e). Relative reliability of mean number of inspiratory and expiratory wheezes was excellent at all airflows (Table 2), though, greater agreement was found at target airflows (Figure 1h and 1i/Figure 2h and 2i).

(Table 2; Figure 1 and 2)

Breathing pattern

At an airflow of 0.7-1L/s, significant higher flows (post hocs $p<0.001$) and tidal volumes (post hocs $p<0.05$) were found (Table 2). Inspiratory and expiratory time were similar across airflows ($p=0.6$ and $p=0.207$). Intra-subject relative reliability of airflow, tidal volume and time was higher at target airflow of 0.4-0.6L/s (ICCs from .73 to .95) when compared to spontaneous airflow (ICCs from .60 to .88) or target airflow of 0.7-1L/s (ICCs from .70 to .84) (Table 2). From Figures 3 and 4, it can also be observed that intra-subject absolute reliability was higher at 0.4-0.6L/s.

(Figure 3 and 4)

From the analysis of RS and breathing pattern parameters, it can be verified that intra-subject reliability was higher at an airflow of 0.4-0.6L/s.

Part two

A total of 63 participants (48 male) were enrolled. Most participants had low risk of exacerbations (A-34.9% and B-36.5%) and all used long-acting bronchodilators. Table 3 provides participants' detailed characteristics.

(Table 3)

Respiratory sounds

Descriptive characteristics of NRS intensity (from 9.41 to 14.71db), mean number of crackles (from 1.43 to 3.46) and mean number of wheezes (from 0.06 to 0.40) across locations are presented in table 4. Inter-subject variability was high in all RS parameters however, the mean number of crackles (CV 0.55-0.92) and wheezes (CV 1.15-2.22) were the parameters presenting the highest variation (Table 4). Inter-subject variability was generally higher during expiration than inspiration for all the RS parameters (NRS intensity 0.12-0.23 vs. 0.15-0.21; mean number of crackles 0.56-0.92 vs. 0.55-0.78; mean number of wheezes 1.36-2.22 vs.1.2-2.17) at most locations, with the exception of trachea.

NRS intensity had an excellent relative and absolute reliability at all anatomic locations (Table 4). The relative and absolute reliability of the mean number of crackles and wheezes was moderate to excellent at all anatomic locations. The only exceptions were the mean number of inspiratory and expiratory crackles at trachea, which relative and absolute reliability was poor (Table 4).

(Table 4)

DISCUSSION

To the best of our knowledge this is the first study investigating inter-subject variability and intra-subject reliability of RS at distinct airflows and anatomic locations in patients with stable COPD. The main findings indicated that RS parameters are i) more reliable at an airflow of 0.4-0.6L/s; ii) highly variable across patients and iii) overall reliable at all standardized anatomic locations.

The NRS intensity increased with higher airflows. The link between sound intensity and airflow has long been recognized.[37] From spontaneous to target airflows, mean number of inspiratory and expiratory crackles had a tendency to decrease. This has also been observed in patients with Interstitial Pulmonary Fibrosis, when comparing crackle rate during normal and deep-breathing maneuvers.[38] This may be related with the effect of lung expansion as recordings

were repeated at short intervals.[39] During the first breathing maneuvers, regions of deflated airways probably opened and in the following maneuvers the production of crackles decreased.[39] The mean number of wheezes had also a tendency to decrease. The consecutive expirations at increased airflows could have been sufficient to decrease the cross-sectional diameter of airways, particularly of the second generation of the airway tree,[5] increase linear velocities and aid secretion movement.[40] This phenomenon could have reduced the narrowing airway and thus the production of wheezes.[5, 41] These findings show that the characteristics of RS are variable at distinct airflows, reinforcing the need of using standardized airflows during computerized auscultation. This will be essential if RS are to become a clinical marker for evaluating the effectiveness of treatments.

Relative reliability of NRS intensity and of mean number of crackles was moderate to excellent at the three airflows. However, ICCs in isolation do not provide a true picture of reliability.[30] Bland and Altman method is independent of the true variability and provide detail regarding the nature of the observed intra-subject variability.[30] The agreement assessed from Bland and Altman method was found to be acceptable for NRS intensity and mean number of crackles at the three airflows. Nevertheless, for these RS parameters, a higher agreement was found at an airflow of 0.4-0.6L/s. Reliability of mean number of wheezes was excellent for all airflows. Forced expiratory wheezes have also been found to be reproducible in normal subjects.[42] No systematic bias was observed at any tested airflow, though, a higher agreement was found at target airflows.

Regarding breathing pattern, the mean inspiratory (0.38±0.18L/s) and expiratory (0.3±0.17L/s) flows at spontaneous airflow were similar to values previously reported.[43-45] Significant higher tidal volumes were observed at airflow of 0.7-1L/s, which was expected due to the direct relationship between airflow and volume.[46] Inspiratory (1.15-1.36s) and expiratory (1.50-1.81s) time were within the commonly reported values in the literature.[47] In patients with COPD, the breathing pattern has also been found to be similar during constant and incremental loaded breathing tests.[47] The intra-subject reliability of breathing pattern parameters was found to be better at target airflows.[48] This might be due to the explicit instructions to breathe at a typical peak airflow, which further reduced the breathing complexity.[15] In accordance to this, breathing pattern was also more reliable at target flows, especially at an airflow of 0.4-0.6L/s. This is

probably explained by the fact that the airflow of 0.7-1L/s was the most demanding for patients to perform and maintain during the 20-second recordings.[48] Therefore, from the analysis of RS and breathing pattern parameters, it can be concluded that the target airflow of 0.4-0.6L/s is the most reliable to characterize NRS, crackles and wheezes in patients with COPD.

At an airflow of 0.4-0.6L/s, the NRS intensity across locations was found to be from 9.41 to 14.71db. These values are slightly lower than those found in healthy individuals at right posterior chest (inspiration 17.17db; expiration 11.50db).[49] Nevertheless, in this previous study healthy individuals breathed at a higher target flow (1.5±0.2L/s).[49] The mean number of crackles was found to be from 1.43 to 3.46, being within the previously range described (0.73 - 5).[8, 50] Wheezes were not frequent across locations (from 0.06 to 0.40), which is in line with a previous study.[8]

Nevertheless, even when recorded with the most reliable airflow, RS parameters exhibited considerable inter-subject variability. Among other factors, differences regarding demographic, anthropometric and clinical (e.g., dyspnea, COPD severity and history of exacerbations) characteristics might contributed for this variability across subjects. High inter-subject variability of RS has also been previously reported in patients with Cystic Fibrosis and Bronchiectasis.[51] However, this inter-subject variability is similar to other biosignals that support clinical decisions (e.g., heart rate variability, electromyography).[52, 53] In a clinical perspective, this inter-subject variability limits inferences at group-level as RS patterns may fail to represent patterns seen in individuals. For example, increased wheezing has been recognized as one of the signs of an acute exacerbation of COPD.[54] Nevertheless, due to the high variability of this RS parameter, a small increase in the mean number of wheezes may indicate a change in the clinical status for one patient, but not to another. This highlights the importance of healthcare professionals supporting their clinical decision in the interpretation of RS changes at an individual level and in combination with other clinical data.

