White Paper: Acoustic Airway Clearance with the Frequencer^{TM} - Clinical Evidence and Markets

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April 07, 2020

TABLE OF CONTENTS

1 ABSTRACT

In addition to significant quality of life issues and burden on the health care system, life-threatening situations and death may arise when clearance of mucus from airways is ineffective. This white paper reviews normal airway clearance mechanisms and the main airway clearance diseases and disorders, along with standard airway clearance therapies. An innovative solution for airway clearance with acoustic (sound) waves at an optimized frequency, the Frequencer, is presented, which has numerous benefits to standard mechanical airway clearance techniques (ACT). *In vitro* and clinical evidence is reviewed to demonstrate that sound produced by the Frequencer easily travels through the chest causing the lungs to vibrate and the viscosity of mucus to decrease, making it more liquid and easier to expectorate. Additionally, multiple case studies and testimonials are discussed showing the power of the Frequencer. Last, global markets for an airway clearance device such as the Frequencer are summarized. It can be concluded that the Frequencer is an extremely useful tool to prevent the immense suffering and fatalities related to airway clearance diseases and disorders.

2 ABBREVIATIONS

Abbreviation	Definition
ACBT	Active Cycle Forced Breathing Technique
ACT	Airway Clearance Technique
AD	Autogenic Drainage
ALS	Amyotrophic Lateral Sclerosis
CF	Cystic Fibrosis
COPD	Chronic Obstructive Pulmonary Disease
CPT	Chest Physiotherapy
FEV1	Forced Expiratory Volume in the first second
FET	Forced Expiration Technique
FVC	Forced Vital Capacity
ICU	Intensive Care Unit
IRB	Institutional Review Board
KOL	Key Opinion Leader
MERS	Middle East Respiratory Syndrome
N	Newton
NMD	Neuromuscular Degenerative Disorder
PCD	Primary Ciliary Dyskinesia
PEP	Positive expiratory pressure
PFTs	Pulmonary Function Tests
POC	Proof-of-Concept
RSV	Respiratory Syncytial Virus
SARS	Severe Acute Respiratory Syndrome
SMA	Spinal Muscular Atrophy

3 INTRODUCTION

In health, mucociliary clearance and cough mechanisms are effective and efficient in defending the lung against inhaled pathogens and particles. Life-threatening situations may arise when there is altered mucociliary function and/or cough proves ineffective. In fact, airway clearance diseases and disorders result in millions of deaths per year and cause severe suffering for hundreds of millions more. Bronchial drainage, using airway clearance techniques (ACTs), is required for individuals whose function of the mucociliary escalator and/or cough mechanics is altered and whose ability to mobilize and expectorate airway secretions is compromised. Clearing the airways helps decrease the occurrence of lung infections, respiratory distress and co-morbidities (recurrent pneumonia, etc.), and consequently helps improves lung function. However, the available ACTs are mechanical in nature, and have many limitations. A novel, convenient, consistent and effective solution for ACT using acoustic waves, the Frequencer, is available.

4 THE PRINCIPALS OF AIRWAY CLEARANCE, DISORDERS & TREATMENTS

4.1 Airway mucociliary clearance

Mucus is a heterogeneous, adhesive and viscoelastic reversible gel composed of water, globular proteins, carbohydrates, lipids, salt, water and antimicrobial factors. Mucins, namely large peptidoglycan biopolymers, are the main constituents of mucus, providing structure and viscoelasticity. In healthy humans, the mucus is quite fluid, and is comprised of 1% mucin (by weight) and 2.5% total protein and glycoprotein.[1, 2] Airway mucus traps inhaled toxins.

The respiratory system is lined with cuboidal epithelia cells bearing hair-like motile structures called cilia. The cilia work to move the mucus up through the airways by means of ciliary beating (the so-called mucociliary escalator mechanism), and with the help of a normal cough, mucus detaches from the lung surface easily. In health, 10–100 mL of airway secretions is continuously produced and cleared by the centripetal movement of the mucociliary escalator, and with the aid of transient increases in expiratory air flow.[2, 3, 4]

An effective cough is essential to clear airway secretions from the more proximal airways. For an effective cough one needs firstly to take a sufficiently deep breath in; the glottis needs to close briefly to allow an increase in intrathoracic pressure; followed by expulsive glottic opening together with abdominal contraction, which results in air being forcibly expelled.[2, 3]

In health, mucociliary clearance and cough mechanisms are effective and efficient in defending the lung against inhaled pathogens and particles. Life-threatening situations may arise when there is altered mucociliary function and/or cough proves ineffective. Disease processes can impair ciliary function, alter secretion production and mucus rheology (i.e., making it thicker), and interfere with the cough reflex.[1, 5] Failure to clear secretions allows microbes and particles to collect in them, and rather than protecting the host, an environment where pathogens are protected and able to proliferate is created, resulting in a vicious cycle of inflammation and infection (i.e., chronic lung infection). Airway clearance therapy has been the cornerstone aimed at minimizing the devastating effects of airway obstruction, infection and inflammation due to mucus stasis on the conducting airways and lung parenchyma.

4.2 Summary of major airway clearance disorders

As indicated above, there are a variety of factors that can interfere with mucociliary clearance and cough, making it difficult to mobilize and evacuate secretions from the airways. It is important to note that although one issue may be the primary cause, the impaired processes (*i.e.,* ciliary function, secretion and cough) are closely intertwined in all airway clearance diseases and conditions discussed below. Pulmonary disorders such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), asthma and bronchiectasis alter the production and composition of mucus and function of the cilia. Mucociliary clearance disorders, such as primary ciliary dyskinesia (PCD), reduce the efficacy of ciliary structure and function, as well as the aging process, tobacco use and environment exposures. Progressive neuromuscular degenerative (NMD) disorders such as amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA) and muscular dystrophies inhibit the normal cough reflex. Last, but not least, acute respiratory infections such as pneumonia, influenza, respiratory syncytial virus (RSV) and novel pandemics (SARS, MERS, Ebola, COVID-19) caused by a variety of bacteria and viruses can cause serious inflammation to the respiratory system and damage the cilia, resulting in severe respiratory distress requiring mobilization of secretions in the hospital ward and Intensive Care Unit (ICU).

