UNIVERSITÁ DEGLI STUDI DI MILANO

DOTTORATO IN SCIENZE FISIOLOGICHE MORFORLOGICHE E DELLO SPORT

Sezione di Fisiologia

CICLO XXVIII

Tesi di Dottorato di Ricerca

DIAPHRAGMATIC MOBILITY, LUNG HYPERINFLATION AND EFFECTS OF THE PULMONARY REHABILITATION

Dottorando:

Camilo Corbellini

Matricola: R10266

Tutor: Dott. Luciano Zocchi

Coordinatore: Prof.Michele Mazzanti

Anno Accademico 2014-2015

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ACKNOWLEDGEMENTS

I want to thanks all my patients for every single breath that I borrowed from their lives.

To Elena and Giacomo, who gives me, everyday, love and strength to keep breathing.

To my dear colleagues who helped me on the data collection

To my beloved mom and dad

"All we need is love…

…love is all we need!"

THE BEATLES

“ Hey ho…let’s go!!!”

RAMONES

INDEX

I. ABREVIATION LIST

ABGA: arterial blood gases analyzes

BMI: body mass index

bpm: beats per minutes

CI: confidence interval

COPD: Chronic Obstructive Pulmonary Disease

CS: mitochondrial activity

D*L*,CO: carbon monoxyde lung difusion

DM: diaphragmatic mobility

EELV: end expiratory lung volume

ERV: expiratory residual volume

f: respiratory frequency

FEF $_{25-75}$: forced expiratory flow on 25% to 75%

FEV$_1$: forced expiratory volume on 1sec; cm

FRC: functional residual capacity

FVC: forced vital capacity; centimeters

GOLD. Global Initiative for Chronic Obstructive Lung Disease

HC: healthy controls

HCO3 : bicarbonate

HR: heart rate

IC: inspiratory capacity

ICC: intraclass correlation coefficient

IRV: inspiratory residual volume

Kg: kilograms

L: liters

LDH: lactate dehydrogenase

LOA: limits of agreement

m: meters

MBS: modified borg scale

MHC: myosin heavy chains

MLC: myosin light chain

mmHg: millimeter of mercury

mpm: movements per minute

PaCO2: Partial arterial pressure of carbon dioxide

pH: Hydrogenionic potential

PR: Pulmonary rehabilitation

RV: residual volume

s: seconds

SD: standard deviation

SDP: slow deep inspiration;

SEM: standard error of measurement

SpO_2: partial oxygen saturation

TGV: total gas volume

TLC: total lung capacity

VC: vital capacity

Vt. Tidal volume

Yrs: years

II. FIGURE LIST

III. TABLE LIST

ABSTRACT

Rationale: The diaphragm pathophysiological changes occurring in chronic obstructive pulmonary disease (COPD) leads to functional inefficiency that strongly correlates to the loss of lung function. Muscle fiber shortening follows lung hyperinflation, resulting to a chronic mechanical disadvantage, which worsens in COPD exacerbations. The diaphragmatic mobility (DM) is mostly assessed with techniques that exposes the patient to risks. The ultrasonography on M-mode is easy to use, safe and measures directly the diaphragmatic dome displacement.

Goals: to determine whether the COPD impairs the DM, and verify improvements after an inpatient pulmonary rehabilitation (PR).

Methods: ultrasonography on M-mode assessed the rest breathing and the slow deep inspiration on 52 patients and 15 healthy controls. Lung functions test, arterial blood gas analyses, six minute walk test were also performed.

Results: after initial screening, 36 COPD patients ended the PR. The DM was lower on the slow deep inspiration on COPD patients and correlated with the COPD severity ($r=0.8$, $p<0.001$). The DM on rest breathing was higher for COPD patients and also correlated to the lung disease severity ($r=0.74$, $p<0.001$). After the PR the DM on the slow deep inspiration increases from 4.58cm±1.83cm to 5.45cm±1.56cm ($p<0.01$).

Conclusions: ultrasonography on M-mode showed the correlation between DM impairment and COPD severity. The PR improves diaphragmatic function.

1. INTRODUCTION

The diaphragm is a unique muscle that differentiates the mammals from the other species. In the human body it has the fundamental role of on the respiration, especially on the inspiration, and it can also participate on the swallowing and emesis(1). It has a dome shape and separates the thoracic from the abdominal cavity. Its contraction expands the lower ribcage(2) and the pleural pressure became more negative permitting the inspiratory airflow. It has great endurance and is capable to adapt its anatomy and muscular structure(3).

The main condition that modifies its anatomy and structure is the Chronic Obstructive Pulmonary Disease (COPD)(4). The COPD features a chronic inflammation on lungs and parenchyma and also leads the patient to a systemic inflammation. The progressive parenchyma destruction contributes to airflow limitation, decreasing on the lung elastic recoil, gas exchange and lung hyperinflation(5–7). Those modifications on airways and parenchyma reduce the diaphragm capacity to generate transdiaphragmatic pressure, placing the muscle at mechanical disadvantage (8).

On severe hyperinflated patients the diaphragm apposition zone to the rib cage is reduced, so the lung volume increases and the diaphragmatic dome descends (9). On COPD patients the oxidative stress and systemic inflammatory process leads to a general muscular atrophy. On the diaphragm, the muscular fibers loss its myosin, its sarcomeres become shorter and the muscular cross sectional area reduces (4). From a condition of the muscular atrophy effects and its influence on the diaphragmatic mobility raises the necessity for the diaphragmatic function assessment (10).

The anatomic diaphragm characteristic permits a very large range of techniques to assess its function. Radiography, fluoroscopy and magnetic resonance are usually used to evaluate this muscle but those techniques, for many reasons, are limited and often offers risk to the patient (11). The ultrasonography is already used to diagnose diaphragmatic rupture or thickness, pleural masses and pulmonary effusions (12). The M-mode can assess the diaphragmatic kinetics, measuring directly the dome cranio caudal displacement of this dome, which has been. It is more visible on the right diaphragmatic side(10,13). On COPD patients, such measurement can be useful to address whereas the lung hyperinflation, the mechanical disadvantage and muscular atrophy can reduce the diaphragmatic mobility when compared to healthy subjects and also verify eventually diaphragmatic function improvements after a period of Pulmonary Rehabilitation (PR).

The PR is a very complex intervention, based on the multidisciplinary patients assessment, focusing on exercise training, education and behavior changing (14). As the COPD has multiple manifestations and many comorbidities the principles of integrated care are adopted to treat these patients. The PR can cover a large range of non-pulmonary problems that aren't covered by the medical therapy (5). The exercise training, despite of its strategy, already demonstrated its validity to reach its goal suh the resistance and strength training for upper and lower limbs(15). Concerning the diaphragmatic function and mobility there is a lack of information on how the PR can affect or even improve its functionality.

Considering the progressive COPD's lung and muscular deterioration, and the nature of the anatomic and structural diaphragm modification on such individuals, it is imperative to determine whether the COPD changes the diaphragmatic mobility according to the disease severity. The functional status is the major PR outcome and can be measured in many different ways, as the spirometry, arterial blood gas analyses and walking test. Those tests are standard exams and its large applicability doesn't exclude the importance to introduce other technologies, especially when harmless and easy to use on the daily clinical practice, to access new functional outcomes. So the ultrasonography on M-mode can accomplish that goal and can go further filling those literature gaps providing information also on the PR effect on the diaphragmatic mobility, determining if the improvement on the functional status can also determine improvements on diaphragmatic mobility and lung function.

2. THE DIAPHRAGM

The diaphragm is the main inspiratory muscle, normally providing an increase in thoracic cavity volume corresponding to > 80 % of tidal volume during resting breathing (16).It is composed of two distinct parts, with different features, actions and innervations: the costal diaphragm and the vertebral (crural) diaphragm (17). The features of the diaphragm are unique among skeletal muscles, because, unlike the fibers of the other muscles, its fibers have only one of their ends (the peripheral ones) inserting on skeletal structures, while the proximal ones insert on a free central tendon. The peripheral ends of the crural fibers insert on the ventrolateral aspect of the first three lumbar vertebrae and on the aponeurotic arcuate ligaments, those of the costal fibers insert on the xiphoid process of the sternum and the upper margins of the lower six ribs. From their costal insertions, before folding radially towards the central tendon, the costal fibers, for a substantial fraction of their length, run axially in the cranial direction, remaining apposed to the inner aspect of the lower rib cage, with the only separation provided by the thin pleural space of the costo-phrenic sinus; the strip of inner surface of the rib cage directly facing the axially directed costal diaphragmatic fibers is defined "zone of apposition" of the diaphragm to the rib cage, and, unlike the more cranial portion of inner rib cage surface, which faces the lungs and is subjected to intrathoracic (pleural) pressure, faces the abdominal cavity and is subjected to abdominal pressure changes (18).

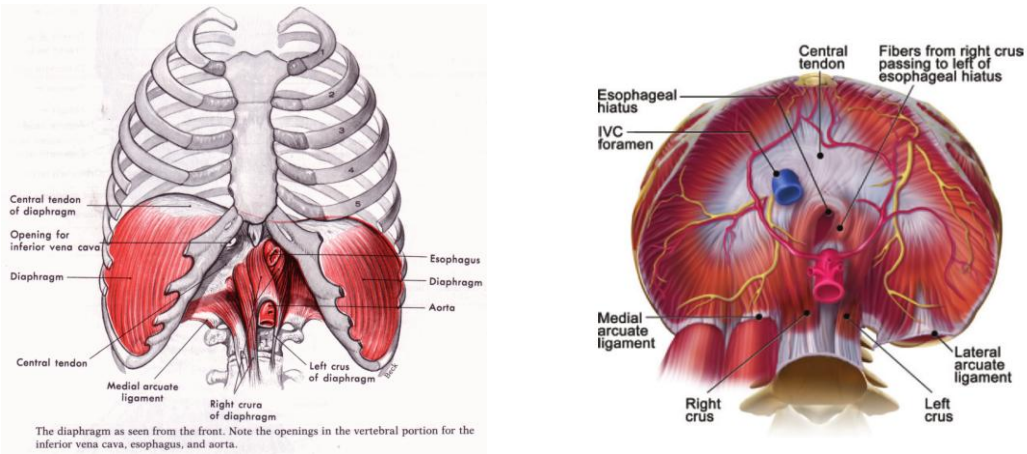


Figure 1: Schematic representation of the human diaphragm . Anterior view (left) and inferior view (right)

Diaphragmatic fibers receive their motor supply through the phrenic nerves, whose motorneurons, in humans, lay in the third, fourth, and fifth cervical segments of the spinal chord. The costal fibers receive somatic motor fibers from the 3rd and 4thcervical segment, while the crural fibers are mostly innervated by motorneurons of the 5th cervical segment (19).

Action of the diaphragm.

The costal and crural fibers of the diaphragm may be separately and selectively activated during non-respiratory tasks involving the muscle (e.g., speech production, cough, vomiting, posture and trunk motion, parturition); however, during normal inspiration they are co-activated. The inspiratory effect of diaphragmatic activation takes place through a combination of multiple actions on the chest wall; they are extensively reviewed in Macklem, 2005, and De Troyer, 2005, and are here briefly summarized. As a result of tension generation and shortening of the diaphragmatic fibers, a cephalad pull is exerted on the costal insertion, and a caudal pull on the central tendineous extremes: the rib insertions are lifted, the axial length of the apposed fibers diminishes and the dome of the diaphragm, essentially corresponding to the central tendon, descends relative to the costal insertions. The size and shape of the dome of the diaphragm remain relatively constant in normal breathing, however the dome's piston-like descent has a double effect: it expands the thoracic cavity along its cranio-caudal axis, and displaces caudally the abdominal viscera. The first effect produces a fall in pleural pressure which, in turn, decreases alveolar pressure and promotes inspiratory flow and lung volume increase, the second one increases abdominal pressure, which, in turn, pushes the ventral abdominal wall outward. In addition, the contracting costal fibers apply a force on the lower six ribs by way of its insertions: due to the axial orientation of these fibers, this force is directed cranially, and has the effect only of lifting the lower ribs, but the features of rib articulation with sternum and spine are such that the force developed also produces their outward rotation. Therefore, the more caudal part of the rib cage is lifted and expanded along its latero-lateral and dorso-ventral axes and the cross sectional area of the lower portion of the rib cage increases. This action of the diaphragm is referred to as the "insertional action". However, the diaphragm contracting alone has an opposing effect on the upper portion of the rib cage: because the diaphragm does not have any direct action on the upper ribs, the fall in pleural pressure caused by its contraction through the thoracic cavity expansion has an expiratory effect on the cranial region of the rib cage, "sucking" it inward, so that the cross-sectional area of the upper portion of the rib cage decreases during isolated diaphragmatic contraction. In normal breathing this effect of diaphragmatic contraction is not observed, because in normal breathing acts the action of the simultaneously contracting rib cage inspiratory muscles (parasternal intercostals and scalenes, invariably co-recruited with the diaphragm), overrides it, expanding also the upper rib cage by lifting and rotating outward the upper ribs (17,20).

Finally, the diaphragm has another inspiratory action on the lower rib cage, related to the existence of the zone of apposition. This zone, in fact, makes the lower rib cage represent a part of the abdominal boundary. In the costo-phrenic pleural recess between the apposed diaphragm and the rib cage, in fact, the changes in pressure occurring during breathing are equal to the changes in abdominal pressure: pressure in this recess rises, rather than falls, during inspiration, indicating that the rise in abdominal pressure is transmitted through the apposed diaphragm to expand the lower rib cage. The magnitude of this "appositional" force developed by the diaphragm on the lower rib cage through the rise in abdominal caused by its descent depends on the size of the zone of apposition: the greater the area of apposed costal fibers, the larger is the lower rib cage expansion observed during inspiration (17). It is likely, therefore, that in COPD, in which chronic pulmonary hyperinflation lowers the diaphragmatic dome, thus reducing the length of the rib cage-apposed costal fibers and, therefore, the appositional surface, the inflationary action of diaphragm contraction be reduced (9).

