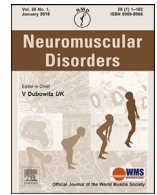




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## 264th ENMC International Workshop: Multi-system involvement in spinal muscular atrophy Hoofddorp, the Netherlands, November 19th – 21st 2021

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### 1. Introduction and overview

On November 19th –21st, 2021, a group of 23 scientists and clinicians, along with two patient advocates, one individual with SMA and three pharma representatives from 11 different countries, convened in a hybrid format in Hoofddorp, The Netherlands. The topic of this workshop was the multi-systemic involvement in Spinal Muscular Atrophy (SMA).

Following a welcome from *Alexandra Breukel*, ENMC representative and director, and the chairpersons of the workshop, *Giovanni Baranello*, *Tom Gillingwater*, *Kathryn Swoboda* and *Rashmi Kothary*, *Baranello* gave an overview of the topic. The hallmark of SMA is the selective degeneration of alpha motor neurons in the brainstem and spinal cord. However, an increasing body of evidence suggests that the SMN protein plays a much wider role across different cell types and physiological systems (e.g.,

cardiovascular, intestinal, vascular and skeletal systems), indicating that SMA may need to be considered as a multi-system disorder [1,2]. Several studies have shown that SMN is ubiquitously expressed in every human and mouse tissue examined, where it is required for viability of all cell types, not only motor neurons. Additionally, several preclinical and clinical studies have demonstrated that non-neuronal manifestations may contribute significantly to SMA pathology and symptoms. However, a clear consensus among clinicians and researchers on how to monitor potential non-neuronal manifestations in SMA patients is still lacking. With the evolving landscape of therapeutics in SMA and the ever-changing natural history of the disease, there is the need for broader systemic monitoring of SMA progression in order to provide a framework for comparative effectiveness studies between central nervous system (CNS) delivered treatments, such as nusinersen, and those delivered systemically (e.g., risdiplam or onasemnogene abeparvovec), but also to monitor and better understand treatment-specific side effects.

These various aspects were at the core of a multidisciplinary workshop sponsored by the ENMC in which clinical, preclinical, translational and therapeutic aspects of the multi-system pathology in SMA were discussed.

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## 2. SMN protein function: molecular perspectives and preclinical studies

### 2.1. Molecular function of the SMN protein - in depth understanding tissue/organ specificity

Brunhilde Wirth pointed out that the SMN protein is multi-functional and that some SMN functions can be attributed to specific domains within the SMN protein structure, with the C-terminus important for multiple protein-protein interactions. However, SMA patient loss-of-function mutations are distributed throughout the *SMN1* gene. Wirth introduced the concept of tissue- and organ-specific versus housekeeping functions of the SMN protein such as its involvement in RNA metabolism. So far, most specific functions of SMN have been identified in the central nervous system (CNS) and motor neurons. These include functions such as axonal mRNA transport and local translation; and neuronal actin dynamics, which in turn is involved in synaptic endocytosis, and regulated by calcium homeostasis [3–5]. Protective genetic modifiers critically interfere with these processes and have helped identify motor neuron-specific SMA disease mechanisms. The polarized nature of motor neurons with its necessity for axonal transport, local translation, synaptic terminal calcium-dependent endocytosis and high energy demand may therefore be an important contributor to the relative susceptibility of this cell type. However, in peripheral cells specific functions of the SMN protein are yet to be identified.

### 2.2. Temporal and tissue-specific variability of SMN protein levels in mouse models of SMA

Ewout Groen pointed out that although the SMN protein is ubiquitously expressed, its spatial and temporal expression vary dramatically between organs, tissues and developmental timepoints [6]. Importantly, there is no clear correlation between SMN mRNA and protein expression highlighting the importance and challenges of SMN protein expression measurements. This is critical for the identification of cells and tissues that are susceptible for a loss of SMN. A standard operating procedure should include a standardized sampling and a fixed time of day since SMN levels vary according to the circadian rhythm [7]. Antibodies used for the detection of the SMN protein should ideally be optimized to the organism in which SMN levels are ought to be investigated. In case of using western blots, a total protein stain rather than housekeeping proteins needs to be included for normalization and a sample-mix for blot-to-blot comparisons [8,9]. This is specifically important if SMN levels are to be compared between organs. Groen provided a specific example from preclinical studies showing high levels of SMN in the kidneys compared to other organs [6,10] and the observation of kidney dysfunction in SMA patients [11].

### 2.3. Defects in myogenesis and synaptic development

Eduardo Tizzano presented data on a muscle-intrinsic SMA pathomechanism which may contribute to the overall phenotype in SMA. Human foetuses predicted to develop type 1 SMA showed reduced diameter of myotubes compared to non-SMA foetuses [12]. Other findings of early muscle involvement in SMA include the differential expression of different markers such as decrease of slow myosin heavy chain (sMHC) expression and increase of fast myosin heavy chain (fMHC) expression. The expression of desmin and vimentin is in general downregulated after birth in muscle from controls whereas is maintained in SMA, and this can represent another indication of delayed maturation. Satellite cells, which are responsible for forming new myofibers during

embryonic development and for muscle regeneration after injury are increased in quadriceps and intercostal muscles (the most affected muscles in SMA) than in the diaphragm, particularly in the atrophic areas of these muscles [13]. Studies in SMA mouse models have also shown a lack of myofiber maturation and specific vulnerability or resistance of muscles to SMA disease [14]. The intrinsic mechanisms causing the involvement of some particular muscles in SMA warrant further investigation. Disaggregation of Acetylcholine Receptors (AChRs) and increased density of presynaptic vesicles have been also described in SMA developing synapses. Furthermore, the loss of innervation can be attributed to defects in synapse maintenance. Since loss of innervation is a post-natal phenomenon, these changes point towards a contribution of the muscle to neuromuscular junction pathology in SMA [15]. These developmental muscle phenotypes are possibly more relevant in SMA type 0 and type 1 since SMN restoration in chronic, adult forms can stabilize neuromuscular junction (NMJ) and muscle function pointing towards SMA type-specific peripheral phenotypes. These findings support the hypothesis of altered neuromuscular development and primary muscle involvement in human SMA.

