



UNIVERSITÉ CATHOLIQUE DE LOUVAIN

FACULTÉ DES SCIENCES DE LA MOTRICITÉ

**LUNG CLEARANCE INDEX FROM NITROGEN WASHOUT IN PATIENTS
WITH CYSTIC FIBROSIS AND NON-CYSTIC FIBROSIS BRONCHIECTASIS:**

**FROM A COMPARISON OF TWO DEVICES
TO THE ASSESSMENT OF CHEST PHYSIOTHERAPY**

William PONCIN, PT

Thesis supervisor: Prof. Patrick LEBECQUE, MD, PhD

Thesis submitted in fulfillment of the requirements for the degree of
« Docteur en sciences de la motricité »

June, 2017

COMPOSITION OF THE JURY

Thesis supervisor:..... Patrick Lebecque, MD, PhD¹

President: Marc Francaux, PhD²

Committee members: Annie Robert, MSc, PhD³
François Vermeulen, MD⁴
Grégory Reychler, PT, PhD^{5,6,7}
Giuseppe Liistro, MD, PhD^{5,6}

Invited member: Benoît Wallaert, MD, PhD⁸

¹ Cystic Fibrosis Unit, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

² Institute of Neuroscience, Université catholique de Louvain, Louvain-la-Neuve, Belgium

³ Institute of Experimental and Clinical Research (IREC), Pole of Epidemiology and Biostatistics (EPID), Université catholique de Louvain, Brussels, Belgium

⁴ Cystic Fibrosis Unit, Pediatric pulmonology, Department of Paediatrics, University Hospitals Leuven, Leuven, Belgium

⁵ Department of Pulmonology, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

⁶ Institute of Experimental and Clinical Research (IREC) - Pole of Pneumology, ENT and Dermatology (PNEU), Université catholique de Louvain, Brussels, Belgium

⁷ Department of Physical Medicine and Rehabilitation, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

⁸ Department of Pulmonology and Immuno-Allergology, University Hospitals of Lille, Lille, France

ACKNOWLEDGMENTS

I have so many people to thank, so diverse feelings and gratitude to express, that the proper formulation of acknowledgements demands the use of my mother tongue.

Je me rappelle mon tout premier marathon. Initiatique, la course fut longue, haletante, épuisante, et jalonnée de moments de doute. Puis, presque sans crier gare, arrive enfin le 42^{ème} km, et la satisfaction, sans égale, de réaliser que l'on arrive au bout du parcours.

L'analogie avec mon parcours doctoral me semble évidente. Seul le niveau de fierté diffère réellement car à l'opposé d'un marathon, épreuve plutôt solitaire, je n'ai pas autant de mérite d'arriver au bout de ce parcours. Concrétiser une thèse, c'est un peu comme marquer le point décisif dans un sport collectif. Alors que celui qui marque ce point récolte l'essentiel des lauriers, il n'est rien sans la construction ni l'appui du jeu de l'équipe à la base de son action. Peut-être, à l'écriture de ces quelques lignes, je réalise que je suis celui qui marque ce point. Pourtant, jamais ce projet n'aurait pu aboutir sans l'aide d'une base solide, bienveillante et stimulante. Ainsi je souhaite remercier très sincèrement tous ceux qui ont contribué, de près ou de loin, à l'élaboration et la conclusion de ce projet de thèse, de vie.

Je remercie mon promoteur, le Professeur Patrick Lebecque, de m'avoir autorisé à entreprendre mon parcours doctoral dans un domaine si exaltant. Érudite et dévoué à ses patients, il a potentialisé ma rigueur scientifique et stimulé mon sens critique envers la recherche clinique. Il m'a également révélé mon opiniâtreté dans les projets qui me tiennent à cœur et je l'en remercie.

J'adresse ma gratitude au Président du Jury, le Professeur Marc Francaux, ainsi qu'aux membres de mon comité d'accompagnement, le Professeur Annie Robert, le Docteur François Vermeulen, le Professeur Giuseppe Liistro et le Professeur Grégory Reyckler. Leurs corrections-suggestions, précieux coups de pouce, conseils et expertises étaient simplement formidables. Mes remerciements vont également au Professeur Benoît Wallaert, membre invité du jury, pour m'avoir accordé sa confiance. Ses remarques m'ont repoussé dans mes retranchements et ont contribué à améliorer le rendu final de cette thèse.

Je tiens à remercier le Professeur Florian Singer (Zurich), une rencontre heureuse au détour d'un congrès. Ses interventions ont été salvatrices dans mon parcours doctoral. Sa grande expertise scientifique et sa gentillesse ont par ailleurs permis d'améliorer drastiquement la qualité de notre article. Dans le même esprit, merci infiniment à Sophie Gohy pour son aide inespérée, son énergie et son temps consacré aux deux articles de ma thèse. Je lui suis très redevable pour ses conseils et encouragements. Merci également à l'unité de Pneumologie, et notamment aux Professeurs Thierry Pieters et Giuseppe Liistro, Mr. Constant Pahulycz, Ghislaine Van de Veken et les techniciens de la fonction respiratoire, sans qui je n'aurais jamais pu mener à bien ma 2^{ème} étude et donc valider mon parcours doctoral. Je n'oublie pas Barbara Dhooghe, Sabrina Noël et le Professeur Térésinha Léal qui ont contribué à collecter l'une des mesures indispensables à cette étude.

« L'argent est le nerf de la guerre ». Lorsque les financements s'interrompent, la poursuite du parcours peut devenir périlleuse, sauf si l'entourage vous soutient... Pour cela (mais c'est loin d'être l'unique raison), je remercie chaleureusement mes parents Claire Molitor et Joël Poncin, ma grand-maman Odette Wyart, ainsi que Yannick Eon, Isabelle Boulanger, Roger et Marie Eon.

Je suis reconnaissant envers toutes les bonnes âmes qui ont lu des petits bouts de thèse ou d'article. Vos corrections furent précieuses, et je vous dois en partie mon niveau de rédaction actuel. Merci à Asha V, Candice N, David G, Frantz D, Krista R, Marie D, Martine R, Michelle T, Morgane F, Nathalie N et mes parents. Mention spéciale pour Eric Theisen et Noémie Leeuwerck, mes 2 relecteurs les plus réguliers.

À toutes ces rencontres d'une richesse infinie, qui ont contribué à construire ma personnalité, à influencer mon parcours professionnel et qui ont cru en moi, merci... Anne-Sophie A et Adrien P, Benjamin S, Fabien C, Fanny T, François G et Adeline P, Grégory R, Grégory S et Aude L, Lynn T, Marie D, Maxime G et Céline D, Nathalie B, Nicolas V et Christelle F, Romain D et Charlotte F, Sébastien G et Johanna G, Thibaud J, et Tifenn E, merci pour tout. Certaines d'entre-elles ont eu une influence notable sur mon aventure doctorale, et je les en remercie tout particulièrement. Sébastien Giot car, par son affection, il m'apporta nombre de répit revigorants et, peut-être sans trop le savoir, il fut l'un des éléments déclencheurs de cette aventure. Il a réussi à attiser et émuler ma curiosité dans un domaine que je n'affectionnais pourtant pas tellement à l'origine. Johanna Gosset pour son amitié et sa confiance qui me vont droit au cœur et m'encouragent à poursuivre certaines voies. Tifenn Eon pour avoir supporté pendant très longtemps de nombreux sacrifices liés à cette thèse, et pour le tournant qu'elle aura donné à ma vie. Nicolas Verniest et Christelle Fortuny pour leur philosophie de vie, me recentrant vers ce qu'il y a de plus important. Grégory Simons pour son empathie et son amour pour le métier, exemplaire et inspirant. Anne-Sophie Aubriot (LGS) pour l'oreille attentive et les conseils adéquats aux moments opportuns, mais aussi pour les questions agaçantes (sur le plan scientifique, bien sûr) mais ô combien utiles pour optimiser la réflexion. Marie Delens pour l'étonnante nouvelle dimension/perspective qu'elle apporte à ma pratique, mes réflexions, ma vie, et bien plus encore. Grégory Reyhler pour les innombrables mains tendues et l'impulsion incroyable qu'il offre à la kinésithérapie respiratoire. Il représente pour moi un modèle professionnel auquel j'aspire.

J'en termine en exprimant ma gratitude à l'égard de l'association Belge de lutte contre la mucoviscidose pour avoir financé le début de mon projet de thèse, ainsi qu'à tous les patients et volontaires sains qui ont gentiment accepté de participer à nos recherches.

« Together, Everyone Achieves More ». Merci à tous.

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LIST OF ABBREVIATIONS

ACT:		Airway clearance techniques
AD:		Autogenic drainage
Ar:		Argon
BMI:		Body mass index
BSI:		Bronchiectasis severity index
CEV:		Cumulated expired volume
CF:		Cystic fibrosis
CFTR:		Cystic fibrosis transmembrane conductance regulator
CO ₂ :		Carbon dioxide
COPD:		Chronic obstructive pulmonary disease
ECFS-CTN:		European Cystic Fibrosis Society – Clinical Trials Network
EM:		Exhalyzer D (EcoMedics AG)
FACED:		acronym for FEV ₁ , Age, chronic Colonization, Extension and Dyspnea
FEF _{25–75} :		Forced expiratory flow between 25% and 75% of forced vital capacity
FEV ₁ :		Forced expiratory volume in 1 second
FRC:		Functional residual capacity
FVC:		Forced vital capacity
HRCT:		High resolution computed tomography
IgG		Immunoglobulin G
IQR:		Interquartile range
LCI:		Lung clearance index
MBNW:		Multiple-breath nitrogen washout
MBW:		Multiple-breath washout
N ₂ :		Nitrogen
NBS:		Newborn screening
NCFB:		Non-cystic fibrosis bronchiectasis
NDD:		EasyOne Pro (ndd Medizintechnik AG)
O ₂ :		Oxygen
Pleth:		Body plethysmography
RV:		Residual volume

SD: Standard deviation
SF6: Sulfur hexafluoride
sGaw: Specific airway conductance
sRaw: Specific airway resistance
SVC: Slow vital capacity
TLC: Total lung capacity

Specific French abbreviations used in text of Chapter 2:

CRF: Capacité résiduelle fonctionnelle
DEM₂₅: Débit expiratoire maximal à 25% de la capacité vitale
DEM₂₅₋₇₅: Débit expiratoire maximal médian entre 25 et 75% de la capacité vitale
ICP: Indice de clairance pulmonaire
MV: Mucoviscidose
RCM: Rinçage d'un gaz sur cycles respiratoires multiples
VEC: Volume expiré cumulé
VEMS: Volume expiratoire maximal en 1 seconde

CHAPTER 1

INTRODUCTION

1.1. Cystic Fibrosis

1.1.1. Definition

Cystic fibrosis (CF) is the most frequent inherited disease in the Caucasian population [1], where approximately 1 in 2000-3000 newborns develop the disease in the European Union [2]. In Belgium, the incidence is estimated to be 1 in 3000 live births or, on average, 30-35 births a year [3]. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. So far over 2000 CFTR putative mutations have been reported, most of them being very rare or privative. All these variants do not necessarily cause CF, yet out of the 322 most common variants representing more than 96% of CF alleles, 281 were identified as disease-causing, and a further 21 as associated with varying clinical consequences [4]. CFTR acts as an ion channel and is essential in transporting chloride ions in and out of the cells, but also in regulating other chloride and sodium channels on the cell membrane. Its high expression on the apical surface epithelium within the airways, pancreas, gastrointestinal tract, sweat glands, liver and seminal ducts in males, elucidates its multi-organic impact [5]. However, progressive lung disease is the main cause of CF morbidity and mortality [6]. When CFTR is not operating normally, water content of the airway surface liquid is decreasing, leading to viscous mucus, ciliary collapse, and decreased mucociliary clearance [7]. Dehydrated mucus accumulates and serves as a favorable environment for the growth of pathogens, triggering a cascade of infection, inflammation and progressive airway destruction [7]. Eventually, this leads to respiratory failure and death.

1.1.2. Diagnosis

Initiation of prophylactic and symptomatic treatments as soon as practicable is imperative in CF patients since lung function deteriorates early in life [8] and late diagnosis is associated with poorer health outcomes [9,10]. Many European countries have thus implemented national newborn screening (NBS) programs [11]. Even though NBS for CF is a cost-effective strategy [12] with proven long-term benefits on nutritional status, respiratory outcomes and survival rates [8,13], some developed countries have not yet implemented this health policy, mainly because of political inertia [11]. NBS protocols vary among countries but all of them measure the immunoreactive trypsinogen blood level [11], which is typically elevated in CF.

In the context of a positive NBS, or recurrent respiratory, digestive troubles and/or parental history, a sweat test is indicated. A sweat chloride concentration of 60 mmol.L⁻¹ or greater is considered as a positive test. In the presence of inconclusive results (sweat chloride value between 30-59mmol.L⁻¹ on 2 separate occasions), CFTR genetic and/or physiologic analysis should be considered. False negatives (< 30mmol.L⁻¹) are rare, although certain CFTR mutations (class IV and V mutations) may lead to normal sweat chloride concentrations. For those patients, other evocative organ abnormalities such as the absence of sperm in the semen of men (nearly all males are azoospermic) may help to identify the disease [14,15].

1.1.3. The role of spirometry and the current challenge

Careful monitoring of the lung disease progression is one of the cornerstones in the management of patients with CF. Regular evaluation of lung function is essential for the clinician to guide therapy and avoid rapid deterioration of lung health, but also for the researcher to demonstrate the benefits of new treatment modalities. The traditional “gold-standard” of lung function testing is spirometry, with forced expiratory volume in one second (FEV₁) as the main readout. However, since CF therapy has considerably improved over the last decades [16], the sensitivity of FEV₁ in monitoring lung disease progression and detecting treatment effects has fairly decreased. Indeed, mean FEV₁ now remains within the normal range until adolescence-adulthood [17] and displays a very slow rate of decline [18-20]. In addition, convincing data repeatedly showed evidence of structural lung damage ascertained by high-resolution computed tomography (HRCT) despite normal spirometric measurements [21-26].

On the other hand, spirometry requires a minimum level of coordination and cooperation, which is not always possible in preschool children [27-29]. In infants, forced flow can be accessed via the raised volume rapid thoracic compression technique, but sedation requirements, low feasibility rates and important variation between tests hamper its application for early intervention trials [30,31]. Though, the first few years of life is conceivably the most critical period for lung health as pulmonary abnormalities develop early in life [32] and progress rapidly into irreversible structural changes such as bronchiectasis [33,34], the latter being associated with accelerated lung function decline [35,36].

There is thus a window of opportunity for early intervention to prevent irreversible structural lung damage. Nonetheless, without feasible and sensitive endpoints, the physician

lacks objective landmarks to adjust treatments and clinical trials would require unrealistic sample sizes and study durations [37,38]. Hence, other sound lung function endpoints need to be established, especially for the relatively well, stable and young patients.

1.2. Non-Cystic Fibrosis Bronchiectasis

1.2.1. Definition

Non-cystic fibrosis bronchiectasis (NCFB) can be the result of a wide range of etiologies, although the disease is classified as idiopathic in the majority of cases [39]. NCFB is a chronic lung disorder characterized by irreversible dilatation of the airways, and is almost always associated with chronic suppuration [40,41]. Pathogenesis of this disorder commonly refers to “Cole’s vicious cycle hypothesis” [42]. In this model, respiratory infection, inflammation, airway destruction and mucus stasis interact in a predisposed host to result in bronchiectasis. Clinical features include chronic cough, bronchorrhea, and recurrent exacerbations with a concurrent impact on health-related quality of life, morbidity, and mortality rates [43-46]. Prevalence of NCFB increases with age, being estimated at 4.2 per 100.000 people in the US for those aged between 18-34 years old and as high as 272 per 100.000 for people above the age of 75 [47]. In children, the incidence rate ranges from 0.5 to 3.7 per 100.000 per year depending on the geographic region considered [48,49].

1.2.2. Diagnosis

In the presence of a chronic productive cough or recurrent respiratory infections, the diagnosis of NCFB should be envisaged [45]. NCFB should also be suggested in the context of enigmatic clinical signs such as unexplained hemoptysis, sputum colonization with *Pseudomonas aeruginosa* or non-productive cough in the absence of other etiological factors [50]. HRCT is the reference test to confirm and assess the extent and distribution of bronchiectasis. Criteria on HRCT include a ratio between the diameters of the bronchus and the accompanying pulmonary artery of > 1 (known as the signet-ring sign), lack of bronchial tapering, visibility of peripheral airways within 1cm of the pleural surface or abutting the mediastinal pleura and bronchial thickening represented as “tram-tracks” [44,51]. Once confirmed, the next step consists in the exclusion of CF diagnosis [45].

1.2.3. The role of spirometry and the current challenge

Among the lung function tests, the role of spirometry with FEV₁ and forced vital capacity (FVC) is especially emphasized by the British Thoracic Society Guideline for NCFB. Recommendations include spirometry measurements at the time of diagnostic evaluation and

annual repeat measures (in most cases) to follow-up patients [50]. Similarly to CF, FEV₁ is of particular importance since it is associated with mortality rates [43,52], is strongly related to structural abnormalities [53] and is embedded into 2 recently validated multidimensional severity scores predicting the course of the disease: the bronchiectasis severity index (BSI) and the FACED¹ score [54,55]. For these reasons, FEV₁ is traditionally used as the primary outcome measurement to monitor and manage patients with NCFB. In general (though not in all cases), NCFB leads to an obstructive disease, with a decreased FEV₁ and a reduced FEV₁/FVC ratio. Some patients may also develop a concomitant restrictive defect (reduced FVC) [56,57].

However, FEV₁ or FVC seem outcomes of limited value to demonstrate a treatment effect in patients with NCFB [58]. The scientific community currently struggles to find an appropriate endpoint able to validate effective intervention, which is critical considering the paucity of evidence-based treatments and management, and the lack of approved therapies in this population [50,59]. Indeed, since NCFB was formerly considered as an orphan disease (< 5 cases per 10.000 people), very little research has been directed towards this condition over the last decades. Consequently, therapeutic options remain largely based on empirical expert opinion [50] and are often extrapolated from CF or chronic obstructive pulmonary disease (COPD) [60]. However, the increasing prevalence [61,62], hospital admissions [63], mortality rates [64] and economic burden [47] owing to bronchiectasis suggest a great need for better clinical and research trajectories. Sensitive and responsive markers of lung function in NCFB are thus urgently required.

¹ FACED is an acronym for **F**EV₁, **A**ge, chronic **C**olonization by *Pseudomonas aeruginosa*, radiological **E**xtension (number of affected lobes) and **D**yspnea.

1.3. Airway clearance techniques

1.3.1. Mucus transport

Due to its constant exposure to the environment, the lung comes into contact with thousands of potentially harmful particles. Fortunately, the airways are endowed with various innate defense mechanisms. One of these involves the mucociliary machinery, which is intended to propel the hazardous substances trapped in the mucus layer to the pharynx thanks to cilia that line the airways [65]. In healthy individuals, the mucociliary apparatus works efficiently, removing about 10-20 mL of mucus per day from the airways [66]. However, in a variety of pulmonary diseases, mucociliary clearance is temporarily or chronically disturbed [67]. In that case, the reliance on expiratory flow transport to remove secretions may increase. In some instances, expiratory flow transport (including cough clearance) is insufficient to guarantee an adequate mucus transport, thereby depriving the patient of one of the most important airway defense mechanisms [66,68].

1.3.2. The aims of airway clearance techniques

Airway clearance techniques (ACT) seek to assist mucus transport by applying physiological principles to the respiratory system. Two-phase gas-liquid flow, equal pressure point with dynamic airway compression, interdependence during deep inspiration, recruitment of collateral ventilation and alteration of mucus rheology are combined to facilitate the cephalad migration of the mucus interactions [69]. Direct stimulation of mucociliary clearance by breathing-induced mechanical stress may also be expected [70-74]. By achieving successful mucus elimination, 2 therapeutic goals are anticipated. The first one is the relief of mechanical consequences owing to mucus obstruction, such as hyperinflation, increased resistance and ventilation maldistribution. The other one is the prevention of the deleterious effect of mucus stasis which sustains the Cole's vicious cycle (*i.e.* favors further infection, inflammation and irreversible tissue damage) [42,75].

1.3.3. The challenge of airway clearance studies

Until the 1960s, chest physiotherapy consisted of one main technique, the postural drainage with clapping [76]. Many more techniques have emerged since, and there is an incredible large amount of comparative studies attempting to answer which is the optimal

treatment regimen in CF or NCFB [69,77]. Astonishingly, even 6 decades later, the level of evidence for a superior technique remains nil [78-82]. The literature is heterogeneous and confusing. Benefits on pulmonary function are contradictory and long term data are scarce [83]. Most studies were short-term trials and underpowered. Patients are often selected irrespective of their pathophysiological status such as disease severity (FEV_1) which, from an intellectual point of view, may affect the efficiency of a given technique. Rare recent well-designed and long-term comparative studies also failed to uncover a gold-standard method among the numerous available techniques. Rather, these studies have highlighted a combination of factors that will continue to hinder any efforts in finding the best treatment regimen. ACT are indeed a research area where strong patient preference, lack of blinding, and the requirement for effortful and demanding participation over long duration all jeopardize study designs [69,83-85]. The lack of appropriate and sensitive outcome measurement makes things even more difficult. For instance, many studies focused on changes in FEV_1 or the amount of collected sputum weight as primary outcomes. However, FEV_1 is now acknowledged as insensitive to ACT [83,85] while collected sputum weight is unreliable [86].

Consequently, research strategy is now shifting from examining the superiority of any technique to customizing ACT in a way that the most appropriate strategy is selected for each individual, according to his particular context. However, without being able to investigate reliably ACT effects, their physiological impacts remain poorly understood, thereby leaving the physiotherapist without evidence-based clues to identify the best therapeutic option in view of the clinical situation. Any outcome measurement able to track chest physiotherapy effects would therefore be very valuable. Over the last decades, new attractive tests such as multiple breath washout, electrical impedance tomography or forced oscillation techniques have become more accessible. Yet, to which extent these tests can help to assess physiotherapy effects and to compare different techniques is not known.

1.4. Ventilation inhomogeneity

The human lung consists of a complex branching structure, with dichotomous divisions from the trachea into a multitude of small exchanging units termed as acini. This distinctive fractal architecture optimizes the gas mixing efficiency within the lungs by providing a large surface of approximately 100m² for gas transfer between the air and pulmonary blood [87]. Yet the trade-off is the emergence of ventilation inhomogeneity, *i.e.* non-uniform distribution of the gas within the lung during inspiration or expiration. In an ideal lung model, air filling and emptying would be perfectly homogeneous in all regions of the lung. In physiologic conditions, however, ventilation distribution unevenness occurs to some degree owing to the effect of gravity, normal mechanical properties differences in smaller lung units and natural geometrical asymmetries predominant in the acini [87]. Pathological processes involving airway abnormalities or parenchymal damage may further exaggerate differences in time-constants for gas filling/emptying between lung regions or structural asymmetries, leading to pathological (raised) ventilation inhomogeneity [88].

The quantitative measurement of gas mixing inefficiency is especially relevant in CF since airway obstruction is non-uniformly distributed [89] and the principal site of gas mixing is located in the small airways (*i.e.* corresponding to generation 8 and beyond [90]), the site where CF lung disease originates [91]. Accordingly, ventilation heterogeneity appears to be a very early event, occurring even in asymptomatic CF infants [92,93]. Whereas conventional lung function tests largely ignore the small airways, the role of ventilation inhomogeneity measurements in detecting peripheral lung regions defects is now well established [88,94]. Measuring ventilation distribution efficiency may also offer a significant added value compared to spirometry in other patchy lung diseases such as NCFB, as suggested by preliminary evidence [95,96]. To summarize, functional markers of ventilation inhomogeneity are attractive in pathological situations where measuring small airways is of particular relevance and where forced flows do not, or no longer, adequately detect treatment effects.

1.5. Thesis aims

Multiple-breath washout (MBW) is a functional, non-invasive radiation-free method of measuring ventilation inhomogeneity, which is feasible in all age ranges. MBW involves the analysis of the clearance rate of a tracer gas at tidal breathing around the functional residual capacity. Different gases may be used, each with advantages and drawbacks, although for both economic and environmental reasons, multiple-breath nitrogen washout (MBNW) has been the approved method for multicenter clinical trials by the European Cystic Fibrosis Society – Clinical Trial Networks (ECFS-CTN). Lung clearance index (LCI) is the main reported outcome derived from MBNW and is considered as one of the most promising measures to receive the surrogate endpoint status in CF [88]. The subsequent development of commercially available devices has propelled the wide use of this technology. There are currently 2 main MBNW apparatus, and because the potential profit is high, somewhat fierce competition divides firms. Given the expectations placed on LCI, whether or not this outcome is interchangeable between both devices and reliably calculated is of great importance when appreciating the effects of therapeutic interventions or tracking lung disease progression.

The primary aim of this PhD project was to compare the performance of 2 MBNW devices in a cohort of healthy and CF participants. The “best” device was subsequently selected to assess the effect of a single physiotherapy intervention on ventilation inhomogeneity in NCFB adults by observing LCI changes after the session. The thesis is organized as follows:

Chapter 2 is a narrative review of the literature addressing the current knowledge of LCI derived from MBNW. This paper will focus on the description, interpretation, clinimetric properties, assets and limits of LCI in the CF context. As we aim to submit the review in a French peer-reviewed journal, Chapter 2 is intentionally written in French. This chapter is formatted to comply with the “Revue des Maladies Respiratoires” requirements.

Chapter 3 reports the agreement of LCI between the 2 main commercially available MBNW setups, the EasyOne Pro (nidd, Zurich, Switzerland) and the Exhalyzer D (EcoMedics, Duernten, Switzerland) in healthy participants and patients with CF.

Chapter 4 addresses the short-term impact of a manual chest physiotherapy technique (autogenic drainage) on ventilation inhomogeneity via LCI in adults with stable NCFB.

Chapter 5 brings perspectives to some issues raised from findings reported in chapters 3 and 4, and concludes the thesis.

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CHAPTER 2

LUNG CLEARANCE INDEX DERIVED FROM NITROGEN WASHOUT IN CYSTIC FIBROSIS

L'indice de clairance pulmonaire mesuré par rinçage de l'azote dans la mucoviscidose

William Poncin, Patrick Lebecque

In preparation for: Revue des Maladies Respiratoires

Résumé

Introduction : C'est dans les voies aériennes périphériques qu'apparaissent les premières anomalies pulmonaires dans la mucoviscidose. Débutant très précocement, leur atteinte est d'abord infra-clinique et évolue insidieusement puisque ces petites voies aériennes sont relativement ignorées par les épreuves fonctionnelles respiratoires conventionnelles.

États des connaissances : Des outils discriminants existent, mais leur complexité et leur aspect invasif restreignent leur utilisation à des fins de recherche et à quelques équipes spécialisées. Par contraste, la technique qui donne accès à l'indice de clairance pulmonaire (ICP) est abordable et non-invasive. L'ICP est nettement plus sensible à une atteinte des petites voies aériennes que les indices dérivés de la spirométrie.

Perspectives : La récente commercialisation des appareils mesurant l'ICP laisse entrevoir son application au domaine clinique, mais soulève en même temps d'autres questions tant les valeurs diffèrent selon l'équipement utilisé. En outre, la standardisation des mesures, qui est un préalable à l'obtention de données multicentriques robustes, reste perfectible.

Conclusions : Malgré la persistance de certaines zones d'ombre susceptibles de limiter son interprétation, l'ICP a sa place en recherche pour détecter précocement les anomalies pulmonaires fonctionnelles et évaluer l'efficacité de nouveaux traitements, notamment chez les patients peu malades. L'intérêt clinique de l'ICP est probable mais reste à préciser.