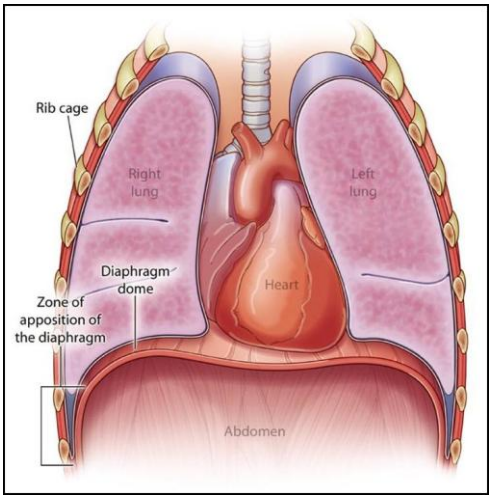


Figure 2 : Schematic representation of the diaphragm apposition zone to the rib cage

If the zone of apposition decreases, the inspiratory action of the diaphragm on the rib cage also decreases. Indeed, in anesthetized eviscerated dogs, it was found that diaphragmatic contraction causes a decrease, rather than an increase, in lower rib cage dimensions: in this experimental setting the abdominal compliance is markedly increased, so that the inspiratory rise in abdominal pressure is very small. In the setting of pulmonary hyperinflation, costal diaphragmatic fibers become oriented transversely inward from their costal insertions to the central tendon the radially, and the area of apposition decreases or, for extreme degrees of hyperinflation, vanishes. A larger fraction of the rib cage becomes exposed to pleural pressure: in these conditions, in addition to the loss of the appositional action of diaphragmatic contraction, the pull exerted on their costal insertions by the radially oriented contracting diaphragmatic fibers has an inward direction, such

that the insertional force decreases, rather than increasing lower rib cage diameters, and an expiratory, rather than an inspiratory, action on the lower rib cage is observed (21,22). These two effects of increasing lung volume account for Hoover's sign observed in subjects with emphysema and severe hyperinflation: an inspiratory decrease in the transverse diameter of the lower rib cage.

Biology and functional properties

The diaphragm is continuously active throughout life, and its fibers are resistant to fatigue, have elevated oxidative capacities and high capillary density. The biological and functional properties have been extensively. Diaphragm muscle mass in normal humans is about 260 g and is correlated positively with body weight, its thickness is about 35 mm, and its area is 750 cm^2 (3,23). The optimal length of the diaphragm for force generation normally occurs at a lung volume below functional residual capacity (FRC), with a sarcomere length reported to be 2.27 ± 0.15 μm in subjects with normal spirometry (24). In specimens obtained postmortem from subjects with normal respiratory function prior to sudden accidental death, the mean relative proportion of type I fibers was about 50%, the remainder being evenly divided between type IIa and type IIb fibers (25). In biopsies obtained from control subjects, the average fiber cross-sectional area in the costal diaphragm was 2,200 μm^2. In biopsy specimens taken during thoracotomy from patients with normal ventilatory function (FRC, TLC, residual volume [RV], forced expiratory volume in 1 second [FEV1]) type I fibers were about 54%, and type IIa and type IIb were 21% each (26). Data obtained brain-dead organ donors, and patients with mild impairment of pulmonary function, the proportions of diaphragm fibers containing myosin heavy chains I and IIa (MHC- and MHC-IIa are similar, while proportion of fibers containing MHC-IIb is low (27). The human diaphragm fibers contain six different isoforms of myosin light chain (MLC), with the same proportions of MLC-2s and MLC-2f. The mean number of capillaries per muscle fiber is about 1.9±0.1, ranging between 1.5 and 2.4. Type I fibers are surrounded by 4-6 capillaries and type IIa and type IIb fibers by only 3-5 capillaries. The diffusion distances between capillaries and muscle fibers are less for type I than for type II fibers (28). The metabolic properties of the costal and crural parts of the diaphragm differ from those of other respiratory muscles, having a higher mitochondrial activity (CS), with the costal part having a 20% higher oxidative capacity than the crural part. Conversely, the lactate dehydrogenase (LDH) activity is very low in the diaphragm compared with the other respiratory muscles. The lower LDH/CS ratio in the diaphragm indicates high oxidative and relatively low glycolytic capacity (29).

3. THE HUMAN BREATHING

The respiratory pump is a mechanical system comprising a number of different muscles cyclically activated and deactivated to provide ventilation, and the chest wall that forms the boundaries of the thoracic cavity. The respiratory muscles move the parts of the chest wall on which they insert, cyclically increasing or reducing the volume of the thoracic cavity and thereby ventilating the lungs

The respiratory muscles.

The main respiratory muscles are (a) the diaphragm, the most important among the primary (i.e., invariably recruited in inspiration) inspiratory muscles, divided into its costal and crural parts, which have different actions, anatomic origins, and spinal cord segmental innervations; (b) the inspiratory muscles of the rib cage, comprising the parasternal intercostal and the scalene muscles, other primary inspiratory muscles being invariably recruited during inspiration, and accessory inspiratory muscles as the external intercostal and sternocleidomastoid muscles; (c) the abdominal muscles, among which the transversus muscle appears to be the most important among the expiratory muscles, and (d) the expiratory muscles of the rib cage, including the internal intercostals and the triangularis sterni muscles. To a first approximation, the compartments displaced by the respiratory muscles are the rib cage and the abdomen. Due to the combined simultaneous contraction of the 3 groups of primary inspiratory muscles, normal inspiration occurs by outward displacements of the abdominal wall and expansion of the rib cage.

The chest wall.

For the purposes of description of the mechanical events taking place during inspiration, it is useful to describe the chest wall as follows (17,30). The chest wall can be modeled as a two compartment (rib cage and abdomen) structure. The two compartments are separated by a thin musculotendinous structure (the diaphragm), and are mechanically arranged in parallel. Expansion of the lungs can be accommodated by expansion of either the rib cage or the abdomen or by a variety of different combinations of individual expansions of the two compartments.

The displacements of the rib cage during breathing are related to the motion of the ribs. When the ribs are displaced in the cranial direction, their ventral ends also move laterally and ventrally, the cartilages rotate cranially around the chondro-sternal junctions, and the sternum is displaced ventrally: therefore, the action of any muscle that elevates the ribs is to increase both the lateral and the dorso-ventral diameters of the rib cage (inspiratory effect on the rib cage). On the other hand, the costovertebral joints of ribs 7 to 10 have less constraint on their motion. Therefore,

the ribs below the level of the xiphisternum (7th to 12th) have some freedom to move independently of the sternum, so that the lower region of the rib cage may be displaced independently of rib cage muscle contraction. Furthermore, this portion of the rib cage forms a part of the abdominal (not the thoracic) boundary (see below). For these reasons, the rib cage too should be considered as partitioned into two compartments, subjected to different forces and separately displaceable: the upper rib cage, exposed on its inner surface to pleural pressure (Ppl), and the lower part of the rib cage, exposed on its inner surface to abdominal pressure. As described later, diaphragmatic contraction causes opposite changes in the two pressures.

The respiratory displacement of the abdominal compartment is easily interpreted. First of all, with the exception of its ventral wall and of its upper limiting surface (the diaphragm), its boundaries (the spine dorsally, the pelvis caudally, and the iliac crests laterally) are virtually fixed and cannot undergo any displacement. Furthermore, since the abdominal content (neglecting 100 - 300 mL of abdominal gas) is liquid and, therefore, incompressible, any local inward displacement of one of its displaceable boundaries must be accompanied by an equal outward displacement of the other one. When the diaphragm contracts during inspiration (see below), its descent usually results in an outward displacement of the ventral abdominal wall; conversely, abdominal muscle contraction causes an inward displacement of the ventral abdominal wall resulting in a cranial push of the diaphragm into the thoracic cavity.

4. CHRONIC OBSTRUCTIV PULMONARY DISEASE

Definition

The definition of Chronic Obstructive Pulmonary Disease (COPD) is not hermetic. Actually this acronym does not fits well enough the complexity of these miscellaneous lung diseases that causeairway obstruction (31). From 1998 the G*lobal Initiative for Chronic Obstructive Lung Disease (GOLD)* initiated to produce recommendations for the diagnosis, prevention and management of COPD. On its last update (2014) the COPD was defined as a common preventable and treatable disease, characterized by persistent airflow limitation. It is defined as progressive and associated with a chronic inflammatory response in the airways and lungs to noxious particles or gases. Its exacerbations and comorbidities contribute to the overall severity in individual patients (5).

These diseases, besides its functional impairments, commonly feature a chronic inflammation on the parenchyma and particularly on the peripheral airways. It is also associated to a systemic inflammation, especially on its frequent exacerbations. The inflammatory response of the COPD comes from either the innate and adaptive immune response, but the basis for this amplification for the immune response is not completely clear and might be associated with genetic factors (32).

Lung Hyperinflation

The association between the progressive parenchyma destruction and the small airway disease contributes to the airflow limitation. But this mixture varies from a person to another one. The chronic inflammation destructs the lung parenchyma, leading to the loss of alveolar attachments to the small airways and decreases the lung elastic recoil. This permanent destructive enlargement of airspaces, from distal to the terminal bronchioles limits the airflow reducing the ability of the airways to remain open during expiration (5,6).

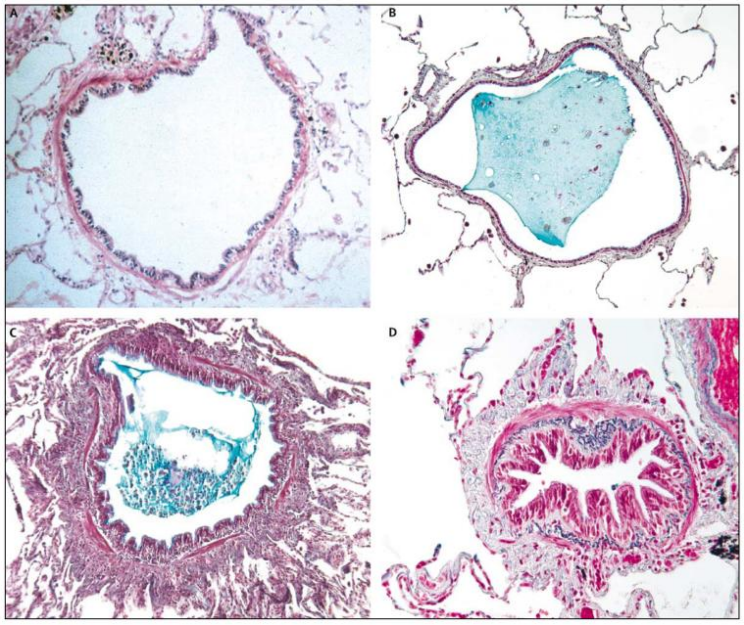


Figure 3: Histopathology of COPD small airways (A) Normal airway with open lumen (B) Small airway lumen filled with mucus (C)Acute inflammation and mucus surrounded by thick airway wall (D) Chronic inflammation: fibrosis and hyperplasia of smooth muscle cells accompanied by fibrosis resulting in the obstruction of the airway. Modified from Hogg (2004) (84).

This mechanism is called "airtrapping" and leads to the dynamic lung hyperinflation. It occurs when patients commence inhalation before full exhalation has been achieved, rapping the air within the lungs with each successive breath(7). This functional modification is correlated but not defined with the reduction in the forced expiratory volume during the first second (FEV_1) and FEV_1/Forced Vital Capacity (FVC) ratio and it can occurs either independent or in addition to static hyperinflation. The hyperinflation also reduces the inspiratory capacity and the functional residual capacity (FRC) increases, especially during exercise (in this case it is called dynamic hyperinflation), resulting in increase of exertional dyspnea and limitation the exercise capacity (5,7).

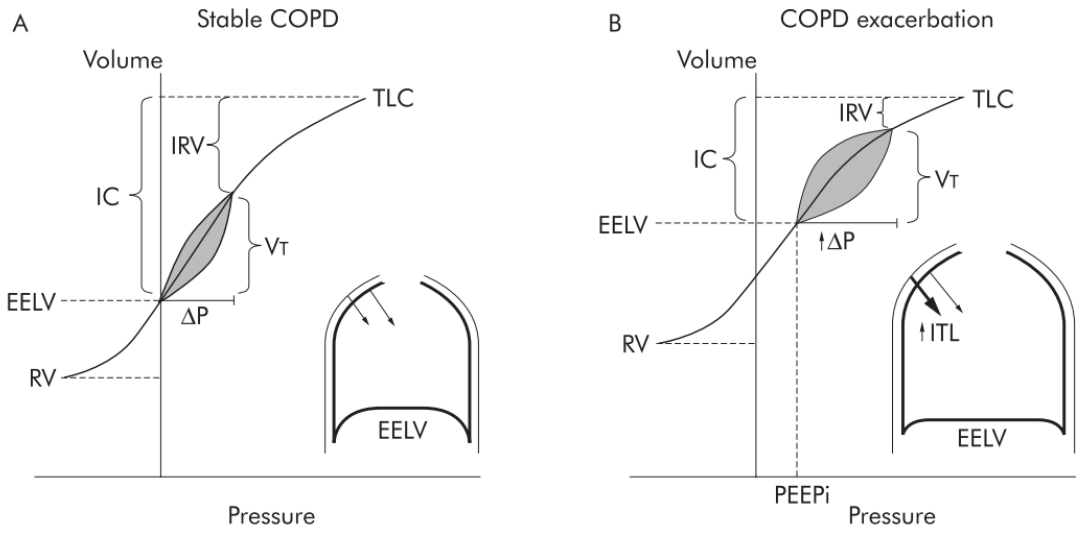


Figure 4: Schematic of mechanical effects of COPD exacerbation. Representative pressure-volume plots during (A) stable COPD and (B) COPD exacerbation. During exacerbation, worsening expiratory flow limitation results in dynamic hyperinflation with increased end expiratory lung volume (EELV) and residual volume (RV). Corresponding reductions occur in inspiratory capacity (IC) and inspiratory reserve volume (IRV). Total lung capacity (TLC) is unchanged. As a result, tidal breathing becomes shifted rightward on the pressure-volume curve, closer to TLC. Mechanically, increased pressures must be generated to maintain tidal volume (VT). At EELV during exacerbation, intrapulmonary pressures do not return to zero, representing the development of intrinsic positive end expiratory pressure (PEEPi) which imposes increased inspiratory threshold loading (ITL) on the inspiratory muscles (inset); during the subsequent respiratory cycle, PEEPi must first be overcome in order to generate inspiratory flow. Modified from O'Donnell & Parker, 2006(36).

