

POLITECNICO DI MILANO
Scuola di Ingegneria dei Sistemi
Corso di Laurea Magistrale in Ingegneria Biomedica



**VOLUNTARY COUGH MECHANICS IN
HEALTH AND DUCHENNE MUSCULAR
DYSTROPHY**

Relatore: Prof. Andrea Aliverti

Tesi di Laurea di:
Antonella Lo Mauro
Matr.722179

Anno Accademico 2011-2012

.... a Peter
“che fu sorpreso
dai suoi 79 anni
e con la vita
avrebbe ancora
giocato”
(F. De Andrè)

Table of contents

Summary		p. 4
Sommario		p. 10
Chapter 1	Introduction	p. 17
Chapter 2	Electromyography and chest wall volumes during cough in healthy subjects	p. 25
Chapter 3	Blood shift during cough in healthy subjects	p. 45
Chapter 4	Determinants of cough efficiency in Duchenne muscular dystrophy	p. 59
Chapter 5	Conclusions and future perspectives	p. 78
Acknowledgments		p. 81

SUMMARY

Cough is a vagally mediated defensive airway reflex whose function is the removal of mucus or any other inhaled foreign materials therefore providing and maintaining airway clearance and lung hygiene. Cough can be initiated either voluntarily or by the stimulation of cough receptors located primarily in the central airways.

Coughing consists of 3 components: an inspiratory phase, a contraction phase and an expulsive phase. During the inspiratory phase, the subject inhales, usually from 60% to 90% of total lung capacity. In the compressive phase, the subject's glottis closes and the expiratory muscles begin to contract. This phase lasts only 0.2 seconds; however, during this time, elevated intrathoracic pressures are reached. During the final (expulsive) phase of a cough, the glottis opens, and there is a sudden expiratory rush of air.

Therefore, the effectiveness of cough clearance depends on the coordinated neural sequence of phases involved in the cough maneuver and on the inspiratory, expiratory, and glottic muscle functioning necessary to produce sufficient intrathoracic pressure and expiratory gas velocity. The respiratory muscles play an important role during cough, being the propellers that generate the adequate pressures to get effective cough.

Putting together all the pieces of information available in literature, we learned that inspiratory muscles extend their electrical activity until the early stages of the expulsive cough phase while a certain degree of abdominal muscles activation is present in the late inspiratory phase. This suggests coactivation of antagonist muscles to control inspiratory volume of cough, flow and pressure generation as well as counteract the chest wall distortion secondary to the violent muscular activity during cough. The problem is that the action of the diaphragm and of the intercostal muscles have been measured with invasive procedures on animals, the former, and poorly studied, the latter.

The pressure variations, resulting from muscles contractions during cough, induce important cardiovascular effects. Since the heart is within the thoracic cavity, it is subjected to the same rapid rise in pressure as the lungs, and it is postulated that this compressive force propels blood forward because of the presence of valves in the heart. In fact, although all cardiac chambers and great vessels are subjected to this rapid increase and release of pressure during cough, the presence of a competent aortic valve and peripheral vascular tone maintain a higher pressure in the aorta between coughs. Criley et

al. found that repeated rhythmic (every 1-3 sec.) coughs were able to maintain consciousness up to 39 seconds in 3 patients developing ventricular fibrillation during coronary arteriography. The arterial pressure wave resulting from a cough exceeded that induced by external chest compression in 2 individuals in whom both techniques were employed and in 5 others treated by external compression alone.

Several factors, however, conspire to diminish the effectiveness of cough. The neuromuscular weakness, like the Duchenne muscular dystrophy (DMD), affects both the inspiratory and expiratory phases of the cough. Inspiratory muscle weakness prevents the patient from taking a deep breath, thus limiting the volume of air available to be expired during the exhalation phase of the cough. The diaphragm is the major muscle of inspiration, and its weakness greatly affects the patient's ability to achieve the adequate lung volume needed for an effective cough. Because the chest wall was not fully inflated at the beginning of the expiratory phase, the already weakened expiratory muscles were not stretched to their point of optimal mechanical advantage to maximize their expiratory contraction and therefore provide the cough flow required for expectoration of airway secretions.

In order to understand the mechanism of voluntary cough in health and Duchenne muscular dystrophy, we aim to investigate and study the following issues:

- the active role of the respiratory muscles during voluntary cough;
- the effect of muscle contraction on chest wall kinematics;
- the identification of specific thoraco-abdominal pattern induced by cough;
- the effect of posture on cough;
- the effect of the operating volume on muscle action and on the cardiovascular activity caused by coughing;
- the quantification of blood shifts during the three cough phases;
- the effect muscular dystrophy progression on cough;
- the alteration in lung and chest wall volumes during cough induced by a weakened respiratory pump;
- the identification of altered thoraco-abdominal pattern in Duchenne muscular dystrophy patients with inefficient cough;
- the identification of non-volitional and noninvasive index discriminating efficient and inefficient cough in Duchenne muscular dystrophy patients.

To pursue these goals, we performed a multifactorial analysis of cough including measurements of the electrical activity of the respiratory muscles assessed by electromyography (EMG), flow measured at the mouth by a pneumotacograph, chest wall volumes derived by opto-electronic plethysmography (OEP), blood shift using the double body plethysmography and pressures measured with piezoresistive transducers.

More detailed, we recorded the electrical activity of the muscles using non-invasive surface transcutaneous electromyography at a sampling rate of 1 KHz (BTS FREEEMG; BTS, Milan, Italy) using 8 wireless miniaturized probes with active electrodes. The probes amplify the EMG signals, digitize them with 16 bit resolution and communicate with the compact and light receiving unit left on the table and not attached to the subject. The EMG signals were then synchronized with OEP volumes, rectified, low-pass filtered (4th order digital Butterworth filter with a cut-off frequency of 166 Hz) and the Root Mean Square (RMS) value was computed for the inspiratory and expiratory cough phases.

We have simultaneously recorded EMG with OEP, a technique which computes three-dimensional tracking of the displacements of reflective markers attached anteriorly and posteriorly to the skin of the subject between the clavicles and the pubis. The volume of the trunk was measured from the marker positions using Gauss' theorem. OEP is based on a motion analyzer that uses charge-coupled device sensors mounted on TV cameras equipped with infrared lighting. The system provides volume variations of the total chest wall and its two compartments: rib cage, under the action of rib cage muscles and the insertional action of both the diaphragm and the abdominal muscles, and the abdomen, under the action of the diaphragm and the expiratory abdominal muscles.

The combination of OEP with variable-flow whole body plethysmograph allows to measure blood shifts between the trunk and the extremities. This technique, known as double body plethysmography (DBP), was developed in our laboratory and the volume of blood shift is obtained as the difference between the body volume and the chest wall volume obtained by OEP.

The body volume was obtained integrating the flow in and out the whole body plethysmograph measured by a pneumotachometer mounted in the top of the box. This flow was anti-filtered in order to correct for the dynamics of the box itself. These methods have been used in three independent experimental studies whose descriptions, results and

conclusions are reported in three different chapters of this thesis work. Each chapter can be summarized as follow:

Chapter 2

Cough is a defensive airway reflex consisting of a modified respiratory act and therefore involves the sequential activation of several laryngeal and respiratory muscles. The contraction of the latter results in thoraco-abdominal volume variations in order to provide enough amount of air available, the operating volume, to be expired during the expulsive cough phase. Because the supine position introduces mechanical changes in the chest wall, we hypothesized that posture and operating volume could influence muscular activation and thoraco-abdominal displacements during the inspiratory and expiratory phases of voluntary cough in 10 healthy subjects (age: 24.8 ± 4.5 yrs). We measured the EMG activity of seven muscles (scalene, sternocleidomastoid, parasternal, intercostal, diaphragm, external abdominal oblique and rectus abdominis) in supine and seated position at four different operating volumes: total lung capacity (TLC), functional residual capacity (FRC), one tidal and two tidal volumes over it (namely, FRC+ and FRC++). The activity of the muscles, assessed by root mean square value, resulted maximal at TLC and poorly affected by postures while thoraco-abdominal compartments, measured by opto-electronic plethysmography, contributed constantly to volume changes regardless the operating volume and posture.

We believe that the voluntary cough motor mechanism activates a synergetic antagonistic contraction of respiratory muscles leading to a specific thoraco-abdominal pattern, in which the rib cage is the prominent compartment, not affected by either operating volume or posture despite the introduced mechanical changes on the chest wall.

Chapter 3

Double Body Plethysmography (DBP), which combines total body plethysmography and opto-electronic plethysmography, has been recently developed to measure the amount of blood displaced from the thorax to the extremities. By using DBP, we have recently shown that significant blood shifts (V_{BS}) occurs during expulsive maneuvers and that abdominal pressure controls the outflow of blood from the splanchnic vasculature.

We hypothesized that also during cough a significant amount of blood can be displaced from the trunk to the extremities. We studied 7 healthy subjects (age: 28.6 ± 2.5 yrs) during series of voluntary coughs at three different operating volumes: total lung capacity (TLC), one tidal volume and two tidal volumes over functional residual capacity (namely, V_T and $2V_T$). V_{BS} from the thorax to the extremities was measured by DBP during quiet breathing and during cough at each operating lung volume. V_{BS} during cough resulted significantly higher than during quiet breathing ($p < 0.05$). During the expulsive phase V_{BS} increased with increasing operating volume, being almost 700 ml at TLC, whereas it was constantly around 200 ml during the compressive phase. Inspiratory phase was affected by the hemodynamic effect of diaphragmatic contraction and therefore V_{BS} was highly variable. We believe that maximal voluntary cough activates the abdominal circulatory pump. These findings might help to better understand the cardiopulmonary interactions during cough and the mechanism by which coughing during asystolic cardiac arrest can maintain consciousness in human subjects.

Chapter 4

With the progression of Duchenne muscular dystrophy (DMD) cough becomes inefficient leading to recurrent chest infections. Several factors determine the effectiveness of cough in DMD patients. The aim of this study was to investigate how weakened inspiratory muscles alter operating lung and thoraco-abdominal volumes and whether they contribute to cough efficiency.

Pulmonary function, respiratory muscle strength and peak cough flow (PCF) were assessed in 36 DMD patients (age 17.0 ± 5.0). Total and compartmental chest wall volumes were measured by Opto-Electronic Plethysmography in the DMD patients and 15 age-matched healthy controls during quiet breathing and maximal voluntary cough maneuvers in supine position. The DMD population was divided into three groups: $PCF < 160$ L/min (inefficient cough), $PCF > 270$ L/min (efficient cough) and $160 < PCF < 270$ L/min (intermediate cough efficiency).

During the inspiration preceding cough, patients with efficient cough presented normal volume variations whereas patients with intermediate cough efficiency showed low abdominal volume variation ($p < 0.01$). Patients with inefficient cough were characterized by reduced total ($p < 0.05$) and compartmental (ribcage: $p < 0.01$; abdomen: $p < 0.001$) chest

wall volumes during the inspiration preceding cough and reduced abdominal contribution to tidal volume during quiet breathing ($V_{ab}(\%V_t)$, $p < 0.001$). ROC analysis revealed that among all spirometric, respiratory muscle strength and chest wall parameters $V_{ab}(\%V_t)$ was the best discriminator between inefficient and efficient cough.

Inefficient cough in DMD is associated to reduced operating lung and chest wall volume secondary to weakened inspiratory muscles. Abdominal contribution to tidal volume during spontaneous breathing represents a non-volitional and noninvasive index able to discriminate efficient and inefficient cough.

In conclusion, cough is a complex mechanism which involves the sequential activation of several laryngeal and respiratory muscles in the generation of intra-thoracic pressure gradient sufficient to generate not only adequate flow rate at the mouth but also important cardiovascular effects. The synergetic antagonistic activity of respiratory muscles leads to a specific pattern, in which the rib cage is the leading compartment, which is not affected by either operating volume or posture despite the mechanical changes introduced. This suggests that the abdominal muscles mainly act to lower the inferior ribs due to their insertions on the rib cage and that a task-specific somatosensory is active during voluntary healthy cough to recruit rib cage to lead cough despite different mechanical intervention on the chest wall. Moreover, the fluctuations in intrathoracic pressure during maximal voluntary cough activate the abdominal circulatory pump that is able to shift 700 ml of splanchnic blood between thorax and to other body tissues and this could be an important haemodynamic factor which can provide an explanation for cardio-pulmonary resuscitation based on cough. Finally, although cough is an expiratory manoeuvre, a crucial role is played by inspiratory muscles not only because they help in stabilizing the chest wall during cough but also because they provide the operating volume which stretches the expiratory muscles to their point of optimal mechanical advantage to maximize their contraction. Inefficient cough in DMD is associated to reduced operating lung and chest wall volume secondary to weakened respiratory pump and the abdominal contribution to tidal volume during spontaneous breathing represents a non-volitional and noninvasive index able to discriminate efficient and inefficient cough.

Sommario

La tosse è un meccanismo riflesso vagale di difesa delle vie aeree la cui funzione è quella di rimuovere muco o qualsiasi altro corpo estraneo inalato al fine di garantire e mantenere pulite le vie aeree ed i polmoni. Il colpo di tosse può essere sia volontario che indotto dalla stimolazione dei recettori tossivi localizzati principalmente nelle vie aeree centrali.

La manovra di tosse consta di 3 fasi: inspiratoria, compressiva ed espulsiva. Nella prima fase, quella inspiratoria, il paziente inspira normalmente tra il 60 ed il 90% della capacità polmonare totale. Nella fase compressiva, la glottide si chiude ed i muscoli espiratori iniziano a contrarsi. Nonostante questa fase sia molto breve, 0.2 secondi, la contrazione muscolare è tale da raggiungere pressioni intratoraciche elevate. Nella fase espulsiva finale della tosse, la glottide si apre con conseguente emissione di elevato flusso di aria.

L'efficacia della clearance del colpo di tosse, quindi, dipende da un'azione neuro mediata e coordinata delle tre fasi appena descritte e dalla funzionalità dei muscoli inspiratori, espiratori e della glottide per generare adeguata pressione intratoracica e velocità espiratoria dell'aria. Il ruolo dei muscoli respiratori è fondamentale nel colpo di tosse, essendo i propulsori che producono le pressioni necessarie per ottenere tosse efficace.

Dalla letteratura, abbiamo imparato che i muscoli inspiratori estendono la loro attività elettrica sino all'inizio della fase espulsiva così come è già presente un certo grado di attivazione dei muscoli espiratori alla fine della fase inspiratoria. Questo suggerisce coattivazione di muscoli antagonisti al fine non solo di controllare la generazione di volume, flusso e pressioni ma anche per bilanciare e stabilizzare la distorsione della parete toraco-addominale conseguente la violenta attivazione muscolare durante il colpo di tosse. Il problema è che l'azione dei muscoli intercostali e del diaframma sono stati studiati rispettivamente poco o solo su animali mediante modi invasivi.

Le variazioni di pressione generate dalla contrazione dei muscoli durante la tosse hanno importanti effetti cardiovascolari. Essendo il cuore nella cavità toracica, è soggetto anch'esso, come i polmoni, allo stesso incremento rapido di pressione e si ipotizza che tale forza compressiva spinge sangue verso il cuore grazie alla presenza delle valvole cardiache. Infatti, nonostante le camere cardiache ed i grandi vasi siano soggetti al rapido aumento e rilascio della pressione durante il colpo di tosse, la presenza di una valvola aortica efficiente ed il tono muscolare periferico mantengono alta la pressione in aorta tra 2 colpi di tosse. Criley e collaboratori hanno trovato che colpi di tosse ripetuti e ritmici (ogni 1-3 secondi) sono in grado di mantenere coscienti per 39 secondi tre pazienti in fibrillazione ventricolare durante coronografia. L'onda di pressione arteriosa risultante dai colpi di tosse è risultata essere superiore a quella indotta da massaggio cardiaco mediante compressioni

esterne della gabbia toracica in due pazienti in cui nei quali sono state misurate entrambe le tecniche ed i 5 altri che però sono stati trattati solo con compressioni esterne.

Molti fattori contribuiscono a diminuire l'efficacia della tosse. Le patologie neuromuscolari, come la distrofia muscolare di Duchenne (DMD), colpiscono sia la fase inspiratoria che quella espiratoria della tosse. La debolezza dei muscoli inspiratori impediscono al paziente di prendere un respiro profondo, riducendo così il volume di aria disponibile per essere espirato durante la fase espulsiva della tosse. Il principale muscolo inspiratorio è il diaframma, quindi la sua debolezza impedisce al paziente di raggiungere l'adeguato volume polmonare necessario per ottenere tosse efficace. Di conseguenza, poiché la parete toraco-addominale non è sufficientemente insufflata all'inizio della fase espiratoria, i muscoli espiratori, già indeboliti dalla malattia, non vengono allungati fino al loro punto ottimale sulla loro curva tensione-lunghezza per massimizzare la loro contrazione quindi garantire il flusso necessario ad espettorare le secrezioni delle vie aeree.

Al fine di capire i meccanismi alla base della tosse volontaria nel sano e nella distrofia muscolare di Duchenne, ci prefiggiamo di studiare i seguenti argomenti:

- il ruolo attivo dei muscoli respiratori durante tosse volontaria;
- l'effetto della contrazione muscolare sulla cinematica della parete toraco-addominale;
- l'identificazione di pattern toraco-addominali specifici del colpo di tosse;
- l'effetto della postura sulla tosse;
- l'effetto del volume di lavoro sull'azione muscolare e sull'attività cardiovascolare della tosse;
- la quantificazione del sangue spostato durante le tre fasi del colpo di tosse;
- l'alterazione nei volumi del polmone e della parete toraco-addominale durante la tosse conseguente all'indebolimento della pompa respiratoria;
- l'identificazione di pattern toraco-addominali alterati in pazienti affetti da distrofia muscolare di Duchenne con tosse inefficace;
- l'identificazione di nuovi indici non volitivi e non invasivi in grado di discriminare la tosse efficace da quella inefficiente in pazienti Duchenne.

Per raggiungere questo obiettivo, abbiamo condotto un'analisi multifattoriale della tosse che includesse le misure del segnale elettrico dei muscoli respiratori tramite l'elettromiografia (EMG), del flusso alla bocca tramite pneumotacografo, dei volumi della parete toraco-addominale tramite pletismografia optoelettronica (OEP), dello spostamento di sangue tramite doppia pletismografia corporea e delle pressioni tramite trasduttori piezoresistivi.

Più in dettaglio, abbiamo misurato l'attività elettrica dei muscoli in maniera non invasiva tramite EMG di superficie acquisendo ad 1 KHz (BTS FREEEMG; BTS, Milano, Italia) usando 8 sonde

miniaturizzate wireless con elettrodi attivi. Le sonde amplificano il segnale elettromiografico, lo digitalizzano con una risoluzione a 16 bit e comunicano con l'unità ricevente lasciata sul tavolo e quindi non attaccata al soggetto. Il segnale EMG è stato poi sincronizzato con i volumi optoelettronici, rettificato, filtrato passa basso (filtro digitale di Butterwoth del 4° ordine con una frequenza di taglio di 166 Hz) ed è stato calcolato il valore di valore effettivo (RMS) per le fasi inspiratoria ed espiratoria della tosse.

L'EMG è stato misurato simultaneamente con l'OEP, una tecnica che fornisce le coordinate tridimensionali di marcatori passivi posti sulla parete toraco-addominale del soggetto, sia frontalmente che posteriormente, su opportuni punti di repere anatomici tra le clavicole e le creste iliache. Il volume del tronco è stato ottenuto applicando il teorema di Gauss alle coordinate dei marcatori. L'OEP è basato su un sistema di analisi del movimento che usa sensori con dispositivi a carica accoppiata montati su telecamere a luce infrarossa. Il sistema fornisce le variazioni di volume totali della parete toraco-addominale e dei suoi due compartimenti: la gabbia toracica, sotto l'azione dei muscoli toracici e della parte inserzionale del diaframma e dei muscoli addominali, e l'addome, sotto l'azione del diaframma e dei muscoli addominali.

La combinazione dell' OEP con un pletismografo corporeo a flusso variabile permette la misura del sangue spostato tra il tronco e le estremità. Questa tecnica, nota come doppia pletismografia corporea (DBP), è stata sviluppata nel nostro laboratorio ed il volume di sangue spostato è ottenuto per differenza tra il volume corporeo e quello della parete toraco-addominale ottenuto tramite OEP. Il volume corporeo si ottiene integrando il flusso entrante ed uscente il pletismografo corporeo misurato da un pneumotacografo montato in cima al pletismografo. Questo flusso viene prima anti-filtrato per correggere la dinamica dello stesso pletismografo corporeo.

Questi metodi sono stati usati in tre studi sperimentali indipendenti. La descrizione, i risultati e le conclusioni di ogni studio sono riportati in questo lavoro di tesi come diversi capitoli che possono essere così riassunti:

Capitolo 2

La tosse è un riflesso di difesa delle vie aeree assimilabile ad un atto respiratorio modificato e quindi presuppone l'attivazione sequenziale e coordinata di molti muscoli laringali e respiratori. La contrazione di questi ultimi provoca variazioni volumetriche della parete toraco-addominale al fine di fornire la giusta quantità d'aria, il volume di lavoro, da essere espirata durante la fase espulsiva. Poiché la posizione supina introduce modifiche meccaniche alla parete toraco-addominale, si ipotizza che la postura ed il volume di lavoro possano influenzare l'attivazione muscolare e gli spostamenti toraco-addominali durante le fasi inspiratorie ed espiratorie di colpi di tosse volontari

su 10 soggetti sani (età: 24.8 ± 4.5 anni). Abbiamo misurato l'attività EMG di sette muscoli (scaleni, sternocleidomastoidei, parasternali, intercostali, diaframma, obliqui esterni ed il retto addominale) in posizione supina e seduta a quattro diversi volumi di lavoro: capacità polmonare totale (TLC), capacità funzionale residua (FRC), un singolo ed un doppio volume corrente sopra esso (FRC+ e FRC++, rispettivamente). L'attività muscolare, valutata tramite il valore efficace, risulta massimo a TLC e poco influenzato dalla postura, mentre i compartimenti toraco-addominali misurati tramite pletismografia optoelettronica, contribuiscono alle variazioni di volume con rapporto costante indipendentemente dal volume di lavoro e dalla postura.

Crediamo che il meccanismo motorio della tosse volontaria attivi una contrazione sinergica ed antagonista dei muscoli respiratori che porta ad uno specifico pattern toraco-addominale in cui la gabbia toracica è il compartimento predominante e che tale pattern non è influenzato né dal volume di lavoro né dalla postura nonostante le modifiche meccaniche introdotte sulla parete toraco-addominale.

