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Evaluation of disease progression and response to therapy in a cohort of late childhood/adulthood SMA patients: Is there room for new markers?

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Introduction, Pathophysiology and Clinical features

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by an upcoming feature of generalized muscle and atrophy that predominate in the limb muscles and the phenotype is classified into four degrees of severity (SMA I, SMAII, SMAIII, SMA IV) based on age of onset and acquired milestones(1).

Genetic linkage studies have mapped all disease subtypes to chromosome 5q13 and the survival motor neuron genes (SMN) were identified as the disease-causing genes in SMA (2–5).

In particular two genes have been identified: SMN1 and SMN2.

The first is SMA-causing gene, due to its homozygous deletion in ~95% of SMA patients (6), SMN2 is a modifier for SMA phenotype with an inverse relationship between SMN2 copy number and disease severity (7).

Most patients with SMA type I have two copies of SMN2, three copies of SMN2 are common in SMA type II, types III and IV generally have three, four or more (8,9).

Spinal muscular atrophies (SMAs) are characterized by degeneration of the anterior horn cells of the spinal cord, leading to progressive symmetrical limb and trunk paralysis associated with muscular atrophy(4).

In the near past a lot of authors reported SMAs as the second most common fatal autosomal recessive disorder after cystic fibrosis (1 in 6000 newborns) (10–12).

Although 5 to 10% of the normal population lacks a centromeric copy of SMN (SMN2), all patients with SMA retain at least one copy of SMN2.

The genomic sequences of SMN1 and SMN2 differ by only 5 nucleotides, but there is one important functional difference:

The translationally silent C→T transition is located in the exonic splicing region of SMN2. This change leads to frequent skipping of exon 7 during splicing of SMN2-derived transcripts (13,14).

Although SMN1 produces full-length transcripts, the majority of mature transcripts that arise from *SMN2* lack exon 7, these transcripts encode a truncated protein (SMNΔ7) (15). This protein is presumably rapidly degraded, and the disease manifestations of spinal muscular atrophy likely result from a deficiency of full-length SMN protein(16) .

Several studies showed that levels of ribonucleic acid (RNA) and full-length SMN messenger protein are reduced in cell lines and tissues derived from patients with SMN1 compared with control groups (16–20).

The S*MN2* copy number varies in the population, and in patients with spinal muscular atrophy this variation has an important modifying effect on SMA type (21).

The relationship between *SMN2* copy number and disease severity has also been observed in SMA murine models.

Mice lacking SMN gene but expressing 2 copies of the human *SMN2* gene develop severe spinal muscular atrophy and die within 1 week of age; however, mice that express 8 copies of *SMN2* do not develop disease (25).

SMN is a widely expressed protein with a molecular weight of 38 kilodaltons (kDa).

It has been highly conserved through evolution and it's present in both the cytoplasm and the nucleus of the cell body (16).

In the nucleus, SMN is concentrated in punctate structures called "gems" that overlap with or are closely apposed to Cajal bodies that in addiction contains high levels of factors involved in transcription and processing of many types of nuclear RNA(16,26).

Gem number in cell lines or tissues from affected patients is related to disease severity, SMA1 patients showing few or no gems(27).

In neurons SMN is also present in granules in the axons of neurons, where it is rapidly transported bi-directionally(28,29), at the growth cone of motor neurons (30) and at the postsynaptic apparatus of the neuromuscular junction in muscle(31).

SMN protein levels are variable during life, with high expression levels in the embryonic period that decrease in the early postnatal period (25,32–34).

It is possible that the spinal muscular atrophy disease process starts when SMN levels fall below a critical threshold and that the severity of disease manifestations depends, in part, on when this decrease occurs during development(16).

The SMN protein has several domains, including a lysine-rich basic region encoded by exon 2, a Tudor motif encoded by exon 3, a polyproline region encoded by exons 4 and 5, and a region with several tyrosine-glycine (Y-G) pairs encoded by exon 6(16).

SMN protein lacking exon 7 (SMNΔ7) is rarely detectable in cells or tissues derived from human patients or from spinal muscular atrophy animal models, despite robust expression levels of SMNΔ7 messenger RNA, indicating that the SMNΔ7 protein is highly unstable (16).

This is probably due to the fact that SMNΔ7 protein has an impaired ability to oligomerize and to associate with its binding partners (35,36).

Presently, the best-characterized function of the SMN complex is regulating the assembly of a specific class of RNA-protein complexes, the uridine-rich small nuclear ribonucleoproteins (37) a critical component of the spliceosome(38).

In addiction in neuronal processes, the SMN protein binds heterogeneous nuclear ribonucleoprotein, which in turn binds to the 3'-untranslated region of β- actin messenger RNA, interaction required for the efficient transport of β-actin messenger RNA to the growth cones of motor neurons(30).

Therefore motor neurons isolated from mice deficient in SMN show shortened axons and small growth cones, which are deficient in β-actin messenger RNA and protein β-actin messenger RNA (30).

Findings from animal models show multisystemic involvement including skeletal pathogenesis, cardiac arrhythmias and defects vascular necrosis of the extremities, muscular dystrophy, hepatic insufficiency and pancreatic pathology (39–43).

Overall, the evidence supports the differential requirement of SMN by organ and tissue type and by time and suggests that SMN deficiency is implicated not only in organ and tissue development but also in their degeneration with age (44).

Adult patients with SMA demonstrate a characteristic pattern of progressive atrophy of selected skeletal muscles over years, which is similar to the muscle atrophy pattern in mouse models of SMA (45).

Myotubes from patients with SMA type 1 are small and disorganized, with delayed maturation and impaired expression of acetylcholine receptors (46,47).

Decreased activities of mitochondrial complexes 1, 2, and 4 have been reported in skeletal muscles from patients with SMA types 1 to 3 (48).

Muscle differentiation and growth are likewise affected in mouse models of SMA, and, more importantly, pathologic changes in muscle occur independent of and before nerve degeneration (43).

In vivo targeted depletion of SMN in mature muscle fibers by HSA-Cre excision of SMN exon 7 in an SMA mouse model leads to muscle degeneration and regeneration resulting in a dystrophic phenotype (49).

In vitro knockdown of SMN in a myoblast cell line results in reduced proliferation and myotubule fusion defects, which confirms the cell-intrinsic function of SMN in muscle(50)

As SMN is involved in gene expression and transcription, another mechanism by which it affects skeletal muscle may be explained by delay of transcription and expression of essential muscle contraction proteins such as ryanodine, sodium channel, and calcium channels, as seen in a presymptomatic SMA mouse model (51).

In addition, two SMA mouse models suggest that induction of proteasomal degradation or autophagy results in skeletal muscle atrophy (52)

Although SMN deficiency directly causes skeletal muscle pathology, it is unclear to what extent skeletal muscle pathology contributes to the overall pathology in SMA.

Coming back to the “threshold” model, SMN expression in milder phenotypes of SMA could already be sufficient for maximum function, although the progressive muscular atrophy in adults with SMA type 3 suggests that muscle pathology progresses with age (44).

Consistent with mouse models, cardiac abnormalities have been found in patients with SMA types 0 and 1.

There are numerous reports of congenital heart defects in SMA types 0 and 1, including atrial and ventricular septal defects, hypoplastic left heart syndrome, hypoplastic aortic arch, aortic valvular stenosis, and coarctation (44,53), similar data are not reported in SMA types 2 and 3 (54).

Oher reported features include symptomatic bradycardia reported in about 15% patients with SMA type 1 and in SMA mouse models (55,56).

Orthostatic intolerance symptoms during tilt table testing have been reported in patients with SMA type 1 and 2 (57) and sympathetic-vagal imbalance on R-R interval analysis in patients with SMA type 1 on artificial ventilation(58).

Vasculature defects and capillary bed depletion have been reported in patients with SMA and animal models, with resulting functional spinal cord hypoxia and defects in the blood-brain barrier thought to contribute to motor neuron pathology (59).

Both autonomic dysfunction and capillary dysfunction have been implicated in distal necrosis, which can be seen in various SMA mouse models and less commonly in patients with SMAs (60,61).

Among gastrointestinal symptoms, including intolerance to bolus feeding and poor motility likely caused by defect in the enteric nervous system, have been reported in SMA patients and in SMA mice (62,63).

SMA mice have obvious defects in liver development, which is reversed with systemic ASO treatment, and also have elevated triglycerides and dyslipidemia resembling non-alcoholic fatty liver disease (64,65).

This datum has been confirmed by autopsies of infants with SMA type 1 that show fatty vacuolization in the liver, which is characteristic of fatty acid oxidation disorders, Lipid

profiling in children with SMA types 1 to 3 showed that one-third had dyslipidemia (65,66).

Significant osteopenia with increase risk of fractures has been reported across the spectrum of patients with SMAs and mouse model (67,68) more than similar disabling disorders suggesting a cell-intrinsic contribution of SMN to bone function (69).

Scoliosis is still highly prevalent in children with SMA 1 and 2, with incidence of 60–90% and initial presentation in early childhood (70,71).

The hypotonic spinal curves continuously progress through childhood, thoracic kyphosis also develops in most patients to a variable degree(72).

As a consequence of poor trunk and thoracic muscular support, children with SMA have an increased incidence of thoracic insufficiency, the result of scoliosis and distortion of the rib cage (73,74).

Collapse of the ribs (similar to closing an umbrella) contributes to “parasol rib” deformity (73,75–78).

Retrospective study of children with hypotonic scoliosis treated with either rib or spine-based growth-friendly instrumentation systems have shown poor efficacy in ameliorating parasol rib deformity or increasing thoracic volume, and therefore are not recommended (73,79).