NRS intensity, mean number of crackles and mean number of wheezes were found to be reliable across all anatomic locations. At trachea, however, the mean number of crackles had poor reliability. This result may be due to low generation of this adventitious sound at this region of the respiratory tract. It has been generally accepted that crackles are generated when an

airway opens during inspiration or closes during expiration.[39, 55] Since trachea is characterized by a large diameter and rigid wall, it is unlikely to open or collapse during tidal breathing.

In addition, NRS intensity had lower variability and higher reliability than mean number of crackles and mean number of wheezes at all anatomic locations. NRS are the sounds that are produced when breathing and can be heard both during inspiration and expiration (nearly silent).[56] Crackles and wheezes are superimposed events on NRS,[56] which timing may not be perfectly repeatable from breath to breath. Health professionals may, thus, more confidently rely on changes in NRS intensity than in the mean number of adventitious RS.

Study limitations

The recording of distinct airflows at the same session and at relatively short intervals may have influenced the results. However, to minimize bias, the order of tests was standardized and patients were instructed to rest as needed. Future studies assessing intra-subject reliability could perform the recordings in different sessions within the same day. It would be also interesting in future studies to explore the intra-subject test-retest reliability of RS to understand their stability and reliability over time. The present study focused in only one parameter per RS. Future studies could investigate the reliability of RS using other parameters which also have clinical relevance.[57] Additionally, the unbalance sample in terms of COPD severity can be another limitation of the present study. The samples were mainly composed of patients with mild and moderate airflow limitation, and thus it was not possible to explore how the disease severity related to the variability/reliability of RS parameters. However, as the breathing pattern at airflow of 0.4-0.6L/s is similar to that found in patients with advanced COPD[47] and airflow variability is not related with COPD severity,[15] the disease severity might not play a significant role. Future studies should however investigate this.

CONCLUSIONS

The main findings suggest that RS parameters are more reliable at an airflow of 0.4-0.6L/s, highly variable across patients with COPD and overall reliable at all standardized anatomic locations. In future, RS should be assessed in patients with COPD using this target airflow and these anatomic locations. More studies are needed to draw definite conclusions on airflow standards for recording RS in patients with COPD and with other respiratory diseases.

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CONFLITS OF INTEREST

None.

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Figure captions

Figure 1 – Bland and Altman plots of inspiratory normal respiratory sounds intensity, mean number of crackles and mean number of wheezes between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). CR, crackles; NRS, normal respiratory sounds; dB, decibels; WH, wheezes.

Figure 2 – Bland and Altman plots of expiratory normal respiratory sounds intensity, mean number of crackles and mean number of wheezes between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). CR, crackles; NRS, normal respiratory sounds; dB, decibels; WH, wheezes.

Figure 3 – Bland and Altman plots of inspiratory airflow, volume and time between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). Ti, inspiratory time; VT, tidal volume.

Figure 4 – Bland and Altman plots of expiratory airflow, volume and time between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). Te, expiratory time; VT, tidal volume.

Computerized respiratory sounds are a reliable marker in COPD

Running head: Respiratory sounds are reliable in COPD

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Contributors

CJ performed data collection and analysis and drafted the manuscript. AM revised it critically for important intellectual content and provided final approval of the version to be published.

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ABSTRACT

Introduction: Computerized respiratory sounds (RS) have shown potential to monitor respiratory status in patients with COPD. However, variability and reliability of this promising marker in COPD are unknown. Therefore, this study assessed the variability and reliability of RS at distinct airflows and standardized anatomic locations in patients with COPD.

Methods: A two-part study was conducted. Part one assessed the intra-subject reliability of RS at spontaneous and target (0.4-0.6L/s and 0.7-1L/s) airflows in 13 outpatients (69.3±8.6yrs; FEV_1 70.9±21.4% predicted). Part two characterized the inter-subject variability and intra-subject reliability of RS at each standardized anatomic location, using the most reliable airflow, in a sample of 63 outpatients (67.3±10.4yrs; FEV_1 75.4±22.9% predicted). RS were recorded simultaneously at seven anatomic locations (trachea, right and left: anterior, lateral and posterior chest). Airflow was recorded with a pneumotachograph. Normal RS intensity, mean number of crackles and wheezes were analyzed with developed algorithms. Inter-subject variability was assessed with the coefficient of variation (CV) and intra-subject reliability with Intraclass Correlation Coefficient (ICC) and Bland and Altman plots.

Results: Relative reliability was moderate to excellent for normal RS intensity and mean number of crackles (ICCs .66-.89) and excellent for mean number of wheezes (ICCs .75-.99) at the three airflows. Absolute reliability was greater at target airflows; especially at 0.4-0.6L/s. Inter-subject variability was high for all RS parameters and across locations (CV .12-2.22). RS parameters had acceptable relative and absolute intra-subject reliability at the different anatomic locations. The only exception was the mean number of crackles at trachea, which relative and absolute reliability was poor.

Conclusions: RS parameters are more reliable at an airflow of 0.4-0.6L/s and overall reliable at all anatomic locations. This should be considered in future studies using computerized auscultation.

Key-words: computerized auscultation; respiratory sounds; normal respiratory sounds; crackles; wheezes; chronic obstructive pulmonary disease; reliability.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is characterized by persistent airflow limitation that is usually progressive.[1] The forced expiratory volume in one second (FEV_1) has been established as the global marker for COPD diagnosis and monitoring.[1] Nevertheless, changes in FEV_1 in response to treatment are small in relation to its repeatability.[2, 3] New clinical markers are therefore needed for evaluating the effectiveness of treatments in COPD.[4] These markers should be simple in terms of measurement, interpretation and resources used, and have acceptable reliability, to ensure that the error involved in measurement is small enough to detect actual changes.[4]

Respiratory sounds (RS) are a simple, objective and non-invasive marker to assess the function of the respiratory system,[5] which do not require special resources beyond those typical of a patient–health professional encounter. However, variation and reliability of this promising marker across and within patients with COPD are still unknown.