The major airway clearance diseases and disorders are summarized below:

- **CF** is an inherited disease that disturbs ion and water homeostasis across epithelia, rendering mucus more sticky, dense and hard to expel. This abnormal mucus can clog the airways, leading to severe problems with breathing and bacterial infections in the lungs, leading to progressive and permanent damage to the respiratory system.[6] Daily therapy (often multiple times daily) to help remove respiratory sections is required[7], with the most common treatment being Chest Physiotherapy (CPT) (i.e., clapping).
- **COPD**, also known as chronic bronchitis and emphysema, is characterized by respiratory symptoms and a persistent reduction of airflow that is due to airway and/or alveolar abnormalities. The primary causes are smoking or exposure to other noxious particles or gases. Chronic cough and sputum production are hallmarks of the disease and exacerbations of serious episodes consisting of increased breathlessness, cough and sputum production lasting from several days to a few weeks are common. These episodes can be seriously disabling and result in the need for hospitalization and sometimes death.[8] Those with severe COPD or undergoing exacerbations require airway clearance therapy.
- **Asthma** is a chronic disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person (several times a day

to 1x per week). During an asthma attack, the lining of the bronchial tubes swells, causing the airways to narrow and reducing the flow of air into and out of the lung. Exacerbations can lead to hospitalization.[9, 10] Subjects with severe asthma have significantly lower number of ciliated cells and higher dyskinesia and cilia motility indices[11], along with inability to mobilize secretions, therefore requiring assistance with airway clearance. Asthma is the most common chronic disease among children (although morbidity and mortality are higher in adults). Childhood asthma has been associated with adult lung function deficits and increased risk of COPD.

- **Bronchiectasis** is a chronic condition where the walls of the bronchi are thickened from inflammation and infection, with decreased ability to clear secretions. Sometimes described as a disease, bronchiectasis is more appropriately thought of as the result of a pathological process involving a vicious cycle of inflammation, recurrent infection and bronchial wall damage that can occur in association with a variety of primary causes including infectious, genetic, inflammatory, environmental and allergic. For example, bronchiectasis is the major lung anomaly of CF, COPD and asthma, and many cases follow a lung infection.[12] Effective clearance of mucus from the airways is one of the most important treatment modalities that can be instituted in patients with bronchiectasis.[13]
- **PCD** is an autosomal recessive genetic disorder that causes defects in the action of cilia lining the respiratory tract, fallopian tube and flagellum of sperm cells. Patients with PCD require lifelong airway clearance therapy. Like CF, PCD is associated with significant illness and quality of life deficits throughout the lifespan.[14]
- **NMDs** are a group of progressive disorders in which there is a decrease in vital capacity of the lung, which is mainly related to muscle weakness and alterations of the mechanical properties of the lungs and chest wall. Respiratory problems are the main cause of death - the decrease in strength and speed of contraction of the expiratory muscles causes a decrease in the maximum speed of expiration (*i.e.,* cough). Examples are: i) **ALS**, a progressive degenerative disease of the motor neurons, leading to the inability of the brain to initiate and control muscle movement[15]; ii) **SMA,** a genetic disorder characterized by weakness and atrophy in skeletal muscles[16]; and iii) **Muscular dystrophies**, a heterogeneous group of genetic muscle diseases (such as Duchenne muscular dystrophy, myotonic muscular dystrophy, facioscapulohumeral dystrophy, limb girdle muscular dystrophy) with variable distribution of weakness and mode of inheritance.[17]
- **Pneumonia** is the most common serious respiratory infection. It is an infection that inflames the alveoli in one or both lungs, causing difficulty with mucus clearance. Pneumonia may lead to chronic respiratory disease such as bronchiectasis. Bacteria (mainly *Streptococcus*) are the most common cause but viruses and fungi can also cause pneumonia. The prevalence increases in patients with diseases such as asthma, COPD or heart disease. [18]
- **Influenza** (seasonal flu) is a viral respiratory infection and **RSV** is the most common cause of acute respiratory infection in children.[19] Both can spread to the lower respiratory tract,

causing pneumonia or bronchiolitis, requiring hospitalization.

- **Global viral pandemics**. As the world is acutely aware, new respiratory pathogens are emerging that cause severe respiratory distress, requiring effective mobilization of secretions in ICUs for successful outcomes. In 2003, severe acute respiratory syndrome (SARS), caused by a previously unrecognised coronavirus (SARS-CoV) rapidly spread throughout the world. It was contained relatively rapidly. Middle East Respiratory Syndrome (MERS; MERS-CoV) is another example of a viral respiratory illness that is new to humans. Most people infected with MERS developed severe respiratory illness, including fever, cough, and shortness of breath. Finally, COVID-19 is a severe respiratory syndrome caused by another novel coronavirus (SARS-CoV-2). Although studies are required to understand the epidemiology of COVID-19, it presents as severe pneumonia on CT scan.[20] Researchers have performed autopsies on deceased patients and found large amounts of sticky mucus in the deep-seated airways.[21]

4.3 Standard of care for airway clearance

Although anti-inflammatory, enzyme and gene therapies, antibiotics and bronchodilators have been optimized in the last 30 years, complementary mechanical procedures for effective bronchial drainage is required for individuals whose function of the mucociliary escalator and/or cough mechanics is altered and whose ability to mobilize and expectorate airway secretions is compromised.[2, 11, 22] Various airway clearance techniques (ACTs) are used to loosen thick, sticky mucus so it can be cleared from the lungs by coughing or huffing. The major ACTs are summarized below.

