Computerised respiratory sounds can differentiate smokers and non- smokers

Ana Oliveira[1,3] MSc, PT, alao@ua.pt; Ipek Sen[2] PhD, EE, ipekbounee@gmail.com; Yasemin P. Kahya[2] PhD, EE, kahya@boun.edu.tr; Vera Afreixo[3,4] PhD, Math, vera@ua.pt; Alda Marques[1, 3] PhD, PT, amarques@ua.pt

1 Lab 3R – Respiratory Research and Rehabilitation Laboratory, School of Health Sciences (ESSUA), University of Aveiro, Aveiro, Portugal

2 Department of Electrical and Electronics Engineering, Bogazici University, Istanbul, Turkey

3 Institute for Research in Biomedicine (iBiMED), University of Aveiro, Aveiro, Portugal

4 Institute of Electronics and Informatics Engineering of Aveiro (IEETA), Aveiro, Portugal

Corresponding author: Alda Marques, PT, MSc, PhD, Senior Lecturer, Lab 3R – Respiratory, Rehabilitation and Research, School of Health Sciences, University of Aveiro (ESSUA), Agras do Crasto - Campus Universitário de Santiago, Edifício 30, 3810-193 Aveiro, Portugal. Email: amarques@ua.pt

ACKNOWLEDGMENTS

This work has been developed within a European Respiratory Society Short Term Fellowship [grant number STRTF 2013-3055] and partially supported by FEDER through COMPETE and FCT under project UID/BIM/04501/2013 and by National Funds through FCT, in the context of the projects UID/CEC/00127/2013 and Incentivo/EEI/UI0127/2014**.** The authors would also like to acknowledge to all participants involved and to Cátia Pinho and José Semedo for their assistance in data analysis.

ABSTRACT

Purpose: Cigarette smoking is often associated with the development of several respiratory diseases however, if diagnosed early, the changes in the lung tissue caused by smoking may be reversible. Computerised respiratory sounds have shown to be sensitive to detect changes within the lung tissue before any other measure, however it is unknown if it is able to detect changes in the lungs of healthy smokers. This study investigated the differences between computerised respiratory sounds of healthy smokers and non-smokers.

Methods: Healthy smokers and non-smokers were recruited from a university campus. Respiratory sounds were recorded simultaneously at 6 chest locations (right and left anterior, lateral and posterior) using air-coupled electret microphones. Airflow (1.0-1.5 l/s) was recorded with a pneumotachograph. Breathing phases were detected using airflow signals and respiratory sounds with validated algorithms.

Results: Forty-four participants were enrolled: 18 smokers (*mean* age 26.2, *SD*= 7 years; *mean* FEV_1 % predicted 104.7, *SD*= 9) and 26 non-smokers (*mean* age 25.9, *SD*= 3.7 years; mean FEV_1 % predicted 96.8, *SD*= 20.2). Smokers presented significantly higher frequency at maximum sound intensity during inspiration [(M=117, SD= 16.2 Hz vs. M= 106.4, SD= 21.6 Hz; $t(43) = -2.62$, $p=0.0081$, $d_z =0.55$)], lower expiratory sound intensities (maximum intensity: [(M= 48.2, SD= 3.8 dB vs. M= 50.9, SD= 3.2 dB; $t(43) = 2.68$, $p=0.001$, $d_z =-0.78$)]; mean intensity: [(M= 31.2, SD= 3.6 dB vs. M= 33.7,SD= 3 dB; $t(43) = 2.42$, $p=0.001$, $d_z =0.75$)] and higher number of inspiratory crackles (*median [interquartile range]* 2.2[1.7-3.7] vs. 1.5 [1.2-2.2], $p=0.0081$, $U= 110$, $r=-0.41$) than non-smokers.

Conclusions: Significant differences between computerised respiratory sounds of smokers and non-smokers have been found. Changes in respiratory sounds are often the earliest sign of

disease. Thus, computerised respiratory sounds might be a promising measure to early detect smoking related respiratory diseases.

Key-words: computerised auscultation; sound spectrum; crackles, early diagnosis; smoking

Abstract word count: 295

INTRODUCTION

Cigarette smoking is often associated with inflammation, obstruction and destruction of the lung parenchyma and airways [1]. These continuous aggressions to the lung tissue place smokers in a vulnerable position to acquire viral and bacterial respiratory infections (e.g., lower respiratory tract infections). If recurrent, respiratory infections potentiate progressive pathological changes in the small airways that may cause airflow obstruction and ultimately chronic respiratory diseases [2]. Nevertheless, it has been shown that initial changes in the pulmonary tissue caused by smoking may be reversible with optimal management [3].

The early diagnosis of most common chronic respiratory diseases is challenging, mainly due to the lack of sensitivity of the current outcome measures (e.g., chest X ray and lung function tests) [4,5]. Thus, it is essential to find sensitive measures to early detect changes in the lungs of smokers that may indicate respiratory complications.

Several studies have been reporting on the potential of computerised respiratory sounds to provide useful clinical information, as they are directly related to movement of air, changes within the lung tissue and position of secretions within the tracheobronchial tree [6-8]. Although few studies exist on the use of computerised techniques to acquire respiratory sounds in smokers, contradictory information is provided. Laird et al., (1974) [9] and Gavriely et al., (1994) [10] studied respiratory sounds of approximately 300 healthy smokers and non-smokers reporting the presence of “abnormal” sound spectrum and wheezes. Conversely, new evidence gathered by Gavriely et al., (1995) [11] and Gross et al., (2000) [12] indicates no differences

between the sound intensity of smokers and non-smokers. However, these studies focused mainly on normal respiratory sounds and little attention has been given to the presence of adventitious respiratory sounds (i.e., crackles and wheezes), which are often the earliest sign of disease, even before any changes being detected in imaging techniques [13]. Further research is therefore warranted.

This study investigated the differences between computerised respiratory sounds, normal and adventitious, of healthy smokers and non-smokers.