Hip instability is common in patients with SMA (72,74,80–82).

Contractures are common in patients with SMA as a result of decreased range of motion, prolonged static positioning, and agonist-antagonist muscle imbalance (72,74,83,84).

For these reasons clinical features in SMAs are unique among neuromuscular disorders in particular we identified some crucial points:

First: muscular pattern is quite unusual for a neurogenic disorder: muscular, neuromuscular junctions and motoneurons are involved.

Second: a lot of aspects o SMA are to date not well known; in particular while there are a lot of data regarding SMA in infant in literature, little is known about late adolescence childhood.

Third: a lot of aspects are known from animal models but have never been evaluated in humans In particular there are heterogeneous aspects among the multiple available models such as zebra fish, fly and mice that are quite different from human for physiopathological aspects.

Even if a lot of evaluation can be provided from animal model the double gene SMN1 and SMN2 is unique in human and nevertheless the attempt to provide realistic model some aspects cannot be explored by animals.

Finally, constellation of multiple organs are involved in this disorder and a lot of features are unexplored in SMAs due to the early death of patients before the era of treatment.

The new therapies available in our country since 2017 (85) have increased the life expectancy of child and is now possible evaluate older patients and there is a lack of impact on non motor symptoms.

Diagnosis

The diagnostic process for SMA remain unchanged since the original consensus statement (80) unless there are previous familial cases, the diagnostic process is generally prompted by the clinical signs (72).

Typically symptoms at onset includes in infant and childhood, hypotonia, progressive symmetric and proximal weakness affecting the legs more than the arms, sparing of the facial muscles but often with bulbar muscle weakness(72).

Respiratory muscles are often involved with weakness of the intercostal muscles and relative sparing of the diaphragm, which results in the typical “bell-shaped” chest and paradoxical breathing pattern (72).

The diagnosis of SMA is based on molecular genetic testing in typical cases(72).

Muscular biopsy and EMG usually are not needed in type 1 and 2 children; this investigations can help in more chronic forms in which the phenotype might be less striking and can mimic a pure myopathic disorder (72,86).

The gold standard of SMA genetic testing is a quantitative analysis of SMN1.

However, knowledge on SMN1 copies is relevant for identification of heterozygous deletions whereas SMN2 copies are important for prognosis and therapeutic approaches (72).

If only 1 full copy is present and clinical phenotype is compatible with SMA, the remaining SMN1 gene should be sequenced looking for other subtle mutations.

If both full SMN1 copies are present, a diagnosis of SMA is highly unlikely but the SMN1 gene should be sequenced if there is a striking typical phenotype or consanguinity. The gold standard of SMA genetic testing is a quantitative analysis of SMN1(72).

However, knowledge on SMN1 copies is relevant for identification of heterozygous deletions whereas SMN2 copies are important for prognosis and therapeutic approaches; the absence of both full SMN1 copies will provide diagnosis of SMA (72).

Neurophysiological tests useful in SMA

Motor conduction studies

Among neurophysiological tests classically SMA have been explored looking for the assessment of motor unit, defined as the first motor neuron and muscle fiber innervated by it(87).

Motor conduction studies consist of stimulating the nerve at two or more points along its course and recording muscle action potentials with a pair of surface electrodes: an active lead (G_1) placed on the belly of the muscle and an indifferent lead (G_2) placed on the tendon(88,89).

Depolarization under the cathode results in the generation of a nerve action potential, whereas hyperpolarization under the anode tends to block the propagation of the nerve impulse(90).

With the cathode at the best stimulating site, increasing the intensity elicits a progressively larger response until it reaches a maximal potential(90).

Increasing the stimulus further should result in no change in the size of the muscle potential (90).

The use of a 20-30 percent supramaximal intensity guarantees the activation of all the nerve axons innervating the recorded muscle (90).

The compound muscle action potential consists of many motor unit action potentials within the the recording radius of the active electrode in the range of 20 mm from the skin surface and its usual measurements include amplitude, duration from the onset to the

negative or positive peak or to the final r turn to the baseline and latency, from the stimulus artifact to the onset of the negative response (90).

Latency consists of three components:

Nerve activation time from application of the stimulus to the generation of action potential, (2) nerve conduction time, from the stimulus point to the nerve terminal, and (3) neuromuscular transmission time, from the axon terminal to the motor end plate, including the time required for generation of muscle action potential (90).

Dividing the distance between the stimulus points by the corresponding latency difference derives the conduction velocity.

$$\frac{D \text{ mm}}{L_p - L_d \text{ ms}} = \frac{D}{L_p - L_d} \text{ m/s}$$

D= Distance between the two stimulus points in millimeters,

Lp= proximal latencies

L_d= distal latencies in milliseconds.

Separation of the two stimulation points by at least several centimeters, and preferably more than 10, improves the accuracy of surface measurement and, consequently, determination of conduction velocity (90).

In general, axonal damage or dysfunction results in loss of amplitude, whereas demyelination leads to prolongation of conduction time.

Assessment of a nerve as a whole, as opposed to individual nerve fibers, usually reveals more complicated features because different types of abnormalities tend to coexist(90).

Sensory conduction studies

For sensory conduction studies in the upper limbs, stimulation of the digital nerves elicits an orthodromic sensory potential at a more proximal site, alternatively, stimulation of the nerve trunk proximally evokes the antidromic digital potential distally and mixed nerve potential proximally (90).

Sensory fibers with large diameters have lower thresholds and conduct faster than motor fibers by about 5-10 percent (91).

Thus, mixed nerve potentials allow determination of the fastest sensory nerve conduction velocity in healthy subjects and in patients with neuropathies affecting motor fibers more than the sensory axons (92).

For routine clinical recordings, surface electrodes provide adequate and reproducible information non invasively (93,94).

The antidromic potentials from digits generally have a greater amplitude than the orthodromic response from the nerve trunk, because the digital nerves lie nearer to the surface (95)

The types of abnormalities described for motor conduction apply in principle to sensory conduction as well. Substantial slowing in conduction velocity implies demyelination of the sensory fibers, whereas axonotmesis results in reduced amplitude of the compound nerve action potentials with stimulation either distally or proximally to the site of the lesion (90).

Sural nerve potential serves as a sensitive measure for length-dependent distal axonal polyneuropathy (96).

Unlike motor latency, which includes neuromuscular transmission, sensory latency consists only of the nerve activation and conduction time from the stimulus point to the recording electrode, therefore, stimulation of the nerve at a single site suffices for calculation of conduction velocity (90).

V= D/LD. V= Velocity D= distance LD= distal Latency.

With the biphasic digital potential recorded antidromically, the onset latency measured to the initial take-off of the negative peak corresponds to the conduction time of the fastest fibers from the cathode to GI(90).

Quantifying Motor units

A variety of techniques provide the means for calculating motor unit number estimates (MUNE)(97,98).

Technical limitation in achieving unbiased selection constitutes a major source of error. In neuromuscular disorders characterized by a loss of lower motor neurons, a patient's strength depends primarily on the number of remaining motor units in a group of muscles (90).

A variety of techniques provide the means for calculating motor unit number estimates (MUNE) (97,98).

Each method relies on dividing an average size of a single motor unit potential into a maximal compound muscle action potential that represents the sum of all motor units. All the methods have certain assumptions relating to the adequacy of sampling in estimating average size (90).

Technical limitation in achieving unbiased selection constitutes a major source of error.

Supramaximal stimulation of a peripheral nerve activates all the amplitude of a maximal compound muscle response directly relates to the total number and size of muscle fibers, providing a rough estimate(99).

Although the maximal amplitude is usually proportional to the number of axons, abnormally large motor unit potentials after reinnervation partially restore the size, thus concealing the loss of axons (90).

In chronic neurogenic processes, the ease of measuring the reduced number of larger potentials compensates for the inaccuracy resulting from an increased size variation of individual units(100).

The same motor unit potential may vary in size from one stimulus to the next, with defects of neuromuscular transmission requiring special interpretation (eg myasthenia gravis, amyotrophic lateral sclerosis, and neurogenic processes with ongoing reinnervation) (90) .

At any given stimulus intensity, different axons may discharge according to their probability of firing.

If two motor axons have similar excitability, a threshold stimulus may activate them together or alternately. This possibility, termed *alternation,* constitutes another source of error(90).

In the last years there is an increasing interest for these measures as markers of response to conventional therapy, in particular a there are some data of cMAP and motor unit analysis in SMA patients in natural history of the disorder (101,102) and in the follow-up of young that received specific therapy(103–106).

In literature is to date available only a paper, just been published , in with authors report on a cohort of adult patients, that cMAP should be further evaluated as potential easy-to-use electrophysiologic marker in assessing and monitoring clinical response to therapy (107).

Needle EMG

During needle EMG, three types of activity are recorded: insertional activity, spontaneous activity, and voluntary activity (108).

Once the muscle location is properly identified and palpated, the patient is asked to relax. The needle is then quickly inserted through the skin into the muscle. Once the correct needle placement has been established, the first part of the examination is to assess insertional and spontaneous activity at rest.(109)

Muscle normally is quiet at rest, except for the potentials seen at the endplate zone(109).

In child with SMA a finding specific to this disease is spontaneous activity in relaxed muscles, this activity could last for hours and persisted during sleep.

The potentials differed from fasciculations and could be activated by voluntary effort(110).

The second part of a typical EMG consist of evaluation of Motor Unit Action Potential (MUAP).

To analyze MUAPs, the examiner asks the patient to slowly contract the muscle of interest; MUAPs are assessed for duration, amplitude, and number of phases. In addition, the number of MUAPs, their relationship to the firing frequency, and the rate of firing itself are measured (109).

