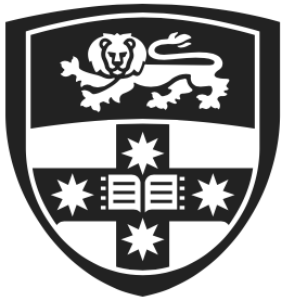
PHYSIOLOGICAL PHENOTYPING OF AIRWAY DISEASE



THE UNIVERSITY OF SYDNEY

WOOLCOCK

LEADERS IN BREATHING & SLEEP RESEARCH

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BSRC, MSRC, RRT
A Thesis Submitted In Fulfillment of The Requirement For The Degree of Doctor of Philosophy (Medicine)
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Statement Of Originality

The work described in this thesis was carried out within the Department of Respiratory Medicine, Royal North Shore Hospital and the Woolcock Institute of Medical Research under the supervision of Professor Gregory G. King, Professor G.Kim Prisk, and Associate Professor Cindy Thamrin.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged. It has neither been presented, nor is it currently being presented, for any other degree.

Hooria Alowiwi

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I contributed to conception and design of the study, analysis of the data, preparation, and writing of the manuscript

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Prof. Gregory G. King

Publications Resulting From This Thesis

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Abstracts

Relationship between concavity of the flow-volume loop and small airway measures in smokers with normal spirometry. Abstract TSANZERS 2021

Effect of bronchodilation response on ventilation distribution in asthma. Abstract TSANZSRS 2023

Variability in peak expiratory flow rates of healthy adults. Abstract TSANZ NSW ASM 2024

Presentations

Oral presentation : TSANZSRS annual scientific meeting 30th April-4th May 2021

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TSANZ NSW ASM 2023

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Research Ethics Approval

Chapter 3

All research protocols were approved by the Northern Sydney Local Health District at the Woolcock Institute of Medical Research (HREC protocol number 2022/ETH00782), and all participants in the study provided written informed consent.

Chapter 4

The original study protocol was approved by the Northern Sydney Central Coast Area Health Service Human Research Ethics Committee (Protocol 1106-209M). Written informed consent was obtained from all recruited patients.

Chapter 5, and 6

All research protocols were approved by the Northern Sydney Local Health District at Royal North Shore Hospital Human Research Ethics (HREC protocol number RESP/18/198), and all participants in the study provided written informed consent.

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List of Abbreviations

ACQ-5 – Asthma control questionnaire-5

AHR – Airway hyperresponsiveness

ASM – Airway smooth muscle

ATS – American Thoracic Society

β-agonist – Beta agonist

BDR – Bronchodilator Response

BMI – Body mass index

CDI – Convection-dependent inhomogeneity

CEV – Cumulative expired volume

COPD – Chronic obstructive pulmonary disease

CT – Computed tomography

DCDI – Diffusion-convection-dependent inhomogeneity

DLCO – Diffusing capacity of carbon monoxide

ERS – European Respiratory Society

FAO – Fixed airflow obstruction

FEV1 – Forced expired volume in 1 second

$FEF_{25-75\%}$ – Forced expiratory flow at 25-75% of FVC

FeNO – Fraction of exhaled nitric oxide

FOT – Forced oscillation technique

FRC – Functional residual capacity

FVC – Forced vital capacity

GINA – Global Initiative for Asthma

ICS – Inhaled corticosteroid

LABA – Long-acting beta agonist

L – Litres

MBNW – Multiple breath nitrogen washout

MEFV – Maximal expiratory flow volume

ml – Millilitres

N_2 – Nitrogen

O_2 – Oxygen

PEF – Peak expiratory flow

ppb – Parts per billion

Rrs – Respiratory system resistance

RV – Residual volume

Sacin – Ventilation heterogeneity in the acinar airways

SABA – Short-acting beta agonist

Scond – Ventilation heterogeneity in the conducting airways

SD – Standard deviation

SnIII – Normalised slope

SPECT – Single photon emission computed tomography

TLC – Total lung capacity

TO – Lung turnover

Xrs – Respiratory system reactance

Zrs – Respiratory system impedance

Table of Contents

Abstract

Introduction

While emerging evidence supports personalised medicine for patients with asthma, there is still much to learn about the physiological measures necessary to effectively enable personalised therapies. The bronchodilator response is an important physiological measure that has traditionally been used to define the presence of asthma, however, the mechanisms of response remain poorly understood. Home monitoring of peak expiratory flow rates (PEF) is a valuable tool for minimising asthma exacerbations, but there is a lack of data on what constitutes normal day-to-day PEF variability. Additionally, small airway dysfunction plays a significant role in the presentation and management of both asthma and smokers with normal spirometry. It is an important characteristic contributing to poor disease outcomes but is often overlooked. Correlating traditional spirometry measures of small airway function with advanced methods could enhance the accessibility of small airway evaluation in routine clinical practice.

Aims

My aim was to characterise physiological measures of airway function that are not commonly, or used at all, in current clinical practice in airways diseases; day-to-day PEF variability in health subjects using clinically relevant indices, an index characterising the shape of the expiratory flow volume curve that putatively reflects small airway function and, bronchodilator responsiveness measured by ventilation imaging, with the overall aim of supporting comprehensive characterisation of a patient and a personalised approach to treatment.

Methods

In smokers with normal spirometry, small airway function measured by multiple breath nitrogen washout (MBNW- ventilation heterogeneity in the convection-dependent conductive (Scond) and diffusion-dependent acinar (Sacin) airways), impulse oscillometry system (IOS) were compared to a recently developed index that characterised the magnitude of concavity of the expiratory flow volume curve, in smokers with normal spirometry. In asthmatic individuals, bronchodilator response was measured using ventilation single photon emission computed tomography (VSPECT/CT) imaging with Technegas to compare changes in total ventilated volume, with changes in spirometry and small airway measurements assessed by the forced oscillation technique (FOT - respiratory system resistance (Rrs) and reactance (Xrs)). Finally, daily peak expiratory flow (PEF) recordings were made by healthy subjects over 3 weeks, and expressed as amplitude percent mean (A%M – mean variability as a percentage of the overall mean value) and amplitude percent highest (A%H – mean variability as a percentage of the highest PEF).

Results

In smokers (n=80), abnormalities in both MBNW and oscillometry parameters are common despite normal spirometry. However, abnormalities in all three measures of small airway function (concavity index, MBNW and IOS) were discordant. In asthmatic subjects (n=22), despite a clearcut response to bronchodilator inhalation in respiratory oscillometry, there was no overall change in response in VSPECT. There was marked variability in changes in total ventilated volume following bronchodilator inhalation which was also discordant with changes in spirometry and oscillatory impedance. However, older subjects had less ventilated lung, and older subjects demonstrated greater increases in total ventilation with bronchodilator. In healthy subjects (n=30), there was a wide range of day-to-day variability in PEF, from almost no variability to surprisingly

high variability. The calculated upper limits of normal (ULN) for A%H and A%M at the 95th percentile were 24% and 30.32%, respectively.

Conclusion

In smokers, the concavity of the expiratory flow volume curve, MBNW and oscillometric impedance measure different aspects of small airway function that are disparate from each other. They therefore cannot be used interchangeably as functional measures, and their clinical utility therefore likely differ. In asthma, bronchodilator responses in small and large airways as measured by oscillometry and spirometry respectively, are not reflected by changes in total lung ventilation measured by VSPECT. Older subjects demonstrated less ventilation but greater increases in ventilation in response to bronchodilator. The mechanisms for this relationship were not investigated but may relate to disease duration and airway/lung remodeling and requires further investigation. In terms of peak flow variability in healthy subjects, the method of quantifying PEF variability has a large effect on day-to-day variability but for A%H, being the simplest and most practical method of measuring day-to-day variability, the finding of 24% being the normal limit of variability is marginally higher than the empirical figure of <20% being used in clinical practice.

General Introduction

Asthma is still a significant individual, community and societal burden. Despite the increasing efforts, and the worldwide guidelines for the diagnosis and management of asthma, asthma mortality remains significant, most of which are potentially avoidable. Globally, it affects over 300 million individuals (1). In Australia alone, over 10% of people have asthma, ranking Australia among the highest in the world (2, 3). In 2022, there has been an increase in the rates of hospitalisation for asthma in Australia, with 467 deaths in just one year which was highest number of asthma deaths since 2016/2017 (4-6).

Current diagnostic, and managements criteria are symptom-driven, but also with a goal of maintaining the best possible lung function (7). For decades, ICS therapy has been the mainstay treatment, with a stepwise approach to achieve control of patient's symptoms (8). Symptoms however are greatly disassociated from objective measures of lung function and of bronchoconstriction, as well as being dissociated with the patients' own perceptions of the severity of their own disease (9). In a study by Ottanelli et al., it was found that patients with airflow obstruction perceive dyspnea less than those without airway obstruction (10). Other studies have also found that the perception of shortness of breath vary significantly among asthma patients, with some experiencing a reduced perception despite having severe airway obstruction (11-13). Some other studies have also found that when using bronchoconstricting agents like methacholine or histamine to induce asthma symptoms, the severity of symptoms varied widely and showed a poor correlation to the degree of airway obstruction, as measured by the fall in FEV_1 (14, 15). Reduced perception of airway obstruction is related to increased risk of exacerbation, and hospitalisation (16). Studies have found that both poor, and high perceivers may under or over use their medication, which is a risk factor for increased asthma mortality (17) (18).

Guidelines advocate using spirometry to manage and monitor patients. Spirometry is used to demonstrate airflow limitation or obstruction, but it has limitations. In general, spirometry has weak relationships with symptoms (19), there being a significant disconnect in many individuals. Thus, it is possible that a patient with controlled symptoms, might in fact have impaired lung function by spirometry and frequent bronchoconstriction measured objectively, and vice versa. Additionally, spirometry is insensitive to small airway dysfunction, which is better measured by respiratory oscillometry for instance. Small airways (<2 mm) are an important site of airflow obstruction in asthma (20), and represents the first site of dysfunction in smoking-related airway diseases (21). Smokers with small airway dysfunction often have chronic respiratory symptoms, a reduced quality of life, and higher rates of exacerbations (22-24). Similarly, in asthma, small airway dysfunction is associated with persistent symptoms (25), poor disease control, and increased exacerbations (26). Early identification of small airway dysfunction, both in smokers with preserved spirometry and in asthma patients may provide some advantages in assessing long-term outcomes. Assessing small airways is therefore crucial, it provides insights into how the disease is manifesting and progressing, as well as its response to treatment. Assessment of small airways is however challenging. In smokers with normal spirometry, small airway dysfunction can precede parenchymal damage (27), and may present with little to no abnormality in spirometry. Similarly, in conditions such as recent-onset asthma or brittle asthma, small airway dysfunction may also be present despite normal spirometry (25, 28).

Assessing small airway function often requires specialised equipment, which is not typically available in primary care settings, where the vast majority of patients are diagnosed and managed. While spirometry may have limitations, it remains more accessible than other methods, and could offer valuable insights into small airway function with improved interpretation. Methods for

assessing small airways in spirometry include mid-expiratory flows ($FEF_{25–75\%}$), and the concave shape of the maximum expiratory curve (29). Despite their high inter-individual variability (30, 31), they remain well known indices of small airway dysfunction and remain in clinical use. There are however, newer tests of small airway function such as oscillatory impedance techniques, like the impulse oscillation technique (IOS) and forced oscillation technique (FOT), as well as multiple breath nitrogen washout (MBNW) (32, 33). Both oscillatory techniques and MBNW are considered sensitive methods for assessing small airways, however they are not routinely performed in a normal clinical settings, and they have mainly been used for research. Information derived from mid expiratory flows and a novel index which characterises the concavity of the expiratory flow volume curve, can be calculated from spirometry. It would therefore be useful to know if these might correlate to oscillatory parameters, and/or MBNW. It's not known whether small airway dysfunction measured by these methods are discordant or whether they capture the same physiological dysfunction of small airways. It might be beneficial to know if the spirometric measures of small airways might offer the same physiological information as the newer tests, and would thus be more freely available for general practice, where newer tests of small airway measurements are not available. Addressing these questions would be helpful in allowing more widespread evaluation of small airway function in clinical practice.

Managing asthma is complex, but still focuses predominantly on symptoms, and guidelines epitomise a "one size fits all" approach, regardless of the individual variations affecting outcomes. Over the years, there has been an increasing move toward personalised medicine that uses certain physiological characteristics or "phenotypes" (34)(35). This approach helps in optimising the treatment outcomes by targeting the individual's clinically relevant characteristics. Phenotyping

ensures that treatment matches the individuals' conditions and needs, ultimately leading to better outcomes.

The bronchodilator response (BDR) is an important characteristic that differentiates individuals with asthma with airway reversibility from those with irreversible airway obstruction. The degree of bronchodilator responsiveness is arguable a clinically important characteristic due to its strong association with asthma outcomes, and its ability to identify patients at risk for exacerbations, and loss of disease control. A bronchodilator response is typically seen in patients with asthma, and it is loosely associated with airway hyperreactivity and reversible airflow obstruction, and reflects increased bronchomotor tone (36, 37). This instability makes patients prone to worsening symptoms and is likely to increase the risk of exacerbations. Studies have found that patients with bronchodilator responsiveness often show reduced lung function at baseline, and are associated with atopic asthma, elevated type 2 inflammation (eosinophilic inflammation) (38, 39) (40), and greater responsiveness to inhaled corticosteroids, as indicated by an improved FEV_1 response (41). In contrast, poor or absent BDR may have neutrophilic inflammation, which is associated with an increased risk of exacerbations (42), and poorer overall lung function (43)(44, 45). Standard treatments may not be effective in these individuals, and they may require higher doses of inhaled corticosteroids (ICS) or even biologic therapy. Response to bronchodilator (BDR) can therefore be a useful characteristic for characterising asthma by identifying patients at higher risk for severe disease or exacerbations, and perhaps may guide personalised treatment.

Despite many studies investigating bronchodilator response in asthma, the underlying mechanisms of this response remain poorly understood. Its known that airway smooth muscle (ASM) is the primary cellular mechanism responsible for regulating airway caliber, and bronchomotor tone, which is why ASM is considered the primary cell responsible for bronchial constriction (36).

However, the extent to what happens to regional ventilation following bronchodilator administration remains unclear. In asthma, airway narrowing occurs non-uniformly, depending on the severity and location of obstruction (large versus small airways), leading to heterogeneous ventilation (46). By extrapolation, the responses to bronchodilators can also be heterogeneous.

Bronchodilator response is routinely measured with spirometry, and defined as improvement in FEV_1 after the inhalation of a short-acting β-adrenergic agonist. Response "thresholds" are however arbitrary and controversial (47). The magnitude of the change in FEV_1 required to define a "positive" or "clinically significant" bronchodilator response differs between studies, and among individual patients. Additionally, FEV_1 BDR has limited sensitivity. It is a common finding to see patients experiencing relief of symptoms after bronchodilator administration even with no significant change in FEV_1. Most importantly, FEV_1 may not be sensitive enough to capture the heterogeneous responses in regional ventilation in different parts of the airway tree. Some regions may improve significantly, while others may show little or no response. FEV_1 being a global measure of function and mainly of larger airways, is unlikely to be able to capture these changes, especially if the distal airways are more affected by asthma than the larger airways. The distal, small airways, which contribute significantly to airflow limitation in asthma do not correlate directly with changes in FEV_1 (48, 49). Bronchodilators can improve obstruction in small airways, improving ventilation distribution even without any measurable change in FEV_1 (50) (49, 51).

Measuring the response of airway obstruction by spirometry remains important in the diagnosis and management of asthma. However, alternative measures of lung function, such as forced oscillation technique (FOT), and imaging modalities such as single photon emission tomography may provide complementary, and clinically relevant information about bronchodilator response in asthma. Ventilation SPECT (VSPECT) might offer unique insights into the bronchodilator

response by visualising changes in regional ventilation before and after bronchodilator. VSPECT is a 3D-ventilation imaging modality that provides spatial, and functional information (52). VSPECT uses Technegas, a radioactive tracer that visualises ventilation and hence areas where there is reduced airflow (53). It might be expected that bronchodilation may or should improve ventilation in regions where it is reduced. Hence VSPECT can be utilised to better understand the physiologic basis of bronchodilator response, in terms of examining the topographical distribution and changes in ventilation in response to bronchodilator inhalation.

The Forced Oscillation Technique (FOT) is a non-invasive method used to assess respiratory mechanics, specifically airway resistance and reactance. FOT is used to assess the changes in the mechanical properties of the small airways by measuring the response of the respiratory system to an oscillating pressure signal (54). FOT has greater sensitivity for detecting BDR than spirometry (55), including responses in the small airways that might not be captured using traditional FEV_1 measurements, making it useful for understanding heterogeneous responses to bronchodilator. The relationship between the bronchodilator response as assessed by SPECT and FOT remains underexplored. The heterogeneity in ventilation could persist even after bronchodilator administration, as bronchodilators might improve airflow in larger airways more effectively than in smaller, more obstructed regions, leaving some areas under-ventilated. Investigating how the bronchodilator response captured by FOT correlates with ventilation changes in SPECT could improve the interpretation of FOT BDR.

Home monitoring peak expiratory flow (PEF) is another important physiologic measurement in asthma (56). After all, acute bronchoconstriction and resolution are the hallmarks of asthma. However, it is underutilised and ability to interpret daily monitoring is poor, but is nevertheless, an important tool that provides objectives measurements to optimise the management of asthma

(57). Increased peak flow variability is associated with increased exacerbations, and asthma deaths (57, 58). Few studies have reported correlations between positive bronchodilator response, and increased peak flow variability (59, 60). Monitoring daily peak flow measurements helps individuals with asthma to recognise the changes or the fluctuations in their airway, allowing timely interventions to minimise exacerbations (57). There are numerous studies on within day variability in peak flow (61-63), but the current use of peak flow in asthma is predicated on the day-to-day variability (not diurnal variability), yet there is no data on what constitute 'normal' day to day PEF variability in healthy individuals.

Asthma management is complex because the clinical manifestations of disease is so heterogenous due to multiple factors. Better understanding the mechanisms underlying bronchodilator responses may help in guiding treatments decisions in the future and possibly also as outcomes in studies of new treatments. Knowing what 'normal' day-to-day peak flow variability will provide an evidence base for how to interpret peak flow monitoring in clinical practice, and if a novel shape index of concavity of the expiratory flow-volume curve correlates with either oscillatory impedance techniques or MBNW, this would support its use as a simple way to measure small airways in clinical practice.

The aims of my thesis, and objectives were as follows:

Aims:

My thesis examines the role of physiological measures in advancing personalised medicine by improving the interpretation of peak flow monitoring in clinical practice, evaluating the utility of a novel shape index in comparison to advanced small airway measures of oscillatory impedance and MBNW, and investigating bronchodilator response in ventilation distribution in 3-dimensional imaging.

Objectives:

1. Measure day-to day variability in PEF using home monitoring peak flow meter
2. Assess the impact of using different methods of quantifying peak expiratory flow variability on day-to-day variability
3. Examine the relationships between the concavity shape of the maximum expiratory curve derived from mid expiratory flows, and the exciting measures of small airway function, the IOS, and MBNW
4. Develop a new method to quantify ventilation SPECT
5. Investigate the accuracy of the currently available method for measuring Technegas activity in quantifying ventilation SPECT
6. Measure bronchodilator response using ventilation SPECT
7. Understand the individual variations in bronchodilator response
8. Determine whether changes in ventilation measured by SEPCT during bronchodilation are related to changes in spirometry
9. Compare changes in ventilation, measured by SPECT, with changes in small airway measurement; respiratory system resistance (Rrs) and reactance (Xrs) measured by FOT
10. Examine the determinants of the bronchodilator response measured by VSPECT

References

1. Vos T, Lim SS, Abbafati C, Abbas KM, Abbasi M, Abbasifard M, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet. 2020;396(10258):1204-22.
2. Australian Institute of Health and Welfare. Asthma. Canberra: AIHW; 2023.
3. Mortimer K, Lesosky M, García-Marcos L, Asher MI, Pearce N, Ellwood E, et al. The burden of asthma, hay fever and eczema in adults in 17 countries: GAN Phase I study. Eur Respir J. 2022;60(3).
4. Statistics . The Australian Bureau of Statistics. Asthma mortality statistics. 2023.
5. National Asthma Council Australia NAC. Asthma Mortality Statistics 2022. 2022.
6. National Asthma Council Australia NAC. Asthma Mortality Statistics 2023. 2023.
7. Mauer Y, Taliercio RM. Managing adult asthma: The 2019 GINA guidelines. Cleve Clin J Med. 2020;87(9):569-75.
8. Levy ML, Bacharier LB, Bateman E, Boulet L-P, Brightling C, Buhl R, et al. Key recommendations for primary care from the 2022 Global Initiative for Asthma (GINA) update. npj Primary Care Respiratory Medicine. 2023;33(1):7.
9. Barnes PJ. Poorly perceived asthma. Thorax. 1992;47(6):408-9.
10. Ottanelli R, Rosi E, Romagnoli I, Ronchi MC, Lanini B, Grazzini M, et al. Perception of bronchoconstriction and bronchial hyper-responsiveness in asthma. Clin Sci (Lond). 2000;98(6):681-7.
11. Bijl-Hofland ID, Cloosterman SG, Folgering HT, Akkermans RP, van Schayck CP. Relation of the perception of airway obstruction to the severity of asthma. Thorax.1999;54(1):15-9.
12. Nannini LJ, Zaietta GA, Guerrera AJ, Varela JA, Fernández OM, Flores DM. Breath-holding test in subjects with near-fatal asthma. A new index for dyspnea perception. Respir Med. 2007;101(2):246-53.
13. Teeter JG, Bleecker ER. Relationship Between Airway Obstruction and Respiratory Symptoms in Adult Asthmatics. Chest. 1998;113(2):272-7.
14. Tetzlaff K, Leplow B, ten Thoren C, Dahme B. Perception of dyspnea during histamine- and methacholine-induced bronchoconstriction. Respiration. 1999;66(5):427-33.

15. Weiner P, Beckerman M, Berar-Yanay N, Magadle R. The perception of dyspnea after bronchoconstriction and bronchodilation in patients with asthma. Respir Med. 2003;97(10):1120-5.
16. O'Loghlen SB, Levesque L, Fisher T, DeWit Y, Whitehead M, To T, et al. Health Services Utilization Is Increased in Poor Perceivers of Bronchoconstriction and Hyperinflation in Asthma. J Allergy Clin Immunol Pract. 2020;8(8):2643-50.e2.
17. Rea HH, Sears MR, Beaglehole R, Fenwick J, Jackson RT, Gillies AJ, et al. Lessons from the national asthma mortality study: circumstances surrounding death. N Z Med J. 1987;100(816):10-3.
18. Spitzer WO, Suissa S, Ernst P, Horwitz RI, Habbick B, Cockcroft D, et al. The use of beta-agonists and the risk of death and near death from asthma. N Engl J Med. 1992;326(8):501-6.
19. Cowie RL, Underwood MF, Field SK. Asthma Symptoms Do not Predict Spirometry. Canadian Respiratory Journal. 2007;14:816132.
20. Yanai M, Sekizawa K, Ohrui T, Sasaki H, Takishima T. Site of airway obstruction in pulmonary disease: direct measurement of intrabronchial pressure. J Appl Physiol (1985). 1992;72(3):1016-23.
21. Gelb AF, Gobel PH, Fairshter R, Zamel N. Predominant site of airway resistance in chronic obstructive pulmonary disease. Chest. 1981;79(3):273-6.
22. Jetmalani K, Thamrin C, Farah CS, Bertolin A, Chapman DG, Berend N, et al. Peripheral airway dysfunction and relationship with symptoms in smokers with preserved spirometry. Respirology. 2018;23(5):512-8.
23. Regan EA, Lynch DA, Curran-Everett D, Curtis JL, Austin JH, Grenier PA, et al. Clinical and Radiologic Disease in Smokers With Normal Spirometry. JAMA Intern Med. 2015;175(9):1539-49.
24. Woodruff PG, Barr RG, Bleecker E, Christenson SA, Couper D, Curtis JL, et al. Clinical Significance of Symptoms in Smokers with Preserved Pulmonary Function. N Engl J Med. 2016;374(19):1811-21.
25. Usmani OS, Singh D, Spinola M, Bizzi A, Barnes PJ. The prevalence of small airways disease in adult asthma: A systematic literature review. Respir Med. 2016;116:19-27.
26. Cottini M, Lombardi C, Micheletto C. Small airway dysfunction and bronchial asthma control : the state of the art. Asthma Res Pract. 2015;1:13.

27. McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, et al. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. N Engl J Med. 2011;365(17):1567-75.
28. Perez T, Chanez P, Dusser D, Devillier P. Small airway impairment in moderate to severe asthmatics without significant proximal airway obstruction. Respiratory Medicine. 2013;107(11):1667-74.
29. Kapp MC, Schachter EN, Beck GJ, Maunder LR, Witek TJ, Jr. The shape of the maximum expiratory flow volume curve. Chest. 1988;94(4):799-806.
30. Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW. Measurement of FEF25-75% and FEF75% does not contribute to clinical decision making. Eur Respir J. 2014;43(4):1051-8.
31. Gelb AF, Williams AJ, Zamel N. Spirometry. FEV1 vs FEF25-75 percent. Chest. 1983;84(4):473-4.
32. Kjeldgaard JM, Hyde RW, Speers DM, Reichert WW. Frequency dependence of total respiratory resistance in early airway disease. Am Rev Respir Dis. 1976;114(3):501-8.
33. Verbanck S, Schuermans D, Meysman M, Paiva M, Vincken W. Noninvasive assessment of airway alterations in smokers: the small airways revisited. Am J Respir Crit Care Med. 2004;170(4):414-9.
34. Chung KF. Defining phenotypes in asthma: a step towards personalized medicine. Drugs. 2014;74(7):719-28.
35. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181(4):315-23.
36. Black JL, Panettieri RA, Jr., Banerjee A, Berger P. Airway smooth muscle in asthma: just a target for bronchodilation? Clin Chest Med. 2012;33(3):543-58.
37. Gibbons WJ, Sharma A, Lougheed D, Macklem PT. Detection of excessive bronchoconstriction in asthma. Am J Respir Crit Care Med. 1996;153(2):582-9.
38. Busse WW, Holgate ST, Wenzel SW, Klekotka P, Chon Y, Feng J, et al. Biomarker Profiles in Asthma With High vs Low Airway Reversibility and Poor Disease Control. Chest. 2015;148(6):1489-96.

39. Wei J, Ma L, Wang J, Xu Q, Chen M, Jiang M, et al. Airway reversibility in asthma and phenotypes of Th2-biomarkers, lung function and disease control. Allergy Asthma Clin Immunol. 2018;14:89.

40. Denlinger LC, Phillips BR, Ramratnam S, Ross K, Bhakta NR, Cardet JC, et al. Inflammatory and Comorbid Features of Patients with Severe Asthma and Frequent Exacerbations. Am J Respir Crit Care Med. 2017;195(3):302-13.

41. Szefler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. J Allergy Clin Immunol. 2002;109(3):410-8.

42. Liu L, Zhang X, Zhang L, Liu Y, Zhang HP, Zhao SZ, et al. Reduced bronchodilator reversibility correlates with non-type 2 high asthma and future exacerbations: A prospective cohort study. Respir Med. 2022;200:106924.

43. Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. Am J Respir Crit Care Med. 1997;156(3 Pt 1):737-43.

44. Shaw DE, Berry MA, Hargadon B, McKenna S, Shelley MJ, Green RH, et al. Association between neutrophilic airway inflammation and airflow limitation in adults with asthma. Chest. 2007;132(6):1871-5.

45. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma : evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest. 2001;119(5):1329-36.

46. Downie SR, Salome CM, Verbanck S, Thompson B, Berend N, King GG. Ventilation heterogeneity is a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation. Thorax. 2007;62(8):684-9.

47. Silvestri IC, Pereira CA, Rodrigues SC. Comparison of spirometric changes in the response to bronchodilators of patients with asthma or chronic obstructive pulmonary disease. J Bras Pneumol. 2008;34(9):675-82.

48. Abdo M, Pedersen F, Kirsten AM, Veith V, Biller H, Trinkmann F, et al. Longitudinal Impact of Sputum Inflammatory Phenotypes on Small Airway Dysfunction and Disease Outcomes in Asthma. J Allergy Clin Immunol Pract. 2022;10(6):1545-53.e2.

49. Pisi R, Tzani P, Aiello M, Martinelli E, Marangio E, Nicolini G, et al. Small airway dysfunction by impulse oscillometry in asthmatic patients with normal forced expiratory volume in the 1st second values. Allergy Asthma Proc. 2013;34(1):e14-20.

50. Svenningsen S, Eddy RL, Lim HF, Cox PG, Nair P, Parraga G. Sputum Eosinophilia and Magnetic Resonance Imaging Ventilation Heterogeneity in Severe Asthma. American Journal of Respiratory and Critical Care Medicine. 2018;197(7):876-84.

51. Verbanck S, Schuermans D, Paiva M, Vincken W. Nonreversible conductive airway ventilation heterogeneity in mild asthma. Journal of Applied Physiology. 2003;94(4):1380-6.

52. Farrow C, King G. SPECT Ventilation Imaging in Asthma. Seminars in Nuclear Medicine. 2019;49(1):11-5.

53. King GG, Eberl S, Salome CM, Meikle SR, Woolcock AJ. Airway closure measured by a technegas bolus and SPECT. Am J Respir Crit Care Med. 1997;155(2):682-8.

54. Oostveen E, Macleod D, Lorino H, Farre R, Hantos Z, Desager K, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. European Respiratory Journal. 2003;22(6):1026-41.

55. Yaegashi M, Yalamanchili VA, Kaza V, Weedon J, Heurich AE, Akerman MJ. The utility of the forced oscillation technique in assessing bronchodilator responsiveness in patients with asthma. Respir Med. 2007;101(5):995-1000.

56. Global Initiative for Asthma. Global strategy for asthma management and prevention Global Initiative for Asthma. Global strategy for asthma management and prevention, 2017. http://www.ginasthma.org.

57. Cross D, Nelson HS. The role of the peak flow meter in the diagnosis and management of asthma. J Allergy Clin Immunol. 1991;87(1 Pt 1):120-8.

58. Hetzel MR, Clark TJ, Branthwaite MA. Asthma: analysis of sudden deaths and ventilatory arrests in hospital. Br Med J. 1977;1(6064):808-11.

59. Ryan G, Latimer KM, Dolovich J, Hargreave FE. Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator, and airway calibre. Thorax. 1982;37(6):423-9.

60. Stein RT, Holberg CJ, Morgan WJ, Wright AL, Lombardi E, Taussig L, et al. Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. Thorax. 1997;52(11):946-52.

61. Boezen HM, Schouten JP, Postma DS, Rijcken B. Distribution of peak expiratory flow variability by age, gender and smoking habits in a random population sample aged 20-70 yrs. Eur Respir J. 1994;7(10):1814-20.
62. Aggarwal AN, Gupta D, Chaganti S, Jindal SK. Diurnal variation in peak expiratory flow in healthy young adults. Indian J Chest Dis Allied Sci. 2000;42(1):15-9.
63. Gannon PF, Newton DT, Pantin CF, Burge PS. Effect of the number of peak expiratory flow readings per day on the estimation of diurnal variation. Thorax. 1998;53(9):790-2.

Chapter 1. Literature Review

1.1 Asthma Definition

Asthma is a heterogenous respiratory disease characterised by chronic inflammation, and airflow limitation (1). Symptoms of asthma are often variable, but involve wheezing, chest tightness, and shortness of breath (2). Both symptoms, and airflow limitation are often reversed, either spontaneously or following bronchodilator treatment (3).

1.2 Prevalence And Burden Of Asthma

Asthma is one of the most common chronic diseases worldwide, often considered a lifelong condition that carries a significant burden (4), leading to poor quality of life (5), disability (6, 7), and high healthcare utilisation for those affected (8). In Australia, the prevalence of asthma is one of the highest (9, 10), affecting 10.8% of the population (11). In 2023, there were 467 asthma deaths recorded, the highest number since 2016 (12). Asthma is more commonly reported among women and older adults, though it affects individuals of all ages. The prevalence of asthma is higher in boys (aged 0–14 years), whereas in individuals over the age of 15, it is more prevalent in females than males (13). Asthma continues to impose a significant burden, in 2023, asthma represented 2.5% of total disease burden, and a total of $851.7 million of the health expenditure in the Australian health care system (9). Asthma rates are particularly high for people living with disabilities, those in low socioeconomically areas, and Aboriginal and Torres Strait Islander (13)

1.3 Asthma Diagnosis

There is no gold standard test for asthma diagnosis, and to date, diagnosis is based on a combination of symptoms, and patient's medical history (14). Clinical presentation of asthma often vary, and symptoms occur intermittently, in terms of frequency and intensity. Because of that, published guidelines recommend that asthma should not be diagnosed based on symptoms alone

(15). Patients must also show evidence of variable expiratory airflow limitation, or airway hyper-reactivity using spirometry or peak expiratory flow (16, 17).

1.4 Treatment

Asthma treatment falls into two main classes, short acting "reliever", and long acting "controller" medications. Reliever medications refer to the short acting β2-agonist (18), and the anticholinergics agents (19, 20). This class of medications improve symptoms, and provide immediate relief during exacerbations by relaxing the constriction in the bronchial smooth muscle. The controller medications refer to the long acting bronchodilators, the anti-inflammatory agents, and biologic therapies. This includes inhaled corticosteroids (ICSs) (12, 21, 22), long-acting β2-agonists (LABAs) (23, 24) and long-acting muscarinic antagonists (LAMAs) (25), leukotriene receptor antagonists (LTRAs) (26), mast cell stabilizers (27), and monoclonal antibody immune-modulating agents (28, 29). Controllers, especially inhaled corticosteroids (ICSs), decrease the risk of exacerbations, improve asthma control (30), reduce the risk of asthma-related death (31), and improve long-term lung function (32).

1.5 Managements

Inhaled corticosteroids (ICSs) are the first line treatments for patients with asthma, and it is the most effective treatment currently available. Asthma management protocols follow a stepwise approach to control symptoms, and to reduce future risk of poor outcomes (33). The approach includes escalation in the treatment as asthma becomes less well-controlled, and more severe. This approach may include the addition of high-dose ICSs, LABAs and LAMAs, leukotriene receptor antagonists, and/or biologics, such as anti-IgE, anti-IL-5, or anti-IL-4/IL-13 therapies (33).

This approach has two key drawbacks: first, the management solely focuses on symptom control.

Asthma is characterised as being episodic in nature, symptoms are highly variable, even within the same patient, at different times. The variability in symptoms can result in either under, or overestimation of the severity of the disease, often leading to inadequate treatment. Symptomatic patients might choose to increase their medication, while asymptomatic patients might regard their condition as managed, and consider their medications as unnecessary. Furthermore, in asthma, inaccurate perception of symptoms is often common, and not in concordance with the actual bronchoconstriction (34). Individuals with asthma may have different interpretations of what they consider symptoms. Individuals who overperceive their symptoms tend to overuse medications, potentially increasing the risk of side effects, and diminishing the effectiveness of treatment (35). In contrast, poor perceivers delay intervention, which make them more susceptible to severe exacerbations. Abnormal perception in both is associated with an increased risk of severe exacerbation, emergency room visits, and hospitalisations (36).

The second drawback; it is now evident that asthma is highly heterogeneous, and the "one size fits all" approach does not fit with all asthmatic patients. Patients with asthma differ significantly in a number of features, including the complexity of symptoms, clinical presentation, and comorbidities (37-39) (40). Asthma also varies in terms of severity (41), degree and pattern of the underlying airflow obstruction, small airway involvement (40), and response to inhaled therapies, including bronchodilators, and inhaled corticosteroids (ICSs) (42, 43).

Asthma management is complex, guidelines that are currently used certainly facilitate improve symptoms in the long term overall, but do not adequately account for the heterogeneity that characterises asthma. In more recent years, there have been increasing efforts to provide personalised care, tailored to the individual characteristics (44). This involves phenotyping patients based on specific characteristics or features (45). Personalised care based on phenotyping in

asthma holds the potential to revolutionise the management of the disease, allowing health care providers to customise care, and deliver treatment based on the set of characteristics of each individual patient (46).

1.6 Phenotypes

The concept of asthma phenotypes is still evolving (47), however the initial approach incorporates three categories. First, phenotypes that are defined by a clinical and/or physiological features, including but not limited to, the age of asthma onset (48), symptoms and exacerbations (49, 50), the presence, and severity of airflow obstruction (51), degree of reversibility and response to inhaled therapy (52, 53). Second, phenotypes that are related to the environment, such as exercise induced asthma (54), and occupational asthma (55). Finally, phenotypes that are defined by the presence or absence of inflammation (56), such as eosinophilic or neutrophilic asthma (56-58). There are many other phenotypes that have been described in the literature (59), however further research is required to understand the specific mechanisms underlying asthma phenotypes before they can be fully addressed.

1.7 Measurements Of Lung Function

Accurate asthma diagnosis, and monitoring are fundamental steps in the disease control. Once asthma is suspected, lung function measurement is essential for assessing airflow limitation and identifying reversible airway obstruction (14). This can be done through the use of spirometry, and/or home monitoring peak expiratory flow (60). These tools remain important in ongoing disease monitoring, to prevent exacerbations, and further decline in lung function.

1.8 Spirometry

Spirometry is a fundamental pulmonary function test used to measure airflow and lung volumes. It is primarily used for diagnosing and monitoring respiratory conditions, such as asthma, and for identifying airflow obstruction and classifying the severity of the disease. Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and their ratio (FEV_1/FVC) are the most commonly reported spirometric parameters (61, 62). The normal range for FEV_1/FVC is typically defined using the lower limit of normal (LLN) and upper limit of normal (ULN), which correspond to the 5^{th} and 95^{th} percentiles (or −1.645 and +1.645 z-scores), respectively(63). The degree or severity of airway obstruction is assessed using FEV_1, which is expressed as a percentage of the predicted FEV_1 (% pred). Spirometric values are compared to reference (predicted) values derived from studies of 'normal' or 'healthy' subjects in the general population, typically using a representative sample with the same anthropometric characteristics (63). Both the ERS and ATS recommend using reference equations that are specific to the population being tested (64-67).

FEV_1 has long been used as a maker of airway obstruction (68), reduced FEV_1 indicates poorly controlled disease, and has been found to predict mortality (69)(70). Impaired values are associated with increased risk for asthma attacks, and exacerbations (71) (72). Reduced FEV_1 values are also associated with poor quality of life, higher risks for acute care visits, hospitalisations (73), and increase use of inhaled therapies (74, 75). Improvements in FEV_1 are also paralleled by better quality of life for asthma patients, improvements in FEV_1 reflect reduced reliance on medications, and decreased healthcare visits (76, 77).

Despite the importance of spirometry testing, results from studies show that spirometry testing is uncommon in primary care practices (78, 79). One significant barrier is the lack of knowledge in conducting, and interpreting the results (80). Normal spirometry does not rule out asthma, as

airflow obstruction can be intermittent, occurring only during specific times of the day or in reaction to allergens. Many primary care providers also lack awareness of the utility of spirometry (81-83), and may not fully understand its clinical significance in the diagnosis and monitoring of asthma. Studies have shown that neglecting spirometry often results in diagnostic errors, and mismanagement (84), both of which contribute to poor clinical outcomes (85, 86).

1.9 Bronchodilator Response

In patients with suspected asthma, bronchodilator response refers to an improvements in lung function following the administration of short acting β2-agonist. This improvement is an indicator of variable expiratory airflow limitation, using spirometry. According to the American Thoracic Society/European Respiratory Society (ATS/ERS), previous and very long standing criteria for a positive bronchodilator response (BDR) was defined as an increase in FEV_1 (or FVC) of ≥12% and ≥200 ml from the baseline (87). A standard dose of short acting β2-agonist, 200–400 mcg of salbutamol or equivalent is given, and the measurements are repeated 10 to 15 minutes after (88). This criterion however is in itself arbitrary. Cut-off values ranging from 10% to 25% have also been suggested by other studies (89-91). More recently, in 2021, ERS/ATS task force recommended a new threshold for defining bronchodilator responsiveness; an increase in FEV_1 and/or FVC > 10% of their predicted value is now considered a positive BDR. This new criterion was intended to reduce the influence of sex, height, and baseline lung function (87). In addition to that, the term "reversibility", which has been traditionally used to describe the change in FEV_1 post bronchodilator, has now been replaced by "responsiveness" to avoid the implication that there is complete reversal of airways obstruction (87).

Airway obstruction in asthma is traditionally considered reversible, however, there are individuals with asthma who exhibit features of fixed or irreversible airflow obstruction (FAO). FAO is

traditionally associated with chronic obstructive pulmonary disease (COPD), but there are cases of asthma presenting with persistent airflow obstruction, even among individuals who have never smoked (92, 93). FAO is typically characterised by a post-bronchodilator FEV_1/FVC ratio < 75% (51, 94), although alternative cutoff values have also been suggested (95, 96) and currently, the Global Lung Initiative (GLI) definition of FEV_1/FVC < lower limit of normal is the most widely used (97). The prevalence of FAO is high, especially in those with severe asthma, reported rates of FAO range from 16% in mild asthma (92), 23% in non-smoking asthmatics (93), to as high as 49% in severe asthma (94).

1.9.1. Significance Of Bronchodilator Response

Bronchodilator response is an important physiological phenotype, yet the precise mechanisms underlying this response are not entirely clear. It is known that bronchodilators, such as β2 agonists like Salbutamol, alleviate bronchoconstriction by binding with β2 receptors. β2 receptors are located on bronchial smooth muscle, and are part of the sympathomimetic nervous system. The bronchodilation effect is caused by the stimulation of β2 receptors, which sets off a chain reaction that leads to increased synthesis of cyclic adenosine monophosphate (cAMP), which causes smooth muscle relaxation (98). However, the mechanisms contributing to the airway response, or the specific reasons as to why some asthmatic patients show a significant bronchodilator response while others do not, remain poorly understood.

Spirometry is the standard for determining the response to bronchodilator, however the threshold for a positive bronchodilator responsiveness varies (89) (99). Furthermore, Spirometry mainly reflects the function of larger airways and may remain unchanged even after significant improvements in small airway function. The small airways are the narrow, distal (farther down) in

the respiratory tree, and although improvements in their function may be less evident in routine testing, they significantly impact overall lung function (100, 101).

In clinical settings, asthma's variability in symptoms and airway obstruction over time makes the clinical interpretation of BDR complex and patient-specific. While a positive BDR indicates reversibility of airway narrowing, some patients may not exhibit a significant BDR despite having other features of asthma. This is particularly true in individuals with well-controlled asthma or those with chronic airway remodeling. In well-controlled asthma, airway inflammation and bronchoconstriction are controlled through treatment, such as inhaled corticosteroids. As a result, the airways are less likely to show significant BDR during testing. In contrast, poorly controlled asthma, characterised by persistent inflammation and frequent symptoms, is more likely to show a pronounced BDR. A positive bronchodilator response in asthma patients has been associated with symptoms of wheeze (102), atopy (103), lower baseline FEV_1 values, and bronchial hyperreactivity (103), with E. Heffler et al. reporting that a bronchodilator response (BDR) despite treatment correlates with poor disease control, even in patients with normal spirometry (104). Reduced or absent bronchodilator response however, is more complex. The lack of a significant response may indicate effective disease management rather than a sign of disease severity, or it can signal more chronic forms of asthma, such as fixed airway obstruction (FAO) or the presence of airway remodeling (105). In FAO, patients may or may not demonstrate BDR, or may vary over time but the fact that by definition, they do not normalize their spirometry post bronchodilator (105), indicates the need for a greater understanding of this causes and alternative therapeutic approaches.

Understanding an individual's bronchodilator response enables healthcare providers to personalise asthma care. However, BDR requires careful clinical interpretation and like any physiological test,

should be integrated into the overall clinical picture, considering the patient's treatment history, disease duration, and asthma control, rather than being used in isolation.

1.10 Home Monitoring Peak Expiratory Flow

Peak expiratory flow (PEF) monitoring is recommended by asthma guidelines if the patient's history or examination is suggestive of asthma. Though it's not as reliable as spirometry, and should not be used as a substitute (106), PEF monitoring can provide valuable information. PEF monitoring can be used to document variable expiratory flow limitation, assess asthma severity, monitor response to treatment, and identify or detect early exacerbations (16, 107, 108).

PEF measures the maximal flow rate that can be expired during forceful expiration. It's simple, and commonly performed at home using a small, hand-held peak flow meter (109). Monitoring variability is perhaps the most important aspect of PEF measurements in asthma. PEF variability has been used as a marker of airway responsiveness (110, 111), it has been shown that the magnitude of PEF variability correlates well with asthma severity (112). It is also recommended for use in patients with poor perception of their symptoms, as it can be a good indicator of asthma control and response to therapy (113). By contrast, increased PEF variability can be a sign of reduced lung function (114), and is associated with increased morbidity and mortality due to asthma (115, 116), Aggarwal et al. found that in chronic stable asthma, patients with higher PEF variability required more emergency room visits, and hospitalisation (117).

1.10.1. Peak Expiratory Flow Variability

PEF variability is naturally observed in healthy individuals, but it becomes exaggerated in individuals with asthma (118) (119). Variability in PEF can be measured within a day, and over several days. PEF follows a circadian rhythm; minimum values are observed late at night, or early

in the morning, and maximum values peak in the late afternoon (111, 118). PEF values are influenced by age, height, gender, and ethnicity. Therefore, interpretation of PEF values should be compared with "normals", with similar anthropometric characteristics (120, 121) (122).

Most asthma guidelines recommend that variability should be calculated as the difference between the minimum, and the maximum, from at least twice daily PEF measurements. The monitoring period however is left for healthcare providers to decide, but general recommendations being at least two to three weeks of monitoring. There are several studies that have adopted an intensive approach, such as recording PEF measurements at regular intervals, such as every 2 hours throughout the day, or three to more than five measurements in a day, over a short period of time (110) (123, 124) (117), while other studies included fewer measurements, but over a longer period of time (125) (126) (127) (128). To provide an accurate estimation of PEF variability, Gannon et al. recommends that PEF measurements should be taken at least four times per day, spaced evenly throughout the day (129). In clinical practice however, the practicality of recording frequent PEF measurements in modern, affluent societies is challenged due to lifestyles, daily routines, and work commitments (129, 130).

1.10.2. Normal Values Of Peak Expiratory Flow Variability

The normal value of diurnal variability of PEF in healthy individuals varies, and to date, evidence suggests that there is much overlap between PEF variability in healthy individuals and that in asthma. According to current practice guidelines, diurnal, i.e. within day variability of 20% and more is suggestive of asthma (14, 107). Several other studies reported different values for diurnal variability. Higgins et al. have investigated the diurnal variability of PEF in a random sample, primarily consisting of Caucasians, from the general population. PEF measurements were obtained from 342 subjects, at 2-hour intervals over a 7-day period. The group of 121 individuals, which

included current, and ex-smokers reported a mean diurnal variability of 8.7%. While the subgroup of 221 individuals with history of wheeze, and asthma reported a mean variability of 13. 5% (131). Boezen et al. also reported a diurnal variability of 3.66% in a random population of 520 individuals, which included lifelong non-smokers, current, and ex-smokers (132). There are other studies that reported diurnal variability in asymptomatic, healthy nonsmoker adults, with a mean of 6.26%, and 7.23%, respectively (133, 134). The wide range of values observed in PEF primarily is due to variations in both the methods used for recording PEF, and the mathematical calculations applied.

