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MORPHOLOGICAL AND FUNCTIONAL CHRACTERIZATION OF THE DIAPHRAGM IN PATIENTS ON THE WAITING LIST FOR LUNG TRANSPLANTATION

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SUMMARY

The respiratory system allows the gas exchange between our body and the external environment. This gas exchange is the vital function that we call respiration, and it allows the entrance of O_2 inside the body and the emission of CO_2. This exchange is performed into the lungs, two organs placed inside the thoracic cavity. These organs can be inflated or deflated depending on the action of the respiratory muscles that constitute the respiratory pump that moves air into the lungs.

Several pathologies can affect the respiratory system and many of them lead to the premature death of the patient. The causes of lung disease are several and different; they can be genetic, infective or smoking induced.

The most common pathologies are Idiopathic Pulmonary Fibrosis (PF), Cystic Fibrosis (CF) and Chronic Obstructive Pulmonary Disease (COPD). These pathologies affect the lungs in different ways: 1) PF is a progressive fibrosing interstitial pneumonia that causes a reduction of lung compliance and the formation of scar tissue. The latter makes difficult the lung to expand, the former impairs gas exchange. The origin of this disease has not been found yet even if some risk factors are known like, for example, genetic factors, smoking, virus infection; 2) CF is a genetic disease that makes all secretions become thick causing mucus build-up in the airway with resulting recurrent inflammation and infection that lead to structural changes of the lungs. As a result, airway obstruction is frequent in cystic fibrosis and the lung compliance decreases becoming more rigid. Damaged genes are the responsible of cystic fibrosis; these genes are the ones coding for the CFTR protein (Cystic Fibrosis Transmembrane Conductance Regulator) whose malfunctioning leads to a modification of the exocrine excretion. As a result, we have the production of sweat with a high salt content and a really viscous mucous secretion; 3) COPD causes airways obstruction or emphysema (rupture of the inner walls of alveolar sacs with creation of larger air spaces with trapped air), or a combination of both, thus leading to lung hyperinflation. This disease is caused mainly by tobacco smoke, but also to air pollution and even occupational exposures to dust and chemicals.

All of the three pathologies lead to chronical respiratory failure, and until now no definitive cure are known but lung transplantation that improves quality of life of patients and increases their life expectancy.

In order to enter in the waiting list for the lung transplant, patients must undergo a deep clinical examination about their cardio-respiratory conditions and infective state; respiratory physiotherapeutic evaluations are also necessary. On the basis of statistical considerations which take into account the patient clinical evaluation, the probability of survival after the lung transplant is computed. In order to be inserted in the lung transplant waiting list all the following criteria must be met:

1- the risk of death from lung disease within 2 years if lung transplantation is not performed must be higher than 50%;

2- the likelihood of surviving at least 90 days after lung transplantation must be higher than 80%;

3- the likelihood of 5-year post-transplant survival from a general medical perspective must be greater than 80%, provided that there is an adequate graft function.

After the transplant, all the respiratory system including the respiratory muscles and the diaphragm must adapt to the new condition.

Post-transplant complications risks are always present. They can be subdivided in early or tardive complications. The earliest complications which may arise right after lung transplantation intervention is related to the impossibility to weaning patients from the life-support system or from the mechanical ventilation. This condition may be related to a malfunctioning of the new transplanted lung (mainly due to primary graft dysfunction or infections) or to a malfunctioning diaphragm, which may also result to be temporary or permanently paralyzed and it does not contribute to the ventilator pump to inflate the new lungs to guarantee gas exchange.

Tardive complications, on the other hand, are related to infections or to other problems that appear after some time from the weaning from mechanical ventilation.

Because the diaphragm is the most important respiratory muscle and it plays an important role in the weaning process from mechanical ventilation after the transplant, we aim to study it with a multidisciplinary approach in order to understand if and how it copes with severe lung diseases.

A collaboration between the Thoracic Surgery Unit of the 'IRCCS Fondazione Cà Granda Policlinico Maggiore di Milano' and the 'Dipartimento di Elettronica Informatica e Bioingegneria' of Politecnico di Milano was established in order to study all patients on the lung transplant waiting list of Policlinico di Milano.

The main aim of our study was to investigate the diaphragm of these patients from several points of view with both a functional and a morphological analysis in order to understand if it is affected by the severe lung diseases, too. None of the three considered diseases, namely PF, CF, COPD, affects directly the diaphragm, but all of them may induce a morphological and functional change in this muscle due to the abnormal shape of lungs and their impaired mechanical properties.

First of all, a functional general characterization of the respiratory system of the patient was assessed by different clinical tests, like spirometry, DLCO test, Sniff Nasal Inspiratory Pressure (SNIP), Maximal Inspiratory Pressure (MIP), Maximal Expiratory Pressure (MEP). Spirometry evaluate the ability of lungs to change their volume during forced maneuvers; Diffusing Capacity of Lung for Carbon Dioxide (DLCO) is considered in order to evaluate the quality of gas exchanges in the alveolar sacs; MIP and MEP assess the strength of respiratory muscles by measuring the pressures they are able to generate.

Spirometry, MIP, MEP, SNIP tests are not able to differentiate which of the different part of the respiratory system is impaired in case of reduced test results; altered values of one of them may be caused by chest wall abnormalities or by a disease of the lungs or by an impairment of the ventilatory pump. That is the reason why we used more specific tests in order to evaluate diaphragm characteristics performing a multifactorial approach.

The morphological characterization of the diaphragm was performed by the analysis of the images acquired with the Computerized Tomography (CT) technique. Starting from the CT images, we segmented the left and right hemidiaphragms as the lower boundaries of lungs in order to compute their length and radius of curvature. The measures were taken both in the coronal and in sagittal plane in order to have a better characterization. We also analyzed Ultrasonography (US) images in order to assess the diaphragmatic thickness, thickening and excursion during breaths. Thickness measurement was performed during quite breathing, so at end inspiration (EI) and end expiration (EE), but also at the end of a forced expiration (FE) and a forced inspiration (FI).

The force of the diaphragm is assessed by Phrenic Nerve Stimulation (PNS). It is a test which induces the maximal non-volitional diaphragm contraction. Electromyography (EMG) and Electroneurography (ENG) are performed during PNS in order to give information about the diaphragm contractility and phrenic nerve conduction.
Synchronously to most of the previously mentioned tests, Optoelectronic Plethysmography has also been performed. This technique allows to assess the thoraco-abdominal volume changes induced by respiration. One of the OEP advantages is the possibility to evaluate the contribution of the different compartments of the chest wall: Pulmonary Rib Cage (RCp), Abdominal Rib Cage (RCa), Abdomen (Ab). In order to characterize the diaphragm from the functional point of view, we focus our attention on the abdominal compartment as it is mainly affected by the diaphragm action: it expands only after diaphragmatic contraction.
The innovation introduced in this work is the synchronism between most of the test present in clinical literature and the OEP technique. This permits us to add volume change information to measurements made with the other clinical tests.

All the 28 patients in the lung transplant waiting list of the 'IRCCS Fondazione Cà Granda Policlinico Maggiore di Milano' in our period of collaboration were included in the study. We subdivided them by pathology: 9 pulmonary fibrosis (PF), 14 cystic fibrosis (CF) and 5 Chronic Obstructive Pulmonary Disease (COPD). We compared the results not only among the different groups of patients but also with a control group of healthy subjects. When possible, we directly acquired data from healthy subjects; when it was not possible, we used data coming from other works, taken from the reference database of the TBM Lab, Politecnico di Milano.
Spirometry shows a reduced FVC comparing to the predicted values, revealing restrictive characteristics. Lower values of FEV_1 highlight the obstructive nature of the COPD pathology while higher FEV_1 values points out the restrictive nature of PF. CF patients have intermediate FEV_1 values which demonstrate the fact that the pathology is a mixture of a restrictive and obstructive disease.
As regards DLCO test, it is shown that almost all patients have lower values than predicted and particularly PF patients present the worst values.
MIP and MEP data reveals that PF and CF patients have median percentage values of mouth pressure both for the inspiratory and for the expiratory maneuver which are almost equal to

the predicted values. COPD patients, on the other hand, present much lower values than the reference ones.

The SNIP test points out that the two fibrosis population, contrary to the COPD group, are able to generate higher values of pressure with respect to the reference value.

As morphology regards, in our analysis, we found different shapes of the diaphragm according to the different pathologies: COPD patients were characterized by a flatter and lengthier diaphragm compared to healthy controls, while pulmonary fibrosis group shows a more curved diaphragm.

Regarding the thickening and thickness of the muscle there are no differences between patients and healthy groups. This may lead to the conclusion that the diaphragm of these patients is not hypotonic.

Concerning the non-volitional stimulation of the phrenic nerve that provides a maximal contraction of the diaphragm, there are no differences in both electromyography and elettroneurography results; on the other hand, whereas cystic fibrosis patients show a significant reduction in the abdominal volume variation.

The analysis of the breathing pattern of patients shows no differences in the tidal volume, while respiratory frequency was significantly higher in all of them. As a consequence, patients show higher values of the Rapid and Shallow Breathing Index, thus leading to conclude that the ventilator pump works even more than in the normal subjects because it has to overcome the lack of the gas exchange efficiency.

During the inspiratory capacity maneuver, the whole group of patients show reduced inspiratory capacity volume, particularly for the CF group. This reduction in volume is caused by a reduction of the rib cage contribution; the maximal and forced inspiration is impaired and this might be due to a reduced action of the rib cage inspiratory muscle that cannot expand lungs as in healthy condition.

After the above descripted results, it seems that although the morphological changes induced by the lung disease, the diaphragm of these patients is preserved. In fact, during quiet breathing it does not show any hypotonic sings and its contractility, motion, thickening and action are similar to the healthy control group. The reduced contribution during inspiratory capacity may be due to the increased load that it has to sustain because of the impaired lungs of the patients.

We believe that this multidisciplinary approach should be extended also after the lung transplant in order to study how the diaphragm adapts to the changes induced by the transplantation. Some preliminary data confirm this assumption. In fact, during the period of our collaboration, we have performed this analysis in 6 patients after the lung transplant. Several indexes result significantly different after the operation: for example, MIP and MEP measurements show a significant decrease in the post-operative group, the percentage of the abdominal compartment in the tidal volume is lower after the transplant than before it, while the pulmonary rib cage shows a higher percentage; latency of the phrenic nerve is higher after the transplant.

The reduction in MIP and MEP results and the different contribution of the compartments to the tidal volume can be explained by the physiological conditions of the patient: the surgical wound after few days is not yet well healed and suture at the level of the abdomen could still induce pain in the subjects. As a consequence, patients make use in a higher fraction of the rib cages muscles to allow lung expansion.

The higher latency might be due to the stretching that the phrenic nerve had to undergo during the surgical intervention which leads to a worse conductivity and consequently to a longer stimulus latency.

Because of the short period elapsed between the transplant and our analysis, considering the physiological condition of the patient, resulting benefits coming from the new lungs could be hidden. For this reason, it is recommended to perform the identical analysis also after 6 months from the surgical operation in order to see the adaptation of the muscle to the new lungs and the improvements in the breathing pattern. Unfortunately, in our period of collaboration, we were able to do it just for one patient, but results are remarkable.

For example, both the percentage contribution of abdominal compartment to the tidal volume and breathing frequency reaches normal values, comparable to those of healthy subjects.

These considerations, even if they are possible only for one subject, highlight the importance to longitudinally extend the work in order find out the late onset benefits induced by the transplant.

The present work is organized in five chapters.

In the first chapter, we introduced the respiratory system subdivided in upper airways, lungs and respiratory muscles, mainly diaphragm. We presented rapidly the three pathologies that can affect the lungs and that the only treatment possible is lung transplantation.

In this chapter it is also explained the conditions to be matched for the patients in order to be selected as a potential lung recipient and how the waiting list is composed. We presented the main post-transplant complications including the diaphragmatic paralysis.

For this reason, we described deeply the diaphragm anatomy and its contribution in the different respiratory phase and the most widely used tests in order to assess its morphology and functionality.

In chapter two we introduced the patients of our study, subdividing them by their pathology; we introduced the methods that we utilized for the in-depth analysis of the diaphragm: spirometry, respiratory muscle tests, CT imaging, US imaging, PNS and OEP.

We explained the protocol of our work, what the patient had to do and which tests were performed. Once that the protocol is fully described, we showed in which way we elaborated the data of each test. We deeply explained data elaboration from the OEP acquisition during quiet breathing and during other different maneuvers that we asked the patient to perform.

In chapter three we show the statistical method used to compare groups. Results coming from the comparison between pathological subject data and the control group data are also shown per any performed test. We also included a case study of the 5 patients who had performed CT images both at maximal and minimal lung volumes (TLC and RV) to underline the different conformations of the diaphragm for the different pathologies.

In chapter four we discussed critically the main results, finding relations between knowledges from the literature, the results of our analysis and the clinical considerations of the patients.

In chapter five, the future prospective of the present work are reported and we showed the preliminary results coming from the six transplanted patients that we could analyze during our thesis period. All their data were acquired few days after the transplant, in order to detect the immediate changing in functionality and morphology due to the surgical operations.

We showed also results coming from the only patient who underwent the analysis also after 6 months from the lung transplant. Parameters comparison highlight the importance to longitudinally extend the study in order to reveal all the transplant benefits.

SOMMARIO

L’apparato respiratorio è l’insieme di organi che permette lo scambio di gas tra il nostro corpo e l’ambiente esterno. Questo scambio di gas è chiamato respirazione e permette l’ingresso di O_2 nel sangue e la fuoriuscita di CO_2. Tale scambio di gas avviene nei polmoni, due organi presenti all’interno della cavità toracica. Questi organi possono essere riempiti e svuotati di aria a seconda dei muscoli respiratori attivati, i quali costituiscono la pompa ventilatoria.

Le patologie che possono colpire l’apparato respiratorio sono diverse e, per la funzione primaria e vitale che svolge tale sistema, la maggior parte di queste causano morte prematura. Le cause di queste patologie sono diverse: possono essere malattie genetiche, infettive o causate da agenti esterni come il fumo.

Tra le più comuni individuiamo la Fibrosi Polmonare Idiopatica (PF), la Fibrosi Cistica (CF) e la Broncopneumopatia Cronica Ostruttiva (COPD). Queste tre patologie colpiscono i polmoni in maniera differente: 1) la fibrosi polmonare idiopatica è una malattia polmonare interstiziale che causa una riduzione dei volumi polmonari e un aumento della rigidità degli stessi, insieme alla formazione di tessuto fibrotico cicatriziale. Mentre la riduzione dei volumi polmonari provoca una alterazione nel meccanismo di scambio dei gas a livello alveolare, il tessuto fibrotico e l’aumentata rigidità dei polmoni causano una maggior difficoltà di espansione dei polmoni stessi. La causa principale di questa patologia non è ancora nota, anche se si conoscono alcuni fattori di rischio come, per esempio, fattori genetici, infezioni virali, agenti esterni; 2) la fibrosi cistica è una malattia genetica che colpisce i polmoni facendone aumentare le secrezioni mucose e provocando, inoltre, infiammazioni ricorrenti e cambiamenti della conformazione dei polmoni. A seguito di questi effetti, i pazienti affetti da fibrosi cistica presentano ostruzioni delle vie aeree superiori e polmoni rigidi, il che causa una elevata difficoltà di espansione degli stessi. La causa della malattia è dovuta a un difetto nel gene che codifica per la proteina CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), la cui diversa conformazione provoca alterazione nelle escrezioni esocrine; 3) la COPD causa ostruzioni delle vie aeree e/o enfisema polmonare (rottura degli alveoli con conseguente distruzione della parete alveolare e creazione di sacche di gas) che portano a iperinflazione polmonare. Questa patologia ha tra

le maggiori cause il fumo, l'esposizione prolungata ad ambienti altamente inquinati o ricchi di polveri e gas tossici.

Tutte le patologie sopra citate portano, col tempo, a una insufficienza respiratoria cronica; in aggiunta, al momento non si conoscono cure in grado di risolvere definitivamente la malattia. Per questi motivi l'unica soluzione che può offrire al paziente una miglior qualità di vita e una prolungata sopravvivenza è il trapianto di polmoni.

Per decidere dell'inserimento di un paziente nella lista d'attesa per il trapianto di polmoni, è necessario che esso si sottoponga a una serie di test clinici che hanno lo scopo di valutarne la condizione cardiorespiratoria e la presenza di eventuali infezioni dei tessuti; sono necessarie anche valutazioni di tipo fisioterapeutico. Sulla base di considerazioni statistiche che tengono conto della condizione clinica del paziente, è possibile calcolare la probabilità di sopravvivenza del paziente a seguito del trapianto. Per essere inseriti nella lista d'attesa per il trapianto di polmoni, le condizioni che devono essere verificate sono le seguenti:

1- un rischio di morte per malattia polmonare entro 2 anni se non viene effettuato il trapianto superiore al 50%;

2- una probabilità superiore all'80% di sopravvivere almeno 90 giorni dopo il trapianto di polmoni;

3- una probabilità superiore all'80% di sopravvivere 5 anni dopo il trapianto nel caso il trapianto dovesse andare a buon fine.

Una volta effettuato il trapianto, il corpo umano necessita di un periodo di adattamento al nuovo organo; in particolare il diaframma e gli altri muscoli respiratori della gabbia toracica devono essere in grado di adattarsi alla presenza dei nuovi polmoni.

L'adattamento è un processo critico e non sempre va a buon fine. Il rischio di complicanze a seguito del trapianto polmonari è sempre presente; si possono suddividere tale complicazioni in precoci e tardive. Le prime sono quelle che si presentano a una breve distanza di tempo dal trapianto; un esempio è l'impossibilita di svezzare il paziente dalla ventilazione meccanica. Tale condizione può essere dovuta ad un mal funzionamento dell'organo trapiantato o ad un malfunzionamento del diaframma il quale può risultare permanentemente o temporaneamente paralizzato e quindi non essere più in grado di contribuire alla funzione della pompa ventilatoria per garantire gli scambi di gas. Le cause che portano a una paralisi diaframmatica non sono ancora note: potrebbe essere dovuta a una

lesione a livello del sistema nervoso o ad un mancato adattamento del diaframma a seguito dell'operazione.

Le complicanze tardive sono quelle che si verificano più avanti nel tempo, e possono essere legate allo sviluppo di infezioni o ad altri problemi che si verificano dopo lo svezzamento del paziente dalla ventilazione meccanica.

Dato che il diaframma è il muscolo respiratorio principale ed ha un ruolo fondamentale nel processo di svezzamento dalla ventilazione meccanica post trapianto, il nostro scopo è quello di analizzare il diaframma dei pazienti prima del trapianto con un approccio multidisciplinare per vedere come esso riesce a far fronte alle patologie citate sopra.

All'interno dell'IRCCS Fondazione Cà Granda Policlinico Maggiore di Milano è stato istituito un gruppo di studio in collaborazione con il Dipartimento di Elettronica Informatica e Bioingegneria del Politecnico di Milano che ha il compito di analizzare le condizioni del diaframma dei pazienti in lista d'attesa per il trapianto di polmoni.

Lo scopo principale dello studio è quello di indagare le condizioni del diaframma da diversi punti di vista, analizzandone sia la funzionalità che la morfologia, per valutare se anch'esso subisce delle alterazioni a causa della patologia. Nessuna tra Fibrosi Cistica, Fibrosi Polmonare Idiopatica e Broncopneumopatia Cronica Ostruttiva colpisce direttamente il diaframma, ma le alterate proprietà meccaniche del polmone possono causare variazioni nella morfologia e nella funzionalità del diaframma stesso.

Per prima cosa è stata fatta una caratterizzazione del sistema respiratorio dei pazienti tramite diversi test clinici: spirometria, Diffusione Alvelo-Capillare del Monossido di Carbonio (DLCO), Sniff Test (SNIP), Massima Pressione Inspiratoria (MIP), Massima Pressione Espiratoria (MEP). La prima valuta la funzionalità polmonare, misurandone le variazioni di volume durante manovre forzate, la seconda valuta l'efficienza degli scambi gassosi a livello alveolare, mentre le altre valutano la forza dei muscoli inspiratori ed espiratori misurandone le pressioni massime generate in diverse manovre.

Risultati anomali dei test precedentemente elencati possono essere dovuti a tre differenti cause: ridotte capacità polmonari, alterate proprietà meccaniche della gabbia toracica o un malfunzionamento della pompa ventilatoria. Per questo motivo abbiamo svolto altri test più specifici per valutare le caratteristiche del diaframma.

Per quanto riguarda la caratterizzazione morfologica, abbiamo utilizzato immagini di Tomografia Computerizzata (CT) dalle quali abbiamo selezionato l'emidiaframma destro e

sinistro per calcolarne il raggio di curvatura e la lunghezza. Questa operazione è stata effettuata sia per le viste sul piano sagittale che sul piano coronale, per avere una caratterizzazione più completa. Abbiamo anche analizzato immagini ecografiche per la misura di parametri come lo spessore, l'inspessimento e l'escursione diaframmatica durante la respirazione. Le misure di spessore sono state effettuate sia durante il respiro spontaneo, a fine inspirazione ed espirazione (EI, EE rispettivamente), che durante inspirazioni ed espirazioni forzate (FI, FE rispettivamente).

Diversi parametri riguardanti la contrattilità del diaframma sono misurati attraverso la stimolazione del nervo frenico (PNS). Quest'ultimo è un test non volitivo che induce la massima contrazione del diaframma. Le tecniche di elettromiografia (EMG) ed elettroneurografia (ENG) sono state usate per valutare la contrattilità del principale muscolo inspiratorio e la conduzione del nervo che comanda il muscolo stesso.

Parallelamente ai test sopra citati, abbiamo utilizzato una tecnica chiamata Pletismografia Optoelettronica che permette il calcolo del cambiamento di volume della parete toraco-addominale indotto dalla respirazione. Uno dei vantaggi della OEP è dato dalla possibilità di valutare il comportamento dei diversi compartimenti di cui è composto il torace: la gabbia toracica polmonare (RCp), la gabbia toracica addominale (RCa) e la parete addominale (Ab). Per caratterizzare il diaframma da un punto di vista funzionale abbiamo focalizzato la nostra attenzione sul compartimento addominale poiché è lì che il diaframma agisce maggiormente. L'innovazione introdotta da questo lavoro è il sincronismo tra la maggior parte dei test sopra elencati e la OEP. Questo permette di aggiungere valutazioni di tipo volumetrico alle misure derivanti dai test clinici.

Tutti i 28 pazienti in lista d'attesa per il trapianto di polmone presso l'IRCCS Fondazione Cà Granda Policlinico Maggiore di Milano nel periodo in cui abbiamo collaborato con l'ospedale sono stati inseriti nel nostro studio.

Abbiamo suddiviso la popolazione di pazienti sulla base della loro patologia di partenza: 9 pazienti affetti da PF, 5 da COPD e 14 da CF. I risultati estratti, relativi ai pazienti, sono stati confrontati con i parametri di un gruppo di controllo costituito da soggetti sani. Quando ci è stato possibile abbiamo acquisito noi stessi i dati dei soggetti sani; qualora ciò non fosse possibile abbiamo preso i dati dal database di riferimento del TBM Lab del Politecnico di Milano.

I dati di spirometria rivelano una riduzione significativa della capacità vitale (FVC) rispetto ai valori predetti, mostrando un pattern respiratorio ristretto. I risultati di FEV_1 mettono in luce come i soggetti affetti da COPD risultino ostruiti, mentre pazienti affetti da PF risultino ristretti. Soggetti affetti da fibrosi cistica, invece, hanno valori di FEV_1 intermedi, maggiori di quelli di pazienti affetti da COPD e minori di quelli di pazienti affetti da PF a dimostrazione del fatto che la fibrosi cistica presenta aspetti tipici sia di una patologia ostruttiva che di una patologia restrittiva.

Un'altra informazione utile per una caratterizzazione del paziente è quella relativa alla qualità degli scambi di gas all'interno dei polmoni (DLCO). Da questi valori, i pazienti affetti da PF sono quelli con una peggior efficienza negli scambi di gas a livello alveolare.

I test di MIP e MEP hanno rivelato che i PF e CF hanno valori molto simili alla normalità, mentre i COPD decisamente inferiori.

Lo SNIP test presenta valori comparabili o addirittura maggiori del predetto per i due tipi di pazienti fibrotici, mentre valori al di sotto del predetto per i COPD.

Per quanto riguarda la morfologia, abbiamo riscontrato differenze significative tra sani e soggetti patologici: i pazienti affetti da COPD rivelano un diaframma più piatto rispetto ai sani e le altre patologie, mentre i pazienti affetti da PF mostrano un diaframma significativamente più curvo.

Per quanto riguarda la misura di spessore ed inspessimento del muscolo, non vi sono differenze tra soggetti patologici e sani. Questo ci può portare a dire che il diaframma non è affetto da miotonia.

Valutando la stimolazione elettrica del nervo frenico, non sono presenti differenze nelle misure di EMG e ENG; pazienti affetti da CF mostrano una minor variazione di volume addominale durante la stimolazione.

L'analisi del pattern respiratorio dei pazienti non mostra differenze per quanto riguarda il volume corrente, mentre la frequenza respiratoria risulta maggiore per tutte e tre le patologie. Di conseguenza i pazienti mostrano un indice di Rapid and Shallow Breathing (RSBi) più elevato del normale il che ci porta a concludere che la pompa ventilatoria lavora in modo adeguato, svolgendo anche un lavoro superiore a quello dei soggetti sani, probabilmente per compensare la minore efficienza in termini di scambi di gas indotta dalle malattie. Questa ipotesi è avvalorata dai valori di DLCO dei pazienti che abbiamo analizzato: essendo praticamente tutti molto al di sotto del 100% del predetto, questa scarsa efficienza di scambi

gassosi a livello alveolare porta i pazienti ad una ventilazione più elevata per mantenere costanti i livelli di pH nel sangue. I volumi polmonari durante la capacità inspiratoria mostrano una riduzione significativa, in particolare per i pazienti affetti da PF. Questa riduzione è prevalentemente dovuta alla riduzione del contributo della parte alta della gabbia toracica; una inspirazione massimale alterata è dovuta alla ridotta azione muscolare degli intercostali che non riescono ad espandere i polmoni come accade invece nei sani.

Dopo i risultati sopra descritti, si può affermare che, nonostante i cambiamenti morfologici indotti dalla patologia polmonare, il diaframma dei pazienti considerati in questo studio sembra essere preservato. Infatti, a riposo, non ci sono segni di ipotonia e la contrazione, l'escursione, l'inspessimento e la forza del diaframma sono comparabili a quelle dei sani. Il ridotto contributo mostrato nella capacità inspiratoria può essere dovuto al maggior carico che il diaframma deve sostenere a causa dell'alterazione delle proprietà dei polmoni del paziente.

Pensiamo che questo approccio multidisciplinare debba essere ripetuto anche in seguito al trapianto di polmoni per vedere come il diaframma si adatta ai cambiamenti indotti dal trapianto stesso. I dati preliminari confermano questa affermazione. Infatti, durante il periodo della nostra collaborazione, abbiamo effettuato l'analisi precedentemente descritta su 6 pazienti dopo il trapianto.

Alcuni indici risultano diversi rispetto ai dati ottenuti prima dell'intervento: per esempio MIP e MEP hanno valori decisamente più bassi nel postoperatorio; il compartimento addominale ha una componente molto ridotta nel volume corrente dopo l'operazione mentre quella della gabbia toracica pettorale è molto aumentata; la latenza del nervo frenico è più elevata dopo il trapianto.

La riduzione di MIP e MEP e la differente compartimentalizzazione del volume corrente possono essere spiegati dalle condizioni fisiologiche dei soggetti: le incisioni effettuate a livello addominale durante l'operazione erano ancora lontane dall'essere totalmente guarite quindi potevano provocare dolore durante gli sforzi e comportare, quindi una ridotta espansione del compartimento addominale. Il contributo della gabbia toracica alta risulta, di conseguenza, aumentato.

La latenza maggiore può essere dovuta allo stress meccanico che subisce il nervo frenico durante l'operazione, che ne deteriora le condizioni rendendo più lenta la conduzione nervosa.

Dato il breve periodo intercorso tra il trapianto e la nostra analisi post-operatoria e considerando le condizioni cliniche del paziente, i benefici del trapianto potrebbero non essere visibili a pochi giorni dall'operazione. Per questo motivo si raccomanda di prolungare questo tipo di analisi anche dopo un periodo di 6 mesi dal trapianto, per vedere meglio gli adattamenti del muscolo ai nuovi organi e i miglioramenti del pattern respiratorio del paziente. Per nostra sfortuna, durante il periodo di collaborazione con l'ospedale, ci è stato possibile acquisire solamente un paziente dopo 6 mesi dal trapianto, ma i risultati sono comunque notevoli. Ad esempio sia la percentuale del contributo addominale del volume corrente che la frequenza raggiungono valori comparabili ai soggetti sani.
Queste considerazioni, nonostante si basino sull'analisi dei dati di un solo paziente, sottolineano l'importanza di uno studio longitudinale per la detezione dei benefici tardivi indotti dal trapianto.

Il presente lavoro di tesi è organizzato in cinque capitoli.
Nel primo capitolo introduciamo il sistema respiratorio, suddiviso in polmoni, vie aeree e muscoli respiratori, sottolineando l'importanza del diaframma. Presentiamo rapidamente le tre patologie polmonari che portano al trapianto di polmoni. In questo capitolo spieghiamo anche le condizioni che devono essere verificate affinché un paziente sia inserito nella lista d'attesa per il trapianto. Di questa operazione spieghiamo brevemente quali sono le principali complicanze, tra cui la paralisi del diaframma.
Per questo motivo descriviamo più dettagliatamente il diaframma, la sua anatomia e il suo contributo nelle diverse fasi respiratorie, oltre ai vari test per misurarne la funzionalità e morfologia.
Nel capitolo due presentiamo i pazienti presi in considerazione nel nostro lavoro, suddividendoli sulla base della loro patologia, spieghiamo nel dettaglio i metodi utilizzati per l'analisi morfologica e funzionale del diaframma: spirometria, test dei muscoli respiratori, immagini CT, ecografia, stimolazione del nervo frenico e OEP.
Spieghiamo, quindi, il protocollo seguito per tutti i pazienti, quali test sono stati effettuati e in che modo. Una volta spiegato il protocollo ci soffermiamo sull'elaborazione dei diversi dati acquisiti. Nel capitolo tre mostriamo i risultati del nostro lavoro, includendo un case study di 5 pazienti di cui abbiamo valutato le immagini tomografiche sia a TLC che RV, mostrando le diverse conformazioni diaframmatiche.

Nel capitolo quattro abbiamo discusso i nostri risultati comparandoli a conoscenze provenienti dalla letteratura e considerazioni cliniche.

Nel capitolo cinque abbiamo descritto i possibili sviluppi futuri del nostro lavoro, riportando i risultati preliminari dei 6 pazienti post trapianto acquisiti. Abbiamo spiegato come tutti i loro dati fossero stati acquisiti a pochi giorni dal trapianto e che quindi questi dati potrebbero essere fuorvianti e nascondere alcuni benefici indotti dall'operazione. Per questo motivo mostriamo anche il risultato del paziente acquisito a 6 mesi dal trapianto.

Chapter 1

Introduction

1.1 The Respiratory System

The respiratory system is that system constituted by organs which allow a continuous gas exchange between blood and the external environment with the final purpose to supply oxygen to any structure of the body and to remove carbon dioxide coming from cell metabolism. Breathing is the action which makes it possible.

Actually breathing consists of four different processes:

-pulmonary ventilation: air is moved into and out of the lungs;

-external respiration: gas exchange between lungs and blood;

-transport: transport of oxygen and carbon dioxide between lungs and tissues;

-internal respiration: gas exchange between systemic blood vessels and tissues.

It is possible to distinguish two different components within the respiratory system: the upper and the lower respiratory system. The first one includes mouth, nose, nasopharynx and pharynx; the second one is constituted by trachea and lungs with bronchi and their ramifications.

Respiration is achieved through the mouth, nose, trachea and lungs. Oxygen enters the respiratory system through the mouth and the nose. Then it passes through the larynx and the trachea which is a tube that enters the chest cavity. Here the trachea splits into two smaller tubes called bronchi. Each bronchus then divides again forming the bronchial tubes. The bronchial tubes lead directly into the lungs where they divide into many smaller tubes which

connect to tiny sacs called alveoli. The inhaled oxygen passes into the alveoli and then diffuses through the capillaries into the arterial blood. Meanwhile, the waste-rich blood coming from the veins releases its carbon dioxide into the alveoli and it follows the same path out of the lungs when you exhale.

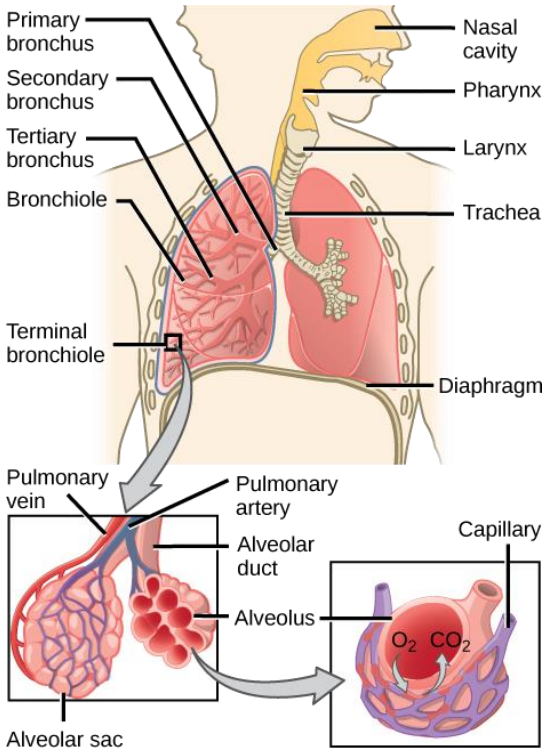

Figure 1.1.1 – Air flow pathway from the nasal cavity through the pharynx, larynx, trachea, lungs till the alveoli where gases are exchanged with capillaries.