2.1. Introduction

La mucoviscidose (MV) est causée par la mutation d'un gène codant pour une protéine exprimée par de nombreux organes, la protéine CFTR (Cystic Fibrosis Transmembrane conductance Regulator) [1]. Parmi les organes touchés, le système respiratoire est celui où se manifestent les modifications les plus sévères et invalidantes, et malgré les avancées scientifiques majeures de ces dernières décennies, la progression de la maladie pulmonaire reste inéluctable. En l'absence d'un traitement qui permettrait de corriger l'anomalie génétique à la base de la MV, la prise en charge est symptomatique, l'objectif étant de ralentir et retarder le déclin de la fonction pulmonaire aussitôt le diagnostic prononcé [2]. Mais l'atteinte pulmonaire est d'apparition précoce et de progression insidieuse : inflammation, infection et anomalies structurelles s'expriment déjà pendant les premières années de vie, et ce même chez le nourrisson asymptomatique [3-6]. Par conséquent, il apparaît fondamental d'être en mesure de surveiller la fonction pulmonaire dès le plus jeune âge afin d'identifier les nourrissons ou jeunes enfants MV qui pourraient éventuellement bénéficier d'une intervention thérapeutique plus agressive [7]. Le challenge est donc de trouver un outil de mesure faisable dès la petite enfance, sensible aux premières anomalies pulmonaires et n'impliquant idéalement aucun effet secondaire. L'indice de clairance pulmonaire (ICP) dérivé d'un test de rinçage d'un gaz sur cycles respiratoires multiples (RCM) est actuellement l'une des mesures les plus prometteuses dans ce contexte. L'objet de cette revue est de décrire la méthode RCM ainsi que la signification de l'ICP qui en découle. L'azote étant le gaz de rinçage qui semble s'imposer dans les études multicentriques, la revue insistera volontairement sur les propriétés clinimétriques, atouts et limites de l'ICP dérivé du rinçage de l'azote dans le cadre de la mucoviscidose.

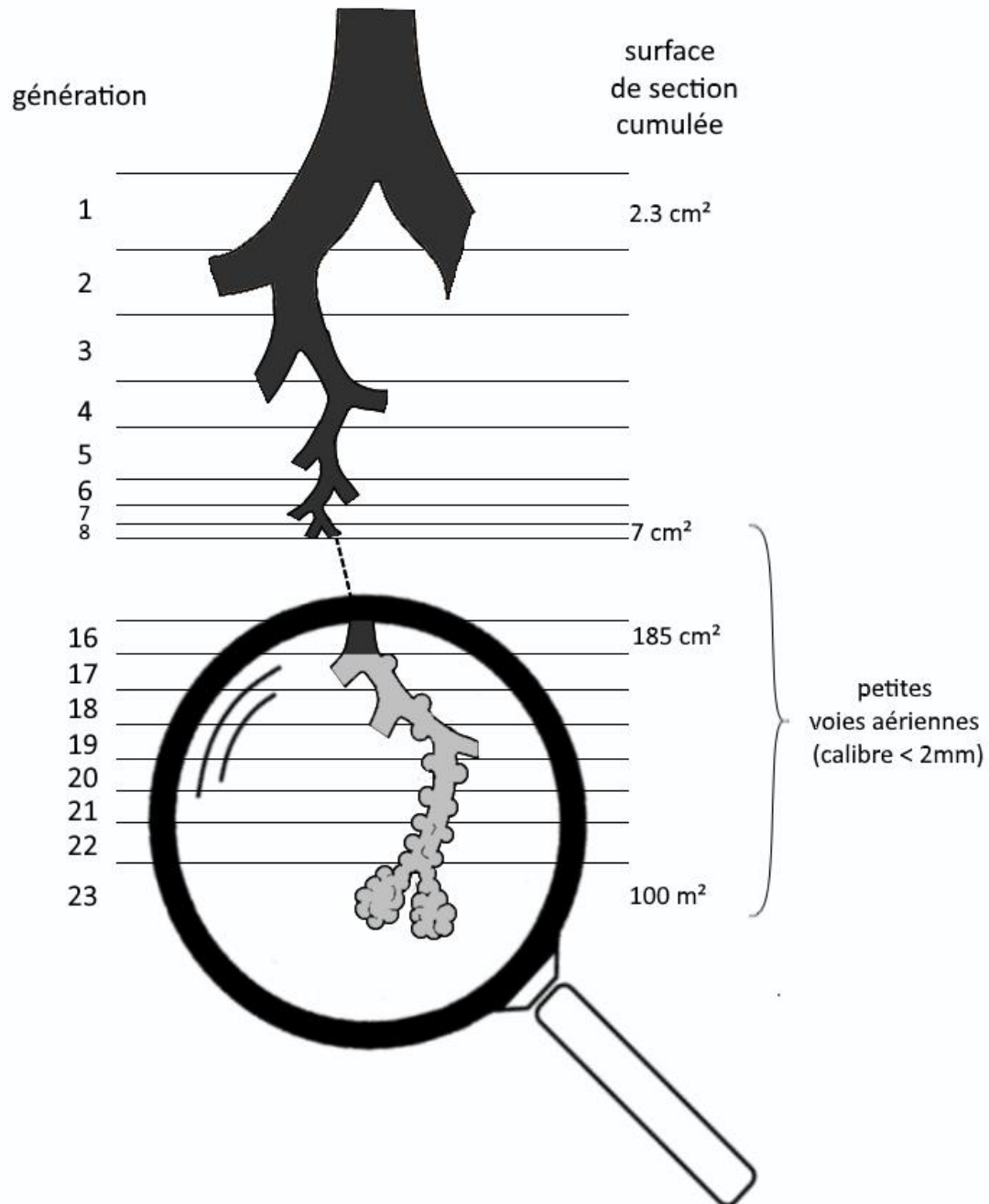
2.2. Les limites de la spirométrie : les voies aériennes distales et l'âge de l'enfant

Actuellement, la spirométrie reste l'outil de mesure de la fonction pulmonaire central et incontournable, à la base du suivi clinique des patients MV. Outre le fait que cette technique soit non-invasive, peu onéreuse, opérateur-accommodant et bien standardisée [8], son marqueur historique, le volume expiratoire maximal en 1 seconde (VEMS), est hautement reproductible et considéré comme le prédicteur le plus significatif de la mortalité chez les patients MV [9]. Pour le clinicien, il garde une valeur de guide tout au long de l'évolution du patient. En revanche, cet indice s'avère peu contributif pour évaluer l'état des petites voies aériennes et c'est pourtant à leur niveau que siègent les premières atteintes de la maladie pulmonaire dans la MV [10]. Ces dernières sont définies comme ayant un diamètre interne de moins de 2mm chez l'adulte, ce qui correspond approximativement aux générations bronchiques 8 et ultérieures [11]. En effet, le VEMS reflète principalement des limitations de débit, fortement dépendantes des résistances/calibres des voies aériennes. Or, l'ampleur de la surface de section totale des générations distales comparativement aux régions proximales est telle que ces petites voies aériennes contribuent très faiblement à la résistance totale des voies respiratoires (*Fig. 1*). Chez le sujet sain, on estime que moins de 15% de la résistance totale de l'arbre aérien peut être attribué aux voies aériennes distales [12].

Le débit expiratoire maximal médian entre 25 et 75% de la capacité vitale (DEM₂₅₋₇₅) et le débit expiratoire maximal à 25% de la capacité vitale (DEM₂₅) sont classiquement reconnus comme étant plus représentatifs de la fonction des petites voies aériennes ; ce sont par ailleurs les premières variables spirométriques à décliner chez le patient MV [13,14]. Mais comparativement au VEMS, leur variabilité intra- et inter-individuelle sont beaucoup plus importantes. D'autre part, l'interprétation de ces indices devient hasardeuse lorsque la capacité vitale est anormale [15,16]. Enfin, dans les premiers stades de développement de la maladie, l'obstruction périphérique hétérogène peut être masquée par l'augmentation compensatoire des flux provenant des voies aériennes parallèles, non débit-restreintes [17]. Toutes ces raisons pourraient expliquer la sous-estimation notoire des premières anomalies pulmonaires dans la MV par la spirométrie. À cela s'ajoute l'inconvénient d'un certain degré de coopération et coordination imposé par la spirométrie, expliquant sa relative infaisabilité

avant l'âge scolaire. Bien que certains centres pédiatriques très expérimentés reportent des données techniquement satisfaisantes chez l'enfant entre 3 et 6 ans moyennant l'utilisation de critères d'acceptabilité modifiés [18,19], la réalisation correcte de la technique est plus sûrement atteinte vers l'âge de 5-6 ans.

Figure 1. Segmentation fractale des voies aériennes pulmonaires



Les régions pulmonaires foncées représentent les voies aériennes de conduction tandis que les zones claires désignent les voies aériennes intra-acinaires.

L'intérêt semble donc évident de développer un outil à la fois capable de détecter les anomalies régnant dans les voies aériennes périphériques, « région silencieuse » au regard de la spirométrie, mais également de témoigner de l'efficacité potentielle d'un nouveau traitement endiguant les dommages pulmonaires précoces, idéalement avant que ceux-ci ne deviennent irréversibles. Il n'en demeure pas moins que la répétition des mesures qu'impose le suivi clinique ou scientifique nécessite une approche méthodologique réaliste, c'est-à-dire que l'outil doit pouvoir être applicable à tous les âges, tout en restant confortable pour le patient et sans effet secondaire. L'évaluation de l'inhomogénéité de la ventilation via le test RCM rend possible la combinaison de toutes ces caractéristiques, légitimant son intérêt dans la MV.

- La spirométrie sous-estime l'état des voies aériennes distales, siège des premiers dysfonctionnements pulmonaires.

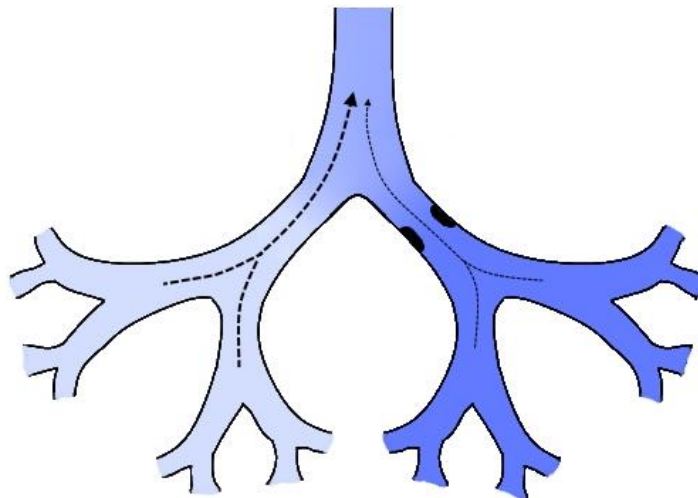
2.3. L'inhomogénéité de la ventilation

Le poumon peut être considéré comme un organe à forme géométrique de type fractale : de la trachée (génération 0) aux sacs alvéolaires (génération 23), chaque branche mère issue de n'importe quelle génération bronchique donnera presque systématiquement naissance à deux nouvelles branches filles homothétiques (*Fig. 1*). Cette conception architecturale particulière, multiplicative, aboutira à des millions de petites unités dont le rôle capital est l'échange gazeux, les alvéoles. Anatomiquement, le poumon peut être subdivisé en deux régions distinctes. La première région, nommée zone de conduction, s'étend des générations bronchiques 1 (bronches souches) à 16 (bronchioles terminales), et n'assure qu'un rôle de conduction de l'air entre le milieu ambiant et la deuxième région, les acini. Un acinus est une entité anatomique reliant la génération 17, où l'on voit naître les premières alvéoles sur les parois des bronchioles (bronchioles respiratoires), à la génération 23 (sacs alvéolaires) (*Fig. 1*). Cette région combine donc les rôles de conduction de l'air et d'échange air/sang.

Dans les zones de conduction, la vitesse linéaire de l'air est relativement élevée. Les déplacements et mélanges des molécules gazeuses au sein de ces zones sont majoritairement

générés par les forces mécaniques inhérentes à la respiration (gradient de pression). C'est la convection. Dans un modèle pulmonaire où l'air est distribué de manière parfaitement homogène, les régions pulmonaires descendantes de générations parallèles devraient accueillir et refouler un volume d'air strictement égal au cours de chaque cycle respiratoire. En réalité, cette distribution idéale n'est jamais observée, même chez le sujet sain. En effet la gravité modifie la compliance régionale du poumon et assure de ce fait une meilleure ventilation des zones pulmonaires dépendantes, comparativement aux zones non-dépendantes [20]. D'autre part, la structure du parenchyme pulmonaire n'est pas en tout point identique, ce qui provoque des inégalités mécaniques (compliance, résistance) iso-gravitationnelles normales entre différentes unités pulmonaires [21,22]. Les inhomogénéités ventilatoires décrites dans ce paragraphe sont dites « liées à la convection » et sont associées à des vitesses de remplissage ou vidange séquentielles entre plusieurs larges unités pulmonaires (*Fig. 2*).

Figure 2. Inhomogénéité de la ventilation dans les voies aériennes de conduction

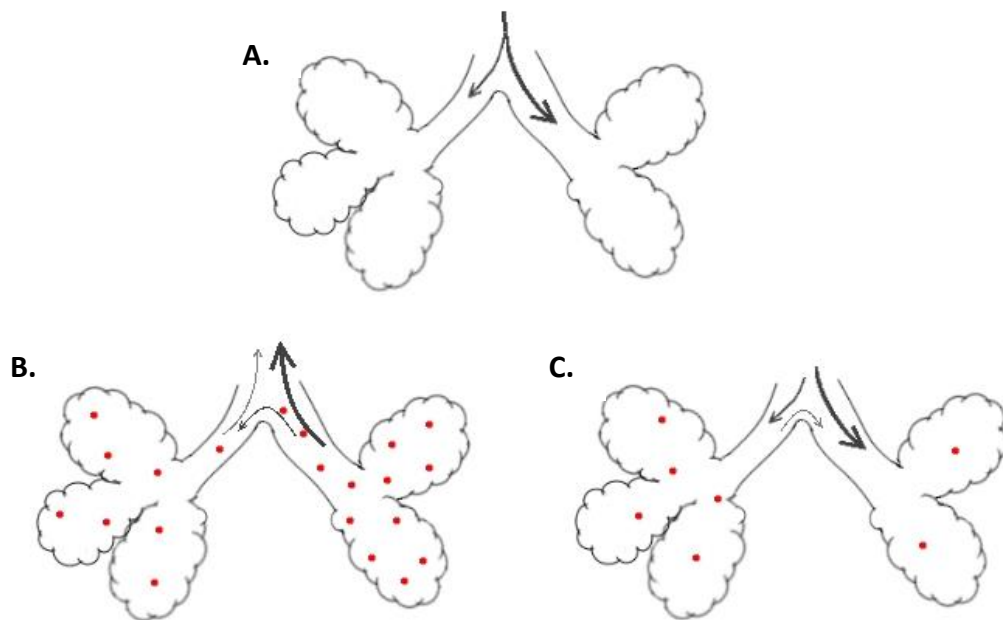


Une différence mécanique (physiologique ou pathologique) entre 2 sous-unités situées dans la zone de conduction désaccouple leur constante de temps de remplissage/vidange lors de la ventilation. À l'expiration, la concentration gazeuse de la branche parente reflète le mélange des inégalités des concentrations provenant des deux branches filles.

Dans la région intra-acinaire, l'étroitesse du calibre des petites voies aériennes combinée à l'augmentation exponentielle de leur surface de section totale rend la vitesse linéaire de plus en plus faible au fur et à mesure des générations successives, si bien que le transport des gaz devient progressivement dominé par le phénomène de diffusion

(déplacement des molécules qui tend à homogénéiser les concentrations ; gradient de concentration). Lorsque la part des échanges gazeux convectifs et diffusifs est de magnitude semblable, on parle de « front de convection-diffusion ». Ce dernier est estimé avoir lieu à l'entrée des acini chez le sujet sain. Toute asymétrie géométrique intra-acinaire (différence de volume ou de surface de section entre 2 régions intra-acinaires desservies par une même branche mère) conduira à un renouvellement gazeux préférentiel vers l'unité dont le volume est plus faible et/ou avec un calibre plus large [23]. À chaque expiration, une partie du volume de l'unité la plus riche en gaz ira combler la zone voisine moins concentrée par effet de diffusion. Les phases inspiratoires subséquentes entraîneront un mouvement diffusif inverse puisque le gradient de concentration sera alors transitoirement permuté. Ce phénomène de va-et-vient est dit de « diffusion pendulaire » et témoigne des inhomogénéités ventilatoires « liées à l'interaction convection-diffusion » (*Fig. 3*). À noter qu'ici encore les asymétries architecturales intra-acinaires sont communes chez le sujet sain [24], et sont donc en partie responsables d'une distribution naturellement inégale de la ventilation [22,25].

Figure 3 : Inhomogénéité de la ventilation dans les voies aériennes intra-acinaires.



A) À l'inspiration d'un gaz donné, l'unité sub-acinaire dont la surface de section est plus grande et le volume plus petit sera préférentiellement ventilée, elle accumulera donc une plus grande concentration gazeuse. **B)** Au début de la phase expiratoire suivante, une partie du gaz de l'unité la plus concentrée diffusera vers l'unité parallèle plus diluée (diffusion pendulaire). **C)** L'inverse est constaté lors de l'inspiration subséquente.

L'hétérogénéité ventilatoire physiologique, qu'elle soit liée à la convection ou à la diffusion, est néanmoins nettement plus prononcée dans le poumon malade. Des anomalies survenant dans les voies aériennes de conduction sont susceptibles de modifier davantage les relations pression-volume entre de larges unités pulmonaires, tandis que leurs manifestations plus périphériques accentueront les irrégularités structurelles intra-acinaires [26-29]. L'aggravation consécutive de l'inhomogénéité ventilatoire rendra le brassage des gaz intra-pulmonaires moins efficient.

L'ICP est la simple quantification du degré d'inhomogénéité ventilatoire globale du poumon ; au plus la distribution de la ventilation est inégale, au plus sa valeur est élevée. L'ICP est sensible aux inhomogénéités ventilatoires survenant à tous les étages pulmonaires (zones de conduction et intra-acinaire) mais ne peut toutefois pas discerner les zones topographiques où siègent préférentiellement ces inhomogénéités [28,30]. D'autres méthodes permettent de préciser la localisation des régions principalement atteintes [23,30,31], mais leur description sort du cadre de cette revue. L'ICP reste l'indice d'inhomogénéité ventilatoire le plus fréquemment reporté car il est non seulement conceptuellement simple à comprendre et à calculer (voir ci-dessous), mais est aussi considéré comme le paramètre RCM le plus robuste et probablement le plus informatif [32-34].

Mesurer l'inhomogénéité de la ventilation est pertinent dans les pathologies où la topographie des lésions est variable et hétérogène [35,36], surtout si – comme c'est le cas dans la MV – l'atteinte initiale concerne les petites voies aériennes [10]. Dans la MV, l'ICP est effectivement un indice détectant très précocement les premiers signes de la maladie pulmonaire, et ce même chez l'enfant dont les variables spirométriques restent normales [37-40]. Ne requérant qu'une collaboration passive pour être mesuré, l'ICP est en outre faisable chez le très jeune enfant, ce qui lui confère un avantage certain par rapport aux mesures fonctionnelles conventionnelles [39,41]. En fait, l'ICP est même accessible chez le nourrisson sous sédation voire pendant son sommeil [42-45], bien que sa faisabilité dans ce contexte reste pour le moment limitée à quelques équipes de recherche bien entraînées et équipées étant donné les obstacles technologiques et méthodologiques spécifiques liés à l'immaturation du système respiratoire du nourrisson [46-48].

À noter enfin que l'inhomogénéité de la ventilation n'est pas l'apanage de la MV, ce qui explique que le champ d'application de l'ICP ne soit pas restreint au cadre de cette pathologie. Le potentiel de cet outil est en effet exploré dans d'autres maladies respiratoires

comme l'asthme [49,50], la dyskinésie ciliaire [51,52], les bronchiectasies non liées à la mucoviscidose [53,54] ou encore la bronchiolite aiguë du nourrisson [55], mais les données sont encore insuffisamment étayées que pour pouvoir préjuger de la réelle plus-value scientifique ou clinique de l'ICP dans ces pathologies.

- L'inhomogénéité de la ventilation est physiologique mais est nettement plus marquée dans la mucoviscidose et ce dès les premières années de vie.
- Les anomalies survenant dans les voies aériennes de conduction ou les régions intra-acinaires sont susceptibles d'exagérer l'inhomogénéité ventilatoire.
- Faisable à tous les âges, l'ICP quantifie, de manière non-invasive et sans recours à un isotope radioactif, l'inhomogénéité globale de la ventilation.

2.4. Description d'un test de rinçage sur cycles respiratoires multiples

Puisque l'inégalité de la distribution de la ventilation altère l'efficacité du mélange des gaz intra-pulmonaires, la mesure de l'inhomogénéité de la ventilation repose sur l'étude de la relative aisance d'élimination, ou rinçage, d'un gaz inerte intra-pulmonaire. Dans le cas du RCM, le comportement du rinçage est étudié sur plusieurs respirations, à volume courant et au niveau de la capacité résiduelle fonctionnelle (CRF). La réalisation de la procédure implique le raccord du sujet à un débitmètre, un analyseur de gaz et un système permettant l'administration des gaz. Le gaz inerte est approprié s'il est à la fois non toxique, s'il ne participe pas aux échanges air/sang et s'il est peu soluble dans le sang ou les tissus. Les 3 gaz les plus utilisés dans ce contexte sont l'azote, l'hélium et l'hexafluorure de soufre (SF₆) [40]. Parmi ces gaz, l'azote est le seul à résider naturellement dans le poumon ; son rinçage est obtenu en administrant 100% d'oxygène au sujet. Dans les deux autres cas, le sujet doit préalablement inhaler ces gaz.

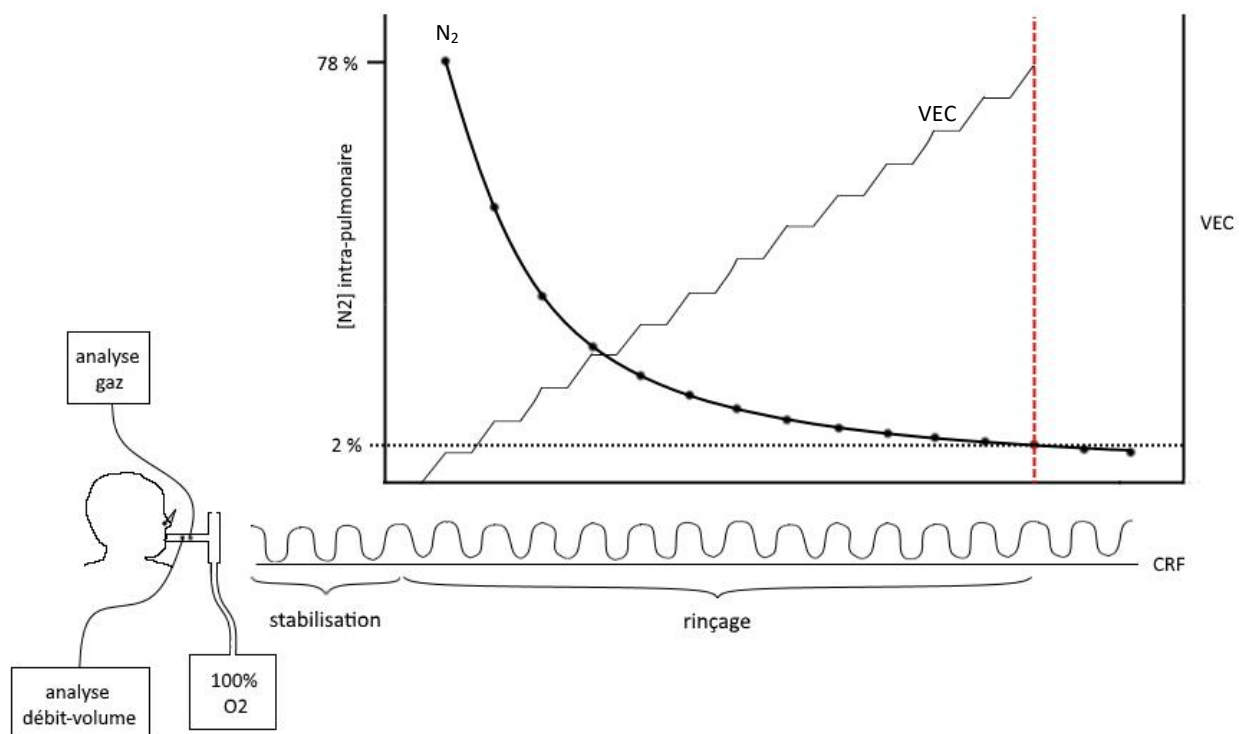
La procédure peut être divisée en deux parties : les phases de remplissage (« wash-in ») et de rinçage (« washout ») du gaz inerte. Pendant la première phase, le sujet respire, de manière détendue et à volume courant, un mélange gazeux comportant le gaz marqueur. Jusqu'il y a peu, cette mixture contenait souvent 4% de SF₆, 21% d'oxygène, l'azote comblant la fraction manquante. Lorsque cet équilibre est atteint dans le poumon, le sujet est

déconnecté du système lui administrant le mélange gazeux et respire à nouveau l'air atmosphérique ambiant ; la phase de rinçage débute alors.

Lorsque c'est l'azote qui est utilisé comme gaz traceur, la phase de remplissage est remplacée par une phase de stabilisation (« pre-washout ») pendant laquelle le sujet respire de l'air atmosphérique tout en étant raccordé aux analyseurs. Cette étape permet de contrôler la stabilité de la respiration courante et la capacité résiduelle fonctionnelle (CRF) du sujet, garante de la qualité des mesures. Le cas échéant, la phase de rinçage de l'azote débute en administrant au sujet de l'oxygène pur.

La phase de rinçage perdure jusqu'à ce que la concentration du gaz soit réduite à 1/40^{ème} (2.5%) de sa concentration initiale sur trois respirations consécutives. Pendant l'entièreté de la phase de rinçage, la ventilation et l'évolution de l'élimination intra-pulmonaire du gaz marqueur sont analysées sur les cycles respiratoires successifs. La figure 4 schématise une manœuvre utilisant l'azote comme gaz marqueur.

Figure 4. Représentation schématique du rinçage de l'azote sur cycles respiratoires multiples.



Le graphique représente l'analyse simultanée de la concentration intra-pulmonaire d'azote et du volume expiré cumulé au cours des cycles respiratoires successifs de l'enfant. Les cycles respiratoires sont représentés en dessous du graphique, l'expiration étant caractérisée par l'ascension de la courbe. L'analyse de la concentration d'azote a lieu à la fin de chaque expiration. Le trait vertical en pointillé délimite la fin du test. Liste des abréviations : VEC, volume expiré cumulé ; CRF, capacité résiduelle fonctionnelle ; N₂, azote ; O₂, oxygène.