1.10.3. Indices Of Peak Expiratory Flow Variability

There are many mathematical equations that have been described to measure PEF variability. Commonly used methods include, amplitude percent mean "A%M" [(highest PEF-lowest PEF)/mean PEF]x100, amplitude percent highest "A%H" [(highest PEF-lowest PEF)/highest PEF)]x100, and standard deviation percent mean (standard deviation of PEF /mean PEF)x100 (131, 133, 135). Although not as common, there are other methods described by other investigators including amplitude percent minimum [(highest PEF-lowest PEF)/lowest PEF] x100, lowest percent personal best [lowest PEF/patient's best PEF]x100, lowest percent predicted [lowest PEF/patient's predicted PEF]x100, and amplitude of cosinor analysis (125, 134, 136).

1.10.4. Peak Expiratory Flow Variability And Response To Treatment

The ability of PEF variability to accurately predict the response to inhaled therapy is variable, however, guidelines recommend using PEF monitoring with other markers to establish a more comprehensive profile of treatment response. Few studies have shown association between diurnal PEF variability and the response to corticosteroids. In a study involving 14 patients recovering from acute severe asthma, fluctuations in both the rate of PEF and diurnal variability were

investigated as indicators of treatment response. The study found that improvements in PEF and reductions in diurnal variability occurred in parallel with the clinical treatment response, with PEF improving and diurnal variability decreasing within the first 24 hours of therapy. The same results were also observed in another study, following an acute exacerbation of asthma in 26 individuals aged 18 to 69 (127, 128). Additionally, in a study involving 141 patients receiving combination therapy with a β2 agonist and inhaled corticosteroid, diurnal PEF variability was found to significantly decrease from 18.0% to 10.2% over the three months of treatment (137). These findings highlight the importance of incorporating PEF measurement in asthma, where variability in PEF can serve as valuable tool for proactive management, for both, health care providers, and for patients.

1.11 Small Airways

The respiratory tree consists of 23 airway generations (Figure 1). The large, and proximal airways are referred to as the conducting airways, which comprises airway generations 1-15.

Starting from the 16th generation onward, the remaining airways are now within the primary pulmonary lobule or acinus. They are thus commonly referred to as the acinar airways, or airways within the respiratory acinar zone because gas exchange occurs in these airways (Figure 1). They consist of respiratory bronchioles, alveolar ducts, and sacs (138, 139). As the airway generations progress, the cross sectional surface area increases, and the airflow resistance reduces. Small airways have also been define by being less than <2mm in inner diameter, and although they make up large sectional surface area, they contribute very little to the total airway resistance (140). For this reason, small airways are known as the “quiet zone” (141). Dysfunction in these airways is not easily detected until a considerable portion of the airways has already been affected by the disease.

It is now recognised that small airways are a major site of airway obstruction, and inflammation in patients with asthma (142-144). Small airway dysfunction is prevalent in asthma, affecting around 50 to 60% of patients (145). Numerous studies consistently showed that small airway dysfunction is associated with poorer asthma control, and higher exacerbation rates (146, 147). Conventional physiological tests such as spirometry are unable to sensitively evaluate small airways, thus more sensitive methods are required. Since early disease may manifest only by small airway dysfunction, small airway measurements may have clinical utility in early detection of disease and thus help in preventing disease progression, prevent irreversible structural damage, and improve asthma control.

To assess small airway function, several measurement tools have been utilised. Traditionally, indices derived from spirometry during the FVC manoeuvre, such as the forced expiratory flow at 25-75% of FVC ($FEF_{25-75\%}$) and the concave shape of the maximal expiratory flow volume curve (MEFV), have been used. However, more sensitive techniques are now available, including the forced oscillation technique (FOT) and the multiple breath nitrogen washout test (MBNW).

1.12 The Forced Expiratory Flow ($FEF_{25-75\%}$)

The forced expiratory flow (FEF) between 25% and 75% of FVC ($FEF_{25-75\%}$) is a measure of the mean airflow rate during the middle, and the end portion of the FVC manoeuvre. It represents the flow rate between the 25% and 75% points of the forced expiration. Reduced $FEF_{25-75\%}$, less than <65% of predicted is considered abnormal (148, 149), and often thought of as a sensitive marker of small airway obstruction (68, 150, 151). Reduction of $FEF_{25-75\%}$ results in a concave shape in the MEFV curve. ATS/ERS acknowledge that the earliest changes associated with small airway obstruction is reflected in the shape of the MEFV curve (68). The MEFV reflects the relationship between the volume of air expired, and the corresponding flow rates during the FVC manoeuvre.

The curve typically has three distinct phases, an initial steep rise, a plateau, and a decline. The presence of airway disease can change the shape of MEFV by causing a more pronounced concave shape toward the terminal portion of the spirogram (Figure 2) (152). The frequent use of $FEF_{25-75\%}$, and MEFV is based on the understanding that flows towards the end of a forced expiratory manoeuvre are at low lung volumes and are effort independent and flow-limited in small airways. At low lung volumes, lung elastic recoil is relatively reduced, and the size of airways are smaller. Therefore, obstruction primarily thought to reflect dysfunction in the small airways (153-155).

$FEF_{25-75\%}$, and the MEFV curve are standard parameters reported in spirometry. However, guidelines recommend disregarding $FEF_{25-75\%}$ and MEFV in the presence of a normal FEV_1/FVC ratio (156, 157). Additionally, guidelines typically do not provide specific recommendations on how abnormalities in these measures should be interpreted, leading to uncertainty regarding their clinical significance (158-160). $FEF_{25-75\%}$ is related to the FVC manoeuvre, which can be highly variable, influenced by factors such as the patient's effort, and technique (161-163). Additionally, the accuracy of $FEF_{25-75\%}$ values is also influenced by the lung volume; if lung volumes are abnormally low or high, then $FEF_{25-75\%}$ values may be artificially reduced or elevated. Furthermore, $FEF_{25-75\%}$ also lacks reproducibility, and sensitivity for diagnosing obstructive airway disease (164, 165). Poor correlation has been also shown between $FEF_{25-75\%}$, and histologic measures of lung tissue inflammation (166).

Despite the above considerations, there is evidence supporting its use, especially when FEV_1 values are normal. In asthma, reduced $FEF_{25-75\%}$ with normal FEV_1 is associated with bronchial hyperreactivity (167), and correlates with bronchodilator responsiveness (148). Reduced $FEF_{25-75\%}$ has been found related to poor asthma outcomes, in both, children, and in adults with asthma (168). Patients with reduced $FEF_{25-75\%}$ are found to have more severe asthma, with increased

exacerbations, higher healthcare utilisation, and greater steroid use (169, 170). Reduced $FEF_{25-75\%}$ has also been shown to indicate high FeNO levels (171).

In smokers, $FEF_{25-75\%}$ has been found useful in early diagnosis, reduced $FEF_{25-75\%}$ is frequently observed in smokers with normal lung function, even in those without history of respiratory symptoms, or disease (172-174). Studies on $FEF_{25-75\%}$, and its outcomes in smokers are limited, however recent research by Kwon et al. and collogues found that in individuals with reduced $FEF_{25-75\%}$ levels, the rate of developing COPD was higher. Additionally, another study involving 2978 individuals, including smokers with and without COPD across different severities, found that those with lower $FEF_{25-75\%}$ values had more severe disease, dyspnea, and greater emphysema (175).

1.13 The Maximal Expiratory Flow Volume Curve (MEFV)

The MEFV curve can be a useful tool in potentially detecting early airway obstruction. The "scooping" out or the appearance of a concave shape in the curve is indicative of heterogonous lung emptying (176). The concave shape appears during healthy aging, but also often seen in obstructive airway disease, such as in asthma, and COPD patients (177-180). In a study conducted by Schachter et al. involving 477 cotton textile workers, including both current and never smokers, it was observed that abnormalities in spirometry were present, but the concave pattern was only seen in those who were current cigarette smokers. (181). In another study, Kapp et al. examined 5,140 individuals, including smokers, individuals with asthma, chronic bronchitis, and those with symptoms of dyspnea and wheezing. The study found that the degree of concavity was greater in smokers, and in individuals with asthma or symptoms, compared to those without (182).

There are several methods for quantifying the concavity shape of the MEFV curves. 1) the slope ratio (SR) index was introduced by Mead et al. to detect the MEFV shape due to emphysema or

asthma (176). This method quantifies the instantaneous slope measured at desired points along the MEFV curves. 2) The Beta angle (β◦) method by Kapp et al. which determines an angle around the flow at 50% of vital capacity (18). Consequently, the resulting angle serves as an indicator of the overall curvature of the entire MEFV curve (182). 3) global and peripheral indices by Johns et al., based on ($FEF_{50\%}$) and ($FEF_{75\%}$), where the degree of concavity is obtained by calculating the percentage decrease of the measured flows, from the corresponding idealised reference flows (179). There are other methods that have been described in the literature, such as Kmax (183), the flow-ratio (FR) technique (184), and area under the MEFV curve (185). All of the aforementioned methods, however, have proven complex, and have not been widely adopted in clinical practice.

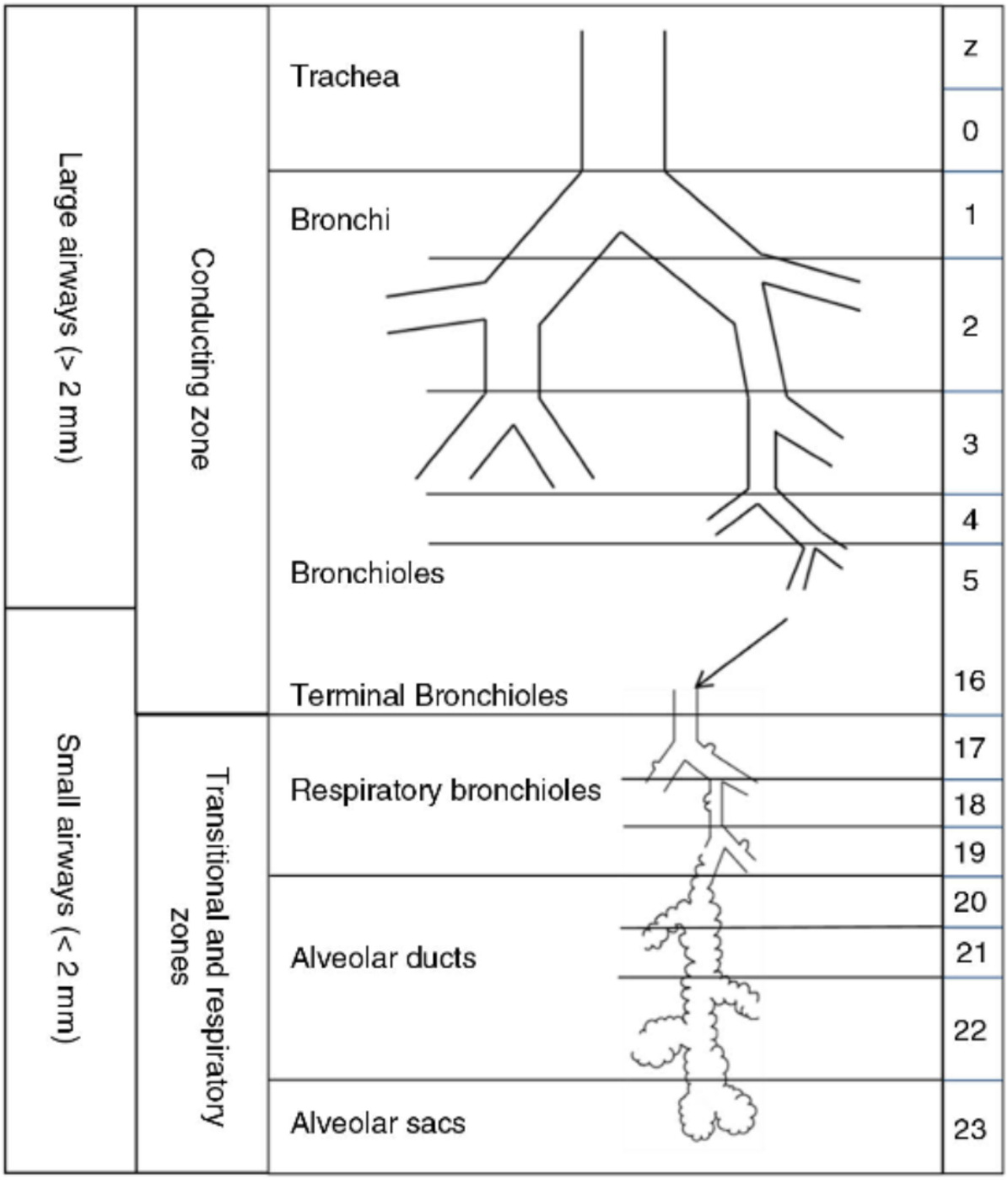


Figure 1 : From Weibel et al., 1963 (186)

Bronchial tree: 23 airway generations.

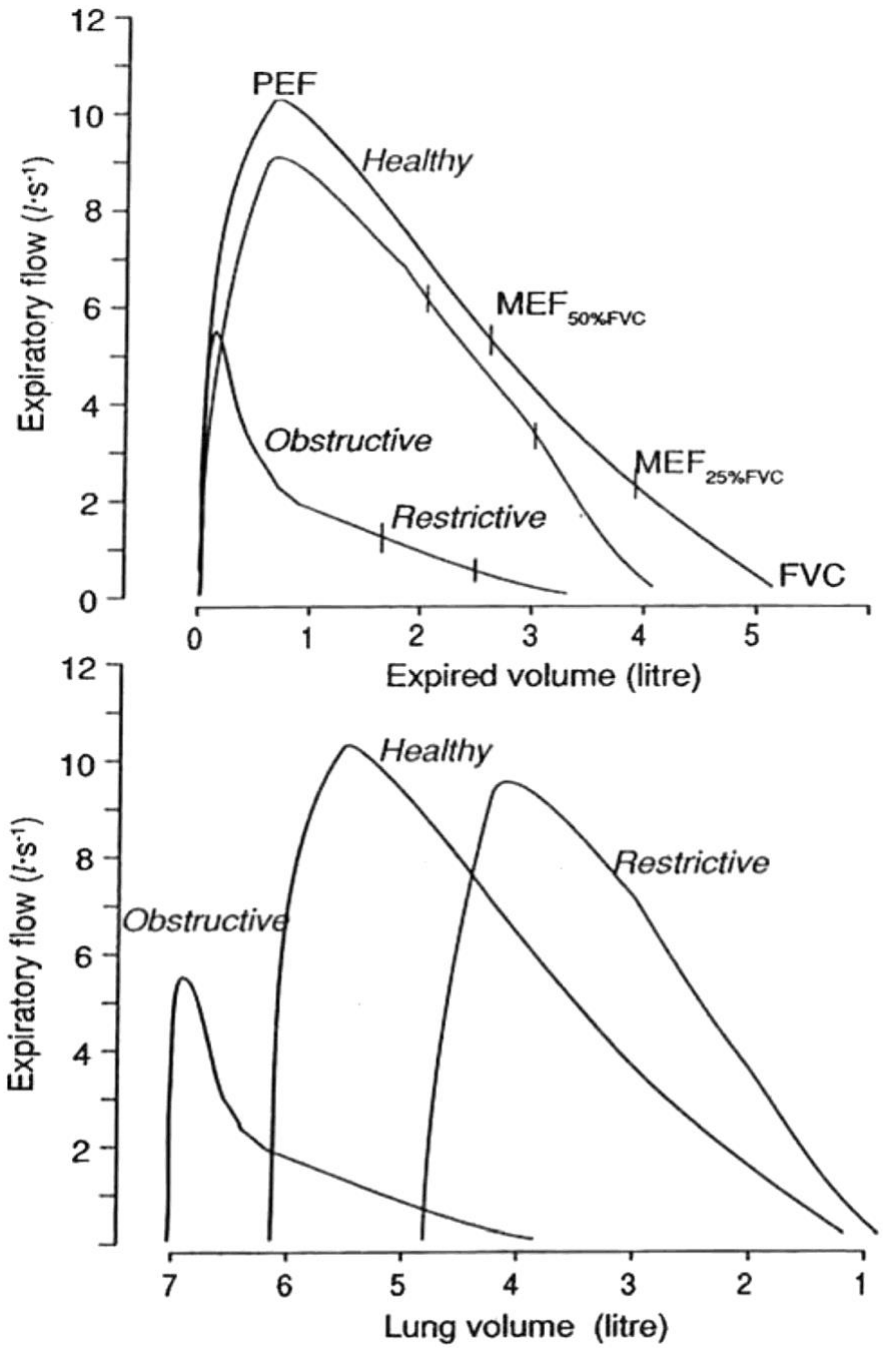


Figure 2 : From Ph.h Quanjer et al., 1993 (153)

Maximal expiratory flow curves (MEFV)

Small airway dysfunction can also be characterised by ventilation heterogeneity. Ventilation heterogeneity refers to the uneven airflow distribution in the lungs. In healthy lungs, the branching structure of the airways is not perfectly symmetrical, there are also anatomical differences in the structure of lung lobes (187, 188), and gravitational effects, all of which contribute to some degree to ventilation heterogeneity. However, this heterogeneity is normal, and does not impair lung function. In the presence of airway diseases, like in asthma, disease processes affecting airways and lung, such as inflammation and remodeling, can exacerbate ventilation heterogeneity, contributing to respiratory symptoms, and decreased lung function (138, 139, 189, 190). Furthermore, differences in the mechanical properties of the lungs, particularly related to the resistance of the airflow within the airways, and elastic properties (ability of the lung to stretch and recoil) also contribute to ventilation heterogeneity. Lung units with better ventilation or less obstruction tend to empty quickly, while those with increased resistance or decreased elasticity may empty more slowly (187, 188, 191). Several measurements are now used to assess ventilation heterogeneity in clinical practice and research, these include multiple breath nitrogen washout (MBNW), forced oscillation technique (FOT), and ventilation lung imaging.

1.14 The Multiple Breath Nitrogen Washout (MBNW)

MBNW test is a non-invasive technique for assessing lung function, it is particularly sensitive to abnormalities in small airways, and ventilation heterogeneity within the lungs (192). It also helps in evaluating disease severity, monitor disease progression, and guide therapeutic decisions (193). The test involves breathing a gas mixture containing a tracer gas, typically nitrogen or helium, and then measuring the concentration of this gas as it is washed out over multiple breaths. Over time, there has been advancements and refinements in the MBNW technique. Reference equations for predicted values now exist, consensus guidelines have also been developed outlining

recommendations for equipment specification, validation procedures, and measurement techniques (194-197).

1.14.1. MBNW Indices

The MBNW test was first developed to assess lung volumes (FRC). During the test, patients breathe 100% oxygen continuously, over which time the concentration of expired nitrogen steadily decreases, from about 78%, to below 2%, indicating that the lungs are washed out of nitrogen. FRC can be then be determined by analysing the cumulative expired volume of nitrogen, from the corresponding nitrogen concentrations for each breath during the test (194, 198).

Breath by breath analysis of MBNW test also provide information about the anatomical site of the pathology, which is based on principles of gas transport and distribution. Ventilation heterogeneity reflects ventilation within either the conducting airways, or the acinar airways. In the conducting "proximal" airways, the ventilation or the gas transport occurs by convection i.e. along pressure gradients, and inhomogeneity (or heterogeneity) has been termed convection dependent inhomogeneity (CDI). In the smaller airways, gas transport occurs by diffusion i.e. along concentration gradients, and termed diffusion-convection-dependent inhomogeneity (DCDI) because of the interaction at the diffusion front where convection stops and diffusive gas movement starts. These zones are also referred to as Scond, and Sacin, respectively (199, 200). These indices have been meticulously derived through a combination of theoretical, experimental, and lung modeling analysis (201, 202). Methods of calculating Scond, and Sacin are provided in chapter 2

1.14.2. Clinical Significance Of Scond And Sacin

Ventilation heterogeneity in Scond, and in Sacin is increased in asthma, with higher values indicating worse ventilation heterogeneity (203). Scond, and Sacin are also more abnormally "increased" in uncontrolled compared to controlled asthma (204). Both Scond, and Sacin correlate with markers of airway inflammation (205), and airway hyperresponsiveness (18) (206). In asthma, both Scond, and Sacin have been shown to be useful in monitoring the response to therapy, to bronchodilators, and to inhaled corticosteroids (203, 207) (204, 208). In smokers, Scond, and Sacin are more sensitive than spirometry in detecting early smoking related damage. Jetmalani et al. (107) has shown that small airway dysfunction, as measured by MBNW, is prevalent among smokers, even when spirometry is normal. Additionally, they also showed a correlation between reported respiratory symptoms in smokers, and small airway dysfunction. Individuals reporting symptoms indicative of chronic bronchitis exhibited higher Scond values, suggesting that increased ventilation heterogeneity within the conduction airways contribute to symptoms in smokers (209). Verbanck et al. also investigated small airway dysfunction in 169 smokers, in which they also found abnormalities in Scond and Sacin were prevalent in smokers, even in those who had no spirometric evidence of airway obstruction (210). These findings highlight the utility of MBNW in therapy monitoring, and in detecting early disease changes, especially when spirometry may not be as sensitive.

1.15 Forced Oscillation Technique (FOT)

The forced oscillation technique (FOT) is another non-invasive technique used to assess lung function by measuring the mechanical properties of the respiratory system (191). FOT involves applying external pressure oscillations to the lungs, during normal tidal breathing. The external oscillations, which consist of different frequencies are typically small pressure signals generated

by a loudspeaker (211). FOT measures respiratory impedance (Zrs), by characterising how the respiratory system responds in terms of flow, to pressure changes, at different rates or frequencies of oscillation. The lowest frequencies can be <5Hz , and highest >20Hz (212) (211). Measurements obtained at low frequencies are believed to predominantly capture the small airways, as these signals penetrate deeper into the lungs before being absorbed. Measurements or signals at higher frequencies, exceeding >20 Hz are primarily thought to reflect characteristics of the larger airways. (213) (211, 212).

Respiratory impedance consist of two components, resistance (Rrs), and reactance (Xrs). The resistance (Rrs), is the 'in-phase' or 'real part', where the pressure is in phase with flow, and describes the resistance of airflow, and thought to reflect airway caliber. Reactance (Xrs) is the 'out-of-phase' or the 'imaginary part', where pressure is out of phase with flow (214). Reactance describes the elastic properties, or the stiffness of the respiratory system under oscillatory conditions. The concept of Xrs is complex, however it incorporates the concepts of inertance and capacitance (211). Inertance represents the mass-inertive forces of the moving air in the conducting airways, while capacitance represent the elastic, energy storage properties of the lung (211) hence most commonly referred to as elastance. The inertance component is associated with positive sign, and dominates at higher frequencies, while the elastance component is associated with a negative sign and dominates at lower frequencies (211) (191). Methods of calculating Rrs, and Xrs are described in chapter 2. Ongoing research is being conducted on predictive and reference equations for FOT (215). However, current recommendations and consensus regarding the application, interpretation, and standardisation of FOT are available (216). FOT parameters are generally reported as z-scores, which are corrected for age, height, and gender (217) (218).

1.15.1. Clinical Significance Of FOT

FOT measured at lower frequencies is sensitive to small airway obstruction, even when conventional measures such as FEV_1 are normal (219, 220). Both resistance (Rrs) and reactance (Xrs) have been shown to increase following bronchoprovocation tests in individuals with suspected asthma (221), indicating their sensitivity in detecting airway obstruction. Studies examining the effect of inhaled therapies on oscillatory parameters have consistently shown that they are more sensitive than spirometry in detecting improvements. For instance, several studies have demonstrated greater sensitivity in detecting bronchodilator responsiveness in asthma, with the difference in Rrs and Xrs between baseline measurement and after salbutamol inhalation, proving more useful than FEV_1 (25)(222). Other studies have shown that these parameters are more sensitive in detecting improvements following therapy with inhaled corticosteroids and/or long-acting β-agonists (LABA) (223, 224) (225). In addition, a few studies have also shown that oscillatory parameters may be superior to spirometry in detecting early smoking-related damages (226), their role as tools for identifying small airway changes in smokers at an early stage has been demonstrated in numerous studies (227) (228-230).

1.16 Ventilation Lung Imaging

Ventilation lung imaging also provides a regional assessment of ventilation heterogeneity, but differs fundamentally from the lung function tests discussed above, by offering insights into the spatial distribution of ventilation within the lungs. There are several different imaging modalities available, these include computed tomography (CT), single photon emission computed tomography (SPECT), positron emission tomography (PET), and helium (^{3}He) and xenon (^{129}Xe) magnetic resonance imaging (MRI). A key focus of this thesis is measuring bronchodilator response using ventilation SPECT/CT (VSPECT/CT) with the goal of understanding the

differences in response in asthma patients, as well as refining the image analysis technique for VSPECT data.

1.16.1. Single Photon Emission Computed Tomography

Single photon emission computed tomography (SPECT) is a nuclear medicine imaging technique that is able to provide three dimensional (3D) imaging information. SPECT measures gamma radiation (photons) that is emitted by a radiotracer administered to the patient. These photons are detected by a gamma camera, which rotates around the patients lungs during the imaging process. The gamma camera captures the emitted photons from different angles, allowing for the reconstruction of three-dimensional (3D) images (231, 232). Over the years, SPECT has undergone significant advancements and improvements, one of which has been the development of hybrid SPECT systems combined with CT, known as VSPECT/CT imaging (232) . VSPECT/CT quantifies the functional/physiologic data, which is correlated topographically with the underlying anatomical structures.

In VSPECT/CT imaging, the radioactive tracer commonly used is technetium-99m (^{99m}Tc), which is incorporated into a graphite nanoparticulate aerosol (Technegas) (233). Technegas is widely utilised in VSPECT images due to its greater availability, and low cost compared to other radioactive isotopes. It also has a relatively short half-life (about 6 hours), and the radiation dose from ^{99m}Tc is generally considered to be low, and poses minimal risk to patients. Additionally, Technegas is inhaled while the patient is in an upright position, followed by supine SPECT images, with the Technegas remaining in the same deposition pattern. When Technegas is inhaled by the patient, particles remain fixed for at least 20 minutes, allowing for the visualisation of ventilation patterns (234) (235). Additional details for the equipment, the procedures for generating and administering Technegas to patients are discussed in chapter 2.

1.16.2. Finding From Ventilation Studies Of Asthma

The ventilation pattern in asthmatic patients has been an area of significant interest in clinical research and practice. Visualising ventilation patterns provides insight into how airway obstruction and narrowing affect the distribution of ventilation. Assessing ventilation patterns can serve as a marker of asthma severity, by identifying areas or regions of the lungs where ventilation is compromised or absent (236). Moreover, assessing changes in ventilation before and after treatment, including bronchodilators or corticosteroids helps characterise the individual response, and potentially guide further treatment decisions. Understanding the individual ventilation patterns, and how they respond to different treatments could represent a significant step forward in personalised treatment for asthma.

In asthma, ventilation distribution has been shown to be heterogeneous (Figure 3), and in severe cases, absent (237, 238). SPECT imaging with Technegas in asthma shows that ventilation is significantly heterogeneous during bronchoconstriction (Figure 4) (238) (239). Recent studies by Farrow et al. in mild to moderate asthma have shown that areas of airway closure, measured as absent ventilation on VSPECT, were predicted by ventilation heterogeneity in convection dependent airways (Scond), and severe baseline airway hyperresponsiveness. Airway narrowing, on the other hand measured on VSPECT as reduced ventilation, was predicted by diffusion dependent airways (Sacin), as measured by MBNW (240) (241). These findings indicate that small airway dysfunction is an important determinant in the mechanisms of airway closure, and airway narrowing in asthma.

The evidence using ventilation MRI imaging in asthma is more extensive. In MRI studies, the gas is delivered while the patient is in a supine position, in a breath-hold technique. Therefore, MRI imaging reflects the distribution of ventilation while in a supine position. MRI studies in asthma

have shown that ventilation defects occur during induced bronchoconstriction (242) (243), and reduce in volume with acute bronchodilator inhalation (244) (245). Ventilation defects also correlate with markers of airway inflammation, Svenningsen et al. and collogues found that ventilation defects in patients with asthma, both those with uncontrolled and controlled eosinophilic bronchitis tend to improve post bronchodilator administration, however the improvement was found to be less when there is uncontrolled sputum eosinophilia (246). Ventilation defects have been found to correlate with measures of asthma severity (247). In patients with moderate-to-severe persistent asthma, ventilation defects were found larger, compared to those with mild-to-moderate disease. Furthermore, individuals with asthma who have large ventilation defects are often found to visit emergency rooms more frequently and require hospitalisations, suggesting that ventilation defects could serve as predictor for exacerbations (248).

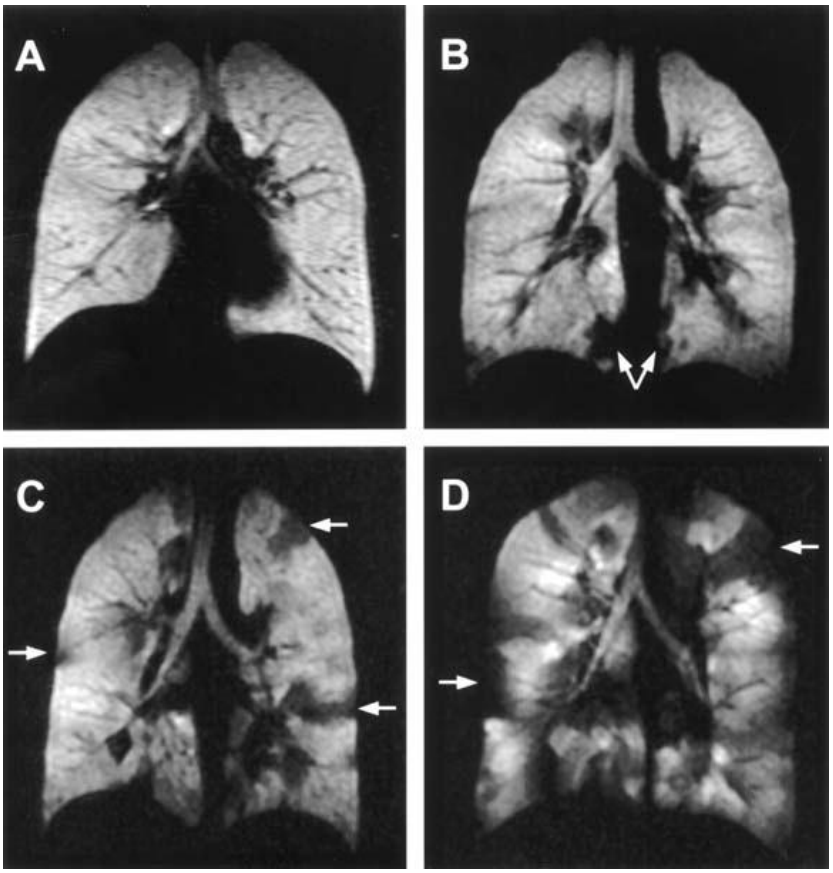


Figure 3: From Samee S et al., 2003 (242)

Imaging of heterogeneous ventilation by hyperpolarised helium (^{3}He)-MRI.

Shown in a healthy subject (A), mild (B), moderate (C), and severe asthma (D).

*The arrows point to areas with defects.

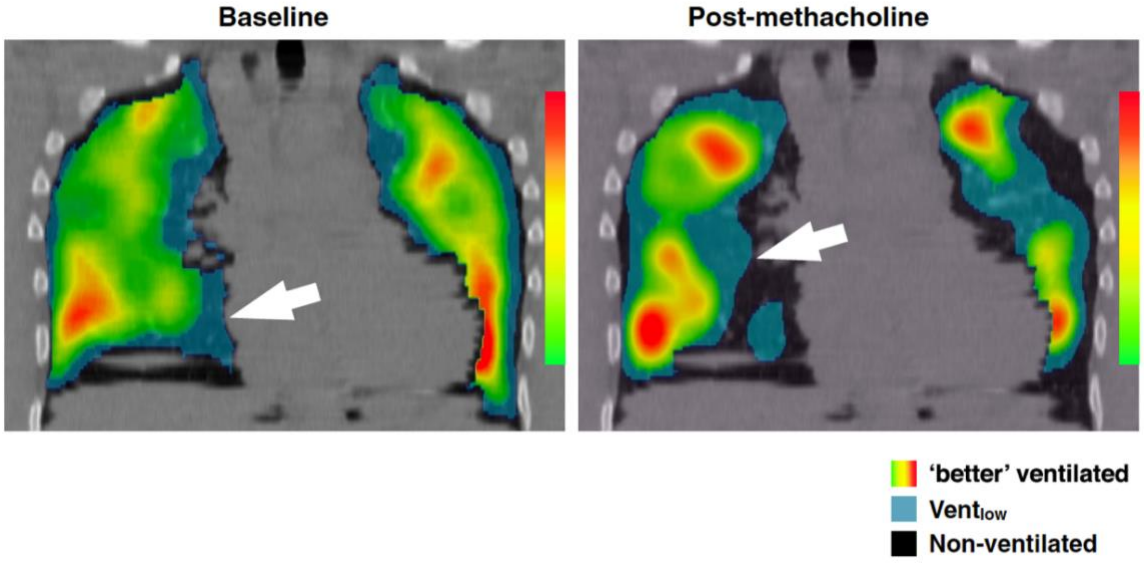


Figure 4: From Farrow CE et al., 2017 (241)

Ventilation single-photon-emission computed tomography/computed tomography (VSPECT/CT).

Images of an asthma subject, at baseline, and post methacholine challenge test.

1.16.3. Analysis Of SPECT Images

Quantification of SPECT nuclear ventilation images involves a combination of image reconstruction, segmentation, and quantitative analysis. Reconstruction process involves the removal of noise, due to photon scatter. Photons scatter occurs due to the interaction of gamma rays with the surrounding soft tissues, which is dependent on tissue density. Attenuation of photons (absorption by tissues) is another source of noise, which cause reduction in signal intensity of the SPECT image (249). Thus, scatter and attenuation corrections are essential for reducing noise, and to optimize the accuracy of SPECT image data. Scatter and attenuation corrections can be performed by applying filtering and reconstruction algorithms using specialised imaging software (Figure 5). The selection of the type of filter, and reconstruction technique depend on the type of the SPECT study, however, in lung studies, the most commonly used filters are the Butterworth and Hanning filters (250), and an iterative reconstruction method using the ordered subset expectation maximization (OSEM) (251).

Segmentation involves dividing or separating the lungs from the surrounding tissues and structures. This involves using the CT to identify the lung borders and then overlying the ‘lung map’ onto the SPECT ventilation image. This also can be performed through fully and semi-automated segmentation algorithms (Figure 6) (252). Once segmentation is complete, the SPECT image can be analysed further to quantify regional ventilation distribution. This is achieved using a 'threshold', which is a cutoff value derived from the frequency distribution of Technegas radioactivity. Technegas particles may impact onto the airways, more typically in the larger airways due to high particle velocity. This results in localised areas of high Technegas activity, or what is known as “hot spots” (253). In obstructive airway disease, such as in asthma, “hotspots” occur in the presence of severe airway narrowing and appear as extreme values in frequency

histograms of Technegas activity and do not represent true ventilation, but rather regions of highly turbulent flow. In both healthy and diseased states, some lung regions do not receive any ventilation due to airway closure (239) (239). The proportion of the lungs affected by closure increases with age and with disease. Images analysis thus requires a method to define ventilation versus non-ventilation, and is achieved using a threshold value whereby values above a threshold are considered to be ventilation and those below the threshold to be non-ventilated.

Traditional thresholds are highly influenced or biased by hotspots, which are calculated as an arbitrary percentage of the maximum count (254). In more recent studies, Farrow et al. developed a threshold method that was based on the average radioactivity between 5% and 80% of the maximum (240), thus avoiding any influence of hotspots (values above 80% of the maximal count). The 'mean 5% to 80% technique' was developed to avoid potential bias of hotspots, which occur particularly in conditions like asthma when airways may be severely narrowed, which cause ventilation to be highly heterogenous. Other methods for assessing Technegas ventilation distribution includes qualitative or visual techniques based on the assessment of experienced nuclear medicine physicians (255). However, to date, analysis of VSPECT images in the lungs remains a challenge.

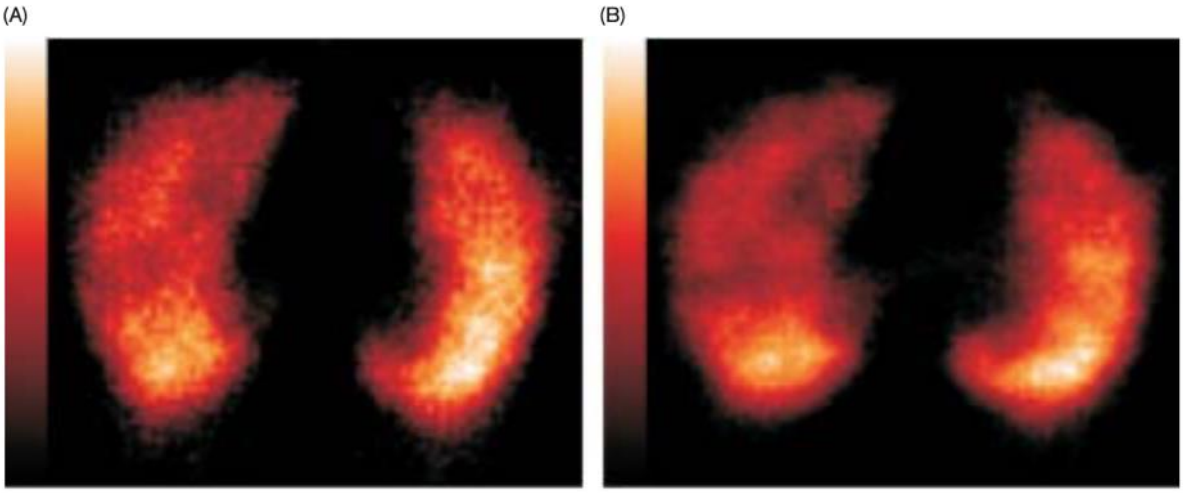


Figure 5: From Gustafsson et al., 2003 (256)

Example of lung ventilation image of a healthy subject.

No attenuation correction (A), and with attenuation correction (B).

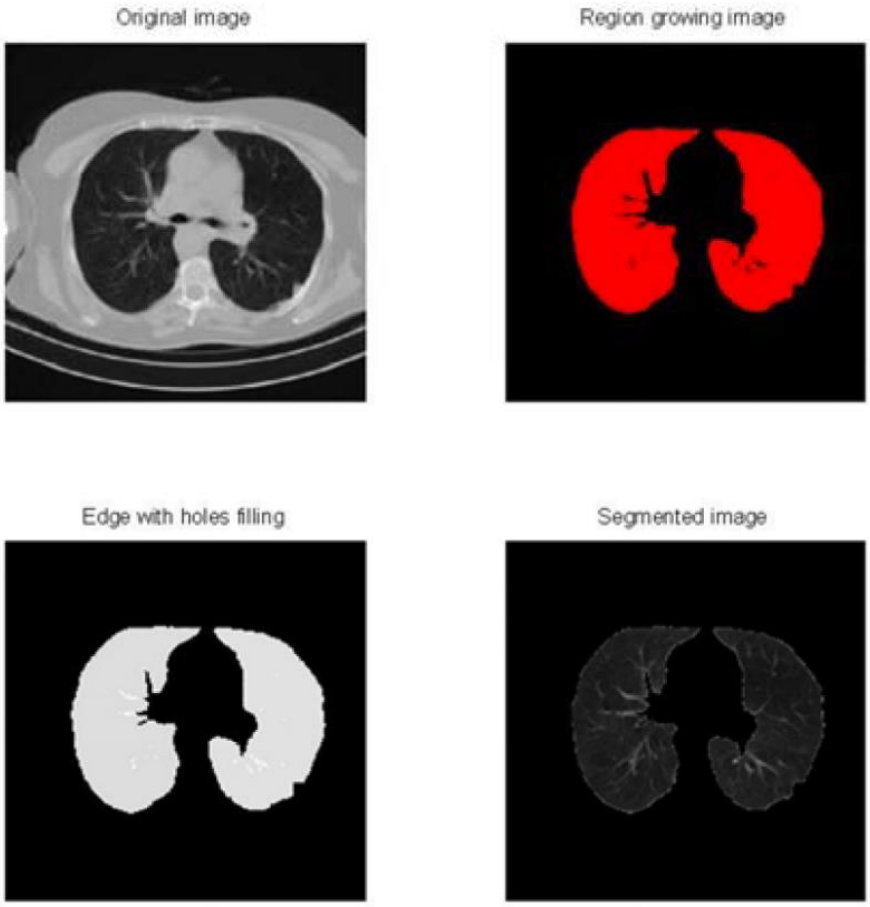


Figure 6: From N.Mesanovic et al., 2011 (257)

Example of lung segmentation using region growing algorithm

1.17 Summary

Personalised care in asthma tailored to the individual phenotypes has the potential to significantly improve disease outcomes. Physiological small airway tests have the potential to detect changes in the small airways earlier than traditional spirometry. Early detection allow earlier diagnosis, and intervention. Additionally, while the bronchodilator response serves as a key physiological measure, the determinants are unclear. Traditional spirometry may not adequately capture the response. Instead, techniques such as SPECT/CT ventilation imaging could identify regional ventilation defects, and identify areas of improvement or persistence following treatment. Moreover, despite being a valuable tool for advancing personalise asthma care, PEF monitoring is underutilised. Providing data on PEF variability in healthy subjects would help clinicians interpret PEF monitoring data and could increase its utilisation and improve asthma outcomes.

1.18 References

1. Sockrider M, Fussner L. What Is Asthma? American journal of respiratory and critical care medicine. 2020;202(9):P25-P6.
2. Patadia MO, Murrill LL, Corey J. Asthma: symptoms and presentation. Otolaryngologic clinics of North America. 2014;47(1):23-32.
3. Barnes PJ. Drugs for asthma. British Journal of Pharmacology. 2006;147(S1):S297-S303.
4. Tai A, Tran H, Roberts M, Clarke N, Gibson A-M, Vidmar S, et al. Outcomes of childhood asthma to the age of 50 years. Journal of Allergy and Clinical Immunology. 2014;133(6):1572-8
5. McDonald VM, Hiles SA, Jones KA, Clark VL, Yorke J. Health-related quality of life burden in severe asthma. Med J Aust. 2018;209(S2):S28-s33.
6. Sadatsafavi M, McTaggart-Cowan H, Chen W, Mark FitzGerald J. Quality of Life and Asthma Symptom Control: Room for Improvement in Care and Measurement. Value Health. 2015;18(8):1043-9.
7. Gonzalez-Barcala FJ, de la Fuente-Cid R, Tafalla M, Nuevo J, Caamaño-Isorna F. Factors associated with health-related quality of life in adults with asthma. A cross-sectional study. Multidiscip Respir Med. 2012;7(1):32.
8. Ferreira de Magalhães M, Amaral R, Pereira AM, Sá-Sousa A, Azevedo I, Azevedo LF, et al. Cost of asthma in children: A nationwide, population-based, cost-of-illness study. Pediatric Allergy and Immunology. 2017;28(7):683-91.
9. Australian Institute of Health and Welfare. Asthma. Canberra: AIHW; 2023.
10. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health. 2012;12(1):204.
11. https://www.abs.gov.au/statistics/health/health-conditions-and-risks/asthma/latest-release. Australian Bureau of Statistics. Available from: https://www.abs.gov.au/statistics/health/health-conditions-and-risks/asthma/latest-release.
12. National Asthma Council Australia NAC. Asthma Mortality Statistics 2023. 2023.
13. Australian Institute of Health and Welfare. Asthma. Canberra: AIHW; 2024
14. Guidelines for the diagnosis and management of asthma. National Heart, Lung, and Blood Institute. National Asthma Education Program. Expert Panel Report. J Allergy Clin Immunol. 1991;88(3 Pt 2):425-534.

15. Reddel HK, Bacharier LB, Bateman ED, Brightling CE, Brusselle GG, Buhl R, et al. Global Initiative for Asthma Strategy 2021: Executive Summary and Rationale for Key Changes. Am J Respir Crit Care Med. 2022;205(1):17-35.

16. Global Initiative for Asthma. Global strategy for asthma management and prevention. Global Initiative for Asthma. Global strategy for asthma management and prevention, 2017. http://www.ginasthma.org.

17. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. 2017.

18. Vos T, Lim SS, Abbafati C, Abbas KM, Abbasi M, Abbasifard M, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet. 2020;396(10258):1204-22.

19. Soler X, Ramsdell J. Anticholinergics/antimuscarinic drugs in asthma. Curr Allergy Asthma Rep. 2014;14(12):484.

20. Plotnick LH, Ducharme FM. Acute asthma in children and adolescents: should inhaled anticholinergics be added to beta(2)-agonists? Am J Respir Med. 2003;2(2):109-15.

21. Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. Thorax. 1999;54(2):108-14.

22. Wasserfallen JB, Baraniuk JN. Clinical use of inhaled corticosteroids in asthma. J Allergy Clin Immunol. 1996;97(1 Pt 2):177-82.

23. Bensch G, Lapidus RJ, Levine BE, Lumry W, Yegen Ü, Kiselev P, et al. A randomized, 12-week, double-blind, placebo-controlled study comparing formoterol dry powder inhaler with albuterol metered-dose inhaler. Annals of Allergy, Asthma & Immunology. 2001;86(1):19-27.

24. FitzGerald JM, Chapman KR, Della Cioppa G, Stubbing D, Fairbarn MS, Till MD, et al. Sustained bronchoprotection, bronchodilatation, and symptom control during regular formoterol use in asthma of moderate or greater severity. The Canadian FO/OD1 Study Group. J Allergy Clin Immunol. 1999;103(3 Pt 1):427-35.

25. Yaegashi M, Yalamanchili VA, Kaza V, Weedon J, Heurich AE, Akerman MJ. The utility of the forced oscillation technique in assessing bronchodilator responsiveness in patients with asthma. Respir Med. 2007;101(5):995-1000.

26. Matsuse H, Kohno S. Leukotriene receptor antagonists pranlukast and montelukast for treating asthma. Expert Opin Pharmacother. 2014;15(3):353-63.
27. Leung KB, Flint KC, Brostoff J, Hudspith BN, Johnson NM, Lau HY, et al. Effects of sodium cromoglycate and nedocromil sodium on histamine secretion from human lung mast cells. Thorax. 1988;43(10):756-61.
28. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med. 2009;360(10):973-84.
29. Humbert M, Beasley R, Ayres J, Slavin R, Hébert J, Bousquet J, et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. Allergy. 2005;60(3):309-16.
30. Pauwels RA, Pedersen S, Busse WW, Tan WC, Chen YZ, Ohlsson SV, et al. Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial. Lancet. 2003;361(9363):1071-6.
31. Suissa S, Ernst P, Benayoun S, Baltzan M, Cai B. Low-dose inhaled corticosteroids and the prevention of death from asthma. N Engl J Med. 2000;343(5):332-6.
32. Tan DJ, Bui DS, Dai X, Lodge CJ, Lowe AJ, Thomas PS, et al. Does the use of inhaled corticosteroids in asthma benefit lung function in the long-term? A systematic review and meta-analysis. European Respiratory Review. 2021;30(159):200185.
33. REPORT GM, 2022 GINA Report. 2022 GINA MAIN REPORT. 2022 GINA Report, Global Strategy for Asthma Management and Prevention. 2022.
34. Barnes PJ. Poorly perceived asthma. Thorax. 1992;47(6):408-9.
35. Spitzer WO, Suissa S, Ernst P, Horwitz RI, Habbick B, Cockcroft D, et al. The use of beta-agonists and the risk of death and near death from asthma. N Engl J Med. 1992;326(8):501-6.
36. O'Loghlen SB, Levesque L, Fisher T, DeWit Y, Whitehead M, To T, et al. Health Services Utilization Is Increased in Poor Perceivers of Bronchoconstriction and Hyperinflation in Asthma. J Allergy Clin Immunol Pract. 2020;8(8):2643-50.e2.
37. Frey U, Suki B. Complexity of chronic asthma and chronic obstructive pulmonary disease: implications for risk assessment, and disease progression and control. Lancet. 2008;372(9643):1088-99.