The respiratory muscles play a fundamental role for the correct breathing occurrence: they help to pump the carbon dioxide out of the lungs and pull the oxygen into the lungs. These muscles can be classified on the basis of their function: inspiration or expiration.

The inspiratory muscles elevate the ribs and sternum in order to give lungs the possibility to expand and accommodate as much air as possible.

The primary inspiratory muscles are the external intercostal muscles and the diaphragm. There are also some accessory inspiratory muscles, as for example the sternomastoid, the anterior scalenus and the pectoralis major and minor which support the primary inspiratory muscles activity. The most important inspiratory muscle is diaphragm which is a sheet of internal skeletal muscle that extends across the bottom of the rib cage. It separates the thoracic cavity from the abdominal cavity. Thanks to its changing shape, it makes

possible inspiration: when it contracts, the volume of the thoracic cavity increases and air is drawn into the lungs.

Differently from the inspiratory muscles, which are always necessary to assure inspiration, the expiratory muscles action is negligible during quiet expiration as the elastic recoil of lungs and ribs plays the major role as far as air exhalation is concerned. On the other side, expiratory muscles result to be necessary during forced expiration; they contract to depress ribs and sternum in order to provide the maximum air exhalation.

The primary expiratory muscles are the internal intercostals, intercostalis intimi and subcostals. Even for expiration there are some accessory muscles like, for example, the abdominal muscles.

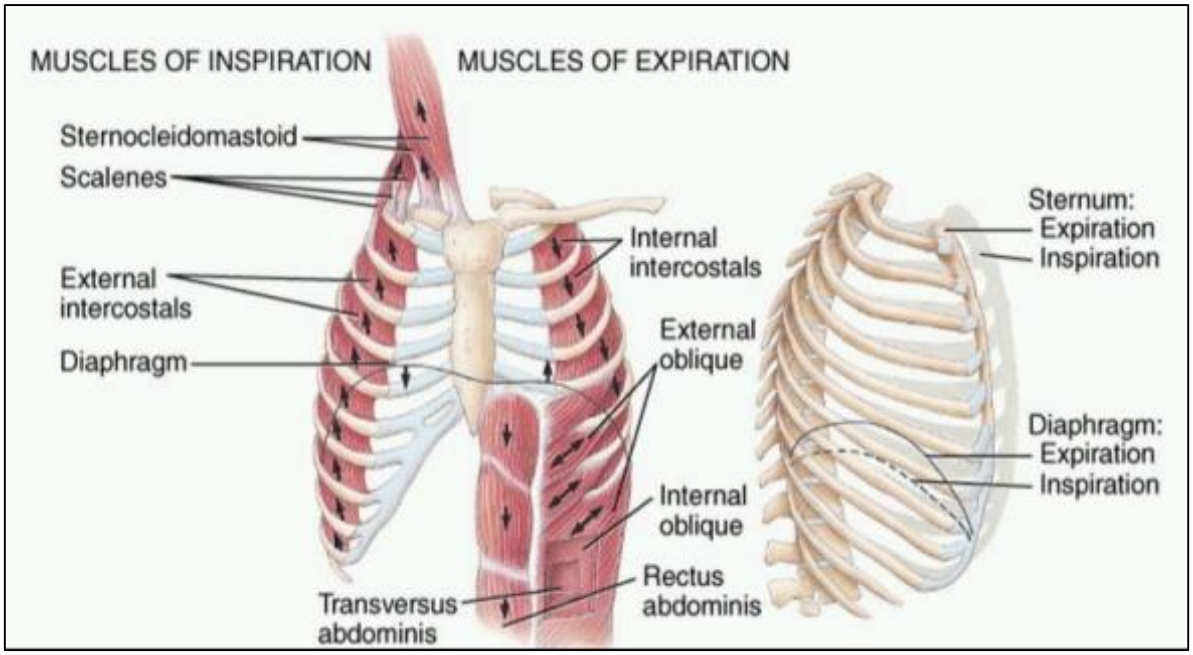

Figure 1.1.2 – Respiratory muscles. In the figure on the left inspiratory and expiratory muscles are shown; in the figure on the right it is possible to see the diaphragm during inspiration (dotted line) and during expiration (continuous line).

Even if the gas exchanges between blood and the external environment is the main function of the respiratory system, it is not the only one. Actually it has many other important functions as protecting respiratory surfaces from dehydration and temperature variation, defending itself and other tissues against pathogenic micro-organisms attacks, assisting the regulation of blood volume and pressure and the pH control. [1]

1.1.1 The Lungs

Lungs are the organs where respiration takes place. They are two, located near the backbone in both sides of the heart, in the upper thorax. Lungs are enclosed within a sac called pleural sac. It has the function to avoid friction during the lung movements due to respiration.

Each of the lung is subdivided into sections called lobes. The right lung has three lobes and the left one has two lobes; these are further divided into bronchopulmonary segments and sublobes.

Lungs do not have a unique blood supply; they receive deoxygenated blood from the heart with the purposes of oxygenating it (pulmonary circulation), while a separated supply of oxygenated blood (bronchial circulation) is used for the lung cell respiration.

The function of the lungs is exchanging O_2 and CO_2 between external environment and blood. In order to generate a gas flow, a pressure gradient is required between the two different regions. A pressure variation of about -5 cmH_2O within the alveoli (during quite breathing) makes the generation of the inspiratory flow possible; particularly, the variation in pressure is induced by an expansion of the volume of the thoracic cavity under the action of the inspiratory muscles [2]. The expiratory flow is generated when the intra-alveolar pressure becomes higher than the atmospheric one; in this way gas flows out through the upper airway structures.

There are several parameters used to describe the lung movement, which is strongly linked to the volume, and the respiration phases:

- **Total Lung Capacity** (TLC), the maximum volume at which lungs can be expanded;
- **Residual Volume** (RV), the volume of air remaining within the lungs after a forced expiration;
- **Tidal Volume** (TV), the volume of air moved into or out of the lungs during quiet breathing;
- **Functional Residual Capacity** (FRC), the volume of air in the lungs at the end of an expiration during quiet breathing;
- **Vital Capacity** (VC), the maximum amount of air that can be expired starting from the end of a forced inspiration till the end of a forced expiration: VC = TLC - RV;
- **Inspiratory Capacity** (IC), the maximum volume of air that can be inspired starting from the functional residual capacity position: IC = TLC - FRC;

- **Inspiratory Reserve Volume** (IRV), the maximal additional air volume that can be inhaled starting from the end-inspiratory level during quiet breathing: IRV = IC - TV;
- **Expiratory Reserve Volume** (ERV), the maximum volume of additional air that can be expired from the end of an expiration during quiet breathing: ERV = FRC - RV.

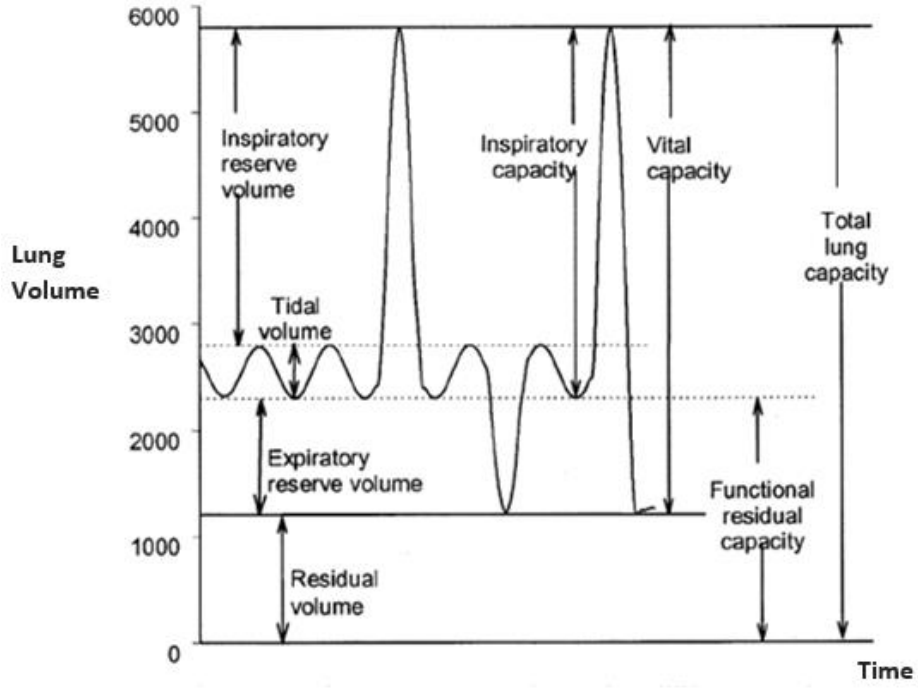

Figure 1.1.1.1 – Schematic view of volume parameters. Numerical values refer approximatively to an adult male.

Lungs are not the only constituent part of the respiratory system, but they are the most affected by diseases.

1.2 Most Common Lung Diseases

The three most common lung disease are: Idiopathic Pulmonary Fibrosis (PF), Chronic Obstructive Pulmonary Disease (COPD) and Cystic Fibrosis (CF).

These diseases affect the lungs in different ways, but all of them lead to chronic respiratory failure and premature death of the pathological subjects.

1.2.1 Idiopathic Pulmonary Fibrosis

It is a chronic and progressive pathology occurring in 0,03-0,04% of the global population [3]. This data is continuously increasing. Moreover, idiopathic pulmonary fibrosis (PF) diagnosis are not so much and they are tardive in most of the cases; this fact causes the death of a lot of PF patients after 3-6 years from the appearing of the first symptoms [4].

The idiopathic pulmonary fibrosis is a pathology which affects only the lungs and leads to a decreased lung tissues elasticity. It comes from an abnormal alveolar tissue repairing process with a resulting excessive production of fibrous tissue. Further consequences of this pathology are lung hypertension and right cardiac deficiency [5].

Even though a lot of improvements about the knowledges of the pathology have been made, many aspects are still unknown, like for example, the main cause of the disease. A lot of risk factors have been found, anyway: genetic factors, responsible of the lung cell behavior alteration; natural aging, as we can look at the idiopathic pulmonary fibrosis as the lung precocious aging; environmental factors, that means the presence of toxic substances into the air [4]. Some other risk factors like smoking, virus infections, chronic tissue damages induce a worsening of the pathology.

No definitive therapy has been found until now; anyway some treatments are able to improve temporarily symptoms and to slow the pathology progress.

Lung transplantation is an option which is often taken into consideration.

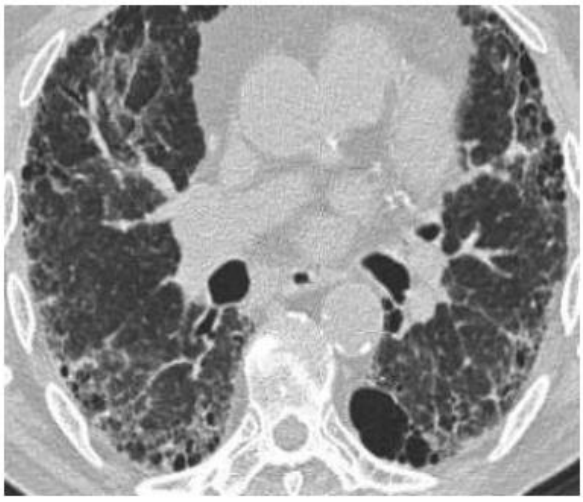

Figure 1.2.1.1 – CT of pulmonary fibrotic lungs.

1.2.2 Cystic Fibrosis

Cystic fibrosis is a really rare pathology affecting one person over 8000-10000 in Europe (the incidence is about 0,03-0,04% in Italy) [6]. This kind of patients have a life expectancy of about 40 years (4% of the population goes beyond this age). [7]

Differently from the idiopathic pulmonary fibrosis, cystic fibrosis is a multi-organ pathology which affects not only the respiratory system but also the digestive system (particularly it can cause diseases to the pancreas, to the intestine, to the liver). Actually all internal organs may be affected from the pathology.

Damaged genes are the responsible of cystic fibrosis; these genes are the ones coding for the CFTR protein (Cystic Fibrosis Transmembrane Conductance Regulator). Such a protein regulates transmembrane hydroelectric flow; its malfunctioning leads to a modification of the exocrine excretion. As a result, we have the production of sweat with a high salt content and a really viscous mucous secretion. [8]

As far as the respiratory system is regard, cystic fibrosis consequences are:

-airway obstruction;

-bronchial infection;

-lung chronic inflammation.

These phenomena lead to a gradual deterioration of the respiratory function.

Improvements in nutrition, mucus clearance, treatment of inflammation and infection have led to dramatic improvements in cystic fibrosis respiratory morbidity and mortality.

Lung transplantation may be taken into account.

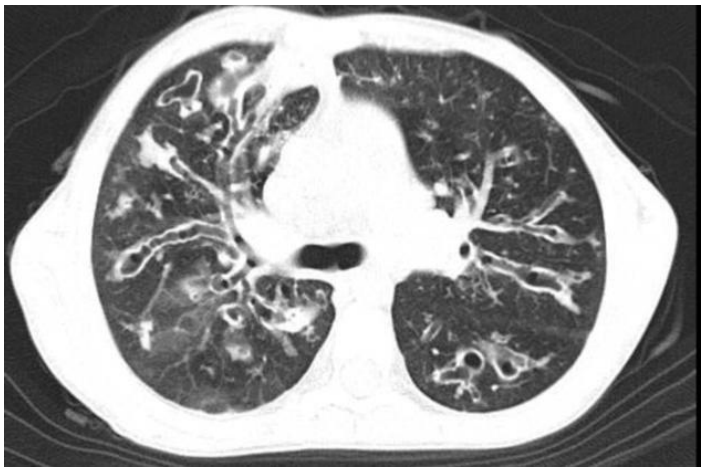

Figure 1.2.2.1 – CT image of cystic fibrotic lungs. Mucus deposits are visible (red arrow).

1.2.3 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a respiratory syndrome characterized by progressive, partially reversible airway obstruction and lung hyperinflation. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines COPD as a disease that is ‘‘usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases’’.

Worldwide, 64 million people were estimated by World Health Organization in 2004 to have COPD. It is estimated that prevalence of COPD will increase within the next 10 years, while mortality will remain at the current rate [9].

The term COPD actually describes a group of lung conditions (diseases) that make it difficult to empty the air out of the lungs causing shortness of breath (also called breathlessness), limitation daily activities and worsening health related quality of life [10].

Anatomically, the abnormal processes in the airways of patients with COPD develop as poorly reversible or irreversible airway obstruction, or emphysema (rupture of the inner walls of alveolar sacs with creation of larger air spaces with trapped air), or a combination of both. The disease is frequently accompanied by mucus hypersecretion that leads to productive cough and may impair bacterial clearances.

Chronic obstructive pulmonary disease is mainly a result of direct damaging effects of inhaled particles and gases and subsequent cellular inflammatory processes in the airway. These noxious particles or gases are inhaled with cigarette smoke or smoke from burning biomass fuel. Genetic factors may also cause the COPD onset [9].

COPD has no cure yet and doctors are not able to cure damages of the airways and lungs. However, treatments and lifestyle changes can help in feeling better.

Lung transplantation is a possibility to consider [11].

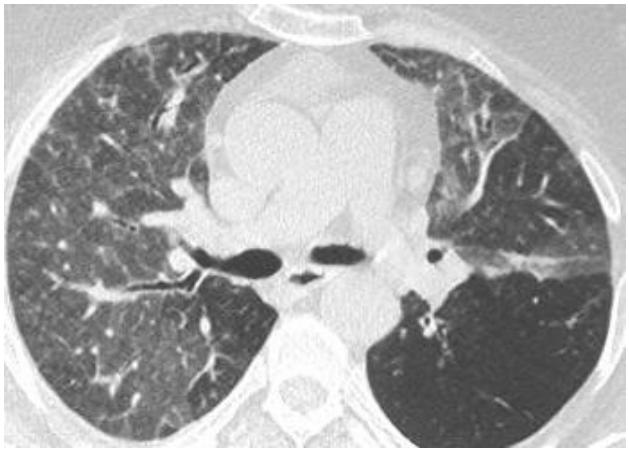

Figure 1.2.3.1 – CT of lungs affected by COPD.

What is important to underline, is that all the descripted pathologies, are pathologies which do not affect directly the diaphragm. Anyway, different lung properties, like for example shape and elasticity, may cause an alteration in the diaphragm functionality; moreover, the oxygen therapy and the non-invasive ventilation which patients with lung disease may have been undergone may lead to a diaphragm weakening. Also the fact that these kind of patients have respiratory failure, thus they do not practice any physical activity, affects muscle condition.

1.3 Lung Transplantation

Because, as mentioned before, the three above descripted pathologies lead to premature death, the solution to significantly improve quality of life in that kind of patients is lung transplantation.

Lung transplantation is the most complex one, considering the solid organs (heart, liver, kidney), and it consists in the substitution of one or both of the lungs with healthy ones from a dead donor.

It should be considered for adult patients suffering from chronic end-stage lung disease. Not any patients can undergo lung transplantation; some general criteria have to be satisfied:

- High risk of death (> 50%) from lung disease within 2 years if lung transplantation is not performed;
- High likelihood (> 80%) of surviving at least 90 days after lung transplantation;
- High likelihood (> 80%) of 5-year post-transplant survival from a general medical perspective provided that there is adequate graft function.

What should be taken into account is that not any donated organ can be received by any patients; for physiological reasons, the received lungs must have dimensions comparable to the sick ones in order to fit the space in the thorax and be in an optimal position to avoid an alteration in the diaphragm shape, curvature and length which can lead to a variation in the diaphragm functionality and contractility ability.

In order to be selected as potential lung recipients, patients' situation has to be carefully analyzed by qualified clinicians.

The decision to place the patient in the active waiting list is critical. Particularly, clinicians have to assess the timing of entrance in the waiting list for the patients and to identify who is more likely to benefit from a lung transplant, in their specific conditions at that time. From 2014, the International Society of Heart and Lung Transplantation drew up a new guideline for physicians who have to deal with waiting list patients, in order to standardize and set up a common line for the evaluation, inserting new parameters and considerations compared to the previous guidelines.

In the document "A consensus document for the selection of lung transplant candidates: 2014 - An update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation" by Weil et al [12] are explained indications, contraindications (relative and absolute) and specific disease criteria that has to be followed to correctly place the patients in the waiting list. All of these criteria are supported by scientific data.

One of the characteristic indexes that are reliable and internationally used in lung transplantation waiting lists is the Lung Allocation Score (LAS); it is an index which is calculated to infer the statistical probability of the patient's survival in the first year after the transplantation and the projected length of survival post-transplant. This score is calculated by a complex formula that takes into account several clinical parameters of the patient like, for example, age, kind of disease, body mass index (BMI), pulmonary arterial pressure, forced vital capacity and other indexes.

Moreover, patients have to undergo to a long series of tests which aim at assessing their cardio-respiratory functionality but also their infectious condition, their status by a physiotherapeutic point of view…

Once that patients' clinical situation is clearly analyzed and taken into account, considering LAS and all other criteria, the candidates are finally included in the waiting list and

continuously monitored by clinicians in order to update, if necessary, their position in the waiting list.

Lung transplantation is nowadays a well-established treatment that improves survival and quality of life in patients with advanced chronic lung diseases. Of course probabilities of success are high, but mortality during the operation are around 5-10% [13].
It is most often indicated in cases of COPD, cystic fibrosis, interstitial lung disease, non-cystic fibrosis bronchiectasis, and pulmonary hypertension.

1.3.1 Main Post-Operative Complications

Like any other surgical intervention, also lung transplantation may have some post-operative complications which must be taken into account in order to assure the success of the operation.
Acute rejection is one of the primary causes of transplantation failure. Actually there are two types of acute rejection: acute cellular rejection (the most common), characterized by perivascular and interstitial mononuclear cell infiltrates; and acute humoral rejection, which is also referred to as acute antibody-mediated rejection. Humoral rejection is more closely related to hyperacute rejection, which occurs immediately after the surgical procedure (but it can also occur later). An active surveillance has to be performed.
Another possible complication due to lung transplantation is infection. The prevalence of pathogens depends on the time elapsed since transplantation. Until the first postoperative month, the most common infections are those related to the surgical procedure, to the donor, or to the recipient. From the first postoperative month to the sixth postoperative month, activation of latent infections is common. After the sixth postoperative month, the prevalence of community-acquired infections (pneumonia and urinary tract infection) increases [14].
Furthermore, patients receiving immunosuppressant are at an increased risk of malignancies. The most common types of malignancies are skin cancer and lymphoproliferative disorders. Primary graft dysfunction and chronic allograft dysfunction represent other two possible transplantation failure causes.
Finally, surgical complications may also occur. They include dehiscence, necrosis and bronchial anastomotic stenosis. Vascular complications such as venous stenosis are rare,

occurring in 1-2% of cases. Paralytic ileus is the most common abdominal complication, occurring in 30-50% of patients. Gastroparesis, acute cholecystitis, and intestinal perforation can also occur.

The postoperative complications we talked about until now refer to organ transplantation, in general.

As far as lung transplantation specifically regards, the arising of early complications makes impossible the weaning of the patient from the life-support system or from the mechanical ventilation. It may be caused by a malfunctioning of the new transplanted lung (mainly due to primary graft dysfunction or infections) or to a compromised diaphragm, which may also result to be paralyzed and which does not allow to the lungs to work as they should.

The cause of the diaphragmatic failure is not yet explained; anyway, possible causes may be related to a resection of the phrenic nerve, low temperature reached and phrenic nerve stretching during the operation, but also to prolonged mechanical ventilation after the transplantation causing muscle hypotonia.

1.4 The Diaphragm

1.4.1 Diaphragm Structure

Lungs are enclosed in the thoracic cavity by the rib cage on the front, back, and sides, with the diaphragm forming the floor of the cavity.

The diaphragm is a double dome-shaped sheet of muscle and tendon that serves as the main muscle of respiration and it plays a vital role in the breathing process: as the diaphragm contracts, the volume of the thoracic cavity increases and air is drawn into the lungs. Moreover, it serves as an important anatomical landmark that separates the thorax, or chest, from the abdomen.

Structurally, it consists of two parts: the peripheral muscle and central tendon.

The peripheral muscle is made up of many radial muscle fibers – originating on the ribs, sternum, and spine – that converge on the central tendon. The central tendon – a flat aponeurosis made of dense collagen fibers – acts as the tough insertion point of the muscles. When air is drawn into the lungs, the muscles in the diaphragm contract, and pull the central tendon inferiorly into the abdominal cavity. This enlarges the thorax and allows air to inflate the lungs.

The peripheral muscle can be further divided by its origins into the sternal, costal, and lumbar regions. The sternal region is made up of two small muscular segments that attach to the posterior aspect of the xiphoid process. The costal region is made up of several wide muscle segments whose origins are found on the internal surface of the inferior six ribs and costal cartilages. The lumbar region has its origins on the lumbar vertebra through two pillars of tendon called the musculotendinous crura, which fixes the diaphragm at the back. Actually two crura exist: the right crura and the left crura and they are joined by a fibrous median arcuate ligament. Additional posterior attachments include also the paired medial and lateral arcuate ligaments. The medial arcuate ligaments extend over the anterior psoas muscles as fibrous attachments between the L1 or L2 vertebral body and the transverse processes of L1. The lateral arcuate ligaments are instead thickened fascial bands covering the quadratus lumborum muscle and they extend from the transverse processes of T12 laterally to the mid-portion of the 12th ribs.

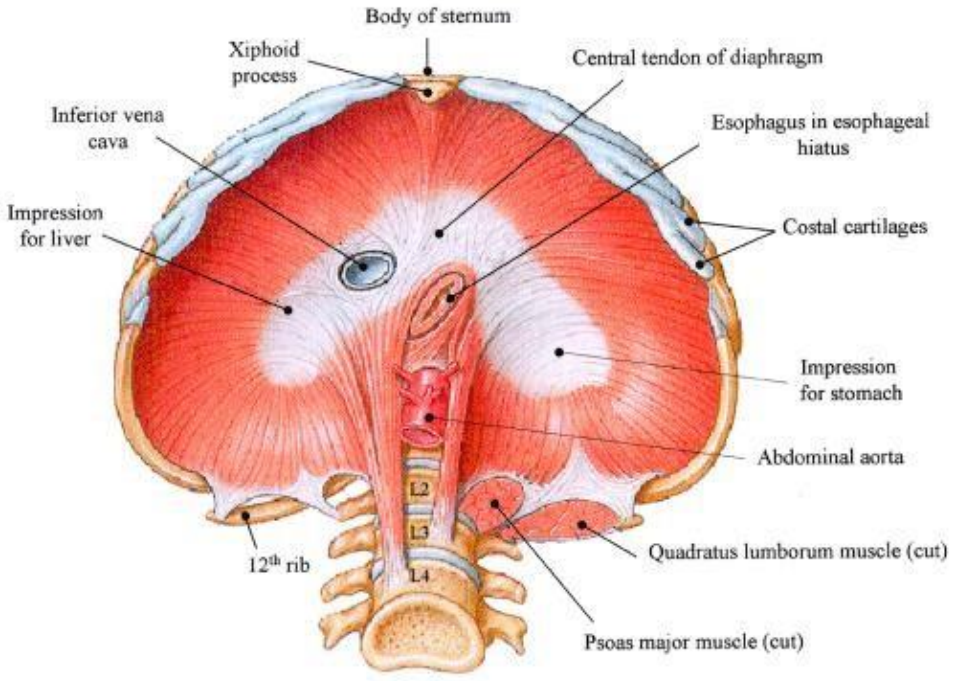

Figure 1.4.1.1 – Anatomical and functional structure of the diaphragm.

Another important functional and anatomical characteristics of the diaphragm is the presence of three main openings (hiatuses) that allow important structures to pass between the thorax and abdomen.

There are three large openings - the caval, the esophageal, and the aortic opening - plus a series of smaller ones.

The caval opening allows the passage of the Inferior Vena Cava (IVC). It is at the T8 level and contains the IVC and branches of the right phrenic nerve. It passes through the mid-portion of the central tendon. As it is surrounded by tendons, the caval hiatus is enlarged any time inspiration occurs. Since thoracic pressure decreases upon inspiration and draws the caval blood upwards toward the right atrium, increasing the size of the opening allows more blood to return to the heart, maximizing the efficacy of lowered thoracic pressure returning blood to the heart.

The esophageal hiatus is at the T10 level and it contains the esophagus, vagus nerve and sympathetic nerve branches.

The aorta does not pierce the diaphragm but rather passes behind it between the left and right crus.

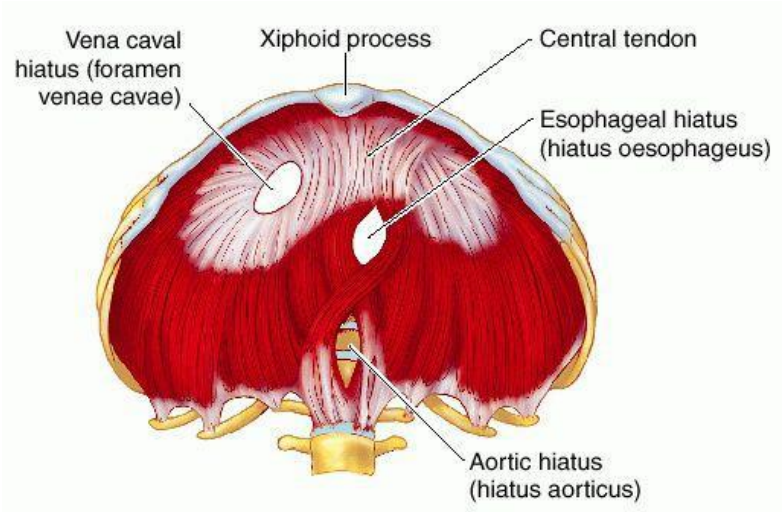

Figure 1.4.1.2 – Anatomical structure of the diaphragm showing its openings.

The diaphragm is primarily innervated by the phrenic nerve which is formed from the cervical nerves C3, C4 and C5. [15]

1.4.2 Diaphragm Function

From a functional point of view, the diaphragm is the primary muscle of ventilation.

During inspiration it contracts flattening itself; the accessory muscles of respiration, external intercostal, sternocleidomastoid and scalene muscles tense too so that the anterior rib cage is elevated. [15] The overall contraction results in an increasing of the lung volume which leads to a decreasing of the intrathoracic pressure. In this way the air flow from the external environment into the lungs is promoted.

It is also important to mention that the inspiratory muscles, including the diaphragm, are stronger in man than in women and that strength tends to reduce with the increment of the age. Therefore, the inspiratory muscles of the elderly can have resistance problems with increased mechanical loads due to severe respiratory disease. Generally, the diaphragm subjected to a heavy load reaches fatigue only in exceptional physiological circumstances in healthy subjects.

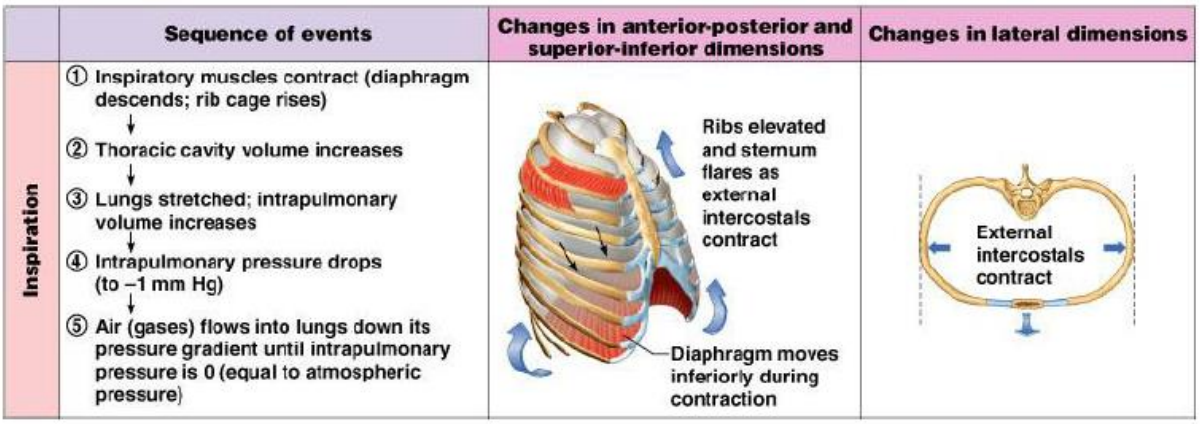

Figure 1.4.2.1 – Schematic view of the inspiratory mechanism

During a spontaneous exhalation, the rib cage drops to its resting position while the diaphragm relaxes and elevates to its dome-shaped position in the thorax. Air within the lungs is forced out of the body as the size of the thoracic cavity decreases.

Forced exhalation involves the internal intercostal muscles used in conjunction with the abdominal muscles, which act as antagonist paired with the diaphragm's contraction.

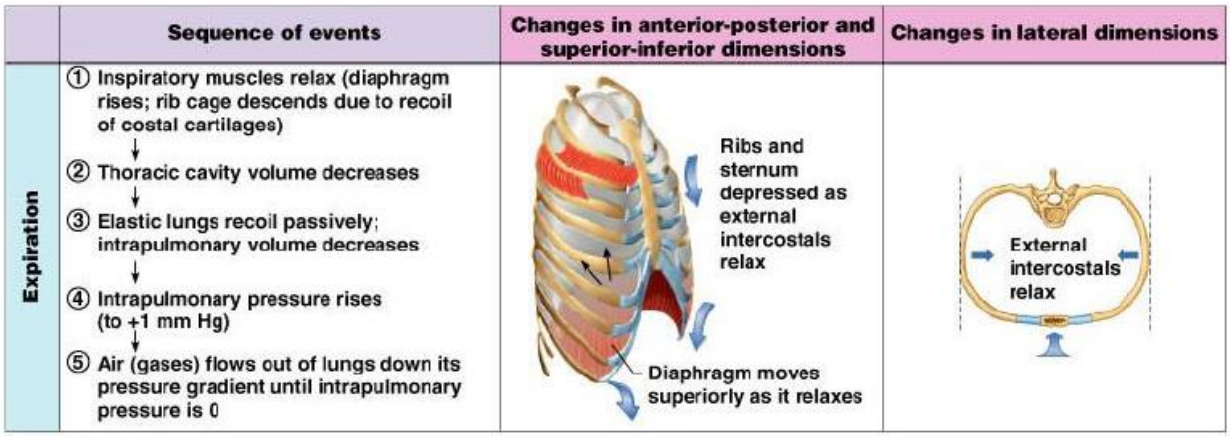

Figure 1.4.2.2 – Schematic view of the expiratory mechanism

The diaphragm muscle not only plays a role in respiration but also has many roles affecting the health of the body. Diaphragm aids in emesis, urination, defecation and, for women, in childbirth. It is important for posture, for proper organ function, for the cervical spine and trigeminal system, as well as for the thoracic outlet. It is also of vital importance in the vascular and lymphatic systems [16].
From a functional perspective, the crural and the costal region act with different purpose; the crural region is responsible for a correct breathing whereas the costal region prevents gastroesophageal reflux. The two regions are required to work at different times and with different innervation during deglutition, esophageal distension and vomiting.