The concept of lung hyperinflation deserves a special chapter in the setting and diagnoses of the chronic lung disease. It has been known for centuries, specifically during the half of the nineteenth century doctor Willian stokes described: "repetition of the inspiratory efforts causes such an accumulation of air in the disease portion of the lung as ultimately to nearly prevent its further expansion". Then the clinical importance of the lung hyperinflation was overshadowed by the use of the FEV_1 and FEV_1/FVC to categorized the severity and progression of disease. However, the patients symptoms and exercise capabilities has better correlation with changes in lungs volumes than with measures if airflow. The dyspnea is more likely a sign of worsening hyperinflation and reduction of inspiratory capacity (IC) than other spirometric measurements(33).

There are differences between the definition of static and dynamic lung hyperinflation. The static lung hyperinflation refers to the increase in end expiratory lung volume (EELV) above the predicted normal value. The resetting of the respiratory system volume at rest is due to permanent parenchymal destruction. In presence of expiratory flow limitation, the EELV is dynamic determined by some factors: the constant for empty the respiratory system that is determined by the product of resistance and compliance, the inspiratory tidal volume and the expiratory time available(34,35).

Assessment and severity

The gold standard measurement for the dynamic hyperinflation is the body plethismography. The changes in the inspiratory capacity (IC) can also reflect changes in EELV, representing the limit for tidal volume expansion in patients with airflow limitation.(35).The figure XX shows the differences on IC between healthy subjects, and COPD patients. The EELV remains stable when the minute ventilation increases to accommodate increase respiratory demands and the Vt expands into the IC. On COPD patients, the airflow limitation increases the resistance to expiratory flows prolonging the time required to exhale a given volume of air reducing the IC. The onset of the dynamic hyperinflation will occur at ever lower minute ventilation as disease severity worsens, even occurring in quiet breathing in severe or exacerbated patients (7). The reduction in the IC is also strongly correlated with the perception of dyspnea (36).

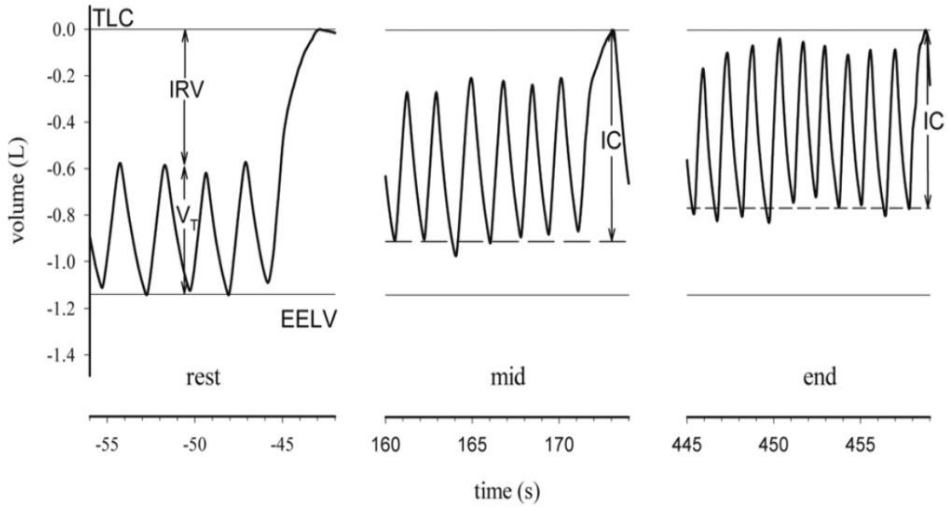


Figure 5: Volume tracing from a patient with severe COPD who demonstrated ventilatory dependent dynamic hyperinflation. Inspiratory capacity (IC) decreases and EELV increases as ventilation increases during exercise. Modified from Dolmage et al, 2013 (34)

The manifestation of COPD, ranges from dyspnea, poor exercise tolerance, chronic cough with or without sputum production to respiratory failure or *cor pulmonale*. Its diagnosis is confirmed when a patient who has this symptoms, and/or history of exposure to risk factors for the disease and also presents airflow obstruction measured by a spirometry. In such cases the airflow obstruction must not be fully reversible (5,37). The spirometric criteria for airflow limitation is a fixed FEV_1/FVC ratio<0.70, post bronchodilator and the airflow limitation severity has specific cut points for simplicity purposes. Its conventionally stated that when the FEV_1 post bronchodilator, represented on its percentage over predicted is equal or higher than 80% the airflow limitation is mild. When the FEV_1 is between 50% to 80% is moderate. Severe for FEV_1 between% 30 and 50% and very severe for FEV_1 bellow 30% (5).

5 THE DIAPHRAGM IN COPD.

The increase in FRC is one of the causes of the increased mechanical load imposed on the diaphragm in COPD. The great mechanical impedance in COPD requires an increase in inspiratory muscle recruitment, in order to maintain minute ventilation. The pattern of inspiratory muscle activation includes 1) an enhanced activation of the diaphragm, and 2) the shift towards a preeminent activation of the extra diaphragmatic rib cage muscles, both the primary (parasternal intercostals and scalenes), and accessory muscles of inspiration, like the sternocleidomastoid. The abdominal muscles are also frequently active during the expiratory cycle, aiding the first part of ensuing inspiration by actively displacing the respiratory system below FRC (38).

The modification on the muscle structure and function is the most preeminent and relevant issue on the functionality of COPD's patients deteriorating the quality of life, whereas the lower limb muscles are essential having also direct clinical consequences since respiratory muscles are needed for achieving an appropriate level of alveolar ventilation. Muscle dysfunction is defined as the loss of strength, endurance or both, and the diaphragm impairment may contribute to hypercapnic respiratory failure and exercise limitation (4)

The diaphragm is the most disadvantaged inspiratory muscle by the hyperinflation of COPD, first of all because its muscle fiber length is reduced, but also because the flattening that it undergoes makes it a less effective inspiratory muscle because of the reduction in area of apposition to the rib cage and of the expiratory action of its transversally oriented costal fibers. However, as suggested by studies of diaphragmatic function in patients with COPD, its mechanical responses to stimulation may be well preserved, due to adaptive responses that partially compensate for the length changes caused by hyperinflation. In any case, hyperinflation is associated with an increased energy cost of breathing and disadvantaged inspiratory muscle function (39)

Since the diaphragm is at a greater mechanical disadvantage than rib cage muscles, the increased activation of the diaphragm and increased recruitment of rib cage muscles brings about changes in the strategy of respiratory muscles use and recruitment, resulting in a pattern of compartmental chest wall displacement characterized by an increased contribution of rib cage expansion to tidal volume, similar to that observed during exercise in normal subjects. The respiratory muscles on such individuals work chronically against an increased load, requiring the generation of more force or energy to move air in to the lungs. This detrimental effect of the lung hyperinflation is caused by an increasing on the airflow resistance and by a reduction of the dynamic pulmonary compliance. Nevertheless, several studies have not found any correlation

between airflow obstruction and muscle dysfunction (8).The acute hyperinflation may reduce 30 to 40% of the diaphragm length when the lung volume increase from the residual volume (RV) to the total lung capacity (TLC). The pressure generating ability is closely dependent on lung volume. The diaphragm dome becomes flatter near TLC, when the muscle virtually ceases to generate an inspiratory pressure(9).

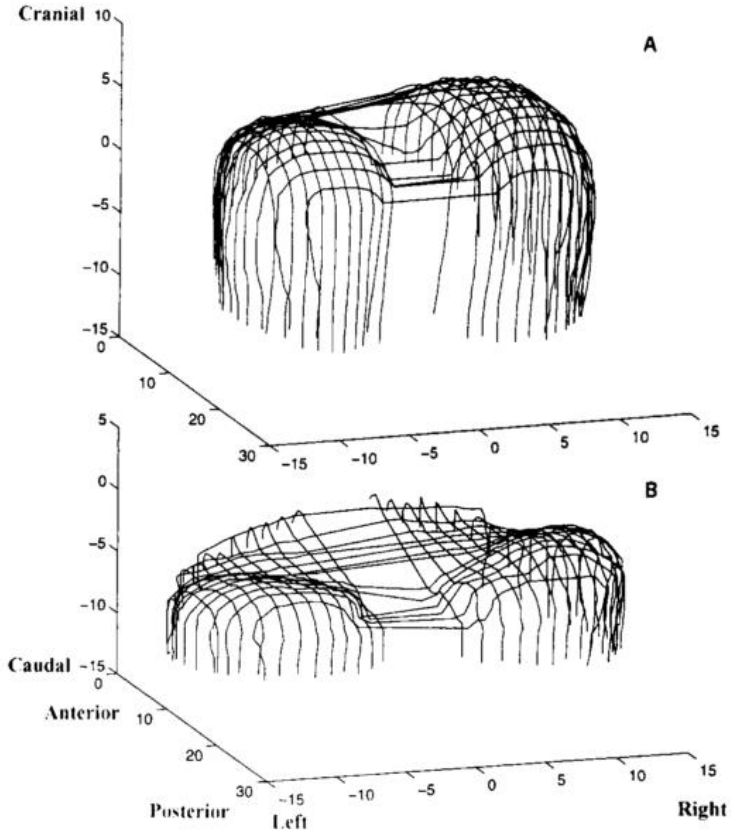


Figure 6: Three-dimensional reconstruction of diaphragm in one control subject (A) and one patient with COPD (B) at FRC. Reconstruction is shown from posterior-superior left lateral perspective. There is marked reduction in muscle surface area in the patient. *Modified from Cassart et al 1997 (82).*

Patients with COPD have a reduced transdiaphragmatic pressure generating capacity than healthy subjects (40). The diaphragm must work against an increased work load due to airflow limitation, but the geometrical changes in the thorax resulting from pulmonary hyperinflation have dramatic effects on its performance: its fiber length is reduced, and, in addition, the flattening that it undergoes decreases the area of apposition, resulting in it being a less effective inspiratory muscle. Indeed, the results of experimental studies suggest that the diaphragm may become expiratory in action at very high lung volumes (21,22). Furthermore, during exercise, FRC increases even more in many COPD patients, as a result of the reduction in expiratory time caused by the increased breathing frequency required to increase minute ventilation (41). The dynamic hyperinflation occurring in exercising subjects with COPD may result in an end-inspiratory lung volume

approaching total lung capacity and is often associated with intense dyspnea and a marked degree of activation of the diaphragm (42).

On the other hand, some aid, although of limited effectiveness, is provided to the diaphragm by abdominal muscle contraction. The abdominal muscles are usually not active during quiet breathing, but they are recruited during resting breathing in subjects with COPD. The recruitment of the abdominal muscles, by increasing abdominal pressure, which exerts a cranially directed force on the diaphragm, tends to improve diaphragmatic configuration by decreasing its radius of curvature and causing diaphragm fiber lengthening (41). However, the association of expiratory muscle recruitment in COPD with more effective generation of pressure by the diaphragm has not been confirmed: the pressure output of the diaphragm for a given electrical activation (measured by the electromyography) was unchanged in COPD subjects during tidal breathing with and without phasic abdominal muscle contraction (20). Phasic expiratory contraction of the abdominal muscles may have another beneficial effect on diaphragmatic function in COPD, reducing its workload: a strong contraction of the abdominal muscles, which actively deflates the lungs, not only reduces expiratory time, but also pushes the respiratory system below its relaxation volume so that, at the onset of the subsequent inspiration, the sudden relaxation of abdominal muscles promotes the passive descent of the diaphragm so that an initial increase in lung volume occurs merely by the elastic recoil of the system, before the inspiratory muscles become activated, thus reducing the work they must actively perform (17).

The diaphragm in COPD: Biology and functional properties

In addition to the mechanical explanation for diaphragm weakness in COPD, several studies showed altered functional, structural and metabolic characteristics in diaphragm biopsies of patients with COPD (3). Adaptive phenomena taking place are in part compensatory of the mechanical disadvantage, but some of their effects is vanified or counterproductive. As an example, the shift towards fiber type I(see below), oxidative and fatigue resistant, allows an increase in endurance but reduces the force generating capacity.

Respiratory muscles, like other skeletal muscles, are plastic and can adapt their function in response to environmental circumstances or disease by modifying many of their contractile properties (muscle mass, dimension and proportion of fibers, length and number of sarcomeres, myosin heavy-chain isoforms), capillary density, and metabolic features (energy production, mitochondrial amount, content and activity of the oxidative and glycolytic enzymes). Adaptations occurring in the

diaphragm in response to chronic obstructive pulmonary disease (COPD) have been studied to a certain extent, and the main findings are the following. (3,23).

Dimensions. Studies of diaphragm dimensions in COPD patients have yielded variable results. Diaphragm mass and thickness have been reported to be greater in COPD patients with normal body weight than in chronically ill underweight patients without COPD but, in contrast, diaphragm volume and thickness were reduced in underweight patients with chronic bronchitis compared with normal-weight patients without COPD. However, according to another study (43), the gross dimensions of the diaphragm were similar to those inpatients without chronic lung disease.

Length-tension properties and sarcomere Length: When lung volume is chronically increased by hyperinflation, the diaphragm is chronically shortened, and, therefore, placed at a mechanical disadvantage: adaptations have been shown to occur, restoring optimal length. Sarcomere length in the diaphragms of COPD patients compared to subjects with normal spirometry was found to be reduced, and to correlate with the degree of hyperinflation, such that an inverse correlation was found with %TLC and %RV. This indicates that adaptive factors reduce sarcomere length when the operational muscle length is chronically reduced. This structural change is thought to preserve the force generating capacity of the diaphragm in a condition of chronic fiber shortening by displacing the diaphragmatic length–tension curve, see Figure 23-1). In fact, the diaphragms of COPD patients were found to generate similar, or even greater forces than those generated by the diaphragms of normal subjects at similar lung volumes (24,39,44).

