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The importance of SMA screening tests in newborns – review

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Abstract

Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease caused by the loss of alfa motor neurons occuring with a frequency of 1 of 11000 births. It leads to muscular weakness and is cause of high rate of mortality in children. 95% cases of SMA are caused by homozygous deletion in SMN1 which is identified as the SMA disease-determining gene. The number of copies of SMN2 determines the phenotype of the disease. Screening tests for SMA can detect 95-98% of the mutations in SMN1, but cannot detect point mutations. The SMA genetic tests are characterized by high sensitivity and specificity, they are inexpensive and therefore can be used on a large scale. Generally DNA is isolated from the dry blood spot and then subjected to PCR analysis. The SMN2 copy number is determined using the MLPA technique. Screening tests allow the diagnosis of SMA even before the onset of symptoms. Numerous scientific studies show that early treatment in most patients with detected SMA allows for better neuromotor development in children. The most beneficial effects of the SMA therapy are visible in patients treated pre-symptomatically - and this is only possible thanks to the use of screening tests. In this review we present the importance of SMA screening tests in newborns according to the latest scientific reports.

Key words: SMA; spinal muscular atrophy; newborn screening; SMN1; SMN2

Introduction

Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease caused by the loss of alfa motor neurons. It occures with a frequency of 1 of 11000 births. SMA leads to muscular weakness and atrophy and is cause of high rate of mortality in children [1,2]. The first descriptions of SMA date back to the 1890s by Guido Werdnig and then by Johan Hoffmann [3].

95% cases of SMA are caused by mutation in SMN1 (survival of motor neuron 1) by homozygous deletion at region 5q13 (exon 7). A smaller percentage of mutations within SMN1 include heterozygous mutations, as well as point mutations associated with exon 7 deletion. However, even though SMN1 was identified as the SMA disease-determining gene (one of the key molecules of pathogenesis of SMA) and main SMA therapies are concentrated at 5q13, researches also indicate the role of mutations in the SMN2 (survival of motor neuron 2) and NAIP (neuronal apoptosis inhibitory protein) genes in the emergence of the disease. SMN2 is homologous centromeric copy of SMN1 (inversely duplicated gene on chromosome 5q13) and is disease-modifying gene because of the correlation between copy number of SMN2 and disease severity. According to some scientists (Eun-Ji et al., Qu et al.) the NAIP (and its deletion) may be linked to the severity of the SMA but more research needs to be done [1,3,4,5,6].

SMA is clasified into five subtypes – type 0,I,II,III and IV, according to age of onset and level of acquired motor skills. Earlier onset of the disease is usually associated with poorer motor function and shorter life expectancy. Subtype 0 of SMA appears already in the prenatal period, and a newborn has a survival time of less than one month. Subtype I (also named as a Werdnig-Hoffmann disease) is the most common subtype of SMA (more than 50% of cases) and, next to subtype 0, it is the most severe form of the disease with a high mortality rate – without medication child's life expectancy is 8-24 months. It appears within 6 months of birth, requires respiratory assistance and the child is generally unable to sit unsupported and has problem with feeding. Subtype II of SMA (Dubowitz disease) appears by 18 months of age, people are not walking independently but can sit unaided, but without any tratment usually live into early adulthood (20s-30s). Subtype III (Kugelberg-Welander disease) appears after 18 months of age, people are able to move independently, and life expectancy is similar to that of healthy individuals. The last, subtype IV of SMA, is very rare and appears generally after the

age of 20 (usually during second or third decade of living), but has no effect on reducing life expectancy and is the mildest form of SMA [1,3,5,7,8].

Here we present, the role of the SMA screening tests in newborn and its importance in the treatment of this disease.

Screening tests

Screening tests for SMA can detect 95-98% of SMN1 mutations, but are not able to detect point mutations occurring in some patients. The tests are characterized by high sensitivity and specificity, they are inexpensive and therefore can be used on a large scale. The tests are performed with DNA from a dry blood spot (DBS), which provides the right amount of DNA material for analysis (Czibere et al.). The most common method to detect SMA mutations is real-time PCR [9,10]. A non-invasive screening system using dried saliva spots (DSS) has also been developed. The isolated DNA is then subjected to PCR analysis, as in the case of using dry blood drop. However, the DSS method has some limitations - the amount of amplifying DNA obtained from liquid saliva is lower than in blood, and PCR inhibitors contained in saliva may distort the results (Wijaya et al. – 1/61 test was failure out of DSS samples -1.6%; using DBS showed no failure of the test) [11]. The use of the new Intelligent Ratio (IR) method using FII as a reference gene in the analysis by Cavdarli et al. allowed the user and the amount of DNA used in the extraction to provide quick and accurate results, completely consistent with the MLPA analysis [12].

