Diaphragm Ultrasound in Lung Transplant Patients: a prospective observational study of early diaphragmatic dysfunction

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Elise Jane Crothers declare that this thesis, is submitted in fulfilment of the requirements for the award of Master of Physiotherapy Thesis, in the Graduate School of Health at the University of Technology Sydney.

This is a conventional thesis which is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Abstract

Diaphragmatic dysfunction is a well-known complication after cardiothoracic surgery, but few studies have documented its incidence and consequences after lung transplantation. Previous research has demonstrated that patients with postoperative diaphragmatic dysfunction frequently require increased duration of mechanical ventilation, longer length of stay in Intensive Care Units, and longer length of stay in hospital due to compromised pulmonary function. Point-of-care ultrasound is emerging as a convenient, accurate, and non-invasive tool for assessing diaphragmatic function at the bedside. The aim of this thesis was to use of point-of-care ultrasound to prospectively report the incidence of diaphragmatic dysfunction after lung transplantation up to three months postoperatively, and evaluate its impact on clinical outcomes.

In our prospective observational study we documented the prevalence and natural history of diaphragmatic dysfunction in 27 lung transplant recipients using ultrasound preoperatively; then at one day, one week, one month, and three months postoperatively. The ultrasound methods used were diaphragmatic excursion during quiet breathing, deep breathing, voluntary sniff, and thickening fraction. Patients with and without diaphragmatic dysfunction according to each of these methods were compared for differences in clinical outcomes: duration of mechanical ventilation, length of stay (LOS) in Intensive Care (ICU), hospital LOS and discharge destination.

The prevalence of diaphragmatic dysfunction on all four outcome measures was highest at one day after transplant and then reduced over time. Diaphragmatic dysfunction, at three months after transplant was similar to preoperative measures, suggesting good recovery of diaphragmatic function within three months. No statistically significant differences in clinical

outcomes were found between those with diaphragmatic dysfunction compared to those without. However, the increase in hospital length of stay is likely clinically significant.

In conclusion, this thesis has demonstrated that early diaphragmatic dysfunction is common, but mostly recovers within three months after surgery. Ultrasound examination of diaphragmatic excursion during quiet breathing is the most useful method for clinical practice because it is valid, reliable, independent of patient effort, and can discriminate between those with and without dysfunction. Although our study did not find a negative impact on clinical outcomes of statistical significance, patients with diaphragmatic dysfunction had an increased hospital length of stay which is of clinical importance to investigate further. To fully understand whether diaphragmatic dysfunction is important in this cohort, future research will need to consider patient-centred outcomes and be across multiple sites to examine more of the lung transplant population.

CHAPTER 1: INTRODUCTION

The diaphragm is the primary muscle of inspiration, and its function is critical for optimal respiration. It is a dome-shaped musculotendinous membrane that divides the abdomen from the chest. The diaphragm is comprised of two halves, a right and left 'hemidiaphragm'. Each hemidiaphragm is supplied by its own phrenic nerve, arising from the C3-C5 nerve roots. As the muscle contracts it descends and flattens, generating a negative intrathoracic pressure which draws air into the lungs. As it relaxes it rises again causing passive exhalation. Weakness or 'dysfunction' of the diaphragm can therefore lead to ventilatory compromise.

1.1 What is diaphragmatic dysfunction?

By definition, diaphragmatic dysfunction can be viewed as weakness or paralysis of the diaphragm[1]. Physiologically, it is defined as a reduced ability of the diaphragm to generate a negative intrathoracic pressure sufficient for respiration[2]. Depending on the cause, diaphragmatic dysfunction may be partial or complete, unilateral or bilateral, temporary or permanent.

One of the earliest case reports of diaphragmatic paralysis was described by Comroe and colleagues in 1951[3]. The hallmark symptom of severe diaphragmatic dysfunction was dyspnoea in the supine position (orthopnoea). Subsequent reports of patients with severe diaphragmatic dysfunction all reported orthopnoea as the primary complaint[4]. In supine, the increased pressure of the abdominal contents on the weakened diaphragm, coupled with the mechanical inefficiency of the diaphragm in this position leads to an increased work of breathing, and thus, dyspnoea[4].

Diaphragmatic dysfunction can be caused by any lesion or disease process that interferes with diaphragmatic innervation, contractile muscle function, or mechanical coupling to the chest wall[5]. Principal causes of unilateral diaphragmatic dysfunction can be classified as traumatic lesions (such as surgical injury to the phrenic nerve); compression or infiltrative processes (e.g., cervical arthrosis and malignancy); inflammatory disease (e.g., vasculitis and shingles); central neurological disease (e.g., multiple sclerosis), and idiopathic[6,7]. The principal causes of bilateral diaphragm dysfunction can be classified as neurological disease (e.g., poliomyelitis and Guillain-Barre syndrome); myopathy (e.g., muscular dystrophies, amyloidosis, critical illness, and ventilator induced diaphragm dysfunction); connective tissue diseases (e.g., systemic lupus erythematosus), and idiopathic[7].

This thesis focuses on ultrasound assessment of diaphragmatic dysfunction arising after cardiothoracic surgery and in particular, lung transplantation. Dysfunction of the diaphragm after cardiothoracic surgery could be attributed to several causes including surgical injury to the diaphragm muscle or phrenic nerve, postoperative diaphragmatic fatigue or inhibition, altered chest wall mechanics, and extended periods of postoperative mechanical ventilation[7-11]. Injury to the ipsilateral phrenic nerve can result from accidental transection or traction[12] during surgical dissection, retraction of the mediastinum, and manipulation of the pericardium[13]. Transection leads to a paralysed diaphragm with paradoxical motion. Traction[3,10] may cause neuropraxis, leading to transient dysfunction characterised by either dyskinesia (paradoxical movement), akinesia (no movement), or hypokinesia (reduced movement) of the respective diaphragm[12]. Although ultrasound assessment will not provide information on the cause of dysfunction, it will identify whether the movement or contraction of one or both hemidiaphragms have been affected by the surgical process and allow us to monitor its progress during the postoperative period of recovery.

1.2 Incidence

The incidence of diaphragmatic dysfunction after a bilateral lung transplant has been previously reported to be as high as 41%[14]. However, the exact incidence with current medical and surgical management is unknown. Most previous research in this cohort has either been conducted retrospectively, or relied on traditional diagnostic methods (e.g., fluoroscopy) that are difficult to perform in the acute postoperative phase. In retrospective investigations, diaphragmatic function was only investigated upon clinical suspicion of dysfunction, and therefore cases of mild or early diaphragmatic dysfunction could have been missed. It was not until 2009[14] that standardised assessment methods using ultrasound were developed, and to date, these methods have not been used in the acute postoperative period; thus, the true incidence after lung transplant is unknown.

To date, five publications have investigated the incidence of diaphragmatic dysfunction after lung transplantation. First, Sheridan Jr and colleagues (1995)[15] conducted a prospective study of 27 lung transplant recipients (10 single and 17 double lung transplants) in the first postoperative week and diagnosed diaphragmatic dysfunction with nerve conduction studies, then confirmed with fluoroscopy. The overall incidence of diaphragmatic dysfunction was 8/27 patients (29.6%), with an incidence of 7/17 (41%) after bilateral lung transplant surgery. The diagnostic criteria for diaphragmatic dysfunction using nerve conduction studies was based on a wide value of 10ms for the diagnosis of abnormal phrenic latency[16]. Mean phrenic nerve latency in normal subjects is reported to be between 6.6 and 8.2ms with an upper limit between 9.0 and 10.0ms[16]. Mild diaphragmatic dysfunction which would have resulted in only slight prolongation of the phrenic nerve latency may have been missed by the generous upper limit, potentially underestimating the incidence of dysfunction.

Diagnosis using bedside ultrasound was first used in 1997 during a prospective evaluation of 27 lung transplant recipients (15 single lung and 12 double lung recipients) and 33 heart transplant recipients[17]. Patients were assessed three hours after extubation. Dysfunction was diagnosed if movement of the hemidiaphragm on ultrasound during a voluntary sniff maneuver (a forced inspiration through the nose with a closed mouth) was less than 2cm. The diagnosis was confirmed with fluoroscopy. Only 2 of the 27 lung transplant patients (7.4%) were diagnosed with diaphragmatic dysfunction. One explanation for this low incidence could be the ultrasound method used for diagnosis, which was to image diaphragmatic movement in the coronal plane (in B-Mode, described in detail in Chapter 2) during a voluntary sniff maneuver. This method has not been standardized or validated, so it is possible that this method or reference value is not sensitive enough to detect abnormal motion of the diaphragm.

A low incidence of diaphragmatic dysfunction (9.3%) was also reported in a retrospective chart evaluation of 97 (59 single and 52 double) lung transplantations[18]. Diaphragmatic dysfunction was only suspected when patients were unsuccessfully weaned from respiratory support, and then investigated with nerve conduction studies. However, mild degrees of diaphragmatic dysfunction may not have warranted investigation based on the criteria of unsuccessful weaning from respiratory support. A similar conclusion was reported in another retrospective review of 185 single and double lung transplantations[19]. Only six patients were identified (3.2%) as having a diagnosis of 'diaphragmatic paralysis' in the physician notes, which were confirmed by fluoroscopy or ultrasound. They reported that the true incidence of diaphragmatic dysfunction in their sample may be higher, as patients who were asymptomatic or had only a brief period of diaphragmatic dysfunction may not have warranted investigation.

Most recently, a prospective study of 30 bilateral lung transplant patients was published whereby diaphragmatic function was assessed using a comprehensive, multimodal protocol[13]. This protocol included ultrasonography of the right hemidiaphragm (measuring excursion, thickness and thickening fraction), electromyography (EMG) phrenic nerve stimulation, spirometry, mean inspiratory pressure, and 6-minute Walk Test. Assessments were conducted at four time points: within one year prior to transplant surgery, at discharge from hospital, six months post-transplant, and 12 months post-transplant. They found that all patients had significantly reduced diaphragmatic function at discharge from hospital compared to their baseline assessment using measures of force, strength, electrical activity, and kinematics. 100% of patients displayed altered phrenic nerve function in terms of a prolonged latency at the time of discharge from hospital compared to their preoperative assessment. On ultrasound assessment, however, only end-inspiratory thickness was significantly lower at discharge from hospital compared to baseline assessment. The ultrasound measurements they obtained where not categorised as having dysfunction versus no dysfunction according to predetermined cut-offs, rather, any significant decrease in measurement was considered dysfunctional.

From the current body of research examining diaphragmatic dysfunction after lung transplantation, the incidence can range from 3.2% to 100% depending on the method and time of assessment. Only two authors have attempted to examine diaphragm function in the immediate postoperative phase, Sheridan et al.[15] reporting an incidence of 29.6%, and Ferdinande et al[18]. reporting an incidence of 7.4%. The use of validated ultrasound methods and current definitions in this thesis will assist to determine the true incidence of diaphragmatic dysfunction in the acute postoperative period.

1.3 Disease Burden

Previous research has demonstrated that patients with postoperative diaphragmatic dysfunction after cardiothoracic and abdominal surgery frequently require increased duration of mechanical ventilation[17,18,20,21], longer length of stay in the Intensive Care Units (ICU)[18,19,22], and longer length of stay in hospital[19] due to compromised pulmonary function. In addition, lung transplant patients with diaphragmatic dysfunction require more frequent reintubation for respiratory failure[18,19], have an increased need for non-invasive ventilation, and tracheostomy[18]; have reduced lung function as measured with spirometry, and are more susceptible to the development of nosocomial pneumonia[17]. In the long term, patients with persistent diaphragmatic dysfunction after coronary artery bypass graft surgery have more readmissions to hospital for respiratory complications, reduced exercise tolerance due to dyspnoea on exertion, and reduced self-reported quality of life[23]. All of these events consume health care resources, increase patient mortality, and reduce the health care benefits of the surgical procedure. However, in one report, reduced diaphragmatic function measured at the time of discharge from hospital had no adverse effects on ICU length of stay, hospital length of stay, or duration of mechanical ventilation after bilateral lung transplant[13]. To evaluate this discrepancy, this thesis prospectively investigated the impact of postoperative diaphragmatic dysfunction on the following acute clinical outcomes: ICU length of stay, hospital length of stay, duration of mechanical ventilation, and discharge destination in patients after lung transplant.

1.4 Risk Factors

Risk factors associated with the development of diaphragmatic dysfunction after lung transplant are not clear. Two surgery-specific risk factors for the development of diaphragmatic dysfunction during open cardiac surgery include the use of an ice slush for

topical cardiac cooling which exposes the phrenic nerve to low temperatures[8,12,13,18] and prolonged duration on cardiopulmonary bypass[8,24]. In contrast, during lung transplantation, patients are not exposed to any ice slush or cooling agents so this risk is eliminated. Aside from prolonged duration on cardiopulmonary bypass, one possible risk factor specific to lung transplantation is the use of surgical retractors to retract the mediastinum[15,18] which can place traction of the phrenic nerve. However, the use of surgical retractors is not common.

Another potential risk factor is the duration of mechanical ventilation. Studies of critically ill patients have looked at the impact of prolonged mechanical ventilation on diaphragmatic function using ultrasound, and have demonstrated atrophy of the diaphragm[25,26]. Atrophy of the diaphragm has been associated with delayed liberation from mechanical ventilation[2,10,11,25-28], and mechanical ventilation is therefore both a risk factor for the development of diaphragmatic dysfunction and a consequence of diaphragmatic dysfunction.

In addition to prolonged cardiopulmonary bypass (CPB) and mechanical ventilation, there are a number of other potential risk factors which are commonly considered in research evaluating postoperative diaphragmatic dysfunction. These include the surgical approach used (e.g., bilateral thoracotomy vs clamshell), the primary diagnosis for transplant, sex, age and body mass index (BMI)[9,13,15,29]. As the lung transplant population is small, consideration of these variables was included to allow for future pooling of data or meta-analysis. This thesis evaluated the influence of these variables to identify whether or not they are associated with the development of postoperative diaphragmatic dysfunction.