It has been shown, using computerized auscultation, that in stable patients with COPD, adventitious RS are mainly characterized by inspiratory crackles and expiratory wheezes.[6-9] More recently, RS were suggested to be useful to diagnose community-acquired pneumonia in this population.[10] These recent studies showed that RS might have potential to monitor the respiratory status of patients with COPD. However, inter-subject variability and intra-subject reliability was not explored, hindering the interpretation of actual changes. In addition, RS have been recorded with no control over patients' airflows, despite the well-known influence of airflow on respiratory acoustic and breathing pattern.[11-13]

Computerized respiratory sound analysis (CORSA) guidelines recommend recordings with an inspiratory and expiratory peak airflow of 1–1.5L/s or 10–15% of the predicted maximum peak expiratory airflow.[14] However, it is unknown if the airflow recommended suit the breathing pattern specificities of patients with COPD. It has been shown that breathing pattern in patients with COPD has reduced complexity compared with healthy subjects,[15] which may affect RS reliability at different airflows. CORSA guidelines also standardized seven anatomic locations (trachea; right and left: anterior, lateral and posterior chest) to record RS.[14] Nevertheless, inter-subject variability and intra-subject reliability of RS at each anatomic location in patients with COPD has never been investigated. To address these relevant research needs, this study

assessed the i) intra-subject reliability of breathing pattern and RS at distinct airflows and ii) inter-subject variability and intra-subject reliability of RS at each standardized anatomic location in patients with COPD.

METHODS

Study design

A two-part study was conducted. Part one assessed the intra-subject reliability of breathing pattern and RS at three distinct airflows, using a small sample of outpatients with COPD. Part two characterized the inter-subject variability and intra-subject reliability of RS at each anatomic location, using the most reliable airflow from part 1 and a larger sample of outpatients with COPD.

Participants

Outpatients with COPD were recruited from two primary care centers. Inclusion criteria were diagnosis of COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (presence of a post-bronchodilator FEV1/FVC<0.70)[1] and clinical stability for 1 month prior to the study (no hospital admissions, exacerbations as defined by the GOLD[1] or changes in medication for the respiratory system). Patients were excluded if they presented co-existing respiratory diseases or had severe neurological, musculoskeletal or psychiatric impairments. Approval for this study was obtained from the ethics committee of the Center Health Regional Administration (2013-05-02) and from the National Data Protection Committee (3292/2013). Eligible patients were identified via clinicians and then contacted by the researchers, who explained the purpose of the study and asked about their willingness to participate. When patients agreed to participate, an appointment with the researchers was scheduled. Written informed consent was obtained prior to data collection.

Data collection

Socio-demographic, anthropometric (height and weight) and clinical (smoking habits, dyspnea, exacerbations in the past 3 months and in the previous year, medication) data were first recorded in the two study parts. Then, airflow and RS were collected. Lung function was assessed with spirometry (MicroLab 3500, CareFusion, Kent, UK) according to standardized guidelines.[16] Patients were classified in 4 groups (A, B, C, D) using the GOLD combined

assessment (symptoms-mMRC, spirometry and risk of exacerbations).[1] All assessments were performed by two physiotherapists and the order was standardized.

Part one

Airflow and RS were acquired simultaneously. Recordings were performed at spontaneous airflow, at a peak of 0.4-0.6L/s (typical tidal airflow range), and at a peak of 0.7-1L/s (modestly increased airflow). Similar target airflows have been used in previous research.[17] After 5-min of quiet sitting, the three distinct airflows were acquired following the standardized order: spontaneous, 0.4-0.6L/s and 0.7-1L/s. Spontaneous breathing was tested first, so it would not be influenced by the target airflows and the order of the two target airflows was selected based on the increased airflow demand. Patients were in a seated-upright position, wearing a nose clip and breathing through a mouthpiece connected to a heated pneumotachograph (3830, Hans Rudolph, Inc., Shawnee, KS, USA). For each airflow, patients performed three trials of 20 seconds each[18], followed by a 2-min recovery period. During spontaneous airflow, patients were instructed to breathe normally and biofeedback of the flow signal was not presented. During target flows, patients had visual biofeedback of the flow signal (RSS 100R Research Pneumotach System, Hans Rudolph, Shawnee, KS, USA) and were instructed to maintain the flow between two horizontal lines. Recording of each target flow was preceded by a training phase of at least 3 breathing cycles.

RS recordings followed CORSA guidelines for short-term acquisitions[14] and were performed simultaneously at seven anatomic locations (trachea; right and left anterior chest; right and left lateral chest; right and left posterior chest)[19] using the LungSounds@UA interface.[20] Seven stethoscopes (Classic II S.E., Littmann®, 3M, St. Paul, MN, USA), with a microphone (frequency response between 20Hz and 19kHz - TOM-1545P-R, Projects Unlimited, Inc.®, Dayton, OH, USA) and preamplifier circuit (Intelligent Sensing Anywhere®, Coimbra, PT) in the main tube, were attached to the patient's skin with adhesive tape (Soft Cloth Surgical Tape, 3M, St. Paul, MN, USA). The analogue sound signals were further amplified and converted to digital by an audio interface (M-Audio® ProFire 2626, Irwindale, CA, USA). The signal was converted with a 24-bit resolution at a sampling rate of 44.1kHz and recorded in .wav format.

Part two

Airflow and RS were acquired simultaneously at the most reliable airflow identified in part one of the study. The same procedures from part one were followed.

Signal processing

All files were processed using algorithms written in Matlab®R2009a (Mathworks, Natick, MA, USA). Breathing phases were automatically detected using the positive and negative airflow signals. Mean inspiratory and expiratory time were then calculated. The mean airflows and tidal volumes were calculated per breathing phase using flow and volume raw signals. To combine the detected breathing phases with sound signals, the flow signals were timed synchronized with tracheal sound signals. Due to the simultaneous acquisition of RS at the seven locations, the breathing phases detected on tracheal sounds were applied to the other six locations.

Crackles were detected using a multi-algorithm technique based on established algorithms.[21-23] This multi-algorithm technique showed a 7% performance improvement over the best individual algorithm.[24] Wheezes were detected using an algorithm based on time-frequency analysis.[25] The mean number of crackles and wheezes per breathing phase was extracted. After excluding these adventitious sounds, normal respiratory sounds (NRS) were analyzed based on the methodology proposed by Pasterkamp[26] and the mean intensity was determined within a frequency band of 100 to 2000Hz.[26, 27]

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY, USA). The level of significance was set at 0.05.

