UK National Screening Committee

# Screening for spinal muscular atrophy

## External review against programme appraisal criteria for the UK National Screening Committee (UK NSC)

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Conditions are reviewed against evidence review criteria according to the UK NSC's evidence review process.

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# Plain English summary

SMA is a genetic disease that makes muscles weak. It can get worse over time. SMA can be fatal if it affects the muscles that control breathing. There are 5 different types of SMA, from type 0 (the most severe, affects newborn babies and it is often fatal before the age of 18 months) to type 4 (stable and mild disease, affects adults and usually only causes mild problems). About half of all SMA patients have type 1 SMA.

Screening for SMA has been suggested. There are three ways that SMA screening could be carried out:

- screening adults to see if they have the gene mutation most commonly linked to SMA, but who are not affected by it, i.e. they are 'carriers'. If two parents have the gene, there is a 1 in 4 chance that their baby will have SMA
- screening babies before they are born to see if they have SMA
- screening newborn babies to see if they have SMA

The UK National Screening Committee (NSC) last looked at the evidence about SMA in 2013. They decided that there was not enough evidence to introduce a screening programme in the UK.

This was because:

- there was not enough information on the number of people affected by SMA in the UK
- there was very limited evidence about how acceptable a screening programme would be
- there was no evidence on how to support individuals who need to make difficult decisions following screening
- there was a lack of information on the reliability of screening tests for SMA
- no effective treatments for SMA were identified

The aim of this review was to look at new evidence and decide whether the current recommendation should change. The review looked at evidence for patients with 5q SMA only, which is the most common form of SMA. Evidence on a new SMA treatment called nusinersen was identified. Studies have shown that nusinersen can improve symptoms in children with SMA. However, this review was not able to assess evidence on the effectiveness of nusinersen in children without symptoms. This is because the evidence in children without symptoms is still emerging and has not yet been published in high quality article types that can be included in this review. There is also no evidence on the long term effects of nusinersen. In general, the evidence found in this review is not enough to change the previous recommendation. There is still not enough evidence that screening for SMA would be more helpful than harmful.

# Executive summary

## Purpose of the review

This rapid review examines the evidence concerning screening for spinal muscular atrophy (SMA) in the UK. Specifically, the review examines evidence relating to 5q SMA only (i.e. forms of SMA not associated with *SMN* mutations are not assessed within the scope of this review). The review is an update to a previous UK NSC review, which was published in 2013.

## Background

SMA is an autosomal recessive disease, the second most common of this kind after cystic fibrosis.[1] It involves degeneration of the alpha motor neurons in the spinal cord, leading to symmetrical muscle weakness, atrophy and paralysis in late-stage disease of the most severe types.[2] The impact upon the muscles used to support breathing can have lethal consequences.[3]

SMA is traditionally categorised into 5 different types, spanning from type 0 (the most severe) to type 4 (stable and mild disease). Type 1, also referred to as Werdnig-Hoffman disease, is the most common, accounting for approximately 50% of incident cases of SMA.[2] In spite of this categorisation system, there remains a large degree of overlap between the types.

Most cases of SMA (95%) are caused by mutations in survival motor neuron *(SMN)*[*] genes, which code for the SMN protein. The vast majority can be attributed to a homozygous deletion of the *SMN1* gene in exons 7 (and exon 8 in the majority of cases).[2] Other possible causes include *SMN1* mutation, or "compound heterozygosity" where one copy of *SMN1* is deleted and the other has a mutation leading to loss of function.[4-6] Overall, these genetic changes lead to a decrease in functional SMN protein and ultimately lead to patients developing SMA.[4-6] The related *SMN2* gene can partially compensate for deletions or mutations in *SMN1*, with a higher number of *SMN2* copies generally correlating with reduced disease severity.

In the UK, there are currently no disease-modifying treatments routinely available for use by the National Health Service (NHS) for SMA, and management involves a holistic approach to disease symptoms. However, the Food and Drug Administration (FDA) in the US and the European Medicines Agency (EMA) in Europe recently approved nusinersen (Spinraza™), an antisense

[*] Note that, in line with convention, italics are used to denote names of genes (e.g. *SMN1* and *SMN2*), whereas regular font is used to denote names of proteins (e.g. SMN)

oligonucleotide (ASO)† for the treatment of both mild and severe SMA.[7, 8] Phase 3 data for this intervention has been recently published, demonstrating that nusinersen showed statistically significant improvements on motor function compared to sham control.[9, 10] In 2018 a UK Expanded Access Programme (EAP) was set up to provide access to nusinersen for eligible children with SMA type I.[11] Currently, all infants diagnosed with SMA type 1 (caused by mutations in *SMN1* only) before 7 months will be treated with nusinersen. In May 2018, the Scottish Medicines Consortium (SMC) reimbursed nusinersen for restricted use in patients with type 1 SMA.[12] The National Institute for Health and Care Excellence (NICE) is conducting a Single Technology Appraisal of nusinersen for treating SMA, with the aim of assessing the clinical and cost-effectiveness of nusinersen within its marketing authorisation. Following a public consultation in August 2018, the committee proposed that nusinersen should not be recommended, within its marketing authorisation, for treating 5q SMA.[13] After the consultation, NICE informed the stakeholders in November 2018 that the appraisal committee was not in a position to provide the outcome of their deliberations. Discussions with Biogen and NHS England remain ongoing and NICE will keep all those involved up to date regarding progress.[14]

Three different approaches have been proposed for SMA screening: carrier screening in adults of child-bearing age, antenatal screening (involving both carrier screening and diagnostic testing of the foetus where indicated) and newborn screening. Screening of adults of child-bearing age aims to identify individuals that carry a mutated *SMN1* gene, but are not affected by the disease themselves. If both parents are SMA carriers then the risk of their offspring having SMA, being an asymptomatic carrier, or being unaffected (not a carrier) are 25%, 50% and 25%, respectively. Antenatal and newborn screening, however, both aim to identify babies and fetuses who have SMA, rather than carriers. All current approaches to screening are limited in their ability to predict the type of SMA that an individual will develop and the severity of the disease. Whilst studies have shown that a higher *SMN2* copy number correlates with a milder clinical phenotype, it is not currently possible to accurately predict phenotype severity from genetic information alone, and various modifying factors have been proposed as having a role in determining SMA severity, e.g. plastin 3.[15, 16] However, treatment algorithms have been developed for SMA patients using *SMN2* copy number.[17] It is important that tests are accurate so that individuals can make informed decisions about their pregnancies or treatments.

## Focus of the review

The current review update aims to synthesise and appraise the available evidence published since August 2012 (when the previous UK NSC review search was completed) concerning the viability,

† As an ASO, nusinersen binds to the pre-messenger ribonucleic acid (pre-mRNA) for *SMN2* and modifies its splicing to promote increased production of full-length SMN protein

effectiveness and appropriateness of any of the screening pathways for 5q SMA in a UK population. The review searched for evidence under 7 key questions, each relating to specific UK NSC criteria for screening recommendation:

- What is the prevalence of SMA in the UK? (criterion 1)
- What is the optimal test for carrier screening for SMA? (criterion 4)
- What are the reported outcomes of SMA carrier screening programmes? (criterion 11)
- What are the reported outcomes of SMA antenatal screening programmes? (criterion 11)
- Is there a simple, precise and validated screening test for newborn screening for SMA? (criterion 4)
- What is the optimal diagnostic pathway for screen-detected SMA newborns? (criterion 9)
- What is the reported effectiveness of pharmacological treatment for SMA? (criterion 10)

*Initially, as with the 2013 UK NSC review, the scope of this review update included all types of SMA. However, in response to comments received during the stakeholder consultation process, the UK NSC agreed to amend the scope of the review to consider evidence relating to 5q SMA only (i.e. forms of SMA not associated with SMN mutations are not assessed within the scope of this review).*

## Recommendation under review

A previous review in 2013 summarised the available evidence concerning screening in SMA.[18] It found that there was insufficient evidence on the epidemiology of SMA, including the number of people affected by the disease and its specific types. There were also no reliable carrier screening methods available at the time, and there was a lack of information about the acceptability of any type of screening programme and the psychosocial implications of screen-detected carrier status. Concerns about the inability of antenatal and neonatal tests to identify the severity of the disease were also discussed as this could substantially impact the prognosis of an affected individual. The authors concluded that if reliable carrier screening methods were not available, screening may not help people make decisions about whether or not to have children and may make it difficult for health professionals to offer advice.

The 2013 review found no effective treatments and no cure for SMA of any type; however, the first consensus statement for SMA in 2007 was identified,[19] which recognised management of the symptoms of SMA as the current standard of care. Finally, evidence regarding the effectiveness of SMA screening was scarce, with no RCTs and only a small number of population-based studies on this topic.

Based on the findings of the 2013 review, the UK NSC determined that a national screening programme for SMA should not be recommended in either adults or pregnant women.

## Findings and gaps in the evidence of this review

Within the scope of this review, no studies were identified relating to questions 3, 4 or 6 on the outcomes of different screening programmes and optimal diagnostic pathways for SMA. One study was relevant to question 1, 2 studies to question 2, 4 studies to question 5 and 5 studies to question 7.

*Criterion 1: The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease.*

Criterion 1 is not met following this review update. A single study reported the incidence of SMA in the UK as 10.9 cases per 100,000 live births. However, the incidence was not consistent with the incidence reported by a previous study conducted in the north-east of England (4.15 per 100,000 births). It is unclear if the results from this study are more valid than the previous study.

Therefore, in line with the findings of the previous review, there is still insufficient information about the total number of people affected by SMA or how many people are affected by each type of SMA. It is also not yet possible to accurately determine from an individual's genotype whether they will be mildly or severely affected by SMA.

*Criterion 4: There should be a simple, safe, precise and validated screening test.*

Criterion 4 is not met following this review update for either carrier screening or newborn screening. Two studies reporting on the accuracy of SMA carrier screening tests found that, although methods for the identification of *SMN1* copy numbers in potential carriers are promising, there are limitations to the identification of all SMA carriers. Limitations include concern about the applicability of the studies to the review questions due to the inclusion of non-randomly recruited study populations.

Four studies reported on SMA newborn screening tests. Two studies found that mCOP-PCR and HRM analysis are highly sensitive and specific newborn SMA screening methods. These studies were not of high quality as they did not use an adequate reference standard and they did not test populations representative of a general population screening programme. Therefore, the evidence base for criterion 4 indicates it is not possible to robustly quantify the accuracy of screening methods for SMA carriers and neonates.

*Criterion 9: There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared*

*with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme shouldn't be further considered*

*Criterion 10: There should be agreed evidence based policies covering which individuals should be offered interventions and the appropriate intervention to be offered*

Five RCTs reported outcomes of treatment for SMA. Olesoxime, valproic acid and somatropin were investigated in one RCT each but were not found to be effective treatments for SMA. Two RCTs investigated the use of nusinersen compared to sham control. Despite the small volume of evidence identified, there is now data suggesting that nusinersen is effective in improving outcomes for patients with SMA.[10, 20] However, there is still insufficient evidence that pre-symptomatic treatment is more beneficial than normal care at the current time and there is a lack of long-term efficacy and safety data. Therefore, criteria 9 and 10 are not yet met.

This review did not identify any prospective studies relating to carrier or antenatal screening programmes for SMA. Therefore, there is little robust evidence to show positive outcomes of screening programmes, apart from the small population studies identified in the previous review.

## Recommendations on screening

This updated analysis of the evidence for a population-wide carrier screening programme for SMA against the UK NSC criteria did not identify sufficient evidence to support a change in the previous recommendation. The main reasons for this are poor-quality evidence as to the effectiveness of screening programmes in the UK population, and the lack of a clear diagnostic and treatment pathway involving a screening programme.

### Limitations

This review was limited to peer-reviewed literature published in English since August 2012 that was freely available. For articles that were not freely available, additional publications were acquired, where possible, from the Cambridge University Library. The previous UK NSC review did not investigate newborn screening, so studies on newborn screening published prior to August 2012 have not been captured as part of this review (as the database searches only identified literature published after August 2012).

Articles were screened by a single reviewer. A second reviewer examined all included articles, 10% of excluded articles and any articles where there was uncertainty about inclusion or exclusion. Although less thorough than a full systematic review, this should still ensure that any articles where the eligibility was unclear were reviewed twice.

## Evidence uncertainties

Overall, the evidence base on the epidemiology of SMA is still limited. There is a substantial evidence gap related to the number of people affected by SMA and each of its types in the UK. There is also a lack of evidence on the relationship between SMA genotype and clinical prognosis.

Although there are encouraging results for the identification of carriers (with tests showing high sensitivity and specificity for the identification of some types of carriers), the evidence identified is limited, particularly as the proposed methods have an inherent risk of false negative test results. Therefore, more research is needed to extend the ability of screening tests to identify all types of carrier status.

Similarly, newborn screening tests are also unable to identify all types of SMA, and tests that cover all types need to be developed. Furthermore, the volume of evidence on the screening tests in newborns was small and many of the studies did not evaluate screening tests in a randomly recruited and representative population. High-quality prospective studies are needed to evaluate newborn screening tests in the general population.

There was encouraging evidence that a new drug, nusinersen, is effective in improving outcomes for patients with SMA. However, the volume of evidence is low, the long-term efficacy and safety of nusinersen is currently unknown and there is a lack of information on the effectiveness of nusinersen specifically on screen-detected populations.

No evidence was found evaluating the outcomes of different screening programmes for carrier status, and one study (conducted in Taiwan) evaluated outcomes of newborn screening. Further interventional and prospective observational studies, in populations with similar demographics to the UK, are therefore needed to evaluate the effectiveness and impact of such screening programmes.

# Introduction and approach

## Background

### Disease background

Spinal muscular atrophy (SMA) is a neurodegenerative disease in which the most severe types are associated with symmetrical muscle weakness, atrophy and paralysis in late-stage disease.[2] The burden of disease for patients can be high, and can also impact the family of the affected individual, especially in patients with more severe types of SMA, which are nearly always fatal in infancy. Family members may be affected emotionally and financially due to caring for a child with SMA. For example, seeing their child's health deteriorate as well as employment and social constraints can dramatically reduce the quality of life of carers and families.[21]

SMA patients experience muscle weakness which can dramatically limit mobility and daily activities, and increase the risk of pain from joint contractures and other orthopaedic complications (scoliosis).[22] For example, patients often have difficulty with activities such as walking, eating and writing, often requiring assistance from parents or caregivers.[23, 24] Perhaps most importantly, the impact upon the muscles used to support breathing can be substantial, leading to lethality in the most severe types and a risk of chest infections across all types.[3] These symptoms generally occur due to progressive degeneration of nerve cells called alpha motor neurons in the spinal cord, which are required to transmit impulses from the brain to the muscles in the torso and limbs.[25]

SMA is an autosomal recessive disorder, the second most common disease of this kind after cystic fibrosis.[1] Among autosomal recessive diseases, SMA is the most common early fatal disorder, affecting around 1 in 6,000 live births in the UK, and has an estimated carrier prevalence of 1 in 34.[26] Whilst SMA is seen as a pan-ethnic disease, carrier frequencies can vary significantly between ethnic groups, with one review reporting carrier frequencies of approximately 1 in 50 in Caucasian and Asian populations, 1 in 76 in Hispanic populations and 1 in 100 in Black populations.[27]

SMA is traditionally categorised into 5 different types according to age of onset and disease severity (Table 1).[28] These span from type 0 (the most severe) to type 4 (stable and mild disease).[2] Type 1, also referred to as Werdnig-Hoffman disease, is the most common, accounting for approximately half of all incident cases of SMA.[2] Although both genotype and phenotype are used to diagnose a specific type of SMA, the disease is a continuous spectrum of symptoms with complex and interacting genetic causes. Therefore, there can be a large degree of overlap between SMA types and each type can have highly variable symptoms and prognoses.[29]

## Table 1: Summary of the types of spinal muscular atrophy

| SMA type (alternative disease name) | Age of onset | Clinical symptoms | Prognosis |
|---|---|---|---|
| Type 0 | *In utero*[2] | • Failure to swallow and breathe at birth, facial diplegia, joint contractures[2] | Death a few weeks after birth |
| Type 1 (Wernig-Hoffman) | 4 to 6 months[2, 22] | • Lack of head control, weak cry and cough[19]<br>• Feeding compromised by age of 1[19]<br>• Severe hypotonia and weakness in the trunk, limbs and eventually intercostal (rib) muscles[19] | Death from respiratory failure, or ventilation within 2 years[30] |
| Type 2 (Dubowitz disease) | 6 to 18 months[30] | • Slow to reach developmental milestones[2]<br>• Children can sit and sometimes stand but cannot walk unaided | Survival into adulthood[30] |
| Type 3 (Kugelberg-Welander) | 18 months to 3 years in 3a, after 3 years in 3b[2] | • Variable – from requiring a wheelchair to walking unaided, deterioration in puberty[31]<br>• Proximal muscles primarily affected by atrophy and weakness | Normal life expectancy[32] |
| Type 4 (adult-onset) | Second or third decade[2] | • Stable and mild disease, no respiratory or gastrointestinal problems[19]<br>• Flaccid hypotonia, fasciculations, muscular atrophy or deep-tendon reflexes[2]<br>• Ambulation throughout adulthood[33] | Normal life expectancy[33] |

## Genetics

Most cases of SMA (95%) are caused by mutations in survival motor neuron *(SMN)* genes,‡ which code for the SMN protein. These genes are found on human chromosome 5, with 2 genes (*SMN1* and *SMN2*) found at the chromosomal end (telomeric) and centre (centromeric) respectively. The main function of SMN appears to be in the splicing of pre-messenger ribonucleic acid (pre-mRNA). Although the underlying genetic changes occur in all cell types and SMN expression is ubiquitous, the process of pre-mRNA splicing is particularly important in motor neurons. This may explain why motor neurons appear to be preferentially affected in SMA.[34] The involvement of the motor neurons is the underlying cause of the neuromuscular symptoms that characterise SMA and lead to poor survival outcomes, particularly in the severe types.