38. Novelli F, Bacci E, Latorre M, Seccia V, Bartoli ML, Cianchetti S, et al. Comorbidities are associated with different features of severe asthma. Clin Mol Allergy. 2018;16:25.

39. Boulet L-P. Influence of comorbid conditions on asthma. European Respiratory Journal. 2009;33(4):897-906.

40. Lemanske RF, Jr., Busse WW. Asthma: clinical expression and molecular mechanisms. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S95-102.

41. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. J Allergy Clin Immunol. 2007;119(2):405-13.

42. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. Am J Respir Crit Care Med. 2003;168(11):1308-11.

43. Peters-Golden M, Swern A, Bird SS, Hustad CM, Grant E, Edelman JM. Influence of body mass index on the response to asthma controller agents. Eur Respir J. 2006;27(3):495-503.

44. Kaur R, Chupp G. Phenotypes and endotypes of adult asthma: Moving toward precision medicine. J Allergy Clin Immunol. 2019;144(1):1-12.

45. Koczulla AR, Vogelmeier CF, Garn H, Renz H. New concepts in asthma: clinical phenotypes and pathophysiological mechanisms. Drug Discov Today. 2017;22(2):388-96.

46. Guilleminault L, Ouksel H, Belleguic C, Guen YL, Germaud P, Desfleurs E, et al. Personalised medicine in asthma: from curative to preventive medicine. European Respiratory Review. 2017;26(143):160010.

47. Hekking PP, Bel EH. Developing and emerging clinical asthma phenotypes. J Allergy Clin Immunol Pract. 2014;2(6):671-80; quiz 81.

48. Tan DJ, Walters EH, Perret JL, Lodge CJ, Lowe AJ, Matheson MC, et al. Age-of-asthma onset as a determinant of different asthma phenotypes in adults: a systematic review and meta-analysis of the literature. Expert Rev Respir Med. 2015;9(1):109-23.

49. Kupczyk M, ten Brinke A, Sterk PJ, Bel EH, Papi A, Chanez P, et al. Frequent exacerbators--a distinct phenotype of severe asthma. Clin Exp Allergy. 2014;44(2):212-21.

50. Jain N, Satish K, Abhyankar N, Velayudhan N, Gurunathan J. Repeated exacerbation of asthma: An intrinsic phenotype of uncontrolled asthma. Lung India. 2019;36(2):131-8.

51. Konstantellou E, Papaioannou AI, Loukides S, Patentalakis G, Papaporfyriou A, Hillas G, et al. Persistent airflow obstruction in patients with asthma: Characteristics of a distinct clinical phenotype. Respir Med. 2015;109(11):1404-9.
52. Zedan M, Attia G, Zedan MM, Osman A, Abo-Elkheir N, Maysara N, et al. Clinical asthma phenotypes and therapeutic responses. ISRN Pediatr. 2013;2013:824781.
53. Wei J, Ma L, Wang J, Xu Q, Chen M, Jiang M, et al. Airway reversibility in asthma and phenotypes of Th2-biomarkers, lung function and disease control. Allergy Asthma Clin Immunol. 2018;14:89.
54. Hallstrand TS, Moody MW, Aitken ML, Henderson WR, Jr. Airway immunopathology of asthma with exercise-induced bronchoconstriction. J Allergy Clin Immunol. 2005;116(3):586-93.
55. Quirce S, Sastre J. Occupational asthma: clinical phenotypes, biomarkers, and management. Curr Opin Pulm Med. 2019;25(1):59-63.
56. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med. 1999;160(3):1001-8.
57. Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. Thorax. 2002;57(7):643-8.
58. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, et al. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. J Allergy Clin Immunol. 2010;125(5):1028-36.e13.
59. Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet. 2006;368(9537):804-13.
60. Gallucci M, Carbonara P, Pacilli AMG, di Palmo E, Ricci G, Nava S. Use of Symptoms Scores, Spirometry, and Other Pulmonary Function Testing for Asthma Monitoring. Front Pediatr. 2019;7:54.
61. Brusasco V, Crapo R, Viegi G. Series ATS/ERS task force: Standardisation of lung function testing. Eur respir J. 2005;26(2):319-38.
62. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. European Respiratory Journal. 2005;26(5):948-68.

63. Stanojevic S, Kaminsky DA, Miller MR, Thompson B, Aliverti A, Barjaktarevic I, et al. ERS/ATS technical standard on interpretive strategies for routine lung function tests. European Respiratory Journal. 2022;60(1):2101499.
64. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. Am Rev Respir Dis. 1991;144(5):1202-18.
65. Roca J, Burgos F, Sunyer J, Saez M, Chinn S, Antó JM, et al. References values for forced spirometry. Group of the European Community Respiratory Health Survey. Eur Respir J. 1998;11(6):1354-62.
66. Glindmeyer HW, Lefante JJ, McColloster C, Jones RN, Weill H. Blue-collar normative spirometric values for Caucasian and African-American men and women aged 18 to 65. Am J Respir Crit Care Med. 1995;151(2 Pt 1):412-22.
67. Korotzer B, Ong S, Hansen JE. Ethnic differences in pulmonary function in healthy nonsmoking Asian-Americans and European-Americans. Am J Respir Crit Care Med. 2000;161(4 Pt 1):1101-8.
68. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005;26(5):948-68.
69. Hansen EF, Vestbo J, Phanareth K, Kok-Jensen A, Dirksen A. Peak flow as predictor of overall mortality in asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001;163(3 Pt 1):690-3.
70. Lange P, Nyboe J, Appleyard M, Jensen G, Schnohr P. Relation of ventilatory impairment and of chronic mucus hypersecretion to mortality from obstructive lung disease and from all causes. Thorax. 1990;45(8):579-85.
71. Kitch BT, Paltiel AD, Kuntz KM, Dockery DW, Schouten JP, Weiss ST, et al. A single measure of FEV1 is associated with risk of asthma attacks in long-term follow-up. Chest. 2004;126(6):1875-82.
72. Covar RA, Fuhlbrigge AL, Williams P, Kelly HW. The Childhood Asthma Management Program (CAMP): Contributions to the Understanding of Therapy and the Natural History of Childhood Asthma. Curr Respir Care Rep. 2012;1(4):243-50.
73. Osborne ML, Pedula KL, O'Hollaren M, Ettinger KM, Stibolt T, Buist AS, et al. Assessing future need for acute care in adult asthmatics: the Profile of Asthma Risk Study: a prospective health maintenance organization-based study. Chest. 2007;132(4):1151-61.

74. Chipps BE, Zeiger RS, Borish L, Wenzel SE, Yegin A, Hayden ML, et al. Key findings and clinical implications from The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study. J Allergy Clin Immunol. 2012;130(2):332-42.e10.
75. Rabe KF, Vermeire PA, Soriano JB, Maier WC. Clinical management of asthma in 1999: the Asthma Insights and Reality in Europe (AIRE) study. Eur Respir J. 2000;16(5):802-7.
76. Rathinam KK, Abraham JJ, Vijayakumar TM. Dupilumab in the Treatment of Moderate to Severe Asthma: An Evidence-Based Review. Current Therapeutic Research. 2019;91:45-51.
77. Raphael GD, Lanier RQ, Baker J, Edwards L, Rickard K, Lincourt WR. A comparison of multiple doses of fluticasone propionate and beclomethasone dipropionate in subjects with persistent asthma. J Allergy Clin Immunol. 1999;103(5 Pt 1):796-803.
78. Weidinger P, Nilsson JL, Lindblad U. Adherence to diagnostic guidelines and quality indicators in asthma and COPD in Swedish primary care. Pharmacoepidemiol Drug Saf. 2009;18(5):393-400.
79. Barton C, Proudfoot J, Amoroso C, Ramsay E, Holton C, Bubner T, et al. Management of asthma in Australian general practice: care is still not in line with clinical practice guidelines. Primary Care Respiratory Journal. 2009;18(2):100-5.
80. Hueto J, Cebollero P, Pascal I, Cascante JA, Eguía VM, Teruel F, et al. [Spirometry in primary care in Navarre, Spain]. Arch Bronconeumol. 2006;42(7):326-31.
81. Abramson MJ, Schattner RL, Sulaiman ND, Del Colle EA, Aroni R, Thien F. Accuracy of asthma and COPD diagnosis in Australian general practice: a mixed methods study. Prim Care Respir J. 2012;21(2):167-73.
82. Wickstrom GC, Kolar MM, Keyserling TC, Kelley DK, Xie SX, Bognar BA, et al. Confidence of graduating internal medicine residents to perform ambulatory procedures. J Gen Intern Med. 2000;15(6):361-5.
83. Petty TL. Benefits of and barriers to the widespread use of spirometry. Curr Opin Pulm Med. 2005;11(2):115-20.
84. Sichletidis L, Chloros D, Spyratos D, Chatzidimitriou N, Chatziiliadis P, Protopappas N, et al. The validity of the diagnosis of chronic obstructive pulmonary disease in general practice. Prim Care Respir J. 2007;16(2):82-8.

85. Buffels J, Degryse J, Liistro G. Diagnostic certainty, co-morbidity and medication in a primary care population with presumed airway obstruction: the DIDASCO2 study. Prim Care Respir J. 2009;18(1):34-40.
86. Backer V, Harmsen L, Lund T, Pedersen L, Porsbjerg C, Rasmussen L, et al. A 3-year longitudinal study of asthma quality of life in undiagnosed and diagnosed asthma patients. Int J Tuberc Lung Dis. 2007;11(4):463-9.
87. Stanojevic S, Kaminsky DA, Miller MR, Thompson B, Aliverti A, Barjaktarevic I, et al. ERS/ATS technical standard on interpretive strategies for routine lung function tests. Eur Respir J. 2022;60(1).
88. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med. 2019;200(8):e70-e88.
89. Eliasson O, Degraff AC, Jr. The use of criteria for reversibility and obstruction to define patient groups for bronchodilator trials. Influence of clinical diagnosis, spirometric, and anthropometric variables. Am Rev Respir Dis. 1985;132(4):858-64.
90. Criteria for the assessment of reversibility in airways obstruction. Report of the Committee on Emphysema American College of Chest Physicians. Chest. 1974;65(5):552-3.
91. Anthonisen NR, Wright EC. Bronchodilator response in chronic obstructive pulmonary disease. Am Rev Respir Dis. 1986;133(5):814-9.
92. Vonk JM, Jongepier H, Panhuysen CI, Schouten JP, Bleecker ER, Postma DS. Risk factors associated with the presence of irreversible airflow limitation and reduced transfer coefficient in patients with asthma after 26 years of follow up. Thorax. 2003;58(4):322-7.
93. Ulrik CS, Backer V. Nonreversible airflow obstruction in life-long nonsmokers with moderate to severe asthma. Eur Respir J. 1999;14(4):892-6.
94. ten Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. Factors associated with persistent airflow limitation in severe asthma. Am J Respir Crit Care Med. 2001;164(5):744-8.
95. Tashkin DP, Moore GE, Trudo F, DePietro M, Chipps BE. Assessment of Consistency of Fixed Airflow Obstruction Status during Budesonide/Formoterol Treatment and Its Effects on Treatment Outcomes in Patients with Asthma. J Allergy Clin Immunol Pract. 2016;4(4):705-12.

96. Zhang L, He L, Gong J, Liu C. Risk Factors Associated with Irreversible Airway Obstruction in Asthma: A Systematic Review and Meta-Analysis. Biomed Res Int. 2016;2016:9868704.
97. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. European Respiratory Journal. 2012;40(6):1324-43.
98. Billington CK PR, Hall IP. β2 Agonists. Handb Exp Pharmacol. 2017;237:23–40. Billington CK, Penn RB, Hall IP. β2 Agonists. Handb Exp Pharmacol. 2017;237:23–40. 217.
99. Tuomisto LE, Ilmarinen P, Lehtimäki L, Niemelä O, Tommola M, Kankaanranta H. Clinical value of bronchodilator response for diagnosing asthma in steroid-naïve adults. ERJ Open Res. 2021;7(4).
100. Wagner EM, Liu MC, Weinmann GG, Permutt S, Bleecker ER. Peripheral lung resistance in normal and asthmatic subjects. Am Rev Respir Dis. 1990;141(3):584-8.
101. Wagner EM, Bleecker ER, Permutt S, Liu MC. Direct assessment of small airways reactivity in human subjects. Am J Respir Crit Care Med. 1998;157(2):447-52.
102. Janson C, Malinovschi A, Amaral AFS, Accordini S, Bousquet J, Buist AS, et al. Bronchodilator reversibility in asthma and COPD: findings from three large population studies. Eur Respir J. 2019;54(3).
103. Busse WW, Holgate ST, Wenzel SW, Klekotka P, Chon Y, Feng J, et al. Biomarker Profiles in Asthma With High vs Low Airway Reversibility and Poor Disease Control. Chest. 2015;148(6):1489-96.
104. Heffler E, Crimi C, Campisi R, Sichili S, Nicolosi G, Porto M, et al. Bronchodilator response as a marker of poor asthma control. Respiratory medicine. 2016;112:45-50.
105. Rutting S, Thamrin C, Cross TJ, King GG, Tonga KO. Fixed Airflow Obstruction in Asthma: A Problem of the Whole Lung Not of Just the Airways. Front Physiol. 2022;13:898208.
106. Gautrin D, D'Aquino LC, Gagnon G, Malo JL, Cartier A. Comparison between peak expiratory flow rates (PEFR) and FEV1 in the monitoring of asthmatic subjects at an outpatient clinic. Chest. 1994;106(5):1419-26.
107. 2023 Global Initiative for Asthma (GINA) Report: Global Strategy for Asthma Management and Prevention. www.ginasthma.org/2023-gina-main-report.

108. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol. 2007;120(5 Suppl):S94-138.
109. Dobra R, Equi A. How to use peak expiratory flow rate. Arch Dis Child Educ Pract Ed. 2018;103(3):158-62.
110. Neukirch F, Liard R, Segala C, Korobaeff M, Henry C, Cooreman J. Peak expiratory flow variability and bronchial responsiveness to methacholine. An epidemiologic study in 117 workers. Am Rev Respir Dis. 1992;146(1):71-5.
111. Troyanov S, Ghezzo H, Cartier A, Malo JL. Comparison of circadian variations using FEV1 and peak expiratory flow rates among normal and asthmatic subjects. Thorax. 1994;49(8):775-80.
112. ten Hacken NH, Timens W, Smith M, Drok G, Kraan J, Postma DS. Increased peak expiratory flow variation in asthma: severe persistent increase but not nocturnal worsening of airway inflammation. Eur Respir J. 1998;12(3):546-50.
113. Tsurikisawa N, Oshikata C, Sato T, Kimura G, Mizuki M, Tsuburai T, et al. Low Variability in Peak Expiratory Flow Predicts Successful Inhaled Corticosteroid Step-Down in Adults with Asthma. J Allergy Clin Immunol Pract. 2018;6(3):972-9.
114. Wijnhoven HA, Kriegsman DM, Hesselink AE, Penninx BW, de Haan M. Determinants of different dimensions of disease severity in asthma and COPD : pulmonary function and health-related quality of life. Chest. 2001;119(4):1034-42.
115. Jones KP, Bain DJ, Middleton M, Mullee MA. Correlates of asthma morbidity in primary care. Bmj. 1992;304(6823):361-4.
116. Hetzel MR, Clark TJ, Branthwaite MA. Asthma: analysis of sudden deaths and ventilatory arrests in hospital. Br Med J. 1977;1(6064):808-11.
117. Aggarwal AN, Gupta D, Kumar V, Jindal SK. Assessment of diurnal variability of peak expiratory flow in stable asthmatics. J Asthma. 2002;39(6):487-91.
118. Hetzel MR, Clark TJ. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. Thorax. 1980;35(10):732-8.
119. Higgins BG, Britton JR, Chinn S, Cooper S, Burney PG, Tattersfield AE. Comparison of bronchial reactivity and peak expiratory flow variability measurements for epidemiologic studies. Am Rev Respir Dis. 1992;145(3):588-93.

120. Radeos MS, Camargo CA. Predicted peak expiratory flow: Differences across formulae in the literature. The American journal of emergency medicine. 2004;22(7):516-21.
121. Tsanakas JN, Primhak RA, Milner RD, Hatzimichael A, Karpouzas JG. Unexpectedly high peak expiratory flow rates in normal Greek children. Eur J Pediatr. 1983;141(1):46-9.
122. Graff-Lonnevig V, Harfi H, Tipirneni P. Peak expiratory flow rates in healthy Saudi Arabian children living in Riyadh. Ann Allergy. 1993;71(5):446-50.
123. Singh V, Meena P, Sharma BB. Asthma-like peak flow variability in various lung diseases. Lung India. 2012;29(1):15-8.
124. Jena S, Mohanty A, Mania R, Pal A. Diurnal variation of peak expiratory flow rate in healthy young adults: A gender-based study. Journal of Medical Society. 2017;31:104.
125. Brand PL, Duiverman EJ, Postma DS, Waalkens HJ, Kerrebijn KF, van Essen-Zandvliet EE. Peak flow variation in childhood asthma: relationship to symptoms, atopy, airways obstruction and hyperresponsiveness. Dutch CNSLD Study Group. Eur Respir J. 1997;10(6):1242-7.
126. Reddel HK, Salome CM, Peat JK, Woolcock AJ. Which index of peak expiratory flow is most useful in the management of stable asthma? American Journal of Respiratory and Critical Care Medicine. 1995;151(5):1320-5.
127. Boulet LP, Milot J, Turcotte H. Relationship between changes in diurnal variation of expiratory flows, lung volumes and respiratory symptoms after acute asthma. Respir Med. 1991;85(6):487-93.
128. Packe GE, Freeman W, Cayton RM. Rates of change in peak expiratory flow and in diurnal variation in peak flow in patients recovering from acute severe asthma. Clin Sci (Lond). 1994;86(1):59-65.
129. Gannon PF, Newton DT, Pantin CF, Burge PS. Effect of the number of peak expiratory flow readings per day on the estimation of diurnal variation. Thorax. 1998;53(9):790-2.
130. Quirce S, Contreras G, Dybuncio A, Chan-Yeung M. Peak expiratory flow monitoring is not a reliable method for establishing the diagnosis of occupational asthma. Am J Respir Crit Care Med. 1995;152(3):1100-2.
131. Higgins BG, Britton JR, Chinn S, Jones TD, Jenkinson D, Burney PG, et al. The distribution of peak expiratory flow variability in a population sample. Am Rev Respir Dis. 1989;140(5):1368-72.

132. Boezen HM, Schouten JP, Postma DS, Rijcken B. Distribution of peak expiratory flow variability by age, gender and smoking habits in a random population sample aged 20-70 yrs. Eur Respir J. 1994;7(10):1814-20.
133. Aggarwal AN, Gupta D, Chaganti S, Jindal SK. Diurnal variation in peak expiratory flow in healthy young adults. Indian J Chest Dis Allied Sci. 2000;42(1):15-9.
134. Casale R, Pasqualetti P. Cosinor analysis of circadian peak expiratory flow variability in normal subjects, passive smokers, heavy smokers, patients with chronic obstructive pulmonary disease and patients with interstitial lung disease. Respiration. 1997;64(4):251-6.
135. Künzli N, Stutz EZ, Perruchoud AP, Brändli O, Tschopp JM, Bolognini G, et al. Peak flow variability in the SAPALDIA study and its validity in screening for asthma-related conditions. The SPALDIA Team. Am J Respir Crit Care Med. 1999;160(2):427-34.
136. Reddel HK, Salome CM, Peat JK, Woolcock AJ. Which index of peak expiratory flow is most useful in the management of stable asthma? Am J Respir Crit Care Med. 1995;151(5):1320-5.
137. Kerstjens HA, Brand PL, de Jong PM, Koëter GH, Postma DS. Influence of treatment on peak expiratory flow and its relation to airway hyperresponsiveness and symptoms. The Dutch CNSLD Study Group. Thorax. 1994;49(11):1109-15.
138. Weibel ER, Gomez DM. Architecture of the human lung. Use of quantitative methods establishes fundamental relations between size and number of lung structures. Science. 1962;137(3530):577-85.
139. Weibel ER. Geometric and Dimensional Airway Models of Conductive, Transitory and Respiratory Zones of the Human Lung. In: Weibel ER, editor. Morphometry of the Human Lung. Berlin, Heidelberg: Springer Berlin Heidelberg; 1963. p. 136-42.
140. Macklem PT, Mead J. Resistance of central and peripheral airways measured by a retrograde catheter. J Appl Physiol. 1967;22(3):395-401.
141. Mead J. The lung's "quiet zone". N Engl J Med. 1970;282(23):1318-9.
142. Yanai M, Sekizawa K, Ohrui T, Sasaki H, Takishima T. Site of airway obstruction in pulmonary disease: direct measurement of intrabronchial pressure. J Appl Physiol (1985). 1992;72(3):1016-23.

143. Haley KJ, Sunday ME, Wiggs BR, Kozakewich HP, Reilly JJ, Mentzer SJ, et al. Inflammatory cell distribution within and along asthmatic airways. Am J Respir Crit Care Med. 1998;158(2):565-72.
144. Carroll N, Carello S, Cooke C, James A. Airway structure and inflammatory cells in fatal attacks of asthma. Eur Respir J. 1996;9(4):709-15.
145. Usmani OS, Singh D, Spinola M, Bizzi A, Barnes PJ. The prevalence of small airways disease in adult asthma: A systematic literature review. Respir Med. 2016;116:19-27.
146. Postma DS, Brightling C, Baldi S, Van den Berge M, Fabbri LM, Gagnatelli A, et al. Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS): baseline data from a prospective cohort study. Lancet Respir Med. 2019;7(5):402-16.
147. van der Wiel E, ten Hacken NH, Postma DS, van den Berge M. Small-airways dysfunction associates with respiratory symptoms and clinical features of asthma: a systematic review. J Allergy Clin Immunol. 2013;131(3):646-57.
148. Simon MR, Chinchilli VM, Phillips BR, Sorkness CA, Lemanske RF, Jr., Szefler SJ, et al. Forced expiratory flow between 25% and 75% of vital capacity and FEV1/forced vital capacity ratio in relation to clinical and physiological parameters in asthmatic children with normal FEV1 values. J Allergy Clin Immunol. 2010;126(3):527-34.e1-8.
149. Ciprandi G, Capasso M, Tosca M, Salpietro C, Salpietro A, Marseglia G, et al. A forced expiratory flow at 25-75% value <65% of predicted should be considered abnormal: a real-world, cross-sectional study. Allergy Asthma Proc. 2012;33(1):e5-8.
150. Lebecque P, Kiakulanda P, Coates AL. Spirometry in the asthmatic child: is FEF25-75 a more sensitive test than FEV1/FVC? Pediatr Pulmonol. 1993;16(1):19-22.
151. McFadden ER, Jr., Linden DA. A reduction in maximum mid-expiratory flow rate. A spirographic manifestation of small airway disease. Am J Med. 1972;52(6):725-37.
152. O'Donnell CR, Castile RG, Mead J. Changes in flow-volume curve configuration with bronchoconstriction and bronchodilation. J Appl Physiol (1985). 1986;61(6):2243-51.
153. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault J-C. Lung volumes and forced ventilatory flows. European Respiratory Journal. 1993;6(Suppl 16):5-40.
154. Mead J, Turner JM, Macklem PT, Little JB. Significance of the relationship between lung recoil and maximum expiratory flow. J Appl Physiol. 1967;22(1):95-108.

155. Pride NB. Macklem PT. Lung mechanics in disease. In: Macklem PT MJ, eds. Handbook of Physiology. The Respiratory System. Mechanics of Breathing. Section 3 VI, and III, part 2. Bethesda, American Physiological Society,1986, 659–692p.
156. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl. 1993;16:5-40.
157. Pellegrino R. Interpretative strategies for lung function tests. European Respiratory Journal. 2005;26(5):948-68.
158. Pellegrino R, Brusasco V, Miller MR. Question everything. Eur Respir J. 2014;43(4):947-8.
159. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
160. Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW. Measurement of FEF25-75% and FEF75% does not contribute to clinical decision making. Eur Respir J. 2014;43(4):1051-8.
161. Park SS. Effect of effort versus volume on forced expiratory flow measurement. Am Rev Respir Dis. 1988;138(4):1002-5.
162. Suratt PM, Hooe DM, Owens DA, Anne A. Effect of maximal versus submaximal expiratory effort on spirometric values. Respiration. 1981;42(4):233-6.
163. Sobol BJ, Emirgil C. SUBJECT EFFORT AND THE EXPIRATORY FLOW RATE. Am Rev Respir Dis. 1964;89:402-8.
164. Boggs PB, Bhat KD, Vekovius WA, Debo MS. Volume-adjusted maximal mid-expiratory flow (Iso-volume FEF25-75%): definition of "Significant" responsiveness in healthy, normal subjects. Ann Allergy. 1982;48(3):137-8.
165. Sherter CB, Connolly JJ, Schilder DP. The significance of volume-adjusting the maximal midexpiratory flow in assessing the response to a bronchodilator drug. Chest. 1978;73(5):568-71.
166. Sutherland ER, Martin RJ, Bowler RP, Zhang Y, Rex MD, Kraft M. Physiologic correlates of distal lung inflammation in asthma. J Allergy Clin Immunol. 2004;113(6):1046-50.
167. Malerba M, Radaeli A, Olivini A, Damiani G, Ragnoli B, Sorbello V, et al. Association of FEF25-75% Impairment with Bronchial Hyperresponsiveness and Airway Inflammation in Subjects with Asthma-Like Symptoms. Respiration. 2016;91(3):206-14.

168. Siroux V, Boudier A, Dolgopoloff M, Chanoine S, Bousquet J, Gormand F, et al. Forced midexpiratory flow between 25% and 75% of forced vital capacity is associated with long-term persistence of asthma and poor asthma outcomes. J Allergy Clin Immunol. 2016;137(6):1709-16.e6.
169. Riley CM, Wenzel SE, Castro M, Erzurum SC, Chung KF, Fitzpatrick AM, et al. Clinical Implications of Having Reduced Mid Forced Expiratory Flow Rates (FEF25-75), Independently of FEV1, in Adult Patients with Asthma. PLoS One. 2015;10(12):e0145476.
170. Rao DR, Gaffin JM, Baxi SN, Sheehan WJ, Hoffman EB, Phipatanakul W. The utility of forced expiratory flow between 25% and 75% of vital capacity in predicting childhood asthma morbidity and severity. J Asthma. 2012;49(6):586-92.
171. Ciprandi G, Tosca MA, Cirillo I, Lionetti E, Leonardi S, Miraglia Del Giudice M, et al. Impaired FEF25-75 may predict high exhaled nitric oxide values in children with allergic rhinitis and/or asthma. J Biol Regul Homeost Agents. 2012;26(1 Suppl):S27-33.
172. Tantisuwat A, Thaveeratitham P. Effects of smoking on chest expansion, lung function, and respiratory muscle strength of youths. J Phys Ther Sci. 2014;26(2):167-70.
173. Gold DR, Wang X, Wypij D, Speizer FE, Ware JH, Dockery DW. Effects of cigarette smoking on lung function in adolescent boys and girls. N Engl J Med. 1996;335(13):931-7.
174. Wafy S, Agamy G, Ali A. Early spirometric changes in asymptomatic smokers; is it a time dependent? European Respiratory Journal. 2016;48(suppl 60):PA4320.
175. Ronish BE, Couper DJ, Barjaktarevic IZ, Cooper CB, Kanner RE, Pirozzi CS, et al. Forced Expiratory Flow at 25%-75% Links COPD Physiology to Emphysema and Disease Severity in the SPIROMICS Cohort. Chronic Obstr Pulm Dis. 2022;9(2):111-21.
176. Mead J. Analysis of the configuration of maximum expiratory flow-volume curves. J Appl Physiol Respir Environ Exerc Physiol. 1978;44(2):156-65.
177. Ohwada A, Takahashi K. Concave pattern of a maximal expiratory flow-volume curve: a sign of airflow limitation in adult bronchial asthma. Pulm Med. 2012;2012:797495.
178. Healy F, Wilson AF, Fairshter RD. Physiologic correlates of airway collapse in chronic airflow obstruction. Chest. 1984;85(4):476-81.
179. Johns DP, Das A, Toelle BG, Abramson MJ, Marks GB, Wood-Baker R, et al. Improved spirometric detection of small airway narrowing: concavity in the expiratory flow-volume curve in people aged over 40 years. Int J Chron Obstruct Pulmon Dis. 2017;12:3567-77.

180. Dominelli PB, Foster GE, Guenette JA, Haverkamp HC, Eves ND, Dominelli GS, et al. Quantifying the shape of the maximal expiratory flow-volume curve in mild COPD. Respir Physiol Neurobiol. 2015;219:30-5.
181. Schachter EN, Kapp MC, Maunder LR, Beck G, Witek TJ. Smoking and cotton dust effects in cotton textile workers: an analysis of the shape of the maximum expiratory flow volume curve. Environ Health Perspect. 1986;66:145-8.
182. Kapp MC, Schachter EN, Beck GJ, Maunder LR, Witek TJ, Jr. The shape of the maximum expiratory flow volume curve. Chest. 1988;94(4):799-806.
183. Zheng CJ, Adams AB, McGrail MP, Marini JJ, Greaves IA. A proposed curvilinearity index for quantifying airflow obstruction. Respir Care. 2006;51(1):40-5.
184. O'Donnell CR, Rose RM. The flow-ratio index. An approach for measuring the influence of age and cigarette smoking on maximum expiratory flow-volume curve configuration. Chest. 1990;98(3):643-6.
185. Vermaak JC, Bunn AE, de Kock MA. A new lung function index: the area under the maximum expiratory flow-volume curve. Respiration. 1979;37(2):61-5.
186. Weibel ER. Geometric and Dimensional Airway Models of Conductive, Transitory and Respiratory Zones of the Human Lung. In: Weibel ER, editor. Morphometry of the Human Lung. Berlin, Heidelberg: Springer Berlin Heidelberg; 1963. p. 136-42.
187. Engel LA, Paiva M. Analyses of sequential filling and emptying of the lung. Respir Physiol. 1981;45(3):309-21.
188. Fukuchi Y, Cosio M, Murphy B, Engel LA. Intraregional basis for sequential filling and emptying of the lung. Respir Physiol. 1980;41(3):253-66.
189. Fowler WS. Lung function studies; uneven pulmonary ventilation in normal subjects and in patients with pulmonary disease. J Appl Physiol. 1949;2(6):283-99.
190. Robertson JS, Siri WE, Jones HB. Lung ventilation patterns determined by analysis of nitrogen elimination rates; use of mass spectrometer as a continuous gas analyzer. J Clin Invest. 1950;29(5):577-90.
191. Oostveen E, MacLeod D, Lorino H, Farré R, Hantos Z, Desager K, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. Eur Respir J. 2003;22(6):1026-41.

192. Fowler WS. Lung function studies; the respiratory dead space. Am J Physiol. 1948;154(3):405-16.
193. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. Eur Respir J. 2013;41(3):507-22.
194. Robinson PD, Latzin P, Verbanck S, Hall G, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. European Respiratory Journal. 2013;41(1399-3003 (Electronic)):507-22.
195. Verbanck S, Van Muylem A, Schuermans D, Bautmans I, Thompson B, Vincken W. Transfer factor, lung volumes, resistance and ventilation distribution in healthy adults. Eur Respir J. 2016;47(1):166-76.
196. Verbanck S, Thompson BR, Schuermans D, Kalsi H, Biddiscombe M, Stuart-Andrews C, et al. Ventilation heterogeneity in the acinar and conductive zones of the normal ageing lung. Thorax. 2012;67(9):789-95.
197. Tonga KO, Robinson PD, Farah CS, King GG, Thamrin C. In vitro and in vivo functional residual capacity comparisons between multiple-breath nitrogen washout devices. ERJ Open Res. 2017;3(4).
198. Robinson PD, Goldman MD, Gustafsson PM. Inert gas washout: theoretical background and clinical utility in respiratory disease. Respiration. 2009;78(3):339-55.
199. Crawford AB, Makowska M, Paiva M, Engel LA. Convection- and diffusion-dependent ventilation maldistribution in normal subjects. J Appl Physiol (1985). 1985;59(3):838-46.
200. Paiva M. Two new pulmonary functional indexes suggested by a simple mathematical model. Respiration. 1975;32(5):389-403.
201. Verbanck S, Paiva M. Model simulations of gas mixing and ventilation distribution in the human lung. J Appl Physiol (1985). 1990;69(6):2269-79.
202. Paiva M, van Muylem A, Engel LA. Slope of phase III in multibreath nitrogen washout and washin. Bull Eur Physiopathol Respir. 1982;18(2):273-80.
203. Verbanck S, Schuermans D, Noppen M, Van Muylem A, Paiva M, Vincken W. Evidence of acinar airway involvement in asthma. Am J Respir Crit Care Med. 1999;159(5 Pt 1):1545-50.

204. Farah CS, King GG, Brown NJ, Downie SR, Kermode JA, Hardaker KM, et al. The role of the small airways in the clinical expression of asthma in adults. J Allergy Clin Immunol. 2012;129(2):381-7, 7.e1.

205. Farah CS, Keulers LA, Hardaker KM, Peters MJ, Berend N, Postma DS, et al. Association between peripheral airway function and neutrophilic inflammation in asthma. Respirology. 2015;20(6):975-81.

206. Hardaker KM, Downie SR, Kermode JA, Farah CS, Brown NJ, Berend N, et al. Predictors of airway hyperresponsiveness differ between old and young patients with asthma. Chest. 2011;139(6):1395-401.

207. Farah CS, King GG, Brown NJ, Peters MJ, Berend N, Salome CM. Ventilation heterogeneity predicts asthma control in adults following inhaled corticosteroid dose titration. J Allergy Clin Immunol. 2012;130(1):61-8.

208. Verbanck S, Schuermans D, Paiva M, Vincken W. The functional benefit of anti-inflammatory aerosols in the lung periphery. J Allergy Clin Immunol. 2006;118(2):340-6.

209. Jetmalani K, Thamrin C, Farah CS, Bertolin A, Chapman DG, Berend N, et al. Peripheral airway dysfunction and relationship with symptoms in smokers with preserved spirometry. Respirology. 2018;23(5):512-8.

210. Verbanck S, Schuermans D, Meysman M, Paiva M, Vincken W. Noninvasive assessment of airway alterations in smokers: the small airways revisited. Am J Respir Crit Care Med. 2004;170(4):414-9.

211. Smith H, Reinhold P, Goldman M. Forced oscillation technique and impulse oscillometry. European Respiratory Monograph. 2005;31.

212. Desiraju K, Agrawal A. Impulse oscillometry: The state-of-art for lung function testing. Lung India. 2016;33(4):410-6.

213. Goldman MD, Saadeh C, Ross D. Clinical applications of forced oscillation to assess peripheral airway function. Respir Physiol Neurobiol. 2005;148(1-2):179-94.

214. Bates JH, Irvin CG, Farré R, Hantos Z. Oscillation mechanics of the respiratory system. Compr Physiol. 2011;1(3):1233-72.

215. Deprato A, Ferrara G, Bhutani M, Melenka L, Murgia N, Usmani OS, et al. Reference equations for oscillometry and their differences among populations: a systematic scoping review. Eur Respir Rev. 2022;31(165).

216. King GG, Bates J, Berger KI, Calverley P, Melo PLd, Dellacà RL, et al. Technical standards for respiratory oscillometry. European Respiratory Journal. 2020;55(2):1900753.
217. Oostveen E, Boda K, Van Der Grinten CPM, James AL, Young S, Nieland H, et al. Respiratory impedance in healthy subjects: baseline values and bronchodilator response. The European respiratory journal. 2013;42(6):1513-23.
218. Brown NJ, Xuan W, Salome CM, Berend N, Hunter ML, Musk AW, et al. Reference equations for respiratory system resistance and reactance in adults. Respiratory Physiology & Neurobiology. 2010;172(3):162-8.
219. Goldman MD. Clinical Application of Forced Oscillation. Pulmonary Pharmacology & Therapeutics. 2001;14(5):341-50.
220. Clément J, Làndsér FJ, Van de Woestijne KP. Total resistance and reactance in patients with respiratory complaints with and without airways obstruction. Chest. 1983;83(2):215-20.
221. Wouters EF, Polko AH, Schouten HJ, Visser BF. Contribution of impedance measurement of the respiratory system to bronchial challenge tests. J Asthma. 1988;25(5):259-67.
222. Cottee AM, Seccombe LM, Thamrin C, King GG, Peters MJ, Farah CS. Bronchodilator Response Assessed by the Forced Oscillation Technique Identifies Poor Asthma Control With Greater Sensitivity Than Spirometry. Chest. 2020;157(6):1435-41.
223. Manoharan A, von Wilamowitz-Moellendorff A, Morrison A, Lipworth BJ. Effects of formoterol or salmeterol on impulse oscillometry in patients with persistent asthma. J Allergy Clin Immunol. 2016;137(3):727-33.e1.
224. Yamaguchi M, Niimi A, Ueda T, Takemura M, Matsuoka H, Jinnai M, et al. Effect of inhaled corticosteroids on small airways in asthma: investigation using impulse oscillometry. Pulm Pharmacol Ther. 2009;22(4):326-32.
225. Timmins SC, Diba C, Schoeffel RE, Salome CM, King GG, Thamrin C. Changes in oscillatory impedance and nitrogen washout with combination fluticasone/salmeterol therapy in COPD. Respir Med. 2014;108(2):344-50.
226. Faria AC, Costa AA, Lopes AJ, Jansen JM, Melo PL. Forced oscillation technique in the detection of smoking-induced respiratory alterations: diagnostic accuracy and comparison with spirometry. Clinics (Sao Paulo). 2010;65(12):1295-304.

227. Dellacà RL, Santus P, Aliverti A, Stevenson N, Centanni S, Macklem PT, et al. Detection of expiratory flow limitation in COPD using the forced oscillation technique. Eur Respir J. 2004;23(2):232-40.
228. Frantz S, Nihlén U, Dencker M, Engström G, Löfdahl CG, Wollmer P. Impulse oscillometry may be of value in detecting early manifestations of COPD. Respiratory Medicine. 2012;106(8):1116-23.
229. Pisi R, Aiello M, Frizzelli A, Calzetta L, Marchi L, Bertorelli G, et al. Detection of Small Airway Dysfunction in Asymptomatic Smokers with Preserved Spirometry: The Value of the Impulse Oscillometry System. Int J Chron Obstruct Pulmon Dis. 2021;16:2585-90.
230. Su ZQ, Guan WJ, Li SY, Ding M, Chen Y, Jiang M, et al. Significances of spirometry and impulse oscillometry for detecting small airway disorders assessed with endobronchial optical coherence tomography in COPD. Int J Chron Obstruct Pulmon Dis. 2018;13:3031-44.
231. Jaszczak RJ, Coleman RE. Single photon emission computed tomography (SPECT). Principles and instrumentation. Invest Radiol. 1985;20(9):897-910.
232. Roach PJ, Gradinscak DJ, Schembri GP, Bailey EA, Willowson KP, Bailey DL. SPECT/CT in V/Q scanning. Semin Nucl Med. 2010;40(6):455-66.
233. Burch WM, Sullivan PJ, McLaren CJ. Technegas--a new ventilation agent for lung scanning. Nucl Med Commun. 1986;7(12):865-71.
234. Wiebe L, Burch W, Abrams D. Review: Technegas - 99mTc-Metal Core Graphite Nanoparticles for Pulmonary Ventilation Imaging. Current Radiopharmaceuticals. 2010;3:49-59.
235. Currie GM, Bailey DL. A Technical Overview of Technegas as a Lung Ventilation Agent. Journal of Nuclear Medicine Technology. 2021;49(4):313-9.
236. Svenningsen S, Kirby M, Starr D, Coxson HO, Paterson NA, McCormack DG, et al. What are ventilation defects in asthma? Thorax. 2014;69(1):63-71.
237. Tzeng YS, Lutchen K, Albert M. The difference in ventilation heterogeneity between asthmatic and healthy subjects quantified using hyperpolarized 3He MRI. J Appl Physiol (1985). 2009;106(3):813-22.
238. King GG, Eberl S, Salome CM, Young IH, Woolcock AJ. Differences in airway closure between normal and asthmatic subjects measured with single-photon emission computed tomography and technegas. Am J Respir Crit Care Med. 1998;158(6):1900-6.

239. King GG, Eberl S, Salome CM, Meikle SR, Woolcock AJ. Airway closure measured by a technegas bolus and SPECT. Am J Respir Crit Care Med. 1997;155(2):682-8.
240. Farrow CE, Salome CM, Harris BE, Bailey DL, Bailey E, Berend N, et al. Airway closure on imaging relates to airway hyperresponsiveness and peripheral airway disease in asthma. J Appl Physiol (1985). 2012;113(6):958-66.
241. Farrow CE, Salome CM, Harris BE, Bailey DL, Berend N, King GG. Peripheral ventilation heterogeneity determines the extent of bronchoconstriction in asthma. J Appl Physiol (1985). 2017;123(5):1188-94.
242. Samee S, Altes T, Powers P, de Lange EE, Knight-Scott J, Rakes G, et al. Imaging the lungs in asthmatic patients by using hyperpolarized helium-3 magnetic resonance: assessment of response to methacholine and exercise challenge. J Allergy Clin Immunol. 2003;111(6):1205-11.
243. Costella S, Kirby M, Maksym GN, McCormack DG, Paterson NA, Parraga G. Regional pulmonary response to a methacholine challenge using hyperpolarized (3)He magnetic resonance imaging. Respirology. 2012;17(8):1237-46.
244. Zha N, Pike D, Svenningsen S, Capaldi DP, McCormack DG, Parraga G. Second-order Texture Measurements of (3)He Ventilation MRI: Proof-of-concept Evaluation of Asthma Bronchodilator Response. Acad Radiol. 2016;23(2):176-85.
245. Svenningsen S, Kirby M, Starr D, Leary D, Wheatley A, Maksym GN, et al. Hyperpolarized (3) He and (129) Xe MRI: differences in asthma before bronchodilation. J Magn Reson Imaging. 2013;38(6):1521-30.
246. Svenningsen S, Eddy RL, Lim HF, Cox PG, Nair P, Parraga G. Sputum Eosinophilia and Magnetic Resonance Imaging Ventilation Heterogeneity in Severe Asthma. American Journal of Respiratory and Critical Care Medicine. 2018;197(7):876-84.
247. Svenningsen S, Nair P, Guo F, McCormack DG, Parraga G. Is ventilation heterogeneity related to asthma control? European Respiratory Journal. 2016;48(2):370-9.
248. Mummy DG, Kruger SJ, Zha W, Sorkness RL, Jarjour NN, Schiebler ML, et al. Ventilation defect percent in helium-3 magnetic resonance imaging as a biomarker of severe outcomes in asthma. J Allergy Clin Immunol. 2018;141(3):1140-1.e4.
249. Tsui BM. The AAPM/RSNA physics tutorial for residents. Physics of SPECT. Radiographics. 1996;16(1):173-83.

250. Van Laere K, Koole M, Lemahieu I, Dierckx R. Image filtering in single-photon emission computed tomography: principles and applications. Comput Med Imaging Graph. 2001;25(2):127-33.
251. Bruyant PP. Analytic and Iterative Reconstruction Algorithms in SPECT. Journal of Nuclear Medicine. 2002;43(10):1343-58.
252. Mesanovic N, Grgic M, Huseinagic H, Males M, Skejic E, Smajlovic M. Automatic CT Image Segmentation of the Lungs with Region Growing Algorithm. 2021
253. Khatib I, Young PM. Technegas, A Universal Technique for Lung Imaging in Nuclear Medicine: Technology, Physicochemical Properties, and Clinical Applications. Pharmaceutics. 2023;15(4).
254. King GG, Carroll JD, Müller NL, Whittall KP, Gao M, Nakano Y, et al. Heterogeneity of narrowing in normal and asthmatic airways measured by HRCT. Eur Respir J. 2004;24(2):211-8.
255. Bajc M, Markstad H, Jarenbäck L, Tufvesson E, Bjermer L, Jögi J. Grading obstructive lung disease using tomographic pulmonary scintigraphy in patients with chronic obstructive pulmonary disease (COPD) and long-term smokers. Ann Nucl Med. 2015;29(1):91-9.
256. Gustafsson A, Jacobsson L, Johansson A, Moonen M, Tylen U, Bake B. Evaluation of various attenuation corrections in lung SPECT in healthy subjects. Nucl Med Commun. 2003;24(10):1087-95.
257. Mešanović N, Grgic M, Huseinagić H, Males M, Skejic E, Smajlovic M, editors. Automatic CT Image Segmentation of the Lungs with Region Growing Algorithm2011.

Chapter 2. Research Methods

2.1 Introduction

This chapter introduces the research methodologies that have been used to achieve the objectives of my thesis. The following describe the different lung function tests used to measure airway physiology and lung mechanics. This chapter provides an overview of the methods, with further details described in the subsequent individual chapters.

2.2 Spirometry

Spirometry is the most commonly performed lung function test in respiratory laboratories. It is considered the "gold standard" test for diagnosing and monitoring respiratory diseases. It is often the first test performed to screen for the presence of airway diseases. Spirometry is also used to measure the response to therapy, and monitor disease activity (1, 2).

2.2.1. Brief Description Of The Test

In my thesis, spirometry was performed using a Medgraphics CPFS/D USB spirometer (Medgraphics, St. Paul, MN), Medisoft BodyBox 5500 (Medisoft Corp, Sorrines, Belgium), and VyntasTM body plethysmography (Vyaire Medical, Inc.). Spirometry is often referred to as the FVC manoeuvre, which is the maximum volume of air that can be expired after maximal inspiration. The FVC manoeuvre is extremely effort dependent, and requires consistent coaching to ensure that patient exhales as forcefully, rapidly, and completely into the spirometer as possible (3). FEV_1 is the measurements that is often reported with FVC, and defined as the volume of air that is expired at the first second of the FVC manoeuvre. FEV_1 and FVC are be reported as the largest values of three acceptable manoeuvres. Both, FVC and FEV_1 are reported in litres, and percentage of predicted, corrected for age, height, and ethnicity (4). FEV_1/FVC is the ratio of FEV_1 to FVC, expressed as a percentage. It's commonly reported with FEV_1 and FVC, and most

widely used to detect for airway obstruction. Interpretations of spirometry measurements are done in accordance with ATS/ERS recommendations (4).

2.3 Flow Volume Loop

The graph generated from the FVC manoeuvre displays two curves, the maximal expiration flow-volume curve (MEFV), and the maximal inspiration flow-volume curve (MIFV). The MEFV curve shows the expiratory flow from maximal inspiration to maximal expiration, where MIFV shows the opposite, the inspiratory flow, which is from maximal expiration to the maximal inspiration (5). The reported flow volume loop is the result of MEFV and MIFV plotted together (Figure 1). The graph represents the flow (in litres per second) on the y-axis, and the volume (in litres) on the x-axis, taken from the single best FVC manoeuvre.

It has been suggested that the shape of MEFV curve is indicative of early obstructive airway disease, and small airway dysfunction (5). Obstructive airway disease causes the MEFV curve to have a concave appearance or "scooped out" curve (Figure 2). The large airways obstruction is thought to occur in the first portion of the FVC manoeuvre, where small airways (less than 2mm in diameter) contribute little to the total airway resistance. As the lung continues to empty, the end tail of the MEFV becomes largely effort-independent, and it's thought to reflect the resistance in the small airways (6). Although ATS/ERS recognise the concavity of the curve as an early sign of airway obstruction, many clinicians only use it to assess patient's effort during the FVC manoeuvre.