Part one

Descriptive statistics were used to characterize the sample. Inspiratory and expiratory mean airflow, tidal volume and time were determined by computing the mean of the three recordings at each airflow. The mean NRS intensity, mean number of crackles and mean number of wheezes per breathing phase were determined by computing the mean of the three recordings at all anatomic locations. One-way repeated measures ANOVA was used to analyze differences in the breathing pattern and RS across airflows. When a statistically significant difference was found, Bonferroni post hoc tests were performed. Statistical analysis was completed with the estimation of effect sizes. The effect size was computed via Partial eta-squared as it is the index

more commonly reported for analysis of variance with repeated measures.[28] Partial eta-squared was interpreted as small ($\eta^2 \geq 0.01$), medium ($\eta^2 \geq 0.06$) or large ($\eta^2 \geq 0.14$) effect.[29]

As recommended for intra-subject reliability,[30] both relative, with Intraclass Correlation Coefficient (ICC), and absolute reliability, with Bland and Altman method, were used. The ICC equation (1, K) was used, where k=3 since three recordings were performed for each airflow. ICC was interpreted as excellent (>0.75), moderate to good (0.4-0.75) or poor (<0.4).[31] Bland and Altman method assesses the agreement between two sets of measures.[32] Thus, random numbers were generated in Matlab to delete one recording. Bland and Altman plots were created to analyze the distribution of results (GraphPad Prism version 5.01, GraphPad Software, Inc., La Jolla, CA, USA).[32]

Sample size was determined as described by Bonett.[33] A sample size of 13 subjects was required to estimate an ICC of 0.9 with a 95% confidence interval width of 0.2 (α=0.05 and k=3).[33]

Part two

Descriptive statistics were used to characterize the sample. The mean NRS intensity, mean number of crackles and mean number of wheezes per breathing phase were determined by computing the mean of the three recordings for each anatomic location (trachea, anterior right and left, lateral right and left, posterior right and left). The inter-subject variability in RS parameters was measured with the coefficient of variation (CV), as it is useful for analyzing the variability of measures, independently of the magnitude of the data.[34] It is defined as the standard deviation divided by the mean.[35] The relative and absolute intra-subject reliability of RS parameters were computed, as described above, per anatomic location.

Sample size for the CV was estimated using the approach of Kelley.[36] Using data from part one, it was found that the CV of NRS intensity was between 0.17 and 0.25. It was determined that a minimum of 59 individuals was needed for a CV of 0.25 with a 95% confidence interval width of 0.1 (α=0.05).[36]

RESULTS

Part one

Thirteen participants (10 male) were enrolled. Four participants had mild, six moderate and three severe-to-very-severe airflow limitation. All patients used long-acting bronchodilators. Table 1 provides participants' characteristics.

(Table 1)

Respiratory sounds

Intensity of NRS during inspiration and expiration was higher at an airflow of 0.7-1L/s (post hocs $p<0.001$) (Table 2). No significant differences were seen in the mean number of crackles (inspiratory $p=0.451$; expiratory $p=0.066$) and wheezes (inspiratory $p=0.296$; expiratory $p=0.121$). Relative reliability of NRS intensity was moderate to excellent at the three airflows (Table 2). Bland and Altman plots indicated greater agreement for NRS intensity at an airflow of 0.4-0.6L/s (Figure 1b and 2b). Relative reliability of the mean number of inspiratory and expiratory crackles was found to be moderate to excellent for the three airflows (Table 2). However, a higher level of agreement existed at an airflow of 0.4-0.6L/s, with narrower limits of agreement (Figure 1e and 2e). Relative reliability of mean number of inspiratory and expiratory wheezes was excellent at all airflows (Table 2), though, greater agreement was found at target airflows (Figure 1h and 1i/Figure 2h and 2i).

(Table 2; Figure 1 and 2)

Breathing pattern

At an airflow of 0.7-1L/s, significant higher flows (post hocs $p<0.001$) and tidal volumes (post hocs $p<0.05$) were found (Table 2). Inspiratory and expiratory time were similar across airflows ($p=0.6$ and $p=0.207$). Intra-subject relative reliability of airflow, tidal volume and time was higher at target airflow of 0.4-0.6L/s (ICCs from .73 to .95) when compared to spontaneous airflow (ICCs from .60 to .88) or target airflow of 0.7-1L/s (ICCs from .70 to .84) (Table 2). From Figures 3 and 4, it can also be observed that intra-subject absolute reliability was higher at 0.4-0.6L/s.

(Figure 3 and 4)

From the analysis of RS and breathing pattern parameters, it can be verified that intra-subject reliability was higher at an airflow of 0.4-0.6L/s.

Part two

A total of 63 participants (48 male) were enrolled. Most participants had low risk of exacerbations (A-34.9% and B-36.5%) and all used long-acting bronchodilators. Table 3 provides participants' detailed characteristics.

(Table 3)

Respiratory sounds

Descriptive characteristics of NRS intensity (from 9.41 to 14.71db), mean number of crackles (from 1.43 to 3.46) and mean number of wheezes (from 0.06 to 0.40) across locations are presented in table 4. Inter-subject variability was high in all RS parameters however, the mean number of crackles (CV 0.55-0.92) and wheezes (CV 1.15-2.22) were the parameters presenting the highest variation (Table 4). Inter-subject variability was generally higher during expiration than inspiration for all the RS parameters (NRS intensity 0.12-0.23 vs. 0.15-0.21; mean number of crackles 0.56-0.92 vs. 0.55-0.78; mean number of wheezes 1.36-2.22 vs.1.2-2.17) at most locations, with the exception of trachea.

NRS intensity had an excellent relative and absolute reliability at all anatomic locations (Table 4). The relative and absolute reliability of the mean number of crackles and wheezes was moderate to excellent at all anatomic locations. The only exceptions were the mean number of inspiratory and expiratory crackles at trachea, which relative and absolute reliability was poor (Table 4).

(Table 4)

DISCUSSION

To the best of our knowledge this is the first study investigating inter-subject variability and intra-subject reliability of RS at distinct airflows and anatomic locations in patients with stable COPD. The main findings indicated that RS parameters are i) more reliable at an airflow of 0.4-0.6L/s; ii) highly variable across patients and iii) overall reliable at all standardized anatomic locations.

The NRS intensity increased with higher airflows. The link between sound intensity and airflow has long been recognized.[37] From spontaneous to target airflows, mean number of inspiratory and expiratory crackles had a tendency to decrease. This has also been observed in patients with Interstitial Pulmonary Fibrosis, when comparing crackle rate during normal and deep-breathing maneuvers.[38] This may be related with the effect of lung expansion as recordings

were repeated at short intervals.[39] During the first breathing maneuvers, regions of deflated airways probably opened and in the following maneuvers the production of crackles decreased.[39] The mean number of wheezes had also a tendency to decrease. The consecutive expirations at increased airflows could have been sufficient to decrease the cross-sectional diameter of airways, particularly of the second generation of the airway tree,[5] increase linear velocities and aid secretion movement.[40] This phenomenon could have reduced the narrowing airway and thus the production of wheezes.[5, 41] These findings show that the characteristics of RS are variable at distinct airflows, reinforcing the need of using standardized airflows during computerized auscultation. This will be essential if RS are to become a clinical marker for evaluating the effectiveness of treatments.

