Evolution of respiratory function in Duchenne muscular dystrophy from childhood to adulthood

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ABSTRACT In Duchenne muscular dystrophy (DMD), it is still to be determined if specific timepoints can be identified during the natural evolution of respiratory dysfunction from childhood to adulthood and if scoliosis, steroid therapy and nocturnal noninvasive mechanical ventilation (NIMV) have any effect on it.

In a 7-year retrospective study performed on 115 DMD patients (6–24 years), evaluated once or twice per year, with 574 visits in total, evolution mean curves of spirometry, lung volumes, spontaneous breathing and thoraco-abdominal pattern (measured by optoelectronic plethysmography) parameters were obtained by nonlinear regression model analysis.

While predicted values of forced vital capacity, forced expiratory volume in 1 s, and peak expiratory flow decline continuously since childhood, during spontaneous breathing the following parameters become significantly different than normal in sequence: abdominal contribution to tidal volume (lower after 14.8 years), tidal volume (lower after 17.2 years), minute ventilation (lower after 18.1 years) and respiratory rate (higher after 22.1 years). Restrictive lung pattern and diaphragmatic impairment are exacerbated by scoliosis severity, slowed by steroids treatment and significantly affected by NIMV.

Spirometry, lung volumes, breathing pattern and thoraco-abdominal contributions show different evolution curves over time. Specific timepoints of respiratory impairment are identified during disease progression. These should be considered when defining outcome measures in clinical trials and treatment strategies in DMD.

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Introduction

Duchenne muscular dystrophy (DMD) is an X-linked myopathy resulting in progressive wasting of locomotor and respiratory muscles, with consequent chronic ventilatory failure that is the main cause of death. In these patients it is extremely important, therefore, to measure lung function and respiratory muscle action in order to monitor the progression of the disease, to identify early signs of ventilatory insufficiency, to plan optimal interventions for improving the quality of life and to quantify the effects of novel gene-modifying strategies and pharmacological therapies [1–9].

At present, the outcome measures consider mainly motor function, namely of lower and upper limbs. Specific respiratory outcome measures are needed to objectively evaluate the effects of interventions in DMD, not only regarding spirometric and lung volume indexes but also respiratory muscle function. Forced vital capacity (FVC), when expressed in litres, follows a pathognomonic pattern characterised by an ascending phase, a plateau and a descending phase during the course of the disease [10–15]. When FVC is instead expressed as percentage of predicted value, it linearly declines with age indicating a progressive increase of lung restriction [10–13, 15–17]. The deterioration of lung function can be stabilised with steroid therapy [5, 18–21], which helps in delaying the loss of ambulation and the consequent development of scoliosis, an additional contributor to the restrictive lung pattern [5, 21–27].

Spirometry is recommended by current guidelines for routine lung function evaluation in DMD [1, 2], since FVC has prognostic value for survival [11] and provides useful guidance for treatment [11, 15, 28, 29]. Nevertheless, spirometry has inherent limitations. A high level of patient cooperation is needed; thus, it cannot be applied in early childhood and it becomes difficult in adulthood due to the fatigue induced by repeated maximal manoeuvres and/or the presence of macroglossia [30–32]. It provides only a global evaluation of lung restriction, irrespectively of its possible causes, such as alterations in lung, chest wall and respiratory muscles or a combination thereof. Moreover, it does not provide any specific information on the impairment of ribcage muscles, diaphragm and abdominal muscles.

The detailed analysis of spontaneous breathing at rest including thoraco-abdominal contributions to tidal volume (breathing pattern), represents a useful approach for noninvasive and non-volitional assessment of respiratory function feasible in all patients [10, 33]. Abdominal contribution to tidal volume progressively decays with age [33] being a strong predictor of nocturnal hypoxaemia [34] and inefficient cough [35].

We hypothesised that breathing pattern can provide information complementary to spirometry, regarding the natural course of the disease and the effects of given treatments. The specific aims were to study the natural evolution of respiratory function in terms of spirometry, lung volumes and breathing pattern from childhood to adulthood, to identify possible key points and to investigate possible effects of scoliosis, nocturnal noninvasive mechanical ventilation (NIMV) and steroid therapy.

Materials and methods

Patients

This is a 7-year retrospective study of respiratory function in 115 patients, with a defined diagnosis of DMD [3], ranging in age from 6 to 24 years and with data collected on least at three different visits, out of the 167 patients followed at the IRCCS (*Istituto di Ricovero e Cura a Carattere Scientifico*) "E.Medea". Patients were evaluated once per year until wheelchair bounding and thereafter twice per year, for 574 visits. At each visit, anthropometric and clinical (scoliosis, ambulation, steroid therapy and use of assistive respiratory devices) data were documented. All patients or parents signed a consent form, approved by the local ethics committee according to the declaration of Helsinki.

56 age-matched healthy male subjects were enrolled as control group. The control group was composed of healthy brothers of DMD patients, relatives of the researches and students of the laboratory who volunteered to take part to the study.

Pulmonary function test

At each visit, FVC, forced expiratory volume in 1 s (FEV_1), peak expiratory flow (PEF) and subdivisions of lung volume (functional residual capacity (FRC), residual volume (RV) and total lung capacity (TLC)) by the nitrogen washout technique were measured (Vmax series 22, SensorMedics, Yorba Linda, CA, USA). Spirometric data were presented both as absolute and expressed as percentage of the predicted values [36]. Nocturnal oxygen saturation (S_{PO_2}) was measured in all patients not under nocturnal NIMV by pulse oximetry (Nonin, 8500, Quitman, TX, USA) and only recordings >8 h were considered acceptable.

Assessment of spontaneous breathing pattern at rest

Breathing pattern was measured in supine position using opto-electronic plethysmography (OEP System; BTS, Milan, Italy) and a geometrical model based on 52 markers [37, 38].

After a short period of adaptation to the recording conditions, total and compartmental volumes were continuously measured during five minutes of quiet breathing. An average period of 90 s of stable breathing was then selected during which the following parameters were calculated breath-by-breath: tidal volume (V_T), respiratory rate (RR), minute ventilation, rapid and shallow breathing index (RSBi; calculated as RR/V_T) and ribcage and abdominal tidal volumes (ΔV_{RC} and ΔV_{AB}, respectively, expressed both in litres and as percentage contribution to V_T). Tidal volume was analysed also normalised according to weight. The ribcage was in turn split into compartments, namely pulmonary ribcage and abdominal ribcage [37, 38]. The volume variations of the two ribcage components were presented both as absolute and percentage values.

Effects of scoliosis, steroids and NIMV

To study the effect of scoliosis, patients were grouped accordingly to the severity of scoliosis: null, mild (Cobb angle <20°), moderate (20–40°), severe (>40°) and after spinal fusion. To study the effect of steroids, patients were grouped as "naive" (never treated or treated for <1 year), "current" (under treatment or stopped since <1 year before the visit) or "past" (not under treatment, but previously treated for >1 year). To study the effect of NIMV, patients were grouped as "NIMV" (currently under treatment) and "no-NIMV" (patients never treated with NIMV within the period of the study). Patients who underwent NIMV treatment within the period of the study were identified and the data before the start of the treatment constituted a third group called "pre-NIMV".