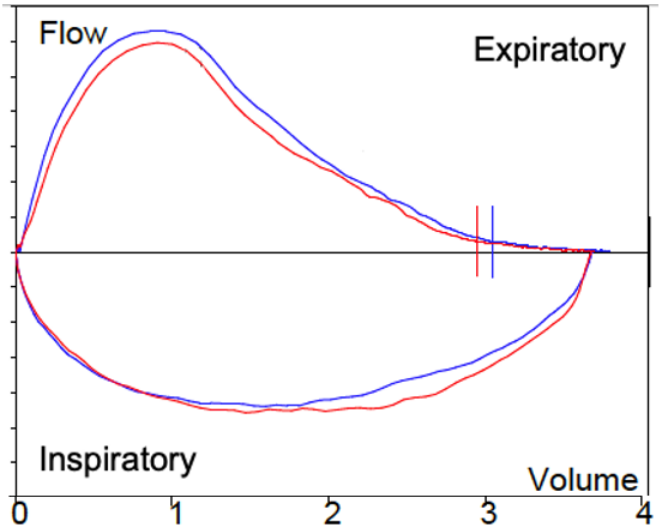


Figure 1: Example of normal flow volume curve taken from the dataset.

FEV_1/FVC ratio (z-scores< 1.64).

Spirometry performed acceptably and reproducibly according to ERS/ATS criteria

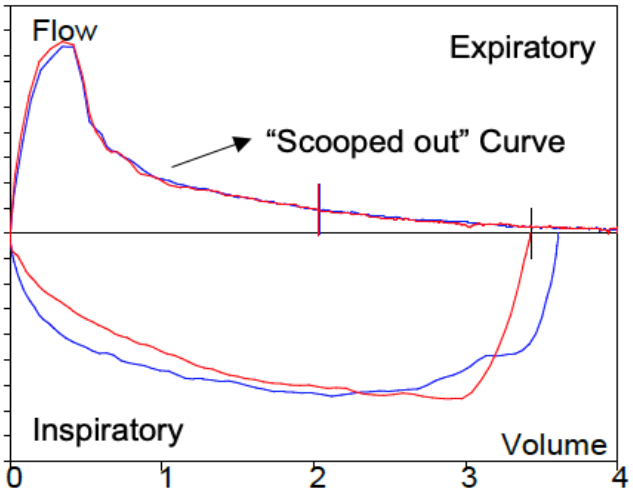


Figure 2: Example of flow volume curve showing concavity "scooped out"

shape taken from the dataset. FEV_1/FVC ratio (z-scores >1.64).

Spirometry performed acceptably and reproducibly according to ERS/ATS criteria

2.4 Home Monitoring Peak Expiratory Flow

Peak expiratory flow (PEF) is the maximum flow that can be expired after a maximal inspiration. PEF can be reported with FVC manoeuvre, but also can be measured alone during a brief, forced manoeuvre from full lung inflation, with maximal effort, using a peak flow meter (7). PEF varies throughout the day, and this variation is increased in patients with asthma (8). For that reason, measurements of PEF are commonly used in asthma management, for diagnosis, and for monitoring disease (9).

2.4.1. Brief Description Of The Test

Measurements of PEF were performed according to ATS/ERS standards (10), using a hand-held device Vitalograph (PEF meter, Ennis, Ireland), and reported in litres per minute. After the PEF meter was set to zero, subjects were asked to inhale maximally, and exhale as fast and as hard as possible into the meter, for one second. The exhalation should be performed with maximal effort, ensuring that neither the tongue nor the teeth obstructs the meter (10). Subjects were asked to perform daily PEF measurements and record them in a personal PEF diary for three weeks.

2.5 Bronchodilator Response

Bronchodilator response (BDR) is a test used to assess variable airway obstruction. An improvement in airway obstruction after bronchodilator has been considered a feature of asthma. Increased bronchodilator response has been found to be associated with the disease control, increased exacerbation rates (11), and mortality (12). However, in the last decade, it has become increasingly clear that BDR is highly variable in asthma, and can occur in other airway diseases, such as COPD (13).

2.5.1. Brief Description Of The Test

Performing spirometry before and after bronchodilator inhalation has been used to assess airway responsiveness/reversibility. Current ATS/ERS guidelines define positive bronchodilator response as a change of >10% of predicted value in FEV_1 or FVC, and also using the former criteria, ≥12% and ≥200ml increase in FEV_1 or FVC (4). Subjects performing BDR tests are routinely asked to withhold taking reliever medications for eight hours, and any combination or controller medication for 24 hours prior to the test. ATS/ERS recommend short acting β2-agonist, such as salbutamol delivered as four inhalations, for a total of 400 mcg using a metered-dose inhale (MDI) and a spacer (4). In my thesis, bronchodilator response was defined positive if subjects had ≥200 ml and ≥12% increase in FEV_1 or FVC, and also >10% of predicted value.

Measurements of FEV_1, and FVC were made before and after the bronchodilator inhalation, and the absolute and relative changes in FEV_1 and FVC were calculated as follows (2, 3):

Relative change (%) = (post-bronchodilator value − initial value)/predicted value × 100

Absolute volume change (ml) = (post-bronchodilator value – pre bronchodilator value)

2.6 Multiple Breath Nitrogen Washout Test

MBNW test is a non-invasive lung function measurements that has been described for the measurements of FRC for decades. More recently, through experiments, and lung modeling studies, MBNW is now more utilised for providing information about the degree of ventilation heterogeneity within the lungs (14). Analysis of MBNW test allows insights into the heterogeneity in conducting airways, where ventilation is predominately by convection (Scond), and in acinar airways where ventilation occurs by diffusion only (Sacin) (15).

2.6.1. Brief Description Of The Test

MBNW was performed according to ATS/ERS recommendations (15) using open-circuit Exhalyzer D with spiroware v3.3.3 (Eco Medics AG, Dürnten Switzerland). The MBNW device used in my thesis at Royal North Shore Hospital is shown in Figure 3.

The test involves breathing 100% oxygen (O_2), while measuring decreasing nitrogen concentration (N_2) over a series of expired breaths. Subjects breathe pure O_2 until the N_2 falls from 78%, to below 2% (Figure 4) (15). Prior to each test, volume calibration was conducted using a 1 litre syringe, and gas calibration using 100% O_2 (0% N_2) and room air (78% N_2) to ensure accurate measurements of gas concentrations. Additionally, a calibration using a 3 litre syringe simulating lung model that was developed in house, was performed to ensure the measured FRC volumes fell within an acceptable range.

During the test, subjects were instructed to sit up straight, and breathe on a scuba-style mouthpiece, and wear nose clip, maintaining a tidal volume of 1 to 1.3 litres, and a breathing rate of 9-12 per minute. Initially, subjects breathed in room air to establish stable tidal breathing; once the breathing was stable, subjects were switched to breathing 100% O_2. At the start of the test, N_2 concentration in the lungs is approximately 78%, as the subjects breathe in 100% O_2 for the reminder of the test, N_2 concentration falls quasi-exponentially (Figure 4). The test was terminated when the N_2 falls below 2% of its starting concentration.

At the end of the test, FRC was calculated from the total expired nitrogen volume, calculated from the cumulative expired volumes, and expired N_2 concentration for each breath.

Three technically acceptable trials with FRC values within ±10% of the mean were collected from each subject. If subjects fail to produce 3 acceptable tests, then the mean of two tests was reported.

Tests were excluded if there was any signs of leak, tidal volumes fell outside 1-1.3 litres for the majority of the breaths, and/or N_2 concentration failed to reach 2% and lower (16).

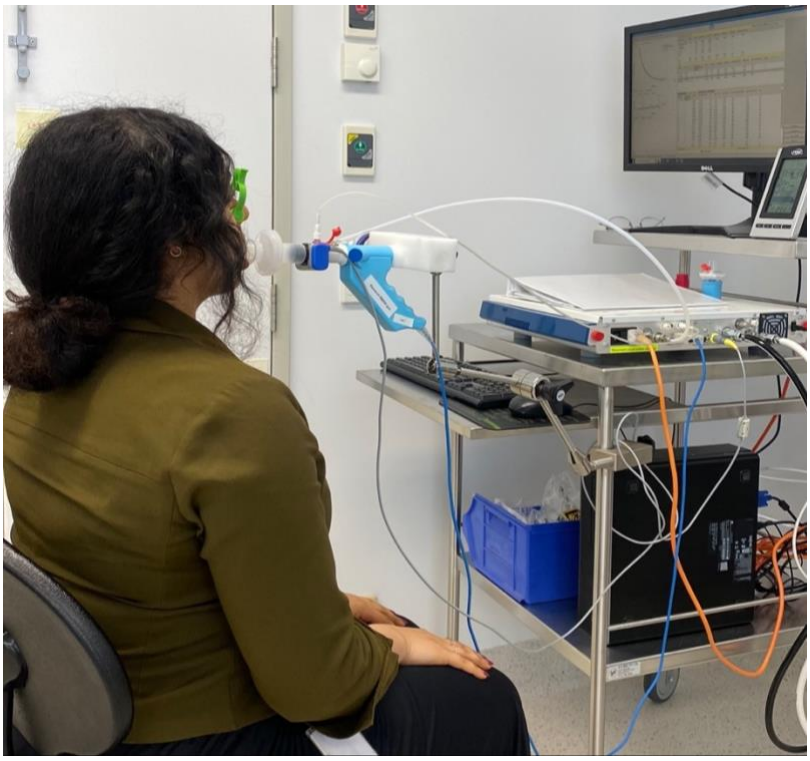


Figure 3: Photograph of MBNW device used in my thesis.

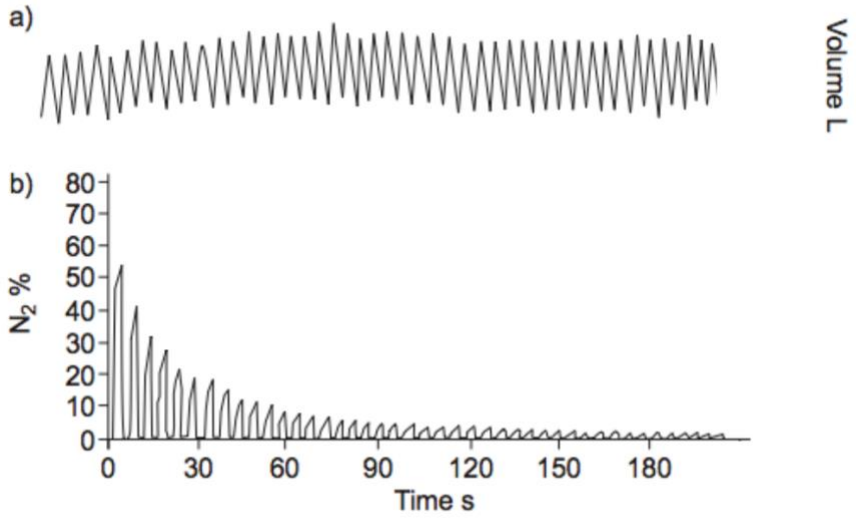


Figure 4: From Robinson et al (16), Example of the washout trace .

a) Volume trace in the upper window, shows the tidal breathing during the MBNW test, and

b) the N_2 washout trace in the lower window that shows the gradual reduction in N_2 concentration.

2.6.2. Heterogeneity Indices

The MBNW expirogram consists of four phases, occurring as the N_2 gradually decreases over consecutive breaths, reflecting the properties of gas exchange in the lungs. Phase I represents the anatomical dead space and contains O_2. Phase II consists of a mixture of dead space and alveolar gas. Phase III is the alveolar phase, containing expired alveolar gas from alveolar units as they continue to empty. Finally, phase IV reflects the rapid increase in N_2 concentration and thought to reflect airway closure (16, 17). Ventilation heterogeneity is calculated from phase III slope of each breath of the washout (Figure 5). Throughout the test, series of N_2 expirograms are generated for each breath. Since the N_2 concentration gradually falls, phase III slope is then normalised (SnIII) by the mean expired N_2 (phase III slope/ mean expired N_2).

Lung turn over (TO) is calculated for each breath as the cumulative expired volume divided by FRC of the washout (CEV/FRC). For example, if the breaths from 1-5 had a cumulative total of 5L (5 breaths x 1L/breath), and the FRC was determined to be 5 L, then the 5^{th} breath would have a TO of 1. Normalised phase III slope is then plotted against TO (Figure 6).

Scond is calculated as the regression line between TO 1.5, and 6, representing ventilation heterogeneity in convection dependent airways. Sacin is calculated as the slope of the first breath minus Scond component, representing ventilation heterogeneity in diffusion dependent airway (acinar airway). The Scond component is calculated as Scond x TO of the 1^{st} breath.

All indices from MBNW test were calculated using software program Exhalyzer D with spiroware version 3.3.3 (Eco Medics AG, Dürnten Switzerland). References values, and z-scores were calculated using Verbanck et al published equations (18).

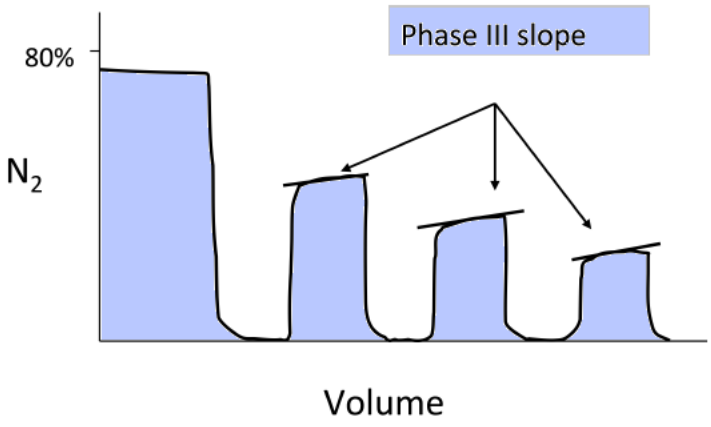


Figure 5: Example of Phase III slopes

For each expired breath, expired N_2 concentration is plotted against its expired volume. Phase III slope is then calculated by linear regression. To make each slope comparable, phase III slope is normalised (SnIII) by dividing the slopes to the mean N_2 of that breath (phase III slope/ mean expired N_2).

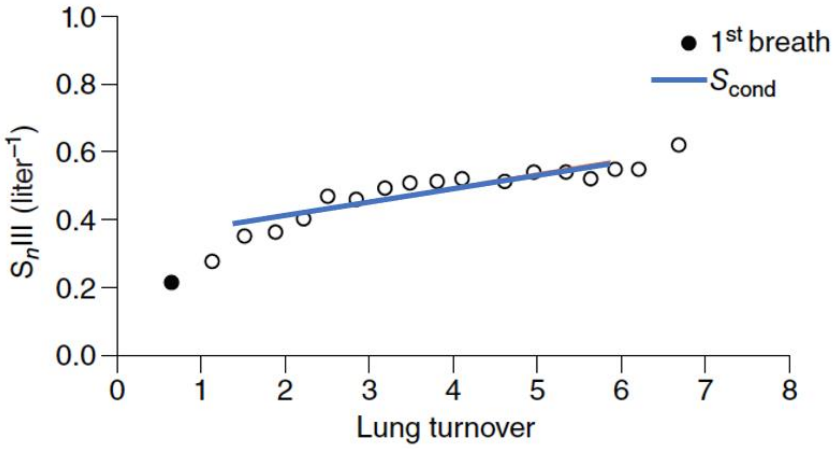


Figure 6: Example of normalised slope of an asthmatic subject taken from the dataset.

Normalised slope (SnIII) plotted against lung turnover to calculate Scond (regression line between turnover 1.5 and turnover 6), and Sacin (as the SnIII of the first breath minus Scond component, equal to slope phase III of the first breath - Scond × lung turnover of the 1st breath). Each breath represent the mean value of that breath from all the three acceptable washouts

2.7 Forced Oscillation Technique

The method of applying pressure oscillations to the respiratory system to characterise respiratory system mechanics, is referred to as the forced oscillation technique (also known as respiratory oscillometry) (19). The advantages of FOT is that its non-invasive, rapid, and unlike spirometry it is effort independent. FOT method involves applying oscillations at either single or a number of combined frequencies, at the airway opening, usually during relaxed stable breathing. The oscillation is commonly generated using a loud-speaker but other hardware may be used for signal generation (19).

The primary measurement of FOT is the respiratory impedance (Zrs), which embodies respiratory system resistance (Rrs) and respiratory system reactance (Xrs). Rrs is thought to reflect airway caliber, where Xrs reflects the dynamic (non-static) elastic properties of the lung (20).

2.7.1. Brief Description Of The Test

FOT was measured using the TremoFlo C-100 (Thorasys, Thoracic Medical Systems) as per ERS recommendations (21). Prior to each testing, the device was calibrated using a calibration load of known Rrs and Xrs supplied by Thorasys, (Thoracic Medical Systems).

In all studies in my thesis, FOT was performed before the spirometry. Subjects were seated in upright position, connected to a mouthpiece with head in a neutral position, and with nose clip on (Figure 7). During the test, subjects were asked to support their cheeks with their hands, and instructed to breathe quiet tidal breathing. The measurements were checked for signs of leak, coughs, or any other artifacts; tests containing artifacts were automatically rejected and the test was repeated until three acceptable trials were achieved. The mean of three 60-second acceptable measurements was recorded, and the mean of respiratory system resistance at 5 Hs (Rrs_{5hs}) and

respiratory system reactance (Xrs_{5hs}) were included in the analyses. Reference values and cutoffs were derived from Oostveen et al (22).

Figure 7 : Photograph of a subject performing FOT.

2.7.2. FOT Indices

Respiratory impedance (Zrs) is determined from the overall opposition to the flow of air in the respiratory system, which is the relationship between the pressure (*P*), and airflow (*V'*). It embodies both, the resistance (Rrs) (in phase with *P*, and *V'*), and the reactance (Xrs) (out of phase with *P*, and *V'*). Resistance and reactance are both frequency-dependent in FOT, which means that impedance will change with different frequencies of oscillation applied (19-21).

Impedance is typically calculated using the following formula:

$Z = P / V' = \text{Rrs} + j\,\text{Xrs}$

Where *P* is the pressure, *V'* is the flow, and $j = j = \sqrt{(-1)}$ (Figure 8).

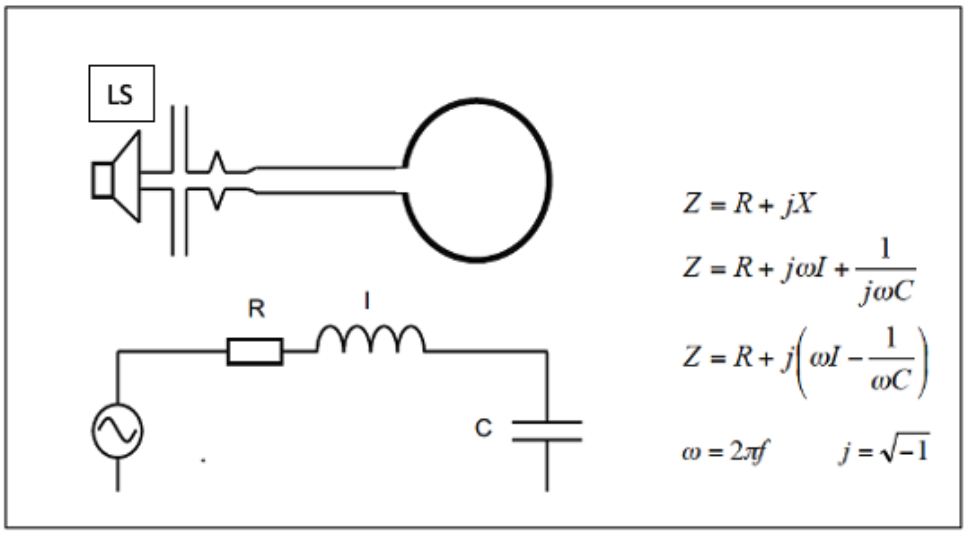


Figure 8: Reproduced with courtesy of Professor GG King. Schematic diagram showing how the respiratory impedance (Z) is measured.

Loud speaker (LS), resistance (R), $j = \sqrt{(-1)}$, reactance (X), inertia (I), capacitance (C), frequency (f), $\omega = 2\pi f$

2.8 Single Photon Emission Computed Tomography

Single photon emission computed tomography (SPECT) is a nuclear medicine imaging technique that uses gamma rays to provide 3-dimensional images. SPECT is widely used to measure ventilation and perfusion, most commonly in the investigation of pulmonary embolism in adults patients (23).

Ventilation SPECT (VSPECT) imaging in Australia and several other countries involves the use of Technegas as the radiotracer agent (CycloMedica Australia, Sydney), which is an ultrafine carbon particle aerosol labelled with Technetium-99m (^{99m}Tc) (24). The advantages of using Technegas is that it can be inhaled in upright position, and it adheres to the lung structures and remains fixed for 20 min or more. Though it is not a true gas, it has a gas like behavior which thus provides a way to measure regional distribution ventilation throughout the lung (25).

SPECT is commonly combined with low dose, non-contrast computed tomography scans (CT) to provide both anatomical colocation, and an attenuation map for attenuation correction of the SPECT image data.

2.8.1. Brief Description Of The Test

SPECT/CT imaging was performed at the Department of Nuclear Medicine, Royal North Shore Hospital, using a Siemens Symbia T16 SPECT/CT system. Technegas was prepared by a nuclear medicine technologist on site, the process involving rapidly heating technetium-99m at high temperatures in a graphite crucible, which produces the Technegas. Subjects were given precise breathing instructions to ensure optimal deposition (Figure 9).

The proprietary inhalation tubing was connected to a pneumotach so that the subjects' inhalation rate and volumes could be monitored in real time and to provide visual feedback to both, the subject and to the administrator. Five-hundred to 900mls volumes of Technegas were slowly inhaled from

FRC at ~30-60 l/min in a sitting position, followed by 5 seconds breath hold. Two to four inhalations were required to achieve a minimum radioactivity of ~1.5 kilocounts required for imaging. VSPECT was acquired during tidal breathing, with subjects lying supine inside the scanner with arms above heads. At the end of VSPECT, a CT scan was acquired immediately, in the same position. The CT scan was acquired during a breath hold at FRC. After breathing in, subjects were instructed to breathe out normally and to hold their breath for 20 seconds. (Figure 10).

SPECT images were processed using a 128 x128 matrix, at 15 second per stop, with a total of 120 views (60 stops) resulted in a total acquisition time of 15 min. Low-dose CT was performed without contrast, with a low-dose protocol (30 mAs, 110kVp, pitch 1.5, slice thickness 3 mm). Total radiation dose for the entire studies was 4.5 mSv per subject.

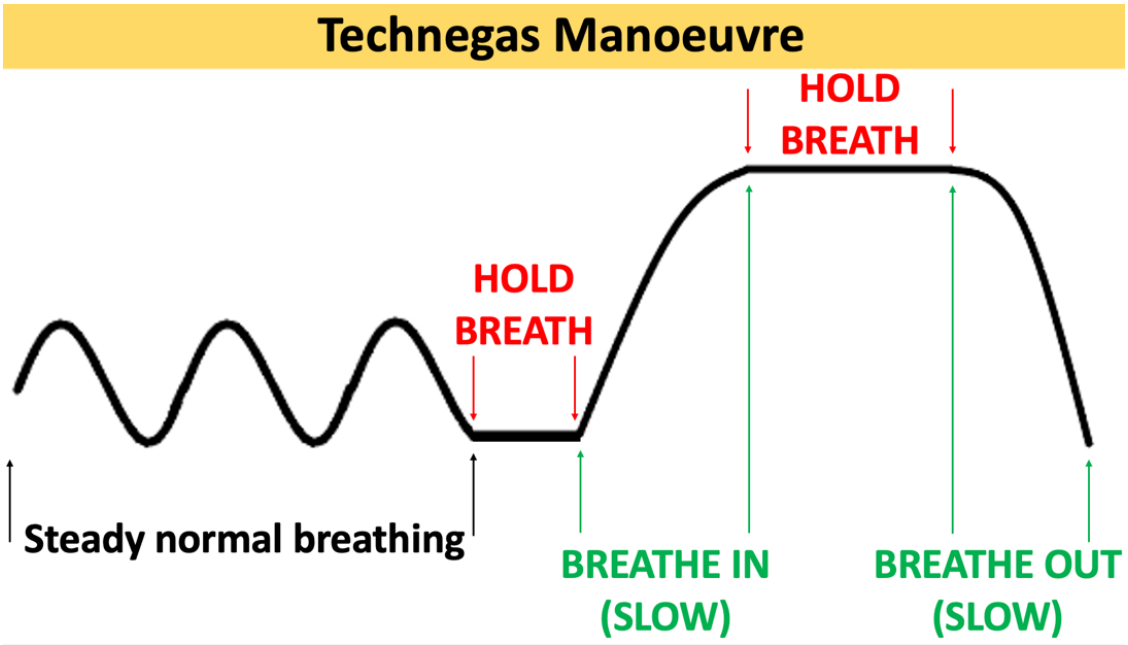


Figure 9: Technegas inhalation technique.

Subjects start the test with normal tidal breathing, and then instructed to breathe out and hold breath. Technegas then slowly inhaled from FRC, followed by 5 seconds breathe hold.

Figure 10: Photograph of a subject during VSPECT Scanning.

VSPECT and CT were acquired in the same position with subjects lying supine, with arms above heads.

2.8.2. Image Processing And Reconstruction

VSPECT images were reconstructed with attenuation correction, using ordered subset expectation maximization (OSEM), with 128 slices and Gaussian filter (iterations = 2, order-subsets = 8, full-width at half maximum (FWHM)= 8). CT images were reconstructed using 512 x 512 matrix with a Siemens algorithm and transferred to a HERMES (Hermes Medical Solutions, Stockholm, Sweden) workstation in DICOM format.

Registration of SPECT images to CT scans was performed using a HERMES multimodality software package (Hermes Medical Solutions). Due to the difference in matrix sizes between SPECT, and CT images, firstly, the matrix of SPECT had to be increased from 128 x128 to 512 x 512. After matching the matrix sizes, manual registration was performed by visually aligning SPECT, with the edges of the lungs on CT (Figure 11).

Fiducial markers were also used to establish a chest frame of reference, and to enhance the accuracy of image registration between SPECT, and CT images. The Technologist prepared these markers by using pieces of medical tape that were dipped in technetium-99m solution. These pieces were measured 2mm in diameter, and then were placed on each subject's chest, at sternal notch, and 12th rib (Figure 12). The markers were thus visible in both the SPECT and CT images.

2.8.3. Image Analysis: Threshold And Measurements Of Ventilation

In my thesis, a new method named 'Slope' was developed, and validated against a physical lung phantom model (this method is described in chapter 5). This image analysis method consisted of the following steps:

1). CT processing: all CT scans were processed using custom written code within MATLAB (MathWorks, Natick, MA, USA). A seeded region growing algorithm within MATLAB allowed the mask of the lung to be created and exported in DICOM format (Figure 13).

2). VSPECT processing: CT masks were overlayed into VSPECT. Technegas activity in VSPECT image outside the CT lung mask were then set to zero, and therefore excluded from the analysis (Figure 14). The custom-written MATLAB code for processing CT scans and VSPECT images was written by a biomedical engineer who was part of our research team, the Airway Physiology and Imaging Group.

3). Thresholding: the 'Slope' method was used to classify the lung into ventilated and non-ventilated regions, which represents the total CT lung volume and sum to 100%. In this method, a cumulative frequency histogram of the SPECT image data was constructed. The instantaneous slopes were then calculated for each data point. The first data point at 90% cumulative frequency, with a slope equal or less than 0.010, was selected as the 'slope point'. The activity of this 'slope point' was then multiplied by 30% to get the final threshold.

The thresholding analysis to categorise VSPECT was developed and characterised using a gold standard: an in-house built, lung phantom model (detailed explanation of lung phantom model and thresholding method is provided in chapter 5).

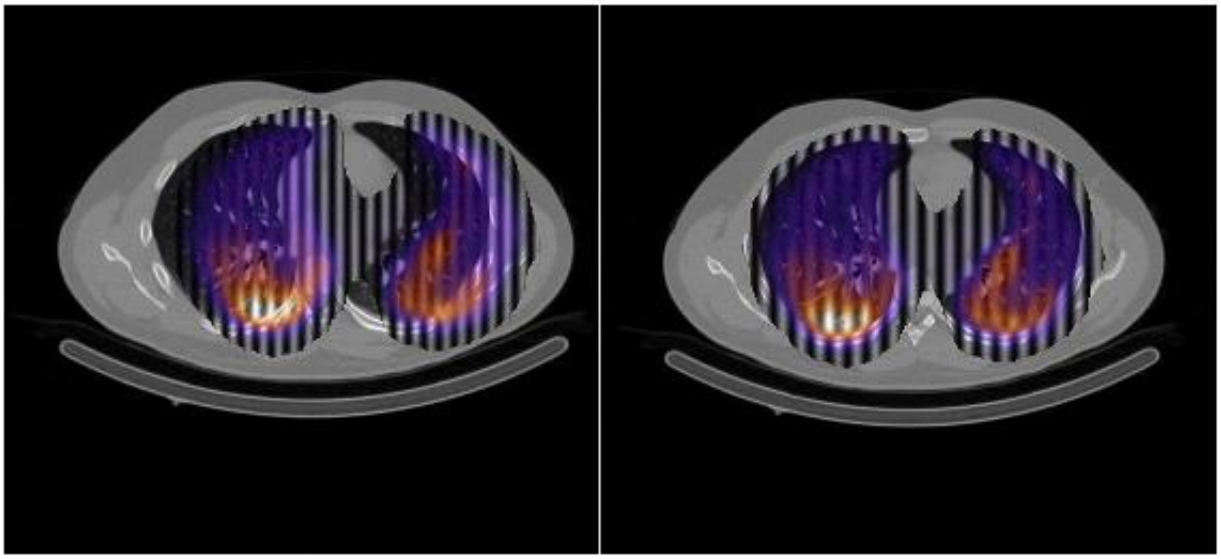


Figure 11 : VSPECT and CT images taken from the dataset for an asthmatic subject to show the process of registration. Image registration is performed to ensure that VSPECT and CT images were well-aligned. Left image shows CT and VSPECT before registration. Right image shows CT, and VSPECT after registration.

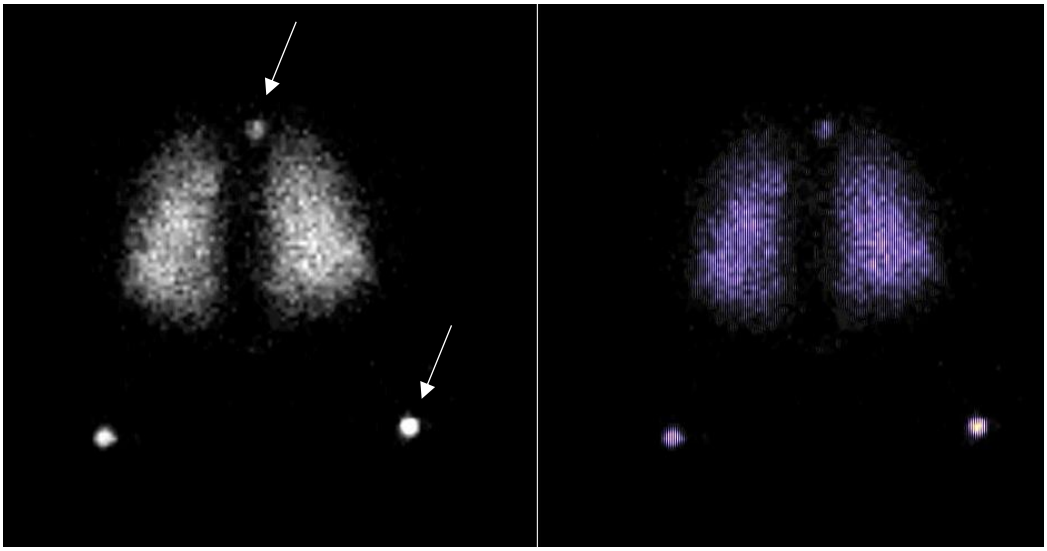


Figure 12: VSPECT image taken form the data set.

Figure is showing the fiducial markers that were used to establish chest frame of reference.

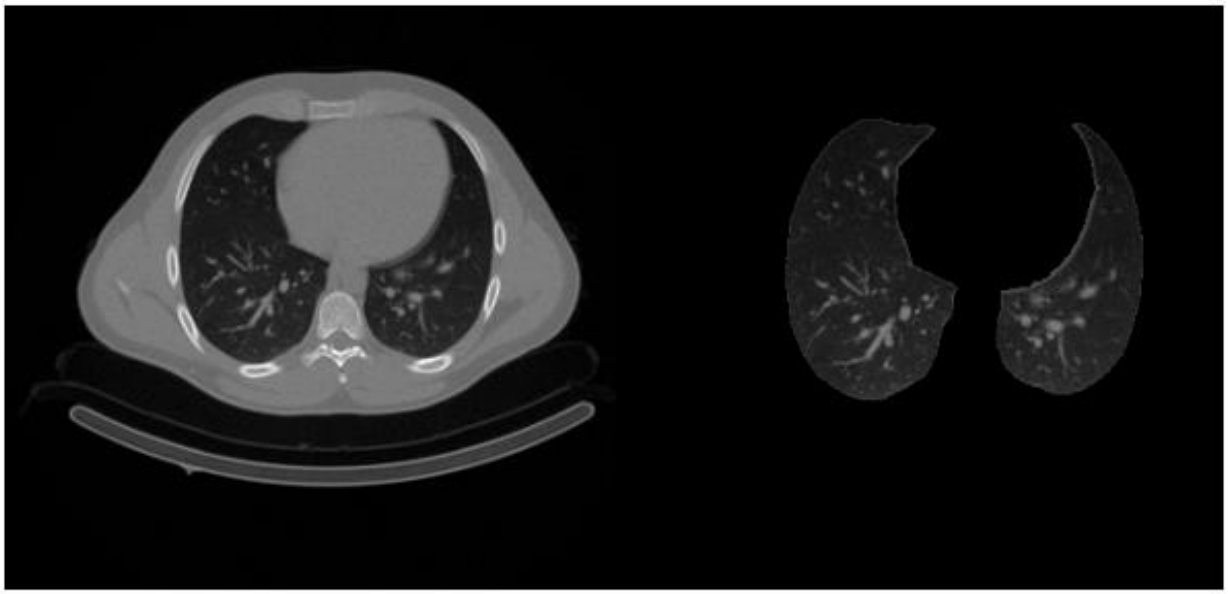


Figure 13: CT, and mask processing

CT slice taken from the dataset, and the corresponding CT mask created.

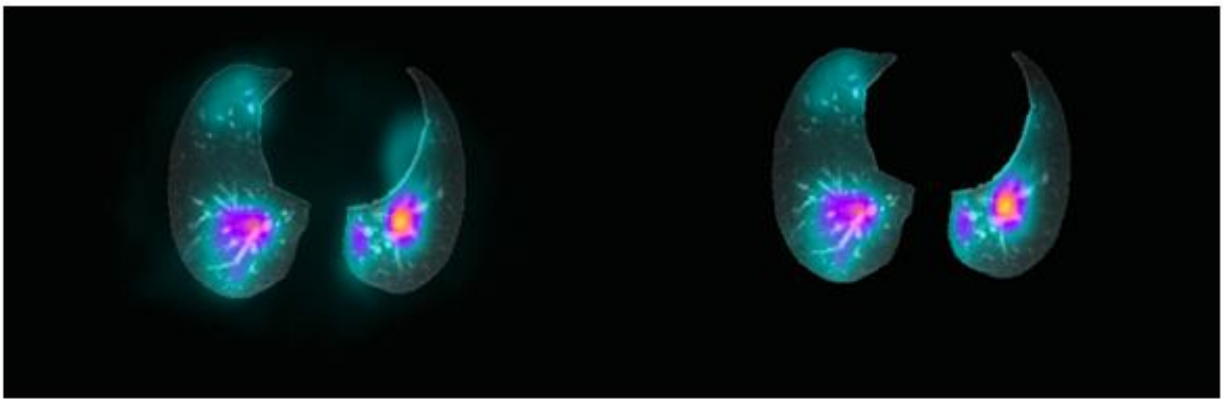


Figure 14: CT masks are overlayed into VSPECT.

To define lung outline, CT masks are overlayed into VSPECT to exclude any activity outside of the lungs.

2.9 References

1. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. American journal of respiratory and critical care medicine. 2019;200(8):e70-e88.
2. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med. 2019;200(8):e70-e88.
3. Levy ML, Quanjer PH, Rachel B, Cooper BG, Holmes S, Small IR. Diagnostic Spirometry in Primary Care: Proposed standards for general practice compliant with American Thoracic Society and European Respiratory Society recommendations. Primary Care Respiratory Journal. 2009;18(3):130-47.
4. Stanojevic S, Kaminsky DA, Miller MR, Thompson B, Aliverti A, Barjaktarevic I, et al. ERS/ATS technical standard on interpretive strategies for routine lung function tests. European Respiratory Journal. 2022;60(1):2101499.
5. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005;26(5):948-68.
6. Mead J. Analysis of the configuration of maximum expiratory flow-volume curves. J Appl Physiol Respir Environ Exerc Physiol. 1978;44(2):156-65.
7. D'Alonzo GE, Smolensky MH. PEF flow meter plays important role in asthma therapy. J Am Osteopath Assoc. 1991;91(9):858, 60.
8. Hetzel MR, Clark TJ. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. Thorax. 1980;35(10):732-8.
9. Guidelines for the diagnosis and management of asthma. National Heart, Lung, and Blood Institute. National Asthma Education Program. Expert Panel Report. J Allergy Clin Immunol. 1991;88(3 Pt 2):425-534.
10. Brusasco V, Crapo R, Viegi G. Series ATS/ERS task force: Standardisation of lung function testing. Eur respir J. 2005;26(2):319-38.
11. Pongracic JA, Krouse RZ, Babineau DC, Zoratti EM, Cohen RT, Wood RA, et al. Distinguishing characteristics of difficult-to-control asthma in inner-city children and adolescents. J Allergy Clin Immunol. 2016;138(4):1030-41.

12. Ulrik CS, Frederiksen J. Mortality and markers of risk of asthma death among 1,075 outpatients with asthma. Chest. 1995;108(1):10-5.

13. Marín JM, Ciudad M, Moya V, Carrizo S, Bello S, Piras B, et al. Airflow reversibility and long-term outcomes in patients with COPD without comorbidities. Respir Med. 2014;108(8):1180-8.

14. Robinson PD, Goldman MD, Gustafsson PM. Inert gas washout: theoretical background and clinical utility in respiratory disease. Respiration. 2009;78(3):339-55.

15. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. The European respiratory journal. 2013;41(3):507-22.

16. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. Eur Respir J. 2013;41(3):507-22.

17. Stuart-Andrews CR, Kelly VJ, Sands SA, Lewis AJ, Ellis MJ, Thompson BR. Automated detection of the phase III slope during inert gas washout testing. Journal of Applied Physiology. 2012;112(6):1073-81.

18. Verbanck S, Van Muylem A, Schuermans D, Bautmans I, Thompson B, Vincken W. Transfer factor, lung volumes, resistance and ventilation distribution in healthy adults. Eur Respir J. 2016;47(1):166-76.

19. Oostveen E, MacLeod D, Lorino H, Farré R, Hantos Z, Desager K, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. European Respiratory Journal. 2003;22(6):1026-41.

20. Peslin R, Fredberg J, Macklem P, Meads J. Handbook of physiology: The respiratory system III. Mechanism of breathing. Bethesda: American Physiological Society. 1986.

21. King GG, Bates J, Berger KI, Calverley P, Melo PLd, Dellacà RL, et al. Technical standards for respiratory oscillometry. European Respiratory Journal. 2020;55(2):1900753.

22. Oostveen E, Boda K, van der Grinten CP, James AL, Young S, Nieland H, et al. Respiratory impedance in healthy subjects: baseline values and bronchodilator response. Eur Respir J. 2013;42(6):1513-23.

23. King GG, Harris B, Mahadev S, editors. V/Q SPECT: utility for investigation of pulmonary physiology. Seminars in nuclear medicine; 2010: Elsevier.

24. BURCH WM, Sullivan PJ, McLaren CJ. Technegas-a new ventilation agent for lung scanning. Nuclear medicine communications. 1986;7(12):865-72.

25. Crawford A, Davison A, Amis T, Engel L. Intrapulmonary distribution of 99mtechnetium labelled ultrafine carbon aerosol (Technegas) in severe airflow obstruction. European respiratory journal. 1990;3(6):686-92.

Chapter 3. Day to Day Peak Expiratory Flow Variability

What is the clear-cut division for healthy “non-asthmatics” individuals?

3.1 Abstract

Introduction

Home monitoring peak expiratory flow rates (PEF) is an important clinical tool in the day-to-day management of asthma. Furthermore, several methods of expressing day-to-day variability numerically, have been proposed , although there is no clear consensus or standardised protocol. There are no data on what normal day to day PEF variability is, using methods to express variability that are practical for routine clinical practice.

Methods

Healthy, non-smoking adults performed spirometry, and were provided with a Vitalograph peak flow meter to measure their PEF upon waking, for 21 consecutive days. Within session, and day-to-day PEF variability were expressed as amplitude percent mean (A%M), calculated as (highest PEF – lowest PEF)/(mean of highest + lowest PEF)*100%, averaged over 21 days; and amplitude percent highest (A%H), calculated as (highest PEF – lowest PEF)x100/ highest PEF), respectively. Univariate analysis was used to examine relationships between PEF and age, height, and sex. The upper limit of normal (ULN) of PEF variability was defined as the 95th percentile for the group as a whole.

Results

A total of 30 subjects (16 males; mean ±SD age, 33±13 years) were analysed. Absolute mean PEF was 506.96±144.5 L/min. Within session variability "A%M" was (5.24%±2.74), with ULN of 12.88% at the 95th percentile. Day-to-day PEF variability, expressed as A%H, and A%M was (10.64%±5.81), and (12.13±7.22), respectively. The ULN of A%H, and A%M at the 95th percentile was 24%, and 30.32%, respectively. Within session, and day to day PEF variability were not related to age or height, or sex.

Conclusion

The upper limits for day-to-day PEF variability are helpful for the interpretation of PEF recordings in disease. Day-to-day PEF variability >24% and within-session variability >12% should be considered useful screening for asthma. Furthermore, guidelines should reconsider establishing a protocol for variability measurements for comparable results.

3.2 Introduction

Peak expiratory flow (PEF) is the maximal expiratory flow at which an individual can expire from maximal inhalation. The measurements are relatively simple, easy, and can be performed with a simple and inexpensive device. Variability of PEF measurements over a period of time has been used as marker of airway lability (1). Asthma guidelines recommend the use of PEF variability in the screening and diagnosis of asthma, as well as for assessing disease control, and monitoring (1). Increased PEF variability is associated with higher burden of symptoms (2), increased risk of exacerbations, hospitalisation (3, 4), and asthma deaths (4). According to The National Heart Lung and Blood Institute(5), and GINA (6) guidelines, diurnal variability of 20 % is diagnostic of asthma. This criterion however, was originally suggested by M R Hetzel et al., as a cut-point value for diurnal variability (7). Current guidelines emphasise asthma control on a day-to-day basis, however, no clear definition of what constitutes day-to-day variability in PEF has ever been reported. It is not known what constitutes day-to-day variability in healthy "normal-non-asthmatic" versus those with asthma. Several large studies have reported wide ranges of diurnal variability, in random populations (3, 8, 9), in healthy subjects but including smokers (10) (11, 12), and in asthmatic individuals (7, 13), however, they all addressed within-day, i.e. diurnal variability. Several indices have been proposed for measuring PEF variability, however, no single index, or protocol has been established as the ideal one for clinical practice. Guidelines do not specify a method for calculating PEF variability, although different numerical expressions have been proposed (14, 15) (16). The most common methods are the amplitude percent mean (highest-lowest/mean) (9), amplitude percent highest (highest-lowest/ highest) (17), and the standard deviation percent mean (SD% mean or COV) (8). These indices have been utilised by previous early investigators, and have been favored for epidemiological purposes. Guidelines also have not

specified timing of testing, frequency of the measurements, and the duration of monitoring. Evidence suggest one to two measurements per day, over 2 weeks is the best length for monitoring variability (2, 18), however other investigators recommended more frequent measurements, but a shorter number of days (19). The inconsistency in the number of measurements, frequency, and the calculations involved, have led to variations in variability measurements, even within the same populations.

Anecdotal observations and discussions suggested that unusually high or low values occasionally occurred in asthma patients' peak flow recordings. These recordings may be considered aberrant and inaccurate and were usually ignored, if they were not correlated with any change in clinical symptoms and were isolated events. Therefore, in clinical practice, there may be some filtering of peak flow graphs or diaries, that is dependent on the physicians' practices and clinical thinking.

PEF variability is an important clinical measurement that is closely associated with asthma outcomes. Despite many studies looking at diurnal, or circadian PEF variability in asthma, there are no studies addressing day-to-day variability in healthy subjects. Therefore, the first aim of this study was to measure day-to-day variability of PEF rates in healthy "non-asthmatic" adults, and examine its relationships with sex, age, and height. Furthermore, due to the lack of a standardised numerical expression for calculating PEF variability, the second aim was to compute PEF variability using the most common methods.

3.3 Methods

Study design and subjects

Adults who were current non-smokers and/or with a smoking history of <5 pack/years, aged 18 – 80 years, were recruited from staff and students of the Woolcock Institute of Medical Research, Sydney, Australia. After obtaining written informed consent, subjects attended the respiratory

laboratory for a single visit, where they first underwent lung function testing, including lung volumes by body plethysmography to confirm they had normal lung function. Subjects also completed the Woolcock clinical questionnaire to confirm the absence of any chronic lung diseases, symptoms or comorbidities that may affect lung function, including smoking history. At the end of the visit, each subject received a personal peak flow meter, and a diary to use at home for 3 weeks. This study has been approved by Northern Sydney Local Health District at Royal North Shore Hospital Human Research Ethics, and Woolcock Institute of Medical Research (HREC protocol number 2022/ETH00782)

Lung function testing

Spirometry and lung volumes were performed according to American Thoracic Society/European Respiratory Society (ATS/ERS) quality criteria (20) using Medisoft BodyBox 5500 (Medisoft Corp, Sorrines, Belgium). All lung function indices were expressed as z-scores, using published equations (21).

Peak expiratory flow measurements

Each Subjects received a personal PEF meter, Vitalograph (Model-4300, Ennis, Co. Clare, Ireland), and were instructed to record PEF measurements at home, every morning, upon waking, for 3 weeks (21 consecutive days). Subjects were instructed by a trained scientist who demonstrated the correct use of the meter. After the demonstration, subjects had to be able to perform the PEF correctly before they were sent home. In addition, they also received a recording diary that had the following instructions written on it: 1) perform the test in upright position; 2) take a deep breathe outside the meter; 3) seal your lips tightly around the mouthpiece; 4) blow into the PEF meter, as fast and as hard as you can; 5) read the value at the red-indicator and write this down as your first PEF measurement; 6) return the red-indicator to zero; 7) repeat the same step

twice more, and record your 2nd, and 3rd measurement; 8) write down the highest of the three recordings. Furthermore, in instances of missing a scheduled PEF measurement, subjects were explicitly instructed to refrain from adding any fabricated value. Instead, they were instructed to leave it blank.