1.5 Diagnosis

Reported symptoms of bilateral diaphragmatic dysfunction include orthopnoea, dyspnoea on exertion[1], coughing, chest pain, and sleep-disordered breathing (nocturnal hypoventilation)[7].

Individuals with severe unilateral dysfunction may also experience the same symptoms. However, approximately half of patients with unilateral dysfunction are asymptomatic[6]. Mild or unilateral dysfunction may not be detected because accessory muscles can often compensate for the paretic or paralysed diaphragm[12]. In the presence of underlying cardiorespiratory disease or obesity, these symptoms will be exacerbated.

Clinically, diaphragmatic dysfunction might first be suspected if there is elevation of one or both hemidiaphragms on chest x-ray [1], difficulty weaning from the ventilator, unexplained failed extubation, respiratory insufficiency, or observed abdominal paradox (inward drawing of the abdomen during inspiration), which is suggestive of severe bilateral diaphragmatic dysfunction[12,30]. In severe cases, bilateral diaphragmatic dysfunction can lead to hypoventilation and hypercapnic respiratory failure[1].

Historically, diaphragmatic dysfunction has been diagnosed using chest x-ray, fluoroscopy, dynamic medical resonance imaging (MRI), and transdiaphragmatic pressure monitoring, electromyography (EMG), or nerve conduction studies, in response to phrenic nerve stimulation. All of these diagnostic tests have their limitations because they can produce false-positive (e.g., fluoroscopy) and false-negative (e.g., chest x-ray) findings[31], are invasive, associated with radiation and require the patient to be moved, time-consuming, uncomfortable, highly complex, and expensive.

1.5.1 Radiology

Radiologically, the affected hemidiaphragm will appear higher than the unaffected hemidiaphragm, either on chest x-ray or computed tomography (CT) scan of the chest. Only severe diaphragmatic dysfunction, as demonstrated by paradoxical movement, can be detected reliably with chest x-ray[17]. Chest x-ray is highly sensitive (sensitivity 90%) but not

specific (specificity 44%) in detecting unilateral paralysis of the diaphragm[1]. Elevation of a hemidiaphragm is not always a result of diaphragmatic paralysis, and commonly after surgery it could be attributed to atelectasis. Dynamic MRI may allow evaluation of diaphragmatic motion. However, its use is limited by cost, portability and the special expertise required to interpret the images[1]. For these reasons it is not widely used in clinical practice.

1.5.2 Fluoroscopy

For many years fluoroscopy has been the most commonly used diagnostic test for the evaluation of possible diaphragmatic dysfunction[5,7,12]. Fluoroscopy uses a continuous x-ray to obtain moving images of internal body parts and organs. It is easy to use, easy to interpret and has good inter-rater reliability. During this test, the patient is instructed to breathe in and out during tidal (quiet) breathing, deep breathing, and a sniff manoeuvre while the diaphragms are assessed fluoroscopically. With diaphragmatic dysfunction, the affected hemidiaphragm will show reduced or delayed movement. In more severe dysfunction of the hemidiaphragm it will paradoxically be pulled upwards during inspiration[6], however, this is not highly specific as 6% of normal subjects demonstrate paradoxical motion (false-positive result)[32]. Fluoroscopy is only useful in detecting unilateral diaphragmatic dysfunction[15] because findings for bilateral dysfunction can be interpreted as normal, resulting in false-negatives[1,5,7]. Fluoroscopy typically assesses the anterior dome of the diaphragm which moves 40% less than the posterior dome assessed by ultrasound. Other disadvantages of fluoroscopy are that it requires patients to be off positive-pressure ventilation, and thus, it cannot be used for patients who are mechanically ventilated, is dependent on patient effort and cooperation to perform the deep breathing and sniff manoeuvres, requires transportation out of the intensive care unit to a radiology suite, and uses ionising radiation which is potentially harmful. Thus, the use of fluoroscopy for the diagnosis of diaphragmatic dysfunction is time consuming, and potentially unsafe for the assessment of critically ill patients[12,14].

1.5.3 Phrenic Nerve Stimulation

During phrenic nerve stimulation, transdiaphragmatic pressure, electromyography, and phrenic nerve conduction can be recorded to reveal information about diaphragmatic function. Phrenic nerve stimulation can be performed transcutaneously at the level of the neck to elicit contraction of the diaphragm muscle. Phrenic nerve stimulation techniques are rarely used in the clinical setting because they are invasive, time consuming, can be uncomfortable for the patient, less widely available, and require considerable expertise[7].

1.5.3.1 Transdiaphragmatic Pressure

The reference method to quantify bilateral diaphragmatic function is to measure transdiaphragmatic pressure (Pdi) in response to stimulation of the phrenic nerve[5,7,22]. During stimulation, the negative pressure can be monitored by calculating the difference between oesophageal and gastric pressures measured by catheters in the oesophagus and stomach.

1.5.3.2 Electromyography

Electromyography is performed by recording electrical activity in the muscle in response to stimulation or activation of the muscle. Electrical activity is recorded by a needle electrode inserted into the diaphragm via the intercostal space. It requires an experienced examiner and is invasive but provides highly accurate information regarding the diagnosis and prognosis of phrenic nerve disorders[5,18].

1.5.3.3 Nerve Conduction Studies

Phrenic nerve integrity can be evaluated by recording compound motor action potential (CMAP) amplitudes and latencies with chest surface electrodes in response to phrenic nerve

stimulation[33]. Nerve conduction studies require an experienced examiner but can be performed at the bedside[18]. The main limitation of nerve conduction testing is that it can only detect abnormalities in neural conduction, which does not rule out dysfunction due to pathology of the diaphragm muscle[34]. Nerve conduction studies were used to assess diaphragmatic function of lung transplant recipients in the study by Sheridan et al [15]. Of note, four of the seven patients diagnosed with phrenic nerve dysfunction declined repeat testing due to discomfort caused by the test.

1.5.4 Ultrasound

Due to the constraints imposed by the diagnostic methods outlined above, point-of-care ultrasound is emerging as the modality of choice to examine diaphragm function. Ultrasound is non-invasive, devoid of radiation, readily available at the bedside, and relatively fast and easy to use[35,36]. Ultrasound is used to take static measures of diaphragm thickness and dynamic measures of excursion and thickening fraction, which are detailed in Chapter 2. Ultrasonographic diaphragm assessment techniques have high intra- and inter-observer reproducibility[14] and has diagnostic superiority over fluoroscopy[12,21,37-40]. There is a significant reduction in the mean time between clinical suspicion and diagnostic testing, with only 15 minutes required for diagnostic ultrasound as opposed to 17 hours for fluoroscopy[12]. Additionally, this technique can be learned after a relatively short period of training[12] which means allied health professionals can acquire the skill and conduct the assessments. Diagnostic ultrasound, therefore, has the potential to be a clinically valuable tool for investigating the incidence and time course of diaphragmatic dysfunction after lung transplantation.

1.6 Natural History

The natural history of diaphragmatic dysfunction after lung transplantation is unclear, largely due to the variety of assessment methods used for diagnosis and variable periods of follow-up. To date, the time course of recovery of diaphragmatic dysfunction after lung transplantation has been reported in four studies with their assessment methods and results outlined below.

First, seven patients with phrenic nerve dysfunction were followed at one- to three-month intervals with nerve conduction studies and fluoroscopy [15]. However, only three patients agreed to repeat testing with nerve conduction studies. All seven patients had consistently abnormal fluoroscopic studies from 2-8 months postoperatively.

Second, diaphragmatic dysfunction in lung transplant patients were followed up using monthly chest x-rays over a period of two years postoperatively[17]. In the four patients identified as having paradoxical movement of their hemidiaphragm, diaphragmatic elevation persisted on chest x-ray during the entire two-year follow-up period. In the two patients with restricted movement, chest x-ray results returned to normal within seven days after transplantation. As previously mentioned, chest x-ray has poor specificity in detecting unilateral, or mild dysfunction of the diaphragm, so these results may not necessarily be a true reflection of the amount of residual dysfunction.

Third, for a group of lung transplant patients with known phrenic nerve dysfunction, the time interval to recovery was 524.5 ± 241.8 days (range 126 to 882 days, median 492 days)[18]. This study considered full recovery of diaphragmatic function as a predicted forced vital capacity (FVC) of at least 90%. FVC is the the maximal volume of gas that can be exhaled from full inhalation by exhaling as forcefully and rapidly as possible during a pulmonary function test. In this study, four of the nine patients with phrenic nerve dysfunction after lung transplant

recovered, but five never reached the predicted FVC of >90%. The author did not specify when baseline spirometry was established. Although reduced lung function might be a surrogate for diaphragmatic dysfunction, spirometry is not an accurate indicator of diaphragmatic function because there are many limiting factors that influence spirometry such as pain, pre-existing lung disease, and donor lung age which will influence postoperative lung function. Furthermore, the accuracy and reproducibility of pulmonary function tests are limited by dependence on lung volumes, patient effort, and a wide range of variability within the normal range[41].

Finally, a recent study used a multimodal assessment protocol (ultrasonography of the right hemidiaphragm, EMG phrenic nerve stimulation, spirometry, mean inspiratory pressure, and 6-minute Walk Test) to assess diaphragm function up to 12 months after lung transplant[13]. They reported significantly reduced diaphragmatic function in all of their patients on at least one outcome measure at the time of hospital discharge which persisted for 3-6 months, and then returned to baseline by 12 months postoperative.

In summary, data on the natural history of diaphragmatic dysfunction after lung transplantation is scarce and conflicting, taking anywhere between 7 days and 2 years to recover. The use of diagnostic ultrasound to assess diaphragmatic function pre- and postoperatively has the capacity to improve this knowledge deficit.

1.7 Rationale and Aims of this Thesis:

This thesis aimed to improve our knowledge of the incidence, natural history, and clinical impact of diaphragmatic dysfunction after lung transplantation by addressing the knowledge gaps identified above. By means of a prospective observational cohort study, the objectives of this thesis were to:

1) Document the incidence of diaphragmatic dysfunction by comparing pre- and post-operative diaphragmatic function in lung transplant patients using point-of-care ultrasound.
2) Examine the time course of diaphragmatic function up to three months postoperatively.
3) Evaluate the potential impact of diaphragmatic dysfunction on acute clinical outcomes.
4) Identify pre-, intra- and post-operative risk factors that may contribute to the development of postoperative diaphragmatic dysfunction.
5) Demonstrate inter-rater reliability of taking measurements from saved ultrasound images in retrospect.

2 CHAPTER 2: METHODOLOGY

2.1 Background

As stated in the previous chapter, the primary aim of this thesis was to use point-of-care ultrasound to diagnose diaphragmatic dysfunction in lung transplant patients. There are many different methods to examine diaphragmatic function using point-of-care ultrasound and no single standard for the diagnosis with ultrasound exists. The two main approaches are to (1) measure diaphragmatic excursion and (2) measure diaphragm thickness and calculate what is known as the 'thickening fraction'. This chapter will describe the different assessment methods reported in the literature, and outline the methods adopted for the evaluation of diaphragmatic dysfunction in this thesis.

2.2 Diaphragm Excursion

The first publication to evaluate diaphragm motion was by Haber et al.[42] in 1975. Excursion of the diaphragm was assessed using a qualitative approach with ultrasound in B-Mode[42,43]. B-Mode, or Brightness Modulation Mode, is the display of a two-dimensional image. Based on brightness, the image displays strong and weak echoes, in relation to their depth[44]. In these early studies, subjects were imaged in prone or sitting, using a posterior scan position[42,43]. Due to its impracticality, the posterior approach was later abandoned for preference of a subcostal or coronal intercostal probe placement in the supine position [40]. In this position there is also less variability and greater reproducibility[26,40].

To obtain images of diaphragmatic excursion for quantitative assessment, an anterior, subcostal, horizontal probe position is used, placing a low frequency curvilinear or phased-array probe between the anterior and mid axillary lines as shown in Figure 2.1. B-Mode is used

to select the exploration line of each hemidiaphragm using the liver as an acoustic window on the right (Figure 2.2) and the spleen on the left[14,36]. M-Mode is then selected to show movement of the diaphragm along the line of exploration, and measure the amount of excursion (or displacement) during each respiratory phase. M-Mode, or Motion Mode, records a one-dimensional image through the amplitude and speed of movement over time, creating lines across the screen[23]. To enhance reproducibility of M-Mode sonography, the diaphragmatic delineation in B-mode must be as perpendicular as possible to the diaphragmatic excursion line before applying the M-mode [35].

The ultrasound beam (in B-Mode) approximately intercepts the mid-posterior portion of the diaphragm, which corresponds to the part of the diaphragm with the greatest diaphragmatic excursion during spontaneous breathing [36]. There is little difference in the diaphragmatic excursion between the middle and posterior part of the diaphragm [45].

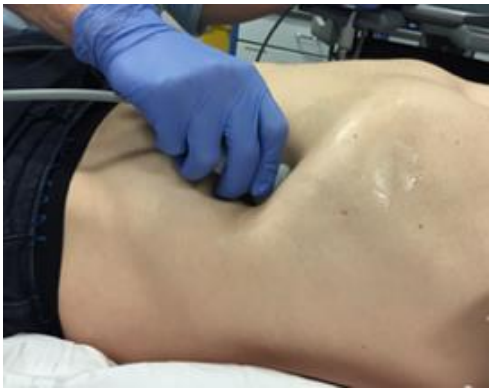

Figure 2.1 Anterior, subcostal, horizontal probe position for excursion measures.

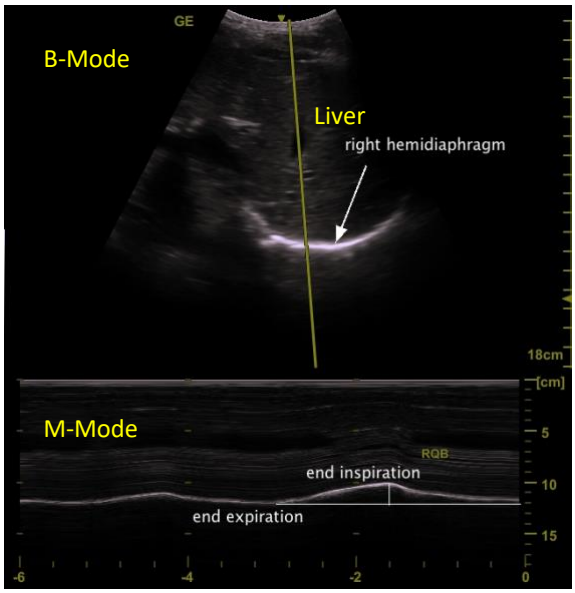

Figure 2.2 Using B-Mode to select the exploration line of the right hemidiaphragm using the liver as an acoustic window.