METHODS

Study design and Participants

A cross-sectional study with healthy smokers and non-smokers recruited from a university campus was conducted between March and May 2014. Participants were included if they were healthy non-smokers defined as life-long non-smokers, with no medical history suggestive of airway disease (i.e., no known diagnosis of a respiratory disease or chronic medication for the respiratory system) and normal spirometry. Healthy smokers were included using the same criteria as healthy non-smokers, except they were current smokers. Healthy non-smokers were excluded if they were past smokers. Participants, both smokers and non-smokers, were excluded if they were taking medication for the respiratory system, had an acute respiratory disease or presented any acute respiratory symptom (i.e., cough, dyspnoea, sputum) up to one month before data collection, presented chronic respiratory diseases, cardiac diseases, neurological impairments, current/previous history of pulmonary lobectomy, neoplasic disease or immunological disease and/or significant musculoskeletal disorders (e.g., kyphoescoliosis) that could affect respiratory acoustics.

Approval for this study was obtained from a national Ethics Committee in compliance with the Declaration of Helsinki. The study was advertised in flyers and distributed in the community.

Volunteers willing to participate contacted the researchers. In this first contact, researchers provided detailed explanations about the study, verified participants' eligibility and scheduled an appointment to perform data collection. Written informed consent was obtained from all individual participants included in the study, prior to any data collection.

Data collection procedures

Socio-demographics (gender, age), anthropometric (height, weight and body mass index - BMI) and clinical (smoking habits in pack-years) data were collected first with a structured questionnaire. The activities limitation resulting from dyspnoea were assessed with the modified Medical Research Council questionnaire – mMRC [14]. Then, computerised respiratory sounds and lung function, assessed according to the European Respiratory Society guidelines [15], with a portable spirometer (Micro 1, CareFusion, Kent, UK), were recorded. The parameters extracted from the spirometer were the percentage predicted of forced expiratory volume in one second (FEV_1 % predicted), the percentage predicted of forced vital capacity (FVC % predicted) and the ratio between both (FEV_1 % predicted/FVC % predicted). All assessments were performed by two researchers in a standardised order.

Respiratory sounds and airflows were recorded simultaneously and synchronized via a multi-channel respiratory sounds data acquisition device custom-developed in the Lung Acoustic Laboratory at Bogazici University, Istanbul, Turkey (see [16] for the technical details of a five-channel version of the same device). The system included six air-coupled electret microphones (SONY ECM-44 BPT), a Fleisch type pneumotachograph (Validyne CD379, Northridge, Calif), an analog amplifier-filter unit, an analog-to-digital converter and a laptop computer running a graphical user interface program implemented in LabVIEW. Microphones, located into capsules made of Teflon [17], were attached on the participant's skin with adhesive tapes (CPK Roll-fix 10cm-width) at six chest locations (right and left: anterior, lateral and posterior) [18].

The analog amplifier-filter unit used has an overall gain of 200 and a pass-band of 80 - 4000 Hz (a sixth-order Bessel high-pass filter with 80 Hz cut-off to filter out heart and muscle noise, followed by an eight-order Butterworth low-pass filter with 4000 Hz cut-off for anti-aliasing). The airflow signal was passed through a second order unity-gain Butterworth low-pass filter with a cut-off frequency of 35 Hz. The pre-processed analog data (six-channel sound signals and the airflow signal) were simultaneously converted into digital form via a data acquisition card (NI DAQCard-6024E) in 12-bit resolution and a sample frequency in each channel of 11025 samples per second, and transferred to the laptop computer. The recorded data were later converted to .wav format.

Each data acquisition session lasted for 20-seconds [19]. Environmental conditions were kept under tight control to prevent external noise during recordings. Participants were in a seated-upright position, wearing a nose clip and breathing through a mouthpiece at a standardised airflow (1.0-1.5L/s). Visual feedback regarding airflows and respiratory sounds was obtained via the graphical user interface program.

Signal processing

Firstly, breathing phases (i.e., inspiration and expiration) were automatically detected using the positive and negative portions of the flow signals recorded, and the sound signals were separated and labelled accordingly. Secondly, for each inspiration and expiration, the power spectra of the normal respiratory sound signals was estimated via the Welch's method adopting 256-point Hamming windows with 50% overlap, and 2^14-point fast Fourier transformation. Thirdly, for each estimated spectrum, the following parameters were calculated: percentile frequencies f25, f50, and f75, maximum intensity (Imax), frequency at maximum intensity (Fmax), mean intensity over the whole frequency range (Imean), and the mean intensities over the following frequency intervals: 100-300 Hz, 300-600 Hz, 600-1200 Hz and 1200-2000 Hz. Finally, the values of each parameter calculated for inspirations were summed and dived by the

total number of inspirations; and the values of each parameter calculated for expirations were summed and dived by the total number of expirations. The sound intensities were calculated in dB, and the reference used was the baseline noise of the data acquisition system (1.5 * 10-10 W).

Crackles were detected using a validated algorithm based on the combination of fractal dimension and box filtering techniques [20]. Wheezes were detected using an algorithm based on time-frequency analysis [21]. The number of crackles, initial deflection width (IDW), largest deflection width (LWD), two-cycle duration (2CD), wheeze occupation rate and frequency (Hz) of crackles and wheezes per breathing phase were extracted. Crackle and wheeze detection were performed using Matlab®R2011a.

Statistical analysis

Descriptive statistics were used to describe the sample. Socio-demographic and clinical characteristics between groups were compared with Independent Samples t-test for continuous normally distributed data (FEV_1 % predicted/FVC % predicted ratio), Mann-Whitney U test for continuous non-normally distributed and ordinal data (age, FEV_1 % predicted, FVC % predicted, BMI, pack-years, mMRC) and Fisher's exact test for categorical data (gender).

