Spontaneous Breathing Pattern as Respiratory Functional Outcome in Children with Spinal Muscular Atrophy (SMA)

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Abstract

Introduction

SMA is characterised by progressive motor and respiratory muscle weakness. We aimed to verify if in SMA children 1)each form is characterized by specific ventilatory and thoraco-abdominal pattern(VTAp) during quiet breathing(QB); 2)VTAp is affected by salbutamol therapy, currently suggested as standard treatment, or by the natural history(NH) of SMA; 3)the severity of global motor impairment linearly correlates with VTAp.

Materials and methods

VTAp was analysed on 32 SMA type I (SMA1,the most severe form), 51 type II (SMA2,the moderate), 8 type III (SMA3,the mildest) and 20 healthy (HC) using opto-electronic plethysmography. Spirometry, cough and motor function were measured in a subgroup of patients.

Results

In SMA1, a normal ventilation is obtained in supine position by rapid and shallow breathing with paradoxical ribcage motion. In SMA2, ventilation is within a normal range in seated position due to an increased respiratory rate($p < 0.05$) with reduced tidal volume($p < 0.05$) secondary to a poor contribution of pulmonary ribcage($\%\Delta V_{RC,P}$, $p < 0.001$). Salbutamol therapy had no effect on VTAp during QB($p > 0.05$) while tachypnea occurred in type I NH. A linear correlation($p < 0.001$) was found between motor function scales and VTAp.

Conclusion

A negative or reduced $\%\Delta V_{RC,P}$, indicative of ribcage muscle weakness, is a distinctive feature of SMA1 and SMA2 since infancy. Its quantitative assessment represents a non-invasive, non-volitional index that can be obtained in all children, even uncollaborative, and provides useful information on the action of ribcage muscles that are known to be affected

by the disease.Low values of motor function scales indicate impairment of motor but also of respiratory function.

Introduction

Spinal Muscular Atrophy (SMA) is characterized by degeneration of α motoneurons in the spinal cord, resulting in progressive motor and respiratory muscle weakness and paralysis[1–5]. According to severity and age of onset, clinical phenotypes have been grouped into the most severe type I (SMA1), the moderate type II (SMA2) and the mildest type III (SMA3) forms[6–8].

Respiratory problems are the major causes of hospitalization, morbidity and mortality in SMA children[9]. Over the last decade, proactive management of respiratory complications has rapidly increased, thus improving survival and quality of life.[10–14] Additionally, an increasing number of potential therapeutic strategies are entering or have already entered clinical phases[5,15,16]. Specific respiratory outcome measures are therefore needed to objectively evaluate the effects of interventions in all SMA children.

Respiratory function can be assessed by invasive and non-invasive techniques[17–21]. Invasive measurements are generally not well tolerated by children, and non-invasive techniques should be considered. These include the measure of spirometric parameters, peak cough flow (PCF)[22], ventilatory pattern and thoraco-abdominal contribution during spontaneous quiet breathing at rest (QB).

Since the early childhood, in SMA1 and SMA2 spirometric indexes are reduced and show a relatively slow rate of decline, eventually worsened by scoliosis[23–28]. Cough is inefficient and requires assistance since the first years of life[29–31]. Both spirometry and PCF measurement are volitional tests requiring high collaboration and therefore being consistently performed since the age when the child understands the operation. On the other hand, non-volitional measurement during QB is feasible in almost all patients, even those uncooperative.

SMA children are at risk of hypoventilation, and ventilatory support is needed to reverse the resulting reduced arterial oxyhemoglobin saturation and microatelectasis.[14] Rapid and shallow breathing ensues in awake SMA2, hypothesized to be a respiratory strategy these children adopt to minimize their work of breathing[23].

None of the above techniques or parameters provides specific information on inspiratory ribcage muscles (iRCM) status. This would be useful, as in SMA the phrenic motoneurons, and consequently the diaphragm, are preserved[32], while progressive weakness affects iRCM[9].

The assessment of thoraco-abdominal contribution during QB allows to selectively study the involvement of the different respiratory muscle groups. Thoraco-abdominal asynchrony and reduced ribcage expansion have been reported in SMA1 and SMA2 children but only lying supine[17–19]. In this position, the diaphragm lengthens, becoming the leading respiratory muscle with increased tension development. In supine position, therefore, a possible deficit of iRCM can be masked by the action of the diaphragm. For this reason, it would be of clinical interest to study these children also in seated position when iRCM and the diaphragm almost equally contribute to tidal volume[33]

We therefore hypothesized that 1) the three forms of SMA are characterized by different and specific ventilatory and thoraco-abdominal patterns during QB and 2) the rib cage contribution to tidal volume provides specific information on iRCM, and is affected by posture. To verify this, the respiratory pattern of young children affected by the three forms of SMA was

separately described in seated and supine position and compared with healthy children. In addition to these two main hypotheses, we also verified if 3) the breathing pattern is affected by salbutamol therapy, being proposed to have beneficial effect on motor function and spirometry in SMA by increasing the survival motorneuron transcript levels in leucocytes, and currently suggested as standard supportive treatment for children with SMA in many neuromuscular centres[34–36]; and 4) respiratory outcomes are linearly correlated with the severity of global motor impairment, assessed by functional motor scales, as already reported for adult SMA patients[37]. Finally, as parents of SMA1 infants are called to choose between leaving the pure natural history of the disease to take its course or to use respiratory supports, we also investigated if the breathing pattern differs between these two groups of SMA1 infants[10,11,38,39].

Materials and Methods

Subjects and clinical features

This is a prospective cross-sectional study, approved by the Research Ethics Board of the Carlo Besta Neurological Research Institute (registration number: CE: 20/2014), on SMA children enrolled according to the following criteria: genetically proven diagnosis of SMA; age<8 years; absence of previous spinal surgery, acute respiratory failure, airway infections and 24-hours mechanical ventilation dependence.