Relative reliability of NRS intensity and of mean number of crackles was moderate to excellent at the three airflows. However, ICCs in isolation do not provide a true picture of reliability.[30] Bland and Altman method is independent of the true variability and provide detail regarding the nature of the observed intra-subject variability.[30] The agreement assessed from Bland and Altman method was found to be acceptable for NRS intensity and mean number of crackles at the three airflows. Nevertheless, for these RS parameters, a higher agreement was found at an airflow of 0.4-0.6L/s. Reliability of mean number of wheezes was excellent for all airflows. Forced expiratory wheezes have also been found to be reproducible in normal subjects.[42] No systematic bias was observed at any tested airflow, though, a higher agreement was found at target airflows.

Regarding breathing pattern, the mean inspiratory (0.38±0.18L/s) and expiratory (0.3±0.17L/s) flows at spontaneous airflow were similar to values previously reported.[43-45] Significant higher tidal volumes were observed at airflow of 0.7-1L/s, which was expected due to the direct relationship between airflow and volume.[46] Inspiratory (1.15-1.36s) and expiratory (1.50-1.81s) time were within the commonly reported values in the literature.[47] In patients with COPD, the breathing pattern has also been found to be similar during constant and incremental loaded breathing tests.[47] The intra-subject reliability of breathing pattern parameters was found to be better at target airflows.[48] This might be due to the explicit instructions to breathe at a typical peak airflow, which further reduced the breathing complexity.[15] In accordance to this, breathing pattern was also more reliable at target flows, especially at an airflow of 0.4-0.6L/s. This is

probably explained by the fact that the airflow of 0.7-1L/s was the most demanding for patients to perform and maintain during the 20-second recordings.[48] Therefore, from the analysis of RS and breathing pattern parameters, it can be concluded that the target airflow of 0.4-0.6L/s is the most reliable to characterize NRS, crackles and wheezes in patients with COPD.

At an airflow of 0.4-0.6L/s, the NRS intensity across locations was found to be from 9.41 to 14.71db. These values are slightly lower than those found in healthy individuals at right posterior chest (inspiration 17.17db; expiration 11.50db).[49] Nevertheless, in this previous study healthy individuals breathed at a higher target flow (1.5±0.2L/s).[49] The mean number of crackles was found to be from 1.43 to 3.46, being within the previously range described (0.73 - 5).[8, 50] Wheezes were not frequent across locations (from 0.06 to 0.40), which is in line with a previous study.[8]

Nevertheless, even when recorded with the most reliable airflow, RS parameters exhibited considerable inter-subject variability. Among other factors, differences regarding demographic, anthropometric and clinical (e.g., dyspnea, COPD severity and history of exacerbations) characteristics might contributed for this variability across subjects. High inter-subject variability of RS has also been previously reported in patients with Cystic Fibrosis and Bronchiectasis.[51] However, this inter-subject variability is similar to other biosignals that support clinical decisions (e.g., heart rate variability, electromyography).[52, 53] In a clinical perspective, this inter-subject variability limits inferences at group-level as RS patterns may fail to represent patterns seen in individuals. For example, increased wheezing has been recognized as one of the signs of an acute exacerbation of COPD.[54] Nevertheless, due to the high variability of this RS parameter, a small increase in the mean number of wheezes may indicate a change in the clinical status for one patient, but not to another. This highlights the importance of healthcare professionals supporting their clinical decision in the interpretation of RS changes at an individual level and in combination with other clinical data.

NRS intensity, mean number of crackles and mean number of wheezes were found to be reliable across all anatomic locations. At trachea, however, the mean number of crackles had poor reliability. This result may be due to low generation of this adventitious sound at this region of the respiratory tract. It has been generally accepted that crackles are generated when an

airway opens during inspiration or closes during expiration.[39, 55] Since trachea is characterized by a large diameter and rigid wall, it is unlikely to open or collapse during tidal breathing.

In addition, NRS intensity had lower variability and higher reliability than mean number of crackles and mean number of wheezes at all anatomic locations. NRS are the sounds that are produced when breathing and can be heard both during inspiration and expiration (nearly silent).[56] Crackles and wheezes are superimposed events on NRS,[56] which timing may not be perfectly repeatable from breath to breath. Health professionals may, thus, more confidently rely on changes in NRS intensity than in the mean number of adventitious RS.

Study limitations

The recording of distinct airflows at the same session and at relatively short intervals may have influenced the results. However, to minimize bias, the order of tests was standardized and patients were instructed to rest as needed. Future studies assessing intra-subject reliability could perform the recordings in different sessions within the same day. It would be also interesting in future studies to explore the intra-subject test-retest reliability of RS to understand their stability and reliability over time. The present study focused in only one parameter per RS. Future studies could investigate the reliability of RS using other parameters which also have clinical relevance.[57] Additionally, the unbalance sample in terms of COPD severity can be another limitation of the present study. The samples were mainly composed of patients with mild and moderate airflow limitation, and thus it was not possible to explore how the disease severity related to the variability/reliability of RS parameters. However, as the breathing pattern at airflow of 0.4-0.6L/s is similar to that found in patients with advanced COPD[47] and airflow variability is not related with COPD severity,[15] the disease severity might not play a significant role. Future studies should however investigate this.

CONCLUSIONS

The main findings suggest that RS parameters are more reliable at an airflow of 0.4-0.6L/s, highly variable across patients with COPD and overall reliable at all standardized anatomic locations. In future, RS should be assessed in patients with COPD using this target airflow and these anatomic locations. More studies are needed to draw definite conclusions on airflow standards for recording RS in patients with COPD and with other respiratory diseases.

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CONFLITS OF INTEREST

None.

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Figure captions

Figure 1 – Bland and Altman plots of inspiratory normal respiratory sounds intensity, mean number of crackles and mean number of wheezes between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). CR, crackles; NRS, normal respiratory sounds; dB, decibels; WH, wheezes.

Figure 2 – Bland and Altman plots of expiratory normal respiratory sounds intensity, mean number of crackles and mean number of wheezes between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). CR, crackles; NRS, normal respiratory sounds; dB, decibels; WH, wheezes.

Figure 3 – Bland and Altman plots of inspiratory airflow, volume and time between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). Ti, inspiratory time; VT, tidal volume.

Figure 4 – Bland and Altman plots of expiratory airflow, volume and time between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). Te, expiratory time; VT, tidal volume.

Table 1 - Socio-demographic, anthropometric and clinical characteristics of participants (n=13).