To simplify the interpretability of the findings, for each chest region (anterior, lateral and posterior), the results from the right and left locations were pooled by a simple polling method (i.e., the individual values acquired from right and left locations were averaged and placed in the database under the headings of anterior, lateral and posterior, according to the region from which they have been taken and then the analysis comparing regions between smokers and non-smokers was performed as if the data of each region were derived from a single sample) [22,23]. Then, the number of participants with crackles and wheezes in each chest region was calculated

and Fisher's exact test was used to investigate the groups' differences on the number of participants presenting crackles and wheezes.

Finally, comparisons between smokers and non-smokers at anterior, lateral and posterior chest regions were performed using Independent Samples t-test for normally distributed data (i.e., normal respiratory sound parameters) and Mann-Whitney U test for non-normally distributed data (i.e., adventitious respiratory sound parameters). Bonferroni corrections were applied for the number of comparisons performed per respiratory sound parameter (i.e., 6 comparisons per respiratory sound parameter) between smokers and non-smokers. Thus, the level of significance was set at 0.0083.

Statistical analyses were completed with the estimation of effect sizes via Cohen's d for Independent Samples t-test [24] and *r* for Mann-Whitney U test [25]. Cohen's d_z effect sizes were interpreted as a small (≥0.2), medium (≥0.5) or large (≥0.8) [24] and *r* as small (*r* ≥0.2), medium (*r* ≥0.3) or large (*r* ≥0.5) effects [24]. Statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY, USA) and plots created using Matlab®R2011a and GraphPad Prism version 5.01 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Participants

Fifty-four volunteers agreed to participate in the study. However, 1 participant did not complete the assessment and 8 were excluded due to: asthma (n=1), presence of acute respiratory symptoms (i.e., cough, sputum and fatigue) two days prior to data collection (n=1) and past history of smoking habits (n=6). One participant was posteriorly excluded from the data analysis due to poor quality of the sound recording (i.e., movement artefacts and external noise). In total, 44 healthy participants were enrolled: 18 smokers (*mean* age 26.2, *SD*= 7 years; *mean* FEV_1 % predicted 104.7, *SD*= 9) and 26 non-smokers (*mean* age 25.9, *SD*= 3.7 years; *mean* FEV_1 %

predicted 96.8, *SD*= 20.2). All participants presented normal body composition and lung function, however, smokers reported significantly higher levels of dyspnoea at the mMRC questionnaire (*median [interquartile range]* 0.5 [0-1] vs. 0 [0], *U*= 135, *p*=0.002, *r*=-0.47). No differences were observed for the remaining variables (Table 1).

(Please insert table 1 about here)

Computerised respiratory sounds

Differences in normal respiratory sounds between smokers and non-smokers were only observed at the posterior region. Smokers presented significantly higher inspiratory Fmax [(M=117, SD= 16.2 Hz vs. M= 106.4, SD= 21.6 Hz; *t*(43) = -2.62, *p*=0.0081, d_z =0.55)] and lower expiratory intensity, both Imax [(M= 48.2, SD= 3.8 dB vs. M= 50.9, SD= 3.2 dB; *t*(43) = 2.68, *p*=0.001, d_z =-0.78)] and Imean [(M= 31.2, SD= 3.6 dB vs. M= 33.7,SD= 3 dB; *t*(43) = 2.42, *p*=0.001, d_z =0.75)] (Figure 1). Figure 2 shows the average spectrum of the respiratory sounds recorded form all participants at anterior, lateral and posterior chest.

(Please insert figure 1 about here)

(Please insert figure 2 about here)

Most participants presented at least one crackle per breathing cycle in all the assessed chest locations, however no differences were found between groups for the number of participants with crackles (p>0.05). Significant differences in the number of crackles were found at the anterior/upper region, where smokers presented significantly higher number of inspiratory crackles (2.2 [1.8-3.7] vs. 1.5 [1.2-2.2], *p*=0.0081, *U*= 110, *r*=-0.41) than non-smokers (Figure 3). No differences were found for the remaining crackle parameters. Fig. 4 shows a respiratory sound presenting a crackle acquired at posterior chest from a smoker participant.

(Please insert figure 3 about here)

(Please insert figure 4 about here)

Wheezes were observed in a reduced number of participants, mainly at the posterior regions of smokers [n= 10 (56%)]. No differences were found between groups regarding the number of participants with wheezes or the characteristics of wheezes ($p>0.05$). Fig. 5 shows spectrograms of RS recorded at anterior chest from a smoker, presenting wheezes, and a non-smoker participants.

(Please insert figure 5 about here)

A detail description of normal respiratory sounds, crackles and wheezes characteristics in each group can be found in the online supplementary material.

DISCUSSION

According to the authors' best knowledge, this is the first study comparing normal and adventitious computerised respiratory sounds between healthy smokers and non-smokers. The main findings indicated that smokers present significantly higher inspiratory Fmax, lower expiratory sound intensity and higher number of inspiratory crackles than non-smokers.

Frequency at maximum power provides information on the frequency range of the sound activity [26], and its increase during histamine challenges is often related with airway narrowing in patients with asthma and COPD [27]. In the present study, such increase was already found during normal breathing manoeuvres in the posterior lower lung of very light smokers (i.e., less than 5 pack-years), which may indicate some degree of early bronchial constriction in the smaller airways of this population. Nevertheless, previous literature, has reported no differences in this variable between healthy and diseased populations [12,28,27]. The differences between these previous studies and the present one, may be explained by

methodologic and recruitment dissimilarities. It is known that Fmax depends mainly on the cut-off frequency of the high-pass filter [12], which in the present study was lower (80Hz) than in the previous literature (100Hz) [28,27] in order to also analyse adventitious respiratory sounds [29]. Also, previous studies tended to have older populations [12,28] and to include light smokers in the non-smokers group [12]. The present results should however be interpreted with caution and further studies with similar standardised protocols are yet needed.