As the patient slowly increases force, both the firing frequency and the number of MUAPs normally increase. After the MUAPs are assessed at one location, the needle is moved slightly within the muscle to a different site, and the process is repeated.

Ideally, 10 to 20 different MUAPs should be studied (109).

Typically in SMA patients the amplitude of the activity during effort and the amplitude and duration of the motor unit potentials are markedly increased (110).

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Follow-up

According to recent guideline for diagnosis and treatment of SMA, management of patients diagnosed with SMA requires specific and multidisciplinary skills (70,72,80). Different topics should be evaluated for follow-up and due to the complexity of the disorder an individualized approach is needed.

Basically a complete neuromotor and clinical examination is required every 6 months (111,112).

Neurological assessment

Clinical assessment in SMA includes performing a physical examination, with a focus on the musculoskeletal system and related functional impairments(72).

These should include different means of assessments of strength and range of joint motion, relevant motor functional scales and timed tests to monitor those aspects of function that reflect activities of daily living(113–117).

These assessments should be performed routinely by trained examiners every 6 months, unless there are special circumstances requiring different follow up (72).

Regular monitoring of these aspects will allow to monitor possible changes over time, to identify aspects requiring intervention and response to intervention and allows to compare individual results to the trajectories of progression reported in recent studies (118,119).

Until now, because of their limited survival, spinal management was rarely discussed as a possible option in non-sitters, unless they had stable respiratory and nutritional function (112,120).

Specific rigid braces allowing stable sitting position may be used, provided they do not compromise pulmonary function.

Supine Cobb angle or that obtained in the sitting position using a trunk brace may be used in their follow up (120).

The advent of new therapies leading to increased survival and overall functional improvements, is rapidly changing the scenario of spinal management in these patients (121,122).

The hypotonic spinal curves continuously progress through childhood, thoracic kyphosis also develops in most patients to a variable degree: inspection of the spine should be conducted as part of the routine clinical examination (72).

When kyphoscoliosis is suspected on forward bend test in sitting or standing posture, anteroposterior and lateral projection spine radiographs should be performed in the most upright position independently attainable by the patient (i.e. sitting in children who can sit independently, standing in SMA 3) to define and quantify the extent of spinal deformity in both coronal and sagittal planes (72).

For SMA 1 and 2 patients, scoliosis >20° should be monitored every 6 months until skeletal maturity and yearly after skeletal maturity.

Management with spinal orthoses is often advocated to support the hypotonic trunk and treat scoliosis >20°, especially in a child with significant growth remaining (123,124) .

Bracing is palliative and unable to halt progression of spinal deformity(74,123).

As a result, spinal instrumentation is frequently indicated to preserve trunk balance in sitting, re-align the distorted thorax to facilitate respiratory function and improve overall quality of life (81,125–127).

The decision to surgically instrument the spine is predicated mainly on curve magnitude (i.e. major curve Cobb angle ≥50°) and rate of progression (≥10° per year).

Other factors, such as decreasing respiratory function, parasol rib deformity, hyperkyphosis and adverse effects on functional mobility, pelvic obliquity, and trunk imbalance should also be considered (72).

Pulmonary function tests should be considered as part of the pre-operative evaluation to determine surgical risk and post-operative respiratory management.

In skeletally immature patients younger than 8 to 10 years, "growth-friendly" instrumentation, that stabilizes and improves spinal deformity, but allows for continued spine growth should be considered (74,112,127–131).

To decrease the need for repeated surgery, magnetically controlled growing rods have recently been advocated(132) as an alternative to traditional growing rods that require sequential surgical lengthenings(133–136).

For children between the ages 8 to 12 years, there was variability in practice among members of the expert panel; the surgical approach depended on clinical variables, especially skeletal maturity and spine growth remaining(72).

In nearly skeletally mature patients 12 years of age or older, definitive posterior spine fusion using dual rod, multi-segmental constructs should be implemented with or without extension to the pelvis, depending on whether the pelvis is part of the scoliotic curve (137).

While there were no published studies on how to accommodate for intrathecal access in patients undergoing spinal instrumentation, there was consensus that one or two mid-lumbar levels should be left unexposed in the midline to accommodate intrathecal access, necessary for the administration of recently approved drugs such as Nusinersen, and antisense oligonucleotide which does not cross the blood brain barrier(72).

Clinical Scales

A lot of clinic scales have been developed to evaluate patients with SMA.

Currently in our country is required by regulatory autorithy AIFA (138) that almost two scales among CHOP INTEND, Motor milestones HINE sez. 2, HFMSE, MFM 32 RULM must be administrated every 6 months for Risdiplam administration.

Among these scales CHOP INTEND and HINE sez 2 have been validated for SMA 1 infants and anyway for child younger than 1 year (139,140).

We usually use RULM and HFMSE to evaluate our patient because of a part of our patient come from other centers and these two scales are the most common among other Italian Neuromuscular units.

RULM

The original Upper Limb Module (ULM) was developed as an international effort by clinicians, physical therapists, researchers, and patient advocacy groups in an attempt to address these shortcomings(141).

The original module was designed specifically to assess upper limb function in non-ambulatory SMA patients, mainly targeting young children (142).

This scale have been reviewed many times main were ULM2 and the currently used RULM(142).

The RULM now has a total of 20 items with an entry item that serves as functional class identification and does not contribute to the total score (142,143).

The remaining 19 scorable items reflect different functional domains and are graded on a 3- point system with a score of 0 (unable), 1 (able, with modification), and a maximum of 2 (able, no difficulty) (142,143).

There is only 1 item (item I) that is scored as a can/cannot score, with 1 as the highest score.

The maximum total score is 37 (142,143).

A scoring sheet and manual with detailed graphics that provide better, standardized administration procedures related to general testing guidelines, patient positioning, equipment, item instructions, start and stop positions, and detailed scoring options were a part of the RULM revision (142).

Assessments are performed unilaterally on the preferred arm chosen by the individual (142,143).

Details of the items included in the final version of the Revised Upper Limb Module
Entry item
Bring hands from lap to table
Complete the path bringing the car to the finish line without stopping or taking pencil off of paper?
Pick up coins/tokens
Place coin/token into cup : On table: horizontal At shoulder height: vertical
Reach to the side and touch the coin/token: Bring hand at shoulder height and above
Push button light with one hand
Tearing paper
Open Ziploc container
Raise 200-g cup to mouth
Lift 200-g weight and bring it from 1 circle to the other (midline to outer circle on tested side) without sliding
Lift 500-g weight and bring it from 1 circle to the other (midline to outer circle on tested side) without sliding
Lift 200-g weight and bring it from one circle to the other (inner to outer circle on opposite side) without sliding across midline
Bring 500-g sand weight from lap to table or eye level
Bring both arms above head - *Shoulder abduction*
Bring 500-g weight above shoulder height - *Shoulder abduction*
Bring 1-kg weight above shoulder height - *Shoulder abduction*
Bring hand above shoulder height - *Shoulder flexion*
Bring 500-g weight above shoulder height - *Shoulder flexion*
Bring 1-kg weight above shoulder height - *Shoulder flexion*

Fig1. RULM Items

HFMSE

The Hammersmith Functional Motor Scale Expanded (HFMSE), a motor function scale specifically designed for SMA, is widely used in patients (144–146).

The activities included in the original Hammersmith scale and in the expanded version were chosen by clinicians because of their functional relevance after careful observation and evaluation of many SMA patients (144–146).

Motor skills are scored on 20 items using a 3 point (0–2),it is feasible (taking approximately 15 min to administer), requires minimal equipment, is clinically meaningful, and has good inter–rater reliability (145–147).

Table 2 The Hammersmith SMA functional motor scale.

Name	Date	Age	Hosp. no.
Score 2 points	Score 1 point	Score 0	Score
(1) Frog/chair sitting no hand support	1 hand support	2 hand support	( )
(2) Long sitting, no hands	1 hand support	2 hand support	( )
(3) 1/2 roll from supine, both ways	One way (R/L?)	Unable	( )
(4) Touches one hand to head (R/L?) (in sitting)	Flexes head to hand	Unable	( )
(5) Touches two hands to head (in sitting)	Flexes head to hands	Unable	( )
(6) Rolls prone to supine over R	Pushes on hand	Unable	( )
(7) Rolls prone to supine over L	Pushes on hand	Unable	( )
(8) Rolls supine to prone over R	Pulls on hand	Unable	( )
(9) Rolls supine to prone over L	Pulls on hand	Unable	( )
(10) Gets to lying from sitting (safely, not accidentally)		Unable	( )
(11) Achieves prop on forearms-head up	Holds position when placed	Unable	( )
(12) Lifts head from prone (arms down by sides)		Unable	( )
(13) Achieves four point kneeling-head up	Holds position when placed	Unable	( )
(14) Achieves prop on extended arms-head up	Hold position when placed	Unable	( )
(15) Gets to sitting from lying through side lying	Through prone	Unable	( )
(16)Crawls	Crawls, head up	Unable	( )
(17) Lifts head from supine	Through side flexion	Unable	( )
(18) Stands holding on with one hand	Stands with MINIMAL trunk support	Knee/hip support needed	( )
(19) Stands independently: count >3	Stands independently count of 3	Stands momentarily	( )
(20) Takes >4 steps unaided	Takes 2–4 steps unaided	Unable	( )
			Total ( )

All tests done without jackets/orthoses.

Fig 2. HFMSE items (146).

Respiratory assessment

Spirometry

It is well known that spinal muscular atrophy has an impact on the respiratory system that is dependent in large part on the type of SMA or more precisely the severity of loss of muscle function(148).