As any other skeletal muscle diaphragm contraction generate force. The contractile force that it can generate is related to the length of its fibers at the initial moment of the contraction, to the stimulation frequency and to the shortening velocity of its fibers.

We know that a skeletal muscle is able to generate the maximal contractile force when it is in its optimal resting length; a stretching or a foreshortening of muscular fibers lead to a submaximal generated strength.
In the specific case of respiratory muscles, we can distinguish two different behaviors:

- As far as inspiratory muscles concern, they are foreshortened when lung volume becomes higher than the FRC level; this lead to a reduction in the generated contractile forced.
- As far as the expiratory muscles regards, a reduction in the generated force verifies when lung volumes are lower than the FRC level.

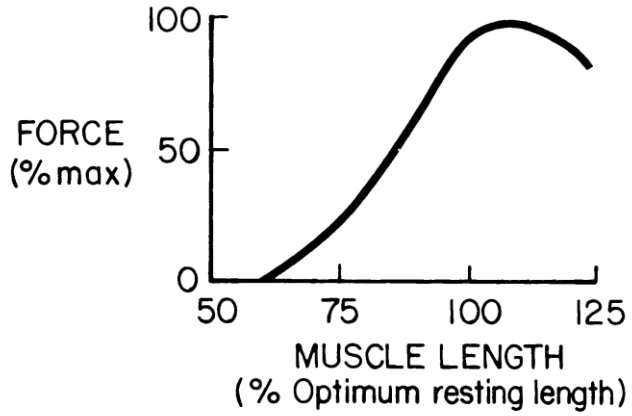

Figure 1.4.2.3 – Length-Forced relationship.

Beyond muscle length, also the velocity of shortening of the muscle itself determines the generated force. By speeding up fiber shortening, the generated contractile force is reduced.

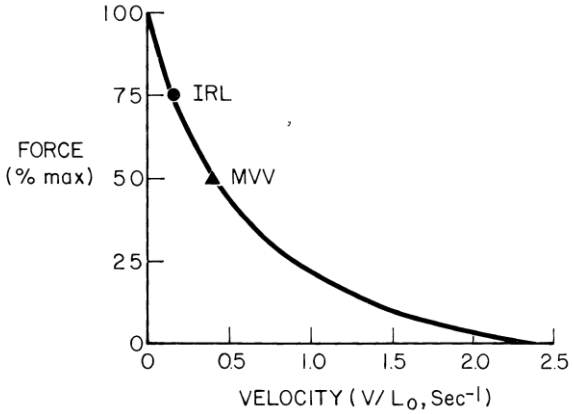

Figure 1.4.2.4 – Shortening velocity-force relationship. IRL stands for Inspiratory Resistive Loading while MVV for Maximum Voluntary Ventilation.

Looking at inspiratory muscles, previous works have shown that when a resistive load is applied, fibers velocity of shortening decreases thus generating a higher contractile force [17].

At last, also the frequency of stimuli influences the ability of the muscles of generating force. In particular, by augmenting the stimulation frequency, it is possible to generate higher force values.

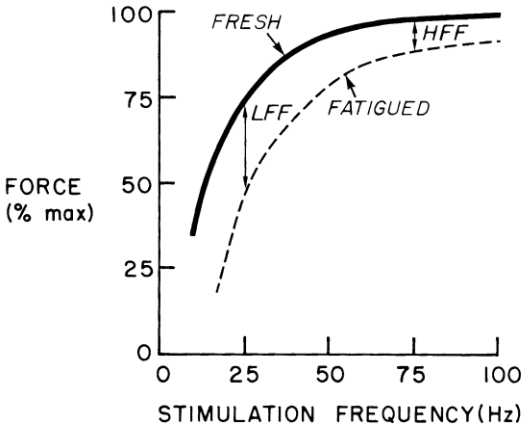

Figure 1.4.2.5 – Stimulation frequency-force relationship.

Possible causes of respiratory muscle weakness are related to damages to motor nerves, to neuromuscular junction, to muscle cell per se; also central or peripheral neuropathies may induce muscular weakness [17].

The muscle fatigue is defined as the inability of muscles to generate a contractile force.
It can be caused by an inhibition of neural drive, failure of transmission across neuromuscular junction, excessive force and duration of contraction, impaired muscle blood supply, impaired excitation-contraction coupling and depletion of muscle energy stores.
Muscular fatigue is mainly related to the stimulation frequency, to the duration of the stimulation and to the duration and the force of the muscle contraction.
Human inspiratory muscles become fatigued during maximal voluntary efforts such as repeated inspiration through a severe airflow resistance. A fatigued muscle is able to generate lower force.

Even if pathologies like idiopathic pulmonary fibrosis, cystic fibrosis and chronic obstructive pulmonary disease do not directly affect diaphragm, as a consequence of the impaired lungs, it may change its morphology and consequently its functionality and its capability to generate force.
In patients affected by pulmonary fibrosis, for example, diaphragm results to be lengthened; the increased fiber length is responsible for a lower generated force and the effort which is required to expand lungs is higher.
In COPD patients, the augmented resistance of the airways, FRC and respiratory rate affect inspiratory muscles function. Both the increase in airway resistance and hyperinflation of the lung increase the work of breathing; the increase in respiratory rate is such that at the end of an expiration, the inspiratory muscles contract before expiration has proceeded long enough for the lung and chest wall to reach the relaxation volume. This increase in the lung volume at rest causes a reduction in the length of the diaphragm fibers which consequently causes a reduction in the force generation and an increase in the required breathing work.
As a final result, even if none of the cited pathologies affect directly the diaphragm, on the basis of the severity of the disease a diaphragm weakness or fatigue may be diagnosed.

In the same way as lung diseases lead to a modified breathing pattern, also muscles malfunctioning, weakness and fatigue may lead to wrong respiratory mechanisms and compromise the quality of ventilation.

To assess the presence of respiratory muscles weakness, what is usually done is to evaluate the strength that muscles are able to generate through some clinical tests. Tests we refer to are called Mouth Inspiratory Pressure test (MIP) and Mouth Expiratory Pressure test (MEP) and they measure the pressure which the subject is able to generate during a forced inspiration and expiration, respectively, when a resistive load is applied to the mouth, with no air flow. The higher is the pressure value, the higher is the force which muscles are able to generate.

MIP and MEP resulting values may be corrected to the lung volume at which the maneuver has been performed (at RV for the MIP test and TLC for the MEP one) in order to make possible comparisons between measures coming from different subjects [17].

1.5 Morphological and Functional Evaluation of the Diaphragm: most Common Measurements and Tests

Clinicians are allowed to use a lot of test to measure the morphology and the functionality of the diaphragmatic muscle. A morphological study of the diaphragm aims at having a measure of two different parameters regarding the shape: diaphragm length and curvature; on the other side, the study of the diaphragm functionality aims at knowing how it works, its strength and its kinematic.

1.5.1 Diaphragm Morphology

In order to assess the diaphragm morphology, clinicians use three different kinds of imaging: Computerized Tomography, Ultrasonography and Magnetic Resonance Imaging. An image elaboration process allows us to extract whatever measures we are interested in.

Computerized Tomography is the most invasive method, as it is based on ionizing radiations; more specifically photons with an energy in the range of X-Rays are used. This technique allows us to display internal tissues of the body and to distinguish them on the basis of their density. Although the Computerized Tomography is an invasive technique, it is one of the most used digital device as far as the diagnosis regards because of its good resolution and its relatively low cost.

Magnetic Resonance uses the magnetic spin of the hydrogen atoms in tissues to map the density of molecules in which hydrogen is present. The output image is a map of the

hydrogen density in the tissue, so it is more selective for soft tissue than hard ones. Magnetic Resonance is less invasive than CT and more repeatable, but also less precise.

Ultrasonography is a clinical test which can be used to assess both the morphology of the diaphragm, in terms of thickness [18], and its functionality in terms of dome motion.

Ultrasonography makes use of ultrasonic pulses which are generated by emitting crystals and which are directed into the body tissues. The ultrasonic wave travels within the tissue until the moment in which it senses a change in the acoustic impedance. In correspondence of this point, part of its power is reflected back producing an echo, which is detected by receiver crystals. The echo power is the physical quantity used to create images.

Using these three different kinds of imaging techniques it is possible to display shape and length of the diaphragm; hemi-diaphragm paresis or other abnormalities can be revealed without using any other test [18].

1.5.2 Diaphragm Functionality

Looking at functional tests, we can distinguish between invasive and non-invasive tests and between volitional and non-volitional ones.

Invasiveness is defined on the basis of the ability of a test to penetrate the immune system of the human organism; volitional tests, contrary to non-volitional tests, are the ones which require patient collaboration.

The first and most used test, in order to assess the functionality of the overall respiratory system is the so called **spirometry**. It is a non-invasive volitional test which requires the patient to perform a maximal inspiration (till reaching the Total Lung Capacity) and a maximal expiration (till reaching the Residual Volume). During this maneuver the patient is attached by a mouthpiece to an instrument called spirometer which measures the air flow passing through it. The instrument is able to calculate some parameters during the spirometry, and all of them are well known in literature. One of these parameters is the Forced Vital Capacity (FVC) that is the maximal change in volume between a maximal inspiration and a forced expiration; when the FVC value is different from the predicted one, it reveals the presence of some pathology. Another parameter is called FEV_1, that measures the volume of exhaled air during a forced expiration in the first second of the spirometry maneuver. When this parameter is different from the 80-120% of the predicted value, it is

an index of abnormality of the airways (it points out an obstructive or a restrictive pathology).

Considering volitional and non-invasive tests, **Maximal Inspiratory Pressure (MIP)** is the most widely used measure to evaluate the inspiratory muscle strength in patients with suspected muscle weakness. It is determined by measuring upper airway pressure (mouth or trachea for intubated or tracheostomized patients) during a maximal voluntary inspiratory effort. The measured pressure is a composite of the pressure generated by the inspiratory muscles and the elastic recoil pressure of the lungs and chest wall [18].

Linked to the MIP, on the other side, there is the **Maximal Expiratory Pressure (MEP)** test which is based on the same principle but the patient is asked to perform a maximal expiration. The device used for the measurement, which is attached to the patient through a mouthpiece, measures the maximal pressure generated during the maneuver. This quantity is not linked to the diaphragm but it helps to understand the functionality of the expiratory muscles.

Another volitional and non-invasive method is the measurement of **Sniff Nasal Inspiratory Pressure (SNIP)**. It measures the activity of the inspiratory muscles and it accurately reflects esophageal pressure (Pes), having the advantage of being noninvasive [18]. It is performed by inserting an eartip in one nostril and by measuring the pressure through the contralateral one. The patient is asked to perform a short and sharp sniff with closed mouth starting from the end expiratory volume after a quiet breath.

Because sniff is a natural effort that many patients find easier to perform than static efforts, the sniff nasal inspiratory pressure (SNIP) was recently proposed as an alternative to the MIP [19,20]. However, SNIP can underestimate esophageal pressure swing in subjects with nasal obstruction, patients with chronic obstructive pulmonary disease and severe neuromuscular patients. Nevertheless, since SNIP maneuver has predicted normal values, it is noninvasive and it is easier to perform than maximal inspiratory pressure (MIP) maneuver, it could be considered as the first simple test to use in order to assess inspiratory muscle weakness [21].

Spirometry, MIP, MEP, SNIP tests are not able to differentiate which of the different part of the respiratory system is impaired; altered values of one of them may be caused by chest wall abnormalities or by a disease of the lungs or by an impairment of the ventilatory pump.

That is the reason why other clinical tests are necessary in order to find the causes of altered values.

An invasive, non-volitional method to assess diaphragm functionality is the measurement of the **Transdiaphragmatic Pressure (Pdi).** This pressure is the difference between gastric pressure (Pga) and the Esophageal Pressure (Pes) (Pdi = Pga – Pes) and it represents the force generated by the diaphragm rather than by the other respiratory muscles. It is invasive as it makes use of a catheter through nose and mouth to place the pressure sensors into the esophagus and stomach. The advantage of this method is that the pressure is measured directly in the position of our interest, it is not an approximation. The disadvantage is its invasiveness, as a catheter passing through mouth and nose is not comfortable for pathologic subjects. It also requires special instruments that are not always easily available and placement requires expert clinicians too.

As regards the non-volitional tests, one of the most used is the **Phrenic Nerve Stimulation (PNS)**. This kind of stimulation can be performed electrically or magnetically; the former is more painful for the patient but it is also more selective, the latter is not painful and so the patient is more comfortable. They are both based on stimulating the cervical phrenic nerve, which is sufficiently superficial in the neck region. The two stimulations induce the rapid maximal contraction of the diaphragm. During the stimulation clinicians could measure different parameters, depending on what they are interested in. The most common are electromyographic quantities and pressure measurements. Three quantities are usually measured during the test: latency of the response, its maximal amplitude, the area of the negative peak of the response. While the maximal amplitude response is an index of the number of axons activated in the nerve, the area of the negative peak gives back information about the conductivity of the phrenic nerve itself. In addition, if a pressure sensor is used, it is also possible to extract a measure of the Trans-diaphragmatic pressure (Ptdi) which increases as a step during the stimulus and decreases exponentially after that.

The advantages of the PNS are that it allows the measurement of the main inspiratory muscle strength, irrespective of patient cooperation. The diaphragm strength can be measured thanks to the fact that it is innervated exclusively by the phrenic nerve, and this enables the overall muscle stimulation.

The magnetic phrenic nerve stimulation creates a magnetic field in the cervical region by placing small coils over it, while the electrical one makes use of surface electrodes to induce the nerve action potential.

Magnetic stimulation easily penetrates tissues and bones, preferentially activating larger neural fibers rather than smaller fibers, which are responsible for mediating pain. Moreover, it provides greater safety and comfort with respect to the electrical stimulation.

However, since the magnetic field can stimulate other cervical nerves and muscles, its use is usually less specific for the measurement of diaphragm strength than is that of electrical stimulation, although this difference does not appear to be clinically relevant [22,23].

Moreover, magnetic stimulators are very expensive and not readily available [18].

1.6 Aim of the Study

Starting from the knowledges we have from the literature, we took most of the shown tests in order to assess the functionality and morphology of the diaphragm in a multifactorial approach. At the same time of the clinical tests, we performed an Optoelectronic Plethysmography analysis in order to evaluate the volume changes during the mentioned test, or the breathing pattern of the subjects under our analysis.

Because a diaphragmatic paralysis after a lung transplantation has been frequently observed and because of the fact that no specific causes have yet been found, a collaboration between the Thoracic Surgery Unit of 'IRCCS Fondazione Cà Granda Policlinico di Milano' and the TBM Lab at Politecnico di Milano has been set in order to in-depth analyze the diaphragm. We wanted to collect as much information as possible and investigate if there are some abnormalities that can predict the failure of the muscle after the transplant.

Chapter 2

Materials and Methods

2.1 Patients

The present study has been conducted with the cooperation of the Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico in Milano.

Patients taking part in our study were afferent to the Thoracic Surgery Unit of the previously cited hospital. Inclusion criteria to be met in order to take part in the study are the following:

- patients included on the waiting list for lung transplant;
- age > 18;
- informed consent.

Pathologies affecting patient participating in our study were three: pulmonary fibrosis, cystic fibrosis, chronic obstructive pulmonary disease.

To compare parameters of patients we have collected also parameters of a different number healthy volunteers.

2.2 Methods of Investigation

2.2.1 Spirometry

The spirometry test measures the airflow passing through a spirometer; by its integration it is possible to calculate the air volume inhaled or exhaled by a patient. The spirometer is equipped with two different pressure sensors divided by a grid which constitutes an

obstruction to the air flow. The resistance offered by the grid is known by the producer. By measuring the two pressures and knowing the grid resistance it is possible to calculate the air flow following the equation: $flow = \Delta P / Resistance$. The flow measurement is, indeed, indirectly computed.

Spirometry tests are the most widely used in order to evaluate respiratory functionality as they are able to assess lung function by indirectly measuring volume changes during time.

The spirometer which has been used in our study is Spirolab II® (MIR) and it is shown in Figure 2.2.1.1.

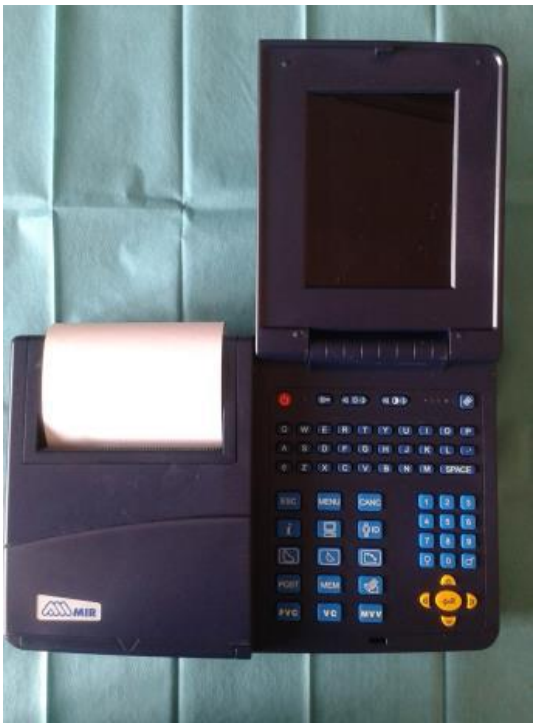

Figure 2.2.1.1 – Spirometer Spirolab II®

The advantages of this kind of test are related both to the easily portability of the instrumentation and to the fact that it can be performed bedside, without requiring a specific and particularly equipped room.

We worked together with the hospital physiotherapist specialized in the respiratory system. Spirometry tests have been performed according to the criteria of the American Thoracic and European Respiratory Society [24].

2.2.2 Diffusion Capacity of the Lung for Carbon Monoxide (DLCO)

Together with the two spirometry indexes we wanted to characterize the rate of impairment of the gas exchange of the different pathologies, so we collected the DLCO values of patients from their medical records. It is an index of the quality of gas exchange in the lungs [25].

It is measured in a non-invasive way by clinicians. The test involves measuring the partial pressure difference between inspired and expired carbon monoxide. It relies on the strong affinity and large absorption capacity of erythrocytes for carbon monoxide and thus demonstrates gas uptake by the capillaries that are less dependent on cardiac output.

2.2.3 Maximal Inspiratory Pressure and Maximal Expiratory Pressure

Maximal inspiratory pressure (MIP) and Maximal Expiratory Pressure (MEP) are simple, convenient, and noninvasive indices of respiratory muscle strength, but standards are not clearly established [26].

The instrumentation needed consists of a well-fitting mouthpiece connected to a small chamber, to which a pressure gauge is connected. Thanks to the fact that air flow is prevented by a high resistance of the instrument, a sensor measures the pressure generated at the mouth during maximal inspiratory (MIP) or expiratory (MEP) efforts.

A small leak in the chamber prevents the patient from using mouth muscles to generate higher pressure, mainly in the MEP test [26].

MIP and MEP measurements are both expressed in cmH_2O.

Because lung volume cannot change significantly during the tests, results are to a large extent independent of the properties of the lungs. They are general tests of neuromuscular function of the combined diaphragm, abdominal, intercostal, and accessory muscles [26].

The clinician always incited the patient vocally, as it has been proved that a maximal vocal encouragement motivates subjects in doing their better [27].

Figure 2.2.3.1 – MicroRPM by Carefusion

2.2.4 Sniff Nasal Inspiratory Pressure

Another measurement performed by the physiotherapist in order to have an evaluation of the whole inspiratory muscles strength is the Sniff Nasal Inspiratory Pressure test.

Sniff Nasal Inspiratory Pressure (SNIP) test is used to estimate in an accurate and non-invasive way the esophageal pressure swing during sniff maneuvers [21] and it is performed by occluding a nostril and by getting the measure through the contralateral one. Sniff measurements are expressed in cmH_2O.

The instrumentation used in our work is the same used for the MIP and MEP measurements, just switching the final mouthpiece with a nostril pressure sensor.

Similarly to the spirometer, also the SNIP instrumentation is easily portable and it can be performed bedside, without requiring a specific and particularly equipped room; as a disadvantage, SNIP underestimates the esophageal pressure in patients with nasal obstruction, chronic respiratory obstruction and probably also in patients with neuromuscular disease [21].

2.2.5 Computerized Tomography

The Computerized Tomography (CT) is an imaging technique which makes use of ionizing radiation (X-rays) and which allows to reproduce a sectional view of the internal tissues of a body. Furthermore, specific processing technique of the CT-images could provide a 3-dimensional representation of the internal body.

It is probably the most used digital device as far as the diagnosis regards also because of its relatively low cost.

Another advantage related to the CT imaging technique is related to the fact that it can be performed without any kind of limitations; any part of the body can be investigated, no matter about hard or soft tissues, presence of metallic prosthesis or presence of air inside organs.

What is difficult, as in other imaging technique, is the interpretation of the images once they are provided by the machine. Considerations about the CT are usually given by doctor with a long experience who can distinguish abnormalities in tissues just by looking at a CT image.

Moreover, X-Rays constitutes themselves the disadvantage of the technique. Actually, ionizing radiations are dangerous for the human body as they could break atomic bonds in tissue and so create charged ions that can destroy DNA chains and induce cancer.

In our study, the use of CT is trivial to assess the shape of the diaphragm, in terms of length and radius of curvature.

CT slices, as they are, can not give us an overall view of the main respiratory muscle as just a sequence of images from the axial view is provided. For this reason, some elaboration processes have been necessary.

2.2.6 Ultrasonography

Ultrasonography is a diagnostic imaging technique used for the visualization of the internal body structures by recording the reflections of ultrasonic pulses directed into the tissues [28]. This kind of imaging technique is worldwide used to examine soft tissues, bone surfaces and muscles.

The physical principle that allows us to display images is the reflection of ultrasound waves in correspondence of those points where there is a change of the acoustic impedance.

Ultrasounds are mechanical waves provided by an emitter, usually based on a piezo-electric crystal. This kind of crystal has the property of expanding and contracting if a voltage drop is applied to its surface. Mechanical waves are produced in such a way.

For diagnostic purposes, the range of frequency is from 2 to 15 MHz; just for special applications it could reach 40MHz. It must be taken into account that the higher is the frequency and the higher is the image resolution, but, conversely, the ability to penetrate the tissue decreases with high frequency. For this reason, the right tradeoff between depth and resolution has to be chosen.

An ultrasonic wave travels through a medium until its acoustic impedance is constant; when there is a change in the acoustic impedance, part of the power of the wave is transmitted and the rest of the power is reflected back to the emitter. The percentage of the reflected and transmitted wave is related to the change in acoustic impedance.

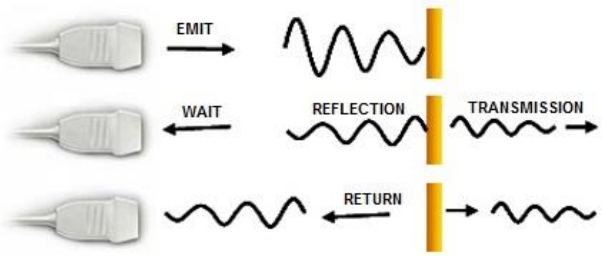

Figure 2.2.6.1– Simplification of principle of work of US.

What is used for displaying the images is the power of the reflected waves, which ,hitting the piezo-electric crystals of the transmitter/receiver, induces a voltage drop between the surfaces of the crystal. There are different modes of representing the echoes: A-Mode, B-Mode, M-Mode and Doppler Mode.

- The A-Mode (Amplitude Mode) is a mono-dimensional imaging visualization: the echo is represented by peaks whose amplitude is proportional to its intensity while its depth is proportional to the distance of the interfaces which generated the echo;
- the B-Mode (Brightness Mode) is a bi-dimensional imaging visualization. Echoes are represented along a scan line on the basis of their distance from the source (this distance is determined by taking into account the time they need to go back to the receiver); their intensity is indeed represented through a grey scale: white

corresponds to a maximal intensity while black points out the absence of echoes. Intermediate grey levels are related to intermediate levels of power of the echoes.

- An M-Mode (Motion Mode) image shows the motion of different points of tissue along a single scan line as a function of time. To generate the M-Mode images, the B-Mode data from subsequent acquisitions of the same scan line are placed side by side in an array as an image. The image is updated in real-time as newer data become available.

In our analysis, only B and M-Mode are used.

B-Mode ultrasound approach provide a 2-dimensional image of the muscle allowing evaluation of its thickness [20].

On the other hand, M-Mode allows evaluation of diaphragm kinetic, motion and excursion, speed of contraction and duration of the cycle [29]; they are shown to be replicable and reliable measurements [30].

The advantages of ultrasonography are a lot: it can be used easily bedside in hospital because of the relatively small dimension; it does not use any kind of radiation so no limitations on the frequency of use or parts of the body scanned; it provides real time images, so it can be commonly used in ICU units, and finally it has a relatively low costs compared to other imaging techniques.

On the other hand, the technique has some disadvantages: the presence of air or bone structures always limits the quality of the images; the quality of the image is strictly related to the operator; positioning the probe and recognizing the structures is not trivial and operators need time to make their own experience.

Using US imaging, two kinds of measurements related to the diaphragm can be performed: thickness at different lung volume and the whole diaphragm motion during several breaths.

Many works in literature explain how to assess the functionality of the diaphragm using US by using these two measurements [31].

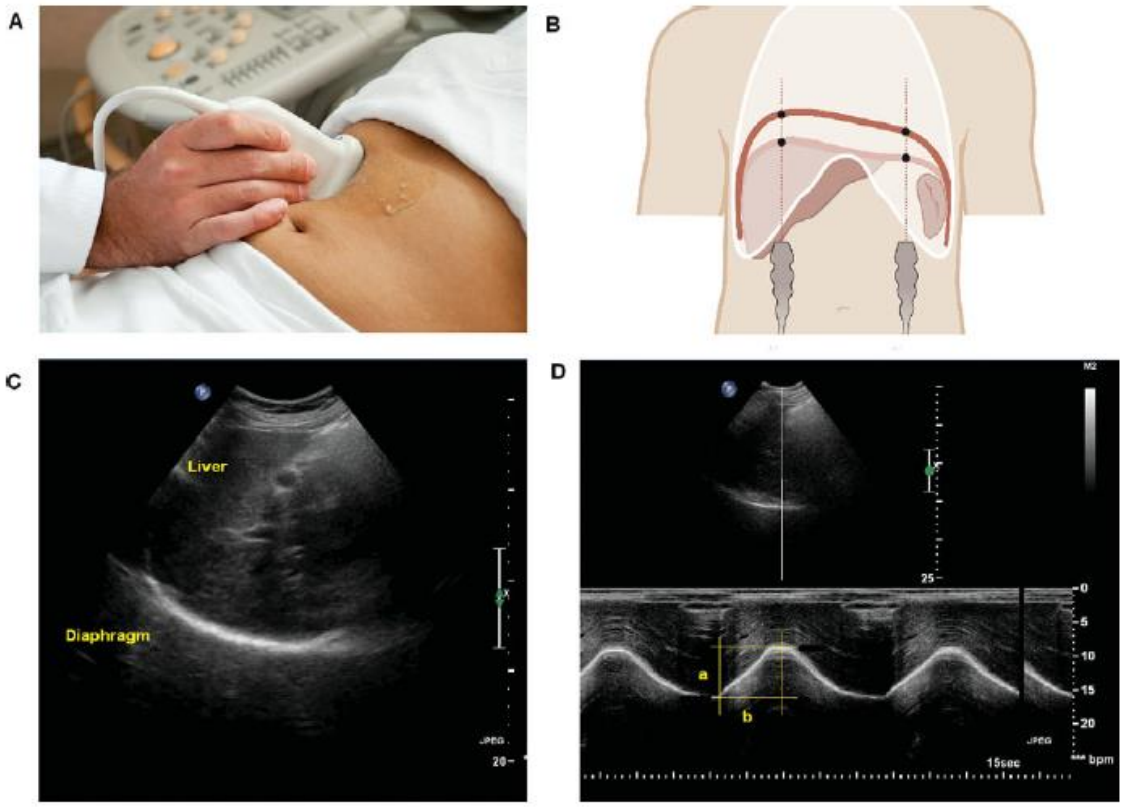

Figure 2.2.6.2 – Curvilinear transducer placement for the anterior subcostal view, with the transducer positioned below the costal margin in the midclavicular line. (B) Scheme of US beam path. (C) Diaphragm dome B-Mode image. (D) M-mode image: anatomical structures motion along the exploration line.

2.2.7 Electromyography and Electroneurography during Phrenic Nerve Stimulation

Electromyography is a clinical test that allows the clinicians to investigate the muscular activity in terms of action potential. Electrodes placed on the skin, in correspondence of known positions, record and measure the muscular action potentials after a proper stimulus. The measurements are indexes of the integrity of the muscle, and indirectly of the force generated during the stimulus.

Phrenic nerve stimulation is a kind of stimulation that allows the clinician to induce a maximal contraction of the diaphragm whenever it is needed. This non-volitional contraction is caused by an electrical current provided in the phrenic nerve by a specific stimulator.

Electroneurography is a clinical test which records the neural activity, in terms of latency, amplitude and velocity of the stimulus. Latency is the time interval that elapses from the time instant in which the stimulus is provided to the detection of its effect in a known

anatomical point. Amplitude is a measure of the neural action potential; velocity is the speed of conduction of the action potential through the nerve.
In the case of our interest it is used a percutaneous stimulator, because it is less invasive, more practical and tests are easily implementable.

2.2.8 Plethysmography

The term plethysmography (from ancient greek: plethysmos = multiplication, increase) refers to the measure of the volume variations of a specific part of the body.
In respiratory applications, plethysmography can be applied in two different ways:

- Measuring the effects of volume variations in the thorax, due to the presence of gas in a plethysmograph chamber;
- Measuring the volume variation from geometrical variations of the thorax, due to some relevant point movements, through magnetometers, variable inductance plethysmographs or more recently Optoelectronic Plethysmography (OEP, BTS system, Milano, Italy).

The latter technique is the one we used in our study because of its several advantages.

2.2.8.1 Optoelectronic Plethysmography (OEP)

Optoelectronic Plethysmography (OEP system, BTS, Milano, Italy) is a technique able to non-invasively measure chest wall volume changes without any kind of connection to the patient. OEP measures the changes of the complex shape of the chest wall during breathing by modeling the thoraco-abdominal surface with a large number of points belonging to selected anatomical reference sites of the rib cage and abdomen. The three-dimensional positions and displacements of each point are measured by using passive markers (plastic hemisphere covered by a thin film of retro-reflective paper) placed on the skin.
Acquisitions are performed by the SMART optoelectronic system (BTS, Italy). It is based on an automatic and versatile movement analysis system. It is composed by 8 TVcameras placed at 2.5 meters height, with 3 degrees of freedom in order to move their orientation in space when needed, focusing on the zone of positioning of patient.

Figure 2.2.8.1.1 – Reconstruction of TV cameras and patient's trunk. Black dots are markers placed on the patient and used as reference points.

LEDs surrounding the TV camera work as lightning elements; they irradiate the retroreflective markers in the near infrared wavelength (880nm) and markers reflect it. By their reflection, markers are recognized by the cameras and reported as white points in the PC screen.

Figure 2.2.8.1.2 – On the left, one of the cameras placed in our laboratory. On the right, the two different kinds of retroreflective markers (the spherical one and the hemi-spherical).

A dedicated parallel processor executes real-time pattern recognition algorithms to identify the two-dimensional position of each marker recorded by each camera and transform it into the three-dimensional coordinates thanks to the system calibration previously performed. Once the three-dimensional co-ordinates of the points belonging to the chest wall surface are acquired, a closed surface is defined by connecting the points to form triangles. For each

triangle, the area and the direction of the normal related vector are determined. Successively, the internal volume of the shape is computed using Gauss' Theorem.

The plethysmographic approach of the study using optoelectronic plethysmography (OEP) is reliable as shown in the study of Massaroni et al. in which they assessed OEP accuracy compared to a chest wall simulator [32].

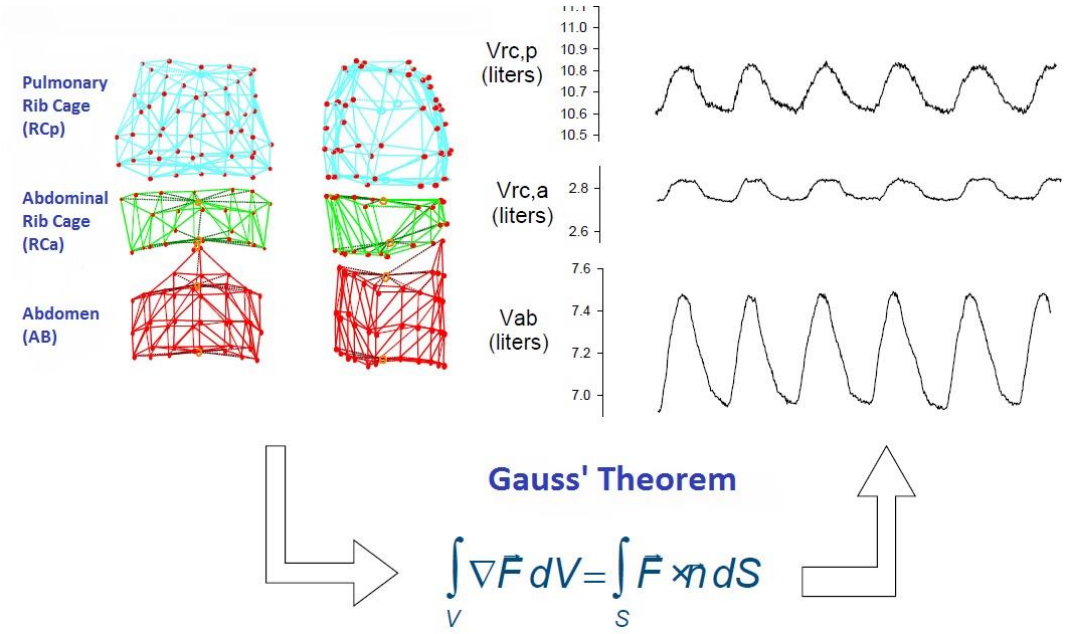

Figure 2.2.8.1.3 – On the left: Result of the reconstruction and identification of markers and reconstruction of the geometrical reference model. On the right: the variation of the three compartments volume during time. The track has been obtained through the computation of the plethysmographic data.