*SMN1* and *SMN2* have very similar sequences, and the resultant SMN protein has the same function in each case. However, a single base pair difference in the genetic sequence between *SMN1* and *SMN2* means that *SMN2* is usually missing coding region (exon) 7 due to alternative splicing, and therefore mainly encodes a truncated protein.[35, 36] Therefore, only 10 to 20% of *SMN2* transcripts result in a fully functional protein.[37]

Up to 95% of all cases of SMA can be attributed to a homozygous deletion of the *SMN1* gene in exons 7 and 8.[2] Other possible causes include a mutation in *SMN1* that converts the gene into an *SMN2*-like gene (known as “gene conversion”), or a trait known as “compound heterozygosity” where one copy of *SMN1* is deleted and the other has a mutation leading to loss of function.[4-6]

Importantly, whatever the genetic cause, the result is a decrease in functional SMN protein. Due to the truncated nature of SMN encoded by *SMN2*, this protein is unable to completely compensate for the lack of SMN encoded by *SMN1*;[38] however the number of *SMN2* copies in the genome is known to inversely correlate with disease severity, suggesting some rescue of protein function by *SMN2* may occur.[39, 40] As a result, increasing copy numbers of *SMN2* are associated with milder types of SMA (Table 2).[37, 41]

‡ Note that, in line with convention, italics are used to denote names of genes (e.g. *SMN1* and *SMN2*), whereas regular font is used to denote names of proteins (e.g. SMN)

**Table 2: *SMN2* copy number incidence within each spinal muscular atrophy type**

| SMA type | *SMN2* copy number |
|---|---|
| Type 0 | 1 copy of *SMN2* |
| Type 1 (Wernig-Hoffman) | 1 or 2 copies of *SMN2* in 80% of patients |
| Type 2 (Dubowitz disease) | 3 copies of *SMN2* in <80% of patients |
| Type 3 (Kugel-Welander) | 3 or 4 copies of *SMN2* in 96% of patients |
| Type 4 (adult-onset) | 4 or more copies of *SMN2* |

## Treatment

Current therapies for SMA focus on management of disease symptoms in a holistic approach, depending on whether a patient is deemed a 'non-sitter', 'sitter' or 'walker'. Management can be categorised into pulmonary care; gastrointestinal and nutritional care; orthopaedic care and rehabilitation; and palliative care for the end of life.[19]

Finding a cure for SMA rather than simply managing the symptoms remains an active area of research worldwide. Avenues of investigation include gene therapy, molecular therapy and small molecule drugs, as well as muscular strength enhancers, neuroprotective factors and stem cell therapy.[2]

Recently, the Food and Drug Administration (FDA) in the US and the European Medicines Agency (EMA) in Europe approved nusinersen (Spinraza™), an antisense oligonucleotide (ASO) for the treatment of both mild and severe SMA.[7, 8] This therapy modulates the splicing of the *SMN2* gene, allowing inclusion of exon 7 and compensating for the lack of SMN protein that contributes to the symptoms of the disease. In 2018 a UK Expanded Access Programme (EAP) was set up to provide access to nusinersen for eligible children with SMA type I.[11] Currently, all infants diagnosed with SMA type 1 (caused by mutations in *SMN1* only) before 7 months will be treated with nusinersen. In May 2018, the Scottish Medicines Consortium (SMC) reimbursed nusinersen for restricted use in patients with type 1 SMA.[12] The National Institute for Health and Care Excellence (NICE) is conducting a Single Technology Appraisal of nusinersen for treating SMA, with the aim of assessing the clinical and cost-effectiveness of nusinersen within its marketing authorisation. Following a public consultation in August 2018, the committee proposed that nusinersen should not be recommended, within its marketing authorisation, for treating 5q SMA. After the consultation, NICE informed the stakeholders in November 2018 that the appraisal committee was not in a position to provide the outcome of their deliberations. Discussions with Biogen and NHS England remain ongoing and NICE will keep all those involved up to date regarding progress.[14]

Historically, the majority of clinical studies have involved reducing the severity of SMA in patients where degeneration is already apparent, with the aim of prolonging life and improving motor

function.[43] However, a recent trial (NURTURE [ClinicalTrials.gov registration NCT02386553]) is assessing infants with genetically diagnosed, pre-symptomatic SMA to prevent degeneration before it begins (evidence from the NURTURE trial is not included in the review as it is currently only published in conference abstracts, and this review only considers peer-reviewed publications).[44, 45] Interim results from the Phase II NURTURE study assessing the efficacy and safety of nusinersen in infants with pre-symptomatic SMA have shown there were improvements in mean Hammersmith Infant Neurological Examination (HINE) motor milestones scores verses baseline.[46] As this was a single-arm study, it was not designed to compare pre-symptomatic treatment to treatment that is initiated after symptoms start.

Phase 3 data for nusinersen has recently been published from the ENDEAR and CHERISH trials for both infantile-onset and later-onset SMA, respectively. In both trials, interim analysis revealed significant benefits of nusinersen versus sham control in terms of the primary endpoints. In results from the ENDEAR trial (Finkel et al [2017]) a significantly higher percentage of infants in the nusinersen group had a motor milestone response than in the control group (21/51 [41%] versus 0/27 [0%]; p<0.001) at interim analysis conducted on 15th June 2016 with all study participants having been enrolled for at least 6 months.[9] Similarly, there was a significant between-group difference favouring nusinersen in the CHERISH trial (Mercuri et al [2018]) when measuring least-squares mean change in the Hammersmith Functional Motor Scale Expanded (HFMSE) score at interim analysis, when all children had been enrolled for at least 6 months and at least 39 had completed a 15-month assessment (least-squares mean difference in change, 5.9 points; p<0.001).[10] This led to early termination of the trials and initiation of open-label extension studies.[9, 10]

## Screening

Three different approaches have been proposed for SMA screening:

- Carrier screening in adults of child-bearing age, also known as pre-conception genetic screening (PCGS)
- Antenatal screening
- Newborn screening

Each of these approaches is summarised below.

## Carrier screening

Among adults of child-bearing age, the aim of screening is to detect whether an individual is a carrier of SMA. The term ‘carrier’ is applicable due to the recessive nature of SMA, meaning that a person can carry the genetic risk factor for SMA, such as an *SMN1* deletion on one copy of

chromosome 5, but retain a functional copy of *SMN1* on their other chromosome 5. This functional copy means the individual will not be affected by the disease themselves. Carrier screening is typically carried out using gene dosage analysis, which can determine how many functional copies of *SMN1* an individual has. If both parents are SMA carriers then the risk of their offspring having SMA, being an asymptomatic carrier, or being unaffected (not a carrier) are 25%, 50% and 25% respectively. Carrier screening can enable adults to understand the risk of their offspring having SMA, and to make informed choices about reproduction accordingly. If both parents are found to be carriers of SMA, there are multiple options to consider. These include deciding not to conceive a child; being aware of the risk of a conceived child developing SMA, so that antenatal or newborn testing and treatment can be initiated early where applicable; choosing to use donor gametes from a non-carrier; pre-implantation genetic diagnosis (in vitro fertilisation followed by genetic analysis of embryos); or terminating an existing pregnancy (following prenatal diagnosis). Compared to prenatal screening, carrier screening provides couples with more reproductive options, such as preimplantation genetic diagnosis or adoption.

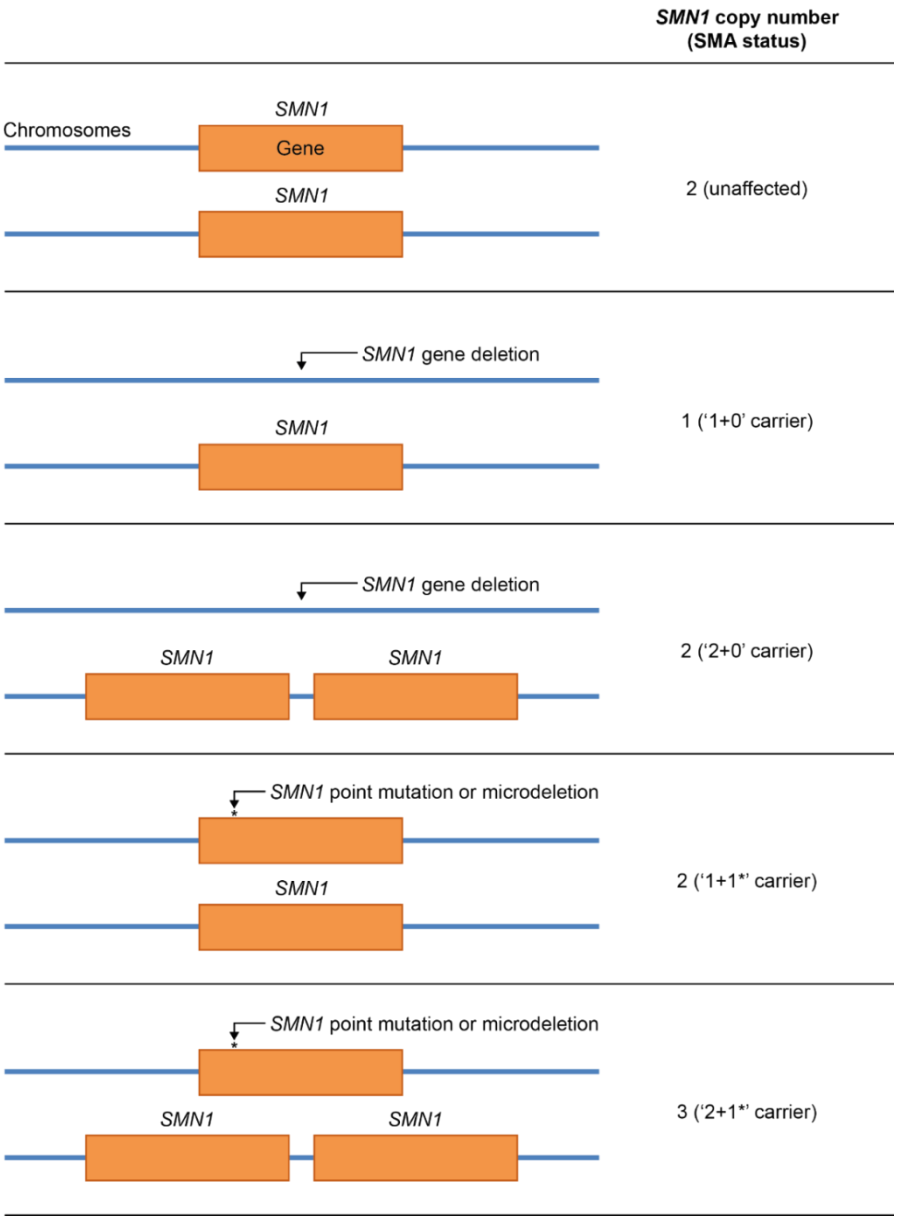
SMA carrier screening has limitations due to the genetic complexity underlying the disease. Carrier tests may not reveal individuals with rarer genotypes, for example, when there is more than one *SMN1* allele on the same chromosome and none on the other chromosome. Moreover, although 2 parents may be informed that they are both carriers and that there is therefore a 25% chance of their offspring being affected by SMA, it is not currently possible to predict SMA type on an individual basis.[47-49] Index cases[§] are often used to predict disease severity of future affected offspring in SMA-affected families. However, this also has limitations, for example variability of type within sibling groups is not uncommon. In relation to a general population screening programme, the lack of an index case would make the prognostic uncertainty particularly problematic.

Carrier testing also has disadvantages when conducted within some ethnic groups as the carrier detection rate may vary depending on the carrier genotype. There are 4 main carrier genotype arrangements for SMA (Figure 1).[50, 51] The most common of these is the ‘1+0’ genotype where there is a deletion of *SMN1* on one chromosome and therefore only one functional copy. The ‘2+0’ genotype describes when an individual has 2 functional copies of *SMN1* on one chromosome and none on the other. This genotype is rarer than the ‘1+0’ genotype, but prevalence varies depending on ethnicity. Lastly, in the ‘1+1*’ or ‘2+1*’ genotypes, there is a non-functional *SMN1* (indicated by the * after the copy number) due to microdeletion or point mutation alongside one or two functional copies, though both of these cases are rare.[52] The ‘2+0’ genotype is the main cause for discrepancy in screening results. If gene dosage analysis reveals a 2-copy result, then an individual could be a ‘1+1’ non-carrier or a ‘2+0’ carrier. In one particular meta-analysis, carrier screening usefulness was analysed within populations of various ethnicities. The sensitivity of

[§]Index case is defined as the first identified case in a group of related cases

detecting carrier status in the black population was only 71% due to a high incidence of '2+0' genotype carriers and subsequently high levels of false-negative results.[52]

**Figure 1: Carrier genotypes in spinal muscular atrophy (adapted from MacDonald et al 2014)[52]**




SMA, spinal muscular atrophy; *SMN,* survival motor neuron.

## Antenatal screening

Antenatal screening aims to identify babies and fetuses who have SMA, rather than carriers. Screening tests therefore typically look for homozygous loss (loss of both copies) of *SMN1*, the most common genetic change underlying the condition. Additionally, the test should ideally be able to detect compound heterozygosity, where individuals have a mutation in one copy of *SMN1* and a deletion of the other copy.

The review only identified evidence on antenatal screening that involves invasive procedures to gather fetal DNA, followed by genetic analysis in the form of restriction fragment length polymorphism (RFLP) testing, multiplex ligation-dependent probe amplification (MLPA) or quantitative polymerase chain reaction (qPCR).[53] However, these invasive processes carry a 0.5 to 1% chance of miscarriage.[54, 55] More recently, research suggests that screening using non-invasive procedures on maternal plasma samples known to contain cell-free fetal DNA (cffDNA) may be feasible for determining gene dosage and therefore reduce the risk of miscarriage. Although this may be an option for families already affected by SMA due to the presence of an index case, it may be some time before this can be used in general population screening. It is important to consider though, that diagnosis at this stage cannot accurately determine how the disease will clinically manifest; the same number of copies of *SMN2* may translate to disease of differing severity (that is, different types) in different patients, limiting the predictive power of antenatal analysis.[2] This is important when considering the implications of antenatal screening as decisions concerning termination of pregnancy may rest on the potentially ambiguous results of a molecular test.

A survey of families affected by SMA found that 75% of families were in favour of screening in some form. However, they had concerns including carrier stigmatisation, social engineering and, for antenatal screening in particular, the risk of termination when a high quality of life could still potentially be achieved.[25] Therefore, if screening were to be implemented, the provision of genetic counselling should be carefully timed and given appropriately.[56]

## Newborn screening

As with antenatal screening, newborn screening aims to identify babies and fetuses who have SMA, rather than SMA carriers. Therefore, tests typically look for homozygous loss of *SMN1*.

Screening of newborns has the benefit of potentially identifying individuals with SMA before disease onset, allowing for identification of those who would benefit from novel molecular and genetic therapies.[57] Newborn dried blood spot (DBS) screening is one technique that can be used to detect SMA at this stage, but a subsequent diagnostic test is required for confirmation in all cases. Such tests include single-strand conformation polymorphism (SSCP), RFLP, denaturing high-performance liquid chromatography (DHPLC), MLPA and competitive PCR.[56]

Various ethical implications could result from the introduction of a screening programme, whether this is carrier screening, antenatal screening or newborn screening; therefore, population-based pilot studies have been conducted investigating these social issues. One survey showed overwhelming support from expectant couples for newborn DBS screening for SMA, even considering a lack of treatment development.[58] Additionally, a further study reported that 84% of individuals surveyed from a total of 232 members of the general public, who had no prior relationship to SMA, were in favour of newborn screening.[59]

Evidence from one study suggests that the majority of parents would still want to know if their child had the disease at birth, even if it would affect their child's health and shorten their lifespan.[58] At present, as there is no cure for SMA and no way to accurately predict prognosis, for example using *SMN2* copy number, there is currently no clear consensus on the impact of parents receiving the news that their child is affected by SMA at birth, through newborn screening programmes, compared to at diagnosis, following the onset of symptoms.[25, 59] One study has suggested that a diagnosis at birth hastens parental grief and allows the family to more quickly come to terms with their child's condition, and the implications it has for their family's future.[60] Other evidence has reported that a diagnosis through newborn screening, before symptom onset, may have a negative impact on parents and that the first weeks and months following their child's birth can be overshadowed by anxiety, shock and grief which can interfere with the bonding process.[61]

An additional consideration regarding the implementation of SMA newborn screening would be whether or not this should be an opt-in or opt-out programme. Evidence from one study suggests that there is support for an opt-out approach considering the UK newborn screening programme already utilises this approach.[62] Additionally, there was support that, given there are finite resources within public services, an opt-in approach may require more resource through further education and time commitments from healthcare professionals, and therefore an opt-out approach was preferred. A newborn screening programme would also not require additional blood draws or burdensome procedures for the babies and their families, as samples would already be taken for the other newborn screening tests. One implication of newborn screening could be the risk of a false positive test and the impact this may have on families and the initiation of any unnecessary, and potentially invasive, treatment. However, a number of studies have also used second-tier screening methods to confirm positive results. For example, Chien et al. 2017 used real-time PCR (RT-PCR) to screen for SMA-positive patients, with all positive screening results confirmed using droplet digital PCR (ddPCR).[63]

Newborn or antenatal tests also have limitations. Parents would immediately become aware that they are SMA carriers following a positive newborn or antenatal screening test. This may cause anxiety and stress to parents when planning future pregnancies. However it should be noted that, even in the absence of newborn screening tests, parents would likely become aware that there is a high probability they are carriers upon diagnosis of their child, following symptom onset. However,

parents being aware of their carrier status allows them to consider other reproductive options, such as pre-conception genetic screening, for any future pregnancies.[64]

Additionally, individuals carrying a fetus diagnosed with SMA may choose to terminate the pregnancy even though it is unclear how severe the disease would be in the child. For example, some individuals with homozygous deletions or gene conversions in *SMN1* are unaffected by SMA symptoms, due to adequate expression of SMN protein encoded by *SMN2*.[49, 65] There is also a small risk of overdiagnosis, which is the diagnosis of a condition that would not have caused symptoms during an individual's lifetime. A newborn diagnosed with SMA may be treated immediately after birth with invasive treatments such as nusinersen, which is administered via spinal injections. For a small proportion of patients that have a less severe form of the disease, such as type 4 patients (approximately <5%),[27] they might receive invasive treatments but only develop a milder form of the disease that does not require extensive treatment. Approximately 2% of patients with *de novo* mutations in *SMN1*,[15] (that is, mutations that occur for the first time as a result of a variant in a germ cell or shortly after fertilisation, and so present for the first time in one family member), are at risk of false negative results from antenatal or newborn tests.