The respiratory muscles are also involved with a weakness of the intercostal muscles and a relatively spared diaphragm(149,150).

This respiratory muscle weakness translates into a cough impairment, resulting in poor clearance of airway secretions and recurrent pulmonary infections with an increased risk of pulmonary atelectasis, restrictive lung disease due to a poor or insufficient chest wall and lung growth, nocturnal hypoventilation, and finally respiratory failure(150).

Over the last decade, the approach to treating the pulmonary manifestations of SMA has shifted from a reactive approach, of starting treatment to support airway clearance and ventilation only when there is a clear indication, to a proactive approach of introducing these therapies earlier in the disease process (151,152).

The assessment of the respiratory muscles is challenging because these muscles are not directly accessible as is the case for peripheral muscles (150).

As the function of muscles is to develop force and shorten, in the respiratory system, force is generally estimated as pressure and shortening as lung volume change or displacement of chest wall structures (150).

Tests evaluating respiratory muscles are separated in "volitional" or "nonvolitional" tests and "noninvasive" or "invasive" tests (i.e. requiring the measurement of the oesophageal (Poes) and gastric pressures (Pgas)) (153–155).

This separation is of major importance for the clinical assessment of patients.

Indeed, it is easily understandable that volitional and noninvasive tests are the most commonly used in clinical practice because of their simplicity, availability and ease(155).

These tests include vital capacity (VC), maximal static pressures, sniff nasal inspiratory pressure (SNIP), peak expiratory flow (PEF) and peak cough flow (PCF)). (155).

Vital capacity (VC) is the simplest test that is widely used in children with NMD. Technical quality standards have been published which may be difficult to fulfill for young children(155).

Thus, simpler maneuvers have been used, such as slow VC and maximal inspiratory capacity(156).

Mask spirometry may circumvent the inability to seal lips around a mouthpiece for patients with facial weakness, which is very common in children with NMD.

VC in the sitting and supine position is recommended in case of diaphragm dysfunction as a > 25% drop in the supine position is associated with diaphragm weakness(157).

In children with SMA II, VC% predicted is already low at the age of 6-8 years and decreases thereafter by about 10% per year (149). VC is preserved in patients with SMA III until early adulthood with about a 5% decrease thereafter(155).

Maximal static inspiratory (MIP) and expiratory (MEP) pressures are also used for routine monitoring of respiratory strength.

Peak inspiratory and expiratory pressures have been used as simpler tests and have shown their usefulness in predicting severe chest infection in children with NMD (158).

In infants, mouth pressures generated during crying efforts may provide an index of global respiratory muscle strength (159).

The measurement of the sniff nasal inspiratory pressure (SNIP) is a simple and noninvasive way to assess inspiratory muscle strength in children.

A sniff is a natural maneuver which many children over the age of 3-4 years find much easier to perform than maximal static pressures, especially when the test is associated with visual feedback on a computer screen(153,160)

Because of its simplicity, SNIP should be part of the routine evaluation of muscle strength in children with NMD (154,161).

Indeed, even though SNIP and MIP are not interchangeable, SNIP is easier to perform. The main limitation of SNIP is the underestimation of inspiratory muscle strength in case

of nasal obstruction, because of adenoids or nasal polyps, or in patients with severe respiratory muscle weakness(160).

PEF and PCF are routinely used in adult patients with NMD for whom thresholds associated with an impaired coughing ability have been validated PEF and PCF can be performed in children as young as 4 years old (155).

Standard values for PCF have been published for children but thresholds associated with or predictive of respiratory complications are not available for young children(162).

In young or non-cooperative children, other tests are available, these tests have the great advantage of allowing the calculation of numerous informative parameters but they are inconvenient in that they require the placement of esophageal and gastric balloon catheters or catheter-mounted pressure transducers and for this reason are more invasive and not routinely used (153–155,161)

For sitters and standers, there is consensus that all patients able to perform spirometry should do so during each visit and clinic visits are recommended, every 6 months for sitters (151).

Most ambulant patients with SMA type 3 have normal pulmonary function, but with a small decline noted over a 4-year span in one natural history study(102,148).

Nonetheless, the clinical assessment of these patients should include careful review of cough effectiveness with an upper respiratory infection, and search for any symptoms of sleep apnea or hypoventilation (snoring, arousals, morning headaches, daytime somnolence) (151).

The presence of any such concerns should prompt an assessment by a pulmonologist with consideration of pulmonary function testing and sleep study. Pre-operative assessment is also important (151).

No pro-active interventions are indicated for ambulant patients with SMA. Supportive care should be provided when there are specific concerns identified in the clinical assessment. Immunizations are the same as for sitters (151).

A respiratory therapist should be involved to initiate and support assisted airway clearance and respiratory range of motion therapy (151).

Airway clearance

Manual chest physiotherapy combined with mechanical insufflation–exsufflation (e.g., Cough Assist® orVitalCough®) should be the primary mode of airway clearance therapy and should be made available to all non-sitters (151).

Because of the importance of aggressive management of respiratory illnesses (163–168), airway clearance techniques should be introduced proactively in patients based on either clinical assessment of cough effectiveness or by measuring peak cough flow (not a routinely performed test in infants) (163).

When initiating cough assist devices, the insufflation and exsufflation pressures should be increased gradually to 30–40 cm H2O of positive or negative pressure, respectively, or instead increase them to the maximal tolerated pressure (166).

While there are case reports suggesting the use of mechanical insufflation or NIV to help prevent chest wall distortion there was less consensus whether this is always a reasonable expectation and on the specifics of how to best accomplish this (166,169,170).

Oral suctioning with a mechanical suction pump and catheter is a critical part of airway clearance in non-sitters and should be used with any patient with an ineffective cough (151).

While for sitters and standers there is consensus that all patients able to perform spirometry should do so during each visit, there was no clear consensus on the value of peak cough flow measurement or when a sleep study should be performed in the management of sitters (151).

Airway clearance, manual chest physiotherapy combined with mechanical insufflation-exsufflation (e.g., Cough Assist® or VitalCough®) should be made available to all patients with an ineffective cough (151).

It should be introduced proactively in patients using either clinical assessment of cough effectiveness or by measuring peak cough flow (163).

Sleep

Screening non-sitters for respiratory failure should include assessment with pulse oximetry and capnography (end tidal CO2 (EtCO2) or transcutaneous CO2 (TcCO2)) when awake), and using sleep study or pneumogram with CO2 recording when there is even minimal suspicion of hypoventilation (151).

Data from the literature and expert opinion supports using a sleep study to confirm when a patient has sleep disordered breathing or respiratory failure and needs to use non-invasive positive pressure ventilation (NIV) (163).

Clinic visits are recommended initially for every 3 months for non-sitting patients with SMA (151).

A sleep study should always be performed, however, in symptomatic patients or when there is even a minimal suspicion of nocturnal hypoventilation to determine when a patient has sleep disordered breathing or respiratory insufficiency and needs to use bilevel NIV(163).

A polysomnography or respiratory polygraph is a noninvasive and non volitional way to detect respiratory muscle weakness during sleep and confirm the need for NIV(155). Screening for sleep disorder breathing is important because it precedes daytime symptoms of respiratory failure (170).

However, the scoring of respiratory events during sleep in patients with NMD requires specific expertise.

Indeed, instead of apneic and hypopneic events, these patients usually present a progressive simultaneous decrease in airflow and thoracic and abdominal movements accompanied or not by a decrease in pulse oximetry (SpO2) and/or an increase in transcutaneous carbon dioxide (PtcCO2), suggestive of global inspiratory muscle weakness (171).

Paradoxical breathing with opposition phase on the thoracic and abdominal belts may be the consequence of diaphragmatic dysfunction or weakness of the intercostal muscles and should not be falsely interpreted as “obstructive events” (171,172).

In clinical practice, periods of “reduced ventilation” or paradoxical breathing, especially during REM sleep, associated with a minimal SpO2 < 90% and/or a maximal PtcCO2 value > 50 mmHg are markers of respiratory muscle weakness and justify the initiation of long term NIV in children with NMD(155)**.**

Ventilation

Non-invasive positive pressure ventilation (NIV) should be used in all symptomatic infants and in non-sitters prior to signs of respiratory failure, to be "prepared" for respiratory failure, prevent/minimize chest wall distortion, and palliate dyspnea (164,166,170,173,174).

Continuous positive airway pressure (CPAP) should not be used to treat chronic respiratory failure, but may be used with caution temporarily to help maintain resting lung volume (functional residual capacity (FRC) in younger patients who are unable to synchronize with the ventilator in NIV mode, and who are not markedly hypercapnic. This applies also to weak non- sitters.

It should be recognized that CPAP may fatigue SMA patients and could interfere with weaning from full time use(151).

Interface selection and fitting to the patient by an experienced clinician is strongly recommended, as was using at least two comfortable interfaces with different facial contact points, and using a nasal interface initially.

In non-sitters there is strong support for initiating NIV using clinical titration with focus on correcting gas exchange and reducing the work of breathing (151).

Tracheotomy ventilation according to clinical status, prognosis, and quality of life an option in selected patients in whom NIV is insufficient or fails, or if there is no effective interface for providing ventilation (151).

Similar to non-sitters, non-invasive positive pressure ventilation (NIV) should be used in all symptomatic patients (164,166,170,173,174).

The best approach is individualized to each patient's need and quality of life. A sleep study should be used to determine when a patient has sleep disordered breathing or respiratory failure and needs to use bilevel NIV, and to titrate settings(163).

Why is SMA a lesson among neuromuscular disorders?