Variability calculations

Within session variability

Expressed as the amplitude % mean ($A\%M_{within\text{-}session}$), calculated as (highest PEF – lowest PEF) x100/(mean value of the two) calculated for each day, then averaged over 21 days. (Figure 1)

Day-to-day variability

1) Amplitude as a percentage of the highest ($A\%H_{day\text{-}to\text{-}day}$), calculated as (highest PEF – lowest PEF)x100/ highest PEF), over 21 days. (Figure 1)

2) Amplitude as a percentage of the mean of consecutive days ($A\%M_{day\text{-}to\text{-}day}$), calculated as (highest PEF – Lowest PEF)x100/ (mean PEF), over 21 days. (Figure 1)

3) Exclusion of any PEF values that fell outside ±3SD range of the mean (Outlier-Excluder) then calculated as A%H (highest PEF – lowest PEF)x100/ highest PEF), and A%M
(highest PEF – lowest PEF)x100/ mean PEF)

4) Variation of consecutive morning PEF's ($A\%_{Consecutive}$), calculated as (highest morning PEF – highest morning PEF of the day before)x100/(mean value of the two), calculated for each day, then averaged over 21 days

5) Coefficient of variation (22) calculated as (SD/ mean PEF) x 100 (Figure 1).

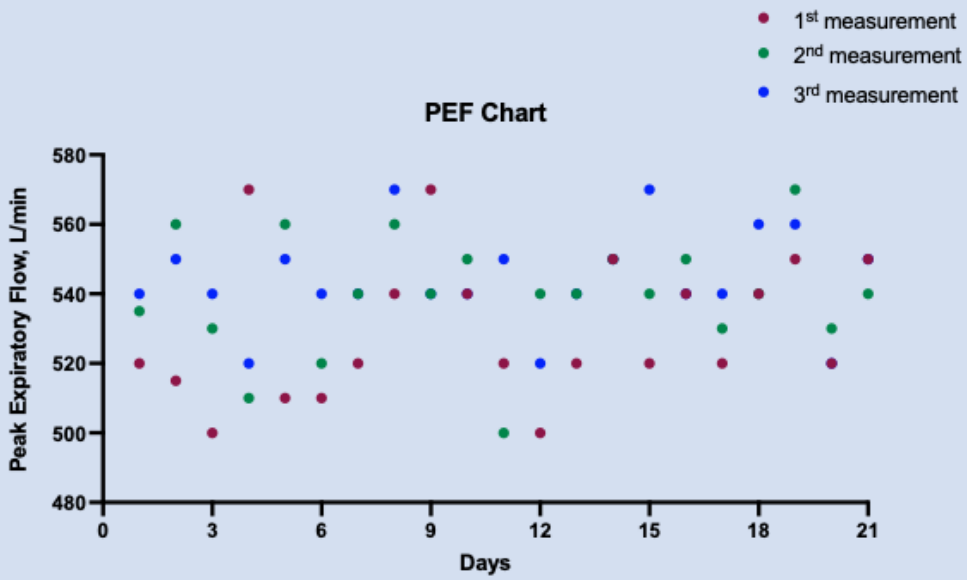
6) Physician-led approach. Given the lack of standardised guidelines for calculating PEF variability, we explored the influence of clinical expertise in estimating the PEF variability. Three respiratory physicians, with varying level of experience were provided with PEF charts of all

participants. The daily highest PEF measurements were plotted on a graph, and physicians then visually estimated the variability by selecting the highest, and the lowest PEF. Variability was then calculated as follows: (highest PEF – lowest PEF)x100/ highest PEF). Physician 1 had the most experience, with more than 25 years in respiratory medicine, physician 2 had >10 years, and physician 3 had < 10 years of experience.

3.4 Statistical Analysis

Statistical analysis was carried out with SPSS (IBM, SPSS, v26), and graphs were prepared with GraphPad Prism 8 (GraphPad Software Inc). The distribution of PEF values, and variability were inspected by frequency distribution, and the Shapiro–Wilk test was used to assess the distribution for normality. Parametric data were expressed as means ±SD, and nonparametric data were expressed as median. Differences between within-session variability, and day-to-day variability were examined by a paired Student's t test for parametric variables or Wilcoxon's signed-rank test for non-parametric variables. Correlations between PEF parameters, and PEF variability with lung function, and demographics were examined using Pearson's correlation coefficient or Spearman's rank correlation for parametric and nonparametric variables, respectively. Additionally, the association of age, height, and sex with PEF variability was analysed by Spearman's correlation coefficient, and linear regression. All tests were performed two-sided, and P value of <0.05 was considered statistically significant.

A)



Calculations of peak flow indices

Within session variability

$$A\%M_{within\text{-}session} = \frac{(540-520)X100}{Avg\ (540:520)} + \frac{(560-515)X100}{Avg\ (560:515)} + \frac{(540-500)x100}{Avg\ (540:500)} +$$

$$\frac{(570-510)x100}{Avg\ (570:510)} + \frac{(560-510)x100}{Avg\ (560:510)} + \frac{(540-510)x100}{Avg\ (540:510)} + \frac{(540-520)X100}{Avg\ (540:520)} + \ldots / 21\ days$$

$$= (3.77+8.37+7.69+11.11+9.34+5.71+3.77 + \ldots) / 21\ days$$

$$A\%M_{within\text{-}session} = \mathbf{5.19\%}$$

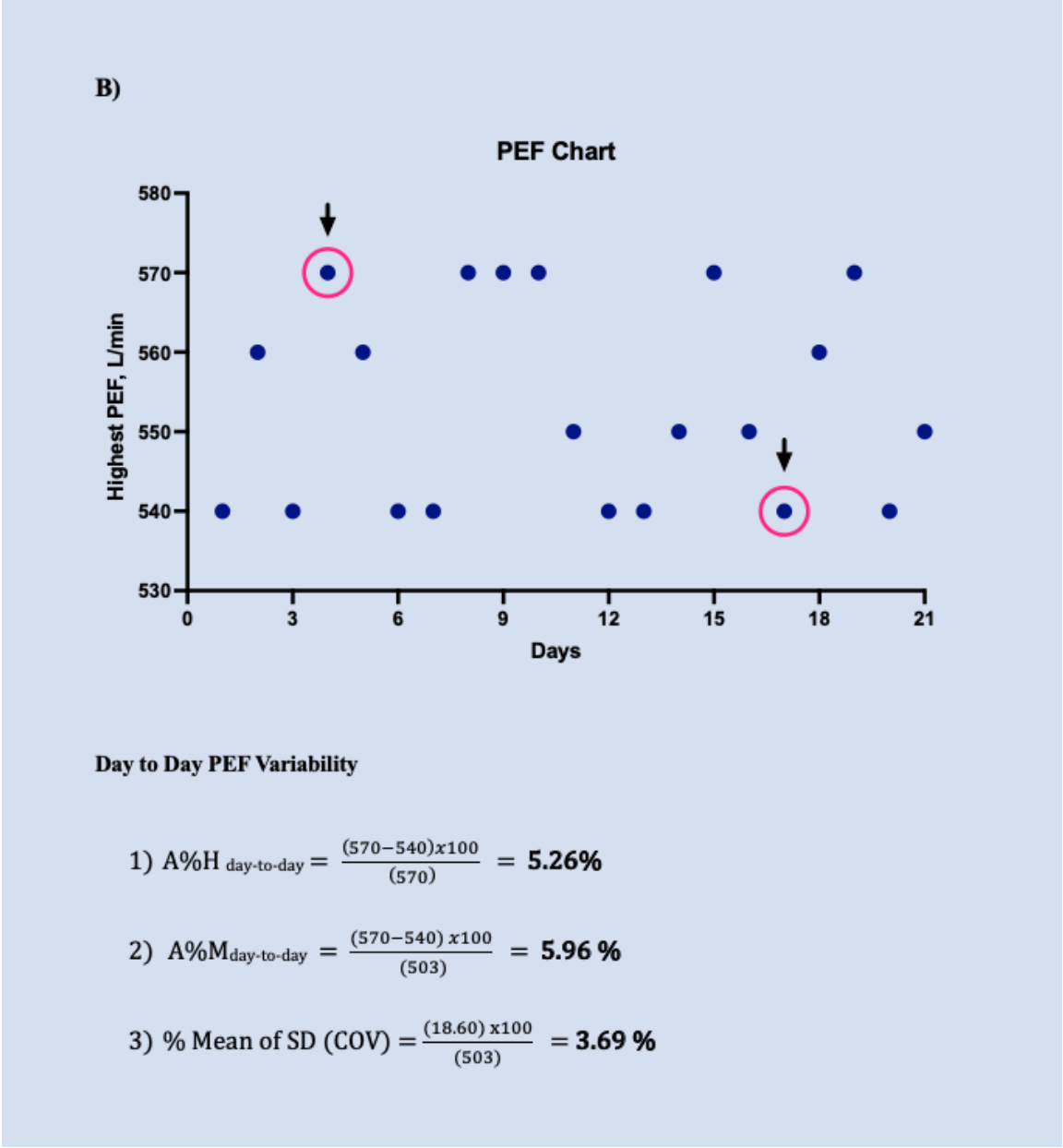


Figure 1: Example of peak expiratory flow chart for 22 yr male showing how the variability was calculated. Within session, as the amplitude % mean, calculated for each day, then averaged over 21 days. Day to day variability, calculated as the amplitude % highest (1), amplitude % mean (2), and COV (3), *circled PEF are for the highest, and lowest PEF over 21 consecutive days.

3.5 Results

Subject characteristics and PEF data

A total of 30 subjects completed the study with no withdrawals. Each participant provided complete measurements, and all data were included in the analysis. Satisfactory recordings were assessed by reviewing the data to ensure the completeness of each participant's diary entries. The readings were analysed on a day-to-day basis to identify any suspicious data patterns. If any issues were found, participants were contacted for clarification. Three participants missed one day of recording due to illness, and their variability was assessed over 20 days instead of 21.

30 subjects were included in the analysis (16 males, mean age of 37±13.69 years), the demographic description, and baseline lung function measurements are shown in Table 1. All subjects had normal lung function test, including lung volumes and FEV_1/FVC ratio (z-scores >1.64).

The group mean PEF was 506.96 L/min ±144.5 (Table 2). Male subjects produced higher values of peak expiratory flows than females, the absolute mean PEF was 600 L/min±112.71 in males, compared to 415 L/min±86.25 in females (mean difference ±SD,184±36.40 L/min, $p<0.0001$).

Table 1. Demographics, & baseline lung function measurements	
Parameters	
Participants, n	30
Male/Female,n	16/14
Age, yr#	37 ± 13.69
Height, cm	171.86 ± 10.49
BMI, kg/m2	26.01 ± 5.23
FEV_1, % predicted	102.65 ± 10.42
FEV_1, Z-scores	0.18 ± 0.75
FVC, % predicted	102.17 ± 8.69
FVC, Z-scores	0.34± 0.96
FEV_1/FVC, %	83.27 ± 6.64
FEV_1/FVC, Z-scores	0.05 ± 1.19
TLC, % predicted	97.91 ± 11.57
TLC, Z-scores	-0.29 ± 0.96
FRCPleth, % predicted	99.57 ± 19.67
FRCPleth, Z-scores	-0.09 ± 0.96
VC, % predicted	98.01 ± 13.99
VC, Z-scores	-0.50 ± 1.01
RV/TLC, % predicted	103.91 ± 24.97
RV/TLC, Z-scores	0.47 ± 0.92

#Data are presented as means ± SD. BMI, body mass index. FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity, vital capacity; RV, residual volume. N=30.

PEF variability

The results of PEF variability, calculated using the different methods, are summarised in Table 2. Mean within session variability "A%M" was 5.24%±2.74. The ULN of within session variability at the 95th percentile was 12.88%. There was no significant difference between females and males in within session PEF variability (mean difference ±SD, 0.46±1.01%, p=0.651).

Mean day to day PEF variability, expressed as $A\%H_{day\text{-}to\text{-}day}$, was 10.64%±5.81, and the ULN at the 95th percentile was 24% (Table 2). There was no significant difference between females and males in $A\%H_{day\text{-}to\text{-}day}$ PEF variability (mean difference ±SD, 0.86±2.16%, p=0.867).

Mean day to day PEF variability, expressed as $A\%M_{day\text{-}to\text{-}day}$ was 12.13±7.22, and the ULN at the 95th percentile was 30.32%. There was also no significant difference between females and males in $A\%M_{day\text{-}to\text{-}day}$ PEF variability (mean difference ±SD, 0.81±2.71%, p=0.766).

Paired t-tests were conducted to determine if there is a difference between PEF variability calculated by $A\%H_{day\text{-}to\text{-}day}$, or $A\%M_{day\text{-}to\text{-}day}$ .The result showed that there was statistically significant increase in PEF variability when calculated by $A\%M_{day\text{-}to\text{-}day}$ (mean difference ±SD, +1.43±1.65%, p<0.0001).

Within session variability A%M was significantly lower compared to day to day PEF variability expressed in either $A\%H_{day\text{-}to\text{-}day}$, or $A\%M_{day\text{-}to\text{-}day}$ (mean difference ±SD, -5.40±5.29, p<0.0001), and (mean difference ±SD, -6.89±6.59, p<0.0001), respectively (Figure 2).

Variation between consecutive days ($A\%_{Consecutive}$) PEF was 0.47%±1.82, with no significant difference between males and females (mean difference ±SD, 0.19±0.65%, p=0.775).

Using the Outlier-Excluder method which excluded outliers, the mean day-to-day variability expressed as $A\%H_{day\text{-}to\text{-}day}$ was 10.64% ±5.81, and $A\%M_{day\text{-}to\text{-}day}$ was 12.13% ±7.22. The mean COV was 5.02 % ±2.28. That is, excluding points >3SD had no effect on calculations.

Day-to-day variability expressed as $A\%H_{day\text{-}to\text{-}day}$, calculated from the physician-led approach, differed across the three physicians; 7.38% ±3.31, 11.16%±5.65, and 8.29%±3.19, respectively. The difference was statistically significant (all p values <0.05) (Figure 3).

Table 2. Peak expiratory flow variability measurements

Parameter	Mean	ULN	LLN
PEF, L/min	506.96±144.5		
$A\%M_{within\text{-}session}$,%	5.24%±2.74	12.88	1.30
$A\%H_{day\text{-}to\text{-}day}$,%	10.64±5.81	24.03	0.68
$A\%M_{day\text{-}to\text{-}day}$,%	12.13±7.28	30.32	0.69
$A\%_{Consecutive}$,%	0.47±1.82	6.60	1.42
Outlier-Excluder, “A%H” ,%	10.64±5.81	24.03	0.68
Outlier-Excluder, “A%M” ,%	12.13±7.22	30.32	0.69
Physician-led approach, %			
Physician1	7.38±3.31	14.70	0.62
Physician2	11.16±5.65	24.39	3.08
Physician3	8.29±3.19	15.06	2.32
COV, %	5.02±2.28	10.49	2.49

#Data are presented as means ± SD, and ULN, upper normal limit; PEF, peak expiratory flow; L/min, Liter per minute; A%M, amplitude percent mean; A%H, amplitude percent highest. N=30.

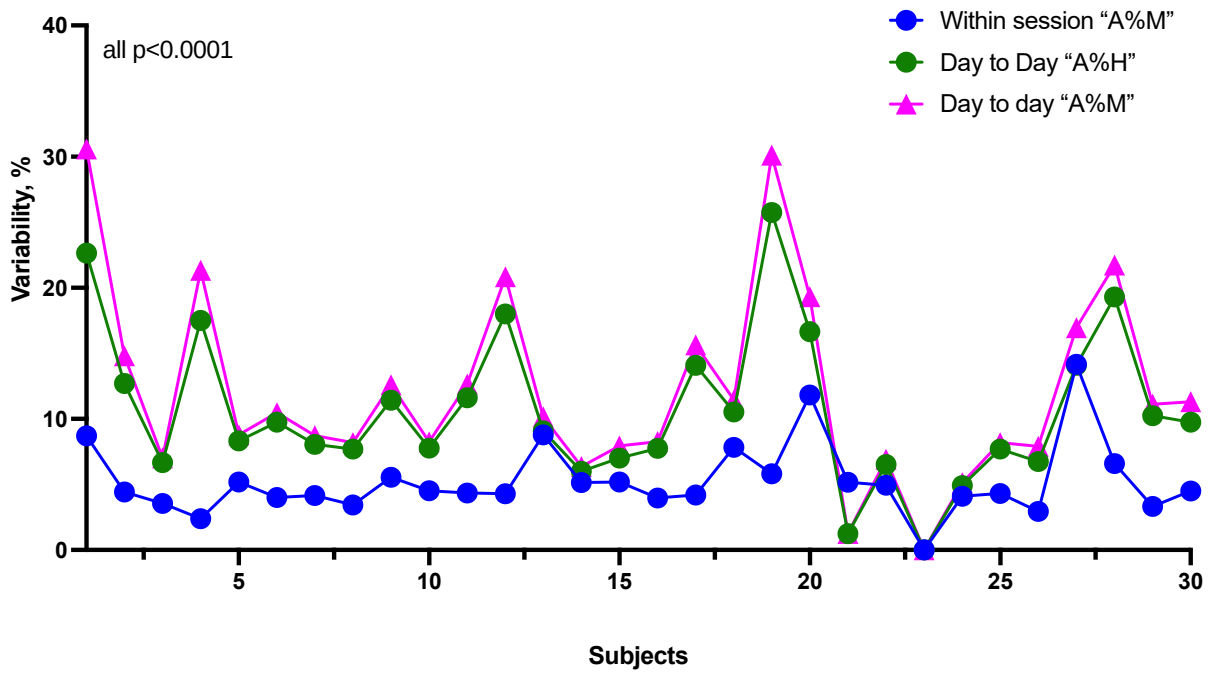


Figure 2: Within session and day-to-day PEF variability in all subjects. Within session produced lower variability %, compared to day-to-day (paired t-test, $p<0.0001$). Within session PEF variability was expressed as amplitude percent mean %; and day to day PEF variability expressed as amplitude percent highest, A%H, and amplitude percent mean A%M.

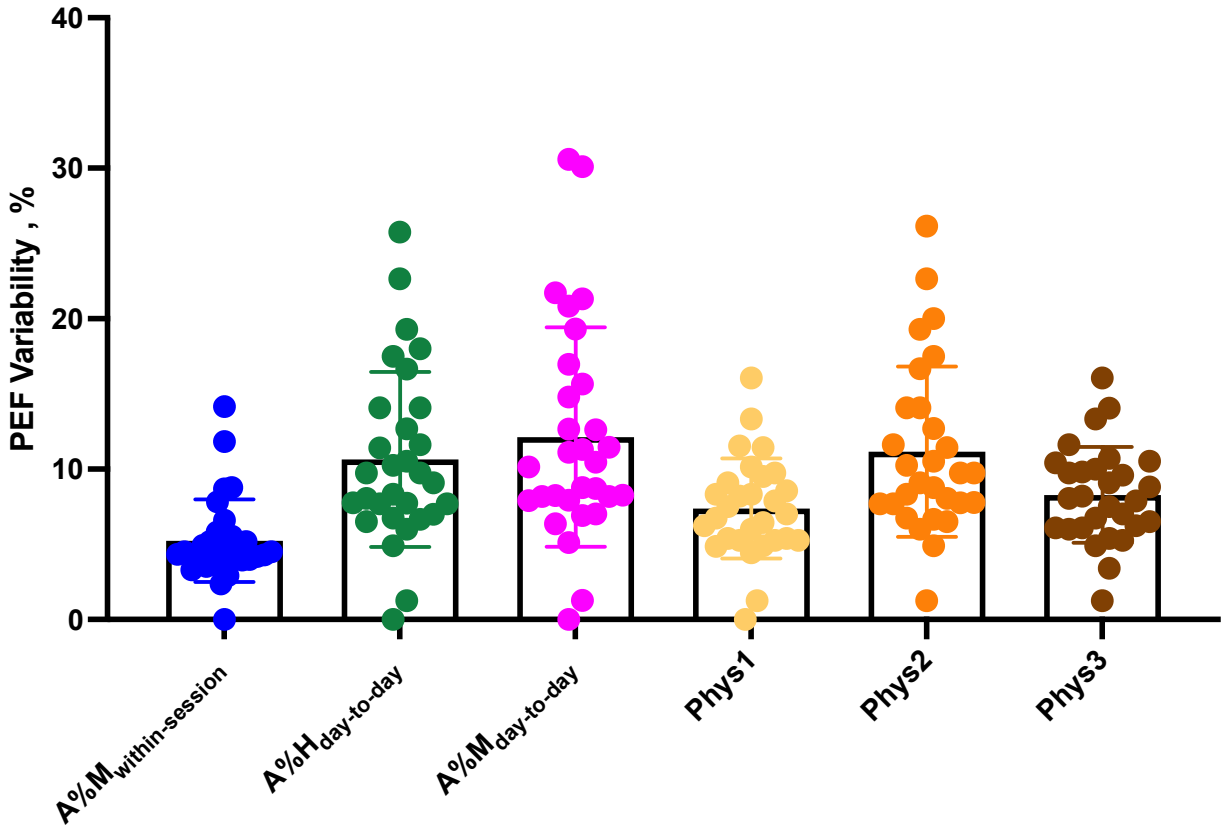


Figure 3: Scatter dot plots displaying day-to-day PEF variability, with mean ±SD.

PEF Variability: within session ($A\%M_{within\text{-}session}$), day-to-day using the highest and lowest expressed as % of highest ($A\%H_{day\text{-}to\text{-}day}$) and as % of the mean ($A\%M_{day\text{-}to\text{-}day}$), and physician led $A\%H_{day\text{-}to\text{-}day}$ for the three physicians (Phys1, Phys2 and Phys3).

There were significant differences in mean day to day PEF variability in $A\%H_{day\text{-}to\text{-}day}$ among the three physicians (paired t-test, all p values <0.05), N=30.

Correlations of PEF data, and anthropometric measurements

The mean absolute PEF was related to BMI ($p=0.002$), sex ($p<0.0001$), and height ($p<0.0001$) (Table 3).

There were no statistically significant correlations between within session variability $A\%M_{day\text{-}to\text{-}day}$, and age, height, or sex (all $p>0.05$). Day-to-day PEF variability expressed as $A\%H_{day\text{-}to\text{-}day}$, was not related to age, height, or sex (all $p>0.05$), however there were correlations with FEV_1 z-scores ($p=0.039$), and FEV_1/FVC z-scores ($p=0.027$) (Table 3).

Day-to-day PEF variability expressed as $A\%M_{day\text{-}to\text{-}day}$, was also unrelated to age, height, or sex (all $p>0.05$), but there were again significant correlations with FEV_1 z-scores ($p=0.04$), and FEV_1/FVC z-scores ($p=0.027$).

Table 3. Univariate correlations between PEF Data and anthropometric characteristics and spirometry

	Absolute PEF, L/min		Within Session, A%M %		Day-to-Day, A%H	
Parameter	rho	P-value	rho	P-value	rho	P-value
Age, yr	0.048	0.802	-0.152	0.422	0.095	0.616
Sex	-0.702	<0.0001	-0.170	0.370	0.008	0.968
BMI, kg/m2	0.579	0.002	-0.019	0.925	-0.051	0.802
Height, cm	0.710	<0.0001	0.019	0.924	-.102	0.606
FEV_1, z-score	0394	0.063	-0.216	0.321	-0.433	0.039
FVC, z-score	0.362	0.090	-0.116	0.599	-0.126	0.598
FEV_1/FVC, z-score	0.302	0.161	-0.093	0.673	-0.461	0.027

#Univariate correlations using Spearman's correlation coefficient. BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity. Within session variability was expressed as amplitude percent mean; and day to day as amplitude percent highest.

3.6 Discussion

In this study, day-to-day variability in PEF was measured in 30 healthy non-smoker adults, using 3 different methods that have been used in past published studies, Additionally, the relationships of PEF variability with sex, age, and height were also examined. The results showed that the upper limit of normal for day-to-day variability in PEF expressed as amplitude percent highest ($A\%H_{day\text{-}to\text{-}day}$), a method that is used commonly in clinic practice, is 24%. The magnitude of variability is highly dependent on methodology used for calculation e.g. ULN varying from 6.6% for variability between consecutive days ($A\%_{consecutive}$) up to 30% of best calculated for highest versus lowest as % of overall mean ($A\%M_{day\text{-}to\text{-}day}$). Variability also differs between the way 3 physicians normally determine variability in routine clinical practice, and varies greatly between healthy individuals. It was also found that the day-to-day variability in PEF is unrelated by sex, age, and height, but is related to FEV_1 and FEV_1/FVC z-scores.

To the best of our knowledge, this is the first study of data on day-to-day variability in PEF, in healthy non-smoker adults, calculated in a manner that is used in routine clinical practice i.e. $A\%H_{day\text{-}to\text{-}day}$ as well as with other methods that have been described in the literature. The findings of the present study showed that when using amplitude percent highest $A\%H_{day\text{-}to\text{-}day}$, variability in PEF resulted in a mean of 10.64% , and an upper limit of normal of 24%. Within session variability resulted in a mean of 5.24%, with an upper limit of normal of 12.88% . The results of the present study offer potential insights in using PEF recording to help distinguish asthma from non-asthma. A day-to-day to day variability in PEF of 24% of the highest value or less, suggests normal fluctuations in airway caliber. It's reasonable to expect that day to day variability could be even higher in asthmatic individuals in whom asthma is active or uncontrolled, since asthma is

characterised by airway hyperresponsiveness, and spontaneous bronchoconstriction, which could lead to even more pronounced changes in daily PEF values.

The day-to-day variability in the present study was calculated and expressed as amplitude percent highest, as well as by other methods. One study has suggested that calculating PEF variability by averaging the daily difference from the overall mean value, or COV were the preferred method since it provided greatest separation between 221 participants with wheeze or asthma diagnosis, from 121 participants that had neither(8). In the current study, variability was expressed in 6 different ways; 4 of which have been used in published studies ($A\%M_{day\text{-}to\text{-}day}$, $A\%H_{day\text{-}to\text{-}day}$, $A\%_{Consecutive}$ and COV). We also had 3 respiratory physicians who had clinical interests in the management of airways diseases, calculate $A\%H_{day\text{-}to\text{-}day}$ as they normally would in clinical practice, based on paper graphs. The mean $A\%H_{day\text{-}to\text{-}day}$ for the three physicians differed significantly (7.38%, 11.16% and 8.29%), with the upper limits of normal of (14.7%, 24.4% and 15.1%, respectively). The differences between the physicians were due to their exclusion of values that they felt were outlying or 'non-representative' values. Boezen et al. found that the first PEF recording to be consistently lower than the remainder, which they suggested was likely a training effect (9). The recordings from the first day or two could thus have a significant effect on variability calculated as $A\%H_{day\text{-}to\text{-}day}$. This suggests that at least the first one or two recordings should be ignored. This possibly or probably typifies the variations and subjectivity in interpretation of graphical PEF data between health care professionals. Figure 3 suggests that physician 2 did not sensor any data at all, since there is no apparent effect on the range of $A\%H_{day\text{-}to\text{-}day}$, compared to the objective calculations. Since the 'Outlier-Excluder' analysis had no effect on variability, this suggests that physician's 1 and 3 were excluding values within a ±3SD range. $A\%H_{day\text{-}to\text{-}day}$ is solely dependent on 2 values and the fact that this will therefore, be strongly affected by single outlying

values, should be recognised by any clinicians who use this method of analysis. It is thus, the reasoning behind subjectively censoring peak flow diary data during clinical interpretation, in an attempt to eliminate 'aberrant or unreliable' measurements, albeit subjectively.

Using an averaging method ($A\%M_{day\text{-}to\text{-}day}$) to quantify variability uses all of the data and minimises the effects of extreme values. It is thus less affected by any data censoring by a clinicians. However, the calculation is onerous and impractical for medical staff to calculate from hardcopy diaries, which is the routine practice for PEF recordings. Electronic PEF recordings with electronic devices are too expensive for single patient use and so it is unlikely that $A\%M_{day\text{-}to\text{-}day}$ is going to be used outside the research setting.

In the current study, day-to-day variation in the highest morning PEF was also reported to assess the fluctuations in the highest recorded PEF across mornings. H.M. Boezen et al. reported mean day-to-day variation in morning PEF of 0 0.08%, in a random population sample of 511 participants over 7 days (9). In the current study, we found mean day-to-day variation in the highest morning PEF to be 0.47% in healthy adults. The variability in the Boezen et al. study seems very low but this may reflect the large numbers and short duration of recordings.

There was wide range of day-to-day variability between participants, which was not predicted by demographics or by spirometry. We found that variability was unrelated to sex, height, or age. The published data on the effect of age on PEF variability is however, conflicting. There is no data on day-to-day PEF variability, however, 2 studies reported associations between diurnal variability, and age (3, 23), while 2 others did not (3, 24) . Evidence suggests that diurnal PEF variability starts to increase with age, in individuals of 70 years of age and older (23) (25). In this study, we did not observe any possible trend towards increasing day-to-day variability with age, however, the

dissociation with age in the current study might be explained by the small numbers and the fact that the participants were, on average 10 years younger when compared with previous studies.

To explore the impact of sample size on the precision of results for peak expiratory flow (PEF) variability measurements, a simulation was created using a random number generator to produce values simulating variation in PEF readings (e.g., 500, 450, 400, and 425) and reflecting the day-to-day variability seen in PEF values. These simulated ranges were then used to calculate variability as a percentage of the best PEF value. The coefficient of repeatability (CR), which measures how much an individual's repeated measures vary (in this case, peak expiratory flow (PEF), was assessed along with the effect of sample size on the precision of the 95% confidence interval (CI). The CR for a sample size of n = 17 was found as 15, and this value remained unchanged at 15 when the sample size was doubled to n = 34. This indicates that the CR is stable and not influenced by increasing the number of participants. Furthermore, despite the increase in sample size, the observed 95% CI for the variability percentage (approximately 20%) remained unchanged (did not significantly narrow). This suggests that increasing the sample size does not significantly affect the variability of PEF measurements.

Figure 3 indicates the wide range of variability between subjects. Whether this reflects true variability in airway caliber or whether it is variation in technique or effort is uncertain. This wide range in healthy subjects is also clinically relevant since A%H of 25% might be abnormal in one individual whose ‘inherent’ or ‘normal’ variability might be 5%. This is another characteristic of PEF variability that should be borne in mind by clinicians using PEF diaries in their patients.

Day to day variability in PEF was also found to be unaffected by sex. Sex related differences in PEF diurnal variability have been found similar between females, and males in some studies (11) (26), while other studies observed females having higher diurnal variability than males (9, 24). In

those studies however, they studied diurnal variability in random population of adults, and also may have been the result of females having a history of asthma and being older compared to males.

3.7 Limitations

The choice to sample from Woolcock institute staff and students was driven by accessibility, and practicability over a short period of time. This sampling may introduce potential biases, participants may be more hesitant to disclose sensitive behaviors, such as smoking weed or vaping, due to concerns about social or professional implications. However, Smoking history was screened for all participants to ensure that only current non-smokers or those with less than a 5-pack-year history were included. Additionally, Woolcock institute staff and students may be more familiar with the techniques and methods of peak flow measurements due to their background, which could also lead to more accurate or consistent data collection compared to participants with less experience. The sample may not fully represent the general, and future studies may benefit from expanding the sample to include individuals outside of the academic environment (reduce biases associated with familiarity with the methods).

The time at which peak flow is recorded, and the number of measurements taken per day may have an impact on the calculated variability. In healthy individuals, and in asthma, PEF follows circadian rhythm, where the peak occurring at mid, and late afternoons, and the lowest at early mornings (7, 27). In the current study, participants measured PEF upon waking, which it is acknowledged that might have resulted in lowest PEF values being recorded, as PEF is generally lowest in the early morning, however, one study showed that measurements taken upon waking up give a good approximation of true PEF variability (7). It was also observed that students generally woke up one to two hours later than senior clinicians and staff, who maintained earlier waking times even on weekends. However, this timing should not significantly impact PEF variability. For

assessing day-to-day variability, single, time-standardised measurements are optimal, participants were instructed to standardise their recording times to ensure that observed changes reflect actual physiological change rather than the effects of timing. Additionally, The frequency of PEF measurements per day to calculate variability is still not definitive, and studies on diurnal variability have varied from two to more than ten readings per day (7, 9). There are also certain limitations to keeping personal peak flow diary at home, such as potential errors in technique or inconsistency by individuals. However, all diaries provided to subjects had the instructions written on them and one on one education was provided to all participants. All subjects returned their PEF diary, with satisfactory recordings, however we can never discount the possibility of fabricated entries. Finally, we also acknowledge the relatively small sample size.

3.8 Conclusion

Home monitoring peak expiratory flow is a valuable tool for monitoring, and managing asthma. Obtaining reference values for day to day variability of PEF in healthy adults is useful, it provides a baseline for assessing and interpreting PEF data for individuals with asthma. The findings from the study showed that day-to-day PEF variability greater than 24% and within-session variability above 12% can be useful for screening asthma, these could help identify individuals with asthma-related variability in clinical settings. It will be necessary however to establish a consensus or protocol on PEF monitoring to ensure reliability, and comparability of results across studies. The method of quantifying PEF variability in routine clinical practice is limited by practical considerations i.e. the time required to manually calculate $A\%M_{day\text{-}to\text{-}day}$ versus the ease and quickness of $A\%H_{day\text{-}to\text{-}day}$, and has a large effect on the quantification on day-to-day variability and on differences between individuals. There is also a large range of variability in peak flow in health individuals, which is relevant to clinical practice.

3.9 References

1. Cross D, Nelson HS. The role of the peak flow meter in the diagnosis and management of asthma. J Allergy Clin Immunol. 1991;87(1 Pt 1):120-8.
2. Quackenboss JJ, Lebowitz MD, Krzyzanowski M. The normal range of diurnal changes in peak expiratory flow rates. Relationship to symptoms and respiratory disease. Am Rev Respir Dis. 1991;143(2):323-30.
3. Higgins BG, Britton JR, Chinn S, Lai KK, Burney PG, Tattersfield AE. Factors affecting peak expiratory flow variability and bronchial reactivity in a random population sample. Thorax. 1993;48(9):899-905.
4. Bateman JR, Clarke SW. Sudden death in asthma. Thorax. 1979;34(1):40-4.
5. Guidelines for the diagnosis and management of asthma. National Heart, Lung, and Blood Institute. National Asthma Education Program. Expert Panel Report. J Allergy Clin Immunol. 1991;88(3 Pt 2):425-534.
6. Mauer Y, Taliercio RM. Managing adult asthma: The 2019 GINA guidelines. Cleve Clin J Med. 2020;87(9):569-75.
7. Hetzel MR, Clark TJ. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. Thorax. 1980;35(10):732-8.
8. Higgins BG, Britton JR, Chinn S, Jones TD, Jenkinson D, Burney PG, et al. The distribution of peak expiratory flow variability in a population sample. Am Rev Respir Dis. 1989;140(5):1368-72.
9. Boezen HM, Schouten JP, Postma DS, Rijcken B. Distribution of peak expiratory flow variability by age, gender and smoking habits in a random population sample aged 20-70 yrs. Eur Respir J. 1994;7(10):1814-20.
10. Neukirch F, Liard R, Segala C, Korobaeff M, Henry C, Cooreman J. Peak expiratory flow variability and bronchial responsiveness to methacholine. An epidemiologic study in 117 workers. Am Rev Respir Dis. 1992;146(1):71-5.
11. Aggarwal AN, Gupta D, Chaganti S, Jindal SK. Diurnal variation in peak expiratory flow in healthy young adults. Indian J Chest Dis Allied Sci. 2000;42(1):15-9.
12. Casale R, Pasqualetti P. Cosinor analysis of circadian peak expiratory flow variability in normal subjects, passive smokers, heavy smokers, patients with chronic obstructive pulmonary disease and patients with interstitial lung disease. Respiration. 1997;64(4):251-6.

13. Clark TJ, Hetzel MR. Diurnal variation of asthma. Br J Dis Chest. 1977;71(2):87-92.

14. Gannon PF, Newton DT, Pantin CF, Burge PS. Effect of the number of peak expiratory flow readings per day on the estimation of diurnal variation. Thorax. 1998;53(9):790-2.

15. Gibson PG, Wlodarczyk J, Hensley MJ, Murree-Allen K, Olson LG, Saltos N. Using quality-control analysis of peak expiratory flow recordings to guide therapy for asthma. Ann Intern Med. 1995;123(7):488-92.

16. Lambert PC, Burton PR, Abrams KR, Brooke AM. The analysis of peak expiratory flow data using a three-level hierarchical model. Stat Med. 2004;23(24):3821-39.

17. Brand PL, Duiverman EJ, Waalkens HJ, van Essen-Zandvliet EE, Kerrebijn KF. Peak flow variation in childhood asthma: correlation with symptoms, airways obstruction, and hyperresponsiveness during long-term treatment with inhaled corticosteroids. Dutch CNSLD Study Group. Thorax. 1999;54(2):103-7.

18. Enright PL, Lebowitz MD, Cockroft DW. Physiologic measures: pulmonary function tests. Asthma outcome. Am J Respir Crit Care Med. 1994;149(2 Pt 2):S9-18; discussion S9-20.

19. Venables KM, Burge PS, Davison AG, Newman Taylor AJ. Peak flow rate records in surveys: reproducibility of observers' reports. Thorax. 1984;39(11):828-32.

20. Stanojevic S, Kaminsky DA, Miller MR, Thompson B, Aliverti A, Barjaktarevic I, et al. ERS/ATS technical standard on interpretive strategies for routine lung function tests. European Respiratory Journal. 2022;60(1):2101499.

21. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. European Respiratory Journal. 2005;26(5):948-68.

22. Covar RA, Szefler SJ, Martin RJ, Sundstrom DA, Silkoff PE, Murphy J, et al. Relations between exhaled nitric oxide and measures of disease activity among children with mild-to-moderate asthma. J Pediatr. 2003;142(5):469-75.

23. Boezen HM, Schouten JP, Postma DS, Rijcken B. Relation between respiratory symptoms, pulmonary function and peak flow variability in adults. Thorax. 1995;50(2):121-6.

24. Künzli N, Stutz EZ, Perruchoud AP, Brändli O, Tschopp JM, Bolognini G, et al. Peak flow variability in the SAPALDIA study and its validity in screening for asthma-related conditions. The SPALDIA Team. Am J Respir Crit Care Med. 1999;160(2):427-34.

25. Higgins BG, Britton JR, Chinn S, Cooper S, Burney PG, Tattersfield AE. Comparison of bronchial reactivity and peak expiratory flow variability measurements for epidemiologic studies. Am Rev Respir Dis. 1992;145(3):588-93.
26. Borsboom GJ, van Pelt W, van Houwelingen HC, van Vianen BG, Schouten JP, Quanjer PH. Diurnal variation in lung function in subgroups from two Dutch populations: consequences for longitudinal analysis. Am J Respir Crit Care Med. 1999;159(4 Pt 1):1163-71.
27. Iwasaki Y, Kubota Y, Yokomura I, Ueda M, Hashimoto S, Hara H, et al. Timing of lowest and highest peak expiratory flow in patients with asthma: influence of anti-inflammatory treatment. Respir Med. 2000;94(4):385-90.

Chapter 4. Relationship Between Concavity Of The Flow-Volume Loop And Small Airway Measures

4.1 Abstract

Background

There is increasing evidence of small airway abnormalities in smokers despite normal spirometry. The concavity in the descending limb of the maximum expiratory flow curve (MEFV) is a recognised feature of obstruction and can provide information beyond FEV_1, and potentially early smoking-related damage. We aimed to evaluate concavity measures compared to known small airway measurements.

Methods

Eighty smokers with normal spirometry had small airway function assessed: multiple breath nitrogen washout (MBNW) from which ventilation heterogeneity in the diffusion-dependent acinar (Sacin) and convection-dependent conductive (Scond) airways were assessed, and impulse oscillometry system (IOS) from which respiratory resistance and reactance at 5 Hz (Rrs and Xrs) were measured. Concavity measures were calculated from the MEFV, partitioned into global and peripheral concavity.

Results

We found abnormal peripheral and global concavity as well as acinar ventilation heterogeneity are common in “normal” smokers. Concavity measures were not related to either MBNW or IOS measurements.

Conclusion

Abnormalities in concavity indices and MBNW or oscillometry parameters are common in smokers despite normal spirometry. However, these measures likely reflect different mechanisms of peripheral airway dysfunction.

4.2 Introduction

Spirometry is the "gold standard" measure of airflow obstruction, which is defined as a FEV_1/FVC less than the lower limit of normal (1). In early chronic obstructive pulmonary disease (COPD), terminal bronchiole obliteration is the initial abnormality, but will not affect the FEV_1/FVC until around 2/3 of these small airways are lost since the small airways (less than 2 mm in diameter) account for little of the total airflow resistance (2).

Johns and colleagues, described two indices for estimating concavity of the expiratory limb of the flow volume curve as potential clinically useful measures of small airways disease. They defined a global index as the forced expiratory flows at 50% of the FVC (FEF50), and a peripheral index based on the FEF75, both expressed as ratios of the theoretical flows at 50% and 75% of FVC respectively, (fig.1) interpolated from a straight line between peak flow and RV (3). Since there are currently complex measures of lung function that are putatively sensitive to small airway function, in particular oscillometry and multiple breath nitrogen washout (MBNW), it would be useful to know how these may relate to these global and peripheral spirometric indices.

MBNW is a complex measures of ventilation heterogeneity, where Scond is an index of this in peripheral conducting (convection dependent) airways and Sacin is an index related to the even more peripheral acinar (convection-diffusion dependent) airways. Oscillometry is another complex measure, of the respiratory system resistance (4) and reactance (Xrs), reflecting especially airway caliber and oscillatory stiffness of the respiratory system, respectively. Both Rrs and Xrs, but in particular Xrs are sensitive to small airway dysfunction (4). Results from a recent study show that 75% of smokers with normal spirometry have an abnormality in at least one MBNW or oscillometry index (5).

Our aim was to examine relationships between concavity measures and both MBNW and oscillometry parameters in current or ex-smokers with normal FEV_1/FVC ratio from the aforementioned study (5). We also examined concordance in abnormal results between these tests. We hypothesized that the global concavity index related to Scond and Rrs, while the peripheral index related to Sacin and Xrs.

4.3 Methods

Study design and subjects

We analyzed previously published data of eighty current or ex-smokers (≤ 50 years of age) who had>5 pack/year smoking history but normal spirometry. The original study protocol was approved by the Northern Sydney Central Coast Area Health Service Human Research Ethics Committee (Protocol 1106- 209M). Written informed consent was obtained from all recruited patients. No clinical patient data were used and therefore no administrative permissions were required.

At enrollment, inclusion criteria were no history of past/present respiratory diseases or use of respiratory medications and having normal spirometry defined by post bronchodilator FEV_1/ FVC ratio > LLN and FEV_1 > LLN.

Respiratory symptoms including cough, shortness of breath, and wheeze were documented via questionnaire. Each subject was tested in a single session at the Woolcock Institute of Medical Research or Royal North Shore Hospital, Sydney, Australia.

Lung function testing

Subjects performed spirometry according to the ATS/ERS guidelines from which global and peripheral concavity indices were calculated from the maximum expiratory flow-volume curve (MEFV), as described above (3). The upper limit of normal for post bronchodilator central concavity index is 34.8% in males and 26.3% in females; the upper limits of normal for peripheral concavity index is 61.2% in males and 63.1% in females (3).

Respiratory system impedance measures were made using a Jaeger Masterscope CT IOS (CareFusion, Hoechberg, Germany), from which Rrs and Xrs at 5 Hz (R5 and X5) were calculated. The MBNW test was performed using an in-house-built device as previously described (5). We

used published upper and lower limits of normal to define abnormality of MBNW (6), oscillometry (7) and spirometry (8) indices.

4.4 Statistical Analysis

Statistical analysis was carried out with SPSS (IBM, SPSS, v26), relationships between parameters were examined by Spearman correlation coefficients. Concordance in abnormal function was evaluated using Cohen's kappa. A p value of <0.05 was considered statistically significant.

4.5 Results

Subject characteristics

The demographics data for males (n=51) and females (n=29) are summarized in Table 1. Overall, abnormal peripheral concavity was found in 19/80 (23.8%) smokers and abnormal global concavity in 22/80 (27.5%) smokers.

Abnormal Sacin was found in 33 (41.3%) smokers and abnormal Scond in 19 (23.8%) smokers. Only 1 and 5 participants had abnormal Rrs and Xrs, respectively.

Correlations between concavity indices, small airway measures, spirometry, age, and smoking history

Global concavity was unrelated to either MBNW (rs =0.10, p=0.34 and rs =0.08, p=0.47 for Scond and Sacin, respectively) or impedance parameters (rs =0.10, p=0.35 and rs =0.02, p=0.84 for Rrs and Xrs, respectively) (Table 2, Figure 2).

Peripheral concavity was also unrelated to MBNW (rs =0.09, p=0.39 and rs =0.20, p=0.07 for Scond and Sacin, respectively). Peripheral concavity was unrelated to Xrs (rs = -0.09, p=0.38), but weakly correlated with Rrs (rs =0.27, p=0.01) (Table 2, Figure 2).

Global concavity was related to age (rs =0.27, p=0.01). Peripheral concavity was related to both height (rs = -0.26, p=0.02) and age (rs =0.58, p<0.001). Global concavity was unrelated to smoking history (rs =0.04, p=0.67), while peripheral concavity was related (rs =0.38, p=0.001). There was poor concordance between concavity indices and either MBNW parameters or IOS parameters, with kappa values ranging between -0.09 and 0.25 (Table 3).

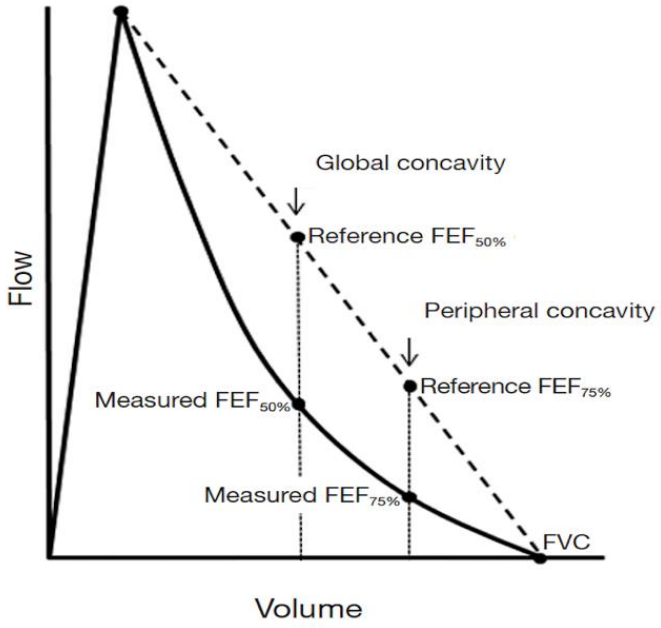


Figure 1: Global and peripheral concavity indices are calculated from the maximum expiratory flow-volume curve (MEFV).

Global concavity is based on forced expiratory flow at 50%, and peripheral concavity is based on forced expiratory flow at 75%, of the expired FVC. The degree of concavity is compared to the "normal" reference flows at $EFE_{50\%}$ and $EFE_{75\%}$.