Diaphragm excursion has been studied during quiet breathing, during a voluntary sniff manoeuvre (a quick nasal inspiration with a closed mouth), and during deep breathing. Diaphragmatic excursion during quiet breathing in spontaneous ventilation is the main ultrasound parameter for assessing diaphragmatic function because it has been well correlated with transdiaphragmatic pressure measurements[21,22,46] and is more precise than conventional fluoroscopy[37,47], both of which have previously been considered the gold standard methods of assessment. Measurements of excursion taken during quiet breathing are most reliable as they are independent of patient effort. On the other hand, measurements during deep breathing or voluntary sniff depend on maximal voluntary inspiratory effort from the patient, requiring full cooperation from the patient[21]. Thus, these techniques may be imprecise if the patient is drowsy or unable to cooperate at the time of assessment. Another limitation of assessing diaphragm motion during deep inspiration is that the left hemidiaphragm can be obscured by the expanding lung. Indeed, the left hemidiaphragm was obscured in 65% of diaphragm assessments (n = 15/23) in a study of diaphragmatic motion by Gerscovich et al.[40] The voluntary sniff manoeuvre has traditionally been used in fluoroscopic diaphragm assessments as it can be useful in differentiating a diminished diaphragmatic response (neuropraxia) from a completely absent response (paralysis)[23]. In diaphragm ultrasound assessments, excursion of the diaphragm during a voluntary sniff has been evaluated in the assessment of patients after heart and lung transplantation[17], and in normal volunteers[14]. Taken together, it is unclear which breathing manoeuvre (quiet breathing, deep breathing, or voluntary sniff) may be most affected by, and most useful for the diagnosis of diaphragmatic dysfunction after lung transplant surgery, therefore, this thesis has examined all of them.

Anterior subcostal transverse scanning on a semi-recumbent patient is a safe, feasible, reliable, fast, and reproducible way to assess diaphragmatic excursion[36]. This technique has

low interobserver variability for experienced (3.9-6%) and inexperienced (7.1-7.7%) operators[36]. Experienced operators during quiet breathing had intraobserver variability of 6% (18.4 $\pm$ 7.6mm p< 0.01) whereas, inexperienced operators had intraobserver variability of 7.1% (21.7 $\pm$ 8.6mm p< 0.01). For deep breathing excursion, experienced operators had intraobserver variability of 3.9% (78.8 $\pm$ 13.3mm p<0.001) compared to inexperienced operators, who had intraobserver variability of 7.7% (69.1 $\pm$ 13.7mm p<0.001). In support of this finding, Boussuges et al.[14] also reported high intra- and inter-observer reproducibility of ultrasound during quiet breathing in healthy volunteers. Intraobserver reproducibility was 96% for the right hemidiaphragm and 94% for the left hemidiaphragm, and the interobserver reproducibility 95% for the right and 91% for the left hemidiaphragm. Furthermore, the intra- and inter-observer reproducibility of excursion measures of ICU patients were found to be in a similar range 88%-99%[21,34]. Whether the operator is experienced or inexperienced, measuring excursion during quiet breathing or deep breathing, and in healthy subjects or ICU patients, this method of assessing diaphragmatic excursion has proven reliability.

To establish reference values for normal diaphragmatic movement in men and women, Boussuges and colleagues[14] evaluated 210 healthy adult volunteers (150 men, 60 women; age 50 $\pm$ 14 years). Cut-off values for the normal range of diaphragmatic excursion during quiet breathing were 0.9-2.4cm for women and 1.0-2.6cm for men; during deep breathing was 3.6-8.4cm for women and 4.7-9.3cm for men; and during voluntary sniff was 1.6-3.7cm for women and 1.8-4.4cm for men. Hence, it is accepted that the lower limit values of "normal" diaphragmatic excursion are 0.9cm and 1cm during quiet breathing; 3.7cm and 4.7cm during deep breathing; and 1.6cm and 1.8cm during voluntary sniff for women and men, respectively. Subsequent to this publication, other authors have deemed excursion less than 1cm during quiet breathing as a clinical indicator of diaphragmatic dysfunction[6,15,19].

Although measurement of diaphragmatic excursion with M-mode sonography is a valid and reliable tool to quantitatively assess diaphragmatic function after surgery, it is not accurate if the patient is mechanically ventilated[44]. In this situation, the amount of diaphragmatic excursion is invalid because the sum of active contraction of the diaphragm is masked by the addition of passive force applied by the positive pressure from the ventilator, increasing the overall excursion measured[44]. In cases where patients are mechanically ventilated, an assessment of diaphragm thickening is likely a better indicator of diaphragmatic function.

2.3 Diaphragm Thickening

Thickening of the diaphragm indicates a shortening of the diaphragm during muscular contraction, and therefore, the degree of thickening is proposed to reflect the magnitude of diaphragmatic effort[48]. Images of diaphragm thickness are obtained during B-Mode with a high frequency (>10MHz) linear probe in the 'zone of apposition' where the diaphragm attaches to the rib cage. This can be located by placing the ultrasound probe between the 8^{th}-9^{th} ribs in the mid-axillary line as shown in Figure 2.3. In this area, the diaphragm appears as a structure with three distinct layers (Figure 2.4). A non-echogenic (dark) central layer of muscle, bordered by two echogenic (bright white) layers: the peritoneum and the pleura. Diaphragm thickness is the perpendicular distance between the pleural and peritoneal layers[35]. The thickness of the central layer can be measured at the end of maximal inspiration (Total Lung Capacity) and the end of expiration (Functional Residual Capacity). B-Mode ultrasound imaging of diaphragm thickness and thickening is 93% sensitive and 100% specific for the diagnosis of neuromuscular diaphragmatic dysfunction[31]. The average change in diaphragm thickness from resting expiration to resting inspiration in healthy subject is 20% on the right and 23% on the left. However, almost one third of healthy subjects have no or minimal diaphragm thickening with tidal breathing so it is important that subjects are able to take a deep breath[49]. Poor patient

effort will prevent maximal thickening of the diaphragm and could, therefore, give false positive results[50].

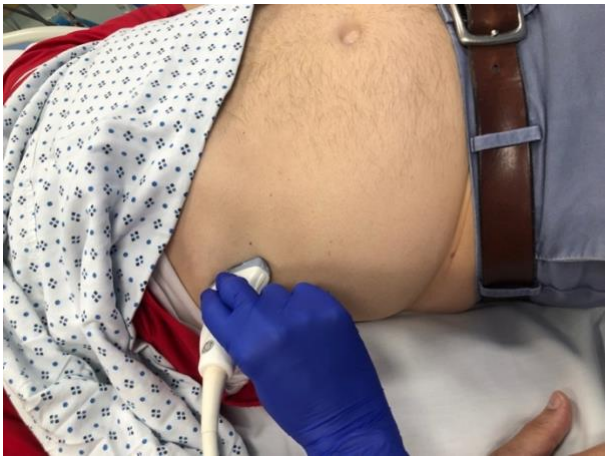

Figure 2.3 Probe placement in the 'zone of apposition' where the diaphragm attaches to the rib cage between the 8th-9th ribs in the mid-axillary line. Patient is positioned in supine.

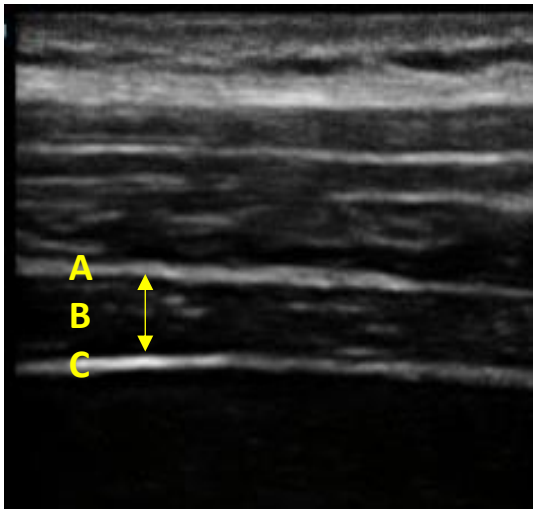

Figure 2.4 Ultrasound image of diaphragm thickness in the zone of apposition showing three distinct layers A. pleural layer, B. diaphragm layer, C. peritoneal layer. Diaphragm thickness is the perpendicular distance between the pleural and peritoneal layers.

The inter- and intra-rater agreement of diaphragm thickness measures is high, so long as the assessor marks the site for all subsequent measurements[26,50]. This is important because diaphragm thickness is highly variable (up to 6mm difference) depending on the chosen intercostal space for imaging[50]. Marking the site for repeated measures for a longitudinal postoperative study is not practical or feasible, so in this case authors recommend measuring the degree of thickening or 'thickening fraction' as a more reliable measure of dysfunction[46]. The intra- and inter-rater reliability of measuring thickening fraction is high. For inter-rater reliability: the intraclass correlation coefficient (ICC) is 0.95 (95%CI 0.78-0.99); and intra-rater reliability ICC is 0.98 (95% CI 0.83-0.99)[51].

The thickening fraction is equal to the thickness at the end of inspiration, minus the thickness at the end of expiration, divided by the thickness at the end of expiration[35].

$$TF = \frac{\text{thickness at end-inspiration} - \text{thickness at end-expiration}}{\text{thickness at end-expiration}}$$

Thickening fraction has been correlated with diaphragm electrical activity and transdiaphragmatic pressure during inspiratory manoeuvres[26]. Gottesman and McCool[20] used ultrasound to evaluate diaphragmatic thickness of 15 healthy volunteers and 15 patients with known diaphragmatic dysfunction. They concluded that an end-expiratory thickness of less than 2mm, combined with a thickening fraction less than 20% can differentiate between a paralysed and functioning diaphragm. The upper and lower limits of normal thickening fraction were 21 to 57%. Similarly, in 150 normal subjects aged 20-83 years (mean=50.6± 17.8) diaphragm thickening fraction was 20% at maximal inspiration[50]. A thickening fraction less than 20% has subsequently been used as a clinical indicator of diaphragmatic dysfunction.

Diaphragmatic dysfunction may therefore be evaluated with ultrasound in terms of the amount of excursion, and it's thickening fraction. We acknowledge that there is a methodological challenge in choosing previously published criteria for dysfunction, especially as these values have been established in small groups of normal, healthy subjects. As both approaches have their merits and limitations, this thesis has documented the incidence of diaphragmatic dysfunction using both methods in lung transplant.

2.4 Materials and methods used in this thesis

To evaluate these methods in the lung transplant population, a prospective, observational cohort study was conducted on 27 lung transplant recipients at St Vincent's Hospital in Sydney. A sample size of 27 was selected to match the sample size used in previous prospective lung

transplant studies outlined in Section 1.2[13,15,17] This sample was also a realistic and feasible size to achieve within the recruitment timeframe allocated for this study.

2.4.1 Ethics and Consent

Approval to conduct this study was granted by the St Vincent's Hospital Health Research Ethics Committee (HREC/14/SVH/203) and the study was registered with the Australian New Zealand Clinical Trials Registry ACTRN12615001371583. The principles of the Declaration of Helsinki formulated by the World Medical Association, the Declaration of Istanbul, and the ISHLT Statement of Transplant Ethics have been adhered to. Written informed consent was obtained from eligible participants on the lung transplant waiting list.

2.4.2 Study Protocol

Ultrasonographic assessment of both hemidiaphragms was conducted preoperatively, then one day after transplant in the Intensive Care Unit (ICU), one week after transplant in hospital, one month, and three months after transplant, either in the hospital or in the outpatient clinic. A follow-up period of three months was selected as we expected to examine the incidence of dysfunction, and any potential for recovery, during the acute postoperative phase. The three-month period was also a realistic time frame for the aims of a Masters thesis.

Demographic and other data were collected preoperatively from the medical record including patient age, sex, body mass index (BMI), and primary diagnosis for lung transplant. Intraoperative variables considered as possible predictors for diaphragmatic dysfunction included the type of transplant received, type of incision used and time on cardiopulmonary bypass.

2.4.3 Procedure

In the lead up to the study, bedside training of Elise Crothers (EC) by George Ntoumenopoulos (GN) was undertaken in a cohort of patients in the Intensive Care Unit which included lung transplant patients. The training included imaging by EC with supervision by GN to ensure that consistency in technique and procedures were maintained. During the training period 49 patients were imaged together; a combination of preoperative and postoperative Cardiothoracic surgical patients, and general Intensive Care patients.

All ultrasonographic examinations were conducted using either the FujiFilm SonoSite M-Turbo (Fujifilm, Bothell, WA, USA) or GE Healthcare Venue 50 (GE Healthcare Australia, NSW, Australia) point-of-care ultrasound machines. To minimise the time-cost to the patient, all still images were recorded on a computer for subsequent analysis using Image J software (Rasband, W.S., Image J, U.S. National Institutes of Health, Beseda, Marylands, USA. https://imagej.nih.gov/ij/, 1997-2018).

Ultrasound examinations were performed with the patient in the semirecumbent position, with head up 30-45 degrees determined by patient comfort. At each assessment, the right hemidiaphragm was assessed first, recording excursion images during quiet breathing, deep breathing, and voluntary sniff for at least 3 breath cycles, and recording thickness images at the end of expiration and maximal inspiration. This was repeated for the left side.