Le degré d'inhomogénéité ventilatoire global (désigné par l'ICP) peut alors être déduit en divisant le volume expiré cumulé (VEC) au fil du rinçage par le volume pulmonaire (CRF).

$$\text{ICP} = \text{VEC} / \text{CRF}$$

Le volume pulmonaire est donné en divisant le volume rincé du gaz par la différence entre les concentrations alvéolaires initiale et finale de ce gaz.

$$\text{CRF} = V_{\text{traceur}} / ([\text{gaz}]_{\text{init}} - [\text{gaz}]_{\text{fin}})$$

Où V_{traceur} est le volume du gaz inerte rincé, $[\text{gaz}]_{\text{init}}$ et $[\text{gaz}]_{\text{fin}}$ les concentrations en début et à la fin de la manœuvre de rinçage, respectivement. Il est assumé que la concentration alvéolaire correspond à celle calculée à la fin de chaque expiration. L'ICP n'a donc pas d'unité mais est plutôt exprimé en « turnover », c'est-à-dire le nombre de renouvellements du volume pulmonaire requis pour réaliser l'entière du rinçage. Comme le sujet respire à volume courant, le volume pulmonaire en question correspond à la CRF. Chez un sujet sain, l'ICP est typiquement de l'ordre de 6-7,5 turnovers [40]. En vulgarisant, cet indice reflète donc « la facilité » avec laquelle un sujet parvient à se débarrasser d'un gaz inerte contenu dans ses poumons.

Un test complet demande actuellement la réalisation d'une série de 3 manœuvres techniquement acceptables [56]. Entre chaque test, il convient de respecter un intervalle de temps afin d'assurer la restitution complète des concentrations gazeuses alvéolaires initiales. Un temps d'arrêt conservateur correspondant à 2x la durée du rinçage précédent est actuellement recommandé. Les critères définissant la recevabilité d'un test ont fait l'objet d'un consensus [56] qui évoluera si des modalités moins contraignantes se révèlent aussi performantes [57]. L'ICP reporté sera calculé en moyennant les valeurs observées sur les 3 premières manœuvres acceptables.

- Le test RCM se réalise à volume courant, au niveau de la CRF. Il nécessite l'analyse du rinçage d'un gaz inerte.
- L'ICP décrit la relative aisance d'un sujet à se débarrasser d'un gaz inerte intra-pulmonaire ; il représente le nombre de fois que le sujet doit expirer un volume égal à sa CRF pour éliminer ce gaz.

2.5. Les gaz traceurs et l'intérêt de l'azote

Le gaz de rinçage utilisé a une incidence sur les mesures du RCM, si bien que les valeurs d'ICP obtenues en utilisant l'un ou l'autre gaz ne sont pas interchangeables [41,58,59]. En effet, les propriétés physiques des gaz inertes ne sont pas comparables et influent inéluctablement sur leur mode de transport au sein des différentes générations bronchiques. Par exemple, l'étage pulmonaire où prédominent les phénomènes de diffusion dépend directement de la diffusivité du gaz, elle-même inversement proportionnelle à la densité de ce gaz [56]. Pour l'hélium, gaz très léger ($4\text{g}\cdot\text{mol}^{-1}$), on estime que l'endroit où s'équilibrent les phénomènes de transport par convection et diffusion se situe légèrement en amont de l'entrée des acini. Le SF₆ étant bien plus dense que l'hélium ($146\text{g}\cdot\text{mol}^{-1}$), cette transition est présumée avoir lieu dans des régions pulmonaires plus profondes [31]. Chez le sujet sain, les différences de volume découlant de deux branches sœurs intra-acinaires sont de plus en plus marquées au fur et à mesure que l'on atteint les dernières générations pulmonaires [24]. Ainsi, naturellement, les inégalités de concentration seront accentuées avec le SF₆ et atténuées avec l'hélium [23]. La présence d'anomalies pulmonaires au sein des régions intra-acinaires exagèrera davantage les inégalités de concentration du SF₆. À l'inverse, les inhomogénéités ventilatoires siégeant préférentiellement dans les zones plus proximales s'exprimeront plutôt par le rinçage contrarié de l'hélium [31].

Indépendamment de la localisation topographique des lésions, l'origine endo- ou exogène des gaz peut également influencer l'ICP. En effet lors de la phase de remplissage, les gaz exogènes pourraient ne jamais atteindre la concentration alvéolaire recherchée dans les zones très mal ventilées, masquant la contribution de ces régions à l'inhomogénéité ventilatoire globale pendant la phase de rinçage [58]. L'azote, gaz endogène, pourrait donc être l'option la plus attrayante, mais certains inconvénients peuvent lui être reprochés. L'azote n'est par exemple pas un gaz totalement insoluble dans le sang ou les tissus. Par conséquent, une fraction de ce gaz peut transiter du sang vers les alvéoles (rétrodiffusion) et contaminer (augmenter) la valeur de l'ICP, même si cette contribution semble en réalité assez faible [56,60]. Un autre obstacle à son utilisation, plus gênant, relève de la stimulation hyperoxique – liée au recours d'oxygène pur pour la phase de rinçage – des chémorécepteurs périphériques du petit enfant [61]. Bien que cela soit sans conséquence chez l'enfant de plus de 6 ans, l'hyperoxie semble perturber les centres respiratoires du nourrisson, voire de

l'enfant d'âge préscolaire, ce qui modifie leur pattern respiratoire [46,62,63]. Or, d'une part un pattern respiratoire régulier est un prérequis à la qualité de la mesure [56] et, d'autre part, l'ICP peut varier significativement si le volume courant ou la CRF diffèrent substantiellement [64]. En conséquence, un protocole adapté incluant une préexposition à 40% d'O₂ afin d'induire une tolérance à l'hyperoxie a été proposé et semble être une option encourageante [47], permettant de pallier la modification du pattern respiratoire. Jusqu'il y a peu, cette préoccupation ne concernait que le rinçage de l'azote. En réalité, il a récemment été démontré que le SF₆, qui s'imposait comme l'alternative la plus robuste chez le nourrisson, modifiait également le pattern respiratoire de celui-ci. Ce phénomène est surtout attribué à la haute densité du SF₆ et son effet désavantageux sur la charge respiratoire du petit enfant [65,66].

Au-delà de ces observations physiologiques, des considérations d'ordre plus pragmatique peuvent conduire à choisir l'un ou l'autre gaz. Concernant l'hélium, gaz hautement volatil, il est difficile de maintenir l'étanchéité parfaite des circuits, ce qui limite beaucoup son utilisation dans les études appliquant le RCM [32]. Le SF₆ apparaît être une meilleure alternative, mais son coût est élevé et il participe à l'effet de serre. L'utilisation de ce gaz pour les examens médicaux n'est en outre pas agréée dans certains pays, comme la France. L'azote contourne par contre ces grandes limitations puisque l'oxygène est aisément et largement disponible à des fins médicales. La possibilité de respirer l'air libre entre deux rinçages pulmonaires successifs constitue par ailleurs un petit avantage pratique appréciable. Pour ces raisons, l'azote est probablement le gaz traceur qui s'imposera pour des fins cliniques ou les études multicentriques. Une étude récente lui accorde d'ailleurs un profil plus favorable que le SF₆ pour évaluer les effets d'un traitement, confortant donc le choix de l'azote [67].

- Les gaz traceurs se comportent différemment dans le poumon et influencent la valeur de l'ICP.
- L'azote apparaît raisonnablement comme le gaz qui s'imposera dans les études multicentriques ou pour des fins cliniques.

2.6. L'indice de clairance pulmonaire dans la mucoviscidose

Le RCM est une technique ancienne, décrite pour la première fois en 1950 [68]. L'intérêt de l'ICP est rapporté deux ans plus tard [69]. La technique va rester longtemps confinée à des laboratoires de recherche de physiologie respiratoire, principalement parce qu'elle nécessite alors un spectrographe de masse (encombrant et coûteux, à l'achat comme à l'entretien) pour l'analyse en temps réel de la concentration du gaz traceur [70]. La résurgence de l'intérêt pour la technique RCM chez les praticiens dédiés à la MV est surtout expliquée par les progrès technologiques de ces dernières années. L'introduction d'appareils disponibles dans le commerce a en effet permis de s'acquitter du besoin d'un spectrographe de masse, contribuant largement à améliorer l'accessibilité à la technique.

Gustafsson *et al.* ont publié en 2003 la première étude démontrant, chez des enfants MV de 3 à 18 ans, l'avantage de sensibilité de l'ICP par rapport au VEMS pour détecter les anomalies pulmonaires [37]. Ces résultats, confirmés depuis par de nombreuses autres études [31,38,39,71-74], ont débouché vers l'exploration plus large des propriétés clinimétriques et de la faisabilité de l'ICP dans la MV. Une revue récente énumère ces données [40]. La plupart ont été établies sur base d'études utilisant un spectrographe de masse et le SF₆ comme gaz traceur. Les lignes qui suivent les résument très succinctement tout en tenant compte des données issues de plus récentes publications. Les études ayant mesuré l'ICP via le rinçage de l'azote avec un appareil commercialement disponible sont répertoriées et résumées dans les tableaux 1 et 2. Étant donné certaines réserves concernant l'intérêt de l'ICP pour les essais cliniques chez le nourrisson [75-78], les obstacles technologiques liés aux spécificités ventilatoires du tout petit (petit volume courant, fréquence respiratoire élevée, espace mort relativement important, soupirs) [48,79,80], les doutes soulevés par les algorithmes actuellement proposés [81-83] et l'effet des gaz traceurs sur leur pattern respiratoire [46,47,65], ce chapitre se focalisera surtout sur ce qui est connu chez l'enfant de plus de 2 ans.

2.6.1. Reproductibilité

L'ICP est une mesure très reproductible chez un même sujet. Le coefficient de variation intra- ou inter-test, que les sessions soient séparées de quelques heures ou quelques mois, fluctue entre 4 à 8% chez le sujet sain ou MV [40,84-88]. Néanmoins, la sélection exclusive de tests basés sur des critères de qualité plus stricts permet d'améliorer davantage la reproductibilité de l'ICP, ce qui peut se justifier dans les études longitudinales ou lors d'essais cliniques [89,90]. Son coefficient de répétabilité (il représente 95% de la variabilité physiologique et technique de l'ICP) semble indépendant de l'âge du patient et oscille entre 0.96 et 1.40 [67,74,85,91,92]. Autrement dit, une modification de la valeur de l'ICP d'au moins 1 unité doit être obtenue avant d'estimer la variation du paramètre comme pertinente.

2.6.2. Validité

L'habileté de l'ICP à discriminer les patients MV des cohortes contemporaines saines est invariablement démontrée. Chez l'enfant MV âgé de 2 à 10 ans, une valeur élevée (anormale) est observée dans 58 à 77% des patients [39,86,89,93,94]. La prévalence d'un ICP élevé augmente avec l'âge jusqu'à devenir presque systématiquement anormal chez le patient adulte [40,76,85,95,96]. L'indice peut également différencier des sous-groupes de patients ; la valeur diffère selon le statut infectieux (principalement absence ou présence de *Pseudomonas aeruginosa*), la présence d'un épisode de toux, d'une exacerbation pulmonaire ou de certaines anomalies structurelles [40,74,86]. Ces dernières sont en outre bien mieux détectées par l'ICP que par la spirométrie [71,73,97].

2.6.3. Corrélation avec d'autres mesures

L'ICP est corrélé avec toute une série d'indicateurs liés à la sévérité de l'atteinte pulmonaire, comme les variables dérivées de la spirométrie [40], le score clinique [85,98], certains marqueurs inflammatoires [88,99], la charge bactérienne [88,100] ou encore le statut infectieux à *Pseudomonas aeruginosa* [74]. Surtout, cet indice est bien corrélé avec les scores obtenus par l'imagerie par résonance magnétique [76,101] ou la tomodensitométrie thoracique – le « gold standard » pour évaluer la présence et l'étendue des dommages pulmonaires structurels dans la mucoviscidose – chez l'adulte comme chez l'enfant MV [71,73,93,97,102,103], du moment qu'il ait plus de 2 ans [93,104].

2.6.4. Validité prédictive

Peu d'études documentent la valeur pronostique de l'indice. Aurora *et al.* établissent la validité de l'ICP mesuré à l'âge préscolaire à prédire la fonction pulmonaire sur environ 4 ans [91]. Par ailleurs, Vermeulen *et al.* démontrent que la valeur de l'ICP obtenue chez le patient MV âgé de 5 à 19 ans pronostique le risque de développer une exacerbation pulmonaire endéans l'année [98]. Ces derniers résultats contrastent cependant avec de nouvelles données obtenues chez l'enfant d'âge préscolaire, où l'ICP ne présume pas la prochaine exacerbation à 1 an [86]. D'autres travaux sur cet aspect clinimétrique sont donc encore requis.

2.6.5. Sensibilité au traitement

L'ICP semble être un outil prometteur pour évaluer l'efficacité de certaines interventions thérapeutiques dans la MV, notamment chez les patients dont l'atteinte pulmonaire reste relativement légère. Il apparaît en effet plus sensible que le VEMS pour détecter l'effet des traitements inhalés comme le sérum salin hypertonique [67,105,106] ou la désoxyribonucléase recombinante humaine [107], ainsi que l'Ivacaftor (chez des enfants avec au moins une mutation de classe III) [108,109]. Chez le petit enfant (1 – 4,5 ans), l'ICP met aussi en évidence l'effet thérapeutique du sérum salin hypertonique sur 48 semaines [110]. L'intérêt de la mesure pour évaluer l'effet d'une cure intraveineuse d'antibiotique est en revanche plus nuancé. Bien que les dernières études montrent une nette amélioration de l'ICP après le traitement d'une exacerbation pulmonaire [76,111], la plupart des auteurs reportent une réponse généralement moins franche, hétérogène, et parfois non significative, malgré l'amélioration significative du VEMS [99,112-115]. Lorsque les études sont regroupées, le changement de l'ICP est statistiquement significatif mais la taille de l'effet est modeste (amélioration de 0.4 turnover) et moindre que pour le VEMS [116]. Les différents aspects physiologiques que représentent l'ICP et le VEMS peuvent expliquer ces résultats discordants, et seront davantage discutés dans la section « limites de l'ICP ». Des conclusions similaires peuvent être tirées envers la kinésithérapie respiratoire où la réponse de l'ICP est également imprévisible individuellement, et tandis que certains auteurs rapportent une amélioration en moyenne significative après la kinésithérapie, d'autres ne démontrent pas d'effet sur l'indice [117-121]. La variabilité et l'inconstance des réponses de l'ICP à certaines interventions soulignent la nécessité de mener d'autres études sur ce sujet.

2.6.6. Valeurs de référence

Les valeurs de référence sont spécifiques à l'appareil RCM et au gaz de rinçage. Le tableau 1 liste les valeurs normatives obtenues chez les sujets sains avec un appareil commercialement disponible et l'azote comme gaz traceur. Les études imposant un volume courant fixe lors du rinçage ne sont pas reprises dans ce tableau. La grande majorité des données a été collectée avec l'Exhalyzer D (Eco Medics AG, Dürnten, Suisse). Avec cet appareil et l'azote, l'ICP moyen s'étend de 6.1 à 7.4 unités, mais oscille le plus souvent entre 6.7 et 7.1 unités avec une limite supérieure de la normalité n'excédant d'ordinaire pas 8 turnovers pulmonaires. Considérant toujours cet équipement, les études comprenant les plus larges cohortes montrent que l'ICP moyen est stable de 3 à 18 ans, puis augmente linéairement avec l'âge (0.0211 unité/an) [76,93,122].

Tableau 1. Valeurs prédites et limite supérieure de normalité de l'ICP mesuré par rinçage de l'azote

N	Groupe d'âge	Appareil	Nbre de mesures	ICP			Référence
				Moyenne	Écart-type	LSN	
33	Enfants et Adultes 8-60 ans	EasyOne Pro	2-3	6.3	0.6	7.5	Fuchs [123]
51	Adultes 18-65 ans	EasyOne Pro	2	6.42	0.54	7.50	Poncin [95]
51	Enfants 5-18 ans	EasyOne Pro	2	6.78	0.63	8.04	Poncin [95]
50	Enfants 4-16 ans	Exhalyzer D	≥ 1	6.1	0.9	7.9	Singer [74]
24	Enfants 6-17 ans	Exhalyzer D	3	6.44	0.57	7.58	Yammine [124]
64	Enfants et Adultes > 6 ans	Exhalyzer D	≥ 2	6.60	0.34	7.28	Uhlving [125]
74	Enfants et Adultes 23 ± 7.3 ans	Exhalyzer D	3	6.67	0.35	7.37	Gustafsson [126]
10	Adultes n.r.	Exhalyzer D	3	6.69	0.42	7.53	Benseler [41]
44	Enfants 5-16 ans	Exhalyzer D	3	6.7	1.1	8.9	Singer [31]
72	Enfants 3-15 ans	Exhalyzer D	≥ 2	6.71	0.51	7.73	Ramsey [93]
48	Enfants 5-18 ans	Exhalyzer D	3	6.72	0.37	7.46	Green [84]
25	Enfants 6-18 ans	Exhalyzer D	≥ 2	6.8	0.4	7.6	Jensen [90]
20	Enfants 7-17 ans	Exhalyzer D	3	6.8	0.5	7.8	Yammine [64]
62	Enfants 3-18 ans	Exhalyzer D	≥ 2	6.81	n.r.	n.r.	Jensen [58]

79	Enfants et Adultes 4-21 ans	Exhalyzer D	≥ 2	6.9	0.6	8.1	Stahl [76]
51	Adultes 18-65 ans	Exhalyzer D	2	6.92	0.35	7.62	Poncin [95]
25	Enfants 6-18 ans	Exhalyzer D	≥ 2	7.00	0.49	7.98	Ramsey [103]
46	Enfants 6-16 ans	Exhalyzer D	≥ 2	7.04	0.52	8.08	Yammine [127]
20	Enfants 2.5-6 ans	Exhalyzer D	3	7.05	0.39	7.83	Benseler [41]
32	Enfants 5-19 ans	Exhalyzer D	2-3	7.05	0.45	7.95	Yammine [128]
50	Enfants 5-18 ans	Exhalyzer D	2	7.05	0.55	8.15	Poncin [95]
52	Enfants 2.5-6 ans	Exhalyzer D	≥ 2	7.1	(6.1-8.1)*	n.r.	Stanojevic [86]
400	Adultes 17-71 ans	Exhalyzer D	3	7.13	0.57	8.27	Kjellberg [122]
57	Enfants 4-18 ans	Exhalyzer D	3 (2 – 4)	7.30	0.50	8.3	Vermeulen [98]
17	Enfants 4-5 ans	Exhalyzer D	≥ 2	7.3	0.6	8.5	Mandaliya [129]
20	Enfants 7-18 ans	Exhalyzer D	3	7.4	0.7	8.8	Nyilas [130]

EasyOne Pro : ndd Medical Technologies, Zurich, Suisse

Exhalyzer D : EcoMedics AG, Dürnten, Suisse

LSN : limite supérieure de la normalité (moyenne + 2x écart-type) ; n.r. : non reporté ; * étendue.

2.6.7. Faisabilité

La faisabilité varie globalement avec l'âge. Les lignes qui suivent rapportent le taux de succès d'un test RCM en considérant le pourcentage de patients MV ayant réalisé au moins 2 mesures valides, sauf mention explicite. Chez le tout petit (0 – 4 ans) et sans recours à la sédation, Bakker *et al.* rapportent un taux de succès de 40% [44]. Les raisons des échecs sont, par ordre d'importance, l'enfant qui pleure ou parle pendant le test, le rejet du masque facial, les critères de qualité non respectés et une respiration irrégulière. La faisabilité grimpe chez l'enfant d'âge préscolaire, avec un taux de succès allant de 75% (pour 3 mesures) à 83% [39,86]. Stanojevic *et al.* montrent qu'en moyenne 5 essais – mais cela peut aller jusqu'à 12 – sont nécessaires pour obtenir 2 mesures valides, et que le taux de succès augmente à chaque visite (66% lors de la première visite, 75% 1 mois plus tard, 86% à 3 mois, 90% à 6 mois, 88% à 9 mois, 96% à 1 an) [86]. Chez les patients MV de plus de 6 ans, le taux de succès s'étend entre 80 et 100% [40,127]. Un taux d'échec conservateur d'au moins 20% est donc à prévoir lors du recrutement pour les essais cliniques.

La faisabilité clinique a également été testée en imposant une contrainte de temps de 20 minutes à des enfants naïfs au test RCM. Le taux de succès chute alors à 60% chez les enfants âgés de 3 à 6 ans et 41% chez les enfants de 4-16 ans [74,131]. Les raisons d'échec les plus fréquentes sont une fuite, un test prématurément arrêté par l'enfant, et un pattern respiratoire irrégulier. Cela souligne l'importance de la familiarisation préalable des enfants au RCM et la nécessité d'aménager un temps plus long chez les patients n'ayant jamais réalisé le test. Chez les adultes MV, le taux de succès est excellent mais comme l'atteinte pulmonaire est plus sévère, le test peut devenir très chronophage [40]. Ainsi, lorsque la durée du test est restreinte à 25 minutes, près d'un adulte MV sur 4 n'atteint pas l'objectif [95].

- L'ICP présente des propriétés clinimétriques attractives dans la MV.
- La validité prédictive de l'indice mérite d'être davantage explorée.
- La variabilité et l'inconstance des réponses de l'ICP à certaines interventions justifient le besoin d'autres essais thérapeutiques plus larges.
- Dans le cadre de la recherche, la faisabilité de la mesure est satisfaisante, surtout chez le patient ayant déjà eu une expérience avec le RCM.
- Dans un contexte clinique, il est nécessaire d'aménager un temps de test plus important lors de la première visite.

Tableau 2. Propriétés clinimétriques de l'ICP mesuré par l'Exhalyzer D et le rinçage de l'azote dans la mucoviscidose

Propriétés clinimétriques	Auteur, année	Résumé
Reproductibilité	Yammine 2013 [124] Singer 2013 [74] Singer 2013 [31] Jensen 2013 [58] Green 2016 [84] Amin 2016 [67] Stanojevic 2017 [86] Poncin 2017 [95]	Le coefficient de variation intra- ou inter-session est similaire chez les individus sains ou patients MV en état stable, enfants comme adultes. Il s'étend entre 3.5 et 7.4. Entre plusieurs tests répétés endéans 24h, le coefficient de répétabilité est estimé entre 0.96 et 1.24 pour le patient MV [67,74].
Validité		
% patients MV avec ICP anormal	Yammine 2013 [124] Singer 2013 [74] Singer 2013 [31] Vermeulen 2014 [98] Gustafsson 2014 [126] Ramsey 2016 [93] Kane 2016 [109] Green 2016 [84] Nyilas 2016 [130] Poncin 2017 [95] Stahl 2017 [76] Stanojevic 2017 [86] Ramsey 2017 [103]	Chez l'enfant d'âge préscolaire, la sensibilité de l'indice est estimée entre 58-67% [86,93]. Entre l'enfance (> 4 ans) et l'âge adulte, la sensibilité augmente et fluctue entre 80-92% [31,74,76,84,98,124,130]. Ramsey <i>et al.</i> (2016, 2017) reportent des taux plus faibles (58 et 71%), liés probablement à l'inclusion d'un nombre important de patients ayant un VEMS normal. Le Z-score moyen du VEMS (95% IC) était de -0.13 (-0.51 ; 0.26) et -0.17 (-0.74 ; 0.40) respectivement [93,103]. Chez l'adulte MV, la probabilité d'obtenir un ICP anormal est très élevée (90-100%) [95,109,126].
% patients MV avec ICP anormal et VEMS normal	Singer 2013[31] Vermeulen 2014 [98] Gustafsson 2014 [126] Poncin 2017 [95]	Entre l'enfance (> 4 ans) et l'âge adulte, 80-92% des enfants avec un VEMS normal ont un ICP anormal [31,98]. Lorsque la cohorte comprend également, voire uniquement des adultes, les taux sont légèrement plus élevés (86-96%) [95,126].
Corrélations		
	Singer 2013 [74] Vermeulen [98] Bigler 2015 [132] Green 2016 [84] Ramsey 2016 [93] Poncin 2017 [95] Stahl 2017 [76] Stanojevic 2017 [86] Ramsey2017 [103]	L'ICP est corrélé avec les variables spirométriques (VEMS, VEMS/CVF, DEM ₂₅₋₇₅), l'âge [76], Scond [132], la charge bactérienne [74], la PaO ₂ [74] ainsi que certains scores cliniques (CFQ-Rresp et CFCS) [98]. L'indice est également corrélé avec les scores d'imagerie pulmonaire quantifiés via le scanner [93,103] ou l'imagerie par résonance magnétique [76]. Ramsey <i>et al.</i> (2016) montrent que la force de corrélation de l'indice avec les scores tomодensitométriques augmente avec la catégorie d'âge [93].

Tableau 2. Suite

Propriétés clinimétriques	Auteur, année	Résumé
Validité prédictive (pronostique)	Vermeulen 2014 [98] Stanojevic 2017 [86]	Chez les enfants de 4 à 18 ans, l'ICP prédit à la fois le risque de développer une exacerbation pulmonaire dans l'année et le temps avant la prochaine exacerbation [98]. À l'inverse chez l'enfant d'âge préscolaire, l'ICP ne permet pas de pronostiquer le risque d'une exacerbation pulmonaire contractée dans l'année qui suit la mesure [86].
	Welsch 2014 [114] Yammine 2014 [115] Pfleger 2015 [120] Hatziaorou 2015 [111] Amin 2016 [67] Kane 2016 [109] Stahl 2017 [76]	La réponse de l'ICP à une cure d'antibiotique administré par voie intraveineuse est inconstante. Deux études montrent une amélioration significative de l'ICP après le traitement d'une exacerbation pulmonaire [76,111], tandis que la réponse moyenne n'est pas significative dans 2 autres études [114,115]. La réponse de l'ICP à la suite d'une seule séance de kinésithérapie respiratoire est hétérogène, et en moyenne non significative [120]. Chez un groupe de 18 patients, la nébulisation d'une dose unique de sérum salin hypertonique n'entraîne pas de modification réellement significative sur 24H [67]. L'étude de seulement 10 patients porteurs d'une mutation de classe III suffit à démontrer une amélioration significative de l'ICP, évidente dès le premier contrôle à 1 mois et qui persiste jusqu'à au moins 6 mois [109].
Faisabilité	Yammine 2013 [124] Singer 2013 [74] Singer 2013 [31] Jensen 2013 [58] Vermeulen 2014 [98] Benseler 2015 [41] Pfleger 2015 [120] Green 2016 [84] Poncin 2017 [95] Stanojevic 2017 [86]	Voir texte. Au moins deux essais valides sont obtenus chez 80-95% des patients. Ce taux peut diminuer sensiblement si une contrainte de temps de 20-25min est appliquée chez des patients naïfs à la technique RCM [74,95].

Abréviations : CFCS, score clinique de la mucoviscidose ; CFQ-Rresp, domaine respiratoire du questionnaire révisé pour la mucoviscidose ; CVF, capacité vitale forcée ; DEM₂₅₋₇₅, débit expiratoire maximal médian entre 25 et 75% de la capacité vitale ; IC, intervalle de confiance ; ICP, indice de clairance pulmonaire ; MV, mucoviscidose ; PaO₂, pression artérielle en oxygène ; RCM, rinçage sur cycles respiratoires multiples ; Scnd, inhomogénéité ventilatoire spécifique aux voies aériennes de conduction ; VEMS, volume expiratoire maximal en 1 seconde.

2.7. Les limites de l'indice de clairance pulmonaire

2.7.1. Limites techniques

Le RCM repose sur la mesure simultanée des débits et des concentrations gazeuses. Lorsqu'il s'agit par exemple de la concentration d'azote, les appareils commerciaux ne la mesurent pas directement : ils en offrent une approximation indirecte à partir d'autres signaux [72,133]. Chacun de ces signaux doit être mesuré très précisément. Ils nécessitent des capteurs performants avec un temps de réponse minime et un ratio signal/bruit optimisé, surtout chez les petits enfants avec un rythme respiratoire élevé et notamment à la fin du rinçage où les variations des concentrations gazeuses deviennent très minces [56,79]. Plus critique encore est la phase de synchronisation, où tous les signaux (gaz et débit) sont alignés et combinés afin d'obtenir les volumes gazeux expiratoires, précurseurs des mesures de CRF et d'ICP. Toute erreur de synchronisation, aussi subtile soit-elle, aura une influence notable sur ces mesures [79,134,135].

