Diaphragmatic dysfunction in Collagen VI myopathies

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Abstract

Collagen VI-related myopathies are hereditary disorders causing progressive restrictive respiratory insufficiency. Specific diaphragm involvement has been suggested by a drop in supine volumes. This pilot study aimed at characterizing the respiratory muscle phenotype in patients with COL6A1-3 genes mutations. Lung function, blood gases, muscle strength and respiratory mechanics were measured in 7 patients between 2002 and 2012. Patients were classified as Early-Severe (n = 3), Moderate-Progressive (n = 2) and Mild (n = 2) according to clinical disease presentation. Seven patients (aged 6–28) were evaluated. Forced vital capacity distinguished the Mild group (>60% predicted) from the two other groups (<50% predicted). This distinction was also possible using the motor function measure scale. Diaphragmatic dysfunction at rest was observed in all the Early-Severe and Moderate-Progressive patients. During a voluntary sniff maneuver diaphragmatic dysfunction was observed in all patients, as assessed by a negative gastric pressure. All patients had diaphragmatic fatigue assessed by a tension-time index over the threshold of 0.15. Diaphragmatic dysfunction during a maximal voluntary maneuver and diaphragmatic fatigue are constant features in Collagen VI myopathies. These observations can assist the diagnosis and should be taken in account for the clinical management, with the early detection of sleep-disordered breathing. © 2013 Elsevier B.V. All rights reserved.

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1. Introduction

Collagen VI (COLVI)-related myopathies, characterized by mutations in the COL6A1-3 genes, are nowadays recognized as a continuous clinical spectrum going from Bethlem myopathy, the milder form, to Ullrich
congenital muscular dystrophy (UCMD), the most severe form, with intermediate phenotypes [1]. UCMD, as described for the first time in 1930, leads to a ‘sclero-atonic’ phenotype characterized by hypotonia, muscle weakness of early onset with axial and proximal joint contractures and hyperelasticity of distal joints [2]. Respiratory impairment usually develops in the first or second decade and is a common cause of death if not treated with ventilatory support. Arrest of motor milestones with no acquisition of walking ability is seen in a subset of patients, but most children are able to walk. Among those, some show a later progression of muscle weakness with loss of ambulation around 10 years of age, and most of them require mechanical ventilation in childhood or young adulthood [3]. Bethlem myopathy, first described in 1976, is characterized by early contractures of finger flexors, wrists, elbows and ankles [4], and usually begins in the first or second decade. Respiratory failure and distal hyperlaxity may be absent or milder than in UCMD. The disease course is usually slow, with a majority of patients remaining ambulatory. However, progression of muscle weakness often occurs in the fifth decade, with about half of the patients needing walking support or a wheelchair. Patients with intermediate forms display a lesser degree of weakness and a longer period of ambulation.

COLVI myopathies emerge as an important group of disorders, somewhat under-recognized until recently, probably because of a difficult diagnostic approach due to a large clinical variability and the overlapping presentation with other muscular disorders. Therefore, many challenges remain to overcome in order to understand the genetic, biochemical and pathophysiological basis [1].

Respiratory muscle involvement varies according to the neuromuscular disorder. Indeed, the diaphragm and expiratory muscles are preferentially affected in Duchenne muscular dystrophy, whereas intercostals muscles are the weakest respiratory muscles in patients with spinal muscular atrophy [5]. In patients with COLVI-related myopathy, a progressive restrictive respiratory insufficiency may be observed [6,7]. Although pulmonary complications are the most important cause of morbidity and mortality in this disorder, data characterizing the precise respiratory muscle involvement are not available and data regarding respiratory function are only recent. We have previously reported the occurrence of a constant drop in the supine forced vital capacity (FVCsup) in a large series of patients with early-onset COLVI-related myopathy [8], suggesting a selective diaphragmatic dysfunction [9]. Moreover, the pathogenesis of muscle degeneration in COLVI deficiency was only recently partially clarified [10]. The first animal model, Col6al−/− mice, was fundamental in understanding the cellular pathways involved in these diseases [11]. Interestingly, the diaphragm appeared to be the most affected muscle, with evident signs of necrosis [11]. A latent mitochondrial dysfunction accompanied by ultrastructural alterations of mitochondria and the sarcoplasmic reticulum, resulting in spontaneous apoptosis was found in about one-third of muscle fibers of the mouse model [10]. Reduced contractile strength of the diaphragm and other muscle groups was also reported [10]. However, up to now, no study has assessed specifically the strength of diaphragm and other respiratory muscles in children with these disorders.