Maximum and mean respiratory sound intensities have been reported as an objective indicator of regional pulmonary ventilation, with high sound intensities indicating strong pulmonary ventilation [30,8]. Nevertheless, this variable is also dependent on the respiratory flows achieved during the breathing manoeuvre [30,8], and for that reason each participant's inspiratory and expiratory flow was standardized at 1-1.5 l/s. This respiratory flow should result in a lung volume of approximately 30% of vital capacity, which, in young subjects, is the lung volume at which all airways are open and when breath sound intensity corresponds to pulmonary ventilation [31]. In the present study, smokers presented lower Imax and Imean than non-smokers for the same airflow, which might already indicate poorer pulmonary ventilation.
Crackles have been associated with a variety of lung diseases, such as pulmonary fibrosis, congestive heart failure and pneumonia [7,32] and one recent systematic review has indicated that particularly inspiratory crackles are often reported in the computerised auscultation of patients with COPD [33]. This is particularly relevant for the present study, as smokers presented significantly more inspiratory crackles than non-smokers. It is known that tobacco smoking is the leading cause of COPD [34], and thus the detection of inspiratory crackles in smokers may be a promising method for the early diagnosis of this disease. Nevertheless, these results should be interpreted with caution, as no significant differences in the number of crackles were found between smokers and non-smokers in the study of Alzahrani, M. (2011)[35]. There are methodological reasons that might explain the dissimilarities observed. In the study of Alzahrani, M. (2011), the airflow was not controlled, respiratory sounds were acquired with a digital

stethoscope and crackles were analysed with the Vannuccini, et al (1998)[36] algorithm, whereas in the present study, airflow was controlled, respiratory sounds were acquired with a multichannel microphones equipment and crackles were analysed with Pinho, et al algorithm[20]. Therefore, these methodological options may have influenced the results and consequent differences observed. More research with larges samples, respiratory volume/flow control and similar acquisition and analysis methods are needed to draw stronger conclusions about crackles ability to distinguish between smokers and non-smokers.

The absence of wheezes in the present study was expected as it is known that the effects of smoking are firstly detected in the smaller airways [37] and wheezes are only generated by airflow limitation in larger airways (until the 7^{th} generation of the tracheobronchial tree) [38]. Nevertheless, it should also be noticed that wheezes might occur when there is flow limitation, but flow limitation is not necessarily accompanied by wheezes [38], and thus it could have happened that the respiratory flow used in this study was not enough to generate wheezes. Studies involving forced manoeuvres and higher flow levels are needed to further explore the presence of wheezes in smokers.

The mechanisms for changes in respiratory sounds caused by tobacco smoking are not well understood however, it may be attributed to the development of inflammatory processes in the smaller airways. Tobacco smoke contains toxic gases which when inhaled into the lungs might originate both local and systemic inflammatory responses by stimulating alveolar macrophages to release inflammatory mediators [1]. Consequently, these macrophage cells release protease to break down connective tissue in the lung parenchyma and also stimulate mucus hypersecretion around damaged tissue, ultimately leading to airway obstruction and emphysema. Airway obstruction is likely to affect the distribution and extent of turbulence and flow within the bronchi, causing poor regional ventilation, and consequently lower respiratory sound intensity [11]. Emphysema might have an impact on the air movement in the distal

airways causing some of them to close and open irregularly depending on the air volume, generating crackles.

Differences in sound amplitude and in the number of crackles are often audible by the human ear in severe respiratory conditions, such as pneumonia and interstitial lung diseases [30]. Although statistically significant and with medium effect sizes, the differences between smokers and non-smokers were of approximately 2dB and 1 crackle per breathing cycle which would probably not be detected by human ear. This highlights the potential of using computerised respiratory sounds especially in primary care settings for the early diagnosis of respiratory diseases among smokers.

It should also be noticed that smokers already presented some degree of activities limitation resulting from dyspnoea. Dyspnoea is the most common symptom limiting activities performance and the major reason for referral to respiratory rehabilitation programs in patients with chronic respiratory diseases [39]. The fact that such light and young smokers already exhibit this symptom should be a call for early intervention in educational fields (i.e., universities, colleges, high schools) to advice for the effects of tobacco smoking. Further studies involving dyspnoea reports during exercise are needed to confirm this finding.

This study has some limitations that need to be acknowledged. Firstly, respiratory sounds from right and left regions were pooled to compute comparisons. Differences in lung acoustics between right and left lung are well documented in the literature [26,40,41], however the main aim of this study was to detect differences between two populations and not to establish reference values. Thus, because the same methodology was used for both groups, it is not believed that this could have affected the results. Nevertheless, health professionals should be aware of these differences when assessing patients in clinical practice. Moreover, the three chest regions were studied separately however, it is still unknown if the same sound was being captured in the different regions, as respiratory sounds were collected simultaneously. This

could have influenced our findings. Nevertheless, this methodology was used in both groups and this is a relatively unexplored area where further research is yet needed to understand its clinical implications.

Secondly, "healthy smokers'" were selected based on a pure clinical definition and although this criteria has been used by others [11,42,35], it is recognised that it presents some limitations, as it cannot be guaranteed that these participants do not have an undiagnosed respiratory condition or will not develop one in the near future. However, smoker participants were very light smokers compared to those enrolled in previous research (pack-years 2.2 [0.9-5.5] vs. 25±17 [11] vs. 5.6 ± 4.9 [42]), and presented similar spirometry values than non-smokers, which provides confidence to rely that a respiratory condition was absent.

Thirdly, many comparisons were conducted on the respiratory data and this could have increased type I error, i.e., rejecting the null hypothesis when it should not be rejected. However, because comparisons were only carried out between smokers and non-smokers (no comparisons were conducted among regions and respiratory phases within each group), we believe that the likelihood of increasing this error was controlled. Nonetheless, Bonferroni corrections have also been applied to control for this type of error.

Fourthly, the sample size used in this study was exploratory and might not have been sufficient to detect truly significant changes between smokers and non-smokers in other parameters. Thus, studies with sample size estimations are needed. This exploratory study is a first step towards the use of computerised respiratory sounds in the screening of respiratory diseases among smokers and could be used as a pilot study to compute sample sizes in future studies.