There are two main advantages in using OEP instead of other methods which measure ventilatory parameters: the absolute absence of connections to the patients and the possibility to subdivide the subject's trunk into different compartments.

The lack of physical instrument of connection between the patient and the motion capture system results to be an advantage as it makes possible to get rid of all the measurement errors provided by any system connected to the patient (for example an incorrect connection leads to air dispersion making us obtain distorted results). No connection is an advantage also for the patient because he has to stand or lay down in a bed without anything that makes him uncomfortable or limits his freedom. OEP makes sure that measurement is made by the software which evaluates markers position and follows their movements during time.

Afterwards the possibility to separate the different compartments of the subject's trunk is an advantage because it allows us to evaluate the behavior of the different trunk compartments by themselves and to calculate which is their contribute on the total volume variation. The subdivision gives us information about different respiratory muscles functionality and, particularly, the variation of abdomen volume is an index of diaphragmatic muscle mobility. Moreover, we can consider separately the right and the left side of each compartment, in order to underline any asymmetries or differences between the two parts of the considered compartment.

Further advantages are related to the fact that a near infrared wavelength is used. Indeed, making use of an infrared wavelength, it is possible to operate both in dark rooms or illuminated ones, without any kind of problem with phosphorescent objects which emits small amount of light above 730 nm. Moreover, the frequency of considered wavelength is not effective on human body and not perceptible by human eye; in this way the patient does not feel uncomfortable and movements are kept as natural as possible [33].

2.3 Protocol of the Data Acquisition

2.3.1 Spirometry and SNIP test

As regards lung functions measurements, the physiotherapist of the Thoracic Surgery Unit asked the patients to perform a Vital Capacity maneuver both in supine and seated position. The maneuver is constituted by an initial maximal inspiration (until reaching the Total Lung Capacity) followed by a forced maximal expiration (until reaching the Residual Volume).

The parameters measured by the clinicians were the classical spirometry measurements (FVC and FEV1) both for supine and seated position.

After the Vital Capacity maneuver the clinicians let the patients rest a bit because of the maximal effort that they were asked to perform; then a SNIP test was planned. Patients were lying supine on the bed and an earplug was placed in a nostril while the other one was kept closed by the clinician. the patient is asked to perform some rapid and sharp inspirations (SNIFF) with the nose spaced out by a period of quiet breathing. The pressure sensor contained in the earplug measures the pressure generated by inspiratory muscles during the sniff maneuver.

In order to be sure to measure the maximal effort, repetition of the maneuver was needed, usually three or four times; the best result, that means the maximal value of pressure, was taken as final result.

2.3.2 DLCO Measurements

DLCO tests require very little effort compared to spirometry tests. The subject inhales a test gas containing 0.3% CO and a tracer, and a measurement is made of the exhaled concentration of CO and the tracer after a breath-hold of about 10 seconds.
The DLCO is the ratio between the rate of disappearance of CO and the difference of alveolar concentration of CO and the partial pressure of CO in the blood.

2.3.3 MIP and MEP Measurements

The Thoracic Surgery Unit physiotherapist performed two different measurements on the patients: Maximal Inspiratory Pressure and Maximal Expiratory Pressure.
Both of them are simple tests in which patients generate as much inspiratory or expiratory pressure as possible against a blocked mouthpiece where it is inserted a pressure sensor.
For the MIP test, after some seconds of quite breathing, the patients are asked to sustain a maximal inspiratory effort starting from the residual volume (that means at the end of an expiration). As far as the MEP test regards, the starting volume is usually TLC (Total Lung Capacity), and positive pressure has to be generated by a forced expiration.
Both of the test are performed in seated position and under the supervision of the clinician, whose presence was helpful to provide the proper vocal encouragement in order to help the patient to perform the maximal effort.
The test has to be repeated more than once, usually three or four times, in order to give the patient the possibility to learn the proper execution of the maneuver; the final considered results are always the one related to the best performance.

2.3.4 CT Acquisition

CT protocol included a chest CT scan of the patients at total lung capacity (TLC). CT scanner in use was “SIEMENS VA0 COAD” by Siemens. CT slice thickness was 3mm and voxel

height and width were 0.76mm. Each CT image had a 512x512 pixels resolution. For the images taken into account by our thesis, patient did not need any contrast medium.
The CT images considered in our study were acquired right after the patients were included in the waiting list for the transplantation.

2.3.5 US Imaging

Ultrasound imaging technique, differently from CT one, took place in our lab.
For the two different measurements performed, two probes were needed. For the measure of the diaphragmatic thickness, the surgeon needed a linear type probe, while for the diaphragmatic excursion a curved one was necessary.
The positioning of the ultrasonic probe was always on the right side of the patient thorax. For the thickness measure, the probe was placed in correspondence of the seventh intercostal space looking at the insertion point of the diaphragm in the rib cage. The placement of the probe to detect the dome excursion was on the abdominal zone, craniocaudally directed.

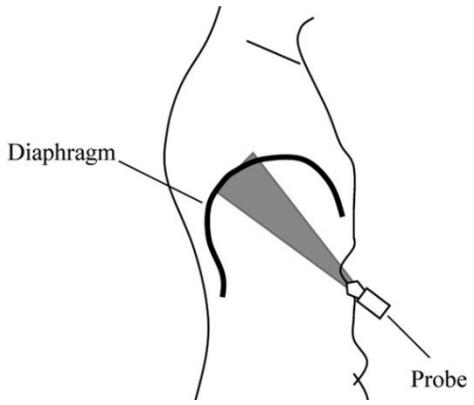

Figure 2.3.5.1 – Positioning of the probe during diaphragm excursion acquisition.

This kind of measurements were performed during the quite breathing, so the clinician measured thickness at end inspiration (EI) and end expiration (EE), but also at the end of a forced expiration (FE) and a forced inspiration (FI).
During the acquisition of these images, we used a system developed by other engineering students at Politecnico di Milano to capture the screened images as they are and save them in PC folders, using a frame grabber and an Arduino Nano by Arduino (Ivrea, Italy) as a

breadboard with its ATmega328 microcontroller; this allowed us an offline elaboration of the data.

In our study, a surgeon of the Thoracic Surgery Operative Unit has taken all the ultrasonography images, in order to have a more reliable measure.

2.3.6 Phrenic Nerve Stimulation

Another test in collaboration with personnel in the hospital was the Phrenic Nerve Stimulation (PNS) performed by a specialized neuro-pathophysiology technician.

The protocol of the test implied the positioning of two surface electrodes: the active one is applied in correspondence of the xiphoid process, while the reference electrode above the costal margin. The ground electrode was placed on the chest wall at the level of the sternum.

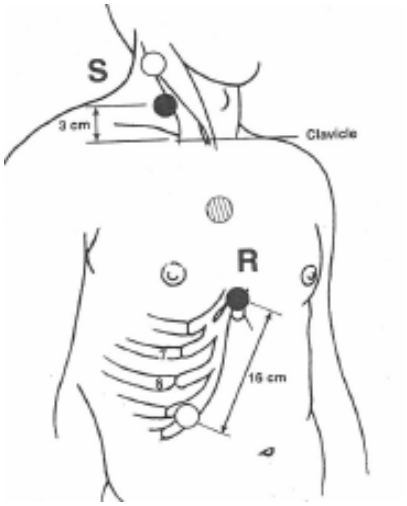

Figure 2.3.6.1 – Placement of the surface electrodes on the patient's thorax (Bolton method).

In our study, stimulation has been always performed with subjects lying supine and the phrenic nerve was transcutaneously stimulated at the posterior border of the sternomastoid muscle in the supraclavicular fossa just above the clavicle.

The standard protocol was to let the patients breathe for at least 30 seconds, then stimulate the nerve and record EMG/ENG parameters; then a second nerve stimulation was performed after 10/15 seconds of quiet breathing in the same modalities.

The procedure was repeated twice because the protocol includes both the stimulation of the right and left phrenic nerve.

The difference between the left and right stimulation is the positioning of the sensing electrode that had to be moved from one side to the other one, and of course the neck stimulation side.

Parameters which was possible to set by clinicians in the period of acquisition are basically two:

1. Duration of the stimulus: between 0.1 e 0.3 msec;
2. Intensity of the stimulus: always between 20mA and 90mA.

Both these two parameters are set in order to obtain the supramaximal stimulation, because the aim is to induce the maximal contraction of the muscle. In order to reach the supramaximal stimulation of the nerve, more than one stimulation was needed as trials, in increasing order of intensity.

2.3.7 OEP Standard Protocol

2.3.7.1 Preliminary Operations

The OEP acquisition system needed preliminary operations in order to properly acquire data: system calibration and patient preparation.

The goal of the calibration procedure is to create a relation between pixels in the images displayed by the system and centimeters in the real space.

In order to do this operation, it is necessary that cameras focus on three little bars that together form a right-handed tern. A different number of markers is placed on every bar and the distances between markers is known and fixed. Thanks to the fact that we know the mutual distances of markers, the calibration system converts the markers distances from centimeters to pixels finding the link between the two units of measurement. This link will be used to reproduce and to show us on the PC screen all the images acquired by the cameras in the right proportions.

A second step of the calibration procedure is to define the space volume within which cameras have to work. In order to do this, we simply moved one of the three little bar earlier used within the space we are interested in, trying to pass through every possible point of the volume of interest. This procedure takes a few minutes, and it has to take into account the difference in height and waistline between patients.

Once we concluded this second step, system was able to converge in the defined volume and to reconstruct accurately markers 3D data within this space.

We performed the calibration procedure of the OEP system every time we had a session of acquisition with patients.

As regards the patient preparation, we had to place markers on their thorax following a reference model.

Patients are asked to undress their upper body: female patients usually keep on their bras; male ones are naked from waist to head.

We had to place 52 markers on the patient's trunk. The possibility to study volume changes is based on the idea that the trunk can be considered as composed by different compartments using markers as reference points. These compartments are well defined and in order to have the right subdivision we have to follow a unique and replicable placements of the markers on the trunk surface.

Our patients were laying in supine position on the bed during the whole acquisition so we had to cover up with markers only the frontal surface; they are attached using a two label adhesive film on the patients' skin. For the marker placement we followed the guidelines in literature [34].

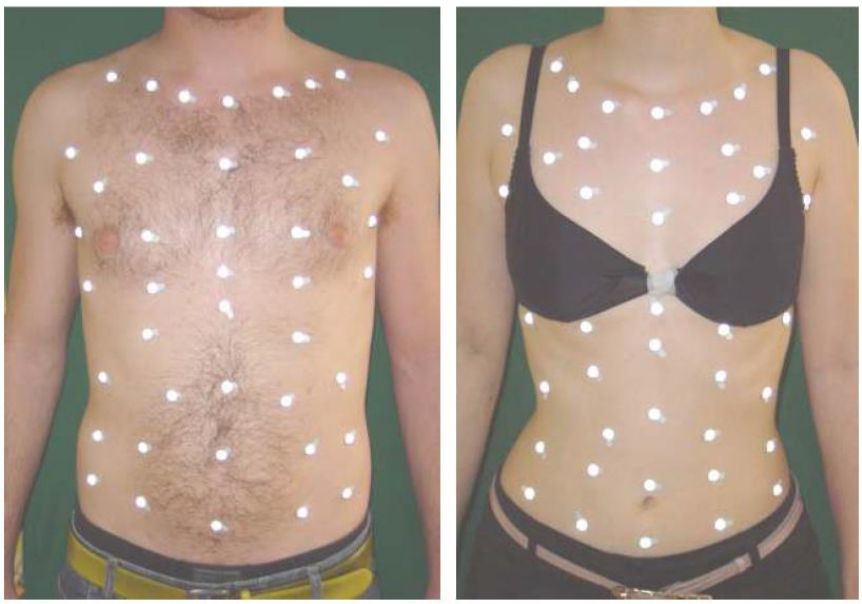

Figure 2.3.7.1.1– Marker positions on a male and female trunk.

Once that all the 52 markers are attached to the patients skin we were able to start the measurements.

2.3.7.2 OEP Measurements

The protocol for the OEP acquisition has been performed during the following patient's maneuvers:

- supine spirometry maneuver.
- phrenic nerve stimulation.
- Quiet breathing: the patients were asked to breathe for four minutes without interacting or speaking with anyone and without moving.
- Inspiratory capacity maneuver: patients were asked to inspire as much as they can and then deflate their lungs to the normal condition.

Because of the synchronous acquisition of the OEP system and the several test performed by the clinicians it is trivial the positioning of the personnel within the lab. In case someone interposed between OEP cameras and the patients, the OEP acquisition could be compromised.

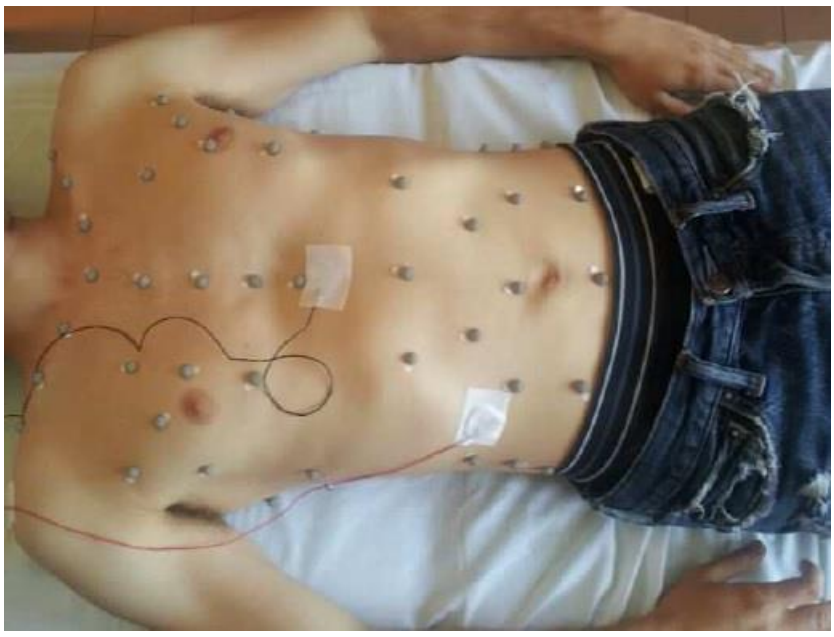

Figure 2.3.7.2.1 – Example of synchronous test preparation: placement of the OEP markers and PNS electrodes

2.4 Data Elaboration

Once that all the data were acquired, different elaboration were needed in order to implement a deep analysis on every kind of tests.

2.4.1 Spirometry

As regards spirometry data, we had to compare the FVC and FEV1 measurements of the different subjects. Given the great differences in height and size of the patients, we referred all the FVC volumes to the predicted ones. These predicted values come from the prediction equation present in literature, which calculate the expected FVC value of a healthy subject given his height and weight. The same operation has been performed for the FEV1 values; predicted values has been calculated for each subject and then the ratio of the two.
In this way, all the measures were expressed as the percentage of the predicted, and the statistical analysis was possible.

2.4.2 DLCO Data Elaborations

Because of the great variability between the data coming from the DLCO test of the different patients we expressed the values as percentage of the predicted on the basis of clinical evaluations.

2.4.3 MIP and MEP Data Analysis

For the comparison of MIP and MEP results there are not standardized values for healthy subjects and different studies show different methodologies to estimate normal values. For physiological reasons it is shown that age affects MIP and MEP: decreasing pressure values are seen with increasing age [26].
Because of the impossibility to find reasonable and replicable normal values, we selected a work in literature in which reference values are present: this review was presented by John A. Evans et al. [26]. The equations taken into account are set after a comparison of the results of several different works.
Regarding MIP equations, Evans et al. implemented the following ones:

- For male patients: $MIP(cmH_20) = 120 - (0.41 * age)$
- For female patients: $MIP(cmH_20) = 108 - (0.61 * age)$

As shown in Evans et al article, female values always start from a lower limit, and the decreasing rate connected to the age is bigger.
The equations for MEP are:

- For male patients: $MEP\ (cmH_20) = 174 - (0.83 * age)$

- For female patients: $MEP\ (cmH_20) = 131 - (0.86 * age)$

The same evaluations on age dependence ratio and starting point values underlined for MIP values are present in MEP equations.

What we performed as analysis of the pressure values given by the physiotherapist is the calculation of the predicted values for each subject, taking into account sex and age, and then the computation of the ratio between those values and the measured ones.

2.4.4 SNIP Measurements

We elaborated data coming from the SNIP test in the same way as MIP and MEP data. Because of the impossibility to recruit healthy subjects and perform the test, we used the normality values coming from literature works. There are reliable works that show that SNIP values are different from male to female subjects and decrease with age, for the same reason as MIP and MEP [21].

In literature there are cut off values for the SNIP test: pressure values higher than 70cmH_2O for men and 60 cmH_2O for women are considered unlikely to be associated with inspiratory muscle weakness.

In order to find predicted values, we used the ones stated by Araújo et al. [35].

They found equations to predict SNIP normality value: as MIP and MEP there is a negative proportionality with age.

Normality values for male subjects are: $SNIP\ (cmH_20) = 135.6 - (0.47 * age)$

Normality values for female subjects are: $SNIP\ (cmH_20) = 110.1 - (0.36 * age)$

In order to deal with the magnitude of different values we calculated for every subject the predicted value through these equations and then we divided the measurement taken by the physiotherapist by the predicted value. In this way we performed the calculation of the fraction of SNIP predicted. After that, we statistically analyzed the differences between the groups of patients.

2.4.5 CT Images Data Analysis

What we wanted to extract from CT images are information about the curvature and the length of the diaphragm both from a coronal view and from a sagittal one and also both at

the end of an inspiration and at the end of an expiration (if images related to the two different lung volumes were available).

We performed a manual segmentation for each image of our interest because there was no possibility to automatize the selection: PF and CF lungs showed a large amount of fibrotic tissue makes impossible the automatized distinction between them and healthy tissues belonging to other organs. This did not happen for COPD patients but for a more homogeneous analysis we performed the same kind of data elaboration for the three groups.

In order to conduct our analysis, the first necessary step is to obtain coronal and sagittal images of lungs starting from the axial one, provided by the CT scan.

RadiAnt DICOM Viewer is an image visualization software able to automatically display CT images both on the coronal and on the sagittal plane.

Once we got the necessary sequences of images, we had to extract the diaphragm profile (segmentation process). As a simplification, what is usually done is to identify the diaphragm as the inferior boundary of lungs. In this way, the task is just a matter of distinguishing lungs from all the other tissues and organs.

By using the software RadiAnt, we have selected the slice in which the diaphragm showed its maximal apex. This image, the previous two and the following two, have been selected and converted from the Dicom format into the JPEG one. This format change was performed in order to allow the further manual segmentation of the diaphragm making use of the image processing software ImageJ.

For each patient, 15 images have been saved and analyzed: 5 slices related to the right lung in the sagittal plane, 5 slices related to the left lung in the sagittal plane and 5 slices in the coronal plane displaying both the lungs. This selection operation has been done both for images related to an end inspiratory volume and to an end expiratory one only when it was possible.

In order to operate the segmentation, using the software ImageJ we selected several points along the diaphragm profile and we made an interpolation thanks to an automatic function of the software. Once we had the points on the lung boundary we saved the coordinates of the interpolated points in a .txt file to allow parametrical analysis we were interested in.

In order to compute parameters of our interest we used some existing MATLAB codes which have been slightly modified to be adapted to our requirements.

2.4.5.1 Curvature Computation

For determination of the mean radius of curvature of the diaphragm, its dome was approximated to a hemisphere. The radius of this hemisphere was calculated both in the coronal and sagittal CT planes.

By convention, a positive curvature sign was given to curvatures that were concave toward the abdomen, and a negative sign was given to curvatures that were convex toward the abdomen.

Starting from the silhouette of the diaphragm the first operative step is the computation of the mid-point E between the extremes A and B of the silhouette itself. The intersection between the silhouette and the straight-line perpendicular to the segment AB and passing through E was then defined as point D. The radius of curvature R was finally calculated:

$$R^2 = EB^2 + (R - ED)^2$$

$$R = \frac{(ED^2 + EB^2)}{2 * ED}$$

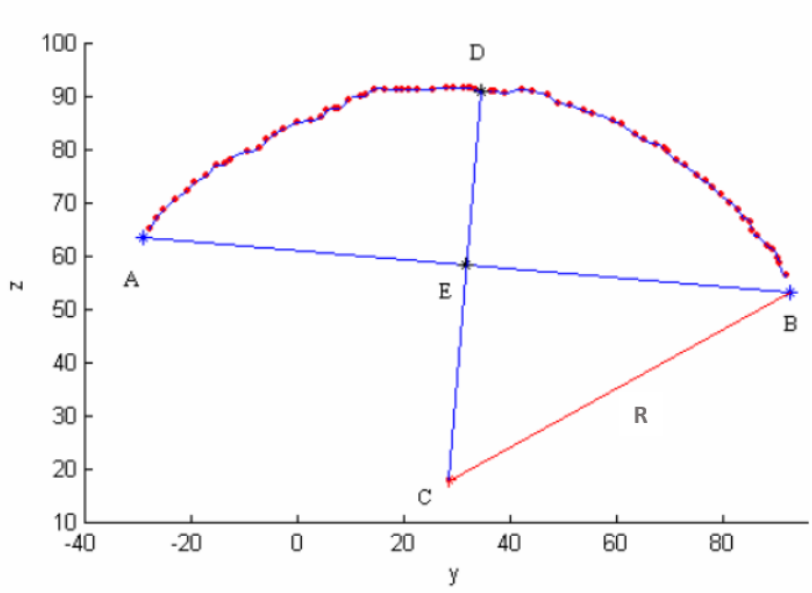

Figure 2.4.5.1.1 – Example of radius of curvature of a selection of points

Actually the radius of curvature was computed as the mean of the radius of curvature of the 5 slices selected for both coronal and sagittal plane. A distinction between the right and the left hemidiaphragm has been taken into account because of the anatomical differences between them, related to the presence of different organs in the right and left side of the body

(the presence of the liver in the right part of the abdomen influences the diaphragm shape and curvature).
Of course, the radius of curvature is initially expressed in pixels because of the nature of the images; a conversion from pixels to millimeters was needed.

2.4.5.2 Length Measurement

The length of the two hemidiaphragm was directly measured both for the coronal and the sagittal views in the selected slices.
The proper measurement was done considering the whole number of pixels of the interpolated shape of the diaphragm that we already selected manually for the curvature, and then multiplying it for the ratio mm/pixel present in the Dicom Info file. In the end, we measured four different lengths: left hemidiaphragm length in the coronal and sagittal planes, and right one in the same two planes.

2.4.5.3 Images Overlapping: a Case Study

As actually we got the expiratory CT images just for few patients, we decide to treat them as a case study.
What we wanted to show was the different radius of curvature and length of the diaphragm between RV and TLC conditions. In order to compare in a qualitative way the two situations, we overlapped two CT images of the same patient, sliced in the same plane at the same level. This superimposition has been performed using an Open Source software called 'VV the 4D Slicer' (by David Sarrut. The two overlapped images are colored in different ways in order to highlight the differences between them.
This visual analysis has not been possible for all of the patients but just for 5 of them as only these subgroup of patients underwent two different Computerized Tomography at different lung volumes.

2.4.6 US Imaging

The data coming from US imaging are basically divided in two groups:

- Diaphragmatic thickness measurements
- Diaphragmatic excursion

2.4.6.1 Diaphragm Thickness and Thickening

The measurement of diaphragmatic thickness is one of the more direct test in order to evaluate the morphology of the muscle.

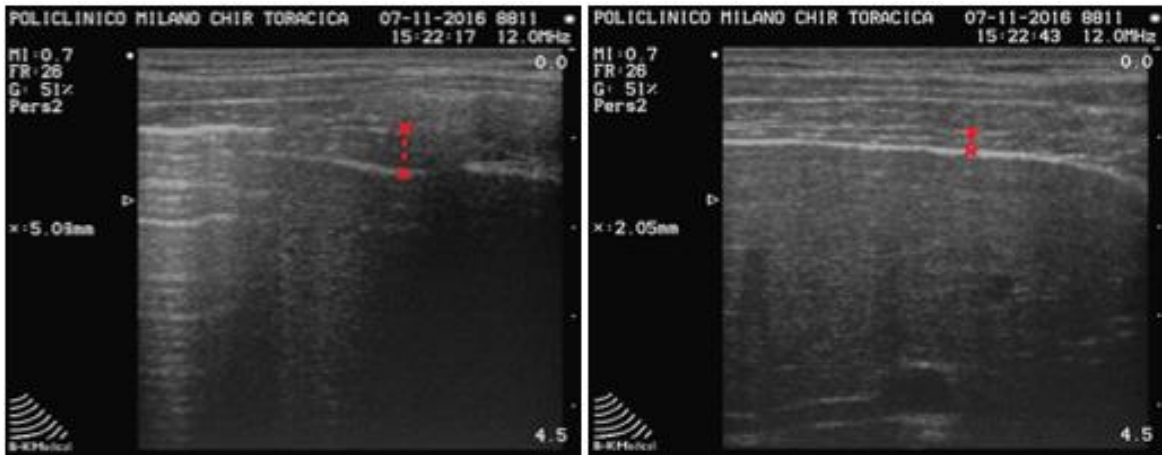

Figure 2.4.6.1.1 – Two different measurements of the diaphragm thickness: the left image is at end inspiration (EI), the right one is at end expiration (EE).

Regarding these measurements, we only recorded the values, grouped them for each pathology and analyzed them statistically.

Because of the huge variability of the values, due to the different height and size of the patients we calculated the percentage change in diaphragmatic thickness (% Δ_{Ti}) both for the quiet condition and for the forced one by using the corresponding values of thickness:

$$\%\Delta_{Ti,quiet} = \frac{Thickness\ at\ EI - Thickness\ at\ EE}{Thickness\ at\ EE} * 100$$

$$\%\Delta_{Ti,forced} = \frac{Thickness\ at\ FI - Thickness\ at\ FE}{Thickness\ at\ FE} * 100$$

We choose this index $\%\Delta_{Ti}$ as it was taken into account in most of the works present in literature, as for example in “Monitoring Recovery from Diaphragm Paralysis With Ultrasound” by Summerhill et al [34], or in “Change in Diaphragmatic Thickness During the Respiratory Cycle Predicts Extubation Success at Various Levels of Pressure Support Ventilation” by Scott Blumhof et al [36].

In literature, we found several works that assess mean values of diaphragmatic thickness, and we have taken into account the study proposed by Carrillo-Esper et al. [37]. After the evaluation of more than 100 healthy subjects, subdivided by age and sex, they concluded that the mean value of thickness for male is 1.8 mm and for female is 1.4 mm [37].

Regarding the pathologies in which we were interested in, one of the most interesting paper about CF is [38]. This article shows much more parameters in addition to thickness in CF patients, as respiratory indexes or body composition, and shows how they are related. For example, diaphragm muscle mass is related to an individual height and weight, and loss of as much as 30% of predicted body weight has been found to be associated with a reduction in diaphragm mass in terms of thickness. [38].

For COPD patients, the article written by Baria et al., shows no significant differences between patients and healthy subjects [20].

2.4.6.2 Diaphragm Excursion

Another important useful analysis in order to evaluate the diaphragmatic condition is the measure of the excursion of the muscle during the respiration. This kind of evaluation is usually performed by ultrasonography, especially using an M-Mode analysis.

The result of an ultrasonography test is a series of images, as in the ultrasonography screen, sampled at a frequency of 10Hz, in order to detect eventually very rapid movements of the structures under analysis.

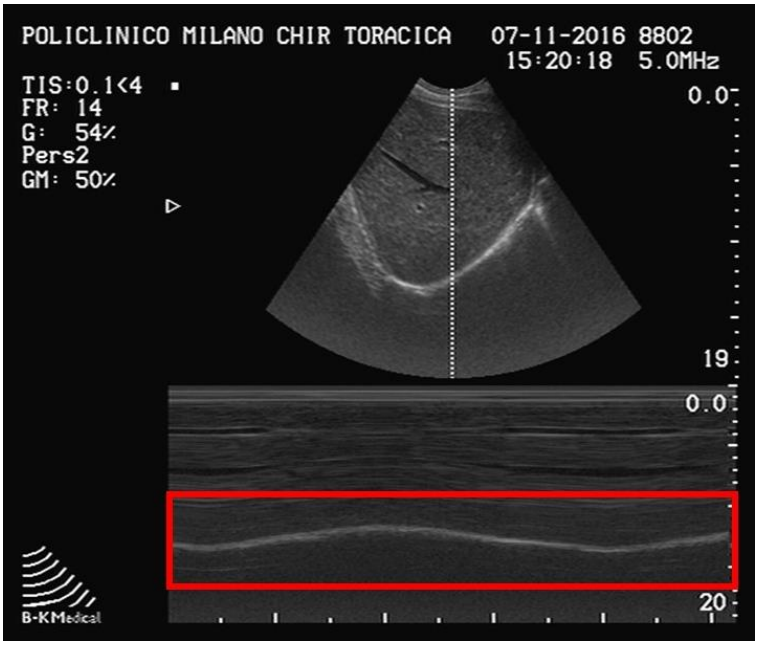

Figure 2.4.6.2.1 – Ultrasonography of the diaphragm in M mode, in the red rectangle is highlighted the diaphragmatic motion during the breaths.

Once that the images have been saved and stored, we wanted to analyze the motion in a simple but efficient way. We wrote a Matlab function that deals with these images making an initial calibration in order to detect the relations between pixels on the images and centimeters in the real world. Once that this calibration is performed, knowing the distance in cm in the reference scale placed on the right side of the screen, we selected the points of the positive and negative edges of the sinusoidal shape that the diaphragm motion creates on the screen using the function 'ginput'. This function saves the coordinates of the point manually selected in two arrays, one for the X and one for the Y ones. After the proper selection of the points, we analyzed the mean difference in the Y coordinate between positive and negative edges sinusoidal shape that is the envelop of diaphragmatic excursion, and divided it by the calibration factor, in order to have the mean range of motion in centimeters. Because of the impossibility to perform ultrasonography to healthy subjects, due to several reasons, we compared the values already present in the dataset of the TBM Lab at the Politecnico di Milano.

2.4.7 EMG/ENG Data from Phrenic Nerve Stimulation

From the EMG/ENG acquisition during the phrenic nerve stimulation, two different values were recorder for every patient: latency and negative peak area of the Compound Muscular Action Potential (CMAP).

Latency of onset is the time interposed between the instant in which the stimulus is provided and the moment in which a muscle response is recordable, that means when the CMAP signal becomes different from zero. This delay is measured in milliseconds. It is an indication of the conduction velocity of the phrenic nerve. In case of nerve injury this latency becomes longer than normal and PNS is the best way to diagnose it.

The area of the negative peak is the integral of the CMAP graph. It is automatically calculated by the software used by clinicians on the basis of the signal sensed by the receiver electrode. The area is measured in mV*ms. This quantity is related to the amount of recruited muscular fibers.

CMAP peak amplitude and area describe the number and characteristics of bioelectrical signal generators, like for example the muscle fibers, and they are expected to reflect the capacity of muscle to generate force.

Most of the studies present in literature about PNS regard patients who had neuromuscular diseases or mechanical damages to the phrenic nerve, and only few works deal with the kind of patient that we are studying. This lack of in-depth analysis is mainly due to a reason: none of the three pathologies affect directly the diaphragm, or the phrenic nerve.

Only in COPD patients, a study by Podnar et al. [39] demonstrated that CMAP latencies are significantly longer than healthy subjects' ones and that also CMAP amplitudes are higher than healthy subjects. On the other hand, CMAP duration results to be shorter in COPD patients compared with the control group.

Although phrenic CMAP area has been shown to be more robust than amplitude [40] as it is less sensitive to phase cancellation of potentials generated by individual muscle fibers, the latter is more often used in clinical practice. Furthermore, results from previous studies suggest that phrenic CMAP amplitude may be better than area to differentiate disorders of the diaphragm and its innervation from lung disorders.

In our study the peak amplitude of the CMAP is not taken into account.

No further elaboration was needed for the CMAP area and latency in our study; we only subdivided values for the different pathology groups and then we performed a statistical analysis in order to find differences between group of patients and healthy subjects.

2.4.8 OEP Data Elaboration

Data coming from the different test synchronous to the OEP acquisition required the same initial geometrical models to reconstruct the volume and then several different methods to analyze the volume changes.