In the patient population, cough assistance device was routinely used by 57% of SMA1 and 90% of SMA2 children, nocturnal ventilation respectively by 40% (two SMA1 patients were tracheostomized) and 29%, spinal bracing by 50% and 82%, and the 41% of SMA2 were on salbutamol at a stable dosage from at least 12 months. Eleven (34%) SMA1 and ten (19.5%) SMA2 children had at least one hospitalization for acute respiratory illness within the previous year (or since birth for infants) before the acquisition.

Parents of 10 SMA1 infants refused any respiratory support.

A control group of healthy children was also included.

All parents signed a written informed consent.

Motor and lung function assessment

Global motor function was assessed by applying the 16-item scale Children's Hospital of Philadelphia Infant Test for Neuromuscular Disorders (CHOP INTEND) scale[40,41] for SMA1, while the 33-items Hammersmith functional motor scale expanded (HFMSE)[42] and the 9-items Upper Limb Module (ULM) were used in SMA2 and SMA3. The HFMSE assesses motor function (e.g. lying, rolling, sitting, crawling, attaining standing, walking, running and jumping) in order of progressive difficulty, with higher values showing higher function abilities. It incorporates 13 relevant items to eliminate the "ceiling" effect of the original scale when applied to ambulant SMA patients. The 9 items of ULM were specifically developed to assess upper limb function in non-ambulant SMA patients, including young low functioning children [42]. The total score is calculated by summing the scores of the individual items and it can range from 0 (all activities failed) to 64, 66 and 18 (all activities achieved unaided), for the CHOP INTEND, HFMSE and ULM respectively. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), their ratio (FEV_1/FVC) and peak cough flow (PCF) were measured (Pony FX PNT, Cosmed, Rome-Italy) when children were able to reasonably understand and follow instructions. The criteria used to determine if the spirometry tests were performed to an acceptable degree followed the Thoracic Society/European Respiratory Society Statement [43]. Predicted spirometric values were calculated by applying the global lungs initiative equations for spirometry[44,45] while predicted values of PCF according to Bianchi and Baiardi

[46]. The numerosity of the percentage values was reduced compared to absolute values because there are no prediction values for children younger than the age of 4.

Breathing pattern and thoraco-abdominal contribution during QB

The volume variations of the chest wall (ΔV_{CW}) and its compartments, namely pulmonary ribcage ($\Delta V_{RC,P}$), abdominal ribcage ($\Delta V_{RC,A}$) and abdomen (ΔV_{AB}) were measured by opto-electronic plethysmography (OEP, BTS, Milan, Italy)[47,48] during at least 5 minutes of spontaneous QB before in seated and then other 5 minutes in supine position with the child awake (Brazelton stage 4)[49] while watching a cartoon (Fig 1). If the child was drowsy or slept or cried all the time during the acquisition, and therefore intervals of QB were impossible to be identified, he/she was excluded from the study.

The following parameters were calculated during inspiration of a 40-seconds period of QB: respiratory rate (RR), tidal volume (V_T), minute ventilation ($V'_E = RR^*V_T$), rapid and shallow breathing index (RSBi = RR/V_T), the percentage contribution of each compartment to V_T (%$\Delta V_{RC,P}$, index of iRCM action; %$\Delta V_{RC,A}$, index of the expansion of the appositional zone of the diaphragm and %ΔV_{AB}, index of diaphragmatic action) and the phase shift angle between $\Delta V_{RC,P}$ and ΔV_{AB} (ϕ_{TA}). V_T was calculated as the difference between body-weight normalized ΔV_{CW} and the total anatomic dead space body-weight normalized.[50] The accuracy of OEP system during quiet breathing has been previously tested by simultaneous measurements with
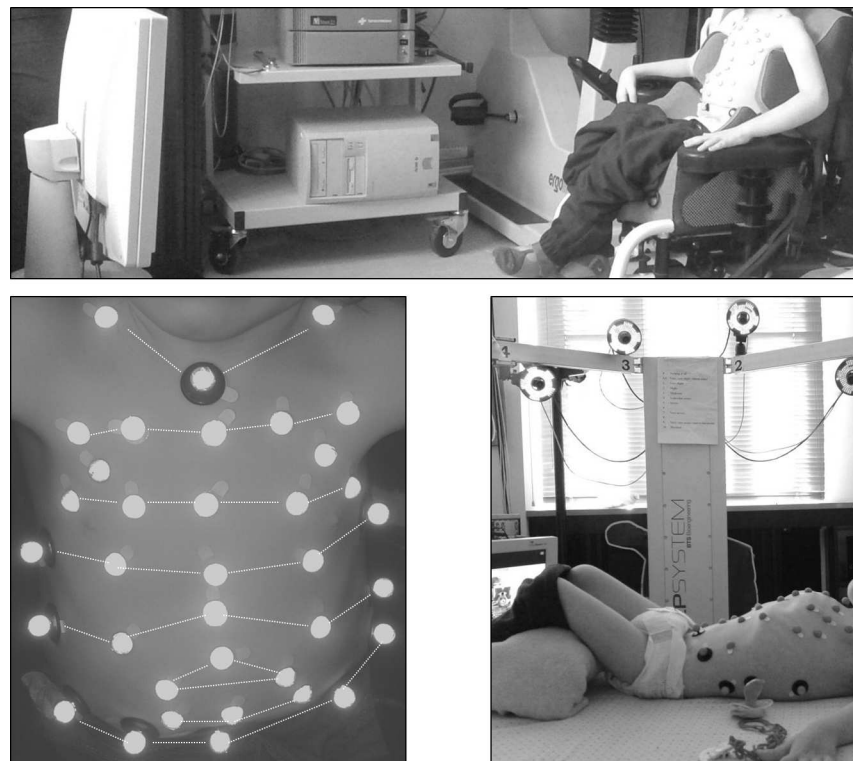
Fig 1. Experimental set-up for the analysis of chest wall volumes *via* opto-electronic plethysmography of a SMA child in seated (top) and supine (bottom right) positions while watching a cartoon. SMA3 and HC could sit without support and therefore 89 and 52 markers were respectively used in seated and supine position[44,45,48,50]. SMA2 children were all wheelchair bound and in both postures a new model was used with five horizontal rows of five markers, three rows of three markers with two additional markers making a total of 36 (bottom left). SMA1 were analyzed only in supine position, using the 36 model as well.