Statistical data analysis

Data were analysed using the following regression model:

$$y_{iG}(t) = \sum_{k=1}^{S} \beta_{kG}\phi_k(t) + \sum_{k=1}^{S} b_{kiG}\phi_k(t) + \varepsilon_{iG}(t) \quad (1)$$

where: i) $y_{iG}(t)$ is the datum that one would have recorded if the i-th subject in the group G were measured at age $t \in (6,\ 24)$. The group G can be one of the following: the whole population of DMD patients, the whole population of healthy controls, or one of the categories of the steroid or the scoliosis subgroups; ii) $\sum_{k=1}^{S} \beta_{kG}\phi_k(t)$ indicates the population mean curve; iii) $\sum_{k=1}^{S} b_{kiG}\phi_k(t)$ indicates the subject-specific correction such that $\sum_{k=1}^{S} \beta_{kG}\phi_k(t) + \sum_{k=1}^{S} \beta b_{kiG}\phi_k(t)$ indicates the subject-specific evolution curve, and finally iv) $\varepsilon_{iG}(t)$ indicates the session-specific measurement error.

TABLE 1 Anthropometric and clinical data of Duchenne muscular dystrophy patients

Age years	Visits n	Patients n	Weight kg	Height cm	Wheelchair bound %	Using CAD %	Under NIMV %	Current steroid users %	Scoliosis %				
									NU	M	MO	S	SF
6	23	23	21.1 (20.6–24.9)	116.5 (112.6–121.5)	9	0	0	78	100	0	0	0	0
7	31	28	25.0 (22.5–30.5)	121.0 (118.5–125.0)	11	4	0	79	96	4	0	0	0
8	28	25	29.0 (25.2–35.0)	127.0 (122.0–131.0)	24	12	0	80	88	12	0	0	0
9	31	28	31.0 (27.0–36.0)	132.0 (129.8–135.5)	43	4	4	68	79	14	4	4	0
10	33	28	37.0 (31.5–45.0)	140.0 (134.0–142.0)	68	11	4	50	54	32	11	4	0
11	37	35	45.0 (38.0–51.5)	144.0 (138.0–149.0)	80	17	0	49	40	34	20	6	0
12	39	34	49.0 (38.0–57.0)	151.0 (138.0–155.5)	85	24	0	26	29	32	18	18	3
13	41	35	54.5 (44.0–66.5)	155.0 (148.0–158.5)	97	29	0	20	26	29	17	23	6
14	42	35	55.0 (50.0–65.0)	160.0 (155.0–165.0)	97	31	3	9	14	17	20	26	23
15	35	27	57.0 (48.3–65.0)	162.0 (157.0–165.0)	96	41	11	4	4	26	33	11	26
16	34	28	56.5 (42.0–68.8)	164.0 (159.3–167.3)	96	57	21	4	7	18	29	25	21
17	39	26	60.0 (47.5–70.0)	165.0 (160.5–168.0)	96	54	27	4	8	19	31	12	31
18	37	26	53.0 (44.0–63.0)	165.0 (160.0–167.0)	100	46	35	4	8	12	23	27	31
19	27	20	58.0 (45.3–73.5)	166.0 (162.8–171.0)	100	60	35	5	10	10	25	35	20
20	37	24	58.0 (46.0–67.9)	165.5 (164.0–168.3)	100	54	33	4	4	17	13	50	17
21	26	18	57.0 (50.0–72.3)	166.5 (165.0–170.3)	100	33	17	6	6	11	17	56	11
22	27	18	54.0 (46.1–64.0)	165.0 (164.0–168.8)	100	83	50	6	0	17	22	44	17
23	19	15	58.0 (42.0–78.0)	167.0 (164.5–176.5)	100	73	33	0	0	27	27	47	0
24	11	8	64.5 (46.5–78.8)	174.5 (165.0–177.3)	100	75	63	0	0	13	38	38	13

Data are presented as median (interquartile range), unless otherwise stated. CAD: cough assistive device; NIMV: noninvasive mechanical ventilation; NU: null; M: mild; MO: moderate; S: severe; SF: spinal fusion.

To estimate the effects of scoliosis, steroid therapy and NIMV, we separately performed asymptotic likelihood-based tests to pairwise compare the population means of all the categories of the steroid and the scoliosis subgroups. Finally, for every acquired datum and for every group G, we assessed the significance of the random effects related to the subject with a likelihood ratio test, which compared the full model with the one that excluded all the random effects. Significance was determined by $p<0.05$. The proposed model was implemented in R (version 3.2.3 R Core Team (2015). A language and environment for