Using a proper software, we transformed the markers positioning during time into the corresponding volume changes during the acquisition period. The software in use, automatically subdivided the tracks of the volume in the three compartments: pectoral rib cage (V_{RCp}), abdominal rib cage (V_{RCa}), and abdomen volume (V_{Ab}). Using the same software, it is possible to subdivide each of them into the right and left side, in order to detect asymmetrical movements of the thoracic compartments.

2.4.8.1 Quiet Breathing Elaboration

For the quiet breathing pattern, using a dedicated software, we selected the maxima and minima of 50 second of breathing in the chest wall volume tracks. After that, we used a Matlab function which calculates several indexes from the selected points. The main indexes are:

- respiratory time: duration of the inspiratory phase, duration of the expiratory phase, total breath duration;
- duty cycle (*DC*), computed as the ratio between the inspiratory time and the total breath duration;
- respiratory frequency (*f*), that is the number of breathing per minute;
- ventilation ($\dot{V}$), that represents how many air liters enter the lungs per minute. It has also been computed its value normalized by the weight of the considered subject;
- tidal volume (V_t), total air liters which enter the lungs per breath. It coincides with the chest wall (*CW*) volume. Also its weight normalized value has been considered;

- pulmonary rib cage (RC_p) volume, which quantifies the contribution to the tidal volume of the upper rib cage. It has been computed both as an absolute value and as percentage with respect to the entire chest wall volume;

- abdominal rib cage (RC_a) volume, which quantifies the contribution to the tidal volume of the lower rib cage. It has been computed both as an absolute value and as percentage with respect to the entire chest wall volume;

- abdominal (*Ab*) volume, which quantifies the contribution to the tidal volume of the abdomen. It has been computed both as an absolute value and as percentage with respect to the entire chest wall volume;

- inspiratory and expiratory air flow (*FLOW_I*, *FLOW_E*);

- rapid shallow breathing index, computed as the ratio between the respiratory frequency and tidal volume; also its weight normalized value has been considered.

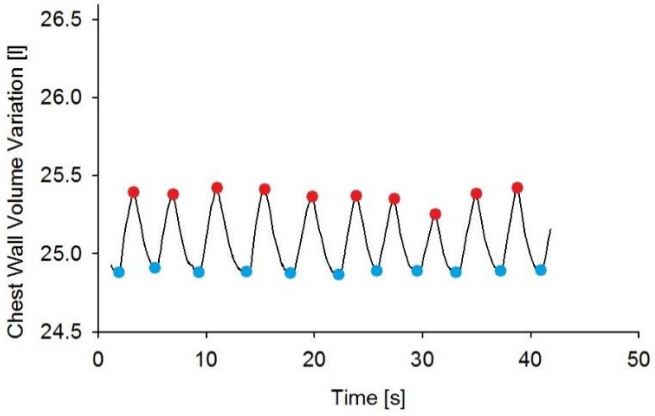

Figure 2.4.8.1.1 – Example of selection of maxima (red points) and minima (blue points) during quite breathing.

2.4.8.2 Vital Capacity

Regarding the vital capacity maneuver acquired by OEP, point selection on the volume tracks was the same as already mentioned for the inspiratory capacity, but the parameters were slightly different because of the more complex maneuver:

- chest wall vital capacity (VC_{cw}) volume;

- pulmonary rib cage vital capacity volume (VC_{RCp}), abdominal rib cage vital capacity volume (VC_{RCa}), abdominal vital capacity (VC_{ab}) volume both as absolute values and as percentage of the chest wall volume;

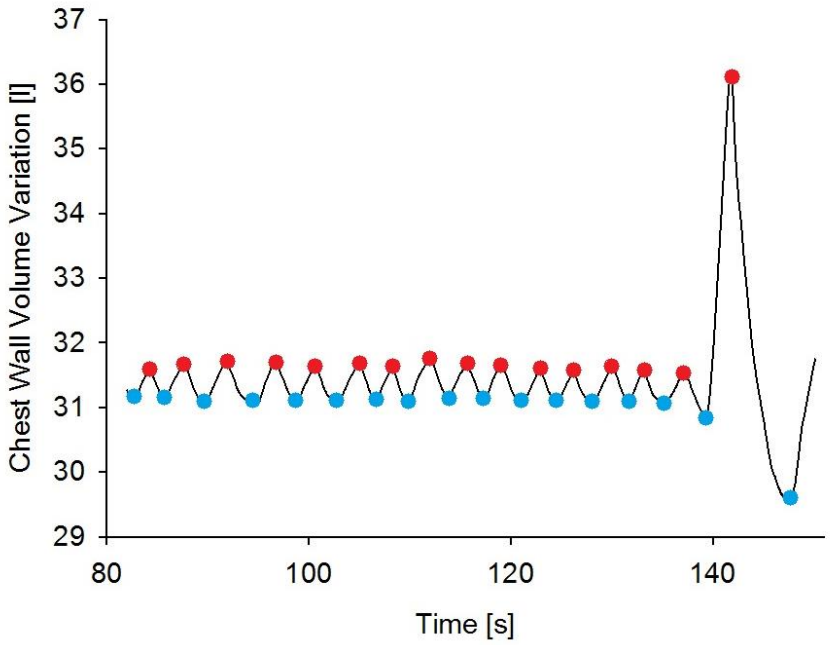

Figure 2.4.8.2.1 – Example of selection of maxima (red points) and minima (blue points) in a vital capacity maneuver

2.4.8.3 Inspiratory Capacity

For the inspiratory capacity files almost the same kind of points selection are performed, but the last maxima and the last minima of the selection had to be the ones corresponding to the considered maneuver.

Parameters measured from the Matlab elaboration are:

- chest wall inspiratory capacity (IC_{cw}) volume;

- pulmonary rib cage inspiratory capacity volume (IC_{RCp}), abdominal rib cage inspiratory capacity volume (IC_{RCa}), abdominal inspiratory capacity (IC_{ab}) volume both as absolute values and as percentage of the chest wall volume.

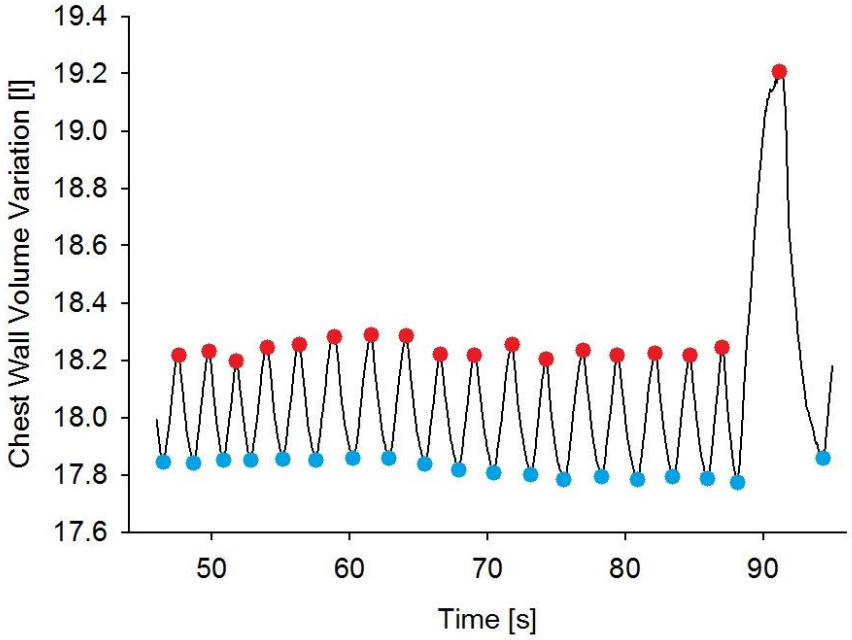

Figure 2.4.8.3.1 – Example of selection of maxima (red points) and minima (blue points) in an inspiratory capacity maneuver.

2.4.8.4 PNS Analysis

This study uses for the first time OEP while is performed Phrenic Nerve Stimulation (PNS). From the volumetric point of view, no analysis has ever been matched with PNS so until now there were only physiological considerations on how the thorax volume could change during non-volitional diaphragm activation.

Because of this, as a first step, we found useful to evaluate the thorax volume changes of healthy subjects and compare the actual behavior of the different compartments with some

theoretical considerations. In order to do this, we enrolled a group of 12 healthy subjects, and asked them to participate in our study.

From the OEP point of view, we elaborated the files as it was a normal acquisition, so the same geometrical model has been applied and the same differentiation in sub-compartment has been performed by the software. In order to perform a proper evaluation of the volume tracks during the stimulus we selected the breaths in which it is applied. Comparing several healthy subjects' tracks we found that volume changes are always more visible in the abdominal compartment, as we expected. This is the reason why we focus our analysis on the abdominal compartments.

After a quick comparison of normal breaths and stimulated ones, we decided the selection of the following points starting from the track of the abdominal compartment:

- The first point was selected in correspondence of the volume at the end of expiration, within the breath containing the stimulus;
- The second point was chosen in correspondence of volume at the end of the quiet inspiration, always within the breath containing the stimulus;
- The third point was in correspondence of the instant time in which the effect of the stimulus on the volume is visible, so the volume at which started the steeper slope;
- The fourth point was the apex of the volume changes induced by the stimulus;
- The fifth point was in correspondence of the instant time in which the effect of the stimulus is no more recognizable.

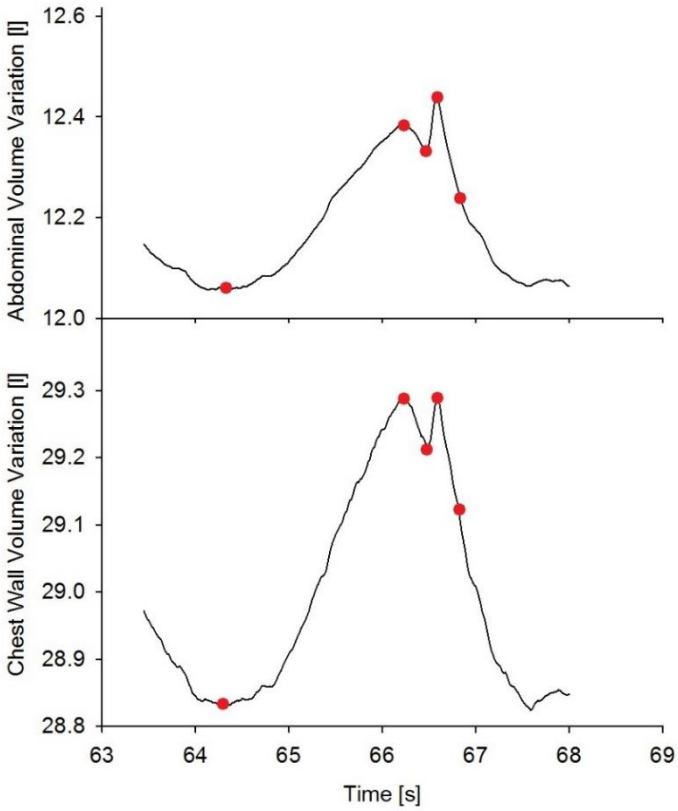

Figure 2.4.8.4.1 – Volume changes during PNS in the whole chest wall (under) and the only abdominal compartment (above). Red points are the ones selected as reference points.

Starting from the selected points that we have just described, we wrote a Matlab function to elaborate them. Information related to the point selection are saved in a '.dmv' file; in each row of this file the following indexes are saved for each chosen sample:

- Time;
- Frame;
- Volume of the pulmonary rib cage;
- Volume of the abdominal rib cage;
- Volume of the abdomen;
- Volume of the chest wall;
- Volume of the right and left contribution of the pulmonary rib cages;
- Volume of the right and left contribution of the abdominal rib cage;
- Volume of the right and left contribution of the abdominal compartments.

This information is the starting point for our elaboration.

We decided to compare the breaths and the stimulus amplitude.

The first step has been the computation of the difference between volumes related to the second selected point and the first one; in this way it has been possible having a measure of the tidal volume of that single breath. The remaining three points have been useful to visualize the breathing mechanics during the stimulation. In particular, we interpolated the initial and final point of stimulation effect (the third and the fifth saved points) in order to find the straight line passing through them; we then looked for the projection on this straight line of the fourth selected point. The actual stimulation volume change (ΔS) is finally computed through a difference of the volume value in correspondence of the fourth saved point and the found projection. That is a method to operate a correction for the drift effect so that the delta volume computed during the stimulation is not influenced by the changes in volume due to the breath phase; this is a way to make the stimulation delta volume independent from the moment in which the stimulation occurs (during the inspiratory or the expiratory phase). The drift effect correction was done both for the abdominal compartment and for the whole chest wall.

We finally extracted some indexes in order to describe the abdominal compartment behavior which reflects the diaphragm action. The extracted indices are:

- $\Delta S_{ab}/\Delta T_{ab}$, where ΔS_{ab} is the abdominal volume change due to the stimulation and ΔT_{ab} is the tidal volume referred to the abdominal compartment. It is useful to know how great is the percentage contribution of the stimulation looking at the only abdominal compartment;

- $\Delta S_{ab}/\Delta T_{cw}$, where ΔT_{cw} is the tidal volume referred to the whole chest wall. This is an index about how much the stimulus was working on the only abdominal compartment compared to the tidal volume in order to have a normalized index for every subject;

- $\Delta S_{cw}/\Delta T_{cw}$, where ΔS_{cw} is the chest wall volume change due to the stimulation and ΔT_{cw} is the tidal volume referred to the chest wall itself. It quantifies the effect of the stimulation looking at the whole chest wall;

- $\Delta S_{ab}/FRC$ and $\Delta S_{cw}/FRC$, where FRC refers to the chest wall Functional Residual Capacity;

- $\Delta S_{ab} / \Delta T_{QB_{ab}}$, where $\Delta T_{QB_{ab}}$ refers to the tidal volume related to the abdominal compartment computed by analyzing the track recorded during quiet breathing. For the same

reasons we computed also $\Delta S_{ab} \,/\, \Delta T_{QB_{cw}}$, where $\Delta T_{QB_{cw}}$ quantify the tidal volume of the chest wall during quiet breathing maneuver.

- ΔRC_p and ΔRC_a are two indices which describe how the volume of the pulmonary rib cage and of the abdominal rib cage change during the stimulation;

- the percentage of volume at which the stimulation starts is also calculated, by the ratio of the delta volume between stimulation starting point and the end expiratory volume in the abdominal compartment, and the tidal volume of the abdomen. It is also declared if the stimulus comes during the inspiration or expiration.

- the drift speed is another interesting index which allows us to know how rapidly the abdominal volume increases as a consequence of the stimulation. It is easily calculated by making the difference between the volume in correspondence of the starting point of the stimulation and the maximal volume value reached thanks to the stimulation; this difference is finally divided by the corresponding time interval. This index is directly related to the velocity of contraction of the diaphragm.

The phrenic nerve stimulation is performed both on the right and on the left side; all the previous cited indexes have been computed separately during both the stimulations.

Once that the healthy subject parameters have been analyzed by the point of view of the volume changes, we applied the same protocol on pathological subjects, in our case patients in waiting list for lung transplantation, in order to detect the differences in the diaphragmatic action during the phrenic nerve stimulation and compare it even between the different pathologies.

Chapter 3

Results

3.1 Anthropometric Results

Patients who took part in our study were 28 and all of them were afferent to the Thoracic Surgery and Lung Transplant Operative Unit of the foundation IRCCS Ca' Granda, Ospedale Maggiore Policlinico in Milan.

All of them have been previously inserted in the lung transplant waiting list after an evaluation of their diaphragm condition. They were affected by three different pathologies: pulmonary fibrosis, cystic fibrosis, chronic obstructive pulmonary disease; particularly, before the lung transplant, we met: 9 patients affected by pulmonary fibrosis, 14 patients affected by cystic fibrosis and 5 of them were affected by chronic obstructive pulmonary disease.

In the following tables we have presented the anthropometric data of our patients; in order to respect their privacy we referred to them with increasing number. In the table, their age, weight, height, BMI, LAS and their pathology (FP refers to pulmonary fibrosis, FC refers to cystic fibrosis, COPD is used to indicate a chronic obstructive pulmonary disease) have been reported.

PATIENT	AGE (yrs)	WEIGHT (kg)	HEIGHT (cm)	BMI (kg*m-2)	LAS	PATHOLOGY
Subject 1	60	75	168	26.57	41.62	FP
Subject 2	43	62	174	20.48	31.49	FP
Subject 3	64	65	167	23.31	36.2	FP
Subject 4	55	54	164	20.08	41.37	FP
Subject 5	48	79	170	27.34	30.05	FP
Subject 6	61	63	156	25.89	37.25	FP
Subject 7	62	85	177	27.13	ND	FP
Subject 8	51	80	165	29.39	38.1	FP
Subject 9	54	56	154	23.61	40.5	FP
MEAN ± SD	55.11 ± 7.08	68.78 ± 11.22	166.11 ± 7.54	24.87 ± 3.20		

Table 3.1.1 – Anthropometric data of patients affected by Pulmonary Fibrosis. Pre-operative condition.

PATIENT	AGE (yrs)	WEIGHT (kg)	HEIGHT (cm)	BMI (kg*m-2)	LAS	PATHOLOGY
Subject 10	37	50	164	18.59	33.6	FC
Subject 11	20	50	166	18.14	ND	FC
Subject 12	26	78	183	23.14	32.4	FC
Subject 13	30	61	170	21.11	33.78	FC
Subject 14	22	62	157	25.15	32.53	FC
Subject 15	21	47	165	17.26	44	FC
Subject 16	52	68	171	23.26	34.92	FC
Subject 17	40	65	158	26.04	35.4	FC
Subject 18	22	47	151	20.48	32.76	FC
Subject 19	18	42	150	18.67	33.7	FC

Subject 20	36	60	167	21.51	34.01	FC
Subject 21	32	46	161	17.75	31.57	FC
Subject 22	23	49	163	18.29	ND	FC
Subject 23	19	41	150	18.22	34.74	FC
MEAN ± SD	28.43 ± 9.91	55.09 ± 11.23	162.57 ± 9.15	20.54 ± 2.89		

Table 3.1.2 – Anthropometric data of patients affected by Cystic Fibrosis. Pre-operative condition.

PATIENT	AGE (yrs)	WEIGHT (kg)	HEIGHT (cm)	BMI (kg*m-2)	LAS	PATHOLOGY
Subject 24	55	58	173	19.38	31.6	COPD
Subject 25	64	70	178	22.09	32.89	COPD
Subject 26	56	78	174	25.76	ND	COPD
Subject 27	63	41	160	16.02	34.02	COPD
Subject 28	36	98	195	25.78	ND	COPD
MEAN ± SD	54.70 ± 11.22	69.00 ± 21.38	176.00 ± 12.59	21.80 ± 4.21		

Table 3.1.3 – Anthropometric data of patients affected by Chronic Obstructive Pulmonary Disease. Pre-operative condition.

Some evaluations have been done on the anthropometric data in order to test the homogeneity of the different populations in terms of age, weight, height as these parameters may influence lung volumes and their ability to expand or the diaphragmatic length.

Parameters of pathologic subjects have been compared to healthy subject parameters. Unfortunately, we did not deal with a unique control group as we could not perform all of the clinical test. Values of some parameters of healthy subjects have been taken from the reference TBM Lab database of the Politecnico di Milano.

Quiet breathing control group is constituted by 23 healthy subjects. For 13 of them we made acquisitions at the foundation IRCCS Ca' Granda, Ospedale Maggiore Policlinico in Milan; the remaining 10 subjects data are taken from the TBM Lab database.

The control group was composed by 11 males and 12 females. Their mean age is 32 ± 7 yrs; their mean weight is 71 ± 4.5 kg; their mean height is 178.7 ± 5.7 cm and their mean BMI is 22.3 ± 1.21 $kg*m^{-2}$.

Regarding the computerized tomography, data were taken from the TBM Lab database. The control group was composed by 2 males and 3 females. Their mean age is 48 ± 4 yrs; their mean weight is 79 ± 21 kg; their mean height is 176 ± 15 cm and their mean BMI is 25.5 ± 2.02 $kg*m^{-2}$.

As far as the ultrasonography regards we got data of healthy subjects from the TBM Lab database. The control group was composed by 14 subjects. Their mean age is 26 ± 5 yrs; their mean weight is 76.9 ± 10.8; their mean height 1.80 ± 0.05m and their mean BMI was 23.8 ± 2.8 $kg*m^{-2}$.

For the phrenic nerve stimulation test, we got data from 12 healthy subjects whose data were acquired by us at Policlinico di Milano. The control group mean age is 30 ± 9 yrs; its mean weight is 67 ± 13 kg; its mean height is 173 ± 9 cm and their mean BMI is 22 ± 2.7 $kg*m^{-2}$.

For the inspiratory capacity and the vital capacity we made acquisition on 9 healthy subjects, 1 male and 8 females. Their mean age is 30 ± 11 yrs; their mean weight is 67 ± 16 kg; their mean height is 173 ± 12 cm and their mean BMI is 22 ± 2.52 $kg*m^{-2}$.

3.2 Analysis Methodology

In the elaboration phase we calculated multiple parameters, widely descripted in the previous chapter, for each pathology and also for healthy subjects group, in order to compare them by group.

The software used to compute a proper statistical analysis was SigmaStat (Systat Software Inc).

Results are subdivided on the basis of the diaphragm characteristics they refer to: morphology, nerve conduction, contraction, action on the thorax and strength.

Each index calculated in the chapter undergoes a Kolmogorov Smirnoff test in order to test the normality distribution of the samples: its null hypothesis is that samples come from a Gaussian shaped distribution; if the *p-value* is lower than 0.05 this hypothesis is rejected.

After that, in order to compare the groups, it has been performed a One Way ANOVA test, whose null hypothesis is that the means and standard deviations of the compared population data are equal to each other. If the *p-value* is lower than 0.05 the pairs of groups have different distributions.

In case of non-Gaussian distributions, it was performed the corresponding test for non-normal distributions, one-way ANOVA on Ranks.

In case of significant differences between pairs of groups, the symbols used are the following:

- $0.01 < \text{p-value} \leq 0.05 \Rightarrow *$
- $0.001 \leq \text{p-value} \leq 0.01 \Rightarrow **$
- $\text{p-value} < 0.001 \Rightarrow ***$

Actually different symbols are used to indicate which populations result to be statistically different one from each other. Furthermore, a horizontal curly bracket is used to point out differences between the group constituted by the all the patients and the group constituted by healthy subjects.

In some cases, where the measure was performed twice at different times, and our aim was to detect the differences between the two, we used the ANOVA RM (Repeated Measurements), setting as repeated variable the subjects, and as independent variables the different measures taken at different time instant.

3.3 Spirometry and Pulmonary Function Test Results

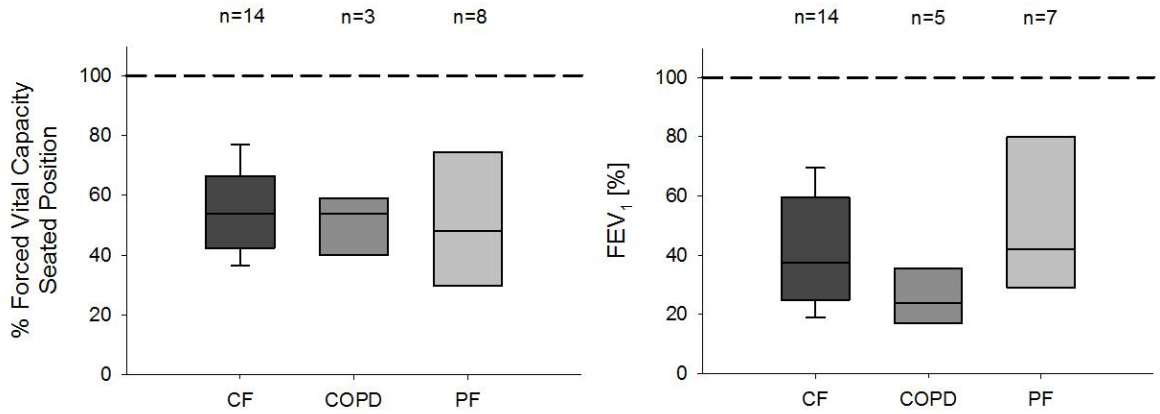

Figure 3.3.1 – Results from maneuvers of Forced Vital Capacity are shown. The dotted lines refer to the reference value of 100%.

In Figure 3.3.1, results obtained from spirometry show that patients have percentage values of FVC lower than predicted. Values of FEV_1 result to be reduced for all the three group of patients, too.

We also considered the drop in Forced Vital Capacity between values obtained in the seated position and values obtained in supine position as it has been demonstrated that a drop higher than 25% is an index of diaphragmatic weakness. Results are shown in Figure 3.3.2.

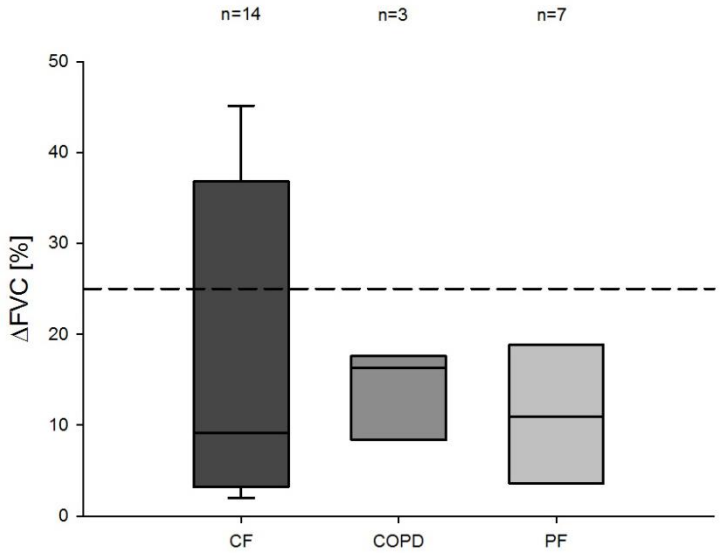

Figure 3.3.2 –Percentage of FVC drop between seated and supine position.

COPD and PF patients do not have a drop in the forced vital capacity values higher than 25% while some CF patients show values higher than the threshold.

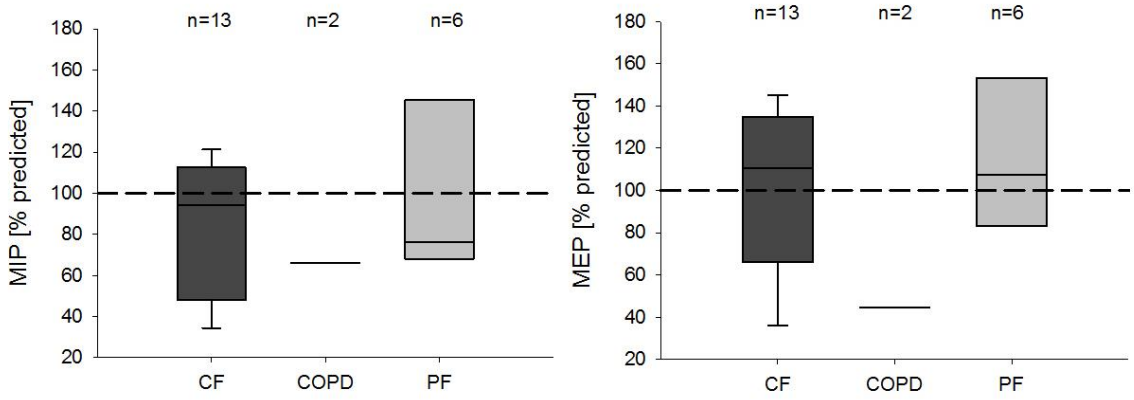

Figure 3.3.3 – Results from MIP and MEP tests expressed as the percentage of the predicted values. The dotted lines refer to the reference value of 100%.

In Figure 3.3.3, results related to MIP and MEP tests reveals that pulmonary fibrosis patients such as cystic fibrosis patients have median percentage values of mouth pressure both for the inspiratory and for the expiratory maneuver which are almost equal to the 100%.
COPD patients, on the other hand, present values much lower than 100%. Anyway, results related to COPD patients may not be reliable, as the population was constituted just by two subjects.

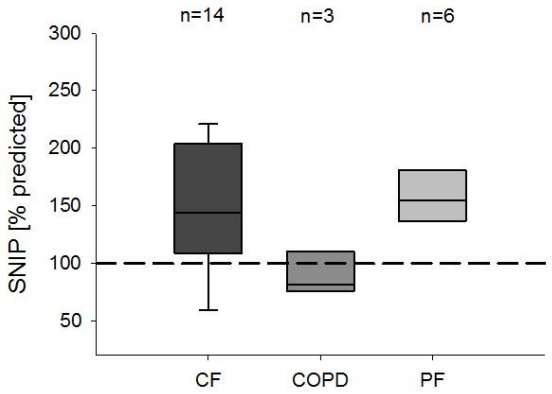

Figure 3.3.4 – Results from SNIP tests. The dotted lines refer to the reference value of 100%.

The SNIP test points out that the two fibrosis population are able to generate higher values of pressure with respect to the reference value of 100%.

Values related to COPD patients are lower than the 100% (Figure 3.3.4).

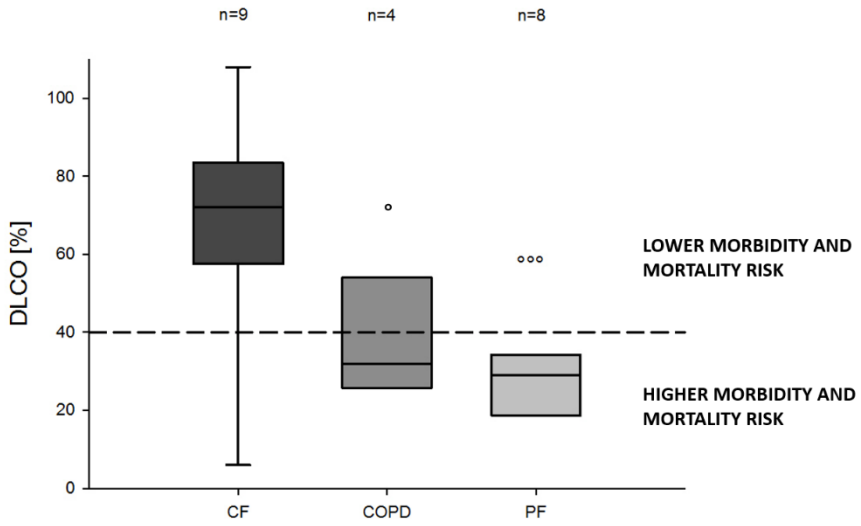

Figure 3.3.5 – Percentage of predicted values for DLCO test. The symbol ° points out the difference with CF group.

In Figure 3.3.5 DLCO is expressed as percentage of the predicted values and the difference between groups are shown.

The dotted line at 40% indicates the threshold above which there is a high risk of morbidity and mortality due to an impairment of the gas exchange mechanism.

The CF group reveals higher median value with respect to the other two groups. All the PF values are under 40%.

3.4 CT Imaging Results

3.4.1 Diaphragm Length and Curvature

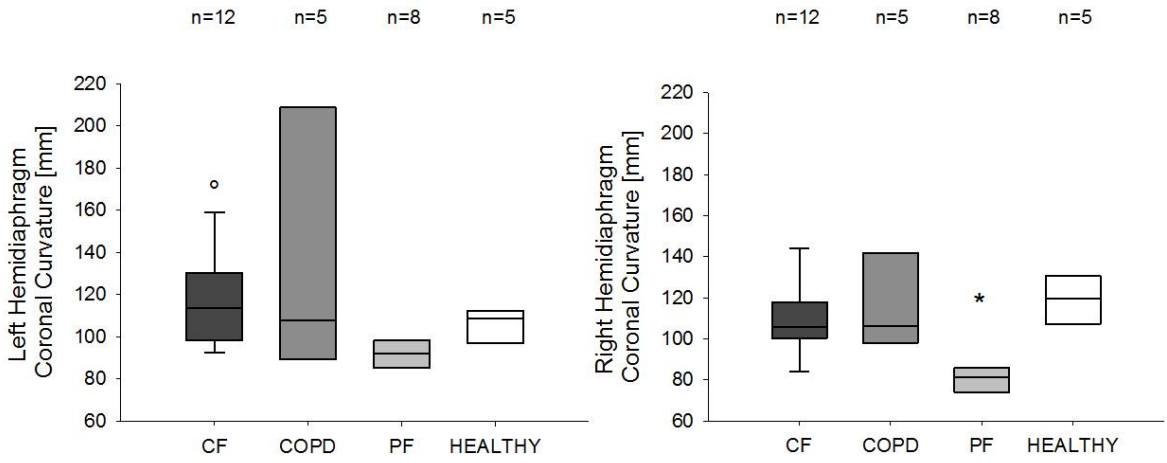

Figure 3.4.1.1 – Results of the computation of the left and right hemidiaphragm curvature in the coronal plane. The symbol * points out a difference with respect to the healthy group, while ° refers to the PF group.

In Figure 3.4.1.1 the results of the computation of the right and left hemidiaphragm curvature in the coronal plane are shown. Differences are statistically significant between pulmonary fibrosis patients and healthy subjects in the right hemidiaphragm, resulting lower in the PF patients; on the other hand, for the left hemidiaphragm, pulmonary fibrosis patients show a smaller curvature compared to the cystic fibrosis ones.