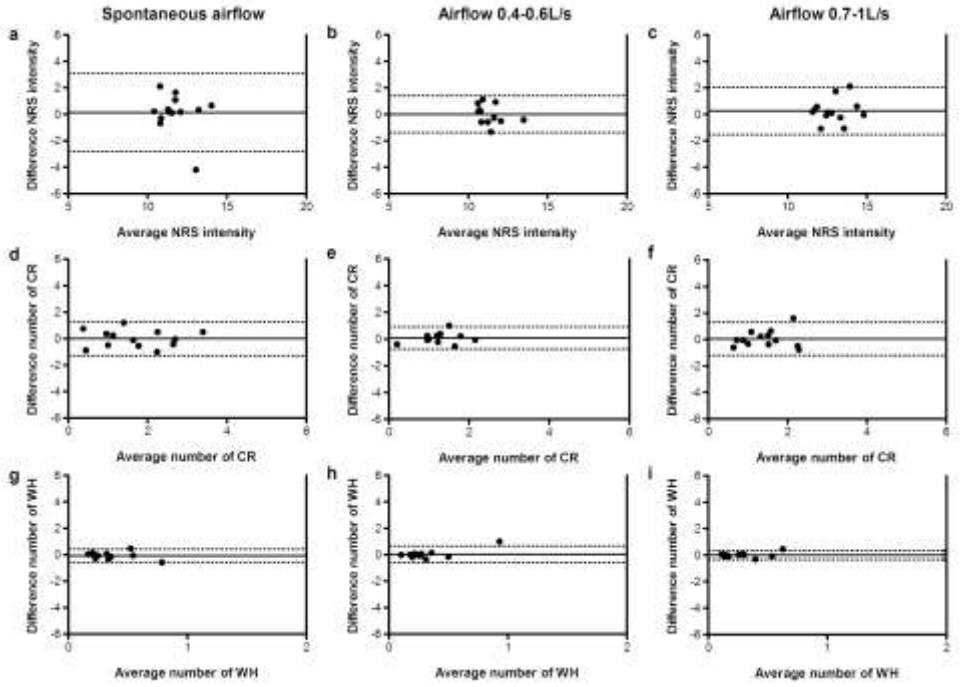
Age (years)	69.3 ± 8.6
Gender (male/female)	10/3
Current smokers	0
mMRC, M[IQR]	1 [1, 2]
BMI (kg/m^2)	29.5 ± 3.4
Exacerbations past 3 months	
0	5
1	6
≥ 2	2
FEV_1 (L)	1.8 ± 0.6
FEV_1 (% predicted)	70.9 ± 21.4
FEV_1/FVC	65.7 ± 8.6
GOLD airflow limitation, n(%)	
Mild	4
Moderate	6
Severe-to-very-severe	3
GOLD combined assessment, n(%)	
A – low risk, less symptoms	3
B – low risk, more symptoms	7
C – high risk, less symptoms	1
D – high risk, more symptoms	2

Values are shown as mean±standard deviation unless otherwise indicated. mMRC, modified British Medical Research Council questionnaire; M, median; IQR, interquartile range; BMI, body mass index; FEV_1, forced expiratory volume in one second; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

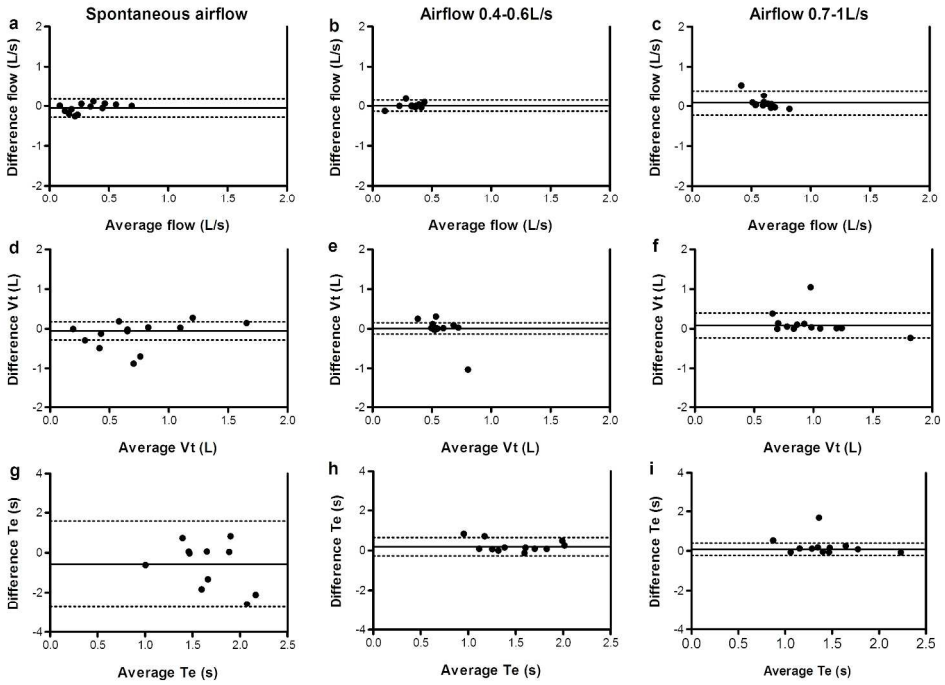
Table 2 – Descriptive characteristics and intra-subject relative reliability of respiratory sounds and breathing pattern parameters at three airflows (n=13).

	Spontaneous		**0.4-0.6L/s**		**0.7-1L/s**		**p-value**	**η^2**
	mean±SD	$ICC_{1,3}$ (95%CI)	mean±SD	$ICC_{1,3}$ (95%CI)	mean±SD	$ICC_{1,3}$ (95%CI)		
Inspiratory NRS intensity (dB)	11.8 ± 2.16	.74 (.35→.91)	11.32 ± 1.88	.88 (.7→.96)	12.98 ±2.33	.89 (.73→.96)	<.001	.634
Expiratory NRS intensity (dB)	10.49 ± 2.05	.66 (.14→.89)	10.30 ± 1.82	.65 (.13→.88)	12.06 ± 2.96	.74 (.36→.91)	<.001	.757
Mean number of crackles per inspiration	1.57 ± 0.78	.75 (.38→.92)	1.30 ± 0.60	.71 (.27→.90)	1.38 ± 0.50	.81 (.52→.94)	.451	.064
Mean number of crackles per expiration	2.49 ± 1.35	.78 (.44→.93)	1.47 ± 1.05	.89 (.74→.97)	1.34 ± 0.64	.75 (.39→.92)	.066	.203
Mean number of wheezes per inspiration	0.35 ± 0.49	.79 (.46→.93)	0.31 ± 0.55	.78 (.46→.93)	0.25 ± 0.31	.75 (.37→.92)	.296	.096
Mean number of wheezes per expiration	0.59 ± 0.91	.89 (.72→.96)	0.72 ± 1.72	.99 (.96→.99)	0.30 ± 0.39	.78 (.46→.93)	.121	.161
Inspiratory flow (L/s)	0.38 ± 0.18	.73 (.32→.91)	0.44 ± 0.14	.95 (.88→.98)	0.7 ± 0.11	.74 (.34→.91)	<.001	.648
Expiratory flow (L/s)	0.30 ± 0.17	.88 (.70→.96)	0.33 ± 0.09	.92 (.81→.97)	0.60 ± 0.09	.77 (.42→.92)	<.001	.751
Inspiratory V_T (L)	0.54 ± 0.18	.76 (.37→.93)	0.57 ± 0.1	.85 (.63→.95)	0.96 ± 0.22	.84 (.61→.95)	.001	.431
Expiratory V_T (L)	0.56 ± 0.25	.60 (.01→.87)	0.56 ± 0.11	.73 (.31→.91)	0.95 ± 0.24	.70 (.25→.90)	.001	.525
Ti (s)	1.36 ± 0.41	.64 (.02→.89)	1.15 ± 0.28	.85 (.60→.96)	1.24 ± 0.34	.84 (.59→.95)	.600	.097
Te (s)	1.81 ± 0.53	.72 (.29→.91)	1.71 ± 0.85	.80 (.50→.93)	1.50 ± 0.40	.77 (.42→.92)	.207	.123