Capitolo 3

La doppia pletismografia corporea (DBP), che combina la pletismografia corporea con quella optoelettronica, è stata sviluppata recentemente per misurare il quantitativo di sangue spostato tra il torace e le estremità. Usando la DBP, abbiamo dimostrato che vi è un significativo scambio di sangue (V_{BS}) durante manovre espulsive e che è la pressione addominale che controlla il deflusso di sangue dal circolo splancnico.

Si ipotizza che anche durante un colpo di tosse vi sia un significativo quantitativo di sangue che viene spostato dal tronco alle estremità. Sette soggetti sani (età: 28.6 ± 2.5 anni) sono stati studiati durante una serie di singoli colpi di tosse a tre diversi volumi di lavoro: capacità polmonare totale (TLC), un singolo ed un doppio volume corrente sopra capacità funzionale residua (V_T e $2V_T$, rispettivamente). V_{BS} durante tosse è risultato significativamente maggiore di quello misurato a respiro spontaneo ($p < 0.05$). Durante la fase espulsiva V_{BS} aumenta all'aumentare del volume di lavoro, essendo circa 700 ml a TLC, mentre risulta costante intorno 200 ml durante la fase compressiva. La fase inspiratoria risente dell'effetto emodinamico della contrazione diaframmatica e questo rende il V_{BS} altamente variabile.

Crediamo che colpi di tosse volontaria massimali attivino la pompa circolatoria addominale. Questi risultati possano aiutare nel comprendere meglio le interazioni cardiopolmonari indotte dalla tosse ed il meccanismo per cui tossire durante un arresto cardiaco sia in grado di mantenere il paziente in condizione di coscienza.

Capitolo 4

Con il progredire della distrofia muscolare di Duchenne (DMD) la tosse diventa inefficace portando a frequenti infezioni toraciche. Molti fattori contribuiscono all'efficacia della tosse nei pazienti DMD. Lo scopo di questo studio è di investigare come i muscoli inspiratori indeboliti dalla malattia alterino i volumi di lavoro polmonari e toraco-addominali e se contribuissero all'efficacia della tosse.

Abbiamo misurato la funzionalità polmonare, la forza dei muscoli respiratori ed il picco di flusso durante la tosse (PCF) in 36 pazienti Duchenne (età: 17.0 ± 5.0 anni). I volumi totali e compartimentali della parete toraco-addominali sono stati misurati tramite pletismografia optoelettronica su pazienti DMD e su 15 coetanei maschi sani durante respiro spontaneo e colpi di tosse volontaria massimali. I pazienti DMD sono stati suddivisi in tre gruppi: $PCF < 160$ L/min (tosse inefficace), $PCF > 270$ L/min (tosse efficace) e $160 < PCF < 270$ L/min (tosse di efficacia intermedia). Durante l'inspirazione che precede il colpo di tosse, le variazioni volumetriche nei pazienti con tosse efficace sono normali, mentre pazienti con tosse di efficacia intermedia presentano ridotte variazioni addominali ($p < 0.01$). I pazienti con tosse inefficiente sono caratterizzati da sia ridotto volume totale ($p < 0.05$) e compartimentale (gabbia toracica: $p < 0.01$; addome: $p < 0.001$) durante la fase inspiratoria che da un ridotto contributo addominale durante respiro spontaneo, espresso come percentuale del volume corrente ($V_{ab}(\% V_t)$, $p < 0.001$). L'analisi ROC ha rivelato che tra tutti i parametri spirometrici, di forza muscolare respiratoria e optoelettronici, $V_{ab}(\% V_t)$ risulta essere il miglior predittore che discrimini tra tosse efficace ed inefficace.

La tosse inefficace nella DMD è associata a volumi di lavoro polmonari e toraco-addominali conseguenti all'indebolimento dei muscoli inspiratori. Il contributo addominale al volume corrente durante respiro spontaneo in posizione supina rappresenta un indice non volitivo e non invasivo in grado di discriminare tra tosse efficace ed inefficace.

In conclusione, la tosse è un meccanismo complesso che implica l'attivazione sequenziale di muscoli laringei e respiratori per produrre un gradiente pressorio intratoracico sufficiente a generare non solo flussi adeguati, ma anche importanti effetti cardiovascolari. L'attivazione sinergica ed antagonista dei muscoli respiratori porta ad un pattern specifico, in cui la gabbia toracica è il compartimento prevalente, e tale pattern non è condizionato né dal volume di lavoro né dalla postura nonostante introducano delle alterazioni meccaniche. Questo suggerisce che i muscoli addominali, a causa della loro inserzione sulla gabbia toracica, lavorano soprattutto per abbassare le

coste inferiori e che un comando somatosensoriale dedicato si attivi durante tosse volontaria nel soggetto sano che recluti la gabbia toracica a condurre il colpo di tosse nonostante possibili interventi meccanici sulla parete toraco-addominale. Inoltre, le fluttuazioni di pressione intratoracica durante colpi di tosse volontaria massimali attivano la pompa circolatoria addominale che è in grado di spostare fino a 700 ml di sangue splancnico tra torace e gli altri tessuti corporei e questo può essere un importante fattore emodinamico che possa ulteriormente spiegare il meccanismo di rianimazione cardio-polmonare basato sulla tosse. Infine, nonostante la tosse sia una manovra espiratoria, un ruolo cruciale spetta ai muscoli inspiratori non solo perché aiutano a stabilizzare la parete toraco-addominale durante il colpo di tosse, ma anche perché forniscono il volume di lavoro che allunga i muscoli espiratori fino al loro punto meccanico ottimale che ne massimizza la contrazione. La tosse inefficace nella DMD è associata a ridotti volumi di lavoro polmonari e toraco-addominali come conseguenza dell'indebolimento della pompa muscolare respiratoria e il contributo percentuale addominale al volume corrente durante respiro spontaneo in posizione supina rappresenta un indice non volitivo e non invasivo in grado di discriminare tra tosse efficace ed inefficace.

*“ Una domanda è come un coltello che squarcia
la tela di un fondale dipinto per permetterci di
dare un’occhiata a ciò che si nasconde dietro ”*

Milan Kundera

Chapter 1 - Introduction

Cough is a vagally mediated defensive airway reflex whose function is the removal of mucus or any other inhaled foreign materials therefore providing and maintaining airway clearance and lung hygiene. Cough can be initiated either voluntarily or by the stimulation of cough receptors located primarily in the central airways^[1].

The sequence of phases in a cough maneuver can be classified as inspiratory, compressive and expulsive, with the latter two constituting the expiratory phase (figure 1.1).

At the beginning of the inspiratory cough phase active abduction of the glottis supports a rapid inspiratory airflow and the subject inhales, usually, 60% to 90% of total lung capacity. The inspiratory phase is marked by contraction of the diaphragm and accessory inspiratory muscles to reach an adequate operating volume, *i.e.* the volume of air at the end of the inspiratory phase. Inhaling a larger volume of air produces a greater degree of lengthening in the expiratory muscles, therefore increasing the force-generation by the muscles involved in cough by improving the muscle length-tension relationship. Increasing expiratory muscle length with a greater inspired air volume generates a greater positive intrathoracic pressure and a greater expiratory flow and volume.

In the compressive phase there is an almost simultaneous adduction of the glottis with the onset of expiratory muscle contraction. The duration of this phase is approximately 0.2 seconds. Glottic closure is reinforced by the supraglottic ventricular folds and may also involve the epiglottis. The expiratory muscles of the rib cage and abdomen contract in a coordinated effort, compressing the alveolar gas volume. Glottic closure limits the degree of expiratory muscle shortening during contraction and increases the amount of positive intrathoracic pressure that can be generated during the compression phase. Intrathoracic pressure during the compression phase may be as great as 300 cm H₂O^[2].

At the initiation of the expiration phase the glottis opens abruptly, within 20 – 40 ms. With the release of compression the central airway pressure drops to or below atmospheric pressure. Pleural pressure and alveolar pressure continue to rise with expiratory-muscle contraction. The peak cough flow (PCF) ranges from 360 to 1000 L/minute to obtain a normal cough. PCF is the result of the combined effect of pressurized gas released from the alveolar space and airway gas displaced by dynamic airway compression the expiratory airflow in the peripheral airways depends on the volume of air initially inspired^[3]. The initial peak expiratory phase is followed by a longer expiratory phase, which lasts 0.2– 0.5

seconds. Expiratory flow and dynamic compression, therefore, are the primary factors that produce an effective cough.

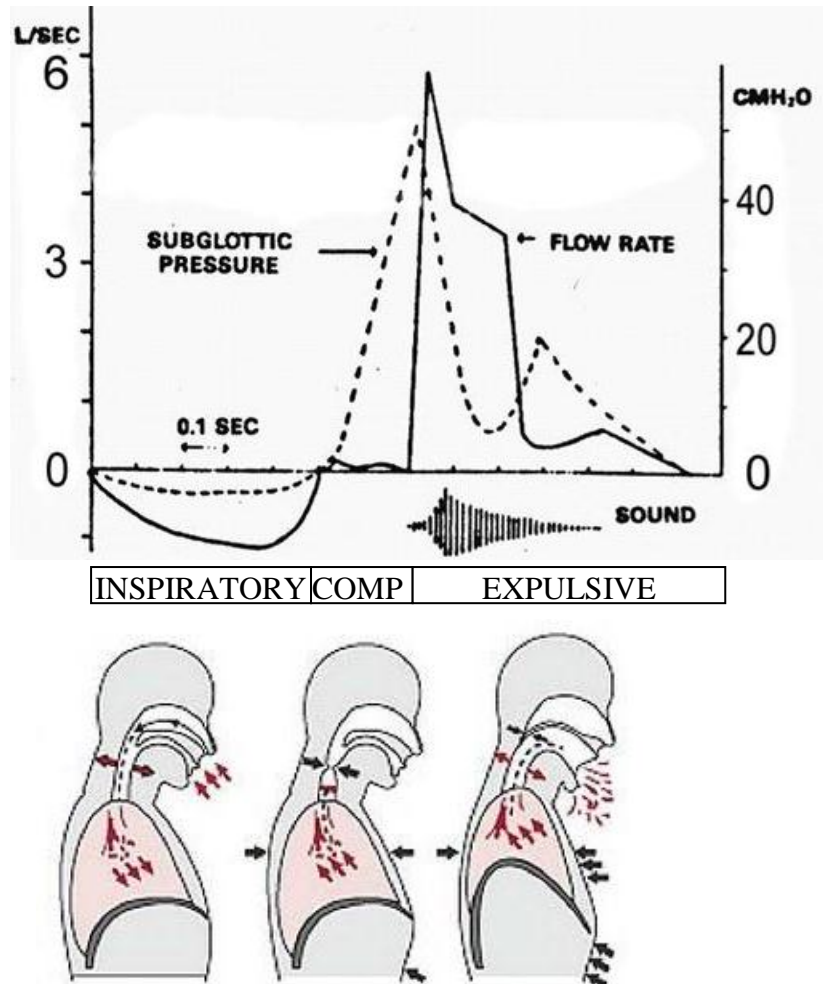


Figure 1.1: Schematic diagram depicting changes in flow and subglottic pressure during the inspiratory, compressive (COMP), and expulsive phases of cough^[4].

Because during a cough maneuver an important role is played by the volume variations, Smith et al^[5]. proved that opto-electronic plethysmography (OEP) provides important information during cough. OEP is a technique which computes three-dimensional tracking of the displacements of reflective markers attached anteriorly and posteriorly to the skin of the subject between the clavicles and the pubis. The volume of the trunk is measured from the marker positions using Gauss' theorem. OEP is based on a motion analyzer that uses charge-coupled device sensors mounted on TV cameras equipped with infrared lighting (figure 1.2). The system provides volume variations of the total chest wall and its two compartments: rib cage, under the action of rib cage muscles and the insertional action of

both the diaphragm and the abdominal muscles, and the abdomen, under the action of the diaphragm and the expiratory abdominal muscles^[6].

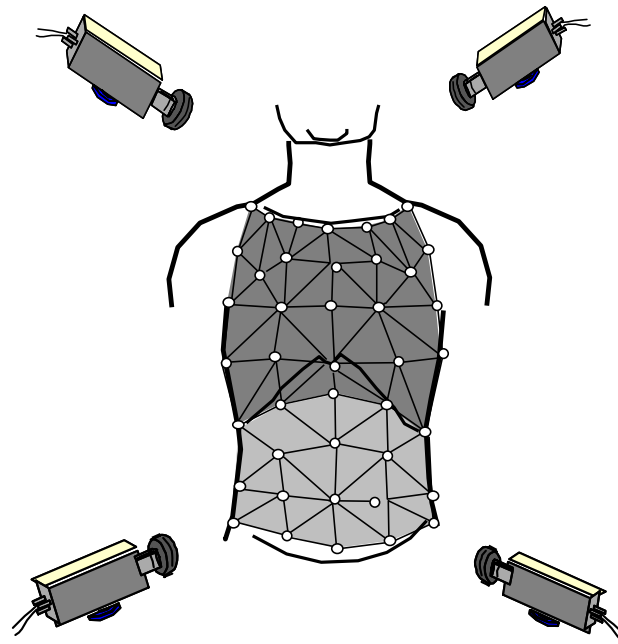


Figure 1.2: schematic representation of opto-electronic plethysmography including IR charge-coupled device (CCD) cameras and chest wall subdivision in ribcage (dark grey) and abdomen (light grey)

The effectiveness of cough clearance depends on the coordinated neural sequence of phases involved in the cough maneuver and the inspiratory, expiratory and glottic muscle functioning necessary to produce sufficient intrathoracic pressure and expiratory gas velocity. Therefore the respiratory muscles play an important role during cough, being the propellers that generate the adequate pressures to get effective cough. The electromyography of respiratory muscles has been studied on animals and humans mainly during induced cough by several nebulized agents. The diaphragm, the most important respiratory muscles, have been studied only with invasive procedures on animals, the intercostals muscles have been poorly studied whereas more information are available on the electrical activity of abdominal muscles in humans. Putting together all these pieces of information, we learned that inspiratory muscles extend their electrical activity until the early stages of the expulsive cough phase while a certain degree of abdominal muscles activation is present in the late inspiratory phase. This suggests coactivation of antagonist muscles to control inspiratory volume of cough, flow and pressure generation as well as counteract the chest wall distortion secondary to the violent muscular activity during cough^[7].

The pressure variations, resulting from muscles contractions during cough, induce important cardiovascular effects. In fact, during the inspiratory phase, pressure in the abdominal cavity rises owing to the contraction of the diaphragm, while it falls in the thoracic cavity. As a result, blood is abruptly drawn in from the vena cava, so that the right atrium and right ventricle fill to capacity. Consequently, the cardiac output increases. During the compressive phase, intrathoracic pressure rises and blood is squeezed out from the intra-thoracic organs and forced to the periphery, thus increasing systemic blood pressure. The drop in intrathoracic pressure that occurs at the end of the expulsive phase of cough has the reverse effects: cardiac output and systemic blood pressure markedly decrease, while systemic venous pressure rises^[7].

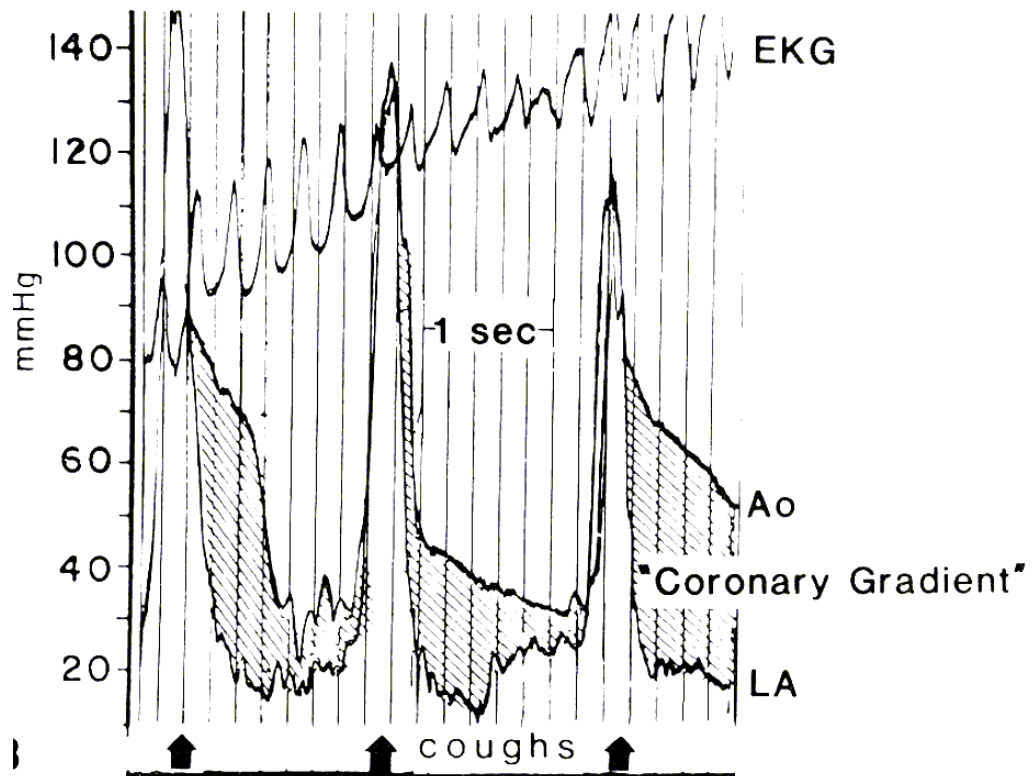


Figure 1.3: Electrocardiogram (EKG), aortic (Ao) and left atrial (LA) pressures in a 50 years old male with diffuse coronary artery disease. Following ventricular injection the patient was asked to cough 3 times. Ventricular fibrillation occurred between the second and third cough. The aortic and left atrial pressure rose to 130-150 mmHg with each cough but the aortic pressure remained higher (shaded area) during relaxation between coughs due to the competent aortic valve and the peripheral vascular resistance^[8].

The changes in cardiovascular activity caused by coughing have important effects: Criley et al^[8-10] have shown that patients developing ventricular fibrillation during cardiac catheterization may carry out their own positive intrathoracic pressure resuscitation (figure

1.3) and maintain consciousness by coughing in a forceful, rhythmic fashion, and these authors proposed voluntary coughing as a form of cardiopulmonary resuscitation.

Up to now cough maneuver has been considered in normal condition, with an efficient respiratory pump, but several factors conspire to diminish the effectiveness of cough. The neuromuscular weakness, for example, affects both the inspiratory and expiratory phases of the cough. Inspiratory muscle weakness prevents the patient from taking a deep breath, thus limiting the volume of air available to be expired during the exhalation phase of the cough. The diaphragm is the major muscle of inspiration, and its weakness greatly affects the patient's ability to achieve the adequate lung volume needed for an effective cough. The exhalation phase is compromised with the patient being unable to generate enough force to properly expel secretions present in the airway. Because the chest wall is not fully inflated at the beginning of the expiratory phase, the muscles of expiration are not stretched to their point of optimal mechanical advantage to maximize their expiratory contraction. The lungs themselves are not fully inflated as to allow for maximal passive elastic recoil during expiration. These factors further limit the effectiveness of the expiratory muscles. Patients with Duchenne muscular dystrophy (DMD), a recessive X-linked form of muscular dystrophy, may have sufficient ventilation and yet be at risk for pulmonary congestion and acute respiratory failure associated with weak cough^[3].

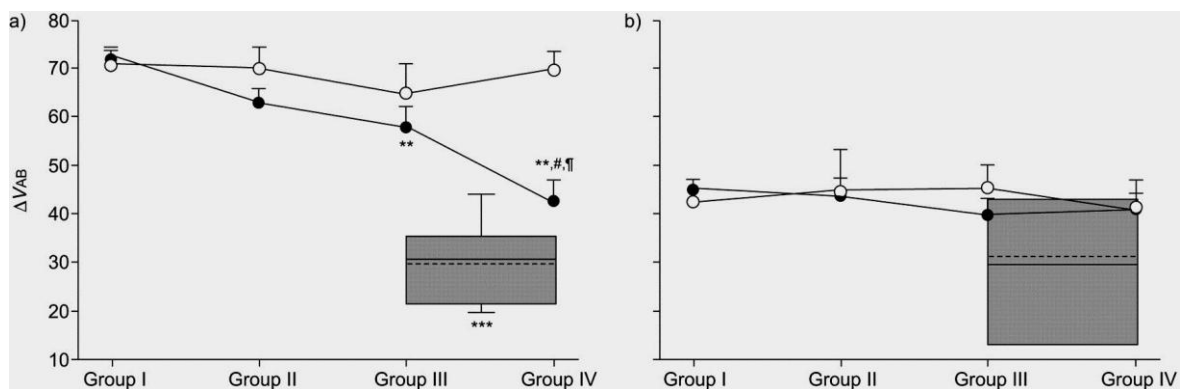


Figure 1.4 : Mean±standard error (○ and ●) of abdominal volume (ΔV_{AB}), expressed as percentage of total tidal volume of the different age groups (Group I: ≤ 7 yrs, Group II: ≥ 8 and ≤ 12 yrs, Group III: ≥ 13 and ≤ 16 yrs, Group IV: ≥ 17 yrs) in a) supine and b) seated positions. ●: Duchenne muscular dystrophy (DMD) patients; ○: healthy subjects. Box plots indicate the distribution of ΔV_{AB} in DMD patients belonging to groups III and IV, spending $\geq 10\%$ of the night with arterial oxygen saturation measured by pulse oximetry $< 95\%$ (DeSat group). Boxes show the 25th and 75th percentiles, the median (—) and the mean (---); whiskers present the 10th and 90th percentiles. **: $p < 0.01$ versus group I; #: $p = 0.001$ versus group II; $p < 0.01$ versus group III; ***: $p < 0.001$ versus groups III and IV^[11].

In these patients, it has been shown that in supine position during quiet breathing the lowering of the abdominal contribution to tidal volume (figure 1.4), suggesting the impairment of diaphragm^[11], is an important marker of the progression of the disease and an early indicator of nocturnal hypoxemia^[12]. This is markedly in adults DMD patients in which ribcage compartment contribution is significantly higher than abdominal therefore they cope with the progressive impairment of the diaphragm by increasing the recruitment of the inspiratory rib cage muscles which, presumably, are also impaired as well. This suggests that the adopted strategy might not sustain maximal and explosive maneuvers like voluntary cough.