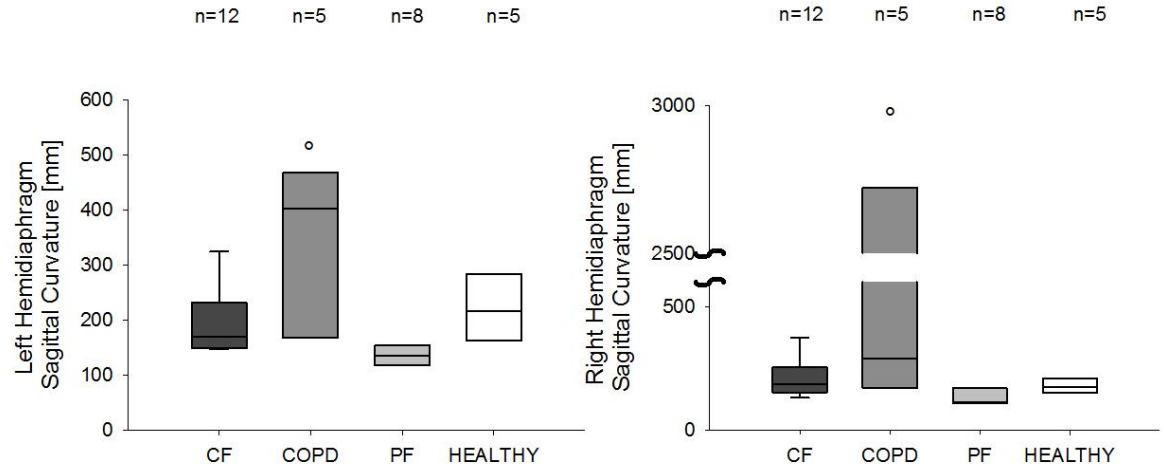

Figure 3.4.1.2 – Results of the computation of the left and right hemidiaphragm curvature in the sagittal plane. The symbol ° points out a difference with respect to pulmonary fibrosis group.

In figure 3.4.1.2 results related to the diaphragm curvature in the sagittal plane are shown. Both for the right and left hemidiaphragm, the curvature results to be higher in COPD patients with respect to PF ones.

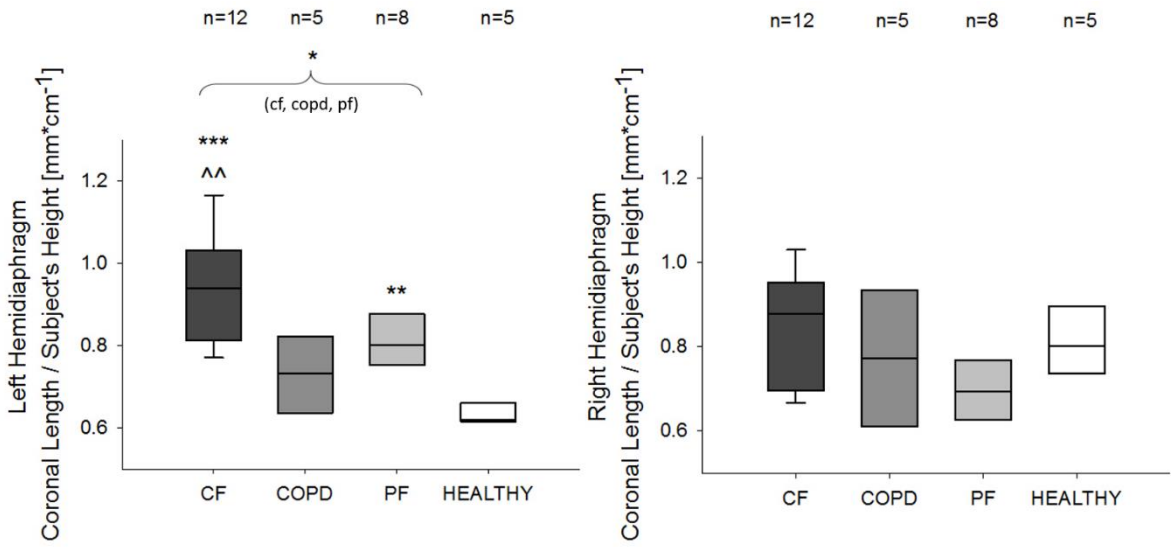

Figure 3.4.1.3 – Results of the left and right hemidiaphragm length normalized on the subject's height measurements in the coronal view. The symbol * points out a difference with respect to the healthy group, while ^ refers to the COPD group.

In Figure 3.4.1.3 are represented the results of the measurements of left and right hemidiaphragm length normalized on the subject's height, in the coronal plane. The left ones result to be higher for cystic fibrosis patients with respect both to the heathy group and to the COPD group. Also patients affected by pulmonary fibrosis show higher values of the represented measurements with respect to healthy subjects. Also a global difference between patients and the control group results from a statistical analysis (Figure3.4.1.3).

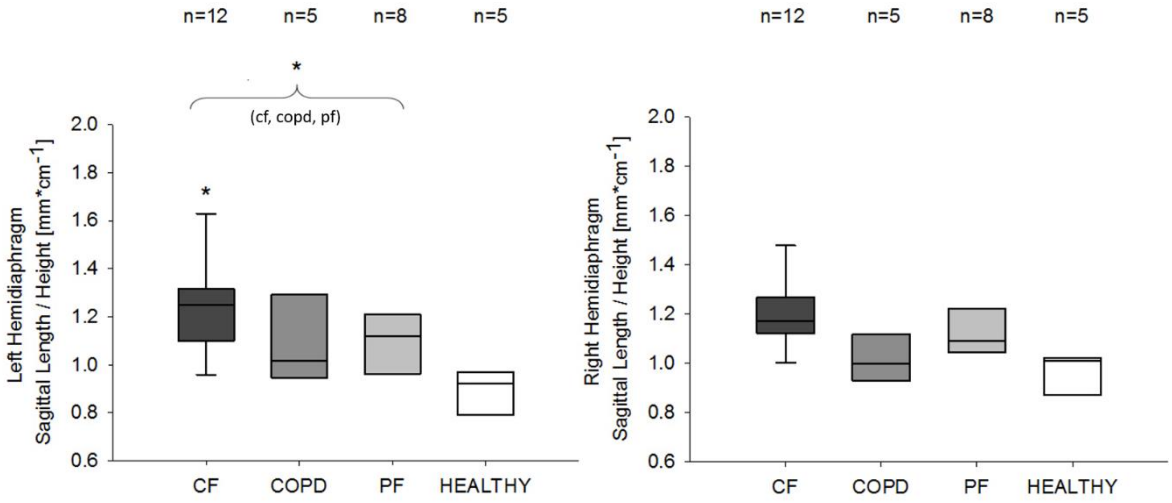

Figure 3.4.1.4 – Results of the left and right hemidiaphragm length normalized by the subject's height in the sagittal plane. The symbol * points out a difference with respect to the healthy group.

In Figure 3.4.1.4 is shown the result of the length measurement of the two hemidiaphragms normalized by the subject's height in the sagittal plane.

As far as the left hemidiaphragm concerns, results show that cystic fibrosis patients have a higher value of the represented measurements with respect to the healthy group. Also a difference between the whole group of patients and the healthy group results from a statistical analysis. No statistical differences are pointed out as far as the right hemidiaphragm concerns, but PF and CF group seems to have greater length values compared to COPD and healthy subjects.

3.4.2 Case Study: Comparison between TLC and RV

We had the possibility to show CT images taken at the two different lung volumes of five 5 different patients.

3.4.2.1 Case Study: PF Patients

As regards the pulmonary fibrosis patients, only one of them underwent the computerized tomography both at TLC and RV.

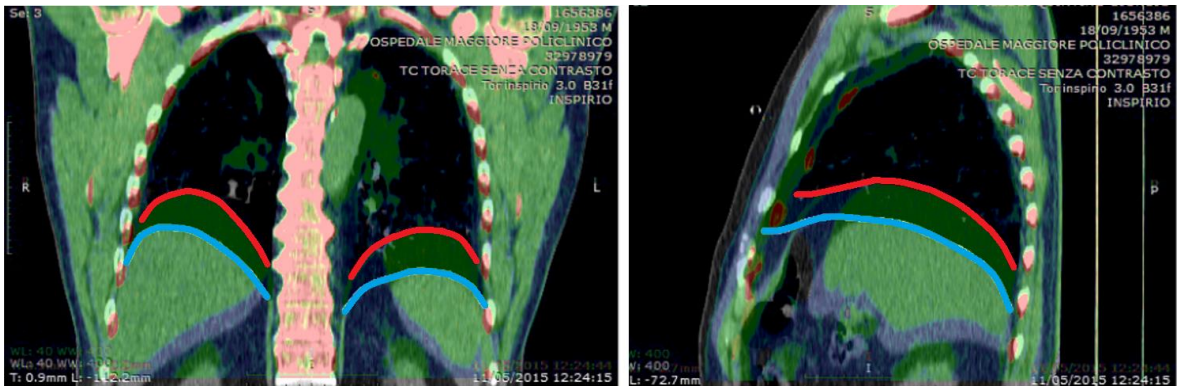

Figure 3.4.2.1.1 – Overlapping of CT images taken at TLC (green) and RV (red) of a pulmonary fibrosis patient.

	Coronal Plane				Sagittal Plane			
LUNG VOLUME	Right Curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)	Right curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)
TLC	72.2	73.3	120.1	133.4	110.8	131.7	184.3	188.2
RV	70.0	62.9	121.1	129.9	84.2	119.8	183.1	173.5

Table 3.4.2.1.1 – Table showing morphological parameters of the diaphragm of a PF patient at two different lung volumes.

3.4.2.2 Case Study: CF Patients

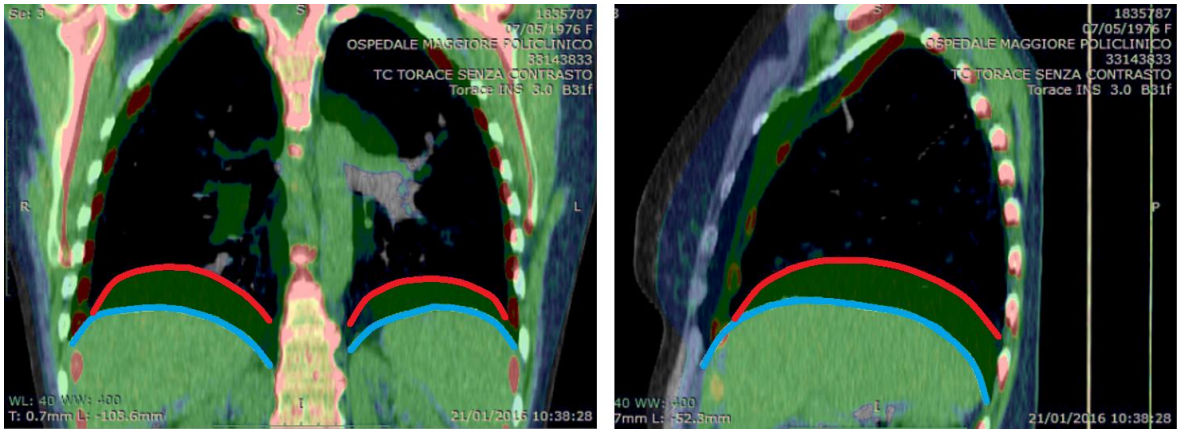

Figure 3.4.2.2.1 – Overlapping of CT images taken at TLC (green) and RV (red) of a pulmonary fibrosis patient.

	Coronal Plane				Sagittal Plane			
LUNG VOLUME	Right Curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)	Right Curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)
TLC	106.6	132.2	150.3	168.8	126.1	147.4	200.9	267.4
RV	81.5	94.45	142.9	157.9	119.3	138.4	186.1	236.4

Table 3.4.2.2.1 – Table showing morphological parameters of the diaphragm of a PF patient at two different lung volumes.

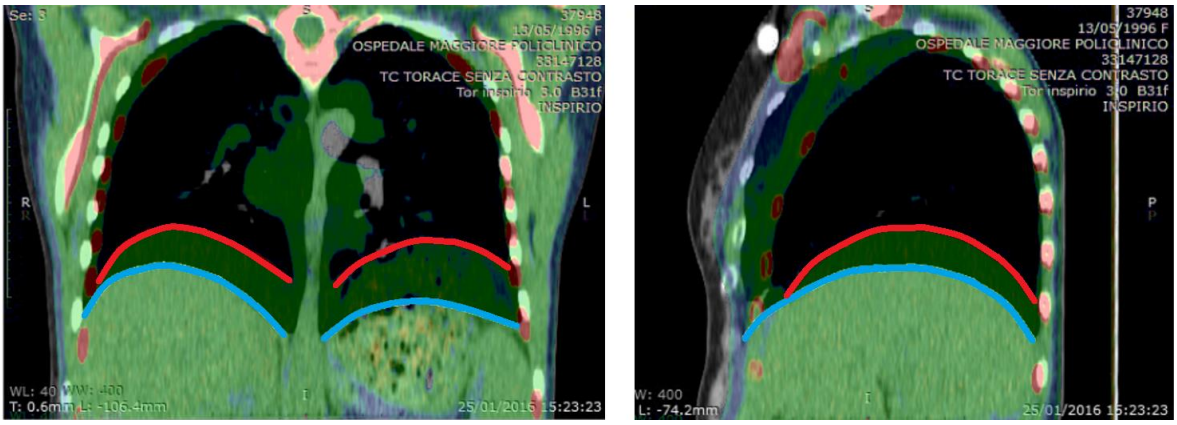

Figure 3.4.2.2.2 – Overlapping of CT images taken at TLC (green) and RV (red) of a pulmonary fibrosis patient.

	Coronal Plane				Sagittal Plane			
LUNG VOLUME	Right Curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)	Right Curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)
TLC	143.4	101.8	156.4	180.8	175.5	146.5	232.3	222.4
RV	99.5	90.0	138.8	165.0	119.2	120.7	211.0	217.3

Table 3.4.2.2.2 – Table showing morphological parameters of the diaphragm of a PF patient at two different lung volumes.

3.4.2.3 Case Study: COPD Patients

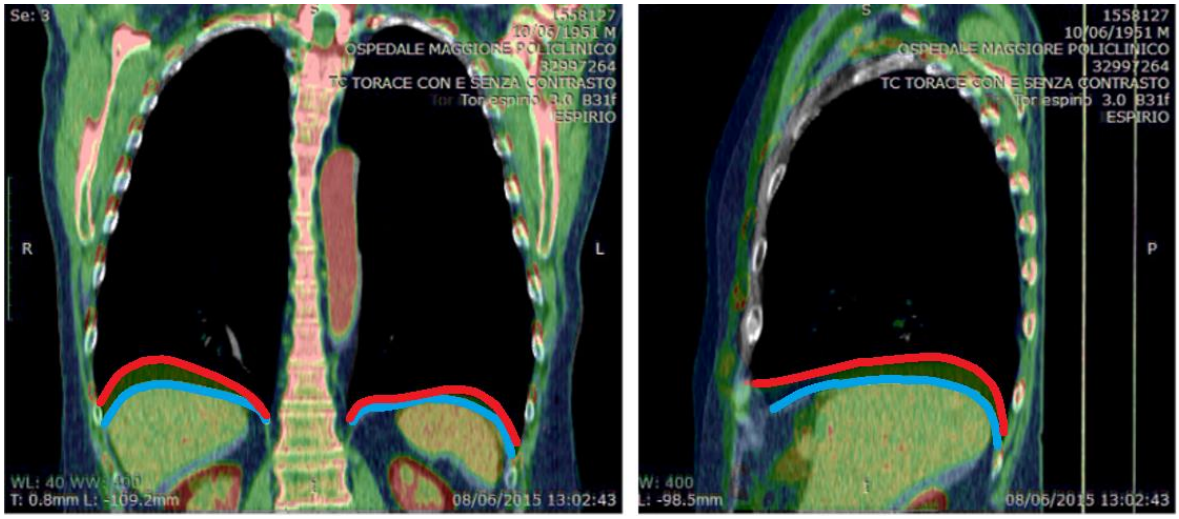

Figure 3.4.2.3.1 – Overlapping of CT images taken at TLC (green) and RV (red) of a COPD patient.

	Coronal Plane				Sagittal Plane			
LUNG VOLUME	Right curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)	Right curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)
TLC	103.2	92.9	149.5	147.8	289.8	176.6	177.5	201.9
RV	91.8	86.7	142.5	149.6	231.5	148.9	169.4	192.3

Table 3.4.2.3.1 – Table showing morphological parameters of the diaphragm of a COPD patient at two different lung volumes.

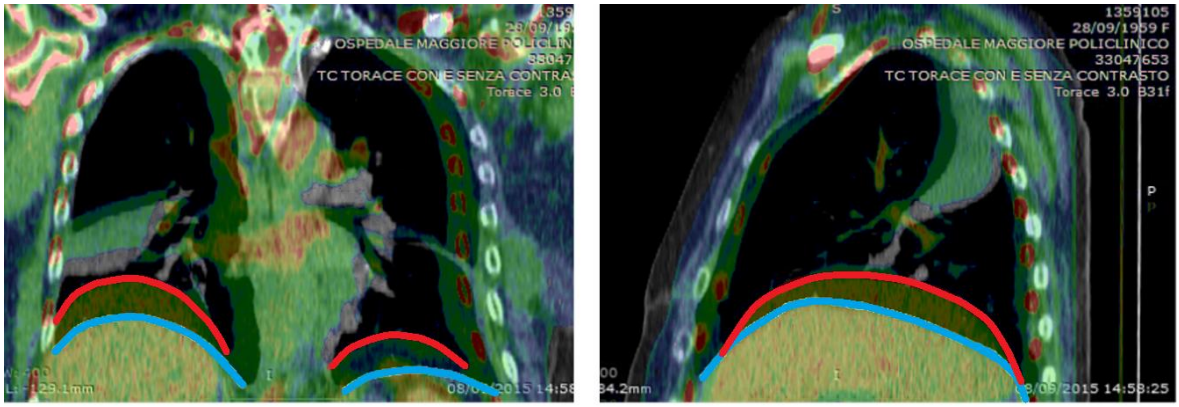

Figure 3.4.2.3.2 – Overlapping of CT images taken at TLC (green) and RV (red) of a COPD patient.

	Coronal Plane				Sagittal Plane			
LUNG VOLUME	Right curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)	Right curvature (mm)	Left Curvature (mm)	Right Length (mm)	Left Length (mm)
TLC	112.0	85.6	108.2	141.4	192.4	157.7	177.5	112.0
RV	67.0	74.5	100.8	137.5	163.4	127.9	252.0	249.6

Table 3.4.2.3.2 – Table showing morphological parameters of the diaphragm of a COPD patient at two different lung volumes.

3.4.2.4 Comparison between Pathologies

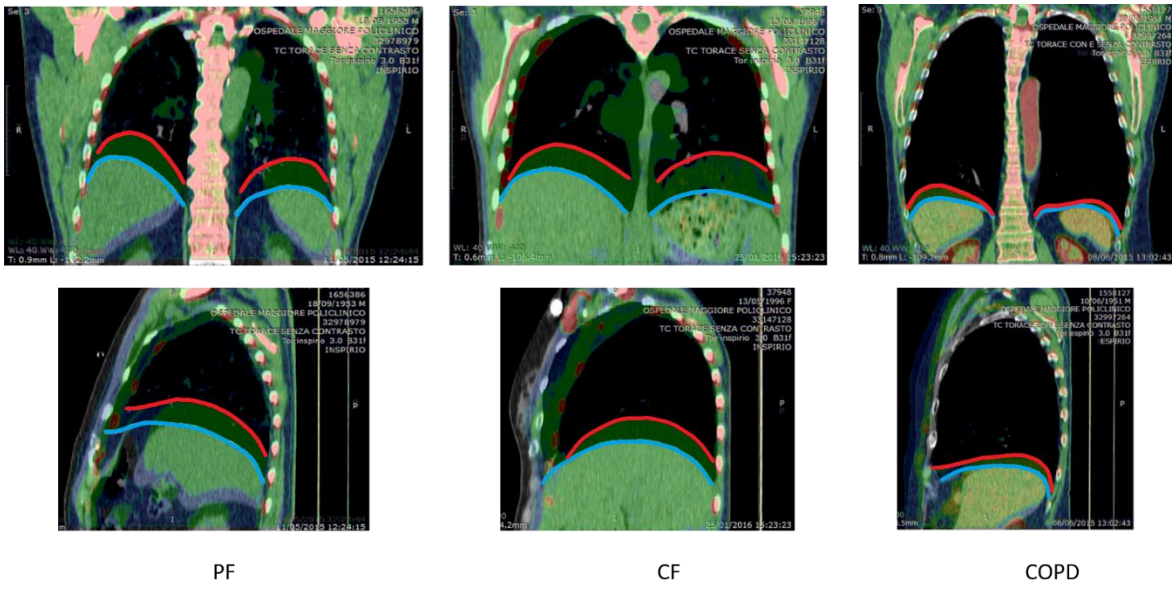

Figure 3.4.2.3.1 from the left: PF, CF and COPD CT images acquired at TLC and RV, in the coronal plane (on the top) and sagittal plane (at the bottom)

In Figure 3.4.2.3.1 it is possible to see differences of lungs and diaphragms of patients affected by the three different diseases. COPD patients show a flatter diaphragm and larger lungs; PF patients have reduced lung volumes and a more curved diaphragm; finally, CF patients show intermediate characteristics, both in terms of lung volumes and diaphragm curvature.

3.5 Ultrasonographic Imaging Results

3.5.1 Diaphragm Motion

In order to assess the motion ability of the diaphragm, ultrasound images of patients have been collected and analyzed.

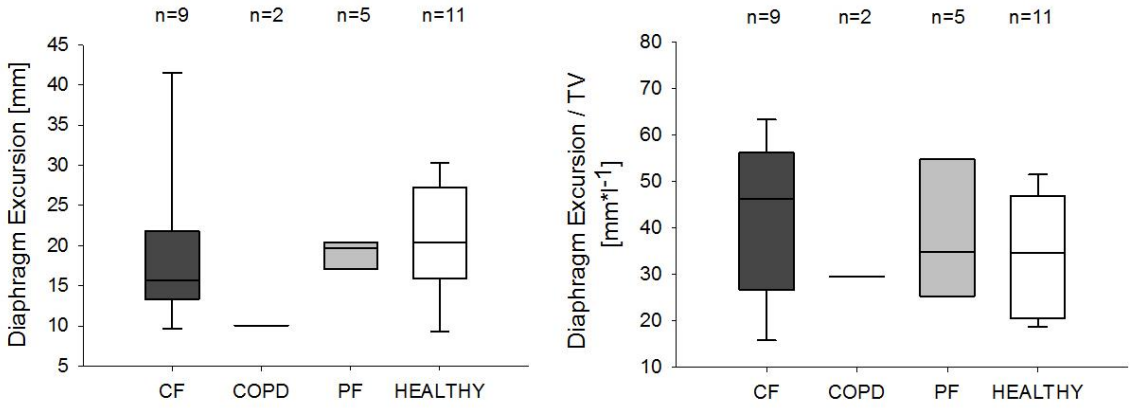

Figure 3.5.1.1 – Box plot related to the diaphragm motion; the one on the left show the dome excursion while on the right, the measure of motion has been normalized through the tidal volume during quiet breathing.

No meaningful differences have been found from a statistical point of view in the diaphragmatic excursion, nor in the same index normalized by the tidal volumes of the subjects. Only COPD seem to show a lower excursion but data are collected for only two patients.

3.5.2 Diaphragm Thickness and Thickening

Ultrasound images acquisition offered us also the possibility to investigate the thickness of the diaphragm both during quiet breathing and during forced respiratory maneuvers.

Diaphragm thickness has been measured at the end of an expiratory act and at the end of an inspiratory one (during the forced maneuver, measures have been taken at the end of an inspiratory capacity maneuver and at the end of a forced expiratory act). Relative results are shown in Figure 3.5.2.1 and Figure 3.5.2.2, which respectively refers to a quiet breathing maneuver and to a forced respiratory one.

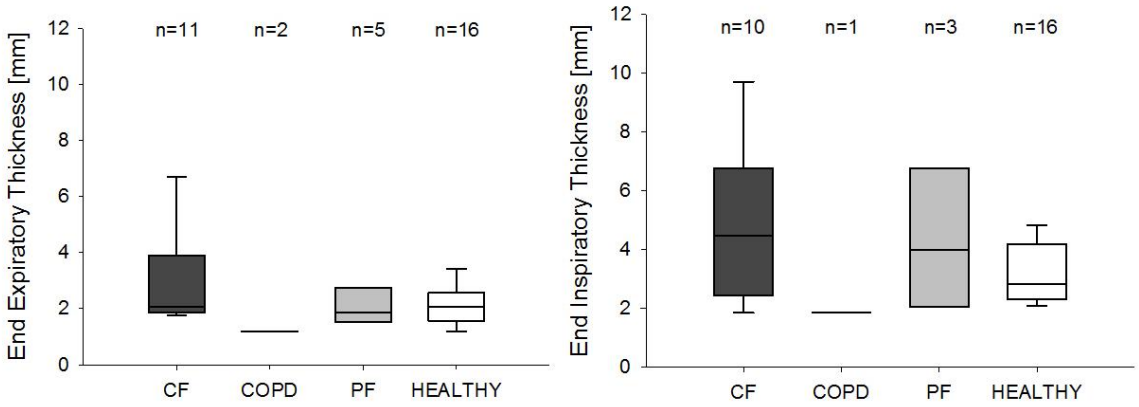

Figure 3.5.2.1 – Thickness results referring to quiet breathing.

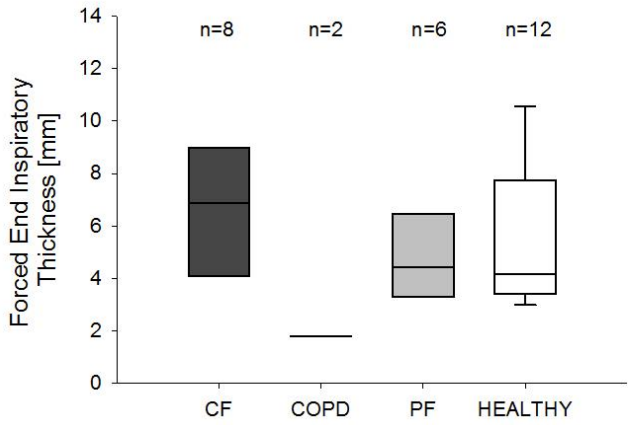

Figure 3.5.2.2 – Thickness results referring to forced respiratory maneuvers.

By combining the diaphragm thickness values, it is possible to extract the percentage of thickening of the diaphragm. Thickening values are shown in Figure 3.5.2.3 where, on the left, it is possible to see the ones related to a quiet breathing condition while, on the right, the thickening related to the forced maneuver are presented.

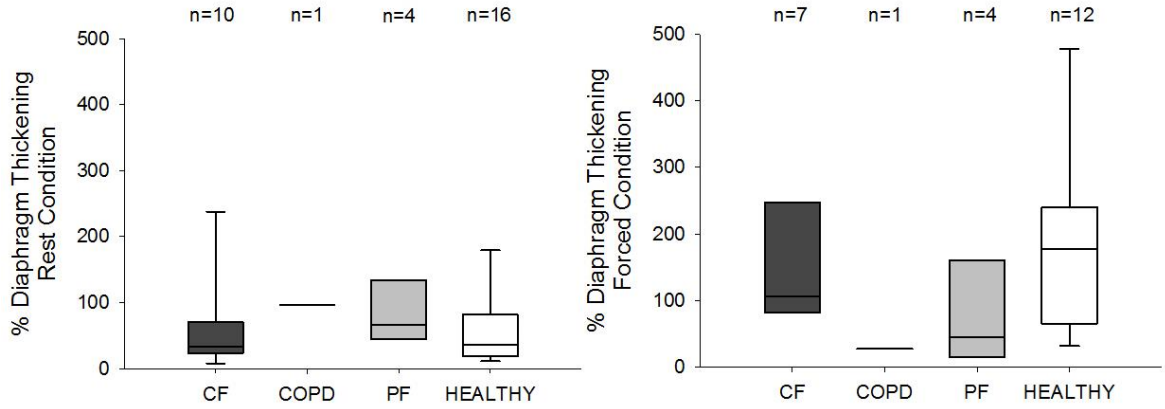

Figure 3.5.2.3 – Thickening results related to a quiet breathing condition (on the left) and to a forced respiratory maneuver (on the right).

No significant differences have been revealed between pathological subjects and healthy ones as far as the thickening index regards.

3.6 Phrenic Nerve Stimulation

3.6.1 Nerve Latency and CMAP Area

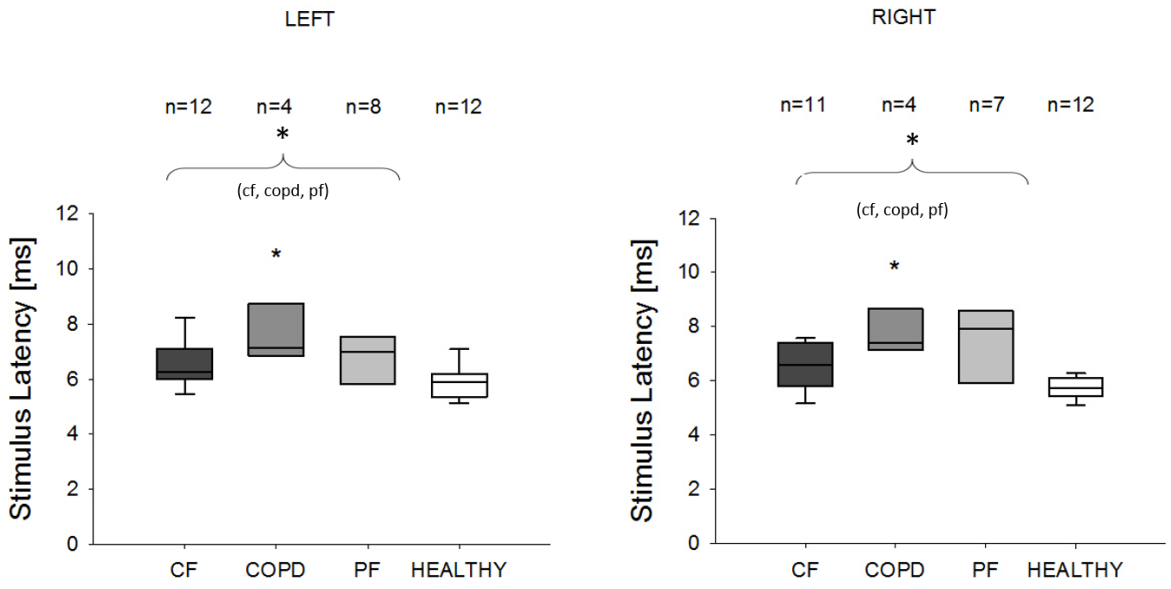

Figure 3.6.1.1 – Results of the ratio between latency of the stimulus and height of the subject in left and right phrenic nerve. The symbol * points out a difference with respect to the healthy group.

Data related to the stimulus latency are shown in Figure 3.6.1.1. Significant differences are present between patients and the control group; in particular, the stimulus latency of COPD patients is higher than healthy subjects one.

As far as the CMAP area regards, in Figure 3.6.1.2 results are shown. No differences are visible between patients and healthy subject values.

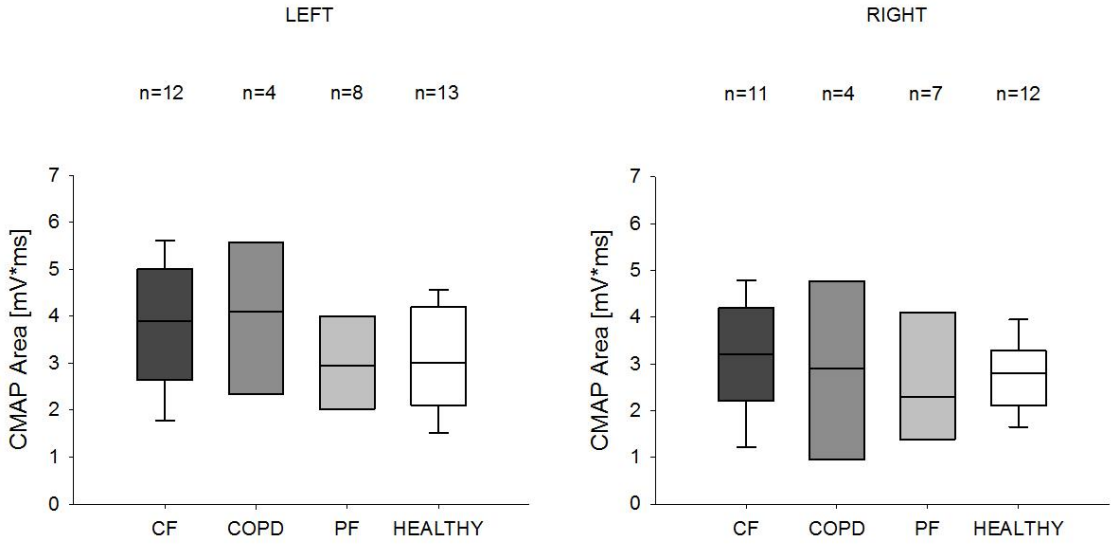

Figure 3.6.1.2 – Different values of the CMAP area in patients and healthy subjects.