While being a monogenetic neuromuscular disease, the resulting phenotypic spectrum is complex and SMA is generally perceived as a systemic disease (175).

Accordingly, caring for patients with SMA requires the interdisciplinary management of respiratory, nutritional and gastroenterological, orthopedic, and psychosocial issues. General treatment recommendations were published in 2007 in the first consensus statement on standards of care in SMA (176).

Nevertheless, the implementation of standards of care is highly variable and is influenced by cultural perspectives, socioeconomic factors, and the availability of regional resources (111).

Due to advances and improvements in care over the last decade, an updated version of recommendations on diagnosing SMA and patient care was published only recently (111,151).

The first drug approved for SMA treatment was Nusinersen (former IONIS-SMNRX), an antisense-oligonucleotide (ASO) that enhances the inclusion of exon 7 in mRNA transcripts of SMN2.

Nusinersen binds to an intronic splice-silencing-site in intron 7 of SMN2 and thereby suppresses the binding of other splice-factors (177).

This results in an increased proportion of SMN2-mRNA with included exon 7 and consecutively more functional full-length SMN2 protein (178,179).

After promising results for Nusinersen in phase I and phase II studies with children with SMA type 2 and 3(122,180), three phase III studies were initiated subsequently: In the ENDEAR study, 121 infants with SMA type 1 and younger than 7 months of age underwent either repeated intrathecal injections of Nusinersen or a sham-intervention entailing no drug application (181)

Those receiving Nusinersen demonstrated a prolonged time to death or need for permanent ventilation compared to the sham-control group (182).

The criteria for being a "motor-milestone-responder" (achievement of motor milestones in HINE-2 scale; Hammersmith Infant Neurological Examination) were fulfilled by 51% in the verum group but by 0% in the sham-control group.

Although the verum group's motor development differed strongly from the disease's natural history, only a minority of patients (6/73) achieved independent sitting during the Nusinersen treatment period lasting about one year (182).

In the CHERISH study, the effects of Nusinersen were studied in 126 older children (median age 4 years) with SMA type 2 and onset of symptoms after the age of 6 months. Again, the Nusinersen group exhibited a gain in motor functions (mean+4.0 points in HFMSE scale; Hammersmith Functional Motor Scale Expanded version), whereas the sham control group deteriorated slightly (–1.9 points in HFMSE scale) (183).

Both studies were terminated prematurely after these results became apparent in interim analysis and all participants were switched to the treatment group.

The effects of pre-symptomatic Nusinersen treatment were studied in the NURTURE study in 25 infants under 6 weeks of age with 2 (n=15) or 3 (n=10) SMN2 copies. All 25 patients acquired the ability to sit independently and 22/25 achieved independent walking(106,184).

Nusinersen was approved by the Federal Drug Agency (FDA) in December 2016 and by the European Medicines Agency (EMA) in May 2017. The first patients with SMA type 1 had been treated beforehand within an Expanded Access Program (EAP) in some countries (181).

Onasemnogene abeparvovec-xioi is an intravenously administered adeno-associated viral vector-based gene replacement therapy approved in the U.S. in 2019 for the treatment of pediatric patients who are < 2 years old with bi-allelic mutations in the *SMN1* gene (185).

Approved for use in the E.U. in 2020, Onasemnogene abeparvovec-xioi gene therapy delivers a copy of the gene encoding human SMN protein in patients with SMA(186).

An open-label, single arm, ascending dose clinical trial assessed the safety and efficacy of onasemnogene abeparvovec-xioi in subjects < 2 years old with genetically confirmed bi-allelic *SMN1* gene deletions, two copies of the *SMN2* gene, and absence of the c.859G > C modification in exon 7 of the *SMN2* gene, and with SMA symptom-onset before 6 months of age.

Onasemnogene abeparvovec-xioi was administered as a single intravenous infusion to low-dose ($n = 3$) and high-dose groups ($n = 12$).

After 24 months, one subject in the low-dose cohort required permanent ventilation while all subjects in the high-dose group were alive and without permanent ventilation.

None of the subjects in the low-dose group were able to sit without support, stand or walk. In the high-dose group, nine subjects (75.0%) could sit without support for ≥ 30 s, and two (16.7%) could stand and walk without assistance.

The most frequent adverse events with an incidence > 5 observed in four open-label studies of 44 subjects receiving intravenous (IV) infusion, were elevated aminotransferases exceeding the upper limit of normal (27.3%) and vomiting (6.8%)(185).

A phase 3 open-label, single-arm, single-dose trial enrolled symptomatic subjects < 6-months-old ($N = 22$) with SMA due to biallelic *SMN1* mutations (deletion or point mutations) and one or two copies of *SMN2* (187).

Subjects received a single 30–60 min IV infusion of onasemnogene abeparvovec-xioi (1.1×10^{14} vg/kg) and were then assessed once weekly for 4 weeks, and then monthly until age 18 months or early termination.

Coprimary efficacy outcomes were independent sitting for ≥ 30 s (Bayley-III item 26) at 18 months of age and freedom from permanent ventilation at age 14 months. By the data cutoff, 13 of the 19 subjects continuing in the trial reached 14 months of age without permanent ventilation, one of the study's coprimary efficacy endpoints (187).

In addition to survival, assessment of the other coprimary efficacy endpoints found that 10 of the 21 subjects (47.6%) achieved the ability to sit without support for ≥ 30 s between

9.2 and 16.9 months of age (mean age was 12.1 months). Based on the natural history of the disease, subjects who met the study entry criteria would not be expected to attain the ability to sit without support, and only approximately 25% of these subjects would be expected to survive (i.e., being alive without permanent ventilation) beyond 14 months of age. In addition, 16 of the 19 subjects had not required daily non-invasive ventilation (NIV) use. (187).

Serious adverse events (n = 10, 45%) were most commonly consequences of the underlying disease, including some form of respiratory tract infection.

Other events included transient transaminase elevation (n = 7, 32%), of which two (9%) developed severe elevation of transaminases that responded to steroids. Two subjects (9%) developed low platelet counts (≤ 75,000 /μL) that were not associated with clinical sequelae and resolved spontaneously(187).

The manufacturer has reported that 1,400 doses of *onasemnogene abeparvovec-xioi* have been administered worldwide since it received marketing authorization (188).

An approach to altering the splicing of SMN2 and thus increasing the amount of functional SMN-protein is also taken by small molecules such as RG7916 (Risdiplam) and LMI070 (Branaplam) (181).

These compounds are taken orally, cross the blood-brain barrier, and have been shown to increase the amount of full length SMN-protein (189).

The label indication in the USA was expanded in 2022 to include patients < 2 months of age, based on interim efficacy and safety data from the RAINBOWFISH study showing that pre-symptomatic babies reached key motor milestones after 12 months of Risdiplam treatment (190).

Risdiplam modifies *SMN2* pre-mRNA splicing to promote the inclusion of exon 7 and increase levels of functional SMN protein (191).

In SMA mouse models, Risdiplam treatment led to a robust increase in functional SMN protein in the central nervous system and in peripheral tissues (192,193).

The efficacy of Risdiplam has been demonstrated in infants with type 1 SMA (194) and in individuals with type 2 and type 3 SMA (195).

SUNFISH (196) is an ongoing, multi-center, randomized, double-blind, placebo-controlled, two-part, Phase 2/3 study that assessed the efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of Risdiplam in a broad patient population of children, teenagers, and adults aged 2–25 years with type 2 or type 3 SMA; the study did not exclude patients with low baseline motor function or hallmarks of more advanced disease, such as severe scoliosis, contractures, impaired bulbar function, and a need for enteral feeding or non-invasive ventilation.

Part 1 was a dose-finding study in patients with type 2 or type 3 SMA (ambulant and non-ambulant) to inform the dose for Part 2. In Part 1, Risdiplam treatment led to a sustained increase in SMN protein in the blood, and exploratory efficacy analyses showed improvement or stabilization in motor function (195).

Confirmatory Part 2 investigated the efficacy of Risdiplam in individuals with type 2 or non-ambulant type 3 SMA at the dose selected in Part 1.

SUNFISH Part 2 met the primary endpoint, demonstrating a statistically significant difference between patients treated with Risdiplam and those treated with placebo in the change from baseline in the 32-item Motor Function Measure (MFM32) total score at month 12(197).

Of instance in the last 4 years 3 new therapies with different mechanisms of action have been developed for these disorders (121,140,198).

The majority of data about these conditions derive from experience in child, with specific clinical scores that evaluate the motor impairment and in particular proximal motor weakness typical of this stage of disease, while progression and response to treatment is still unclear for adult patients(199).

Central to successful development of Nusinersen, Onasemnogene abeparvovec, and Risdiplam have been well-designed and executed clinical trials (200).

Because of the variable clinical severity of SMA, trials were designed to test therapeutic efficacy in separate but parallel studies of infants and older children using age-appropriate outcome measures, including well-validated motor functional scales (117,142,201–204).

In addition, because preclinical data in SMA mouse models had repeatedly demonstrated that earlier intervention was significantly more efficacious, each drug development program included a study to test drug efficacy in at least a small number of very young, presymptomatic patients.

Because older patients progress very slowly, they were not included in these controlled trials, although observational studies of Nusinersen efficacy in adults are now being reported (205).

Interestingly, despite the distinctions in type, administration route, and biodistribution of the three therapeutics, the trial results across the drugs are more similar than discrepant. In each case, presymptomatic or very early initiation of the drug resulted in marked reductions in mortality as well as near normal achievement of early motor milestones, including sitting and walking in some cases (106,182,183,206).