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a spirometer in healthy adults[47,51], newborns[52] and infants[53]. In all these studies, the discrepancy between the two measurements was always <4%. The intraclass correlation coefficient values of OEP was shown to be higher than 0.75 while the coefficient of variation less than 10%. These parameters indicate that OEP presents adequate intra- and inter-rater reliability[54].

Statistical analysis

Differences in anthropometric data, respiratory parameters and motor function were tested applying a one-way Analysis of Variance(ANOVA) with disease as independent factor. When normality test failed, a Kruskal-Wallis one-way ANOVA on Ranks was performed. Post-hoc tests based on Holm–Sidak and Dunn's methods were respectively used for parametric and non-parametric ANOVA.

The same analysis was performed, considering medication or the family choice as independent factor, to test if motor and respiratory function of SMA2 under salbutamol therapy were different than not-treated children or if differences were present between SMA1 infants whose parents had chosen to follow the natural history of SMA or to support ventilation.

The least-square linear regression analysis was performed to verify if motor functional scales linearly correlated with the respiratory function parameters significantly affected by the disease.

Data are expressed as median and interquartile range (IQR). Significance was determined by p<0.05.

Results

Anthropometric, spirometric and cough data

One-hundred-fifteen children were enrolled and data on 111 reported. Four children were excluded because it was not possible to obtain at least 40 seconds of QB (Fig 2).

Age was similar between SMA2, SMA3 and healthy (HC, median: 62.4, IQR: 52.5–72.0 months) children, while SMA1 were younger and characterized by lower weight (HC, 18.0,15.5–22.0 Kg) and height (HC, 114.5, 110.0–120.0 cm), as shown in Table 1.

SMA2 children showed reduced absolute spirometric and PCF values compared to SMA3 whose PCF was lower than predicted (Table 1).
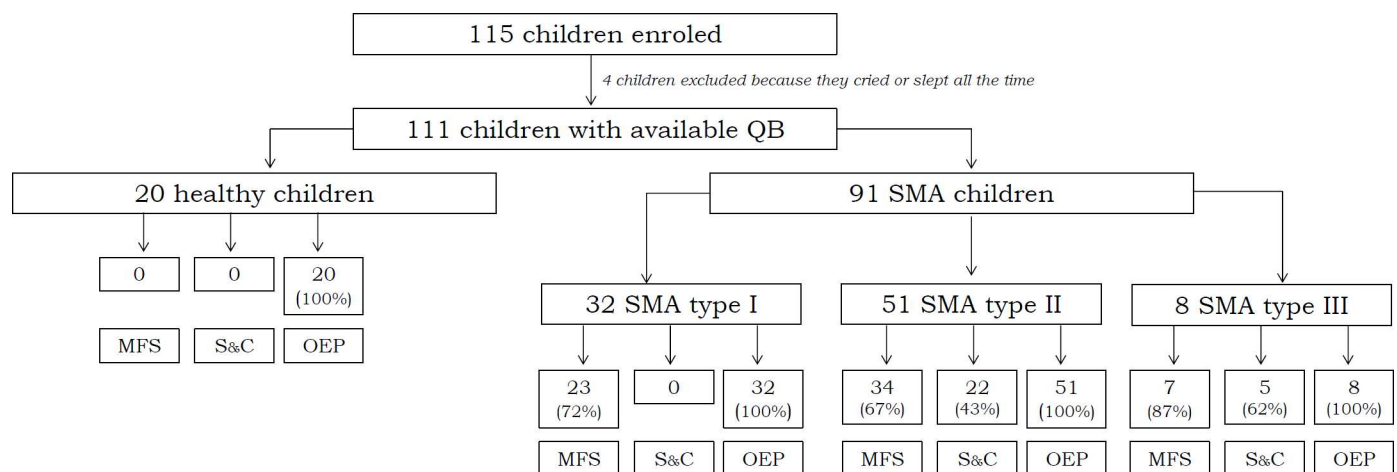

Fig 2. Flow diagram of recruited children and functional tests. QB: quiet breathing; MFS: motor functional scale; S&C: spirometry and cough tests; OEP: opto-electronic plethysmography

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Table 1. median and interquartile range (IQR) of anthropometric, motor function, spirometric and cough data in SMA types I (SMA1), II (SMA2) and III (SMA3) children.

	SMA1		SMA2		SMA3	
	median	**IQR**	**median**	**IQR**	**median**	**IQR**
Anthropometry						
n	32		51		8	
age (months)	9.8	(6.2–16.0)°	44.8	(36.4–63.8)	64.8	(54.4–79.8)
height (cm)	72.0	(66.1–85.0)°	101.8	(91.5–109.7)	120.0	(101.5–122.0)
weight (Kg)	7.6	(6.8–9.6)°	14.4	(12.2–17.0)	18.5	(15.2–21.5)
Motor function assessment						
n	23		34		7	
CHOP INTEND (/64)	22.0	(21.0–32.5)	-		-	
HFMSE (/66)	-		16.5	(12.0–24.9)#	49.0	(45.5–55.0)
ULM (/18)	-		10.0	(6.0–12.8) ###	18.0	(17.5–18.0)
Spirometry and cough measurement						
n			22		5	
FVC (L)	-		0.55	(0.47–0.89) ###	1.34	(1.16–1.63)
FVC (%pred)	-		69.4	(47.2–90.9)	94.3	(89.5–102.2)
FEV_1 (L)	-		0.53	(0.40–0.71) #	1.06	(1.03–1.53)
FEV_1 (%pred)	-		61.5	(50.7–82.9)	103.7	(91.8–105.3)
FEV_1/FVC (%)	-		89.6	(81.7–98.5)	93.0	(90.3–96.0)
PCF (L/min)	-		97.2	(59.85–112.2) #	154.5	(116.1–216.2)
PCF (%pred)	-		59.8	(43.5–70.0) #	73.3	(63.8–89.2)

°: p<0.05 vs SMA2,SMA3 and healthy children

#, ###: p<0.05, 0.001 vs SMA3.