The aim of our study was therefore to characterize the respiratory muscle phenotype of patients harboring mutations in one of the COLVI-encoding genes.

2. Materials and methods

2.1. Patients

The charts of all the patients with a genetically confirmed COLVI-related myopathy who were followed at our multidisciplinary neuromuscular clinic between 2002 and 2012 were retrospectively reviewed. Molecular studies and clinical examination data were collected. Complementary data, including motor function measure scale (MFM), whole body muscle magnetic resonance imaging (WBMRI), and skin and muscle biopsy findings were collected when available. Patients were classified in three groups of clinical severity, according to maximal motor ability and progression [12]. Patients who never walked were classified as ‘Early-Severe’, those who acquired walking ability but lost it in the course of the disease as ‘Moderate-Progressive’ and those ambulatory with no major loss of motor function as ‘Mild’ [13]. Scoliosis surgery was decided during a multidisciplinary discussion, according to the current standards of care [14]. Post-surgery pulmonary function tests were performed at least 6 months after fusion surgery. We also collected the use of intermittent positive pressure breathing (IPPB) and the use of trunk orthosis.

Noninvasive ventilation (NIV) was initiated in case of diurnal hypercapnia (arterialized carbon dioxide tension PaCO2 >45 mmHg), or nocturnal hypercapnia (transcutaneous carbon dioxide (PtcCO2) >50 mmHg for at least 2% of night time) and/or if minimal pulse oximetry (SpO2) was <90% for at least 2% of night time [15,16]. Invasive ventilation was indicated in case of persistent hypercapnia despite a NIV use over 20/24 h.

The study was approved by the Institutional Review Board of the French learned society for respiratory medicine “Société de Pneumologie de Langue Française”, and all patients and parents gave their informed consent.

3. Procedures

3.1. Immunohistochemical studies

COLVI immunolabeling in skin fibroblasts was carried out as previously described [12] or using the refined
protocol reported by Hicks et al. [17]. Slides were observed with an Axiosplan 2 microscope equipped with a HBO100 mercury lamp (Zeiss) and representative images were obtained using the Metaview software (Roper Scientific).

3.2. COL6A1-3 molecular studies

The coding regions of the genes were amplified from fibroblasts-derived cDNA with overlapping primers as described by Brinas et al. [12].

3.3. Lung function and respiratory muscle tests

Lung function and respiratory muscle tests were recorded in a stable condition in the upright position, with the exception of FVC in supine position (FVCsup). Data were expressed as a percentage predicted for gender, age and height or arm span.

3.3.1. Noninvasive non volitional tests

 Functional residual capacity was measured by the Helium dilution technique (FRCHe%pr). Respiratory rate (f), tidal volume (Vt) and minute ventilation (Ve) were measured and the rapid shallow breathing index (f/Vt) was calculated [18]. Capillary arterialized blood gases were determined [19].

3.3.2. Noninvasive volitional tests

 Forced vital capacity (FVCup) (Morgan Spiroflow spirometer) and maximal sniff nasal inspiratory pressure (SNIP) were measured in the upright position as described elsewhere [20,21]. FVCsup was reported when available.

3.3.3. Invasive non volitional tests

 Subsequently, an oesogastric catheter (Gaeltec, Dunvegan, Isle of Skye, UK) was inserted pernasally [22]. Transdiaphragmatic pressure (Pdi) was obtained by subtracting online the oesophageal pressure (Pes) signal from the gastric pressure (Pgas) signal. Paradoxical breathing was defined by a negative Pgas/Pes ratio [23] and a Pgas – Pdi swing ratio (∆Pgas/∆Pdi) shifting to less negative values than −1 [24–26]. A value ranging between −1 and 1 indicates an ever-increasing contribution of the rib cage and expiratory muscles, as compared with the diaphragm, to tidal breathing. With complete diaphragmatic paralysis, the ratio becomes equal to 1 [26]. The patient’s inspiratory effort was assessed as the oesophageal (PTPes) and diaphragmatic pressure–time products (PTPdi) [27]. Dynamic lung compliance (Cdyn) was measured as previously described [28].