3.6.2 OEP-PNS Results

3.6.2.1 Healthy Subject Analysis

As already stated, no one has ever performed an optoelectronic analysis contemporaneously to an electrical stimulation of the phrenic nerve, so no standardized methods were present in literature. For this reason, the first analysis we wanted to conduct, was a qualitative analysis on the tracks of healthy subjects, in order to understand which should be the response of the whole system to the stimulation.

We analyzed tracks coming from 12 healthy subjects in order to find, if existing, a repeatable behavior of the system response and to take it as a gold standard; we wanted also to find out some reliable indexes to make possible a comparison between healthy and pathological subjects.

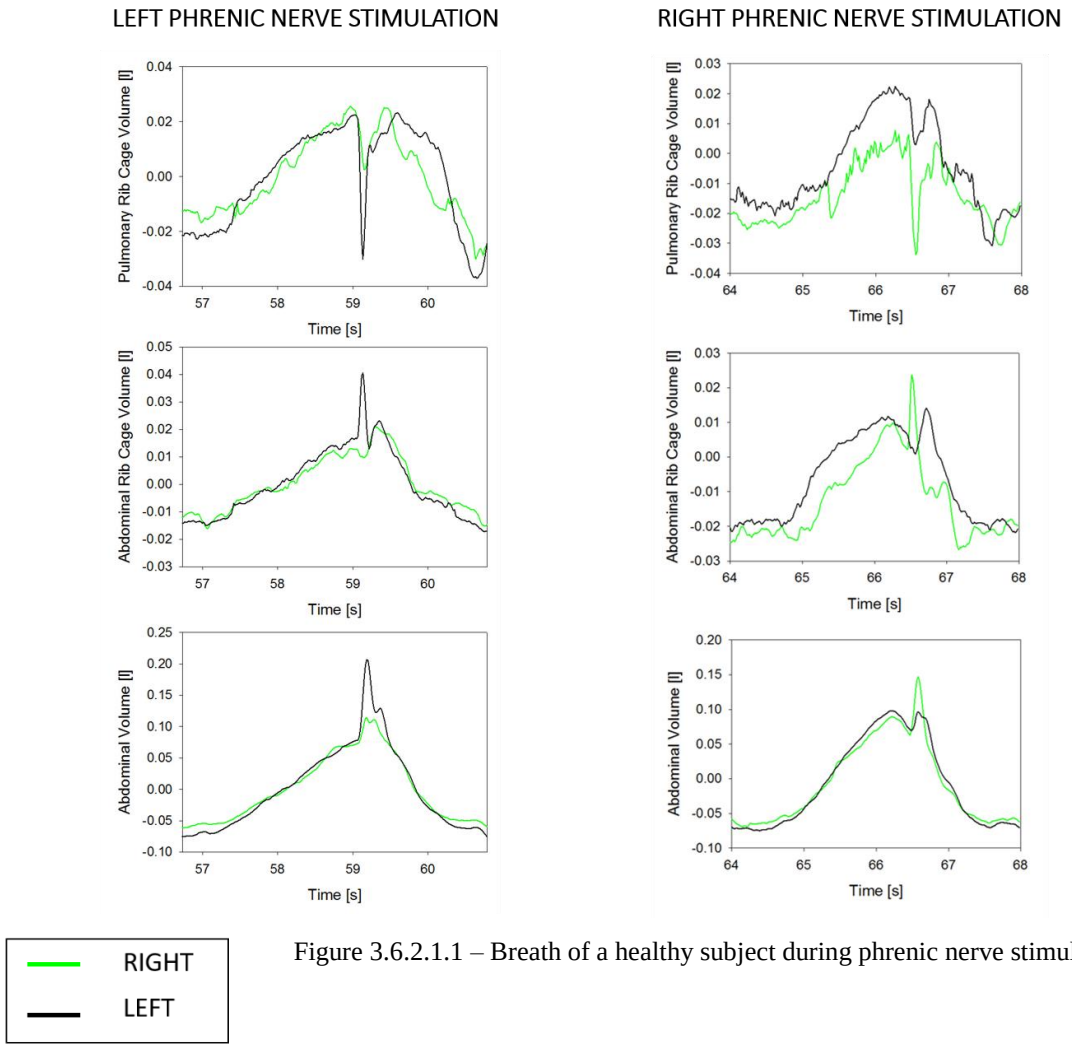

Figure 3.6.2.1.1 – Breath of a healthy subject during phrenic nerve stimulation.

Of the 24 tracks we analyzed (2 tracks per each subject), we found out a repeatable behavior in 9 of them, whose main characteristics are shown in Figure3.6.2.1.1.

When the stimulus is provided, it generates some effects on the different thorax compartments. Looking at the 9 tracks we were referring previously, what is visible and recognizable is that the stimulus provokes a depression of the pulmonary rib cage and an expansion of the abdominal rib cage, without any dependence on the stimulated phrenic nerve. What does change is the magnitude of the response: the ipsilateral hemithorax moves more than the contralateral one does. Looking at the abdominal behavior we can see a rapid increasing of the volume in correspondence of the stimulation, both for the ipsilateral and for the contralateral hemithorax. The response of the three compartments reflects in the chest wall track where it is possible to see an increasing volume similar to the one of the abdominal compartment but lower in amplitude.

Beyond an analysis of the amplitude of the changing volume, we conducted also a temporal analysis. What we have found in 9 tracks is that the ipsilateral and the contralateral parts of the pulmonary rib cage depress contemporaneously. We can not say the same thing for the abdominal rib cage: the ipsilateral part of the thorax expands earlier than the contralateral one does. As far as the timing of the abdominal response, we notice that the left and the right part of the compartment react at the same time instant, without any dependence on the stimulated nerve.

Even if just 9 tracks presented almost identical behaviors, one of the most interesting thing we found out is that the abdominal behavior is equal in any subject, that means that in presence of an electrical nerve stimulation, the abdomen always reacts increasing its volume. The greater impact of the PNS on the abdominal compartment and the fact that it always reacts in the same way, in any acquired subject, allowed us to focus our attention on the abdominal content itself to extract some significant indexes both for healthy and for pathological subjects.

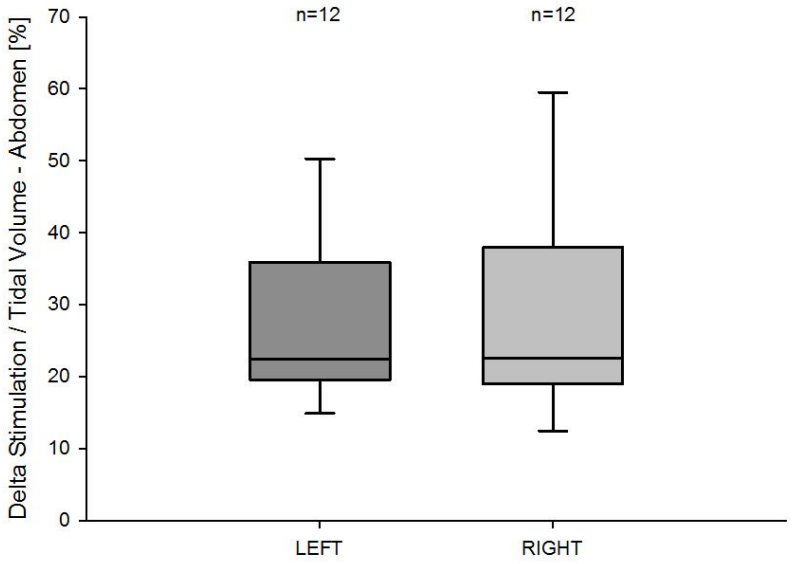

Figure 3.6.2.1.2 – Abdominal volume change induced by left or right stimulation

In Figure 3.6.2.1.2 it is shown the abdominal volume change induced by the stimulation normalized by the tidal volume of the patient. The total abdominal expansion result to be comparable, whichever phrenic nerve is stimulated (the left or the right one).

3.6.2.2 Patients and Healthy Subjects Comparison

Because of the considerations that has been stated in the previous paragraph, we choose to compare indexes that regard only the abdominal compartment for the statistical analysis with pathological subjects.

Here are shown the most significant results.

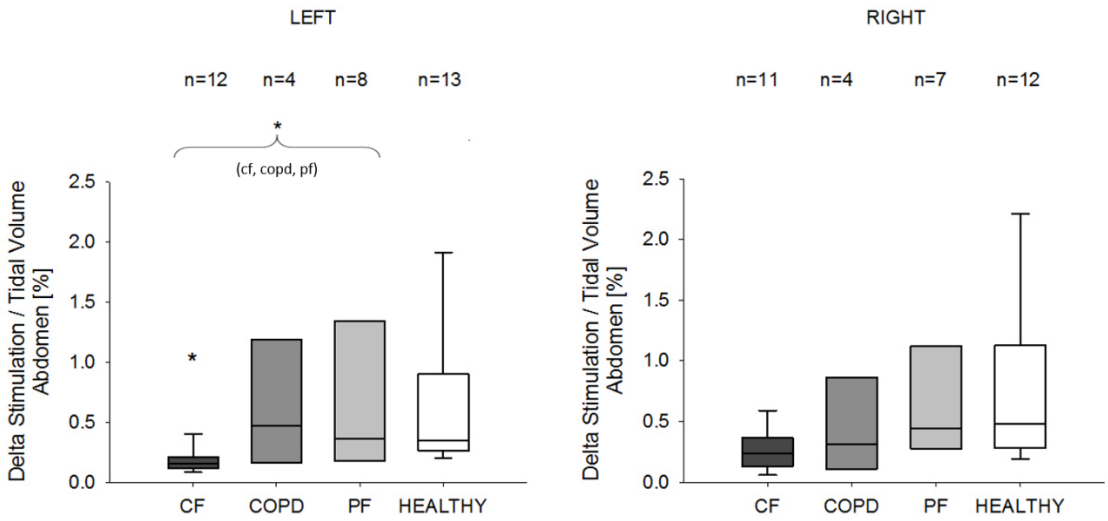

Figure 3.6.2.2.1 – Ratio between abdominal volume change during stimulation and abdominal tidal volume when the stimulus was given to the right or left phrenic nerve. The symbol * points out a difference with respect to the healthy group.

The Figure 3.6.2.2.1 shows the differences between healthy group and patients in the abdominal volume change induced by the PNS normalized by the abdominal tidal volume. The values of abdominal tidal volume are taken from the quiet breathing tracks. This is done in order avoid the effects of a muscular tension caused by the uncomfortable condition of the patients induced by the stimulation which may modify the system response.

On the left, results related to the left phrenic nerve stimulation are shown. Here, a statistically lower volume change between the group of patients compared to the healthy ones results. By performing a comparison by pairs, the cystic fibrosis group showed a reduced value for this variable.

When the right phrenic nerve is stimulated, no statistical difference is present, even if the three group of patients seems to have lower value of the represented index.

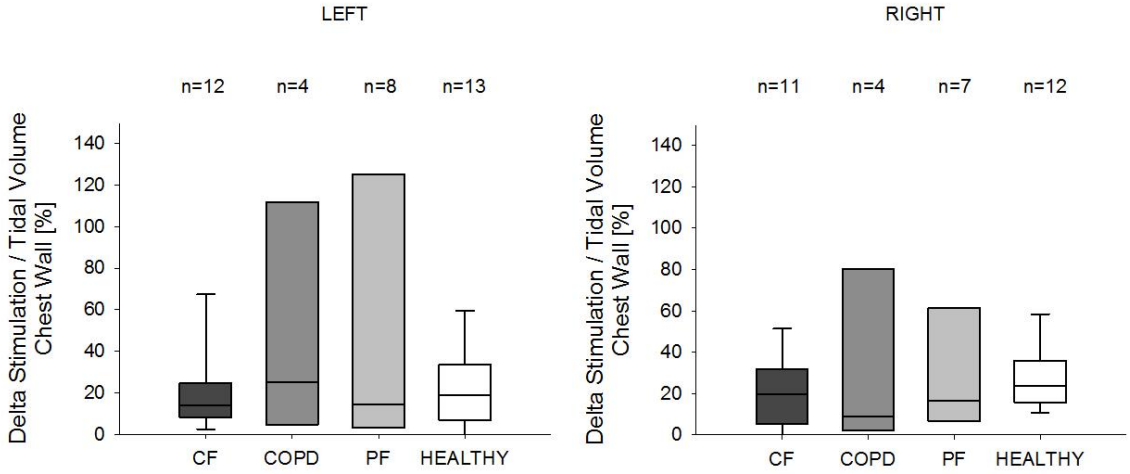

Figure 3.6.2.2.2 – Results of the volume change induced by the stimulation in the thorax volume and the tidal volume of the subjects.

The figure above shows that there are no statistically differences in the ratio between the volume change induced in the thorax by the stimulus and the tidal volume in the patients. The values of tidal volume are taken from the quiet breathing tracks. As in the previous case, the analysis was performed both for the right and for left phrenic nerve separately.

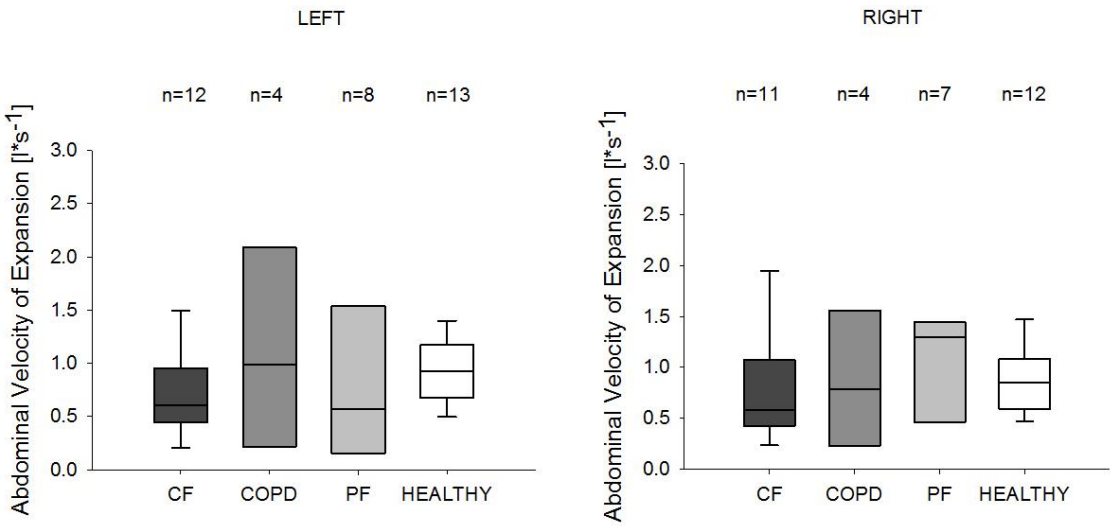

Figure 3.6.2.2.3 – Velocity of expansion of the abdomen results, both for right and left phrenic nerve stimulation.

The velocity of expansion of the abdomen shows no differences between the four analyzed groups. In figure are represented values for both right and left phrenic nerve stimulation.

3.7 Optoelectronic Plethysmography Results

3.7.1 Quiet Breathing

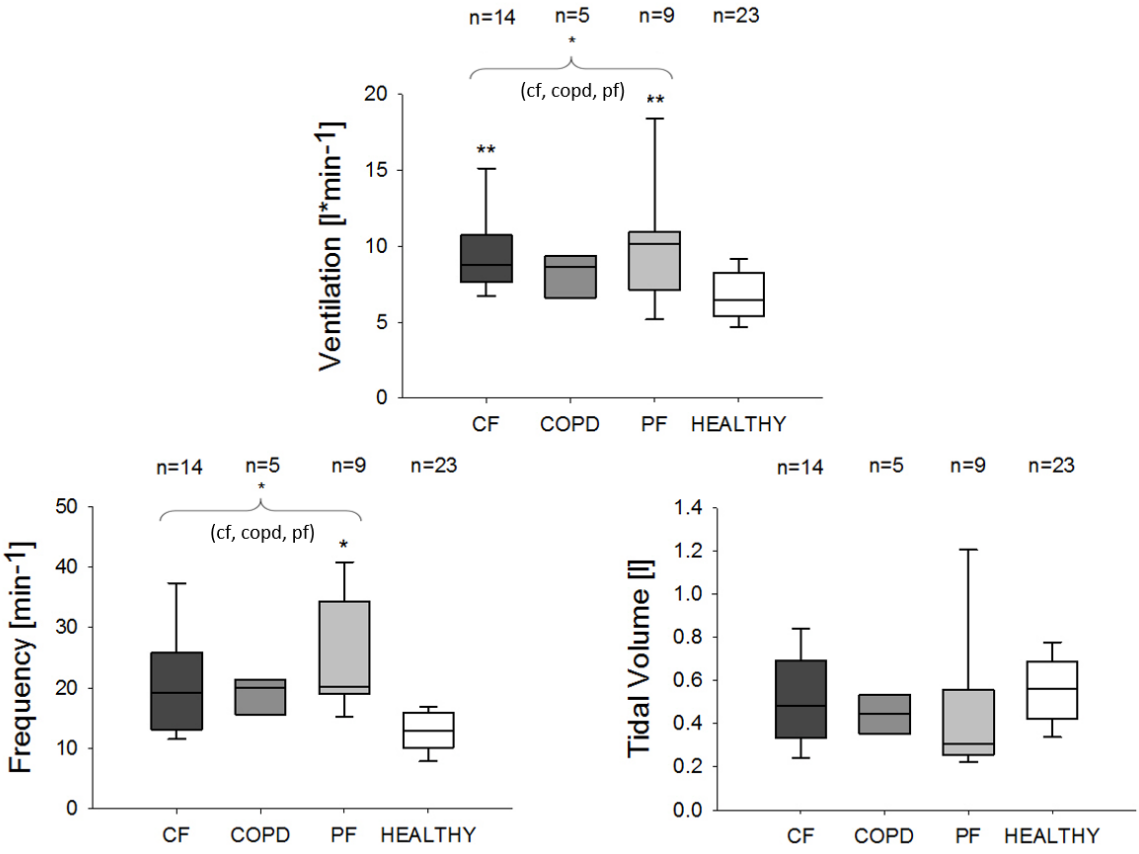

Figure 3.7.1.1 – The plot on the top represents values of minute ventilation. The two plots on the bottom show the contribution of frequency and tidal volume to the minute ventilation. The symbol * points out a difference with respect to the healthy group.

As far as the minute ventilation regards, increased values result both for PF patients and for CF ones. Moreover, a global difference between the three pathological groups and the healthy population is pointed out. The increased minute ventilation is mainly due to an increased breathing frequency, as far as PF patients regards and also considering the patients as a unique group. Patients tidal volume does not reveal significant difference with respect to the healthy group; although, the box plot shows a slightly reduced tidal volume for patients (Figure3.7.1.1).

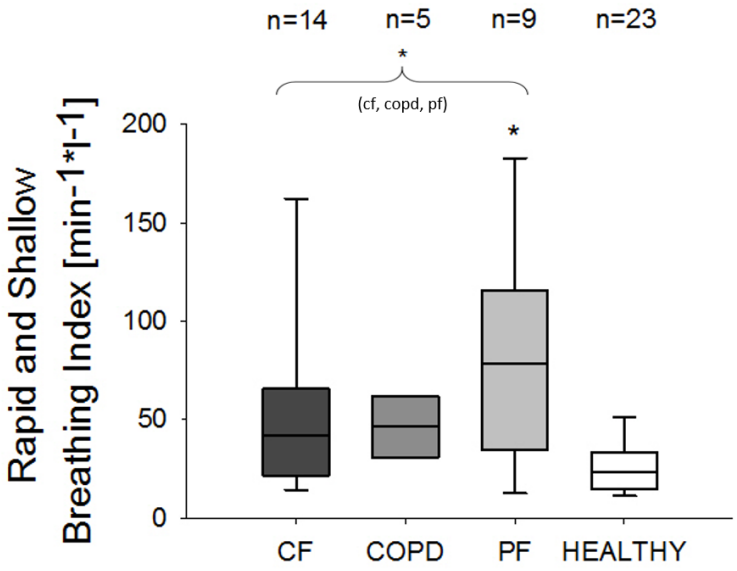

Figure 3.7.1.2 – Box plot related to the Rapid and Shallow Breathing Index (RSB Index).
The symbol * points out a difference with respect to the healthy group.

The box plot related to the RSB index (Figure 3.7.1.2) shows a difference between the PF patients and the healthy subjects and a difference between the whole group of patients with respect to the healthy population. Particularly, patients seem to have higher values of Rapid and Shallow Breathing.

Another important analysis we made was about the contribution of each thorax compartment to the breathing and to the total volume change. Figure 3.7.1.3 highlights the contribution of the Pulmonary Rib Cage (RCp), of the Abdominal Rib Cage (RCa) and of the Abdominal (Ab) compartment as a percentage of the whole chest wall volume change.

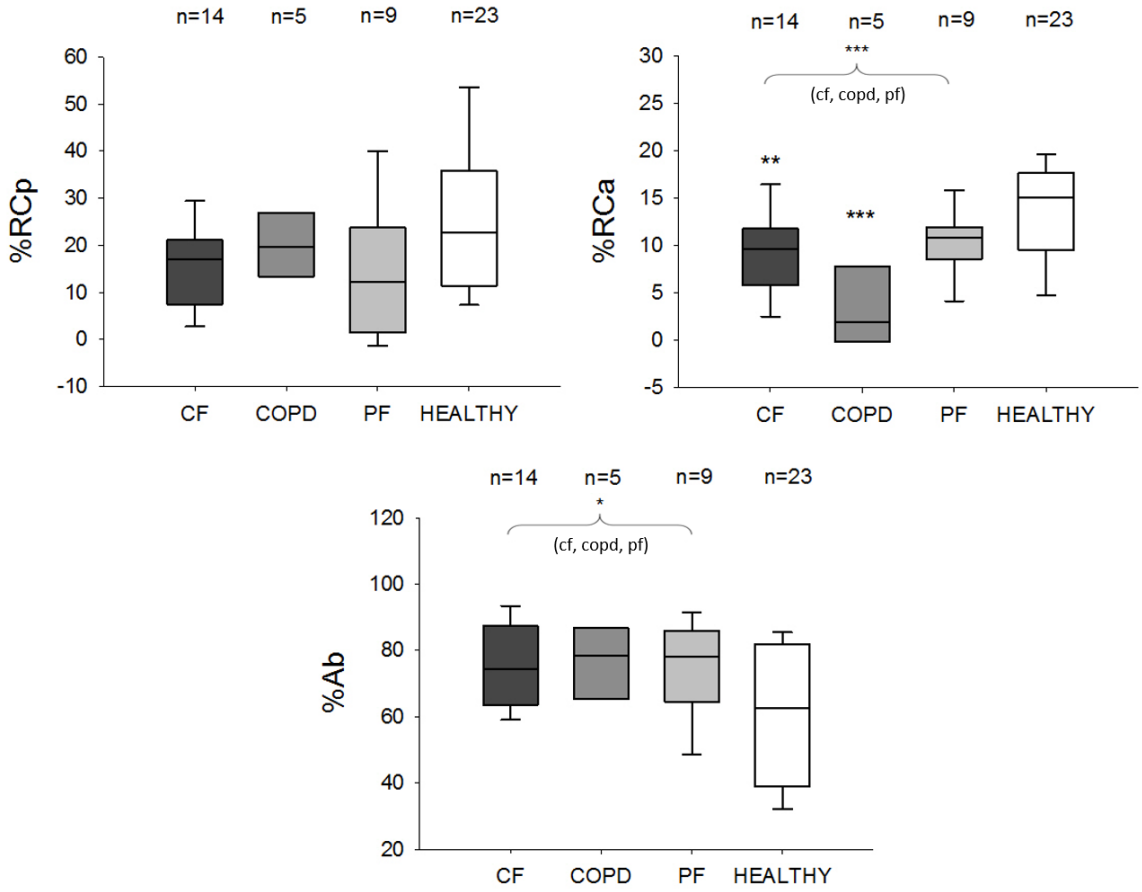

Figure 3.7.1.3 – The figure shows the contribution of the Pulmonary Rib Rage (RCp), of the Abdominal Rib Cage (RCa) and of Abdomen (Ab) as a percentage of the chest wall volume changes. The symbol * points out a difference with respect to the healthy group.

A significant difference, statistically speaking, can be seen in the contribution of the abdominal rib cage. In particular, its contribution in CF and in COPD patients results to be significantly lower than the contribution related to healthy subjects. A global difference between the whole patient group and the healthy population can be seen both in the abdominal rib cage and in the abdomen contribution; patients, globally, show a reduced abdominal rib cage contribution and a higher abdominal contribution (Figure 3.7.1.3).

3.7.2 Vital Capacity

Results related to the Vital Capacity maneuver are shown in Figure 3.7.2.1.

We have shown the contribution of each compartment on the chest wall volume variations. CF and PF patients reveal to have a significantly reduced volume variation during the maneuver with respect to the control group. Also globally, patients result to have a diminished chest wall volume change with respect to healthy subjects and it is mainly due to the reduced contribution of the pulmonary rib cage, which actually is lower in PF and CF patients.

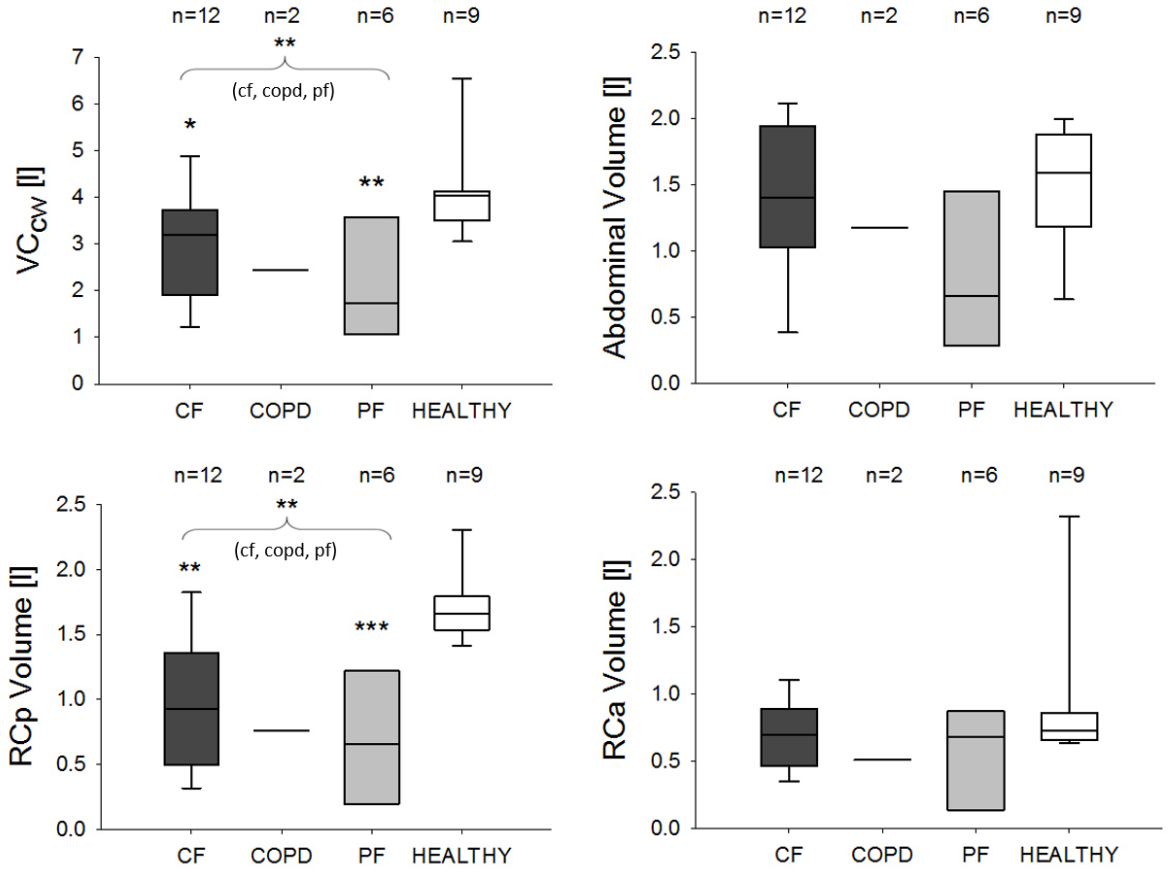

Figure 3.7.2.1 – The figure shows the volume variation due to a vital capacity maneuver for the chest wall (VCcw) and the percentage contribution of the three compartments to the chest wall volume variations itself (pulmoanry rib cage, RCp, abdominal rib cage, RCa, abdomen, Ab). The symbol * points out a difference with respect to the healthy group.

3.7.3 Inspiratory Capacity

Also maneuvers of inspiratory capacity have been performed. Related results are shown in Figure 3.7.3.1 such as the single contribution of each thorax compartment influences the global volume variations.

Statistically significant differences have been found in the whole chest wall volume change where patients show reduced values. Lower chest wall volume variations are mainly due to a reduced contribution of the abdomen and of the pulmonary rib cage. In particular, CF and PF patients have a significant lower contribution of the RCp compartment with respect to the healthy group.

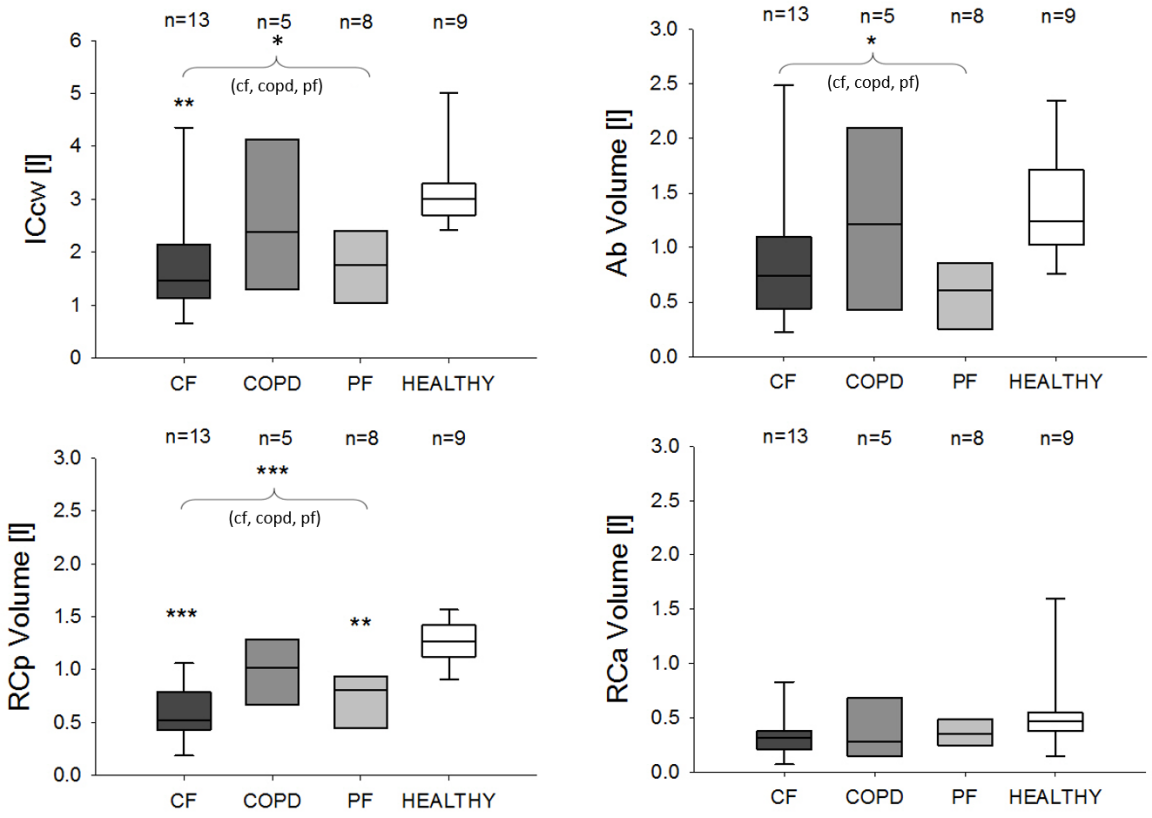

Figure 3.7.3.1 – The figure shows the volume variation due to an inspiratory capacity maneuver for the chest wall (ICcw) and the percentage contribution of the three compartments to the chest wall volume variations itself (pulmoanry rib cage, RCp, abdominal rib cage, RCa, abdomen, Ab). The symbol * points out a difference with respect to the healthy group.

Chapter 4

Discussion

In a group of 28 patients on the waiting list for lung transplant, we collected data from several points of view, in order to evaluate the morphology and functionality of their diaphragmatic muscle.

Patients were subdivided into three different groups according to their pathology. The first group was composed by patients affected by pulmonary fibrosis (PF), the second one by cystic fibrosis (CF) and the third one by chronic obstructive pulmonary disease (COPD). PF and COPD groups show a higher mean age because of the later onset of the disease while the cystic fibrosis is a congenital pathology so the onset is earlier. The lower number of patients in the COPD is due to the fact that the disease worsening is slower compared to the worsening of the other two pathologies [3,6,10]. This fact, together with the late onset of COPD, makes the patients more likely to live even without a lung transplant, considering only oxygen therapy.

Spirometry results show reduced lung volumes during forced vital capacity maneuver, both in supine and in seated position, thus indicating restrictive characteristics of the lung function. These results are supported by previous works in literature [41].

Also FEV1 values reflects literature results, as the COPD show a lower value compared to the two other groups. This reduction points out the presence of an obstructed condition.