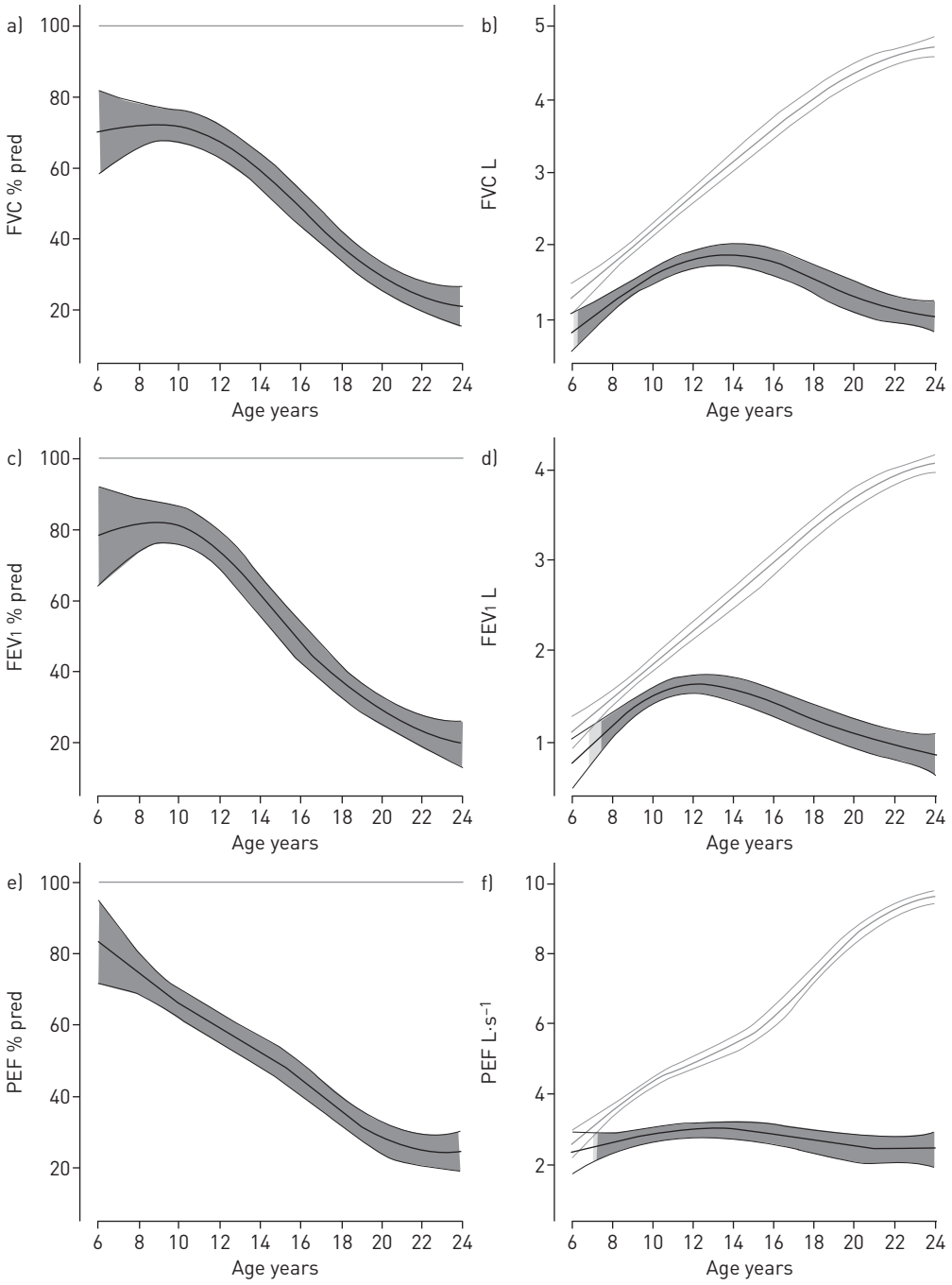

FIGURE 1 Evolution with age of the maximum-likelihood estimation population mean curve (thick line) and its 95% pointwise asymptotic confidence intervals (thinner lines) of forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and peak expiratory flow (PEF) expressed as percentage predicted (a, c, e, respectively) and absolute values (b, d, f, respectively) in Duchenne muscular dystrophy patients. Black lines: measured values; grey lines: predicted values; light grey areas: values significantly different from predicted with $0.01<p<0.05$; dark grey areas: values significantly different from predicted with $p<0.01$.

statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) with the package "lme4"[39]. Additional details are reported on the online supplement.

Results

Patients

In table 1, anthropometric and clinical data of the 115 enrolled DMD patients are reported for each age. At the age of 13 years, all patients but one were wheelchair bound. More than one-third of the patients older than 14 years were using cough assistive-devices. In the study group, 28 patients were regularly using nocturnal NIMV (since a mean age of 19.1±3.5 years). The youngest patient using NIMV was 15.6 years old. Scoliosis worsened with age. Steroid treatment was prevalent among younger patients.

In the course of the study, nine patients died: four due to respiratory and/or swallowing insufficiency (mean age 16.7±2.3 years); and five to cardiac failure (mean age 17.6±4.5 years).

Median (interquartile range) age of the control group age was 16.1 (7.7–22.7) years, height was 1.7 (1.3–1.8) m, weight was 60 (29–75) kg and body mass index was 19.6 (16–23) kg·m^{-2}.

Spirometry, lung volumes and nocturnal oxygen saturation

Evolutions with age of FVC, FEV1 and PEF are shown in figure 1. These parameters, when expressed as percentage of the predicted values, linearly declined by 4.6% per year (age range 11–22 years), 5.4% per

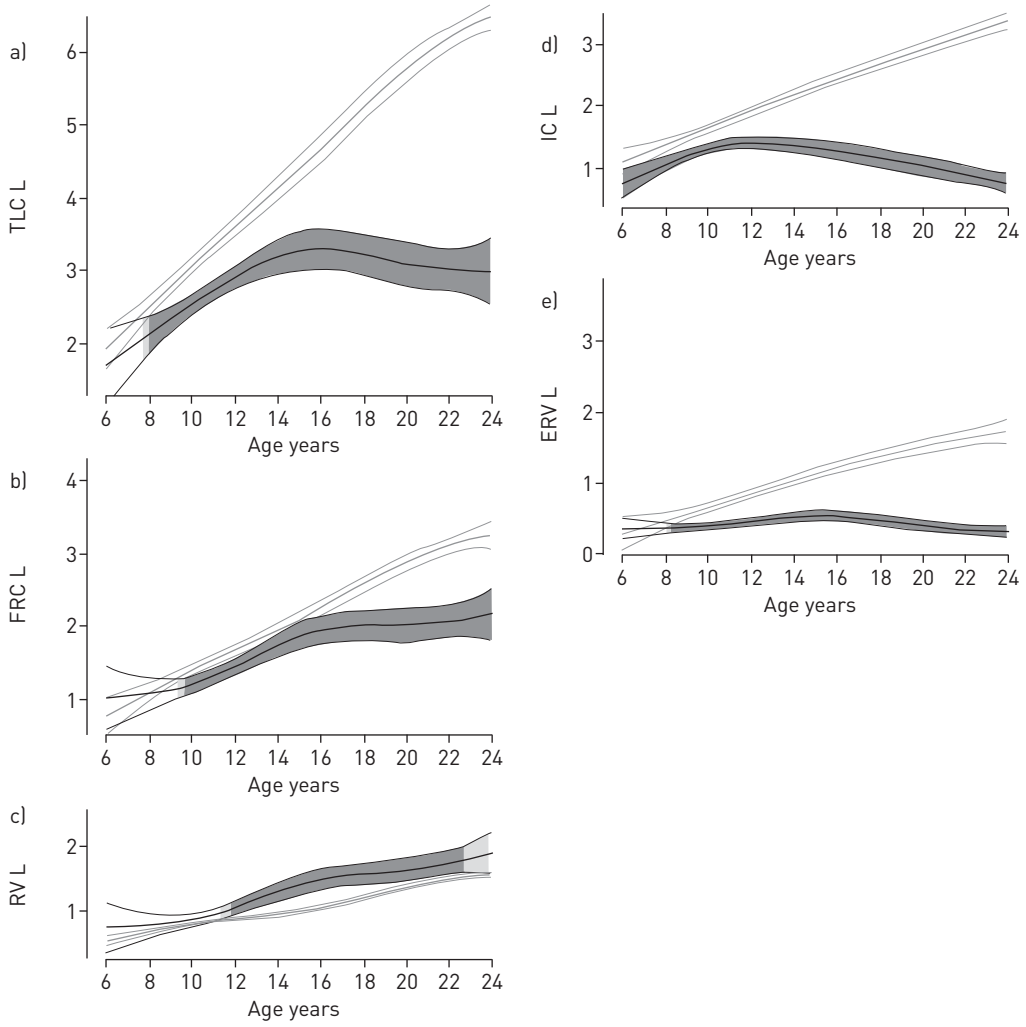

FIGURE 2 Evolution with age of the maximum-likelihood estimation population mean curve (thick line) and its 95% pointwise asymptotic confidence intervals (thinner lines) of total lung capacity (TLC; a), functional residual capacity (FRC; b), residual volume (RV; c), inspiratory capacity (IC, d) and expiratory reserve volume (ERV, e) expressed as absolute values in Duchenne muscular dystrophy patients. Black lines: measured values; grey lines: predicted values; light grey areas: values significantly different from predicted with $0.01<p<0.05$; dark grey areas: values significantly different from predicted with $p<0.01$.

year (age range 11–22 years) and 3.8% per year (age range 6–20 years), respectively. All absolute and predicted values were significantly reduced after the age of 7 years. The reduction of FVC was due to both inspiratory capacity and expiratory reserve volume, being significantly lower than predicted after the age of 6 and 8 years, respectively (figure 2). The reduction of TLC and FRC began at the age of 7.7 and 9.3 years, respectively, while residual volume became greater than predicted after the age of 11.3 years (figure 2).