BIBLIOGRAPHY

1. Hegland KW, Bolser DC, Davenport PW. Volitional control of reflex cough. *J Appl Physiol*. 2012 Jul;113(1):39-46
2. Boitano LJ. Management of airway clearance in neuromuscular disease. *Respir Care*. 2006 Aug;51(8):913-22
3. Kravitz RM. Airway clearance in Duchenne muscular dystrophy. *Pediatrics*. 2009;123 Suppl 4:S231-5.
4. The Thorax Vol. 85 by C. Roussos (1995, Dekker Incorporated, Marcel)
5. Smith JA, Aliverti A, Quaranta M, McGuinness K, Kelsall A, Earis J, Calverley PM (2012) Chest wall dynamics during voluntary and induced cough in healthy volunteers. *J Physiol* 590(Pt 3): 563-574.
6. Cala SJ, Kenyon CM, Ferrigno G, Carnevali P, Aliverti A, Pedotti A, Macklem PT, Rochester DF. Chest wall and lung volume estimation by optical reflectance motion analysis. *J Appl Physiol*. 1996 Dec;81(6):2680-9
7. Fontana GA, Lavorini F (2006) Cough motor mechanisms. *Respir Physiol Neurobiol* 152(3): 266-81.
8. Criley JM, Niemann JT, Rosborough JP, Hausknecht M. Modifications of cardiopulmonary resuscitation based on the cough. *Circulation*. 1986 Dec;74(6 Pt 2):IV42-50
9. Criley JM, Blaufuss AH, Kissel GL. Cough-induced cardiac compression. Self-administered form of cardiopulmonary resuscitation. *JAMA*. 1976 Sep 13;236(11):1246-50
10. Criley JM, Blaufuss AH, Kissel GL. Self-administered cardiopulmonary resuscitation by cough-induced cardiac compression. *Trans Am Clin Climatol Assoc*. 1976;87:138-46.
11. Lo Mauro A, D'Angelo MG, Romei M et al. Abdominal volume contribution to tidal volume as an early indicator of respiratory impairment in Duchenne muscular dystrophy. *Eur Respir J*. 2010;35(5):1118-25.
12. Romei M, D'Angelo MG, LoMauro A et al. Low abdominal contribution to breathing as daytime predictor of nocturnal desaturation in adolescents and young adults with Duchenne Muscular Dystrophy. *Respir Med*. 2012;106(2):276-83.

Chapter 2 - Electromyography and chest wall kinematics during cough in healthy subjects

Keywords: cough, EMG, opto-electronic plethysmography, respiratory muscles

2.1 INTRODUCTION

Cough is an important defence mechanism: its main function is to maintain airway clearance by removing mucus and/or foreign bodies from the lower respiratory tract. Cough is vagally mediated and it is the result of the coordinated activity of various respiratory muscles. Inspiratory muscles, the diaphragm and the external intercostal muscles, contract to increase lung volume needed to generate the high-velocity of expiratory flow^[1,2]. The contraction of the expiratory muscles builds up high positive intrapleural and intra-airway pressures to develop the adequate peak expiratory flow rates. The electrical activity of the muscles can be recorded using non-invasive surface transcutaneous electromyography (EMG) to determine muscle capacity in terms of activation and contractile function^[3-5]. The electromyography of respiratory muscles has been studied on animals and humans mainly during induced cough by several nebulized agents. During cough, the diaphragm, the most important respiratory muscles, have been studied only with invasive procedures on animals, the intercostals muscles have been poorly studied^[6] whereas more information are available on the electrical activity of abdominal muscles in humans^[6-9]. Putting together all these pieces of information, we learned that inspiratory muscles extend their electrical activity until the early stages of the expulsive cough phase^[2] while a certain degree of abdominal muscles activation is present in the late inspiratory phase. This suggests coactivation of antagonist muscles to control inspiratory volume of cough, flow and pressure generation as well as counteract the chest wall distortion secondary to the violent muscular activity during cough^[6].

The operating volume, *i.e.* the volume inspired at the end of the inspiratory cough phase, is the most important determinant of peak cough flow as it affects the expiratory muscle length and therefore their efficiency of contraction^[10]. Because direct comparison of the respiratory muscles activation and volume variations during cough have not been made, the purpose of this study was to combine measurements of EMG and chest wall volumes variations in order to get a more comprehensive evaluation of the action of respiratory muscles during cough. We used opto-electronic plethysmography^[11] because it provides not only total but also compartmental chest wall volume variations resulting from muscles contraction.

We hypothesized that muscular activity and therefore the thoraco-abdominal displacement would be influenced by the operating volumes. In addition, we explored whether posture could effect as well, because in the supine position the hydrostatic forces displace the diaphragm cranially, lung volume decreases, expiratory muscles lengthens and abdominal compliance reduces.

2.2 METHODS

Subjects and Ethics Statement

We studied ten healthy subjects (age: 24.8 ± 4.5 yrs), five female, who were students or collaborators in our laboratory and therefore all have experience in respiratory maneuvers. They signed a written informed consent form, as approved by the Local Ethical Committee of IRCCS “E. Medea” Institute. The study was approved by the Institute’s Human Ethics Committee according to the declaration of Helsinki.

Flow and volumes measurements

The flow at the mouth was measured with the subject wearing a nose clip and breathing through a pneumotacograph (Heated Pneumotach 800 L; Hans Rudolph®, Shawnee, USA) attached to a snorkel rubber mouthpiece. By definition, peak cough flow was the maximum values reached during the expiratory cough phase. Synchronized measurements of rib cage, abdominal and chest wall volume were performed using Opto-Electronic Plethysmography (OEP System; BTS, Milan, Italy). The system provides the 3D coordinates of retro-reflective markers placed on the trunk of the subject: 52 on the anterior chest wall surface if the subject lied supine, 47 more markers would be added in the posterior chest wall surface if the subject sat without back support^[11,12]. The combination of these coordinates with specific geometrical models and the Gauss’ theorem provides the computation of rib cage (V_{RC} : the volume enclosed from the clavicles to the costal margin), abdominal (V_{AB} : the volume enclosed from the costal margin to the iliac crest) and chest wall (V_{CW} : the sum of the two compartments) volumes. The point of start cough, end inspiration and end cough have been selected on the chest wall trace to identify the inspiratory and the expiratory cough phase and the thoraco-abdominal contribution was computed for each phase as percentage of V_{CW} variations.

EMG recordings

Respiratory muscles activity was recorded at a sampling rate of 1 KHz using a wireless surface electromyography (BTS FREEEMG; BTS, Milan, Italy) using 8 miniaturized probes with active electrodes. The probes amplify the EMG signals, digitize them with 16 bit resolution and communicate with the compact and light receiving unit left on the table and not attached to the subject. The system can be interfaced with OEP.

The skin of the subject was prepared by frictioning it with alcohol in order to improve the adherence of the 40 mm solid gel surface electrodes positioned as followed:

- one pair on the muscle belly of the left sternocleidomastoid muscle (SCM)
- one pair on the right scalene muscles (SCA)
- one pair on the right 1st intercostal space, 2 cm apart from the Lewis' angle, to measure the activity of parasternal muscles (PAR)
- one pair on the right 8th intercostal space, 4 cm apart from the nipple line, which is the most suggested place to measure diaphragmatic activity (IC8)
- one pair 2 cm below the xiphoid process in the abdominal wall (AXP)
- one pair on the right side between the lower costal margin and the iliac crest 4 cm apart from the nipple line to measure the activity of the external abdominal oblique muscle (EOB)
- one pair on the right side, 2 cm apart, at the height of the umbilicus to measure the activity of the rectus abdominis (RAB)

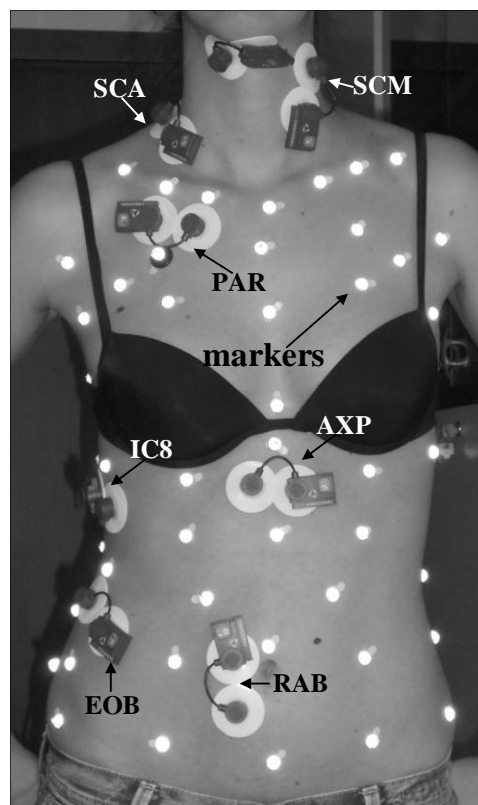


Figure 2.1: EMG electrodes positions – Photograph of a subject wearing markers and electrodes for EMG measurements of the sternocleidomastoid (SCM), the scalene (SCA), the parasternal (PAR), the intercostal (IC8), the external abdominal oblique (EOB) and the rectus abdominis (RAB) muscles. The AXP electrodes should measure the activity of the costal insertion of the abdominal muscles and of the descending diaphragmatic dome.

Figure 2.1 shows a photograph of a subject with the positioning of the EMG probes and the markers on the anterior chest wall surface. The EMG signals were then synchronized with OEP volumes, rectified, low-pass filtered (4th order digital Butterworth filter with a cut-off frequency of 166 Hz) and the Root Mean Square (RMS) value was computed (figure 2.2). Simultaneous recordings of chest wall volumes and EMG signals during four coughs starting from total lung capacity are illustrated in figure 2.3, while the inspiratory and expiratory cough phases identified from chest wall volume trace are shown on figure 2.4 representing chest wall and EMG traces during a single voluntary cough.

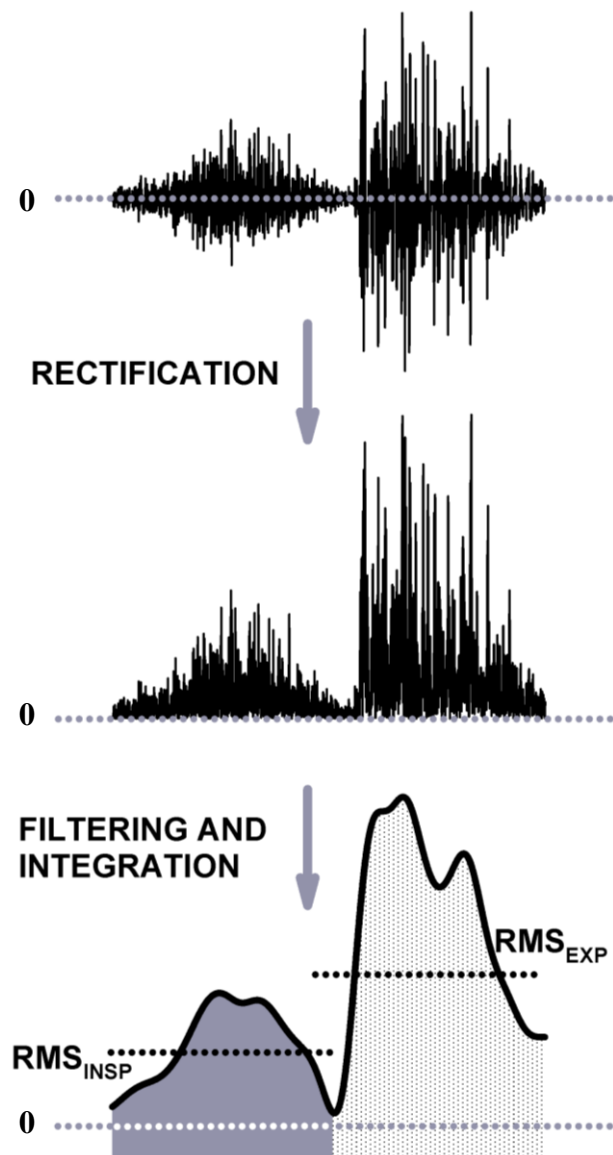


Figure 2.2: EMG data processing – The raw data (top panel) is rectified (middle panel) and integrated; then the root mean square value (RMS) is computed for the inspiratory (grey area, RMS_{INSP}) and expiratory (dotted area, RMS_{EXP}) cough phases (bottom panel)

Protocol

After a few minutes of spontaneous quiet breathing to rest, subjects were asked to perform a slow vital capacity (SVC) and then to cough as forcefully as possible (4 single voluntary coughs at 40 s intervals). Coughs were performed from four different operating volumes in a random order: functional residual capacity (FRC), one tidal and two tidal volumes over it (FRC+ and FRC++, respectively) and total lung capacity (TLC) without providing any visual feedback. The same manoeuvres were performed before in seated position and then in supine position. We focused on the inspiratory and expiratory phase, since we want to investigate the role of both inspiratory and expiratory muscles during voluntary cough.

Statistical analysis

Peak cough flow, chest wall volumes and EMG activity were compared using a two-way Analysis of Variance (ANOVA) with the operating volume and posture as independent factors. Post-hoc tests were based on Holm-Sidak and Dunn's method. Data are expressed as mean±standard deviation. Significance was determined by $p < 0.05$.

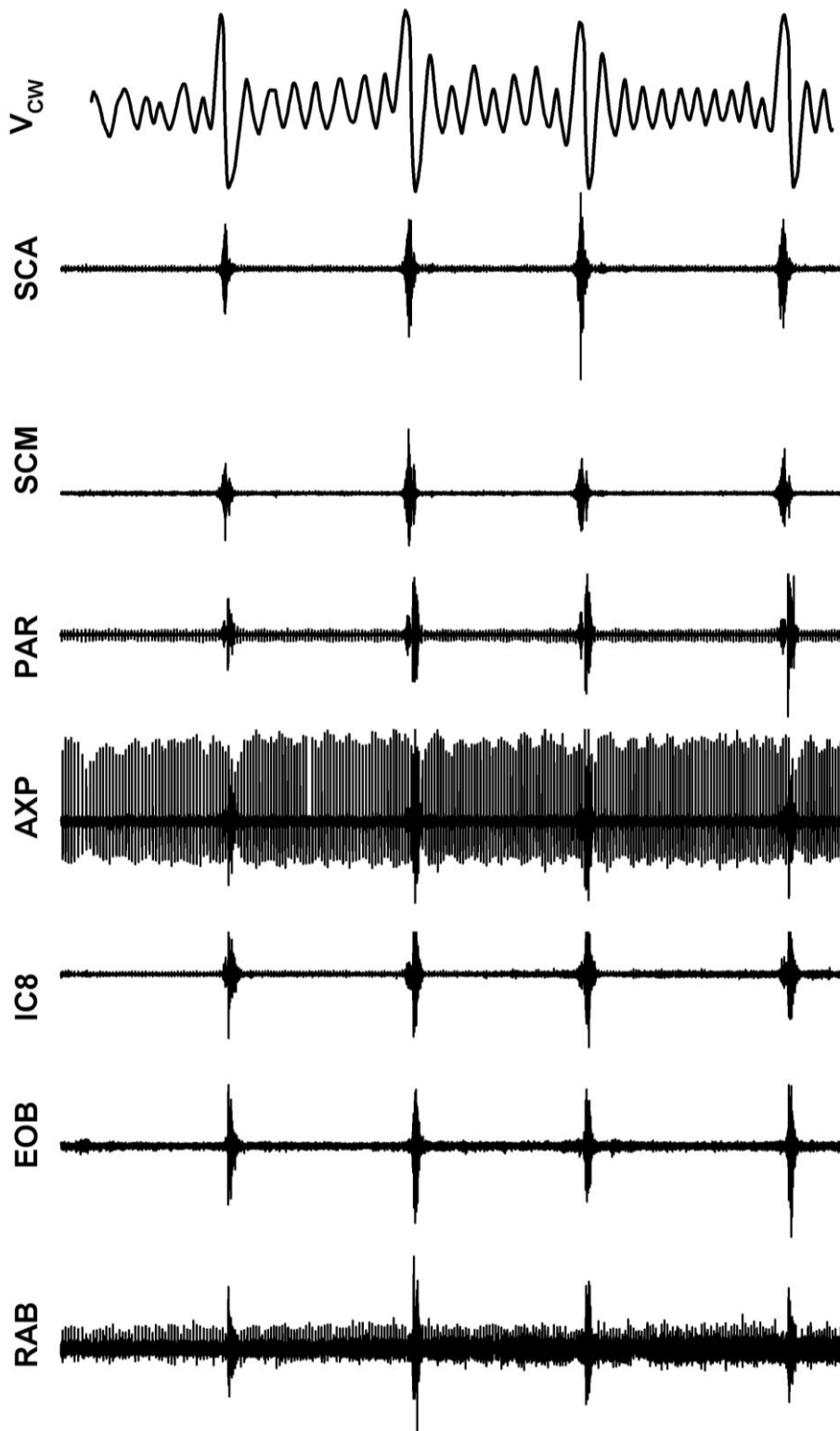


Figure 2.3: Volume and EMG recordings – Chest wall volume and EMG tracings of the sternocleidomastoid (SCM), the scalene (SCA), the parasternal (PAR), the intercostal (IC8), the external abdominal oblique (EOB) and the rectus abdominis (RAB) muscles during four coughs starting at total lung capacity. The AXP trace should be the measurement of the activity of the costal insertion of the abdominal muscles and of the descending diaphragmatic dome.

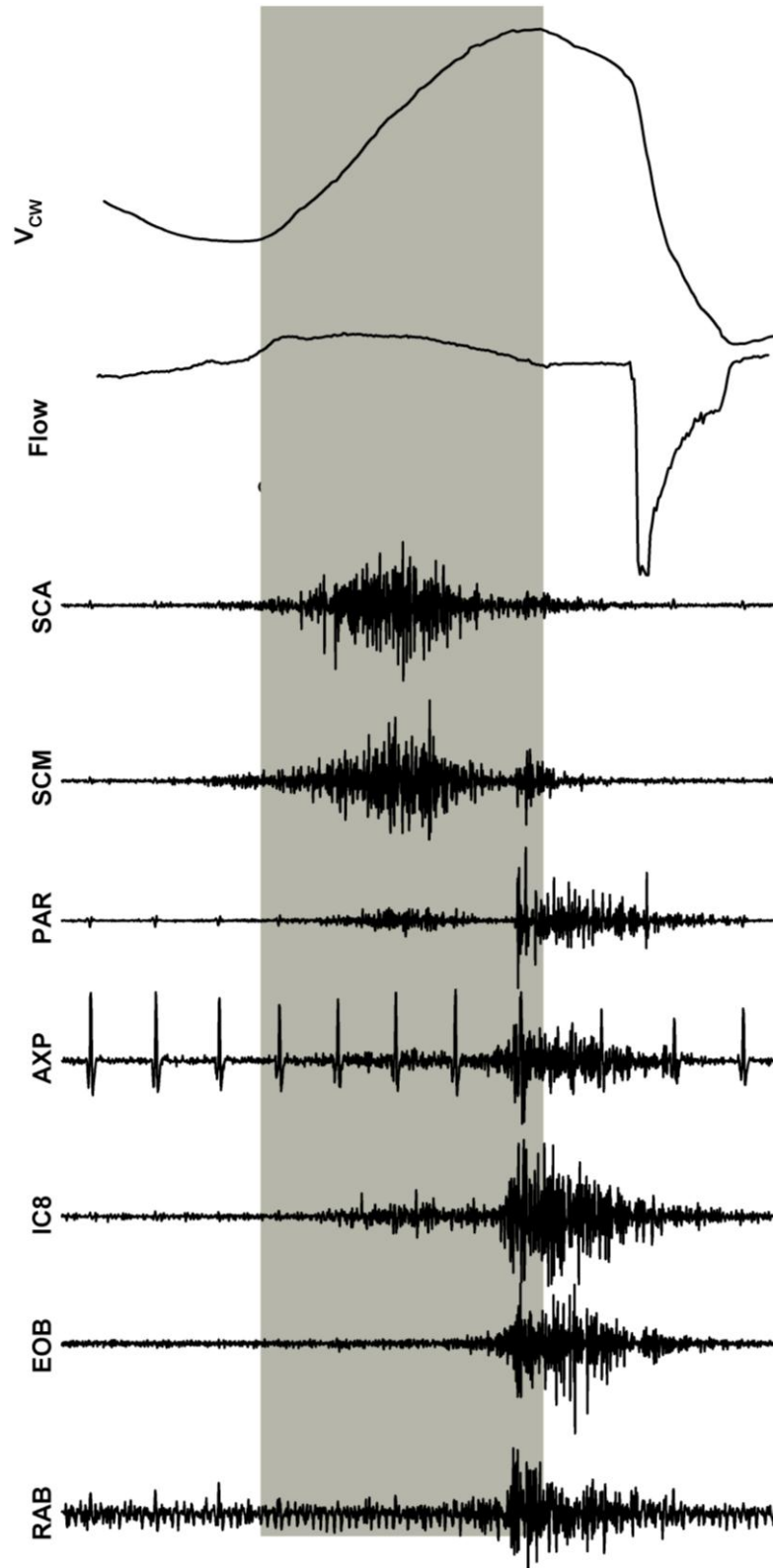


Figure 2.4: Volume, flow and EMG recordings – Chest wall volume, flow and EMG tracings of the sternocleidomastoid (SCM), the scalene (SCA), the parasternal (PAR), the intercostal (IC8), the external abdominal oblique (EOB) and the rectus abdominis (RAB) muscles during four coughs starting at total lung capacity. The AXP trace should be the measurement of the activity of the costal insertion of the abdominal muscles and of the descending diaphragmatic dome. The grey area represent the inspiratory cough phase.

2.3 RESULTS

Operating volumes and peak cough flow

Despite the lack of visual feedback, subjects performed four different operating volumes, similar within the two postures, as shown in the top panel of figure 2.5 in which the operating volumes are expressed as percentage of total lung capacity reached during SVC. Accordingly, peak cough flows augmented with increasing operating volume and were comparable in seated and supine positions (figure 2.5, bottom panel).