Table 1: Baseline characteristics, spirometry of smokers (n=80)

Parameters	
Sex (M/F)	51/29
Age (years)	43 (11)
Height (cm)	175 (10)
BMI (kg/m2)	25.2 (4.4)
Smoking (pack-years)	17.7 (10.3)
Past/current smokers	21/59
FEV_1, (% predicted)	98 (10)
FEV_1, (Z-score)	-0.13 (0.81)
FVC, (% predicted)	105 (12)
FVC, (Z-score)	0.37 (0.90)
FEV_1/FVC ,Ratio	76 (4)
FEV_1/FVC, (Z-score)	-0.80 (0.90)
FEF_{25-75}, (% predicted)	90 (23)
FEF_{25-75} ,(Z-score)	-0.40 (0.81)
Global concavity, (%)	17 (25)
Peripheral concavity, (%)	45 (24)

Data are presented as mean ± (SD).
BMI, body mass index, $FEF_{25–75\%}$, forced expiratory flow between 25 and 75% of FVC; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity

Table 2: Univariate correlations (Spearman correlation coefficients) between concavity indices, small airway measures, spirometry, age, and smoking history (n=80).

Parameters	Global Concavity, %		Peripheral Concavity, %	
	rho	P-value	rho	P-value
Scond	0.10	0.34	0.09	0.39
Sacin	0.08	0.47	0.20	0.07
Rrs	0.10	0.35	0.27	0.01
Xrs	0.02	0.84	-0.09	0.38
FEV_1/FVC	-0.64	<0.001	-0.54	<0.001
Age	0.26	0.01	0.58	<0.0001
Pack/Year	0.04	0.67	0.38	0.001

Scond, convection-dependent airways; Sacin, diffusion-dependent airways; Rrs, resistance; Xrs, reactance; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity. *A p value of <0.05 is statistically significant.

Table 3: Abnormality overlap and concordance using Cohen's Kappa between concavity indices and small airway measures in smokers (n=80)

Parameters	Global Concavity, %			Peripheral Concavity, %		
	Kappa	P-value	Abnormality Overlap	Kappa	P-value	Abnormality Overlap
Scond	0.24	0.02	9	0.25	0.02	8
Sacin	0.05	0.63	10	0.18	0.07	11
Rrs	0.06	0.10	1	0.07	0.07	1
Xrs	0.05	0.51	2	-0.09	0.24	0

Scond, convection-dependent airways; Sacin, diffusion-dependent airways; Rrs, resistance; Xrs, reactance; FEV1, forced expiratory volume in 1s; FVC, forced vital capacity. *A p value of <0.05 is statistically significant.

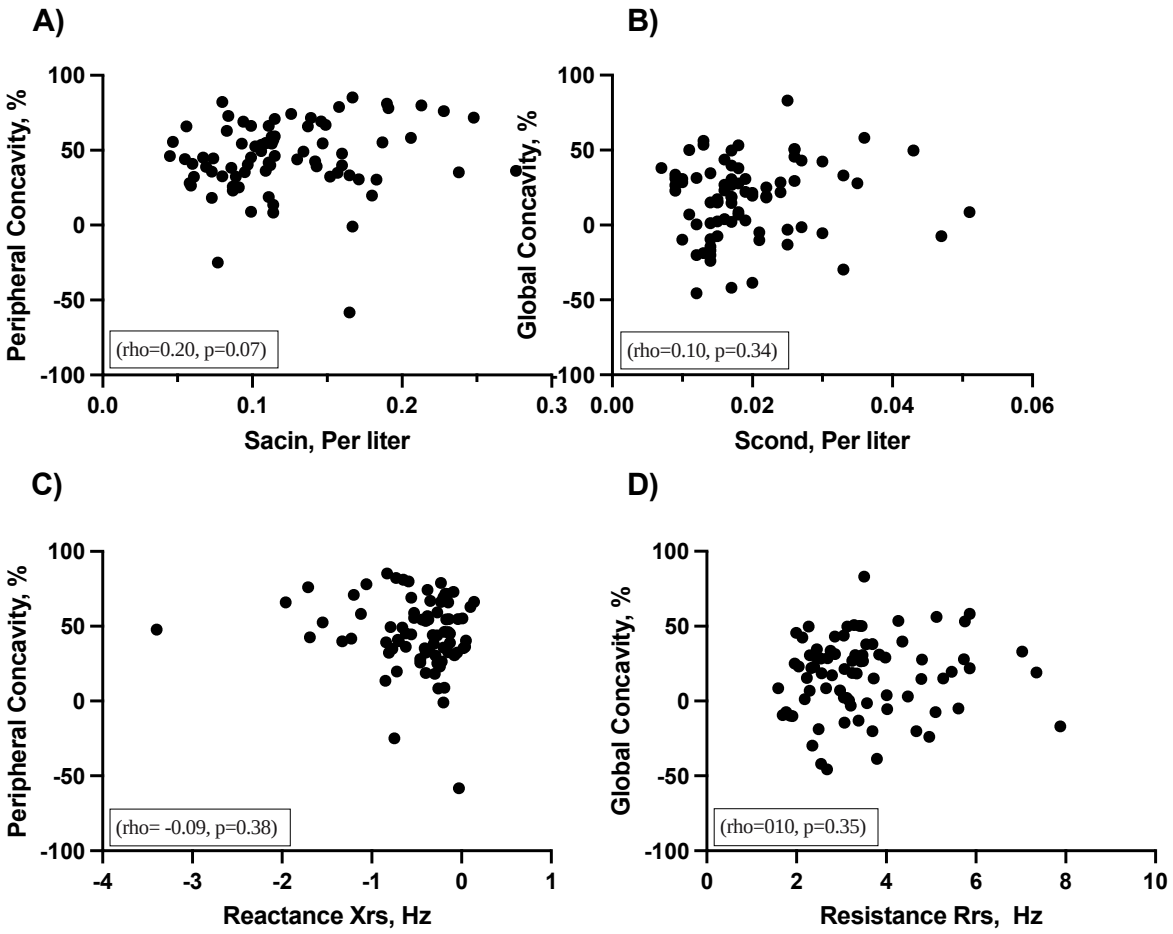


Figure 2: Correlations of concavity indices, and small airway measures

No significant correlation between peripheral concavity and Sacin (A), global concavity and Scond (B), peripheral concavity and reactance (Xrs) (C), global concavity and resistance (Rrs) D).

4.6 Discussion

We found in this cohort of younger smokers with normal FEV_1/FVC ratio, that both global and peripheral concavity indices picked up abnormalities in about a quarter of individuals, which is a similar finding to a previous unrelated evaluation (3). However, concavity of the expiratory limb of the maximal flow-volume was unrelated to MBNW and impedance parameters and consequently there was also poor concordance between these tests in detecting abnormal airway function. Paradoxically, abnormalities in both Sacin and Scond were as prevalent as in global and peripheral indices indicating that they were all sensitive parameters, but the abnormalities did not occur in the same individuals, thus the poor concordance, suggesting that they are picking up different and quite subtle pathophysiological abnormalities. Impedance on the other hand was much less frequently abnormal. Interestingly, peripheral concavity related to smoking history, while global did not.

Ventilation in obstructive airways diseases is characterized by nonuniform lung emptying which results in reduced mid-expiratory flows in spirometry. This is manifest visually by development of concavity of the descending limb of the flow-volume curve (9). Reduced mid-expiratory flows correlate with greater small airways disease, measured histologically in smokers (10) but is also a normal effect of ageing. The increase in lung compliance (11) with ageing explains at least part of the relationships that both concavity indices have with age. In addition, the peripheral, but not global index, correlated with smoking history. However, it is difficult to determine whether this is due partly or wholly to ageing, since age was, not surprisingly, related to pack/year history ($rs = 0.50$, $p < 0.001$).

Further, in multiple regression analysis including age and height, smoking history was no longer a significant predictor of peripheral concavity which suggests perhaps the need to develop age- and height-related prediction equations for these parameters.

There were differences between current and ex-smokers in our dataset, of the 80 smokers, 21 were former smokers. The Scond, Sacin, IOS parameters, and concavity indices were lower (i.e. less deranged) compared to that in the smoker group. Given that current smokers evidence of more peripheral airway dysfunction than ex-smokers, we suggest that abnormalities in these indices may be related ongoing smoking. The only MBNW or IOS parameter that correlated with smoking history was Sacin ($r = 0.23$, $P = 0.03$). These finding are in agreement with the previous work of Verbanck et al. In Verbanck study, she found that abnormities in Scond and Sacin occur in the absences of reduction in FEV_1/FVC ratio and that with increasing pack-years of smoking, increased Scond and Sacin indicate early small airway changes (12).

The clinical usefulness of mid-expiratory flows in obstructive airways disease is uncertain. There is high concordance between $FEF_{25-75\%}$ and FEV_1/FVC ratio in detecting airflow obstruction but only 2.9% having normal $FEF_{25-75\%}$ when the FEV_1/FVC is reduced (13). However, FEF75 was normal in 12.3% despite a reduced FEV_1/FVC (13) though this may be due to abnormality in the more proximal airways and not really true COPD (3).

A strong correlation between global and peripheral indices and FEV_1/FVC was evident in our study ($rs = -0.64$, $p < 0.001$ and $rs = -0.54$, $p = < 0.001$, respectively) (Table 2), which suggests that they are looking at the same phenomenon but concavity is more sensitive. Mid-expiratory flows have not yet been shown to be predictive of COPD. Part of the problem may be due to its high within- and between-individual variability, and its high prevalence of abnormality in smokers (14) (15) most of whom do not develop COPD as currently defined by FEV_1/FVC. In contrast, imaging

measures of small airways disease, lung clearance index, diffusing capacity for carbon monoxide, airway wall thickening and emphysema predict greater loss of FEV_1 (16, 17)

The lack of relationship between concavity indices and MBNW or oscillometry parameters could be because spirometry is a forced manoeuvre, while MBNW and oscillometry are tidal breathing tests. If smoking altered parenchymal interdependence and airway compliance, this might translate to abnormality of forced manoeuvres but not necessarily in oscillometry. The similar prevalence of abnormality in concavity indices and MBNW parameters, but with very little overlap, is difficult to explain but suggests they represent differing functional abnormalities. The prognostic implications in terms of FEV_1 decline of concavity indices, oscillometry and MNBW parameters are yet to be established but are part of ongoing longitudinal cohort research.

4.7 Limitations

Although we found a high prevalence of abnormal concavity indices in this cohort, this was likely due to subject selection i.e. smokers some of whom had symptoms. Secondly, the upper limits for the concavity indices were derived healthy subjects of mean age 59 (range 40–87) years, which is significantly older than our study cohort. There may have also been differences due to geographical location (Tasmania versus Sydney). Arguably, if upper limit of normal values had been determined from a younger cohort, closer in age to this study cohort, the prevalence of abnormality may have been higher. Finally, although we found associations between concavity indices and age, height and smoking history, they were weak in nature and several correlations were explored. However, the lack of associations between concavity indices, oscillometry and MBNW parameters may also be due to the relatively small sample size given the inherent variability between tests. Given the relatively small size of this cohort, it is not possible to generalize these results and associations require further exploration.

4.8 Conclusion

Abnormalities in both concavity indices and MBNW parameters are common in smokers with normal spirometry, but infrequent using oscillometry. However, it is somewhat disappointing that there is poor concordance between these different tests in detecting abnormal function in this group of subjects, suggesting that they likely represent different aspect of lung and airway dysfunction.

4.9 References

1. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. Respirology (Carlton, Vic). 2017;22(3):575-601.
2. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. N Engl J Med. 1968;278(25):1355-60.
3. Johns DP, Das A, Toelle BG, Abramson MJ, Marks GB, Wood-Baker R, et al. Improved spirometric detection of small airway narrowing: concavity in the expiratory flow-volume curve in people aged over 40 years. Int J Chron Obstruct Pulmon Dis. 2017;12:3567-77.
4. Bhatawadekar SA, Leary D, de Lange V, Peters U, Fulton S, Hernandez P, et al. Reactance and elastance as measures of small airways response to bronchodilator in asthma. J Appl Physiol (1985). 2019;127(6):1772-81.
5. Jetmalani K, Thamrin C, Farah CS, Bertolin A, Chapman DG, Berend N, et al. Peripheral airway dysfunction and relationship with symptoms in smokers with preserved spirometry. Respirology. 2018;23(5):512-8.
6. Jetmalani K, Chapman DG, Thamrin C, Farah CS, Berend N, Salome CM, et al. Bronchodilator responsiveness of peripheral airways in smokers with normal spirometry. Respirology. 2016;21(7):1270-6.
7. Oostveen E, Boda K, Van Der Grinten CPM, James AL, Young S, Nieland H, et al. Respiratory impedance in healthy subjects: baseline values and bronchodilator response. The European respiratory journal. 2013;42(6):1513-23.
8. Quanjer P, Stanojevic S, Cole T, Baur X, Hall GL, Culver B, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: The global lung function 2012 equations. The European respiratory journal. 2012;40(6):1324-43.
9. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. European Respiratory Journal. 2005;26(2):319-38.
10. Berend N, Woolcock AJ, Marlin GE. Correlation between the function and structure of the lung in smokers. Am Rev Respir Dis. 1979;119(5):695-705.
11. Mittman C, Edelman NH, Norris AH, Shock NW. Relationship between chest wall and pulmonary compliance and age. Journal of Applied Physiology. 1965;20(6):1211-6.

12. Verbanck S, Schuermans D, Meysman M, Paiva M, Vincken W. Noninvasive assessment of airway alterations in smokers: the small airways revisited. Am J Respir Crit Care Med. 2004;170(4):414-9.
13. Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW. Measurement of FEF25-75% and FEF75% does not contribute to clinical decision making. Eur Respir J. 2014;43(4):1051-8.
14. Kwon DS, Choi YJ, Kim TH, Byun MK, Cho JH, Kim HJ, et al. FEF(25-75%) Values in Patients with Normal Lung Function Can Predict the Development of Chronic Obstructive Pulmonary Disease. Int J Chron Obstruct Pulmon Dis. 2020;15:2913-21.
15. Wafy S, Agamy G, Ali A. Early spirometric changes in asymptomatic smokers; is it a time dependent? European Respiratory Journal. 2016;48:PA4320.
16. Mohamed Hoesein FA, de Jong PA, Lammers JW, Mali WP, Schmidt M, de Koning HJ, et al. Airway wall thickness associated with forced expiratory volume in 1 second decline and development of airflow limitation. Eur Respir J. 2015;45(3):644-51.
17. Zaigham S, Wollmer P, Engström G. The Association of Lung Clearance Index with COPD and FEV(1) Reduction in 'Men Born in 1914'. Copd. 2017;14(3):324-9.

Chapter 5. Phantom Lung Model: Validation Of A Threshold For VSPECT Imaging Technique

5.1 Abstract

Introduction

Asthma is characterised by severe airway narrowing, which appears as an absent ventilation on ventilation imaging. Fusion of single-photon emission computed tomography (SPECT) with computed tomography images (CT) provide measurements of ventilation, with functional and structural information. There are several methods available with which to determine threshold values to separate ventilated and non-ventilated lung regions on ventilation SPECT (VSPECT) images; however, no studies have assessed the accuracy of these methods with a physical lung phantom model

Methods

To simulate ventilated lung, ten lung phantom models were constructed of various sizes, and filled with radioactive water containing Technetium-99m (^{99m}Tc) solution. Non-ventilated lung was simulated by inserting smaller containers inside the ventilated (^{99m}Tc) containing 'lung', which were filled with non-radioactive water. To determine an appropriate threshold method for distinguishing ventilated from non-ventilated lung regions, the published method of Farrow et al. "Mean$_{5\text{-}80}$" (based on 'middle range' activity), and a novel method using curve-fitting of the voxel activity distribution "Slope" were compared.

Results

"Slope", was the more accurate method for separating ventilated and non-ventilated 'lung', with the narrower limits of agreement and no bias.

Conclusion

The method of "Mean$_{5\text{-}80}$" is unsuitable for VSPECT imaging analyses, particularly in asthma with poor or heterogeneous ventilation. The "Slope" method of threshold determination provided highly

accurate separation of ventilated from non-ventilated ‘lung’ and is therefore, the preferred threshold method for VSPECT image analyses.

5.2 Introduction

Single-photon emission computed tomography (SPECT) is a 3-dimensional, nuclear imaging technique that has been utilised to investigate ventilation and perfusion(1). SPECT uses Technegas (CycloMedica, Sydney, Australia), as the ventilation agent, which is made up of ultrafine (~100 nm) Technetium [^{99m}Tc] carbon particles (2). SPECT images are often fused with low-dose computed tomography (CT) to provide co-localisation of structural and functional information. In asthma, airway obstruction, and narrowing result in heterogenous ventilation (3), and regions of absent ventilation within the lungs. Areas of absent ventilation have been suggested to be an important marker of the disease severity (4, 5). It is in this domain that SPECT can be used to provide meaningful information.

Technegas inhalation results in areas with high, low and absent radioactivity. Localised areas of high activity or "hotspots" occur as a result of Technegas impaction within the airways (mostly proximal airways) where there is narrowing and turbulent flow. Accurate determination of areas that are either ventilated or nonventilated, based on the presence or the absence of the radioactivity, require a threshold that is unaffected by hotspots, or by regional variations in Technegas activity (heterogenous ventilation). To date, there is no threshold method for SPECT that has ever been assessed for its accuracy with a physical lung phantom model.

Traditional thresholds have most commonly been based on an arbitrary percentage of the maximum activity and thus, are completely dependent on the presence or absence of "hotspots". Previous studies have determined that different thresholds between 10 to 70% of the maximum value (6-8), are accurate for such analyses. However basing thresholds on the maximum activity will strongly bias because of the very heterogenous ventilation that typically occurs in asthma. For this reason, Farrow et al. (9) developed a SPECT threshold method that was based on the average

Technegas activity, calculated from counts between 5% and 80% of the maximum. By basing the threshold value on the ‘middle values’, it would thus eliminate the effects of hotspots. This threshold was previously verified against FRC derived from MBNW (9) .

In addition to Farrow et al.’s method, there was an opportunity to develop a new thresholding method, to separate ventilated from nonventilated lung in Technegas SPECT images, which also was unaffected by ‘hotspots’ in obstructive airways diseases where ventilation was particularly heterogeneous. Therefore, the first aim of this study was to develop a new threshold to quantify SPECT ventilation, and to validate that with a physical lung phantom model. The second aim was to compare the accuracy of volume measurements between Farrow et al. threshold, and the new developed threshold with the lung phantom model.

5.3 Methods

The lung phantom is a physical model of lungs that is made to develop and assess the measurement accuracy of the imaging analysis technique for VSPECT image data.

Ten lung models of various sizes were made (ranging from 0.4 to 5.1 litres) by tightly filling plastic containers with polystyrene beads, and then filling the remaining space with either radioactive (mimicking ventilated lung) and non-radioactive water (mimicking nonventilated lung). This approximated human lung density seen in CT scans. The radioactive water was made by mixing Technetium-99m (^{99m}Tc) diluted in normal saline, with water. Three concentrations of ^{99m}Tc have been used, ranging from 0.04,0.06, and 0.08 mBq/mL to simulate the typical range of radioactivity that was seen with human studies. The volume of plastic containers was determined from their measured dimensions.

Ten separate, smaller plastic containers were used as 'ventilation defects' or 'non-ventilated' lung to stimulate lung regions that don't receive ventilation (ranging from 0.007 to 2.5 litres). They were filled as above but the water was non-radioactive. They were placed inside the larger containers that had radioactive water. These smaller, non-radioactive containers thus appeared as 'non-ventilated' spaces, within the 'ventilated lung' (Figure 1).

The volumes of the 'ventilated' and 'non-ventilated' container(s) and ^{99m}Tc concentrations are shown in Table 1.

To simulate hotspots, 15 small plastic vials (0.05 litres) were placed on the outer exterior of lung phantoms. Hotspots were filled with higher Technetium-99m concentration eluted in saline and tap water (ranging from 0.18. 0.24, and 0.30 mBq/mL) (Figure 1).

Table 1: Volumes of lung phantoms, and Technetium-99m concentrations.

Phantom lung#	Total volume (L)	VentVol (L)	VentNon (L)	Concentration (mBq/mL)
1	5.13	4.34	0.79	0.04
2	4.44	2.57	1.79	0.04
3	1.25	1.00	0.25	0.04
4	0.41	0.39	0	0.04
5	5.13	2.66	2.47	0.06
6	1.38	1.38	0	0.06
7	0.66	0.45	0.21	0.06
8	5.13	3.16	1.98	0.08
9	2.86	2.49	0.37	0.08
10	0.93	0.86	0.08	0.08

Volume measurements of ten lung phantoms, ventilated volume (VentVol), and non-ventilated volume (VentNon). L = litre; mL = millilitre; mBq= millibecquerel

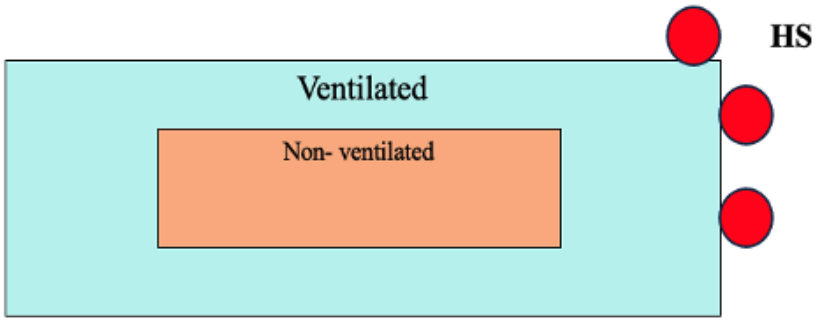


Figure 1: Diagram of lung phantom experiment.

Phantom with hot spots (HS). True ventilated volume of the phantom = (container volume + hot spot) – (volume of defect container). This configuration allow phantoms to be analysed with, and without hotspot included.

VSPECT/CT imaging

All phantom lung imaging took place at the Department of Nuclear Medicine, Royal North Shore Hospital, using a Siemens Symbia T16 SPECT/CT system. The phantom imaging protocol was identical to that used in human studies, see chapter 2 Section 2.8.1. Briefly, SPECT, and CT images were acquired using 128 x 128 matrix and 512 x 512 matrix respectively. CT images were acquired with low dose protocol, and the count rates for each phantom was also identical to that in human studies, being a minimum of 1.5 kilocounts/s required for imaging (Figure 2).

Image processing and reconstruction

The image processing and reconstruction protocols were previously addressed in chapter 2, section 2.8.2. Briefly, SPECT, and CT images were reconstructed using ordered subset expectation maximization (OSEM), with 128 slices and Gaussian filter, and using 512 x 512 matrix respectively. Both SPECT and CT images were then transferred to a HERMES, and converted to DICOM format. Registration of SPECT images with the CT images was performed using a HERMES multimodality software package, by visually aligning the edges of lung phantom, with edges of the containers from CT scans.

Lung phantom analyses

The true volume of each phantom was calculated by measuring the dimensions of the containers. Outlines of the lung phantom and of the 'ventilation defect' containers, and hotspots were defined from the CT images. From these outlines, masks were created manually, which then were applied to the SPECT images to exclude all the Technegas activity outside of the phantom (Figure 3), as would normally be done in image analysis of human studies. All lung phantom analyses, including mask construction was performed using Fiji (National Institutes of Health, USA).

Figure 2 : Photograph of SPECT/CT scan of lung phantom

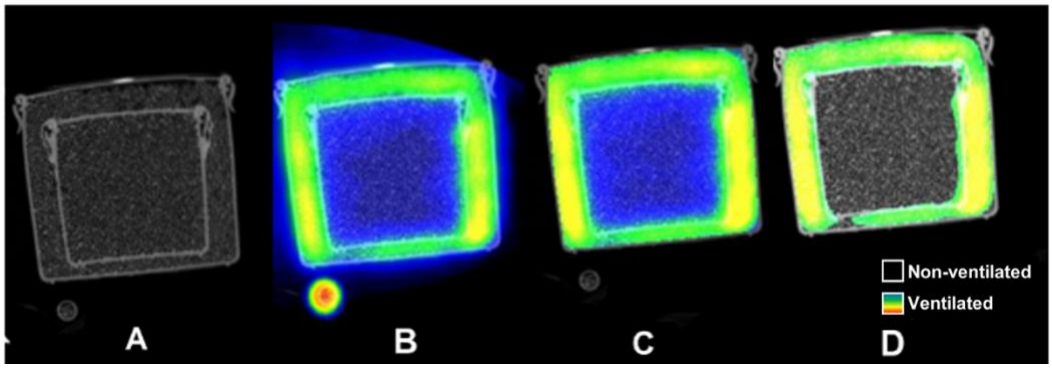


Figure 3: Analysis process for lung phantom.

Lung phantom CT image (A); SPECT overlayed on CT (B); masked SPECT/CT (C); masked SPECT/CT image with a threshold applied (D). The color code corresponding to increasing Technetium-99m activity

Thresholds

A total of 10 phantoms with and without hotspots, were analysed. The true volume of each phantom which contained ^{99m}Tc "$Vent_{Vol}$", was compared with the measured volume, from two thresholds: 1) using $Mean_{5-80}$, method of Farrow et al. (9), and 2) a newly developed threshold method called "Slope".

1) $Mean_{5-80}$ method

The weighted average of all voxels between 5% to 80% of the maximum ($Mean_{5-80}$) was calculated, which thus excluded any influence of the very highest and lowest voxel values. The threshold was then defined as 50% of that average value, as described by Farrow et al. All voxels containing ^{99m}Tc activity $\geq 0.5 \times Mean_{5-80}$ were considered ventilated.

2) "Slope" method

Voxels with the high count intensity are generally also of low frequency and manifest as a tail toward the end of the activity versus frequency distribution plot. These voxels may represent Technegas impaction and clumping at airway branchpoints and locations of severe narrowing, and may form hotspots. The principle behind the "Slope" threshold method was to identify the radioactivity count that separated the hotspot (or the few voxels of the highest count rate), from the remainder of the voxels, that is, to isolate hotspot or the few voxels with the highest values, from the rest. This was done using the cumulative frequency plot (Figure 4), from which the instantaneous slope for points in the rightward region of this distribution was calculated. This most rightward part of the cumulative frequency distribution is flat, consistently across human and phantom studies. By moving leftward from the flat region, the point at which the slope changes from near zero could be identified, using objective criteria. A threshold value could then be calculated, based on the radioactivity value of this point, which excluded the highest radioactivity

values. The threshold value was a percentage of the activity, of that so-identified point. The ideal percentage value which was found by calibrating the measured volumes against the gold standard measurements.

The method of calculating the Slope as a threshold was previously addressed in chapter 2, section 2.8.3. Briefly, a cumulative frequency was constructed and expressed as a percentage against voxel intensity, and then the slopes were calculated for each point. Starting from 100%, the first point which had a slope equal to or greater than 0.010 %/count was found. All points located in this manner were within the upper 10^{th} percentile of radioactivity counts. Following this, the radioactivity count of that point was multiplied by differing percentages to obtain the candidate threshold. All voxels with values above the candidate threshold were considered to contain ^{99m}Tc activity, and determined to be "ventilated" (Figure 4).

Selection of slope point, and the percentage

1). Volume measurements were examined using slopes of 0.001, 0.010, 0.100, and 1. Then multiplying the corresponding radioactivity counts by percentages ranging from 20 to 40% were then used to measure "ventilated" volumes. The values that produced the most accurate measurements of $Vent_{Vol}$ and $Vent_{Non}$ were then used to derive the final threshold.

2). Lin's concordance correlation coefficient (CCC) was used for sensitivity analysis.

Lin's CCC (ρc) measures the strength, and agreement (both precision and accuracy) of a new measurement or method compared to a gold standard. In this case, how well the thresholds, agreed with the known true volume from the phantom. The strength of agreement for ρc is determined as follows: < 0.90 indicates poor strength, 0.90 – 0.95 indicate moderate strength, 0.95 – 0.99 indicate substantial strength, and > 0.99 indicates near perfect agreement (10).

5.4 Statistical Analysis

GraphPad Prism version 10.0.2 was used for statistical analyses, and generating graphs, except for Lin's concordance correlation coefficient analysis, which was done on IBM SPSS statistics version 27. Shapiro–Wilk test was used to assess the distribution for normality. Bland–Altman plots were also generated to assess the errors in volume measurements (true volume minus threshold measured volume), and plotting the mean difference and 95% limits of agreement (95% limits of agreement (LOA)). Linear regression was then performed, of the difference versus gold standard, to determine any proportional bias. The effect of hot spots on thresholds was examined by comparing threshold measurements from phantoms, with and without hotspots, using repeat measures one-way ANOVA for parametric variables or Friedman test for nonparametric variables, with post-hoc Dunnett's multiple comparisons test for both. Data are presented as mean ±SD unless otherwise stated. Statistical significance was set at P-value <0.05.

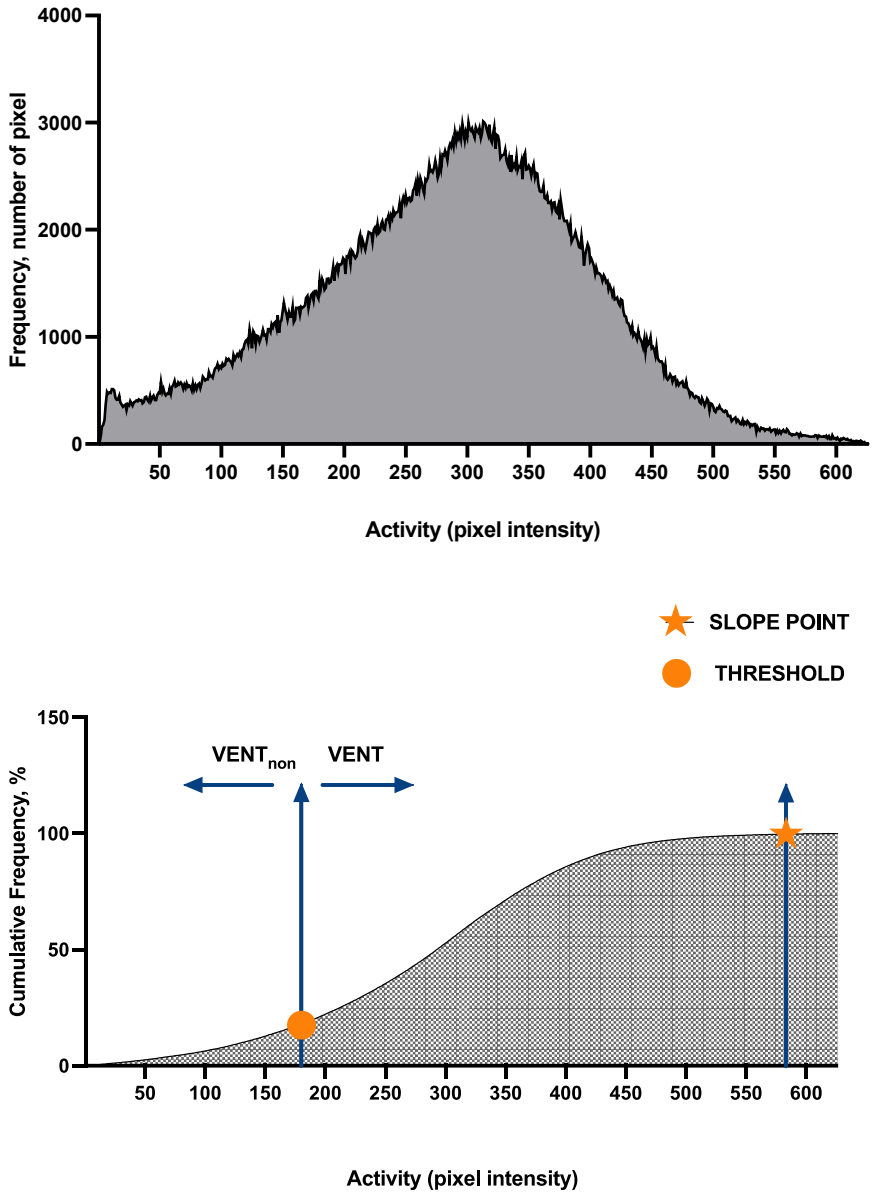


Figure 4: Threshold analysis using “Slope”.

Top Window: Histogram distribution of Technegas from a SPECT image.

Bottom Window: Transformed cumulative frequency, expressed as the % of total frequency, plotted against the pixel intensity. The Initial data point above 90% , with a slope equal or less than 0.010 is selected as the slope point. The radioactivity count (pixel intensity) corresponding to the slope point is then multiplied by 30% to get the final threshold.

*Vent = ventilated activity; $Vent_{Non}$ = non-ventilated activity

5.5 Results

Sensitivity analysis

To determine the slope point cut-off-value, and the percentage, the strength of agreement was compared using ρc from Lin's concordance correlation, between the true volume measurements of $Vent_{Vol}$, and the measured $Vent_{Vol}$'s. A Slope point value of 0.010 %/count multiplied by 30%, was shown to produce the highest level of agreement, with near-perfect agreement for $Vent_{Vol}$ (ρc = 0.997). The strength of agreement between true volume and volume measured from different cutoff values for slope points, 0.001, 0.010, 0.100, and 1 (multiplied by 30%) are shown in Table 2. Table 3 shows the different percentages that were tested (20%, 25%, 30%, 35% and 40%), to derive the final threshold value.

Table 2. Lin's Concordance Coefficient for different slope points

Parameter	True $Vent_{Vol}$ (L)
	ρc
30% of Slope 0.001	0.984
30% of Slope 0.010	0.997
30% of Slope 0.100	0.994
30% of Slope 1.000	0.978

Lin's Concordance Coefficient analysis showing the degree of strength between slope points, and the true measured volume for all phantoms. 30% of slope 0.010 showing near perfect strength. #Data is reported as ρc; concordance correlation coefficient

Table 3. Lin's Concordance Coefficient for different percentages of slope point 0.010

Parameter	True $Vent_{Vol}$ (L)
	ρc
20% of Slope 0.010	0.953
25% of Slope 0.010	0.984
30% of Slope 0.010	0.997
35% of Slope 0.010	0.994
40% of Slope 0.010	0.970

#Lin's Concordance Coefficient analysis showing the degree of strength for 30% of slope, compared to 20%, 25%, 35%, and 40%. 30% of slope 0.010 showing near perfect strength with the measured true volume. #Data is reported as ρc; concordance correlation coefficient

Accuracy of thresholds in measuring $Vent_{Vol}$, and $Vent_{Non}$

Lin's concordance correlation plots were created to show the strength of agreement between true and threshold measured $Vent_{Vol}$ and $Vent_{Non}$ (Figure 5). When the $Mean_{5-80}$ threshold method was applied on the lung phantom, there was substantial strength of agreement for $Vent_{Vol}$ (ρc= 0.969), but poor strength of agreement for $Vent_{Non}$ (ρc =0.897). By contrast, when the "Slope" threshold method was applied, there was near perfect strength of agreement for $Vent_{Vol}$ (ρc = 0.997), and substantial strength of agreement for $Vent_{Non}$ (ρc= 0.988).

The accuracy of thresholds to measure "$Vent_{Non}$"

Figure 6 is Bland–Altman plots, showing the difference between threshold measured $Vent_{Non}$, and true $Vent_{Non}$ from all phantoms.

True volumes versus $Mean_{5-80}$ threshold measured volumes

Bland-Altman plot revealed differences in between $Mean_{5-80}$, and true $Vent_{Non}$, with a size dependent under-estimation of non-ventilated volume, with larger ventilation defects. The mean difference ± SD between true $Vent_{Non}$ and $Mean_{5-80}$ was (0.159 ± 0.316 Litres). The 95% limits of agreement were −0.459 – 0.778 Litres. The proportional bias was confirmed by the significant linear regression (slope = 0.319, $p<0.0001$) (Figure 6).

True volumes versus "Slope" threshold measured volumes

The mean difference between volumes measure by the "Slope" threshold method, and true $Vent_{Non}$ volumes was small, the mean difference ±SD was (0.023 ± 0.129 Litres) and 95% limits of agreement were −0.229 – 0.275 Litres. There was also evidence of proportional bias (slope = 0.094, $p<0.0001$), however, it was small (Figure 6).

Effect of hot spots on thresholds

To show if threshold cut-off values would change with the introduction of hotspots, the phantoms were first analysed without hotspots, then again with hotspots included (0.18. 0.24, and 0.30 mBq/mL).The $Mean_{5\text{-}80}$'s thresholds were lower for phantoms without hotspots, compared to phantoms with 0.18 mBq/mL, and 0.30 mBq/mL hotspots ($p = 0.0097$ and < 0.0001, respectively) (Figure 5). On the other hand, when using the "Slope" method, calculated threshold values were not different (all p values > 0.05) (Figure 7).

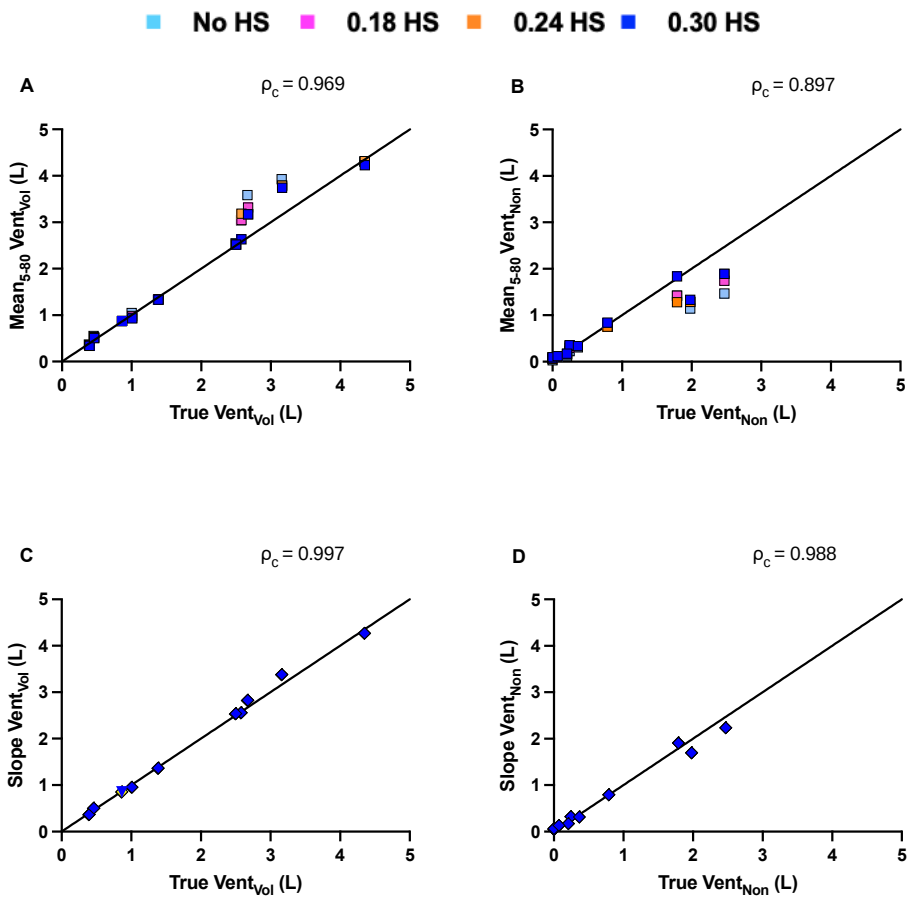


Figure 5: Plots of Lin's concordance correlation coefficient (ρc) showing the degree of strength between true $Vent_{Vol}$, $Vent_{Non}$, and thresholds

$Mean_{5\text{-}80}$, and $Vent_{Vol}$ (A), $Mean_{5\text{-}80}$, and $Vent_{Non}$ (B) Slope, and $Vent_{Vol}$ (C), Slope, and $Vent_{Non}$ (D). The line is the line of identity. Overall, the "Slope" method showed the more accurate measurements for $Vent_{Vol}$ and $Vent_{Non}$.

*Measured volumes for are all the phantoms, are plotted against true volumes, for phantoms with and without hotspots. No HS = no hot spot, 0.18 HS = 0.18 mBq/mL hot spot; 0.24 HS = 0.24 mBq/mL hot spot; 0.30 HS = 0.30 mBq/mL hot spot.

$Vent_{Vol}$, and $Vent_{Non}$, are expressed in litres (L).

#Data is reported as ρc; concordance correlation coefficient

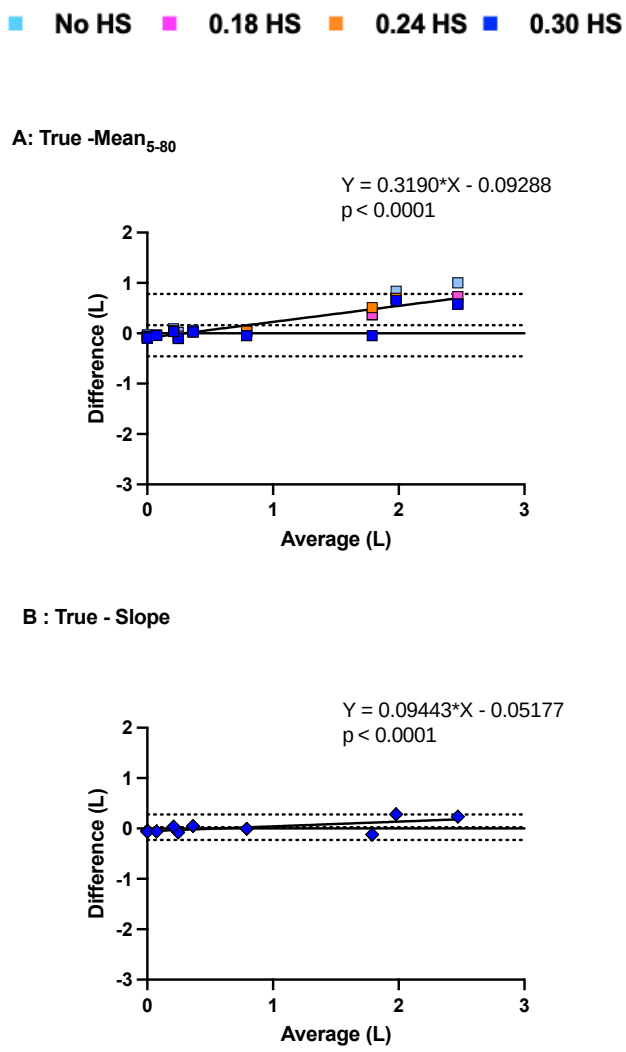


Figure 6: Bland–Altman plot showing the differences between non-ventilated volume ($Vent_{Non}$) measurements from true and threshold methods.

Agreement between volumes measured by $Mean_{5-80}$ method and the gold standard (A), (mean difference (95% limits of agreement) 0.159 (−0.459, 0.778), p<0.0001).

Agreement between volumes measured by the "Slope" method and the gold standard (B), (mean difference (95% limits of agreement) 0.023 (−0.229, 0.275), p<0.0001).

Mean difference and 95% limits of agreement are displayed in dotted lines.

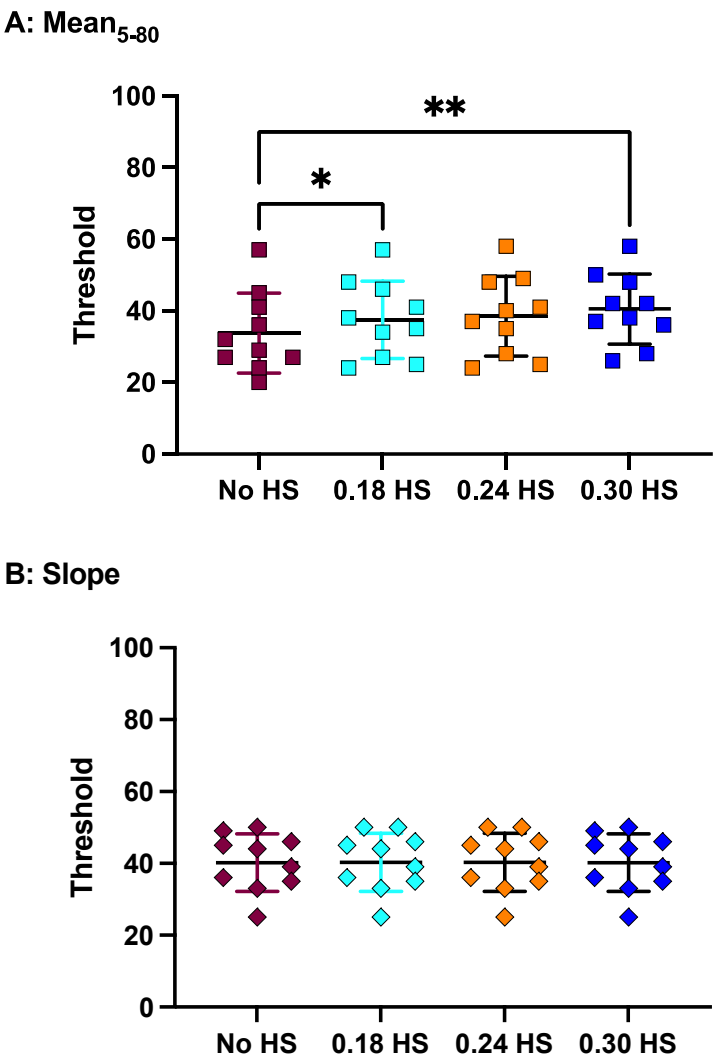


Figure 7: Calculated threshold values for phantoms with and without hotspots (HS).

No hot spots (No HS), a 0.18 mBq/mL ^{99m}Tc hot spot (0.18 HS), 0.24 mBq/mL 99mTc hot spot (0.24 HS), and 0.30 mBq/mL ^{99m}Tc hot spot (0.30 HS).

Mean$_{5\text{-}80}$ (A), thresholds cutoff values derived from Mean$_{5\text{-}80}$ change (increase), as the hotspot values increase. Slope (B), thresholds derived from "Slope" remained unaffected.

RM one-way ANOVA was used for comparisons of threshold cut-off values. Post-hoc analysis for all threshold methods was done with Dunnett's multiple comparisons test, *$p< 0.05$; ** $p< 0.01$.

5.6 Discussion

In this study, two threshold methods were assessed in lung phantoms of different sizes and with different ventilation defects, to identity an accurate imaging analysis for SPECT image data. Thresholds derived from $Mean_{5\text{-}80}$ method that have been used previously, and a new "Slope" method were used, to measure ventilated volumes ($Vent_{Vol}$), and non-ventilated volumes ($Vent_{Non}$) from ten different sized lung phantoms. We also assessed the robustness of these methods against hotspots. It was found that threshold derived from "Slope" method produced the more accurate measurements, with the smallest mean differences compared to the gold standard measurements. Furthermore, it was also found that the $Mean_{5\text{-}80}$ method was minimally affected by hotspots, while the newer "Slope" method was not.

In the current study, it was found that $Mean_{5\text{-}80}$ underestimated $Vent_{Non}$ in lung phantoms, and thresholds values were significantly affected by hotspots. While this method was designed to be more robust in heterogenous ventilation, particularly when radioactivity from Technegas clumping was present, the results of the experiment showed its still affected to a small degree. To derive the threshold, $Mean_{5\text{-}80}$ uses a weighted mean radioactivity value, derived from all voxels with activities between 5% to 80% of the maximum "hotspot". Although the threshold value is not derived directly from the maximal count, it must to some degree, be affected by that maximum value since that value determines what is within the "5% to 80% of maximum" range. With the introduction of the 0.18 mBq/mL and 0.30 mBq/mL "hotspots" to the lung phantoms, there was significant increase in threshold cutoff values. As the "hotspots" get hotter (greater heterogeneity), then the maximum activity count is greater, which consequently leads to an increase in the mean range of 5% to 80%. If the mean range of 5-80% increases, that leads to increased threshold cutoff values and underestimation of non-ventilated volume.