Technologiquement, la précision des mesures est donc fastidieuse et dépend des spécificités de chaque appareil telles que leur compatibilité avec certains gaz inertes, les composants qui les constituent (nombre et emplacement des capteurs, circuits, logiciels, etc.) et les algorithmes de correction (espace mort, gaz ré-inspiré, synchronisation, etc.) bien gardés par les concepteurs. Ainsi, l'ICP mesuré par deux machines n'analysant pas le même gaz varie notamment en raison des composants matériels qu'induit la mesure de ces gaz (analyseurs gazeux direct versus indirect, espace mort différent, etc.) [41,58,136,137]. De manière plus surprenante, les valeurs d'ICP dérivé d'un rinçage de l'azote et obtenu par l'EasyOne Pro (nidd Medical Technologies, Zurich, Suisse) ou l'Exhalyzer D (Eco Medics AG, Dürnten, Suisse), 2 systèmes au design globalement comparable et validés *in vitro* [133,138], ne sont pas interchangeables chez le sujet sain et encore moins chez le patient MV [95,136,139]. Les discordances semblent en partie liées aux algorithmes de mesures, mais aussi à l'interposition d'une « valve à la demande » administrant l'oxygène sur l'une des machines. Cette valve semble influencer le pattern respiratoire des individus, impactant secondairement la CRF et l'ICP [95]. Quelles que soient les raisons, ces différences peuvent être préjudiciables pour les études visant à établir le bénéfice d'un traitement et freinent les collaborations multicentriques.

Afin d'harmoniser et d'implémenter les données ultérieures dans des projets multicentriques, les Réseaux Européen et Américain de recherche clinique en mucoviscidose se sont accordés sur un équipement jugé *a priori* approprié pour mesurer l'ICP. L'Exhalyzer D est l'appareil qui a été retenu, avec la méthode du rinçage de l'azote. Les procédures opérationnelles relatives à cet équipement ont été standardisées chez les adultes et enfants de plus de 6 ans. Il est important de souligner que le travail de standardisation est un processus en permanente évolution, susceptible de se modifier ultérieurement en fonction de nouvelles expertises [57]. Les procédures de standardisation chez le plus jeune enfant ne sont pas encore élaborées.

2.7.2. Limites physiologiques

Il est couramment admis que l'ICP est représentatif de l'état des voies aériennes distales. En réalité, l'ICP est l'indicateur d'un conglomérat de divers signaux survenant à tous les étages pulmonaires, y compris au niveau des voies aériennes centrales. Ainsi, s'il est assez juste de considérer l'indice comme un reflet des petites voies aériennes dans les phases précoces de la MV, cette affirmation n'est plus valide lorsque l'atteinte pulmonaire est plus sévère. Une même valeur peut donc traduire des phénotypes pathologiques très distincts [34,140], et l'interprétation longitudinale de l'ICP devient hasardeuse en présence de bronchiectasies diffuses ou de bouchons muqueux [34,36].

En outre, la technique RCM quantifie les disparités ventilatoires présentes entre toutes les unités pulmonaires en communication avec la bouche. L'air piégé est par conséquent négligé et ne contribue pas à la valeur de l'ICP. Une intervention thérapeutique efficiente pourrait se solder par la réouverture partielle d'un territoire pulmonaire précédemment complètement obstrué, débouchant sur une augmentation paradoxale de l'inhomogénéité de la ventilation [120]. La probabilité d'observer ce phénomène s'accroît avec la sévérité de la maladie pulmonaire, ce qui peut expliquer en partie le comportement hétérogène de l'ICP après un traitement [99,112-115,117-120]. Le test RCM peut également devenir sérieusement chronophage chez ces patients plus gravement atteints pour lesquels l'inhomogénéité ventilatoire globale peut être considérable, limitant sa faisabilité clinique [141,142]. Par ailleurs, l'allongement du temps de rinçage peut rendre substantielle la contribution tissulaire (rétrodiffusion) de l'azote. Pour toutes ces raisons, mesurer l'ICP semble moins pertinent chez

les patients plus sévèrement atteints, pour lesquels le VEMS est d'ailleurs souvent suffisamment informatif.

2.7.3. Considérations logistiques

La mesure de l'ICP paraît simple mais elle nécessite de l'opérateur un investissement important en termes d'apprentissage et d'implication. Celui-ci doit tout d'abord encourager les sujets à respirer selon un mode respiratoire spontané et détendu, en distrayant les plus jeunes enfants de manière appropriée. Pendant le rinçage, un focus particulier doit être accordé aux fuites. En effet, toute fuite, fut-elle brève ou minime, a un impact significatif sur les mesures d'ICP et de CRF et doit conduire à l'élimination systématique du test. L'observation de l'évolution du rinçage à la recherche de toute anomalie laissant suspecter une fuite est donc cruciale [143]. Simultanément, l'opérateur doit pouvoir scruter tous les comportements du sujet et épingler chaque attitude qu'il juge probable d'influencer les mesures. Enfin, dans l'entre deux manœuvres, l'opérateur doit être rapidement capable de déterminer si la qualité du rinçage est conforme aux standards [56]. La fiabilité des mesures exige donc un haut niveau d'aptitude, mais est décisive pour les études multicentriques [33]. Même si elle ne requiert de la part de l'enfant qu'une collaboration passive, la mesure du LCI est donc en réalité un test opérateur-dépendant. À cet effet, l'ECFS-CTN a délégué à un centre (Imperial College London/Royal Brompton Hospital) les compétences nécessaires pour coordonner les procédures de certification assurant la collection de données de haute qualité. Il incombe ainsi à chaque opérateur de suivre l'entraînement recommandé afin de répondre au niveau de qualification qu'impose la technique.

Influencée par l'appareillage, les critères opérationnels retenus (nombre de tests valides) et surtout l'importance de l'atteinte pulmonaire, la durée du test n'est par ailleurs pas négligeable. En utilisant l'Exhalyzer D et l'azote comme gaz traceur, chaque rinçage prend en moyenne 3 minutes chez l'enfant, mais sa durée peut excéder 6-7 minutes chez certains enfants MV [74]. Les recommandations consensuelles vont évoluer [84,89,124,144] mais incluent actuellement la réalisation de 3 manœuvres techniquement acceptables, espacée entre elles d'un intervalle de temps correspondant à 2x la durée du rinçage précédent [56]. En excluant le processus de familiarisation, les erreurs techniques, les mesures techniquement inacceptables et les embarras cliniques (toux, arrêt prématuré du rinçage, hyperventilation, etc.), un test idéal compte approximativement une vingtaine de minutes chez l'enfant MV

avec une atteinte pulmonaire légère. Il n'est donc pas étonnant de constater qu'en conditions réelles, chez des enfants MV inexpérimentés au RCM, seuls 25% d'entre eux parviennent à réaliser au moins 2 manœuvres techniquement acceptables en 20 minutes [74]. L'opérateur doit donc veiller à aménager un temps de test relativement important et adapté à la situation (âge, naïveté du sujet, etc.) afin de multiplier les chances d'obtenir des mesures de haute qualité, cliniquement pertinentes et/ou transposables dans le domaine de la recherche.

- Les limites de la mesure relèvent des domaines techniques, physiologiques et logistiques.
- Les valeurs d'ICP et de CRF sont dépendantes de l'équipement utilisé et en aucun cas interchangeables.
- L'ICP n'est pas spécifique aux petites voies aériennes et néglige les zones pulmonaires totalement obstruées. Son interprétation est plus complexe chez les patients MV dont le VEMS est significativement altéré.
- La technique réclame un investissement important de la part de l'opérateur, elle est en réalité opérateur-dépendant.
- Compte tenu des recommandations actuelles, un test RCM dure idéalement 20 minutes chez des enfants MV dont l'atteinte pulmonaire est légère à modérée. En conditions réelles, ce temps peut aisément doubler.

2.8. Perspectives

La dépendance des variables RCM à la configuration de l'équipement (gaz, machine, logiciel) constitue probablement la pierre d'achoppement majeure de la technique. Cela suggère que l'essentiel des informations existantes, qui ont été enseignées à partir d'un spectrographe de masse et du SF₆, n'est pas simplement transposable aux nouvelles technologies plus accessibles et au rinçage de l'azote. Le même bilan peut être dressé entre différents appareils commerciaux modernes utilisant pourtant le même gaz de rinçage, tant les valeurs d'ICP et de CRF sont discordantes [95,139]. Les valeurs de référence sont donc spécifiques à chaque méthode, ce qui complique les comparaisons multicentriques. La similitude des profils clinimétriques de chaque équipement n'est pas non plus garantie. En ce sens, la validation des équipements *in vitro*, en mesurant la précision des valeurs de CRF via un modèle pulmonaire, constitue un prérequis indispensable, mais ne permet pas de répondre aux incohérences disproportionnelles observées *in vivo* [139]. Une des sources plausibles de ce décalage *in vitro* / *in vivo* pourrait consister en la difficulté des modèles pulmonaires à intégrer tous les facteurs physiologiques conditionnant la performance des tests *in vivo*, comme le CO₂ expiré, les corrections BTPS, ou les modifications du pattern respiratoire survenant pendant le test. La confrontation des mesures de volumes pulmonaires obtenues par chaque machine RCM avec d'autres techniques plus fondamentales comme la pléthysmographie ou, mieux encore, la méthode de dilution à l'hélium, semble être une étape importante à franchir si l'on souhaite décrypter les causes qui les opposent. En attendant, les données actuellement amassées avec l'Exhalyzer D et l'azote suggèrent en tous les cas que cet équipement est au moins aussi prometteur dans la MV que la méthode de référence (spectrographe et SF₆). Il reste toutefois nécessaire de garder à l'esprit que tous ces appareils commerciaux restent dans leur phase de développement. De nombreux efforts restent à fournir de la part de la communauté scientifique afin de parfaire la standardisation des mesures. Peut-être plus critique encore est la part des concepteurs, pour lesquels l'amélioration des appareils et des logiciels est un préalable indispensable à l'obtention de données robustes, stables dans le temps. C'est particulièrement vrai en ce qui concerne les algorithmes de correction, rectifiés au gré des versions du logiciel. Leur modification, leur nombre et les endroits où ils interviennent appellent en ce sens à une plus grande transparence envers le clinicien.

Une autre question importante demeure en suspens : l'ICP a-t-il un intérêt pour le praticien se consacrant à la prise en charge de patients MV ? Il est concevable qu'elle permette d'intensifier utilement le traitement i) à des moments cruciaux, précocement surtout lorsque la clinique reste muette tandis que progresse déjà l'atteinte des petites voies aériennes ii) ou chez des patients chez lesquels elle aurait contribué à identifier un risque distinct de dégradation pulmonaire accélérée. Les modalités à mettre en œuvre dans ces circonstances resteraient cependant à préciser mais on peut imaginer que la mesure de l'ICP aide à en monitorer l'efficacité. Spécifiquement, la prescription de la kinésithérapie, qui reste l'élément le plus encombrant du traitement symptomatique actuel, pourrait par exemple être affinée si cette technique se révélait capable d'enfin permettre une comparaison objective de ses multiples modalités.

En confrontant l'ICP à d'autres informations médicales, certains Centres Européens l'utilisent déjà pour prendre des décisions thérapeutiques [57]. Une différence de 1 unité basée sur le coefficient de répétabilité de l'indice est alors considérée comme seuil d'alarme. Mais il est important de rappeler que ce coefficient reflète le degré de variabilité individuelle de l'ICP ; il ne renseigne en aucun cas sur le changement clinique réellement ressenti par le patient. Ainsi, en l'absence d'un critère seuil informant d'une détérioration ou amélioration cliniquement significative, sa transition confiante dans le monde clinique médical reste ambiguë.

- Comprendre les différences qui opposent les appareils RCM est nécessaire pour éviter les biais d'interprétation de l'ICP.
- La différence minimale cliniquement importante de l'ICP n'est pas encore établie.

2.9. Conclusions

La surveillance régulière du système respiratoire est à la base d'une prise en charge de qualité chez les patients MV. Ces dernières années, la mesure de l'ICP a fait l'objet d'un intérêt considérable dans la MV, sur le plan scientifique surtout, comme outil d'évaluation de nouveaux traitements chez des patients peu malades. Cet engouement est particulièrement justifié en pédiatrie où les atteintes pulmonaires précoces étaient, jusque-là, difficilement décelables. En représentant l'inhomogénéité ventilatoire, l'ICP apparaît être bien plus discriminatoire que les épreuves fonctionnelles respiratoires conventionnelles et est mieux corrélé aux dommages pulmonaires structurels. De nombreuses études ont établi ses qualités clinimétriques dans la MV et plusieurs essais cliniques considèrent désormais l'ICP comme critère d'évaluation principal. Il est également concevable que cet indice devienne utile dans les décisions médicales mais son intérêt pour le clinicien reste à prouver. En clinique en tout cas, l'utilisation de l'azote comme gaz marqueur s'imposera nécessairement. Les appareils actuellement disponibles dans le commerce ne sont pas interchangeables. Les comparer et comprendre les différences qui les opposent, affiner les recommandations de standardisation, explorer davantage l'intérêt d'autres indices d'inhomogénéité de ventilation et généraliser la formation qualitative des opérateurs sont autant d'étapes susceptibles de lever, dans les prochaines années, les zones d'ombre qui persistent autour de l'ICP.

À retenir

- L'ICP désigne l'inhomogénéité globale de la ventilation, il est représentatif des anomalies résidentes dans toutes les générations bronchiques.
- L'atteinte des petites voies aériennes est détectée précocement par l'ICP, au contraire des variables mesurées via la spirométrie.
- Les propriétés clinimétriques de l'indice sont pour la plupart bien établies dans la MV.
- L'ICP semble moins utile lorsque la maladie pulmonaire liée à la MV est trop prononcée.
- Les facteurs techniques, technologiques et logistiques inhérents à l'exécution du test RCM doivent être consciencieusement appréciés afin de limiter les erreurs d'interprétation et produire des données de haute qualité.

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CHAPTER 3

AGREEMENT BETWEEN MULTIPLE-BREATH NITROGEN WASHOUT SYSTEMS IN CHILDREN AND ADULTS

Agreement between multiple-breath nitrogen washout systems in children and adults

William Poncin*, Florian Singer*, Anne-Sophie Aubriot, Patrick Lebecque

* These authors contributed equally to this work

Adapted from: Journal of Cystic Fibrosis 2017; 16(2):258-266

3.1. Abstract

Background: Comparability of multiple-breath washout (MBW) systems has been little explored. We assessed agreement in lung clearance index (LCI) from two similar, commercial nitrogen MBW setups in patients with cystic fibrosis (CF) and controls.

Methods: The EasyOne Pro (NDD) and Exhalyzer D (EM) were randomly applied in 85 adults (34 with CF) and 97 children (47 with CF and normal forced expiratory volume in one second). We assessed differences between setups in LCI, lung volumes and breathing pattern and diagnostic performance for detecting abnormal lung function.

Results: Compared to NDD, EM measured higher LCI, functional residual capacity and cumulative expired volume while respiratory rate was lower. Mean difference (limits of agreement) in LCI was 1.30 (−2.34 to 4.94). In CF, prevalence of abnormal LCI was greater in children and similar in adults using EM compared to NDD.

Conclusions: Agreement of MBW outcomes between setups is poor and explained by nitrogen measurement techniques and breathing pattern.

3.2. Introduction

Nitrogen multiple-breath inert gas washout (MBW) is increasingly adopted within both clinical and research settings [1]. Ease of testing, favorable diagnostic performance in mild lung disease, and increasing availability of setups running on oxygen (O_2) led to renaissance of MBW [2-4]. The derived lung clearance index (LCI) is an established lung function outcome of both central and peripheral airways where early cystic fibrosis (CF) lung disease originates [5]. LCI is a volume ratio, *i.e.* cumulative expired volume over functional residual capacity (lung turnovers), estimating ventilation distribution efficiency [3]. Compared to forced expiratory volume in one second (FEV_1) from spirometry, LCI is more specific and sensitive for structural and functional pathology detected in computed tomography scans and treatment response in mild CF lung disease [6-8]. Accordingly, LCI from different setups is reported from studies targeting early origins of mild CF [4,7,9-11]. Though, agreement between systems is largely unknown. The extent to which MBW outcomes of different devices are interchangeable has been little explored.

We assessed agreement of LCI measurement between two commercially available MBW setups with comparable design, the EasyOne Pro; ndd (NDD) and the Exhalyzer D; Eco Medics AG (EM) [3]. Both apply 100% O_2 via open bypass, indirectly measure nitrogen concentration, have similar sensors, and *in vitro* validation data are available [12-14]. The objective of this study was to establish comparability of setups in children with mild CF lung disease, adults with CF and healthy controls. Primary outcome was agreement of LCI. We hypothesized that there was no clinically relevant difference between setups, *i.e.* LCI differences would not exceed 10%, corresponding to the magnitude of LCI between-test variability [3,12].

3.3. Methods

3.3.1. Study design

We conducted a prospective, cross-sectional study in the CF outpatient setting of the University Hospital Saint-Luc (Brussels, Belgium). From May 2013 to March 2015 healthy adults, adults with CF, healthy children and children with CF were enrolled. Prior to the study manufacturers installed the equipment, provided training in calibration and measurements, and approved operational readiness of the setup. For LCI measurements, all participants were randomly assigned to NDD or EM followed each with the other device. Randomization was established by alternate allocation using the participant's identification number. 5 min for instruction and training followed by maximum 25 min for MBW were allocated for each device. This time frame was considered sufficiently long to allow for at least two high quality tests and to increase protocol adherence in children with short attention spans and adults with advanced lung disease in whom MBW may be time consuming [15]. A single investigator (W.P.) applied MBW in all participants. Following MBW, patients with CF performed spirometry. The study was approved by the local Ethics Committee. Written informed consent was obtained from caregivers and participants.

3.3.2. Participants

All participants were naïve to MBW and studied at least three weeks apart from any symptom suggesting upper respiratory tract infection (*i.e.* nasal congestion, rhinorrhea or sore throat). Patients with CF were enrolled when clinically stable, *i.e.* not fulfilling criteria of pulmonary exacerbation [16], within the last two months as judged by a single clinician (P.L.) and without previous infection with methicillin-resistant *Staphylococcus aureus* or *Burkholderia cepacia*. CF adults with mild to moderate lung disease were enrolled consecutively while all children (6.0 to 17.9 years) with classic CF (sweat chloride concentration $>60 \text{ mmol.L}^{-1}$) were approached. Age-matched healthy controls were recruited by advertisement and selected on the basis of a questionnaire to exclude past or current smoking, history of wheezing, asthma or chronic cough, prematurity (<34 weeks of gestation), and orthopedic, neuromuscular or cardiac diseases likely to affect the respiratory system.

In patients with CF, respiratory symptoms were recorded by structured interviews on the study date. Microbiology, immunoglobulin G (IgG), and high-resolution computed

tomography (HRCT) data determined closest to the study date were also used. At every bimonthly clinic visit, respiratory cultures were obtained from sputum or from deep pharyngeal aspiration after a full session of physiotherapy. Leeds criteria were used to define *Pseudomonas aeruginosa* infection status [17]. *Pseudomonas aeruginosa* serology [18] was checked at least annually. Patients were further characterized by total IgG serum levels and expressed as z-scores [19]. Structural lung disease was quantified using HRCT [20].

3.3.3. Study assessments

3.3.3.1. Nitrogen multiple-breath washout

We used two commercially available, unmodified, stand-alone MBW setups including hard- and software: the EasyOne Pro and WBreath software (version 3.37; ndd Medical Technologies, Zurich, Switzerland), and the Exhalyzer D and Spiroware software (version 3.1; Eco Medics AG, Duernten, Switzerland) [12,15,21]. Both setups include an open bypass delivering pure O₂ (via demand valve in NDD) for nitrogen washout, two ultra-sonic flowmeters for tidal flow and molar mass measurement, and O₂ and carbon dioxide (CO₂) sensors. Both setups measure nitrogen indirectly. NDD estimates nitrogen from molar mass, EM from the remainder gas fraction after subtracting the O₂ and CO₂ fractions (Dalton's law of partial pressures) [12,22]. Linearity of sensors and accuracy of algorithms to measure lung volumes in EM have been tested against the current reference standard [12]. Similar but preliminary data exist for NDD [14].

All recommended acceptability criteria for inert gas washout testing were applied [3]. However we performed two instead of three MBW runs and accepted FRC variability $\leq 15\%$ instead of 10%. We have chosen the more liberal cut-off from two technically acceptable tests because (i) 15% is well below the 25% cut-off beyond which measurements should be discarded, (ii) to decrease testing time and increase success rate, and (iii) since two technically acceptable tests do not significantly influence mean LCI values or its sensitivity to detect abnormal ventilation distribution efficiency [23-25]. Three or more runs were conducted whenever quality standards were not met. Testing conditions were akin: MBW measurements were performed in a sitting position, breathing through a snorkel mouth piece and wearing a nose clip. Subjects watched the same video for distraction facilitating regular breathing. The operator manually ended the washout if end-tidal nitrogen concentration of at least three consecutive breaths fell below 1/40th of initial end-tidal nitrogen concentration. Software

automatically calculated all MBW outcomes as recommended [3]. Functional residual capacity (FRC) is the ratio of net volume of exhaled nitrogen over the difference between initial and final end-tidal nitrogen concentration. Cumulative expired volume is the net cumulative volume required to wash-out nitrogen to 1/40th of its initial concentration. Wait time between tests were determined by the setups' lockout time, *i.e.* at least 1.5 times the previous measurement duration. The primary outcome LCI is the ratio of cumulative expired volume over FRC, units are lung turnover. Secondary outcomes were diagnostic performance, repeatability, tidal volume, tidal volume over functional residual capacity (VT/FRC), respiratory rate, minute ventilation, and measurement duration per test. Immediate success rate determined in children was defined as proportion of those children in whom acceptable MBW tests were obtained from the first two attempts compared to those requiring more attempts. We also assessed CO₂ drift due to changes in breathing pattern. The latter data were however only available from NDD. Central overread of MBW quality was provided at the University Children's Hospital Zurich.

3.3.3.2. Spirometry

Patients with CF performed spirometry using the Jaeger MasterScreen (CareFusion, Germany) according to guidelines [26]. Outcomes were FEV₁ and forced expiratory flow between 25% and 75% of forced vital capacity (FEF_{25–75}) and expressed in z-scores using recommended reference equations [27]. Children with CF who had abnormal pre-bronchodilator FEV₁ (z-score < -1.64) on the study day were excluded from analysis.

3.3.4. Statistical analysis

We estimated sample size based on the arithmetic mean and coefficient of repeatability of LCI, *i.e.* 10% between-test variability, reported for EM [12]. For a one-sample comparison assuming a (i) mean LCI value = 10 changing by 10% to a hypothesized LCI value = 11, (ii) two-sided alpha = 0.05, (iii) power = 0.90, (iv) Standard Deviation (SD) = 4, the estimated required sample size is 169. To allow for 15% drop out, we aimed to enroll 200 subjects.

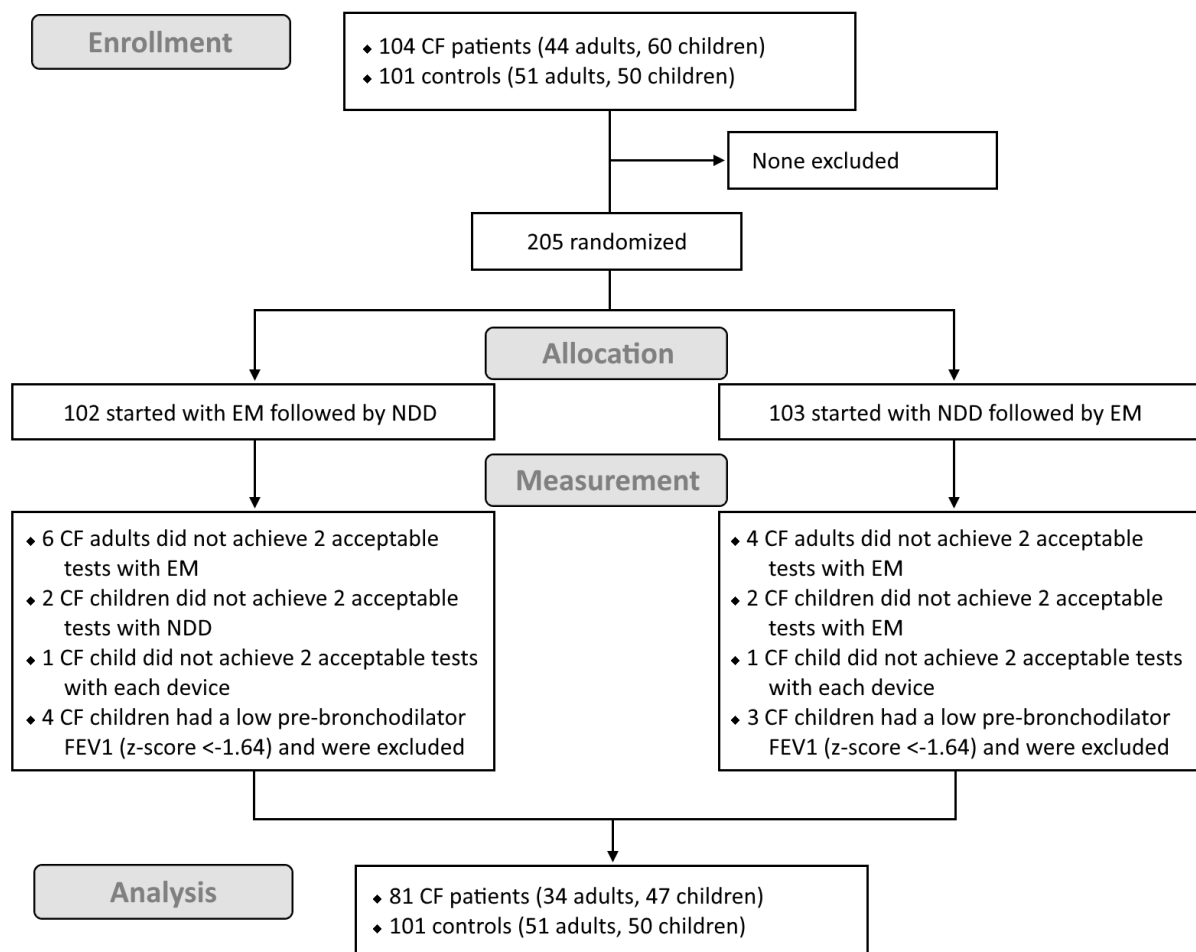
Data were compared by parametric and non-parametric tests allowing for dependent or independent data as appropriate. Stratified analyses were done by disease (healthy vs. CF) and age groups (children 5–18 years vs. adults 18.1–65 years). Agreement in LCI between devices was assessed by Bland Altman plots [28]. Limits of agreement were calculated as mean

difference ± 1.96 times the SD of differences and expected not to exceed $\pm 10\%$ (natural between-test variability). We applied uni- and multi-variable linear regression models to explain LCI variability. Within-test repeatability of LCI and FRC were calculated as ratio of difference (%) between tests over their average. We derived LCI z-scores from healthy controls and defined abnormal LCI in CF patients by z-score >1.64 . We assessed diagnostic performance to detect abnormal lung function (LCI) in CF using Kappa and McNemar's χ^2 (adjusting for dependent data), and the association with FEV₁. For sensitivity analysis we compared MBW outcomes between controls and CF within setups, and we replicated analyses using MBW acceptability criteria with FRC variability $\leq 10\%$ instead of $\leq 15\%$. Analyses were done using Stata (version 14. College Station, TX: StataCorp LP). A p-value <0.05 was considered statistically significant.