Chest physiotherapy (CPT) is an ACT to drain the lungs, and may include percussion (clapping), vibration, deep breathing, and huffing or coughing.[11] Clapping or percussion is the manual external striking of the chest wall with a cupped hand or mechanical device in a rhythmic fashion to loosen secretions from the bronchial walls. The patient is usually positioned in such a way as to enhance mucus drainage (postural drainage). CPT is often prescribed 2-3x/day for 20 min at a time, therefore it is demanding and energy draining for both the patient and caregiver. Additionally, CPT is uncomfortable and can be extremely painful for the patient (58 Newtons (N) of force is utilized). It can also lead to rib cage fractures in the elderly and patients with osteoporosis. Due to these reasons, adherence to CPT is low in adolescent and adult patients. Furthermore, it can be contraindicated for babies, individuals with artificial airways or breathing tubes and individuals who have gone through surgery.[22, 23] However, since CPT has been in use with beneficial effects for roughly 4 decades, since no device is required and since it encourages expectoration of sputum, it has become the “gold standard”.

High frequency chest wall compression (**Vest therapy**) is another form of ACT. An inflatable vest that fits snugly around the thorax is used to apply high frequency, small-volume expiratory pulses to the external chest wall. Negative trans-respiratory pressure is generated by short, rapid expiratory flow pulses (2–25 Hz), thereby mobilizing airway secretions.[11] Vest therapy has been reported to cause pain, chest tightness, chest restriction, migraines and a feeling of claustrophobia. Additionally, as with CPT, Vest therapy cannot be used in babies or in patients with artificial airways.

Positive expiratory pressure (PEP) and **oscillating PEP** airway clearance devices (*i.e.,* Acapella, Flutter, Cornet) can also help prevent complications of respiratory diseases, such as lung infections. These are portable devices and hold the airways open ('stent' them) to facilitate clearance. Some of them vibrate the airways as well, which can help move mucus from smaller airways to larger one where it is easier to cough it out or swallow it.[24] Limitations of these devices are that they are awkward and difficult to hold for an entire treatment, and they cannot be used in neonates or the paediatric population.

Natural techniques such as forced expiration (FET), autogenic drainage (AD) and active cycle forced breathing (ACBT) open the airways and aid in mucus drainage. These require no external equipment, but require practice, can involve dizziness and discomfort, and are impractical for young patients. Exercise is also a very important form of ACT, as it moves mucus and naturally opens the airways, in addition to strengthening muscles associated with breathing and coughing.

Regardless of the technique, clearing the airways is critical to help to decrease lung infections and improve lung function. Personalized medicine must be applied to airway clearance, considering individual patients' lung pathology, clinical, functional, environmental and social factors, as well as the physiological concepts underlying ACTs.[25]

5 THE FREQUENCER™ – ACOUSTIC AIRWAY CLEARANCE TREATMENT

5.1 Introduction to the Frequencer

The available ACTs are mechanical in nature, and as outlined above have many limitations, resulting in inadequate mucus clearance, a decline in condition, and often hospitalization. Mechanical waves cause strong surface effects but diminish rapidly with distance travelled into the chest. The Frequencer was developed as an innovative solution for ACT by promoting bronchial drainage with acoustic (sound) waves at an optimized frequency that easily travels through the chest causing the lungs to vibrate and the viscosity of mucus to decrease, making it more liquid and easier to expectorate. While acoustic waves are weaker at the surface, they penetrate the chest wall more easily than mechanical waves due to their ability to propagate through air and water (see reference 2 for a thorough review of principles behind the Frequencer's effectiveness in clearing mucus from airways).

The Frequencer is non-invasive and digitally controlled, consisting of a control unit and a transducer.[26] The transducer is a disc like device that is manually applied to the front of the chest. The user adjusts the frequency of the transducer such that it causes a sympathetic resonance felt by the patient within the thorax. It can be positioned selectively on different areas, particularly the low lung areas, with no discomfort or pain. Only 1.9 N of force is used to seal the transducer to the chest wall (*vs.* 58 N of force used in clapping). Furthermore, delivery of the treatment is always consistent. The concept and design resulted in Louis Plante, the inventor, winning the 1st Patient Innovation Award in the Patient category for the Frequencer.[27]

5.2 Applied *in vitro* studies with the Frequencer

The Frequencer matches human lung resonance frequency of ~37-42 Hz[28], and Dymedso has validated that repetitive sound waves at 40 Hz is the optimal frequency to reduce the viscosity of mucus.[29, 30] An early internal *in vitro* study (Dymedso, 2004) demonstrated a reduction in mucus viscosity due to vibration. Briefly, mucus was vibrated under various conditions for a given time at specified amplitude and then allowed to flow through a capillary tube under constant pressure. Stickier and higher viscosity fluids take a longer time to flow through than less adherent, thinner fluids. Thus, faster flow times for a given amount of mucus indicate increased mucus mobility. In all cases, agitated mucus flowed more freely than unaltered mucus. When mucus was agitated mechanically, its viscosity changed less than when it was vibrated with the Frequencer. Moreover, direct agitation by the Frequencer was the most effective (40 Hz for 1 min at half power) and flowed an average of 25% faster than unaltered mucus. A manuscript published by Cantin et al.[29] confirmed the original findings. The application of the Frequencer to a simulated mucus preparation of 40 mg/mL mucin resulted in a significant acceleration of the flow of the mucin solution as measured by a capillary rheometer. The Frequencer-treated mucin preparations reached flow rates higher than control and similar to those treated with a vortex mixer (control = 1.02 ± 0.01 mm/sec, vortex = 1.22 ± 0.02 mm/sec, Frequencer = 1.22 ± 0.02 mm/sec, $P < 0.001$ each *vs.* control; $P > 0.05$ vortex *vs.* Frequencer, n = 8).