In contrast, postsymptomatic initiation of treatment in infants or children resulted in more modest improvements in motor function (122,207,208).

Long-term outcome data remain fairly limited, but at least for Nusinersen-treated patients, slow improvement in motor function may continue for many years(209) .

While the success of these clinical trials remains a breakthrough, perhaps their most significant lesson is that SMA is far from being deemed cured.

Further, the observed variability in clinical outcomes is poorly understood.

A lot of variable have been delineated and much more are still unknown that may be limiting efficacy and that can contribute to develop other neurogenetic disease drug development programs (200).

Although there are a lot of clinical and instrumental data, particularly neurological and pneumological, on children affected by SMA 1, many aspects concerning adult patients and particularly with SMA 2 and 3 are not yet completely clarified.

In this subgroup of patients, in addition to the direct aspects of the pathology, there are also complications from inactivity, and the well-known multisystemic aspects in young patients are not completely clarified in more adult patients.

Furthermore, typical pathology markers such as clinical scales, neurophysiological tests of cMAP and the quantitative analysis of motor units are not always applicable in adult patients and in many cases are influenced by multiple aspects of the pathology.

The response to pharmacological therapies is not totally documented nor is it clear if the scales routinely used are powerful enough to identify potential improvement.

To data even if two drugs are available for these patients no predictive indicators are available identifying these ones could help to make therapeutic algorithms.

With these aims we propose a prospective study in with patients are clinically evaluated every six months and related to a minimal invasive neurophysiological assessment with the attempt to find neurophysiological markers predictive of improvement and to explore other than motor aspects.

Patients and methods

We report of an Italian cohort of late adolescent/adult SMA 2 and 3 patients admitted to our department between Gen 2022 and Oct 2023.

Data refers to 20 patients (12 F) with SMA 2 and 3 in late adolescence/adulthood .

Main age at fist evaluation was of 32.6y with a range of 12-65.

Diagnosis was supported by SMN1 analysis with the homozygotes deletion in all patients.

SMN2 was evaluated in late onset patients.

A multidisciplinary clinical evaluation was performed at first evaluation and then every 6 months.

Patients receive clinical neurological evaluation with RULM and Hammersmith clinical scales and Pneumological evaluation with spirometry, emogas analysis and poligraphy every 6 months.

10 target muscles of limbs were evaluated with Medical Research council scale (MRC) (210) at both sides a total score was calculated for the most common muscles involved in SMA, this is a part of the routinely used practice in neuromuscular disorders and we evalutate this score in SMA in the same way of others adulthood neuromuscular conditions(211).

Patients underwent at first evaluation with neurophysiological assessment based on motor and sensitive nerve conduction studies (sural, peroneal, median and ulnar nerves). Blood test samples were performed at every assessment including blood cells count, lipid assessment renal and hepatic functionality indexes, thyroid functionality index.

In case of significative variation of RULM, HFMSE or CV patient underwent to a complete neurological, pneumological and Neurophysiological assessment.

3 patients received previously Evrysdi (5,1mg) in other centers and have been evaluated after two year of therapy as baseline, 2 patients received in the past Nusinersen (for 2 years and for 5 years) that was disrupted because of anatomical difficulties in rachicentesis (scoliosis), a patient was previously involved in a clinical trial with olesoxime but he voluntary dropped out before the end of study ten years before our evaluations . 14 patients haven't received previously any specific treatment.

Al patients received therapy with Evrysdi (5,1 mg/day) in our center.

Patients could communicated to center to report any reaction or possible side effect.

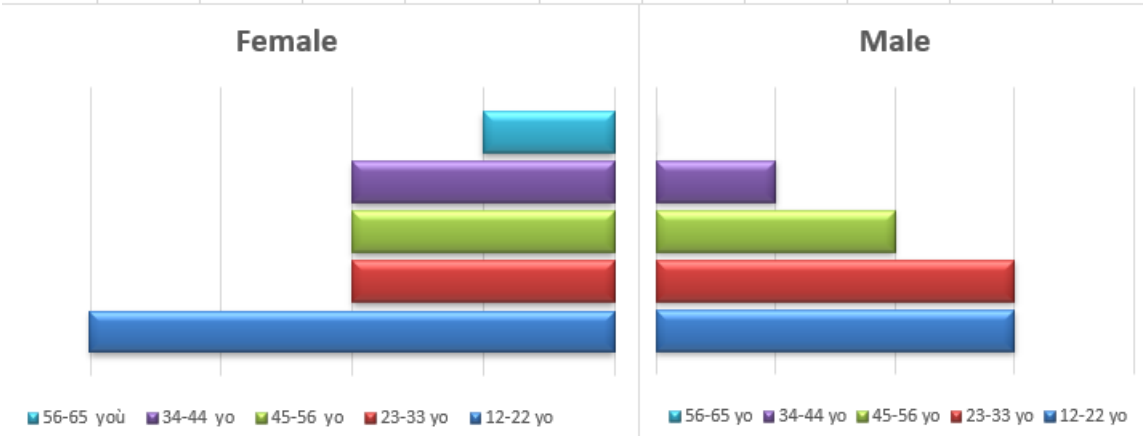
The Shapiro–Wilk test was performed to assess normality for all quantitative variables. Correlation analyses between continuous variables were carried out by using Spearman correlation coefficients (ρ).

Analyses were performed using SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for MacOS, Version 26.0. Armonk, NY: IBM Corp), and the level of significance was set at $p < 0.05$

Results

6 patients showed a SMA3 and 14 patients a SMA2, onset in all patients was before 3 years of age except patient 13 that . had an onset at 12 y.o.

Graph 1 summarize demographic data.



No significative blood abnormality where observed in our patients.

2 patients (n 8 and n 13) were still able to walk at first evaluation of whom a patients whit a tardive onset (12yo) had 3 copies of SMN2 and a patient with onset at 8 yo was still walking at 44 yo and had 4 copies of SMN2.

4 patients with SMA3 lost walking ability at 8-9 yo.

All patients with SMA2 where wheelchair bound.

Neurological examinations shows no sensorial involvement in all patients.

Patient n 15 and n17 underwent to spinal surgical procedure during the period of the study.

Table 1 shows clinical features and involvement in target muscles in all patients:

PT=Previous Treatment Neck F/E = neck flexors /extensor; R/L = MRC right/left; Delt= deltoids, Bic =

ID	SMA Type	P T	Neck F/E	Delt R/L	Bic R/L	Tric R/L	Fing flex R/L	Fing ext R/L	Adduct R/L	Ileop R/L	Quadri R/L	Tib Ant R/L	S T R/L	TS	**Δ TS**
1	2	//	1/1	0/0	0/0	0/0	1/1	1/0	0/0	0/0	0/0	0/0	1/1	6	0
2	2	//	2/2	0/0	0/0/	0/0	0/1	0/1	0/0	0/0	0/0	0/0	0/0	6	0
3	2	//	3/3	0/0	0/0	1/1	1/1	1/1	1/1	0/0	0/0	0/0	0/0	14	0
4	2	//	3/3	2/2	3/3	1/1	2/2	4/4	2/2	0/0	1/1	1/1	2/2	43	0
5	3	//	4-/4-	0/0	2/2	2/2	4-/4-	3/3	0/0	0/0	0/0	0/0	1/1	32	0
6	3	//	4-/3	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/1	0/0	9	0
7	2	//	2/2	0/0	4-/3	3/3	4/4	4/4	0/0	0/0	1/1	2/2	2/2	43	0
8	3	//	5/5	5/5	5/5	5/5	5/5	5/5	5/5	3/3	4/4-	5/5	5/5	104	0
9	2	Nusinersen	2/3	1/3	3/4-	2/3	4/4-	4-/4-	2/2	2/2	1/1	4/4+	5/4+	65	0
10	2	Risdiplam	3/3	0/0	4-/1	3/1	4/4	3/3	1/1	0/0	0/0	0/1	0/1	33	0
11	2	//	3/3	0/0	3/4-	3/4-	1/2	1/1	0/0	0/0	0/0	2/2	2/2	33	0
12	2	//	3/2	0/0	0/0	0/0	2/2	2/2	0/0	0/0	0/0	1/2	1/2	19	0
13	3	//	5/5	5/5	5/5	5/5	5/5	5/5	5/5	3/3	3/3	5/5	5/5	102	0
14	2	Risdiplam	3/3	0/0	4/4	3/3	3/3	4/4	3/3	0/0	0/0	3/0	4-/4	51	//
15	2	Nusinersen	4/3	2/2	4-3	3/3	4/4	4/4	1/1	3/3	3/3	4/4	4/4	70	0
16	2	//	2/2	0/0	3/3	3/3	3/3	3/3	2/1	0/0	2/1	0/0	0/0	34	0
17	2	//	2/2	1/1	3/3	4-/4	4/4	4/4	1/1	1/1	1/1	3/3	3/3	64	
18	2	Risdiplam	2/2	0/0	3/1	2/2	3/1	3/1	1/1	1/1	1/1	1/1	2/2	30	0
19	3	//	4/2	1/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	2/2	1/1	14	0
20	3	//	4-/3	0/0	4+/4-	4-/4-	¾-	4-/4	0/0	0/0	0/0	4/4-	4/4-	54	//

biceps; Tric =triceps; Fing flex = fingers flexor; ,Fing ext = fingers extensors; Adduct= adductor; Iliop= iliopsoas, Quadri = quadriceps; Tib Ant= tibialis anterior; ST= sural triceps; TS= MRC total score; ΔTS= ΔMRC total score after 6 months.

Osteotendineal reflexes where absent at four limbs in all patients except for the two patients still walking in with where absent only at lower limbs.