Finally, the complex set up used to record respiratory sounds and airflow may be perceived as a limitation to the use of computerised respiratory sounds in the clinical practice, namely in

primary care settings. Future research should focus in developing technologies for acquiring high quality data at bedside with minimal setup.

CONCLUSION

Computerised respiratory sounds allowed to detect differences in the sound intensity and number of crackles of healthy smokers and non-smokers, indicating that light smokers already present significantly lower sound intensity and higher number crackles than non-smokers. Thus, it can be suggested that computerised respiratory sounds may be successfully implemented in clinical practice as a useful method for early diagnosis smoking related respiratory diseases.

Funding: This work has been developed within a European Respiratory Society Short Term Fellowship [grant number STRTF 2013-3055]. This work was also partially supported by FEDER through COMPETE and FCT under project UID/BIM/04501/2013 and by National Funds through FCT, in the context of the projects UID/CEC/00127/2013 and Incentivo/EEI/UI0127/2014**.**

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

REFERENCES

1. Milner D (2004) The physiological effects of smoking on the respiratory system. Nurs Times 100 (24):56-59
2. Saldías F, Díaz O (2011) Cigarette Smoking and Lower Respiratory Tract Infection. In: Martin-Loeches I (ed) Bronchitis. InTech. doi:10.5772/17652
3. Beane J, Sebastiani P, Liu G et al. (2007) Reversible and permanent effects of tobacco smoke exposure on airway epithelial gene expression. Genome Biol 8 (9):R201. doi:10.1186/gb-2007-8-9-r201
4. Marques A, Bruton A, Barney A (2006) Clinically useful outcome measures for physiotherapy airway clearance techniques: a review. Physical Therapy Reviews 11 (4):299-307. doi:10.1179/108331906X163441
5. Parkes G, Greenhalgh T, Griffin M et al. (2008) Effect on smoking quit rate of telling patients their lung age: the Step2quit randomised controlled trial, vol 336. vol 7644. doi:10.1136/bmj.39503.582396.25
6. Baughman RP, Loudon RG (1985) Lung sound analysis for continuous evaluation of airflow obstruction in asthma. Chest 88 (3):364-368. doi:10.1378/chest.88.3.364
7. Piirila P (1992) Changes in crackle characteristics during the clinical course of pneumonia. Chest 102 (1):176-183. doi:10.1378/chest.102.1.176
8. Kiyokawa H, Pasterkamp H (2002) Volume-dependent variations of regional lung sound, amplitude, and phase. Journal of applied physiology (Bethesda, Md : 1985) 93 (3):1030-1038. doi:10.1152/japplphysiol.00110.2002
9. Laird CW, Homburger F, Ishikawa S (1974) Letter: Breath-sound changes after cigarette smoking. Lancet 1 (7861):808. doi:10.1016/S0140-6736(74)92876-1
10. Gavriely N, Nissan M, Cugell DW et al. (1994) Respiratory health screening using pulmonary function tests and lung sound analysis. The European respiratory journal 7 (1):35-42. doi:10.1183/09031936.94.07010035
11. Gavriely N, Nissan M, Rubin AH et al. (1995) Spectral characteristics of chest wall breath sounds in normal subjects. Thorax 50 (12):1292-1300. doi:10.1136/thx.50.12.1292
12. Gross V, Dittmar A, Penzel T et al. (2000) The relationship between normal lung sounds, age, and gender. American journal of respiratory and critical care medicine 162 (3 Pt 1):905-909. doi:10.1164/ajrccm.162.3.9905104
13. Bohadana A, Teculescu D, Martinet Y (2004) Mechanisms of chronic airway obstruction in smokers. Respir Med 98 (2):139-151. doi:Mechanisms of chronic airway obstruction in smokers
14. Doherty DE, Belfer MH, Brunton SA et al. (2006) Chronic Obstructive Pulmonary Disease: Consensus Recommendations for Early Diagnosis and Treatment. Journal of Family Practice:S1
15. Miller MR (2005) Standardisation of spirometry. European Respiratory Journal 26 (2):319-338. doi:10.1183/09031936.05.00034805
16. Sen I, Kahya YP (2005) A multi-channel device for respiratory sound data acquisition and transient detection. Conference proceedings : Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Annual Conference 6:6658-6661. doi:10.1109/iembs.2005.1616029
17. Kraman SS, Wodicka GR, Oh Y et al. (1995) Measurement of respiratory acoustic signals. Effect of microphone air cavity width, shape, and venting. Chest 108 (4):1004-1008
18. Rossi M, Sovijarvi ARA, Piirila P et al. (2000) Environmental and subject conditions and breathing manoeuvres for respiratory sound recordings. European Respiratory Review 10 (77):611-615
19. Vyshedskiy A, Murphy R (2012) Crackle Pitch Rises Progressively during Inspiration in Pneumonia, CHF, and IPF Patients. Pulmonary medicine 2012:240160. doi:10.1155/2012/240160