The fact that COPD and PF patients show a ΔFVC index lower than 25% means that they are not affected by diaphragmatic weakness. As regards CF patients, only 4 of them overcome that threshold, therefore a diaphragmatic weakness may be diagnosed.

MIP and MEP median values are shown to be in a normal range of values, so the whole respiratory muscles system seems to work properly, despite the disease, when a maximal effort is required. What is important to underline is the fact that the pathologies affecting the patients we dealt with, are pathologies which do not directly affect muscles; thus respiratory muscles should keep working as expected.

Together with spirometry values, the characterization of the patients is followed by considering the DLCO values. This value is an index of efficiency of the as exchange mechanism in the lungs.
All of the three groups showed median DLCO values lower than 100%. Particularly, as expected from the literature, PF group shows DLCO values much lower than 40%. It is the cut off threshold below which a high risk of morbidity and mortality is associated [25]. The low DLCO values for PF patients may be due to the presence of scar tissue in the lungs induced by the disease; as fibrotic tissues replaced the alveolar wall, gas exchange efficiency is reduced.

Diaphragm morphological analysis focuses on the curvature and length of the left and right hemidiaphragm in the coronal and sagittal view.
What results show, is that COPD patients have higher values of curvature radius, in accordance with what we expected. Actually, what it is known [42], is that diaphragm is flattened by the high load constituted by the augmented lung volume; a flat diaphragm leads to an elevated curvature radius. On the other hand, the reduced lung volume in PF patients, gives the diaphragm the possibility to assume a more curved conformation, that means, a lower curvature radius. CF patients usually have a diaphragm curvature which stays in between the one of COPD patients and the one of PF patients because of the restrictive and obstructive pathology that affects them.
The higher median value of hemidiaphragms length in CF and PF patients may be due to the fact that the rigidity of their lungs prevent the maximal shortening of the diaphragm fibers.

All these considerations allow us to conclude that diaphragm morphology results to be altered in patients, even if the pathologies do not directly affect the diaphragm. The modified diaphragmatic anatomy is due to the modified lung volumes typical of the pathologies we dealt with.

The reported case study shows how diaphragm of one PF patient, two CF patients and two COPD patients moves passing from a forced end inspiratory volume to a forced end expiratory one.

The diaphragm of the reported PF patient is highly curve, due to the reduced lung volumes. The difference in position and curvature is clearly visible.

Similar considerations can be inferred for CF patients; actually, their diaphragm is not as curved as the one of PF patients. Anyway, the difference in position and curvature is clearly visible.

COPD patients, on the other hand, as expected, show a really flat diaphragm. It has a very low range of motion because of the small difference between RV and TLC given by the lung hyperinflation and upper airway obstruction.

Results coming from ultrasonographic imaging, do not point out any difference between patients and healthy subjects. Diaphragm excursion, its thickness and its thickening assume values similar to the ones of healthy subjects. Thickness and thickening have been measured both during quite breathing and during forced maneuvers. The fact that these values do not show any difference with respect to the healthy group, even in forced condition, is not in contradiction with what we have concluded until now; actually the diaphragm is not affected by the pathologies we dealt with and for this reason it should not be neither hypotonic nor deconditioned. Normal values of thickness and thickening are in accordance with these considerations.

Looking at the Phrenic Nerve Stimulation results, in particular to the latency of the stimulus, what does emerges, is that almost no difference is presents. Only COPD patients show a higher latency but, as reported by by Podnar et al. [39], this may probably be caused by their higher mean age which influences the conduction ability of nerves.

The fact that no differences about the CMAP area result, is again an index of the fact that the diaphragm muscle is neither deconditioned nor hypotonic as its action potential is similar to the one of healthy subjects.

Looking at the breathing pattern during the quite breathing, OEP results show a higher ventilation of the whole group of patients compared to the healthy subjects. This increase in ventilation is mainly due to an increased respiratory frequency (results about frequency are supported by Aliverti et al. [33] work in which a comparable group of patients has been analyzed) while the tidal volume does not show any kind of anomaly. In addition, the Rapid and Shallow Breathing Index (RSBi) assumes higher values with respect to healthy subjects one. Actually, as the tidal volume of patient seems to be similar to the healthy subject one, the breathing is rapid, but not seriously shallow.

The augmented frequency together with the higher values of the RSBi may be explained by considering that patients have an impaired gas exchange efficiency from the DLCO results. For this reason, they overcome that lower efficiency with an increased respiratory frequency in order to wash out the residual CO_2 and introduce a higher amount of O_2 within the body.

This increased frequency, together with the unimpaired tidal volume, shows that the ventilatory pump is not affected by the disease, but it works even more than in healthy subjects.

Considering the compartment behavior during quite breathing, a reduced contribution of the rib cage results; particularly, the abdominal rib cage seems to be compromised. This behavior may be caused by the reduced compliance which characterizes PF and CF rib cage that makes difficult the expansion of the rib cage itself. As far as the COPD regards, they usually have higher lung volumes compared to healthy subjects; the constant hyperinflation they are affected by results in a shortening of inspiratory muscles which are not able to contract further during inspiration. As a result, the rib cage does not expand as it should.

On the other hand, the abdominal compartment of single groups does not show any significant differences with respect to the healthy group; this points out that the diaphragm, which mainly induce the abdomen volume changes, is able to cope with the pathology and it guarantees a right respiration during quiet breathing.

Looking at the forced maneuvers, lung vital capacity values results to be significantly lower for PF and CF patients mainly because of the reduced contribution of the pulmonary rib

cage. The causes of this kind of behavior may be found in the reduced compliance that characterizes their lungs that prevents from the rib cage expansion during forced maneuvers. As far as the inspiratory capacity concerns, reduced volumes characterize patients maneuver, particularly the ones of CF patients. This reduction is in part caused, again, by a reduced contribution of the pulmonary rib cage, but a reduction in the abdominal contribution also emerges. The diminished volume changes in the abdominal compartments highlights the inability of the diaphragm to sustain breathing during forced maneuvers, probably due to the fact that a stiff lung prevents diaphragm motion during forced maneuvers.

Phrenic Nerve Stimulation volume tracks coming from healthy subjects show that the three compartments constituting the thorax have symmetrical behaviors.
Considering the pulmonary rib cage, the electrical stimulus provokes a depression of the stimulated side, which is higher in amplitude and synchronous to the contralateral one. For the abdominal rib cage, the stimulus induces an expansion higher in amplitude but delayed with respect to the contralateral one. Finally, for the abdominal compartment, it is induced an expansion, which is higher in amplitude and synchronous with respect to the contralateral side. The trends are similar whichever is the stimulated phrenic nerve.

As the abdominal compartment is the one in which the effect of the stimulus is more clearly recognizable, the comparison between results of pathological and healthy subjects is focused on the abdomen itself.
The difference in the ratio between the volume change induced by the stimulus and the tidal volume which results for CF patients with respect to the healthy group may be due to the fact that cystic fibrosis does not only affect lungs but also internal organs, like liver, pancreas, gastrointestinal structures. These organs are impaired because of the higher secretion of thick mucus that may induce change to their mechanical properties. Depending on the severity of the pathology and of the degree of degradation of these organs, abdomen induced volume changes may be impeded and reduced.
The fact that the velocity of expansion of the abdomen does not show any meaningful difference between patients and healthy subjects means that the velocity of contraction of the diaphragm is comparable between the different groups

The analysis we made and the results we obtained, let us conclude that patients we dealt with have a diaphragm able to cope at least during quiet breathing with the pathology they are affected by. Of course, the progress of the pathology and the increasing severity induce diaphragm weakness and fatigue during time and this may be the reason why some patients undergo a diaphragmatic paralysis after lung transplant.

Chapter 5

Future Developments

During the period we cooperated with the foundation IRCCS Ca' Granda, Ospedale Maggiore Policlinico in Milan, we met 6 patients who underwent lung transplant. 5 of them were acquired also in the pre-operative situation.

Their anthropometrical data are presented in Table 5.1.

PATIENT	AGE (yrs)	WEIGHT (kg)	HEIGHT (cm)	BMI (kg*m-2)	PATHOLOGY
Subject 29	65	61	168	21.61	FP
Subject 30	63	82	176	26.47	FP
Subject 31	55	46	165	16.90	FP
Subject 32	36	56	167	20.08	FC
Subject 33	54	60	154	25.09	FP
Subject 34	20	38	150	16.89	FC
MEAN ± SD	48.76 ± 17.48	57.08 ± 15.03	163.33 ± 9.63	21.17 ± 4.03	

Table 5.1 – Anthropometrical data of patients who underwent lung transplantation.

As we had their data at our disposal, we analyzed them in order to make a comparison between results obtained before and after the lung transplant operation.

Three considerations must be taken into account:

- the post-operative group is composed just by 6 patients, that means that statistical results may not be reliable;

- patients were affected by different pathologies before the surgical intervention, that means that initial conditions were different;
- acquisitions were performed within 20 days from the surgical operation, that means that some results may be altered by the resulting wounds and by the underwent stress.

We decided anyway to perform the comparison in order to have a global and overall view on the immediate effects of lung transplant.

5.1 Results from Transplanted Patients

Following results refer to the entire group of patients acquired before the lung transplant (constituted by PF, CF, COPD patients), the group of patients who were acquired after lung transplant and the control group of healthy subjects.

Spirometry results, in particular FVC values, do not show any significant difference between transplanted patients and preoperative ones. Results of the two groups assume lower values than the predicted ones.

Results from MIP, MEP and SNIP tests are shown in Figure 5.1.1

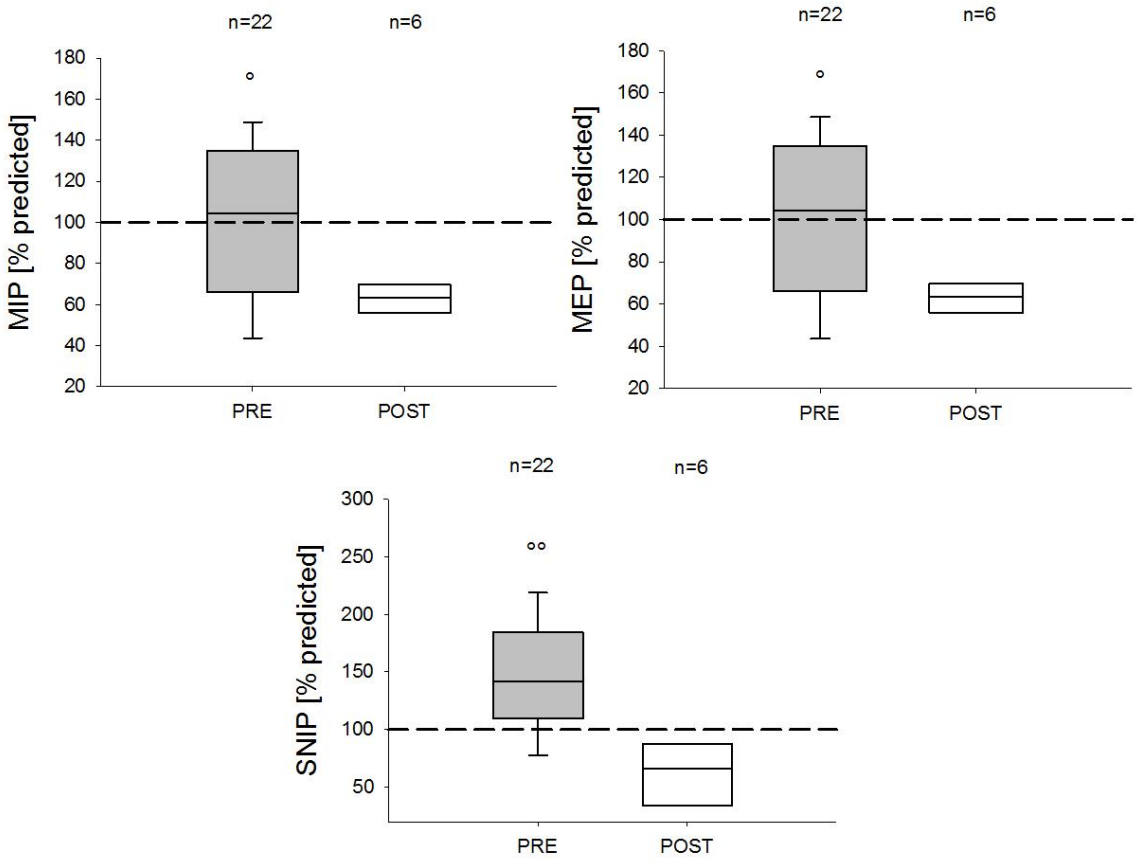

Figure 5.1.1 – Results from MIP, MEP and SNIP tests expressed as the percentage of the predicted values. The dotted lines refer to the reference value of 100%. The symbol ° points out a difference with respect to the post-operative group.

A significant reduction of the MIP, MEP and SNIP values results.

From a morphological point of view, looking at the post-operative group, the left hemidiaphragm length normalized by the subject height, measured in the coronal plane, shows increased values with respect both to the control group and to the pre-operative one. (Figure 5.1.2)

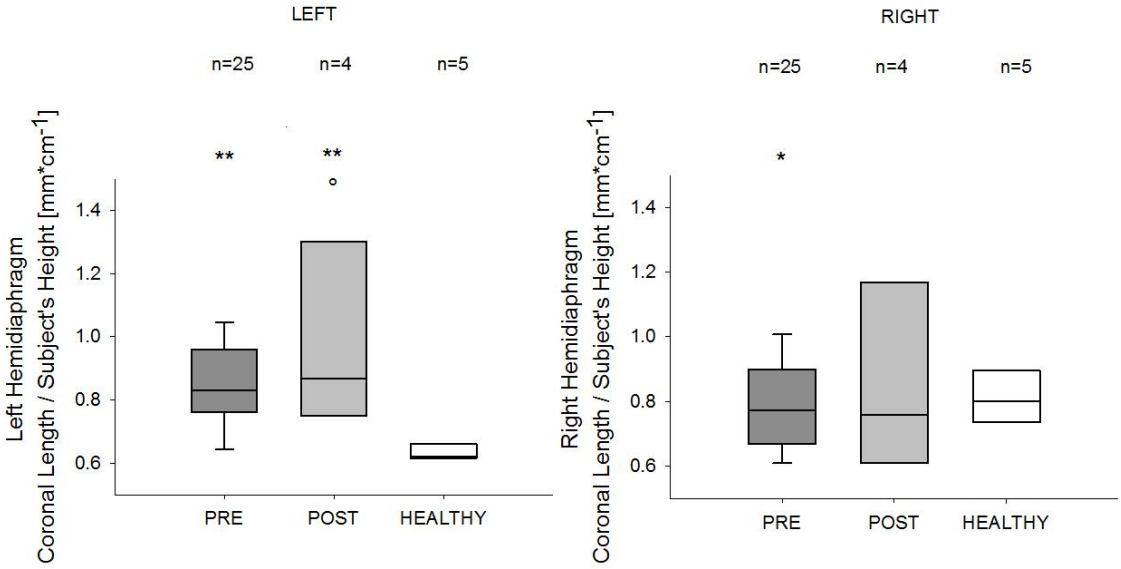

Figure 5.1.2 – Results of the left and right hemidiaphragm length normalized on the subject's height measurements in the coronal view. The symbol * points out a difference with respect to the healthy group, while ° is refers to the pre-operative group.

Also the electrical stimulus latency shows higher values in the post-operative condition with respect to the control group and to the pre-operative one; this is true for the left phrenic nerve stimulation. Anyway, it must be taken into account the fact that we got values of latency just for one patient in the post-operative condition and for this reason results may not be reliable.

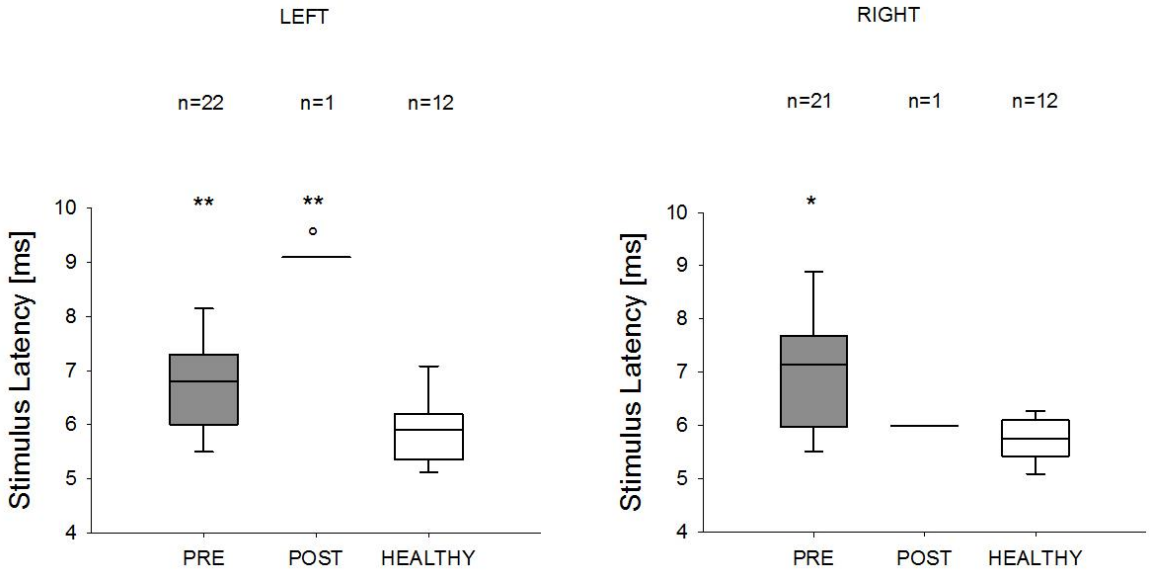

Figure 5.1.3 – Results of the ratio between latency of the stimulus and height of the subject in left and right phrenic nerve. The symbol * points out a difference with respect to the healthy group, while the symbol ° refers to the pre-operative group.

Looking at the breathing pattern, we found out some significant differences. In particular, we can notice that the respiratory frequency of the post-operative group is higher than the healthy subject; moreover, its median value is higher than the pre-operative patient group. A similar consideration may be done for the RSB index (Figure 5.1.).

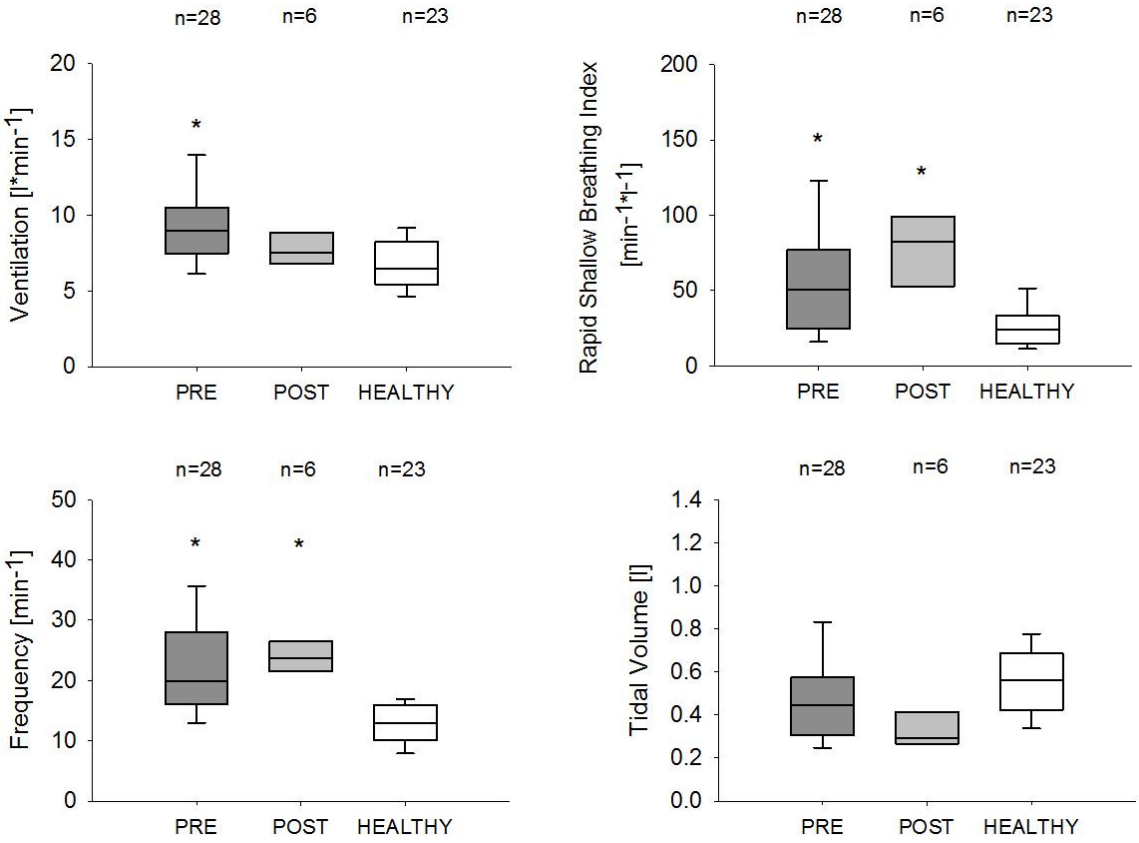

Figure 5.1. – The plot on the top represents values of minute ventilation. The two plots on the bottom show the contribution of frequency and tidal volume to the minute ventilation. The symbol * points out a difference with respect to the healthy group.

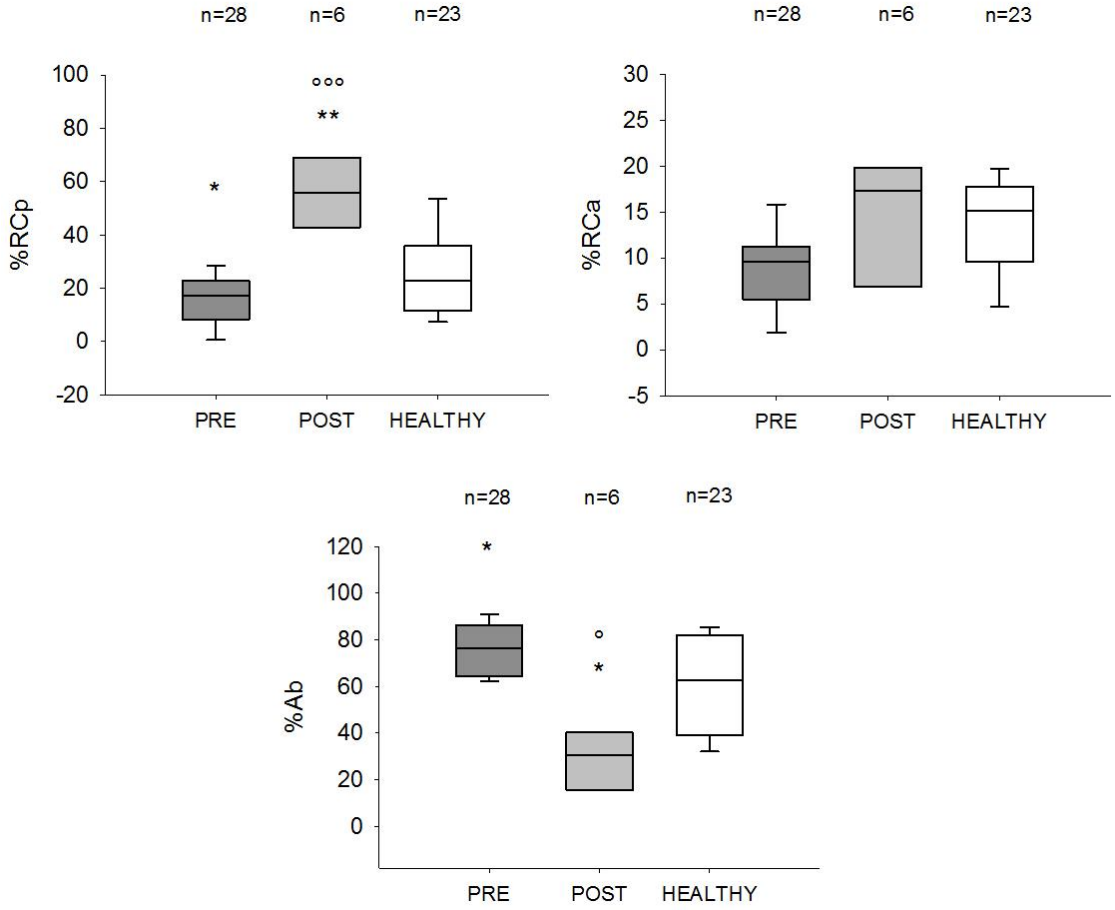

Figure 5.1.5 – The figure shows the contribution of the Pulmonary Rib Rage (RC,p), of the Abdominal Rib Cage (RC,a) and of Abdomen (Ab) as a percentage of the chest wall volume changes. The symbol * points out a difference with respect to the healthy group while the symbol ° points out a difference with respect to the pre-operative group.

An interesting behavior can be seen in the immediate post-operative time. What we can observe in Figure 5.1.5 is a high increase in the pulmonary rib cage contribution and a significant decreasing of the abdomen contribution to the quiet breathing with respect both to the healthy group and to the pre-operative group.

Inspiratory capacity values are shown in Figure 5.1.6.

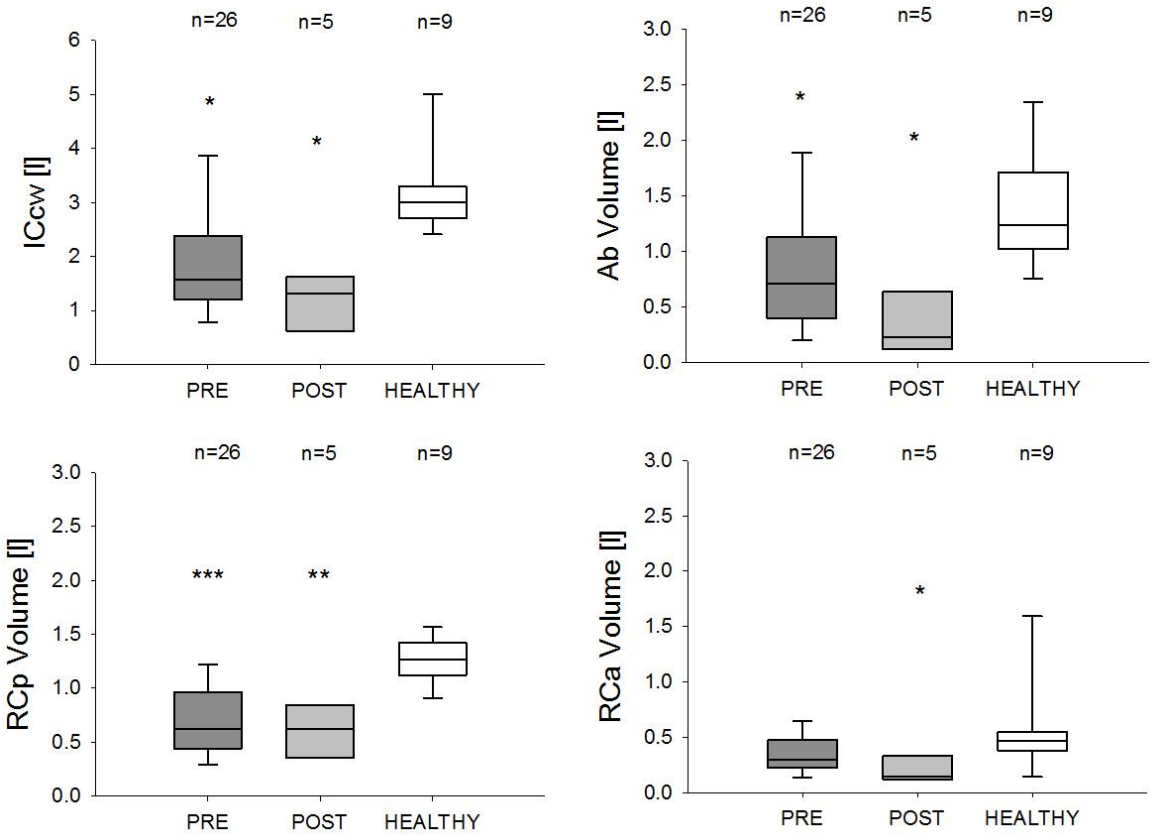

Figure 5.1.6 – The figure shows the volume variation due to an inspiratory capacity maneuver for the chest wall (ICcw) and the percentage contribution of the three compartments to the chest wall volume variations itself (pulmoanry rib cage, RCp, abdominal rib cage, RCa, abdomen, Ab). The symbol * points out a difference with respect to the healthy group.

During an inspiratory capacity maneuver post-operative patients show reduced volume changes with respect to the other groups, due to a diminished contribution of all the three compartments.

5.2 Discussion

Results related to spirometry and to the breathing pattern, both during quiet breathing and the forced maneuver, may lead to the conclusion that the lung transplant did not have the desired effect. This is not always true, of course.

Looking at MIP, MEP, SNIP test results, a reduction in the generated pressure is visible. This may probably be caused by the open wound that patients still present at the moment of acquisition. Performing forced maneuvers causes a stretching of the skin and, consequently,

a stretching of the wound arousing pain. In order to avoid pain, patients may have performed a submaximal maneuver, thus resulting in reduced pressure values.

From a morphological point of view, the differences between the post-operative group and the control group which are still present may point out the that the diaphragm has not yet adapted to the new condition induced by the lung transplant. It may be necessary a longer adaptation period.

A consideration on the stimulus latency may be done. The lung transplant intervention is really invasive. Tissues, muscles, organs placed near to lungs are stressed and may be compromised. The same holds for the phrenic nerve and this may be the reason why latencies result to be higher right after the surgical intervention.

Looking at the breathing pattern, the fact that the abdomen contribution presents a sharp decrease may be connected to the wound: as a consequence of the reduced abdominal supply induced by the surgical wound, the rib cage increases its action to assure a suitable chest wall volume change.
The respiratory frequency is still higher in the post-operative condition with respect to the healthy one and the same holds for the RSB index. These considerations may lead to the conclusion that the gas exchange mechanism is still compromised; although, it may be caused by the fact that the systems, the nervous one and muscular one, have not adapted to the new condition.

Also during the inspiratory capacity maneuver a reduction in the abdominal compartment is visible and it may be due again to the presence of the wound.

Because of the short period elapsed between the transplant and our analysis and considering the physiological condition of the patient, resulting benefits coming from the new lungs seems to be hidden. For this reason, it is recommended to perform the identical analysis also after 6 months from the surgical operation in order to see the adaptation of the muscle to the new lungs and the improvements in the breathing pattern.

Unfortunately, in our period of collaboration with the hospital, we were able to repeat the procedure just for one patient, but results are remarkable.

Looking at the respiratory frequency of the patient under analysis (Figure 5.2.1), we can see how it undergoes an increase right after the surgical intervention but after 6 months from the transplant it reaches lower values compared to the preoperative conditions, almost comparable to the healthy subjects.

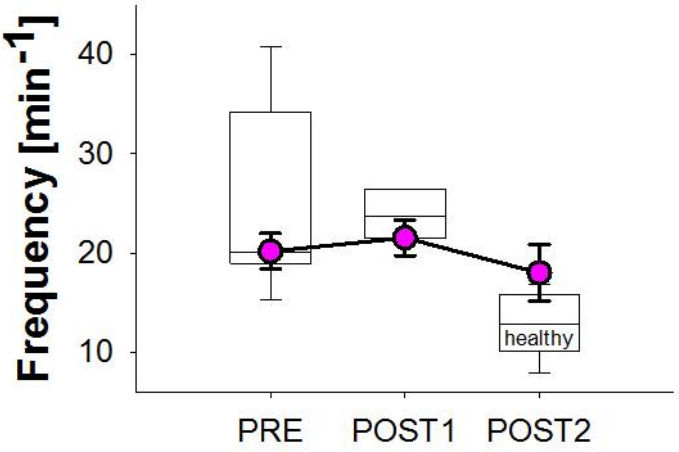

Figure 5.2.1 – Different respiratory frequency of the subject under our analysis before, 15 days and 6 months after the transplantation (in pink). The boxplots (in white) show the population of the data coming from patients (on the left), post transplanted patients (in the middle) and healthy subjects (on the right).

On the other hand, the abdominal contribution to the tidal volume during quiet breathing (Figure 5.2.2) is subjected to a great decrease right after the transplant, but in the second post-transplant acquisition, it reaches higher values, similar to the ones of healthy subjects.

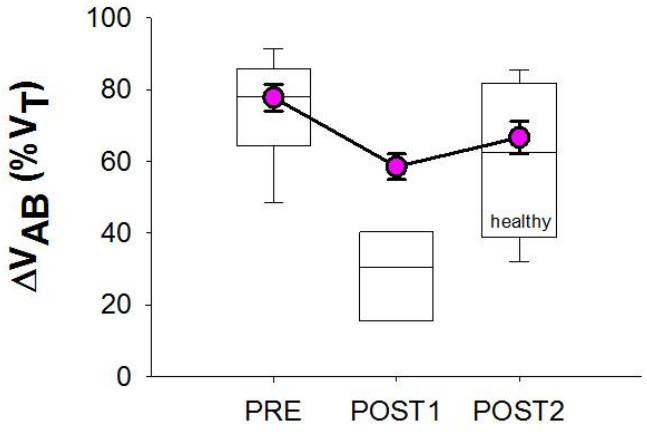

Figure 5.2.2 – Abdominal percentage to the tidal volume during quiet breathing

These considerations, even if they are possible only for one subject, highlight the importance to longitudinally extend the work in order find out the late onset benefits induced by the transplant.

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