### 2.4. Spleen, immune system and liver involvement

Rashmi Kothary emphasized the importance of peripheral pathologies from a perspective of SMA treatments with SMN-enhancing drugs. Many studies in SMA mouse models have shown that systemic treatments are far superior over treatment regimens which focus on the CNS largely omitting the periphery. Moreover, SMA mice which received peripheral SMN-enhancing treatment only display an impressive positive effect on the overall pathophysiology including an impressively increased survival. This may be attributable to the amelioration of peripheral pathology such as liver steatosis which disappears in peripheral only treated animals. While there is evidence from SMA mice that there is a strong contribution of a liver-intrinsic pathomechanism to the liver phenotype, other organs such as muscle and pancreas may have an important influence especially if involved in metabolic regulation. Although there is emerging evidence of subclinical liver pathology in SMA patients, further clinical research is needed to address why some patients are spared from liver pathology while others are not, and whether genetic modifiers influence this aspect.

### 2.5. Defects in pancreatic development and glucose metabolism in SMN-depleted mice

Melissa Bowerman presented data on an interplay between intrinsic genetic factors and external factors such as nutrition and ageing in the shaping of peripheral phenotypes in SMA, specifically in relation to glucose metabolism. SMA mice exhibit glucose intolerance and an altered pancreatic islet cell composition with an increased number of  $\alpha$ -cells and a decreased number of  $\beta$ -islet cells [16]. In milder SMA mice, high fat diet results in an increased glucose intolerance that is further exacerbated by ageing [17]. Indeed, older and milder SMN-depleted mouse models became obese and diabetic over time [17]. Consequently, current treatments for diabetes have the potential to ameliorate the SMA phenotype. Furthermore, these metabolic dysfunctions may have systemic implications as they could affect other peripheral organs and/or be reciprocally impacted by other metabolic and peripheral defects that occur in SMA [18].

### 2.6. Glycaemic metabolism abnormalities in SMA patients

Kathryn Swoboda reported on the clinical picture of SMA patients which is highly diverse regarding metabolic phenotypes,

and that metabolic abnormalities may be strongly confounded by the neuromuscular pathology. Observations include a poor tolerance towards fasting and catabolic states, frequent lactic acidosis, mitochondrial dysfunction, fatty acid oxidation abnormalities, systemic carnitine deficiency, fatty liver, and insulin resistance. The huge variability points out the importance of controlled clinical feeding studies that have revealed an abnormal urine organic acid profile in all enrolled SMA type 1 patients. In SMA type 2 and 3, 94% and 50% of the patients displayed abnormalities after overnight fast, respectively. *Swoboda* also highlighted that the mitochondrial pathology observed in SMA patients may be muscle intrinsic since a knockdown of SMN in cultured myotubes mimics this aspect.

### 2.7. Vascular abnormalities

*Simon Parson* introduced abnormalities in vascularization as a common mechanism of CNS and peripheral organ pathology in SMA. Not only did SMA mice display decreased vascularization in muscle, spinal cord and retina, but resulting hypoxia is also present in many organs [19,44]. Hypoxia may negatively influence SMN pre-mRNA splicing which may represent a negative feedback loop in peripheral SMA pathology. Retina vascularization defects reported in mouse models and patients, may be an accessible outcome measure for the assessment of vascularization deficits in SMA patients, and also a candidate biomarker for clinical studies as demonstrated by observations in a cohort of type 2 SMA patients. It is noteworthy that reduced vascularization in the retina was completely rescued by a systemic, SMN-enhancing antisense approach in SMA mice. Evidence supporting an endothelial-cell autonomous pathology was shown (Zhou et al., in review). The reduced vascularization observed in SMA patients together with circulating biomarkers of endothelial cell damage, suggest blood vessel breakdown. This may also be relevant in peripheral nerves where the vasa nervorum supply axons. Longitudinal data from treated patients are required in this area.

## 3. Translational perspectives

### 3.1. From animal studies to real world data: a translational approach

*Peter Claus* discussed the intrinsic targets and alterations in SMA. *Claus* introduced the key concept that changes in molecular networks caused by the lack of SMN might become irreversible, particularly when disease advances. An important aspect is that such network alterations occur as a secondary effect, hence the restoration of the SMN protein pursued by current treatment strategies could have little beneficial effect once the damage in the molecular networks is established. This has been demonstrated in pre-clinical models in different peripheral organs and spinal cord. It is therefore important to characterize SMN-independent treatment targets, particularly when these are shared by different organs [20].