Myosin and Fiber Types: The data available in the literature support the notion that the chronically increased load faced by the diaphragm in COPD patients results in an adaptive shift of fiber composition towards fiber types with increased resistance to muscle fatigue. Indeed, data obtained from thoracotomy biopsy specimens of diaphragms of COPD patients showed a larger proportion of type I fibers (71±5% vs 42 ±2%), a lower proportion of type IIx fibers (21±3% vs 27±3%), while the proportion of type IIa fibers was similar to that found in control subjects (figure 5). The proportion of fibers containing MHC-I in the diaphragms of COPD patients increased (figure 6) whereas the proportion of the fibers containing the MHC-IIa and MHC-IIb isoforms decreased. In addition, the percent content of the MHC-I isoform in the diaphragms of COPD patients was positively correlated with TLC and FRC and negatively correlated with FEV_1. Furthermore, diaphragmatic fiber diameter was smaller in COPD patients than in subjects with normal lung function (45–47).

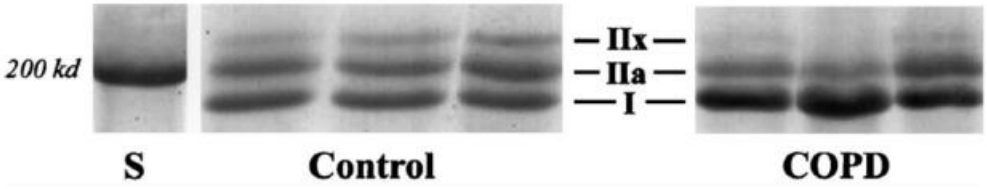


Figure 7 Representative Coomassie-stained SDS-polyacrylamide gels showing the distribution of the I, IIa, and IIx isoforms of the MHC in the costal diaphragm of three control subjects and three patients with severe COPD. A molecular weight standard is shown at the left. Modified from Levine et al 2001 (85)

Capillary Density: The chronically increased load faced by the diaphragm in COPD patients seems to induce a structural remodeling in the vasculature. Indeed, the capillary density of the diaphragm was found to increase with the severity of airway obstruction, correlating with $\%FEV_1$ (45).

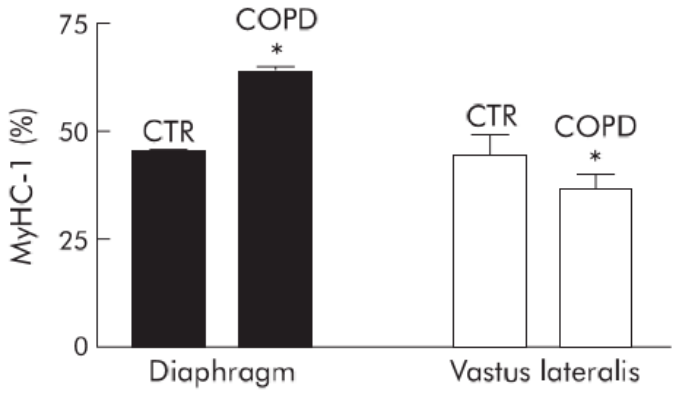


*Figure 8: Relative proportion of MyHC-1 isoform in biopsy samples from the diaphragm and vastus lateralis muscles of healthy subjects (CTR) and patients with chronic obstructive pulmonary disease (COPD). Data from Levine et al168 for the diaphragm muscle and Satta et al163 for the vastus lateralis muscle; *p,0.05. Modified from Polla et al 2004* (86).

Metabolism: A number of studies showed that the metabolic capacity of the diaphragm is enhanced in COPD patients, in whom the mitochondrial concentration resulted to be increased, with a positive correlation with the level of hyperinflation. In particular, in the diaphragms of severe COPD patients oxidative capacity is preserved and glycolytic activity is reduced. The metabolic adaptations to the increased load in severe COPD are similar to those elicited by endurance exercise training in limb muscles, suggesting the occurrence of a long-term endurance training of respiratory muscles in COPD (24).

Furthermore, diaphragm atrophy and injury have been documented in COPD patients, as a consequence of the metabolic derangement accompanying the disease and also of the glucocorticoid

therapy often adopted. Atrophy is characterized by a decrease in protein content and fiber cross sectional area resulting in reduced force production. Diaphragm fiber atrophy is a consistent finding in patients with severe COPD, and is attributed to increased proteolysis. Diaphragm fibers show reduced cross sectional area in patients with severe COPD compared to non-COPD patients. Diaphragm injury is characterized by morphological abnormalities such as disruption of membranous structures (sarcolemma, mitochondria etc), degeneration of the cytoplasm, and disorganization of the contractile apparatus. Recent data demonstrate diaphragm injury in patients with COPD. Increased sarcomere disruption has been found in the diaphragm of patients with moderate-to-severe COPD and to increase with progression of the disease. Increased oxidative stress, reflecting an imbalance between the production and scavenging of free radicals was demonstrated in the diaphragm of patients with severe COPD (24,47–50).

6. DIAPHRAGM ASSESMENT AND M MODE ULTRASONOGRAPHY

The necessity for the diaphragmatic function assessment arises in many clinical situations. Conditions as muscular dystrophy, nervous system disease and phrenic nerve injuries after thoracic or abdominal surgery may impair definitively or temporally the diaphragmatic motion. The neuromuscular characteristic, and the diaphragm anatomic conformation and localization permit the assessment of its function in many different ways. Regarding its contractile function and muscular fatigue with electrical or magnetic phrenic nerve stimulation. Its motion can also be studied by fluoroscopy and this technique until now has an important role on the diagnosis of diaphragmatic paralysis (10).

Those different techniques available for its assessment have its strengths and weaknesses. Chest radiographs can reveal diaphragm elevation on the side of its dysfunction but are insensitive, having poor prediction of normal motion. Fluoroscopy identifies the individual dome excursion either. It requires spontaneous breathing and evolves important radiation exposure and the need to move the patient to a specific unit, limiting the technique forbidden its utilization for critical patients. The magnetic resonance is technologically very evolved but include operator dependence, limited availability and high costs (11).

In this context the ultrasonography can became an important player to evaluate the diaphragmatic function, providing an alternative method for investigating, especially its mobility. This technique is ubiquitous in medical facilities as it doesn't requires radiation and even can be used at the patient bedside (12,51). The use of ultrasonography has been used to diagnose diaphragm rupture, pleural masses, and pulmonary effusions. It is now a validated technique especially for the diaphragmatic excursion analyses (12).

The ultrasonography offer a bidimensional image and the diaphragmatic kinetics assessment can be performed using the M-mode or the B-mode and in both cases the success of the examination depends on the operator experience to find the right angle to do the appropriate diaphragmatic dome visualization. The M-mode measures directly the craniocaudal diaphragmatic excursion of its dome. Its more visible on the right side and it should be performed positioning the transducer in the direction of the dome's muscle movement (10,52).

In the B-mode, that is mostly used to access the left diaphragmatic side, the craniocaudal movement is observed on the longitudinal sections and the transducer must be placed in a position perpendicular to the movement. The problem on this protocol is the fact that the diaphragm

visualization is shadowed by the lung itself and because of the interposition of ribs movement on deep inspirations. On B-mode is also possible to assess the diaphragm structure measuring its thickness(53) and verify the right side kinematics assessing the craniocaudal displacement of the left branches of the portal vein (13,52). Previous studies using B- mode, verified the correlation between the functional status and DM on healthy and COPD patients, stating that there is a positive correlation to the distance walked and the DM and also between the inspiratory capacity measured on the spirometry and the DM (54). Figure 7 shows the correlation between the DM and the lung function test.

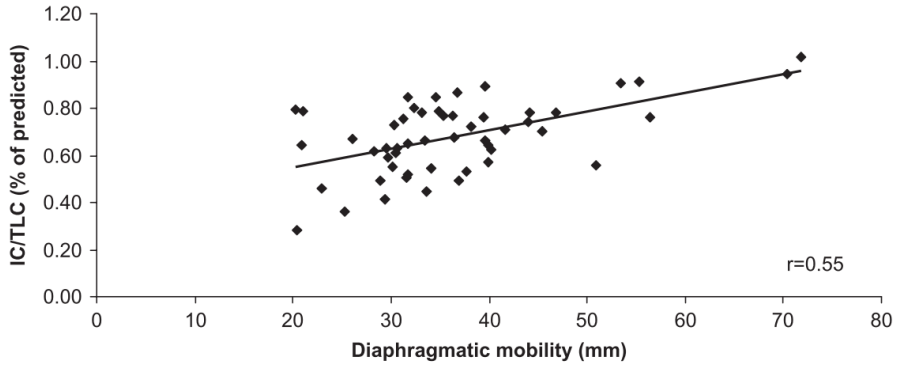


Figure 9: correlation between Inspiratory capacity/ total lung capacity ratio and the diaphragmatic mobility in COPD patients. IC: inspiratory capacity; TLC: total lung capacity; mm= milimeters. *Modified from Paulin et al, 2007)*(54).

The mobility of the diaphragmatic dome, from both sides, has been analyzed in several researches from the eighties until now. Those measurements followed similar protocols and they used the M-mode to directly measure the craniocaudal movement of the right dome (the most visible) and they all assessed healthy subjects. The mobility on resting breath range from 14±2 millimeters (mm) to 21±6 mm, and on forced breath from 42±16 mm to 75±10 mm (55). The Table 1 presented below is a modified from Testa et al 2011 and presents a collection of the reported right hemidiaphragmatic measurements by M- mode ultrasonography.(table 1).

Table 1: Literature reports of right hemidiaphragmatic excursion measurements during resting and forced breathing by M-mode US in normal subjects

	Resting breath excursion (mm)	Forced breath excursion (mm)
Harris et al. 1983		48 ± 16
Houston et al. 1992	17 ± 7	42 ± 16
Cohen et al. 1994a		60 ± 7
Cohen et al. 1994b	15 ± 2	
Targhetta et al. 1995		75 ± 10
Houston et al. 1995a	21 ± 6	
Ayoub et al. 1997		58 ± 4
Ayoub et al. 2001	14 ± 2	60 ± 8
	16 ± 3	61 ± 13
Kantarci et al. 2004		49 ± 11
Scott et al. 2006	15 ± 4	
Boussuges et al. 2009	18 ± 3	66 ± 13

mm= milimiters; modified from Testa et al, 2011 (55)

Besides those findings, the diaphragmatic dysfunction, after major abdominal surgery, on healthy individuals, were reported on a trial that used the M-mode from the preoperative day to the seventh postoperative day. This study stated that the loss on the vital capacity has correlation on the loss of the DM (56). The figure 8 demonstrate the association between the lung function and the loss on the DM.

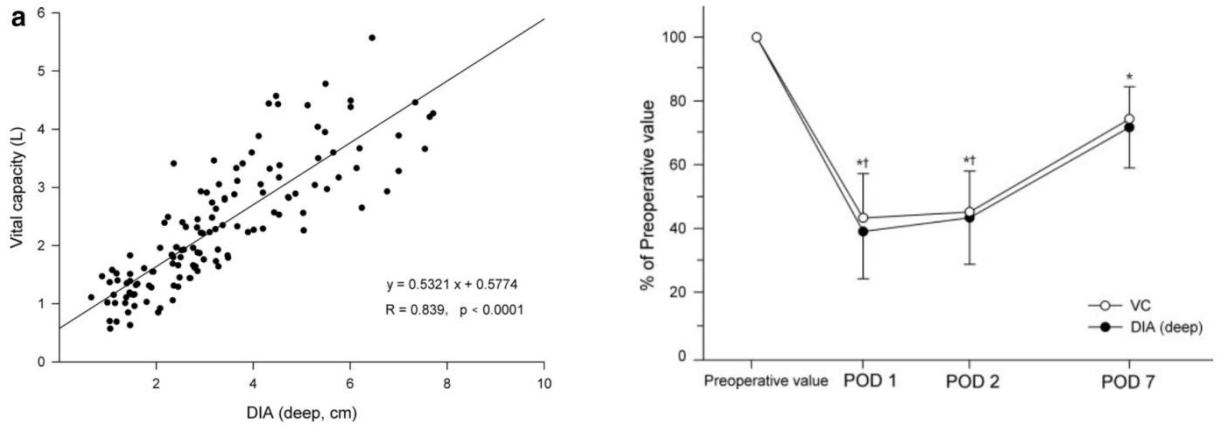


Figure 10: on the left panel the correlation betwen the vital capacity and the diaphragmatic mobilty on deep inpisration (DIA [deep, cm]). On the right panel the DIA (deep) and vital capacity variation according the post operative day. L=liters; cm=centimeters; VC=vital capacity; DIA (deep)= diaphragmatic mobility on deep inspiration; POD= post operative day. Modified from Kim et al (56)

To conclude this section is important to mention that the variability values from the DM at rest or deep breathing was also tested (55). Both intraobserver and interobserver demonstrated low variability. For the rest breathing 6% and 3.9% for the deep breathing with statistic significance

($p<0.01$.). The study demonstrated that the assessment on M-mode can vary from an expert investigator to a inexperienced. In this case the variability interobserver can reach 31.9% confirming the crucial importance for the presence of an experience examiner to avoid errors.

7. PULMONARY REHABILITATION

The definition of pulmonary rehabilitation (PR), as the definition of COPD, has changed over the past ten years. The importance of the PR is clearly demonstrated and the most relevant findings in the current literature are the reduction of dyspnea, increase in exercise capacity and improvement of quality of life. Considering its complexity and relevance, we are going to present it as it is stated: "Pulmonary rehabilitation is a comprehensive intervention based on a thorough patient assessment followed by patient- tailored therapies that include, but are not limited to, exercise training, education, and behavior change, designed to improve the physical and psychological condition of people with chronic respiratory disease and to promote the long-term adherence to health-enhancing behaviors"(57).