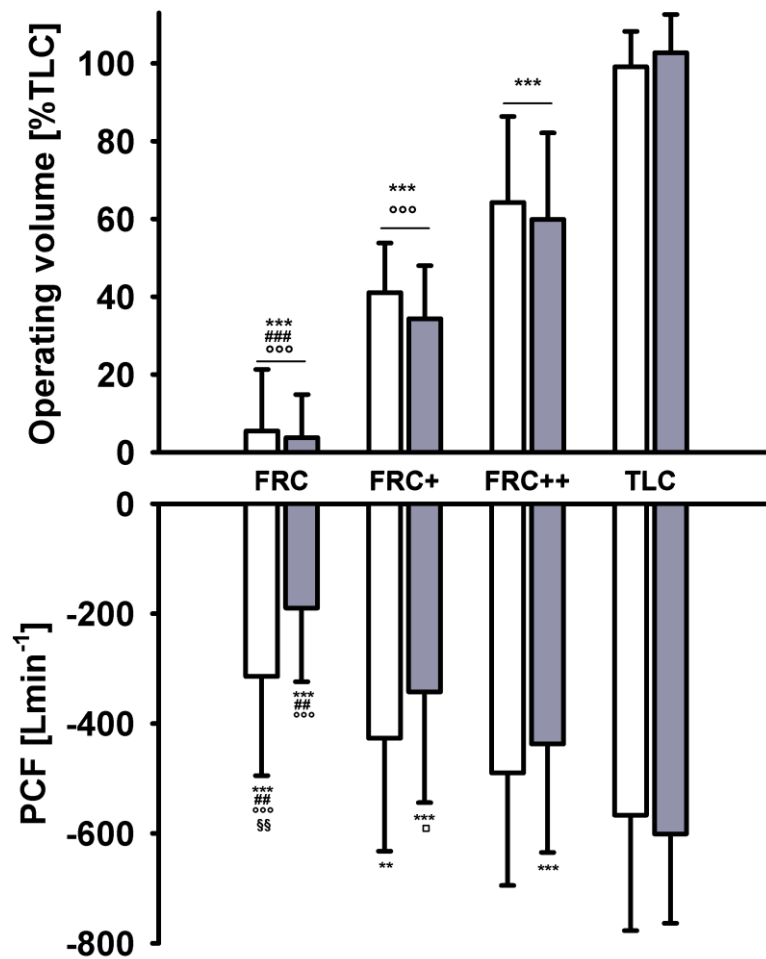


Figure 2.5: Operating volumes and peak cough flow– Average values \pm standard deviation of operating volume expressed as percentage of TLC (top panel) and peak cough flow (bottom panel) during cough at each operating lung volume: functional residual capacity (FRC), FRC+tidal volume (FRC+), FRC+two tidal volumes (FRC++) and total lung capacity (TLC) in seated (white bars) and supine (grey bars) positions. *, **, ***: $p < 0.05, 0.01, 0.001$ vs TLC; °, °°, °°°: $p < 0.05, 0.01, 0.001$ vs FRC++; #, ##, ###: $p < 0.05, 0.01, 0.001$ vs FRC+; §, §§, §§§, §§§§: $p < 0.05, 0.01, 0.001$ vs supine position.

Chest wall volume during cough phases

The absolute chest wall volume variations of the inspiration and expiratory cough phases are reported in figure 2.6. While end inspiratory cough volume increased with increasing operating volume as expected, end expiratory cough volume significantly reduced passing from TLC to FRC, indicating a stronger recruitment of the expiratory reserve volume at lower operating volume. The same pattern was found in both postures.

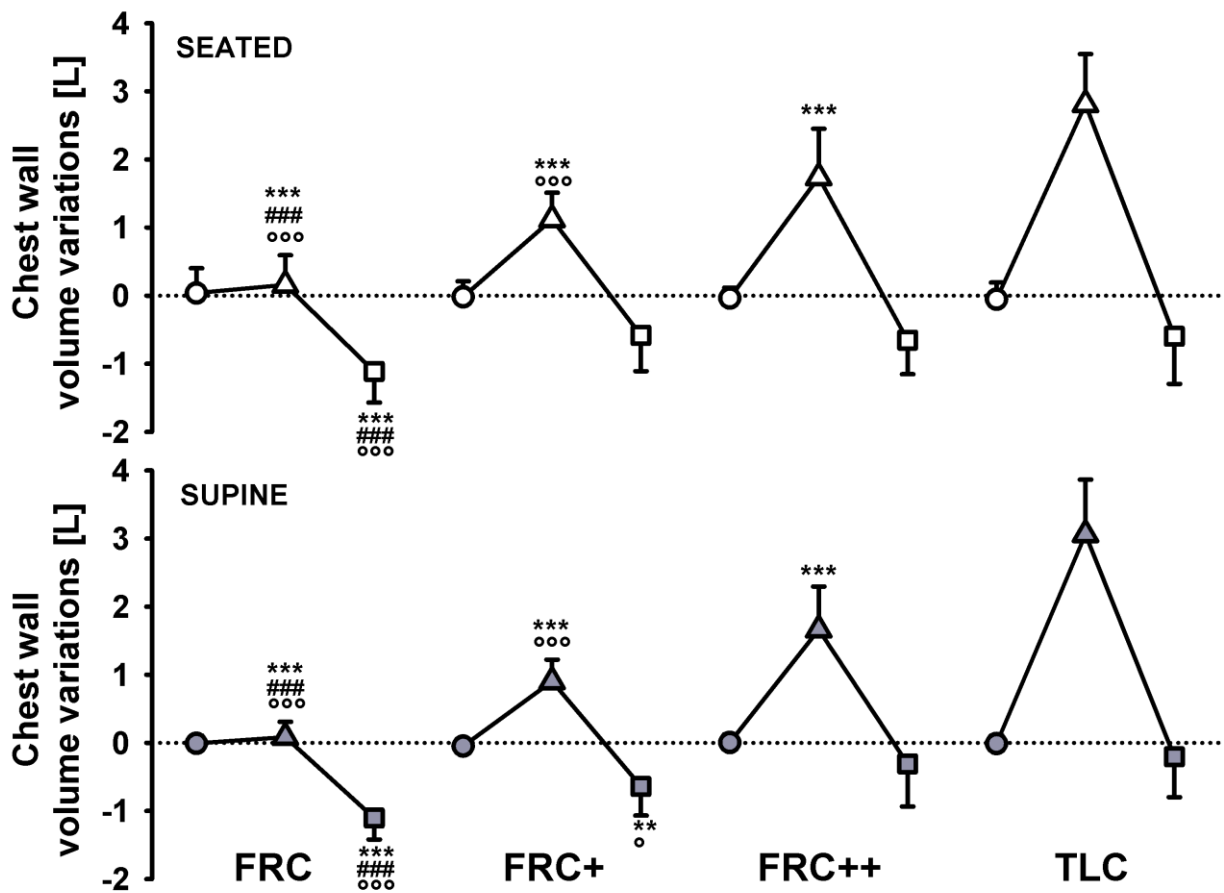


Figure 2.6: Chest wall volumes during cough – Average values \pm standard deviation of chest wall volume at start inspiration (circles), end of inspiration (triangle), end of cough (square) at each operating lung volume: functional residual capacity (FRC), FRC+tidal volume (FRC+), FRC+two tidal volumes (FRC++) and total lung capacity (TLC) in seated (top panel, white symbols) and supine (bottom panel, grey symbols) positions. *, **, ***: $p < 0.05, 0.01, 0.001$ vs TLC; °, °°, °°°: $p < 0.05, 0.01, 0.001$ vs FRC++; #, ##, ###: $p < 0.05, 0.01, 0.001$ vs FRC+; §, §§, §§§: $p < 0.05, 0.01, 0.001$ vs supine position.

Figure 2.7 shows the thoraco-abdominal percentage contribution during inspiratory and expiratory cough phases which both turned to be predominantly thoracic no matter what the posture is.

Interestingly, we found differences on thoraco-abdominal compartmentalization only in the expiratory cough phase in seated position; whereas operating volumes and posture seemed not to have effects on the inspiratory and supine expiratory cough phases. We did not show data of the inspiratory cough phase at FRC because they were highly variable due to their tiny volume variations.

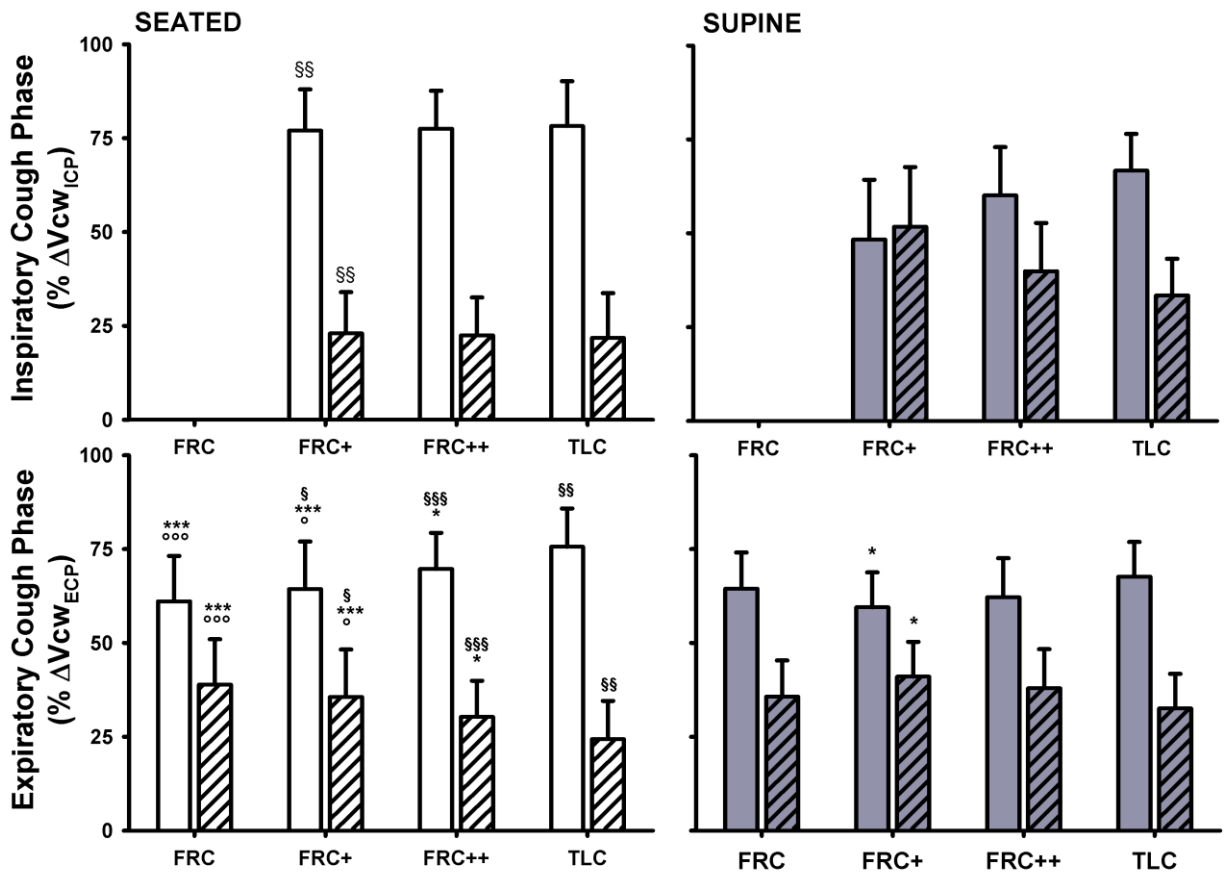


Figure 2.7: Thoraco-abdominal contribution – Average values \pm standard deviation of the percentage contribution of rib cage (open bar) and abdomen (coarse bar) during the inspiratory (top panels) and expiratory (bottom panels) cough phases at each operating lung volume: functional residual capacity (FRC), FRC+tidal volume (FRC+), FRC+two tidal volumes (FRC++) and total lung capacity (TLC) in seated (left panels, white bars) and supine (right panels, grey bars) positions.

*, **, ***: $p < 0.05, 0.01, 0.001$ vs TLC; °, °°, °°°: $p < 0.05, 0.01, 0.001$ vs FRC++; #, ##, ###: $p < 0.05, 0.01, 0.001$ vs FRC+; \$, \$\$, \$\$\$: $p < 0.05, 0.01, 0.001$ vs supine position.

RMS of respiratory muscles during cough

The root mean square value has been used to quantify the electric signal of each muscle during the inspiratory and the expiratory cough phase both in seated and in supine position. It reflects the physiological activity in the motor unit during contraction.

The sternocleidomastoid and the scalene are muscles which lift the ribs and they resulted active during both cough phases being maximal at TLC. In seated position the activation was higher than supine only at TLC (figure 2.8).

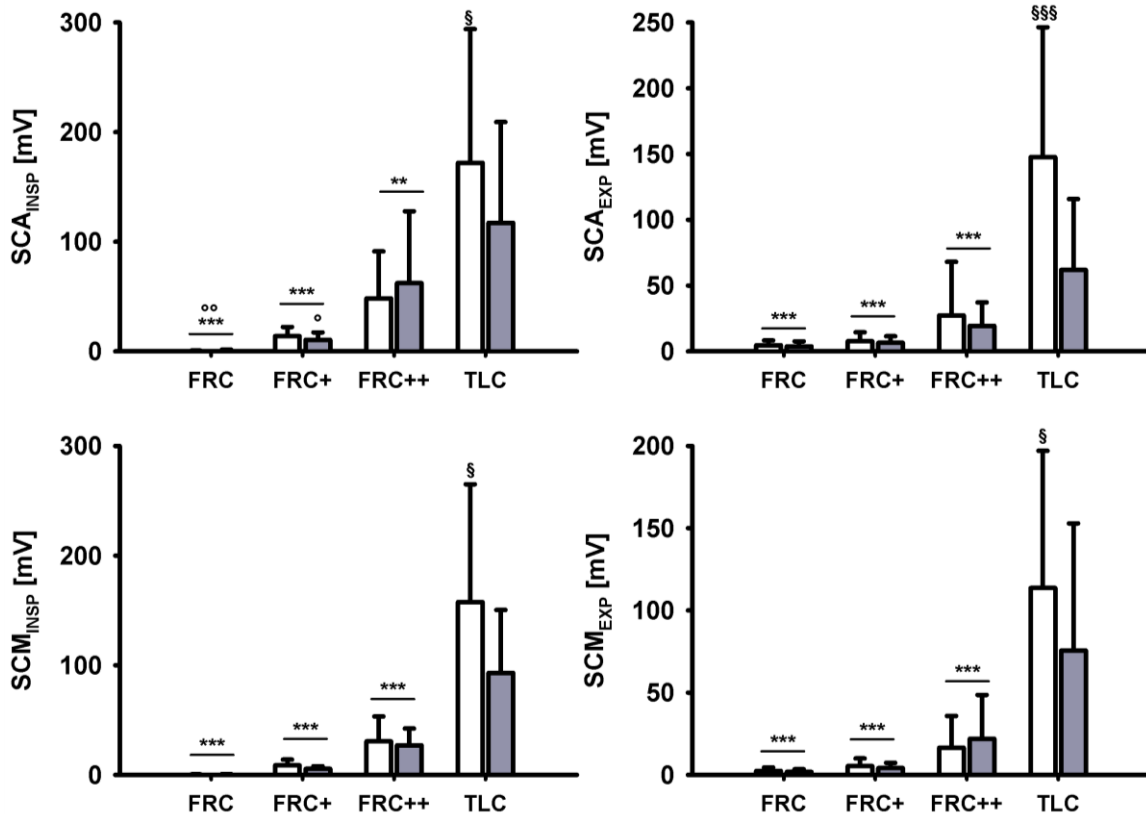


Figure 2.8: RMS of accessory neck muscles – Average values \pm standard deviation of root mean square value of the EMG signals for SCA (top panels) and SCM (bottom panels) muscles in seated (white bars) and supine (grey bars) positions during the inspiratory (left panels) and the expiratory (right panels) cough phase at each operating volumes: functional residual capacity (FRC), FRC+tidal volume (FRC+), FRC+two tidal volumes (FRC++) and total lung capacity (TLC).

, *: $p < 0.01, 0.001$ vs TLC; °, °°: $p < 0.05, 0.01$ vs FRC++; §, §§§: $p < 0.05, 0.001$ vs supine position.

A comparable pattern was found for the signals acquired at the intercostal spaces, the first and the eighth: they resulted active during inspiration and expiration, maximal at TLC and almost equal within postures (figure 2.9).

The RMS of the abdominal signals demonstrated the same activation, but they showed higher values, even though not statistically tested, during the expiratory phase (Figure 2.10). A similar behavior was found only at the eighth intercostal space.

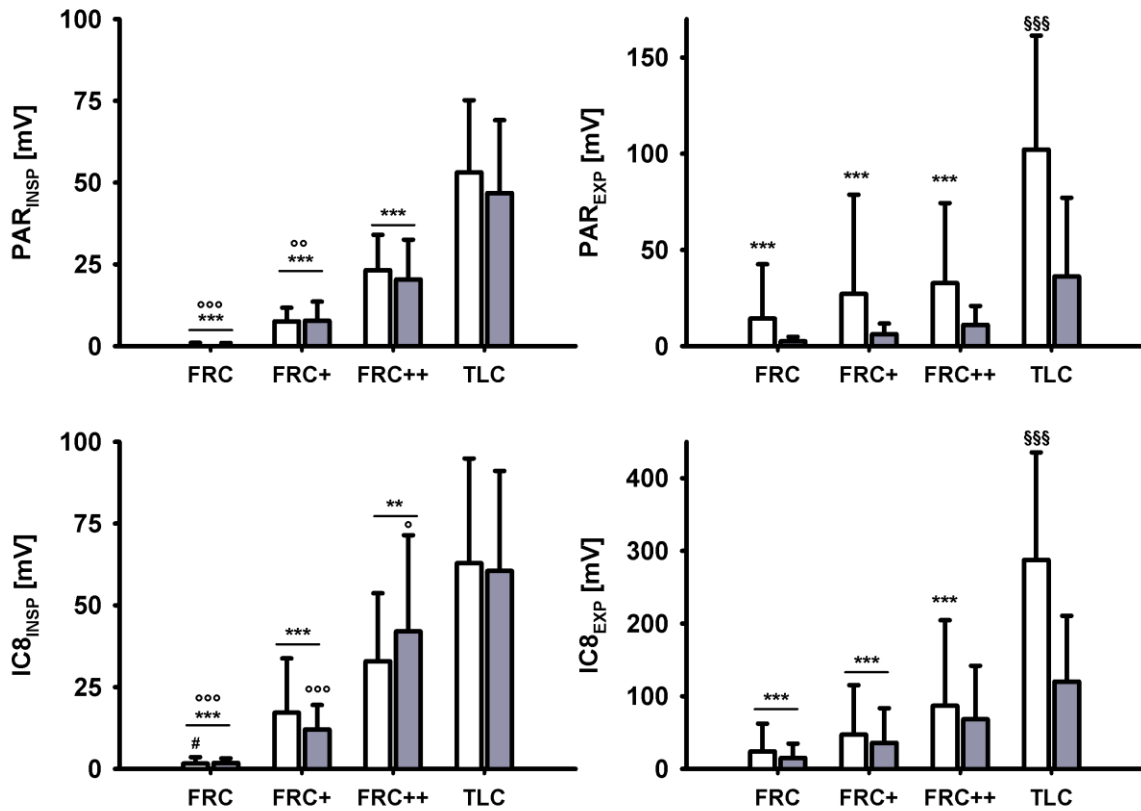


Figure 2.9: RMS of thoracic muscles – Average values \pm standard deviation of root mean square value of the EMG signals for PAR (top panels) and IC8 (bottom panels) muscles in seated (white bars) and supine (grey bars) positions during the inspiratory (left panels) and the expiratory (right panels) cough phase at each operating volumes: functional residual capacity (FRC), FRC+tidal volume (FRC+), FRC+two tidal volumes (FRC++) and total lung capacity (TLC).

***: $p < 0.001$ vs TLC; °°, °°°: $p < 0.01, 0.001$ vs FRC++; #: $p < 0.05$ vs FRC+; \$\$\$: $p < 0.001$ vs supine position.

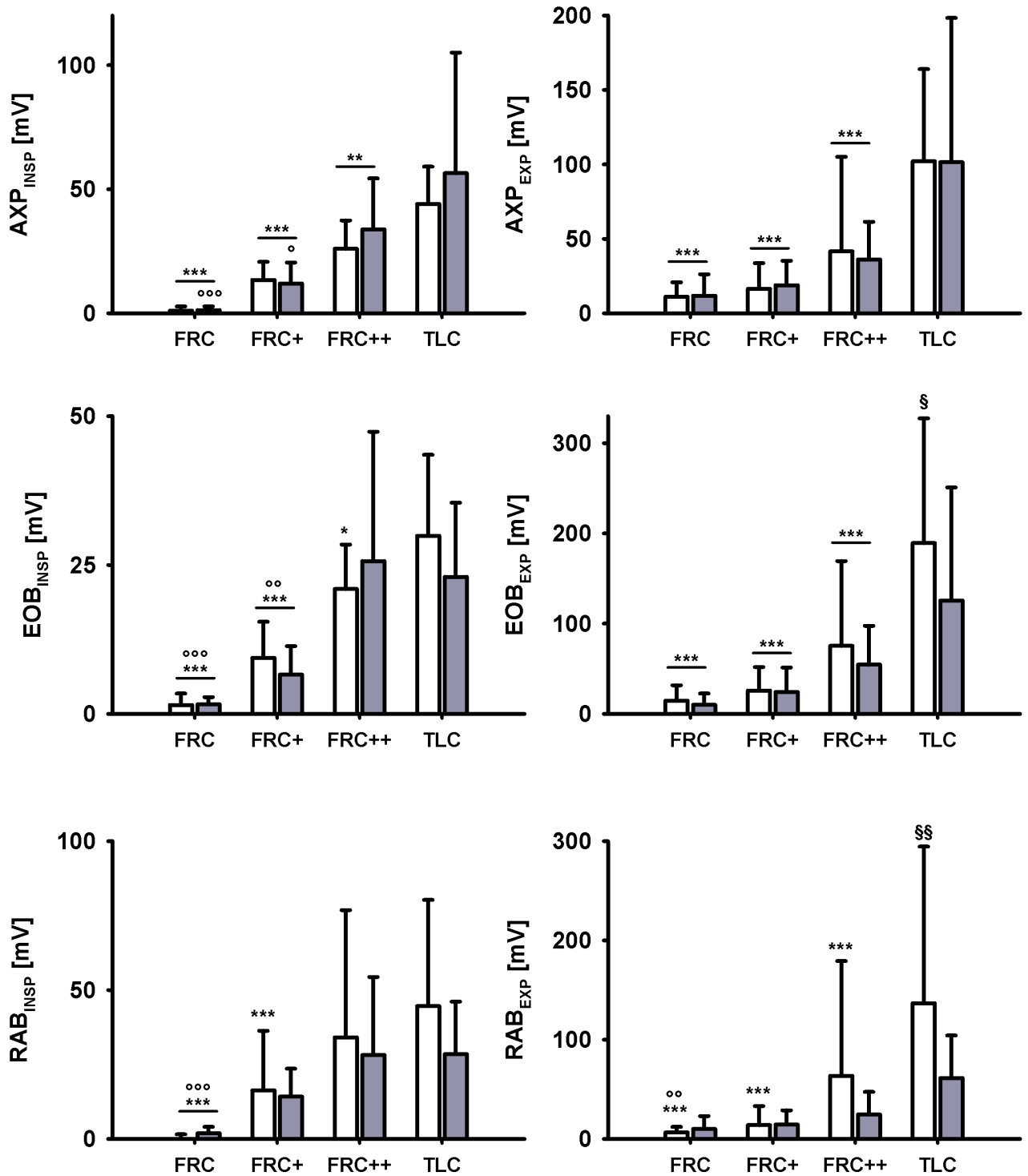


Figure 2.10: RMS of abdominal muscles – Average values \pm standard deviation of root mean square value of the EMG signals for AXP (top panels), EOB (middle panels) and RAB (bottom panels) muscles in seated (white bars) and supine (grey bars) positions during the inspiratory (left panels) and the expiratory (right panels) cough phase at each operating volumes: functional residual capacity (FRC), FRC+tidal volume (FRC+), FRC+two tidal volumes (FRC++) and total lung capacity (TLC).