There are different strategies that could be used to elucidate peripheral defects in SMA in pre-clinical models. To differentiate between intrinsic and secondary defects, a possible strategy is to focus on pre-symptomatic P1 mice (Taiwanese) and compare data with symptomatic P5 mice. This could help to elucidate intrinsic defects before secondary alterations occur.

To determine systematic defects affecting body homeostasis, several types of analyses could be performed. Both an unbiased screening by transcriptomics and proteomics and a biased screening to look for known developmental genes could be alternative or complementary strategies. Importantly, not all changes are necessarily reflected at an expressional level.

Hence, it is important to analyse signalling by phospho-proteomics or antibody-based assays. Efforts should be directed to elucidate whether organ-specific or common pathomechanisms in different organs exist, and this could be pursued by multi-omics bioinformatics.

Another important question is how SMN is mechanistically involved in different networks, an aspect that is still underrepresented in SMA research and that could be explored by analysing the interactome with multi-omics.

Lastly, to determine irreversible targets, drugs could be used to enhance SMN expression administered at different time points, particularly when disease has already manifested. The use of drugs, as pointed out during the discussion, is a crucial tool to decipher whether such defects are a consequence of “intrinsic” phenotypes manifesting later (e.g., due to a different response to varying SMN thresholds) or represent secondary effects.

*Claus's* group is focusing on bone homeostasis, lung and kidney (in collaboration with *Parson's* group; [10]), for which proteomics and/or transcriptomics data have been obtained. An altered bone development has been demonstrated [21] and clusters of dysregulations have been identified (e.g., Collagen IV in the kidney).

STRING network analysis of a phospho-proteome has shown alterations in proteins involved in neuronal structure and processes, in microtubules, and in mRNA splicing in the spinal cord.

Using a BioID2 approach, researchers could characterize proteins interacting with SMN including transient interactions. Using this approach, the group has identified about 300 interactors (in collaboration with *Heidi Fuller's* group, Wolfson Centre for Inherited Neuromuscular Disease, Oswestry, UK) which can now be compared to proteomic changes to assess the mechanistic interaction of SMN.

### 3.2. Preclinical animal models to study multi-system involvement in SMA

*Tom Gillingwater* reviewed animal models of SMA. A vast array of models has been developed for SMA, from *C. elegans* through to pig, playing a critical role in developing and testing treatments for SMA.

More than one model has been used to understand and characterize in-depth what happens in SMA neuronal cells, leading to the identification of dying-back axonopathy, NMJ defects, and the temporal profile of functional changes occurring in motor neurons. Animal models have also played a key role in allowing us to place SMA in the context of other neuromuscular disorders (e.g., comparing NMJ defects in mouse models of SMA and ALS). A crucial question is how similar or dissimilar are neuromuscular structures between humans and mice. For instance, the morphology, structure, and functional correlates of human NMJs are very different compared to mice. Everything we observe in mouse models does not, therefore, necessarily replicate directly in humans. Notably, NMJs of both sheep and pig are considerably more like those in the human. Importantly, we may now need to extend this question of species-specific features to the other systemic aspects of SMA. It was also highlighted that there are species-specific differences in pharmacokinetic responses to many treatments.

*Gillingwater's* group addressed the key question whether organ-specific responses to the lack of SMN occurred before symptom onset or even before birth. In SMA mouse embryos (E14.5), significant molecular changes were found in different organs, such as the liver [22]. Furthermore, different organs had very

different proteomes, suggesting that such changes may be organ-specific.

### 3.3. SMA cerebral organoids as models to investigate functional alterations in the brain of SMA patients

*Stefania Corti* gave an overview of the possible role of organoids to investigate CNS involvement in SMA. Organoids are in vitro 3D cellular clusters derived either by primary tissue, embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) and that are capable of self-renewal and self-organization. While organoids replicating several organ systems have been generated, *Lancaster* and colleagues showed the possibility to study neuronal development with cerebral organoids, one of the different variants generated to study specific structures of the CNS [23].

When it comes to CNS organoids generated from iPSC derived from human SMA type 1 cells, they present both functional, micro- and macrostructural changes compared to control. Specifically, they have altered calcium response and they are less active when measuring electrophysiological activity. Notably, they have a higher response after glutamate induction.

*Corti's* group started a new protocol to generate an organoid to replicate the spinal cord of SMA patients. In such a model they demonstrated that SMA spinal cord-like organoids exhibit a higher rate of cell death and that motor neurons display lower axonal length compared to control.

These findings are interesting, as they also demonstrate that abnormalities occur very early during development. However, several limitations should be considered, for instance the lack of sensory input, the absence of an anatomical structure, and the lack of defined circuits. One way to overcome such limitations is to connect different organoids representing brain, spinal cord and muscle (e.g., human cortico-motor assembloids as described by *Pasca's* group; [24]).

In summary, organoids that replicate CNS and spinal cord of SMA patients partially recapitulate neural tissue cellular complexity and displayed differentiation defects associated with basal activity and hyperexcitability. Cerebral organoids displayed functional deficits similar to those of the spinal cord organoids, revealing the widespread impact of the disease.