## Current policy context and previous reviews

A previous review was conducted for the UK NSC in 2013, with the aim of summarising the available evidence concerning antenatal and carrier screening in SMA.[18]

The 2013 evidence identified dosage analysis as the main method by which to determine carrier status; however, it found that there are limitations associated with this method. These primarily stem from the challenges in distinguishing between different genotypes and identifying specific gene mutations, all of which are known causes of SMA. The concern was raised that if reliable screening methods were not identified, it may not help people make decisions about whether to have children and it will be difficult for health professionals to offer advice.

Evidence regarding the effectiveness of SMA screening was scarce, with no RCTs and only a small number of population-based studies on this topic. Concerns about the inability of tests to identify the severity of the disease were also discussed, since this could substantially impact the prognosis of a child. No effective treatments and no cure for SMA of any type were identified. However, the first consensus statement for SMA in 2007 was identified, which suggested that disease management and treatment have begun to be considered.[19] The consensus statement recognised management of SMA symptoms as the current standard of care. Insufficient evidence was identified on the epidemiology of SMA, including the number of people affected by the disease overall and the specific disease types, the acceptability of screening and the psychosocial implications of screen-detected carrier status.

The stakeholder consultation following publication of the 2013 review also highlighted some areas that would benefit from more clarity. In particular, it was suggested that a very considered and consistent approach to patient education and genetic counselling would be required if a programme were to be implemented due to the complicated molecular genetics of SMA. In particular, time and resources would be required to explain risks and assist with informed decisions for the parents.

Based on the findings of the 2013 review, the UK NSC determined that a national screening programme for SMA should not be recommended in either adults or pregnant women.

## Objectives

Following on from the conclusions in the 2013 review, the update will assess the quality and volume of evidence published since July 2012 (when the searches for the previous review were conducted).

The current review aims to update the 2013 evidence review and assess whether there is any evidence for reconsidering the current screening recommendations for 5q SMA. The review will appraise evidence on the questions presented in table 3, which each relate to the criteria set out by the UK NSC for assessing the suitability of a screening program.

**Table 3: Key questions for the evidence summary, and relationship to UK NSC screening criteria**

| | Criterion | Key questions | Number of publications included |
|---|---|---|---|
| | **THE CONDITION** | | |
| **1** | The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease. | What is the prevalence or incidence of SMA in the UK?<br>• What is the prevalence of mutations in the *SMN1*, *UBA1, DYNCH1*, and *VAPB* genes among patients affected by SMA in the UK? | 0 |
| | **THE TEST** | | |
| **4** | There should be a simple, safe, precise and validated screening test. | What is the optimal test for carrier screening for SMA?<br>• Are there any non-genetic tests being used as carrier tests for SMA? | 2 |
| | | Is there a simple, precise and validated screening test for newborn screening for SMA? | 4 |

| | Criterion | Key questions | Number of publications included |
|---|---|---|---|
| | | This question will examine:<br>• Test accuracy outcomes<br>• Acceptability of the test (screening and diagnostic) | |
| | **THE INTERVENTION** | | |
| **9** | There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme shouldn't be further considered. | What is the optimal diagnostic pathway for screen-detected SMA newborns?<br>• Is the test able to give information on the severity of the condition? | 0 |
| **10** | There should be agreed evidence based policies covering which individuals should be offered interventions and the appropriate intervention to be offered. | What is the reported effectiveness of pharmacological treatment for SMA?<br>• Is the pharmacological treatment equally effective for all SMA types? | 5 |
| | **THE SCREENING PROGRAMME** | | |
| **11** | There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (eg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened. | What are the reported outcomes of SMA carrier screening programmes?<br>This question will examine:<br>• Test accuracy outcomes<br>• Uptake of the test | 0 |
| | | What are the reported outcomes of SMA antenatal screening programmes?<br>This question will examine:<br>• Test accuracy outcomes<br>• Uptake of the test | 0 |

## Methods

The current review was conducted by Costello Medical in collaboration with the UK NSC, in keeping with the UK NSC evidence review process. The search strategy is presented in Appendix 1, and the methods of study selection (including full eligibility criteria and quality assessment checklists used) are detailed below.

## Eligibility for inclusion in the review

The following review process was followed:

1. Each abstract was reviewed against the inclusion/exclusion criteria by one reviewer. Where the applicability of the inclusion criteria was unclear, the article was included at that stage in order to ensure that all potentially relevant studies were captured. A second independent reviewer provided input in cases of uncertainty, and validated the first reviewer's screening decisions for all included studies and 10% of excluded studies. Any disagreements were resolved by discussion until a consensus was met.
2. A search for freely available full-text articles required for the full-text review stage was conducted, and where possible, additional publications were acquired from the Cambridge University Library.
3. Each full-text article was reviewed against the inclusion/exclusion criteria by one reviewer, who determined whether the article was relevant to one or more of the review questions. A second independent reviewer provided input in cases of uncertainty, and validated the first reviewer's screening decisions for all included studies and 10% of excluded studies. Any disagreements were resolved by discussion until a consensus was met.

Eligibility criteria for each question are presented in Table 4 to Table 10 below.

## Table 4: Inclusion and exclusion criteria for question 1

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Question** | What is the prevalence or incidence of SMA in the UK? | |
| **Sub-question** | What is the prevalence of mutations in the *SMN1*, *UBA1*, *DYNC1H1*, and *VAPB* genes among patients affected by SMA in the UK? | |
| **Population** | Individuals with SMA in the UK | • Individuals who do not have SMA<br>• Individuals who do not live in the UK |
| **Intervention** | Any or none | - |
| **Comparator** | Any or none | - |
| **Outcomes** | • Prevalence or incidence of SMA<br>• Prevalence of mutations in the *SMN1*, *UBA1*, *DYNC1H1*, and *VAPB* genes<br>• Association of each mutation with SMA types | - |
| **Study design/publication type** | Peer-reviewed evidence from:<br>• Interventional studies (including RCTs)[a]<br>• Observational studies (prospective or retrospective)<br>• Case control studies[b]<br>• Systematic reviews and meta-analyses of the above study designs | • Narrative reviews, commentaries or letters<br>• Conference abstracts or other publication types that have not been peer-reviewed |
| **Language** | English language | Non-English language |
| **UK NSC criteria** | 1. The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease. | |

RCT: randomised controlled trial; SMA: spinal muscular atrophy
[a]For data on prevalence of mutations within a group of SMA patients only. [b]For data on association of mutations with SMA types only.

## Table 5: Inclusion and exclusion criteria for question 2

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Question** | What is the optimal test for carrier screening for SMA? | |
| **Sub-question** | Are there any non-genetic tests being used as carrier tests for SMA? | |
| **Population** | Pregnant and non-pregnant adults | Children (individuals aged under 18) |
| **Intervention** | Tests using molecular genetic analysis including:<br>• Competitive PCR<br>• Absolute quantitative PCR-relative quantitative PCR<br>• Denaturing high-performance liquid chromatography (DHPLC)<br>• High-resolution melting analysis<br>• Multiplex ligation probe amplification (MLPA)<br>• Digital PCR<br>Tests using molecular markers for SMA | Tests not used to screen for SMA carriers |
| **Reference Standard** | Western blot and immunohistochemical analyses[a] | Tests that are not western blots or immunohistochemical analyses |
| **Outcomes** | Clinical performance measures including:<br>• Sensitivity<br>• Specificity<br>• False positive rate<br>• False negative rate<br>• PPV/NPV | Outcomes that are not clinical performance measures |
| **Study design/publication type** | Peer-reviewed evidence from studies with randomly or consecutively enrolled populations including:<br>• Interventional studies (including RCTs) where the intervention is a screening test<br>• Observational studies (prospective, retrospective or case control)<br>• Systematic reviews and meta-analyses of the above study | • Narrative reviews, commentaries or letters<br>• Conference abstracts or other publication types that have not been peer-reviewed |

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| | designs<br>If the above study types are not available, summary of case control studies | |
| **Language** | English language | Non-English language |
| **UK NSC criteria** | 4. There should be a simple, safe, precise and validated screening test | |

DHPLC: denaturing high-performance liquid chromatography; NPV: negative predictive value; MLPA: Multiplex ligation probe amplification; PCR: polymerase chain reaction; PPV: positive predictive value; RCT: randomised controlled trial; SMA: spinal muscular atrophy

[a]No studies using the specified reference standard were identified; therefore, a post hoc protocol modification was made to include studies using any reference standard

## Table 6: Inclusion and exclusion criteria for question 3

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Question** | What are the reported outcomes of SMA carrier screening programmes? | |
| **Population** | Individuals that are planning pregnancy | • Individuals that are not planning a pregnancy<br>• Individuals that are currently pregnant |
| **Intervention** | Tests using molecular genetic and non-genetic analysis to determine whether the individual is a SMA carrier | Tests that are not used to determine whether the individual is a SMA carrier |
| **Comparator** | Any or none | - |
| **Outcomes** | • Uptake of SMA carrier screening (by gender)<br>• Number/percentage of couples who are both SMA carriers<br><br>Tests using molecular genetic analysis including:<br>• Competitive PCR<br>• Absolute quantitative PCR-relative quantitative PCR<br>• Denaturing high-performance liquid chromatography DHPLC)<br>• High-resolution melting analysis<br>• Multiplex ligation probe amplification (MLPA) | • Outcomes not describing the uptake of SMA carrier screening or the number/percentage of couples who are both SMA carriers<br>• Tests not used to screen for SMA carriers |

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| | • Digital PCR<br>Tests using molecular markers for SMA | |
| **Study design/publication type** | Peer-reviewed evidence from prospective population based studies including:<br>• Interventional studies (including RCTs)<br>• Prospective observational studies<br>• Systematic reviews and meta-analyses of the above study designs | • Retrospective or case control studies<br>• Narrative reviews, commentaries or letters<br>• Conference abstracts or other publication types that have not been peer-reviewed<br>• Retrospective studies |
| **Language** | English language | Non-English language |
| **UK NSC criteria** | 11. There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (such as Down's syndrome or cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened. | |

RCT: randomised controlled trial; SMA: spinal muscular atrophy

## Table 7: Inclusion and exclusion criteria for question 4

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Question** | What are the reported outcomes of SMA antenatal screening programmes? | |
| **Population** | Pregnant individuals | Individuals that are not pregnant |
| **Intervention** | • Tests using molecular genetic and non-genetic analysis to determine whether the individual is a SMA carrier<br>• Antenatal diagnostic tests for SMA | • Tests that are not used to determine whether the individual is a SMA carrier |
| **Comparator** | Any or none | - |
| **Outcomes** | • Uptake of SMA carrier screening (by gender)<br>• Uptake of SMA diagnostic testing<br>• Number of SMA affected fetuses | Outcomes that do not indicate carrier screening uptake, diagnostic testing uptake and fetuses with SMA |

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Study design/publication type** | Peer-reviewed evidence from prospective population-based studies including:<br>• Interventional studies (including RCTs)<br>• Prospective observational studies<br>• Systematic reviews and meta-analyses of the above study designs | • Retrospective or case control studies<br>• Narrative reviews, commentaries or letters<br>• Conference abstracts or other publication types that have not been peer-reviewed |
| **Language** | English language | Non-English language |
| **UK NSC criteria** | 11. There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (such as Down's syndrome or cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened. | |

RCT: randomised controlled trial; SMA: spinal muscular atrophy

## Table 8: Inclusion and exclusion criteria for question 5

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Question** | Is there a simple, precise and validated screening test for newborn screening for SMA? | |
| **Sub-question** | Is the test able to give information on the severity of the condition? | |
| **Population** | Neonates | Individuals that are not neonates |
| **Intervention** | Tests for SMA using DBS methodologies | Tests for SMA that do not use DBS methodologies |
| **Comparator** | Deletion test[a] | Non-deletion tests |
| **Outcomes** | Clinical performance measures including:<br>• Sensitivity<br>• Specificity<br>• False positive rate<br>• False negative rate<br>• PPV/NPV<br>Clinical measures indicating the severity of the condition | Outcomes that are not clinical performance measures or that do not indicate the severity of the condition |

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Study design/publication type** | Peer-reviewed evidence:<br>• Interventional studies (including RCTs) where the intervention is a screening test<br>• Observational studies (prospective, retrospective or case control studies)<br>• Systematic reviews and meta-analyses of the above study designs | • Narrative reviews, commentaries or letters<br>• Conference abstracts or other publication types that have not been peer-reviewed |
| **Language** | English language | Non-English language |
| **UK NSC criteria** | 4. There should be a simple, safe, precise and validated screening test | |

DBS: dried blood spot testing; NPV: negative predictive value; PPV: positive predictive value; RCT: randomised controlled trial; SMA: spinal muscular atrophy
[a]No studies using the specified reference standard were identified; therefore, a post hoc protocol modification was made to include studies using any reference standard

## Table 9: Inclusion and exclusion criteria for question 6

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Question** | What is the optimal diagnostic pathway for screen detected SMA newborns? | |
| **Population** | Individuals who had SMA detected through screening as a neonate | Individuals who were not detected with SMA during screening as neonates |
| **Intervention** | • Pharmacological interventions<br>• Non-pharmacological interventions<br>• None | - |
| **Comparator** | Individuals with SMA who are not screened, and receive normal care | • Individuals without SMA<br>• Individuals with screen detected SMA |
| **Outcomes** | Clinical and safety outcomes including:<br>• Quality of life<br>• Improved mobility (preventing joint stiffness, and improving flexibility and range of movement)<br>• Nutrition and feeding (avoiding problems such as dehydration and ensuring healthy | Outcomes that are not clinical or safety measures |

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| | development)<br>• Improved breathing<br>• Decrease in respiratory complications (fatal breathing problems caused by a weakening of the respiratory muscles and respiratory tract infections)<br>• Increased life expectancy | |
| **Study design/publication type** | Peer-reviewed evidence:<br>• Interventional studies (RCTs to be prioritised)<br>• Prospective comparative observational studies<br>• Systematic reviews and meta-analyses of the above study designs | • Retrospective or case control studies<br>• Narrative reviews, commentaries or letters<br>• Conference abstracts or other publication types that have not been peer-reviewed |
| **Language** | English language | Non-English language |
| **UK NSC criteria** | 9. There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme shouldn't be further considered. | |

RCT: randomised controlled trial; SMA: spinal muscular atrophy

## Table 10: Inclusion and exclusion criteria for question 7

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Question** | What is the reported effectiveness of pharmacological treatment for SMA? | |
| **Sub-question** | Is the pharmacological treatment for SMA equally effective for all SMA types? | |
| **Population** | Individuals with SMA | Individuals who do not have SMA |
| **Intervention** | Pharmacological interventions | Non-pharmacological interventions or none |
| **Comparator** | Normal care | - |

| Domain | Inclusion criteria | Exclusion criteria |
|---|---|---|
| **Outcomes** | Clinical and safety outcomes including:<br>• Quality of life<br>• Improved mobility (preventing joint stiffness, and improving flexibility and range of movement)<br>• Improved breathing<br>• Nutrition and feeding (avoiding problems such as dehydration and ensuring healthy development)<br>• Improved breathing<br>• Decrease in respiratory complications (fatal breathing problems caused by a weakening of the respiratory muscles and respiratory tract infections)<br>• Increased life expectancy | Outcomes that are not clinical or safety measures |
| **Study design/publication type** | Peer-reviewed evidence:<br>• Interventional studies (RCTs to be prioritised)<br>• Prospective comparative observational studies<br>• Systematic reviews and meta-analyses of the above study designs | • Retrospective or case control studies<br>• Narrative reviews, commentaries or letters<br>• Conference abstracts or other publication types that have not been peer-reviewed |
| **Language** | English language | Non-English language |
| **UK NSC criteria** | 10. There should be agreed evidence-based policies covering which individuals should be offered interventions and the appropriate intervention to be offered. | |

RCT: randomised controlled trial; SMA: spinal muscular atrophy

## Appraisal for quality/risk of bias tool

The following tools were used to assess the quality and risk of bias of each study included in the review:

- Epidemiology studies: JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data[66]
- RCTs: Critical Appraisal Skills Programme (CASP) Randomised Controlled Trials Checklist[67]
- Studies reporting the accuracy of screening methods: Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool[68]

It was pre-specified that other study designs (e.g. cohort studies) would be assessed using the appropriate checklists provided by CASP; however, all identified studies were prevalence studies, RCTs or screening studies.

Results of the quality assessments are presented in Appendix 3.

## Databases/sources searched

The following electronic databases were searched:

- MEDLINE, including MEDLINE In-Process, MEDLINE Daily and Epub Ahead of Print
- Embase
- The Cochrane Library, including the following:
    - Cochrane Database of Systematic Reviews (CDSR)
    - Cochrane Central Register of Controlled Trials (CENTRAL)
    - Database of Abstracts of Reviews of Effects (DARE)

Searches were conducted in August 2017, with an updated literature search conducted in February 2018. Full details of the searches, including the search strategy for each database, are presented in Appendix 1.

# Question level synthesis

Database searches yielded 2179 results, of which 11 publications were ultimately selected for extraction and data synthesis across all questions. Appendix 2 contains a full PRISMA flow diagram (Figure 2), along with a table of the included publications and details of which questions these publications were relevant to (Table 21).

A study-level summary of data extracted from each included publication is presented in a 'Summary and appraisal of individual studies' in Appendix 3. Here, publications are stratified by question.

## *Criterion 1 – Epidemiology of spinal muscular atrophy*

Criterion 1 of the UK NSC Screening Criteria states that:

- 'The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease'

The epidemiology of SMA was examined in the previous UK NSC review, where they identified one study reporting disease incidence in the north-east of England as 1 in 24,119 live births, and carrier prevalence between 1 in 76 and 1 in 111.[18, 69] However, one conclusion of the review was that there is not enough information about the total number of people affected and how many people are affected by each type of SMA.[70] A recent literature review noted that most studies were performed in small populations, so a small change in the number of cases can have a large impact on the estimated prevalence; in addition, older studies used clinical (rather than genetic) diagnosis of SMA, which may have led to classification inaccuracies.[71] Therefore, the evidence base on the epidemiology of SMA was limited.

In this update, the reviewers searched for relevant data published since the 2013 review, specifically relating to the following questions:

*Question 1 – What is the prevalence or incidence of SMA in the UK?*
*Sub-question – What is the prevalence of mutations in the SMN1, UBA1, DYNC1H1, and VAPB genes among patients affected by SMA in the UK?*

## Epidemiology – eligibility for inclusion in the review

This review looked for interventional and observational studies, as well as systematic reviews and meta-analyses relevant to question 1. Interventional studies were only relevant if they provided data on the prevalence of mutations within a group of SMA patients. Publications were eligible for inclusion if they included information about the prevalence or incidence of SMA, mutations leading to SMA, or association of mutations to SMA types in UK populations.