For the excursion measures, an anterior, subcostal, horizontal probe position was chosen, placing a low frequency curvilinear probe between the anterior and mid axillary lines (Venue 50 4C 2.5-6MHz or SonoSite C60xi 5-2MHz transducer) as shown in Figure 2.1. B-Mode was used to select the exploration line of each hemidiaphragm using the liver as an acoustic window on the right (Figure 2.2) and the spleen on the left[14,36]. The transducer was angled

medially, cranially, and dorsally to visualise the posterior third of the hemidiaphragm[14,41]. For images of quiet breathing the patient was instructed to relax and breathe normally. For images of deep breathing, the patient was asked to breathe in as deeply as possible and then breathe out. For images of voluntary sniff the patient was asked to do a short, sharp sniff through their nose, and this was demonstrated by the assessor. Images of diaphragm inspiratory amplitudes (excursions) were recorded from M-mode sonography, placing the ultrasound beam as perpendicular as possible to each hemidiaphragm[35].

For the thickness measures, images were obtained during B-Mode with a high frequency (>10MHz) linear probe placed between the 8th-9th ribs in the mid-axillary line [46] (Figure 2.3). The patient was instructed to breathe in as deeply as possible and then breathe out. Images of diaphragm thickness were recorded at the end of maximal inspiration and at the end of expiration.

2.4.4 Outcome Measures

Ultrasound Outcome Measures

The primary outcome measures used in this study were the presence of diaphragmatic dysfunction on ultrasound measures of:

(i) excursion during quiet breathing

(ii) excursion during deep breathing

(iii) excursion during a voluntary sniff

(iv) thickening fraction

(i) Excursion during quiet breathing

The presence of ultrasonographic diaphragmatic dysfunction was defined by diaphragmatic excursion measurement <1.0cm for men and <0.9cm for women[14].

The amplitude of excursion was measured on the vertical axis of the tracing from the end of expiration of the previous breath to the end of tidal inspiration as marked on the ultrasound (Fig. 2.5). To reduce the measurement error, measures were averaged from up to 3 consecutive breath cycles[29] and then repeated by a blind assessor for reliability analyses.

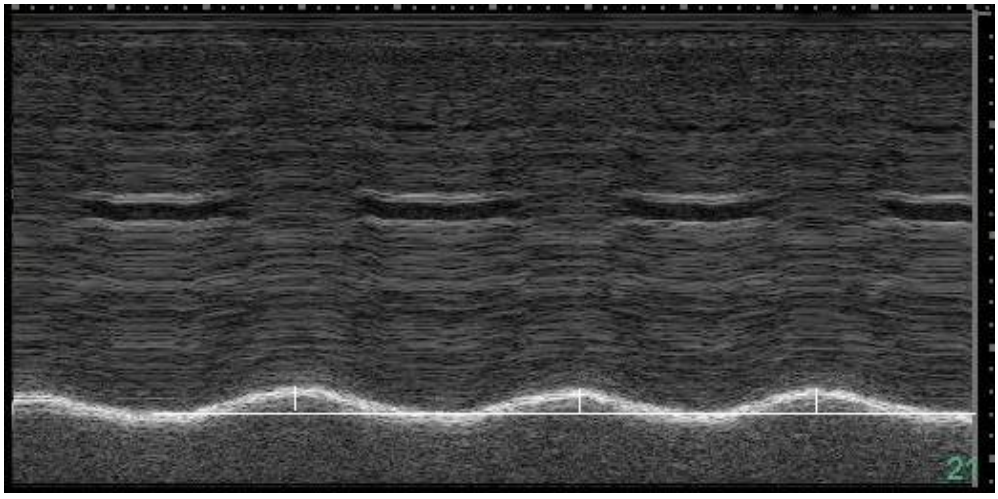

Figure 2.5 Measurement of excursion during three quiet breaths.

(ii) Excursion during deep breathing

The presence of ultrasonographic diaphragmatic dysfunction was defined by diaphragmatic excursion measurement <4.7 cm for men and <3.6 cm for women [14]. The amplitude of excursion was measured on the vertical axis of the tracing from the end of expiration of the previous breath to the end of maximal inspiration as marked on the ultrasound (Fig. 2.6). Measures were averaged from up to 3 breath cycles and then repeated by a blind assessor for reliability analyses.

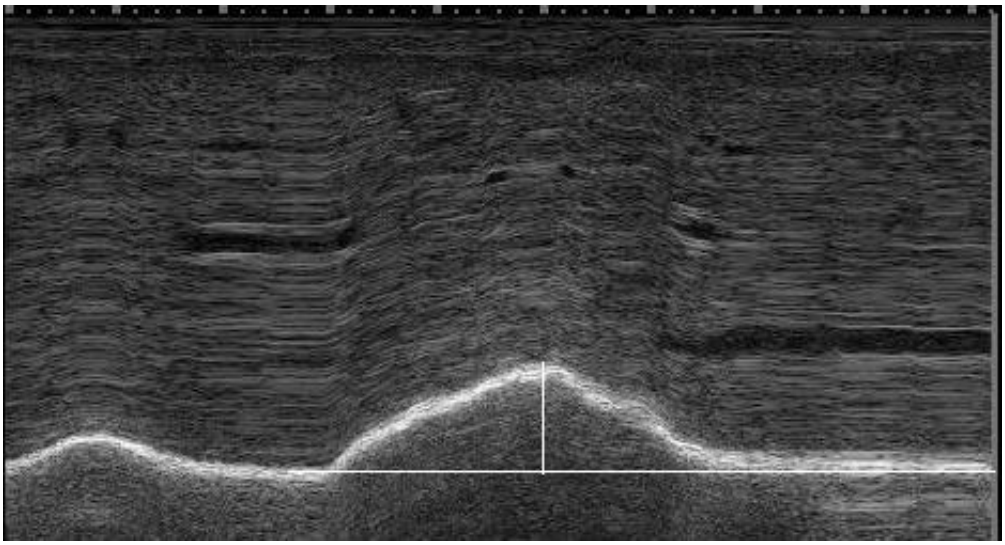

Figure 2.6 Measurement of excursion during a single deep breath.

(iii) Excursion during voluntary sniff

The presence of ultrasonographic diaphragmatic dysfunction was defined by diaphragmatic excursion measurement <1.8cm for men and <1.6cm for women during voluntary sniff[14]. The amplitude of excursion was measured on the vertical axis of the tracing from the end of expiration of the previous breath to the end of a short, sharp nasal inspiration as marked on the ultrasound (Fig. 2.7). Measures were averaged from up to 3 breath cycles and then repeated by a blind assessor for reliability analyses.

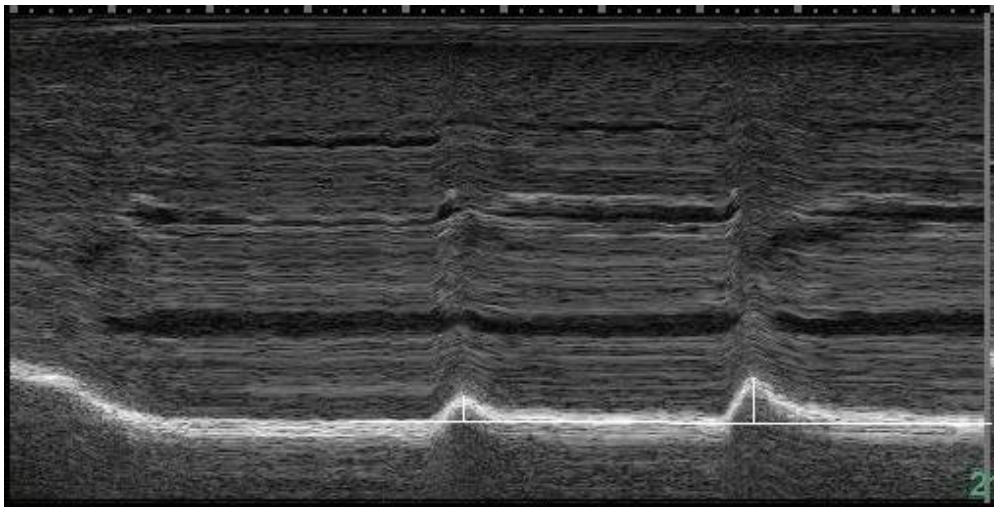

Figure 2.7 Measurement of excursion during two voluntary sniff manoeuvres.

(iv) Thickening fraction

Diaphragmatic dysfunction was defined as a diaphragmatic thickening fraction <0.2[20]. Diaphragm thickness was averaged from three measures of the thickness between the two echogenic lines on an image of end-expiration and from an image of end of deep inspiration (Fig 2.8). Thickening Fraction was calculated as TF = thickness at end-inspiration - thickness at end-expiration/thickness at end-expiration[35].

(a) **(b)**

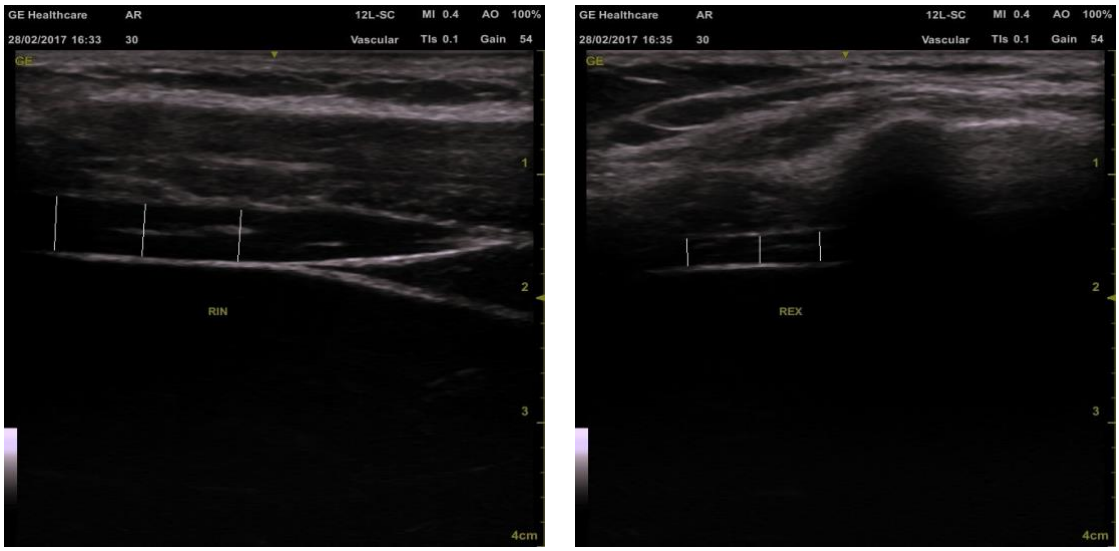

Figure 2.8 Measurement of hemidiaphragm thickness (a) at end of deep inspiration (b) at end of expiration

Clinical Outcome Measures

The secondary aims of this thesis were to evaluate the potential impact of diaphragmatic dysfunction on clinical outcomes and identify pre-, intra- and postoperative risk factors that may contribute to the development of postoperative diaphragmatic dysfunction. To ascertain the possible effect of diaphragmatic dysfunction on clinical recovery, the following postoperative outcome measures were obtained retrospectively from the patient's medical record or from the hospital's transplant record database:

1. the duration of mechanical ventilation (hours)
2. need for extracorporeal membrane oxygenation (ECMO) (days)
3. length of stay in intensive care (hours)
4. length of stay in hospital (days)
5. discharge destination

To identify possible risk factors associated with the development of diaphragmatic dysfunction in lung transplant patients, the following baseline and perioperative variables were recorded from the patient's medical record:

1. Age
2. Sex
3. Body Mass Index
4. Primary diagnosis for lung transplant
5. Incision type
6. Transplant type
7. Cardiopulmonary bypass time

2.5 Statistical Analysis

We reported the incidence of diaphragmatic dysfunction for each ultrasound outcome measure at each point in time. The chi-squared test was used to compare categorical variables, and the Mann-Whitney U test was used to compare continuous variables between groups (i.e., those patients with diaphragmatic dysfunction vs those without dysfunction). A two-tailed P value < 0.05 was considered statistically significant. Inter-observer repeatability of the ultrasound measures taken in retrospect was tested using intraclass correlation coefficient. Intraclass correlation estimates and their 95% confident intervals were calculated using SPSS based on average measures, consistency, two-way mixed-effects model. The results for excursion on quiet breathing are reported in Chapter 3: the publication. Results for the excursion measures on deep breathing and voluntary sniff, and thickening fraction, are reported in Chapter 4.

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ORIGINAL ARTICLE

Incidence of early diaphragmatic dysfunction after lung transplantation: results of a prospective observational study

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Abstract

Background: Diaphragmatic dysfunction is common after cardiothoracic surgery, but few studies report its incidence and consequences after lung transplantation. We aimed to estimate the incidence of diaphragmatic dysfunction using ultrasound in lung transplant patients up to 3 months postoperatively and evaluated the impact on clinical outcomes.

Methods: This was a single-center prospective observational cohort study of 27 lung transplant recipients using diaphragmatic ultrasound preoperatively, at 1 day, 1 week, 1 month, and 3 months postoperatively. Diaphragmatic dysfunction was defined as excursion < 10 mm in men and < 9 mm in women during quiet breathing. Clinical outcomes measured included duration of mechanical ventilation, length of stay (LOS) in Intensive Care (ICU), and hospital LOS.

Results: Sixty-two percentage of recipients experienced new, postoperative diaphragmatic dysfunction, but the prevalence fell to 22% at 3 months. No differences in clinical outcomes were found between those with diaphragmatic dysfunction compared to those without. Patients who experienced diaphragmatic dysfunction at 1 day postoperatively were younger and had a lower BMI than those who did not.

Conclusions: Diaphragmatic dysfunction is common after lung transplant, improves significantly within 3 months, and did not impact negatively on duration of mechanical ventilation, LOS in ICU or hospital, or discharge destination.

KEYWORDS
diaphragmatic dysfunction, intensive care, lung transplantation, ultrasound

4 CHAPTER 4: ANALYSIS OF DIAPHRAGMATIC DYSFUNCTION USING ADDITIONAL ULTRASOUND OUTCOME MEASURES

This chapter presents the results of the remaining ultrasound examinations collected during the prospective observational study described in Chapter 3. These additional examinations were to establish the incidence and time course of diaphragmatic dysfunction identified on excursion during deep breathing; excursion during voluntary sniff; and the diaphragmatic thickening fraction.