Another published study[30] tested normal and pathological synthetic mucin solutions (1 % and 4 % by weight, respectively) *in vitro* with the Frequencer. Forty-eight (48) experiments were conducted by adding mucus to a plastic cylindrical reactor with an external diameter corresponding to that of the opening of the Frequencer adaptor. The frequency applied was varied from 20 Hz to 60 Hz and the amplitude from 50% to 100% intensity. Additionally, the effect of NaCl on mucus rehydration was assessed to determine the optimal frequency and intensity for optimal mucus rehydration and rheology. The Frequencer proved to be effective in the homogenization of a synthetic mucin solution in 20 min. A working frequency of 40 Hz and a 0.5 gL^{-1} NaCl solution were the optimal parameters to obtain a partial rehydration of mucus, regardless the amplitude selected. Additionally, the treatment of a 4% mucus solution with 0.5 gL^{-1} NaCl at 40 Hz provided adhesion values 17 % and 25 % lower than those obtained for a 1% by weight mucus solution, indicating that the Frequencer elicited improved effectiveness when the environment was richer in mucins. The authors concluded that with an operating frequency of 20 Hz to 65 Hz, the Frequencer provides an efficient and gentler therapy than traditional CPT.

In addition to the reduction of mucus viscosity through repetitive vibrations as described above, other potential mechanisms of action of the Frequencer, reviewed by Cantin and Bacon[2] and Cantin et al.[29] include shearing at the mucus/airway interface induced by resonant energy targeted to specific locations in the lungs, a combination of mechanical and acoustical coupling from the chest wall that transmits vibrations to small and large airways, peristaltic action due to longitudinal waves, and acoustic streaming and related phenomena thus reducing mucus adhesion.

Several aspects of the Frequencer indicate that it is a safe device. First, the sound pressure level was measured at 56 – 78 dB in free air at a frequency range of 25 - 70 Hz at maximal power, which is well below the threshold for hearing damage.[31] Second, stray magnetic fields are minimal, due to the neodymium motor structure. Third, the unit is isolated from electrical shock, and the transducer operates at 20 VAC or less. Finally, the constant force applied to the body is about 16 N in order to produce an acoustic seal, yet the Frequencer can provide peak pressures of about twice that of the high frequency chest wall oscillation (HFCWO) device. The dynamic force applied by the Frequencer's mechanical and acoustical vibrations is between ~0.4 N and 3 N, depending on the degree of coupling between the apparatus and the body.

5.3 Applied clinical science of the Frequencer

Two Institutional Research Board (IRB)-approved published clinical studies provide evidence that the vibrations generated by amplified low-frequency audio at the chest wall induces sputum production in patients with CF. Additionally, smaller proof-of-concept (POC) clinical trials of the Frequencer have been performed on specific patient populations in clinics and hospitals with excellent outcomes. Two such trials are highlighted below: i) on adult patients with severe chronic pulmonary disease in a Physiotherapy Clinic and ii) on premature neonates and children with severe pulmonary issues in ICUs and the Paediatric Ward.

The first published clinical study[29] compared the Frequencer to conventional CPT using expectorated sputum weight as the main outcome measurement. Twenty-two (22) stable adult patients with an established diagnosis of CF and an FEV1 ≥ 35% of predicted were included in the study. CPT and Frequencer therapies were separated by 12 – 24 hours in each patient, with 11 receiving the CPT first, and 11 the Frequencer therapy first. CPT was delivered for 20 min with clapping and postural drainage. Frequencer therapy was applied for 20 min, with applications of 5 min to the anterior and posterior areas of each hemi-thorax. Frequencer treatments were performed with the patient sitting, and no attempt was made to combine the Frequencer with postural drainage. For both treatments, sputum was collected during the 20 min of treatment and for 5 min following the end of treatment and weighed. The amount of sputum produced during CPT was statistically similar to that produced with the Frequencer (CPT = 9.27 ± 1.20 g *vs.* Frequencer =9.24 ± 1.62 g, $P=0.97$, $n=22$). The authors concluded that both therapies provided equivalent efficacy. Additional benefits included the ease of use of the Frequencer and the potential to use without the aid of a caregiver.

The second published clinical study[32] involved adding the Frequencer to the University of California San Diego Medical Center's inventory of airway secretion clearance devices for CF. Sputum from 12 adults was collected (84 treatments). The majority (75%) of the treatments were productive (n=17 for 1 to 4 mL of sputum, 23 for 5 to 10 mL of sputum, 3 of 11 to 15 mL of sputum and 20 swallowed sputum), while 25% of the treatments were unproductive (n=19 no sputum and 2 scant sputum). The authors considered these acceptable results of sputum production and concluded that the Frequencer could safely be considered as one of the preferred or primary choices by the patient and the Respiratory Care Practitioner.