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Breathing pattern and thoraco-abdominal contribution during QB

SMA1 children were characterized by normal V_E, greater RSBi and RR, reduced V_T (Fig 3) with negative %$\Delta V_{RC,P}$ compensated by higher %ΔV_{AB} leading to paradoxical thoraco-abdominal motion close to complete out of phase (Fig 4). They also showed lower (p<0.001) values of %$\Delta V_{RC,A}$ (median: -2.1%; IQR: -8.7–3.2) compared to SMA2 (12.0%; 8.1–15.7), SMA3 (18.1%; 16.6–19.1) and HC (12.2%; 8.6–15.8).

Lying supine, SMA2 breathed like healthy peers whereas differences emerged when seated, with normal V'_E, higher RR and reduced V_T resulting in rapid and shallowed breathing (Fig 5). Despite the increased %ΔV_{AB}, V_T was lower because of the reduced %$\Delta V_{RC,P}$ (Fig 6) with %$\Delta V_{RC,A}$ not differing among SMA2 (15.6%; 12.2–18.5), SMA3 (21.0; 17.0–28.1) and HC (20.3%; 16.7–23.5).

%$\Delta V_{RC,P}$ was negative in 14 and 6 SMA2 children, respectively in supine and seated position. No differences were found between SMA3 and HC children.

Fig 7 shows representative thoraco-abdominal volume traces for each considered group.

Effect of salbutamol therapy

In SMA2, HMFSE, ULM, FVC, FEV_1, PCF, breathing pattern and thoraco-abdominal contribution during QB in seated and supine position did not differ (p>0.05) between children on salbutamol at a stable dosage from at least 12 months and untreated children.
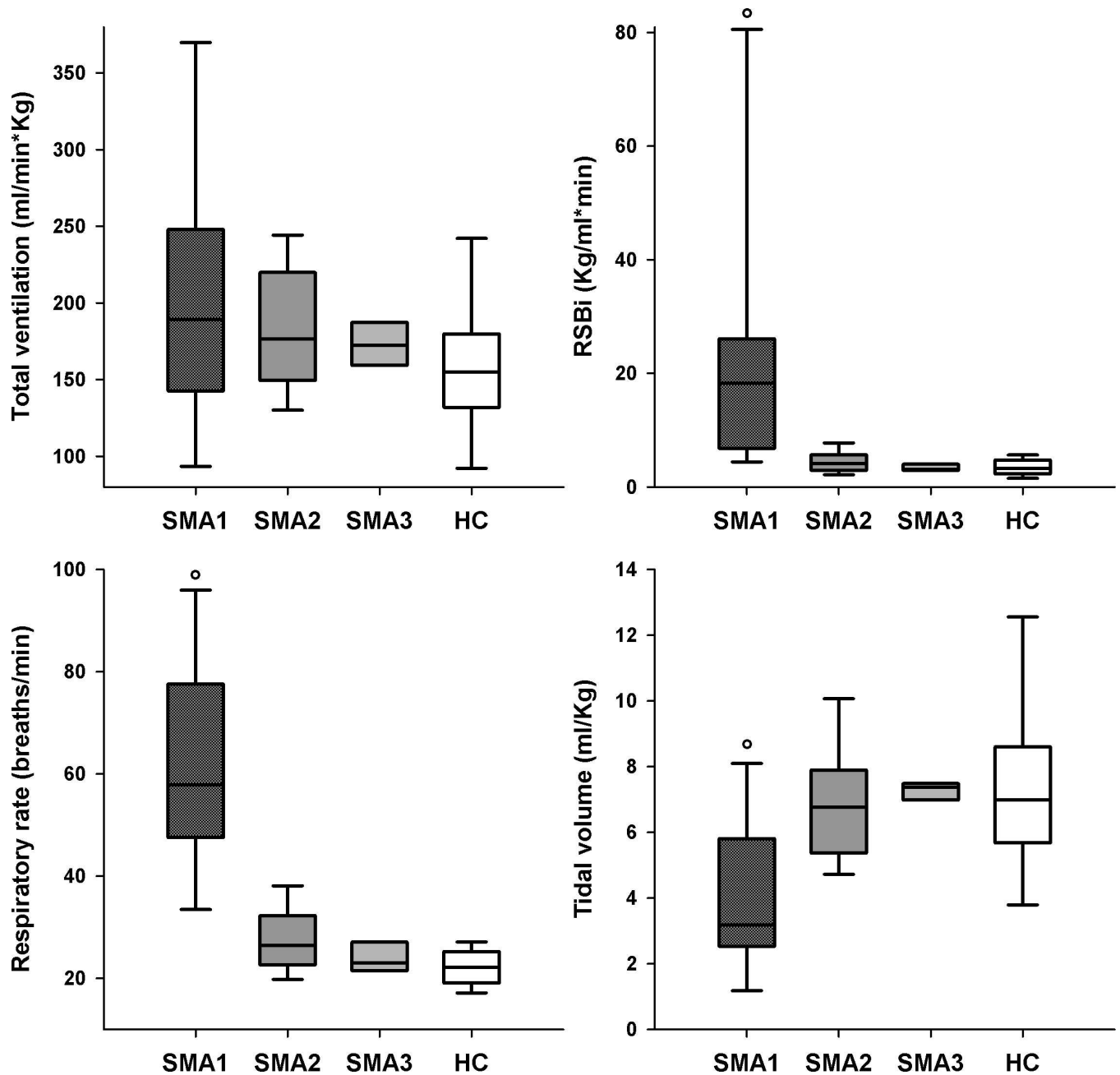
Fig 3. Box-and-whisker plot representing the median (line within the box), the IQR (length of the box), the 90th and the 10th percentiles (whiskers above and below the box) of total ventilation (V'_E, top left panel), rapid and shallow breathing index (RSBi, top right panel), respiratory rate (bottom left panel) and tidal volume (V_T, bottom right panel) in SMA types I, II, III and healthy children (SMA1, SMA2, SMA3 and HC, respectively) at rest during spontaneous quiet breathing in supine position. ˚: p<0.05 vs SMA2, SMA3 and HC