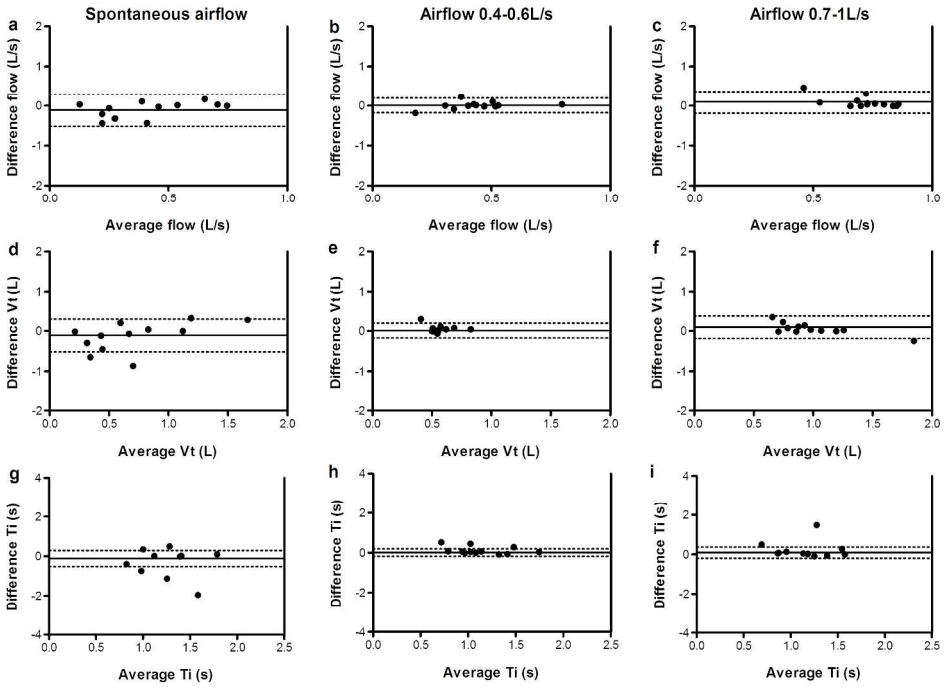
CI, confidence interval; ICC, Intraclass correlation coefficient; NRS, normal respiratory sounds; Te, expiratory time; Ti, inspiratory time; V_T, tidal volume; η^2, Partial eta-squared.



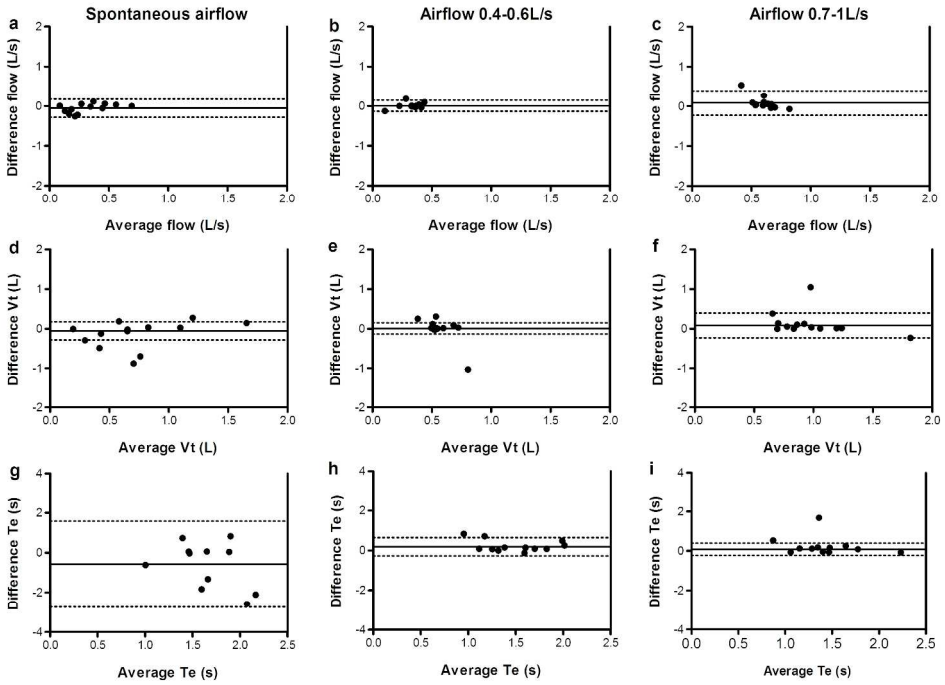
Bland and Altman plots of inspiratory normal respiratory sounds intensity, number of crackles and number of wheezes between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). CR, crackles; NRS, normal respiratory sounds; dB, decibels; WH, wheezes.
297x210mm (300 x 300 DPI)



Bland and Altman plots of expiratory normal respiratory sounds intensity, mean number of crackles and mean number of wheezes between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). CR, crackles; NRS, normal respiratory sounds; dB, decibels; WH, wheezes.
299x212mm (300 x 300 DPI)



Bland and Altman plots of inspiratory airflow, volume and time between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). Ti, inspiratory time; VT, tidal volume.
298x212mm (300 x 300 DPI)



Bland and Altman plots of expiratory airflow, volume and time between two recordings at three distinct airflows: spontaneous; 0.4-0.6L/s and 0.7-1L/s. The bold line represents the mean difference and the dotted lines the 95% limits of agreement (95%LA). Te, expiratory time; VT, tidal volume.
299x212mm (300 x 300 DPI)

Table 3 - Socio-demographic, anthropometric and clinical characteristics of participants (n=63).