Tab2 summarize data of RULM and Hammersmith scores and their Δ after 6 months of therapy.

Patient	RULM TOT	HFMSE TOT	Δ RULM	Δ HFMSE
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	7	2	8	0
5	13	5	+2	0
6	0	1	0	0
7	13	1	0	0
8	37	52	0	0
9	22	5	0	+2
10	5	0	1	0
11	0	0	0	0
12	0	0	0	0
13	38	50	0	0
14	17	3	//	//
15	23	11	-1	5
16	8	0	-1	0
17	24	3	0	0
18	5	2	0	0

19	0	0	0	0
20	16	2	//	//

RULM= Revised Upper Limb Module; HFMSE= Hammersmith Functional Motor Scale.

At neurological and neurophysiological evaluation we commonly observed in all our patients as typical feature of the disease reduced or absents compound motor potentials (cMAPs) when recorded cMAPs shows normal velocity and latency except for patient 9 in whom a moderate carpal tunnel syndrome was found.

Patient n 10 and n 17 refused to undergo to neurophysiological assessment.

cMAP Amplitudes are summarized in tab 3; for each nerve is reported the best side.

Patient *ID*	Median cMAP mV	Ulnar cMAP mV	Proneal (SPE) cMAP_mV
1	0,3	0	0
2	1,5	0	0
3	0,4	0,3	0
4	2.9	0,6	1
5	7	1.3	0
6	4,3	1,1	0
7	3,3	1,3	0
8	5,5	9,1	5
9	3,8	3,9	0
10	//	//	//
11	1,7	0,5	0,5
12	0,4	0	0
13	2,7	6,7	7,9

14	2,1	0,9	0,4
15	3,6	0,7	0,7
16	0.8	0,5	0,3
17	//	//	//
18	2,2	1	0
19	1,4	0,8	0,3
20	3.7	1.8	0

. cMAP=compound Motor Pontential Amplitude, mV= milliVolts, SPE=external sciatic popliteal nerve

When recorded SAPs shows normal velocity and latency, patient 9 in with a moderate carpal tunnel syndrome was found.

Even if Sural Nerve Potential sometimes could be technically difficult to record in this population there are few difficulties due to muscle atrophy of leg and a supportive and reduced impendence.

SAP Amplitudes are summarized in tab 4 for each nerve is reported the best side.

Patient	**_Med S amp µV**	**UlnS_ amp µV**	**_Sur amp_µV**
1	46	31	0
2	39	31	6,1
3	25	38	7,3
4	41	35	48
5	92	50	0
6	42	29	0
7	28	67	23
8	5	45	13

9	46	60	24
10	//	//	//
11	98	51	11
12	15	11	0
13	31	34	11
14	85	73	0
15	47	40	32
16	30	84	0
17	//	//	//
18	74	41	3,7
19	60	0,9	0
20	77	37	0

µV= micro Volts; Med S amp = sensitive median action potential amplitude; UlnS_ amp= Sensitve Ulnar Action Potential amplitude; Sur amp= sural nerve action potential amplitude.

Respiratory data are summarized in table 5, data of patient n 3 are not available because of permanently ventilated.

	AHI	SpO 2m	T90 %	O DI	NAD IR	FVC_ ab	FVC %	SN IP	SNIP %	MI P	MIP %	ME P	MEP %

1	2	97	0	0	94	0,94	25	36	32,8	12	11,5	10	7,1
2	22,2	97	1,9	8,9	81	1,03	27	67	63,3	36	67,3	20	15
3	//	//	//	//	//	//	//	//	//	//	//	//	//
4	22	94	12	20	54	2,28	56	60	54	57	34,2	33	22,8
5	10	95	2,7	4,2	66	1,6	53	51	61,4	56	41,3	32	37,8
6	6,4	97	0,1	2,3	88	1,98	52	52	48,7	55	54,7	31	23,1
7	7,8	97	0	2,3	91	0,97	30	61	52,1	38	57,6	25	16,2
8	18,2	94	11,5	21	71	3,13	98	75	88,2	75	80,7	80	86,6
9	6	97	0,3	4,3	86	2,09	64	68	75,1	31	32,3	31	27,2
10	6,7	96	0	0	91	0,43	10	32	28,3	32	29,9	12	8,1
11	7,2	95	10,4	11	50	0,5	12	40	35	9	8,3	7	4,6
12	7,2	94	0	1,4	90	0,53	20	25	28,3	10	11,1	8	7,6
13	5,2	98	0	0,4	90	4,39	104	84	70	74	74,3	68	42,2
14	6	97	4,7	7,5	82	0,59	17	46	50,4	21	19,4	19	16,3
15	6,5	98	0	1,4	90	0,49	18	36	39,3	21	47,1	31	26
16	7	96	2,2	5,1	67	0,83	29	45	51,2	21	50,9	19	18,3

17	5,2	98	0,3	5,1	80	0,75	22	41	44,5	34	41	18	15
18	12,7	98	0,1	2,5	85	0,8	27	41	48,2	10	50,5	14	15
19	8,8	96	0	2,6	89	1,03	37	74	92,1	20	29,7	18	24,5
20	8	94	1,3	5,1	85	2,11	67	65	77,3	46	81,8	30	33

AHI= apnea ipopnea Index, SpO2m = Main periferic nocturna saturomety ; FVC ab forced vital capacity absolute; MEP= ; MEP maximal static mouth expiratory pressure; MIP = maximal static mouth inspiratory pressure; ODI=oxygen desaturation index (number of oxygen desaturations of 4% or more per hour of total recording time);T90%= % time spent with SaO2 below 90%; NADIR=lowest oxygen saturation ; SNIP= Sniff nasal inspiratory pressure.

Tolerability of drug is good in our population.

Two patients showed side effect after therapy onset.

A patient reported nausea. Before the onset of treatment patient experienced frequent nausea but he reported an increase frequency and intensity after drugs onset and decided to disrupt Risdiplam

Another patient reported headache (experienced before) of mild intensity that resolved after a week.

The same patient reported mouth ulcers, here, we recommended to take a cup of water just after drug intake, (according to literature) and lesions disappeared in 5 days (212).

The MRC SUM Score shows a significative correlation with the RULM (ρ=0.932) and with the HFMSE (ρ=0.787).

A correlation was found between the duration of the disease and the clinical scales (TS ρ= -0.526, RULM ρ= -0.450, HFSME ρ= -0.274), this data is also found for pneumological aspects with a correlation with AHI (ρ=0.620) and from a neurophysiological point of view with the sensory amplitude of the sural nerve (ρ= -0.497) but not with the remaining sensory and motor potentials.

For all three of these clinical scores, correlation is observed with the cMAP of all nerves examined TS (Median amplitude ρ= 0.567, Ulnar amplitude ρ=0.693, SPE amplitude ρ=0.611) RULM (Median amplitude ρ= 0.628, Ulnar amplitude ρ= 0.753, SPE Amplitude ρ=0.481), HFSME (median amplitude ρ= 0.755, ulnar amplitude ρ=0.800, SPE Amplitude ρ=0.428).

The sural nerve SAP shows a correlation with the clinical scales (TS ρ= 0.631) but not with other scales , RULM ρ= 0, 473 HFMSE ρ= 0.458 a less strong correlation is also found between the amplitude of the SAP of the ulnar nerve and the TS scale ρ=0.501 and RULM ρ=0.492 but not with HFSME ρ=0.299).

A restrictive respiratory pulmonary syndrome was very common in our cohort of patients excluding data for ambulant patients was found in all cases..

A statistically significant correlation is also found between MIP and scales (TS ρ=0.410 RULM ρ=0.512, HFSME ρ= 0.555) and with the CMAPs of the ulnar and median nerves (ρ=0.692 Median and ρ=0.648 Ulnar).

Patients n 4, 5, 9, 15 and 16 according to methods because of a variation of RULM and HFMSE underwent to a second complete assessment that not differs from baseline.

Discussion

In our country, the introduction of therapies also for adult patients was very close to the beginning of the SARS CoV2 pandemic.

For neuromuscular patients this period was particularly hard and involved restrictions in order to contain the risk of infection.

On the other hand, for years this particular type of patients has not had an effective treatment and patients who have passed adolescence/adulthood are in a very slow worsening condition.

Before 2022, in our region there was only one centre able to taking care of SMA patients, almost 300 km away from the city of Palermo.

Therefore, for these aspects, patients often for social, and for the disability itself chose not to carry out follow-up checks or underwent follow-up checks very rarely.

Although Nusinersen had also been authorized since some years in adult patients due to the intrathecal administration and since patients had to travel considerable distances in our region and often also stay one day every 4 months in Hospital, it was rejected as a therapeutic option in some of our patients who arrived at 2022 (the time in which Risdiplam was approved) without having undergone a specific treatment.

At a first analysis it would seem that 2 ambulatory patients (patient n°8 and n°13) have better clinical and neurophysiological data; this could follow for one case to a recent onset of the disease while in the other one it could be likely due to the genetic background (4 copies of SMN2) which is fundamentally in agreement with the literature.

The analysis of the copy number of SMN2 in our entire population could have provide further insights, however, at the moment, it is not easy to perform in our centre.

Patients who received previous treatment would appear to have a better clinical and neurophysiological profile, but the same patients are also the youngest in the population and this could therefore represent a bias.

Still in the same group of patients, a confounding factor is given by the surgical treatment of scoliosis in two patients, which involves a notable postural improvement with greater ability to perform daily activities and better clinical scores.

We evaluated the MRC SUM Score in this cohort of patients similarly to what was carried out in other adult neuromuscular pathologies we attempted to apply this system to adult SMA patients (211).

