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Spinal muscular atrophy — new therapies, new challenges

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ABSTRACT

Spinal muscular atrophy (SMA) is a progressive neurodegenerative disease with an autosomal recessive trait of inheritance and great variability of its clinical course – from the lethal congenital type (SMA0) to the adult-onset form (SMA4). The disease is associated with a deficiency of SMN protein, which is encoded by two genes *SMN1* and *SMN2*.

Clinical symptoms depend on mutations in the *SMN1* gene. The number of copies of twin similar *SMN2* gene, which produces small amounts of SMN protein, is the main phenotype modifier, which determines the clinical severity of the disease. Until recently, it was considered that spinal cord motoneurons undergo selective loss. Recent studies have shown the role of SMN protein in various cellular processes and the multisystemic character of SMA. The aim of the therapeutic strategies developed so far has been to increase the expression of SMN protein by modifying the splicing of SMN2 gene (intrathecally administered antisense oligonucleotide – nusinersen; orally available small molecules: RG7916 and LMI070 or *SMN1* gene replacement therapy (AAV9-SMN).

The first SMN2-directed antisense oligonucleotide (nusinersen) has demonstrated in clinical trials high efficiency, and it has now been registered. The best effects were obtained in patients who were introduced to the drug in the pre-symptomatic period. Studies on other substances are ongoing. The great advances in SMA therapy and increased understanding of the pathogenesis of the disease raise hopes for changes to the natural history of the disease. Simultaneously, it increases awareness of the need to improve the standard of patient care and early diagnosis (newborn screening). Many questions (e.g. emerging phenotypes, combined therapies, systemic vs. intrathecal administration, long-term consequences, and complications of the therapy) will require further studies and observations.

Key words: **s**pinal muscular atrophy, *SMN1* gene, SMN protein, nusinersen, gene therapy

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Introduction

Spinal muscular atrophy belongs to a group of diseases characterised by a loss of spinal cord motoneurons, which leads to skeletal muscle atrophy and weakness.

The most common form is proximal spinal muscular atrophy infantile and juvenile type, associated with mutations in the *SMN1* gene. In most cases, SMA is a severe, progressive and incurable disease, which leads to paralysis and respiratory failure. Despite improved standards of care, it remains one of the most frequent causes of death in children affected with genetic disorders.

Since the identification of the *SMN1* gene, associated with pathogenesis of the diseases, intensive work has been undertaken with the aim of expanding knowledge of the molecular defect and pathogenesis of the disease, its natural history, and potential therapeutic methods [1]. Recently, studies on the first drug in SMA – the antisense oligonucleotide nusinersen – have been successfully completed [2, 3]. Research into, it is to be hoped, equally effective substances with various mechanisms of action is underway (gene therapy - AVXS-101, micromolecules targeting alternative splicing of *SMN2:* RO7034067, LMI070) [4]. Encouraging results from preliminary tests have raised hopes for their effectiveness, and thereby changing the

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natural history of this severe and incurable disease, albeit many questions remain to be answered.

Classification of SMA

The currently approved division of SMA expands the classic classification by Munsat (into SMA1, 2 and 3) by the addition of two further forms: SMA0 with prenatal onset of clinical symptoms; and the adult form SMA4 [5].

SMA0 is a rare form, with symptoms visible already during foetal life and a poor prognosis [6]. Weak foetal movements are noted by mothers-to-be in the prenatal period. After birth, hypotonia, areflexia, a total or almost total lack of active movement, and very often arthrogryposis are observed. Respiratory failure develops in the first minutes or hours of life. The disease is associated with an extreme deficit of the SMN protein.

SMA1 patients present within the first six months of life with progressive generalised muscle weakness, atrophy and areflexia, and patients are never able to sit unsupported. SMA1 leads to respiratory insufficiency within the first year of life.

In SMA2, the first symptoms are seen between the ages of 7–18 months; the child is never able to stand but can sit unsupported. Later in life, severe scoliosis and contractures develop.