3.3.4. Invasive volitional tests

 Maximal sniff Pes (SniffPes), Pgas (SniffPgas), and Pdi (SniffPdi) were measured. Gastric pressure during a maximal cough (Pgas cough) was determined to assess the strength of the expiratory muscles [29]. Fatigue of the diaphragm and the global inspiratory muscles was assessed by the diaphragmatic tension-time index (TTdi) and oesophageal tension-time index (TTes), respectively [30].

4. Results

4.1. Clinical and complementary findings

The data of seven patients (5 males) were analyzed (Table 1). All patients harbored at least one mutation in one of the three genes encoding the COLVI subunits.

Clinically, all patients had an early onset with hypotonia or motor delay and a typical association of distal hyperlaxity and axial and proximal limb contractures. However clinical severity was very variable. Two patients of the Mild group were ambulatory, 2 patients of the Moderate-Progressive group acquired walking but lost this ability during the course of the disease and the 3 patients of the Early-Severe group were never able to walk.

Measurements of MFM and WBMRI findings were not available for the Moderate-Progressive patients at the time of the study. MFM was over 60% in the Mild patients and below 45% in the Early-Severe patients. WBMRI findings were available in 5 patients and showed a typical distribution of muscle involvement, most with alternating bands (tigroid pattern) within multiple muscles. The intercostal muscles, but not the diaphragm, were identifiable and showed marked fatty changes in the Early-Severe group.

Scoliosis was observed in four patients. Scoliosis was mild in two patients (#6, #7) and severe in the other patient (#5) of the Early-Severe group. One Mild patient (#2) developed a late onset scoliosis requiring spinal fusion.

In the Early-Severe patient #7, NIV was started 3 years prior to the respiratory muscles testing. In another Early-Severe patient (#5), invasive ventilation by tracheotomy was required 6 months prior to the testing.

4.2. Lung function and respiratory muscle data

Age at first visit for the respiratory muscle study was ranged between 6 and 29 years old. In patient #5, lung testing was performed during spontaneous breathing with the tracheotomy cannula closed.

4.2.1. Noninvasive non volitional tests

Only 3 patients had an FRCHe measurement. Predicted FRCHe values were below 60% in 2 patients (1 Moderate-Progressive and 1 Early-Severe) and within the normal range in the other patient (Moderate-Progressive).

Only the tracheotomized patient of the Early-Severe group (#5) had an abnormal daytime PaCO₂ above 45 mmHg.
The breathing pattern parameters displayed a large inter-individual variability. $f_i$, $V_i$ and $f_d/V_i$ were high in the Early-Severe group and in the patient #4 of the Moderate-Progressive group, indicating a rapid shallow breathing.

4.2.2. Noninvasive volitional tests

Simultaneous FVCup and FVCSup measurements were available in 5 patients. Predicted FVCup were over 60% in the Mild group and below 50% in the 2 other groups (Fig. 1). All the 5 patients, in whom positional FVC was available, had a drop in FVCup as compared to FVCup (mean drop 18 ± 8%, range 9–29%).

SNIP values were below normality in all patients (Fig. 2). The lowest values were observed in the Early-Severe group. The breathing pattern of an Early-Severe patient (#5), in whom Pes and Pgas are in phase, reflecting diaphragmatic dysfunction, as opposed to the normal breathing pattern of a Mild patient (#1).

PTPes values were available in all patients and PTPdi values in all but patient #2. PTPes values were above normality, ranged between 125 and 227 cmH$_2$O s/min, with no clear distinction between groups while PTPdi values were variable (range 30–154 cmH$_2$O s/min). Values of $C_i$ dyn ranged between 19 and 140 ml/cmH$_2$O in all patients.

**4.2.3. Invasive non volitional tests**

The $\Delta$Pgas/$\Delta$Pdi index was negative in all patients except in patient #1 of the Mild group. The negative deflection in Pgas was not related to a relaxation of the abdominal muscles at the onset of expiration as the abdominal section did not increase. The $\Delta$Pgas/$\Delta$Pes index was comprised between −0.4 and 0.8, indicating an increased ribcage and expiratory muscle contribution as compared with the diaphragm in all the patients. The patients with the most negative $\Delta$Pgas/$\Delta$Pdi values had also the highest $\Delta$Pgas/$\Delta$Pes value. Fig. 3A and B show the breathing pattern of an Early-Severe patient (#5), in whom Pes and Pgas are in phase, reflecting diaphragmatic dysfunction, as opposed to the normal breathing pattern of a Mild patient (#1).
4.2.4. Invasive volitional tests

SniffPes values were below normality in all patients (Table 2). The most striking observation was that the SniffPgas values were negative in all the patients (except for patient #2 in whom Pgas was not available) despite an initial positive deflection (Table 2 and Figs. 2, 3C and D). SniffPdi and PgasCough values were very low in all the patients (Table 2 and Fig. 2). Surprisingly, SniffPes, SniffPdi and PgasCough values of patient #6 were comparable to the values of the Moderate-Progressive patients (Table 2).

