Summary

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The first part of this thesis describes our present knowledge about the diaphragm: In chapter 2 the embryology, anatomy and physiology are described, together with the assessment of its function. Impairment of diaphragmatic function can have major physiological consequences. Many of these impairments are amenable for surgical correction, but correct diagnosis and treatment require thorough understanding of form and function.

In chapter 3 we evaluated the functional disorders. A paralysed, elevated diaphragm is thought to be the result of either eventration or an acquired paralysis. Although these conditions are considered to be two separate entities, they are discussed together, because symptoms and treatment are similar. This chapter describes some arguments why eventration and paralysis might even be different stages in the same process.

The clinical presentation of diaphragm dysfunction is highly variable and depends on the degree of paralysis, whether it is unilateral or bilateral and on the presence of concomitant pulmonary disease. In unilateral paralysis up to half of the patients are asymptomatic and their paralysis is often detected by chest imaging done for other reasons. The symptomatic ones present with diminished exercise tolerance and dyspnea in supine position, which is called orthopnea. In all patients the dyspnea can be explained by either increased work of breathing and/or by the compression of the lung, leading to atelectasis and shunting. A new, only recently understood, factor that can contribute to the diminished exercise tolerance is the compression of the heart by an elevated diaphragm, resulting in circulatory impairment.

Pulmonary function tests are helpful, but only if done in both an upright and supine position. However, pulmonary function tests do not always correlate well with the severity of the dyspnea, but should be obtained to check for concomitant lung disease and to have a base-line for further testing post-operatively.

A combination of imaging procedures is often needed to establish the correct diagnosis. Dynamic MR imaging may be the best, since this imaging includes both the lung and heart, allowing for the imaging of the dynamic function of the heart in relation to ventilation. Although cardiac
compression can be found in some patients, this not necessarily means that this has a functional consequence. In symptomatic patients with unilateral disease, surgical correction may be indicated. Substantial and durable improvement can be expected. Also in diaphragm surgery there has been a shift to less invasive procedures. Muscle-sparing anterior thoracotomy, mini-thoracotomy with thoracoscopic vision, full thoracoscopic and laparoscopic procedures have all been described. All reported methods of surgical intervention seem to give good results in experienced hands, but randomized investigations are lacking, due to the rarity of the disease. The laparoscopic approach seems promising. Caution is needed in recent idiopathic paralysis, because spontaneous improvement might occur and in bilateral disease, because improvements are limited and complications frequent.

PART II: Effects of Surgical Intervention on Diaphragm Disorders

In Chapter 4 we describe the results of cardio-pulmonary tests in symptomatic patients with a hemidiaphragm paralysis who have symptoms and who were listed for plication. Often the spirometric and static lung volumes are within the normal range before surgery and following plication the changes are small and thus do not explain the improvement of dyspnea relief in these patients. It is clear that pulmonary function tests will improve after the procedure but this improvement is not always correlated to clinical or functional improvements. Furthermore, it remains unknown whether symptomatic relief of symptoms also translates into an improvement of exercise tolerance. Cardiopulmonary exercise testing might provide additional insights to unravel the mechanisms that underlie the relief of exercise induced dyspnea, since this test enables to study the effects of surgery on ventilatory, circulatory and gas exchange parameters at exercise. For this reason, we performed cardiopulmonary exercise tests in a group of patients diagnosed with hemidiaphragm paralysis before and after plication of the diaphragm. We found in patients with hemidiaphragm paralysis who underwent a diaphragm plication that, exercise tidal volumes increased and the ventilatory frequency decreased. Despite this improvement, maximal exercise capacity remained unaltered. Since our study strongly suggest that increase in tidal volume and decrease in
breathing frequency are the most likely explanations for the symptomatic relief of patients, exercise tidal volume might help to preselect patients who will benefit from surgery.