The activity values within non-ventilated containers is very low but not zero, due to photon scatter artefacts, and consists of a large number of voxels, as occurs in human studies. The activity of these voxels is usually greater than 5% of maximum, and therefore, also significantly affects the weighted average. If the non-ventilated space is large, then this will effectively lower the calculated threshold value, which would consequently lead to overestimation of the size of non-ventilated volume, and it would demonstrate a size-dependent bias. However, given that the volume of the non-ventilated space is underestimated, even without "hotspots", the size of the non-ventilated space has no-significant influence on its final measurement.

In contrast, the "Slope" method isn't determined from the maximum count, nor the average activity. The typical "Slope point" has a cumulative percentile value of 94 – 98%, and count values of 500 – 550 (typical maximum counts are about 600). As an example, if the "Slope point" was 550 counts at 95th percentile, voxels of values 550 – 560 would add <0.1% to the cumulative total (slope <0.01 %/ count). Thus, the "Slope" method identifies that 'hottest' voxel that is still somewhat 'numerous', but above this radioactivity level, voxels are very few in number or 'rare'. Once the "slope point" is identified, it is then multiplied by a fixed percentage, which defines the threshold. Given the narrower 95% limits of agreement and lack of effect of "hotspots", these results suggest that it preferred as the thresholding method for calculating non-ventilation volume.

5.7 Limitations

The study acknowledges that this work was limited by the small number of phantoms and the simulation of completely homogeneous ventilation but with "hotspots". The simulation did not include 'low ventilation', which would require several concentrations of technetium solution. A variety of sizes were analysed; however, they were similar in size to human lungs. Additionally, hotspots were placed external to the phantoms, and findings might be different if the hotspots had

been placed inside the lung models. This was not done due to the practical difficulties of doing this in the nuclear medicine lab and handling of radioactive solutions. Future studies could explore the threshold performance on a more diverse set of phantom models, including phantoms with hotspots placed internally.

5.8 Conclusion

In this study, the accuracy of two threshold methods for measuring non-ventilated volumes for SPECT imaging data were compared. The "Slope" method is more accurate than the $Mean_{5-80}$ method, probably because of the inherent nature of the calculation makes it more prone to the distribution of radioactivity and in particular, radioactivity clumping seen as "hotspots".

5.9 References

1. King GG, Harris B, Mahadev S. V/Q SPECT: Utility for investigation of pulmonary physiology. Seminars in Nuclear Medicine. 2010;40(6):467-73.
2. Amis TC, Crawford AB, Davison A, Engel LA. Distribution of inhaled 99mtechnetium labelled ultrafine carbon particle aerosol (Technegas) in human lungs. Eur Respir J. 1990;3(6):679-85.
3. King GG, Eberl S, Salome CM, Meikle SR, Woolcock AJ. Airway closure measured by a technegas bolus and SPECT. Am J Respir Crit Care Med. 1997;155(2):682-8.
4. Svenningsen S, Nair P, Guo F, McCormack DG, Parraga G. Is ventilation heterogeneity related to asthma control? European Respiratory Journal. 2016;48(2):370-9.
5. Mummy DG, Kruger SJ, Zha W, Sorkness RL, Jarjour NN, Schiebler ML, et al. Ventilation defect percent in helium-3 magnetic resonance imaging as a biomarker of severe outcomes in asthma. J Allergy Clin Immunol. 2018;141(3):1140-1.e4.
6. Lee SU, Yoon Chung S, Park RH. A comparative performance study of several global thresholding techniques for segmentation. Computer Vision, Graphics, and Image Processing. 1990;52(2):171-90.
7. Zhang X, Hirano H, Yamamoto K, Kusaka Y, Sugimoto K, Kimoto T, et al. Technegas ventilation SPECT for evaluating silicosis in comparison with computed tomography. Ann Nucl Med. 1996;10(2):165-70.
8. Satoh K, Tanabe M, Takahashi K, Kobayashi T, Nishiyama Y, Yamamoto Y, et al. Assessment of technetium-99m Technegas scintigraphy for ventilatory impairment in pulmonary emphysema: comparison of planar and SPECT images. Ann Nucl Med. 1997;11(2):109-13.
9. Farrow CE, Salome CM, Harris BE, Bailey DL, Bailey E, Berend N, et al. Airway closure on imaging relates to airway hyperresponsiveness and peripheral airway disease in asthma. J Appl Physiol (1985). 2012;113(6):958-66.
10. McBride G. A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. NIWA client report: HAM2005-062. 2005;45:307-10.

Chapter 6. Effect of Bronchodilator Response On Ventilation Distribution Measured By VSPECT/CT Imaging In Asthma

6.1 Abstract

Background

In asthma, mechanisms of bronchodilator response are poorly understood. While clinically relevant, changes in forced expiratory volume in 1 s (FEV_1) after the administration of a short-acting bronchodilator are highly variable, and are not always seen in patients with asthma. Single-photon emission computed tomography (SPECT) ventilation imaging can be used to visualise, and quantify functional responses in asthma. Therefore, the objectives were to measure bronchodilator responsiveness using ventilation SPECT, and to compare the changes in ventilation SPECT with changes in spirometry, and small airway measurements.

Methods

Twenty-two participants with asthma underwent VSPECT/CT imaging with Technegas, before and after bronchodilator administration. Lung regions that were classified as ventilated, and nonventilated "$Vent_{non}$" were calculated using a threshold method and were expressed as a percentage of total lung volume. Multiple-breath nitrogen washout (MBNW) was used to measure large- and small-scale airways function (Scond and Sacin, respectively). Forced oscillation technique (FOT) was used to measure respiratory system resistance (Rrs_{5hz}) and reactance (Xrs_{5hz}).

Results

In total, twenty-two participants with asthma (13 females; mean age, 53±22 years, FEV_1= 80% ± 20%) showed improved FEV_1, FEV_1/FVC, Rrs_{5hz}, and Xrs_{5hz}, post bronchodilator (all $p<0.0001$). Overall, there was no significant change in SPECT measured $Vent_{non}$, however, there was wide range of individual responses. $Vent_{non}$ at baseline was unrelated to spirometry, FOT, or any MBNW indices. Post bronchodilator changes in $Vent_{non}$ were predicted by older age, and correlated with less change in oscillatory resistance.

Conclusion

In asthma, non-ventilated regions are unchanged by bronchodilator inhalation, despite large changes in spirometry and oscillatory impedance. The changes in non-ventilated regions is likely affected by age. The apparently paradoxical relationship between changes in Rrs_{5hz} and $Vent_{non}$ might be explained by the possibility that lung regions that were 'opened to ventilation' had abnormal function which could have paradoxically worsened Rrs_{5hz}.

6.2 Introduction

Asthma is characterised by airways that can narrow too easily and by too much {airway hyperresponsiveness} but which is responsive to short acting bronchodilators. Positive bronchodilator responsiveness is useful for diagnosis of asthma in the presence of a consistent clinical picture and indicative of the important role of airway smooth muscle tone. Positive bronchodilator response is associated with asthma death (1) and greater decline in FEV_1 (2) but also with greater response to higher dose inhaled corticosteroid {ICS} treatment (3). Despite the significance of bronchodilator responsiveness in asthma, the underlying mechanisms are poorly understood. Bronchodilator responses are measured by FEV_1, which is a summation of airways that narrow and close but which are distributed in a heterogeneous manner across the lung. Bronchodilator inhalation in asthma increases both FEV_1 and FVC (4, 5) which suggests the release of bronchomotor tone allows closed airways to reopen, is an important mechanism of action.

Airway closure has been visualised as non-ventilated regions ($Vent_{non}$) in asthma, using 3 dimensional ventilation imaging techniques. These include single photon emission computed tomography (SPECT) using Technegas as a ventilation marker (6), and from magnetic resonance imaging (MRI) using inert gas ventilation tracers (hyperpolarised ^{3}He and ^{129}Xe) (7, 8). MRI studies indicate that non-ventilated regions decrease with bronchodilator inhalation (9, 10). The determinants of bronchodilator responsiveness, as measured by 3-dimensional imaging are unknown, and to date it appears that changes in lung function and in $Vent_{non}$ with bronchodilator inhalation, are dissociated (11). Peripheral airway dysfunction is an important determinant of airway hyperresponsiveness (12) and of closure (13) in asthma. It therefore follows, that peripheral airways may also determine bronchodilator responsiveness in $Vent_{non}$.

Bronchodilator responsiveness has not been examined before using SPECT Technegas imaging. It is logistically, a simple imaging technique because of greater camera availability (being used routinely for pulmonary embolism diagnosis), and also because the ventilation imaging agent Technegas, is generated on-demand and onsite. Technegas is an ultrafine aerosol of around 100nm, its distribution is 'gas-like', however, once the Technegas particles are inhaled, its deposition is fixed i.e. the particles do not move after inhalation (14). This allows subjects to inhale the Technegas in upright posture, and then be imaged supine and yet the distribution of Technegas reflects breathing in upright posture. Ventilation distributions therefore differ from MRI inert gas studies, which are of supine ventilation. Since posture affects the distribution of Ventnon (15), the magnitude of $Vent_{non}$, its distribution, relationships with lung function and responses to bronchodilator will likely differ from those seen in inert gas MRI studies.

the aims for the current study were to measure bronchodilator response using SPECT/CT ventilation imaging, in participants with asthma ranging from mild to severe disease, and to evaluate the relationship of response with peripheral airway function and spirometry. It was hypothesised that $Vent_{non}$ would decrease with bronchodilator inhalation, and that this response would be predicted from peripheral airway function measured by oscillometry and multiple breath nitrogen washout.

6.3 Methods

Study design

Subjects attended the laboratory on two separate visits. At the initial visit (baseline), all subjects completed the asthma control questionnaire 7-items (ACQ7) and had a skin prick test for atopy. All underwent baseline lung function measurements including exhaled nitric oxide (FeNO), spirometry, lung volumes by plethysmography, diffusing capacity, forced oscillation technique (FOT), multiple breath nitrogen washout (MBNW), and a baseline VSPECT/CT scan.

The second visit to measure bronchodilator response, was scheduled 1–7 days after the initial visit, in which spirometry, and FOT were performed before and after the administration of bronchodilator (400 mg of salbutamol delivered by metered dose inhaler and spacer). VSPECT scans were then acquired immediately. All Subjects were asked to withhold reliever medication for six hours, and any combination or controller medication for 24 hours prior to each visits.

Subjects

Twenty-two subjects with asthma were recruited from the Department of Respiratory Medicine at Royal North Shore Hospital and the volunteer database at the Woolcock Institute of Medical Research. Subjects were referred through respiratory physicians or respiratory scientists, who obtained consent for the patients to be contacted for research purposes. Eligibility required participants to be between 18 and 85 years old with a physician diagnosis of asthma. All subjects were also required to be current non-smokers, and/or ex-smokers with less than 5 pack-years smoking history. Subjects completed the Woolcock clinical questionnaire, which provided detailed information on each participant's asthma history and other related health factors, including medication use, asthma history, exacerbation rates, hospitalisations, and comorbidities.

Written informed consent was obtained from each participant before enrolment. This study has been approved by the Human Research Ethics Committee (HREC reference number RESP/18/198) of the Northern Sydney Local Health District.

Lung function testing and assessment of asthma status

Spirometry and lung volumes were performed according to American Thoracic Society/European Respiratory Society (ATS/ERS) quality criteria (16) using a Medgraphics CPFS/D USB spirometer (Medgraphics, St. Paul, MN), and Vyntus PFT system (Vyair medical, Inc) respectively. All lung function indices were expressed as z-scores where possible using published predicted equations (17). Positive bronchodilator responsiveness was defined as a change >10% of the predicted value, and/or ≥ 200 mL and ≥ 12% improvement from baseline FEV_1 and/or FVC after bronchodilator administration as per previous ATS/ERS criteria (18).

Asthma control was categorised as either well controlled (score <0.75), partly controlled (score >0.75 but <1.5), or poorly controlled (score >1.5) (19). FeNO was measured using FeNOBreathTM portable exhaled NO system. The recommended cut-off value for normal FeNO levels is <25 parts per billion (ppb) and for elevated FeNO is >50ppb (20). The skin prick test (21) involved placing a small droplets of aeroallergens on an individual's forearm (alternaria alternata, weed mix, mixed feathers, mold mix, dematophagoides farinae, dermatophagoides pteronyssinus, dog hair, cat hair, aspergillus fumigatus, perennial ryegrass, and grass mixture). A positive skin reaction was defined as a mean wheal of ≥3 mm in diameter. (21). Subjects also had blood drawn for a full blood count and total immunoglobulin E (22).

Diffusing capacity of lung for carbon monoxide (DLCO)

Diffusing capacity of the lung was assessed, using a single-breath DLCO technique, in accordance with the ATS/ERS standards (23). DLCO, and the DLCO/VA, known as the transfer coefficient (KCO) values were corrected for hemoglobin concentration. DLCO, and KCO expressed as percentage of predicated values, and z-scores using GLI published predicted equations (24).

Forced oscillation technique (FOT)

FOT was performed during quiet tidal breathing, in a sitting position, using the TremoFlo C-100 (Thorasys, Thoracic Medical Systems) as per ERS recommendations (25). FOT was measured before, and after bronchodilator administration. The mean of three 60 s measurements was recorded for respiratory system resistance at 5 Hz, and respiratory system reactance at 5 Hz. Reference values and BDR cut-offs were derived from Oostveen et al (26). Positive bronchodilator was defined as an absolute decrease in R5 ≥1.37 cmH2O.s/L, and increase in X5 ≥ 0.55 cmH2O.s/L (26).

Multiple breath nitrogen washout (MBNW)

MBNW was performed according to ATS/ERS recommendations (27), using the Exhalyzer D with spiroware v3.3.3 (Eco Medics AG, Duernten, Switzerland). After establishing stable tidal breathing, participants breathed 100% oxygen with a tidal volume of 1.0–1.3 L and frequency of 9–12 breaths per minute until the nitrogen concentration decreased to 1/40th of the starting concentration. Scond and Sacin were calculated as indices of convection-dependent (Scond) and diffusion-dependent (Sacin) ventilation heterogeneity. References values, and z-scores were calculated using published reference equations (28).

Ventilation lung imaging

VSPECT/CT was performed using Siemens Symbia T16 SPECT/CT system, with Technegas as the ventilation agent (Cyclomedica Australia, Sydney). Technegas is an ultrafine carbon particle aerosol labelled with Technetium-99m (^{99m}Tc), with a mass median aerodynamic diameter of around 100 - 500 nm (14). 500 to 900 mls volumes of Technegas were slowly inhaled from FRC at ~30-60 l/min in a sitting position with spirometric monitoring. Two to four inhalations were required to achieve a minimum radioactivity of ~1.5 kilocounts required for imaging.

VSPECT was acquired during tidal breathing with subjects lying supine inside the scanner with arms above heads, using a 128 x128 matrix, at 15 s per stop, with a total of 120 views (60 stops) resulting in a total acquisition time of 15 min. The CT scan was acquired immediately after SPECT, in the same position, but during breath hold at FRC. CT was performed without contrast, with a low-dose protocol (30 mAs, 110kVp, slice thickness 3 mm). Total dose for the entire study was 4.5 mSv per subject.

Beads containing Technetium (^{99m}Tc) were taped to the sternal notch, ends of the clavicles and lateral aspects of the lower ribs, to act as fiducial markers to ensure accurate VSPECT and CT image registration.

Image reconstruction and registration

VSPECT images were reconstructed using ordered subset expectation maximization (OSEM), with 128 slices and Gaussian filter (iterations=2, subsets = 8, full-width at half maximum (FWHM) =8). CT images were reconstructed using 512 x 512 matrix with a Siemens algorithm and transferred to a HERMES (Hermes Medical Solutions, Stockholm, Sweden) workstation in DICOM format. Registration of the SPECT ventilation images to CT was performed manually to

ensure that VSPECT and CT images were well-aligned using a HERMES multimodality software package (Hermes Medical Solutions).

Image analysis

The VSPECT/CT image analysis (Figure 1) was a two-stage procedure. The steps were:

1) The lung outlines were located from the CT images. Following binarisation using a pre-determined global threshold (29), a seeded region growing algorithm within MATLAB was applied (30) (MathWorks, Natick, MA, USA) The CT outlines were overlain onto the VSPECT and all radioactivity counts outside the lung borders were removed (Figure 1).

2) Calculating an activity threshold for each study, to define $Vent_{non}$. A method for image analysis, which we recently developed and called "Slope" was used to avoid any bias due to Technegas 'hotspots' due to aggregation at points of flow limitation. This method was developed and validated using an in-house built, phantom lung model (chapter 5). The "Slope" was calculated as follows: a cumulative frequency histogram of the SPECT data was created, and then instantaneous slopes were calculated for each data point, starting with the 90^{th} percentile datapoint. The slope for the next data point greater than the 90^{th} was then calculated, and so on, until a slope equal to or less than 0.010 was found. The activity of this slope point was then multiplied by 30% to establish the final threshold. In using this method, Slope produced accurate volumes, and extreme values "hot spots" had negligible effect on the threshold.

Non-ventilated lung volumes "$Vent_{non}$" were defined as all voxels with Technegas activity values less than the defined threshold and was expressed as a percentage of the CT defined lung volume. The change in $Vent_{non}$ post bronchodilator administration was determined by the absolute difference between $Vent_{non}$ post bronchodilator and Ventnon at baseline (Figure 2).

6.4 Statistical Analysis

Statistical analysis was carried out with SPSS (IBM, SPSS, v26), and graphs were prepared with GraphPad Prism 8 (GraphPad Software Inc). VSPECT images were analysed and processed using Fiji (Fiji.net.v2.9). Normality of distributions for all variables was assessed using the Shapiro–Wilk test. Parametric data are expressed as means ±SD, and nonparametric data are expressed as median (interquartile range). Differences between baseline and post bronchodilator variables were examined by a paired Student's t test for parametric variables or Wilcoxon's signed-rank test for nonparametric variables. Correlations between clinical and ventilation parameters were examined using Pearson's correlation coefficient or Spearman's rank correlation for parametric and nonparametric variables, respectively. Linear regression was used to determine the relationships between baseline VSPECT measures and clinical parameters. A p value of <0.05 was considered statistically significant.

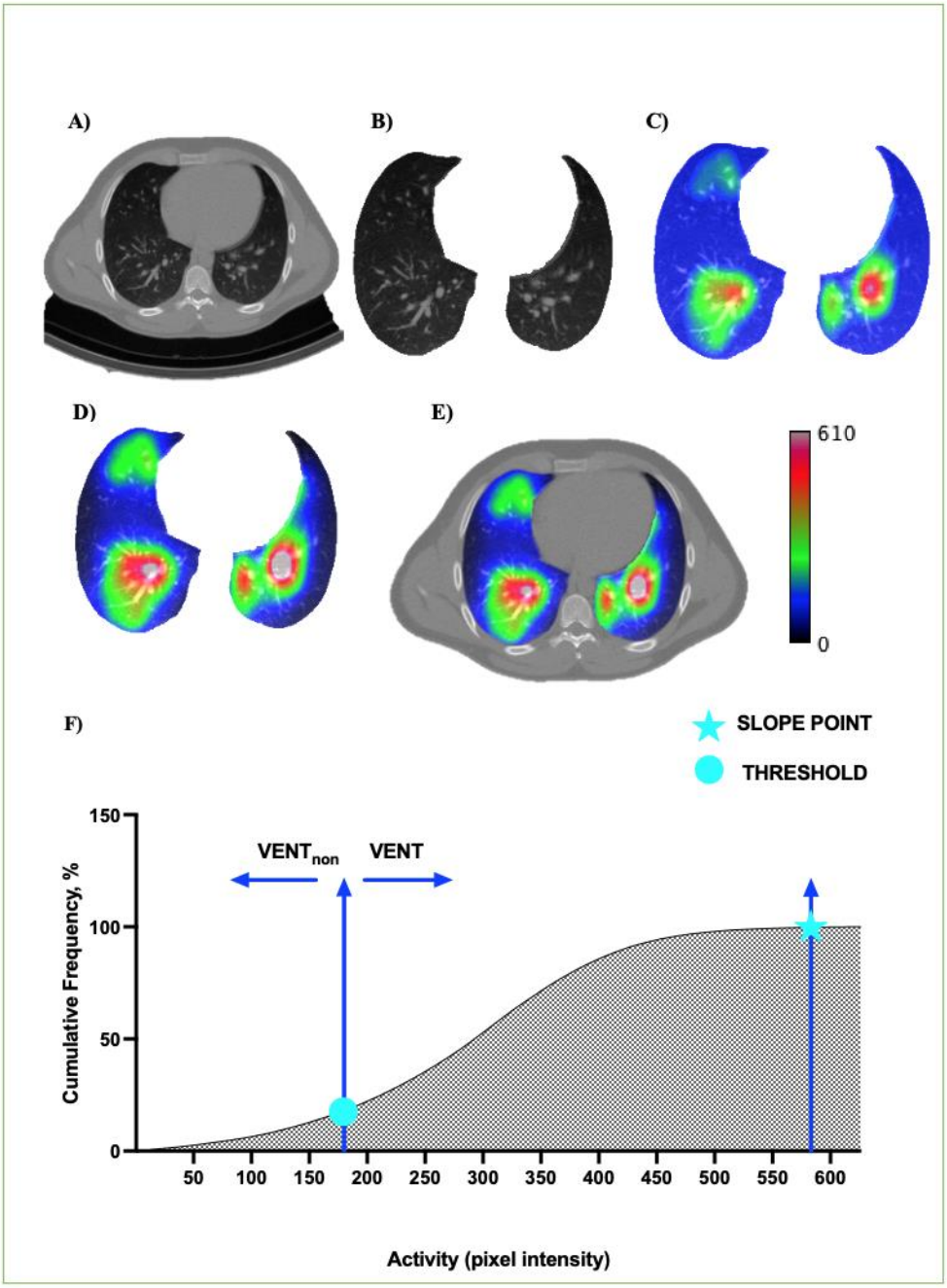


Figure 1: Imaging analysis technique:

CT of the lung (A); CT Mask of the lung (B); VSPECT ventilation overlaid with CT mask, with pixels outside set to zero (C); VSPECT with the threshold applied (D); VSPECT fused with CT (E). The colour codes are for illustrative purposes to demonstrate the image analysis principle.

Regional ventilation is represented by the colour bar on the right, with the greatest ventilation (Vent) represented by bright colours (red). Absent ventilation ($Vent_{non}$) is represented by black.

Histogram distribution, taken from a subject's VSPECT scan after processing (F)

Frequency is transformed to a cumulative frequency percentage % to calculate slope point. Final threshold is then used to divide VSPECT data into, ventilated lung (Vent), and non-ventilated ($Vent_{non}$).

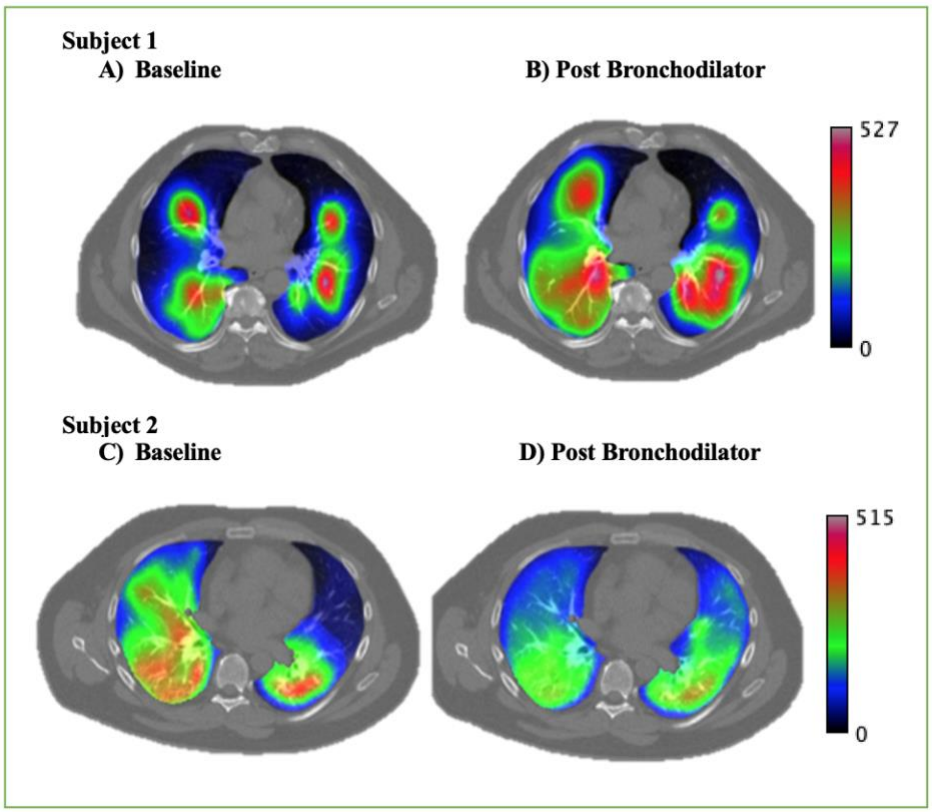


Figure 2. This figure shows the difference in responses between subjects.

(VSPECT/CT) fusion images of two subjects at baseline (A, C) and corresponding images post bronchodilator (B,D) are shown. The colour bars on the images indicate increasing relative Technegas activity, from black to bright red. The colour key indicates colours corresponding to ventilation (Vent) represented by bright red colour, and absent ventilation ($Vent_{non}$) is represented by black colour.

A, and B are images of subject 1, who has severe airway obstruction (as measured by spirometry); after bronchodilator, there was significant improvement in FEV_1, and reduction in $Vent_{non}$.

C, and D are images of a subject 2, who has normal airway function. After bronchodilator, there were improvements, and even more homogamous distribution, as well as a reduction in $Vent_{non}$ (represented by black), but there was not any improvement in spirometry.

6.5 Results

Participant demographics

Participant demographics are summarized in Table 1. Twenty-two patients with asthma were enrolled with a mean ACQ7 score of 1.27±0.93. All Participants were atopic, and 22/22 reported daily use of inhaled corticosteroids and/or inhaled corticosteroid with long-acting beta-2 agonist. Seventeen Subjects were lifelong non-smokers, and five, were past smokers with <5 pack/year history. Five had increased blood IgE levels (183.36 kU/L± 156.15) and nine has blood eosinophil counts of 0.3 x109/L.

Table 2 shows that there was a wide range of airway obstruction and small airways dysfunction, as determined by spirometry, MBNW, and FOT parameters. At baseline, eleven subjects (50%) had an abnormal FEV_1 and eleven (50%) had airflow obstruction as measured by abnormal FEV_1/FVC ratio (z-scores <-1.64). Seven subjects (31%) had an abnormal Scond, and ten (45%) had an abnormal Sacin. Also, ten (45%) patients had abnormal Rrs_{5hz}, and fourteen (63 %) had abnormal Xrs_{5hz}

Table 1. Patient demographics

Participants, n	22
Male/Female,n	9/13
Age, yr#	53 ± 22.81
Height, cm	168.6 ± 11.45
BMI, kg/m2	29.2 ± 8.65
ACQ7,score #	1.27 ± 0.93
Smoking, Pack-years	0.64 ± 1.33
FeNO, ppb	24.59 ± 28.22
Atopy, n	22/22

#Data are presented as means ± SD. BMI, body mass index;ACQ7, 7-item asthma control questionnaire; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion.

Table 2. Baseline lung function measurements

Parameter	Mean ± SD
FEV_1, % predicted	80.05 ± 20.08
FEV_1, z-scores	-1.26 ± 1.30
FVC, % predicted	96.36 ± 15.56
FVC, z-scores	-0.22 ± 1.12
FEV_1/FVC, %	82.41 ± 15.18
FEV_1/FVC, z-scores	-1.69 ± 1.33
TLC, % predicted	101.18 ± 14.60
TLC, z-scores	0.07 ± 1.09
FRCPleth, % predicted	114.05 ± 21.28
FRCPleth, z-scores	0.69 ± 1.04
FRC/TLC, % predicted	108.77 ± 15.41
FRC/TLC, z-scores	0.76 ± 1.29
RV/TLC, % predicted	115.18 ± 29.52
RV/TLC, z-scores	0.67 ± 1.32
$DLCO_{cSB}$, % predicted	94.14 ± 22.14
$DLCO_{cSB}$ z-scores	-0.42 ± 1.31
KCO_{cSB} ,% predicted	101.36±16.46
KCO_{cSB} , z-scores	0.08± 1.14
Rrs_{5hz},cmH2O·s/L	4.80 ± 1.92
Rrs_{5hz}, z-scores	1.20 ± 1.52
Xrs_{5hz}, cmH2O·s/L	-2.74 ± 2.14
Xrs_{5hz}, z-scores	2.50 ± 2.77
Scond, L-1	0.04 ± 0.02
Scond, z-scores	0.47 ± 3.57
Sacin, L-1	0.18 ±0.16
Sacin, z-scores	0.35 ± 2.28

#Data are presented as means ± SD. FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity;FRC functional residual capacity; RV, residual volume; DLCOcSB, single-breath diffusion capacity for carbon monoxide.Rrs_{5hz}, respiratory system resistance at 5Hz; Xrs_{5hz}, respiratory system reactance at 5Hz;Scond, convection-dependent ventilation heterogeneity; Sacin, diffusion-dependent ventilation heterogeneity. Abnormal parameters are in bold.

Changes in lung function and VSPECT post bronchodilator

Changes in lung function are shown in Figure 3. There was improvement in FEV_1 ($P< 0.0001$) and FEV_1/FVC ($P< 0.0001$), but no change in FVC ($P=0.608$). Of the 22 subjects, eight (31%) had a positive bronchodilator response as measured by FEV_1. Also, there was an improvement, i.e., reduction in Rrs_{5hz} ($P< 0.0001$), and Xrs_{5hz} became less negative, i.e., improved ($P< 0.001$). Nine subjects (27%) met BDR criteria by Xrs_{5hz}, compared to only five (18%) in Rrs_{5hz}.

Only three subjects overlapped.

Baseline and post bronchodilator VSPECT measurements are shown in Figure 3. At baseline, $Vent_{non}$ was 35.46%±17.60.. There was no significant change in $Vent_{non}$ post bronchodilator, although, there was a wide range of responses.

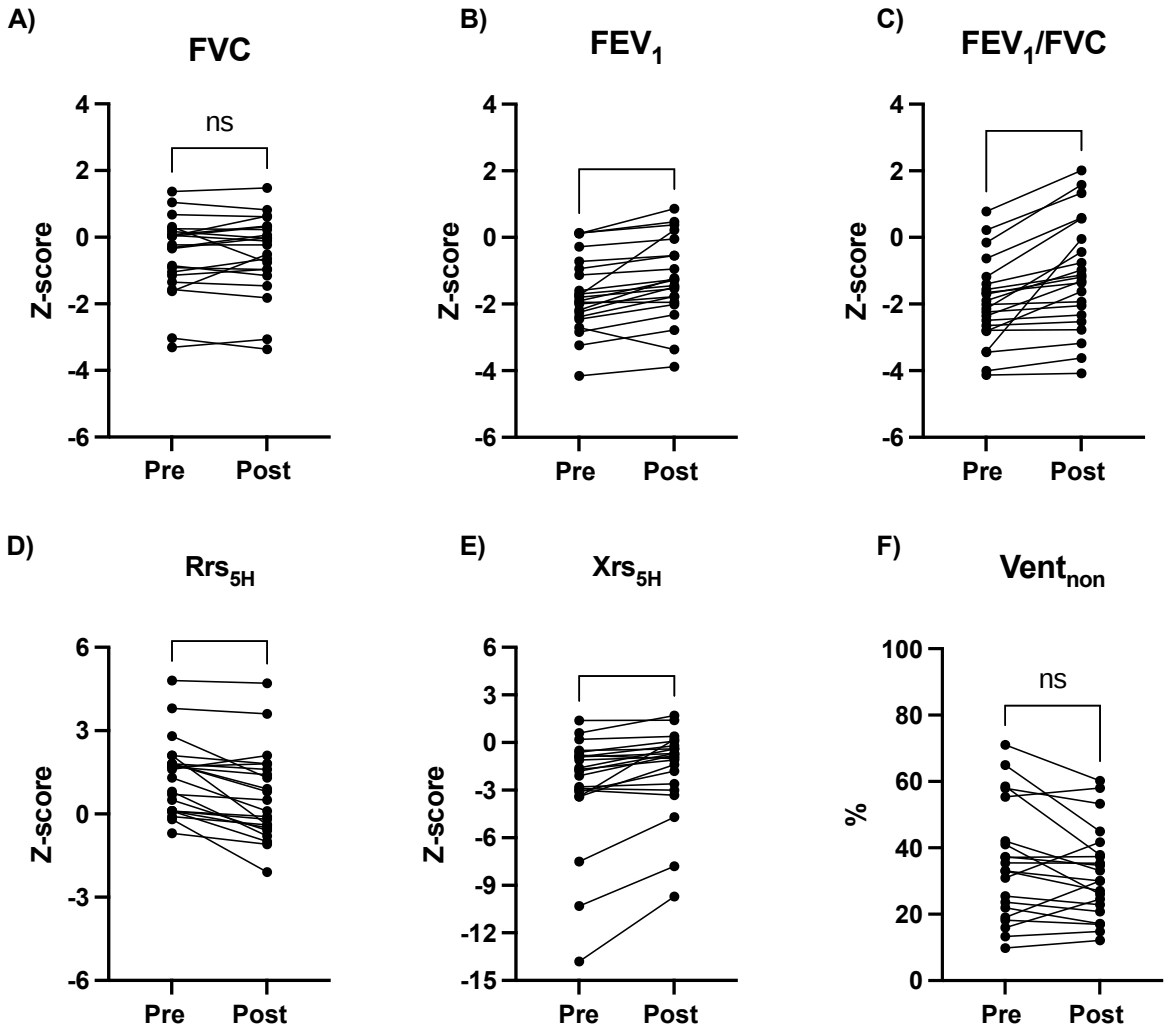


Figure 3. Pre and post bronchodilator lung function measurements.

Mean difference ±SDs for FVC (A) (-0.04 ±0.42), FEV_1 (B)(-0.42 ±0.46), FEV_1/FVC (C) (0.83 ± 0.81), Rrs_{5hz} (D) (0.69 ± 0.72), Xrs_{5hz} (E) (-1.05 ± 1.23), the percentage of the total lung volume that and non-ventilated "$Vent_{non}$"(F) (-3.15 ± 8.59).

Comparisons between baseline and post bronchodilator measurements were carried out using a paired Student's T-test. There were improvements in FEV_1, FEV_1/FVC , Rrs_{5hz}, and Xrs_{5hz}. Volume percentage of $Vent_{non}$ did not significantly change post bronchodilator.

A P value **** <0.0001, *** >0.0002 but <0.001

Relationships between lung function and VSPECT

Table 3. shows the univariate correlations of pre, and post bronchodilator $Vent_{non}$ with baseline lung function parameters.

Baseline $Vent_{non}$ was related to age (p=0.005), i.e. older subjects had much larger $Vent_{non}$ compared to younger subjects. Baseline $Vent_{non}$ was not related to any spirometric, lung volumes, MBNW or FOT indices. There was a negative relationship with KCO (p=0.004) with higher values being associated with lower $Vent_{non}$.

Post bronchodilator, mean $Vent_{non}$ for the group did not change compared with baseline, and did not correlate to any post bronchodilator spirometry, or FOT indices (Table 4). Post bronchodilator $Vent_{non}$ was only predicted by older age (p=0.028), baseline KCO (p=0.004), and baseline Scond (p=0.034).

Figure 4. Shows the relationships between the changes in $Vent_{non}$ ($\Delta Vent_{non}$) , and the changes in lung function measurements post bronchodilator. The changes in $Vent_{non}$ ($\Delta Vent_{non}$) were related to age (p=0.021), (Figure 4.1), and ΔRrs_{5hz} (p=0.001), but not with ΔXrs_{5hz} .(Figure 4.2).

Table 3. Univariate correlations between baseline lung function and pre and post bronchodilator $Vent_{non}$

	Baseline $Vent_{non}$		Post BD $Vent_{non}$	
Parameter	rho	P value	rho	P value
Age, yr	0.586	0.005	0.480	0.028
BMI, kg/m2	-0.108	0.642	-0.211	0.358
ACQ7,score #	-0.176	0.446	-0.087	0.707
Smoking, Pack-years	-0.011	0.961	-0.068	0.768
FeNO, ppb	-0.207	0.368	-0.037	0.873
FEV_1, % predicted	-0.192	0.404	-0.236	0.304
FEV_1, z-score	-0.049	0.832	-0.135	0.559
FVC, % predicted	-0.041	0.860	0.046	0.842
FVC, z-score	-0.060	0.795	0.028	0.904
FEV_1/FVC, %	-0.284	0.210	-0.373	0.904
FEV_1/FVC, z-score	-0.084	0.716	-0.259	0.257
RV/TLC, %	-0.363	0.106	-0.295	0.194
RV/TLC, z-score	-0.034	0.884	-0.040	0.862
FRC/TLC, %	0.259	0.258	0.062	0.790
FRC/TLC, z-score	-0.091	0.659	-0.249	0.277
$DLCO_{cSB}$, mL/min/mm Hg	-0.176	0.445	-0.204	0.375
$DLCO_{cSB}$, z-score	-0.415	0.061	-0.345	0.126
KCO_{cSB}, mL/min/mm Hg	-0.601	0.004	-0.607	0.004
KCO_{cSB}, z-score	-0.447	0.042	-0.437	0.048
Rrs_{5hz}, cmH2O·s/L	-0.002	0.993	0.656	0.127
Rrs_{5hz}, z-score	-0.055	0.813	0.127	0.582
Xrs_{5hz}, cmH2O·s/L	0.301	0.185	-0.292	0.199
Xrs_{5hz}, z-score	0.209	0.364	-0.112	0.628
Scond, L-1	0.250	0.302	-0.380	0.109
Scond, z-score	0.394	0.584	-0.489	0.034
Sacin, L-1	-0.134	0.584	0.269	0.266
Sacin, z-score	0.040	0.870	0.149	0.542

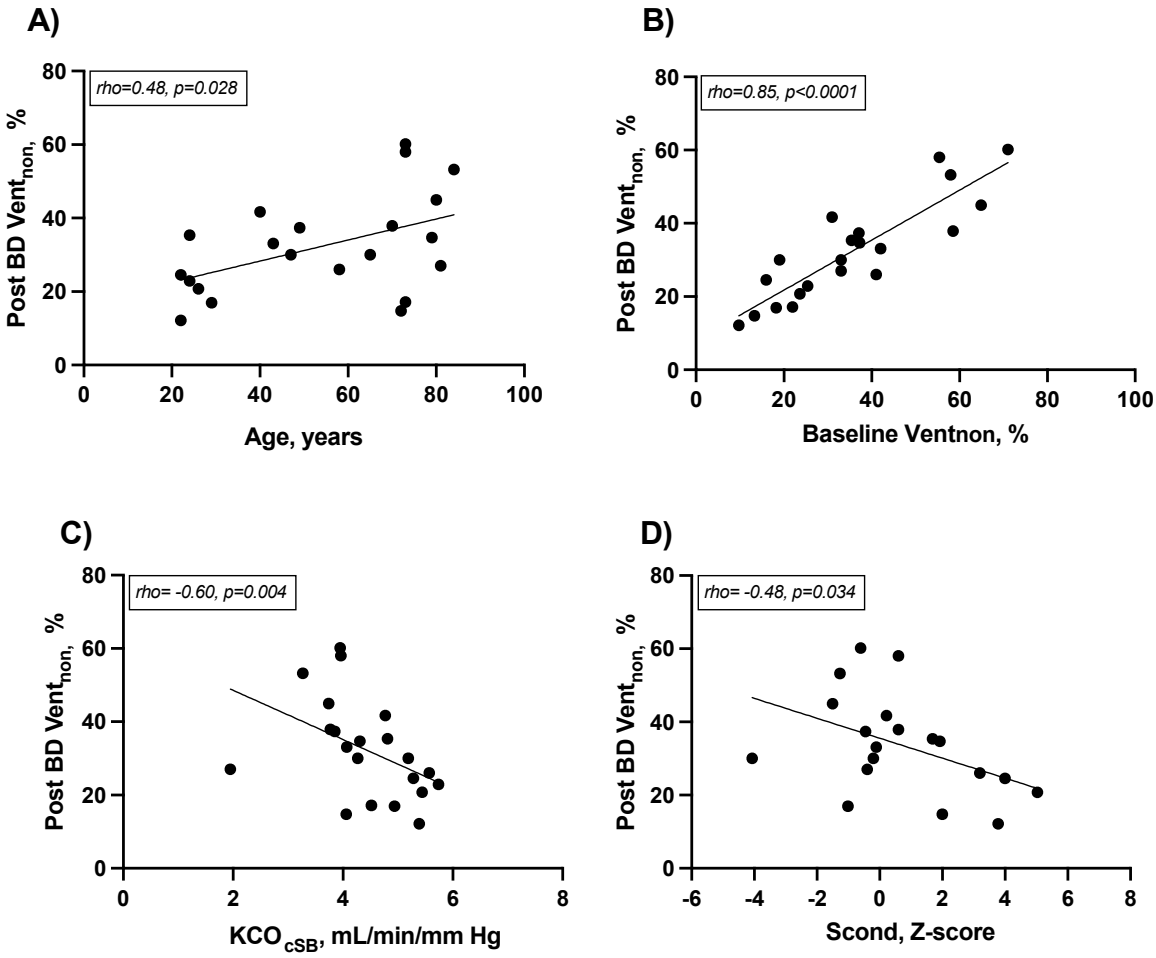
#Univariate correlations between baseline lung function and pre and post nonventilated lung volume ($Vent_{non}$) using Spearman's correlation coefficient. BMI, body mass index;ACQ7, 7-item asthma control questionnaire; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; RV, residual volume, TLC, total lung capacity;FRC functional residual capacity; $DLCO_{cSB}$, single-breath diffusion capacity for carbon monoxide; KCO_{cSB}, Carbon monoxide transfer coefficient;Rrs_{5hz}, respiratory system resistance at 5Hz; Xrs_{5hz}, respiratory system reactance at 5Hz; Scond, convection-dependent ventilation heterogeneity; Sacin, diffusion-dependent ventilation heterogeneity. Significant correlations are in bold. A P value of <0.05 was considered statistically significant.

Table 4. Univariate correlations between post bronchodilator lung function and pre and post bronchodilator $Vent_{non}$

	Baseline $Vent_{non}$		Post BD $Vent_{non}$	
Parameter	rho	P value	rho	P value
Post BD FEV_1, % Pred	0.237	0.301	-0.187	0.685
Post BD FEV_1, z-score	-0.116	0.618	-0.094	0.685
Post BD FVC, % Pred	0.70	0.762	0.289	0.204
Post BD FVC, z-score	0.054	0.816	0.289	0.204
Post BD FEV_1/FVC, %	-0.353	0.116	-0.370	0.098
Post BD FEV_1/FVC, z-scores	-0.220	0.337	-0.341	0.130
Post BD Rrs_{5hz},cmH2O·s/L	-0.034	0.992	-0.086	0.712
Post BD Rrs_{5hz} ,z-score	0.097	0.677	-0.039	0.866
Post BD Xrs_{5hz}, cmH2O·s/L	-0.110	0.636	0.016	0.947
Post BD Xrs_{5hz},z-score	-0.222	0.333	0.033	0.888

#Univariate correlations between post bronchodilator lung function and nonventilated lung volume ($Vent_{non}$) using Spearman's correlation coefficient.FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; Rrs_{5hz}, respiratory system resistance at 5Hz; Xrs_{5hz}, respiratory system reactance at 5Hz; Scond, convection-dependent ventilation heterogeneity; Sacin, diffusion-dependent ventilation heterogeneity.

1)


A)
rho=0.48, p=0.028
Post BD Ventnon, %
Age, years
B)
rho=0.85, p<0.0001
Post BD Ventnon, %
Baseline Ventnon, %
C)
rho= -0.60, p=0.004
Post BD Ventnon, %
KCOcSB, mL/min/mm Hg
D)
rho= -0.48, p=0.034
Post BD Ventnon, %
Scond, Z-score

2)

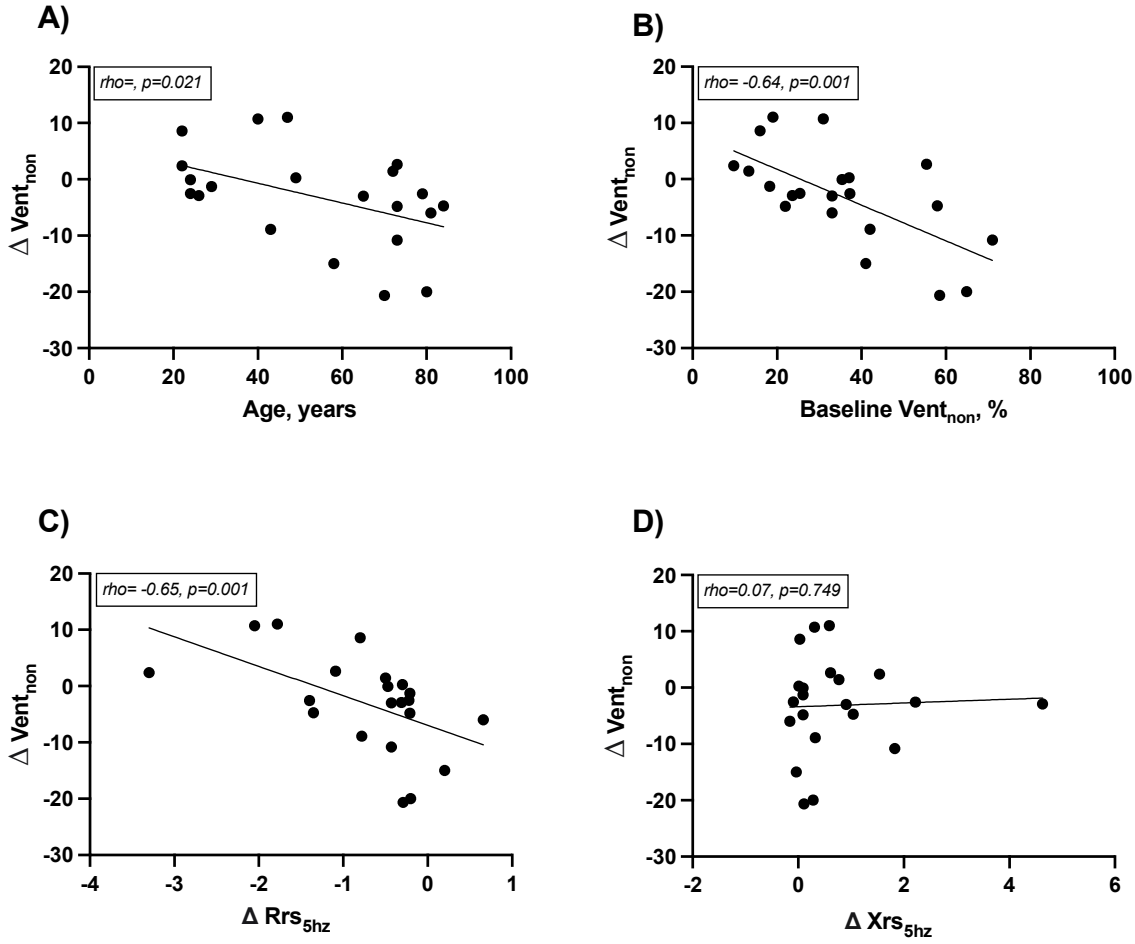


Figure 4. Univariate correlations between lung function, and nonventilated lung volume ($Vent_{non}$) using Spearman's correlation coefficient. A P value of <0.05 was considered statistically significant Post bronchodilator $Vent_{non}$ % was predicted by older age (A), baseline $Vent_{non}$ (B), baseline KCO (C), and worse baseline Scond (D)

Changes in nonventilated lung volume ($\Delta Vent_{non}$) correlated with age (A), baseline $Vent_{non}$ (B), changes in Rrs_{5hz} (ΔRrs_{5hz}) (C), but not with changes in Xrs_{5hz} (D).