*, ***: $p < 0.05, 0.001$ vs TLC; °, °°, °°°: $p < 0.05, 0.01, 0.001$ vs FRC++; §, §§: $p < 0.05, 0.01$ vs supine position.

2.4 DISCUSSION

As far as we know, these data are the first to combine electromyography and chest wall kinematics with the effect of operating volume and posture during voluntary cough. We have shown that posture does not influence volume variations and muscles activation which, as expected, was maximal at TLC. Moreover, thoraco-abdominal compartments contributed constantly to volume changes during inspiratory and expiratory cough phases regardless of the operating volume and posture.

Because it is well known that posture influences thoraco-abdominal kinematics during quiet breathing, being predominantly abdominal in supine position and thoracic in seated^[12], we would have expected two complete different cough patterns in the two postures as well. The supine position, in fact, introduces mechanical changes in the chest wall (*i.e.* cranially displacement of the diaphragm, lung volume reduction, stretching of the expiratory muscles and lowering of the abdominal compliance), but, surprisingly, peak cough flow, chest wall volumes and muscular activation resulted similar with posture in both the considered cough phases. In addition, rib cage and abdomen constantly contributed to cough irrespective of the operating volume and posture. These results suggested that under normal physiological conditions, in order to provide efficient expulsive flow, voluntary cough is associated with a specific co-ordinate muscle activity resulting in a constant thoraco-abdominal contribution no matter the quantity of inhaled air and/or the posture are. We can speculate that the neural structures involved in voluntary cough^[13] are able to bypass the mechanical changes introduced by posture and by increased lung volume with the latter resulting in maximal passive elastic recoil during expiration. Interestingly, the same pattern was maintained also coughing at FRC, with a reduced amount of air in the lung to be compressed and exhaled and the expiratory muscles length to be less optimal, by strongly recruiting the expiratory reserve volume. It would be very interestingly to study whether this pattern is maintained also during reflex and evoked cough^[14].

Even though cough is an expiratory maneuver and the principal expiratory muscles lie in the ventro-lateral aspect of the abdominal wall, the rib cage resulted the leading compartment during cough, indicating an important contribution of the intercostal muscles and the accessory muscles descending from the head and the neck which lift the ribs. Our data confirm that the sternocleidomastoid, the scalene, parasternal and intercostal muscles

are active during the two considered cough phases. In addition, the expiratory activity found at AXP level suggested that during cough the muscles of the anterolateral abdominal wall helped depressing the ribs to which they are attached and compressing the lower thoracic cage^[15].

Because of the lesser amount of publications regarding the activity of intercostal muscles during cough, our results could add a piece of information on the respiratory functions of these muscles.

The EMG measured at the eighth intercostal space during cough has been measured by another group^[8], but they focused on the expiratory phase during capsaicin administration, a tussive agent. The IC8 is the suggested place to measure the activity of the diaphragm *via* transcutaneous electromyography. Moreover, the inspiratory activity measured at the AXP electrodes could be the measurements of the electrical activity of the descending diaphragm during its contraction. This is just an assumption and further investigations, maybe using simultaneous invasive measurement of diaphragmatic activity by transesophageal method^[16], should be needed to verify this hypothesis and isolate the effective contribution of the diaphragm from the cross talk of the inspiratory rib cage muscles. Considering that in literature only animal studies, using invasive wire-electrodes, investigated the role of the diaphragm during induced cough, the present paper could be consider a first attempt to non-invasively study the action of this muscle in humans during voluntary cough.

On the other hand, the role of the abdominal muscles during cough in healthy subjects have been already studied even though not as function of operating volume and posture^[7-9]. Our data confirmed the important role of the external abdominal oblique muscles and the rectus abdominis, but we cannot say which muscles make the largest mechanical contribution to volume variations because we did not compare the degree of activation of the different muscular groups.

Finally, the inspiratory activity of the abdominal muscles, as well as the expiratory activity of the inspiratory accessory neck muscles indicated coactivation of antagonist muscles during cough in order to control the operating volume, the transmission of pressure from abdomen to the thorax^[17] and to stabilize the chest wall, lungs and airways during the cough expulsive phase^[18].

Critique of the methods.

We considered the root mean square, *i.e.* the integration of the total electrical activity during contraction in both the considered cough phases, instead of the “moving average” of the activity which takes in consideration also the duration of the muscular effort. Moreover, we did not normalize the RMS values to the peak amplitude of maximal muscle activation. Because, a part from the diaphragm, all the other considered muscles are not “purely” respiratory but they all have other function, mainly postural, the choice of the maximal reference values is crucial. In fact, it has been shown that abdominal and intercostal accessory muscles can be driven to a greater degree during voluntary postural than respiratory efforts^[1]. We are also aware of the limitation of the transcutaneous EMG measurements which do not reflect the activation of a single muscle, but rather, that of different muscular layers with an important cross-talk of the adjacent muscles. On the other hand, our results are in line with published data.

In conclusion, we combined transcutaneous EMG measurements with the kinematics of the chest wall to study the inspiratory and the expiratory phase of voluntary cough in healthy subjects. The synergetic antagonistic activity of respiratory muscles leads to a specific pattern, in which the rib cage is the leading compartment indicating that the abdominal muscles mainly act to lower the inferior ribs due to their insertions on the rib cage. This thoracic pattern is not affected by either operating volume or posture despite the mechanical changes introduced and this suggest a task-specific somatosensory activation during voluntary cough.

2.5 REFERENCES

1. Gandevia SC, McKenzie DK, Plassman BL (1990) Activation of human respiratory muscles during different voluntary manoeuvres. *J Physiol.* 428: 387-403
2. Kobayashi I, Kondo T, Suzuki H, Ohta Y, Yamabayashi H (1992) Expiratory activity of the inspiratory muscles during cough. *Jpn J Physiol.* 42(6): 905-16.
3. Hutten GJ, van Thuijl HF, van Bellegem AC, van Eykern LA, van Aalderen WM (2010) A literature review of the methodology of EMG recordings of the diaphragm. *J Electromyogr Kinesiol.* 20(2): 185-90.
4. Maarsingh EJ, van Eykern LA, Sprickelman AB, Hoekstra MO, van Aalderen WM (2000) Respiratory muscle activity measured with a noninvasive EMG technique: technical aspects and reproducibility. *J Appl Physiol.* 88(6): 1955-61.
5. Duiverman ML, van Eykern LA, Vennik PW, Koëter GH, Maarsingh EJ, Wijkstra PJ (2004) Reproducibility and responsiveness of a noninvasive EMG technique of the respiratory muscles in COPD patients and in healthy subjects. *J Appl Physiol.* 96(5): 1723-9.
6. Fontana GA, Lavorini F (2006) Cough motor mechanisms. *Respir Physiol Neurobiol* 152(3): 266-81.
7. Fontana GA, Pantaleo T, Lavorini F, Boddi V, Panuccio P (1997) A non invasive electromyographic study on threshold and intensity of cough in humans. *Eur Respir J.* 10(5): 983-9.
8. Vovk A, Bolser DC, Hey JA, Danzig M, Vickroy T, Berry R, Martin AD, Davenport PW (2007) Capsaicin exposure elicits complex airway defensive motor patterns in normal humans in a concentration-dependent manner. 20(4): 423-32.
9. Cox ID, Wallis PJ, Apps MC, Hughes DT, Empey DW, Osman RC, Burke CA (1984) An electromyographic method of objectively assessing cough intensity and use of the method to assess effects of codeine on the dose-response curve to citric acid. *Br J Clin Pharmacol.* 18(3): 377-82.
10. Smith JA, Aliverti A, Quaranta M, McGuinness K, Kelsall A, Earis J, Calverley PM (2012) Chest wall dynamics during voluntary and induced cough in healthy volunteers. *J Physiol* 590(Pt 3): 563-574.

11. Cala SJ, Kenyon CM, Ferrigno G, Carnevali P, Aliverti A, Pedotti A, Macklem PT, Rochester DF. Chest wall and lung volume estimation by optical reflectance motion analysis. *J Appl Physiol.* 1996 Dec;81(6):2680-9
12. Romei M, Lo Mauro A, D'Angelo MG, Turconi AC, Bresolin N, Pedotti A, Aliverti A (2010) Effects of gender and posture on thoraco-abdominal kinematics during quiet breathing in healthy adults. *Respir Physiol Neurobiol.* 172(3): 184-91.
13. Simonyan K, Saad ZS, Loucks TM, Poletto CJ, Ludlow CL (2007) Functional neuroanatomy of human voluntary cough and sniff production. *Neuroimage.* 37(2): 401-9.
14. Hegland KW, Bolser DC, Davenport PW (2012) Volitional control of reflex cough. *J Appl Physiol.* 113(1): 39-46.
15. Roussos C. The thorax, part A: physiology vol 85, Marcel Dekker, Inc
16. Agostoni E, Sant'Ambrogio G, Del Portillo Carrasco H (1960) Electromyography of the diaphragm in man and transdiaphragmatic pressure. *J Appl Physiol.* 15: 1093-7.
17. Tomori Z, Widdicombe JG. (1969) Muscular, bronchomotor and cardiovascular reflexes elicited by mechanical stimulation of the respiratory tract. *J Physiol.* 200(1):25-49.
18. Fontana G.A. (2003) Motor mechanisms and the mechanics of cough In: K.F. Chung, J.G. Widdicombe, H. Boushey (Eds.), *Cough: Causes, Mechanisms and Therapy*, Blackwell Publishing (2003), pp. 193–205

Chapter 3 - **Blood shift during cough in healthy subjects**

Keywords: cough, blood shift, double plethysmography

3.1 INTRODUCTION

In the literature, there are some case reports and clinical studies reporting how forceful rhythmic coughing can provide effective blood flow during ventricular fibrillation without direct chest compression for up to 40 seconds^[1-5]. This mechanism of cough-assisted cardiopulmonary resuscitation constitutes a form of “cardiac massage” secondary to the intrathoracic and intra-abdominal pressure changes during cough phases^[6-10].

On the other hand, we have previously demonstrated that increases in abdominal pressure can produce a circulatory output as great as resting cardiac output^[11,12]. We quantified this blood shifts displaced from the splanchnic vasculature to the extremities during expulsive maneuvers with abdominal pressure (P_{AB}) reaching up to ~ 100 cmH₂O for 0.5-1 s. These conditions are similar to the expiratory cough phase during which abdominal pressure suddenly increases due to a strong respiratory muscles contraction against a closed glottis followed by a forceful expiratory flow when the glottis reopens.

We believe that coughing activates what we have called the abdominal circulatory pump and that this could be the mechanism resulting in a circulatory output sufficient to maintain consciousness in a patient with a non-beating heart as reported in the literature. To test this hypothesis we have used the double-body plethysmography technique, the method we have developed to measure the amount of blood exchanged between the trunk and the extremities^[11]. We were able to quantify the blood shifts during the three phases of voluntary cough, namely: inspiratory, compressive and expulsive. Moreover, because it was shown that the operating volume, the volume inspired prior to coughing, significantly influences peak cough flow but not the pressures generated^[13], we have also investigated if the operating volume could have some influence on the cardiopulmonary interactions during coughs.

3.2 METHODS

Ethics Statement

The research protocol was approved by the local ethics committee of the INRCA Hospital, Casatenovo, LC, Italy and written informed consent was obtained from all the subjects who volunteered for the experiment.

Double Plethysmography

The method was previously tested and described in detail^[11,12].

In synthesis, we simultaneously measured body volume (ΔV_B) variation through a homemade, transparent, variable-flow whole body plethysmograph and chest wall volume variation (ΔV_{CW}) using Opto-Electronic Plethysmography (OEP System; BTS, Milan, Italy)^[14]. Both measures are sensitive to gas compression and heating in the lungs, but the latter also changes in case of blood shifts from the trunk to the extremities. Therefore, such blood shift volume (V_{BS}) is calculated as $\Delta V_{CW} - \Delta V_B$.

The body volume was obtained integrating the flow in and out the whole body plethysmograph measured by a pneumotachometer mounted in the top of the box. This flow was anti-filtered in order to correct for the dynamics of the box itself.

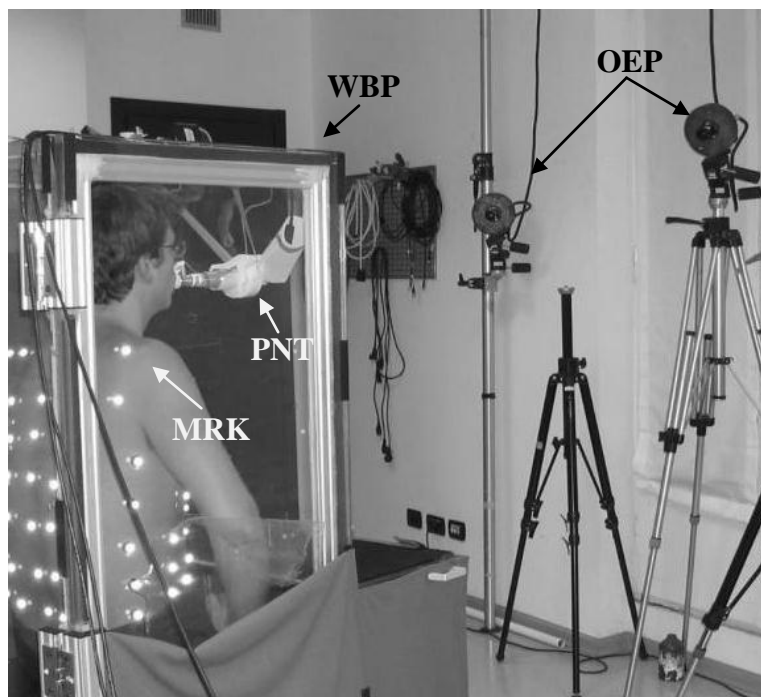


Figure 3.1: Double plethysmograph – Experimental set-up of the double plethysmograph: the transparent homemade body plethysmograph (WBP) plus the cameras and the markers (MRK) of the optoelectronic plethysmography (OEP). The pneumotacograph (PNT) to measure the flow at the mouth is also indicated.

Chest wall volume was measured using the Gauss' theorem and a dedicated geometrical model applied to 3D coordinates of 89 retro-reflective markers placed on the trunk of the subject according to specific anatomical points from clavicles to pubis. The OEP system is based on special infrared video cameras working at a sampling rate of 60 Hz.

Figure 3.1 is a photograph of a subject inside the transparent body box with markers on his trunk and three of the eight cameras recording motion of the anterior markers.

Representative tracings of chest wall and body volumes as well as their difference, the blood shift, are illustrated in figure 3.2.

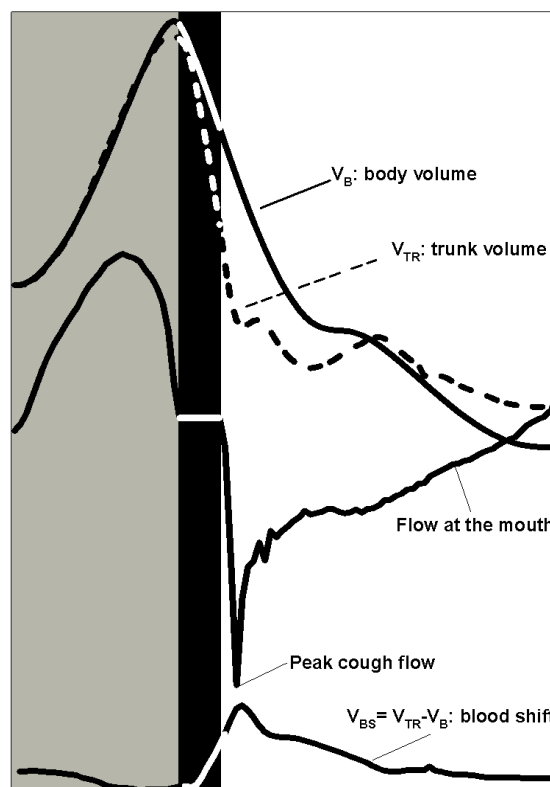


Figure 3.2: Volumes, flow and blood shift during cough - Time courses of the body and trunk volumes, flow measured at the mouth and blood shift during a single maximal voluntary cough. Grey area: inspiratory cough phase; black area: compressive cough phase; white area: expulsive cough phase.

Flow and cough phases

Flow was measured at the mouth using a pneumotachometer (Heated Pneumotach 800 L; Hans Rudolph®, Shawnee, USA) which connected the subject sat inside the whole body plethysmograph to the exterior of the box. The signal flow was used to distinguish the cough phase as shown in figure 3.2. Positive flow identifies the inspiratory cough phase, then the glottis closes and the flow is zero during the compressive phase in which expiratory muscles contract in a coordinated effort increasing the intrathoracic pressure.

Finally, glottis abruptly opens and the flow becomes negative, identifying the expulsive phase, and the peak cough flow is reached. The duration of the compressive and expulsive cough phases has been computed as well.

Oesophageal and gastric pressures

We measured oesophageal and gastric pressures only in one subject by standard balloon-tipped catheter in the esophagus and the stomach attached to piezoresistive transducers (ASDX005D44D-A, full range scale ± 351 cmH₂O, Sensortech Munich, Germany).

Subjects and protocols

We studied seven healthy volunteers (age: 28.6 ± 2.5 yrs), two women, who have experience in respiratory maneuvers.

After a period of five minutes of spontaneous quiet breathing to familiarize with the instrumentation, subjects were asked to performed single maximal voluntary coughs every 40 seconds for three times. Coughs were performed starting from three different and randomized operating volumes: a tidal volume over functional residual capacity (FRC), two tidal volumes over FRC and total lung capacity (V_T , $2V_T$ and TLC, respectively). No visual feedback was provided to the subjects to control the operating volume. Figure 3.3 illustrates representative tracing of chest wall volume and blood shift for each operating volume.

Statistical analysis

To investigate the effects of the three operating volumes on flow, chest wall volume, blood shifts and the duration of expiratory cough phase, a one-way Analysis of Variance (ANOVA) was performed with the operating volume as independent factor. Post-hoc tests were based on Holm-Sidak and Dunn's method. Data are expressed as mean \pm standard deviation. Significance was determined by $p < 0.05$.

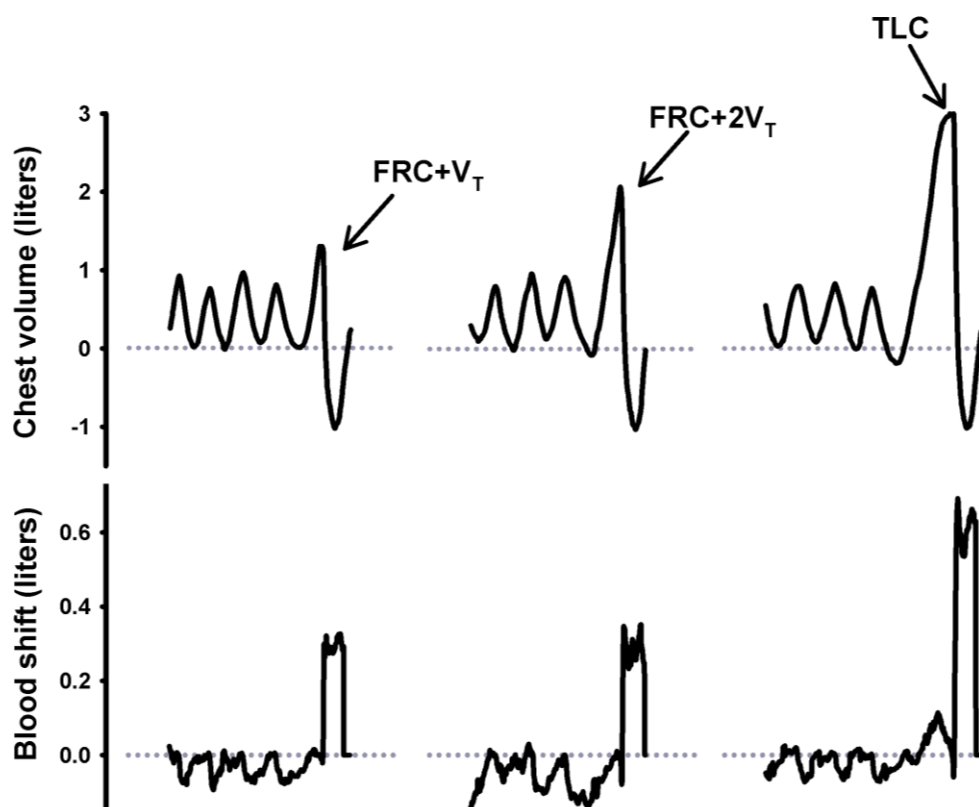


Figure 3.3: Time course of chest wall volume and blood shift— Representative case of chest wall volume changes (top panels) and blood shift (bottom panels) during spontaneous breathing and a single cough maneuver at each operating lung volume: FRC+tidal volume (left panel), FRC+two tidal volumes (middle panel) and total lung capacity (right panel).

