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A novel technique for diaphragm biopsies in human patients

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ABSTRACT

Background: The diaphragm is difficult to biopsy because of its anatomic location. We describe a new laparoscopic diaphragm biopsy technique.
Material and methods: Fifty one patients with amyotrophic lateral sclerosis gave their consent to diaphragm biopsy in the context of an implanted phrenic nerve stimulation protocol (NCT01583088). The biopsy was taken from the costal diaphragm, after opening the parietal peritoneum with scissors, and by grasping the diaphragmatic muscle over the rib with toothed laparoscopy forceps.
Results: The first four electrocoagulation biopsies were unsuitable for morphologic examination. The following 47 biopsies were therefore performed without electrocoagulation. The mean size of the biopsy fragments obtained after preparation was $3 \pm 1 \times 2 \pm 1 \times 1 \pm 1$ mm (maximum: $4 \times 3 \times 2$ mm). A diaphragmatic injury occurred during the section in three cases requiring immediate suture without causing pneumothorax. A small pleural effusion was observed on the postoperative chest x-ray in one patient with a spontaneously favorable outcome. Numerous stains were able to be performed on the fragments obtained.
Conclusions: Diaphragm biopsy can be safely performed by laparoscopy and yields tissue suitable for our future histologic evaluation.

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1. Introduction

The diaphragm is the principal muscle of resting ventilation in humans but the anatomic location of the diaphragm makes it difficult to obtain biopsy material both for research and in other settings. Levine *et al.* [1,2] described a thoracoscopic diaphragm biopsy technique that has subsequently been used by other teams [1,3,4]. The disadvantage of this approach is that it is invasive, it requires thoracoscopy, and postbiopsy repair of the diaphragm [1,2,4]. We describe the methodology and morbidity of a new laparoscopic diaphragm biopsy technique. This technique was developed in the context of a clinical trial currently underway in France designed to evaluate implanted intradiaphragmatic phrenic nerve stimulation in amyotrophic lateral sclerosis (RespiStimSLA; NCT01583088).

2. Materials and methods

After approval by the appropriate ethical and regulatory authority (CPP Ile-de-France 6 La Pitié-Salpêtrière, decision no. 2012-A0017835), all biopsies were obtained from patients who had signed a specific informed consent form.

Fifty-three patients were included in the "RespiStimSLA" protocol between September 24, 2012 and September 1, 2014, and 51 of these patients gave their consent to biopsy, which was performed according to the technique described in the following 47 cases (biopsies performed with electrocoagulation in the first 4 patients were unsuitable for morphologic examination). Patient characteristics are summarized in Table.

2.1. Description of the surgical technique

Biopsy was performed at the end of the surgical procedure, after implantation of stimulation electrodes (i.e., 1 h after induction of anesthesia and start of mechanical ventilation; Fig. 1 and Video 1 online supplement). The biopsy was taken from the left hemidiaphragm, adjacent to its insertion on the 10th rib. The procedure (Fig. 1) started with opening of the parietal peritoneum with scissors introduced via the trocar in the right hypochondrium, followed by grasping of a 5-mm-thick piece of the diaphragmatic muscle over the rib using 5-mm toothed laparoscopy forceps introduced via the epigastric trocar. The diaphragmatic tissue was sectioned with scissors without coagulation on either side of the forceps to obtain a sample of about 1 cm of tissue. The biopsy was extracted through the epigastric trocar, and muscle hemostasis was ensured immediately by bipolar electrocautery (Fig. 1 and Online Supplement). The mean dimensions of the final biopsies were $3 \pm 1 \times 2 \pm 1 \times 1 \pm 1$ mm (maximum: $4 \times 3 \times 2$ mm).

Table – Characteristics of amyotrophic lateral sclerosis patients at biopsy.

Characteristics	Mean ± SD or median (IQR)
Age (y)	57.3 ± 11.3
Gender, *n* of males (%)	29 (61.7)
Time from onset of symptoms to surgery (mo)	22.5 (16.9–35)
Spinal/bulbar onset	37/10
BMI (kg/m^2)	24.6 ± 4.1
ALSFRS	34 (27–40)
PaO_2	87 (81.6–99.3)
$PaCO_2$	38 (35.1–40.8)
FVC (seated/supine) liters and % predicted	Seated: 3 (2.4–3.8), 81.5 (77–95) supine: 2.5 (2.1–2.9), 67 (63–75)
Pi_{max} % predicted	67.5 (55–83.5)
SNIP % predicted	55.5 (46.5–61.5)
% of time with SpO_2 <90%	0.7 (0–4)

BMI = body mass index; FVC = forced vital capacity; IQR = interquartile range; PI_{max} = maximal inspiratory pressure, % predicted; SD = standard deviation; SNIP = sniff nasal inspiratory pressure, % predicted.
TS90: time spent with SpO_2 below 90%, as % of total recording time.

2.2. Morbidity

A diaphragmatic injury occurred in three cases during the section of the fragment grasped by the forceps requiring immediate suture (Video 2, online supplement).

This incident did not cause pneumothorax detectable in the postoperative period despite probably pneumothorax during the laparoscopic surgery, using pneumoperitoneum. No other incident was observed. All patients were extubated and weaned from ventilation on the operating table and were discharged home on the first or second postoperative day.

2.3. Sample management

In the operating room, the biopsy was placed in a dry tube stored in crushed ice and then immediately transferred, prepared, and frozen within a maximum of 1 h.

The biopsy was placed on a glass dish on a bed of crushed ice. The biopsy was then mounted on a cork using tragacanth gum, then immersed in 2-methylbutane chilled in liquid nitrogen for 2 min while applying a circular movement. When freezing was complete, the biopsy was transferred to a chilled cryotube and stored in a freezer at −80°C.

Within 2 d after the biopsy, the samples were transferred to the histopathology laboratory in dry ice, then installed in a cryostat adjusted to −20°C to be sectioned on the same day.

To ensure more reliable interpretation, serial sections were performed, comprising two 10 μ sections for each stain. The following stains were performed in the order of the series: hematoxylin-eosin; cytochrome c oxidase; succinate dehydrogenase; myosin fluorescence immunolabeling; neonatal myosin; and developmental myosin (Fig. 2). Any remaining material was then frozen for subsequent storage.

3. Discussion

Access to the diaphragm via its inferior surface during laparoscopy has already been described for repair of traumatic lesions of the diaphragm [5,6], diaphragm plication procedures [7], resection of diaphragmatic endometriosis lesions [8], partial diaphragmatic resections for cancer invasion [9], or