**Chapter 5** describes the laparoscopic diaphragmatic plication in patients with unilateral diaphragm paralysis. Diaphragm paralysis is an uncommon condition that is usually discovered incidentally in patients who are asymptomatic and who have an elevated hemidiaphragm on chest X-ray. The most common symptom is dyspnea. In symptomatic patients with unilateral diaphragm paralysis and dyspnea disproportionate to the degree of physical activity, diaphragm plication is the treatment of choice to relieve dyspnea. Minimally invasive diaphragm plication techniques are now effective alternatives to open plication. A variety of open and minimally invasive diaphragm plication techniques have been described to reduce symptomatic dysfunctional diaphragm excursion during respiration. We report on our experiences in laparoscopic plication of the paralysed hemidiaphragm in seven patients. In this chapter, we present our modified technique in laparoscopic plication of the paralysed diaphragm. This technique provides excellent field of vision during surgery, postoperative recovery with minimal pain and early discharge from the hospital. The laparoscopic approach is an attractive surgical alternative for the treatment of phrenic nerve palsy and should be considered in all suitable patients.

In **Chapter 6** we describe an unusual treatment of patent foramen ovale after pneumonectomy in three patients. Pneumonectomy is a major procedure with frequent perioperative complications such as empyema, fistula, cardiac problems or respiratory insufficiency. Besides frequent postoperative cardiac and respiratory complications, long-term sequelae are also seen.

After pneumonectomy, anatomical adaptations occur with repositioning of intrathoracic structures. Common changes are elevation of the hemidiaphragm (especially after phrenic nerve damage), mediastinal shift, diminished intercostal space and filling of the postpneumonectomy space with fluid. Infrequently, these adaptations may lead to invalidating complications. In this last chapter of part II, we will focus on a rare complication: shunting through a patent foramen ovale (PFO), as a long-term complication of right-sided pneumonectomy or bilobectomy. This chapter describes three patients who were diagnosed with intermittent shunting through a PFO following lung resection.
Treatment of interatrial shunting is preferable done by percutaneous transcatheter closure. After a percutaneous closure, the shunt may cease, but the right ventricle compression by the diaphragm has not been stopped and neither will the right atrial pressure go down. Therefore, we postulated it may be more logical to remove the cause of the shunting when the condition of the patient allows surgery.

In these patients, right-to-left shunting through a PFO occurred because of an outflow obstruction of the right ventricle due to an elevated diaphragm and surgical plication of the right hemidiaphragm was sufficient to close the PFO.

**PART III: Effects of Diaphragm Inactivity on Muscle Fiber Function, Structure and Gene Expression**

In Chapter 7 we investigated the effect of surgery on the functioning of the diaphragm, the main muscle of inspiration by evaluating diaphragm muscle fiber function after contractile inactivity during thoracic surgery. Postoperative pulmonary complications are significant contributors to morbidity in patients who have undergone upper abdominal, thoracic, or cardiac surgery. The pathophysiology of these complications might involve postoperative inspiratory muscle weakness. The nature of postoperative inspiratory muscle weakness is unknown. Several reports suggest that these pulmonary complications after surgery are related to postoperative inspiratory muscle weakness.

The objective of the present study was to investigate for the first time whether a short-term interruption of normal inspiratory muscle activity during surgery causes weakness of diaphragm fibers. To this end, we used a longitudinal approach and determined the contractile performance of single diaphragm muscle fibers at the end of thoracic surgery and compared this with that at the start of surgery. Serial biopsies from the diaphragm and the latissimus dorsi muscle were obtained from 6 patients during thoracotomy for resection of a tumor in the right lung. Biopsies were taken as soon as the diaphragm had been exposed and again after two hours. The contractile performance of demembranated muscle fibers, as well as fiber morphology and markers for proteolysis, was determined. In all patients, the force-generating capacity of diaphragm muscle fibers at t(2) was significantly reduced compared with that at t(0), with a more pronounced force loss in type 2
fibers compared with type 1 fibers. Diaphragm weakness was not part of a generalized muscle weakness as contractile performance of latissimus dorsi fibers was preserved at t(2). Diaphragm fiber size and myofibrillar structure were not different at t(2) compared with t0, but myosin heavy chain type 2 was significantly reduced at t(2) and MuRF-1 mRNA and protein levels were elevated at t(2).