3.3 RESULTS

Duration of the expiratory cough phase and peak cough flow

The durations of the two components of the expiratory phase, namely the compressive and the expulsive, are reported in the x-axis of figure 3.4. When cough starts from V_T and $2V_T$, the duration of the compressive and the expulsive phases were approximately 200 ms and 700 ms respectively. On the other hand, when cough starts at TLC, both compressive and expulsive phases significantly prolonged and, accordingly, also peak cough flow occurred later. Not surprisingly, peak cough flow increased with the operating volume, passing from 6 Lmin^{-1} at V_T to 12 Lmin^{-1} at TLC.

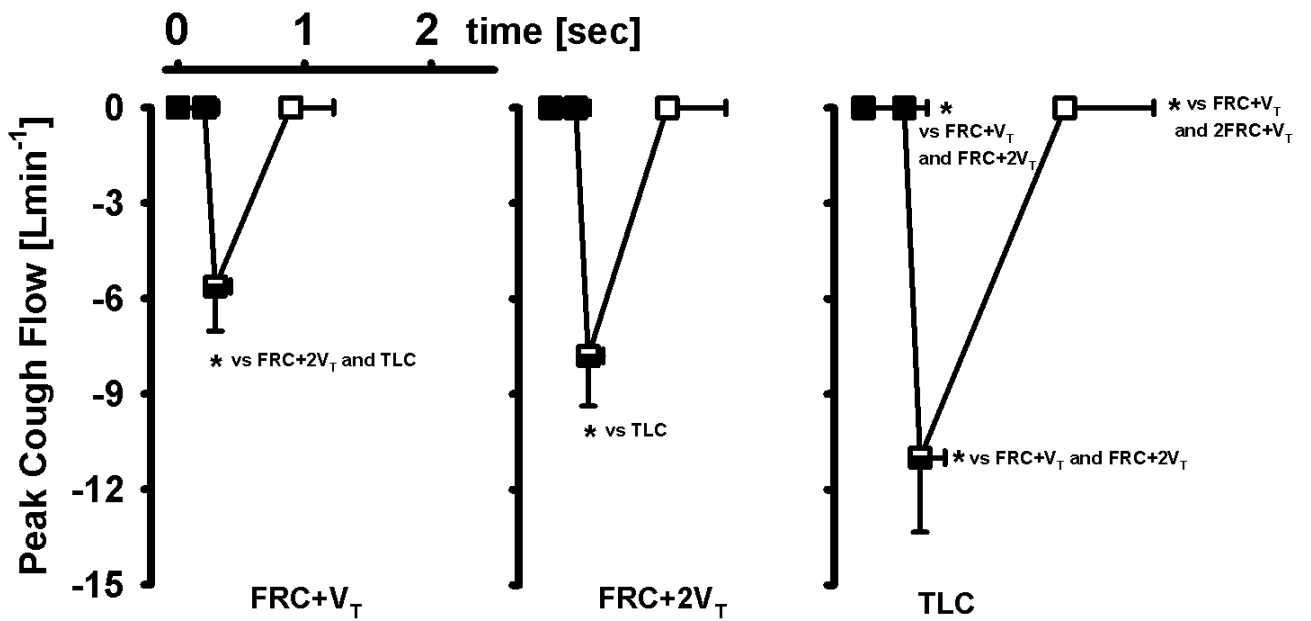


Figure 3.4: Peak flow and expiratory cough duration– Average values \pm standard deviation of peak cough flow (semi-filled bottom symbol) during cough at each operating lung volume: FRC+tidal volume (left panel), FRC+two tidal volumes (middle panel) and total lung capacity (right panel). The x-axis displays the average values \pm standard deviation of the duration of the compressive (close symbols) and the expulsive (open symbol) cough phases.

*: $p < 0.05$

Chest wall volume during cough phases

The changes of chest wall volume during the three cough phases (inspiratory, compressive and expulsive) are reported in figure 3.5.

Although no visual feedback was provided, the subjects were able to reach three different chest wall volumes at the end of the inspiratory phase therefore providing three increasing operating volumes. Accordingly, the compressive phase started from higher volumes as the operating volume increase. The chest wall volume at the end of cough decreased more at the lowest operating volume indicating a stronger recruitment of the expiratory reserve volume.

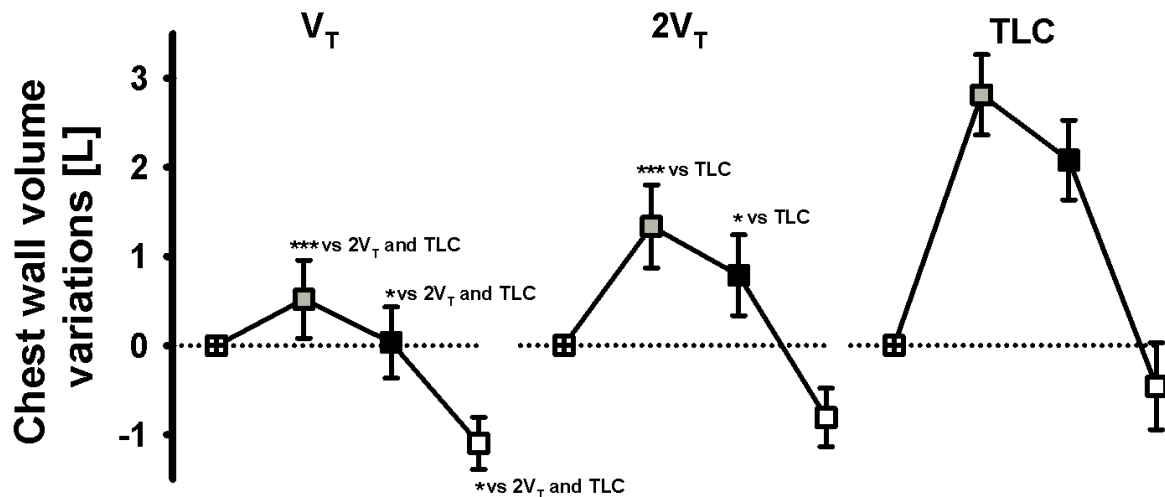


Figure 3.5: Chest wall volumes during cough phases – Average values \pm standard deviation of chest wall volume at start inspiration (crossed symbol), end of inspiration (grey symbol), end of compression (close symbol) and end of expulsion (open symbol) at each operating lung volume: FRC+tidal volume (left panel), FRC+two tidal volumes (middle panel) and total lung capacity (right panel).

*, ***: $p < 0.05$, $p < 0.001$

Blood shift during cough phases

The blood shifted between the extremities and the trunk during the three cough phases and the quiet breathing preceding the maneuvers is shown in figure 3.6.

We considered V_{BS} positive when the blood moved from the trunk to the periphery, negative flowing in the opposite direction.

During expiratory tidal breathing, blood shift was *circa* 51 ml and it was significantly lower than the values found during the compressive and expulsive cough phases. In fact, during the compressive phase V_{BS} was almost constantly around 200 ml independently on the volumes at the end of the inspiratory phase. On the other hand, the amount of blood propelled forward significantly increased with the operating volume during the expulsive phase reaching a mean value of 700 ml at TLC. No differences have been found ($p=0.836$) during the inspiratory cough phase, presumably due to the high variability found.

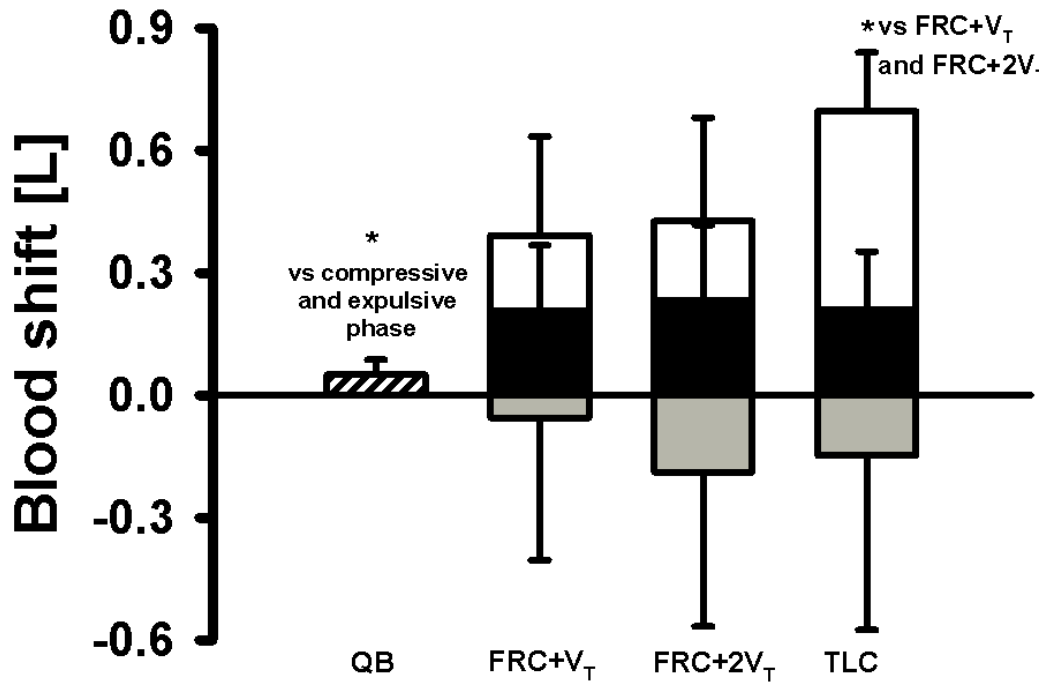


Figure 3.6: Blood shift during cough phases – Average values \pm standard deviation of blood shift during expiratory quit breathing (QB) and cough at each operating volumes [FRC+tidal volume (V_T), FRC+two tidal volumes ($2V_T$) and total lung capacity (TLC)] during inspiratory cough phase (grey bars), compressive cough phase (black bars) and expulsive cough phase (white bars).
*: $p < 0.05$

Pressures during expiratory cough phase

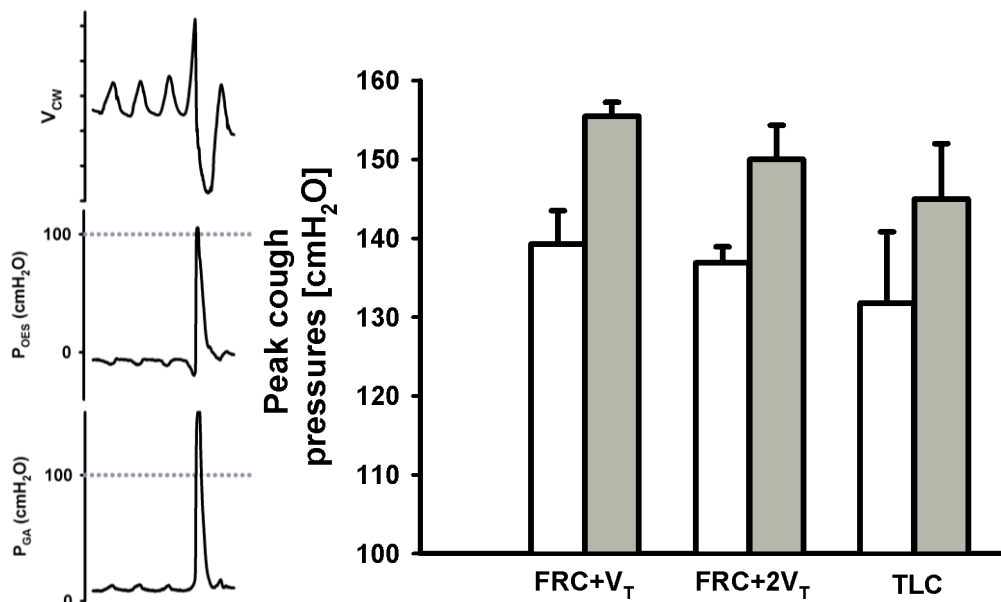


Figure 3.7: Expiratory peak pressures – Representative traces (left panels) of chest wall volume variation (top panel), oesophageal (middle panel) and gastric (bottom panel) pressures during cough at total lung capacity (TLC). 100 cmH₂O is the indicated threshold that activate the abdominal circulatory pump^[11]. Average values \pm standard deviation (right panel) of oesophageal (white bars) and gastric (grey bars) pressure during the expiratory cough phase at each operating volumes: FRC+tidal volume (V_T), FRC+two tidal volumes ($2V_T$) and TLC.

As shown in figure 3.7, the peak oesophageal and gastric pressures did not significantly change with increasing operating volume in the only subject who swallowed the balloon catheter therefore allowing pressure measurements. During cough at all the operating volumes, gastric pressure resulted higher than 100 cmH₂O, the indicated threshold that activate the abdominal circulatory pump^[11].

3.4 DISCUSSION

The current study is the first to quantify the blood exchanged between the trunk and the extremities during the different phases of voluntary cough. We have shown that during maximal coughing 700 ml of blood can be shifted and this could be an important haemodynamic factor which can provide an explanation for cardio-pulmonary resuscitation based on cough proposed by Criley and co-authors^[1-3].

A first measurement of blood pushed out of the trunk during peals of voluntary coughing was published by Smith et al^[13]. They calculate V_{BS} by subtracting the volume of gas compressed in the lungs, estimated from Boyle's law for isothermal transformations, and the volume expired at the mouth from the trunk volume provided by OEP. At the end of the first compressive phase they found 350 ml of blood shift which raised to 613 ml at the end of the peal of cough. Therefore, the amount of blood outflow is likely to be more relevant during prolonged coughing and our results fit with this consideration. In fact, coughing at TLC was associated to lengthened duration of both the compressive and the expulsive phase and the highest blood shift. Our blood shift values during single cough ranged from 400 to 700 ml and they are comparable with the ones found by Smith et al. Moreover, the value of 51 ml of blood forced out the trunk during quiet breathing is similar to results previously published^[11] in the literature.. Interestingly, the amount of blood outflow during the compressive phase of voluntary cough resulted to be almost constant within the three different operating volumes. This can be explained by the fact that the peak gastric and oesophageal pressures, being respectively indices of pleural and abdominal pressure, did not significantly change with increasing operating volume. Therefore, because it was found that increases in abdominal pressure augmented the splanchnic blood output^[11] we can explain the different behaviour found in V_{BS} during the compressive and the expulsive phases with their durations. Even though the compressive

phase lengthened with increasing operating volume up to 322 msec, it is still too short compared to the emptying time constant of the whole splanchnic vascular bed^[11]. On the other hand, the expulsive phase prolonged more than 1.5 sec at TLC and therefore the same driving pressure has the time to displace augmented quantities of blood out of the trunk with increasing operating volume. During inspiration, negative intrathoracic pressure is generated by the contraction of the intercostal muscles and the diaphragm which descends making the pleural pressure to fall and the abdominal to increase. The diaphragm has also a circulatory function producing an oscillatory composition of inferior vena caval blood which favours the splanchnic venous return during inspiration and the venous return of blood below the entry of the hepatic vein during expiration. Maybe such hemodynamic effect of diaphragm contractions and the effects of high pleural pressure on venous return could explain the high variability we found in V_{BS} during the inspiratory cough phase. In fact, we found high values of pleural pressure which increase both during inspiration and the compressive phase which can be considered a short Valsava maneuver because muscles contract against a closed glottis, compressing the alveolar gas and reaching intrathoracic pressure value as great as 300 cmH₂O^[14]. These considerations on the driving pressures during cough could be criticized because we have measured oesophageal and gastric pressures only in one subject, but the absence of correlation between peak pressures and operating volume had already been published^[13]. It's also interesting to notice how the same expiratory driving pressures had different effects on chest wall volume variations at the end of cough being lower when cough starts from diminished lung volumes. Therefore, in order to provide efficient cough, the muscle contraction acted in order to recruit the expiratory reserve volume of about 1 liter.

In conclusion, we believe that the expiratory peak pressure values and timing during single voluntary cough are adequate to activate the abdominal circulatory pump which, under specific circumstances, was proved to produce from the splanchnic bed a cardiac output higher than the resting one. Unfortunately, we did not make any arterial pressure wave or cardiac output measurements, although compatible with the double body plethysmography^[12], that could have helped understanding the important hemodynamic cough effect on the heart able to ensure blood flow during ventricular fibrillation. We are aware that our experimental setup was completely different than the studies on cough resuscitation because our subjects were healthy, seated and not during ventricular

fibrillation. Our findings suggested that vigorous coughing and the consequent fluctuations in pleural and abdominal pressures not only activate the thoracic pump mechanism, which leads the low-resistance pulmonary veins to empty into the left heart, but also the abdominal pump, which displaces splanchnic blood to other body tissues.

Moreover, because cough cardiopulmonary resuscitation required rhythmic coughing every one to three seconds, future studies could measure V_{BS} during peaks of cough. As previously shown^[13], we would expect blood shift even to increase because the presence of a competent aortic valve and peripheral vascular tone maintains a higher pressure in the aorta between coughs.

To summarize, double body plethysmography allows to measure the volume of blood shifted from the trunk to the periphery in normal subjects during the different cough phases. We have investigated the effect of operating volume and cough duration as well. Operating volumes do not influence the amount of blood outflow the compressive phase which was around 250 ml, whereas during the expulsive phase blood shift ranged from 400 to 700 ml with increasing operating volume. The inspiratory cough phase resulted highly variable because of the hemodynamic effects of the diaphragm. Vigorous voluntary cough activate the abdominal circulatory pump which can have an important role during cough cardiopulmonary resuscitation.

3.5 REFERENCES

1. Criley JM, Blaufuss AH, Kissel GL. Self-administered cardiopulmonary resuscitation by cough-induced cardiac compression. *Trans Am Clin Climatol Assoc.* 1976;87:138-46.
2. Criley JM, Blaufuss AH, Kissel GL. Cough-induced cardiac compression. Self-administered form of cardiopulmonary resuscitation. *JAMA.* 1976 Sep 13;236(11):1246-50
3. Niemann JT, Rosborough JP, Niskanen RA, Alferness C, Criley JM. Mechanical "cough" cardiopulmonary resuscitation during cardiac arrest in dogs. *Am J Cardiol.* 1985 Jan 1;55(1):199-204.
4. Miller B, Cohen A, Serio A, Bettock D. Hemodynamics of cough cardiopulmonary resuscitation in a patient with sustained torsades de pointes/ventricular flutter. *J Emerg Med.* 1994 Sep-Oct;12(5):627-32.
5. Jafary FH. Cough-assisted maintenance of perfusion during asystole. *Can J Cardiol.* 2008 Oct;24(10):e76.
6. Criley JM, Niemann JT, Rosborough JP, Hausknecht M. Modifications of cardiopulmonary resuscitation based on the cough. *Circulation.* 1986 Dec;74(6 Pt 2):IV42-50
7. McLachlan CS, Yow SZ, Al-Anazi M, El Oakley RM. Letter by McLachlan et al regarding article, "Cough cardiopulmonary resuscitation revisited". *Circulation.* 2007 May 15;115(19):e460; author reply e461
8. Girsky MJ, Criley JM. Images in cardiovascular medicine. Cough cardiopulmonary resuscitation revisited. *Circulation.* 2006 Oct 10;114(15):e530-1.
9. Cohen A, Gottdiener J, Wish M, Fletcher R. Limitations of cough in maintaining blood flow during asystole: assessment by two-dimensional and Doppler echocardiography. *Am Heart J.* 1989 Sep;118(3):474-9.
10. Mauer D, Wolcke B, Dick W. Alternative methods of mechanical cardiopulmonary resuscitation. *Resuscitation.* 2000 Apr;44(2):81-95. Review.
11. Aliverti A, Bovio D, Fullin I, Dellacà RL, Lo Mauro A, Pedotti A, Macklem PT. The abdominal circulatory pump. *PLoS One.* 2009;4(5).
12. Aliverti A, Uva B, Laviola M, Bovio D, Lo Mauro A, Tarperi C, Colombo E, Loomas B, Pedotti A, Similowski T, Macklem PT. Concomitant ventilatory and

- circulatory functions of the diaphragm and abdominal muscles. *J Appl Physiol*. 2010 Nov;109(5):1432-40.
13. Smith JA, Aliverti A, Quaranta M, McGuinness K, Kelsall A, Earis J, Calverley PM. Chest wall dynamics during voluntary and induced cough in healthy volunteers. *J Physiol*. 2012 Feb 1;590(Pt 3):563-74
 13. Cala SJ, Kenyon CM, Ferrigno G, Carnevali P, Aliverti A, Pedotti A, Macklem PT, Rochester DF. Chest wall and lung volume estimation by optical reflectance motion analysis. *J Appl Physiol*. 1996 Dec;81(6):2680-9
 14. Boitano LJ. Management of airway clearance in neuromuscular disease. *Respir Care*. 2006 Aug;51(8):913-22

Chapter 4: **Determinants of cough efficiency in Duchenne muscular dystrophy**

Keywords: cough, Duchenne muscular dystrophy, respiratory muscles, abdomen, chest wall

4.1 INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked recessive hereditary disease that affects both skeletal and cardiac muscle over time. With the progression of the disease and the deterioration of respiratory muscles function, cough, i.e. the ability to clear the airway of secretions, becomes critical. Respiratory muscle weakness, impaired secretion clearance and mucus plugging further compromise chest infections resulting in recurrent episodes of atelectasis, pneumonia and respiratory insufficiency^[1,2]. Several factors conspire to diminish the effectiveness of cough in DMD patients: the neuromuscular weakness of inspiratory muscles, limiting the depth of pre-cough inspiration, and expiratory muscles in association with chest wall distortion from scoliosis resulting in reduced intrathoracic expiratory pressures and flows^[3]. The assessment of cough effectiveness consider a set of different functional parameters obtained from both noninvasive and invasive volitional tests. The former include the assessment of peak cough flow (PCF), spirometric indexes as FVC and FEV₁^[4], maximal inspiratory and expiratory pressures (MIP and MEP)^[5,6], the latter maximal cough gastric pressure^[7].

Among these parameters, PCF is the most commonly used in the clinical practice. DMD patients with PCF >270 L/min are felt to have an adequate cough whereas PCF <160 L/min is associated with inefficient cough^[8], unable to provide enough mucociliary clearance. When PCF is 160-270 L/min, cough is considered adequate when patients are well, but is likely to fall below 160 during chest infection and viral illness.

Despite the clinical relevance of the problem, however, there is scanty literature describing the mechanisms that determine inefficient cough in DMD patients.