Correlation between motor and respiratory function assessments

While thoraco-abdominal measurements linearly correlated with CHOP INTEND, HFMSE and ULM scales, the latter two also correlated with FVC, FEV_1 and PCF and V_T/Kg (Table 2).
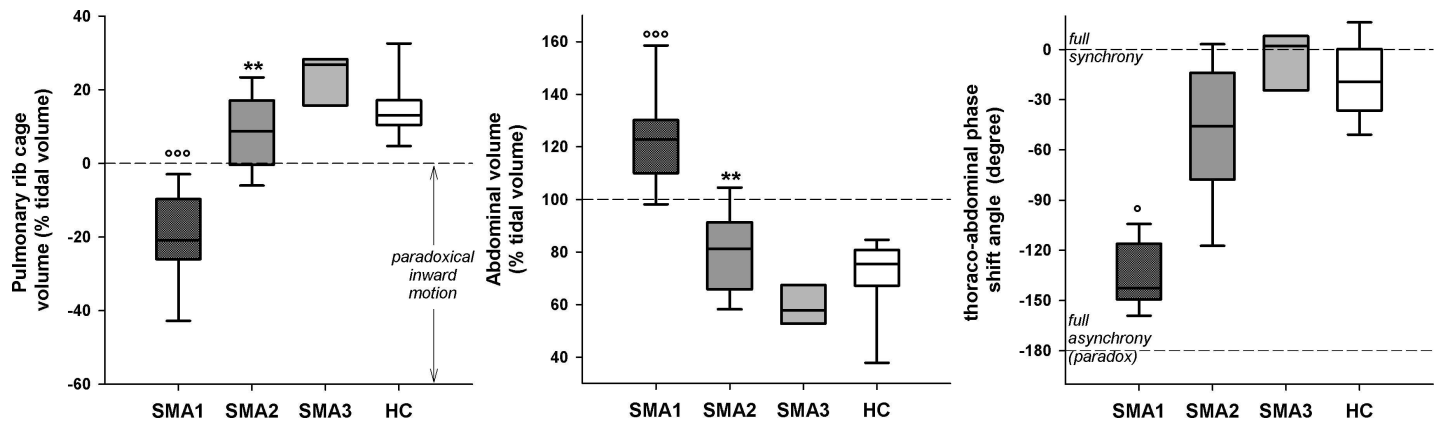
Fig 4. Box-and-whisker plot representing the median (line within the box), the IQR (length of the box), the 90th and the 10th percentiles (whiskers above and below the box) of pulmonary ribcage percentage contribution to tidal volume (left panel), abdominal percentage contribution to tidal volume (middle panel) and thoraco-abdominal phase shift angle (right panel) in SMA types I, II, III and healthy children (SMA1, SMA2, SMA3 and HC, respectively) at rest during spontaneous quiet breathing in supine position. Negative values of pulmonary ribcage percentage contribution to tidal volume indicate paradoxical inward movement of the compartment during inspiration. Phase shift angle varies between 0, when the two compartments are fully in phase, and -180˚ when one compartment is completely out of phase respect the other with consequent paradoxical movement. ˚,˚˚˚: $p<0.05$, 0.001 vs SMA2, SMA3 and HC; **: $p<0.01$ vs SMA3

SMA1 infants on natural history

The age range of the SMA1 group on natural history (NH) was between 4 and 12 months. In order to avoid bias due to age, and therefore to the evolution of the disease, 9 patients receiving respiratory support were excluded because older than 12 months. The remaining 13 infants receiving respiratory support showed significantly lower RR compared to the group on natural history (IQR: 50–66 and 58–84, respectively, $p = 0.041$).

Discussion

In the present study the detailed description of the respiratory function in a large cohort of young (<8 yrs), and therefore hardly cooperative, children affected by the three forms of SMA is provided and compared. The assessment of the breathing pattern at rest was feasible, well tolerated and significantly different according to the severity of the disease. Our findings show that 1) SMA1 and SMA2 are characterized by weakened inspiratory ribcage muscles and spared diaphragm since childhood, as confirmed by accurate measurements of percentage contribution of rib cage and abdomen to tidal volume; 2) when evaluating respiratory muscles action, posture plays a crucial role, with ribcage muscle weakness of SMA2 emerging only in the seated position; 3) a 12-months stable dosage of salbutamol does not affect motor and respiratory functions in our cohort of SMA2; 4) in children with SMA, motor function, assessed by different disease-specific functional scales, is linearly correlated with several spirometric and respiratory function parameters during spontaneous QB; and 5) SMA1 children for whom the spontaneous course of the disease was chosen were more tachypneic compared to those receiving respiratory support.

In these children, respiratory involvement differs according to the severity of the disease, therefore, it is important to differentiate the respiratory treatment since early childhood.