The result of the SMA screening test requires confirmation and evaluation for the SMN1 and SMN2 gene copy number (usually by MLPA technique - multiplex ligation probe amplification). The diagnosis of SMA is confirmed by the loss of two SMN1 alleles, and in the case of loss of one allele, further tests are carried out for point mutations. The assessment of the number of SMN2 copies allows to determine the phenotype of the SMA - acute disease with 1-2 copies, mild disease with 2-3 copies, and mild disease with 3-4 copies. Sometimes as many as 5-6 copies of SMN2 may occur. Moreover, the determination of the number of SMN2 copies allows the prediction of possible disease evolution. Many centers are screening for SMA combined with testing for SCID [9,13].

The current possibility of using drugs modifying the course of SMA (Nusinersen, Risdiplam, Onasemnogene abeparvovec-xioi) emphasizes the important role of early detection of the disease, which will enable early treatment, even before the disease symptoms appear – which showed the most beneficial results. Genereally, the diagnosis of SMA is delayed, so

screening for the mutations responsible for the development of SMA is essential to provide patients with adequate therapies and thereby modify disease progression [1,14].

Dangouloff et al. sent questionnaires to SMA and NBS (Newborn Screening) experts, in which they were asked to evaluate the availability of SMA-modifying drugs and access to newborn screening. Researches received responses from 87 experts from 82 different countries (among the questionnaires sent to experts from 152 countries - out of 197 countries in the world). According to questionnaires, NBS for SMA was implemented in 9 countries – Taiwan (81–90% newborns screened), USA (61–70%), Germany (11–20%), Belgium (45%), Australia (21–40%), Italy (11–20%), Russia (< 10%), Canada (31–40%) and Japan (< 10%). The screening program in these countries allowed a total of 288 newborns to be diagnosed with SMA out of 3674277 newborns. Taiwan, as a pioneer in SMA screening, is the only country which screening all newborns. Moreover in mentioned countries no false negative result have been reported – this shows that the NBT for SMA are highly reliable. Scientists are of the opinion that the use of NBS allows for significant savings in the treatment costs (reducing social costs) of the previously very expensive SMA treatment, thanks to the rapid introduction of the therapy [14].

The introduction of screening for SMA according to Butterfield et al. demonstrated benefits for motor development in children with SMA through early diagnosis and treatment. In the first weeks of a child's life, there is a rapid loss of motor neurons, so it is important to start treatment as soon as possible to slow down the progression of the disease. In 2018, ADHCNS (Secretary's Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children) recommended the implementation of research on SMA do recommended uniform screening panel (RUSP) - by May 2021, the program covered 74% of children in the USA. The recommendations included neurophysiological examinations - including electromyography and nerve conduction, assessment of motor functions and neurological examination every 3-6 months (until the second year of life) and then every 6-12 months. After approval of onasemnogene abeparvovec ADHCNS added a recommendation for immediate treatment in newborns with 4 copies of the SMN2 gene. However, the expert group report on screening for SMA showed that early diagnosis of the disease may be difficult in centers with less experience in the care of patients with SMA and due to insurance authorization for treatment, which also may further delay initiation of treatment. The authors of the publication emphasize that each day of delay worsens the effects of treatment and therefore reduces the chances of achieving motor skills by children [15,16].

Jędrzejowska points out that the success of treatment depends largely on the age of the patient who starts treatment - hence a better prognosis for patients with a shorter duration of the disease (therefore the important role of screening). The scientist points out that the introduction of treatment must be introduced with the viability of the motor neurons, which will allow to recreate the physiological processes in which SMN participates. The duration of treatment depends on the amount of SMN protein. Jędzejowska mentions that, depending on the age of treatment implementation in children with type 1 SMA, even 30–60% of patients treated after the onset of disease symptoms achieve the ability to sit independently, while the implementation of treatment before the onset of symptoms allows for full motor development in most children. Therefore, screening and prompt treatment after mutation detection allows for the proper development of people with SMA. According to the author, it is also important to consider research on SMA carrier status in the future [9].