### 3.4. Brain and cognition

*Giovanni Baranello* discussed brain and cognitive involvement in SMA. *Baranello* mentioned that most of the studies investigating intellectual abilities in SMA have been performed in less severely affected patients and have shown that patients with SMA types 2 and 3 obtained normal to higher than normal IQ and speech/language abilities scores compared to their peers. More recent studies focusing on SMA 1 patients using adapted assessments, have shown poorer performances than controls, particularly in the attention and executive function domains. Speech and language development is also affected, with published data showing that functional and intelligible speech is rarely achieved in untreated SMA 1 children [25]. This is also reflected by a number of neuropathological and neuroimaging findings. Although the number of studies is limited, progressive neuronal degeneration has been demonstrated in particular in SMA type 0 patients, and grey matter volume loss was described also in the cerebellum (in adult patients with no clinical correlation), hippocampus, basal ganglia, and thalami.

Some SMA type 1 patients display reduced performance in executive functions. High expression of SMN is observed in the brain, particularly during the prenatal life, hence suggesting once again the importance of SMN for neuronal development [26].

*Baranello* also reported of increasing concerns among clinicians regarding cognitive and neurobehavioral abnormalities in treated SMA 1. The overall development of different domains including social communication, executive functions, language and memory in growing treated SMA 1 children will require detailed investigation.

It was therefore discussed that not only timing at starting treatment can be crucial to tackle possible brain-related comorbidities, but also the biodistribution of the different SMN-enhancing medications within the CNS.

Lastly, another crucial aspect that was brought to the discussion is the need to fully characterize the overall burden of neurodevelopmental disorders, such as autistic spectrum disorder (ASD). This is usually poorly investigated, and children seldom undergo specific assessments for ASD. Several factors should be taken into consideration, including the environment and the possibility of the co-occurrence of other comorbidities (e.g., fragile X syndrome, etc.). Interestingly, the presence of ASD-like behavior in children affected by SMA type 2 suggests that the pathophysiology can be more complex, and not necessarily related to a certain SMN threshold.

Overall, there is an unmet need to systematically investigate the presence of neurodevelopmental and speech/social communication delay (and its correlation with bulbar dysfunction) using appropriate tools. This would enable to determine which aspects of the neurocognitive domain are impaired and which are the underlying neurobiological basis.

### 3.5. Endocrine abnormalities

*Enrico Bertini* reviewed endocrine abnormalities and nutritional aspects. Individuals affected by SMA, particularly those with type 1 and 2, show decreased lean body mass (because of loss of muscle bulk) and increased fat mass, the latter progressively increasing with age. Hence, the calculation of body mass index (BMI) through the comparison with the general paediatric population might not reflect the actual nutritional status of SMA patients. When considering WHO references, type 2 patients tend to be at the lower end of normal, but growth charts should be confronted with different standards, such as the very recently published data from *De Amicis* and colleagues [27]. Issues of underweight increase with disease duration and it is related to the initial measurement of BMI as well. *Bertini* mentioned studies addressing more in depth endocrinological aspects. Real-life clinical and laboratory data of 62 SMA patients (age range 3 months to 31 years, 24 type 1, 21 type 2, 17 type 3) were collected by *Brener* and colleagues including weight-status, self-reported information on puberty, current pubertal stage, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), basal gonadotropin and androgen levels [28]. Precocious pubarche (mean age at onset  $3.9 \pm 2.8$  years) was found in 24% (15/62) of the SMA cohort [45.9% (11/24) type 1 and 19% (4/21) type 2]. Early onset insulin-resistance was related to the severity of muscular phenotype. Interestingly, insulin resistance may be related to defective mitochondrial biogenesis. In order to clarify this point, studies should be directed at the systematic screening of insulin-resistance in SMA patients and its correlation with muscle function and body mass.

During exercise, *Montes* and colleagues observed diminished muscle oxygen uptake in patients affected by SMA type 3 [29] and increased fatigue. While *Bertini* suggested that mitochondrial dysfunction could be a possible explanation for both, *John Vissing* argued that the latter would be more likely connected to the paucity of motor neurons. If SMN-restoring treatments are not able to correct a putative mitochondrial dysfunction (a reduction in mitochondrial muscle mass was observed in one study-[29]), a more targeted therapy may be considered.

### 3.6. Metabolic abnormalities in adults

John Vissing talked about metabolic disturbances observed in older patients with SMA. Impaired muscle mitochondrial biogenesis [30] and abnormal fatty acid metabolism may predispose to perioperative risk when undergoing surgery. To explore this hypothesis in humans, a study comparing a short fasting period (15–18 h) response of different neuromuscular conditions has been performed by his group. SMA patients seemed more prone to fasting-induced hypoglycemia compared to individuals affected by Duchenne Muscular Dystrophy [31]. Furthermore, fasting consistently induced hyperketosis, hypoglycemia, and impaired fat oxidation in children and adult patients with SMA type 2. Notably, an increase in pyruvate and lactate serum levels was also observed. Taken together, these biochemical changes suggest that in SMA additional factors in addition to reduced muscle mass are playing a detrimental effect on the ability to provide a response to fasting. Clinical protocols to design strategies to avoid perioperative metabolic complications are crucial but still debated (e.g., the use of IV glucose and/or amino acids). However, participants agreed that efforts should be directed at finding a consensus and potentially evaluate the results of different protocols on patients undergoing routine surgery.

### 3.7. Metabolomics in animal models - How this tool can support the investigation of multi-system pathology in SMA

Alexander Van Nuijs discussed the role of metabolomics in the assessment of multisystem pathology in SMA. The main challenge of its application is the plethora of molecules that need to be studied for data interpretation. On the one hand, metabolomics is the omic technique closer to the phenotype, on the other hand it is relatively new and still in development. There are two possible strategies that can be used in metabolomics: (i) an untargeted hypothesis-generating approach or (ii) a targeted hypothesis-driven validation. The former has advantages including an unbiased approach but also limitations such as the complexity of both instrumentation and data analysis. The latter allows quantitative analysis and reduced complexity but is focused on a selected small subset of molecules, not capturing the overall picture.