In figure 3, the mean curves of nocturnal saturation data are reported in terms of night time spent with S_{pO_2} in the ranges 95–100%, 90–94% and <90%, number and average time of desaturation events. Peripheral oxygenation during sleep worsened with age. Desaturation events were already present in childhood.

Spontaneous breathing pattern at rest

The evolution curves of minute ventilation, RSBi and their two components (*i.e.* respiratory rate and V_T) are shown in figure 4. When compared with healthy peers, DMD patients started to hypoventilate at the age of 18.1 years because of a reduced V_T (after the age of 17.2 years), being respiratory rate similar between the two groups almost all throughout the considered age span. Respiratory rate became significantly higher than normal after 22.1 years and rapid and shallow breathing occurred after the age of 20.7 years. When normalised to actual body weight, V_T was similar between DMD and healthy peers in the whole considered age range.

The mean curves of ΔV_{RC} and ΔV_{AB}, both expressed in litres and as $\%V_T$, are shown in figure 5. When expressed in litres, ΔV_{RC} was similar between DMD and healthy controls at all ages, whereas ΔV_{AB} became lower in DMD after the age of 16 years. Thoraco-abdominal volume variations in DMD, when instead expressed as $\%V_T$, showed a pattern starting to be significantly different than normal after the age of 14.8 years, when ribcage and abdominal contribution became significantly greater and lower than normal. The predominance of rib cage compared with abdomen in the relative contribution to V_T progressively increased with age.

Similarly to the ribcage considered as a whole, the expansion of both pulmonary and abdominal ribcage in DMD was almost similar to healthy subjects. The expansion of the abdominal ribcage became lower than controls starting from the age of 22 years. The percentage contribution of pulmonary and abdominal ribcage started to be significantly higher than the control group at the age of 13.2 and 19 years, respectively (see supplementary data).

Figure 6 shows the relationship between the night time spent with S_{pO_2} <95% and FVC (% pred) and ΔV_{AB} ($\%V_T$).

Effect of scoliosis

The mean curves of FVC (% pred) and ΔV_{AB} ($\%V_T$), grouped by the severity of scoliosis, are shown in figure 7. Both parameters were negatively associated with the severity of scoliosis, with patients with severe scoliosis and spinal fusion showing the lowest values.

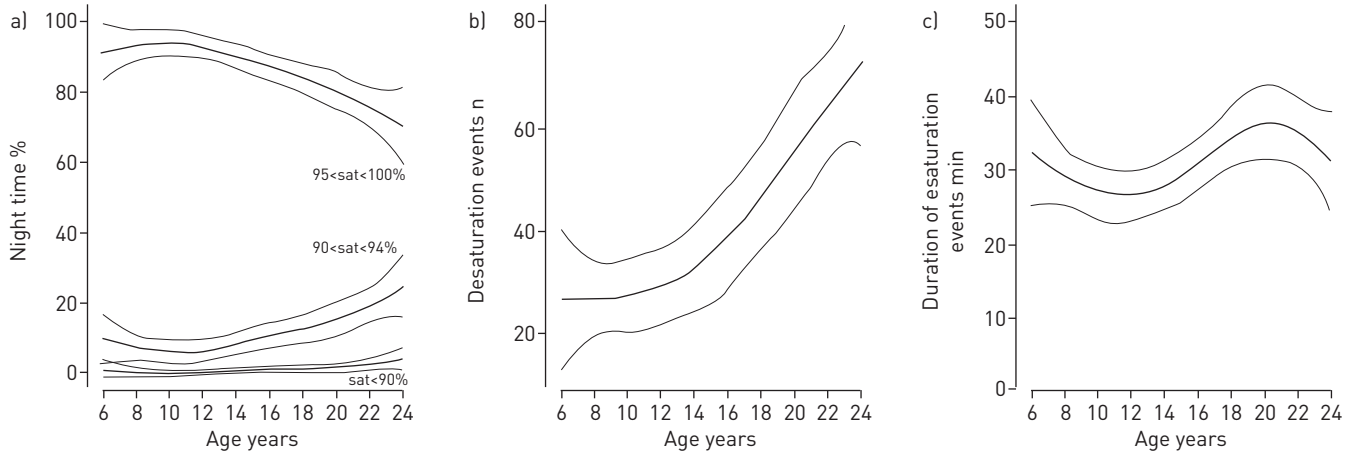

FIGURE 3 Evolution with age of the maximum-likelihood estimation population mean curve (thick line) and its 95% pointwise asymptotic confidence intervals (thinner lines) of the night time spent with nocturnal saturation in the ranges 95–100%, 90–94% and <90% (a), of the number (b) and the average time (c) of desaturation events in Duchenne muscular dystrophy patients.