SMA type I–Without any postural support, SMA1 patients can only adopt the supine position. In this situation, without any ventilatory assistance, while awake and in stable condition, SMA1 children are able to maintain normal levels of V´$_E$ only by adopting a rapid and shallow breathing pattern, mostly due to high RR with a slightly reduced V$_T$. Since RR is known to
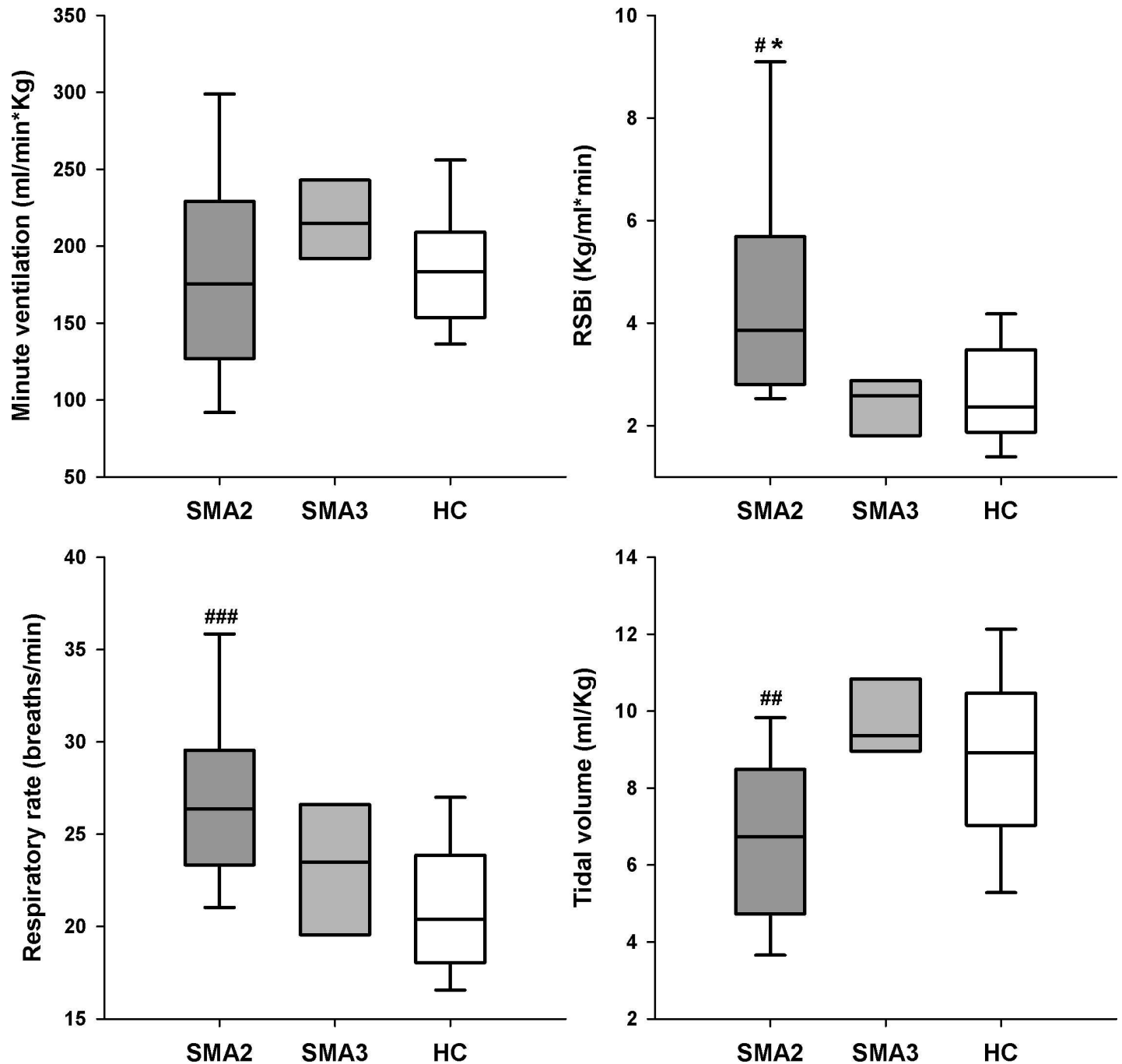
Fig 5. Box-and-whisker plot representing the median (line within the box), the IQR (length of the box), the 90th and the 10th percentiles (whiskers above and below the box) of minute ventilation (V´E, top left panel), rapid and shallow breathing index (RSBi, top right panel), respiratory rate (bottom left panel) and tidal volume (VT, bottom right panel) in SMA types II, III and healthy children (SMA2, SMA3 and HC, respectively) at rest during spontaneous quiet breathing in seated position. *: p<0.05 vs SMA3; #, ##, ###: p<0.05, 0.01, 0.001 vs HC

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inversely correlate with age, being its variability larger in the first months of life, it is important to understand whether the high rate of RR observed in SMA1 is either a consequence of the disease or of the youngest age. Reported reference values for RR in healthy children range from 47 breath/min respectively from 2 to 36 months of age[55–57], whereas in our population of
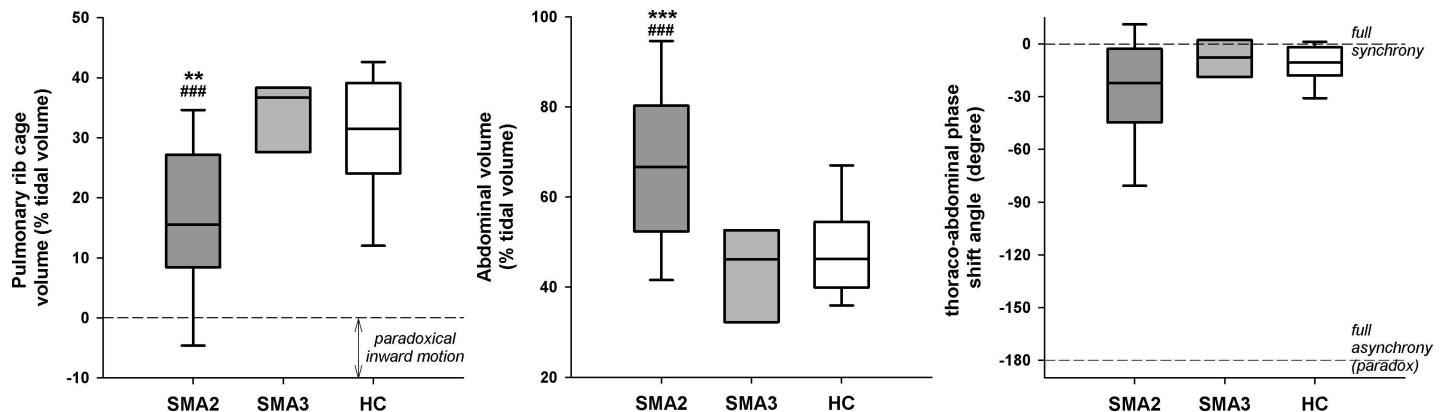
Fig 6. Box-and-whisker plot representing the median (line within the box), the IQR (length of the box), the 90th and the 10th percentiles (whiskers above and below the box) of pulmonary ribcage percentage contribution to tidal volume (left panel), abdominal percentage contribution to tidal volume (middle panel) and thoraco-abdominal phase shift angle (right panel) in SMA types II, III and healthy children (SMA2, SMA3 and HC, respectively) at rest during spontaneous quiet breathing in seated position. **,***: $p<0.01, 0.001$ vs SMA3; ###: $p<0.001$ vs HC