## Epidemiology – description of the evidence

One study from the database searches was relevant to this question.[71] This was a registry study aiming to estimate the worldwide incidence of SMA.

## Epidemiology – summary of findings

The one study identified for this question recruited genetic testing laboratories to report the number of SMA cases they had diagnosed from 2011 to 2015, and combined this data with the birth rate from national statistics to calculate the birth prevalence of SMA in various European countries, including the UK.[72]

### Quality assessment

This study had several strengths: it was a large study of the general population, the investigators used various complementary approaches to ensure that all genetic laboratories testing for *SMN1* were identified, and the response rate from laboratories was high (11 of 12 UK laboratories provided data). However, there were a number of limitations in the methodology: in particular, it is unclear how each laboratory received samples from the population and the methods of testing used by each laboratory are not reported.[71] It should be noted that whilst the tests used in the study are able to identify patients with homozygous deletion of *SMN1* (~95% SMA patients), the remaining ~5% of patients with *de novo* mutations or point mutations may not have been identified and are therefore not included in the incidence calculations.

Furthermore, it may not be accurate to combine the annual number of genetically-confirmed SMA cases with the annual UK birth rate. This is because different populations are being considered in each case: the annual number of genetically-confirmed SMA cases includes a proportion of patients who were diagnosed later in life, but the UK birth rate only includes individuals born within the year in question. The authors also note that some laboratories (including those in the UK) test samples from abroad; therefore, the numerator (SMA cases) and denominator (UK births) are not from the same populations. Although this study provides useful UK-specific data, the results should be interpreted with caution.[71]

Results

In 2015, there were 88 cases of genetically-confirmed SMA and 804,083 live births, giving an incidence of 10.9 per 100,000 births (95% CI 8.8 to 13.5).[71] The five-year results (2011 to 2015) were similar, with 438 cases and 4,020,416 live births, giving an incidence of 10.9 per 100,000 births (95% CI 9.9 to 12.0).[71]

## Summary of Findings Relevant to Criterion 1: Criterion not met

As with the 2013 UK NSC review, this review update found that there is not enough information about the total number of people affected and how many people are affected by each type of SMA. Whilst increasing copy number of *SMN2* is associated with milder types of SMA, it is not yet possible to accurately determine from an individual's genotype whether they will be mildly or severely affected by SMA.

Only one study was included in this review update that identified SMA patients with homozygous deletion of *SMN1*. Although this was a large study the evidence base remains limited. The study was generally well-designed, however, there were a number of limitations due to uncertainties on how the data were reported by the laboratories, including how each laboratory received samples from the population and the methods of testing used by each laboratory are not reported.

This study reported an incidence of 10.9 cases per 100,000 live births, which is not consistent with the incidence of 1 in 24,119 births (calculated as 4.15 per 100,000 births) reported by the study from north-east England identified in the previous UK NSC review. Although the current study is larger and much more recent, the unclear methodology means that it is uncertain whether this finding represents an accurate estimation of the incidence of SMA in the UK.

Overall, there are substantial limitations in the evidence base for this question, and there was no further evidence identified to indicate that it is possible to determine an individual's prognosis from their genotype. Therefore, this criterion is not met.

## *Criterion 4 – Screening tests for spinal muscular atrophy*

Criterion 4 of the UK NSC Screening Criteria states that:

- 'There should be a simple, safe, precise and validated screening test.'

The previous UK NSC review identified that molecular diagnosis and determination of carrier status is possible, with dosage analysis identified as the main method by which carrier status is determined. However, a number of limitations to this method were discussed. These included a risk of false negative test results because:

- SMA carriers that have two or more *SMN1* copies located on a single chromosome would not be detected. This was a particular concern in the African-American population in the US, but it is unclear if any subgroups of the UK population would be similarly affected
- *SMN1 de novo* mutations, which occur in approximately 2% of SMA patients (1% of parents) would not be detected
- 3% to 4% percent of patients, i.e. 1% to 2% of carriers have small intragenic mutations in the *SMN1* gene and when paired with *SMN1* deletion, this genotype cannot also be identified by quantitative analysis of *SMN* gene copies

Conversely, some individuals have 0 copies of *SMN1* but are unaffected and at risk of a false positive diagnosis.

The previous review did not include studies assessing neonatal populations.

This update searched for relevant data published since the 2013 review, and assessed questions relating to carrier screening and newborn screening separately, using questions 2 and 5 below.

*Question 2 – What is the optimal test for carrier screening for SMA?*
*Sub question – Are there any non-genetic tests being used as carrier tests for SMA?*

### Carrier screening – eligibility for inclusion in the review

This review looked for both interventional and observational studies, as well as systematic reviews and meta-analyses of these designs, for screening tests used to identify SMA carriers using molecular genetic analysis. Studies had to assess the clinical performance of the screening test in adult populations, compared to a western blot or immunohistochemical reference standard. Both pregnant and non-pregnant populations were eligible for inclusion.

Due to a lack of relevant evidence, a *post hoc* modification was made to the eligibility criteria, to include studies that used different reference standards to those pre-specified in the review eligibility

criteria (western blot or immunohistochemical staining), but which would otherwise be eligible for inclusion in the review.

## Carrier screening – description of the evidence

Two studies from the database searches were ultimately judged to be relevant to this question. These were cohort studies assessing 2 different screening tests to identify SMA carriers.

## Carrier screening – summary of findings

As was identified in the previous review, these studies investigate dosage carrier screening methods. No studies investigating non-genetic tests to screen for SMA carriers were identified in this review.

### Quality assessment

The risk of bias in the 2 included studies as assessed using the QUADAS-2 quality assessment checklist is presented in Table 11.

**Table 11: Summary of QUADAS-2 assessments for SMA carrier screening studies**

| Question | Feng 2017[73] | Wang 2015[74] |
|---|---|---|
| **PARTICIPANT SELECTION** | | |
| **Risk of bias** | Unclear | Unclear |
| **Concern about applicability** | Unclear | Unclear |
| **INDEX TESTS** | | |
| **Risk of bias** | Unclear | Unclear |
| **Concern about applicability** | High | High |
| **REFERENCE STANDARD** | | |
| **Risk of bias** | Unclear | Unclear |
| **Concern about applicability** | High | High |
| **PATIENT FLOW** | | |
| **Risk of bias** | Unclear | Low |

Note: For the index test and reference standard, questions on risk of bias were answered based on the study's objective, and questions on applicability were answered based on this review's question. For example, a study that was only aiming to measure *SMN1* copy numbers could have low risk of bias if the study was well-conducted, but would lead to high concern about applicability due to the limitations of *SMN1* copy number analysis for identifying all carriers.

#### *Participant selection*

Neither study reported adequate details were reported on the recruitment of participants or their baseline characteristics. It is unclear whether either study used consecutive or random recruitment

of individuals from the general population for assessing the effectiveness of carrier screening; rather, their aim was to investigate screening test methods in a convenience sample. In Wang et al (2015), SMA patients were excluded from the study; however, this is an appropriate exclusion as they would not be part of a general population carrier screening programme. Some participants in Wang et al (2015) were under the age of 18 (proportion unspecified), but it is unclear whether this would affect the results of carrier screening. Therefore, the risk of bias and applicability to the review question are unclear

*Index tests*

The risk of bias associated with the conduct and interpretation of the index test in both studies is unclear. Whilst the methodology for both tests is clear and seems well-designed, few details of the test conduct were reported, including the order in which the index test and reference standard were conducted. It was also unclear whether thresholds were pre-specified, which may have biased the interpretation of the results.

Index tests in both studies were used to identify the copy numbers of *SMN1* in participants. As is discussed above, there are inherent limitations to this method for true identification of SMA carriers, so the concern about applicability is high. Feng et al (2017) reported an approach designed to help overcome these limitations, which is detailed alongside the results below.

*Reference standard*

The risk of bias associated with the conduct and interpretation of the reference standards in the studies is unclear. Neither study reports whether the results of the reference standard were interpreted without the knowledge of the index test result. Feng et al (2017) used 2 reference standards, and it is unclear which participants received which test. As for the index tests, the reference standards were used to identify the copy numbers of *SMN1* in participants, which may not identify all carriers, so the concern about applicability of the reference standards is high.

*Participant flow*

The risk of bias with regards to participant flow was unclear for Feng et al (2017) and low for Wang et al (2015). Both studies considered all participants in the outcome analysis. In Wang et al (2015) all participants received the same reference standard, whereas 2 reference standards were used in Feng et al (2017). It is unclear which samples received each reference standard and this may have introduced bias.

Results

Both studies reported tests to identify *SMN1* copy number. However, this is not sufficient to identify all SMA carriers. For example, there is potential for 2 or more *SMN1* copies to be located on a single chromosome or for one copy to have an intragenic mutation, such that a negative test result would not necessarily rule out carrier status.

Feng et al (2017) used next generation sequencing (NGS) to identify *SMN1* copy number. The study demonstrated a high sensitivity and specificity of NGS for the detection of one copy of *SMN1* (100% and 99.6% respectively), and also a strong performance for the detection of 2 and ≥3 copies of *SMN1* (Table 12).

**Table 12: Summary of results from Feng et al (2017)**

| | NGS performance (%) | 95% CI |
|---|---|---|
| **1 copy of *SMN1*** | | |
| **Sensitivity (n=90)** | 100.0 | 95.9 to 100 |
| **Specificity (n=6648)** | 99.6 | 99.4 to 99.7 |
| **2 copies of *SMN1*** | | |
| **Sensitivity (n=5480)** | 99.4 | 99.1 to 99.5 |
| **Specificity (n=1258)** | 98.3 | 97.5 to 98.9 |
| **≥3 copies of *SMN1*** | | |
| **Sensitivity (n=1168)** | 98.2 | 97.3 to 98.8 |
| **Specificity (n=5570)** | 99.8 | 99.7 to 99.9 |

As well as identifying *SMN1* copy number, Feng et al (2017) used NGS to identify the g.27134T>G SNP, which is associated with 2+0 SMA carrier status in certain ethnicities. This could allow identification of individuals who would otherwise receive false negative results though dosage screening. Results from the NGS method to detect this SNP were completely concordant with a RFLP assay in the same samples; however, Feng et al (2017) did not report overall test performance characteristics using this method.

Wang et al (2015) evaluated a novel high-resolution melting analysis (HRMA) carrier screening test to validate the copy numbers of *SMN1* and *SMN2,* and the method displayed high sensitivity and specificity (100% and 99%, respectively) for identifying individuals with one copy of *SMN1*. However, this study was not able to distinguish between non-carriers with one or more *SMN1* copies on each chromosome and carriers with 2 or more *SMN1* copies on a single chromosome.

## Summary of Findings Relevant to Criterion 4 for Carrier Screening: Criterion not met

Both studies reported high sensitivity (100%) and specificity (≥99%) for identifying individuals with one copy of *SMN1*. However, the evidence base is weak overall.

*Quantity:* A weak evidence base of only two studies was available to assess criterion 4 in relation to carrier screening.

*Quality:* The risk of bias was generally unclear, particularly with the reference standards used. One study reported using 2 reference standards against which to measure the performance of the index test, but did not report which patients received which reference standard and there is a concern that this may have biased the performance results. It is also unclear if the reference standards in either study have been interpreted without the knowledge of the index test result.

*Applicability:* There is high concern about the applicability of the included studies to the review question, because *SMN1* copy number is not an adequate method for identifying all carriers of SMA. There is also concern regarding the applicability of the populations in each study, since they were not evaluating screening studies in a randomly recruited population.

*Consistency:* The two studies on carrier screening used different tests, so it is not possible to assess the consistency of results.

*Conclusions:* The conclusions that can be drawn regarding carrier screening for SMA are limited by the identification of only a weak evidence base in support of this criterion. The two studies identified in this review indicate that methods for the identification of *SMN1* copy numbers in potential carriers are promising; however, these methods have an inherent risk of false negatives. Due to these concerns, this criterion is not met.

*Question 5 – Is there a simple, precise and validated screening test for newborn screening for SMA?*
*Sub-question – Is the test able to give information on the severity of the condition?*

## Newborn screening – eligibility for inclusion in the review

This review looked for interventional and observational studies investigating screening tests using dried blood spot (DBS) samples in newborn populations. The studies had to report the clinical performance of the tests for identification of SMA compared to deletion tests, and/or relevant outcomes assessing the severity of the condition.

Due to a lack of relevant evidence it was agreed with the UK NSC to include studies that used different reference standards to those pre-specified in the review eligibility criteria (deletion tests), but which would otherwise be eligible for inclusion in the review.

## Newborn screening – description of the evidence

Overall, this review identified 4 publications assessing the performance of screening tests to identify SMA using DBS from neonates. Additionally, 2 of these studies assessed genetic deletions that are associated with the severity of SMA. Two of the publications each reported case-control studies (Ar Rochmah 2017 and Er 2012), one a prospective study (Chien 2017) and the final publication reported a retrospective and prospective study (Liu 2016).[63, 75-77]

## Newborn screening – summary of findings

### Quality assessment

The risk of bias in the 4 included publications as assessed using the QUADAS-2 quality assessment checklist is presented in Table 13.

**Table 13: Summary of QUADAS-2 assessments for neonatal SMA screening studies**

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[a] | |
|---|---|---|---|---|---|
| | | | | Retrospective study | Prospective study |
| **PARTICIPANT SELECTION** | | | | | |
| **Risk of bias** | High | Low | High | High | Low |
| **Concern about applicability** | High | Low | High | High | Low |
| **INDEX TESTS** | | | | | |
| **Risk of bias** | Unclear | Low | Unclear | Unclear | Low |
| **Concern about applicability** | Low | Low | Low | Low | Low |

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[a] | |
|---|---|---|---|---|---|
| | | | | Retrospective study | Prospective study |
| **REFERENCE STANDARD** | | | | | |
| **Risk of bias** | Unclear | High | Unclear | Unclear | High |
| **Concern about applicability** | Low | Low | Low | Low | Unclear |
| **PATIENT FLOW** | | | | | |
| **Risk of bias** | Unclear | High | Unclear | Unclear | High |

[a] Liu 2016 reported a retrospective and a prospective component to their study; the risk of bias of these components was assessed separately.

*Participant selection*

The risk of bias due to patient selection was high in 3 studies (Ar Rochmah et al [2017] and Er et al [2012] as well as the retrospective study by Liu et al [2016]), because these studies use a case-control design. This study design has the potential to bias screening test interpretation as the population was not randomly recruited, and therefore not reflective of the general screening population, which can influence calculations for test clinical performance measures. Furthermore, the retrospective study by Liu et al (2016) included patients with suspected limb movement disorders, increasing the risk of bias. The concern about applicability of these studies to the review questions is also high; it is not possible to determine whether the participants in the study were representative of the general population as they were selected on their perceived SMA positive or negative disease status, rather than being randomly recruited into the study. As a result, the studies may not be robust in their evaluation of screening for the purpose of this review.

The risk of bias and concern about applicability to the review question for the study by Chien et al (2017) and the prospective study by Liu et al (2016) was low. Samples were selected in a consecutive and random manner respectively, only newborns were recruited and no inappropriate exclusions were used

*Index tests*

The risk of bias associated with the conduct and interpretation of index tests was unclear in three studies. In Ar Rochmah et al (2017) and Er et al (2012), there is a concern that the index test results were interpreted with prior knowledge of the reference standard. In the retrospective study by Liu et al (2016) it is also unclear what order the index and reference tests were conducted in. If prior knowledge of the reference test was known before the index test was conducted, this may have biased interpretation of the index test.

A threshold was only stated as being pre-specified in the study by Chien et al (2017) and in the remaining studies it was unclear. If the threshold was only specified after analysis of the results, this may have biased interpretation of the test results. Overall, concern about applicability to the

review question was low in all 4 publications as screening was used to identify homozygous deletion of *SMN1*. It should be noted that whilst the tests used in the studies are able to identify patients with homozygous deletion of *SMN1* (~95% SMA patients), the remaining ~5% of patients with *de novo* mutations or point mutations will not have been identified. Therefore, these ~5% of patients would not be captured in real-world screening studies using the testing methodologies identified in this review.

*Reference standard*

The risk of bias regarding the reference standard was unclear in three studies (Al Rochmah et al [2017], Er et al [2012], and the retrospective study by Liu [2016]). In the studies by Al Rochmah et al and Er et al, the methodology used to conduct the reference standards were not detailed; therefore, it is unclear whether the conduct of the tests could have introduced bias. As mentioned above, in the retrospective study by Liu 2016 it was unclear in what order the index and reference tests were conducted which may have introduced bias. In addition, 2 studies (Chien et al [2017] and Liu et al [2016]) only used the reference standard to confirm positive results from the index test, as opposed to all results, irrespective as to whether they tested positive or negative using the index test. This limits the number of applicable screening performance results that can be calculated from the study data.

Concern about applicability to the review question was low in 3 studies as the reference standard was used to evaluate *SMN1* exon 7 deletion, which would capture 95% of SMA patients.[63, 75, 76] As with the index tests, ~5% of SMA patients with *de novo* or point mutations would not be identified. In the respective and prospective studies, reported by Liu et al (2016), concern about the applicability was unclear.

*Patient flow*

The risk of bias due to patient flow was high for Chien et al (2017) and in the prospective study by Liu et al (2016). In both studies the reference standards were only conducted on samples that had tested positive using the index test, which may have introduced bias. Additionally, it is unclear if all samples tested in the study by Al Rochmah et al (2017) were analysed. If there were inappropriate exclusions of samples from the analyses this may have introduced bias. An additional 20 samples were included in the analyses from the study by Er et al (2012), but these samples were not specified in the methodology, therefore it is unclear whether the introduction of these samples could have introduced bias. Finally, in the retrospective study by Liu et al (2016), as the interval between conducting the index test and reference standard was unknown, the risk of bias is unclear. For example, if the reference standard was conducted at a later point in time than the index test, inappropriate sample storage may result in deterioration of the samples which may affect the test results and therefore introduce bias.

Results

All of the identified studies tested for underlying genetic causes associated with SMA and used DBS samples. Three different screening tests were assessed amongst the studies. Both Chien et al (2017) and the prospective and retrospective study in Liu et al (2016) investigated the screening performance of RT-PCR tests. Neither study measured sufficient data to determine the overall sensitivity and specificity of RT-PCR; however, Chien et al (2017) and the prospective study in Liu et al (2016) demonstrate a high number of false positive results. A total of 8/15 samples were false positives in Chien et al (2017) and 22/23 samples were false positives in the prospective study by Liu et al (2016). In Chien et al (2017), it is noted that this is due to the screening test being designed to identify an absence of homozygous *SMN1* exon 7, so does not differentiate patients with a homozygous deletion of *SMN1* from those having 1 copy of *SMN1*.