Although not published, a POC study was performed in the Physiotherapy Department at Maisonneuve-Rosemont Hospital, Montreal.[33] Adults with severe chronic pulmonary disease participated for 3 weeks. During the first 7 days, expectorated mucus was collected via the usual therapy (Acapella, Flutter, Percussor or clapping treatments) and weighed. Treatment with the Frequencer was started on day 8 for 14 days. The number of daily treatments varied according to individual needs of each patient. The Physiotherapists noted an immediate increase in the amount of mucus collected in patients who had difficulty in expectorating due to lack of coughing strength, thickening of the mucus or for any other physiological reason. Cessation of clapping treatments for the majority of the patients occurred. Two Frequencers were purchased as a result of the POC and subsequently 26 patients with CF and bronchiectasis have benefited from the technology, verbalizing that they feel more air in their lung, it reduces shortness of breath, and they are able to increase their daily activities.

Another POC example is from the California Pacific Medical Center[34] where the Frequencer was utilized on premature neonates to five-year-old children with lung collapse, pneumonia, CF, RSV bronchiolitis and neuromuscular disease in ICUs and the Paediatric Ward. The Respiratory Clinical Coordinator reported that the Frequencer was utilized on the sickest patients with almost immediate results. It assisted with lung recruitment and secretion removal, reducing the length of hospital stays. One specific example is that of a premature baby (500 grams) where the Frequencer opened the patient's collapsed lung and the patient was weaned off mechanical ventilation.

It is interesting to note that numerous clinical trials have been initiated but not completed due to overwhelmingly positive results in the first few patients. For example, an IRB-approved clinical trial was approved at the Children's Hospital Los Angeles in paediatric ICU patients with artificial airways (either endotracheal or tracheostomy). Data from the first 2 patients indicated that treatment with the Frequencer produced significantly more secretions by weight than standard CPT. Parents and nursing staff felt the device was safer and more effective than CPT, and requested continued use of the Frequencer.[35] Therefore, the device was purchase without completing the clinical study.

Additional clinical studies are currently in progress or planned. Representative studies include a 20-patient CF study that is in progress at the National Korányi Pulmonology Institute, Hungary[36], which aims to compare the Frequencer and Vest therapy in hospitalized, mild, moderately serious or severe but stable adults. Respiratory function, oxygen saturation and sputum production will be the primary outcomes. Additionally, a two-stage prospective crossover randomized study (n=60 patients less than 2 years old) has been approved by the IRB (waiting Health Canada approval of prototype) for critically ill infants with respiratory distress in pediatric ICUs in Canadian Academic Children's Hospitals.[37] CPT will be compared to treatment with the Frequencer, with tolerance, feasibility and physiological effects being end-points. This study will provide valuable knowledge regarding secretion management in critically ill children. Having a more efficient and better-tolerated procedure for the airway clearance can exercise a large impact on the respiratory management in pediatric ICUs. It will not only produce evidence for current practice but also contribute to more efficient health care practice and resource utilization (*i.e.,* Physiotherapist) and improved quality of care.

Last, and perhaps most critically, a fast-track IRB review request has been requested for a clinical study for use of the Frequencer in 50 patients admitted to hospital for treatment of COVID-19 (CHU Sainte-Justine, Montreal, Canada).[38] A cross-over randomized clinical trial is proposed that will compare the effectiveness of the Frequencer *vs.* CPT for mobilizing secretions in ICU patients for respiratory distress linked to COVID-19 infection. Rationale for the study include expected improvement in the respiratory status of patients with COVID-19 due to the ability for increased treatments *vs.* CPT, potential rapid adoption at other hospital centers, reduction of the impact of COVID-19 on the health system, decreased exposure of physiotherapists to COVID-19 and improved working conditions in ICUs.

5.4 Case studies and testimonials with the Frequencer

Although there are a limited number of large-scale clinical studies (*i.e.,* more patients would be required to detect smaller differences between treatments), numerous case studies and testimonials exist from every age-group (neonates, pediatrics, adolescent, adult, and geriatric) and on a wide-range of lung diseases and conditions including CF, PCD, COPD, NMD, bronchiectasis, pneumonia, RSV, patients with ventilators/tracheostomies or lung collapse and patients awaiting lung transplant.[39] Initial use of the Frequencer often occurred while the patient was in the hospital, reinforcing the fact that current therapies were not possible or were ineffective due to various reasons such as size / age of the patient (propensity for fractures with other treatments, too young to successfully use other modalities such as Acapella and Flutter), discomfort or severe pain (pressure and vibrations of other treatments causing migraines, chest and abdominal pain) or other conditions which preclude the use (*i.e.,* pregnancy, arthritis, osteoporosis, leg ulcers). All case studies and testimonials indicate that the Frequencer is superior to other existing ACT, with reports of easier mucus clearance and expectoration at the recommended frequencies from 37 Hz to 42 Hz. Furthermore, Key Opinion Leaders (KOLs) in the medical community clearly understand the power of the Frequencer as evidenced by their testimonials, requests for purchase of the device (31 requests on record from 2017-2019) and their desire to perform clinical trials. In fact most insurance requests indicate that no other treatment option exists, the Frequencer is a medically necessary device that will be used for the rest of the patient's life, and the Physician is confident that use will result in reduced hospitalizations and exacerbations.