4.1 Methods

The study design, materials, and methods are identical to that described in Chapter 3 with the addition of ultrasound examinations during deep breathing, voluntary sniff, and thickening fraction as described in Chapter 2. These analyses were conducted on the same 27 participants described in Chapter 3.

4.2 Results

4.2.1 Incidence and time course of diaphragmatic dysfunction

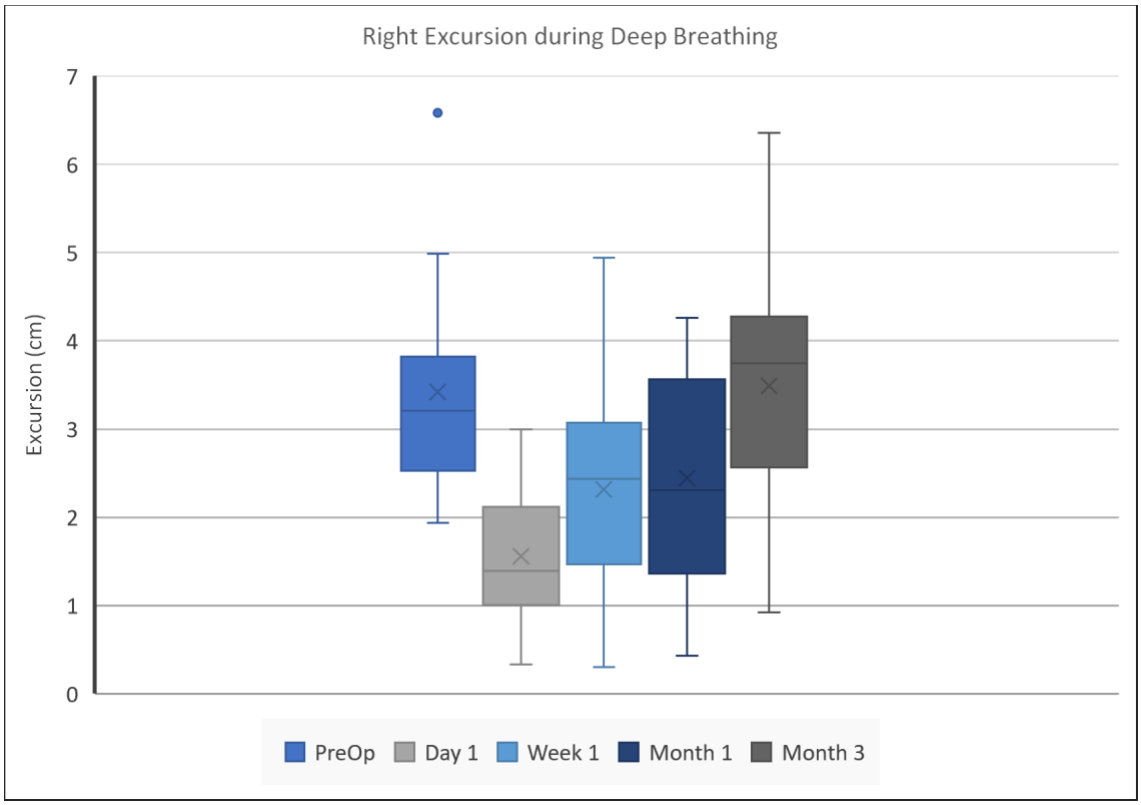
Average diaphragm excursion and thickening fraction measures at each time point for the right hemidiaphragm using the different techniques are shown in Figure 4.1.1. and for the left hemidiaphragm in Figure 4.1.2. Of note, the prevalence of preoperative diaphragmatic dysfunction is much higher on these measurements (deep breathing 89%; voluntary sniff 50%; thickening fraction 32%) than it was for excursion during quiet breathing (11%).

All ultrasound measures, except right thickening fraction, showed a sharp reduction at Day 1 after transplant, followed by a gradual return to baseline (preoperative measurement) on

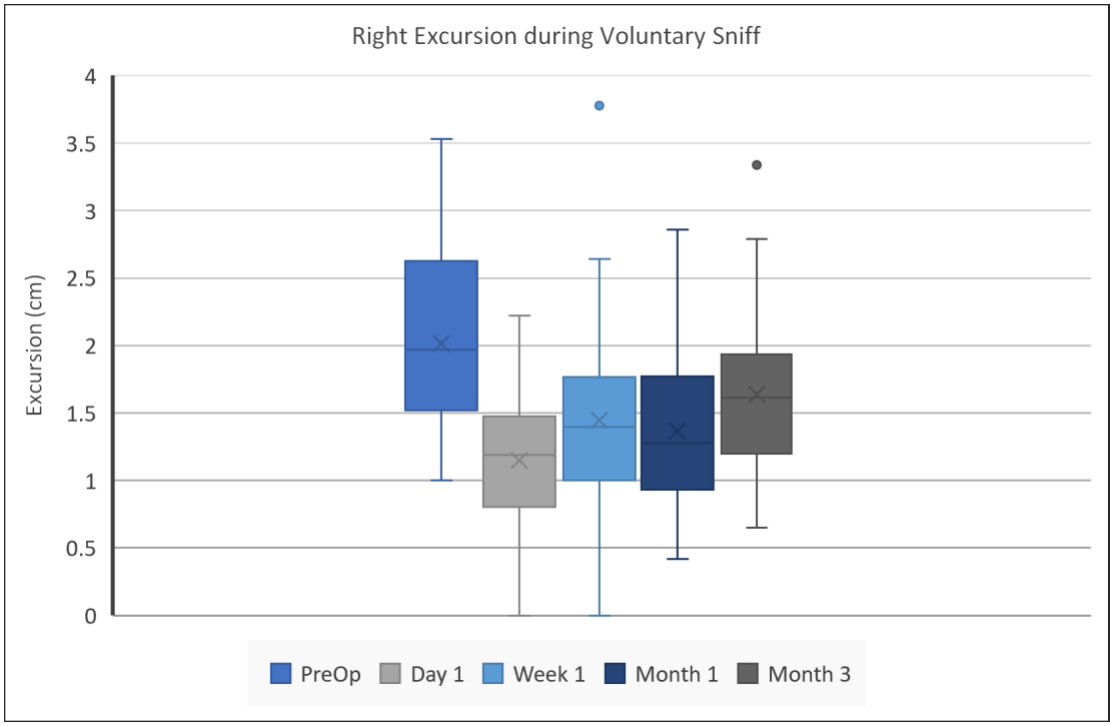
subsequent assessments. This pattern mirrors the results for excursion during quiet breathing, presented in Chapter 3. A two-tailed t-test showed that all ultrasound measures were significantly reduced at Day 1 after transplant for each technique (right voluntary sniff, mean difference 0.86cm, 95% CI = 0.5-1.23cm, $p< 0.0001$; left voluntary sniff, mean difference 0.61cm, 95% CI = 0.10-1.12cm, $p= 0.0181$; right deep breathing, mean difference 1.86cm, 95% CI = 1.22-2.51cm, $p=0.0034$; left deep breathing, mean difference 1.12cm, 95% CI 0.47-1.76, $p= 0.0004$; left thickening fraction, mean difference 0.39; 95% CI 0.16-0.62cm; $p= 0.0007$) compared to the preoperative measurement, except for right thickening fraction (mean difference 0.15; 95% CI -0.11-0.41; $p=0.1267$).

Fig 4.1.1 Median (line), mean (x), interquartile range (box), range (whiskers) and outliers (single dot) of average right hemidiaphragm measures for (a) excursion during deep breathing (b) excursion during voluntary sniff (b) thickening fraction at each assessment time from preoperative to three months postoperative. Only data for the ipsilateral hemidiaphragm of each single lung transplant has been included; data for the non-transplanted side has not been included.

(a)



(b)


Right Excursion during Voluntary Sniff
Excursion (cm)
4
3.5
3
2.5
2
1.5
1
0.5
0
PreOp
Day 1
Week 1
Month 1
Month 3

(c)

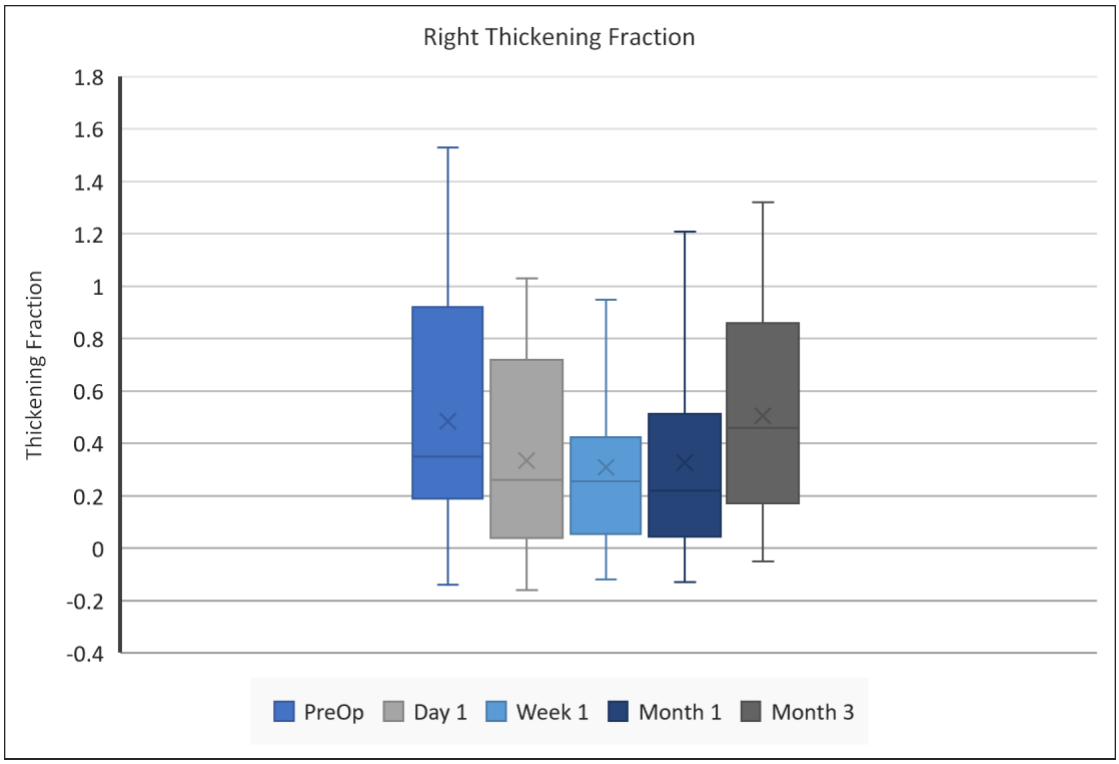
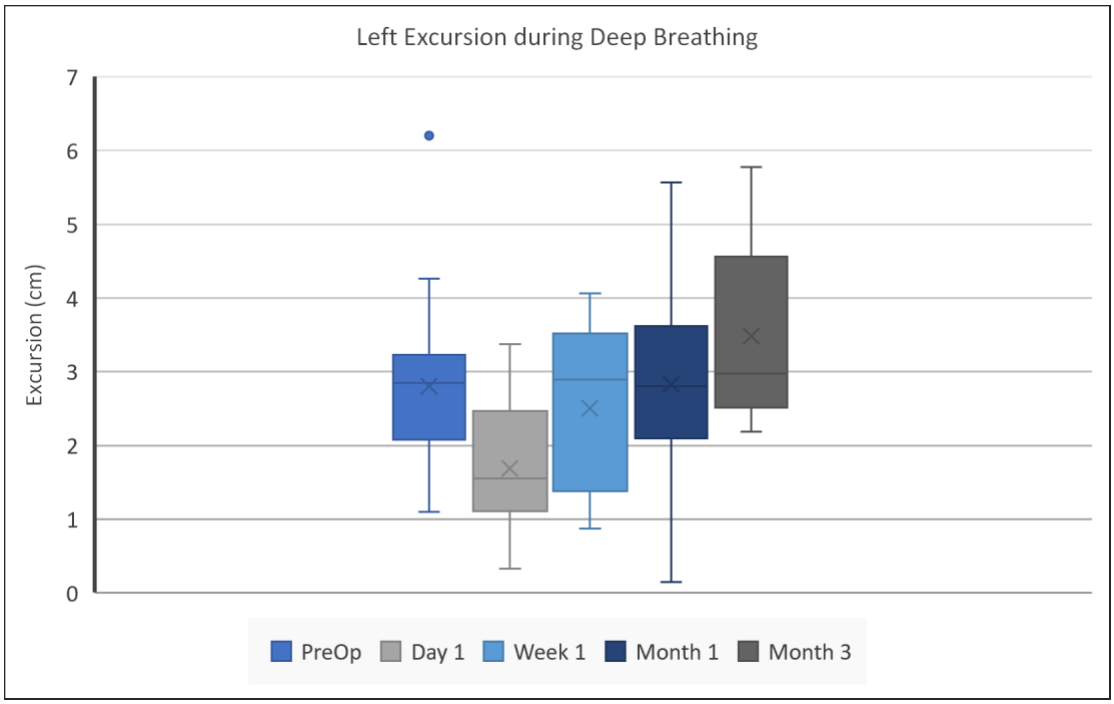
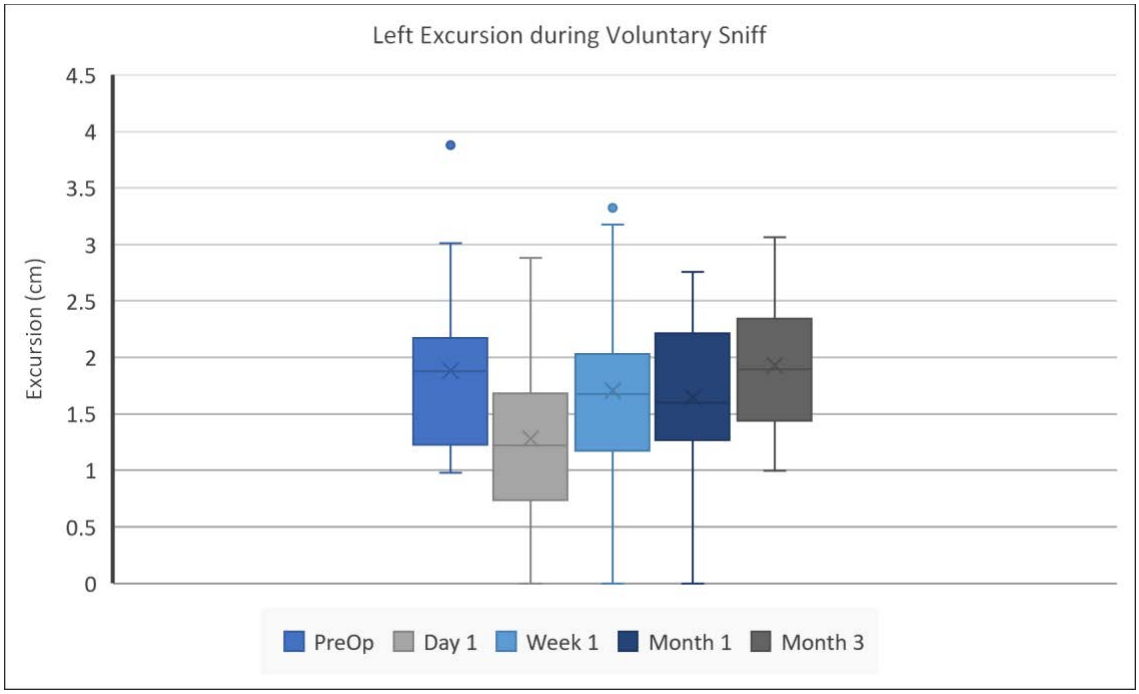

Right Thickening Fraction
Thickening Fraction
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
PreOp
Day 1
Week 1
Month 1
Month 3

Fig 4.1.2 Median (line), mean (x), interquartile range (box), range (whiskers) and outliers (single dot) of average left hemidiaphragm measures for (a) excursion during deep breathing (b) excursion during voluntary sniff (b) thickening fraction at each assessment time from preoperative to three months postoperative. Only data for the ipsilateral hemidiaphragm of each single lung transplant has been included; data for the non-transplanted side has not been included.