Age (years)	67.3 ± 10.4
Gender (male/female)	48/15
Current smokers	16 (25.4%)
mMRC, M[IQR]	1 [1, 2]
BMI (kg/m^2)	29 ± 5
Exacerbations past 3 months	
0	35 (55.6%)
1	17 (27%)
≥ 2	11 (17.4%)
FEV_1 (L)	1.9 ± 0.6
FEV_1 (% predicted)	75.4 ± 22.9
FEV_1/FVC	64.9 ± 9.1
GOLD airflow limitation, n(%)	
Mild	35 (55.6%)
Moderate	22 (34.9%)
Severe-to-very-severe	6 (9.5%)
GOLD combined assessment, n(%)	
A – low risk, less symptoms	22 (34.9%)
B – low risk, more symptoms	23 (36.5%)
C – high risk, less symptoms	8 (12.7%)
D – high risk, more symptoms	10 (15.9%)

Values are shown as mean±standard deviation unless otherwise indicated. mMRC, modified British Medical Research Council questionnaire; M, median; IQR, interquartile range; BMI, body mass index; FEV_1, forced expiratory volume in one second; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

Table 4 – Descriptive characteristics, inter-subject variability, relative and absolute reliability of respiratory sounds per anatomic location at an airflow of 0.4-0.6L/s (n=63).

		mean±SD	CV	$ICC_{1,3}$ (95%CI)	Mean difference (SD)	95%LA
Inspiratory NRS intensity (dB)	**Trachea**	12.94±3.67	0.28	.95 (.92→.97)	-0.28 (1.22)	-2.68→2.12
	Anterior right	12.43±2.00	0.16	.90 (.85→.94)	0.18 (0.91)	-1.62→1.97
	Anterior left	10.43±1.59	0.15	.93 (.89→.95)	-0.12 (0.99)	-2.07→1.83
	Lateral right	12.88±2.73	0.21	.93 (.89→.96)	0.28 (1.48)	-2.61→3.18
	Lateral left	13.65±2.83	0.21	.88 (.82→.92)	0.02 (1.69)	-3.30→3.33
	Posterior right	14.71±2.88	0.20	.93 (.89→.96)	0.16 (0.89)	-1.58→1.91
	Posterior left	12.02±2.25	0.19	.93 (.89→.96)	0.22 (1.34)	-2.40→2.84
Expiratory NRS intensity (dB)	**Trachea**	13.20±3.33	0.25	.93 (.89→.95)	-0.26 (1.47)	-3.14→2.62
	Anterior right	11.16±1.36	0.12	.88 (.81→.92)	0.13 (0.92)	-1.68→1.94
	Anterior left	9.41±1.20	0.13	.91 (.86→.94)	-0.08 (0.80)	-1.65→1.49
	Lateral right	11.68±2.42	0.21	.94 (.90→.96)	-0.07 (1.63)	-3.26→3.11
	Lateral left	12.58±2.90	0.23	.88 (.81→.92)	-0.38 (1.63)	-3.58→2.81
	Posterior right	12.96±2.83	0.22	.89 (.83→.93)	0.14 (0.95)	-1.73→2.00
	Posterior left	10.69±2.01	0.19	.87 (.81→.92)	0.19 (1.66)	-3.06→3.44
Mean number of crackles per inspiration	**Trachea**	1.45±0.90	0.62	-.34 (-1.19→.22)	-1.83 (1.57)	-4.91→1.25
	Anterior right	2.07±1.15	0.55	.79(.69→.87)	0.05 (1.17)	-2.24→2.34
	Anterior left	1.43±0.80	0.56	.55(.32→.72)	0.15 (0.98)	-1.77→2.06
	Lateral right	2.57±1.61	0.63	.59(.37→.74)	0.23(1.72)	-3.14→3.60
	Lateral left	2.24±1.75	0.78	.73(.59→.83)	-0.10(1.36)	-2.77→2.56
	Posterior right	2.86±1.75	0.61	.77(.65→.86)	0.31(1.54)	-2.70→3.33
	Posterior left	2.37±1.77	0.74	.42(.08→.65)	1.45(1.27)	-1.03→3.93
Mean number of crackles per expiration	**Trachea**	1.65±1.11	0.68	.02 (-.61→.43)	-1.75(1.95)	-5.57→2.08
	Anterior right	3.07±1.72	0.56	.78 (.67→.86)	0.22(1.47)	-2.67→3.10
	Anterior left	2.15±1.57	0.73	.90 (.85→.94)	0.25(1.22)	-2.14→2.64
	Lateral right	3.33±2.30	0.69	.52 (.27→.7)	-0.38(2.18)	-4.65→3.89
	Lateral left	2.89±2.06	0.71	.64 (.45→.77)	-0.13(1.28)	-2.64→2.38
	Posterior right	3.46±2.80	0.81	.86 (.79→.91)	0.23(1.70)	-3.10→3.56
	Posterior left	2.99±2.74	0.92	.57 (.31→.74)	1.31(1.24)	-1.12→3.74
Mean number of wheezes per inspiration	**Trachea**	0.35±0.47	1.34	.61 (.41→.75)	0.20(0.63)	-1.04→1.44
	Anterior right	0.16±0.34	2.17	.87 (.81→.92)	0.00(0.18)	-0.36→0.35
	Anterior left	0.06±0.11	1.68	.44 (.15→.64)	0.05(0.20)	-0.33→0.43
	Lateral right	0.20±0.30	1.51	.49 (.23→.68)	-0.01(0.32)	-0.64→0.61
	Lateral left	0.16±0.20	1.20	.42 (.12→.63)	0.05(0.38)	-0.70→0.80
	Posterior right	0.18±0.30	1.65	.80 (.70→.88)	-0.19(0.38)	-0.92→0.55
	Posterior left	0.21±0.27	1.27	.35 (.02→.59)	0.01(0.30)	-0.57→0.59
Mean number of wheezes per expiration	**Trachea**	0.37±0.42	1.15	.63 (.43→.76)	0.14(0.55)	-0.94→1.23
	Anterior right	0.22±0.40	1.82	.84 (.75→.9)	0.03(0.25)	-0.47→0.53
	Anterior left	0.13±0.28	2.22	.83 (.74→.89)	0.04(0.31)	-0.57→0.66
	Lateral right	0.40±0.70	1.75	.67 (.49→.79)	0.06(0.38)	-0.69→0.81
	Lateral left	0.36±0.54	1.48	.64 (.46→.77)	0.02(0.46)	-0.88→0.93
	Posterior right	0.28±0.39	1.36	.65 (.47→.7)	-0.08(0.42)	-0.90→0.73
	Posterior left	0.31±0.53	1.70	.77 (.65→.85)	0.12(0.31)	-0.49→0.74

CI, confidence interval; ICC, Intraclass correlation coefficient; LA, limits of agreement; NRS, normal respiratory sounds; SD, standard deviation.