The CF population has the largest experience with the Frequencer. Thirty (30) case studies and testimonials have been gathered from patients of all genders and ages in 20 centers in the US and Canada. The majority of the patients had exhausted all other treatment means and their lung function was declining. Repeatedly, both patients and Physicians reported greater clearance of pulmonary secretions (*i.e.,* increased sputum production), a dramatic increase in coughing efficiency after treatments, consistent or increased pulmonary health and decreased number of infections and hospitalizations after starting use of the Frequencer. Other advantages reported were more frequent usage, ease of use, autonomous use by patient, comfort, convenient re-positioning (allowing targeting of specific effected lung areas) and better airway clearance position. Five representative CF case studies / testimonials are summarized below:

1) The Physician (Columbia University Medical Clinic) of a 27 year-old female who has been using the Frequencer at home since 2008 reported a significant lung function increase (68% to 81 % PFTs), marked decrease in symptoms and a reduction of antibiotic use in his patient (only required IV antibiotics once in past 3 years *vs.* 4 times in 3 years prior).
2) The Physician (Children's Hospital of Pittsburgh) of a 24-year old male struggling to keep pulmonary status and keep airways clear with declining PFTs had a 3-week home trial of the Frequencer. His physician indicated that his cough was more productive than with the Vest and he had increased sputum production. The patient commented, "I've never had such an effective mode of airway treatment."
3) The mother of a 9-year old male (Quebec, Canada) reported his son's health has been consistent since starting use of the Frequencer at home 3 times daily for 20 min (as it was with the clapping therapy). His FEV is consistent and within the normal range, and since using the Frequencer he has had no hospitalizations. His mother indicates that a huge benefit is autonomy as her son can use it himself, therefore increasing her son's and her family's quality of life.
4) The Physician (Children's Hospital of Philadelphia) of an 18-year old female with coughing, wheezing and low pulmonary function showed superlative improvement of condition after only 10 days of Frequencer use. She had a substantial decrease in cough, chest tightness and overall symptoms and was able to produce clear white sputum (she was not able to produce sputum with CPT). Her FEV1 increased from 2.12 L to 2.35 L and FVC from 2.96 L to 3.37 L. The Physician indicated the Frequencer is the main reason that hospitalization was not required.
5) Last, a blog (A Matter of Life and Breath) from a young women journaling her experience with CF while waiting for a lung transplant quotes the following about the Frequencer: "I

love it! It works like CPT only using sound waves that go through your body. You don't feel squeezing or beating, but then when you speak your voice vibrates just like the vest! You hold the little speaker over each part of the lung for 2 min, just like CPT (front only - it travels through muscle and gets both sides of the lobe), and it works wonders. I cough up tons every time I use it. Highly recommended to try out, especially for people who want a basically silent, tiny, and super effective alternative to the vest."

Additional case studies and testimonials have been gathered from patients with PCD (n = 1), degenerative neuromuscular diseases (n=2+), bronchiectasis (n=4) and neonates with respiratory failure due to multiple birth defects (n=4) from 9 centers in the US and Canada. Both Physicians and patients report benefits similar to that reported above in CF patients. For example:

1) A case study of a 7-year old male (Quebec, Canada) with PCD and repeated hospitalizations due to infection reported that infection completely subsided within 2 days of the onset of infection after daily use of the Frequencer (previously infections would last 1-2 weeks). Coughing efficiency increased dramatically after treatments. The expectorations were large and a yellow color, a result that the patient had never experienced before.
2) A testimonial from a Physician (Sunny Upstate Medical University) reported great success with several NMD patients who have not responded well to the Vest. Specifically, one NMD patient was facing a tracheostomy intervention. Remarkably, use of the Frequencer improved his condition so much that he no longer is a candidate for a tracheostomy.
3) A Physician (Cleveland Clinic) reported that the Frequencer successfully mobilized secretions in a 63-year old male patient with bronchiectasis who could not use a Vest due to worsening of leg ulcers. The Frequencer did not worsen the leg ulcers.
4) A Physician (Stanford) reported use of the Frequencer in a ventilator/tracheostomy dependent infant with chronic respiratory failure due to multiple birth defects and infection. The infant was in the ICU for her first 8 months and used the Frequencer twice daily, which was increased to 4 times daily when she had infections. The Frequencer increased moderate thick white secretions and the infant was allowed out of the hospital at 9 months, but requires home treatment, as no other option is available.

Clearly, the case studies and testimonials are powerful and support the efficiency of the Frequencer. Analysis of 78 patient surveys collected between 2007 to 2020[40] indicates that patients prefer the Frequencer *vs.* their standard CPT. Seventeen (17) questions were included in the survey, and answers were provided on scale of 1 (strongly disagree) to 5 (strongly agree). For all questions, the Frequencer scored higher on the satisfaction scale, and in many cases these values were statistically significant compared to the standard ACT. The Frequencer was preferred for the following reasons: helps to cough up mucus; helps to breath better; helps during illness or infection; helps to maintain lung function over time; effective; simple; can be done anywhere; easy to fit into daily schedule; allows better use of time; convenient; does not cause pain or discomfort; does not cause excessive coughing spells; does not cause difficulty breathing; does not cause other physical problems; well tolerated. Overall, users indicated they were highly satisfied with the Frequencer (satisfaction of 4.6 *vs.* 2.8 with other CPT) and would like to continue with the Frequencer (satisfaction of 4.67 *vs.* 2.43 for other CPT).

It can be concluded from the clinical studies, case studies / testimonials and user surveys that the Frequencer is effective and consistent at clearing pulmonary secretions. Use has been shown to reduce hospitalizations and infections. It is convenient, painless and easy to use, allows targeted treatment to specific problematic lung areas, and increases patient compliance. Additionally, it can be successfully used on a wide range of patient populations (all ages and physical conditions) and in various settings (hospital, clinic, ICU and home).

6 THE MARKET FOR AIRWAY CLEARANCE DEVICES

As reviewed in Section 4.2, there are a vast array of respiratory conditions and infections that require mobilization of secretions. In addition to genetic diseases, threats to lung health are everywhere, and they start at an early age when children are most vunerable. Everyone who breathes is vulnerable to the infectious and toxic agents in the air.