In the literature there are only three published studies exploring an untargeted approach in SMA, but results should be taken with caution, particularly in view of small sample sizes and applied techniques.

The main limitation of this technique is that it requires dedicated methodological designs, and prospective studies are needed. Retrospective analysis of data should be viewed with caution. At this point the method is not fully established to be applied to humans.

### 3.8. Cardiac involvement in SMA

Ludo van der Pol discussed cardiac involvement in SMA. While there are several reports indicating the presence of different cardiac abnormalities in SMA patients, it is not straightforward to discriminate if these are SMN-related, particularly considering that cardiac defects are reported in around 1% of “healthy” newborns.

A systematic review aimed at characterizing not only the incidence but also the type of cardiac defects observed in SMA patients (by the application of specific and approved criteria) was performed in 2017 [32]. Not only did the study outline that the most common abnormalities overall were atrial and ventricular septum defects, but that different types of defects were found in

different SMA subtypes. For instance, in SMA type 1 there was a convergence towards defects of the cardiac septum and of the cardiac outflow tract, the former being overrepresented in patients harbouring a single SMN2 copy. Bradycardia was also commonly observed.

In SMA type 2 and 3 instead, there was a convergence towards cardiac rhythm abnormalities rather than structural defects. Rhythm abnormalities were mainly supraventricular (impulse initiation), occurring at a relatively younger age compared to the healthy population, but impulse conduction abnormalities were also described.

The results above suggest that there is a potential detrimental role played by very low SMN protein level, at specific developmental time points. Moreover, there may be vulnerability of specific cell types or cardiac developmental processes, such as neural crest cells specifically contributing to atrial septum formation.

Notwithstanding the limitation of the data, these findings probably warrant cardiac work-up, specifically prior to initiation of treatment and in view of possible increased vulnerability in the age SMA patients.

### 3.9. Age-dependent SMN expression in disease-relevant tissues and implications for SMA treatment

Charlotte Sumner reported on the advantages of the detailed analysis of human pathology to gather information aimed at directing studies on preclinical models, with the caveat that such approach may select only more severe cases. A study performed in 2019 evaluated SMN expression in spinal cord autopsy samples from SMA patients and controls. On average, there was a developmental decline of SMN levels in non-SMA individuals; however, this was surprisingly variable particularly in prenatal life [26].

Ventral roots were extremely small, but whether this was primarily due to a developmental defect or to neurodegeneration (or both) was not clear. In view of the well-established development of peripheral nerve axons and the full knowledge of its timing in both rodents and humans, these were selected to answer the above question. In humans the maturation of the axon starts around the 10th week of gestation and complete by birth.

In the spinal root samples of SMA patients, there were very few myelinated axons. Interestingly, although both the number and diameter of myelinated axons were reduced, the G-ratio was close to normal (namely, myelin sheath thickness was appropriate for axon diameter). This finding suggested that myelinated axons had not grown to normal diameter and then generated, rather they failed to mature properly. Moreover, there were numerous small, immature axons arrested at different stages of development with a very immature morphology.

Despite the magnitude of histopathological changes, there was far less evidence of large, myelinated axon degeneration (e.g., presence of myelin ovoids) than expected, while small unmyelinated axon degeneration was more abundant than in controls.

In the mouse model (SMNdelta7), slowed motor axon development *in utero* precedes early post-natal degeneration in a topographically specific manner. Specifically, some axons remain unsheathed at E17.5 and subsequently those subsets of axons are more prone to rapid degeneration from P1. Myelinated axons seem much more resistant than abutting axons. Accordingly, neurofilament light chain (NF-L), which is a biomarker of axonal degeneration, consistently increased in the post-natal period [33].

A last take home message was that mice do not recapitulate human pathology completely, particularly concerning muscle, toward which further pathological studies are ongoing.

## 4. Clinical implications

### 4.1. Parent and patient perspective

Being herself affected by SMA, *Rivka Smit* (patient) reported on the difficulties of everyday life. She brought up the main difficulties that she encounters in her daily life, particularly stamina, reduced mouth opening and fatigue during meals. She acknowledged the importance of collecting additional information on the possible multi-system pathology in SMA and offered her availability as a patient to support the scientific community in building up new evidence; however, she also pointed out that it is not always easy for patients to add additional assessments and biosample collections to the already busy schedule of clinical appointments or to accommodate extra research appointments with their active daily life.

To get insights into patients' needs, *Mary Schroth* (Cure SMA) shared data collected from the Cure SMA Annual Community Update Survey which surveys the SMA patient and family community about their experience and daily challenges living with SMA. This information sheds light on comorbidities in SMA, motor function, and future therapies. Of note, there has been an overall decrease in the frequency of hospitalizations from 2018 to 2021. In 2021, the most frequent hospitalization reasons included respiratory distress, surgery or gastrointestinal (GI) tract issues. Frequently reported comorbidities for adults with SMA and independently for children with SMA included contractures and sleep apneas. Adults with SMA reported statistically significant higher rates of anxiety and depression compared to the US adult population. Over time, more people with SMA and their caregivers report increased motor function. The percentage of SMA type 1 individuals having the ability to sit unsupported has increased over time from 13% in 2017 to 54% in 2021. Similarly, for SMA type 2 patients the ability to stand supported or unsupported has increased from 11% in 2017 to 40% in 2021. Unmet needs identified by the SMA community that are important aims of future treatments include increasing muscle strength, achieving new motor function and improving daily living activities. Usage of wheelchair, self-toileting, and self-feeding were identified by adults with SMA as important activities to be maintained or stabilized. Patients demand knowledgeable healthcare professionals and improved communication so that patients and families have access to the best information possible.