Absolute change in $\Delta Vent_{non}$ = (post bd $Vent_{non}$ – pre bd $Vent_{non}$).

6.6 Discussion

In this study of asthmatic subjects, bronchodilator response was measured using ventilation SPECT/CT with Technegas. The study examined relationships between ventilation and lung function, both at baseline, and post bronchodilator inhalation. It was found that bronchodilator administration improved spirometry and oscillatory impedance, but there was no change in mean non-ventilation on VSPECT. However, there was wide range of responses. Furthermore, increasing $Vent_{non}$ was correlated with increasing age, but not with spirometry, oscillatory impedance or MBNW parameters. The change in $Vent_{non}$ was also predicted by age, baseline $Vent_{non}$ and by oscillatory resistance.

The findings in the current study are the first reported measurements of bronchodilator responses measured by VSPECT, in asthmatic subjects. There has been a single report of changes in $Vent_{non}$ in asthma (9), using hyperpolarised ^{3}He MRI, in which the authors reported a significant decrease in $Vent_{non}$ from 6 ±7% to 4 ±5% in 47 asthmatic participants. However, they also reported that only 10/47 participants were bronchodilator 'responders' in terms of $Vent_{non}$. The quantity of $Vent_{non}$ differed greatly compared with that of the current study (35.4 ±17.6%). The differences between the study findings are likely to be due to differences in measurement, including technical differences in ventilation contrast, hardware differences between MRI and SPECT, and to differences in image analysis methods. Radadia et al. (31) showed that image analysis method had the greatest effect on $Vent_{non}$ measurements, in participants undergoing lung cancer resection, who had both ^{129}Xe MRI and Technegas SPECT. Using a SPECT image analysis method (32) produced more than double the $Vent_{non}$ compared with analysis methods commonly used in MRI (23.0 ±14.0 versus 9.4 ±9.4%, respectively), when applied to SPECT image data. Similar differences due to different image analysis method was found with MRI image data (21.0 ±5.2% versus 7.8 ±10%).

Interestingly, despite differences in the physical properties of the ventilation agents, Technegas is an ultra-fine carbon aerosol while Xe is a true but non-inert gas, $Vent_{non}$ measurements were similar (23.0 ±14.0% versus 21.0 ±5.2%).

The method that was used to derive $Vent_{non}$ was rigorously validated using a phantom study, imaged using the same parameters and hardware that we used in our imaging of participants. This is also a novel step in the imaging methodology which provide confidence that our measurements of $Vent_{non}$ are accurate. In contrast, there have not been similar studies with K-means cluster analysis for hyperpolarised gas MRI, presumably due to logistical requirements for an equivalent phantom study. Subjectively, our visual assessments of $Vent_{non}$ correlated with the quantitative measurement i.e. that the non-ventilated volume could be large. Because Technegas is an ultra-fine carbon aerosol (of the order of 100nm diameter), it won't distribute as a true gas. However, the comparison study above (31) suggests strong equivalence in $Vent_{non}$ estimation between Technegas and xenon, albeit in non-obstructed patients.

In the current study, there was marked discordance between $Vent_{non}$ and spirometry. This may be due to the fact that VSPECT is a measurement of ventilation during a 1 litre inhalation from FRC, while spirometry involves a forced vital capacity exhalation. There may also be differences in the way that large and small airways affect VSPECT and spirometry. It is possible that $Vent_{non}$ is determined by small airway function, to which spirometry is insensitive. This is in agreement with our previous SPECT studies, that did not report any correlations between VSPECT, and FEV_1(13, 33). Furthermore, there was no relationship between FVC, and $Vent_{non}$. However, this may be due to the fact that Technegas was inhaled from FRC, whereas FVC occurs during forced exhalation, below FRC. Some non-ventilated areas may be considered 'normal,' while others could be deemed 'abnormal.' Determining which is which requires a comprehensive dataset of healthy individuals,

which currently does not exist. Therefore, a future study could aim to develop a robust library of healthy ventilation data, which would allow for more accurate comparisons and help address the challenges posed by differing breathing manoeuvres.

The relationship between Scond and post-bronchodilator $Vent_{non}$ (although not with pre-bronchodilator $Vent_{non}$), suggests that conductive small airway function is related to phenomenon of airway closure. Higher baseline Scond correlated with greater post BD Ventnon. In a previous VSPECT study of asthma, Scond was strongly correlated with $Vent_{non}$ ($rs = 0.73$, $p<0.001$, $n=22$) (33), and in a different study, predicted the increase in $Vent_{non}$ with methacholine induced bronchoconstriction (32). In healthy participants, Scond correlated strongly with trapped gas volume (34). Thus the relationships between Scond and airway closure or non-ventilation seem to be consistent. The physiologic explanations are uncertain however. Perhaps the $Vent_{non}$ regions receive a some degree of ventilation due to limitations in SPECT resolution and sensitivity, which then contribute to conductive ventilation heterogeneity. Alternatively, $Vent_{non}$ is a marker of ventilation heterogeneity in the rest of the lung that is clearly ventilated according to SPECT.

The ultra-fine particle size of Technegas suggests that its distribution throughout the lungs likely mimics that of a gas, but it's not a true gas. Technegas distribution is likely convection but not diffusion dependent in nature, hence our suggested reasoning that Technegas measurements might also reflect abnormalities in the conductive, small airway zone.

Although Xrs_{5hz} has been found to be sensitive to airway closure, the result of the current study found no significant relationship between Xrs_{5hz}, and $Vent_{non}$ at baseline or post bronchodilator. Furthermore, there was also no significant relationship with baseline or post bronchodilator Rrs_{5hz}, however, the change in Δ $Vent_{non}$ correlated with the change in ΔRrs_{5hz}.

The results showed that subjects with less change in Rrs_{5hz} , showed the greatest change in their $Vent_{non}$. This apparently paradoxical relationship might be explained by bronchodilatation opening up some closed areas but in doing so, worsening mean airway calibre and worsening overall ventilation heterogeneity, if ventilation increased in lung regions that were highly abnormal. This results are in agreement with a previous SPECT study where there was no relationship between VSPECT and Xrs_{5hz} (nor Rrs) post ICS/LABA treatment (33). The relationship with Rrs_{5hz} is novel in relation to SPECT ventilation. The study by Young et al.(35) is one of very few ventilation imaging studies in which there was a weak but significant relationship between MRI VPD and FOT-measured Rrs and Xrs and was a study of 50 asthmatics. However, the magnitude of MRI VPD differs markedly to that measured by VSPECT (as mentioned above). In ^{3}He MRI studies, ^{3}He is inhaled while subjects are positioned supine in the scanner, with the image performed under breath hold conditions. Harris et al. (15) showed that in asthma, the size of ventilation defects tend to increase in supine compared with prone position. In contrast, subjects in this study inhaled Technegas in upright position. This at least means that the body positions for lung function, and FOT, were the same as during Technegas inhalation. Because Technegas adheres to lung structures after which its distribution is unchanged, even though subjects are scanned in supine position, the Technegas distribution still reflects ventilation when upright. Changes in Technegas distribution due to lying down are likely to be small, as suggested by previous SPECT studies (32, 36). Dependent lung tissue may compress in some, which might increase Technegas count density, while reducing it caudal lung regions, however, the exact nature and magnitude of this change cannot be measured given upright SPECT scanning does not exist.

The post-bronchodilator VSPECT visit was scheduled 1-7 days after the baseline, however, based on a ^{3}He MR Imaging study by De Lange et al, it is reasonably safe to assume that distribution of

poorly ventilated lung would not change significantly within a one-week period between VSPECT scans (37). In their study, 67% of defects persisted between studies 1 and 2 (with a median interval of 31 days), 43% persisted between studies 2 and 3 (with a median interval of 41 days), and 38% persisted between studies 1 and 3 (with a median interval of 85 days). The majority of defects (75%) remained in the same location, and most of these did not change in size (37). Additionally, all subjects were asked to withhold their asthma medication according to ERS/ATS withholding times. However, due to logistical problems with visit scheduling, bronchodilator withhold times varied between participants. This may have contributed to greater between-visit variability, particularly with those with severe asthma. It might have also resulted in, or contributed to, the absence of any correlation between VSPECT and FOT indices.

In this study, the relationship between KCO and Ventnon was significant, both at baseline and following bronchodilator administration. Greater $Vent_{non}$ was related to a lower KCO in asthmatic patients is a novel finding. Diffusing capacity of the lungs measures the transfer of carbon monoxide across the alveolar capillary membrane. KCO is a measurement of the rate of CO transport across the alveolar-capillary barrier, per unit of partial pressure gradient, per unit of time and per unit of volume of lung. It can thus, be conceptualised as an index of efficiency of alveolar CO transfer. KCO is sensitive to pulmonary blood flow, and ventilation redistribution (38, 39). Greater $Vent_{non}$ represents greater ventilatory impairment in a regional or topographical sense (i.e. where ventilation is impaired the most), which in turn, may make it more difficult to maintain optimal V/Q matching (hence a lower KCO). Without associated perfusion data, it can only be speculated that V/Q mismatch may be involved. Another possible explanation is alveolar enlargement. There is considerable evidence to support this occurring in asthma, and that it is age related, and if greater $Vent_{non}$ were also a marker of alveolar enlargement (which would make

airway closure more likely and more extensive, then this is a plausible explanation for the relationship between KCO and Ventnon (40, 41).

Age was found related to $Vent_{non}$, both at baseline and post bronchodilator. Age also predicted the changes in Δ $Vent_{non}$. Older participants had larger defects at baseline, but also had the greater change (response) post bronchodilator. Ventilation heterogeneity in known to increase with age, and also increase with asthma. Improvements in ventilation post bronchodilator seen in older subjects could be due to the combined effects of asthma severity, asthma duration, and aging. However, the explanation for this relationship is unknown.

There are no previous VSPECT data on which to base power calculations; therefore, this study was entered as a pilot. Based on an early study by Capaldi et al. (42), which examined 12 patients with severe and poorly controlled asthma (mean age 49 ± 10 years) using MRI to assess ventilation defects (VDP), it was found that VDP significantly decreased post-salbutamol. In their study, baseline VDP was 11.1 ± 10.1%, and post-salbutamol VDP was 8.1 ± 7.6% (P = 0.02). The power calculation suggests that a mean difference (Δ) of 3.0%, and a standard deviation of differences (SDΔ) of 12.65% would require n = 137 participants to detect an effect size of 3% for VDP. However, the study reported statistical significance with only 12 participants, which seems inconsistent with the calculated sample size required for statistical significance. Physiologically, a clinically significant response would need a difference of around 8–10%. Finding an 8% difference in $Vent_{non}$, SD of differences of 12.5%, would require 20 subjects.

6.7 Limitations

The study acknowledges that this work was limited by the small number of subjects, which also means that these results should be interpreted with caution. Asthma participants reported daily use

of inhaled corticosteroids and/or inhaled corticosteroid with long-acting beta-2 agonist suggesting that they were receiving optimal asthma therapy and likely had at least asthma of moderate severity. Future studies could evaluate a larger group of asthmatic subjects with varying disease severity, treatment levels and disease durations. Furthermore, baseline, and bronchodilator SPECT ventilation visits were performed on different days, which could have affected relationships between VSPECT and bronchodilator responses on lung function.

6.8 Conclusion

Bronchodilator response was measured using VSPECT/CT with Technegas, in asthma subjects with mild to severe disease, and the relationships with spirometry and small airway measurements were examined. Bronchodilator administration did not result in a significant improvements in ventilation assessed with functional VSPECT imaging, being disassociated from the improvements in spirometry, and oscillatory impedance. The response in VSPECT is variable, however, the reduction in nonventilated lung observed with older subjects suggest that asthma duration, and severity may influence the response. This significance however require further investigation. This study supports a complementary method (to traditional lung function) of measuring lung function in asthma, which may contribute to improving ‘precision medicine’ i.e. individualised treatment. Future studies should explore VSPECT- derived measures with asthma important clinical outcomes.

6.9 References

1. The Copenhagen City Heart Study G, Lange P, Ulrik CS, Vestbo J, Vestbo J. Mortality in adults with self-reported asthma. The Lancet. 1996;347(9011):1285-9.
2. Hanania NA, Castro M, Bateman E, Pavord ID, Papi A, FitzGerald JM, et al. Efficacy of dupilumab in patients with moderate-to-severe asthma and persistent airflow obstruction. Ann Allergy Asthma Immunol. 2023;130(2):206-14.e2.
3. Lee LA, Bailes Z, Barnes N, Boulet LP, Edwards D, Fowler A, et al. Efficacy and safety of once-daily single-inhaler triple therapy (FF/UMEC/VI) versus FF/VI in patients with inadequately controlled asthma (CAPTAIN): a double-blind, randomised, phase 3A trial. Lancet Respir Med. 2021;9(1):69-84.
4. Quanjer PH, Ruppel GL, Langhammer A, Krishna A, Mertens F, Johannessen A, et al. Bronchodilator Response in FVC Is Larger and More Relevant Than in FEV(1) in Severe Airflow Obstruction. Chest. 2017;151(5):1088-98.
5. Chapman DG, Berend N, King GG, Salome CM. Increased airway closure is a determinant of airway hyperresponsiveness. Eur Respir J. 2008;32(6):1563-9.
6. King GG, Eberl S, Salome CM, Young IH, Woolcock AJ. Differences in airway closure between normal and asthmatic subjects measured with single-photon emission computed tomography and technegas. Am J Respir Crit Care Med. 1998;158(6):1900-6.
7. Harris RS, Winkler T, Tgavalekos N, Musch G, Melo MF, Schroeder T, et al. Regional pulmonary perfusion, inflation, and ventilation defects in bronchoconstricted patients with asthma. Am J Respir Crit Care Med. 2006;174(3):245-53.
8. Samee S, Altes T, Powers P, de Lange EE, Knight-Scott J, Rakes G, et al. Imaging the lungs in asthmatic patients by using hyperpolarized helium-3 magnetic resonance: assessment of response to methacholine and exercise challenge. J Allergy Clin Immunol. 2003;111(6):1205-11.
9. Zha N, Pike D, Svenningsen S, Capaldi DP, McCormack DG, Parraga G. Second-order Texture Measurements of (3)He Ventilation MRI: Proof-of-concept Evaluation of Asthma Bronchodilator Response. Acad Radiol. 2016;23(2):176-85.
10. Svenningsen S, Kirby M, Starr D, Leary D, Wheatley A, Maksym GN, et al. Hyperpolarized (3) He and (129) Xe MRI: differences in asthma before bronchodilation. J Magn Reson Imaging. 2013;38(6):1521-30.

11. Svenningsen S, Eddy RL, Lim HF, Cox PG, Nair P, Parraga G. Sputum Eosinophilia and Magnetic Resonance Imaging Ventilation Heterogeneity in Severe Asthma. American Journal of Respiratory and Critical Care Medicine. 2018;197(7):876-84.
12. Downie SR, Salome CM, Verbanck S, Thompson B, Berend N, King GG. Ventilation heterogeneity is a major determinant of airway hyperresponsiveness in asthma, independent of airway inflammation. Thorax. 2007;62(8):684-9.
13. Farrow CE, Salome CM, Harris BE, Bailey DL, Berend N, King GG. Peripheral ventilation heterogeneity determines the extent of bronchoconstriction in asthma. J Appl Physiol (1985). 2017;123(5):1188-94.
14. Amis TC, Crawford AB, Davison A, Engel LA. Distribution of inhaled 99mtechnetium labelled ultrafine carbon particle aerosol (Technegas) in human lungs. Eur Respir J. 1990;3(6):679-85.
15. Harris RS, Winkler T, Musch G, Melo MFV, Schroeder T, Tgavalekos N, et al. The prone position results in smaller ventilation defects during bronchoconstriction in asthma. Journal of Applied Physiology. 2009;107(1):266-74.
16. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. European Respiratory Journal. 2005;26(2):319-38.
17. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. European Respiratory Journal. 2012;40(6):1324-43.
18. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. European Respiratory Journal. 2005;26(5):948-68.
19. Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. Respir Med. 2006;100(4):616-21.
20. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FeNO) for Clinical Applications. American Journal of Respiratory and Critical Care Medicine. 2011;184(5):602-15.
21. Heinzerling L, Mari A, Bergmann K-C, Bresciani M, Burbach G, Darsow U, et al. The skin prick test – European standards. Clinical and Translational Allergy. 2013;3(1):3.

22. Strunk RC, Szefler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, et al. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. J Allergy Clin Immunol. 2003;112(5):883-92.

23. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A, et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. European Respiratory Journal. 2017;49(1):1600016.

24. Stanojevic S, Graham BL, Cooper BG, Thompson Bruce R, Carter KW, Francis RW, et al. Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians. European Respiratory Journal. 2017;50(3):1700010.

25. Oostveen E, Macleod D, Lorino H, Farre R, Hantos Z, Desager K, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. European Respiratory Journal. 2003;22(6):1026-41.

26. Oostveen E, Boda K, van der Grinten CP, James AL, Young S, Nieland H, et al. Respiratory impedance in healthy subjects: baseline values and bronchodilator response. Eur Respir J. 2013;42(6):1513-23.

27. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. The European respiratory journal. 2013;41(3):507-22.

28. Verbanck S, Van Muylem A, Schuermans D, Bautmans I, Thompson B, Vincken W. Transfer factor, lung volumes, resistance and ventilation distribution in healthy adults. Eur Respir J. 2016;47(1):166-76.

29. Nobuyuki Otsu. A Threshold Selection Method from Gray-Level Histograms. IEEE Trans. Syst. M, Cybern.. 1979. Vol. 9(1):62-66. DOI: 10.1109/TSMC.1979.4310076.

30. D. Kroon RG. D. Kroon, Region Growing. 2021. 2021.

31. Radadia N, Friedlander Y, Priel E, Konyer NB, Huang C, Jamal M, et al. Comparison of ventilation defects quantified by Technegas SPECT and hyperpolarized (129)Xe MRI. Front Physiol. 2023;14:1133334.

32. Farrow CE, Salome CM, Harris BE, Bailey DL, Bailey E, Berend N, et al. Airway closure on imaging relates to airway hyperresponsiveness and peripheral airway disease in asthma. J Appl Physiol (1985). 2012;113(6):958-66.

33. Rutting S, Chapman DG, Thamrin C, Tang FSM, Carroll JRD, Bailey DL, et al. Effect of combination inhaled therapy on ventilation distribution measured by SPECT/CT imaging in uncontrolled asthma. Journal of Applied Physiology. 2021;131(2):621-9.
34. King GG, Downie SR, Verbanck S, Thorpe CW, Berend N, Salome CM, et al. Effects of methacholine on small airway function measured by forced oscillation technique and multiple breath nitrogen washout in normal subjects. Respir Physiol Neurobiol. 2005;148(1-2):165-77.
35. Young HM, Guo F, Eddy RL, Maksym G, Parraga G. Oscillometry and pulmonary MRI measurements of ventilation heterogeneity in obstructive lung disease: relationship to quality of life and disease control. J Appl Physiol (1985). 2018;125(1):73-85.
36. King GG, Eberl S, Salome CM, Meikle SR, Woolcock AJ. Airway closure measured by a technegas bolus and SPECT. Am J Respir Crit Care Med. 1997;155(2):682-8.
37. De Lange EE, Altes T, Patrie JT, Battiston JJ, Juersivich AP, Mugler JP, et al. Changes in Regional Airflow Obstruction over Time in the Lungs of Patients with Asthma: Evaluation with 3He MR Imaging. Radiology. 2009;250(2):567-75.
38. Collard P, Njinou B, Nejadnik B, Keyeux A, Frans A. Single breath diffusing capacity for carbon monoxide in stable asthma. Chest. 1994;105(5):1426-9.
39. Weitzman RH, Wilson AF. Diffusing capacity and over-all ventilation:perfusion in asthma. Am J Med. 1974;57(5):767-74.
40. Gelb AF, Yamamoto A, Verbeken EK, Schein MJ, Moridzadeh R, Tran D, et al. Further Studies of Unsuspected Emphysema in Nonsmoking Patients With Asthma With Persistent Expiratory Airflow Obstruction. Chest. 2018;153(3):618-29.
41. Shimizu K, Tanabe N, Oguma A, Kimura H, Suzuki M, Yokota I, et al. Parenchymal destruction in asthma: Fixed airflow obstruction and lung function trajectory. J Allergy Clin Immunol. 2022;149(3):934-42.e8.
42. Capaldi DPI, Sheikh K, Eddy RL, Guo F, Svenningsen S, Nair P, et al. Free-breathing Functional Pulmonary MRI: Response to Bronchodilator and Bronchoprovocation in Severe Asthma. Acad Radiol. 2017;24(10):1268-76.

Chapter 7. Conclusion And Future Directions

Asthma is common, and complex disease, with clinical and physiological characteristics that vary significantly from one patient to another. Improving disease outcomes is a fundamental goal because it directly impacts the quality of life in individuals with asthma (1). However, achieving optimal control in asthma is challenging, primarily because the heterogeneity, and the complexity makes it difficult to use one-size-fits-all approach (2, 3). The primary aim of my thesis was to utilise, and understand the physiological measurements, that might potentially provide effective tools to enable personalised asthma care in the future. Spirometry was used as a standard lung function test to assess overall lung function. Peak expiatory flow (PEF) was used to assess airway lability. The multiple breath nitrogen washout (MBNW), and the forced oscillation technique (FOT) were used to determine ventilation heterogeneity, and lung mechanics. Regional ventilation was measured using ventilation single photon emission computed tomography/computed tomography (VSPECT/CT). A summary of the main findings from my thesis and future directions are discussed below.

7.1 Day To Day Peak Expiratory Flow Variability

PEF variability is unique to each individual, which means it can be valuable clinical tool for personalised care in asthma management. My thesis has shown the day-to-day variability in peak expiratory flow (PEF) among healthy non-smoker adults is 24% when measured or expressed as amplitude percent highest (A%H). However, there is a wide range of variability between healthy subjects. This finding is important; it provides clinicians with evidence based information for interpreting day to day PEF variability in clinical practice. Additionally, it was found that PEF variability is highly influenced by the method used for calculation. Where health care providers subjectively estimate variability, there is potential for differences between practitioners, who should be aware of the potential discrepancies. Overlooking these differences could lead to

misinterpretation of the information and inaccurate assessment of the patient's disease status. These findings are important because current available guidelines do not have standardised protocols for PEF monitoring. Instead, they typically offer general guidance, leaving the specifics of monitoring, frequency, technique, and interpretation to the healthcare providers. Future research should be directed towards developing evidence-based guidelines or protocols for PEF monitoring.

7.2 Concavity of The Flow-Volume Loop And Small Airway Measures

Small airway measurements, including concavity indices, multiple-breath nitrogen washout (MBNW), and oscillometry, play a crucial role in the early detection of disease. However, these tests are not commonly used in routine clinical practice. My thesis has shown that in smokers, abnormalities in concavity indices, MBNW, and oscillometry are common despite normal lung function by spirometry. It was also found that these abnormalities do not occur in the same individuals, indicating poor concordance between these tests. These findings provide some insight into how different small airway measurements might be used in routine clinical practice since mid-expiratory flows and oscillatory impedance clearly detect different physiologic abnormalities. Future studies could investigate whether changes in small airway function may precede any clinical exacerbations, or worsening symptoms. Spirometry isn't sensitive in detecting changes in lung function, especially in the early stages of the disease (4). Therefore, there is a risk that these abnormalities may not be identified until the damage to the airways has become irreversible i.e. could small airway tests serve as a risk predictor for loss of lung function? Future research could involve different disease cohorts to assess the consistency of these findings and assess the clinical relevance of the concavity indices in predicting disease outcomes. This could involve evaluating the associations between abnormalities of the concavity index, and the development of symptoms,

decline in lung function, and onset of obstructive lung diseases such as chronic obstructive pulmonary disease (COPD) or asthma.

7.3 Validation Of A Threshold For VSPECT Imaging Technique

Ventilation single photon emission computed tomography/computed tomography (VSPECT/CT) measures, and visualises lung ventilation (5). VSPECT/CT can contribute significantly to personalised care by identifying defects regions, and regions of improvements that are specific to each patient. This information can guide targeted interventions to improve overall treatment response. The distribution of ventilation on imaging is difficult to quantify. Until recently, ventilation on SPECT/CT was examined using threshold methods that have not been assessed for their accuracy against a gold standard. In my thesis, a new threshold method was developed and its accuracy assessed against a physical lung model. It was demonstrated that the Slope method accurately measures both ventilated and non-ventilated volumes with precision. The development of the "Slope" method represents a novel, and a significant advancement in the use of VSPECT/CT for assessing lung ventilation. This method will allow accurate detection, as well as improved accuracy and reliability in measuring ventilation defects. These results are also important to facilitate future comparison, and interpretation of results across different clinical and research studies.

7.4 Bronchodilator Response On Ventilation Distribution Measured By SPECT/CT Imaging In Asthma

The present study used ventilation single photon emission computed tomography/computed tomography (VSPECT/CT) to measure bronchodilator response in asthmatic subjects. This is a novel application of VSPECT/CT, and represents the first investigation to measure bronchodilator

response (BDR) using VSPECT/CT in individuals with asthma. Bronchodilator response is a clinically important physiological property in asthma, and VSPECT/CT measurement of it could help in optimise, and personalise treatment regimens. My thesis has shown that in asthma subjects with mild to severe disease, the response in VSPECT is variable post bronchodilator administration, and discordant with improvements in spirometry and oscillatory impedance. Moreover, the volume of ventilation defects and the magnitude of their reduction following bronchodilator administration correlate with age. VSPECT/CT detects ventilation defects that are not captured by traditional lung function tests. Spirometry assesses the function of the entire airways tree but mostly of the large to medium sized airways. Furthermore, the lack of correlation between the change in spirometry, and the change in VSPECT/CT support its use as a complementary modality. VSPECT/CT localises the effects of treatment to lung regions, which is not possible with lung function measurements. Future studies could investigate the relationships between ventilation defects on VSPECT, and clinical asthma outcomes, e.g. rates of exacerbations, and hospital admissions. The volume of ventilation defects on VSPECT, and the response to treatment could potentially relate to asthma duration, and disease progression which could have implications for tailoring treatment strategies in older asthma patients.

7.5 References

1. 2023 Global Initiative for Asthma (GINA) Report: Global Strategy for Asthma Management and Prevention. www.ginasthma.org/2023-gina-main-report

2. Carr TF, Bleecker E. Asthma heterogeneity and severity. World Allergy Organ J. 2016;9(1):41.

3. Peters SP. Heterogeneity in the pathology and treatment of asthma. Am J Med. 2003;115 Suppl 3A:49s-54s.

4. Verbanck S, Schuermans D, Meysman M, Paiva M, Vincken W. Noninvasive assessment of airway alterations in smokers: the small airways revisited. Am J Respir Crit Care Med. 2004;170(4):414-9.

5. Farrow C, King G. SPECT Ventilation Imaging in Asthma. Seminars in Nuclear Medicine. 2019;49(1):11-5.

8. Appendix

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RESEARCH ARTICLE Open Access

Relationship between concavity of the flow-volume loop and small airway measures in smokers with normal spirometry

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Abstract

Background: There is increasing evidence of small airway abnormalities in smokers despite normal spirometry. The concavity in the descending limb of the maximum expiratory flow curve (MEFV) is a recognised feature of obstruction and can provide information beyond FEV_1, and potentially early smoking-related damage. We aimed to evaluate concavity measures compared to known small airway measurements.

Methods: Eighty smokers with normal spirometry had small airway function assessed: multiple breath nitrogen washout (MBNW) from which ventilation heterogeneity in the diffusion-dependent acinar (Sacin) and convection-dependent conductive (Scond) airways were assessed, and impulse oscillometry system (IOS) from which respiratory resistance and reactance at 5 Hz (R5 and X5) were measured. Concavity measures were calculated from the MEFV, partitioned into global and peripheral concavity.

Results: We found abnormal peripheral and global concavity as well as acinar ventilation heterogeneity are common in "normal" smokers. Concavity measures were not related to either MBNW or IOS measurements.

Conclusion: Abnormalities in concavity indices and MBNW or oscillometry parameters are common in smokers despite normal spirometry. However, these measures likely reflect different mechanisms of peripheral airway dysfunction.

Keywords: Small airways, Smokers, COPD, Early disease, Airflow obstruction, Physiology

Background

Spirometry is the "gold standard" measure of airflow obstruction, which is defined as a FEV1/FVC less than the lower limit of normal [1]. In early chronic obstructive pulmonary disease (COPD), terminal bronchiole obliteration is the initial abnormality, but will not affect the FEV1/FVC until around 2/3 of these small airways are lost since the small airways (less than 2 mm in diameter) account for little of the total airflow resistance [2]. Johns and colleagues, described two indices for estimating concavity of the expiratory limb of the flow volume curve as potential clinically useful measures of small airways disease. They defined a global index as the forced expiratory flows at 50% of the FVC (FEF50), and a peripheral index based on the FEF75, both expressed as ratios of the theoretical flows at 50% and 75% of FVC respectively, (Fig. 1) interpolated from a straight line between peak flow and RV [3]. Since there are currently complex measures of lung function that are putatively sensitive to small airway function, in particular oscillometry and multiple breath nitrogen washout (MBNW), it would be useful to know how these may relate to these global and peripheral spirometric indices.

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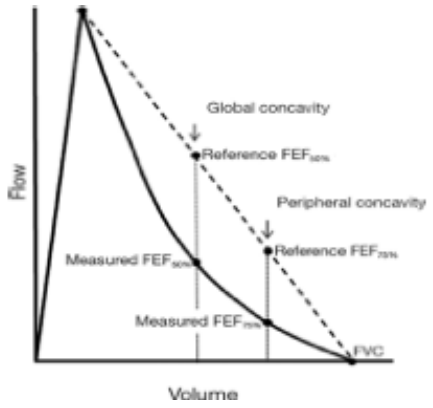

Fig. 1 Global and peripheral concavity indices are calculated from the maximum expiratory flow-volume curve (MEFV). Global concavity is based on forced expiratory flow at 50%, and peripheral concavity is based on forced expiratory flow at 75%, of the expired FVC. The degree of concavity is compared to the "normal" reference flows at EFE50% and EFE75%

MBNW is a complex measures of ventilation heterogeneity, where Scond is an index of this in peripheral conducting (convection dependent) airways and Sacin is an index related to the even more peripheral acinar (convection–diffusion dependent) airways. Oscillometry is another complex measure, of the respiratory system resistance (Rrs) and reactance (Xrs), reflecting especially airway caliber and oscillatory stiffness of the respiratory system, respectively. Both Rrs and Xrs, but in particular Xrs are sensitive to small airway dysfunction [4]. Results from a recent study show that 75% of smokers with normal spirometry have an abnormality in at least one MBNW or oscillometry index [5]. Our aim was to examine relationships between concavity measures and both MBNW and oscillometry parameters in current or ex-smokers with normal.

FEV1/FVC ratio from the aforementioned study [5]. We also examined concordance in abnormal results between these tests. We hypothesized that the global concavity index related to Scond and Rrs, while the peripheral index related to Sacin and Xrs.

Methods

We analyzed previously published data of eighty current or ex-smokers (≤50 years of age) who had>5 pack/year smoking history but normal spirometry. At enrollment, inclusion criteria were no history of past/present respiratory diseases or use of respiratory medications and having normal spirometry defined by post bronchodilator FEV1/FVC ratio>LLN and FEV1>LLN. Respiratory symptoms including cough, shortness of breath, and wheeze were documented via questionnaire. Each subject was tested in a single session at the Woolcock Institute of Medical Research or Royal North Shore Hospital, Sydney, Australia. Subjects performed spirometry according to the ATS/ERS guidelines from which global and peripheral concavity indices were calculated from the maximum expiratory flow-volume curve (MEFV), as described above [3]. The upper limit of normal for post bronchodilator central concavity index is 34.8% in males and 26.3% in females; the upper limits of normal for peripheral concavity index is 61.2% in males and 63.1% in females [3]. Respiratory system impedance measures were made using a Jaeger Masterscope CT IOS (CareFusion, Hoechberg, Germany), from which Rrs and Xrs at 5 Hz (R5 and X5) were calculated. The MBNW test was preformed using an in-house-built device as previously described [5]. We used published upper and lower limits of normal to define abnormality of MBNW [6], oscillometry [7] and spirometry [8] indices. Relationships between parameters were examined by Spearman correlation coefficients. Concordance in abnormal function was evaluated using Cohen's kappa.

Results

The demographics data for males (n=51) and females (n=29) are summarized in Table 1. Overall, abnormal peripheral concavity was found in 19/80 (23.8%) smokers and abnormal global concavity in 22/80 (27.5%) smokers. Abnormal Sacin was found in 33 (41.3%) smokers and abnormal Scond in 19 (23.8%) smokers. Only 1 and 5 participants had abnormal R5 and X5, respectively. Global concavity was unrelated to either MBNW ($r_s=0.10$, $p=0.34$ and $r_s=0.08$, $p=0.47$ for Scond and Sacin, respectively) or impedance parameters ($r_s=0.10$, $p=0.35$ and $r_s=0.02$, $p=0.84$ for Rrs and Xrs, respectively) (Table 2, Fig. 2). Peripheral concavity was also unrelated to MBNW ($r_s=0.09$, $p=0.39$ and $r_s=0.20$, $p=0.07$ for Scond and Sacin, respectively) Peripheral concavity was unrelated to Xrs ($r_s=-0.09$, $p=0.38$), but weakly correlated with Rrs ($r_s=0.27$, $p=0.01$) (Table 2, Fig. 2). Global concavity was related to age ($r_s=0.27$, $p=0.01$). Peripheral concavity was related to both height ($r_s=-0.26$, $p=0.02$) and age ($r_s=0.58$, $p<0.001$). Global concavity was unrelated to smoking history ($r_s=0.04$, $p=0.67$), while peripheral concavity was related ($r_s=0.38$, $p=0.001$). There was poor concordance between concavity indices and either MBNW parameters or IOS parameters, with kappa values ranging between − 0.09 and 0.25 (Table 3).

Discussion

We found in this cohort of younger smokers with normal FEV1/FVC ratio, that both global and peripheral concavity indices picked up abnormalities in about a quarter of

Table 1 Baseline characteristics, spirometry of smokers (n = 80)

Sex (M/F)	51/29
Age (years)	43 (11)
Height (cm)	175 (10)
BMI (kg/m.^{2})	25.2 (4.4)
Smoking (pack-years)	17.7 (10.3)
Past/current smokers	21/59
FEV_1 (% predicted)	98 (10)
FEV_1 (Z-score)	− 0.13 (0.81)
FVC (% predicted)	105 (12)
FVC (Z-score)	0.37 (0.90)
FEV_1/FVC Ratio	76 (4)
FEV_1/FVC (Z-score)	− 0.80 (0.90)
FEF_{25-75} (% predicted)	90 (23)
FEF_{25-75} (Z-score)	− 0.40 (0.81)
Global concavity (%)	17 (25)
Peripheral concavity (%)	45 (24)

There is one missing value for peripheral

Data are presented as mean (SD)

BMI, body mass index, FEF25–75, forced expiratory flow between 25 and 75% of FVC, FEV1, forced expiratory volume in

1 s; FVC, forced vital capacity

Table 2 Univariate correlations (Spearman correlation coefficients) between concavity indices, small airway measures, spirometry, age, and smoking history (n = 80)

Variables	Global concavity%		Peripheral concavity%	
	r_s	*P* value	r_s	*P* value
Scond	0.10	0.34	0.09	0.39
Sacin	0.08	0.47	0.20	0.07
R5	0.10	0.35	0.27	0.01
X5	0.02	0.84	− 0.09	0.38
FEV_1/FVC	− 0.64	< 0.001	− 0.54	< 0.001
Age	0.27	0.01	0.58	< 0.0001
Pack/Year	0.04	0.67	0.38	0.001

There is one missing value for peripheral

R5 resistance, *X5* reactance

individuals, which is a similar finding to a previous unrelated evaluation[3]. However, concavity of the expiratory limb of the maximal flow-volume was unrelated to MBNW and impedance parameters and consequently there was also poor concordance between these tests in detecting abnormal airway function. Paradoxically, abnormalities in both Sacin and Scond were as prevalent as in global and peripheral indices indicating that they were all sensitive parameters, but the abnormalities did not occur in the same individuals, thus the poor concordance, suggesting that they are picking up different and quite subtle pathophysiological abnormalities. Impedance on the other hand was much less frequently abnormal. Interestingly, peripheral concavity related to smoking history, while global did not.

Ventilation in obstructive airways diseases is characterized by nonuniform lung emptying which results in reduced mid-expiratory flows in spirometry. This is manifest visually by development of concavity of the descending limb of the flow-volume curve [9]. Reduced mid-expiratory flows correlate with greater small airways disease, measured histologically in smokers [10] but is also a normal effect of ageing. The increase in lung compliance(11) with ageing explains at least part of the relationships that both concavity indices have with age. In addition, the peripheral, but not global index, correlated with smoking history. However, it is difficult to determine whether this is due partly or wholly to ageing, since age was, not surprisingly, related to pack/year history ($r_s = 0.50$, $p < 0.001$). Further, in multiple regression analysis including age and height, smoking history was no longer a significant predictor of peripheral concavity which suggests perhaps the need to develop age- and height-related prediction equations for these parameters.

There were differences between current and ex-smokers in our dataset, of the 80 smokers, 21 were former smokers. The Scond, Sacin, IOS parameters, and concavity indices were lower (i.e. less deranged) compared to that in the smoker group. Given that current smokers evidence of more peripheral airway dysfunction than ex-smokers, we suggest that abnormalities in these indices

Table 3 Abnormality overlap and concordance using Cohen's Kappa between concavity indices and small airway measures in smokers (n = 80)

Variables	Global concavity%			Peripheral concavity%		
	Kappa	*P* value	Abnormality overlap	Kappa	*P* value	Abnormality overlap
Scond	0.24	0.02	9	0.25	0.02	8
Sacin	0.05	0.63	10	0.18	0.07	11
R5	0.06	0.10	1	0.07	0.07	1
X5	0.05	0.51	2	− 0.09	0.24	0

There is one missing value for peripheral

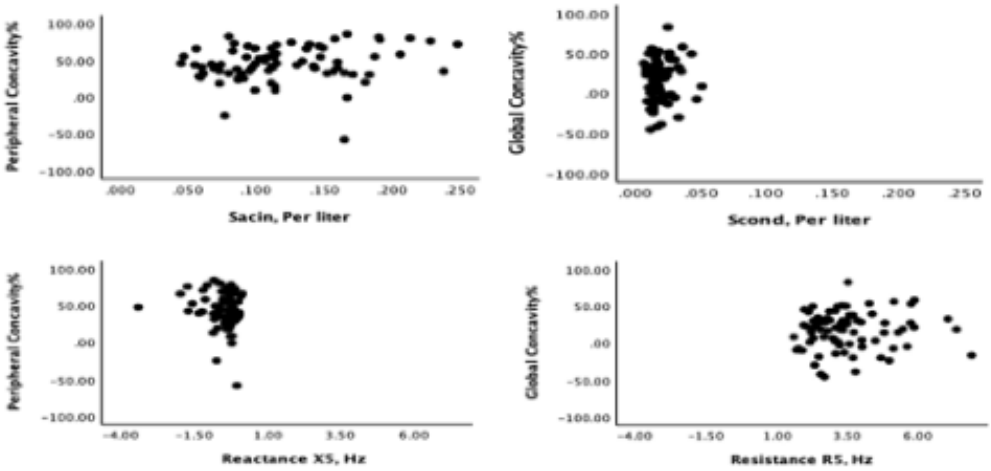

Fig. 2 No significant correlation between peripheral concavity and Sacin ($r = 0.20$, $p = 0.07$), global concavity and Scond ($r = 0.10$, $p = 0.34$), peripheral concavity and reactance (X5) ($r = -0.09$, $p = 0.38$), global concavity and resistance (R5) ($r = 0.10$, $p = 0.35$)

may be related ongoing smoking. The only MBNW or IOS parameter that correlated with smoking history was Sacin ($r = 0.23$, $P = 0.03$). These finding are in agreement with the previous work of Verbanck et al. In Verbanck study, she found that abnormities in Scond and Sacin occur in the absences of reduction in FEV1/FVC ratio and that with increasing pack-years of smoking, increased Scond and Sacin indicate early small airway changes [12].

The clinical usefulness of mid-expiratory flows in obstructive airways disease is uncertain. There is high concordance between FEF25-75 and FEV1/FVC ratio in detecting airflow obstruction but only 2.9% having normal FEF25-75 when the FEV1/FVC is reduced [13]. However, FEF75 was normal in 12.3% despite a reduced FEV1/FVC [13] though this may be due to abnormality in the more proximal airways and not really true COPD [3]. A strong correlation between global and peripheral indices and FEV1/FVC was evident in our study ($r_s = -0.64$, $p < 0.001$ and $r_s = -0.54$, $p = < 0.001$, respectively) (Table 2), which suggests that they are looking at the same phenomenon but concavity is more sensitive. Mid-expiratory flows have not yet been shown to be predictive of COPD. Part of the problem may be due to its high within- and between-individual variability, and its high prevalence of abnormality in smokers [14, 15] most of whom do not develop COPD as currently defined by FEV/FVC. In contrast, imaging measures of small airways disease, lung clearance index, diffusing capacity for carbon monoxide, airway wall thickening and emphysema predict greater loss of FEV1 [16, 17].

The lack of relationship between concavity indices and MBNW or oscillometry parameters could be because spirometry is a forced manoeuvre, while MBNW and oscillometry are tidal breathing tests. If smoking altered parenchymal interdependence and airway compliance, this might translate to abnormality of forced manoeuvres but not necessarily in oscillometry. The similar prevalence of abnormality in concavity indices and MBNW parameters, but with very little overlap, is difficult to explain but suggests they represent differing functional abnormalities. The prognostic implications in terms of FEV1 decline of concavity indices, oscillometry and MNBW parameters are yet to be established but are part of ongoing longitudinal cohort research.

Although we found a high prevalence of abnormal concavity indices in this cohort, this was likely due to subject selection i.e. smokers some of whom had symptoms. Secondly, the upper limits for the concavity indices were derived healthy subjects of mean age 59 (range 40–87) years, which is significantly older than our study cohort. There may have also been differences due to geographical location (Tasmania versus Sydney). Arguably, if upper limit of normal values had been determined from a younger cohort, closer in age to this study cohort, the prevalence of abnormality may have been higher. Finally, although we found associations between concavity indices and age, height and smoking history, they were weak in nature and several

correlations were explored. However, the lack of associations between concavity indices, oscillometry and MBNW parameters may also be due to the relatively small sample size given the inherent variability between tests. Given the relatively small size of this cohort, it is not possible to generalize these results and associations require further exploration.

Conclusion

Abnormalities in both concavity indices and MBNW parameters are common in smokers with normal spirometry, but infrequent using oscillometry. However, it is somewhat disappointing that there is poor concordance between these different tests in detecting abnormal function in this group of subjects, suggesting that they likely represent different aspect of lung and airway dysfunction.

Abbreviations

COPD: Chronic obstructive pulmonary disease; FEV$_1$: Forced expiratory volume in 1 s; FEF$_{50\%}$: Forced expiratory flow at 50%; FEF$_{75\%}$: Forced expiratory flow at 75%; FVC: Forced volume capacity; ICOS: Impulse oscillometry system; MBNW: Multiple breath nitrogen washout; MEFV: Maximum expiratory flow curve; R5: Resistance at 5 Hz; X5: Reactance at 5 Hz; RV: Residual volume.

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Author contributions

H.A. contributed to conception and design of the study, analysis and interpretation of data and preparation of the manuscript. S.W., and K.J. contributed to the study analysis and interpretation. C.T. contributed to conception and design of the study, analysis and interpretation of data and preparation of the manuscript. D.P.J., and E.H.W. contributed to data collection and analysis. G.G.K. contributed to conception and design of the study, analysis and interpretation of data and preparation of the manuscript. All above authors have read and approved the final manuscript.

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Availability of data and materials

The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The original study protocol was approved by the Northern Sydney Central Coast Area Health Service Human Research Ethics Committee (Protocol 1106-209M). Written informed consent was obtained from all recruited patients. No clinical patient data were used and therefore no administrative permissions were required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global strategy for the diagnosis, management and prevention of chronic obstructive lung disease 2017 report: GOLD executive summary. Respirology (Carlton, Vic). 2017;22(3):575–601.
2. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. N Engl J Med. 1968;278(25):1355–60.
3. Johns DP, Das A, Toelle BG, Abramson MJ, Marks GB, Wood-Baker R, et al. Improved spirometric detection of small airway narrowing: concavity in the expiratory flow-volume curve in people aged over 40 years. Int J Chron Obstruct Pulmon Dis. 2017;12:3567–77.
4. Bhatawadekar SA, Leary D, de Lange V, Peters U, Fulton S, Hernandez P, et al. Reactance and elastance as measures of small airways response to bronchodilator in asthma. J Appl Physiol (1985). 2019;127(5):1772–81.
5. Jetmalani K, Thamrin C, Farah CS, Bertolin A, Chapman DG, Berend N, et al. Peripheral airway dysfunction and relationship with symptoms in smokers with preserved spirometry. Respirology. 2018;23(5):512–8.
6. Jetmalani K, Chapman DG, Thamrin C, Farah CS, Berend N, Salome CM, et al. Bronchodilator responsiveness of peripheral airways in smokers with normal spirometry. Respirology. 2016;21(7):1270–6.
7. Oostveen E, Boda K, Van Der Grinten CPM, James AL, Young S, Nieland H, et al. Respiratory impedance in healthy subjects: baseline values and bronchodilator response. Eur Respir J. 2013;42(6):1513–23.
8. Quanjer P, Stanojevic S, Cole T, Baur X, Hall GL, Culver B, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324–43.
9. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319–38.
10. Berend N, Woolcock AJ, Marlin GE. Correlation between the function and structure of the lung in smokers. Am Rev Respir Dis. 1979;119(5):695–705.
11. Mittman C, Edelman NH, Norris AH, Shock NW. Relationship between chest wall and pulmonary compliance and age. J Appl Physiol. 1965;20(6):1211–6.
12. Verbanck S, Schuermans D, Meysman M, Paiva M, Vincken W. Noninvasive assessment of airway alterations in smokers: the small airways revisited. Am J Respir Crit Care Med. 2004;170(4):414–9.
13. Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW. Measurement of FEF25–75% and FEF75% does not contribute to clinical decision making. Eur Respir J. 2014;43(4):1051–8.
14. Kwon DS, Choi YJ, Kim TH, Byun MK, Cho JH, Kim HJ, et al. FEF25–75% values in patients with normal lung function can predict the development of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2020;15:2913–21.
15. Wafy S, Agamy G, Ali A. Early spirometric changes in asymptomatic smokers; is it a time dependent? Eur Respir J. 2016;48(suppl 60):PA4320.
16. Hoesein FAAM, Jong PAD, Lammers JWJ, Mali WP, Schmidt M, Koning HJD, et al. Airway wall thickness associated with forced expiratory volume in 1 second decline and development of airflow limitation. Eur Respir J. 2015;45(3):644–51.
17. Zaigham S, Wollmer P, Engström G. The Association of lung clearance index with COPD and FEV(1) Reduction In "Men Born in 1914." COPD. 2017;14(3):324–9.

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