3.4. Results

In total, 104 CF patients (44 adults, 60 children) and 101 controls (51 adults, 50 children) were enrolled. All controls achieved two valid MBW runs with both devices. From total 104 CF patients, 23 were excluded as 16 did not obtain valid measurements and seven children had abnormal spirometry (*Fig. 1*). For final analysis, 728 MBW measurements from 182 participants, 101 controls (50 children), and 81 with CF (47 children) remained (*Table 1*).

Figure 1: Flow Chart of Study Participants



Ten adult CF patients were unable to achieve two MBW runs with EM due to prolonged washout duration. These ten patients were older and had lower FEV₁ compared to the remaining adult CF cohort (data not shown). Thirteen CF children were excluded from analysis: 6 failed to get 2 acceptable measurements with one or both devices and 7 had a low pre-bronchodilator FEV₁ (z-score < -1.64). In the latter group, LCI from EM was always abnormal while LCI from NDD was abnormal in 5 out of 7. EM - Exhalyzer D (Eco Medics AG); NDD - Easy One Pro (nnd).

Table 1. Study Population

	Healthy Controls		Cystic Fibrosis	
	Children	Adults	Children	Adults
Subjects	50	51	47	34
Males / females	27/23	22/29	25/22	16/18
F508del homozygous	n.a.	n.a.	19 (40%)	24 (65%)
Pancreatic sufficient	n.a.	n.a.	6 (13%)	8 (24%)
Age (years)	11.2 (8.5 - 13.9)	25.1 (23.1 – 28.3)	13.4 (9.5 – 16.1)*	24.9 (21.0 - 32.9)
Height (cm)	146 (130 - 161)	169 (164 - 178)	148 (134 - 161)	163 (159 - 175)*
BMI (kg/m ²)	17.3 (15.8 - 19.7)	21.4 (20 - 23)	18.4 (16.3 - 19.6)	20.8 (19.8 - 23.6)
FEV ₁ (z-score)	n.a.	n.a.	0.12 ±1.06	-1.42 ±1.63
FEF ₂₅₋₇₅ (z-score)	n.a.	n.a.	-1.03 ±1.61	-2.40 ±1.87

Data are presented as n (%), median (interquartile range) or mean ± SD. Variables between health and disease were compared within age groups, statistically significant differences were marked *. Reasons for exclusion were: 2 children with CF did not achieve acceptable tests in both devices due to leaks, 10 adults and 2 children with CF did not achieve more than one test using EM due to time constraints of the study, and 2 children with CF did not meet FRC variability criteria in NDD. BMI - body mass index; FEV₁ - forced expiratory volume in 1 second; FEF₂₅₋₇₅ - forced expiratory flow between 25% and 75% of forced vital capacity; n.a. - not applicable.

3.4.1. Clinical characteristics of patients with cystic fibrosis

Inhaled antibiotics [29], daily chest physiotherapy and inhaled *Dornase alpha* were prescribed in 45, 43 and 42 children respectively. Daily cough was reported from only four (9%) children, 29 (64%) denied any cough. A single child was chronically colonized with *Pseudomonas aeruginosa*, six (13%) were intermittently infected, 20 (45%) were free of infection and 20 (45%) never had been infected. Median *Pseudomonas aeruginosa* antibody level was 0 (interquartile range 0 to 0, range from 0 to 14) arbitrary units. Median IgG serum level was low: 819 (690 to 1006) mg/100ml or -0.7 (-1.73 to -0.47) z-scores. No bronchiectasis was described in 14 children (30%). Children without bronchiectasis were more likely to have a normal LCI using EM (Chi² test p = 0.02) compared to NDD (p = 0.09).

3.4.2. Comparison of LCI between setups

EM measured higher LCI values than NDD. In the whole population (n = 182), LCI from EM was mean (SD; range) 8.98 (3.22; 5.96 to 20.82) and from NDD 7.68 (1.95; 5.02 to 14.57) lung turnover, respectively. Mean difference (95% confidence interval, CI) was 1.30 (1.03 to 1.57) lung turnover, p < 0.001.

This difference in LCI between setups was consistent in controls (*Table 2*). In CF the difference was greater with higher LCI. The difference was associated with the magnitude of LCI: $R^2 = 0.51$ (*Figs. 2-3*). In adults, LCI varied more than 10% between setups in 20 (39%) controls and 31 (91%) with CF ($p < 0.001$); in children in 19 (38%) controls and 31 (66%) with CF ($p = 0.008$).

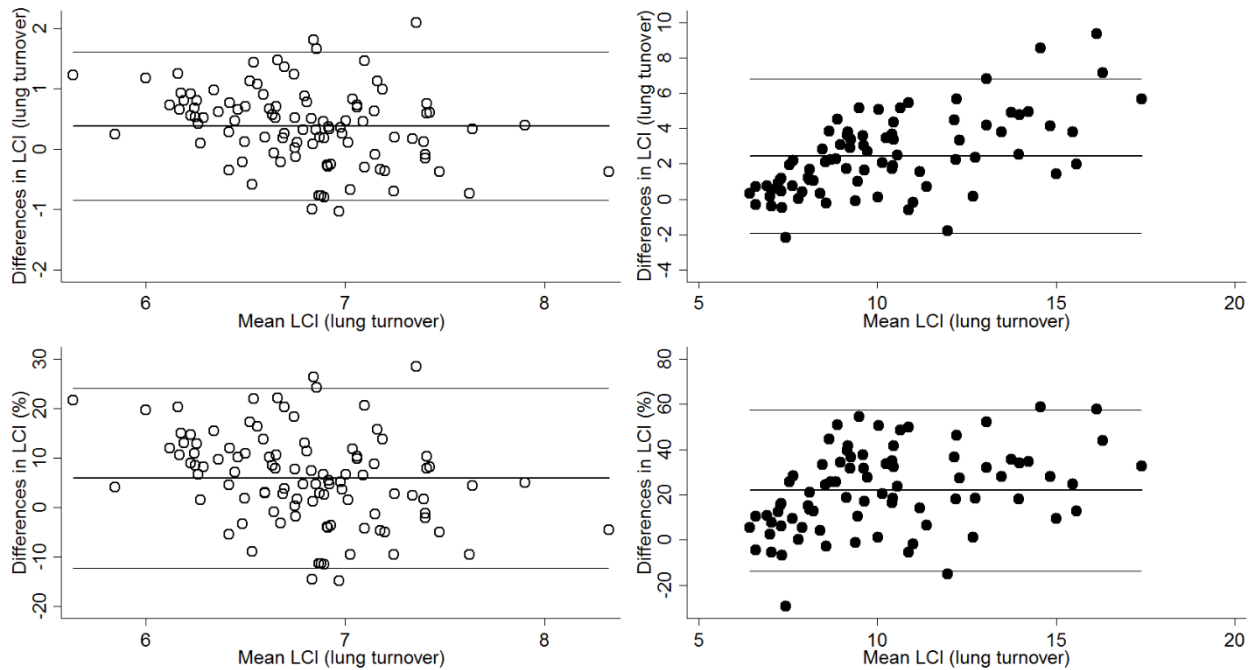
Table 2. Comparison of LCI and FRC between Setups

Adults (n = 85)	Controls (n = 51)			Cystic Fibrosis (n = 34)		
	EM	NDD	p-value	EM	NDD	p-value
LCI	6.92 $\pm$ 0.35	6.42 $\pm$ 0.54	< 0.001	13.73 $\pm$ 3.63	10.01 $\pm$ 2.46	< 0.001
Repeatability (%)	2.4 (1.5 to 5)	7.4 (3.3 to 11.8)	< 0.001	4.5 (1.5 to 8.2)	6.3 (4.1 to 9.5)	0.099
FRC	3.53 $\pm$ 0.93	2.62 $\pm$ 0.69	< 0.001	2.90 $\pm$ 0.90	2.00 $\pm$ 0.80	< 0.001
Repeatability (%)	4.5 (1.5 to 7.8)	5.2 (2.2 to 9.1)	0.390	4.4 (2.7 to 6.9)	6.0 (3.7 to 10.2)	0.180
Children (n = 97)	Controls (n = 50)			Cystic Fibrosis (n = 47)		
	EM	NDD	p-value	EM	NDD	p-value
LCI	7.05 $\pm$ 0.55	6.78 $\pm$ 0.63	0.010	9.76 $\pm$ 2.26	8.32 $\pm$ 1.64	< 0.001
Repeatability (%)	3.9 (1.8 to 5.8)	6.8 (3.4 to 10.5)	0.003	4.3 (1.9 to 7.3)	6.6 (3.0 to 10.6)	0.048
FRC	1.66 $\pm$ 0.86	1.30 $\pm$ 0.69	< 0.001	1.87 $\pm$ 0.97	1.33 $\pm$ 0.64	< 0.001
Repeatability (%)	4.0 (2.2 to 6.7)	7.1 (3.6 to 11.3)	0.027	4.3 (2.1 to 7.7)	3.9 (2.2 to 10.5)	0.140

Data are presented as mean $\pm$ SD or median (interquartile range). EM - Exhalyzer D; FRC - functional residual capacity in litre; LCI - lung clearance index in lung turnover; NDD - EasyOne Pro.

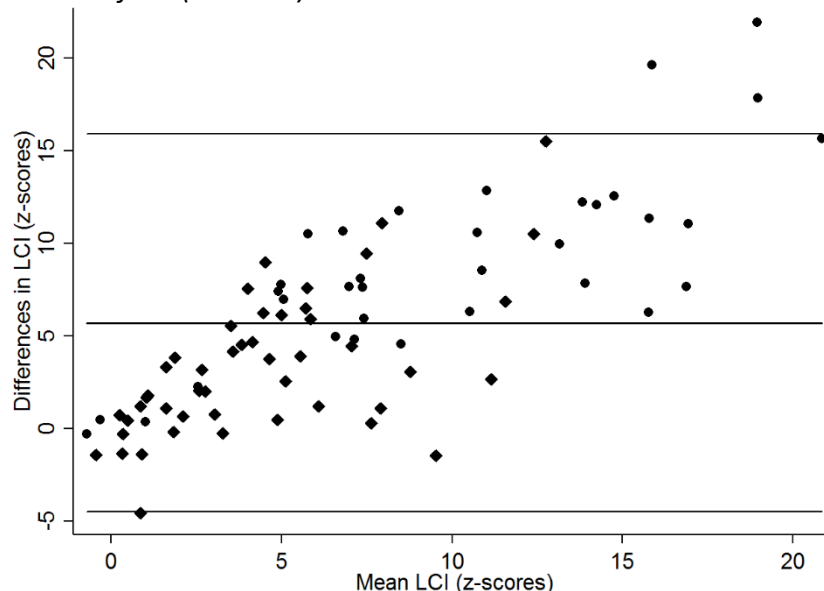
Applying the time point of NDD where LCI was measured to EM source data and to calculate LCI from there did not improve agreement. The new LCI from EM was significantly lower compared to the LCI from NDD, mean difference (95% CI) was -2.07 (-2.32 to -1.82) lung turnover, $p < 0.001$.

Figure 2. Agreement of LCI



Bland Altman plots of lung clearance index (LCI) in 101 controls (50 children) and 81 patients with Cystic Fibrosis (CF) (47 children). Controls are displayed as open circles, patients with CF as closed circles. Differences between nitrogen multiple-breath washout setups were calculated as absolute or relative differences (%) between indices from EM – NDD. The middle line represents the mean difference and the upper and lower lines the upper and lower limits of agreement (mean difference $\pm 1.96 \times \text{SD}$), respectively. EM - Exhalyzer D (Eco Medics AG); NDD - Easy One Pro (nnd).

Figure 3. Agreement of LCI (Z-scores) in CF



Bland Altman plot of differences in Lung Clearance Index (LCI) between nitrogen multiple-breath washout setups, displayed as absolute differences (z-scores) between EM – NDD in Cystic Fibrosis (CF). Z-scores were derived from healthy controls. Children are displayed as diamonds and adults as circles. The middle solid line represents the mean difference and the upper and lower lines the upper and lower limits of agreement (mean difference $\pm 1.96 \times \text{SD}$), respectively. EM - Exhalyzer D (Eco Medics AG); NDD - Easy One Pro (nnd).

3.4.3. Comparison of secondary outcomes

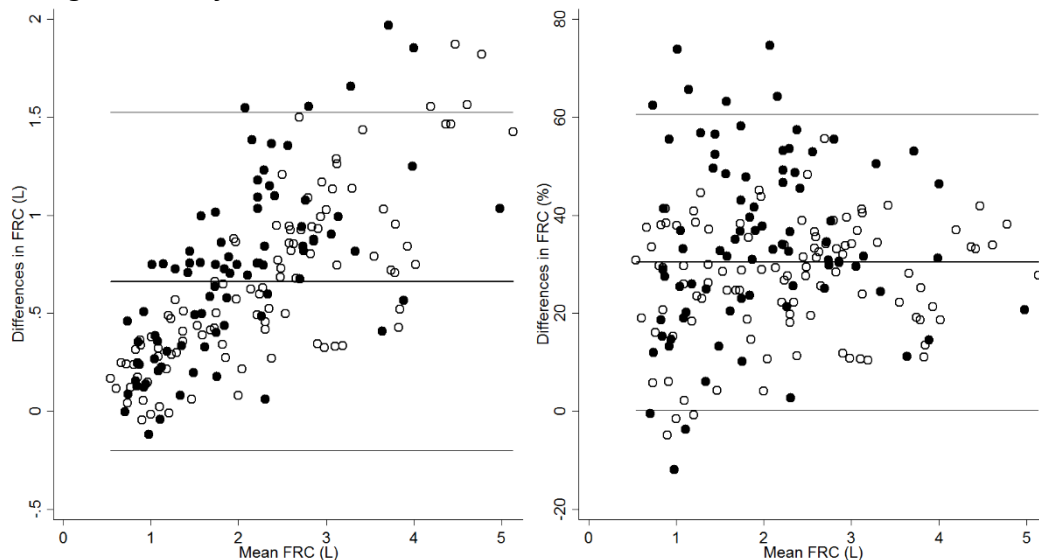
Lung volume and breathing pattern during MBW significantly differed between setups. FRC mean difference was 0.66 L. (30.4%) and limits of agreement were -0.20 to 1.53 L. (0.3 to 60.5%) (Table 3, Fig. 4). Differences in FRC, VT/FRC, cumulative expired volume and respiratory rate were again consistent across age and disease groups, differences in tidal volume and minute ventilation were only consistent in CF (Table 4, Figs. 5–7). A significant nonlinear decay in end-tidal CO₂ was observed using NDD, with a stronger decline over the first ten breaths; relative mean CO₂ change across MBW was -0.6% per breath over the first twenty breaths.

Table 3. Comparison of lung volumes and breathing pattern between setups.

	EM	NDD	Mean diff. (95% CI)
CEV (L)	23.94 ±14.30	13.53 ±6.77	10.41 (9.08 to 11.74)
FRC (L)	2.47 ±1.21	1.81 ±0.89	0.66 (0.60 to 0.73)
Tidal Volume / FRC	0.30 ±0.10	0.35 ±0.13	-0.06 (-0.07 to -0.04)
Respiratory Rate (min ⁻¹)	13.0 ±4.8	18.1 ±7.3	-5.1 (-5.8 to -4.4)
Tidal Volume (mL)	664.9 ±249.8	593.2 ±281.9	71.7 (43.6 to 99.9)
Minute Ventilation (L.min ⁻¹)	7.83 ±2.40	9.24 ±3.23	-1.40 (-1.78 to -1.02)

Data (n = 182) are presented as mean ± SD and compared by paired t-test. All differences were highly statistically significant (p < 0.0001). CEV - cumulative expired volume; EM - Exhalyzer D; FRC - functional residual capacity; NDD - EasyOne Pro; Mean diff. - mean difference; 95% CI - 95% confidence interval.

Figure 4. Agreement of FRC



Bland Altman plots of functional residual capacity (FRC) in 101 healthy controls (50 children) and in 81 patients with Cystic Fibrosis (CF) (47 children). Controls are displayed as open circles, patients with CF as closed circles. Differences between nitrogen multiple-breath washout setups were calculated as absolute or relative differences (%) between indices from EM – NDD. The middle line represents the mean difference and the upper and lower lines the upper and lower limits of agreement (mean difference ±1.96*SD), respectively. EM - Exhalyzer D (Eco Medics AG); NDD - Easy One Pro (nidd).

Table 4. Stratified Comparison of Lung Volumes and Breathing Pattern between Setups.

Adults	Controls (n = 51)				Cystic Fibrosis (n = 34)			
	EM	NDD	Mean Diff (95%CI)	p-value	EM	NDD	Mean Diff (95%CI)	p-value
CEV (L)	25.8 ±6.8	16.7 ±4.5	9.1 (8.1 to 10.1)	<0.001	42.3 ±16.4	19.6 ±7.3	22.6 (18.4 to 26.8)	<0.001
VT (mL)	848 ±198	799 ±285	50 (-22 to 121)	0.173	788 ±150	664 ±186	125 (68 to 181)	<0.001
VT/FRC	0.25 ±0.06	0.31 ±0.10	-0.06 (-0.09 to -0.04)	<0.001	0.29 ±0.09	0.36 ±0.12	-0.07 (-0.10 to -0.04)	<0.001
RR (min ⁻¹)	8.8 ±2.2	9.7 ±3.1	-0.9 (-1.8 to -0.04)	0.042	12.3 ±2.4	16.3 ±3.7	-4.0 (-5.4 to -2.7)	<0.001
MV (L.min ⁻¹)	7.3 ±2.2	7.3 ±2.5	0.0 (-0.6 to 0.7)	0.91	9.5 ±1.8	10.6 ±3.1	-1.1 (-2.1 to -0.1)	0.026
Children	Controls (n = 50)				Cystic Fibrosis (n = 47)			
	EM	NDD	Mean Diff (95%CI)	p-value	EM	NDD	Mean Diff (95%CI)	p-value
CEV (L)	12.4 ±5.6	8.38 ±3.95	4.05 (3.43 to 4.68)	<0.001	20.6 ±11.0	11.0 ±5.4	9.7 (7.7 to 11.6)	<0.001
VT (mL)	484 ±186	465 ±259	19 (-25 to 62)	0.387	569 ±238	455 ±201	114 (69 to 159)	<0.001
VT/FRC	0.32 ±0.10	0.38 ±0.14	-0.06 (-0.09 to -0.02)	0.001	0.33 ±0.12	0.37 ±0.15	-0.04 (-0.08 to -0.01)	0.0274
RR (min ⁻¹)	16.0 ±4.5	23.9 ±5.3	-7.9 (-9.3 to -6.6)	<0.001	15.0 ±5.0	22.4 ±5.0	-7.4 (-8.7 to -6.1)	<0.001
MV (L.min ⁻¹)	7.2 ±2.5	10.0 ±3.6	-2.9 (-3.6 to -2.2)	<0.001	7.9 ±2.4	9.5 ±2.6	-1.6 (-2.2 to -1.1)	<0.001

Data are presented as mean ± standard deviation or mean difference and 95% confidence intervals (Mean Diff (95%CI)). Data were compared between setups stratified by disease and age. CEV - cumulative expired volume, EM - Exhalizer D (Eco Medics AG); MV - minute ventilation; NDD - Easy One Pro (nidd); RR - respiratory rate; VT - tidal volume; VT/FRC - ratio of tidal volume over functional residual capacity.

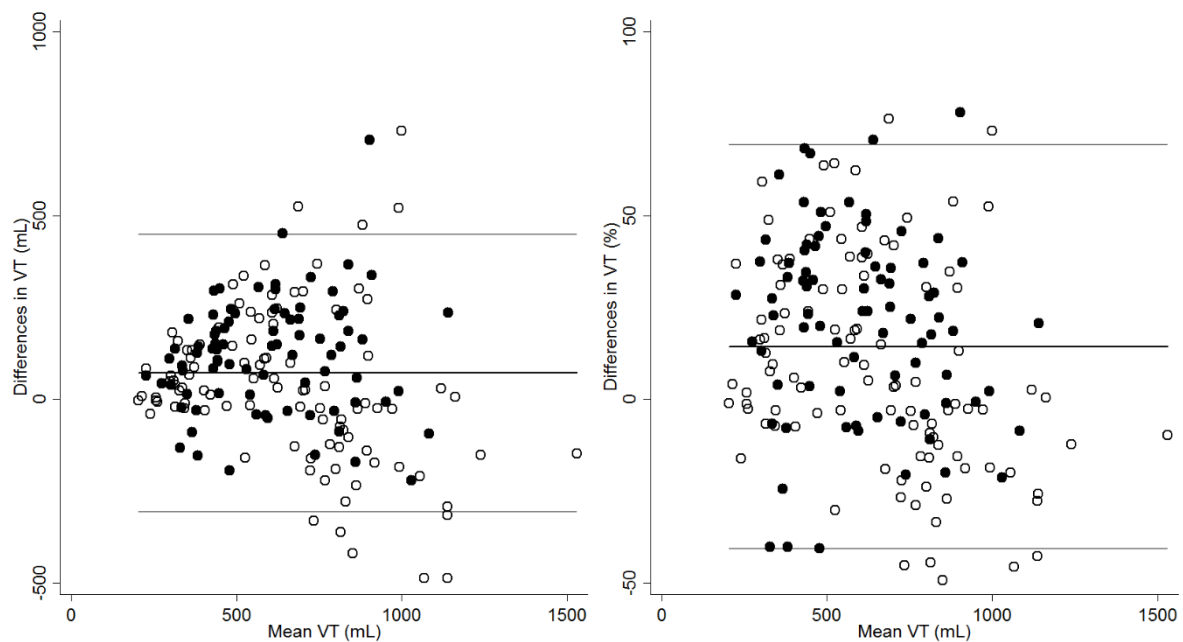
Figure 5. Agreement of Secondary MBW Outcomes (Tidal Volume)

Figure 6. Agreement of Secondary MBW Outcomes (Cumulated Expired Volume)

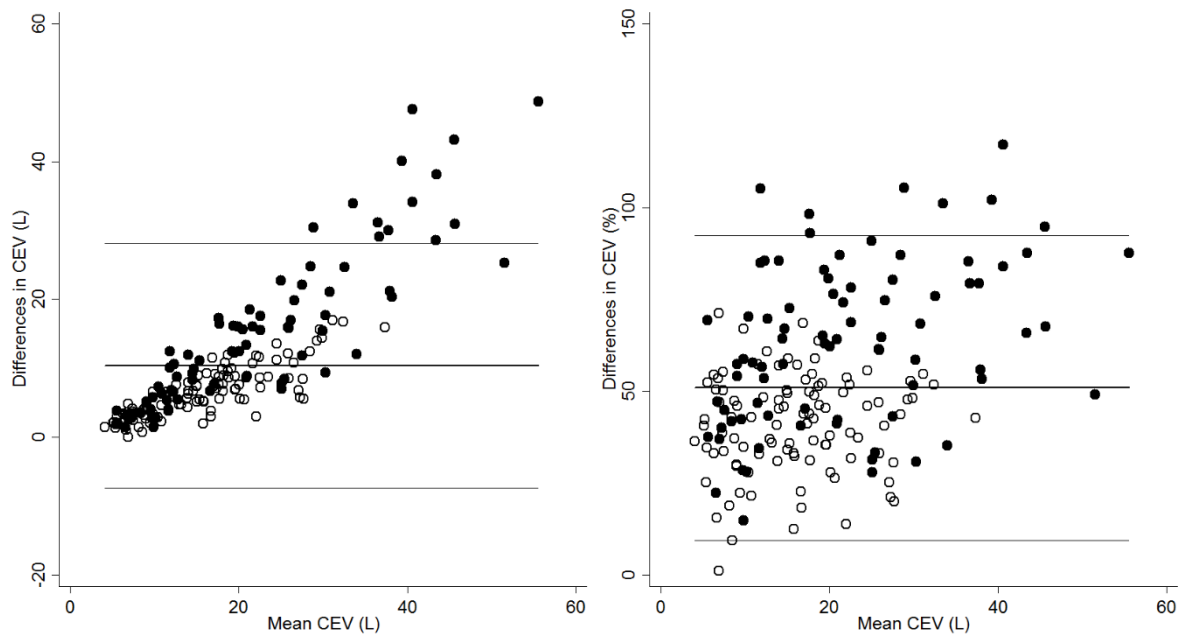
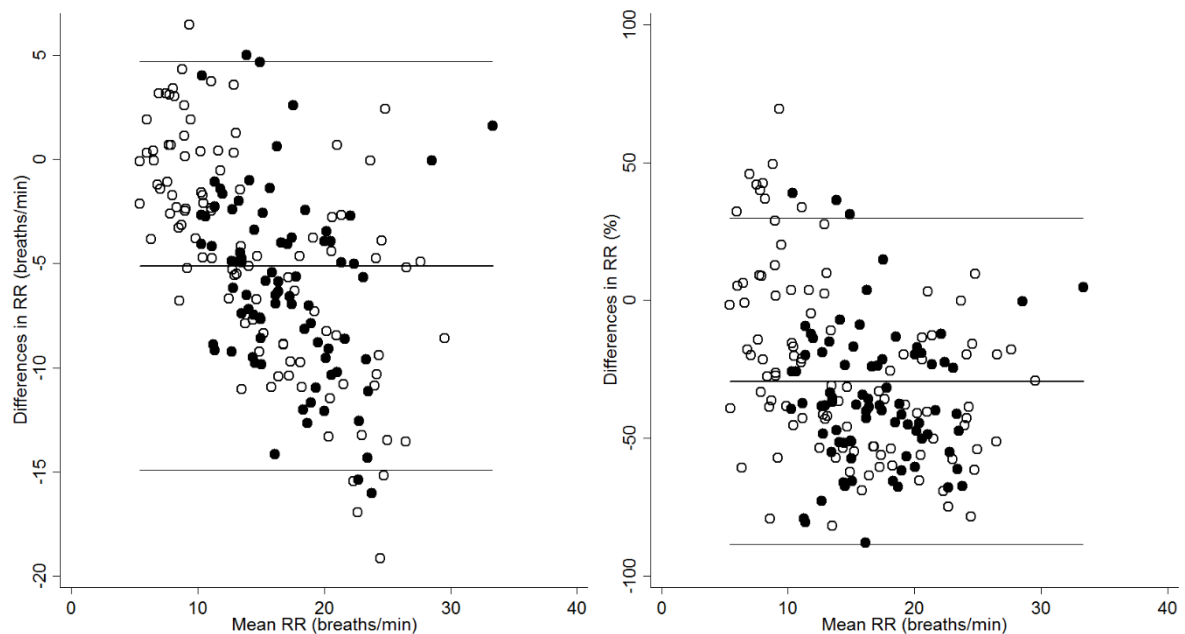


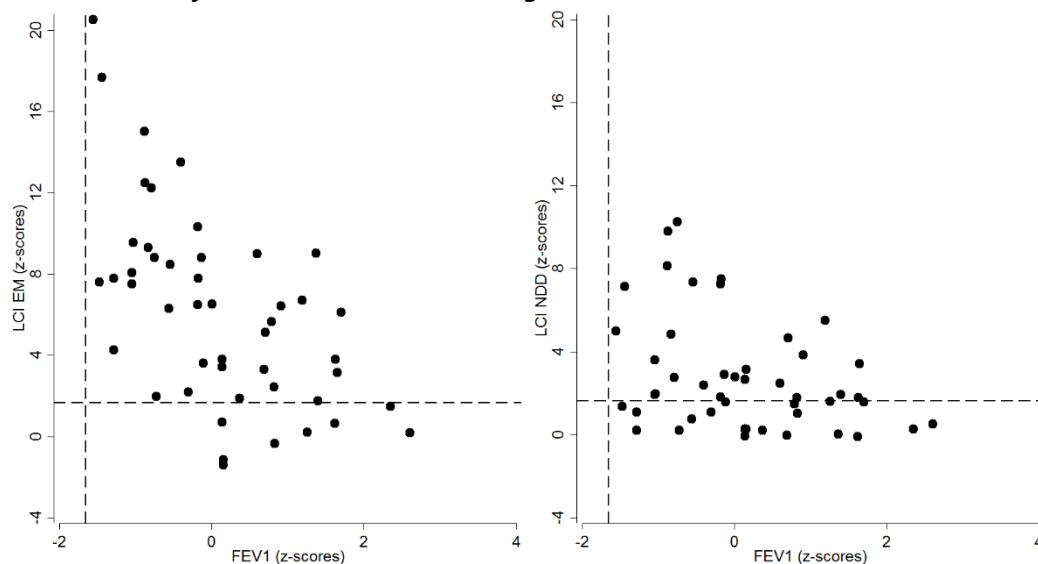
Figure 7. Agreement of Secondary MBW Outcomes (Respiratory Rate)



Bland Altman plots of tidal volume (VT; Figure 5), cumulative expired volume (CEV; Figure 6), and respiratory rate (RR; Figure 7). Controls are displayed as open circles, patients with Cystic Fibrosis as closed circles. Differences between nitrogen multiple-breath washout setups were calculated as absolute or relative differences (%) between indices from EM – NDD. The middle solid line represents the mean difference and the upper and lower lines the upper and lower limits of agreement (mean difference $\pm 1.96 \times \text{SD}$), respectively. EM - Exhalyzer D (Eco Medics AG); NDD - Easy One Pro (nidd).