*Mencia De Lemus* (SMA Europe) further deepened the patients' and family members' perspective. *De Lemus* stated SMA is multi-symptomatic pointing to the role of pain in SMA. Joint contractures and movement of fingers are also an everyday challenge. Now for SMA patients with a milder phenotype, there are several issues apart from pain which are rather still neglected, namely (i) nutrition, (ii) fatigue, (iii) intelligence/cognition and (iv) dental health. She highlighted that from the patient community perspective there is an increasing need for treating other emerging multi-system symptoms apart from the neuromuscular phenotype.

### 4.2. Standard of care-update needs with the multi-system involvement framework

*Mariacristina Scoto* (SMA REACH UK) reported on the multi-disciplinary care for SMA patients. Basic assessments augment over time since treated patients can show multi-system dysfunction requiring additional screenings for organ function, macro- or micronutrients such as Vitamin D.

*Scoto* presented some cases of SMA patients presenting with abnormalities in the liver, gastrointestinal system, bones and the cardiac system. She reported on some experience with young male patients presenting peri-rectal bleeding. To capture

these comorbidities more systematically, the role of national and international registries was emphasized. In treated patients, there is a demand on psychological support addressing the patients' and families' expectations as well as their mental health.

*Lorenzo Maggi* showed data from recent studies on initial evidence of multi-systemic involvement in adults with SMA, in particular asymptomatic cerebellar degeneration in SMA type 3 and 4 patients and lower IQ index scores for working memory and perceptual reasoning in SMA type 2 patients [34,35]. In addition, *Maggi* presented preliminary results on autonomic dysfunction in adult SMA type 3 patients, affecting both sympathetic and parasympathetic systems. *Maggi* pointed out that the current SMA standard of care relates to the most severe phenotypes and the pediatric population [36,37]. In this regard, bone health has been raised as one of the most relevant and feasible topics to be investigated among different non-motor issues in the context of the Italian adult SMA network. *Maggi* agreed with *De Lemus* that there is an increasing need for additional care beyond the neuromuscular phenotype that relates also to everyday life of treated surviving SMA patients that may show milder motor phenotypes associated with more pronounced peripheral symptoms.

During discussion, *Bertini* mentioned bone health assessment and management still not being part of the clinical practice and the difficulty of actionable or non-actionable clinical findings for implementation in research. There should be an endeavor to fill the gap between research and clinic. A well-standardized routine monitoring should address prevention and treatment of possible peripheral issues. The workshop participants agreed this would additionally require more refined individual treatment and care, which may also include pediatrics and internist care. *Vissing*, *Tizzano* and *Susana Quijano-Roy* agreed on physical exercise like swimming that along with physiotherapy can help to improve vascularization. *Van der Pol* suggested that a trial would be needed to tackle the issue of bulbar dysfunction in SMA patients.

There was consensus that patient organizations can help in identifying the needs to prioritize in SMA patient care. Furthermore, a standardized algorithm determining (i) the quantity of multi-disciplinary screening and routine monitoring, and (ii) the type of organs and metabolites needs to be established. It was suggested that a questionnaire prepared by a local patient organization similar to the Cure SMA survey presented by *Schroth* would be helpful if submitted in advance of a doctor appointment. Remote visits may also be a possibility to collect some of the clinical information and could lead to a more individualized and refined patient care, with more focused clinical appointments. Of note, there will be different populations within SMA patients not only in the untreated but also in the treated cohort. *Bertini* argued that the presence of any remaining symptoms after years of treatment is unknown yet. Today, since SMA treatments are available, the SMA patients will live a whole life and adults shall become the focus of any further treatment. It has been highlighted that a balance is required between trying to collect data from patients and respect their daily life.

## 5. Potential impact of treatment on non-neuronal manifestations

### 5.1. Effects on the multi-system pathology in SMA patients receiving treatment

*Ben Tichler* (Biogen) reported on direct and indirect effects associated to SMN deficiency in SMA disease, some of them may also be secondary effects driven by immobility and low muscle mass. This was compared to the considerations of nusinersen administration in its European Product Label (SmPC)