TTes values were normal below 0.30 (range 0.02–0.22), while TTdi values were above the fatigue threshold of 0.15 in all patients (range 0.16–0.73). Of note, the TTdi was high in the patient treated with NIV (0.37, patient #5) and the highest in the tracheotomized patient (0.73, patient #7).

4.3. Respiratory and clinical outcome

Nocturnal NIV was started in 1 patient of the Early-Severe group and 1 patient of the Moderate-Progressive group at the issue of the respiratory tests because of nocturnal hypoventilation on an overnight sleep study. None of the patients died during the study period.

5. Discussion

This study confirms the presence of a particular respiratory muscle phenotype in patients with COLVI myopathies with an elective involvement of the diaphragm, observed during spontaneous breathing in the upright position in all the patients of the Moderate-Progressive and Early Severe groups and during a maximal voluntary maneuver (Sniff) in all the patients [8]. Another major finding of our study was the weakness of expiratory muscles in all patients.

An elective involvement of the diaphragm was found during a maximal voluntary inspiratory maneuver in all patients irrespectively of their clinical severity (Fig. 2). However, as paradoxical motion during a maximal sniff may occur in 6% of normal subjects [31], additional tests are thus required to confirm diaphragmatic dysfunction. The recording of Pes and Pgas during spontaneous breathing is informative within this context as the fall of Pgas during quiet inspiration suggests a greater activity of the intercostal-accessory muscles than of the diaphragm [23–26]. The ΔPgas/ΔPdi ratio has the limitation of giving only qualitative information [23] whereas the ΔPgas/ΔPes ratio has the advantage of giving quantitative information [26]. The ΔPgas/ΔPes ratio showed that all our patients had a less negative ratio than −0.73, which corresponded to the mean value of patients without diaphragmatic dysfunction [32], and reflects a proportionally greater activity of the intercostal-accessory muscles than of the diaphragm. As such, the diaphragmatic dysfunction was less severe in one Mild patient in whom the ΔPgas/ΔPes ratio was −0.29, suggesting that the diaphragmatic function is preserved during spontaneous breathing but that the muscle is not able to cope with a greater demand. This observation is in concordance with the endurance data, which showed a high TTdi index in all the patients with the highest values being observed in patients of the Early-Severe group.

In absence of extensive respiratory muscle tests, positional FVC remains an informative parameter, although it may be difficult to perform in young patients and reproducibility does not guarantee maximality [5]. Indeed, a previous study from our group suggested a predominantly dysfunction of the diaphragm because of a constant drop in FVCsup in patients with a large range of age and clinical severity [8,13].

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**Fig. 1.** Individual values of forced vital capacity (FVC) in absolute values and % predicted according to disease presentation and age. (A) Individual data of absolute value of FVC; (B) individual data of FVC% predicted. Squares: mild group; triangles: moderate-progressive group; circles: early-severe group. White: with nocturnal ventilation; #: tracheotomy; grey: non invasive ventilation at the issue of the respiratory evaluation; black: no nocturnal ventilation. Dashed line indicates the threshold above which values are considered as normal.
Concerning the non-respiratory tests, a marked involvement of intercostals muscles by muscle WBMRI was observed in the patients with an Early-Severe form (with FVC < 50%), not in Mild patients displaying a better FVC [33]. Moreover, the MFM score appeared to be proportional to FVC in this series. Unfortunately measurements were not available for the Moderate-Progressive group.