As in healthy subjects the volume inspired prior to coughing significantly influence the mechanical changes during the expiratory phase of coughing and therefore PCF^[9], one possible cause of inefficient cough in DMD is the reduced operating lung and chest wall volume secondary to weakened inspiratory muscles. The aim of the present study was to investigate if thoraco-abdominal operating volumes during coughing determine the effectiveness of cough in DMD patients. Furthermore, as the abdominal contribution to tidal volume during spontaneous breathing is, in these patients, a strong indicator of diaphragmatic impairment^[10] and a predictor of the onset of nocturnal desaturation^[11], we hypothesized that this parameter could also represent a non-volitional and noninvasive index discriminating efficient and inefficient cough.

4.2 MATERIALS AND METHODS

Subjects

36 DMD subjects attending IRCCS “E. Medea” Institute as inpatients for periodic clinical assessment, and 15 age-matched healthy male subjects recruited as controls were included in the study. In all patients, clinical information including mutations in the DMD gene, use of noninvasive mechanical ventilation and cough assistive devices, lung infection proneness, use of steroids, cardiac dysfunction, severity of scoliosis and spinal fusion were recorded.

All subjects or parents signed a written informed consent form, as approved by the Local Ethical Committee of IRCCS “E. Medea” Institute. The study was approved by the Institute’s Human Ethics Committee according to the declaration of Helsinki.

Measurements and protocol

Pulmonary function tests

Measurements of forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), subdivision of lung volumes (Functional Residual Capacity, FRC; Residual Volume, RV and Total Lung Capacity, TLC) by the nitrogen washout technique were performed (Vmax series 22, SensorMedics, Yorba Linda, CA). Nocturnal oxygen saturation (SpO₂) was measured using a digital pulse oximeter (Nonin, 8500 digital pulse oximeter Quitman, TX).

Respiratory muscle strength

Maximal respiratory pressures were measured at the mouth (MicroRPM; Micro Medical Ltd., Rochester, England). Maximal expiratory pressure (MEP) and maximal inspiratory pressure (MIP) were performed starting respectively from TLC and RV and the effort was maintained for at least one second. The best MEP and MIP values in two or more attempts were chosen.

Peak cough flow (PCF)

Unassisted peak cough flow (PCF) was measured by a portable peak flow-meter (Vitalograph, Ennis, Ireland). Patients were asked to cough as much as possible at least twice and the highest value in each test was chosen.

Optoelectronic Plethysmography

Total and compartmental chest wall volumes were measured by Opto-Electronic Plethysmography (OEP System; BTS, Milan, Italy). The system, based on eight infrared video cameras working at a sampling rate of 60 Hz, computes the 3D coordinates of 52 retro-reflective markers placed, according to specific anatomical points, over the anterior chest wall surface of the subject lying supine (figure 4.1). After a quiet breathing period of stabilization, patients and healthy controls were asked to perform three single maximal voluntary coughs separated by at least 4 or 5 breaths in supine position. No specific instructions regarding operating volume were given to the subjects. For each subject, the cough maneuver with maximal inspired chest wall volume was considered for further analysis.



Figure 4.1: Experimental set-up for the analysis of chest wall volumes in supine position via opto-electronic plethysmography in a DMD patient

The volume of total chest wall (V_{CW}) and its two compartments, rib cage (V_{RC}) and abdomen (V_{AB}), were noninvasively measured by OEP. The points of start inspiration, end

inspiration and end cough were identified on V_{CW} and on the two compartments at the same instants. Tidal volume (V_T), as the average of total V_{CW} variations, and its percentage contribution of V_{AB} were computed during the spontaneous breaths preceding cough (Figure 4.2).

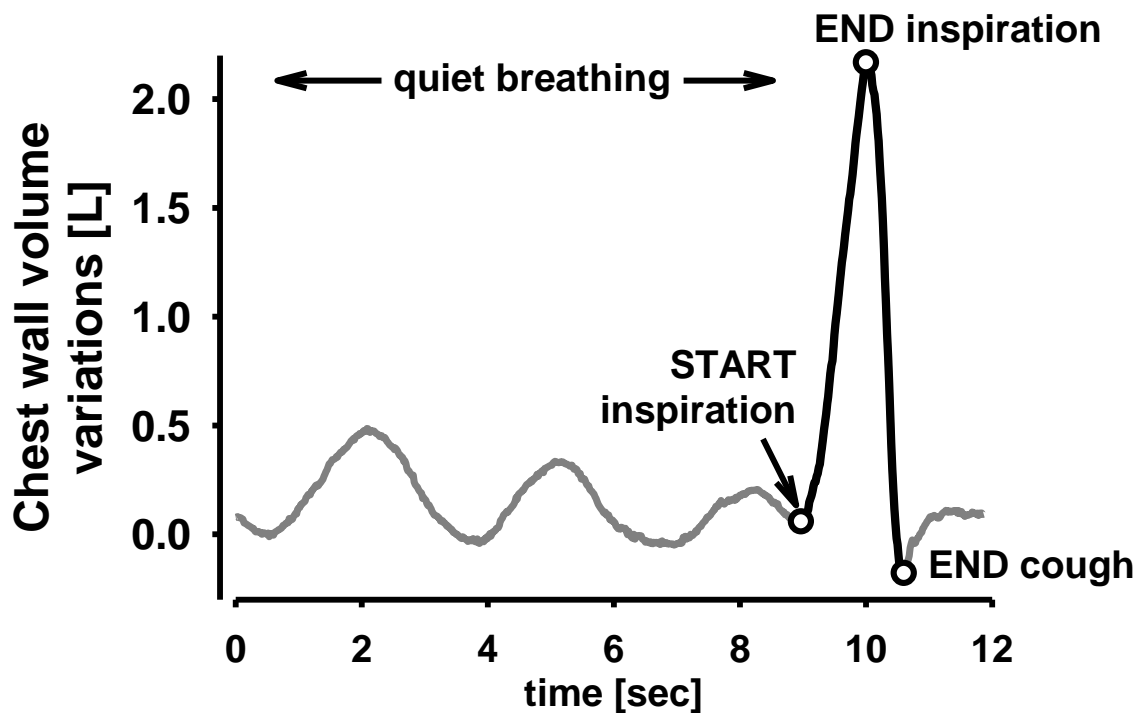


Figure 4.2: Time course of total chest wall volume variation during a period of quiet breathing (grey) followed by a single voluntary cough maneuver (black) in a representative healthy control subject. Open symbols indicate the points of start inspiration, end inspiration and end cough used for the analysis.

Subdivision of DMD patients' group

The DMD population was subdivided into three groups according to PCF: patients with inefficient cough ($PCF < 160$ L/min), adequate cough ($PCF > 270$ L/min) and adequate cough when they are well ($160 < PCF < 270$ L/min).

Statistical analysis

Comparison between groups were conducted using a one-way Analysis of Variance (ANOVA) with cough efficiency as independent factor. Post-hoc tests were based on Holm-Sidak or Dunn's method. Receiver Operating Characteristic (ROC) curve analysis was performed to compare the diagnostic performance of those parameters that resulted significantly different between inefficient and adequate cough. For ROC analysis, adequate

cough was defined when PCF>160 L/min. Data are presented as mean \pm standard deviation (SD) unless otherwise specified. The 95% confidence interval of the area under the normalized ROC curve (AUC) and the optimal cut-off point were also computed as described by Hanley and McNeil^[12]. The level of significance was set at $p<0.05$ for all statistical analyses.

4.3 RESULTS

Clinical data

Patients belonging to the three groups and healthy subjects were similar in age, height and weight (Table 4.1).

	PCF<160 L/min	160<PCF<270 L/min	PCF>270 L/min	healthy subjects
N. patients	16	12	9	15
Age [yrs]	17.6 \pm 4.9	16.9 \pm 6.0	16.1 \pm 4.0	16.3 \pm 5.4
Height [cm]	161.6 \pm 10.0	157.7 \pm 16.4	159.8 \pm 16.8	168.3 \pm 17.6
Weight [Kg]	56.9 \pm 11.8	66.0 \pm 22.1	56.1 \pm 22.2	62.3 \pm 24.4
BMI	21.8 \pm 4.2	25.5 \pm 5.0	21.6 \pm 6.3	21.1 \pm 5.0

Table 4.1: Characteristics of Duchenne muscular dystrophy patients with inefficient cough (PCF<160 L/min), adequate cough when they are well (160<PCF<270 L/min), adequate cough (PCF>270 L/min) and healthy controls Data are expressed as mean \pm S.D. Body Mass Index computed as BMI=weight [Kg]/(height [m])²

Genetic and clinical characteristics of the patients are reported in Table 4.2. Noninvasive mechanical ventilation was used only in patients with inefficient cough (PCF<160 L/min). Cough assistance devices were used in the majority of patients with inefficient cough (13/16), in the half of patient with 160<PCF<270 L/min (6/12) and one third of patients with PCF>270 L/min (3/9). At least one episode of pneumonia or upper airway infection in the year preceding the study occurred in the majority (11/16) of patients with inefficient cough, and in half of the patients belonging to the other two groups. All patients had scoliosis, mostly severe in patients with inefficient cough, moderate in patients with 160<PCF<270 L/min and mild in patients with PCF>270 L/min. Spinal fusion was present only in patients with inefficient cough. The percentages of patients with heart dysfunction and under steroids treatment were similar in the three considered groups.

	PCF<160	160<PCF<270	PCF>270
	L/min	L/min	L/min
Total number of patients	16	12	9
Genetics			
Deletion	14	9	5
Duplication	1	2	2
point mutation	0	1	2
undefined mutation	1	0	0
Noninvasive Mech. Ventilation	6	0	0
Use of cough assisted devices	13	6	3
Pneumoniae/upper airways infections in the last year	11	6	5
Steroids treatment	8	7	5
Heart dysfunction (LVEF<50%)	9	5	5
Scoliosis			
severe (Cobb angle > 40°)	8	1	1
moderate (20° < Cobb angle < 40°)	5	7	3
mild (Cobb angle < 20°)	0	4	5
spinal fusion	3	0	0

Table 4.2: Clinical characteristics of Duchenne muscular dystrophy patients with inefficient (PCF<160 L/min), adequate cough when they are well (160<PCF<270 L/min) and adequate cough (PCF>270 L/min). Each value refers to the number of patients.

Respiratory function data

	PCF<160	160<PCF<270	PCF>270
	L/min	L/min	L/min
Spirometry			
n	15	11	9
FVC [L]	1.24 ± 0.61°	1.52 ± 0.48	2.28 ± 1.07
FVC [% pred]	34.6 ± 19.7°°	47.9 ± 28.0	64.2 ± 28.9
FEV ₁ [L·s ⁻¹]	1.04 ± 0.57°°	1.18 ± 0.48°	1.75 ± 0.69
FEV ₁ [%pred]	35.1 ± 22.7°	44.9 ± 32.3	61.9 ± 35.1
FEV ₁ /FVC [%]	83.7 ± 14.7	77.4 ± 17.2	78.9 ± 17.8
PEF [L·s ⁻¹]	2.21 ± 0.92	2.59 ± 1.08	3.33 ± 1.55
PEF [%pred]	31.3 ± 17.4	41.2 ± 25.2	52.1 ± 31.1
Lung volumes			
n	14	11	8
TLC [%pred]	52.0 ± 24.4°	67.4 ± 48.6	76.0 ± 27.1
RV [%pred]	105.3 ± 62.7	131.4 ± 159.2	111.6 ± 28.0
RV/TLC [%]	48.7 ± 16.2	41.8 ± 14.5	35.7 ± 9.0
FRC N ₂ [%pred]	63.8 ± 33.0	86.6 ± 89.9	86.9 ± 28.9
Nocturnal SpO₂ (% night time)			
n	15	12	8
95%<SpO ₂ <100%	81.7 ± 27.3	88.7 ± 24.6	85.2 ± 27.4
90%<SpO ₂ <94%	11.3 ± 16.6	10.2 ± 23.5	14.7 ± 27.4
SpO ₂ <90%	0.5 ± 1.1	1.0 ± 2.5	0.0 ± 0.0
Respiratory muscles pressures			
n	13	11	8
MIP [cmH ₂ O]	19.2 ± 9.1° [#]	30.2 ± 7.3	35.4 ± 16.5
MEP [cmH ₂ O]	22.0 ± 12.0°	30.1 ± 11.3	36.2 ± 11.7

Table 4.3: Pulmonary function test and respiratory muscles performance in Duchenne muscular dystrophy patients with inefficient cough (PCF<160 L/min), adequate cough when they are well (160<PCF<270 L/min) and adequate cough (PCF>270 L/min). Data are expressed as mean±S.D. FVC: forced vital capacity; FEV₁: forced expiratory volume in 1 s; PEF: peak expiratory flow; TLC: total lung capacity; RV: residual volume; FRC N₂: functional residual capacity measured by N₂ washout; SpO₂: arterial oxygen saturation measured by pulse oxymetry; MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; %pred: percentage of predicted value; n: number of patients with available data

°, °°: p<0.05, p<0.01 (vs PCF>270 L/min)

[#]: p<0.05 (vs 160<PCF<270 L/min)

Inefficient cough was associated to worse spirometric parameters (namely, FVC and FEV₁), reduced lung volumes (IC, ERV and TLC%pred) and maximal pressures (Table 4.3 and Figure 4.3). No differences in the three groups were found in oxygen saturation (SpO₂) during night.

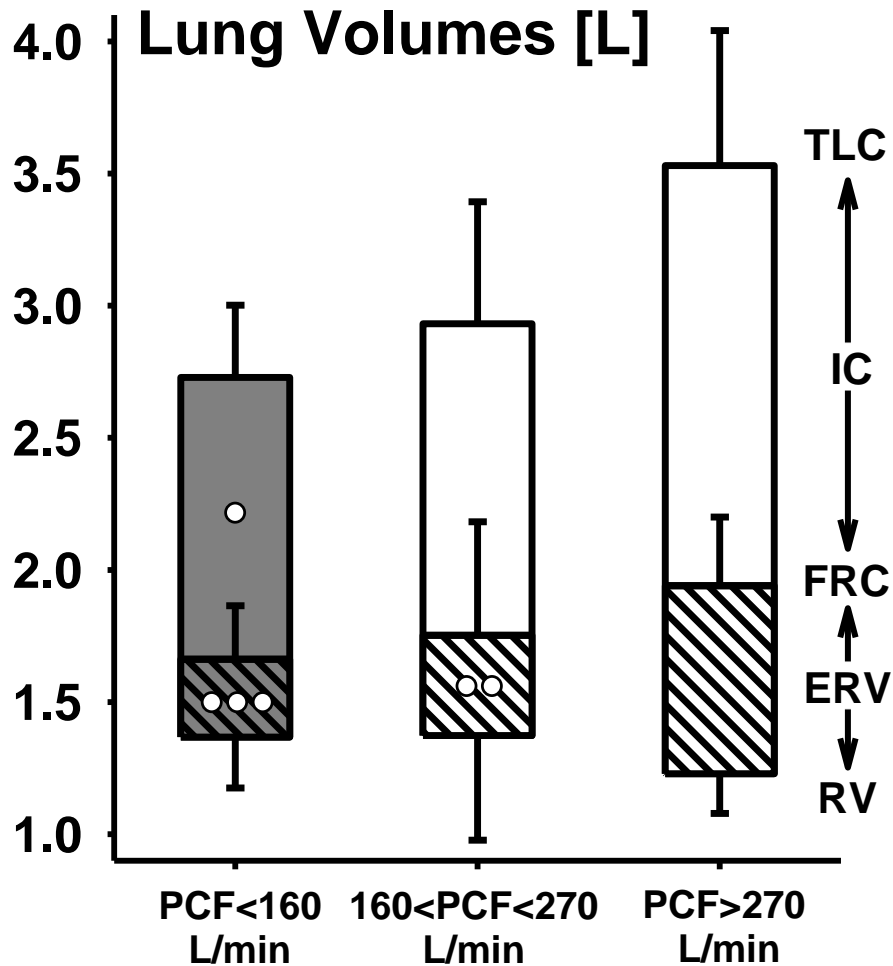


Figure 4.3: Average values \pm SE of absolute (TLC: total lung capacity; FRC: functional residual capacity; RV: residual volume) and relative (IC: inspiratory capacity; ERV: expiratory reserve volume) lung volumes in DMD patients with inefficient (grey) and adequate (white) cough. Dashed areas indicate ERV. °, °°, °°° : $p < 0.05$, < 0.01 and < 0.001 (vs PCF > 270 L/min).

Chest wall volume variations

As shown in Figure 4.4, in patients with inefficient cough, at the end of the inspiration preceding cough maneuver, the volume of the total chest wall was significantly reduced compared to healthy controls and this was due to the reduced inspired volumes of both rib cage and abdomen

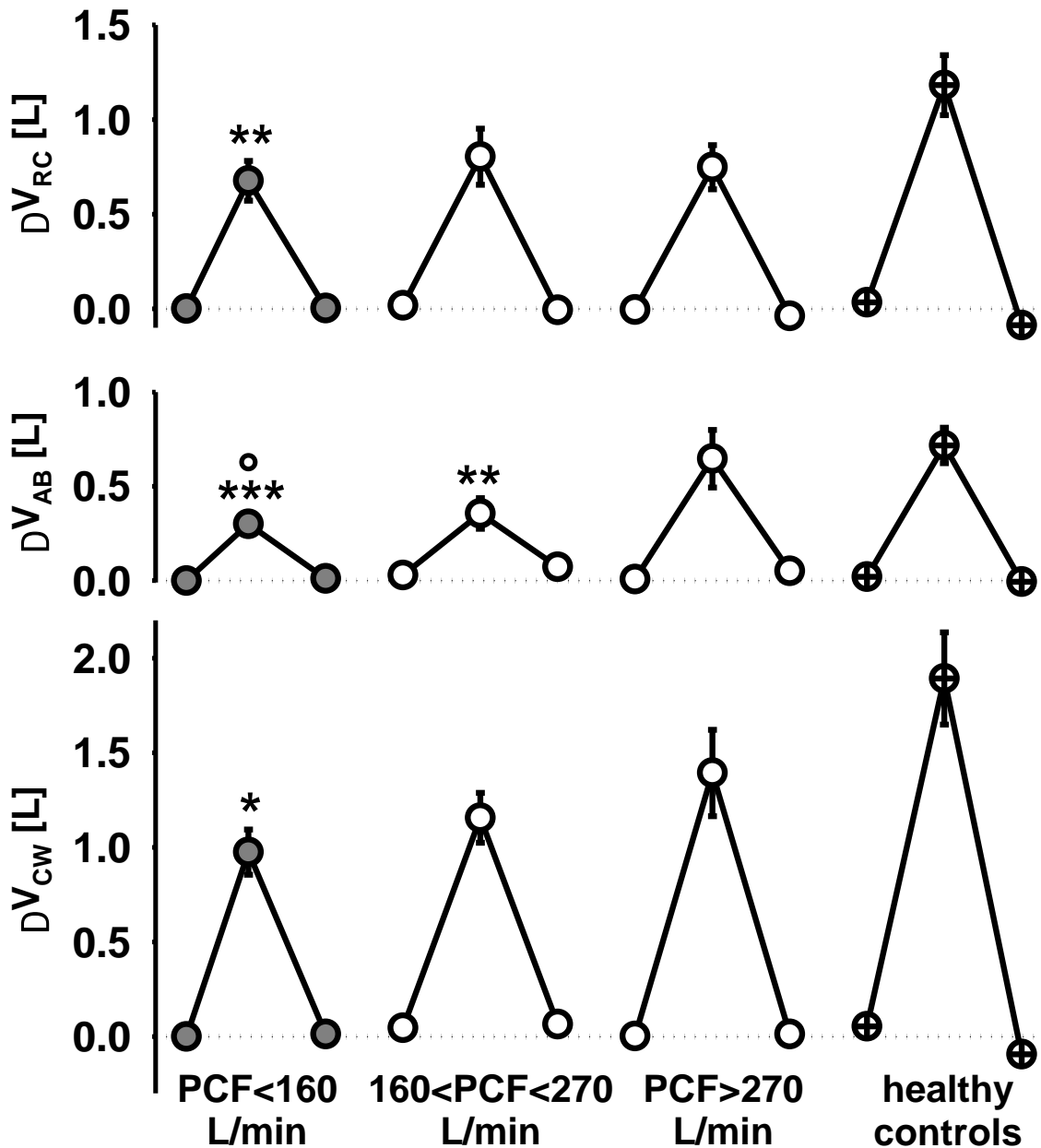


Figure 4.4: Average values \pm SE of rib cage (top panel), abdominal (middle panels) and total chest wall (bottom panel) volume variations at start inspiration, end inspiration and end cough in DMD patients with ineffective cough (close symbols), adequate cough (open symbols) and healthy subjects (crossed symbol). *, **, *** : $p < 0.05$, $p < 0.01$, $p < 0.001$ (vs healthy subjects) ° : $p < 0.05$ (vs PCF > 270 L/min)

Conversely, in patients with PCF>270 L/min total and compartmental inspired volumes preceding cough were not significantly different from controls. In patients with 160<PCF<270 L/min, inspired volume was lower than controls only in abdominal compartment.

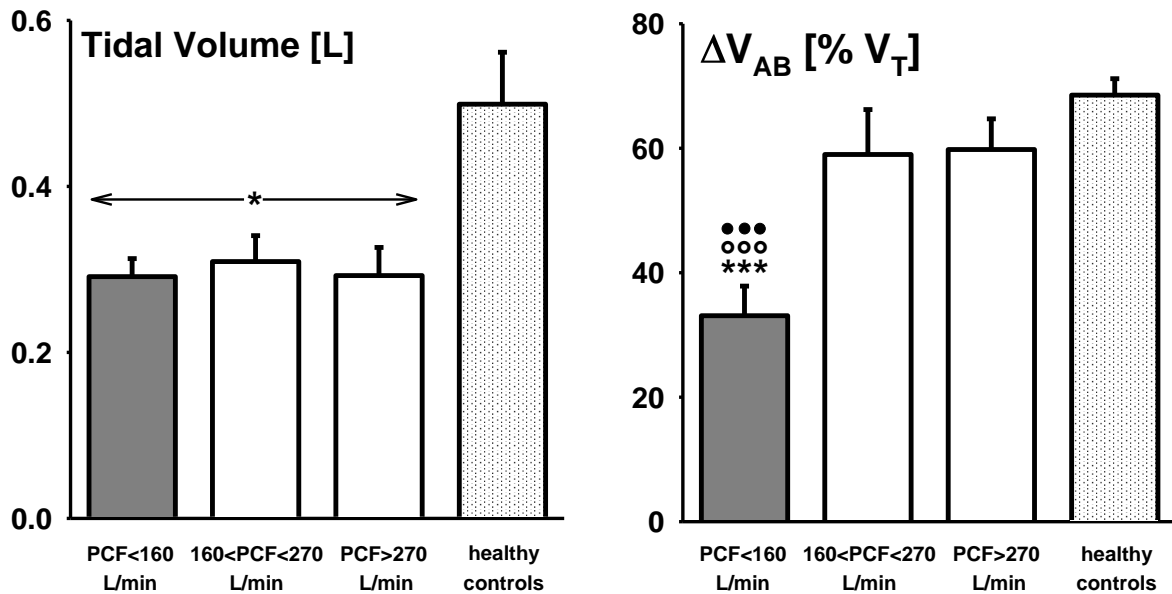


Figure 4.5: Average values±SE of tidal volume (left panel) and its abdominal percentage contribution (right panel) during quiet breathing preceding cough in DMD patients with ineffective cough (grey bars), adequate cough (white bars) and healthy subjects (dotted bars).