LCI measurement took less time in NDD. Time savings (mean difference, 95% CI) were 80 (63 to 97) s per test in controls and 117 (100 to 133) s in CF. Immediate success rate differed only slightly in healthy children but not in children with CF: Comparing EM with NDD, 31 (62%) vs. 20 (40%) controls achieved two valid tests at first try, $p = 0.020$, and 28 (58%) vs. 29 (62%) children with CF, $p > 0.99$. Intra-test repeatability for LCI was good in both devices (*Table 2*). LCI variability was lower in EM except in adults with CF where the difference did not reach statistical significance. LCI from both devices discriminated controls from patients with CF. Diagnostic agreement (normal vs. abnormal LCI) between setups was good (Kappa = 0.82) in adults but poor (Kappa = 0.60) in children. Only 10 (21%) and 20 (43%) children had concordantly normal and abnormal LCI, respectively. In 15 (32%), LCI was only abnormal when using EM compared to 2 (4%) children only when using NDD, $p = 0.002$. In these patients, prevalence of abnormal LCI was greater using EM (35/47; 74%) compared to NDD (22/47; 47%; *Fig. 8*). LCI from EM and NDD was significantly related with FEV₁, but strength of association differed. From both children and adults, R^2 values were 0.54 ($p < 0.001$) and 0.40 ($p < 0.001$) using EM and NDD, respectively. In adults only, R^2 values were respectively 0.36 ($p < 0.001$) and 0.33 ($p < 0.001$). In children, corresponding R^2 values were 0.40 ($p < 0.001$) and 0.12 ($p = 0.02$) (*Fig. 8*). Similar associations were found between LCI from EM and NDD and FEF₂₅₋₇₅.

Figure 8. Association of LCI and FEV₁ in Mild Lung Disease



Scatter plot of lung clearance index (LCI) from Exhalyzer D (EM, left panel) and EasyOnePro (NDD, right panel) vs. forced expiratory volume in one second (FEV₁) in children with CF ($n = 47$): R -squared = 0.40 and 0.12, respectively. LCI z-scores were derived from controls in this study, FEV₁ z-scores from the Global Lung Initiative [23]. Dashed lines represent limits of normal (1.64 z-scores).

3.4.4. Exploring differences in LCI between setups

Relative mean difference (limits of agreement) in LCI was 13.1 (-18.4 to 44.5)%. Lung volume and breathing pattern indices derived during nitrogen MBW were used to explain variability in LCI measurement between EM and NDD setups. The following variables were used from both setups for the linear regression model: cumulative expired volume, FRC, tidal volume, respiratory rate, and minute ventilation. LCI was not introduced into the model as significant collinearity between cumulative expired volume and FRC exists. We used a forward stepwise strategy (cut off p-value < 0.200) to model differences in LCI between setups in a multi-variable regression model including age and disease.

We were able to explain LCI differences between devices. The most significant finding was the net cumulative expired volume determined by nitrogen measurement, which explained 61% (R^2) of LCI variability. In the full multi-variable model adjusting for CF, cumulative expired volume, FRC, tidal volume and respiratory rate (from NDD only) explained 90% of LCI variability (*Table 5*). The effect of age did not reach statistical significance.

Table 5. Multi-Variable Linear Regression Model

Setup	Variable	Unit	Coefficient	95% Confidence Interval	P-value
EM	CEV	L	0.30	0.28 to 0.32	<0.001
	FRC	L	-3.21	-3.60 to -2.84	<0.001
	VT	L	0.74	0.10 to 1.40	0.030
NDD	CEV	L	-0.49	-0.54 to -0.44	<0.001
	FRC	L	4.25	3.71 to 4.79	<0.001
	RR	min ⁻¹	-0.04	-0.06 to -0.02	<0.001
	VT	L	-0.41	-0.98 to 0.16	0.162
	Disease		0.46	0.23 to 0.70	<0.001

Adjusted model R-squared (R^2) = 0.90. CEV - cumulative expired volume; EM - Exhalyzer D (Eco Medics AG); FRC - functional residual capacity; NDD - EasyOne Pro (nidd); VT - tidal volume; RR - respiratory rate.

Washout duration and respiratory rate were positively associated with greater CEV differences in a multi-variable linear regression model (Adj R^2 = 0.57).

3.4.5. Sensitivity analysis

Within each setup, breathing pattern and lung volumes differed between controls and CF patients after adjusting for age. Compared to controls, FRC was smaller only when using NDD, respiratory rate and minute ventilation was greater in CF in both setups (*Table 6*).

Table 6. Sensitivity Analysis: Within Setup Comparison between Controls and Cystic Fibrosis

	EM		NDD	
	Mean Diff. (95% CI)	P-value	Mean Diff. (95% CI)	P-value
FRC (L)	-0.23 (-0.49 to 0.02)	0.069	-0.31 (-0.50 to -0.12)	0.002
RR (min ⁻¹)	1.4 (0.2 to 2.6)	0.025	8.6 (6.8 to 10.4)	< 0.001
VT (mL)	5.3 (-52.1 to 62.8)	0.86	-81.6 (-148.9 to -14.3)	0.018
MV (L.min ⁻¹)	1.38 (0.72 to 2.03)	<0.001	3.59 (2.55 to 4.62)	< 0.001

Data are displayed as age adjusted mean difference, 95% confidence intervals (mean diff. 95% CI) and p-values between controls (n=101) and CF (n=81). EM - Exhalyzer D (Eco Medics AG); FRC - functional residual capacity; MV - minute ventilation; NDD - Easy One Pro (nnd); RR - respiratory rate; VT - tidal volume.

Intra-test variability in FRC and LCI was not associated with study duration (R^2 was < 0.01). We replicated analyses in 115 subjects (63.5%) in whom FRC varied $\leq 10\%$ and confirmed findings: following exclusion of 67 subjects, agreement of LCI measured by the two setups remained poor (*Table 7*). Mean difference (limits of agreement) was 1.31 (-2.38 to 4.99) lung turnover; corresponding relative mean difference was 13.0 (-17.1 to 43.1)%. Differences in LCI between setups was strongly associated with the magnitude of LCI, $R^2 = 0.63$, $p < 0.001$. In adults, LCI varied more than 10% between setups in 12 (36.4%) controls and 19 (86.4%) with CF ($p < 0.001$); in children in 11 (39.3%) controls and 20 (62.5%) with CF ($p = 0.073$).

Among subjects in whom FRC variability was > 10%, 37 (55.2%) were children. FRC variability was >10% in 10 using both setups, 16 only when using EM, and 41 only when using NDD. In both setups FRC variability exceeded 10% in 6 children (one with CF) and 4 adults (two with CF), in EM in 8 children (3 with CF) and 8 adults (2 with CF), and in NDD in 18 children (8 with CF) and 23 adults (11 with CF).

Table 7. Sensitivity Analysis: Between Setup Comparisons Using the Low FRC Variability*Criterion*

Adults	Controls (n = 33)				Cystic Fibrosis (n = 22)			
	EM	NDD	Mean Diff (95%CI)	p-value	EM	NDD	Mean Diff (95%CI)	p-value
LCI (TO)	6.90 ±0.31	6.51 ±0.45	0.39 (0.23 to 0.55)	<0.0001	13.81 ±4.27	10.14 ±2.55	3.67 (2.58 to 4.77)	<0.0001
CEV (L)	25.9 ±7.6	17.2 ±4.9	8.70 (7.35 to 10.04)	<0.0001	42.8 ±15.7	19.4 ±6.3	23.37 (17.9 to 28.8)	<0.0001
FRC (L)	3.56 ±1.04	2.65 ±0.76	0.91 (0.76 to 1.06)	<0.0001	2.96 ±0.88	2.01 ±0.88	0.95 (0.80 to 1.09)	<0.0001
VT (mL)	867 ±211	781 ±301	85.9 (-5.4 to 177.3)	0.064	806 ±158	654 ±195	152.1 (76 to 228)	0.0004
RR (min ⁻¹)	8.8 ±2.4	9.9 ±2.8	-1.1 (-2.2 to 0.1)	0.0595	11.6 ±2.4	16.4 ±3.2	-4.7 (-6.2 to -3.2)	<0.0001
MV (L.min ⁻¹)	7.53 ±2.46	7.30 ±2.52	0.24 (-0.58 to 1.06)	0.56	9.19 ±1.75	10.47 ±2.98	-1.29 (-2.59 to 0.02)	0.054
Children	Controls (n = 28)				Cystic Fibrosis (n = 32)			
	EM	NDD	Mean Diff (95%CI)	p-value	EM	NDD	Mean Diff (95%CI)	p-value
LCI (TO)	6.99 ±0.46	6.69 ±0.53	0.31 (0.06 to 0.55)	0.018	9.19 ±1.68	7.68 ±1.07	1.51 (0.94 to 2.08)	<0.0001
CEV (L)	12.8 ±6.1	8.5 ±4.2	4.39 (3.50 to 5.28)	<0.0001	20.3 ±11.5	10.7 ±5.4	9.51 (7.0 to 12.0)	<0.0001
FRC (L)	1.77 ±0.96	1.37 ±0.79	0.40 (0.29 to 0.50)	<0.0001	1.97 ±1.08	1.41 ±0.70	0.56 (0.39 to 0.72)	<0.0001
VT (mL)	502 ±204	460 ±269	41.4 (-13.7 to 96.6)	0.135	570 ±233	457 ±203	113 (53 to 173)	0.0005
RR (min ⁻¹)	15.7 ±3.6	24.0 ±5.4	-8.3 (-10.3 to -6.3)	<0.0001	15.5 ±5.1	22.9 ±4.9	-7.3 (-9.0 to -5.7)	<0.0001
MV (L.min ⁻¹)	7.23 ±2.39	9.80 ±3.49	-2.57 (-3.39 to -1.76)	<0.0001	8.17 ±2.35	9.63 ±2.47	-1.46 (-2.2 to -0.73)	0.0003

Data from 115 subjects in whom FRC varied ≤10% were compared between setups. 95%CI - 95% confidence interval; CEV - cumulative expired volume; EM - Exhalyzer D (Eco Medics AG); LCI - lung clearance index; Mean Diff - mean difference; MV - minute ventilation; NDD - Easy One Pro (nidd); RR - respiratory rate; TO - turnover; VT - tidal volume.

3.5. Discussion

This is the first study to show that LCI measurement using nitrogen as inert gas depends on the device. There is a significant offset in LCI between available nitrogen MBW setups. LCI difference appears clinically relevant as it exceeds inter-test variability. Reasons for variability between systems are multi-factorial. We were able to identify two important factors explaining LCI differences to a great extent, *i.e.* nitrogen measurement algorithm affecting cumulative expired volume and a demand valve presumably affecting breathing pattern. Agreement in diagnostic performance was good in adults but poor in children.

Agreement in LCI measurement between two currently available MBW setups is poor. We are confident that these findings are reliable. The study was well powered, LCI measurements were repeatable, subgroup and sensitivity analyses from those MBW tests with FRC varying <10% revealed consistent findings. Subjects were naïve to MBW and sequential testing bias was avoided by randomized sequence of testing. In addition, recent *in vitro* and *in vivo* data confirm that FRC from EM exceeds FRC from NDD and from a mass spectrometer [14,30].

Differences in LCI measurement between setups seem clinically relevant as they exceeded the anticipated magnitude of between-test variability (10%) [3]. LCI measurement is complex and influenced by (i) measurement accuracy of volumes, (ii) measurement accuracy of the nitrogen concentration, and (iii) ventilation inhomogeneity per se which can be affected by breathing pattern [31]. In the current study, differences in LCI between setups seem largely explained by breathing pattern and maybe partially by nitrogen measurement.

Differences in cumulative expired volumes reveals that nitrogen measurement significantly differs between the setups. The molar mass algorithm in NDD relatively underestimates exhaled nitrogen compared to composite signal ($1 - O_2 - CO_2$) algorithm in EM (or *vice versa*). End-tidal nitrogen concentration determines the magnitude of cumulative expired volume [3]. *In vitro* data suggest that nitrogen measurement is robust in EM and NDD [12,14]. Yet both setups may be susceptible to tissue nitrogen and variable breathing pattern affecting CO_2 fractions and signal alignment [32]. Current nitrogen measurement techniques may require further improvements to account for physiological drifts [3,14]. However if the main difference between setups would have been the software algorithm determining nitrogen fractions, an earlier time point in EM should have improved agreement. This was not

the case suggesting that differences in breathing pattern mainly determined differences in LCI. Yet we did not have access to source data from NDD so we could not determine possible differences in measurement of nitrogen concentrations or volumes.

Slightly different open bypass systems delivering O₂ most likely account for different breathing pattern between the setups: a demand valve is interposed in the bypass tube only in NDD. We suggest that subjects were not breathing at their normal pattern in NDD as (i) intra-test variability of FRC and LCI was consistently greater, and (ii) we observed a CO₂ drift in children with CF. The interaction between the NDD setup, breathing pattern and MBW outcomes is complex. Observed differences in LCI between setups were probably strongly related to breathing pattern affecting lung volumes and nitrogen measurement. The on-demand gas delivering system in NDD apparently induced changes in setup resistance leading to hyperventilation. We are confident that we observed hyperventilation in NDD as minute ventilation (driven by respiratory rate) was larger in the majority of study subjects compared to EM. During MBW, end-tidal CO₂ declined in children with CF. However, VT, FRC and VT/FRC were smaller in NDD compared to EM probably due to true physiological changes in resting lung volume. Decreased LCI in NDD may relate to both true physiological changes in ventilation and potential nitrogen measurement bias from CO₂ drift. While EM algorithms seem robust under *in vitro* CO₂ drift conditions [12], similar data are currently lacking for NDD. The complex interaction between physiological and technical variables is supported by a previous study reporting that voluntarily breathing at twice the magnitude of physiological VT increases LCI but decreases FRC [31]. Differences in volume measurement are less likely as both setups use similar flow sensors. Our speculations on differences between MBW outcomes have some limitations: While all subjects were breathing pure O₂ via open bypass, differences in the setups' dead space and resistance may have also contributed. Access to breath-by-breath CO₂ and nitrogen signals was constrained in both setups. As others we would like to plead for more transparency in commercial software [33].

3.5.1. Implications

We show that variability in LCI measurement was significantly greater and sensitivity to detect abnormal lung function in CF was lower in NDD compared to EM. Both observations are clearly relevant in the context of trials aiming to identify a benefit in patients with mild lung disease. While both setups are available and in clinical use since a couple of years [1],

hardware and software of both devices seem to remain in development phase. Our data should prompt the manufacturers to revisit and harmonize gas delivery systems and measurement algorithms. However setup modification requires repeat validation work as stated by the consensus group.

This study reinforces current recommendations that age and ethnicity matched healthy controls need to be enrolled and setups should not be changed within ongoing studies [32,34,35]. Based on our own cohort of healthy controls, agreement in diagnostic performance of LCI differed depending on underlying lung disease severity. In addition NDD required significantly less testing time and success rate in adult patients with CF was greater compared to EM.

Our data are one of the first to show that LCI from a prospectively applied duplicate MBW protocol produces repeatable LCI values in children and adults. Several earlier post hoc analyses likewise suggested that MBW can be abbreviated if only the first two tests were considered [23,36]. The latter and our data currently do not support the 10% variability threshold of FRC. Closer inspection of data but not necessarily exclusion may be required [3]. Further studies are necessary to establish feasible and reliable MBW protocols [37].

3.5.2. Methodological considerations

Our LCI data are well within the range of previous studies [38-40]. For example, a recent study using NDD reported a mean LCI of 6.3 in 33 controls (8 to 60 years) whereas it was 6.6 in 101 controls (5 to 65 years) in our study [40]. These data are also reassuring that relevant lung disease was clearly absent in our control population though we did not assess spirometry in the latter.

Comparison of indices estimating ventilation inhomogeneity currently lack a gold standard comparator [30,41,42]. Computed tomography scans may over- or underestimate lung disease in children [42]. Current plastic lung models generate reliable volumes but lack ventilation inhomogeneity parameters and realistic temperature, humidity and gas exchange conditions [12,43,44]. Studies comparing lung volumes from MBW and plethysmography may further elucidate discrepancies. Differences between FRC from EM and NDD compared to FRC from plethysmography may range between -7% to 33% and -36% to -9%, respectively [14]. A previous study comparing LCI from EM with that from a mass spectrometer confirms that differences in LCI from nitrogen MBW rises as LCI increases [30]. Nevertheless, in addition to

between-test variability, comparing lung volumes from different techniques is subject to methodological and physiological confounding, *e.g.* gas-volume vs. pressure-volume measurement, tissue nitrogen, and trapped gas in lung periphery [45,46].

Taken together, LCI from MBW using EM and NDD are currently not interchangeable. Different gas delivery systems and measurement algorithms account for considerable differences in MBW outcomes. Further validation work is warranted.

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CHAPTER 4

SHORT-TERM EFFECT OF AUTOGENIC DRAINAGE ON VENTILATION INHOMOGENEITY IN ADULT PATIENTS WITH STABLE NON-CYSTIC FIBROSIS BRONCHIECTASIS

Short-term effect of autogenic drainage on ventilation inhomogeneity in adult patients with stable non-cystic fibrosis bronchiectasis.

William Poncin, Grégory Reychler, Noémie Leeuwerck, Nathalie Bauwens, Anne-Sophie Aubriot, Candice Nader, Giuseppe Liistro, Sophie Gohy

Adapted from: Respiratory Care 2017; 62(5):524-531

4.1. Abstract

Background & Objective: Lung clearance index (LCI), a measure of ventilation inhomogeneity derived from a multiple-breath washout test, is a promising tool for assessing airway function in patients with non-cystic fibrosis bronchiectasis (NCFB). However, it is unknown whether ventilation inhomogeneity could improve after successful elimination of excessive secretions within bronchiectasis. The objective of this work was to assess the short-term effects of lung secretion clearance using autogenic drainage (AD) technique on standard lung function tests and LCI in patients with NCFB.

Methods: Nitrogen-based multiple-breath washout, spirometry and body plethysmography tests were performed 30 min before AD in adults with stable NCFB. The AD session was followed by a 5-min break, after which the tests were repeated in the same order. Sputum expectorated during AD was quantified as dry weight and correlated with change between post- and pre-measurements (Δ).

Results: Twenty-four subjects were studied (18 female, median age [range]: 65 [21 – 81] y). Mean $\pm$ SD LCI significantly improved after AD (10.88 ± 2.62 vs 10.53 ± 2.35 , $P = 0.042$). However, only 20% of subjects with mucus hyperproduction during AD had a Δ LCI that exceeded measurement variability. The percent of predicted slow vital capacity (SVC%) also slightly improved ($88.7 \pm 19.3\%$ vs $90.0 \pm 19.1\%$, $P = 0.02$). Δ LCI was inversely related to dry sputum weight ($r = -0.48$, $P = 0.02$) and Δ SVC% ($r = -0.64$, $P = 0.001$). Δ SVC% was also correlated with dry sputum weight ($r = 0.46$, $P = 0.02$).

Conclusions: In adults with NCFB and mucus hypersecretion, AD improved ventilation inhomogeneity. LCI change may be the result of the maximal recruited lung volume and the amount of cleared mucus secretion.

4.2. Introduction

Non-cystic fibrosis bronchiectasis (NCFB) is a chronic lung disorder characterized by irreversible dilatation of the airways [1]. Clinical features include chronic cough, bronchorrhea, and recurrent exacerbations with concurrent impact on health-related quality of life, morbidity, and mortality rates [2-4]. Among the current therapeutic arsenal offered to these patients, chest physiotherapy with bronchial drainage is acknowledged as an important element in their regular management [5]. By facilitating discharge of excessive bronchial secretions, chest physiotherapy aims to maintain the patency of the airways to preserve lung function and delay the progression of the disease. However, proving the effectiveness of treatments and especially chest physiotherapy via standard pulmonary function tests is challenging in patients with NCFB [6-8]. Indeed, current lung function end points, such as FEV₁, lack the required sensitivity to allow early detection of treatment effects [9,10].

In this context, the lung clearance index (LCI) derived from a multiple-breath washout (MBW) test is of particular interest. LCI is a noninvasive and radiation-free measurement of ventilation inhomogeneity. Because sputum accumulation can contribute to the uneven distribution of ventilation, the elimination of airway secretions may promote its homogenization and therefore be captured by LCI measurement. In NCFB, LCI has been shown to be repeatable and discriminatory and to be a more sensitive marker of lung function than FEV₁ [11-13], but its role as a potential end point to demonstrate chest physiotherapy efficiency remains little explored [12].

The primary aim of this study was to assess the impact of a 30-min-long autogenic drainage (AD) session on ventilation inhomogeneity via LCI in adults with stable NCFB. We hypothesized that LCI change would inversely relate to the amount of expectorated sputum during the AD session and/or with the severity of bronchiectasis, as measured with the recently developed bronchiectasis severity index (BSI) [14].

4.3. Methods

4.3.1. Study population

Adults with stable NCFB were prospectively recruited in the Cliniques universitaires Saint-Luc (Brussels, Belgium) between March and October 2015. Participation was solicited via a telephone call or during a routine clinical consultation. Subjects were included if they were ≥ 18 y old, had radiological evidence of bronchiectasis confirmed on high-resolution computed tomography, were clinically stable (no antibiotics in the preceding 4 weeks) and if the clinical notes within the year mentioned the presence of a chronic productive cough. Exclusion criteria included a diagnosis of cystic fibrosis, severe obstruction ($FEV_1 < 30\%$ predicted), or inability to refrain from taking short-acting β_2 -adrenoreceptor agonists, if prescribed, the day of the study. Written informed consent was obtained before inclusion. The study was approved by the local ethics committee.

4.3.2. Study design

MBW using nitrogen as inert gas, spirometry, and body plethysmography tests were performed before a 30-min-long AD session. The AD session was followed by a 5-min break, after which the tests were repeated in the same order. During both treatment and the resting period, subjects expectorated into a sterile transparent container. To account for salivary contamination, the secretions were dried in the container without the cap in an oven for at least 72 h at 70°C. The masses of wet and dried secretions were thus measured.

4.3.3. Measurements

2.3.1. Multiple-breath nitrogen washout

A commercially available open-circuit MBW test with a hardware and software package was used for data processing and data analyses (Exhalyzer D and Spiroware 3.1, EcoMedics AG, Duernten, Switzerland). This setup measures nitrogen concentration indirectly from O_2 (measured by a sidestream laser O_2 sensor) and CO_2 fraction (measured by mainstream infrared CO_2 sensor). Flow and derived volumes were measured by a mainstream ultrasonic flow meter. Subjects were examined in a sitting position, wearing a nose clip and breathing through a silicone mouthpiece. MBW tests were performed in accordance with the current consensus [15] albeit we performed 2 technically acceptable MBW runs instead of 3.

We chose 2 runs because this significantly decreases testing time without influencing either mean LCI values or the test's sensitivity to detect abnormal ventilation distribution efficiency [16,17]. Three or more runs were conducted whenever quality standards were not met. A run was considered complete when the nitrogen concentration fell below 2.5% (1/40) of its starting concentration for 3 consecutive end-tidal expirations. LCI was then calculated as the ratio of cumulative expired volume to functional residual capacity (FRC_{MBW}), with cumulative expired volume defined as the sum of all expiratory volumes over the washout. FRC_{MBW} was calculated as the ratio of exhaled nitrogen volume during the washout to the difference in starting and ending end-tidal fraction of that tracer gas. A MBW run was discarded whenever leaks, sighs, or irregular breathing patterns occurred or if the FRC of one run differed by >10% compared with the highest FRC_{MBW} of a valid measurement. Measurements were performed by a single operator certified by the European Cystic Fibrosis Society Clinical Trial Network standardization committee.

2.3.2. Spirometry and body plethysmography

Spirometry and body plethysmography (MasterScreen, Jaeger, Würzburg, Germany) were conducted according to American Thoracic Society/European Respiratory Society standards [18]. Reported spirometry outcomes were slow vital capacity (SVC), forced vital capacity (FVC), FEV_1 , FEV_1/FVC , and forced expiratory flow at 25–75% of the FVC (FEF_{25-75}). Raw data were normalized (% pred and Z-scores) according to the Global Lungs Initiative equations [19] except for SVC, where the predictive equations of Pistelli *et al.* were used [20]. Body plethysmography measurements were FRC (FRC_{pleth}), residual volume (RV), total lung capacity (TLC), RV/TLC, and specific airway resistance and conductance. Raw measurements were converted according to the predictive equations from Stocks and Quanjer [21].

2.3.3. Bronchiectasis Severity Index

BSI is a recently validated multidimensional severity score for predicting future hospital admission and long-term mortality [14,22]. BSI takes into account age, body mass index, FEV_1 , prior hospital admission and exacerbations, Medical Research Council dyspnea score, bacterial colonization, and high-resolution computed tomography score. The latter was carried out within a year of recruitment and scored by 2 independent operators. Otherwise, data were collected the day of the last routine medical visit.

4.3.4. Chest physiotherapy

AD consists in maximizing expiratory air flow during tidal breathing at different lung volumes to mobilize the mucus while minimizing coughing episodes [23]. AD is initiated at the expiratory reserve volume until the inspiratory reserve volume is reached. The treatment session progresses through 3 phases: (i) unsticking the secretions in low volume; (ii) collecting at medium volume; and (iii) evacuation by breathing at high volume [24]. When sufficient mucus has reached the upper airways, the mucus can be expectorated by a cough or a huff [23,24]. AD was performed in a semi-recumbent position (at a 45° angle from the horizontal).

4.3.5. Statistical analysis

We estimated sample size based on the coefficient of repeatability of the LCI previously published [25]. Using a 2-sided α of 0.05 and assuming a mean LCI change of 1 unit and a SD difference of 1.8 [13], we estimated that recruiting 24 subjects would provide a power of 0.80.