High sensitivity and specificity values for the identification of *SMN1* exon 7 deletions were determined for mCOP-PCR and HRM analysis tests. Er et al (2012) reported sensitivity and specificity values of 100% using HRM analysis compared to the reference standard, DHPLC, in 30 patients and 30 controls. In Chien et al (2017), the specificity of RT-PCR was 99.99%, but it was unclear if this was compared to the second-tier assay (ddPCR), which was used to confirm the RT-PCR positive results, or to the reference standard MLPA. Additionally, the sensitivity was estimated (but not calculated) to be 95% as this method only detected the absence of homozygous *SMN1*. Finally, Al Rochmah et al (2017) reported sensitivity and specificity values of 100% using mCOP-PCR compared to the reference standard, PCR-RFLP.

Two of the studies, Ar Rochmah et al (2017) and Liu et al (2016), present screening tests which also have potential to assess the severity of SMA. Ar Rochmah et al (2017) used mCOP-PCR to determine *SMN2* exon 7 deletions with a high sensitivity and specificity of 100% compared to the reference standard, PCR-RFLP. Knowledge of the copy number of *SMN2* can be used to assess prognosis of a SMA patient as a higher number of *SMN2* copies have been shown to correlate with reduced disease severity.[4] Deletions in the *GTF2H2* gene, were assessed by RT-PCR by Liu et al (2016). The authors reported that the *GTF2H2* gene and/or exon deletion may be related to the severity of the SMA, as it can accompany *SMN1* deletion, but its clinical significance is currently unclear, so this evidence was not considered further here.

## Summary of Findings Relevant to Criterion 4 for Newborn Screening: Criterion not met

*Quantity:* A weak evidence base of only 4 studies was available to assess criterion 4 in relation to newborn screening.

*Quality:* Overall, the evidence base has a high or unclear risk of bias. Bias could have been introduced by the case-control design of several studies, as well as the inclusion of patients that were clinically suspected as having limb movement disorders in one study. There was also concern that the index tests were interpreted in the knowledge of the reference test results, which could bias the performance outcome of the screening tests.

*Applicability:* Generally, there is low concern about the applicability of the included studies to the review question. Although none of the studies used reference standards that were pre-specified in the eligibility criteria for this review, the studies did become eligible following the post hoc modification to the eligibility criterial. The index tests and reference standards were designed to identify *SMN1* exon 7 deletion, which would capture 95% of SMA patients.

*Consistency:* For the RT-PCR test, which was assessed by both Chien et al (2017) and Liu et al (2016), a consistently high rate of false positive results was demonstrated across the 2 studies. The other screening tests were each assessed in a single study.

*Conclusions:* There is evidence from two studies that mCOP-PCR and HRM analysis are highly sensitive and specific newborn SMA screening methods; however, in the absence of high-quality prospective screening studies using these methods in the general population, it is not possible to confirm these results. Furthermore, there are also high risk of bias and applicability concerns since many of the studies identified were not evaluating screening studies in a randomly recruited and potentially unrepresentative population.

Overall, there is currently only a small volume of mixed evidence, to assess the availability and efficacy of screening methods for SMA identification in newborns. Therefore, this criterion is not met.

## *Criteria 9 and 10 – Management pathways for spinal muscular atrophy*

Criteria 9 and 10 of the UK NSC Screening Criteria state that:

- 'There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme shouldn't be further considered'
- 'There should be agreed evidence based policies covering which individuals should be offered interventions and the appropriate intervention to be offered'

The previous UK NSC review discussed the diagnosis of SMA, but did not directly aim to determine the optimal diagnostic pathway. The review highlighted 2 studies in which antenatal diagnosis was confirmed in fetuses or newborns and highlighted the overall limitations of diagnostic methods, particularly in terms of false positive results and the implications of this.[47, 78] One study was referenced in which pre-test, interpretation and post-test counselling was provided to advise couples undergoing carrier screening and diagnostic testing.[48]

The review found that there was no effective treatment or cure available for SMA.[18] Management of the symptoms was recognised as the current standard, with advice on pulmonary care, gastrointestinal and nutritional care, orthopaedic care and rehabilitation, and palliative care highlighted in a consensus statement published in 2007.[19] Two SLRs of RCTs were identified, relating to the available drug treatments for (i) type 1 SMA, and (ii) type 2/3 SMA, but there was no statistically significant evidence that any interventions were effective at altering disease course.[79, 80] The review for type 1 disease found only one small RCT (n=10) identified comparing riluzole to placebo. This RCT reported no statistically significant difference between riluzole and placebo.[80] For type 2/3 SMA, the SLR identified a greater number of studies, with 6 RCTs investigating creatine, phenylbutyrate, gabapentin, thyrotropin releasing hormone, hydroxyurea, or combination therapy (valproate and acetyl-L-carnitine).[79] None of the included trials showed statistically significant efficacy for any treatment. Due to the lack of treatments, the previous review stated that the aim of carrier testing would be to allow informed choice during reproductive decision-making, such as whether to terminate a pregnancy or not.

In this update, the reviewers searched for relevant data published since the 2013 review, and assessed questions relating to the diagnostic pathway and pharmacological treatments for SMA separately, using questions 6 and 7 below.

*Question 6 – What is the optimal diagnostic pathway for screen-detected SMA newborns?*
*Sub question – Is the test able to give information on the severity of the condition?*

## Diagnostic pathways – eligibility for inclusion in the review

This review searched for interventional and prospective comparative observational studies, as well as systematic reviews and meta-analyses of the above study designs. Publications were included that compared newborns with SMA identified through neonatal screening to newborns clinically diagnosed with SMA outside of a screening programme. Following diagnosis, affected individuals could receive any pharmacological or non-pharmacological intervention, or no treatment. Outcomes of interest were those relating to clinical and safety measures. Studies were excluded if they did not contain these relevant outcomes or if they reported data relating to individuals without screen-detected SMA as a neonate.

## Diagnostic pathways – description of the evidence

No relevant studies were identified.

## Diagnostic pathways – summary of findings

No relevant studies were identified.

*Question 7 – What is the reported effectiveness of pharmacological treatment for SMA?*
*Sub-question – Is the pharmacological treatment for SMA equally effective for all types?*

## Pharmacological treatments – eligibility for inclusion in the review

This review looked for prospective interventional and observational studies, and systematic reviews and meta-analyses relating to these study types. Publications were eligible for question 7 if they investigated a pharmacological intervention for SMA compared to normal care in individuals of any age with SMA (whether identified through screening or through other methods). Outcomes were deemed relevant if they related to any clinical or safety measure of SMA and its proposed treatments.

## Pharmacological treatments – description of the evidence

The rapid review identified 5 relevant publications reporting on 5 unique cohorts of SMA patients; all 5 of these publications were RCTs, 2 of which had a cross-over design. Although none of the studies were exclusively conducted in a UK setting, 2 studies recruited patients from the UK as well as from other countries.[9, 20] The other 3 studies were carried out in Germany, the USA, and across

multicentre settings across countries but no UK centres.[10, 81, 82] The examined interventions were olesoxime (one study), somatropin (one study), valproic acid (VPA; one study) and nusinersen (two studies).

## Pharmacological treatments – summary of findings

### Quality assessment

Quality assessment tables for studies relevant to question 7 (modified Critical Appraisal Skills Programme [CASP] Randomised Controlled Trials Checklist), can be found in Appendix 3. A summary is shown in Table 14.

**Table 14: Summary of quality assessments for RCTs of pharmacological treatments for SMA**

| Question | Bertini 2017[20] | Finkel 2017[9] | Kirschner 2014[81] | Kissel 2014[82] | Mercuri 2018[10] |
|---|---|---|---|---|---|
| **ARE RESULTS OF THE TRIAL VALID? SCREENING QUESTIONS** | | | | | |
| Did the trial address a clearly focused issue? | Yes | Yes | Yes | Yes | Yes |
| Was the assignment of patients to treatments randomised? | Yes | Yes | Yes | Yes | Yes |
| Were all of the patients who entered the trial properly accounted for at its conclusion? | Yes | Yes | Yes | Yes | Yes |
| **ARE RESULTS OF THE TRIAL VALID? DETAILED QUESTIONS** | | | | | |
| Were patients, health workers and study personnel 'blind' to treatment? | Yes | Yes | Yes | No | Yes |
| Were the groups similar at the start of the trial? | Yes | Unclear | Yes | Yes | Yes |
| Aside from the experimental intervention, were the groups treated equally? | Yes | Yes | Yes | Yes | Unclear |
| **RELEVANCE TO THE RAPID REVIEW** | | | | | |
| Can the results be applied to a UK population? | Yes | Yes | Yes | Yes | Yes |
| Were outcomes of importance to the rapid review considered? | Yes | Yes | Yes | Yes | Yes |

*Validity of results*

Overall, the RCTs included for this criterion were of high quality. In all cases, studies addressed a clearly focused question, randomised patients using appropriate means and accounted for patient flow. Two trials used a cross-over methodology to compensate for low patient numbers.[81, 82] This could influence trial outcomes as patients received both the intervention and placebo over the course of the trial. However, this is beneficial as the same patients receive both treatments and can therefore limit the effects of between-group differences. Furthermore, one trial with this design did report a 2-month wash-out period, which was designed to limit the effects of previously receiving a different treatment. The authors noted that this wash-out period should be sufficient since the effects of growth hormone on muscle strength are short-lived; they also reported that no carry-over effects were seen in primary or secondary outcome measures.[81]

All patients, health workers and study personnel were blinded to treatment in two studies of type 2 or 3 SMA.[20, 81] Kissel et al (2014) reported an unblinded medical monitor in the study.[82] This individual was only responsible for reviewing subjects' blood tests and adverse events, performing dose adjustments and conducting additional testing where necessary. Therefore, it is unlikely that the bias would be introduced as a result. In two studies, nusinersen was administered or the sham procedure was performed by dedicated trial personnel who were aware of the group assignments, but patients, their parents and key trial personnel responsible for outcome assessments were unaware of the group assignments and were not present for the procedure.[9, 10]

Baseline demographics and characteristics were generally similar between groups at the beginning of all trials. However, baseline demographics were not compared in the Kirschner et al (2014) study.[81] Experimental groups were treated equally in all trials.

*Relevance to the review*

In terms of applicability to a UK population, one study included patients treated in a UK setting as well as those from 6 other EU countries, and another included patients from centres in the UK as well as 12 centres worldwide.[9, 20] The other trials were conducted in Germany, the US, and in a worldwide multicentre setting.[10, 81, 82] Ethnicity data is not provided in any case; however, these trials were conducted in comparable high-income countries so it is likely that the results are applicable to the UK.

Overall, the studies reported relevant clinical and safety outcomes; however, the outcomes were not consistently reported across trials. This, along with the fact that the trials investigated treatments for varying SMA types means that comparison between the RCTs is not possible. In addition, only 2 of the 5 trials evaluated an intervention in patients under the age of 3 years. Therefore, there is limited evidence as to whether pharmacological treatments for SMA can have the same degree of benefit in younger patients compared with those evaluated in the majority of the trials identified here.

Results

The primary outcome measures of each study are summarised in Table 15.

**Table 15. Summary of primary outcome measures in RCTs**

| Study | Treatment | n | Primary outcome(s) | Treatment | Placebo | Summary measure |
|---|---|---|---|---|---|---|
| Bertini 2017[20] | Olesoxime | 160 | CFB in MFM D1 + D2 score at 104 weeks | LSM 0.18 (SE 0.717) | LSM -1.82 (SE 0.901) | Difference 2.00 (96% CI -0.25 to 4.25); p=0.0676 |
| Kirschner 2014[81] | Somatropin | 19 | CFB in arm megascore at 12 weeks | Mean -1.05 (SD 6.42) | Mean 0.30 (SD 10.60) | Difference 0.08 (95% CI -3.79 to 3.95; p=0.965) |
| Kissel 2014[82] | VPA | 33 | CFB in total MVICT at 6 months | Mean -0.46 (SD 2.99) | Mean 0.03 (SD 1.55) | p=0.5708 |

| Study | Treatment | n | Primary outcome(s) | Treatment | Placebo | Summary measure |
|---|---|---|---|---|---|---|
| Finkel 2017[9] | Nusinersen | 122 | Motor milestone response, interim analysis[a] | 21/51 (41%) | 0/27 (0%) | p<0.001 |
| | | | Motor milestone response, final analysis | 37/73 (51%) | 0/37 (0%) | p=NR |
| | | | No death or use of permanent assisted ventilation | 49/80 (61%) | 13/41 (32%) | HR 0.53 (95% CI 0.32 to 0.89); p=0.005 |
| Mercuri 2018[10] | Nusinersen | 126 | CFB in HFMSE, interim analysis[b] | LSM 4.0 (95% CI 2.9 to 5.1) | LSM -1.9 (95% CI -3.8 to 0) | Difference 5.9 (95% CI 3.7 to 8.1); p<0.001 |
| | | | CFB in HFMSE, final analysis at 15 months | LSM 3.9 (95% CI 3.0 to 4.9) | LSM -1.0 (95% CI -2.5 to 0.5) | Difference 4.9 (95% CI 3.1 to 6.7); p=NR |

CFB: change from baseline; CI: confidence interval; D1: domain 1; D2: domain 2; HFMSE: Hammersmith Functional Motor Scale-Expanded; LSM: least squares mean; MFM: motor function measure; NR: not reported; SD: standard deviation; SE: standard error; VPA: valproic acid.
[a]The pre-specified interim analysis included the 78 infants (51 in the nusinersen group and 27 in the control group) who had been enrolled for at least 6 months
[b]Interim analysis of the primary endpoint was conducted when all the children had been enrolled for at least 6 months and at least 39 children had completed the 15-month assessment

Three treatments (olesoxime, VPA and somatropin) were not statistically significantly better than placebo. Full details of the 3 RCTs on these treatments can be found in Appendix 3. In summary:

- For olesoxime in type 2 or non-ambulatory type 3 SMA, there was no statistically significant difference between olesoxime and placebo for the primary endpoint (change from baseline in functional domains 1 and 2 [D1 + D2] of the Motor Function Measure [MFM] at Month 24, difference between treatments 2.00 points; 96% CI -0.25 to 4.25; p=0.0676), although the authors noted that this may be due to higher than anticipated variability in the primary outcome measure in the study population, which caused the study to be underpowered.[20] There were also no statistically significant results for most of the secondary endpoints (MFM or Hammersmith Functional Motor Scale [HFMS] endpoints) between the treatment and placebo groups.[20] Adverse event profiles were similar for those receiving olesoxime compared to placebo.[20] There were 2 deaths (one in each study group), although these were not judged to be treatment-related.[20] Olesoxime appears to be safe, but there is currently inconclusive evidence on its efficacy.

- Kissel et al (2014) conducted an RCT investigating the safety and efficacy of VPA in the treatment of adults with ambulatory SMA.[82] A total of 33 subjects were randomised to receive VPA or placebo, and patients crossed over to the other arm at 6 months. Thirty patients completed the study. The primary outcome measure in this study was maximum voluntary isometric contraction testing (MVICT). There were no statistically significant changes from baseline following VPA treatment for any muscle group measure using MVICT at 6 and 12 months. There were also no differences in any of the secondary outcomes at 6 and 12 months. VPA appeared to be well-tolerated, with only 2 adverse events leading to study withdrawal. Two serious adverse events were reported but neither of these were attributed to VPA treatment. Although well-tolerated, VPA appears to be ineffective in improving adult ambulatory SMA.

- Kirschner et al (2014) used a cross-over design to investigate the safety and efficacy of subcutaneous somatropin (growth hormone; GH) for the treatment of type 2/3 SMA.[81] Nineteen

patients were randomised to receive somatropin or placebo for 3 months and then underwent washout for 2 months before treatment switching. For the primary outcome of improvement on upper limb muscle strength, somatropin was not statistically significantly better than placebo (mean difference 0.08 N; 95% CI -3.79 to 3.95; p=0.965). There was also no statistically significant difference between treatment groups in lower limb strength, muscle, pulmonary function or HFMS scores. Adverse events following somatropin generally corresponded with associated side-effects of GH substitution in patients with a GH deficiency, with 5 of moderate intensity (headache, arthralgia, myalgia, peripheral edema, elevated serum thyroid stimulating hormone) and 2 more severe events (myalgia and progressive headache) that led to early trial termination. Therefore, somatropin appears to be an ineffective treatment for SMA and has an unfavourable safety profile.

*Nusinersen*

There were two studies investigating the efficacy and safety of the gene therapy nusinersen in an RCT and open-label extension. Finkel et al's (2017) RCT was in 122 patients with infantile-onset SMA (most likely type 1 disease),[9] while Mercuri et al (2018) studied patients with later-onset SMA (most likely type 2/3 disease).[10] In both RCTs, patients were randomised to receive nusinersen or sham control. Both trials were of high quality.

The primary outcomes of the two RCTs are summarised above in Table 15. Both trials conducted a pre-specified interim efficacy analysis on their primary outcome measure. Finkel et al's (2017) interim analysis was when approximately 80 infants had been enrolled for at least 6 months whereas Mercuri et al's (2018) was when all children had been enrolled for at least 6 months and at least 39 had completed a 15-month assessment. Finkel et al (2017) found a significantly higher percentage of infants receiving nusinersen had a motor-milestone response compared with the control group (21/51 [41%] versus 0/27 [0%]; p<0.001). A motor-milestone response was defined as improvement in at least one of the Hammersmith Infant Neurological Examination, Section 2 (HINE-2) categories and more categories with improvement than categories with worsening. Mercuri et al. (2018) also found a statistically significant between-group difference favouring nusinersen when measuring the least-squares mean Hammersmith Functional Motor Scale Expanded (HFMSE) score relative to baseline (difference between treatments 5.9 points; 95% CI 3.7 to 8.1; p<0.001). These results led to premature termination of the double-blind period of each trial, with all patients invited to enrol in an open-label extension.