including vomiting, headache, back pain (Post Lumbar Puncture Syndrome), meningitis and hydrocephalus (no drug causality demonstrated). *Ksenija Gorni* (Roche) elucidated the adverse events occurring during risdiplam treatment, which will continue to be monitored over time to guarantee the positive risk benefit profile. In current studies, a dose-dependent increase in SMN protein in both CNS and periphery of rats, mice, or primates was documented, additionally an increase in SMN protein on blood was measured in the clinical studies involving all types of SMA and a broad age range (from neonates to adults). However, a combinatorial treatment may be beneficial in addition to existing therapies. For instance, the myostatin pathway may be of special interest, leading to treatment with anti-myostatin additional to risdiplam administration. *Deepa Chand* (Novartis) described the safety profile associated with onasemnogene abeparvovec treatment in children with SMA. Several adverse events have been reported including pyrexia, vomiting, liver toxicity, cardiac adverse events, thrombocytopenia and complement-mediated thrombotic microangiopathy (TMA). Data pertaining to the risks of Hepatotoxicity, TMA and overall safety have been previously published. Hepatic aminotransferase elevations have been reported and, in some patients, elevations were noted prior to treatment, suggesting inherent liver abnormalities may contribute to the hepatotoxicity seen after onasemnogene abeparvovec treatment. An evaluation in conjunction with a paediatric gastroenterologist/hepatologist should be undertaken. TMA treatments used have included increase of corticoid doses and intravenous bolus, fluid and electrolyte management, dialysis, plasmapheresis and eculizumab in some cases. The etiology is not completely clear, and treatment should be individualized after consultation with a paediatric haematologist and/or a paediatric nephrologist [38–40].

The workshop members discussed the adverse events within the current therapy studies. The workshop participants agreed the analysis of SMN level should serve as a standard biomarker due to *SMN2* gene copies being the genetic modifier of SMA. It was mentioned that there is uncertainty if higher doses of nusinersen currently under investigation may lead to accumulation of the compound in peripheral organs like kidneys and liver. Any SMA treatment-related adverse events namely photosensitivity, infertility, or other delayed toxicities developing in multiple organs will be addressed in long-term studies. The risk of dying due to respiratory infections is still not fully prevented. The representatives of the three pharma companies admitted there are still no specific standard assessments related to neither bulbar function such as speech, nor cognitive tests. Data collection on these peripheral symptoms is incomplete and yet ongoing.

There was consensus among participants that treated chronic long-surviving SMA patients may possibly show (i) evidence of peripheral dysfunctions which were masked by the predominant neuromuscular phenotype and (ii) treatment-related effects in specific organs/tissues. Both emerging factors must be considered in future clinical monitoring and in the development of combinatorial treatments.

## 6. Improving identification and monitoring of possible multi-system manifestations in SMA

### 6.1. National and international registry experiences and recommendations

On the last day, *Scoto, Baranello, Bertini, Maggi, Kirschner, Quijano Roy, Desguerre, Van der Pol, Tizzano, Swoboda, De Lemus, and Schroth* gave an overview on the role of different registries to collect multi-system manifestations in SMA and patients' perspective.

At present, there are several registries collecting data of SMA patients according to different criteria and with slightly different aims, including an international collaboration between the UK, Italy and US (iSMAC), and several national registries (e.g., Germany, Netherlands, Italy, France [41], US).

Registries serve both academic purposes and to monitor medium- and long-term complications of newly available treatments (which is mandatory in most), with a few caveats.

First, the main limitation in the context of academic research is that registries are most often not designed to answer specific research questions, this can therefore introduce several biases in the analysis of data and interpretation of results. Well-designed, smaller, but more focused prospective clinical studies supported by academic grants and directed at specific SMA sub-populations are likely to be more informative with regards to the main theme of the workshop. On the other hand, it was pointed out that registries can provide the infrastructure upon which clinical studies are designed, based on the identification of new targets and possible confounding elements.

The compulsory recording of adverse events is crucial to identify both overlooked aspects of the disease (e.g., endocrine abnormalities, fatigue, pain, and nutritional status,) and side effects of new treatments (e.g., neurological, skin, heart, eye, liver, gastro-intestinal, haematological), which can be swiftly discussed at a national level. A clear example is the identification of fatal TMA in one girl treated with onasemnogene abeparvovec in France, complication also reported in additional patients at the international level [42,43]. As a result of this unexpected and severe outcome, national guidelines were reviewed in the following months by the French neuropediatric experts within the therapeutic commission of the neuromuscular network (FILNEMUS), in order to warrant a closer monitoring of blood tests in the first weeks of treatment, when this live-threatening complication may occur (LDH, platelets, transaminases). The implementation of preventive practices and treatments (e.g., avoidance of vaccines in the two weeks to infusion, multiplex viral PCR samples in the peri-therapeutic period, increase in the initial steroid dose to 2 mg/kg/day, use of anti-complement drugs such as eculizumab) and the research of possible genetic underlying defects in affected cases (research of mutations in complement genes) were also included. In parallel, a specific biologic module of haematologic and biochemical parameters was created in 'Registre SMA France' allowing a systematic collection of data, that became available only several months after the first alert. Preliminary results of this collected data were presented. Another example is the identification of photosensitivity in a few patients treated by orally given risdiplam, with areas of different skin coloration in different parts of the body. Concerning eye complications, although eye examinations have not shown abnormal results in the clinical trials nor in real-world follow-up assessments (OCT, funduscopy) in treated patients, occasionally, school children have claimed of some discomfort to computer screens or changes in colour perception, so a closer follow-up of these complaints seems important. Registries should ideally ensure a dynamic setting. New specific and significant queries should be implemented, and patients' awareness should be addressed, as new adverse events are identified, in order to better understand their frequency, origin and relationship with the therapeutic interventions and/or the disease itself.

In the Netherlands, which is favoured by a hub-centred healthcare organization, all patients undergo muscle strength and motor evaluations and are asked to sign biobank consent to allow future analysis of different biological samples (DNA, RNA, blood, CSF, fibroblasts and urine). The quality and consistency of the data that are collected are crucial for both clinical and academic purposes.