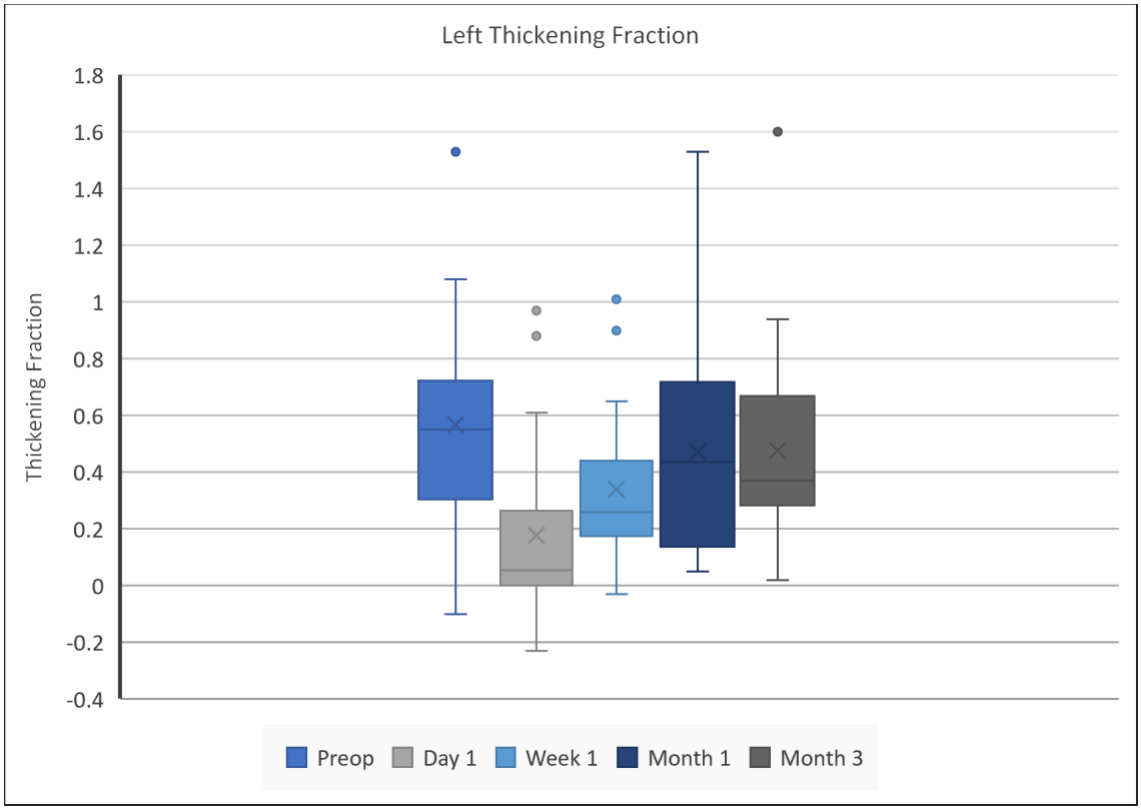
(a)



(b)



(c)



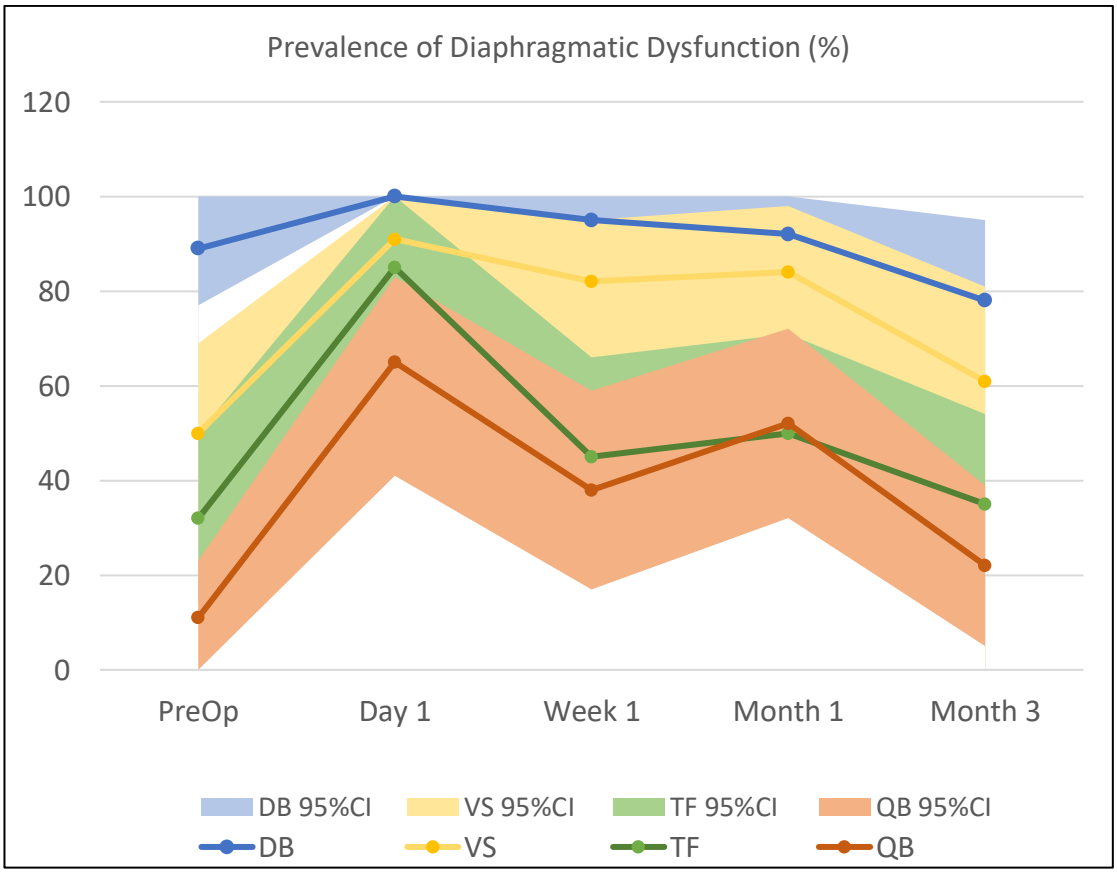
As explained in Chapter 2, the presence of ultrasonographic diaphragmatic dysfunction was defined categorically by a diaphragmatic excursion measurement <4.7cm for men and <3.6cm for women during deep breathing; <1.8cm for men and <1.6cm for women during voluntary sniff; thickening fraction <0.2 for men and women. The prevalence of diaphragmatic dysfunction at each time point is presented in Table 4.1.1. The prevalence, rather than incidence, has been reported as there were a large number of individuals with preoperative dysfunction on most ultrasound measures.

The prevalence of diaphragmatic dysfunction for all ultrasound measurements was highest on Day 1 after transplant and returns close to baseline by Month 3 after transplant. The prevalence of dysfunction on measures of deep breathing was high at all time points, including preoperatively.

Table 4.1 Prevalence of diaphragmatic dysfunction with 95% confidence intervals

	Preop	Day 1	Week 1	Month 1	Month 3
Deep Breathing 95% CI	89% (24/27) 77%-100%	100% (24/24)	95% (21/22) 87%-100%	92% (23/25) 81%-100%	78% (18/23) 61%-95%
Voluntary Sniff 95% CI	50% (13/26) 31%-69%	91% (21/23) 80%-100%	82% (18/22) 66%-98%	84% (21/25) 70%-98%	61% (14/23) 41%-81%
Thickening Fraction 95% CI	32% (8/25) 14%-50%	85% (17/20) 69%-100%	45% (10/22) 25%-66%	50% (11/22) 29%-71%	35% (8/23) 15%-54%

Figure 4.2 Prevalence of diaphragmatic dysfunction showing 95% confidence intervals



Legend:
Abbreviations: QB, dysfunction on quiet breathing; DB, dysfunction on deep breathing; VS, dysfunction on voluntary sniff; TF, dysfunction on thickening fraction; CI, confidence interval.

4.2.2 Influence of diaphragmatic dysfunction on postoperative clinical outcomes

Patients with and without diaphragmatic dysfunction on measures of voluntary sniff and thickening fraction at one day post-transplant were compared with regard to the duration of mechanical ventilation, need for and duration of ECMO, their length of stay in intensive care, their length of stay in hospital and their discharge destination. All patients had diaphragmatic dysfunction on their Day 1 deep breathing assessment, so no comparative analysis could be conducted.

There was no statistically significant difference in clinical outcomes for those patients with or without diaphragmatic dysfunction on voluntary sniff or thickening fraction at one day post-transplant (Table 4.2.1). Patients with persistent diaphragmatic dysfunction (at Month 3 post-transplant) on deep breathing, voluntary sniff, and thickening fraction were compared to those without diaphragmatic dysfunction for the same clinical outcomes (Tables 4.2.1, 4.2.2, 4.2.3) but there was no statistically significant difference in clinical outcomes.

4.2.3 Relationship between perioperative variables and development of diaphragmatic dysfunction

Patients who had diaphragmatic dysfunction on voluntary sniff measurement one day after surgery were younger in age (median [IQR]; 56 [29;83] vs. 65 [62;68], p=0.04) than those who did not (Table 4.2.1). As discussed in Chapter 3, this difference was also observed in patients with diaphragmatic dysfunction on quiet breathing measurement. This difference was not observed at three months post-transplant.

At Month 3 after transplant, patients with and without diaphragmatic dysfunction on measures of thickening fraction showed a significant difference for sex (p=0.04) and primary diagnosis (p=0.03) with the differences depicted in Figure 4.3.

Table 4.2.1 Characteristics of patients with and without diaphragmatic dysfunction on voluntary sniff measures at Day 1 and Month 3

Variables	DD at Day 1	No-DD at Day 1	*p* Value	DD at Month 3	No-DD at Month 3	*p* Value
No. of patients	21	2		14	9	
Age (years)	56 (34-61)	65 (65.5-66.5)	0.04	57 (36.75-61.75)	57 (43-59)	0.55
BMI (kg/m^2)	19.47 (18.12-24.97)	27.95 (24.46-31.45)	0.2	7 (50%)	4 (44%)	1.00
Male	10 (48%)	1 (50%)	1.00	19.35 (18.02-23.10)	26.69 (18.59-28.87)	0.16
Primary Diagnosis			0.19			0.70
Cystic fibrosis	4 (19%)	0		2 (14%)	2	
COPD	5 (24%)	0		4 (29%)	2	
Pulmonary fibrosis	5 (24%)	1 (50%)		4 (29%)	2	
Other	7 (33%)	1 (50%)		4 (29%)	3	
Transplant Type			0.98			0.30
Single Lung	1 (5%)	0		1 (7%)	1	
Bilateral Lung	18 (86%)	2 (100%)		12	6	
Combined Organ	2 (10%)	0		1 (7%)	2	
Incision Type			0.18			0.53
Clamshell	4 (19%)	2 (100%)		4 (29%)	2	
Bilat anterior thoracotomies	15 (71%)	0		8	5	
Sternotomy	1 (5%)	0		1 (7%)	1	
Unilateral thoracotomy	1 (5%)	0		1 (7%)	1	
CPB time (min)	210 (104-233)	210 (183-237)	0.96	202.5 (175.75-227)	205 (201-233)	0.38
ECMO time (days)	0 (0-0)	0 (0-0)	0.64	0 (0-0)	0 (0-0)	0.25
Duration of Mechanical Ventilation (h)	22.5 (12.72-38.9)	26.16 (22.75-29.58)	0.96	19.15 (12.15-33.38)	22.33 (15-27.9)	0.55
ICU length of stay (h)	94.68 (52.9-141.23)	103.12 (86.19-120.1)	0.96	66.5 (50.85-108.97)	94.68 (72.88-165.28)	0.40
Hospital length of stay (days)	24 (14-36)	13.5 (12.75-14.25)	0.19	15.5(13.25-35)	24 (13-29)	0.73
Discharge Destination						
Home	14 (67%)	1 (50%)	0.24	10	6	0.62
Inpatient Rehabilitation	5 (24%)	0		3	3	
Deceased	2 (10%)	1 (50%)		1 (7%)	0	

Legend:

Continuous variables reported as median (interquartile range); percentage data shown as n (%).
*Compared using Mann-Whitney U test. ^Compared using Chi-squared test.
Abbreviations: DD, diaphragmatic dysfunction; BMI; body mass index; COPD, chronic obstructive pulmonary disease; CPB, cardio-pulmonary bypass; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

Table 4.2.2 Characteristics of patients with and without diaphragmatic dysfunction on thickening fraction measurement at Day 1 and Month 3

Variables	DD at Day 1	No-DD at Day 1	*p* Value	DD at Month 3	No-DD at Month 3	*p* Value
No. of patients	17	3		8	15	
Age (years)	55 (30-59)	61 (59.5-63)	0.09	58 (55.75-61)	55 (32-62.5)	0.7
BMI (kg/m^2)	19.23 (18.12-23.11)	19.47 (17.27-26.54)	1.00	18.91 (16.62-21.90)	23.08 (18.77-26.73)	0.12
Male	9 (53%)	1 (33%)	1.00	1	10	0.04
Primary Diagnosis			0.74			0.03
Cystic fibrosis	4 (24%)	0		0	4	
COPD	3 (18%)	2		5	1	
Pulmonary fibrosis	4 (24%)	1		0	6	
Other	6 (35%)	0		3	4	
Transplant Type			0.89			0.50
Single Lung	15 (88%)	3 (100%)		0	2	
Bilateral Lung	2 (12%)	0		8	10	
Combined Organ	0	0		0	3	
Incision Type			0.59			0.62
Clamshell	5 (29%)	0		3	3	
Bilat anterior thoracotomies	11 (65%)	3 (100%)		5	8	
Sternotomy	1 (6%)	0		0	2	
Unilateral thoracotomy	0	0		0	2	
CPB time (min)	210 (201-244)	210 (182-219)	0.56	197.5 (188.5-204.25)	227 (186.5-245)	0.12
ECMO time (days)	0 (0-0)	0 (0-0)	0.61	0 (0-0)	0 (0-0)	0.52
Duration of Mechanical Ventilation, hours	27.9 (12.72-38.9)	18.92 (15.77-20.71)	0.48	22 (17.9430.54)	19.37 (12.31-33.25)	0.54
ICU length of stay (h)	94.68 (52.9-141.23)	94.92 (67.02-95.21)	0.48	95.09 (80.41-154.03)	60.82 (42.69-108.93)	0.13
Hospital length of stay (days)	26 (15-36)	14 (13-40.5)	0.75	33.5 (27-46.75)	15 (13-25)	0.12
Discharge Destination			0.71			
Home	12 (70%)	2 (67%)		4	12	0.14
Inpatient Rehabilitation	3 (18%)	1 (33%)		4	2	
Deceased	2 (12%)	0		0	1	

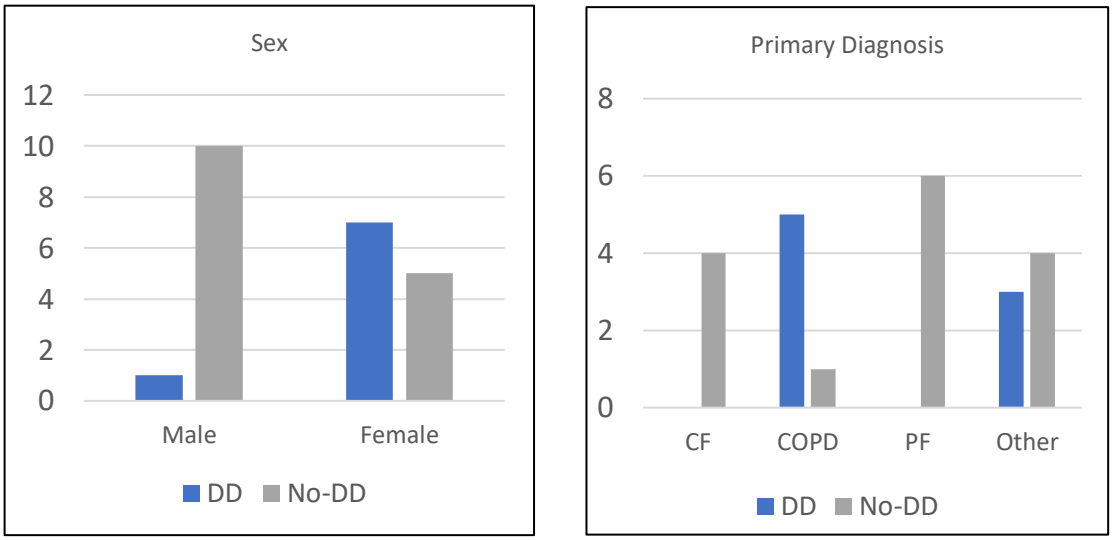
Legend:
Continuous variables reported as median (interquartile range); percentage data shown as n (%).
*Compared using Mann-Whitney U test. ^Compared using Chi-squared test.
Abbreviations: DD, diaphragmatic dysfunction; BMI; body mass index; COPD, chronic obstructive pulmonary disease; CPB, cardio-pulmonary bypass; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

Table 4.2.3 Characteristics of patients with and without diaphragmatic dysfunction on deep breathing measures at month 3 post lung transplantation

Variables	DD at Month 3	No-DD at Month 3	*p* Value
No. of patients	18	5	
Age (years)	56.5 (36.75-60.5)	61 (43-63)	0.43
Male	11	0	0.06
Body Mass Index (kg/m^2)	20.36 (18.22-23.11)	26.77 (19.47-28.87)	0.28
Primary Diagnosis			0.25
Cystic Fibrosis	4	0	
COPD	4	2	
Pulmonary Fibrosis	5	1	
Other	5	2	
Transplant Type			0.35
Single Lung	1	1	
Bilateral Lung	15	3	
Combined Organ	2	1	
Incision Type			0.37
Clamshell	5	1	
Bilat anterior thoracotomies	11	2	
Sternotomy	1	1	
Unilateral thoracotomy	1	1	
CPB time (min)	204.5 (187.5-231.75)	205 (201-210)	0.97
ECMO time (days)	0 (0-0)	0 (0-0)	0.67
Duration of Mechanical Ventilation (h)	19.15 (12.18-31.73)	22.33 (21.08-33.5)	0.48
ICU length of stay (h)	77.67 (50.85-105.64)	108.83 (72.88-170.83)	0.40
Hospital length of stay (days)	21 (13.25-35)	14 (13-24)	0.43
Discharge Destination			
Home	13	3	0.66
Inpatient Rehab Facility	4	2	
Deceased	1	0	

Legend:
Continuous variables reported as median (interquartile range); percentage data shown as n (%).
*Compared using Mann-Whitney U test. ^Compared using Chi-squared test.
Abbreviations: DD, diaphragmatic dysfunction; BMI; body mass index; COPD, chronic obstructive pulmonary disease; CPB, cardio-pulmonary bypass; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

Figure 4.3 Differences in sex and primary diagnosis between those with and without diaphragmatic dysfunction on thickening fraction at 3 months post transplant



Legend:
DD, diaphragmatic dysfunction present; No-DD, diaphragmatic dysfunction absent; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; PF, pulmonary fibrosis.

4.2.4 Reproducibility of image analysis

All the ultrasound images assessed using Image J software were independently measured by two investigators (Elise Crothers and Nikki Molan) to determine accuracy. The intraclass correlation coefficient between the two assessors was calculated for every patient at each time point (preoperative; one day, one week, one month, and three months postoperatively). There was excellent concordance between the two assessors for measures of left and right excursion on deep breathing measures (ranging from 0.851-0.984), and voluntary sniff measures (ranging from 0.903-0.975) demonstrating a high agreement rate between both observers for these measures (see Tables 4.4.1, 4.4.2 and 4.4.3 for results).

For measures of thickening fraction, overall reproducibility of image analysis was more variable (ranging from 0.235-0.941). Agreement between the assessors was excellent for preoperative measures of both hemidiaphragms (0.853-0.886), for the right hemidiaphragm at Week 1 (0.83), and for the left hemidiaphragm at Day 1 (0.941) and good agreement was

achieved for measures of left thickening fraction at Month 3 (0.733). The remaining measures had poor inter-rater concordance with intraclass correlation coefficients < 0.469 (p>0.05).

Table 4.3.1 Inter-rater reliability of excursion measures during Deep Breathing

Right Hemidiaphragm	***Preop***	***Day 1***	***Week 1***	***Month 1***	***Month 3***
Intraclass Correlation Coefficient	0.961	0.980	0.983	0.969	0.984
95% CI Lower Bound	0.913	0.948	0.959	0.929	0.963
Upper Bound	0.983	0.992	0.993	0.986	0.993
Significance	0.000	0.000	0.000	0.000	0.000
Left Hemidiaphragm					
Intraclass Correlation Coefficient	0.932	0.967	0.913	0.932	0.851
95% CI Lower Bound	0.839	0.909	0.740	0.827	0.555
Upper Bound	0.971	0.988	0.971	0.973	0.950
Significance	0.000	0.000	0.000	0.000	0.001

Table 4.3.2 Inter-rater reliability of excursion measures during Voluntary Sniff

Right Hemidiaphragm	***Preop***	***Day 1***	***Week 1***	***Month 1***	***Month 3***
Intraclass Correlation Coefficient	0.964	0.929	0.975	0.910	0.951
95% CI Lower Bound	0.919	0.819	0.937	0.796	0.885
Upper Bound	0.984	0.972	0.990	0.960	0.979
Significance	0.000	0.000	0.000	0.000	0.000
Left Hemidiaphragm					
Intraclass Correlation Coefficient	0.932	0.955	0.948	0.903	0.975
95% CI Lower Bound	0.827	0.870	0.846	0.740	0.923
Upper Bound	0.973	0.984	0.983	0.964	0.992
Significance	0.000	0.000	0.000	0.000	0.000

Table 4.3.3 Inter-rater reliability of Thickening Fraction measures

Right Hemidiaphragm		***Preop***	***Day 1***	***Week 1***	***Month1***	***Month3***
Intraclass Correlation Coefficient		0.853	0.360	0.830	0.411	0.469
95% CI	*Lower Bound*	0.654	-0.768	0.530	-0.451	-0.252
	Upper Bound	0.938	0.768	0.938	0.761	0.775
	Significance	0.000	0.191	0.000	0.123	0.073
Left Hemidiaphragm						
Intraclass Correlation Coefficient		0.886	0.941	0.235	0.245	0.733
95% CI	*Lower Bound*	0.720	0.843	-0.885	-0.907	0.325
	Upper Bound	0.954	0.978	0.690	0.701	0.894
	Significance	0.000	0.000	0.277	0.273	0.003

4.3 Discussion

This chapter investigated the prevalence of diaphragmatic dysfunction according to three different ultrasonographic outcomes measures. This is the first study to report observational data on diaphragmatic dysfunction in the lung transplant population using these outcome measures. To observe the natural history of dysfunction according to each outcome measure, the prevalence of dysfunction was recorded preoperatively; and then one day, one week, one month, and three months after lung transplantation. The characteristics of patients with and without dysfunction at one day, and three months after transplant were then compared for differences. The inter-rater repeatability of retrospective image analysis was also analysed because this has not been evaluated previously.

A summary of the main results follows. All measures of diaphragmatic dysfunction are highest at one day after transplant and demonstrate improvement over time. Although the prevalence

of dysfunction varied greatly between each outcome measure, they all followed the same pattern of prevalence over time, i.e., even when starting with dysfunction preoperatively, diaphragm function worsened at Day 1 and then progressively improved. Diaphragm function, and the prevalence of diaphragmatic dysfunction, at Month 3 after transplant is similar to preoperative measures, suggesting good recovery of diaphragmatic function within three months.

Unlike the prevalence of dysfunction during quiet breathing, the prevalence of dysfunction during voluntary sniff and deep breathing was consistently high at all assessment time points, including preoperatively. This is a new finding because these measurements have previously only been reported for normal individuals[14]. One possible explanation for the high prevalence of dysfunction on these outcome measures is that the voluntary sniff and deep breathing manoeuvres require maximal voluntary inspiratory effort from the patient, which includes full cooperation in patients affected by anaesthesia and analgesia. Thus, these techniques may be imprecise if the patient is drowsy or unable to cooperate at the time of assessment. The high prevalence of dysfunction observed on these outcome measures preoperatively, suggests that on exertion, patients with advanced lung disease do not recruit the diaphragm similarly to normal individuals. For example, in patients with a diagnosis of advanced lung disease such as chronic obstructive pulmonary disease (COPD), it has been demonstrated that relatively little of the increase in lung volume at total lung capacity (TLC) is accounted for by shortening of the diaphragm[52]. The chest wall, diaphragm geometry and respiratory mechanics are altered[52], and accessory muscles compensate to meet an increased ventilatory demand. We would therefore anticipate similar changes to occur in other advanced lung diseases to explain for the reduced diaphragmatic excursion during forced inspiratory manoeuvres.

Unexpectedly, there were no statistically significant differences in clinical outcomes for those patients with versus those without diaphragmatic dysfunction on any of the outcome measures analysed. This result was surprising because previous studies of patients with postoperative diaphragmatic dysfunction after cardiothoracic, abdominal, and lung transplant surgery have reported adverse clinical outcomes[17-22]. This disagreement may be because this study lacked power to detect a difference between the groups. There were, however, some differences observed in hospital length of stay which are of clinical importance. In our study, patients with diaphragmatic dysfunction at Month 3 after transplant on measures of thickening fraction, deep breathing and quiet breathing had a hospital length of stay at least seven days greater than those patients without dysfunction. Patients with diaphragmatic dysfunction identified at Day 1 after transplant on measures of thickening fraction and voluntary sniff had a length of stay more than ten days greater than the patients without dysfunction. A finding of increased hospital length of stay is in accord with findings reported by Maziak et al[19] who found that lung transplant patients with diaphragmatic dysfunction had an average hospital length of stay eight days longer than those without. An increased length of stay is a clinically important finding because it reduces hospital efficiency and can increase patient morbidity[53]. It comes at a considerable cost to hospital resources, obstructs patient flow through the hospital which in turn prevents new admissions and new surgery from being performed, and delays patients' achieving their goal of returning to their home environment.