We concluded that only 2 hours of thoracic surgery causes marked, and selective, diaphragm muscle fiber weakness and we speculate that this selective diaphragm muscle fiber weakness contributes to the development of postoperative pulmonary complications.

In Chapter 8 we investigated the gene expression profile in the diaphragm following contractile inactivity during thoracic surgery. Chapter 7 revealed the development of marked muscle fiber weakness in the diaphragm, but not in the non-respiratory latissimus dorsi muscle, during thoracic surgery. To disentangle the molecular processes that underlie the development of this diaphragm muscle fiber weakness during thoracic surgery, we studied changes in the gene expression profile.

Serial biopsies from the diaphragm and the latissimus dorsi muscle were obtained from four patients during thoracotomy for resection of a tumor in the right lung. Biopsies were taken as soon as the diaphragm had been exposed (t0) and again after two hours (t2). Global differences in gene expression in diaphragm biopsies were assessed by microarray analysis. In this study 346 differentially expressed gene transcripts were found in the diaphragm at t2 vs. t0. Pathway analysis revealed that genes associated with inflammation (83 genes; p<0.0001) and cell death (118 genes, p<0.0001) pathways were significantly upregulated at t2. Of the 346 differentially expressed genes in the diaphragm at t2, 258 were also differential in the latissimus dorsi muscle, with the direction of change being identical for all differentially expressed genes. In addition, latissimus dorsi showed exclusive upregulation of negative regulators of cell death.

We found that two hours of thoracic surgery result in rapid and profound changes in expression of inflammatory response and apoptotic genes in the diaphragm. The apoptotic response was stronger in the diaphragm than in the latissimus dorsi. These findings suggest that the development of selective diaphragm muscle fiber weakness in these patients might be related to an exaggerated apoptotic response. Acute
mediators of apoptotic pathways are rapidly activated during thoracic surgery, and longer durations of mechanical ventilation are likely needed to significantly activate downstream apoptotic pathways.

In Chapter 9 we investigated diaphragm muscle fiber function and structure in humans with hemidiaphragm paralysis. Recent studies proposed that mechanical inactivity of the human diaphragm during mechanical ventilation rapidly causes diaphragm atrophy and weakness. However, conclusive evidence for the notion that diaphragm weakness is a direct consequence of mechanical inactivity is lacking. The paralysed diaphragm can serve as a model for diaphragm muscle fiber inactivity during mechanical ventilation. Studying hemidiaphragm paralysis allows us to investigate the effect of contractile inactivity on diaphragm muscle fiber function and structure, independent of confounding effects related to other major illness or systemic effects.

To study the effect of hemidiaphragm paralysis on diaphragm muscle fiber function and structure in humans, biopsies were obtained from the paralyzed hemidiaphragm in eight patients with hemidiaphragm paralysis. All patients had unilateral paralysis of known duration, caused by en bloc resection of the phrenic nerve with a tumor. Furthermore, diaphragm biopsies were obtained from three control subjects. The contractile performance of demembranated muscle fibers was determined, as well as their fiber ultrastructure and morphology. Finally, expression of E3 ligases and proteasome activity was determined to evaluate activation of the ubiquitin-proteasome pathway. Our results showed that the force-generating capacity, as well as myofibrillar ultrastructure, of diaphragm muscle fibers was preserved up to 8 weeks of paralysis. The cross-sectional area of slow fibers was reduced after 2 weeks of paralysis; that of fast fibers was preserved up to 8 weeks. The expression of the E3 ligases MAFbx and MuRF-1 and proteasome activity was not significantly upregulated in diaphragm fibers following paralysis, not even after 72 and 88 weeks of paralysis, at which time marked atrophy of slow and fast diaphragm fibers had occurred. Our findings reveal that diaphragm muscle fiber atrophy and weakness following hemidiaphragm paralysis develops slowly and takes months to occur. This might indicate that the human diaphragm is resistant to contractile inactivity and we propose that the resistance of the paralyzed diaphragm to contractile inactivity might lie in the passive cyclic stretch
due to the intermittent contractions of the normally innervated contralateral hemidiaphragm.