SMA1 IQR was 48–75 (Fig 2). Tachypnea, therefore, seems to be a clinical sign in SMA1, even in the absence of respiratory infection, representing the ventilatory strategy adopted by these children to compensate for their shallow breathing, in order to guarantee normal ventilation. The reduced V_T, in turn, is due to the inspiratory paradoxical inward movement of pulmonary ribcage (IPIM_RC,p).

Under physiological conditions, in supine position the diaphragm adopts a more favorable geometry, being lengthened by the abdominal content[58–60]. As a consequence, the pressure developed and the resulting abdominal displacement is greater compared to the seated position, even with similar levels of neural drive. This is the reason why in supine position abdominal contribution to tidal volume becomes greater than ribcage[48,61], with the latter not showing any paradox thanks to the action of the iRCM. On the other hand, we observe a consistent presence of IPIM_RCp in SMA1, suggesting that iRCM are presumably not able to contrast the negative pleural pressure swings generated by diaphragmatic contraction. The presence of IPIM_RCp has at least two negative consequences. Firstly, a portion of the lung does not expand properly during inspiration. Secondly, part of diaphragm's work is wasted to distort the chest wall rather than to inflate the lungs.

SMA type II—The breathing pattern of SMA2 is normal in supine, whereas differences emerge in seated position with a rapid and shallow breathing, adopted to guarantee normal ventilation, characterized by reduced $\%\Delta V_{RC,P}$.

Under physiological conditions, the dependence of V_T and thoraco-abdominal pattern on posture is due to its effect on ribcage and abdominal compliances. Compared to supine, in seated position the former increases while the latter decreases because of the tonic activity of abdominal muscles to stabilize the trunk[62]. As a consequence, diaphragm's contraction results more into expanding the inferior ribcage, than in moving the abdominal viscera[63] and the expansion of $\Delta\Delta_{RC,P}$ becomes predominantly due to the contraction of iRCM[64,65]. The reduced $\%\Delta\Delta_{RC,P}$, therefore, suggests that weakened iRCM are not able to fully counterbalance the decrease of pleural pressure determined by the contraction of a preserved diaphragm, also of SMA2, leading to IPIM_RCp in some cases (12% in seated, 27% in supine) and presumably to the poor spirometry.

SMA type III–In our cohort of SMA3, FVC and FEV$_1$ were within predicted values, with slightly lower values of PCF, and normal ventilatory and thoraco-abdominal patterns. Because
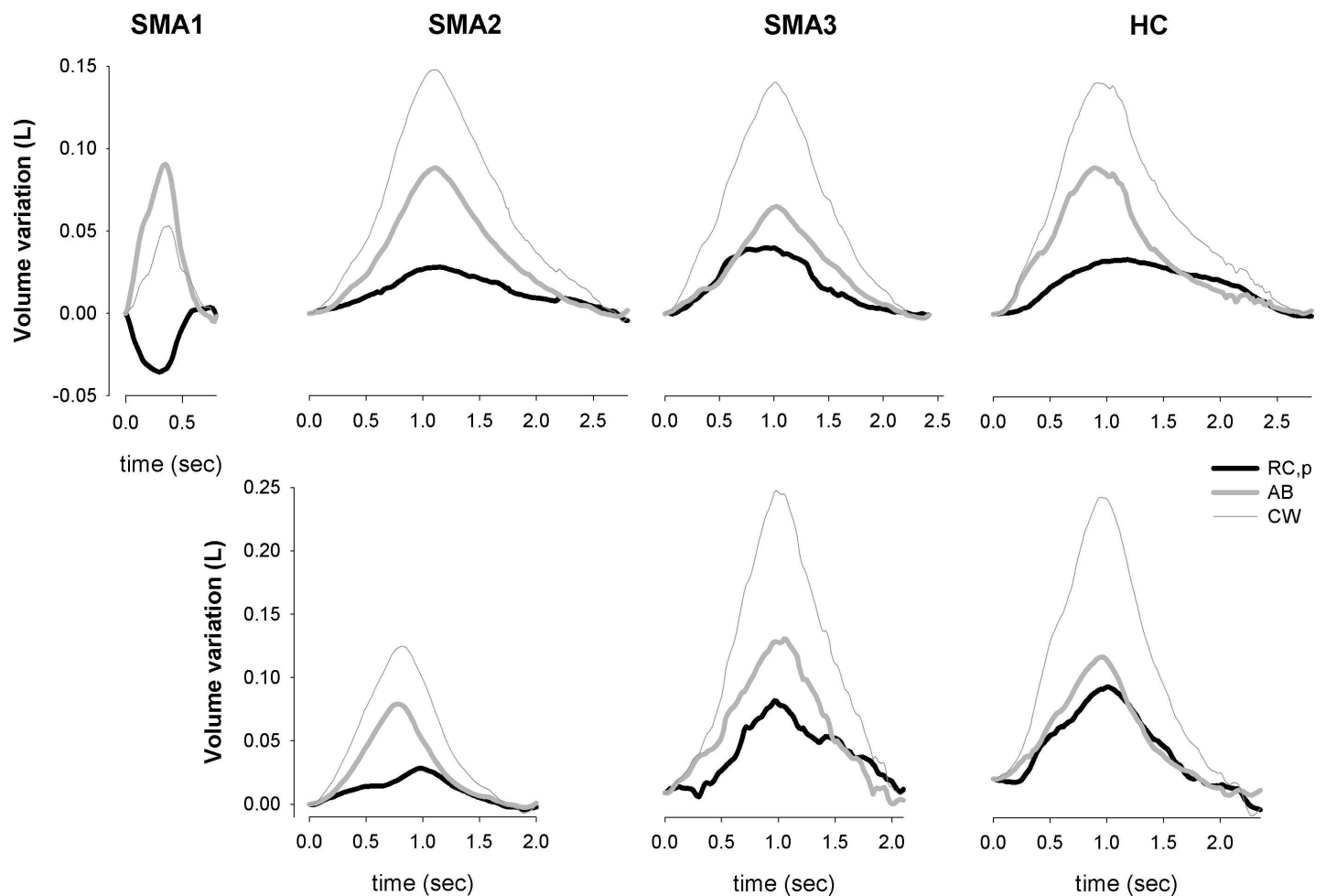
Fig 7. Representative traces of chest wall (CW, thin grey line), pulmonary ribcage (RC,p, black line) and abdominal (AB, bold grey line) volume variations during spontaneous quiet breathing in one represented subject belonging to SMA types I, II, III and healthy children (SMA1, SMA2, SMA3 and HC, respectively). In supine position (upper panels), the reduced tidal volume, the increased respiratory rate and the inspiratory paradoxical indrawing of the pulmonary ribcage compensated by increasing abdominal contribution in SMA1 are evident. Only in seated position (lower panels), in SMA2 total and compartmental volume change are reduced.