Demographically, patients who had diaphragmatic dysfunction with the voluntary sniff measurement at Day 1 postoperatively, were statistically younger in age than those who did not. This relationship was also observed in patients with dysfunction on their measure of excursion during quiet breathing. This finding has not been previously reported. Another new finding from this data was at Month 3 after transplant, patients with and without diaphragmatic dysfunction on measures of thickening fraction showed a significant difference

for sex and primary diagnosis: only one male had diaphragmatic dysfunction, the other ten males did not; and five out of six patients with COPD had diaphragmatic dysfunction, whereas all patients with cystic fibrosis and pulmonary fibrosis did not. Although the group numbers are small, it suggests that male patients, and those with cystic fibrosis or pulmonary fibrosis, have greater potential to increase diaphragm thickness postoperatively compared to their counterparts. To summarise, we found that patients who are younger in age (<65 years) were more likely than older patients to develop diaphragmatic dysfunction Day 1 after transplant based on excursion measures during quiet breathing (Chapter 3) and voluntary sniff. This finding was unexpected and it is difficult to speculate why this may be. In addition, a primary diagnosis of COPD could be a risk factor for persistent diaphragmatic dysfunction at Month 3 if measured by thickening fraction. This association was not observed in the measures of excursion during quiet breathing (reported in Chapter 3), but is not surprising as the correlation between diaphragmatic excursion and thickening fraction is not strong[51]. Interestingly, for patients with COPD, it appears that postoperatively, diaphragmatic excursion during quiet breathing is normalised whereas diaphragmatic thickening is not. As previously mentioned, the degree of thickening reflects the magnitude of diaphragmatic effort[48], therefore for patients with COPD, diaphragmatic recruitment is still limited postoperatively. I propose this could be due to irreversible chest wall remodelling which occurred preoperatively and impacts the length/tension relationship of the diaphragm. However, this theory is only speculation and requires further exploration.

When considering the reliability of image analysis, consistency between clinicians is important if taking measures retrospectively. The inter-rater repeatability of taking these measurements in retrospect has not previously been studied, as most clinicians would take the measurements at the time of image acquisition. The advantage of taking measurements retrospectively is that it minimises the physical assessment time for the patient, allowing them more time for other

interventions, or rest. This data shows that measurements of diaphragmatic excursion during deep breathing and voluntary sniff from saved images can be undertaken retrospectively with high inter-rater reliability. Measures of thickening fraction, however, have poor inter-rater reliability when undertaken retrospectively. It is difficult to speculate the reason for this, as the data did not reveal that one hemidiaphragm, or one particular assessment time-point had worse reliability than the others, and half of the measurements demonstrated good agreement. Thus, the findings suggest that it would be preferable to take these measures contemporaneously at the bedside, in spite of needing more time with the patient.

When evaluating the different measurements used to diagnose diaphragmatic dysfunction in transplant patients, excursion during deep breathing is likely the least useful. The high prevalence of preoperative dysfunction by this method makes tracking of dysfunction in the acute postoperative period and recovery of little use. We found that 89% of patients met the criteria for diaphragmatic dysfunction when using deep breathing preoperatively, all patients met the criteria at day one, and 78% at Month 3. Thus, this measure fails to discriminate between those who may be at risk of poor clinical outcomes versus those who will thrive.

5 CHAPTER 5: DISCUSSION

5.1 Thesis Summary

Diaphragmatic dysfunction represents an important postoperative problem for lung transplant patients but assessing for it can be challenging and thus, clinicians do not fully understand the clinical implications of the problem, or its rate of recovery. Point-of-care ultrasound provides a means to overcome this diagnostic challenge in the acute setting, but it has not been evaluated using standardised methods in the acute postoperative period. The aim of this thesis was to identify the incidence, natural history and clinical impact of diaphragmatic dysfunction in lung transplant patients during the acute postoperative period. This project for the first time reports the prevalence of diaphragmatic dysfunction using a number of different ultrasound measurement techniques against a backdrop of clinical outcome measures to provide insight into its clinical importance in lung transplant patients. To achieve this, we conducted a prospective observational cohort study of 27 lung transplant recipients and used ultrasound to determine the incidence of dysfunction according to four methods: excursion during quiet breathing, deep breathing, voluntary sniff; and thickening fraction. Chapter 2 explains the history of different methods for measuring diaphragmatic dysfunction, forming the basis for the methods used in the study. In addition, we examined the natural history of diaphragmatic dysfunction from Day 1 after transplant up to Month 3, and the impact of dysfunction on clinical outcomes. Moreover, the reliability of taking post hoc measures from saved ultrasound images was for the first time evaluated.

5.2 Summary of Main Findings

Analysis of Diaphragmatic Dysfunction – Identified by Excursion during Quiet Breathing

Using the most commonly reported diaphragm assessment method with ultrasound, excursion during quiet breathing, this study prospectively reported for the first time the incidence and natural history of diaphragmatic dysfunction in lung transplant patients during the acute postoperative period. This study revealed that using this method, the incidence of diaphragmatic dysfunction after lung transplantation was 62% one day post lung transplant. This is much higher than the incidence previously reported by Ferdinande and colleagues[18] (7.4%) who assessed diaphragm function (using ultrasound during sniff) within 3 hours of extubation. Prior to this, the earliest that diaphragm function had been assessed was 'during the first postoperative week', and the incidence of dysfunction at this time was 29.6% as assessed by nerve conduction studies and fluoroscopy[15]. In our study, the prevalence of persistent diaphragmatic dysfunction at three months post-transplant was only 22%, demonstrating good recovery of diaphragm function within this period.

Patients with and without diaphragmatic dysfunction according to excursion during quiet breathing were compared for differences in age, sex, body mass index (BMI), primary diagnosis, transplant type, surgical approach, cardiopulmonary bypass time, need for extracorporeal membranous oxygenation, duration of mechanical ventilation, length of stay in intensive care, length of stay in hospital, and discharge destination. When comparing patients with diaphragmatic dysfunction to those without diaphragmatic dysfunction, there was no statistically significant difference in the postoperative clinical outcomes examined, or any of the predictive perioperative variables. This finding is contrary to findings from early studies [54,55] but supported by more recent findings by LoMauro et al.[56] One possible explanation for the lack of clinical impact in recent studies is that there may be a difference in modern clinical management which masks the effects of dysfunction. Regardless, this speculation should be considered with caution because both our study, and the one by LoMauro and colleagues, had a small sample size.

One other interesting and unexpected finding was that patients who experienced diaphragmatic dysfunction at Day 1 postoperatively were younger in age and had a lower BMI than those who did not. This finding was surprising because it has not been identified in the past, and we cannot speculate why these might be risk factors.

To minimise the assessment time for the patient, the measurements were taken from saved ultrasound images and then repeated by a blind assessor for reliability analyses. When analysing the saved ultrasound images, there was excellent concordance between the two assessors confirming that these measurements can be taken retrospectively to minimise time cost to the patient.

Analysis of Diaphragmatic Dysfunction – Additional Ultrasound Outcome Measures

Other methods available to test for diaphragmatic dysfunction using ultrasound are: measurement of diaphragmatic excursion during deep breathing, excursion during voluntary sniff, and thickening fraction. This thesis also investigated the prevalence and natural history of diaphragmatic dysfunction using these methods. The prevalence of diaphragmatic dysfunction on all three of these additional outcome measures was highest at Day 1 after transplant and then reduced over time. The prevalence of diaphragmatic dysfunction, at Month 3 after transplant was similar to preoperative measures, again suggesting good recovery of diaphragmatic function within three months. Importantly, excursion of the diaphragm involving voluntary actions (i.e., deep breathing and voluntary sniff) showed almost all patients awaiting lung transplant had dysfunction preoperatively. This finding was unexpected, but one that makes sense given that voluntary motions are harder to perform for people with lung disease, recruiting accessory muscles rather than the diaphragm to achieve increased lung volumes. Patients with and without diaphragmatic dysfunction according to

these additional outcome measures (excursion during deep breathing and voluntary sniff, and thickening fraction) were compared for differences in the same baseline and perioperative variables analysed for quiet breathing. There were no statistically significant differences in clinical outcomes for those patients with versus those without diaphragmatic dysfunction, on any of the clinical outcome measures analysed. As we found for dysfunction during quiet breathing, patients with diaphragmatic dysfunction on their voluntary sniff measurement at Day 1 postoperatively, were statistically younger in age than those without. Another new finding was that at Month 3 after transplant, patients with and without diaphragmatic dysfunction on measures of thickening fraction showed a significant difference in sex and primary diagnosis.

These measurements were also taken from saved ultrasound images and then repeated by the blind assessor for reliability analyses. There was excellent concordance between the two assessors for measures of excursion during deep breathing, and voluntary sniff. For measures of thickening fraction, overall reproducibility was much more variable.

5.3 Clinical Implications

The findings of this thesis suggest that ultrasound assessment of diaphragmatic excursion during quiet breathing is likely the most useful in the acute postoperative setting because it can be obtained independent of patient effort, and the outcome measure (based on normative data) is sensitive enough to discriminate between those with and without dysfunction. Assessment of diaphragmatic excursion during deep breathing or voluntary sniff on the other hand, yields a very high prevalence of dysfunction which is probably not clinically helpful (e.g., 100% of patients at Day 1 on measures of excursion during deep breathing). This assessment method is possibly confounded by postoperative factors (such as pain, fatigue and drowsiness)

which impair patient performance when executing a measure dependent on voluntary participation.

Another important clinical finding from this thesis is that patients who are younger in age (<65 years) are more likely than older patients to develop diaphragmatic dysfunction Day 1 after transplant on their excursion measures during quiet breathing and voluntary sniff. Also, a primary diagnosis of COPD is a risk factor for persistent diaphragmatic dysfunction measured by thickening fraction. Considering this, patients with an age less than 65 years and/or a primary diagnosis of COPD could therefore be targeted for prophylactic interventions such as inspiratory muscle training or may require additional respiratory support such high-flow oxygen and non-invasive ventilation. Periodic ultrasound assessment of diaphragmatic excursion during quiet breathing and calculation of their diaphragm thickening fraction could be helpful in the early identification and monitoring of diaphragmatic dysfunction in this subgroup. Future work should consider the value of these targeted interventions.

With regards to conducting an assessment which is efficient for the patient, measurements of diaphragmatic excursion during quiet breathing, deep breathing, and voluntary sniff can be done retrospectively with high inter-rater reliability, allowing the patient more time for other postoperative care interventions. Conversely, measures of thickening fraction have poor inter-rater reliability when done retrospectively, so it would be preferable to take these measures contemporaneously at the bedside, despite requiring more time from the patient.

5.4 Limitations and Directions for Future Research

This thesis has demonstrated that early diaphragmatic dysfunction does not have a significant negative impact on the acute care outcomes we investigated. However, we acknowledge that

we did not assess the impact of diaphragmatic dysfunction on a number of other important clinical and patient-centred outcomes such as postoperative pulmonary function (e.g., spirometry, mean inspiratory and expiratory pressures), physical function (e.g., 6 minute walk test), the development of postoperative pulmonary complications (e.g., atelectasis and pneumonia), need for non-invasive ventilation, readmission to ICU and to hospital, or quality of life measures. Evaluating the relationship between early diaphragmatic dysfunction and these other outcome measures was beyond the scope of this thesis, so further research to evaluate these outcomes measures could provide insight into outcomes of clinical importance and guide supportive interventions.

Another limitation was that data acquisition was not always possible, mainly because it was difficult to obtain a satisfactory acoustic window which resulted in poor quality images. As mentioned in Chapter 3 (analysis of quiet breathing), satisfactory imaging of both hemidiaphragms was not possible in 2/22 patients, and this was one week after transplant. The data for unsuccessful imaging of the other manoeuvres was not recorded. Difficulty in image acquisition has also been reported by other authors, particularly when visualising the left hemidiaphragm[26,57]. Finding a suitable acoustic window in our study cohort was further complicated by the presence of surgical dressings, chest drains, and pneumothorax. While these are known issues, they need to be considered because failure to visualise measurements reduces the sample in an already small population.

We also acknowledge that this project was not powered to establish with definitive precision, the incidence of diaphragm dysfunction. Our sample size was small and heterogenous which is reflective of the typical transplant caseload. To fully understand whether diaphragmatic dysfunction is important in this cohort, future research will need to be across multiple sites and of longer duration to include greater numbers of participants from the lung transplant

population. Now that clinicians have access to a suitable tool for the assessment of early diaphragmatic dysfunction, future directions should focus on pooling data to increase the statistical significance of our clinical outcome data.

Another inclusion for future research would be to consider other perioperative variables which may affect postoperative diaphragm function at different time points such as pain, the presence of chest drains and the use of opioid medications. Some patients experience more pain, require stronger analgesia, or have chest drains for longer than other patients, which may affect diaphragm function and thus, it would be important to examine if these factors have an influence on measures of diaphragmatic function, prolong the duration of diaphragmatic dysfunction, or are related to other clinical outcome measures. Again, evaluating these outcomes was outside the scope of this study. Admittedly these factors do not cause intrinsic muscular dysfunction, but impair diaphragmatic performance nevertheless. Evaluating the influence of these variables on early postoperative diaphragmatic function may provide an explanation for its high incidence in the acute postoperative period. It would be necessary to monitor these variables in the analysis of future studies.

Finally, although there are a number of ultrasound methods available for the assessment of diaphragmatic function, and each has its own diagnostic criteria for diaphragmatic dysfunction, there is no universal agreement on which method is best. This thesis studied each method in isolation, however, there may be a composite measure which is more powerful at identifying those patients who will have worse outcomes. To assist clinicians in the diagnosis of diaphragmatic dysfunction, future research should be directed at developing valid and reliable diagnostic criteria with ultrasound by comparing them with reference methods (such as diaphragmatic twitch pressure) in surgical patient populations. A final consideration would be to develop a grading system for the severity of diaphragmatic dysfunction to see if patients

with severe dysfunction have worse outcomes. Ultimately, we hope to reveal a method of assessment which will accurately identify patients in need of supportive treatments to improve their clinical course.

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