SMA3 is a chronic form, symptomatic after 18 months of life. Independent walking is achieved, but many patients lose ambulation [5, 6].

At the other end of the disease severity spectrum there is its adult form, SMA4, where onset usually occurs in the third decade of life [7]. This is characterised by slowly progressive weakness of proximal muscles, mainly quadriceps, with a good prognosis for life and preserved respiratory function. A novelty in the current classification is the division of the first and third forms into subtypes a, b, and c, depending on age at onset of the first symptoms [8]. It has been noted that earlier onset of the disease usually determines a more severe course. It seems that this classification is a compromise between a too general division into basic forms and a too detailed and difficult in everyday practice classification according to Dubowitz (1.1–1.9, 2.1–2.9, 3.1–3.9, 4) [9]. At the same time, it should be remembered that SMA phenotypes create a clinical continuum from the congenital to adult forms, and even asymptomatic cases. Thus within each form, cases with a milder or a more severe course of the disease will be seen. But there are also borderline cases which do not allow easy and unequivocal qualification to any basic group.

Epidemiology

Recently published study results on SMA epidemiology in Europe indicate an SMA incidence of 1:8,400 births (11.9/100,000) [10]. In 2011–2015 SMA was diagnosed in 4,653 patients in Europe, with 992 cases in 2015 alone. These results are compatible with the largest study on SMA epidemiology ever initiated, which has been conducted in the USA on a multi-ethnic group of 68,478 people [11]. This showed an SMA carrier frequency in the population of 1:54 people and an incidence of 1:11,000 births. A significant difference in carrier frequency was noted as depending on race, with the highest in Caucasians (2.02%) and the lowest in African-Americans (0.98%).

In contrast to SMA incidence, its prevalence is difficult to estimate. This is due to the various ages of onset and clinical courses of SMA which influence lifespan, additionally modified by improved standards of care, especially respiratory support. The global register of patients (the TREAT-NMD Global SMA Patient Registry) provided data from 26 national registries, representing 29 countries and contained a total of 4,526 genetically confirmed patients. This number does not reflect the real number of patients suffering from SMA worldwide [10, 12].

The natural history of SMA

Spinal muscular atrophy is characterised by a very wide spectrum of disease onset, symptom severity, and type of complications. Thus the natural history of the disease might vary in a given patient. Its clinical course depends on the quality of medical care, prophylaxis, and, above all, physiotherapy and appropriate treatment of complications. Recent studies have shown some relationships. In the SMA1 form, the earlier the onset of symptoms, the worse the prognosis and the shorter the survival. A study on the trajectories of SMA1 progression revealed baseline CHOP INTEND scores of below 20 in SMA1a and of 20–40 for both SMA1b and SMA1c [13]. The rate of progression in SMA1a, b and c was significantly different, but none of the children showed any improvement. All infants with the most severe phenotype had rapid declines, and none survived beyond 13 months. Among children with type 1 of SMA, the number of *SMN2* copies is a strong biomarker and disease modifier – the survival curve is decreased compared to patients with two copies. Median survival or age of introduction of mechanical ventilation (> 16 hours a day) is 13.5 months [14]. This period decreases to 10.5 months in children with two copies of *SMN2*. However, it should be noted that the presented data was obtained in a centre which uses the proactive therapeutic approach (i.e. pre-symptomatic mechanical clearing of respiratory tract with cough assistor, NIV, tube feeding or through PEG). Older reports, or reports from centres which focus on palliative treatment, indicate median survival of 6–7 months for SMA1 [15]. According to Australian studies, the chances of survival at 1, 2, 4, and 10 years for children with SMA1 are 40%, 25%, 6%, and 0%, respectively [16]. In turn, a Polish-German study from 2002 indicated that only 10% of SMA1 patients reach the age of 5 years [17]. Respiratory and feeding support increases survival by months and indeed years, but at the price of full dependence on gastrostomy and NIV/IV, with no improvement of motor function [18].