*, *** : $p < 0.05, 0.001$ (vs healthy subjects) ; ○○○: $p < 0.001$ (vs PCF>270 L/min); ###: $p < 0.001$ (vs 160<PCF<270 L/min)

During the period of spontaneous quiet breathing preceding cough maneuvers, all DMD patients showed a reduced tidal volume (Figure 4.5, left), however, only patients with inefficient cough did show an abdominal contribution to tidal volume significantly reduced compared to both patients with adequate cough and healthy controls (Figure 4.5, right).

Figure 4.6 shows rib cage, abdominal and total chest wall volume variations in representative cases of patients' and control groups.

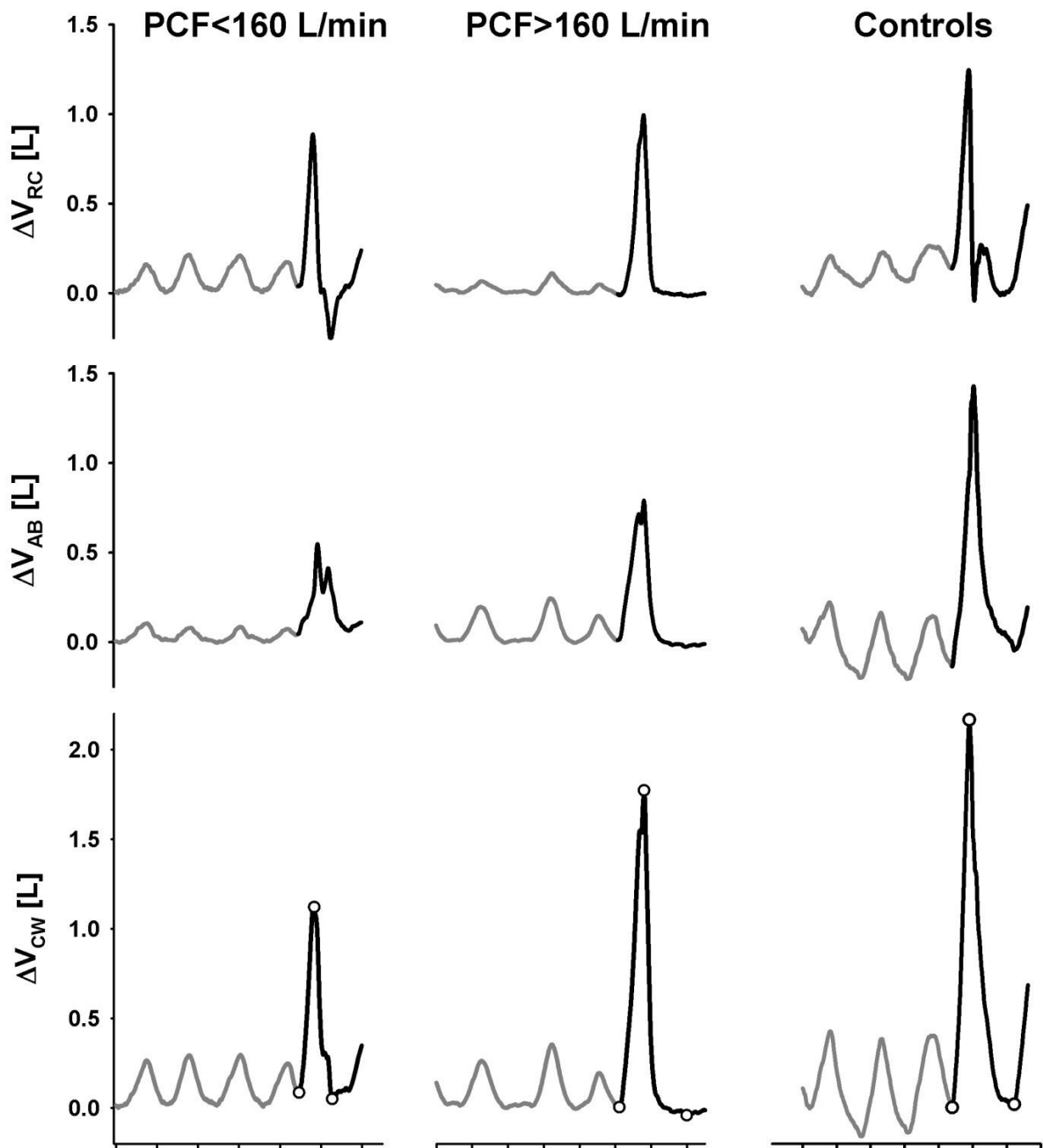


Figure 4.6: Representative cases of thoraco-abdominal volume changes during spontaneous breathing and a single cough maneuver in DMD patients with inefficient cough ($PCF < 160$ L/min), adequate cough ($PCF > 160$ L/min) and healthy controls. Upper panels: volume changes of the ribcage (ΔV_{RC}); middle panels: volume changes of the abdomen (ΔV_{AB}); lower panels: volume changes of the total chest wall (ΔV_{CW}). During quiet breathing, in DMD patients tidal volume is similar but lower than controls. With increasing cough efficiency, abdomen and rib cage volume variations respectively increase and decrease. Inspired volume preceding cough maneuver increases with increasing cough efficiency.

Receiver operating characteristic (ROC) analysis

In Table 4.4 the area under the ROC curve (AUC), the optimal cut-off, the resulting sensitivity and specificity values of the parameters that resulted to be significantly different between inefficient and adequate cough in DMD are shown. These included FVC, FEV₁ and TLC expressed as percentage of predicted value (FVC(%pred), FEV₁(%pred) and TLC(%pred), respectively); MIP and MEP; abdominal percentage contribution to inspiratory cough phase, ΔV_{AB} (%ICP); the abdominal percentage contribution to tidal volume, ΔV_{AB} (%V_T).

Parameter	AUC	Optimal cut-off point	Sensitivity (%)	Specificity (%)
FVC (%pred)	0.73	30.5	76.5	61.5
FEV ₁ (%pred)	0.71	19.0	94.1	46.2
TLC (%pred)	0.64	52.5	68.7	58.3
MIP (cmH ₂ O)	0.82	23.5	82.3	76.9
MEP (cmH ₂ O)	0.75	18.5	93.7	61.5
ΔV_{AB} (%ICP)	0.67	31.2	76.5	69.2
ΔV_{AB} (%V _T)	0.91	46.0	88.2	84.6

Table 4.4: Results of ROC analysis performed on the parameters which resulted different between inefficient and efficient cough, namely: FVC: forced vital capacity; FEV₁: forced expiratory volume in one second; TLC: total lung capacity; %pred: percentage of predicted value, MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; ΔV_{AB} (%ICP): abdominal percentage contribution to inspiratory cough phase; ΔV_{AB} (%V_T): abdominal percentage contribution to tidal volume. Cough was considered adequate when PCF > 160 L/min, inefficient when PCF < 160 L/min. AUC: area under the curve.

The highest AUC was found for the abdominal percentage contribution to tidal volume, ΔV_{AB} (%V_T). Figure 4.7, reporting ROC curves for all the considered parameters, indicates that the curve of ΔV_{AB} (%V_T) was the closest to the upper left corner compared to all the other curves for any value of sensitivity and specificity.

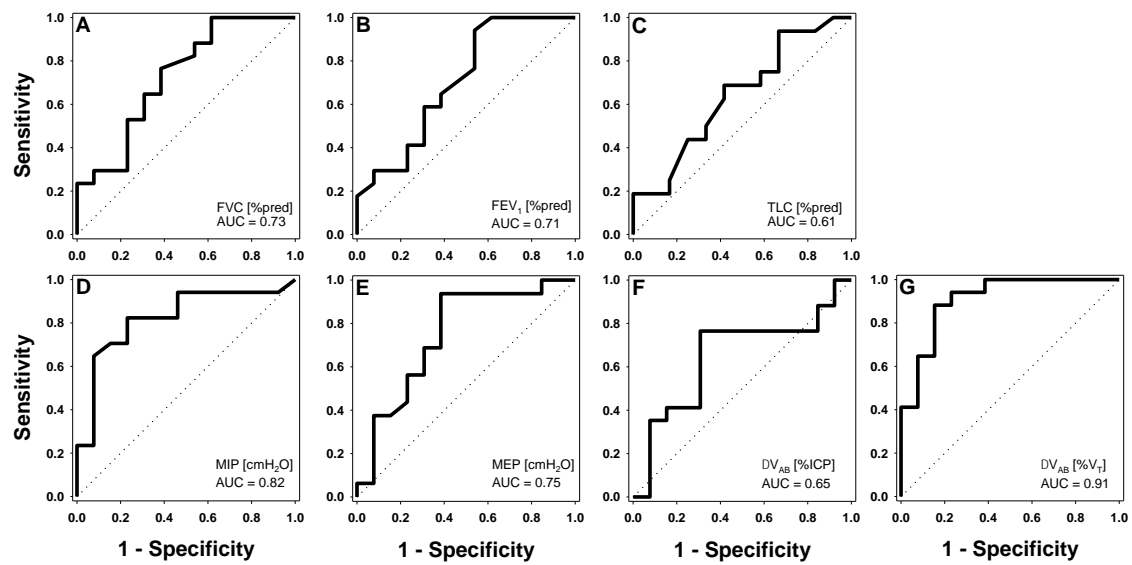


Figure 4.7: ROC curves of predicted Forced Vital Capacity (FVC [%pred]), predicted Forced Expired Volume in one second (FEV₁ [%pred]), predicted Total Lung Capacity (TLC [%pred]), Maximal Inspiratory Pressure (MIP), Maximal Expiratory Pressure (MEP), abdominal percentage contribution to Inspiratory Cough Phase (ΔV_{AB} [%ICP]) and to tidal volume (ΔV_{AB} [%V_T]) considering cough adequate when PCF > 160 L/min, inefficient when PCF < 160 L/min.

4.4 DISCUSSION

To the best of our knowledge, this study is the first that specifically examine how the pattern of total and compartmental chest wall volume variations measured during cough and spontaneous breathing are linked to cough effectiveness in DMD patients. We have shown that the volume inspired prior to coughing is significantly reduced in patients with inefficient cough and that the abdominal contribution to tidal volume during spontaneous breathing is a strong predictor of cough efficiency in DMD patients.

The inspired volume preceding coughing is important because it determines not only the volume of air that can be expelled but also the length-tension relationship of the expiratory muscles, i.e. their ability of producing force. In DMD the weakness of the inspiratory muscles is therefore an important limiting factor of cough efficacy, as it reduces the inspired volume before cough maneuver^[3].

Previous studies have postulated the relationship between inspired volume and cough efficiency on the basis of correlation analysis between peak cough flow and other functional variables as MIP, MEP, FVC and FEV₁^[5,6] made on pooled data. Differently, in the present study we have grouped our cohort of DMD patients according to cough efficiency clinically assessed by PCF. Successively, we studied which parameters significantly differed among groups, considering in our analysis not only the traditional spirometric and MIP/MEP evaluation, but also total and compartmental chest wall volume variations during cough maneuver and spontaneous breathing assessed by opto-electronic plethysmography. Finally, by ROC analysis the best predictors of cough efficiency in DMD were identified.

Our results indicate that the pattern of chest wall volumes changes significantly differ between groups with efficient and inefficient cough, during not only coughing but also spontaneous breathing. While total chest wall volume variation during inspiration preceding cough was significantly lower than controls only in patients with inefficient cough, tidal volume during spontaneous breathing was lower in all DMD patients. This means that total chest wall volume variations (reflecting lung volume variations) can discriminate the efficiency of cough only when considering the cough maneuver *per se*. Conversely, when considering volume variations of the two chest wall compartments, namely the rib cage and the abdomen, significant differences are present also during quiet

breathing. In fact, patients with $PCF < 160$ L/min have a strongly reduced abdominal contribution to tidal volume compared not only to controls but also to patients with efficient cough. In addition, during the inspiration preceding cough abdominal volume changes are helpful to distinguish, with patients with efficient cough, the two groups (with $PCF < 270$ and $PCF > 270$). Patients with efficient cough only when they are well ($PCF < 270$) have reduced contribution of abdomen to inspired volume, suggesting inefficiency of inspiratory muscles.

These results regarding abdominal volume indicate that what really determines cough efficiency is diaphragm contribution to volume displacement both during quiet breathing and inspiration preceding cough.

An important implication of our study is that standard measurements of ventilation at the mouth (e.g. provided by a flowmeter) are not useful in order to discriminate between patients with and without efficient cough. On the other hand, accurate measurements of thoraco-abdominal volume variations are able to predict cough efficiency even during quiet breathing without requiring neither patient's collaboration nor the use of a mouthpiece.

Recently, our group showed that in DMD patients abdominal contribution to volume variations in supine position is a parameter that tracks the progressive diaphragmatic impairment^[10]. In addition, it allows to discriminate between adolescents and adults patients who present either no sign or only mild nocturnal oxygen desaturation^[11] with an optimal cut-off point for $V_{ab}(\%V_t)$ equal to 36.2%. In the present study we have shown that abdominal volume variations are also able to discriminate patients with and without efficient cough (optimal cut-off equal to 46%). The difference in the cut-off values of abdominal contribution can be explained by considering that in the progression of the disease the occurrence of cough inefficiency precedes the early signs of nocturnal oxygen desaturation when the diaphragm, and consequently abdominal volume contribution, is less compromised. In contrast, we found very similar cut-off thresholds for $FVC(\%pred)$, respectively 31% and 30.5%. Interestingly, a cut-off threshold of 30% for $FVC(\%pred)$ has also been found in another recent study to identify patients at high risk for severe chest infections^[1]. All these data suggest that $FVC(\%pred)$, although largely adopted in clinical practice, cannot directly be linked to the mechanisms underlying the progressive loss of cough efficiency and the onset of nocturnal oxygen desaturation, for which the weakness

of inspiratory muscles plays an important role. Abdominal volume contribution to inspired volume, therefore, can help determine when either cough assisting devices or noninvasive ventilation is needed or, at least, further evaluation is required.

The relatively low number of subjects could represent a limitation of this work, although our cohort is composed of only patients with DMD homogeneous in age, anthropometric parameters and clinical history. Another possible limitation is that OEP technique, although widely validated in previous studies in healthy and pathological subjects, is not yet widely diffused into clinics. The great advantage of OEP, however, is that accurate measurements of thoraco-abdominal volumes by OEP can be obtained non-invasively and without volitional tests.

In conclusions, we have shown that inefficient cough in DMD is associated to reduced operating lung and chest wall volume secondary to weakened inspiratory muscles. In addition, abdominal contribution to tidal volume during spontaneous breathing represents a non-volitional and noninvasive index able to discriminate efficient and inefficient cough.

4.5 REFERENCES

1. Dohna-Schwake C, Ragette R, Teschler H, Voit T, Mellies U. Predictors of severe chest infections in pediatric neuromuscular disorders. *Neuromuscul Disord*. 2006 May;16(5):325-8
2. Bushby K, Finkel R, Birnkrant D.J. DMD Care Considerations Working Group. Diagnosis and management of Duchenne muscular dystrophy, part 1: Diagnosis, and pharmacological and psychosocial management. *Lancet Neurol*. 2010;9:77–93.
3. Kravitz RM. Airway clearance in Duchenne muscular dystrophy. *Pediatrics*. 2009;123 Suppl 4:S231-5.
4. Gauld LM, Boynton A. Relationship between peak cough flow and spirometry in Duchenne muscular dystrophy. *Pediatr Pulmonol*. 2005;39(5):457-60
5. Kang SW, Kang YS, Sohn HS et al. Respiratory muscle strength and cough capacity in patients with Duchenne muscular dystrophy. *Yonsei Med J*. 2006;47(2):184-90.
6. Park JH, Kang SW, Lee SC et al. How respiratory muscle strength correlates with cough capacity in patients with respiratory muscle weakness. *Yonsei Med J*. 2010;51(3):392-7.
7. Nicot F, Hart N, Forin V, et al. Respiratory muscle testing: a valuable tool for children with neuromuscular disorders. *Am J Respir Crit Care Med*. 2006;174(1):67-74.
8. Bach JR, Saporito LR. Criteria for extubation and tracheostomy tube removal for patients with ventilatory failure. A different approach to weaning. *Chest*. 1996;110(6):1566-71.
9. Smith JA, Aliverti A, Quaranta M et al. Chest wall dynamics during voluntary and induced cough in healthy volunteers. *J Physiol*. 2012;590(Pt 3):563-74.
10. Lo Mauro A, D'Angelo MG, Romei M et al. Abdominal volume contribution to tidal volume as an early indicator of respiratory impairment in Duchenne muscular dystrophy. *Eur Respir J*. 2010;35(5):1118-25.
11. Romei M, D'Angelo MG, LoMauro A et al. Low abdominal contribution to breathing as daytime predictor of nocturnal desaturation in adolescents and young adults with Duchenne Muscular Dystrophy. *Respir Med*. 2012;106(2):276-83.

12. McNeil BJ, Hanley JA. Statistical approaches to the analysis of receiver operating characteristic (ROC) curves. *Med Decis Making*. 1984;4(2):137-50.

Chapter 5 – Conclusions and future perspectives

We have performed a noninvasive multifactorial study of cough in which we have investigated the respiratory muscular pump, chest wall kinematics and the cardiovascular activity in the different phases of cough in healthy subjects as well as the determinants of its efficiency in Duchenne muscular dystrophy as we can take the following conclusions:

- the synergetic antagonistic activity of respiratory muscles leads to a specific pattern, in which the rib cage is the leading compartment;
- the abdominal muscles mainly act to lower the inferior ribs due to their insertions on the rib cage;
- the thoracic pattern during cough is not affected by either operating volume or posture despite the mechanical changes introduced;
- a task-specific somatosensory is active during voluntary healthy cough to recruit rib cage to lead cough despite different mechanical intervention on the chest wall;
- vigorous coughing and the consequent fluctuations in intrathoracic pressure not only activate the thoracic pump mechanism but also the abdominal pump;
- during maximal coughing 700 ml of splanchnic blood can be shifted between thorax and to other body tissues and this could be an important haemodynamic factor which can provide an explanation for cardio-pulmonary resuscitation based on cough;
- inefficient cough in DMD is associated to reduced operating lung and chest wall volume secondary to weakened inspiratory muscles;
- the abdominal contribution to tidal volume during spontaneous breathing represents a non-volitional and noninvasive index able to discriminate efficient and inefficient cough.

Based on these conclusions, on the methods proposed in the present thesis and in order to further understand the topics that make cough to be such an important mechanism we think that future studies can be focused on:

- the characterization of the respiratory pump, hemodynamic aspects, postural and operating volume influence during reflex and evoked cough as well as during peals of cough in healthy subjects;
- the combination of oesophageal and gastric pressures with all the methods described in order to provide a complete description of the mechanics of the cough in terms of force, displacements and muscular activation;

- the characterization of the respiratory pump on other neuromuscular disease such as Parkinson and various sclerosis in order to find the alterations induced by the pathology;
- the understanding of cough alteration induced by obstructive diseases, like COPD, or by glottis dysfunction;
- the quantification of blood shift and therefore of the hemodynamic cough effects in patients with cardiomyopathy who are the potential user of cough CPR in case of ventricular fibrillation during cardiac catheterization;
- the testing of the efficiency of rehabilitative intervention, both manual and mechanical thorough cough assist device, to improve airway's clearance;
- the correct onset with optimal setting up of cough assist device;
- the development of wearable dispositive to monitor cough events during long periods.

ACKNOWLEDGEMENTS

Alla fine di questo lungo percorso così speciale ci sono molte persone che desidero ringraziare:

- gli “sponsors Lo Mauro” che mi hanno dato la possibilità di raggiungere questo traguardo
- Fabio ed il suo karma: ammetto che funziona!
- Andrea da cui ho imparato tutto quello che mi ha permesso di arrivare fin qua
- LaManù che ha condiviso con me gioie e dolori di una seconda esperienza universitaria ed ora aspettiamo Leon!
- Sabri che anche ora si è rivelata la migliore compagna di tesi che si possa avere
- Jo e le sue “ragazze scatenate”: sei la mia sister
- Chiara per essere così amica e così “divina”
- Francesco ... perché è “GRATIS”
- Vale ufficialmente nominata mia PR
- CatePriskMaryGrape che allietano lo stare in TMB
- Bea, Raffo, Anninkia, Larissa, Andrea P (un regalo l’esserci ritrovati), gli amici dell’OSF e quelli dell’AIL, Joanna, Laura, CriLulù, Grazia (la mia dottoressa preferita) per il loro supporto ed incoraggiamento
- Marianna R, Francesco, il trio e le AMI per le acquisizioni di questa tesi
- i tesisti di questi ultimi 5 anni con i loro preziosissimi consigli ed appunti: Lorenzo, Stefano, Alice, Cristiano, Ilaria, Sebastiano e tutti quelli che ho sicuramente dimenticato
- Stefano per le sue candeline, Fra Andrea per le sue preghiere
- Ale e Veronica per le loro ripetizioni
- la penna di Dario Fo: prezioso amuleto in ogni esame
- i bambini e le famiglie del Dynamo Camp per avermi fatto capire che niente è impossibile

..... c’è un pizzico di ognuno di voi in questa tesi!

GRAZIE DI CUORE

... e quindi uscimmo a riveder le stelle
Dante Alighieri, Inferno XXXIV, 139