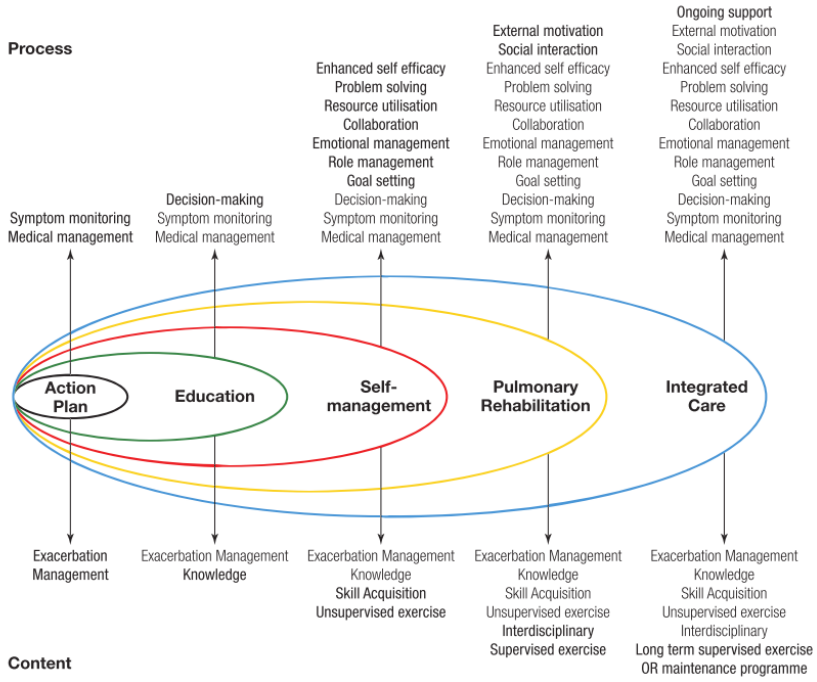


Figure 11: A spectrum of support for chronic obstructive pulmonary disease. Modified from Am J Respir Crit Care Med , 2013. (57).

The COPD has multiple manifestations and a large range of comorbidities, so integrated care principles are adopted to manage these patients. The PR is the core of a process that has the main goalof changing the health behavior and maintenance of its benefits for a long time. It is true that the PR is not the only approach or strategy on the treatment of the chronic lung disease(57). The GOLD statement describes the PR as one of many possible strategies for those patients as: smoke cessation, which is the intervention with the greatest capacity to influence the natural history of COPD. Pharmacologic therapies for stable COPD patients are used to reduce symptoms and

reduce the frequency and severity of exacerbations. Other treatments as oxygen therapy, lung transplantation, lung volume reduction surgery and palliative care are also available with their specific indication. Despite the evidenced effects on the listed outcomes, none of the medications for COPD has been conclusively shown to modify the long-term decline on lung function. Categorized as a non –pharmacologic therapy, the PR is described on the GOLD statement as a therapy that cover a range of non-pulmonary problems that may not be adequately addressed by medical therapy for COPD (5).

The PR is designed and implemented by an interdisciplinary team that includes physicians, physiotherapists, nurses psychologists and other allied health care professionals. Despite its definition as a "program", it can be initiated at any stage of the disease, even in periods of clinical stability, without change its main goals: minimize symptoms, increase exercise performance and enhance quality of life. Taking part in the PR a variety of forms of exercise training, strength training, upper limb training that are currently evidenced to be effective to achieve those goals (14,58).

The exercise training focus is the improvement of the muscular endurance. The current literature describes two strategies o accomplish this objective: the traditional endurance training, where the patient typically work three to five times a week, targeting a maximal level of intensity of continuous exercise on 60% to 70% of the maximal work rate. Usually those exercises are done on cycloergometers and treadmills. The other strategy is called "interval training", which is a modification of the endurance training, where the high-intensity exercise is interrupt regularly with periods of rest or even lower intensity exercise. This modality fits eventually severe or instable COPD patients that couldn't perform the endurance training, and its known that both of the strategies has comparable improvements in exercise capacity and health related quality of life (15,57,59).

As part of the exercise training protocols of the PR, specific activities as resistance /strength training on upper or/and lower limbs, especially where there is an important loss of muscle mass secondary to the illness severity. It also fits patients who present severe dyspnea or the combination of both. The inspiratory muscle training is stated as an adjunctive activity with modest results. It is not associated to improvements on dyspnea or maximal exercise capacity(60). Respiratory physiotherapy and its techniques on the management of COPD patients play a relevant role on the PR, presenting improvements on hypoxemia, airway clearance and management of dyspnea(61).

Concluding, the PR is addressed to both primary and secondary impairments of the COPD. It involves a spectrum of interventions and integrated strategies to promote the lifelong management of patients with chronic pulmonary disease (62)and its effects can be addressed also on the diaphragm. The DM was already evaluated after a PR. A recent study (figure 10 and table 2) evaluated the DM using the fluoroscopy to measure the diaphragmatic area (mm^2) on chest radiographs (63), showing that this intervention, other than the benefits on the quality of life, and exercise resistance leads to improvements on the DM.

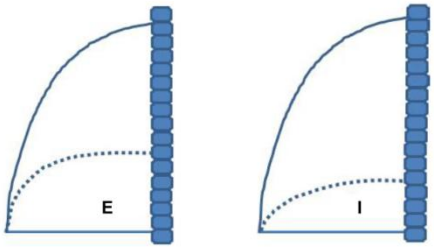


Figure 12 : Diaphragmatic moving area: E= expiratory area; I= inspiratory area. Schematic illustration of the area displaced by diaphragm movement during both inspiration (Area I) and expiration (Area E) in patients with chronic obstructive pulmonary disease. Notes: Horizontal line shows low margin of vertebral body connecting the costal insertion of diaphragm. Vertical brick line shows thoracic vertebral spinous processes. Low curved thick line shows diaphragm shadow. Modified from Chun et al 2015(63).

	Prepulmonary rehabilitation	**Postpulmonary rehabilitation**	***P*-value***
Right (mm^2)	2,022.8±1,548.3	3,010.7±1,495.6	0.001
Left (mm^2)	2,382.4±1,475.9	3,315.9±1,883.5	0.019

Table 2: Changes of diaphragmatic area between expiratory– inspiratory diaphragm motion with fluoroscopy in patients with chronic obstructive pulmonary disease: modified from Chun et al 2015 (63).

8. OBJECTIVES

Main objective

The main objective of this thesis is determine the right diaphragmatic mobility, assessed by M-mode ultrasonography on its cranio caudal displacement on severe COPD patients that underwent a pulmonary rehabilitation program.

Secondary objectives

To assess whether the diaphragmatic mobility on severe COPD patients is different to the diaphragmatic mobility on healthy subjects.

Verify if the diaphragmatic mobility improves after a participation on a Pulmonary Rehabilitation Program.

To correlate the modification on the diaphragmatic mobility with outcomes assessed by the lung functioning test, six minute walk test and arterial blood gas analyses.

9. METHODS

Study design: Cohort study

Patients:

Consecutive COPD inpatients who underwent a Pulmonary Rehabilitation Program and healthy control volunteers. We enrolled 52 COPD patients and 15 healthy controls. All subjects signed an informed consent.

Exclusion criteria:

Subjects with pneumothorax in treatment or treated within the last 12 weeks, active hemoptises, acute heart failure, lung embolia, systemic arterial hypertension, aneurism, hepatic surgery, splenectomy, diaphragmatic paralisys and neuromuscular diseases.

Study methods and protocols:

Every COPD patient comprised a standard evaluation that consisted on the lung function test, six minute walk test and arterial blood gas analyses.

The lung functioning tests were made according to the standards determined by the American Thoracic Society (ATS) and European Respiratory Society (ERS) task force (64,65). The spirometry is a physiologic test that measures how an individual inhales and exhales air volumes as a function of time. It is invaluable for the respiratory status as the blood pressure is important to the cardiovascular health. There are two maneuvers: the assessment of the volumes under a maximal inspiration followed by a maximally forced expiration (blast expiration) that provides the forced vital capacity (FVC), (FEV_1), the forced vital capacity/forced expiratory volume on the first second (FVC/FEV_1). And there is the slow forced maneuver where the patient slowly performs, after a short period of tidal breathing, a slow maximal inspiratory to reach the total lung capacity, followed by a slow forced maximal expiration. From that maneuver we obtained the: expiratory residual volume (ERV), inspiratory capacity (IC), vital capacity (VC), inspiratory residual volume (IRV), tidal volume (V_T) (66).

On the body plethysmograpy cabin it was performed all measurements concerning the absolute lung volumes. It contrasts the simplicity of spirometric volumes because there is large range of techniques to its measurements. In our research it was used the body plethysmography technique where the patient is positioned (seated) in a closed cabin and performed the respiratory maneuvers. The patient was instructed to breath quietly with the mouthpiece attached (three to ten

tidal breaths) and then proceed with a series of gentle pants, with the shutter closed. After that, to conclude the maneuver, the patient performed the slow forced maneuver, just as on the spirometry. The assessment of the lung volumes provided the measurement of the total lung capacity (TLC), total gas volume (TGV), residual volume (RV) and airway resistance (R_{aw})(67).

The lung function test was conclude with the determination of the lung diffusion of carbon monoxide (*D*L,CO). This complementary test measures the rate of Carbon Monoxide uptake, determining the capacity of the lung to exchange gas across the alveolar-capillary interface. The patients were instructed to do a few tidal breathings and then an unforced expiratory maneuver to reach the residual volume followed by a maximal forced inspiration to the total lung capacity. At the end of the maneuver the patients made a 10 seconds apnea and then a maximal exhalation (68). For the lung functioning test it was used the QUARK PFT Plesthismography by COSMED, Italy.

The six minute walking test is a reliable measure of exercise capacity for people with COPD and it is strongly correlated to work capacity and physical activity and it is particularly sensitive to changes after pulmonary rehabilitation. The main outcome on this test is the distance walked on six minutes, that must be compared with a predicted distance (69,70). But in the field of the PR there are others reliable meauseres and outcomes that can be useful on the functional assessment of the COPD patient. The oxyhaemoglobin saturation (SpO_2), measured with pulse oximetry, the heart rate(HR), respiratory frequency (f) and Symptom scores for dyspnea and muscular fatigue by the Modified Borg Scale (MBS). It has a strong correlation with the lung functioning measurements on severe COPD subjects (71).

The arterial blood gas analyses (ABGA) was made by a physician, with a blood sample collected from the radial artery on the pulse. The ABGA measured the hydrogenionic potential (pH), the partial oxygen pressure (PaO_2), partial carbon dioxide pressure ($PaCO_2$), bicarbonate (HCO_3) and oxyhaemoglobin saturation(SpO_2).

Within the second day of recovery the diaphragm mobility ultrasonographic (US) measurement was assessed as follow: the patients were positioned in a semi recumbent position (45 degrees).The US probe was positioned between the anterior and mean axillary line, on the central right subcostal area, cranial and dorsal. The US went perpendicularly on the posterior hemidiaphragm third (it is visualized as the hyperecogenic line behind the liver). The mobility was assessed by M-mode while the patient breaths on tidal volume (rest breathing) and deep slow breathing (to Functional Residual Capacity).

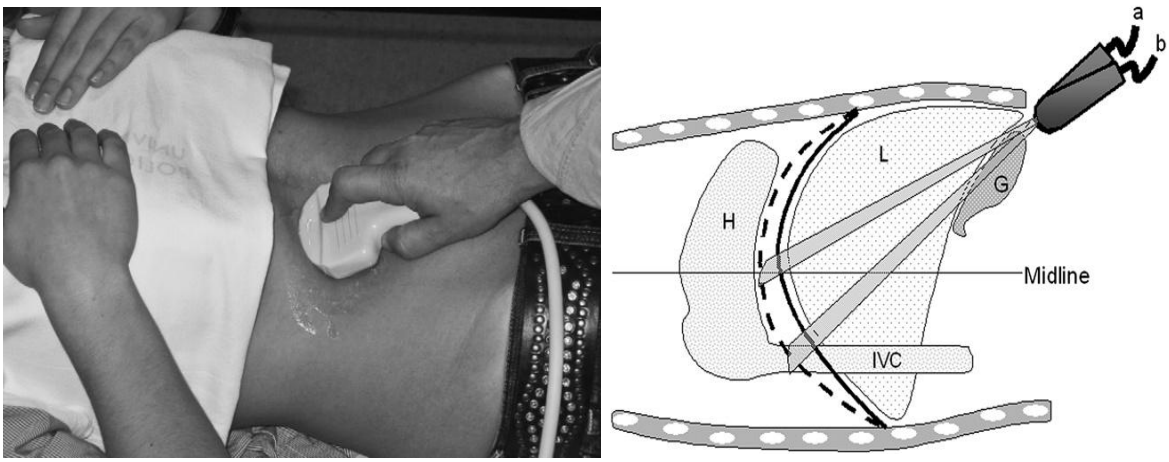


Figure 13: on the left panel the position of the transducer on anterior subcostal abdominal wall at the right midclaviar line. On the left panel, Schematic demonstration of the US technique (a) looking for right hemidiaphragm across liver (L) gallbladder (G) and inferior vena cava (IVC), up to displaying the dome position by cranial steering (b). Diaphragmatic motion toward the transducer from basal relaxation (broken line) to inspiratory contraction (unbroken line).Modified from Testa et al 2011.(55)

The diaphragmatic inspiratory amplitudes, or the craniocaudal excursion of the diaphragmatic dome, were determined placing the first caliper at the foot of the inspiration slope on the diaphragmatic echoic line and the second caliper was placed at the apex of this slope. Rest breathing was measured and recorded several cycles and it was considered the mean slop amplitude. For the slow deep breathing it was also measured and recorded several slopes but it was considered that with the higher distance between the apex to the slop base. On this maneuver it was asked to the patient to do a maximal inspiratory effort, inspiring slowly as deep as possible. For those measurements it was used a portable CX50 compact ultrasound system from PHILIPS.