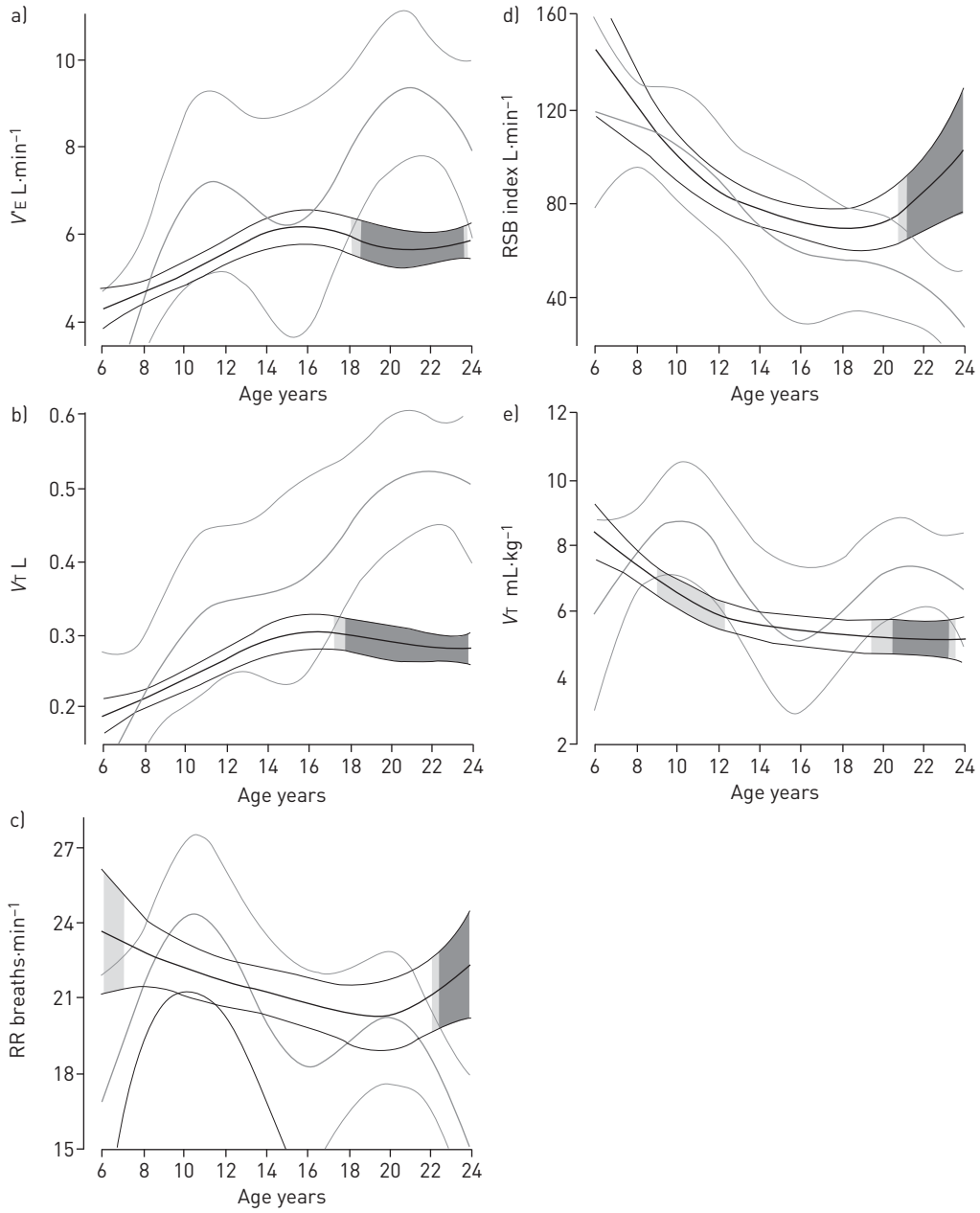

FIGURE 4 Evolution with age of the maximum-likelihood estimation population mean curve (thick line) and its 95% pointwise asymptotic confidence intervals (thinner lines) of minute ventilation (V'_E; a), tidal volume (V_T; b), respiratory rate (RR; c), rapid and shallow breathing index (RSB; d), and tidal volume normalised to body weight (e), during spontaneous breathing in supine position in DMD patients (black lines) and healthy controls (grey lines). Light grey areas: values significantly different from predicted with 0.01<p<0.05; dark grey areas: values significantly different from predicted with p<0.01.

Effect of steroids

The mean curves of FVC (% pred) and ΔV_{AB} (%V_T), in the different steroid therapy groups, are shown in figure 8. In currently steroid-treated patients, FVC (% pred) was significantly higher than naïve and past patients in the age range 15.1–21.3 years and ΔV_{AB} (%V_T) in the age range 13–17.3 years.

Effect of NIMV

The mean curves of FVC (% pred) and ΔV_{AB} (%V_T), grouped by the NIMV use, are shown in figure 9. Compared with the no-NIMV group, patients belonging to pre-NIMV group were characterised by lower values of both FVC (% pred) and ΔV_{AB} (%V_T) from the age of 13 years. After the start of NIMV, FVC (% pred) and ΔV_{AB} (%V_T) in treated (NIMV group) and untreated (no-NIMV) patients remained significantly different until 21.1 and 18.4 years, respectively.

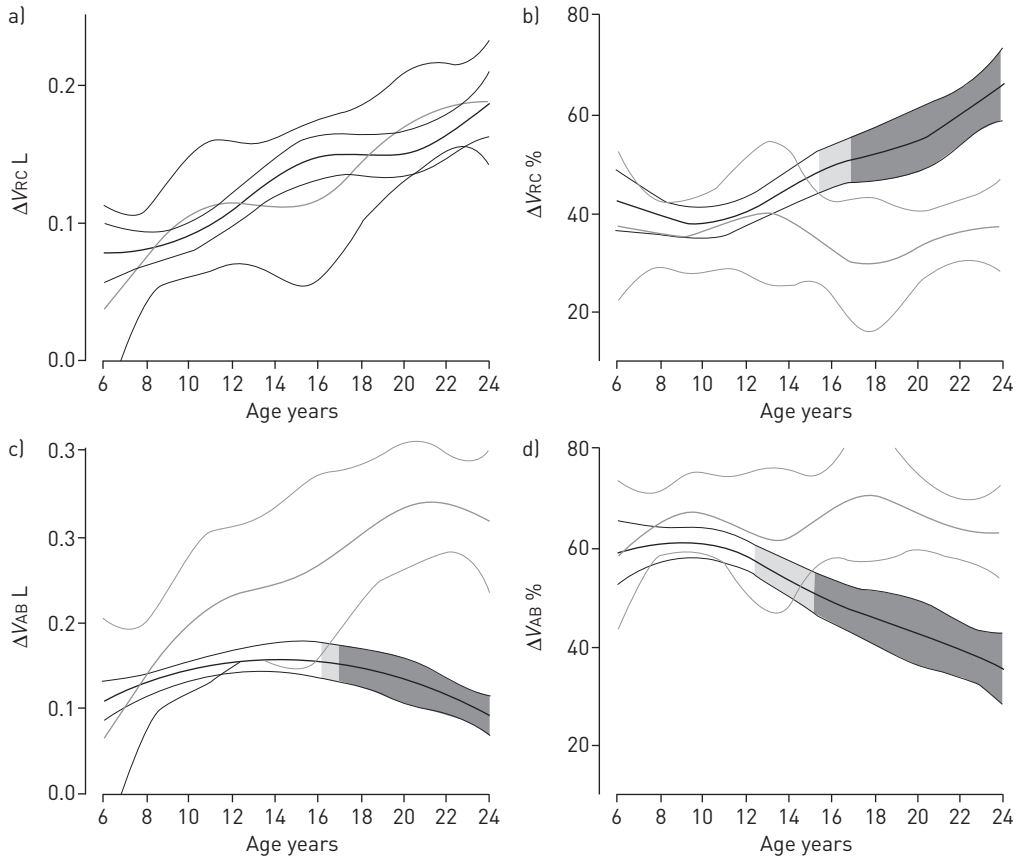

FIGURE 5 Evolution with age of the maximum-likelihood estimation population mean curve (thick line) and its 95% pointwise asymptotic confidence intervals (thinner lines) of ribcage volume variations (ΔV_{RC}) and abdominal volume variation (ΔV_{AB}) expressed in litres (a and c, respectively) and as percentage contribution to tidal volume (b and d, respectively) during spontaneous breathing in supine position in DMD patients (black lines) and in healthy controls (grey lines). Light grey areas: values significantly different from predicted with $0.01<p<0.05$; dark grey areas: values significantly different from predicted with $p<0.01$.

Discussion

In the present study, an original and comprehensive description of the evolution of respiratory function in patients with DMD over the age span 6–24 years is provided. Noninvasive measurements were taken on a group of 115 subjects during 574 observations.