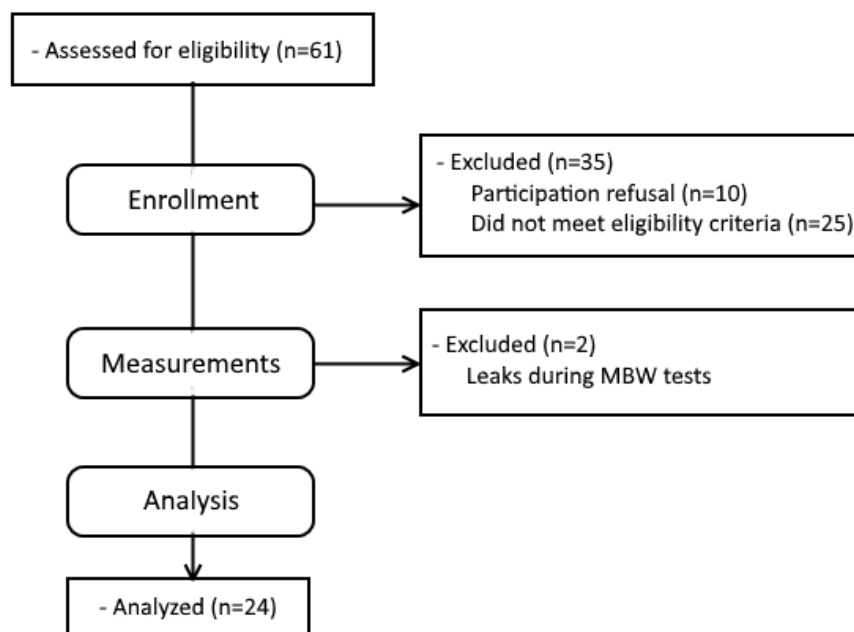
All analyses were performed using SPSS 22 (SPSS, Chicago, Illinois) software. Normality of data were verified with Kolmogorov-Smirnov and Shapiro-Wilk tests. For normally distributed data, values are presented as mean $\pm$ SD. Non-normally distributed values and baseline characteristics are reported as median (interquartile range). Paired t test or Wilcoxon signed-rank tests were used to compare pre- and post-chest physiotherapy measurement outcomes as appropriate. LCI and FRC are reported as the means of 2 technically acceptable MBW runs within one test occasion. Within-test repeatability of LCI and FRC were calculated as the ratio of difference between runs over their average (%). Changes in measurements with AD were expressed as the difference between post- and pre-measurements (Δ). Using Δ LCI of 1 lung turnover as a cutoff, participants were further divided into 2 groups, and the Mann-Whitney U test was used to compare their variables. Relations between Δ LCI and changes in other variables of interest or expectorated sputum weights were investigated by applying Pearson correlation coefficients. Bland-Altman statistics were carried out to assess the mean difference (bias) between FRC_{pleth} and FRC_{MBW} in addition to the upper and lower 95% limits of agreement (bias $\pm$ 1.96 SD of the difference) [26]. Finally, simple linear regression was conducted to model the relationships between FRC bias and lung function parameters. A P value of ≤ 0.05 was considered as statistically significant.

4.4. Results

4.4.1. Study population

Twenty-six subjects were investigated. Two of them were excluded from any analysis because of repeated mouth leaks during consecutive MBW runs, leaving 24 subjects (age 21–81 y) for the final analysis (*Fig. 1*). Baseline characteristics are displayed in *Table 1*.

Figure 1. Flow Chart of Study Participants



Two patients were unable to achieve two multiple-breath washout runs due to repeated mouth leaks.

4.4.2. Sputum weights and lung function measurements

The medians (interquartile ranges) of wet and dry sputum weights were 12.75 (1.24 – 24.04) g and 0.44 (0.1– 1.11) g, respectively. Pre- and post-AD lung function measurements are presented in *Table 2*. LCI and SVC (% pred) statistically improved after the chest physiotherapy session. No other lung function outcome differed statistically. Δ LCI was inversely correlated with spirometry measurement change and expectorated sputum weights (*Fig. 2*). Δ SVC (% pred) was also correlated with wet sputum weight ($r = 0.52$, $P = 0.01$) and dry sputum weight ($r = 0.46$, $P = 0.02$). BSI total score and its categories were not related to any lung function measurements at baseline nor to their respective change with AD. Using an LCI difference of 1 unit as a meaningful cutoff [25], only 5 subjects (21%) had a relevant change (all improved). The bronchiectasis etiologies among those subjects were: primary ciliary dyskinesia (3), idiopathic (1), and post-infectious (1). The amount of cleared sputum and the

associated gain of SVC were significantly more important in these 5 responders compared with the other participants: medians (interquartile ranges) of wet sputum weight, 24.7 (21.4–37.4) g versus 9.6 (0.9–21.9) g; dry sputum weight, 1.3 (0.9–2.3) g versus 0.2 (0.1–0.7) g; SVC (% pred), 5.3 (1.1–5.9)% versus 0.6 (–0.4–1.4)%; $P \leq 0.01$.

Before AD, there was a small but significant bias toward higher FRC_{pleth} when compared with FRC_{MBW} , suggesting trapped gas within the lungs. Mean difference (95% CI) was 0.19 L (0.05–0.34 L), $P = 0.01$. Limits of agreement were –0.47 and 0.86 L; the corresponding relative mean difference was 7.6%, and limits of agreement were –14.5 to 29.6%. Linear regression shows that normalized RV/TLC and RV explained 21% ($R^2 = 0.21$) and 18% ($R^2 = 0.18$) of the variability of trapped gas ($FRC_{pleth} - FRC_{MBW}$) before AD, respectively. Similar results were observed after AD (data not shown).

Table 1. Baseline characteristics of the study population

Characteristic	(n = 24)
Gender	16F / 8M
Age, y	65 [36 to 71]
Aetiology, n (%)	
Idiopathic	12 (50%)
Post-infective	5 (21%)
Primary ciliary dyskinesia	5 (21%)
Immune deficiency	1 (4%)
Severe gastro-oesophageal reflux	1 (4%)
BSI	
Mild, 0–4, n (%)	11 (46%)
Moderate, 5–8, n (%)	6 (25%)
Severe, ≥ 9 , n (%)	7 (28%)
FEV1 (% pred)	87.3 [66.6 to 97.3]
FEV1 (Z-score)	–0.87 [–2.36 to –0.22]
FEV1/FVC	0.72 [0.64 to 0.77]
FEV1/FVC (Z-score)	–0.99 [–2.1 to –0.41]
FEF25–75 (% pred)	60.6 [39 to 90.4]
FEF25–75 (Z-score)	–1.31 [–2.12 to –0.25]
LCI (turnover)	10.28 [8.71 to 12.99]

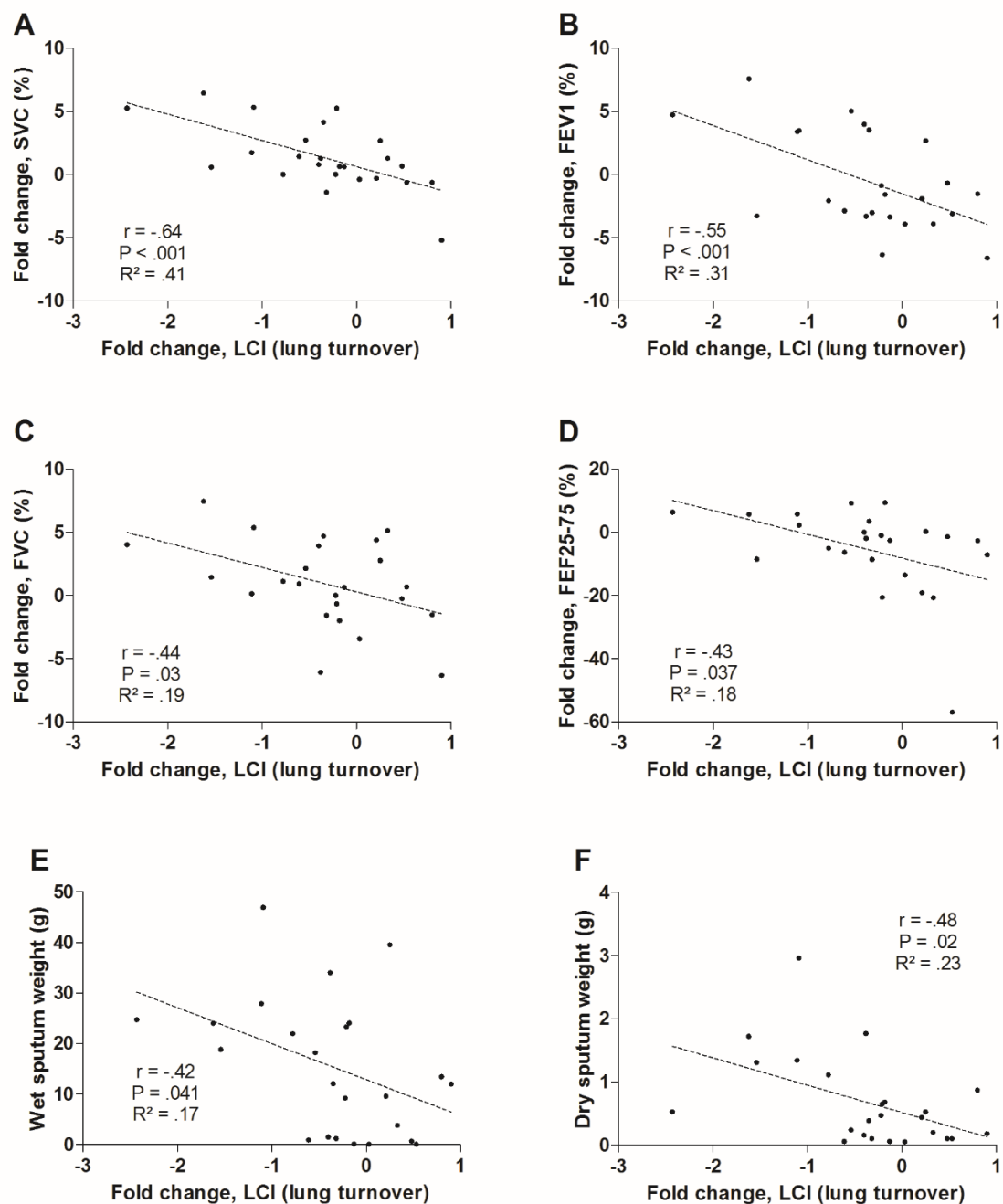
Data are presented as median [IQR]. Definition of abbreviations: BSI, bronchiectasis severity index; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; FEF25–75, forced expiratory flow between 25% and 75% of forced vital capacity; LCI, lung clearance index.

Table 2. Lung function measurement outcomes before and after chest physiotherapy in 24 patients

	Pre-AD	Post-AD	Mean difference [95% CI]	Paired test <i>P</i>
Multiple-Breath Washout				
LCI (turnover)	10.9 ± 0.5	10.5 ± 0.5	+0.4 [+0.0 ; +0.7]	0.04
LCI repeatability (%)	4.2	2.9		
FRC (L)	3.06 ± 0.99	3.07 ± 1.00	-0.01 [-0.11 ; +0.10]	0.91
FRC repeatability (%)	5.0	4.7		
Spirometry				
SVC (% pred)	88.7 ± 19.3	90.0 ± 19.1	-1.3 [-2.4 ; -0.2]	0.02
FEV1 (% pred)	87.3 [66 – 98]	87.0 [67 – 101]	+0.6 [-1.1 ; +2.2]	0.47
FVC (% pred)	94.4 ± 21.6	95.3 ± 21.5	-1.0 [-2.4 ; +0.5]	0.19
FEV1/FVC	0.71 ± 0.10	0.70 ± 0.10	+0.01 [-0.00 ; +0.02]	0.06
FEF25-75 (% pred)	60.6 [37 – 91]	59.2 [38 – 91]	+5.6 [-0.3 ; +11.4]	0.06
Body Plethysmography				
FRC (% pred)	107.0 [103 – 128]	109.0 [99 – 129]	-1.2 [-4.8 ; +2.4]	0.49
FRC (L)	3.25 ± 0.83	3.29 ± 0.87	-0.03 [-0.14 ; +0.07]	0.51
RV (% pred)	121.7 ± 21.4	123.4 ± 23.3	-1.8 [-7.0 ; +3.5]	0.49
TLC (% pred)	105.5 [96 – 111]	108.0 [98 – 114]	-1.6 [-3.4 ; +0.3]	0.09
RV/TLC (% pred)	106.5 [97 – 126]	109.0 [101 – 128]	+0.8 [-3.0 ; +4.6]	0.67
sRaw (kPa.s)	1.44 ± 0.57	1.34 ± 0.55	+0.10 [-0.07 ; +0.27]	0.22
sGaw (1/kPa.s)	0.82 ± 0.41	0.92 ± 0.41	-0.10 [-0.25 ; +0.05]	0.18

Data are presented as mean ± SD or median [IQR]. Definition of abbreviations: AD, autogenic drainage; dif, difference; CI, confidence interval; LCI, lung clearance index; FRC, functional residual capacity; MBW, multiple-breath washout; SVC, slow vital capacity; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FEF25–75, forced expiratory flow between 25% and 75% of forced vital capacity; pleth, body plethysmography; RV, residual volume; TLC, total lung capacity; sRaw, specific airway resistance; sGaw, specific airway conductance.

Figure 2: Relation between lung clearance index (LCI) difference and pulmonary lung function measurement changes or sputum weights, reported as Pearson r and linear regression (R^2)



Shown are the correlations between LCI difference and (A) change in percent predicted of slow vital capacity (SVC), (B) change in percent predicted of forced expiratory volume in one second (FEV₁), (C) change in percent predicted of forced vital capacity (FVC), (D) change in percent predicted of forced expiratory flow between 25% and 75% of forced vital capacity (FEF_{25-75%}), (E) change in wet sputum weight and (F) change in dry sputum weight. Dashed lines represent linear regression lines.

4.5. Discussion

In this study, we show that LCI significantly improved after an airway clearance session. However, only 5 of 24 subjects showed an improvement in ventilation inhomogeneity that exceeded measurement variability. The magnitude of LCI change was independent of disease severity but was rather related to the change of SVC and the amount of cleared sputum. Taken together, our findings suggest that the recruitment of lung volumes following elimination of excessive airway secretions results in a redistribution of ventilation, improving gas mixing efficiency. According to our data, we believe that the quantity of mobilized sputum with treatment should be taken into consideration when assessing the effects of chest physiotherapy on ventilation inhomogeneity.

Our results contrast with previous studies investigating the role of physiotherapy on ventilation inhomogeneity through LCI. Although physiotherapy modalities and the allocated time for airway clearance technique duration differed between studies, their analyses systematically concluded that there was no consistent effect on lung ventilation maldistribution [12,27,28]. However, the mean LCI improvement in our study was rather weak, with a mean relative decrease of 3%, well below the 10% corresponding to the magnitude of between-test variability [25]. Looking for individual physiologically meaningful change (*i.e.* $\Delta\text{LCI} \geq 1$ lung turnover) [25], only 5 subjects (21%) improved. Interestingly, although Pflieger *et al.* [28] did not find mean LCI change after a 30-min-long supervised positive expiratory pressure mask therapy session in CF, 17 of 29 subjects (59%) had a physiologically relevant LCI change (10 had an LCI increase ≥ 1 lung turnover, whereas 7 decreased). The paradoxical rise of LCI after chest physiotherapy is an acknowledged phenomenon attributed to the unveiling of newly partially obstructed airways previously completely plugged and therefore not contributing earlier to ventilation inhomogeneity [15]. In other words, individual relevant alterations in gas mixing were greater in their study. We assume that the underlying pathophysiological condition may partly explain these discrepancies. In CF, the lungs are diffusely and unevenly affected, with chronic sputum hyperproduction being a common clinical sign [29-31]. Likewise, NCFB is a lung disease involving patchy defects, but etiologies are various, and the airways can be either diffusely or locally affected [1]. In the latter forms, the net effect of airway clearance therapy might not be sufficient to provoke demonstrable LCI change. Our responders cleared a greater amount

of sputum during the AD session in comparison with the remainder of the subject cohort. In addition, 3 of the 5 responders had primary ciliary dyskinesia, a disease that shares common features with CF. This suggests that a minimum amount of sputum needs to be mobilized to elicit a detectable effect on ventilation inhomogeneity.

Recently, Lin *et al.* [32] have shown that the direction and magnitude of LCI change varied over time after a chest physiotherapy session in children with an exacerbation of cystic fibrosis. LCI worsened and peaked at 1 h post-chest physiotherapy and then slowly decreased to finally become significantly lower than the baseline value 24 h after chest physiotherapy. On the other hand, the time-course chest physiotherapy effect on FEV₁ in subjects with CF displayed a different pattern of change, with an optimal improvement observed between 30 min and 2 h after the physiotherapy session [33]. In our study, lung function tests were performed nearly immediately after the chest physiotherapy session, whereas Pflieger *et al.* [28] and Grillo *et al.* [12] performed tests 30 and 60 min after the chest physiotherapy session, respectively. Both studies observed a clinically small but significant improvement in FEV₁ with no mean LCI change, the opposite of what we found. Measurement time points are therefore an additional potential issue to consider when assessing chest physiotherapy effectiveness, and future studies attempting to clarify underpinning mechanisms are necessary.

We also observed a significant bias toward lower FRC_{MBW} values as compared with FRC_{pleth}. Body plethysmography uses Boyle's law to determine lung volumes and takes into account all compressible gases within the thorax, whereas inert nitrogen washout uses dilution properties of gases, only considering those in communication with the mouth. The difference between both measurements is therefore expected in obstructive diseases and provides an estimate of gas trapped in the lungs [34]. However, it is also admitted that the commercial nitrogen-MBW device used in this study overestimates true FRC to such an extent that some authors even report higher FRC_{MBW} than FRC_{pleth} [35-38]. The mechanism behind this remains unclear, but because we found a small positive bias toward higher FRC_{pleth}, we assume that substantial volumes of gas were trapped in a number of our subjects. The relatively high values observed in RV and RV/TLC, both markers also traditionally utilized to represent gas trapping [34,39], and their positive associations with the magnitude of the bias (*i.e.* FRC_{pleth} – FRC_{MBW}) further support this hypothesis.

Limitations of this research include the heterogeneity of the disease and the inherent wide spectrum of excessive mucus production between subjects. Another limitation was the

measure of expectorated sputum weight itself. Indeed, sputum can be unintentionally swallowed, therefore inducing error in the calculations. Finally, because the intervention was not controlled, it was also not possible to conclude that the findings were the result of AD alone. Forced expiratory maneuvers associated with spirometry measurements or coughing and huffing episodes interspersing with the AD technique could also account for ventilation redistribution.

In conclusion, we found a small but statistically significant short-term impact on uniformity of gas distribution following an AD session in subjects with NCFB. However, only a small subgroup of subjects with mucus hypersecretion showed an improvement in ventilation inhomogeneity that exceeded measurement variability. It suggests that mucus hyperproduction could be a prerequisite to provoke a physiologically meaningful change in ventilation inhomogeneity with airway clearance techniques.

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CHAPTER 5

PERSPECTIVES & CONCLUSIONS

Although major differences oppose the pathophysiological processes driving cystic fibrosis (CF) and non-CF bronchiectasis (NCFB), both diseases face a common hurdle: the need for a sensitive (and feasible) outcome measurement able to detect subtle clinical changes and treatment effects. To this end, lung clearance index (LCI) derived from multiple-breath nitrogen washout (MBNW) appears to be a very promising alternative. Measuring ventilation inhomogeneity is indeed physiologically insightful in patchy diseases. In CF, the literature assessing the role of LCI is now well furnished. Despite some concerns regarding patients with more advanced disease, LCI displays overall good clinimetric properties. Many clinical trials now consider this index as the primary outcome (ClinicalTrials.gov). In NCFB, data proving the usefulness of LCI are still scarce but some preliminary results are encouraging [1,2]. This last chapter will discuss technological issues, one of the major shortcomings of MBNW tests. Also, the potential role of LCI in assessing chest physiotherapy efficacy will be emphasized.

5.1. Technological issues

Even if outstanding technological progress has been achieved over the last decades, it remains a challenge to combine commercial demands (price, device stability, operator-accessible) with accurate measurements of flow and gas concentration. Current commercial setups running on oxygen circumvent the need for very expensive and bulky equipment by indirectly measuring nitrogen (N_2) concentration [3]. The tradeoff is the added complexity of such technology, which requires more sensors, each with inbuilt characteristics (response time, location on the circuit, sampling rate, sampling volume). This inescapably leads to additional estimation errors, theoretical assumptions and correction algorithms. Especially, the integration of the many signals into one component (flow-gas synchronization) to obtain the desired gas volumes becomes critical.

Robinson *et al.* provide key specifications required to obtain high quality measurements, but underline the unlikelihood for any one system to fulfill all individual specifications [3]. Hence, overall recommendations are provided for manufacturers (*Table 1*). Surprisingly, or rather interestingly, although the devices compared in chapter 3 adhere to the overall recommendations, remarkable LCI and FRC differences were observed [4]. It has to be recalled that each device has been validated using a lung model (*Table 1, Fig. 1*) [5-7]. Nonetheless, *in vitro* performances are not translated into real *in vivo* conditions. Despite

accurate head-to-head comparison between FRC measured with commercial MBNW devices and a lung model (*Fig. 1*), some *in vivo* evidence suggests that the Exhalyzer D (Eco Medics AG) overestimates FRC, while the EasyOne Pro (nidd) underestimates values [7-9]. More embarrassing is that the lower FRC values collected with the EasyOne Pro setup is associated with lesser reliability in LCI, which is actually the primary outcome of interest (Chapter 3). Two factors are presumed to explain such results: the delivery mode of O₂ and N₂ measurement algorithms. See Appendix for a detailed configuration of both devices.

Table 1. Overall recommendations for MBW setups (adapted from Robinson et al. [3])

Instantaneous flow accuracy within 5%, volume accuracy within 3%.

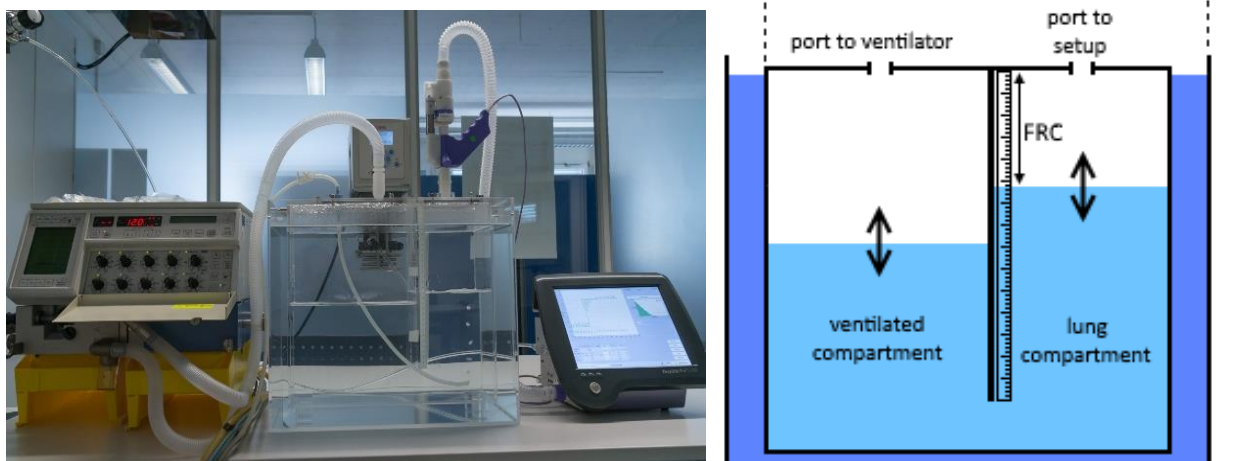
Quality of gas signals allowing determination of FRC and end-tidal gas concentration with sufficient accuracy and resolution.

- Stable end tidal gas concentration within 1% relative to the inert gas concentration at the start of the washout (e.g. 0.8% for 78% N₂).
- Stable end tidal gas concentration within 5% relative to the inert gas concentration at the end of the washout (e.g. 0.1% for 2% N₂).
- Linearity of the gas signal over the full measurement range.

FRC measurement accuracy within 5% of the true FRC value (for 95% of values), using a realistic lung model incorporating BTPS conditions across the intended volume range and breathing pattern of the system.

FRC, functional residual capacity; BTPS, body temperature, ambient pressure, saturated with water vapor.

Figure 1. Lung model validation



Picture and schematic drawing of the lung model used for functional residual capacity (FRC) validation. The lung model consists of two chambers, an inner and an outer tank. The inner tank is partially filled with water and separated into 2 compartments communicating in the lower part. One compartment is attached to the ventilator (ventilation compartment). The outlet of the other compartment (lung compartment) is connected to the setup. Hydraulic pressures generated by ventilator settings through ventilation compartment are transmitted to the lung compartment. FRC is the volume of air contained in the model at end-tidal expiration. The volume is determined by visually reading a vertical tape fixed to the lung compartment (1 millimeter correspond to a predetermined volume). The inner tank was heated by placing it in the outer tank filled with hot water.

The delivery mode of O₂ with the Exhalyzer D consists in a bias flow system. The bias flow provides a continuous fixed flow of pure O₂ during the washout. A sufficient bias flow must be adopted to avoid the risk of ambient air re-inspiration, which would automatically exclude the test. The Exhalyzer D uses a constant bias flow of 1 L.s⁻¹ (regardless of the respiratory phase) to exceed maximum tidal inspiratory flows, and is thus associated with a significant gas waste. The EasyOne Pro is equipped with a demand valve, which provides a two-step flow-gas delivery according to the respiratory phase (2 L.s⁻¹ during inspiration with an overflow valve to drain exceeding flow, 0.25 L.s⁻¹ during expiration). This piece of equipment allows to reduce gas consumption, but has detrimental effects on breathing pattern [3]. In turn, breathing pattern changes during the washout physiologically affect MBW-derived parameters [4].

The synchronization method also differs substantially between devices since it depends of distinct indirect N₂ measurement strategies (*Table 2*). For the Exhalyzer D, the gas signals (CO₂ and O₂) are synchronized to the flow using re-inspiration of expired gas from the post-capillary dead space^a. Gas signals are time shifted in such a way that post-capillary dead space volume correspond to a 50% step change in the gas concentration from its starting inspiratory value [5]. The delay times are calibrated prior to the test based on an average flow rate, which is assumed to be fixed during the subsequent tests. However, if the flow of the subject significantly differs from the calibration procedure, the set synchronization algorithms may become inappropriate. Moreover a small amount of air is steadily vacuumed into the side-stream sampling tube (see appendix *Fig A.1*), thereby leading to subtle delay time change when the subject's flow varies naturally over the washout (personal communication with D. Wendt, Eco Medics AG, Duernten, Switzerland). Yet, a software upgrade was very recently released and now implements a dynamic method for synchronization of gas and respiratory flow, taking into account the variation of the subject's flow while performing the test [10]. On the other hand, the EasyOne Pro device relies on a patented approach. Main-stream and side-stream signals are aligned by cross-correlation, being determined automatically for each MBW trial [11]. Therefore this method does not require prior delay-time calibration and considers each patient own breathing pattern. However, at the time of the study reported in chapter 3, the cross-correlation method did not assume the case where the subject's flow rate

^a Post-capillary dead space corresponds to the volume beyond the gas sampling point. By contrast, pre-capillary dead space is the volume between the mouth and the gas sampling point, see appendix *Fig A.1*.

approached the null value (personal communication with C. Buess, ndd, Zurich, Switzerland). This means flow-gas alignment errors may have occurred if short respiratory pauses, slow end-tidal expiratory flow or swallowing maneuvers occurred during the washout, thereby altering accuracy of end-expiratory gas concentration. New specific cross-correlation correction algorithms have since been implemented, moving from a flow based cross correlation to a volume based cross correlation [12], and MBW values will likely differ from the time we collected our values. Those comments emphasize that change of software versions over time will surely impact LCI and FRC data, as previously demonstrated by Ebdon *et al.* [13]. The ongoing enhancement of hardware and software is of course welcome, but the current data draws attention to significant measurement bias merely related to technological issues. These results should prompt manufacturers to improve (or even harmonize – in a utopian world –) their products and revisit measurement algorithms. Further improvements of devices with, amongst others, greater transparency in commercial software, are necessary.