20. Pinho C, Oliveira A, Jácome C et al. (2015) Automatic Crackle Detection Algorithm Based on Fractal Dimension and Box Filtering. Procedia Computer Science 64:705-712. doi:http://dx.doi.org/10.1016/j.procs.2015.08.592
21. Taplidou SA, Hadjileontiadis LJ (2007) Wheeze detection based on time-frequency analysis of breath sounds. Computers in Biology and Medicine 37 (8):1073-1083. doi:10.1016/j.compbiomed.2006.09.007
22. Jacome C, Oliveira A, Marques A (2015) Computerized respiratory sounds: a comparison between patients with stable and exacerbated COPD. The clinical respiratory journal. doi:10.1111/crj.12392
23. Kompis M, Pasterkamp H, Oh Y et al. Distribution of inspiratory and expiratory respiratory sound intensity on the surface of the human thorax. In: Engineering in Medicine and Biology Society, 1997. Proceedings of the 19th Annual International Conference of the IEEE, 1997 1997. pp 2047-2050 vol.2045. doi:10.1109/IEMBS.1997.758750
24. Cohen J (1988) Statistical Power Analysis for the Behavioral Sciences. 2nd edn. Lawrence Earlbaum Associates, Hellsdale, New Jersey
25. Fritz CO, Morris PE, Richler JJ (2012) Effect size estimates: current use, calculations, and interpretation. Journal of experimental psychology General 141 (1):2-18. doi:10.1037/a0024338
26. Jones A, Jones RD, Kwong K et al. (1999) Effect of positioning on recorded lung sound intensities in subjects without pulmonary dysfunction. Physical therapy 79 (7):682-690
27. Malmberg LP, Sovijarvi AR, Paajanen E et al. (1994) Changes in frequency spectra of breath sounds during histamine challenge test in adult asthmatics and healthy control subjects. Chest 105 (1):122-131. doi:10.1378/chest.105.1.122
28. Malmberg LP, Pesu L, Sovijarvi AR (1995) Significant differences in flow standardised breath sound spectra in patients with chronic obstructive pulmonary disease, stable asthma, and healthy lungs. Thorax 50 (12):1285-1291. doi:10.1136/thx.50.12.1285
29. Vanuccini L, Earis J, Helisto P et al. (2000) Capturing and pre-processing of respiratory sounds. Eur Respir Rev 10 (77):616-620.
30. Pasterkamp H, Kraman SS, Wodicka GR (1997) Respiratory sounds. Advances beyond the stethoscope. American journal of respiratory and critical care medicine 156 (3 Pt 1):974-987. doi:10.1164/ajrccm.156.3.9701115
31. Leblanc P, Macklem PT, Ross WR (1970) Breath sounds and distribution of pulmonary ventilation. Am Rev Respir Dis 102 (1):10-16
32. Vyshedskiy A, Ishikawa S, Murphy RL, Jr. (2011) Crackle pitch and rate do not vary significantly during a single automated-auscultation session in patients with pneumonia, congestive heart failure, or interstitial pulmonary fibrosis. Respiratory care 56 (6):806-817. doi:10.4187/respcare.00999
33. Jacome C, Marques A (2014) Pulmonary rehabilitation for mild COPD: a systematic review. Respiratory care 59 (4):588-594. doi:10.4187/respcare.02742
34. Rennard SI, Drummond MB (2015) Early chronic obstructive pulmonary disease: definition, assessment, and prevention. Lancet 385 (9979):1778-1788. doi:10.1016/s0140-6736(15)60647-x
35. Alzahrani M (2011) Quantifying crackles in the lung of smoking and non-smoking young adults., University of Southampton,
36. Vannuccini L, Rossi M, Pasquali G (1998) A new method to detect crackles in respiratory sounds. Technology and health care : official journal of the European Society for Engineering and Medicine 6 (1):75-79
37. Strulovici-Barel Y, Omberg L, O'Mahony M et al. (2010) Threshold of biologic responses of the small airway epithelium to low levels of tobacco smoke. American journal of respiratory and critical care medicine 182 (12):1524-1532. doi:10.1164/rccm.201002-0294OC
38. Bohadana A, Izbicki G, Kraman SS (2014) Fundamentals of lung auscultation. The New England journal of medicine 370 (8):744-751. doi:10.1056/NEJMra1302901

39. Stendardi L, Binazzi B, Scano G (2007) Exercise dyspnea in patients with COPD. International Journal of Chronic Obstructive Pulmonary Disease 2 (4):429-439
40. Pasterkamp H, Patel S, Wodicka GR (1997) Asymmetry of respiratory sounds and thoracic transmission. Medical & biological engineering & computing 35 (2):103-106. doi:10.1007/BF02534138
41. Kraman S (1983) Lung sounds: Relative sites of origin and comparative amplitudes in normal subjects. Lung 161 (1):57-64. doi:10.1007/BF02713842
42. Kanezaki M, Ebihara S, Nikkuni E et al. (2010) Perception of urge-to-cough and dyspnea in healthy smokers with decreased cough reflex sensitivity. Cough (London, England) 6:1-1. doi:10.1186/1745-9974-6-1