The main result is that a detailed analysis of breathing pattern is able to provide information regarding specific key time points during the natural course of the disease. While predicted values of FVC, FEV_1 and PEF decline since childhood, during spontaneous breathing, the following parameters become significantly different than normal in sequential order: contribution of abdominal compartment to tidal volume (lower after 14.8 years), tidal volume (lower after 17.3 years), minute ventilation (lower after 18.1 years) and respiratory rate (higher after 22 years).

The progressive decline of minute ventilation observed during awake spontaneous breathing at rest, that becomes significant after the age of 18.1 years, is due to progressively reduced V_T caused, in turn, by a decreased abdominal expansion with ribcage volume variations similar to normal all over the considered age span. As a result, the percentage contributions of ribcage and abdomen to tidal volume become increasingly higher and lower, respectively, after the age of 14.8 years. It must be noted that, when normalised to weight, the differences in V_T are no longer present. This is presumably due to the pathological thinness that can develop in adulthood, rather than to a preserved ventilatory pump.

Another novel feature provided by the present study is that both spirometry and lung volumes have been longitudinally measured and analysed in a large cohort of patients. This allowed assessing the determinants of the decay of FVC, namely the relevant reduction of TLC, inspiratory capacity and expiratory reserve volume since childhood, suggesting that the impairment of both inspiratory and expiratory muscles as a whole represents a clear hallmark of DMD.

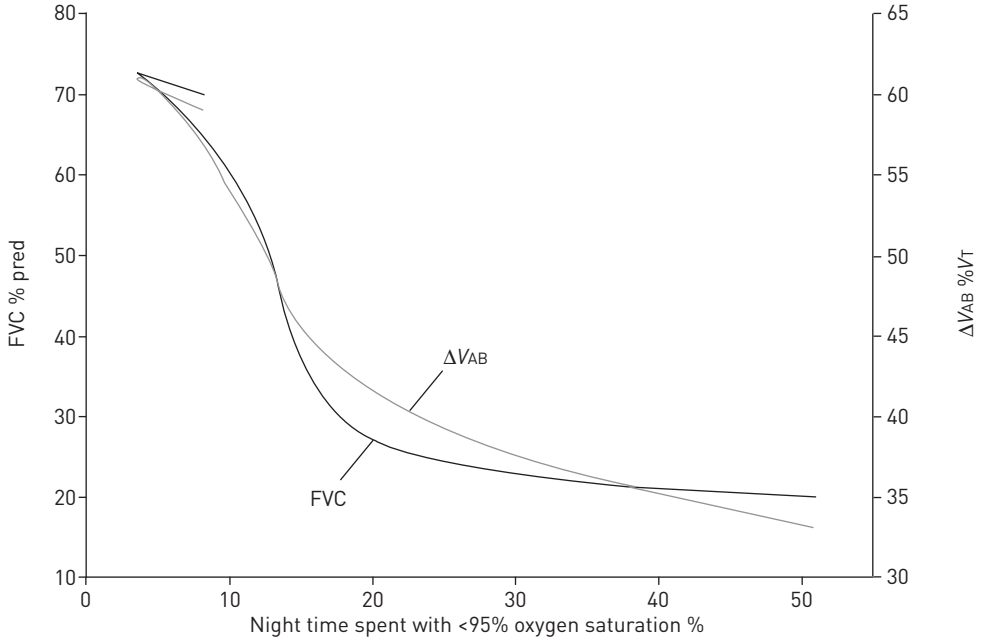

FIGURE 6 Relationship between the maximum-likelihood estimation population mean curves of the night time spent with nocturnal saturation <95% *versus* forced vital capacity expressed as percentage predicted (FVC (% pred), black line, left axis) and abdominal volume variation expressed as percentage contribution to tidal volume during spontaneous breathing in supine position in DMD patients (ΔV_{AB} (%V_T), grey line, right axis).

It is important to emphasise that both spirometry and lung volume assessment, which are traditionally used to evaluate respiratory impairment in DMD patients, represent volitional tests that require a high level of patient's cooperation that is not always possible. In addition, these tests do not evaluate the patient under his normal conditions, represented by spontaneous breathing at rest, but only during maximal manoeuvres. In addition, they provide global indexes of respiratory system function, without being able to differentiate between decreased respiratory system compliance and/or increased respiratory muscle weakness. Finally, they cannot be specific also in differentiating the relative impairment of each respiratory muscle functional group, namely ribcage muscles, diaphragm and abdominal muscles.

During inspiration, rib cage expansion is the result of the action of all inspiratory rib cage muscles, namely the scalene, the external intercostals, the parasternals and the sternocleidomastoid whilst abdominal expansion is due to the action of the diaphragm. Therefore, although in our DMD patients the rib cage expansion remains within the normal range, it can be supposed that inspiratory rib cage muscles are also impaired, being unable to compensate for the insufficient action of the diaphragm that becomes unable to provide an adequate V_T. In this scenario, the impairment of the inspiratory muscles is unbalanced with the diaphragm impacting more substantially than the inspiratory rib cage muscles.

A further evidence of the earlier impairment of the diaphragm with respect to the ribcage muscles derives from the analysis of the abdominal ribcage expansion that becomes significantly lower only at late ages. This is probably due to the fact that this compartment is submitted to both inspiratory ribcage muscles and diaphragm. Therefore, diaphragmatic impairment is masked in the age range 15–22 years by the relatively preserved ribcage muscles becoming evident also in this compartment only after the age of 22 years.

The diaphragmatic action in DMD can be affected in opposite directions by the presence of scoliosis and by corticosteroid therapy. Regarding scoliosis, we have now demonstrated that the worsening of scoliosis exacerbates not only the restrictive lung pattern, but also the action of the diaphragm as well. The reduced contribution of abdominal compartment to tidal volume with increasing severity is a result somehow surprising, as the main effects were expected to be on the rib cage. However, we can speculate that the presence of scoliosis determines a compression of the abdomen and increases diaphragmatic load, shifting chest wall expansion toward the ribcage. Regarding steroids treatment, our results are in agreement with those reported by several recent studies [5, 6, 15, 40] which have demonstrated that current corticosteroid-using patients have better spirometric values than the other two groups. In the present study we report, in addition, that there is a significant effect on abdominal contribution to tidal volume in the age range between 13 and 17.3 years.