In the final analyses, Finkel et al (2017) found that 37/73 (51%) patients in the nusinersen group and 0/37 (0%) patients in the control group had a motor milestone response, with 49/80 (61%) of nusinersen patients and 13/41 (32%) patients on placebo achieving the other primary outcome of 'no death or use or permanent assisted ventilation'. Mercuri et al (2018) found a least-squares mean increase from baseline to month 15 in the HFMSE score in the nusinersen group and a least-

squares mean decrease in the control group (difference between treatments 4.9 points; 95% CI 3.1 to 6.7). Similar efficacy was also observed when sensitivity analyses were conducted in subgroups by *SMN2* copy number.

Secondary outcomes from each trial are summarised in Table 16 and key safety outcomes are summarised in Table 17. Full details of outcomes from each trial are available in Appendix 3 (Table 31 for Finkel et al [2017] and Table 34 for Mercuri et al [2018]).

**Table 16. Key secondary outcomes in RCTs of nusinersen**

| Study | n | Outcomes | Nusinersen, n/N (%) | Placebo, n/N (%) | Summary measure (95% CI) |
|---|---|---|---|---|---|
| Finkel 2017[9] | 122 | Full head control | NR (22) | 0 | NR |
| | | Able to roll over | NR (10) | 0 | NR |
| | | Able to sit independently | NR (8) | 0 | NR |
| | | Able to stand | NR (1) | 0 | NR |
| | | CHOP INTEND response | 52/73 (71) | 1/37 (3) | NR; p<0.001 |
| | | No death | 67/80 (84) | 25/41 (61) | HR 0.37 (0.18 to 0.77) |
| | | No use of permanent assisted ventilation | 62/80 (78) | 28/41 (68) | HR 0.66 (0.32 to 1.37) |
| | | CMAP response | 26/73 (36) | 2/37 (5) | NR |
| Mercuri 2018[10] | 126 | Change in HFMSE score ≥3 points | NR (57) | NR (26) | Difference 30.5% (12.7 to 48.3); OR 6 (2 to 15); p<0.001 |
| | | Achieved ≥1 new WHO motor milestone | 13 (20) | 2 (6) | Difference 14% (-7 to 34); p=0.08 |
| | | CFB number of WHO motor milestones achieved | LSM 0.2 (95% CI 0.1 to 0.3) | LSM -0.2 (-0.4 to 0) | Difference 0.4 (0.2 to 0.7) |
| | | CFB RULM score | LSM 4.2 (3.4 to 5.0) | LSM 0.5 (-0.6 to 1.6) | Difference 3.7 (2.3 to 5.0) |
| | | Able to stand alone | 1 (2) | 1 (3) | Difference -1 (-22 to 19) |
| | | Able to walk with assistance | 1 (2) | 0 | Difference 2 (-19 to 22) |

CFB: change from baseline; CHOP INTEND: Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CI: confidence interval; CMAP: compound muscle action potential; HR, hazard ratio; LSM: least squares mean; OR, odds ratio; RULM, Revised Upper Limb Module; WHO, World Health Organization

**Table 17. Key safety outcomes in RCTs of nusinersen**

| Study | n | Outcomes | Nusinersen, n (%) | Placebo, n (%) |
|---|---|---|---|---|
| Finkel 2017[9] | 122 | Any AE | 77 (96) | 40 (98) |
| | | AE leading to discontinuation | 13 (16) | 16 (39) |
| | | Severe AE | 45 (56) | 33 (80) |
| | | SAE | 61 (76) | 39 (95) |
| Mercuri 2018[10] | 126 | Any AE | 78 (93) | 42 (100) |
| | | Any moderate or severe AE | 39 (46) | 23 (55) |
| | | Any severe AE | 4 (5) | 3 (7) |
| | | Any SAE | 14 (17) | 12 (29) |
| | | Any AE leading to treatment discontinuation | 0 | 0 |
| | | Any AE leading to withdrawal from the trial | 0 | 0 |

AE: adverse event; SAE, serious adverse event
Moderate AEs were defined as events that caused discomfort and interrupted the child's usual daily activities. Severe AEs were defined as symptoms that caused severe discomfort, incapacitation, or substantial effect on daily life. SAEs were defined as any untoward medical occurrence that resulted in death or a risk of death, hospitalisation or prolonged hospitalisation, persistent or substantial disability or incapacity, or a congenital anomaly or birth defect.

With respect to the secondary endpoints, Finkel et al. found that nusinersen outperformed placebo for several motor milestones, as well as response to the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) and peroneal compound muscle action potential (CMAP) tests. The risk of death was 63% lower in the nusinersen group than in the control group. Conversely, there was not a statistically significant between-group difference in the percentage of patients receiving permanent assisted ventilation.

Mercuri et al. found a higher percentage of patients in the nusinersen group who had an increase of at least 3 points in the HFMSE score at Month 15, indicating that statistically significantly more patients achieved a clinically significant improvement following treatment with nusinersen. Conversely, there was no difference in the percentage of children achieving at least one of the six World Health Organization (WHO) motor milestones or in the proportion of children able to stand alone or walk without assistance at analysis. Due to the lack of significance of the secondary endpoint evaluating the percentage of children achieving at least one WHO motor milestone, the remaining secondary endpoints were not statistically analysed.

Both trials reported a comparable overall incidence of adverse events in the nusinersen and control groups.[9, 10] Finkel et al. found that a lower percentage of patients in the nusinersen group compared to the control group experienced a severe adverse event, a serious adverse event or an adverse event that led to discontinuation. Similarly, Mercuri et al. found that the incidence of serious adverse events was lower in the nusinersen group compared to controls while moderate and severe adverse events was also similar between groups; however, the rate of events linked to lumbar puncture (the method of administration of nusinersen and replicated in the sham procedure) within 24, 72, 120, and 168 hours after the assigned procedure was higher in the nusinersen group (9%, 14%, 15%, and 15%, respectively) versus 3% for each time point in the control group.

There are limitations to the evidence on nusinersen. Whilst two well-conducted trails on this treatment were identified as part of the evidence base, there were no studies reporting the long term efficacy or safety of nusinersen and no studies compared the effects of pre-symptomatic verses symptomatic treatment.

## Summary of Findings Relevant to Criteria 9 and 10: Criterion not met

*Quantity:* Overall only a small number of studies published since 2012 were identified for these criteria. No studies were identified as providing relevant information to the diagnostic pathway for SMA and only 5 studies were identified as containing relevant information for pharmacological treatments for SMA. Furthermore, each study had a small number of participants. As a result, only a weak evidence base was available to assess criteria 9 and 10 of this review.

*Quality:* No studies were identified for question 6 within these criteria. The 5 studies identified for question 7 were generally of high quality from a methodological perspective.

*Applicability:* No studies were identified for question 6 within these criteria. The trials identified as relevant to question 7 are highly applicable to the research question. Two of the trials involved individuals from the UK,[9, 20] whilst the other 3 trials were focused on individuals from Germany, the US and multiple countries worldwide in a multicentre setting.[10, 81, 82]

*Consistency:* No studies were identified for question 6 within these criteria. There was only one study on each treatment for olesoxime, VPA and somatropin, so consistency cannot be assessed.[20, 81, 82] Both trials on nusinersen demonstrated that it can have a statistically and clinically significant impact upon motor measures. Comparison between trials is not possible, as they all report different efficacy measures and investigate different interventions, in different SMA types.

*Conclusions:* Three trials found that olesoxime, VPA and somatropin are not effective treatments for improving SMA compared to placebo in the primary endpoints and the majority of secondary endpoints investigated.[20, 81, 82] Despite the small volume of evidence identified, there is now data suggesting that nusinersen is effective in improving outcomes for patients with SMA.[10, 20] Furthermore, the long-term efficacy and safety of nusinersen and effectiveness in pre-symptomatic groups is yet to be evaluated.

## *Criterion 11 – Consequences of screening for spinal muscular atrophy*

Criterion 11 of the UK NSC Screening Criteria states that:

- 'There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (such as Down's syndrome or cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened'

Outcomes of screening programmes were discussed in the previous UK NSC review, with no RCTs identified relating to SMA screening. However, 2 population studies were identified for carrier screening. One of these studies, conducted in Israel, showed acceptability and feasibility of a carrier screening programme following uptake in 93% women already being offered screening for cystic fibrosis and Fragile X syndrome.[83] The second study concluded that SMA screening should be incorporated in Taiwan.[48] In the latter study, it was detailed that genetic counselling was given pre-test, during the interpretation of results, and post-test; however, the uptake and ease of recruitment was not.

Furthermore, although the number of affected couples is reported, it is not clear how this affected subsequent decision making, or how couples rated the overall experience of enrolling in the programme.

In this update to the review, the evidence relating to these criteria was addressed by questions 3 and 4, as discussed here.

*Question 3 – What are the reported outcomes of SMA carrier screening programmes?*

### Carrier screening – eligibility for inclusion in the review

This review searched for interventional and prospective observational studies, as well as systematic reviews and meta-analyses relating to these study types. Publications were included if they reported on the outcomes of carrier screening programmes using molecular genetic and non-genetic techniques in individuals planning pregnancy. Exclusion criteria included publications not relating to carrier screening specifically and the presence of outcomes not relevant to carrier screening or the number or percentage of couples who are both carriers.

## Carrier screening – description of the evidence

No relevant studies were identified.

## Carrier screening – summary of findings

No relevant studies were identified.

*Question 4 – What are the reported outcomes of SMA antenatal screening programmes?*

## Antenatal screening – eligibility for inclusion in the review

This review searched for interventional and prospective observational studies, as well as systematic reviews and meta-analyses relating to these study types. Interventions were to include molecular genetic and non-genetic techniques on pregnant individuals, to determine whether the fetus is a carrier, or to diagnose SMA antenatally. Key exclusion criteria included tests not used to determine carrier status, and outcomes which did not relate to uptake of screening or diagnostic testing or the number of fetuses with SMA.

## Antenatal screening – description of the evidence

No relevant studies were identified.

## Antenatal screening – summary of findings

No relevant studies were identified.

## Summary of Findings Relevant to Criterion 11: Criterion not met

As no studies were found, there are still no RCTs relating to carrier or antenatal screening programmes for SMA. Therefore, the evidence is still insufficient to meet criterion 11.

# Review summary

## Conclusions and implications for policy

This updated analysis of the evidence for a population-wide carrier screening programme for SMA against the UK NSC criteria did not identify sufficient evidence to support a change in the previous recommendation.

The main reasons for this are poor-quality evidence on the epidemiology of SMA, including total prevalence and how many people are affected by each type of SMA, the effectiveness of screening programmes in the UK population, and the optimal diagnostic and treatment pathway following a screening programme. Although UK-based surveys of the general public and families affected by SMA have found support for the idea of a newborn screening programme,[59, 84] this review did not find any studies that implemented a population-based screening programme and reported uptake of the test.

The previous UK NSC review identified limited epidemiological data with little information about the incidence of SMA in the UK. This review update identified a single study reporting UK-specific data for the prevalence of SMA at birth; however, the study did not present data by SMA type within the UK population. Without large, prospective studies of SMA epidemiology in the UK population it is not possible to determine the possible impact of a population screening programme.

Although the sensitivity and specificity of both carrier screening and neonatal screening tests was reported to be high, the studies were not large-scale population screening studies. If the recruitment of patients is not reflective of the general population, then there may be biases in the sensitivity and specificity values reported. Furthermore, for carrier screening, the studies only investigated tests to measure the copy number of *SMN1*, which is not sufficient for identifying all carriers of SMA. Therefore, they were only able to report evidence suggesting that newer tests were as effective as previous tests at identifying carriers with a single copy of *SMN1*, but were not able to report test accuracy measures compared to a gold standard that was able to identify all SMA carriers. For newborn screening, whilst tests were able to identify *SMN1* exon 7 deletions, which occur in ~95% patients, the remaining ~5% of patients who have SMA due to *de novo* mutations or other point mutations were not able to be identified using these testing methodologies. Therefore, these patients would not be identified in screening programmes. The true accuracy of carrier screening or neonatal screening cannot be confirmed without studies comparing the tests to gold standards in well-designed prospective studies.

The previous review found no evidence of effective treatments for SMA. The current review found inconclusive evidence on the efficacy of olesoxime; a single study found no statistically significant

effects for the majority of endpoints, but the authors noted that this may be due to higher than anticipated variability in the primary outcome measure in the study population, causing the study to be underpowered. There is also evidence suggesting that valproic acid and somatropin are not effective treatments for SMA. However, the current review found promising results on nusinersen. Two high-quality RCTs reported better outcomes on measures of motor control in patients with infantile-onset and later-onset SMA given nusinersen compared to sham control.[9, 10] However, the evidence base is still small, and there is a lack of data for the long-term effectiveness and safety as well as evidence in pre-smptomatic patient groups compared to symptomatic groups.

Finally, there is no high-quality evidence for an optimal management pathway for SMA patients identified through screening, so the benefits of pre-symptomatic treatment compared to treatment following symptom onset are unclear. There is also a lack of evidence on the acceptability of screening to the UK population or the expected uptake of a screening programme.

## Limitations

### Limitations of the available evidence

Evidence gaps are detailed in the section above. As well as these gaps, limitations relating to the quality and applicability of some identified evidence are discussed below.

### Limitations of the review methodology

This rapid review was conducted in line with the UK NSC requirements for evidence summaries, as described at https://www.gov.uk/government/publications/uk-nsc-evidence-review-process/appendix-f-requirements-for-uk-nsc-evidence-summaries. All items on the UK NSC Reporting Checklist for Evidence Summaries have been addressed in this report. A summary of the checklist, along with the page or pages where each item can be found in this report, is presented in Table 39 in Appendix 4.

Searches of multiple databases were conducted (see Appendix 1). Database search terms were very broad, and were not restricted by study design, interventions or comparators. Searches were limited to studies published since the previous UK NSC review was conducted.

*Included publication types*

This review only included peer-reviewed journal publications, and excluded not peer-reviewed and grey literature. This may have led to the exclusion of relevant evidence. However, this is an accepted methodological adjustment for a rapid review, and is unlikely to miss any pivotal studies.

The previous UK NSC review did not investigate newborn screening for SMA, only considering screening in the carrier and antenatal setting. Therefore, as this update only includes studies published since 2012, studies on newborn screening published before 2012 have not been considered.

*Language*

Only studies published in English were included. Given that this review was focusing on evidence relevant to the UK setting, this limitation should not have led to the exclusion of any pivotal studies.

*Review methodology*

Articles were reviewed by a single reviewer in the first instance. A second reviewer examined all included articles, 10% of excluded articles, and any articles where there was uncertainty about inclusion. This pragmatic strategy should have minimized the risk of errors.

*Articles not freely available*

Searches for full-text articles were carried out at Cambridge University Library. Some articles were not freely available at this library and were therefore excluded as it was judged that they would not contain any additional pivotal data from relevant populations that would affect the conclusions of this review.

# Appendix 1 – Search strategy

## Electronic databases

The search strategy included searches of the databases shown in Table 18.

**Table 18: Summary of electronic database searches and dates.**

| Database | Platform | Searched on date | Date range of search |
|---|---|---|---|
| **MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print** | Ovid SP | 18th August 2017<br>Update: 22nd February 2018 | 1946 to Present |
| **Embase** | Ovid SP | 18th August 2017<br>Update: 22nd February 2018 | 1974 to 17th August 2017<br>Update: 1974 to 21st February 2018 |
| **The Cochrane Library, including:**<br>- Cochrane Database of Systematic Reviews (CDSR)<br>- Cochrane Central Register of Controlled Trials (CENTRAL)<br>- Database of Abstracts of Reviews of Effects (DARE) | Wiley Online | 18th August 2017<br>Update: 21st February 2018 | - CDSR: Issue 8 of 12, August 2017 (update: Issue 2 of 12, February 2018)<br>- Database of Abstracts of Reviews of Effect: Issue 2 of 4, April 2015<br>- Cochrane Central Register of Controlled Trials: Issue 7 of 12, July 2017 (update: Issue 1 of 12, January 2018) |

## Search terms

Search terms included combinations of free text and subject headings (Medical Subject Headings [MeSH] for MEDLINE, and Emtree terms for Embase), relating to SMA. Search terms for the databases searched through Ovid SP are shown in Table 19, and search terms for the Cochrane Library databases are shown in Table 20.