Upon analysis of the data we find a strong correlation with the RULM (ρ=0.932) and with the HFMSE (ρ=0.787) this score allows a classification of patients using tests routinely used in the clinical evaluation of patients and which do not require any use of accessory equipment.

Even in our cohort, as reported in the introductory part, SMA presents very complex aspects.

Patients with a long history of disease, who are also the majority, provide a unique opportunity for analysis of clinical, respiratory and neurophysiological data.

The literature on this group of patients is severely lacking and relies only on pathology studies.

As already widely discussed, SMA is a pathology not only of motor neurons but theoretically capable of involving every organ and system.

Therefore the administration of a systemic drug can have the advantage of being able to reach every cell of the organism.

These data therefore requires more in-depth considerations.

In our cases, the pathology shows, as expected for a degenerative pathology and as expected from literature data, a greater impairment in older patients.

This is also supported by the statistically significant correlation between the years of pathology and the clinical scales (TS ρ= -0.526, RULM ρ= -0.450, HFSMEρ= -0.274). Similar data are also found from a pneumological point of view with a correlation particularly with the nocturnal ventilation parameters with AHI (ρ=0.620) and from a neurophysiological point of view with the sensory amplitude of the sural nerve (ρ= -0.529 but not with the remaining sensory and motor potentials.

The cMAP allows us to have an estimate of the number of residual motor neurons; it actually represents a very valid approximation of reality as it is not a direct measurement but represents the measurement of a response of an effector (the muscle).

It is a very valid measure in pathologies in which the muscle is not involved in dystrophic-like changes like other motor neuron disorders but as also happens in early forms of SMA; however, in patients with a longer history it could have very insidious aspects.

Furthermore, as can be seen in our case studies, cMAP values very close to 0 millivolts are common.

This data technically implies that the smallest technical error (however present) has a significant percentage impact on the measurements and in any case makes techniques in which many stimuli such as repetitive stimulation and MUNE are to be analyzed, very difficult to perform.

On the other hand, an improvement is possible in patients with cMAP values close to 0 since this data is only partially determined by the loss of motor units but is also due to muscle degeneration which, although irreversible, can undergo partial regeneration.

For all the used clinical scores a strong correlation is observed with the cMAP of all the nerves examined TS (Median Amplitude ρ= 0, Ulnar amplitude ρ=0.693, SPE amplitude ρ=0.611), RULM (Median amplitude ρ= 0.612, Ulnar Amplitude ρ=0.753, SPE Amplidue ρ=0.481), HFSME (Median amplitude ρ= 0.781, Ulnar Amplitude ρ=0.800, SPE Amplidue ρ=0.428).

Furthermore, the ability to carry out a specific activity such as that required on a clinical scale (RULM or the HFMSE) and within certain limits also the MRC requires the functionality not only of the motor unit and therefore of the motor neuron and muscle fibres but also that the entire skeletal muscle system is functional, i.e. without limitations due to the joints, myotendinous retractions or contractures.

These aspects are, as already discussed, characteristic of SMA and are certainly also correlated with the duration of the disease since it is clear that the more inactive motor system is, the more that undergoes to degenerations.

Therefore, although they are aspects that strongly determine disability, they are not due to the primary pathology but represent a direct consequence of it.

Furthermore, in patients with such a long duration of illness the well-known principles widespread for children cannot be applied and therefore one runs the risk of relying on the scales of underestimating or being surprised by an unexpected improvement..

On similar considerations we can understand the results obtained from the correlations with the respiratory data from which it emerges that the FVC significantly correlates only

with cMAP of Ulnar and Median Nerve (ρ=0,585 ulnar and ρ=0,587) but not with clinical scales while MIP and MPE are significantly corelated with both cMAP and clinical scales (MIP-TS ρ=0.410 MIP-RULM ρ=0.512, MIP- HFSME ρ= 0.555; MEP-TS ρ=0.543,MEP-RULM ρ=0.621,MEP- HFSME ρ= 0.755) and CMAPs of the ulnar and median nerves (MIP -Median cMAP ρ=0.698 MIP-Ulnar cMAP ρ=0.648, MEP-Median cMAP ρ= 0.7826 and MEP-Ulnar cMAP ρ= 0,690).

To further support these considerations, the literature reports that FVC is more influenced by skeletal muscle contractures, thoracic deformities and the theoretical loss of parenchymal elasticity of the lung (213,214).

Finally, although the SNIP also allows us to effectively analyse patients with difficulties, common in the population of patients with a long history of SMA, due to the limitations of the temporobandibular joint, it presents different correlations (Ulnar cMAP ρ= 0,665 but not clinical scales) probably due to the fact that, as reported in the literature it is fundamentally determined by the activity of the diaphragm muscle (preserved in these patients) against the MIP and MEP data fundamentally due to the others respiratory muscles (215).

In Our cohort 4 patients have a clear improvement on RULM and HFMSE, but not in MRC or at clinical evaluation and nor at neurophysiological and pneumological evaluation.

This datum may be related to an improvement not related to an increased muscle strength.

Other factors have been related to neuromuscular involvement in SMA such as muscular fatigue and however RULM and HFMSE are measure of a complex activity.

We evaluated also non motor aspects in our patients with clinical and neurophysiological instruments with the aim to provide new markers of drug response and multisystemic involvement, and because of more evident efficacy is reported for the early diagnose to identify new screening strategy.

An unusual and unexpected correlation emerges from the statistical analysis of the SAP of the sural nerve and from its correlation with the clinical score (TS ρ=0,650 RULM ρ= 0.503 HFMSE ρ= 0.489).

Results also showed a correlation, even if less strong, between the SAP amplitude of the ulnar nerve and the TS ρ=0.501 and RULM ρ=0.492 scales but not with HFSME ρ=0.299).

Data available from literature about involvement of sensitive system, have been reported at onset only in SMA 1 and generally do not represent a features of SMA2 and 3, even if reports about this concern only patients at onset of diseases and therefore in young age (216,217).

This datum might be related to a primary involvement of sensitive nerves and has an unclear pathogenesis in SMA 1.

The fact that has not been reported in the past for SMA2 and SMA 3 requires several considerations:

first, the nerve involvement can occur late in patients with type 2 and 3 spinal muscular atrophy,(due to the protective presence of copies of the SMN2 gene), and to date there are no reports relating to populations with age range similar to the our one.

Second, it is often believed that the pathology is predominant in the motor neurons and therefore secondarily in the motor nerves, up to now, the data has only had a diagnostic role and therefore late neurophysiological data are not known.

Finally, a part of patients with SMA2 and SMA 3 did not reach adulthood and therefore some complications, visible in animal models with shorter life cycles than humans, may still not be detectable.

Thus, we could observe new data given the increase of life expectancy induced by new drugs.

The sensory data of our patients correlate with a much more serious neurological and pneumological clinical state.

The absence of SAPS in relation to what is clearly recognized from a neurophysiological point of view is indicative of postganglionic damage.

This statement, however, clashes with the clinic in which a clear sensory deficit is not detectable in our patients.

On the other side the absence of significative blood abnormalities excludes the most common causes of acquire neuropathy and is supportive of a mainly lower limbs neuropathy related to SMN1 gene.

It is possible that these markers could play a prognostic role, however more extensive and thorough checks are needed to understand their true nature.

A prognostic marker is necessary for the pathology since gene therapy has already been approved in children and increasingly complex and expensive treatment options are being studied even for adolescents.

So the ability to identify the patients most at risk of serious disabilities would allow us to develop cost effective containment programs.

However data for neurophysiological changes in adult patients and in particular in treated with risdiplam are not yet available.

Further study might provide us new tools to evaluate efficacy of therapy in motor and non motor involvement of the disorder.

However data of this population could help clarify some aspects of SMA

in particular, the finding of a sensitive neuropathy adds this characteristic to the complexity of the pathology and can be of help to the clinician who must interpret this data in a diagnostic setting.

The analysis of these data in a population of such an advanced age allows considerations on the validity of measures traditionally used in children even in adulthood and allows to see the evolution of the pathology over time.

Finally these observations enable the foundations of an evaluation of the drug's

effectiveness also in this particular context which would ultimately allow an increasingly individualized choice of therapy.

Conclusions:

SMA represents a complex pathology in which many social and disability-related issues must be taken into consideration.

The same patient could have many organs and systems involved and the motor involvement is only one aspect of the pathology.

Furthermore, many times, alongside motor neuron loss and concomitant primitive muscle degeneration, there are concomitant musculoskeletal alterations which contribute to determining disability.

These aspects are particularly evident in our population characterized by a rather high main age.

Furthermore, the MRC sum score based on the MRC of individual muscle groups targeted for the pathology, in this particular group of patients, allows a very realistic estimate of motor involvement and shows results comparable to the use of classic scores for the pathology.

Multiple organs and systems are determined to affect overall disability.

MIP, MEP and SNIP allow a very accurate measurement of respiratory involvement.

A particularly sensitive and axonal neuropathy is a common feature in our group of patients affected by spinal muscular atrophy types II and III and represents the involvement of a clearly non-motor system that can be studied in a simple way in patients affected by SMA.

Classically feature reported in child differ from evidence in adult patients.

cMAP of median, ulnar and SPE nerves is a powerful tool and is related to clinical status

Risdiplam is in our cases a well tolerated drug

During observation period a disease stabilization is common in our adult patients.

In just 6 months 4 patients showed a clinical improvement at RULM and HFMSE.

However data indicate that a longer period of evaluation is e necessary and to essay the benefit and so further studies are necessary to discriminate the effects of drugs in multiple systems..

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