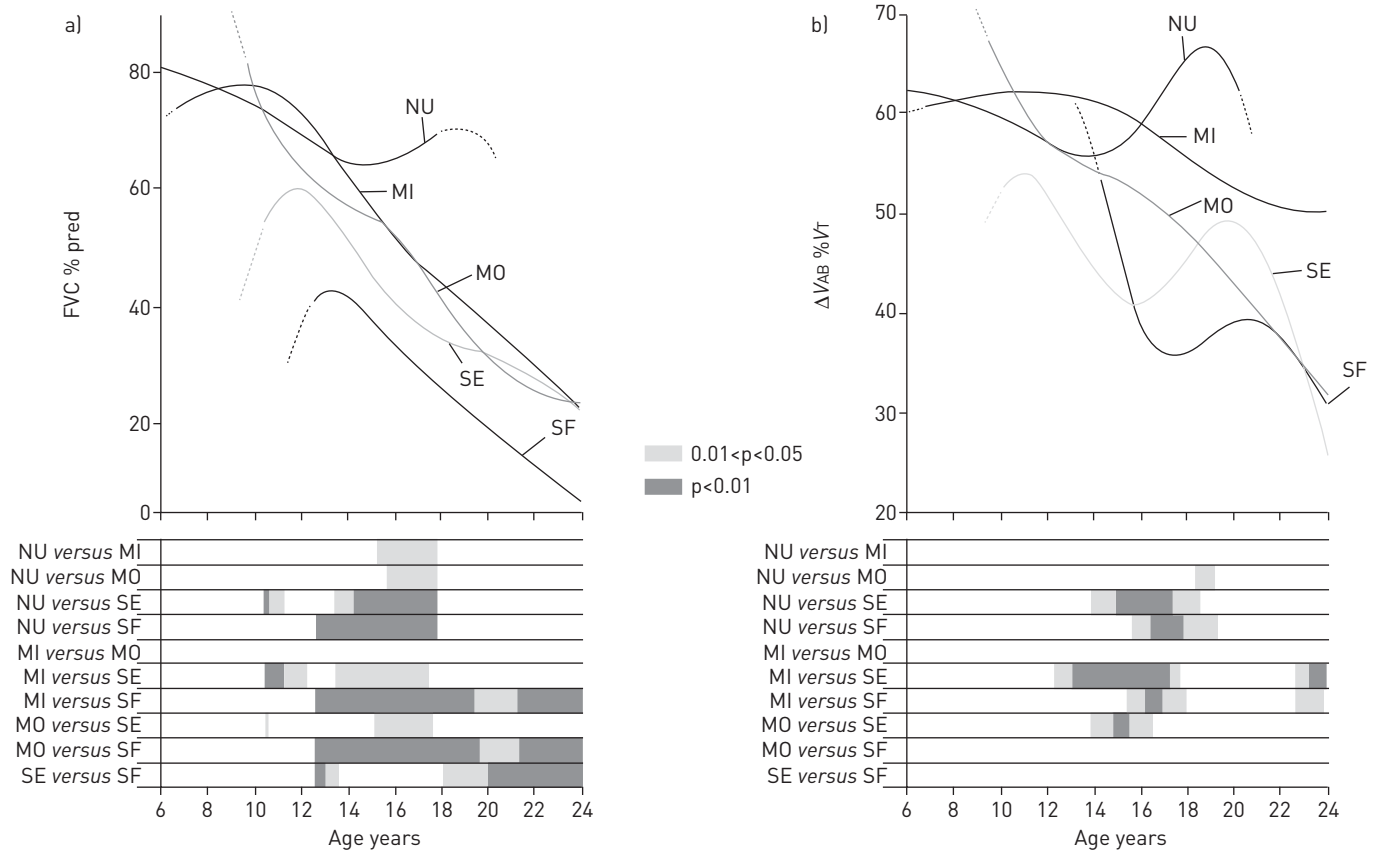

FIGURE 7 Evolution with age of the maximum-likelihood estimation population mean curve of the percentage predicted forced vital capacity (FVC) (a) and percentage abdominal contribution to tidal volume (ΔV_{AB} %V_T) during spontaneous breathing in supine position (b) grouped by scoliosis classification (NU: null; MI: mild; MO: moderate; SE: severe; SF: spinal fusion). Data are reported in the age range in which the asymptotic confidence intervals is <40%. Bottom panels: pairwise comparisons of the population means between all categories of scoliosis (light grey areas: values significantly different from predicted with 0.01<p<0.05. Grey areas: values significantly different from predicted with p<0.01).

A limitation of the study is related to the lack of complete availability of measurements at the extremities of the considered age range of DMD patients, *i.e.* children younger than 6 years and adults older than 24 years. In these patients, although breathing pattern was easily evaluated by opto-electronic plethysmography, the availability and/or reliability of spirometry and lung volumes was very poor due to the lack of collaboration in the youngest and the presence of macroglossia and/or facial muscular weakness in the oldest. In addition, most of the patients under current steroid treatment were the younger ones, with very few old patients. The reason of this bias is that in the clinical practice steroid treatment is gradually stopped after the loss of ambulation because of the side effects [3, 20, 41].

Nevertheless, the study has several strengths. This is the largest data set to date including serial spirometry, lung volumes, nocturnal oxygen saturation and breathing pattern assessment, with data collected at different time points for each subject in the interval 6–24 years. Although this did not allow a direct time-matched data comparison, we dealt with this irregular and subject-specific timing by adopting a regression model based on natural cubic splines with mixed effects. Similar models have been employed in the longitudinal data analysis literature and used in a wide range of applications [42, 43]. Our proposed regression model allows us to obtain estimated values at any time point of our domain taking into account the possible effects of scoliosis and steroids (*i.e.* fixed effects), the specific temporal evolution of each subject (*i.e.* random effects), and the session-specific measurement errors (*i.e.* error term).

The study has clinical implications. Firstly, we have shown that after the age of 14.8 years the diaphragm shows early evidence of weakness, starting to be unable to contribute to an adequate V_T and minute ventilation. Noteworthy, in the same period, the percentage of patients under NIMV becomes significant (table 1). In addition, we have shown that when night time spent with oxygen saturation <95% is >12–13%, its relationship with ΔV_{AB} (%V_T) is more linear than with FVC (% pred). ΔV_{AB} (%V_T), therefore, seems to be more strongly related than FVC (% pred) to the presence of significant nocturnal desaturation, as already shown in a previous paper focusing only a subgroup of adolescent DMD patients [34].