Another area in which registries are likely to be useful is newborn screening. It was highlighted how the detailed collection of the clinical and neurophysiological status of pre-symptomatic patients before treatment in the future will be crucial, hence allowing a better interpretation and comparison of the effects of new treatments.

Data accessibility/sharing, and management differ according to local regulators, and these should be considered. Besides other crucial elements, the funding displays a critical role regarding data handling. While the management, coordination and implementation of large datasets is costly, some of the participants highlighted the importance of being independent from pharma industry, to allow a less-biased approach particularly in view of the possibility to use several products with potential different effects particularly on multisystem aspects in SMA.

## 6.2. Patients' perspective

Lastly, patients' representatives presented patients' perspective and the role of family association. SMA Europe is collecting a series of surveys and in collaboration with Cure SMA is looking into physiotherapy and nutritional aspects. Cure SMA is aiming to improve data collection and elevate the quality of data entry to the dataset via electronic medical records. Regional differences in healthcare system should be considered (e.g., insurance in the US). One important aim for Cure SMA is to finally establish the true incidence of SMA as well as *SMN2* copy number in the US.

A key aspect that emerged from the discussion is that many elements that are important for patients (such as pain, dental care, contractures, fatigability, time spent in care, mobility, nutrition specific requirements and growth parameters, independence, etc.) are often overlooked by academics. At the same time, it may happen that researchers may focus on elements that are not considered impactful from patients. As such, a constructive dialogue between patients, their families and scientific community is crucial to direct the resources not only in terms of funding, but also with regards to the burden that some investigation exert on patients' lives (e.g., number of hospital assessments, etc.).

## 7. Conclusions and workshop deliverables

SMA has been considered a motor neuron-specific disease for decades. The workshop participants agreed that there is a multi-systemic pathology in this condition, due, at least partially, to known or possible interactions and processes related to the lower levels of prenatal SMN protein, which may affect the environment of developing tissues. These interactions may lead to hidden or "latent" post-natal pathological consequences, in particular in patients with less than 3 *SMN2* copies, who in the past died early in life. The identification of prenatal multi-systemic severe complications in patients with 2 *SMN2* copies opens the question of prenatal treatment for this population.

Different aspects and tissue or organ-specific questions emerged during the workshop, warranting further investigation. Novel identified symptoms and dysfunctions require a better understanding and screening in larger populations (e. g. autistic spectrum disorder traits, neurodevelopmental delay and social communication difficulties in babies with 2 *SMN2* copies). The workshop participants summarized scientific evidence for multi-systemic defects, namely impaired myogenesis and synaptic development, spleen, immune system and liver involvement, vascularization defects and hypoxia, mitochondrial defects, collagen dysregulation, cardiac dysfunctions, cognitive impairment, metabolic abnormalities and endocrine alterations. These non-motor neuron manifestations are observed and investigated in multiple preclinical animal models. In this regard, the molecular

function of the SMN protein responsible for these peripheral pathomechanisms requires additional attention in future research. Of note, the additional challenge is that the SMN protein is differentially expressed in different tissues, further changing during development and age. Multi-systemic defects should be promptly addressed and understood in order to allow providing adequate genetic counsel, prenatal diagnosis and post-natal management of SMA patients, and to address the impact and use of new therapies. Real-world data collection and research on specific areas (neurologic, metabolic, intestinal, cardiovascular) will be critical in the near future and will require particular attention in the perspective of worldwide neonatal screening programs and pre-symptomatic treatment.

In summary, the workshop participants endorsed a common strategy to pursue the following objectives in future work: (i) identify the multi-systemic phenotype across different tissues, organs and systems, (ii) understand the underlying molecular pathomechanisms, especially with regard to SMN-enhancing treatments by the use of different preclinical animal models and (iii) develop an interdisciplinary consortium of patients representatives, clinicians, researchers and stakeholders in order to enlighten the multi-faceted peripheral phenotype in SMA through complementary expertise and approaches. Since disease-modifying treatments are now available for SMA, there is the need to refine monitoring, individual care and personalized treatment.

### 7.1. Organizers

Giovanni Baranello (UK), Tom Gillingwater (UK), Kathryn Swoboda (USA), Rashmi Kothary (CAN).

### 7.2. Early career co-organizers

Nora Tula Detering (GER), Niko Hensel (CAN), Alberto Zambon (ITA).

## Participants

Workshop participants: Simon Parson (UK), Mariascristina Scoto (UK), Melissa Bowerman (UK), John Vissing (DK), Brunhilde Wirth (GER), Peter Claus (GER), Janbernd Kirschner (GER), Enrico Bertini (ITA), Stefania Corti (ITA), Lorenzo Maggi (ITA), Ludo van der Pol (NL), Ewout Groen (NL), Charlotte Sumner (USA), Eduardo Tizzano (SPA), Susana Quijano Roy (FRA), Isabelle Desguerre (FRA), Alexander Van Nuijs (BL), Mencia De Lemus (SPA), Rivka Smit (patient), Mary Schroth (Cure SMA).

Industry participants: Ben Tichler (Biogen); Ksenija Gorni (Roche); Deepa Chand (Novartis).

## Declaration of Competing Interest

GB has received speaker and consulting fees from Biogen, Novartis Gene Therapies, Inc. (AveXis), and Roche and has worked as principal investigator of SMA studies sponsored by Novartis Gene Therapies, Inc., and Roche. The other organizers do not have conflicts to declare for this specific workshop.

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