The prognosis for life in patients with SMA2 is much better. According to Australian studies, almost 93% of these patients reach the age of 20 years and approximately 52% survive until the age of at least 40. The Polish-German study from 1997 on a group of 240 SMA2 patients suggested a survival probability of about 75% at the age of 20 years [19]. The survival curve for patients with SMA3 and SMA4 seems to be the same as the average for the general population. In contrast to the acute form of SMA, patients with chronic forms can acquire new motor skills. In patients unable to walk, functional improvement is usually seen until the age of 4–5 years, while between the ages of 5 and 15 years they often experience a gradual deterioration but eventual stabilisation of motor function [20]. Adolescence seems to be the most difficult period for some SMA patients, as many of them lose the ability to walk independently. The probability of preserving the ability to independently walk after 10, 20 and 40 years of the disease is 73%, 44%, and 34%, respectively, for group 3a and 97%, 89%, and 67%, respectively, for group 3b [19].

Standards of care

Spinal muscular atrophy is a progressive and multisystemic disease that leads to many complications: respiratory (impaired cough reflex, respiratory distress), gastrointestinal (gastro-oesophageal reflux, swallowing problems, malnutrition or obesity), musculoskeletal (scoliosis, joint contractures, hip dislocation), and others. Quality of care and prophylaxis of complications definitely prolong lifespan and improve the quality of everyday functioning. Therefore the introduction of uniform criteria of care in SMA worldwide would be of the highest importance. The first standards of care were developed by international experts in SMA and published in 2007 [21]. In 2017, the guidelines of therapy were updated. Recommendations include some aspects (classification and natural history, diagnostics and genetics, management of newly-diagnosed patients, pulmonary and acute care, medications, immunisations, orthopaedic management and rehabilitation, gastroenterological care, symptoms from other organs, ethics and palliative care). Recently published standards present precise recommendations regarding rehabilitation, pulmonary and orthopaedic management, depending on the functional status of the patient (i.e. non-sitters, sitters, and walkers) [22, 23].

Comprehensive care for SMA patients requires the involvement of a multidisciplinary team of specialists from various fields, including neurologists, orthopaedic surgeons, pulmonologists, gastroenterologists, anaesthesiologists, geneticists, physiotherapists and dieticians.

Molecular pathogenesis

The pathogenesis of spinal muscular atrophy is associated with two similar twin genes: *SMN1* and *SMN2*. The clinical symptoms of SMA depend on mutations of the *SMN1* gene, among which 96.5% is biallelic deletion of this gene [24]. Three percent of SMA patients bear point mutations, which occur in a compound heterozygous state with deletion. To date, more than 70 point mutations of the *SMN1* gene have been described. In the Polish population, seven such variants have been identified. The most frequent is mutation T274I in exon 6, which is correlated with chronic forms of SMA [25].

The *SMN2* gene acts as a phenotype modifier. Mutations of the *SMN2* gene are not pathogenic and, as a homozygous deletion, have been found in approximately 10% of a healthy population [26]. However an increased number of *SMN2* copies, as a result of duplication or conversion, alleviates the clinical course of SMA.

SMN1 and *SMN2* genes are twin similar [27]. Their sequence functionally differs by only one substitution C in T in exon 7. In healthy people, the expression of both these genes *SMN1* and *SMN2* leads to the production of the protein SMN. In patients with spinal muscular atrophy, the SMN protein is expressed only from the preserved *SMN2* gene. The *SMN2* gene, due to a single-nucleotide difference in exon 7, undergoes alternative splicing during posttranscriptional processing. Substitution C to T in codon 280 changes the sequence of exonic splice enhancer (ESE), cancelling its action. This in turn leads to the deletion of exon 7 and eventually the practical loss of functional SMN protein. A small amount of *SMN2* transcript (approximately 10–20%) undergoes normal splicing and produces full-length SMN protein, structurally and functionally identical to the product of *SMN1* copy.