The relationship between different airway diseases are often complex, for example, COPD and asthma are often misdiagnosed and, because of the overlapping symptomatology, patients with bronchiectasis are particularly at risk of misdiagnosis. Furthermore, the diseases can co-exist[12] and there may be overlap in the prevalence. This makes it difficult to estimate the entire addressable market. Regardless, the market for a device such as the Frequencer is enormous, as presented in **Table 1** and summarized below. Very conservative estimates indicate that the total global addressable market is between **120 and 134 million** devices.

As can be evidenced by the data presented in **Table 1**, the prevalence of airway clearance diseases with a genetic underpinning is relatively rare (*i.e.,* 1/3200 for CF (Caucasians); 1/16,000 for PCD; between 4 and 8/100,000 for ALS; 2/100,000 for SMA; 5/100,000 for DMD (males); 1.5/100,000 for BMD (males); 16/100,000 for other muscular dystrophies). However, cumulatively, the numbers quickly add up. In the US alone, where good records are available (**Table 1**), ~150,000 people are affected with the diseases listed above. Simply extrapolating to the world's population, this would be a prevalence of **3.4 million** cases. Furthermore, these patients require ACT daily (or multiple times daily) for long periods in their lives, many from early childhood to middle age, and would all benefit from a device that could be used regularly, at home.

The prevalence of COPD and asthma (see **Table 1**), which are largely linked to smoking or exposure to other noxious particles or gases, is enormous. Worldwide, it is estimated that there are ~251 million cases of COPD and between 235 and 339 million cases of asthma. Around 3.71 million death were attributed to COPD in 2015, while asthma results in ~500,000 deaths/year.

Table 1: Prevalence and death rates of the major airway clearance diseases and disorders (references are listed in Section 8.2).

Disease	Prevalence (worldwide)	Prevalence (specific regions)	Survival / Death
CF	• 1/3200 Caucasians[1] • 70,000 cases[2] • 1000 new diagnoses/yr	• US - ~30,000 cases (1/2500 – 1/3500)[2] • Ireland - 1/8000; Finland - 1/25,000[1] • Under-diagnosed in Asia & Africa[2]	• Median predicted survival (2010-14) = 39.3 yrs[3] • Survival rate is increasing • Median predicted survival for those born in 2010 = 56 yrs[4]
COPD	• 251M cases[5, 6] • 65M with severe disease[7] • Under-diagnosed[8] • Increasing worldwide	• US – 16M cases[9, 10]	• 4th leading cause of morbidity and death • 3.17M deaths in 2015 (5% of all deaths globally)[6] • 90% of deaths in low- & middle-income countries[6]
Asthma	• 235[11] - 339.4M[12] cases; 100M more may be effected by 2025[12] • 3.6%[13] to 10%[14] with severe disease (8.46 – 33.9M) • Most common chronic disease in children, > 30% of all paediatric hospitalizations[12, 15] • Increasing worldwide • Under-diagnosed & -treated	• US – 25M cases (7.7% in adults and 8.4% in children)[16]	• Globally ~500,000 deaths/yr[12] • US – 10 people die/day from asthma; in 2017, 3,564 people died [16] • > 80% of deaths occurs in low- & lower to middle-income countries
Bronchiectasis (not CF-related)	• 701/100,000, most in the Western World[17] • No comprehensive prevalence datasets for China, India or Africa • Increasing, especially in US[17]	• US – between 340,000 & 522,000 cases; 139/100,000 adults with up to 812/100,000 (75 yrs+); 70,000 adults diagnosed/yr[18] • UK - 1200/100,000 (65 yrs+)[17]	• Unknown
PCD	• 1/16,000 individuals[20]	• NA	• No reliable demographic data • Anecdotal evidence suggests a reduced lifespan[21]

Disease	Prevalence (worldwide)	Prevalence (specific regions)	Survival / Death
ALS	• Between 4.1/100,000 and 8.4/100,000[22]	• US - 5,000 diagnosed each year; ~16,000 have the disease at any given time[23]	• Average age of diagnosis is 55 yrs • Average survival time is 3 yrs after diagnosis; ~20% survive 5 yrs, 10% live 10 yrs and 5% 20 years or longer[22, 23]
SMA	• 2/100,000[24, 25]	• US - between 10,000 & 25,000 cases	• Type 1 SMA survival only a few years • Other types can survive into adulthood[25]
Muscular Dystrophies	• 16/100,000 combined[26] • 5/100,000 males for DMD[27] • 1.5/100,000 males for BMD[27]	• NA	• Depends on type • All require significant intervention & treatment throughout life
Pneumonia	• 150M new cases/yr among children < 5 yrs, accounting for ~10-20M hospitalizations[28]	• Highest in the developing world • Africa - one of the most frequent reasons for hospital admission[29]	• 18% of all deaths in children = 1.3-1.6M deaths worldwide[30] • 1 in 10 patients in hospital in Africa die[29]
Influenza and RSV	• 3-5M/yr get severe flu[31] • 34M episodes/yr of RSV[29] and 3M hospitalizations[31]	• NA	• As many as 500,000 deaths/yr of flu[31] • 250-000 – 500,000 deaths/yr in US of flu[29] • RSV kills ~66,000-199,000 children/yr[31]
Pandemics	• SARS - 8098 during 2003[32] • COVID-19 - 750,890 confirmed cases (Mar 31/20)[33]; early data indicate ~20% require hospitalization, 13.8% have severe disease with respiratory issues, 6.1% have critical illness with respiratory failure[34,35]	• SARS – 8 in US during 2003 • COVID-19 – see latest WHO Situation report	• SARS – 774 deaths in 2003[32] • COVID-19 – 36,405 deaths (Mar 31/20)[33]
Lung Transplant	• 3500 on waiting list[36]	• 2714 in 2019 in the US[36]	• NA
Preterm Births	• 15M/yr[37]	• US - ~500,000/yr[37]	• ~1M deaths/yr[37] • Preterm birth complications are the leading cause of death in children < 5 yrs • More deaths in low- & middle-income countries

Asthma is the most common chronic disease in children. Both diseases can be severe, and it is proposed that these individuals would be prime candidates for a device such as the Frequencer; there are ~65 million people with severe COPD and up to 34 million people with severe asthma, totaling **100 million** cases worldwide. Bronchiectasis affects ~700/100,000 individuals the Western World and total cases are between 340,000 and 522,000 in the US (**Table 1**). Although the prevalence is high, bronchiectasis is major lung anomaly of COPD and asthma, and it is difficult to separate the prevalence numbers from these diseases.