Figures captions

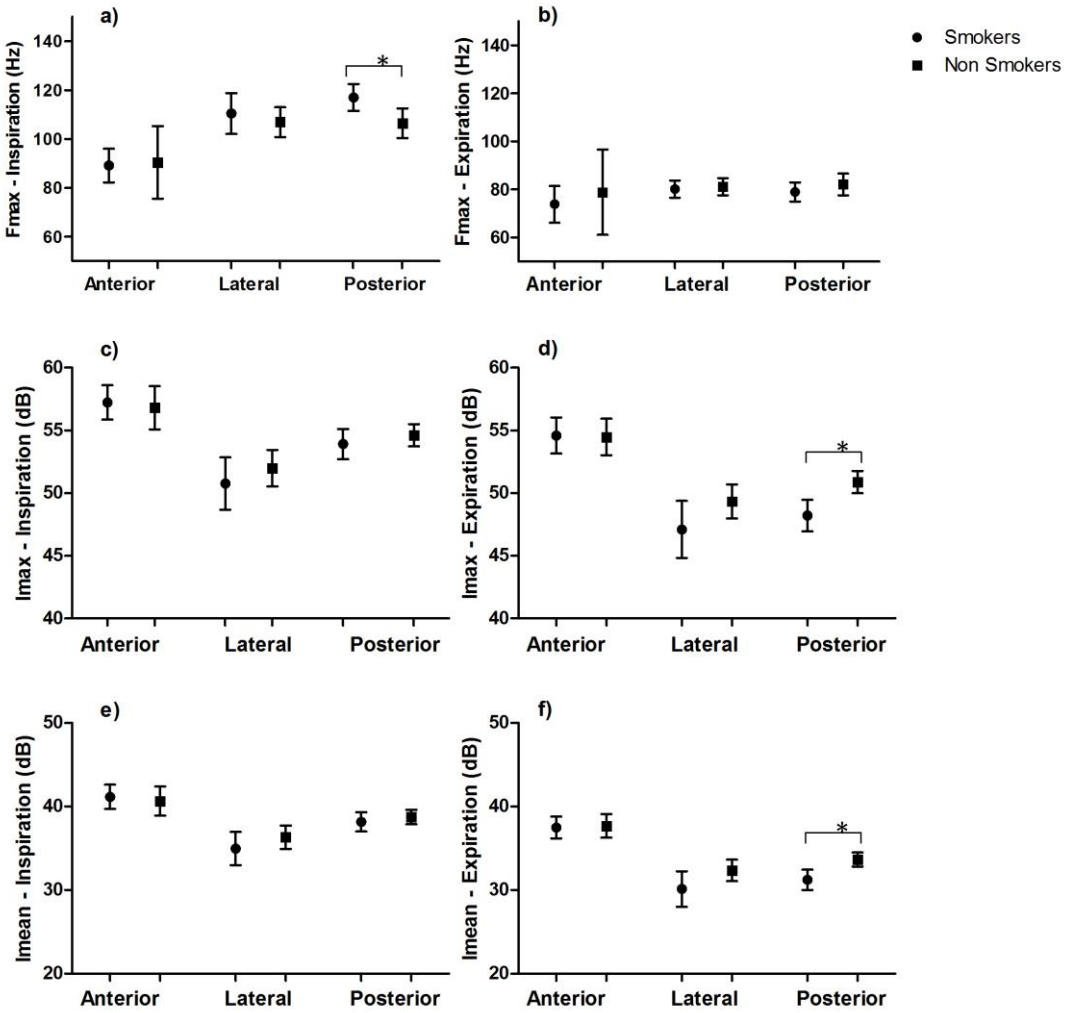


Fig. 1 – Differences in normal computerised respiratory sounds at anterior, lateral and posterior lung between smokers and non-smokers at 100-2000Hz bandwidth. a) Inspiratory Fmax; b) Expiratory Fmax; c) Inspiratory Imax; d) Expiratory Imax; e) inspiratory Imean; f) expiratory Imean. Data are presented as mean and standard deviation. Significant differences are identified with * ($p<0.05$). Fmax - frequency at maximum intensity; Imax – maximum intensity; Imean – mean intensity.

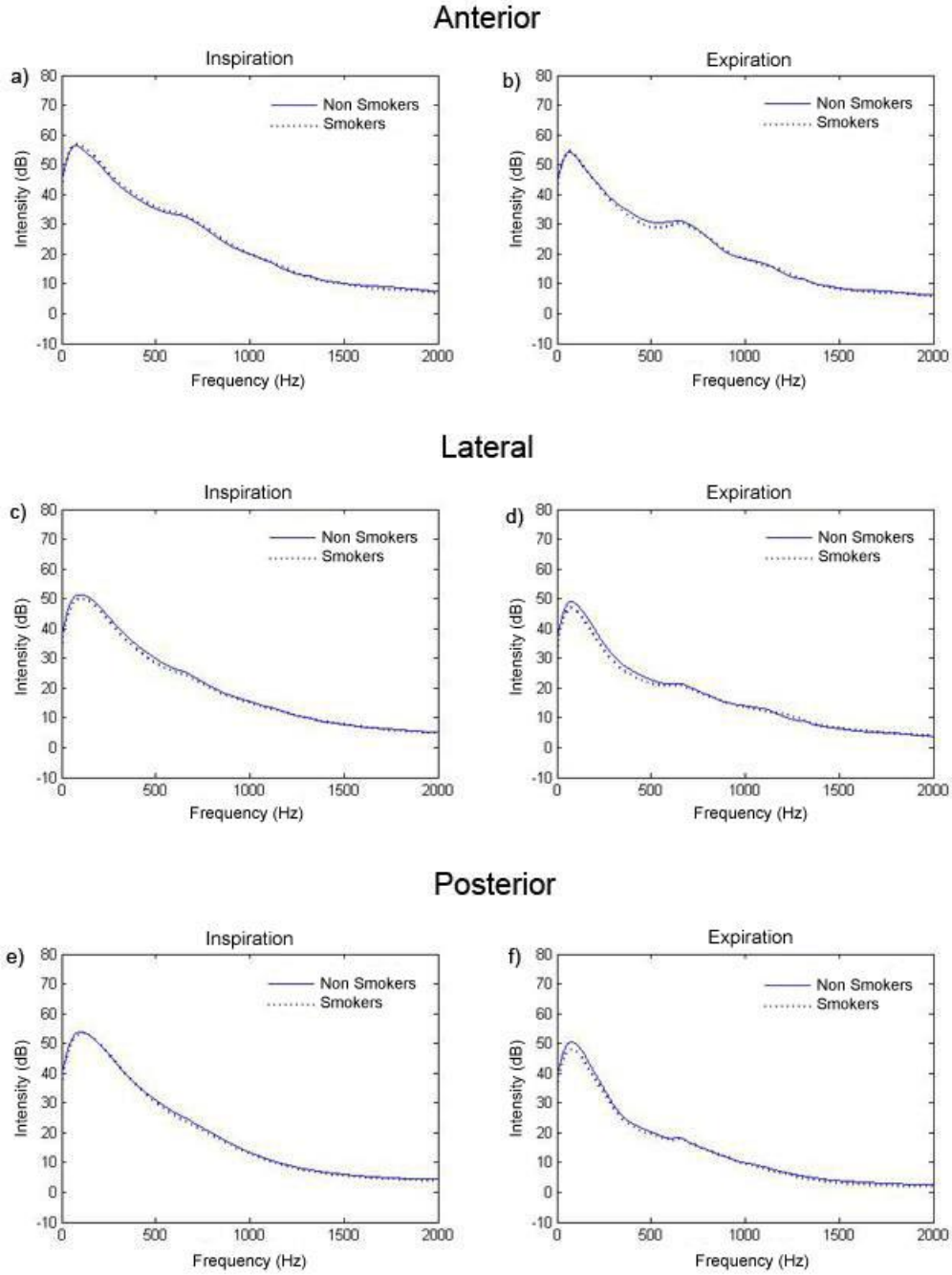


Fig. 2 – Average spectrum of the respiratory sounds recorded form all participants at anterior (a, b), lateral (c, d) and posterior (e, f) chest during inspiration (a, c, e) and expiration (b, d, f).