Interestingly patient #6 of the Early Severe group showed one of the highest ΔPgasp/ΔPes ratio but had a

Table 2
Variations of oesophageal (Pes), gastric (Pgas) and transdiaphragmatic pressure (Pdi) during spontaneous breathing and a sniff maneuver.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical severity</th>
<th>Spontaneous breathing</th>
<th>Volitional maneuver (cmH2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ΔPgas/ΔPdi</td>
<td>ΔPgas/ΔPes</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>0.3</td>
<td>−0.29</td>
</tr>
<tr>
<td>2</td>
<td>Mild</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>3</td>
<td>Moderate progressive</td>
<td>−0.8</td>
<td>0.43</td>
</tr>
<tr>
<td>4</td>
<td>Moderate progressive</td>
<td>−1.2</td>
<td>0.53</td>
</tr>
<tr>
<td>5</td>
<td>Early severe</td>
<td>−0.4</td>
<td>0.29</td>
</tr>
<tr>
<td>6</td>
<td>Early severe</td>
<td>−1.6</td>
<td>0.62</td>
</tr>
<tr>
<td>7</td>
<td>Early severe</td>
<td>−2.4</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Abbreviations: ΔPgasp: gastric pressure swing; ΔPdi: transdiaphragmatic pressure swing; ΔPes: oesophageal pressure swing; SniffPes: maximal oesophageal pressure during a sniff maneuver; SniffPgas: maximal gastric pressure during a sniff maneuver; SniffPdi: maximal transdiaphragmatic pressure during a sniff maneuver.

Fig. 2. Individual data of inspiratory muscle strength (sniff nasal inspiratory and gastric pressures) and expiratory muscle strength (gastric pressure during cough). (A) Evolution of nasal inspiratory pressure during a sniff maneuver (SNIP); (B) evolution of gastric pressure during a sniff maneuver (Sniff Pgas); (C) evolution of gastric pressure during cough (PgasCough). Squares: mild group; triangles: moderate-progressive group; circles: early-severe group. White: with nocturnal ventilation; ▲: tracheotomy; grey: non invasive ventilation at the issue of the respiratory evaluation; black: no nocturnal ventilation. Dashed line indicates the threshold above which absolute values are considered as normal.
Fig. 3. Breathing pattern and Sniff test tracings of patient #5 of the early-severe group (A and C) and patient #1 of the mild group (B and D). Note that Pes and Pgas swings are both negative in the patient of the early-severe group (A), while in patient of the mild group, Pgas swings are positive (B) as normally observed. Sniff measures were low in the patient of the early-severe group (C) and near normality in the patient of the mild group (D). However SniffPgas was negative in both patients, with an initial slight positive deflection, indicating a probable diaphragmatic dysfunction during a volitional maneuver. Pes: oesophageal pressure; Pgas: gastric pressure; Pdi: transdiaphragmatic pressure; I: inspiration; E: expiration; SniffPgas: gastric pressure during a sniff.
relatively preserved respiratory muscle strength and FVC as compared with the 2 other patients of the same group (Table 2 and Figs. 1 and 2). Of note, this patient had a different respiratory management and was the only one who received daily IPPB sessions since infancy. This underlines the necessity to combine several tests in order to better characterize the pathophysiology of respiratory involvement and to extend this pilot study to a larger group of patients taking in account their respiratory management.

Our study has several limitations. First, the number of patients is small and our data need to be confirmed in a larger group. But COLVI myopathies are less common than other neuromuscular diseases such as Duchenne muscular dystrophy or spinal muscular atrophy. Also, diaphragmatic dysfunction is not common as a paradoxical motion of the diaphragm during a sniff was observed only in 4 out of 134 patients with various non-COLVI neuromuscular diseases investigated in our center. Second, we do not have a longitudinal follow-up for this group of patients. Such information is essential to understand the natural history and long-term course of respiratory muscles in COLVI myopathies. Moreover, such an analysis should take into account the different respiratory therapies such as IPPB and NIV. Indeed, these treatments may interfere with the natural history of the disease [34,35]. Finally, sleep studies were not available in all patients; this important point should be considered in future studies, knowing the major risk of nocturnal hypoventilation in patients with diaphragmatic dysfunction [36,37].

To conclude, this study underlines the usefulness of nonvolitional and volitional respiratory muscle tests in children with congenital myopathies. Indeed, the observation of a particular respiratory muscle phenotype as observed in the present study may guide diagnosis. Moreover, the presence of an elective involvement of the diaphragm should lead to early and systematic sleep recordings in order to detect nocturnal hypoventilation in time [36]. At last, further prospective studies of larger groups of patients with different clinical severity including other respiratory muscle tests of the diaphragmatic function and movement by electromyography (electromyography or magnetic stimulation of phrenic nerves) and radiology (ultrasound, dynamic magnetic resonance) should help to increase the knowledge of the pathophysiology of respiratory involvement in COLVI myopathies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2013.11.002.

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