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of the small number in this subgroup, it was not possible to distinguish between the subtypes IIIA and IIIB, who were reported to be characterized by different respiratory features[37].

Taken together, our results confirm the general consensus that, according to the severity of the disease, SMA causes iRCM weakness with a preserved diaphragm, with the following consequences[19,23]: 1) alveolar hypoventilation, 2) poor airway clearance due to reduced cough efficacy, 3) micro-atelectasis reducing lung compliance, 4) bell-shaped chest with sternal depression reducing chest wall compliance, and 5) diaphragmatic fatigue secondary to the increased mechanical load.

$\%\Delta V_{RC,P}$, therefore, represents a potential useful clinical outcome in SMA, that can be obtained by OEP in all children regardless collaboration, severity level and in different postures. OEP may be particularly useful in monitoring the progression of the disease and the effects of possible interventions and/or of new medicinal products in young SMA children. Conversely, active effort-dependent volume measurements, like spirometry and cough, have two intrinsic problems for children: 1) poor availability of reliable values due to the lack of collaboration, and 2) absence of predicted values of children younger than the age of 4 years.

Table 2. Linear regression parameters between CHOP INTEND, HFMSE and ULM scales and the respiratory function parameters significantly affected in SMA types I and II-III, respectively.

	CHOP INTEND		HFMSE		ULM	
	r^2	p	r^2	p	r^2	p
FVC (L)			0.530	**<0.001**	0.599	**<0.001**
FEV$_1$ (L)			0.516	**<0.001**	0.568	**<0.001**
PCF (%pred)			0.229	0.084	0.128	0.231
PCF (L)			0.328	**0.008**	0.323	**0.011**
RR§	0.003	0.796	0.011	0.529	0.052	0.195
RSBi/Kg§	0.007	0.160	0.041	0.228	0.084	0.092
V$_T$/Kg§	0.000	0.966	0.165	**0.012**	0.204	**0.007**
ϕ_{TA}§	0.217	**0.007**	0.441	**<0.001**	0.431	**<0.001**
$\Delta V_{RC,P}$ (%V$_T$) §	0.168	**0.020**	0.419	**<0.001**	0.407	**<0.001**
ΔV_{AB} (%V$_T$) §	0.219	**0.007**	0.530	**<0.001**	0.599	**<0.001**

§ acquired in supine position when correlated with CHOP INTEND and in seated position when correlated with HFMSE and ULM

The correlation coefficient (r^2) quantifies the strength of the association between the variables, when $p>0.05$ there is no significant relationship between the two variables.

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Our study has some limitations: 1) absence of infants in the control group to be compared with SMA1, but normalizing V_T according to body weight and correcting for the total anatomical dead space allowed to take into account the difference in body size that was shown to remain invariant with age[19]; 2) reduced number of available motor function, spirometry and PCF records due either to the young age of patients or to the fact that they were not acquired close to QB analysis; 3) use of a new model of markers, that anyway provided ventilatory parameters comparable with those previously measured by pneumotachography in SMA children[19,23,56,57,66,67].

The strength of this study is that the analysis was performed in a large cohort of young SMA children and controls during awake QB, in the positions commonly adopted in daily life, without any specific requested maneuver. The study has also clinical implications and demonstrated: 1) the importance of posture when evaluating respiratory muscle function; 2) the need for larger prospective randomized, double-blind, placebo controlled trials to better understand the potentiality of salbutamol on motor and respiratory functions in SMA patients (in contrast with other works[34–36] we did not find any difference on motor and respiratory function in SMA2-treated children); 3) the potentiality of CHOP INTEND, HFMSE and ULM scales to predict poor respiratory outcomes, in particular the inefficient action of iRCM, although they do not specifically assess any aspect of respiratory function; and 4) the effect of proactive respiratory assistance in SMA1 infants to help reducing tachypnea. It was not possible to carry out a similar comparison for SMA2 because in these children the respiratory management was performed according to the international guidelines[11,39], and proactive and multidisciplinary management was adopted in all patients. We believe that such respiratory care and assistance may be the reason of the relatively limited incidence of hospitalization in SMA2 children.

Conclusions

A negative or reduced %$\Delta V_{RC,P}$, indicative of ribcage muscle weakness, is a distinctive feature of SMA1 and SMA2 since infancy. Its quantitative assessment represents a non-invasive, non-volitional index that can be used to detect changes on the respiratory function over time and the effects of specific interventions and/or of clinical pharmacological trials in all forms of

SMA. Low values on SMA-specific motor function scales indicate impairment not only of motor but also of respiratory functions.

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Author Contributions

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Investigation: ALM CM PB MTA.

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Visualization: ALM AA CM MTA PB GB.

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