**Table 19: Search strategy for MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print and Embase (searched simultaneously via Ovid SP).**

| Term Group | # | Search terms | Results (2017) | Results (2018) |
|---|---|---|---|---|
| Spinal muscular atrophy | 1 | Spinal muscular atrophies of childhood/ | 1,776 | 1,759 |
| | 2 | Spinal muscular atrophy/ | 9,073 | 9,204 |
| | 3 | werdnig-hoffman.tw. | 159 | 157 |
| | 4 | wohlfart-kugelberg-welander.tw. | 61 | 60 |
| | 5 | Spinal muscular atroph*.tw. | 9,455 | 9,628 |
| | 6 | Or/1-5 | 13,250 | 13,472 |
| Exclusion terms | 7 | exp animals/ not exp humans/ | 9,218,830 | 9,074,130 |
| | 8 | (“conference abstract” or “conference review” or comment or letter or case reports or editorial or note).pt. | 8,200,331 | 8,438,408 |

| Term Group | # | Search terms | Results (2017) | Results (2018) |
|---|---|---|---|---|
| Combined | 9 | 7 or 8 | 17,031,676 | 17,105,403 |
| | 10 | 6 not 9 | 9,588 | 9,693 |
| Date limit (original) | 11 | Limit 10 to dc=20120625-20170818 | 2,785 | - |
| | 12 | Remove duplicates from 11 | 1,919 | - |
| Date limit (update) | 11 | Limit 10 to dd=20170818-20180221 | - | 4,203 |
| | 12 | Limit 11 to ed=20170818-20180221 | - | 196 |
| | 13 | Remove duplicates from 12 | - | 179 |

**Table 20: Search strategy for the Cochrane Library Databases (searched via the Wiley Online platform)**

| Term Group | # | Search terms | Results (2017) | Results (2018) |
|---|---|---|---|---|
| Spinal muscular atrophy | 1 | [mh ^"spinal muscular atrophies of childhood"] | 18 | 20 |
| | 2 | [mh ^"spinal muscular atrophy"] | 25 | 25 |
| | 3 | "werdnig-hoffman":ti,ab,kw | 0 | 1 |
| | 4 | "wohlfart-kugelberg-welander":ti,ab,kw | 0 | 0 |
| | 5 | "spinal muscular atroph*":ti,ab,kw | 87 | 101 |
| | 6 | {or #1-#5} | 97 | 111 |
| Limits | 7 | #6 Publication Year from 2012 to 2017 in Cochrane Reviews (Reviews Only), Other Reviews and Trials | 44 *CSDR: 2; DARE: 1; CENTRAL: 41* | - |
| Limits for update | 7 | #6 Publication Year from 2017 to 2018 in Cochrane Reviews (Reviews Only), Other Reviews and Trials | - | 20 *CDSR: 0; DARE: 0; CENTRAL: 20* |

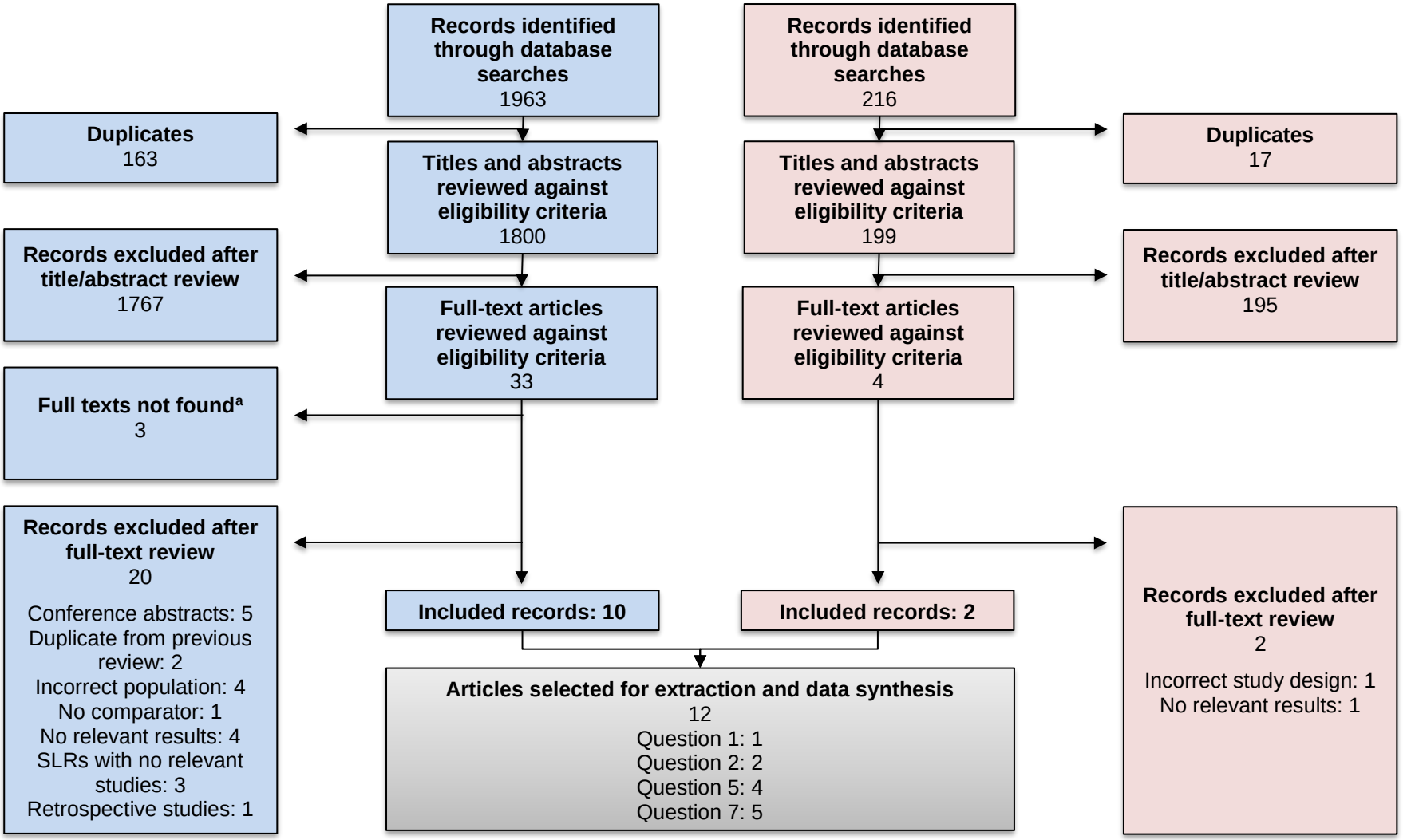
Results were imported into EndNote and de-duplicated.

# Appendix 2 – Included and excluded studies

## PRISMA flowchart

Figure 2 summarises the volume of publications included and excluded at each stage of the review. Eleven publications were ultimately judged to be relevant to one or more review questions and were considered for extraction. Publications that were included or excluded after the review of full-text articles are detailed below.

**Figure 2: Summary of publications included and excluded at each stage of the review**



Blue = August 2017; Red = February 2018. [a] Searches for full-text articles were carried out at Cambridge University Library. Some articles were not freely available at this library. For these articles, it was judged that they would not contain any additional pivotal data from relevant populations that would affect the conclusions of this review. Two articles (Wadman et al [2012], Wadman et al [2012]) were not selected for extraction due to their inclusion in the previous UK NSC review.[79, 80]

## Publications included after review of full-text articles

The 12 publications included after review of full-texts are summarised in Table 21.

**Table 21: Summary of publications included after review of full-text articles, and the question each publication was identified as being relevant to**

| Study | Study design | Country | Year(s) of study | Criterion 1 | Criterion 4 | Criterion 10 |
|---|---|---|---|---|---|---|
| **Ar Rochmah 2017[75]** | Prospective study | Japan* | NR | - | Q5 | - |
| **Bertini 2017[20]** | RCT | Belgium, France, Germany, Italy, Netherlands, Poland, and the UK | 2010 to 2013 | - | - | Q7 |
| **Chien 2017[63]** | Prospective study | Taiwan | 2014 to 2016 | - | Q5 | - |
| **Er 2012[76]** | Prospective, case-control study | Taiwan | NR | - | Q5 | - |
| **Feng 2017[73]** | Cohort study | NR | NR | - | Q2 | - |
| **Finkel 2017[9]** | RCT | Australia, Belgium, Canada, France, Germany, Italy, Japan, Republic of Korea, Spain, Sweden, Turkey, UK and the US | 2014 to 2016 | - | - | Q7 |
| **Kirschner 2014[81]** | RCT | Germany | 2007 to 2010 | - | - | Q7 |
| **Kissel 2014[82]** | RCT | USA* | NR | - | - | Q7 |

| Study | Study design | Country | Year(s) of study | Criterion 1 | Criterion 4 | Criterion 10 |
|---|---|---|---|---|---|---|
| **Liu 2016**[77] | Retrospective and prospective study | China | 2011 to 2014 | - | Q5 | - |
| **Mercuri 2018**[10] | RCT | Canada, France, Germany, Hong Kong, Italy, Japan, Republic of Korea, Spain, Sweden and the US | 2014 to 2017 | - | - | Q7 |
| **Verhaart 2017**[27] | Registry study | Europe, including UK | 2011 to 2015 | Q1 | - | - |
| **Wang 2015**[74] | Cohort study | NR | Patient recruitment from 2012 to 2013 | - | Q2 | - |

* Country assumed from author affiliations of the publication.
N/A, not applicable; NR, not reported; RCT, randomised controlled trial; UK, United Kingdom.

## Publications excluded after review of full-text articles

Of the 37 publications included after the review of titles and abstracts (33 in the 2017 searches and 4 in the 2018 searches), 25 were not included for data extraction. These 25 publications, along with reasons for exclusion, are listed in Table 22.

**Table 22: Publications excluded after review of full-text articles**

| Reference | Reason for exclusion |
|---|---|
| Cali F et al. Journal of genetics. 01 Apr 2014;93(1):179-81. | Does not meet inclusion criteria (includes individuals not necessarily planning pregnancy)<br>Relevant data NR (does not report uptake or the number or percentage of couples who are both carriers, only the number of individuals who are carriers) |
| Chen M et al. Ultrasound in obstetrics & gynecology. 01 Jun 2017;49(6):799-802. | No relevant results |
| Dessaud E et al. Neurology. 2014;83(2):e37 | Conference abstract |
| Dessaud E et al. Neuromuscular disorders. 2014;24(9-10):920-1. | Conference abstract |
| Elsheikh B et al. Neurology. 2012;78(1 Meeting Abstract). | Conference abstract |
| Esposito G et al. Clinical Chemistry and Laboratory Medicine. 01 Dec 2013;51(12):2239-45. | Full text not found |
| Finkel RS et al. The Lancet. 17 Dec 2016;388(10063):3017-26. | No appropriate comparison of intervention to normal care |
| Godinho FMS et al. Genetics and Molecular Biology. 2012;35(4 SUPPL.):955-9. | No relevant results |
| Jiang W et al. Genetic Testing and Molecular Biomarkers. 01 May 2013;17(5):438-42. | Full text not found |
| Kato N et al. Kobe Journal of Medical Sciences. 2014;60(4):E78-E85. | No relevant results<br>Not published in a peer-reviewed journal |
| Khirani S et al. Pediatric Neurology. Aug 2017;73:78-87.e1. | Retrospective study and so not relevant to the specific review question |
| Larson JL et al. BMC Medical Genetics. October 29 2015;16 (1) (no pagination)(100). | No relevant outcomes of screening method |
| Li L et al. Clinical Chemistry and Laboratory Medicine. 01 Mar 2017;55(3):358-67. | Full text not found |
| Macdonald WK et al. Prenatal Diagnosis. 01 Dec 2014;34(12):1219-26. | Relevant information missing (screening tests used in each included study are not specified)<br>Dates of publications all before previous searches were conducted |
| Moore GE et al. Neuromuscular Disorders. 01 Jul 2016;26(7):395-404. | No relevant included studies |
| Parks M et al. European Journal of Human Genetics. 01 Apr 2017;25(4):416-22. | Populations not relevant to review question (2 populations; pregnant women referred owing to a risk of fetal aneuploidy, and pregnant couples who are known carriers of SMA mutations) |
| Petit F et al. Muscle and Nerve. 2011;43(1):26-30. | Patients from incorrect country for inclusion for question 1 (France)<br>Not included for question 5 as not in a relevant population (not newborns) or using relevant methodology (not DBS) |
| Renusch SR et al. Journal of neuromuscular diseases. | No relevant outcomes |

| Reference | Reason for exclusion |
|---|---|
| Jun 04 2015;2(2):119-30. | |
| Shinohara M et al. Kobe Journal of Medical Sciences. 2017;63(2):E37-E40. | No relevant outcomes |
| Sa'adah N et al. Clinical laboratory. 2015;61(5-6):575-80. | Conference abstract |
| Stabley DL et al. Molecular Genetics & Genomic Medicine. Jul 2015;3(4):248-57. | No relevant outcomes |
| Wadman RI et al. Cochrane database of systematic reviews (Online). 2012;4:CD006282. | Duplicate from previous review |
| Wadman RI et al. Cochrane database of systematic reviews (Online). 2012;4:CD006281. | Duplicate from previous review |
| Wood SL et al. American Journal of Perinatology. 01 Oct 2016;33(12):1211-7. | Conference abstract |
| Zanetta C et al. Clinical Therapeutics. 01 Jan 2014;36(1):128-40. | SLR with no relevant studies included |

DBS, dried blood spot; NR, not reported; SLR, systematic literature review.

# Appendix 3 – Summary and appraisal of individual studies

## Data extraction – Criterion 1

One study relating to the epidemiology of SMA is described in Table 23.

**Table 23:**

| **Study reference** | **Verhaart 2017[71]** |
|---|---|
| **Study Design** | Design<br>Registry study<br>Objective<br>To estimate the worldwide incidence of SMA using a combination of multiple sources<br>Dates<br>2011 to 2015<br>Country<br>Various countries in Europe, including UK<br>Setting and Data Sources<br>Data from multiple sources, including genetic laboratories and patient and clinical registries<br>Genetic laboratories testing for *SMN1* were identified using publically available information as well as expert input and validation using the following sources:<br>• the Eurogentest/ Orphanet database of diagnostic laboratories<br>• the European Directory of DNA Diagnostic Laboratories (EDDNAL)<br>• the laboratory database via GeneTests.org<br>• the Genetic Testing Registry (GTR) from NCBI<br>• several country-specific websites<br>• personal communication with patient registry curators and researchers from specific countries.<br>Responses from genetic laboratories were collected via an online survey (http://www.surveymonkey.net) to determine the number of patients with a genetically confirmed diagnosis. The structured survey included questions about diagnostic techniques, total numbers of positive diagnoses, excluding prenatal, in 2015 and in the 5-year period (1 January 2011 to 31 December 2015). The survey was distributed via personalised emails. Two reminders were sent out and up to three further follow-ups were performed fortnightly via telephone and email. In relevant countries, local experts were consulted to determine the important genetic laboratories and their sizes.<br>For consistency purposes, population data for all countries included in the analysis were extracted from the United Nations, which report population numbers per year (as of 1st July) and the number of live births in periods of 5 years (i.e., 2011–2015). To estimate the number of live births for 2015, the |

| Study reference | Verhaart 2017[71] |
|---|---|
| | number of live births for the period 2011–2015 was divided by five. This approximation was used, because not every country has a national statistical office providing accurate data per year.<br>Incidence was calculated by dividing the number of positive tests by the number of live births in the same period and prevalence by dividing the number of patients at the measured timepoint by the total population. "Incidence" here is the proportion of newborns who have confirmed SMA; this is not a true incidence or incidence rate, but rather the prevalence at birth of SMA; however, the authors used the nomenclature of "incidence" in line with other published SMA literature. |
| **Population Characteristics** | Sample Size and Demographics<br>In total, 4653 patients were genetically diagnosed with SMA in the 5-year period 2011 to 2015, of which 992 in 2015 alone, across Europe.<br>In the UK, 11 of 12 invited genetic laboratories responded to the survey |
| **Prevalence and Incidence Outcomes** | Incidence rate from genetic laboratories: |

| Year | No. of SMA patients | No. of live births | Incidence (per 10,000 births) | 95% CI |
|---|---|---|---|---|
| 2015 | 88 | 804,083 | 10.9 | 8.8 to 13.5 |
| 2011 to 2015 | 438 | 4,020,416 | 10.9 | 9.9 to 12.0 |

## Data extraction – Criterion 4

Studies relating to screening tests for SMA are described in Table 24 to Table 29.

### Table 24: Feng 2017 (question 2)

| Study reference | Feng 2017[73] |
|---|---|
| **Study Design** | Design<br>Cohort study<br>Objective<br>To investigate pan-ethnic *SMN1* copy-number and sequence variation by hybridisation-based target enrichment coupled with massively parallel sequencing or next-generation NGS<br>Dates<br>NR<br>Country<br>USA<br>Setting<br>Samples submitted to a genetics laboratory for carrier testing |
| **Population characteristics** | Patient recruitment<br>6738 clinical samples were submitted to the laboratory for carrier testing<br>Data collection |

| **Study reference** | **Feng 2017[73]** |
|---|---|
| | The analyses were performed using de-identified samples submitted to Baylor Genetics Laboratory for carrier testing for a panel of diseases, including SMA, by NGS, qPCR (Fluidigm), and MLPA<br>*SMN1* copy-number results were compared between the NGS and qPCR (Fluidigm) and/or MLPA<br>Sample size and demographics<br>All samples in the study, n=6738<br>5344 with known ethnicity – 2175 Caucasian, 1346 African American, 1359 Hispanic, 53 Ashkenazi Jewish, 411 Asian<br>Among all samples analysed for sequence variants by NGS, 10 individuals were identified with potentially pathogenic single nucleotide variants in the *SMN1* gene. These variants were either previously found in patients with SMA or novel likely pathogenic variants. The NGS results were confirmed using gene-specific PCR followed by amplicon-based sequencing<br>Inclusion/exclusion criteria<br>NR |
| **Screening methods** | Screening methods<br>DNA was extracted from whole blood using commercially available DNA isolation kits (Gentra Systems, Minneapolis, MN)<br>NGS reads aligned to *SMN1* and *SMN2* exon 7 were quantified to determine the total combined copy number of *SMN1* and *SMN2*. The ratio of *SMN1* to *SMN2* was calculated based on a single-nucleotide difference that distinguishes the 2 genes.<br>The NGS data set was also queried for the g.27134T>G SNP and other *SMN1* sequence pathogenic variants<br>**NGS:** genomic DNA was fragmented by sonication, ligated to multiplexing paired-end adapters, amplified by PCR with indexed primers for sequencing, and hybridised to biotin-labelled, custom-designed capture probes in a solution based reaction. Hybridisation was performed at 47°C for at least 16 hours, followed by paired-end sequencing (100 bp) on the Illumina HiSeq 2500 platform<br>Raw-image data conversion and demultiplexing were performed following Illumina's primary data analysis pipeline. The script for the detection of *SMN1* copy numbers using next-generation sequencing coverage depth is deposited at https://sourceforge.net/projects/PGCNARS<br>The methods used to determine *SMN1* and *SMN2* copy numbers were able to determine both the *SMN1:SMN2* copy number ratio and the *SMN1* + *SMN2* copy number sum, and could therefore distinguish between, for example, carriers (with one copy of each) and non-carriers (with two copies of each).<br>Reference standard<br>**MLPA:** *SMN1* copy number was analysed using the MCR-Holland SALSA MLPA Kit P060-B2 or custom-designed MLPA reagents, according to the manufacturer's recommendations. The MLPA reagent contains sequence-specific probes targeted to exons 7 and 8 of both *SMN1* and *SMN2*. The MLPA data were analysed using Coffalyzer software<br>**qPCR** (Fluidigm)**:** *SMN1* copy number was assessed using the TaqMan qPCR assay as part of a panel using the BioMark 96.96 Dynamic Array. Exon 7 from both the *SMN1* and *SMN2* genes was amplified by the following primer pair: 5'-ATAGCTATTTTTTTTAACTTCCTTTATTTTCC-3' and 5'-TGAGCACCTTCCTTCTTTTTGA-3'. A probe that specifically targets the *SMN1* PSV (FAM-TTGTCTGAAACCCTG) was used to detect *SMN1*, whereas *SMN2* was blocked by a probe that targets the *SMN2* PSV (VIC-TTTTGTCTAAAACCC). qPCR (Fluidigm) was performed on the BioMark HD system as previously described, with minor modifications. Copy number was calculated using the ΔΔCt method by normalising to the genomic reference of the case and to the batch reference within the chip |
| **Screening results** | Sensitivity and specificity<br>The test sensitivity and specificity of *SMN1* copy number analysis by a NGS-based computer algorithm compared to Fluidigm (qPCR)/MLPA |

| Study reference | Feng 2017[73] |
|---|---|

| | True positive confirmed by Fluidigm/MLPA | True negative confirmed by Fluidigm/MLPA |
|---|---|---|
| **1 copy of *SMN1*** | | |
| NGS test positive (1 copy of *SMN1*) | 90 | 26 |
| NGS test negative (>1 copy of *SMN1*) | 0 | 6622 |
| **2 copies of *SMN1*** | | |
| NGS test positive (2 copies of *SMN1*) | 5445 | 21 |
| NGS test negative (1 or ≥3 copies of *SMN1*) | 35 | 1237 |
| **≥3 copies of *SMN1*** | | |
| NGS test positive (≥3 copies of *SMN1*) | 1147 | 9 |
| NGS test negative (<3 copies of *SMN1*) | 21 | 5561 |

| | NGS performance (%) | 95% CI |
|---|---|---|
| **1 copy of *SMN1*** | | |
| Sensitivity (n=90) | 100.0 | 95.9 to 100 |
| Specificity (n=6648) | 99.6 | 99.4 to 99.7 |
| **2 copies of *SMN1*** | | |
| Sensitivity (n=5480) | 99.4 | 99.1 to 99.5 |
| Specificity (n=1258) | 98.3 | 97.5 to 98.9 |
| **≥3 copies of *SMN1*** | | |
| Sensitivity (n=1168) | 98.2 | 97.3 to 98.8 |
| Specificity (n=5570) | 99.8 | 99.7 to 99.9 |

bp, base pair; CI, confidence interval; MLPA, multiplex ligation-dependent probe amplification; NGS, next generation sequencing; NR, not reported; PCR, polymerase chain reaction; PSV, paralogous sequence variant; qPCR, quantitative PCR; SMA, spinal muscular atrophy; *SMN*, survival motor neuron; SNP single nucleotide polymorphism.