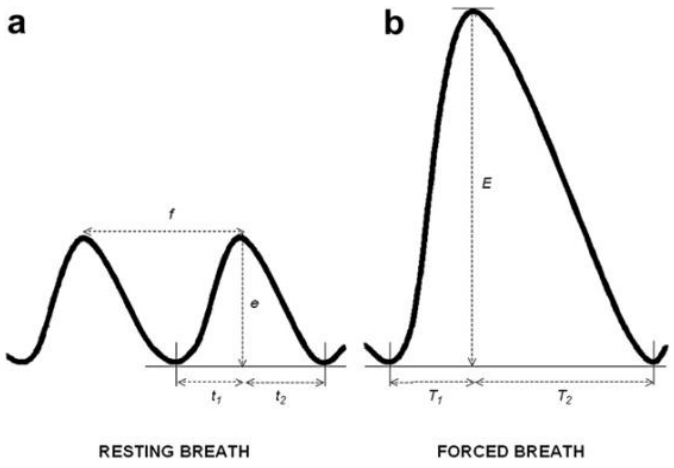


Figure 14: Schematic representation of the measurement technique on resting and forced inspiratory breath.Modified from Testa et al, 2011.(55)

The COPD patients were classified according to the illness severity established by the GOLD criteria (5). Every patient, after the assessment followed the PR as ATS/ERS statement on pulmonary rehabilitation(57). All patients followed a routine of five days a week of physical exercise. The rehabilitation program consisted on a 30 minutes calistenic gymnastic once a day, 20 minutes of cycloergometer training activity twice a day. The aerobic cycloergometer training was set at 60%-70% of the maximum cardiac frequency determined by 220 minus the patients age. The patients also received lower limbs strengths exercises, especially for the quadriceps and hamstring. During the in-hospital stay, the patients received regular prescribed medication and oxygen therapy according to the medical staff evaluation, which was prescribed by the clinician in charge. The patients received also respiratory physiotherapy, regarding the presence of lung secretion retention, and the need to expand unventilated areas.

The control group was composed by healthy volunteers. The volunteers were screened on their lung function and were included those with the FEV1/FVC ratio>0.70. The subjects on the control group were submitted to the same evaluation protocol regarding the spirometry and the M-mode ultrasonography.

Statistical analyses:

The qualitative description of the data will be made with percentages and frequency. The quantitative data is described as means and standard deviation for the symmetrical distributions. We used the Kolmogorov-Smirnov test to determine distributions mean and normality. A One-way analysis of variance (ANOVA) with repeated measurements and Bonferroni test was used as post-hoc test to evaluate statistical significance. Within-group effect sizes were calculated using the Cohen d coefficient interpretation. Cohen considers an effect size greater than 0.8 was considered large, around 0.5 moderate, and less than 0.2 small. For all the data of the study, P values lower than 0.05 were considered significant. We used the Student T- test for independent sample for quantitative data and for comparisons between the COPD patients and control group and ANOVA to the comparisons within COPD group. We used Spearman correlation test to measure the correlation between the diaphragmatic mobility on the deep inspiration and the lung functioning variables. Data were analyzed using SPSS package version 20.0 (SPSS Inc, Chicago, IL, USA).

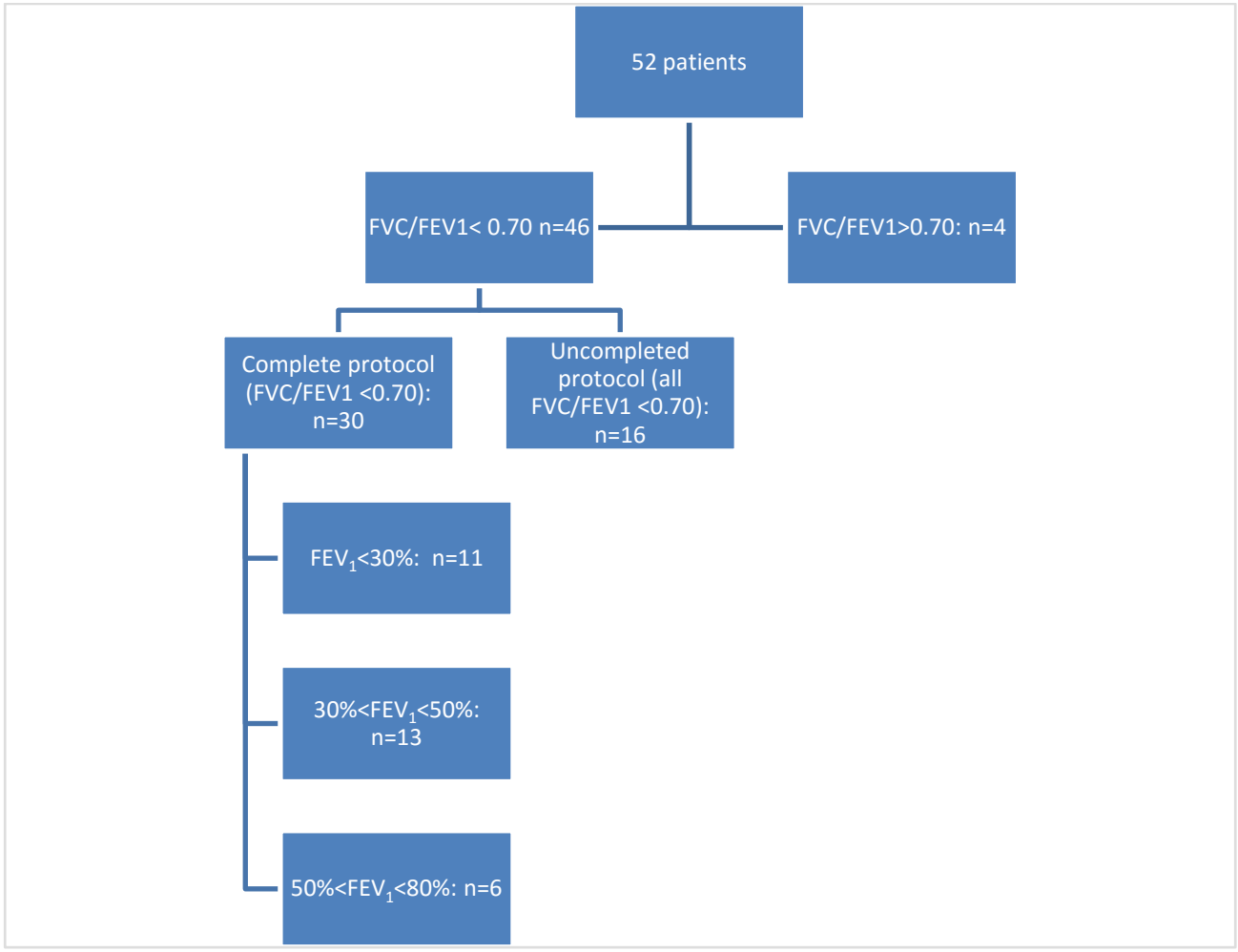
Ethical aspects:

On the present study, the informed consent was obtained from all participants, and the procedures were conducted according to the Declaration of Helsinki (72), and according to the Italian laws for the patients respect on their privacy and data confidentiality (D.L. 196/2003).

10. RESULTS

From September 2014 to July 2015 we assessed 52 consecutive patients (13 females) who underwent pulmonary rehabilitation.46subjects were identified with the inclusion criteria ($FVC/FEV_1 < 0.70$), all patients comprised the first assessment, but among them 16 did not complete the protocol (4 strokes, 6 myocardial infarct, 4 cardiogenic pulmonary edema and 2 thrombophlebitis). Two patients were excluded, one that presented an inverted contraction of the diaphragm and another that died during the hospitalization. The patients that completed the protocol were classified as shown on the flow chart bellow. It was also assessed 15 healthy volunteers that underwent an assessment protocol that comprised the spirometry and the ultrasonography evaluation.

Figure 15 : flow chart presenting the sample characteristics, its classification and outcomes.



The mean hospitalization period comprised 31±8 days. A total of 46 patients (11 women) ended the pulmonary rehabilitation and completed the evaluation protocol. According to the mean spirometric data, our COPD patients presented severe airway obstruction, lung hyperinflation and a

severe reduction on the carbon monoxide lung diffusion. Our patients, at the beginning of the protocol walked 50% of their predicted distance for the six minute walking test.Their mean diaphragmatic mobility (DM) was 2.09±0.8cm for the rest breathing and 4.75±1.58 cm for the slow deep inspiration until the total lung capacity. The average characteristics of the sample for its anthropometry lung functioning tests, six minute walk test and diaphragmatic ultrasonography are described on the table below.

Table 3. (continue on the next page): anthropometric data, lung function, six minute walk test and diaphragmatic ultrasonography for all assessed patients. Mean ± SD

Anthropometric data		**Before PR**
	Subjetc (female)	46(11)
	Age	72 ±10
	Weight *Kg*	61.5±11.5
	High *m*	1.62± 9.3
	BMI $Kg.m^2$	23.5±4.3
Lung function	FVC *L*	2.11±0.6
	FVC *% pred*	72±17
	FEV_1 *L*	0.88±0.36
	FEV_1 *% pred*	40.6±16
	FVC/FEV_1	41.7±11.4
	FEF_{25-75} $L.S^{-1}$	0.33±0.15
	FEF_{25-75} *% pred*	13±6.5
	ERV *L*	0.76±0.31
	IC *L*	1.54±0.41
	VC *L*	2.23±0.62
	VC *% pred*	75±19
	IRV *L*	0.78±0.29
	Vt *L*	0.73±0.23
	*D*L,CO *L*	8±3.15
	*D*L,CO *% pred*	37.3±15.5
	TGV *L*	5.5±1.9
	TGV *% pred*	170±50
	TLC *L*	6.95±2
	TLC *% pred*	123.5±26.9

	RV *L*	4.9±1.7
	RV *%pred*	208±75
	RV/TLC *(%)*	69.4±9.2
Six minute walk test	SpO_2	89±7
	HR *bpm*	108±14
	f *mpm*	26±5
	MBS dyspnea	4±2
	MBS muscle	4±3
	Distance Walked *m*	235±103
	Distance walked *% pred*	50±23
DM		
	Rest breathing (mean) *m*	2.09±0.8
	SDP *cm*	4.75±1.58

PR: Pulmonary rehabilitation; ***Yrs***: years; ***Kg***: Kilograms; ***m***: meters; ***L***: liters; ***S***: seconds; ***BMI***: body mass index; $\boldsymbol{FEV_1}$: Forced expiratory volume on 1sec; cm: ***FVC***: Forced vital capacity; centimeters; $\boldsymbol{FEF_{25-75}}$: forced expiratory flow on 25% to 75%; ***ERV***: expiratory residual volume; ***IC***: inspiratory capacity; ***VC***: vital capacity; inspiratory residual volume; ***Vt.*** Tidal volume; ***DL,CO***: carbon monoxyde lung difusion; ***TGV***: total gas volume; ***TLC***: total lung capacity; ***RV***: residual volume; ***HR***: heart rate; ***bpm:*** beats per minutes; ***f***: respiratory frequency; ***mpm:*** movements per minue; ***DM:*** diaphragmatic mobility; ***SDP:*** slow deep inspiration; ***MBS***: modified borg scale; $\boldsymbol{SpO_2}$: partial oxygen saturation. ¥p=0.02, #p=0.002, *p=0.05, †p≤0.001 for Student t test.

For all the patients (n=46) the mean arterial blood gas analyses (ABGA) presented normoxemia associated with a primary metabolic alkalemia with a concomitant respiratory acidosis. Our patients were hypercapnic with a normal peripheral oxygen saturation. The mean supplementary oxygen flow at the moment of the ABGA were 2±1 liters per minute. Those values did not present clinic or statistical improvements after the PR. Those results are presented in table 4.

Table 4 mean ABGA values on the first assessment.

ABGA	Mean±SD
pH	7,43±0.03
PaO_2 ***(mmHg)***	78±15
$PaCO_2$*(mmHg)*	44±12
HCO_3*(mmHg)*	28±4
SpO_2*(%)*	95±2
Oxygen therapy flow *L.min* $^{-1}$	2±1

ABGA: Arterial blood gases analyzes; **pH:** Hydrogenionic potential; **PaO_2:** Partial arterial oxygen pressure; **$PaCO_2$:** Partial arterial pressure of carbon dioxide; **HCO_3** : bicarbonate; **mmHg**: millimeter of mercury; **SpO_2:** Partial oxygen saturation; : ***L.min*** $^{-1}$liters per minute; ***SD***: standard deviation.

We analyzed the differences for the DM between the healthy controls and the COPD patients, classifying the subjects according to its severity (table 3). The 15 healthy controls (HC) presented normal values for the lung function testing and the DM for the rest breathing was 1.27±0.3 cm and the mean DM for the slow deep inspiration until the total lung capacity was 6.93±1.15 cm. We observed that there was a significant statistical and clinical difference between the mean DM at rest breathing and at the slow deep inspiration between the HC and the others groups when compared using the Student T test for independent sample. The figure 14 demonstrate the correlation of the FEV_1 %predicted and the DM at rest breathing (r=-0.74, with a p <0.001) and the Slow Deep Inspiration (r= 0.796 with a p<0.001), n=45.