An increased number of *SMN2* copies (through duplication or conversion of *SMN1* into *SMN2*) can compensate for deficits caused by loss of the *SMN1* gene, through an increased level of the full-length SMN protein in tissues. A strong correlation has been demonstrated between the number of *SMN2* copies and the phenotype of the disease. In patients with an acute form of SMA 1–2, *SMN2* copies are usually found, while in patients with an intermediate form 2–3 copies are usually found. With a mild form 3–4, and even 5–6 copies, are found [28–30].

The number of *SMN2* copies does not fully explain the phenotype variability in SMA. A recently published study on a large number of patients showed that in a group of 1,627 patients and three *SMN2* copies, SMA1, 2 and 3 were diagnosed in 15%, 54%, and 31% respectively. This is probably due to the fact that SMN2 copies are not functionally equivalent and produce various amount of full-value SMN protein. This could be associated with the role of epigenetic factors, especially with a different pattern of *SMN2* methylation. Significantly lower methylation has been found in patients with the mild form of SMA, compared to patients with the severe form who had the same number of *SMN2* copies. However, considering the different level of methylation in various tissues, drawing final conclusions can be challenging.

Another phenotype modifier is substitution c.859G>C in exon 7 of *SMN2* gene [31]. This has been described in several

patients in whom a mild phenotype did not correlate with the number of *SMN2* copies (one copy of *SMN2* in patients with SMA2 or SMA3*)*. This change creates a sequence of a new ESE in exon 7, therefore increasing the level of the full-length SMN protein and alleviating the symptoms of the disease.

However, this modifier is very rarely identified. In a cohort of 625 Spanish patients it was found in 10 cases with discordant phenotypes (four cases with SMA type 2 and two SMN2 copies, four cases with SMA3 and two SMN2 copies, and two cases with SMA3 and three SMN2 copies) [32]. Among the factors which influence SMA clinical course, sex has also been mentioned. It has been observed that girls are affected by the benign form half as frequently as boys, while cases with asymptomatic biallelic mutation of *SMN1* are more frequent in women [33]. In asymptomatic female carriers of *SMN1* deletion, whose brothers had SMA, overexpression of plastin 3, encoded by *PLS3* gene on chromosome X, has been found [34]. Another newly discovered SMA modifier is NCALD, a Ca^{2+}-dependent negative regulator of endocytosis. Unlike PLS3, which alleviates SMA upon overexpression, NCALD reduction protects individuals with four SMN2 copies from developing SMA. *NCALD* transcript showed a 4- to 5-fold downregulation in the asymptomatic members of a USA family versus SMA1 cases from this family or an independent type 3 SMA group [35].

While the genetic basis of SMA is well understood, the specific molecular pathways underlying SMA are still not completely understood. SMN protein modulates almost every aspect of RNA metabolism, including transcription, splicing, biogenesis of snRNPs, snoRNP assembly, telomerase activity, translation, and mRNA transport. Biogenesis of the spliceosomal snRNP assembly is the best characterised function of SMN. The SMN protein, together with some other proteins such as Gemins 2–8 and Unrip creates a multiprotein complex which is found both in cytoplasm and in nucleus. This complex is involved in the assembly of snRNP, an essential component of pre-mRNA splicing machinery in cells [36].

Independently of the SMN involvement in RNA metabolism, SMN regulates also DNA repair, cell signalling, endocytosis, autophagy and other functions. It is not known which function is crucial for SMA symptoms. It is likely that SMN deficiency simultaneously impacts upon multiple functions.