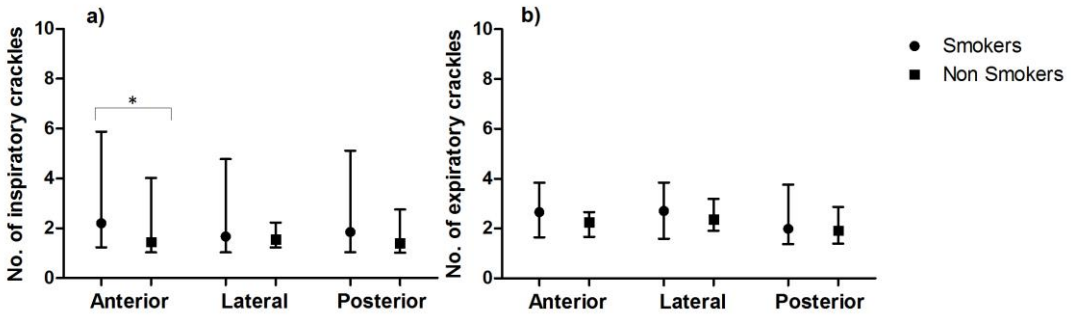
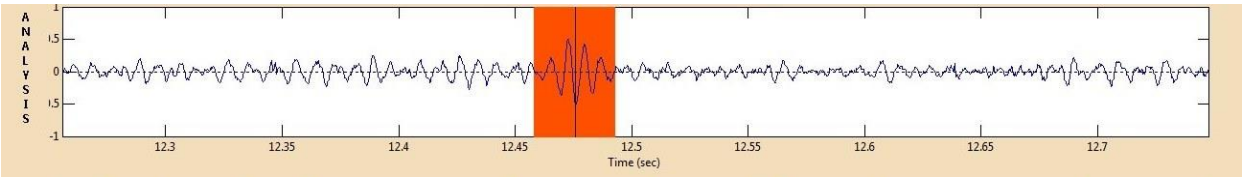


Fig. 3 – Differences in the number of crackles at anterior, lateral and posterior lung between smokers and non-smokers. a) Mean number of inspiratory crackles; b) Mean number of expiratory crackles. Data are presented as median and interquartile range. Significant differences are identified with * ($p<0.05$).

1 

2 **Fig. 4** – Time amplitude plot of a respiratory sound presenting an expiratory crackle, recorded

3 at posterior chest from smoker.

4

5

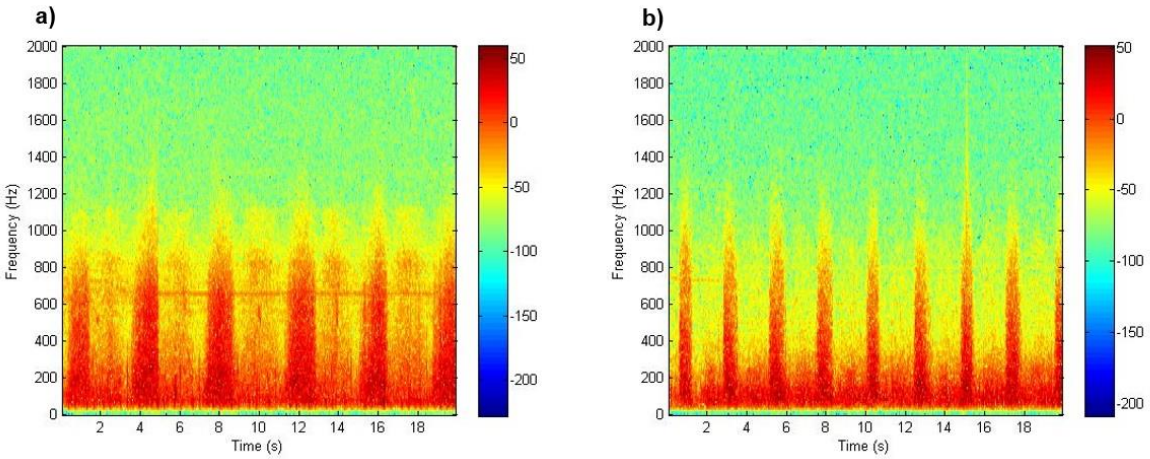


Fig. 5 – Spectrogram of respiratory sounds recorded at anterior chest from (a) a smoker participant presenting expiratory wheezes and (b) a non-smoker participant.

Table captions

Table 1 - Socio-demographic, anthropometric and clinical characteristics of the participants (n=44).

Characteristics	Smokers (n=18)	Non-smokers (n=26)	p
Age (years)	26.2 (7)	25.9 (3.7)	0.40
Gender (male), n(%)	11(61%)	18(69%)	0.75
Pack-years, M[IQR]	2.2 [0.9-5.5]	-	-
mMRC, M[IQR]	0.5 [0-1]	0 [0]	0.002*
BMI (kg/m^2)	23.5 (3.3)	22.3 (2.3)	0.29
FEV_1 (% predicted)	104.7 (9)	96.8 (20.2)	0.11
FVC (% predicted)	102.5 (9.3)	100.1 (10.2)	0.53
FEV_1/FVC (% predicted)	105.1 (7.4)	103.5 (7.2)	0.62

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; FVC, forced vital capacity; * statistical significance for $p<0.05$