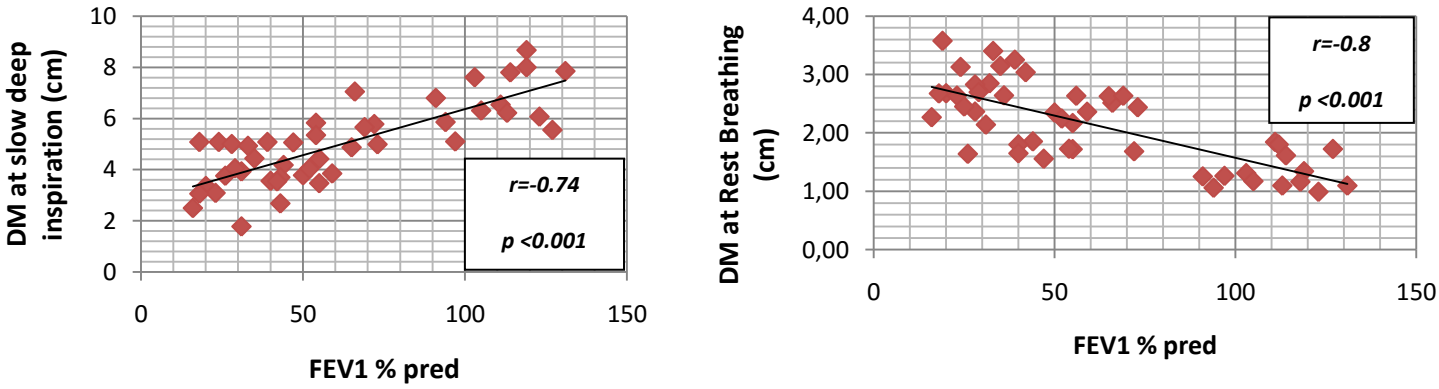


Figure 16: correlation between the diaphragmatic mobility (DM) and the FEV_1%pred. Right panel at rest breathing; left panel slow deep inspiration.

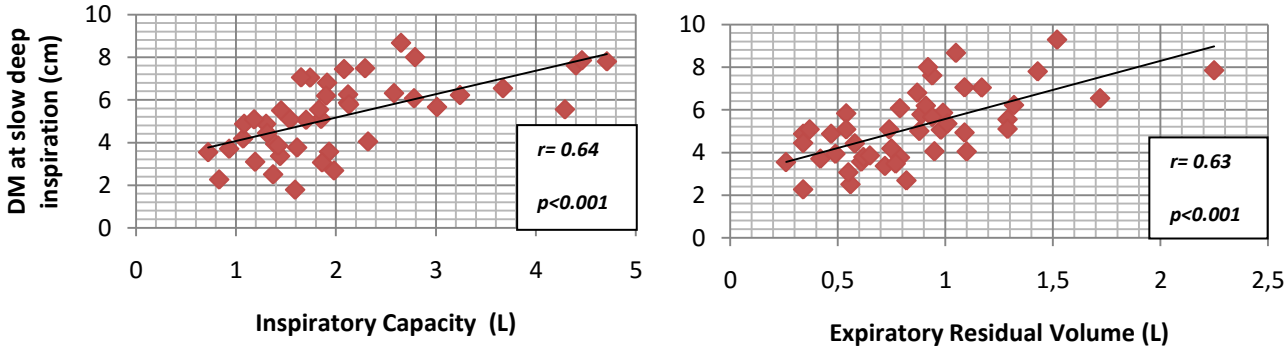


Figure 17: correlation between the diaphragmatic mobility (DM) at slow deep inspiration and the Inspiratory Capacity (left panel) and the Expiratory Residual Volume (right panel). L: liters.

The correlation between the DM and the lung function test was also positive between the Slow Deep Inspiration and the Inspiratory capacity (r= 0.64 with p<0.001) and the Expiratory Residual Volume (r= 0.63 with p<0.001), n=45. The Figures 18 and 19 shows the ultrasonographic images for severe COPD patients and control. The images demonstrates both respiratory movements, rest breathing and slow deep inspiration.

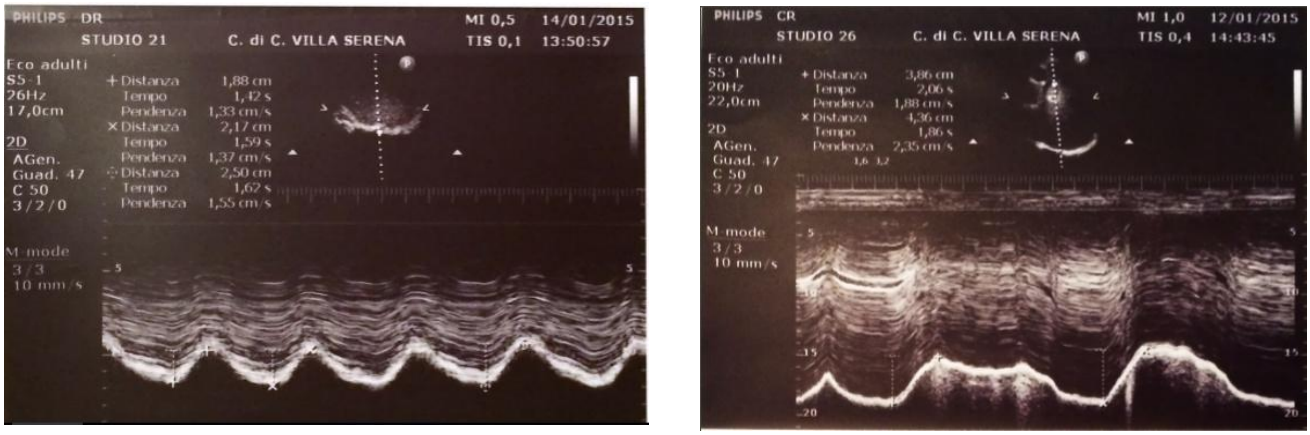


Figure 18: On the left panel diaphragmatic M-mode ultrasonography on rest breathing (mean mobility: 2.18 cm) on a severe COPD patient (FEV_1 =38%/predicted). The right panel show the slow deep inspiration (higher movement: 4.36 cm) from a different a severe COPD patient (FEV_1=50%/predicted).

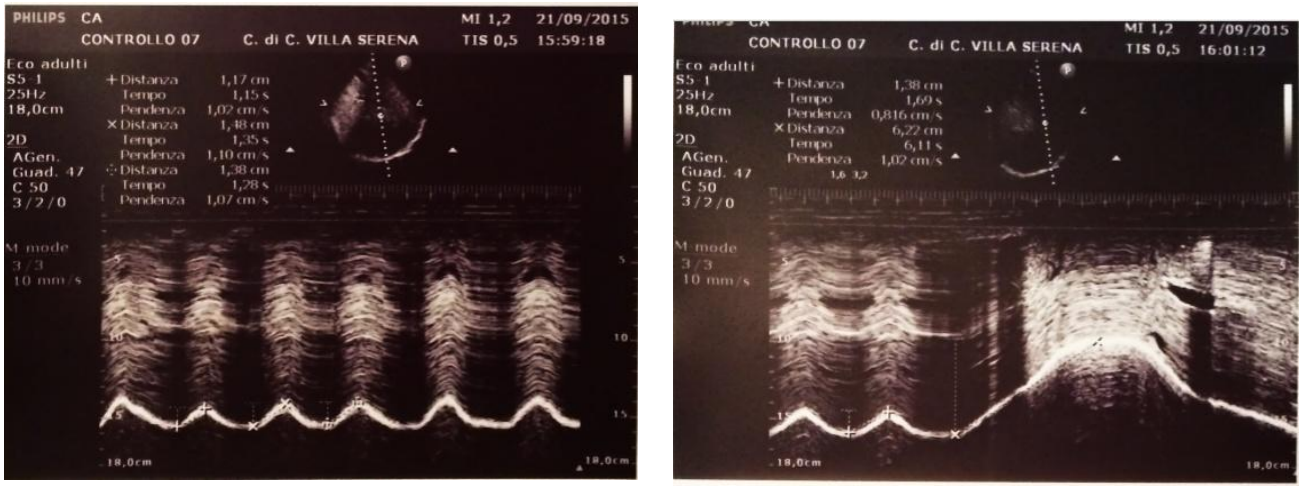


Figure 19: diaphragmatic M-mode ultrasonography on healthy controls. Left panel at rest breathing (mean mobility: 1.25cm), right panel at slow deep inspiration (6.11 cm).

The differences were observed for the DM on rest breathing and on slow deep inspiration. We observed that the diaphragmatic displacement on the rest breathing is higher on all COPD groups if compared to the HC. Otherwise the DM for the slow deep inspiration was lower. The comparisons on the inspiratory capacity measured on the spirometry also showed differences, clinical and statistical between the HC and moderate, severe and very severe COPD patients been lower for the COPD groups (table 5).

Table 5: comparisons according to the COPD severity on the patients who completed the rehabilitation program. Data presented as mean±standard deviation.

	Healthy Controls	Moderate COPD	Severe COPD	Very Severe COPD
Antrophometric data				
Subjetc (female)	15 (9)	6(0)	13 (6)	11 (1)
Age *yrs*	34±9	76.5±7	70±8	65±15
Weight *Kg*	74±32	71.2±13.7	62±15	57.8±10.6
High *m*	162±26	1.66±9	1.60±8	1.61±7.5
BMI *Kg.m^{-2}*	22±6	25.8±5.5	24.1±5	22±4.5
Spirometry				
FVC *L*	4.75±14	2.88±0.56	1.96±0.4	1.8±0.5
FVC *% pred*	106±30	88.6±8.6	71.4±12.8	57±13
FEV_1 *L*	4.04±1.36	1.46±0.2	0.82±0.11	0.5±0.1
FEV_1 *% pred*	103±30	60±7	38.5±5.7	21.7±4
FVC/FEV_1	78±20	51.42±6.5	43.6±9.5	30±4.6
FEF_{25-75} *L.S^{-1}*	4.32±1.55	0.5±0.1	0.32±0.07	0.2±0.05
FEF_{25-75} % pred	92±32	20.6±4.3	12.4±3.3	6.9±1.9
ERV *L*	1.28±0.55	0.96±0.4	0.67±0.3	0.8±0.3
IC *L*	3.26±1.01	2.19±0.3¥	1.53±0.3†	1.24±0.2†
VC *L*	4.52±1.4	2.9±0.5	2.14±0.57	1.9±0.5
VC %	101±32	86.4±5.63	73.8±20.4	58±12
IRV *L*	2.48±0.9	1.13±0.36	0.80±0.3	0.6±0.2
Vt *L*	0.77±0.3	0.97	0.7±0.18	0.63±0.15
D*L,CO *L	-	7.84±6.16	9.09±2.71	5.64±1.46
D*L,CO *% pred	-	35.5±31.5	43.4±13.3	25±5.6
TGV *L*	-	5.39±1.0	5.18±1.9	6.41±1.35
TGV *% pred*	-	168.6±51	172±51	200±40
TLC *L*	-	7.55±1.2	6.72±2	7.6±1.7
TLC *% pred*	-	121±19	127±30	133±24
RV *L*	-	4.9±1.1	4.6±1.6	5.85±1.62
RV *%pred*	-	186±40.6	212±65	262±83
RV/TLC *(%)*	-	64.3±6.3	68±7	76±6
Six minute walk test	-			
SpO_2	-	87±13	90±5	87±8
HR *bpm*	-	104±16	110±9	117±10
f *mpm*	-	25±5	18±3	26±5

MBS dyspnea	-	1.7±1.5	4±3	6±2
MBS muscle	-	3±1.5	3±3	5.7±3
Distance Walked *m*	-	240±93	288±101	160±47
Distance walked % pred	-	52±12	59±25	31±14
DM				
Rest breathing(mean) *cm*	1.27±0.3	2.74±0.7†	1.95±0.8#	2.52±0.6†
SDP *cm*	6.93±1.15	5.61±1.6*	4.4±1.7†	4.6±2#

COPD: Chronic obstructive pulmonary disease; ***PR:*** Pulmonary rehabilitation; ***Yrs***: years; ***Kg***: Kilograms; ***m***: meters; ***L***: liters; ***cm:*** centimeters; ***S***: seconds; ***BMI***: body mass index; FEV_1: Forced expiratory volume on 1sec; cm: ***FVC***: Forced vital capacity; centimeters; FEF_{25-75} : forced expiratory flow on 25% to 75%; ***ERV***: expiratory residual volume; ***IC***: inspiratory capacity; ***VC***: vital capacity; inspiratory residual volume; ***Vt***. Tidal volume; ***DL,CO***: carbon monoxyde lung difusion; ***TGV***: total gas volume; ***TLC***: total lung capacity; ***RV***: residual volume; ***HR***: heart rate; ***bpm:*** beats per minute; ***f***: respiratory frequency; ***mpm:*** movements per minute; ***SDP***: slow deep inspiration; ***MBS***: modified borg scale; SpO_2: partial oxygen saturation. ¥p=0.02, #p=0.002, *p=0.05, †p≤0.001 for Student t test.

The effect of the PR on all COPD patients were assessed comparing the mean variables measured before and after the PR. The comparisons regarding all patients presented an improvement on the DM ($p<0.01$) on the slow deep inspiration maneuver after the PR, simultaneously with improvements on the inspiratory capacity (IC) ($p<0.05$) measured on the lung function test either after the PR. Those results are presented on the table 6:

Table 6: comparisons on lung functioning tests, six minute walking test and diaphragmatic ultrasonography on all COPD patients before and after the rehabilitation program (n=30).

		Before PR	**After PR**
Lung function	**FVC *L***	2.07±0.7	2.16±0.8
	FVC *% pred*	70±18	73±20
	FEV_1 *L*	0.9±0.5	0.97±0.5
	FEV_1 *% pred*	40±18	42±21
	FVC/FEV_1	44±15	44±14
	IC *L*	1.58±0.5†	1.7±0.6†
	VC *L*	2.2±0.7	2.28±0.9
	VC *% pred*	73±19	76±21
	TLC *L*	6.9±2	7±2
	TLC *% pred*	124±27	132±39
Six minute walk test	**SpO_2**	89±8	90±7
	HR *bpm*	110±13	109±12
	f *mpm*	27±7	24±6
	MBS dyspnea	4±3	3.5±2
	MBS muscle	4±3	3±2
	Distance Walked *m*	225±108	283±84
	Distance walked *% pred*	51±22	61±21
DM			
	Rest breathing (mean) *m*	2.25±0.83	2.53±0.82
	SDP *cm*	4.58±1.83#	5.45±1.56#

PR: Pulmonary rehabilitation; ***Yrs***: years; ***Kg***: Kilograms; ***m***: meters; ***L***: liters; ***cm:*** centimeters; ***S***: seconds; ***BMI***: body mass index; ***FEV_1***: Forced expiratory volume on 1sec; cm: ***FVC***: Forced vital capacity; centimeters; ***FEF_{25-75}*** : forced expiratory flow on 25% to 75%; ***ERV***: expiratory residual volume; ***IC***: inspiratory capacity; ***VC***: vital capacity; inspiratory residual volume; ***Vt.*** Tidal volume; ***DL,CO***: carbon monoxyde lung difusion; ***TGV***: total gas volume; ***TLC***: total lung capacity; ***RV***: residual volume; ***HR***: heart rate; ***bpm:*** beats per minute; ***f***: respiratory frequency; ***mpm:*** movements per minute; ***SDP***: slow deep inspiration; ***MBS***: modified borg scale; ***SpO_2***: partial oxygen saturation. † $p<0.05$, #$p<0.01$ for Student T test.