Since the discovery of the basic function of the SMN protein, there has been discussion as to why deficiency of the protein, playing such an important role in the metabolism, causes a near selective loss of motoneurons. SMN protein level in tissues might be critical. Animal models have shown that total loss of SMN protein is lethal in the foetal period. In knock-out mice without their own *SMN* gene, and with introduced human *SMN2,* the severity of the disease depends on the number of introduced copies of the *SMN2* gene. Mice with two copies are affected very severely, yet those with eight copies do not show any symptoms of SMA. A similar result has been obtained by increasing *SMN2* expression through a prion promoter selectively within the central nervous system in mice with two copies of *SMN2*. These mice are healthy. Therefore it seems that the majority of tissues require for normal function only a low level of SMN, while the CNS needs much more.

While SMA mouse models illustrate the importance of SMN in the nervous system, recent studies have indicated a significant effect of SMN protein deficiency on the development and function of other organs i.e. bone, lungs, pancreas, cardiovascular system, liver, and spleen. Observations of symptoms occurring in patients with SMA seem to confirm these results. Thus, as SMA begins to be treated as a multi-systemic disease, it may well require systemic treatment.

New therapies

Recent years have brought dynamic progress in the development of novel SMA therapies. Nusinersen (Spinraza®) is a survival motor neuron-2 (SMN2)-directed antisense oligonucleotide. It binds to the SMN2 pre-mRNA and promotes inclusion of exon 7, resulting in increased SMN protein production. The drug does not cross the blood-brain barrier and is delivered by intrathecal injection.

The safety and efficacy of nusinersen were demonstrated in phase I–III studies leading to early termination of a clinical trial. Nusinersen was licensed for the treatment of all types of SMA in December 2016 in the USA, and a few months later in the EU. The drug has become available to a wide SMA population. Recent reports demonstrate ongoing benefits for patients treated for over three years, as well as for patients who started treatment in the more advanced stages of the disease [37, 38]. Several promising molecules are in phase II or III clinical trials. Risdiplam and branaplam are orally administered small molecules that act as *SMN2* gene splicing modifiers [39-41].

Gene therapy is another emerging therapeutic option. Nonpathogenic, nonreplicating AAV parvovirus has been engineered to become a vector delivering full-length *SMN1* cDNA to the host cells [8]. Preclinical studies have demonstrated the safety and efficacy of AAV9 in animal models of SMA [42]. Single-dose gene replacement resulted in the development of motor milestones in SMA1 children treated with a high dose, as reflected in the CHOP-INTEND scores. Two of the children started walking, and 11/12 were able to sit unassisted. All were alive at 20 months of age, compared to 8% in the historical controls [43]. The pulmonary and nutritional status of the children was also significantly improved [44]. Functional improvement was more pronounced in infants treated before they were three months old [45]. In the light of these results, the USA Food and Drug Administration recently approved onasemnogene abeparvovec-xioi, an adeno-associated virus vector-based as the first ever gene therapy for children with SMA below the age of two years.

All currently conducted clinical trials can be followed on the clinicaltrials.gov website.

Conclusions

SMA is a unique disease, since *SMN2* copy of the gene is present in all patients, being the most promising therapeutic target. The first antisense-based drug targeting intronic splicing silencer N1 in *SMN2* gene, nusinersen, was discovered, tested and registered within a short time. The next emerging therapies are under advanced research and clinical trials. The efficacy of the developed treatment methods is the cause of considerable excitement. But many questions still remain concerning long-term effectiveness, emerging phenotypes, safety profile and tolerance, drug effectiveness in chronic forms, concomitant administration of medicines with various mechanisms of action, administration routes, and the correct time of treatment in the mild forms. Treatment costs can be also a challenge for healthcare systems. Animal studies suggesting the multisystemic character of the disease might indicate that intrathecal administration of the SMA therapies may not be fully effective. The high demand for SMN protein in early life indicates the crucial role of an early diagnosis of this disease for maximum effectiveness. This could be achieved by neonatal screening, although for the severe form this could still be too late. Population screening towards a carrier state of SMA should be considered. At present, from the clinical point of view, the most important seems to be detailed observations of the patients treated with the innovative therapies, and strict compliance with the SMA standards of care.

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