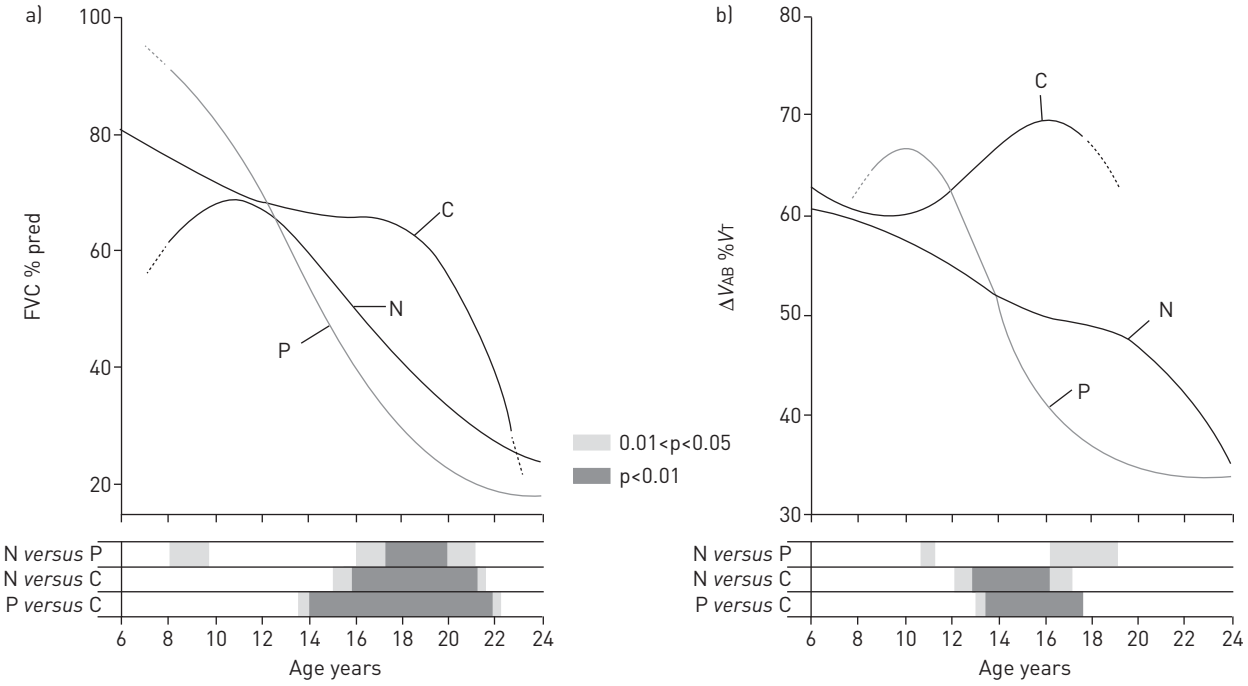

FIGURE 8 Evolution with age of the maximum-likelihood estimation population mean curve of the percentage predicted forced vital capacity (FVC) (a) and percentage abdominal contribution to tidal volume (ΔV_{AB} %V_T) during spontaneous breathing in supine position (b) grouped by corticosteroids treatment groups (C: currently under treatment; P: "past", *i.e.* treated in the past for at least 1 year and not currently receiving steroids; N: naïve, *i.e.* never treated with steroids). Data are reported in the age range in which the asymptotic confidence intervals is <40%. Bottom panels: pairwise comparisons of the population means between all categories of steroid subgroups (light grey areas: values significantly different from predicted with 0.01<p<0.05; dark grey areas: values significantly different from predicted with p<0.01).

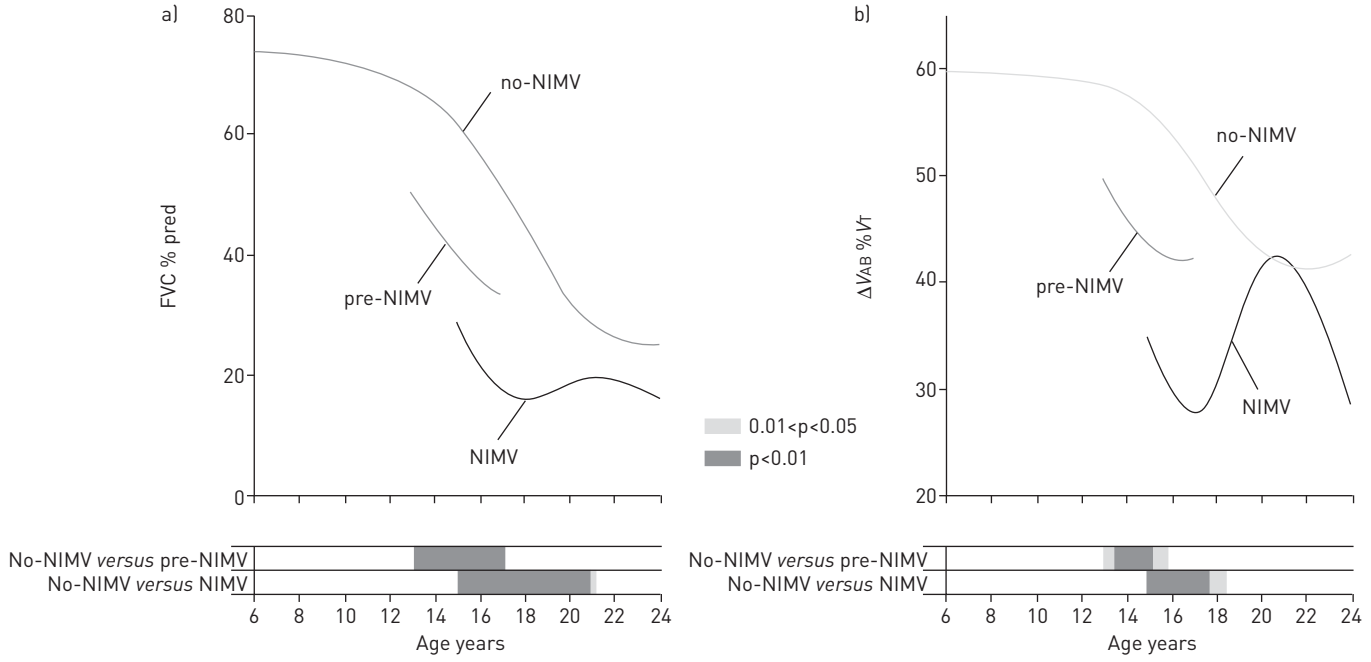

FIGURE 9 Evolution with age of the maximum-likelihood estimation population mean curve of the percentage predicted forced vital capacity (FVC) (a) and percentage abdominal contribution to tidal volume (ΔV_{AB} %V_T) during spontaneous breathing in supine position (b) grouped by nocturnal noninvasive mechanical ventilation (NIMV) groups (NIMV: currently under treatment; pre-NIMV: patients who underwent NIMV treatment within the period of the study. The curve refers to the visits before the start of NIMV; no-NIMV: patients never treated with NIMV within the period of the study). Pre-NIMV data are reported in the range that contains at least six visits in each age. Bottom panels: pairwise comparisons of the population means between pre-NIMV and no-NIMV and between NIMV and no-NIMV (light grey areas: values significantly different from predicted with 0.01<p<0.05; dark grey areas: values significantly different from predicted with p<0.01).

Moreover, we have shown that those patients who started NIMV during the period of the study were characterised by a faster decline of FVC (% pred) and ΔV_{AB} (%V_T). After the start of NIMV, FVC (% pred) and ΔV_{AB} (%V_T) continued to decline for about 3 years, then these parameters increased until the age of 21 years, and then declined again, similarly to untreated patients.

On the basis of these results, we believe that the specific involvement of the diaphragm should be considered in addition to the FVC decline and nocturnal oxygen desaturation for the definition of treatment guidelines, including the timing of starting NIMV [1–4]. Our results, therefore, suggest that technological developments should be addressed in order to provide simple but accurate measurement of abdominal kinematics during spontaneous breathing that could be available in all clinical centres.

In addition, we have shown original evidence that scoliosis represents a burden to the dystrophic diaphragm of these patients and that steroid treatment has efficacy on spirometry and the diaphragm's contribution to tidal volume, supporting the relevance of steroid treatment as part of DMD patients' care.

In conclusion, the evolution curves of spirometry, lung volumes and breathing pattern parameters here presented might be considered to better define outcome measures in the forthcoming clinical trials both in non-ambulant and ambulant DMD patients.

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