The intraclass correlation coefficient(ICCs) for intra-examiner reliability for the measurements of the DM on rest breathing for all patients that completed the PRranged from 0.95 to 0.98 and the standard error of measurement(SEMs) ranged from 1.16cm to 1.85cm respectively for the assessment before and after the PR on comparisons of the mean value for the three movements recorded.

Considering the small size of the sample with very severe patients (n=11), we analyzed the effects of the PR on either severe and very severe patients. We also observed an improvement on the DM followed by an improvement on the inspiratory capacity measured on the spirometry. The table 7 presents the mean values for the lung functioning test and DM on the slow deep inspiration maneuver. Table 8 presents the intraclass correlation coefficient(ICCs) for intra-examiner reliability for the measurements of the DM on rest breathing for those groups. The ICC for severe and very severe patients is lower if compared with analyses of all COPD patients that underwent the PR, 0.95 for all patients against 0.77 for the severe and very severe patients.

Table 7. Comparisons before and after pulmonary rehabilitation, for FEV_1, TLC, IC and for the DM on the deep slow maximal inspiration for the severe and very COPD patients (n=24).

Variable	**Time**		**Effect Size**	**Difference within pre and post**
	Pre PR	Pos PR	Post minus Pre	Post minus Pre
Lung Function			Cohen's d	
FEV_1 *% pred*	35.7± 26.2	37.2± 18.8	0.07	1.5 (-2.5 to 5.5)
TLC *% pred*	131.35± 21.3	131.57± 21.44	0.01	0.22 (-8.68 to 9.11)
IC *L*	1.55± 0.42	1.69± 0.44	0.3	0.14* (0.01 to 0.28)
Ultrasonography				
SDP *cm*	4.58± (1.71)	5.44± (1.45)	0.5	0.86# (0.31 to 1.41)

DM: diaphragmatic mobility; ***FEV_1***: Forced expiratory volume on 1sec; cm; ***TLC***: total lung capacity; ***IC***: inspiratory capacity; ***L***: liters; ***cm:*** centimeters. ***SDP***: slow deep inspiration; Data are expressed as means ± standard deviation. *p <0.05; # p <0.001

Table 8: Test and retest values and index of relative and absolute reliability for the measurements for the DM on rest breathing for the severe and very severe COPD patients (n=24).

Diaphramatic mobility	Test (°)	Retest (°)	Bias (°)	ICC (95% CI)	95% LOA (°)		SEM (°)
					Lower	upper	
rest breathing	1.31 ±0.34	1.37 ±0.36	-0.06	0.77 (0.28; 0.93)	-0.23	0.12	0.47

DM: diaphragmatic mobility; ***Bias***, difference between test and retest; ***ICC***, intraclass correlation coefficient; ***95 % CI***, 95 **%** confidence interval; ***95 % LOA***, 95 % limits of agreement; ***SEM***, standard error of measurement. * Data are expressed as means ± standard deviation (mean±SD).

11. DISCUSSION

The COPD is a chronic and systemic condition that affects different organs and systems. The fact that it is not only a respiratory disease may compromise the subject's motor function and consequently the related quality of life(5). It is observed on severe patients that lung deterioration and loss of its function cause important structural changes on the muscular system, compromising either fiber type I and fibers type II(4,8). Nevertheless the progressive impairments, the COPD patients had benefits and functional improvements when submitted to pulmonary rehabilitation (PR). The PR is a comprehensive and multidisciplinary treatment that focuses on physical training to improve the functional status and reduces the dyspnea(57,58).

The diaphragm as the main respiratory muscle also presents pathophysiological modifications as consequence of the chronic lung disease. There are several changes on its muscular structure, especially due to the modification on the thoracic conformation secondary to lung deterioration on the moderate and severe COPD subject. The diaphragm presents not only modifications on the muscular fiber length, but also on its function, shifting the proportion on its fiber, increasing the number of type II fibers, despite of type I(73,74).

The assessment of the diaphragmatic mobility (DM)can be done with many different techniques, but until now there is no consensus about the best strategy on its assessment (75). The evaluation with the ultrasonography on M-mode is easy and can be performed also at bedside. It had its reproducibility and reliability confirmed on clinical trials(10). But there is a lack of information on subjects with COPD, especially on moderate to severe stages of the chronic lung disease. So we idealize this research, assessing the DM as its cranial-caudal displacement using the ultrasonography on M- mode, on moderate to severe COPD inpatients that underwent a rehabilitation program.

The main results of our research can be summarized as follow: the DM in slow deep breathing on moderate to severe, severe and very severe COPD patients is lower when compared with healthy controls. The patients that ended the rehabilitation program improved the DM on their slow deep breathing, followed by an improvement on the inspiratory capacity. The DM for the rest breathing did not present any improvement after the PR. Surprisingly the rest breathing mobility for all the COPD patients were higher than in all severity groups when compared with the healthy subjects.

Even though current literature regarding the DM on healthy subjects is not consensual to its values for rest breathing and the mobility at the total lung capacity reached after the slow deep

inspiration, it was imperative in this research to verify those outcomes to establish real parameters determining the differences with the COPD subjects. Therefore in our research the healthy controls (HC) presented values similar to those found on previous papers that determine normal values for rest breathing and slow deep inspiration using the same protocol (10,55). Others trials reported different values specially for the DM at the total lung capacity, but those studies had different methods concerning the utilization of the ultrasonographic probe, and different populations (12,75–77). In this context we also confirmed the intra-examiner variability lower than 5%, reported on the research of Testa et al (55) for the measurements of the DM on rest breathing for all COPD patients, demonstrating that the reliability and the reproducibility for this measurement also on such subjects.

Nevertheless it is not a new technique, the assessment of the DM on M-mode has its firsts papers published in the 1990'saimingat describing the benefits, standardizing methods and determining the precision and confidence of such analyses(10,55). Until that moment the utilization of the ultrasound to evaluate the diaphragm was restricted to the utilization of the B- mode technique. The utilization of the B-mode is still controversial in the role of the lung function assessment and was mostly used to measure the diaphragm thickness (53,78). The measurement of the DM with this technique is limited to an indirect measurement of the displacement of the splenic hilum (for the left Hemidiaphragm) and the left intrahepatic branch of the portal vein (for the right hemidiaphragm)(13,52).

Actually the mobility of the diaphragm on the COPD patient with this technique was already described (54), showing that those patients presents a reduced mobility when compared to healthy subjects, but besides the fact that the study measured the DM on B-mode, it failed to describe the DM at rest breathing, determining the DM only for the forced inspiratory and expiratory movements. On the other hand comparisons between COPD patients and healthy controls on spontaneous ventilation and on positive pressure ventilation (PPV) with neuromuscular block did not demonstrate differences on the DM on spontaneous breathing considering the regional diaphragmatic excursion (top, middle and bottom). In this study the differences were observed on PPV either for low and large tidal volumes, being the DM, on such condition, lower on the COPD patients (79).

Those previous findings necessarily did not contrast with ours. Our research demonstrated that the DM at rest (or tidal) breathing is higher on COPD patients when compared with healthy controls having a negative correlation with the FEV_1% predicted ($p< 0.001$). The difference found was not only statistical, but also clinical. The COPD patients, on all their severity subcategories, showed a DM meanly 100% higher when compared to the healthy controls. Our results run parallel

to the results from the studies above. First because of the technique, whereas the data from the others studies were obtained on the B-mode and our measurements were done on M-mode that provided the direct measurement from the displacement of the diaphragmatic dome. Second, the research protocols were completely different. From the study of Paulin et al (54), as mentioned before, we do not have data to compare the DM at rest breathing. The study from Kleiman et al (79) demonstrates that the DM at rest breathing was lower for the COPD patients in a different behavior, with sedated subjects suffering from a neuromuscular block. As the authors stated, in this condition the fact that the diaphragmatic excursion was no longer determined by active contraction prevails, so the regional differences for the DM on the COPD patients were the product of the forces of the PPV over the deteriorated lung parenchyma.

To explain these phenomena we hypothesize that in conditions where there is an increase in the inspiratory load and respiratory frequency is possible the increasing on the DM at rest. The necessity to compensate the increases on ventilatory and metabolic demand is associated to the severe airway obstruction and consequently hypoxia and/or hypercapnia. But, it was described that the COPD diaphragm on such situation does not lose its ability to generate force and resist to fatigue. It was also described that the COPD diaphragm, especially on the severe stage, presents a higher resistance to the fatigue, conserving its capacity to develop force and resistance to load increasing. As a result of its muscular fiber typeshifts, increasing the proportion of fiber type I, slow-twitch, fatigue-resistant fibers, whereas the proportion of fast, fatiguing fibers (type II) is decreased(73). As we know, the COPD is not a disease that deteriorates the diaphragm motor units junctions. It actually changes it by improving its ability to sustain the ventilation under particular conditions as the hypoxia and/or hypercapnia whereas there is an increasing on the recruitment of the units associated to the fiber type I (slow twitch), increasing the transdiaphragmatic pressure(80). Besides that, it was also reported that mechanical inspiratory load on healthy individuals increases the diaphragmatic excursions from 1.7±0.5 cm to 2.1±0.9 cm, increasing also the contraction velocity (81). To conclude, the novel research from Similowski et al (39) demonstrated through the measurement of the transdiaphragmatic pressure that those compensatory mechanism restores the strength of the diaphragm, so it is not so surprisingly that, despite its flattened shape the DM at rest is higher o COPD patients compared to HC.

It is almost consensual that the flattened dome shape is an adaptation of the thoraco-abdominal cavity to the deterioration of the lung parenchyma. In fact three dimensional reconstructions of the diaphragm on healthy and COPD subjects demonstrates that the surface area on the severe stable COPD patients is reduced when the muscle is on the functional residual

capacity, or its resting position, having also a reduced zone of apposition (82). The acute hyperinflation affects the length- tension relationship of the diaphragm, increasing the rest isometric tension and so reducing its pressure generation ability to achieve a maximum active tension, especially to reach total lung volumes (9). The DM on our patients measured on the slow deep inspiration reflected those issues mainly because the COPD patients from all the subcategories presented a lower DM when compared to the HC. Secondly, those differences were higher according to the severity of the COPD. Nevertheless, these results confirmed the previous findings that demonstrate the reduction of the DM on inspiratory and expiratory forced breathing (54). It was also demonstrated that on severe hyperinflated COPD patients has less DM than HC, and this impairment is strongly associated with static air trapping measured by the ratio RV/TLC (83). It is important to note that the mean maximal inspiratory excursions were found with the direct measurement on M-mode, differently from those studies that used the B-mode ultrasonography.

The effect of the PR on the DM is the last main finding on our research. It is known that the PR provides several improvements to the COPD patients, especially on the self related quality of life and tolerance to exercises (71). But there is an important lack of information on the effect of the PR on other functional outcomes. To our knowledge this is the first research that assessed the DM of COPD in the role of the PR, using the ultrasonography on M-mode. Previously these outcomes were assessed with fluoroscopy, where the investigators also found that the DM measured at deep inspirations increases (63). Those findings were expressed in mm^2, so it is impossible to extrapolate their findings to the results that we observed on the direct measurement of the diaphragmatic dome displacement that we measured. In our study the patients that ended the PR increased their DM only at the maximal inspiratory capacity, without any differences at the rest breathing. The increasing was not only statistical, was also clinically significant ranging from 4.58±1.83 cm to 5.45±1.56 cm, increasing more than 1 cm on its maximal excursion, with low effect size, barreling the normal values stated on previous reports (10,55) and confirmed on our study.

12. CONCLUSION

The diaphragm importance on the human physiology seems to be underestimated. The main muscle for the ventilatory pump, as the others tissues or systems, undergoes the same COPD's pathophysiological effects. But fortunately, it adapts in a different way to compensate the lung and airways deterioration and to sustain the energetic and metabolic demands from the whole body. Those adaptations take part either on its shape and cellular conformation.

Surprisingly there is not enough information on the current literature about the diaphragmatic mobility either in normal subjects and in subjects affected by pulmonary diseases. The limits and risks from the traditionally used techniques to assess this outcome may take an important role on this fact. So, the ultrasonography, especially on M-mode could be an interesting player to modify this context, filling those gaps especially due to its reproducibility, feasibility and also portability.

The US on M-mode, in this research provided relevant information regarding the influence of the severe chronic lung disease on the diaphragmatic mobility and also on the improvements of its functionality after the pulmonary rehabilitation. The diaphragm from a patient with a moderate to very severe lung obstruction surprised us, having a higher mobility at rest, when compared to healthy individuals but it demonstrated, as expected, a limited capability to made large excursions at a deep slow inspiration. Finally, patients that completed the pulmonary rehabilitation had improvements on the DM at the slow deep inspiration, confirming that this intervention is important to improve other functional outcomes than those already known as six minute walk test and quality of life.

Our findings were sustained also from other reports. But the fact that we measured the diaphragmatic mobility directly from its dome to its base, made our results more solid, supporting our conclusion that the ultrasonography on M-mode can provide important reproducible information regarding the diaphragm mobility. Our methods and sample size allow us to state that the COPD impair the diaphragmatic function, but this impairment can be in part reverted, and these changes are followed by improvements on lung function. However, our methods did not allow us to establish new parameters, specifically to the assessment of the lung function, neither to made any new cutoff points to determine the respiratory distress, and we think that these limitations may be another frontier to be crossed.

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