Table 2. Specificity of 2 devices in measuring N_2 concentration and synchronizing all signals

	Exhalyzer D	EasyOne Pro
Sensors		
Flow	Ultrasonic flow and molar mass sensor, assessed main-stream	Ultrasonic flow and molar mass sensor, assessed main-stream
Molar mass	Ultrasonic flow and molar mass sensor, assessed main-stream [†]	Ultrasonic flow and molar mass sensors (x2), assessed main-stream <u>and</u> side-stream
CO ₂	Infrared analyzer, assessed main-stream, proximal to the flowmeter	Infrared analyzer, assessed side-stream
O ₂	Laser O ₂ sensor, assessed side-stream	Not assessed directly
N ₂ indirect concentration measurement	Based on Dalton's law of partial pressures $FN_2 = 1 - FO_2 - FCO_2 - FAr$ The FAr (0.93%) is treated as a fixed proportion of the FN_2 ($FAr = FN_2 * 0.0093/0.78$).	Based on side-stream molar mass (which is the sum of all gas concentrations multiplied with the molar mass of each gas), corrected by subtracting FCO_2 from the global molar mass.
Synchronization method	Three different sampling sites. Alignment of O ₂ , CO ₂ and flow is based on re-inspired expiratory gas from post-capillary dead space ‡.	Three different sampling sites. Alignment of main-stream and side-stream molar mass and CO ₂ signals by cross-correlation (patent-protected method).

Main-stream: the sample is not transported and analyzed away from the flow sampling site. The sensor is located either in the hand-held unit where flow is measured, or close to the flow sensor. Side-stream: the sample is diverted from the flow sampling site and is analyzed within the "case" of the device. Abbreviations: F, Fraction; N₂, nitrogen; O₂, oxygen; CO₂, carbon dioxide; Ar, argon. [†]Not processed for the N₂ washout. [‡]Post-capillary dead space corresponds to the volume beyond the gas sampling point, see appendix Fig A.1.

5.2. LCI and chest physiotherapy

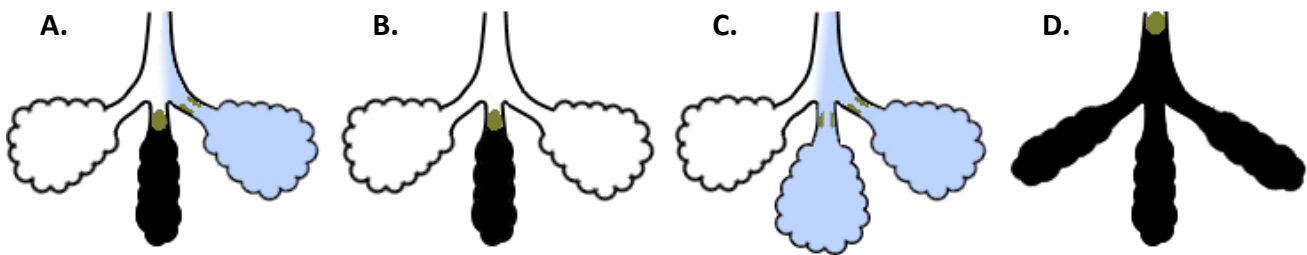
Airway clearance techniques (ACT) account for a substantial part of the treatment in patients with CF and NCFB [14,15]. Even though the large body of scientific literature in physiotherapy has mainly been devoted to compare different airway clearance regimens over the past years, their physiological effects remain poorly understood [16]. This unsatisfactory ascertainment may be partially explained by the lack of an optimal outcome measurement [17-19]. There are famous quotes from Sir William Thomson, also known as Lord Kelvin, saying: *“To measure is to know”; “If you cannot measure it, you cannot improve it”*. In other words, not understanding the physiological mechanisms underlying ACT precludes ways to improve, refine and individualize chest physiotherapy. In this time of evidence-based medicine/practice, responsive and feasible clinical endpoints would therefore be much appreciated to unravel the physiological roots at the basis of physiotherapy treatments.

To this end, assessing the effects of ACT on ventilation inhomogeneity may contribute to improve our knowledge. One of the main objectives of ACT is to dislodge and evacuate mucus sticking on the bronchial wall, hence theoretically preserving the lung from excessive inflammatory response, recurrent infection and rapid deterioration. Most of ACT trials used FEV₁ as the primary outcome measure of lung function. Yet, there is an increasing awareness that FEV₁ is not a suitable endpoint to assess ACT efficacy [17,20]. Besides, FEV₁ or other spirometry-derived parameters do not sensitively detect (change in) small airways (Chapter 2). Therefore, the impact of chest physiotherapy on peripheral lung regions remains broadly ignored. Since changes in peripheral airways will result in ventilation inhomogeneity [21], our study reported in Chapter 4 was intended to investigate the potential effect of a single session of a manual ACT on LCI. Although this study was not controlled, targeted on NCFB patients with a wide range of disease severity and extent, and cannot ensure that the effects were solely localized on small airways, results show at least that the role of LCI to assess chest physiotherapy merits further investigations.

Studies assessing change of LCI following diverse airway clearance regimens have led to contradictory results. Table 3 summarizes findings in patients with CF and NCFB. Out of 7 studies [22-29], only 3 reported a significant decrease (*i.e.* improvement) in LCI with ACT [23,26,27]. Irrespective of the ACT applied, a number of factors may have influenced the outcomes in these studies. Targeted population may differ not only between studies, but also

within studies as etiology of NCFB is very heterogeneous. The range of FEV₁ of study group is often wide. The amount of sputum evacuated is rarely mentioned, though this information may be helpful to anticipate a demonstrable LCI change in short-term studies (chapter 4). Focusing on hyperproductive patients with a mild disease severity (*e.g.* FEV₁ > 70%) might be a good strategy in future ACT studies using LCI as one of the outcome measurements. Further, some authors allowed the administration of salbutamol prior to ACT. Few studies looked at the impact of this drug on LCI of patients with stable asthma or CF and their results are not univocal [30-32] but bronchial hyperactivity is quite common in CF and bronchodilators could theoretically potentiate the effects of an airway clearance regimen. Last but not least is the time interval between the measurement and the treatment. A resting period of 3 hours between the end of the ACT and the first MBNW run is likely optimal to obtain a significant and relevant improvement in ventilation inhomogeneity [33]. This observation may partly results from the ongoing transport of mobilized – albeit not cleared – phlegm owing to the mucociliary clearance [34,35]. Yet, none of the studies fulfilled such a long, impractical, waiting period (*Table 3*).

On the individuals' level, when provided, the size and direction of LCI change was clearly heterogeneous, with some patients showing an increase in ventilation inhomogeneity [23,25,29]. Incidentally, similar findings were found with intravenous antibiotics for a CF pulmonary exacerbation [36-42]. At first sight, an increase of LCI with treatment is counterintuitive since mucus shifting should conceivably improve respiratory mechanics, thereby gas mixing efficiency. Yet it may be useful to recall that LCI measures ventilation inhomogeneity between all lung units communicating directly with the mouth, hence neglecting totally obstructed areas. Therefore an efficient treatment regimen may unplug a previously completely blocked lung zone, thereby unveiling new poorly ventilated units and causing a paradoxical rise of LCI. Alternatively, redistribution of mucus plug shifted, though not cleared, may possibly isolate an area from the mouth, introducing further variability in the response to chest physiotherapy (*Fig. 2*). Those lines emphasize that LCI aggregates different signals within the lungs (including structural or modifiable defects, occurring in the small or larger airways) into a single-dimensional unit. This means it may be difficult to predict or interpret LCI change with physiotherapy in patients with significant advanced disease severity, where mucus is extensively distributed in the entire lung field or where airways may easily clog.

Figure 2: Possible LCI responses due to mucus shifting

A) Baseline situation. The left sub-unit is normally ventilated, the middle sub-unit is completely clogged and the right sub-unit is partly plugged. Black area represents unventilated zones, blue-shaded area represents poorly ventilated lung zones. **B)** The partially obstructed sub-unit has been successfully cleared and is now normally ventilated, thereby decreasing ventilation inhomogeneity and thus LCI. **C)** The previously closed lung unit has been partially opened up, unveiling a poorly ventilated sub-unit which paradoxically increases ventilation inhomogeneity. **D)** The mucus plugs from the middle and right sub-units have been shifted towards the parent branch, becoming now totally closed. The subtended lung zone does not participate anymore in the global measurement of ventilation inhomogeneity.

It seems clear that LCI alone will never be able to provide definitive answers regarding fundamental questions that remain about physiological mechanisms underlying ACT. However, when mixed up with other tests, ventilation inhomogeneity assessments may provide useful information. For instance, using a combination of pulmonary function tests including the single-breath washout method with a double tracer gas mixture, Abbas *et al.* were able to demonstrate specific improvement in peripheral gas mixing with ACT in CF children [43]. Another suggestion is to combine MBW data with other tests such as electrical impedance tomography, a tool that can detect regional ventilation inhomogeneity regardless of whether those are communicating with the mouth or not [44]. Finally, it should be noted that despite LCI response is clearly heterogeneous between individuals, intra-subject response seems much more consistent over time [40,45]. This suggests that a similar intra-subject lung functional unit is prone to repetitive deterioration or improvement, and that LCI may be able to identify responders, *i.e.* patient with modifiable lung defects amenable to change with ACT. This is particularly relevant in a context where identifying patients who are likely to respond well to some airway clearance maneuvers has been a vexing problem for years. Considered together, those perspectives could help to identify and understand why some patients show a response while others simply do not. This is a compulsory preliminary step to overcome before rethinking or refining ACT in an appropriate physiological context.

Table 3. Summary of findings assessing the effects of chest physiotherapy on LCI in patients with CF and NCFB

Study	Design	Tracer Gas, device	Population	Age	FEV ₁ at baseline (Z-score or %pred)	Treatment characteristics	Resting period between CPT & LCI	LCI results
Fuchs (2010) [22]	RCT	SF ₆ , EasyOne Pro	27 CF (stable) IG: n = 16 CG: n = 11	IG: 10 ± 3y CG: 11 ± 4y	IG: -1.0 ± 2.0 Range: -4.6 to 2.4 CG: -0.6 ± 2.1 Range: -4.2 to 1.6	CPT vs no CPT CPT : endurance training + thorax mobilization + ACT (Flutter or PEP) Duration : 30 min Use of salbutamol not reported	nr	IG: 7.76 ± 1.23 vs 7.96 ± 1.04, ns* CG: 8.36 ± 1.57 vs 8.29 ± 1.44, ns*
Pfleger (2015) [25]	Obs	N ₂ , Exhalyzer D	29 CF (stable)	Mean: 22y Range: 7-44y	Mean: 70% Range: 30-116%	Supervised PEP mask Duration : 30 min Use of salbutamol prior to CPT	30 min	Mean (range): 15.00 (7.40–24.20) vs 14.80 (7.14–22.39), P=0.58
Grillo (2015) [29]	Obs	SF ₆ , Modified Innocor	25 NCFB (stable)	58 ± 17y	-1.2 ± 1.4	Usual ACT Duration : until there were 2 clear cycles producing no sputum Use of salbutamol not reported	60 min	Baseline: 10.91 ± 3.39; mean diff = 0.26 ± 0.99, P=0.22
			28 NCFB (PEx)	63 ± 13y	-3.0 ± 1.3			PEx, baseline: 12.76 ± 3.47; mean diff at start of PEx = 0.11 ± 0.85, P=0.51 mean diff at end of PEx = 0.14 ± 0.93, P=0.49
Rodriguez Hortal (2016) [26]	RCT	N ₂ , Vmax 22D	32 CF (stable) IG: n = 16 CG : n = 16	IG: 28 ± 11y CG: 33 ± 9y	IG: 43 ± 12% CG: 55 ± 14%	NIV (IG) vs PEP mask (CG) Each regimen consisted in repeated cycle of HS inhalation, AD, PEP/NIV and huff Duration : 1 h / day, over 3 months Use of salbutamol (+HS) prior to CPT	Measurements are taken prior to CPT	IG: 10.20 ± 2.37 vs 9.20 ± 2.55, P=0.01 CG: 9.69 ± 2.50 vs 9.76 ± 2.50, ns*
O'Neill (2017) [24]	RCT (cross-over)	SF ₆ , Modified Innocor	13 CF (PEx)	33 ± 12	51 ± 22%	HS before Acapella Duet (Group A) vs HS during Acapella Duet (Group B) Duration : 20 min Use of salbutamol (+HS) prior to CPT	0 min and 90 min	From baseline to 90min post treatment: Group A: 14.1 ± 3.6 vs 14.2 ± 3.6, P=0.75 Group B: 13.8 ± 3.4 vs 13.9 ± 3.6, P=0.49 Immediately after CPT : similar results
Grosse-Onnebrink (2017) [23]	RCT	SF ₆ , EasyOne Pro	41 CF (PEx) IG: n = 20 CG: n = 21	IG: 19 ± 8y CG: 24 ± 12y	IG: 51 ± 16% Range: 29-81% CG: 57 ± 20% Range: 25-105%	HFCWO vs no ACT Duration : 30 min No use of salbutamol	0-5 min	Median (range): IG: 11.8 (8.8–19.3) vs 11.1 (8.2–17.8), P=0.002 CG: 11.8 (7.1–15.0) vs 12.3 (7.6–15.2), P=0.64
Poncin (2017) [27]	Obs	N ₂ , Exhalyzer D	24 NCFB (stable)	Median: 65y Range: 21-82y	Median: 87% Range: 39-131%	AD Duration : 30 min No use of salbutamol	5 min	10.88 ± 0.54 vs 10.53 ± 0.48, P=0.042

FEV₁ data are either expressed in z-scores or in % of predicted value. Data are given as means (standard deviation) unless otherwise stated.

Abbreviations: ACT, airway clearance technique; AD, autogenic drainage; CF, cystic fibrosis; CG, control group; CPT, chest physiotherapy; diff, difference; FEV₁, forced expiratory volume in 1 second; HFCWO, high frequency chest wall oscillation; HS, hypertonic saline; IG, intervention group; N₂, nitrogen; NCFB, non-cystic fibrosis bronchiectasis; NIV, non-invasive ventilation (bilevel positive airway pressure); nr, not reported; ns, not significant; PEP, positive expiratory pressure; PEx, pulmonary exacerbation; RCT, randomized controlled trial; SF₆; sulfur hexafluoride; Obs, observational. * P-value not provided

Devices: EasyOne Pro (nidd Medical Technologies, Zurich, Switzerland); Exhalyzer D (EcoMedics AG, Duernten, Switzerland); Modified Innocor (Innovision, Odense, Denmark); Vmax 22D (SensorMedics Corps., Yorba Linda, CA, USA).

5.3. Thesis conclusions

Multiple-breath washout was described for the first time more than 60 years ago. Yet its utility in CF has only been outlined relatively recently. Since the beginning of the decade and the appearance of commercially available MBNW devices, the amount of data has dramatically grown. It soon appeared that clinimetric properties of LCI, the main parameter derived from MBW, were valuable in CF, leading to seriously consider the index to receive the surrogate endpoint status. The biggest outstanding question is maybe the utility of LCI in guiding clinical decision making. However, this thesis showed that clinimetric properties are closely related to the MBW equipment used, in such a way that the derived parameters are clearly not interchangeable between systems. These observations have significant implications since they compel CF centers to adopt the same equipment for multicenter projects. More problematic is that they do not guarantee that LCI computation from different setups has the same ability to appreciate treatment effects. Commercial devices seem to remain in the development phase and further efforts are urgently required from manufacturers. This thesis also questioned the role of LCI in detecting airway clearance effects in NCFB patients. Although the effect size was small, the findings showed the potential of this index in assessing the efficacy of chest physiotherapy, and further data collected in a more well-defined population would be valuable. In the future, combining LCI with other pulmonary function tests may provide long desired information for therapies which still lack a robust rationale, such as chest physiotherapy.

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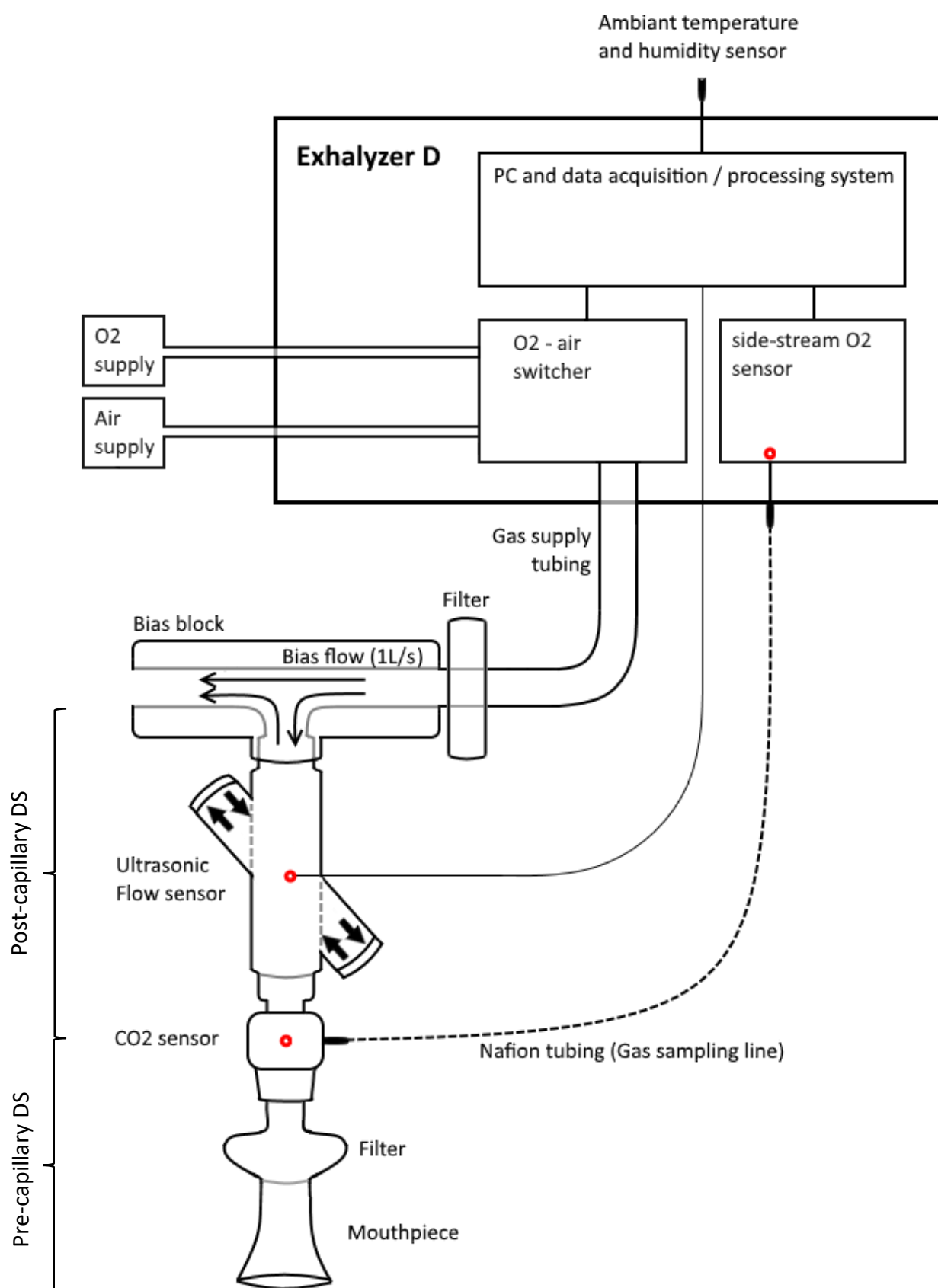
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APPENDIX

A.1. The Exhalyzer D setup



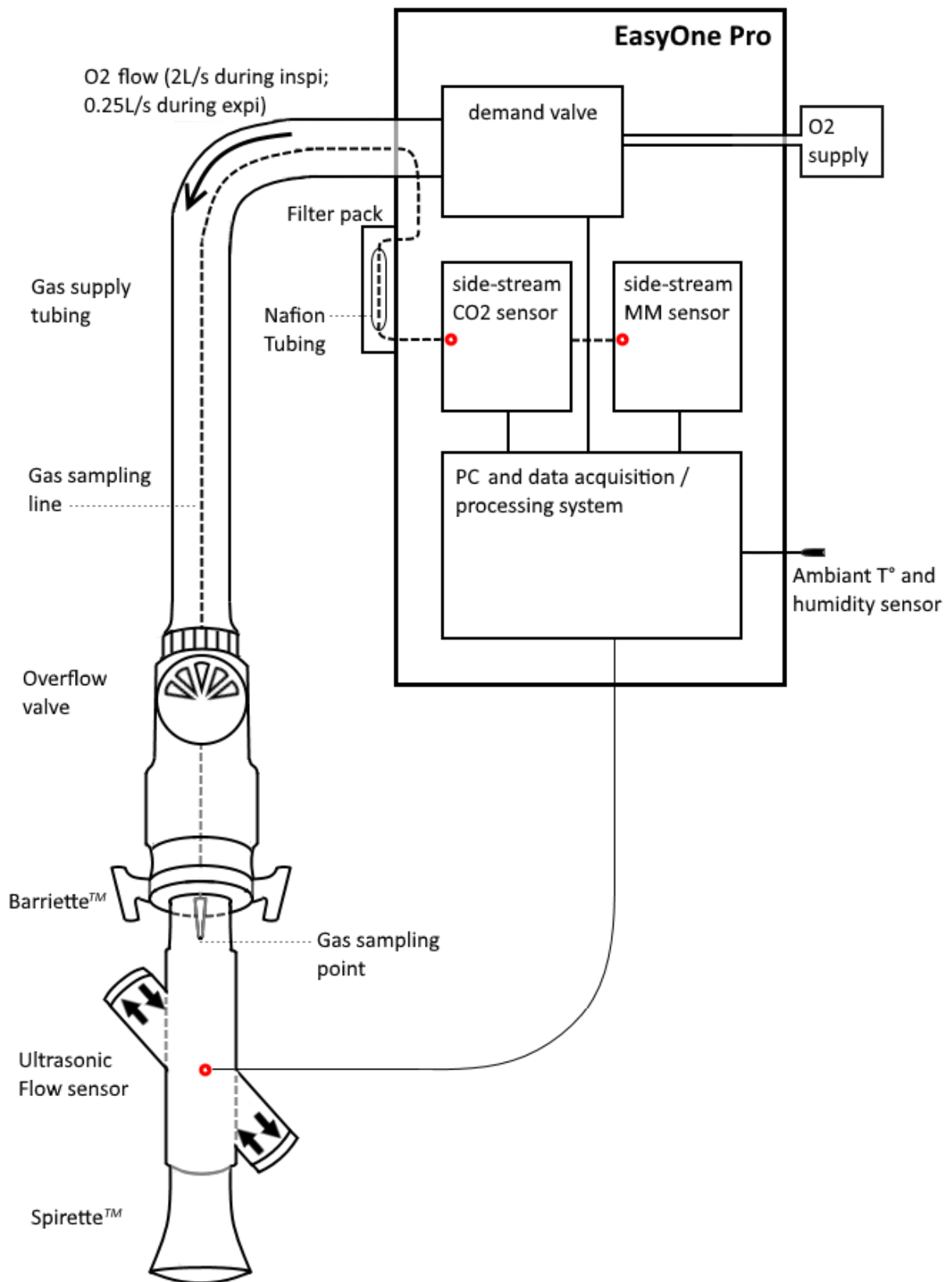
DS: dead space

Flow, CO₂ and O₂ signals are each assessed in a different location (red circles). The flow is assessed main-stream through an ultrasonic flow sensor. Transducers located on either side of the handle cavity emit and receive sounds in alternating directions (short arrows). Hence, during inspiration for instance, a pulse travelling with the flow (diagonally from upper left to lower right on the schematic drawing) will be sped up, reaching the opposite transducer quicker than a pulse travelling against the flow (diagonally from lower right to upper left). The transit-time for each sound pulse is measured, allowing to calculate gas flow (and the derived volume) passing along the handle. CO₂ is assessed main-stream through an infra-red analyzer. O₂ is sampled on the capnograph and is transported towards a side-stream sensor by passing along a nafion tubing. This tubing is semi-permeable to water vapor. O₂ is equilibrating with ambient temperature and humidity while travelling in the nafion tubing. Correction for ambient temperature and humidity measured at the time of testing are subsequently applied. The gas signals (CO₂ and O₂) are synchronized to the flow using re-inspiration of expired gas from the post-capillary dead space. Gas signals are time shifted in such a way that post-capillary dead space volume correspond to a 50% step change (drop for CO₂, rise for O₂) in the gas concentration from its starting inspiratory value.

A bias flow of 1 L.s⁻¹ of pressurized air is constantly supplied during the pre-washout phase^a. The bias flow eliminates re-inspiration of expired gas beyond post-capillary dead space. A bias flow of 1 L.s⁻¹ of pure O₂ is delivered during the washout phase. A peak inspiratory flow exceeding the bias flow will result in an entrainment of room air in the circuit, leading to the exclusion of the test. Disposable filter and mouthpiece are attached at the outlet of the CO₂ port.

^a The pre-washout phase is intended to ensure the stability of the subject's breathing pattern before running the test.

A.2. The EasyOne Pro setup



MM: molar mass; TM: trademarks (owned by ndd Medizintechnik AG)

Flow, molar mass and CO₂ signals are each assessed in a different location (red circles). Flow and derived volume are assessed main-stream through an ultrasonic flow sensor (see above for a technical description). CO₂ is assessed side-stream through an infra-red analyzer. Molar mass is assessed main-stream and side-stream via ultrasonic sensors, but only the side-stream signal is processed to accurately measure molecular weights. Indeed, ultrasonic propagation of sound wave is dependent of the density of the medium, and gas density is proportional to molar mass but inversely proportional to temperature. Therefore temperature must be precisely known and kept constant between both emitting transducers, which is difficult to handle main-stream because of rapid temperature changes within the breathing cycle. Molar mass is thus sampled main-stream by the barriette™, transported along a gas sampling line, transits through a nafion tubing hidden inside a filter pack, and eventually lead to a small side-stream ultrasonic sensor. However, the main-stream molar mass measurement is used to synchronize signals: delay time caused by gas transport towards the side-stream sensors is determined by cross-correlation of the side-stream gas signals (molar mass and CO₂) to the main-stream molar mass signal.

A single patient use spirette™ is inserted inside the handle. Together with the barriette™, also disposable, those inserts provide reliable hygienic safety, thereby avoiding the need for a filter. During the pre-washout phase, the spirette™ and barriette™ are not in contact with each other, so pressurized air supply is not necessary. A motor block disposed above the handle (not represented on the illustration) seal both inserts at the beginning of the washout phase. During the latter, pure O₂ is delivered to the patient through the gas supply tubing. O₂ flow is determined by a demand valve, which provides a two-step delivery according to the respiratory phase (2 L.s⁻¹ during inspiration with an overflow valve to drain exceeding flow, 0.25 L.s⁻¹ during expiration). The O₂ flow exceeding the tidal breathing need is eliminated via the overflow valve. Expired gas volume leave the circuit via the lateral “wings” of the barriette™. The overflow valve may also cover the elimination of expired gas if necessary.