Referring to the publication by Govoni et al. the timing of treatment implementation is especially significant in type I SMA, as most degenerative changes occur in the first months of life. Although the therapeutic window for SMA has not yet been established is considered to be "time is motor neuron" and more extensive newborn screening is needed for faster diagnosis and treatment implementation [17]. Keinath et al. come to similar conclusion - screening for SMA makes it possible to detect mutations and enable treatment when motor neurons have not yet been lost [18].

Vill et al. assessed the impact of newborn screening for SMA in Germany and the introduction of early treatment on the neuromotor development of children. Using the PCR method, 43 patients with a mutation in SMN1 were identified, and then the number of SMN2 copies was determined by the MLPA method. In addition, neurophysiological and physiotherapeutic studies were carried out. In 39.5%, 2 copies of SMN2 were identified, 23% - 3 copies of SMN2, 32.5% - 4 copies, and 4.5% - 5 copies of the SMN2 gene. In patients who were treated before the onset of symptoms, no symptoms of disease development were noticed during the observation. In 47% of patients with two copies of SMN2, early onset of the disease was observed, and therefore motor development was delayed. In untreated children, the course of the disease was more dramatic - children with two copies of SMN2 died, while those with three copies of SMN2 developed proximal weakness within one year of life. Watchful waiting strategies were used in patients with an SMN2 copy count above 4. Two infants (siblings) with 4 copies of SMN2 were identified with a missed diagnosis of type 3 SMA. The results of the study showed that early diagnosis of SMA and implementation of immediate treatment

significantly improved neurodevelopmental outcomes in children. In addition, no false-positive or false-negative results were demonstrated in this study, which proves the high sensitivity and specificity of SMA screening tests. It is important that the interval between diagnosis and treatment is sufficiently short, which will improve the treatment effect. The authors highly recommend introducing genetic screening tests for SMA in more countries and emphasize their importance [19].

The importance of SMA screening tests is also emphasized by Glascock et al. - early identification of mutations in newborns in screening tests maximizes therapeutic effects thanks to treatment already before the onset of symptoms. According to the research group, children with identified 2 or 3 copies of the SMN2 gene should be treated immediately, while children with 4 or more copies of SMN2 for whom treatment is not recommended should be monitored - screened and tested to determine the timing of treatment. - these children have a milder form of SMA than children with fewer copies of SMN2 [20].

Kimizu et al. believe that to maximize the effectiveness of medications for SMA, treatment should be started at the presymptomatic stage of SMA, hence the high recommendation for screening for SMA. The experience of scientists in conducting genetic studies (PCR and/or MLPA) in Japan showed a long delay in the diagnosis of SMA - 515 patients (in age from several days to 63 years) with suspected SMA or other motor neuron disease were examined and 228 of them showed a homozygous SMN1 deletion. Among 221 patients with a homozygous SMN1 deletion (and current clinical information) 42,1% of patients were diagnosed with SMA type I, 32,1% type II, 20,8% type III, and 5,0% type IV. The mean age in the genetic testing for SMA was 11.0 months for type I, 77.3 months for type II and 85.1 months for type III. According to the study, only 20.9% of patients with SMA type II were diagnosed in the proper time (according to scientists „proper" time for SMA type II is earlier than 18 months), while the rate for patients with SMA type I was 65.5% (the proper time, according to scientists, is up to 6 months after birth for SMA type I). For these reasons, scientists call for the implementation of screening for SMA, which will enable early treatment and maximum therapeutic benefit [21].

Conclusion

Newborn screening tests enable early detection and implementation of treatment in people with SMA (also pre-symptomatic). High sensitivity, specificity and low price allow for massive implementation of these tests. The relatively low prevalence of screening tests for SMA

in the world means that many people are diagnosed too late, which makes it difficult to achieve motor skills and proper functioning. Early diagnosis significantly increases the chances of a child's proper development. As Govoni said - "time is motor neuron" - we should diagnose SMA as early as possible, when the motor neurons are not yet completely degenerated.

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