Overall, respiratory infections account for more than 4 million deaths annually and are the leading cause of death in developing countries.[41] Pneumonia, influenza and RSV affect a very large proportion on the population yearly. If one considers that those hospitalized or with severe cases (10-20 million children hospitalized with pneumonia; 3 to 5 million people with severe flu; 3 million hospitalized with RSV; **Table 1**) would be candidates for ACT, a total market of **16 to 30 million** results.

Experience with SARS, MERS, pandemic influenza and other outbreaks has shown that as the epidemic evolves, an urgent need is required to expand treatment options. The appearance of COVID-19 is no exception, and there is critical and immediate need for simple and effective airway clearance devices in untold numbers of hospitalized patients. These never-before-seen zoonotic infections have risen in the past two decades as pandemic potential, due to the smaller distance of reservoirs of disease to humans (i.e., open markets, smaller natural habitats) proximity to humans.[42] Compared with SARS and MERS, COVID-19 has spread more rapidly, due in part to increased globalization and the focus of the epidemic. Another novel virus is not unlikely in the future; unfortunately, this market may not disappear at the end of COVID-19.

Two additional important markets are that of pre-term birth and lung transplant (**Table 1**). In the case of pre-term births, the respiratory system is almost always underdeveloped. There are 15 million pre-term births per year worldwide, with ~1 million deaths. Three-quarters of preterm deaths (**750,000**) could be prevented with current, cost-effective interventions[43], which includes the use of a Frequencer. Last, it can be assumed that those awaiting lung transplant (**3500** worldwide) would require almost daily ACT.

There are a number of factors that indicate that the numbers above are conservative, and the market will rapidly grow. **First**, the survival rate of many diseases has increased. For example, over the past twenty years, the median survival of CF patients has increased[44], and it is predicted that patients born in 2010 will survive to 56 years old.[45] This means that these patients will require ACT longer. **Second,** many of airway clearance conditions are under-diagnosed and under-treated, especially in low and middle-income countries. Overall, COPD tends to be under-diagnosed and more coordinated global criteria for diagnosis are required.[46] Over 80% of asthma deaths occurs in low and lower-middle income countries, but death rates are often attributed to other complications. **Third,** the prevalence of diseases and airway infections are likely to increase due to the global shift in aging, tobacco use, and environmental exposures. For example, COPD is likely to increase in coming years due to higher smoking prevalence and aging populations in many countries.[41] A marked increase in prevalence of bronchiectasis, particularly of severe disease is observed in the elderly.[47] The occurrence of many infectious respiratory diseases is affected by climate and its corollary, air pollution. Climate change affects the incidence and severity of respiratory infections by their affecting vectors and habitats and changing the transmission patterns of viruses.[48] Weather events may alter human host response and susceptibilities to infectious and non-infectious diseases. **Fourth**, one person could require treatment with the device at multiple sites (home, physiotherapy clinic, hospital, ICUs), and justification could easily be made for at least 1 device in every neonatal, paediatric and adult ICU, as well as in every respiratory physiotherapy clinic.

7 CONCLUSIONS

Although drug and gene therapies have undergone significant optimization, complementary mechanical procedures for effective bronchial drainage are required for individuals whose function of the mucociliary escalator and/or cough mechanics is altered. Airway clearance therapy has been the cornerstone aimed at minimizing the devastating effects of airway obstruction, infection and inflammation due to mucus stasis on the conducting airways and lung parenchyma. The available ACTs are mechanical in nature, and have many limitations, resulting in inadequate mucus clearance, a decline in condition, and often hospitalization.

The Frequencer is the only ACT on the market that uses acoustic waves (at 40 Hz) to cause the lungs to vibrate and the viscosity of mucus to decrease. Mechanism, efficacy and practical utility of the device have been validated in a large number of *in vitro* and clinical situations. Clinical studies provide evidence that the vibrations generated by amplified low-frequency audio at the chest wall induces sputum production in patients. Additionally, case studies and testimonials show that the Frequencer offers many benefits over standard ACT including consistency, convenience, comfort, ease of use, targeting of specific problematic lung areas, and overall increase in patient compliance. Real-world use demonstrates that the Frequencer can be successfully used on a wide range of patient populations (all ages and physical conditions) and in various settings (hospital, clinic, ICU and home). In many cases, the Frequencer was the only remaining treatment option.

Significant markets include pulmonary disorders such as CF, COPD, asthma and bronchiectasis, PCD, progressive NMD disorders, and acute respiratory infections such as pneumonia, influenza, RSV and novel viruses (SARS, Ebola, COVID-19). Pre-term / critically ill neonates and lung transplant candidates are other markets. Very conservative estimates indicate that the total global market is between 120 and 134 million devices.

It can be concluded that the Frequencer is a powerful tool to prevent the suffering, hospitalizations, infections and fatalities related to airway clearance diseases and disorders.

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9 APPENDIX – AUTHOR BIOGRAPHY

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