## Table 25: Wang 2015 (question 2)

| Study reference | Wang 2015[74] |
|---|---|
| **Study Design** | Design<br>Cohort study<br>Objective<br>To evaluate and characterise a novel HRMA kit through comparison of the quantitative results of *SMN1* and *SMN2* genes with the current DHPLC assay<br>Dates<br>Patients recruited from November 2012 to March 2013 |

| Study reference | Wang 2015[74] |
|---|---|
| | Country<br>NR<br>Setting<br>NR |
| **Population characteristics** | Patient recruitment<br>A total of 453 participants were recruited from November 2012 to March 2013<br>Informed consent was obtained from each participant or parents of participants aged 18 years or less<br>Data collection<br>Specific details of data collection NR<br>All samples were assessed by the HRMA carrier-screening test and the DHPLC assay to validate the copy numbers of *SMN1* and *SMN2*<br>Sample size and demographics<br>453 participants<br>SMA patients were excluded<br>Other inclusion/exclusion criteria NR<br>Sample population baseline characteristics NR |
| **Screening methods** | Screening methods<br>All of the samples were assessed by the HRMA carrier-screening test to validate the copy numbers of *SMN1* and *SMN2*<br>DNA isolation and PCR:<br>The genomic DNA was isolated from whole blood sample using the TANBead® Blood DNA Auto Kit and Smart LabAssist-32. The DNA was diluted to a final concentration of 20 to 50 ng/ml. The exon 7 and flanking area of the *SMN1* and *SMN2* genes were amplified by PCR. The primers were SMNQ and the internal control KRIT1<br>For the PCR, the 20 µL reaction mixture contained 5 µL of genomic DNA and 15 µL of RI-SMN Master Mix, which contained 10 µM SMNQ primer and 10 µM KIRT1 primer. The PCR reactions were performed in a SelectCycler. The conditions included an initial denaturation at 94°C for 2 min, followed by 25 cycles of 94°C for 1 min, 66°C for 1 min, and 68°C for 1 min, and a final extension at 68°C for 5 min and stopped at 4°C. The length of the SMNQ PCR product was 143 bp and the KRIT1 PCR product was 115 bp<br>Melting curve acquisition and analysis:<br>After the PCR, 1.0 µl LC Green Plus dye was added and incubated at 95°C for 2 min and at 40°C for 2 min. Subsequently, 5 µL of the incubated PCR product were taken into the capillary, The PCR products were placed into the HR-1™ High-Resolution Melter he<br>The samples were first denatured at 95°C and rapidly cooled to 40°C at a rate of 20°C/s, then melted from 60°C to 95°C with a slope of 0.05°C/s. The data were acquired every 1°C for a total of 25 readings. Melting curves were analysed with HR-1 Melt Analysis Tool software<br>Reference standard<br>DHPLC<br>Final validation method<br>All the carriers were validated by MLPA for final confirmation. When the results obtained from HRMA were inconsistent with those observed in |

| Study reference | Wang 2015[74] |
|---|---|
| | DHPLC, the samples were confirmed by MLPA |
| **Screening results** | Sensitivity and specificity<br>• The HRMA method displayed high sensitivity (100%) and specificity (99%) when compared with DHPLC (sensitivity = 93% and specificity = 100%)<br>• The HRMA method had a false-positive rate of 0.8% (3/354)<br>  ○ These 3 false positives (*SMN1/SMN2* = 1:2) were identified to be normal by either the DHPLC or MLPA test *SMN1/SMN2* = 2:3<br>DHPLC (*SMN1/SMN2* = 2:1) failed to recognise 2 participants who displayed *SMN1/SMN2* = 3:1 as identified through HRMA screening and subsequently validated with MLPA |

DHPLC, denaturing high-performance liquid chromatography; DNA, deoxyribonucleic acid; HRMA, high-resolution melting analysis; KRIT1, Krev interaction trapped protein 1; NGS, next generation sequencing; NR, not reported; PCR, polymerase chain reaction; *SMN*, survival motor neuron.

## Table 26: Ar Rochmah 2017 (question 5)

| Study reference | Ar Rochmah 2017[75] |
|---|---|
| **Study design** | Design<br>Case-control study<br>Objective<br>To establish a rapid, accurate, and high throughput *SMN1*-deletion detection system that can be applied to high throughput NBS for SMA<br>Dates<br>NR<br>Country<br>Japan<br>Setting<br>NR |
| **Population characteristics** | Patient recruitment<br>NR<br>Data collection<br>Blood samples were collected from SMA patients and controls and spotted onto filter paper (Thermo Fisher Scientific, Waltham, MA, USA). The storage period of the DBS samples ranged from 1 week to 5 years and were stored in the dark at room temperature (20 to 25°C)<br>Genomic DNA was extracted from DBS and then analysed using modified competitive oligonucleotide priming-PCR (mCOP-PCR)<br>The SMN1 and SMN2 gene profiles of all individuals had been previously analysed by PCR restriction fragment length polymorphism (PCR-RFLP) |

| Study reference | Ar Rochmah 2017[75] |
|---|---|
| | using DNA extracted from freshly collected blood<br><br>Sample size and demographics<br><br>A total of 88 DBS samples from 88 individuals were analysed<br><br>There were 35 controls carrying *SMN1* and *SMN2*, 12 carriers carrying *SMN1* and *SMN2*, 4 controls carrying only *SMN1*, and 37 patients carrying only *SMN2*<br><br>Demographics NR |
| **Screening methods** | Screening methods<br><br>Experimental protocol included the following steps:<br>• Collection of DBS on filter paper<br>• DNA extraction from DBS<br>• Targeted pre-amplification of the sequence containing *SMN1/SMN2* exon 7 using conventional PCR to secure the quality and quantity of each DNA sample<br>• Separation between *SMN1* and *SMN2* exon 7 by PCR-RFLP to test whether the pre-amplification affected the presence or absence of *SMN1*<br>• Gene-specific amplification of *SMN1/SMN2* exon 7 using mCOP-PCR<br><br>Targeted pre-amplification by conventional PCR:<br><br>Targeted pre-amplification of the sequence containing SMN1/2 exon 7 was performed by conventional PCR (Applied Biosytems, Foster City, CA, USA).<br><br>Separation between SMN1 and SMN2 exon 7 by PCR-RFLP:<br><br>PCR-RFLP was performed to enable separation between *SMN1* and *SMN2*<br><br>Real-time mCOP-PCR:<br><br>*SMN1/SMN2* specific amplification was performed by real-time PCR. After a 100-fold dilution of the first round PCR product, 2 µl of template solution was added to PCR mixture. The PCR conditions were: (1) initial denaturation at 94°C for 7 minutes; (2) 15 cycles of denaturation at 94°C for 1 minute, annealing at 37°C for 1 minute, and extension at 72°C for 1 minute; and (3) melting analysis. Fluorescence signals were detected at the end of each extension procedure. Melting curve analysis was performed after PCR amplification, with 10 seconds of denaturation at 95°C, 1 minute of renaturation at 60°C, and then melting, which consisted of a continuous fluorescence reading from 65°C to 97°C at the rate of 5 data acquisitions per °C<br><br>Reference standard<br><br>PCR-RFLP:<br><br>Prior to mCOP-PCR of DBS samples from individuals, all participants had previously been screened for *SMN* genes by PCR-RFLP using DNA extracted from freshly collected blood<br><br>Methodology for RFLP-PCR as a reference standard NR |

| | |
|---|---|
| **Study reference** | **Ar Rochmah 2017[75]** |
| **Screening results** | mCOP-PCR with DBS-DNA versus PCR-RFLP with fresh blood-DNA: detection of SMN1 deletion |

| | | PCR-RFLP (fresh blood-DNA) | | Total |
|---|---|---|---|---|
| | | *SMN1* exon 7 deletion | *SMN1* exon 7 non-deletion | |
| **Real-time mCOP-PCR** | *SMN1* exon 7 deletion | 32 | 0 | 32 |
| | *SMN1* exon 7 non-deletion | 0 | 45 | 45 |
| **Total** | | 32 | 45 | 77 |

mCOP-PCR with DBS-DNA versus PCR-RFLP with fresh blood-DNA: detection of SMN2 deletion

| | | PCR-RFLP (fresh blood-DNA) | | Total |
|---|---|---|---|---|
| | | *SMN2* exon 7 deletion | *SMN2* exon 7 non-deletion | |
| **Real-time mCOP-PCR** | *SMN2* exon 7 deletion | 4 | 0 | 4 |
| | *SMN2* exon 7 non-deletion | 0 | 73 | 73 |
| **Total** | | 4 | 73 | 77 |

- Detection of *SMN1* exon 7 deletion; sensitivity 1.0 [0.87, 1], specificity 1.0 [0.90, 1]
- Detection of *SMN2* exon 7 deletion; sensitivity 1.0 [0.40, 1], specificity 1.0 [0.94, 1]

DBS, dried blood spot; DNA, deoxyribonucleic acid; mCOP-PCR, modified competitive oligonucleotide priming-PCR; NBS, newborn screening; NR, not reported; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SMA, spinal muscular atrophy; *SMN*, survival motor neuron.

## Table 27: Chien 2017 (question 5)

| | |
|---|---|
| **Study reference** | **Chien 2017[63]** |
| **Study design** | Design<br>Prospective screening trial<br>Objective<br>To perform a screening trial to assess the feasibility of pre-symptomatic diagnosis of SMA through newborn screening (NBS) of a dried blood spot (DBS) sample and to assess the reproducibility, false-positive rates, and false-negative rates of the RT-PCR screening method<br>Dates<br>The study was conducted between November 2014 and September 2016<br>Country |

| Study reference | Chien 2017[63] |
|---|---|
| | Taiwan<br><br>Setting<br>The National Taiwan University Hospital Newborn Screening Center |
| **Population characteristics** | Patient recruitment<br>All newborns who underwent routine testing in the National Taiwan University Hospital Newborn Screening Center and who had parental consent for SMA screening. The centre screens approximately 35–37% of newborns born in Taiwan<br><br>Data collection<br>NR<br><br>Sample size and demographics<br>A total of 141,874 participants were potentially eligible. Overall, 21,607 participants were excluded due to lack of consent resulting in 120,267 newborns participating in the study, analysis was completed for more than 98% of samples<br><br>Eligible participants consisted of all newborns who underwent routine newborn metabolic screening at the National Taiwan University Hospital who had received parental consent. Infants from parents who had undergone SMA carrier testing were also eligible |
| **Screening methods** | Screening methods<br>The analysis was performed using DNA-based method to detect the absence of *SMN1* in newborns using an RT-PCR TaqMan SNP genotyping assay on a StepOnePlus RT-PCR 96-Well System<br><br>DNA was extracted from a 3.2-mm punch from each DBS sample. The assay mixture included TaqMan Genotyping Master Mix, primers, and probes targeting c.888+100A (5'-FAM-CAGATGTTAAAAAGTTG-3' MGB) and c.888+100G (5'-VIC-CAGATGTTAGAAAGTTG-3' MGB). The results were interpreted using the gene amplification efficiency, which is represented by ΔRn. Rn (the normalised reporter fluorescence intensity) was obtained from each assay, and ΔRn was the difference between the Rn at the end point and the starting points<br><br>Screening method validation<br>Cutoffs were determined based on ΔRn values of known patients and normal newborns. The screening method was validated by testing 2937 anonymous newborn DBS samples and 9 DNA samples with known *SMN1* and *SMN2* copy numbers and plotting ΔRn of *SMN1* against ΔRn of *SMN2*. Cutoffs were ΔRn<1 for *SMN1* and ΔRn<0.5 for *SMN2*. 77 additional DNA samples with a known SMA affected status were tested using this method and the results were perfectly matched<br><br>The positive screening results were confirmed by a ddPCR assay using the original screening DBS sample<br><br>Reference standard<br>MLPA assay using DNA extracted from recalled whole blood samples. A homozygous deletion of SMN1 exon 7 (with or without deletion of exon 8) confirmed the diagnosis of *SMN*-associated SMA (5q SMA) |

| Study reference | Chien 2017[63] |
|---|---|
| Screening results | Of the 120,267 eligible samples screened using the index test, 15 samples had a positive RT-PCR screening result. Subsequently, it was determined using the ddPCR assay that 7 patients had a homozygous deletion of *SMN1* (patients), whereas the other 8 cases had 1 copy of *SMN1* (carriers)<br>50 samples revealed unsatisfactory results and required repeat testing<br>Sensitivity<br>Sensitivity of RT-PCR was estimated as 95% (this method detected only the absence of homozygous *SMN1)*). It was unclear what reference standard was used to calculate specificity<br>Specificity<br>Specificity of RT-PCR was 99.99%. It was unclear what reference standard was used to calculate specificity<br>PPV<br>• PPV of RT-PCR was 47%. It was unclear what reference standard was used to calculate specificity<br>• Adding ddPCR as a second-tier assay gave a PPV of 100% when compared with MLPA<br>False positive rate<br>• 8/15 samples that tested positive using RT-PCR were false-positive cases (all had 1 copy of *SMN1*), when compared with ddPCR<br>When coupled with a second-tier ddPCR assay, NBS screening revealed no false-positive screening results |

DBS, dried blood spot; ddPCR, droplet digital polymerase chain reaction; DNA, deoxyribonucleic acid; MLPA, multiplex ligation-dependent probe amplification; NBS, newborn screening; NR, not reported; PCR, polymerase chain reaction; PPV, positive predictive value; RT-PCR, real-time PCR; SMA, spinal muscular atrophy; *SMN*, survival motor neuron.

## Table 28: Er 2012 (question 5)

| Study reference | Er 2012[76] |
|---|---|
| Study design | Design<br>Case-control screening study<br>Objective<br>To assess the value of high-resolution melting (HRM) analysis using RT-PCR for a high-throughput screening of SMA<br>Dates<br>NR<br>Country<br>Taiwan<br>Setting |

| Study reference | Er 2012[76] |
|---|---|
| | Chung-Ho Memorial Hospital, Kaohsiung Medical University |
| **Population characteristics** | Patient recruitment<br>A total of 60 DNA samples from SMA patients, carriers and normal individuals were obtained from Chung-Ho Memorial Hospital, Kaohsiung Medical University<br>Data collection<br>DNA samples were obtained from whole blood and dried blood spots, both using the QIAamp® mini DNA extraction kit (QIAGEN) according to the manufacturer's protocol.<br>Sample size and demographics<br>30 patients with SMA and 30 normal subjects reported in publication<br>Demographics NR<br>Results from screening of 80 samples are presented in the sensitivity and specificity analyses of HRM analysis verses denaturing high-performance liquid chromatography (DHPLC,) which is of interest to this review, however the recruitment or characteristics of this population are NR |
| **Screening methods** | Screening methods<br>HRM analysis:<br>(States that technique is described in previous publications)<br>The primers used for HRM analysis were: E1 (forward): 5'-ttcctttattttccttacagggttt-3' and E2 (reverse) 5'-tctgccagcattatgaaagtg-3'. The primer set was used to amplify the region including the substitution of single nucleotide in *SMN1* exon 7.<br>For DBS, 2 µl of DNA sample was added to the PCR mixture. The melting curves were normalized by selecting the areas of pre-melt (initial fluorescence) and post-melt (final fluorescence)<br>The genomic DNA samples obtained from whole blood and DBS were mixed with driver before HRM analysis. The driver was defined as a DNA sample with known *SMN1/SMN2* copy number (*SMN1/SMN2*=0:3)<br>Identical DNAs (sample A and sample B or sample C and D) were analysed repetitively by HRM for confirming whether the melting curve was reproducible for both whole blood and DBS samples<br>Reference standard<br>DHPLC:<br>All the results were previously confirmed by DHPLC<br>Methodology for DHPLC is NR in this publication |

| Study reference | Er 2012[76] |
|---|---|
| Screening results | Sensitivity and specificity<br>When comparing the results of *SMN1* exon 7 deletion by using HRM analysis to the use of DHPLC: |

| | | DHPLC | | |
|---|---|---|---|---|
| | | (+) | (-) | Total |
| HRM analysis | ***SMN1* exon 7 deletion (+)** | 30 | 0 | 30 |
| | ***SMN1* exon 7 deletion (-)** | 0 | 50 | 50 |
| | **Total** | 30 | 50 | 80 |

Sensitivity: 100%
Specificity: 100%

DBS, dried blood spot; DHPLC, denaturing high-performance liquid chromatography; DNA, deoxyribonucleic acid; HRM, high-resolution melting; NBS, newborn screening; NR, not reported; PCR, polymerase chain reaction; RT-PCR, real-time PCR; SMA, spinal muscular atrophy; *SMN*, survival motor neuron.

## Table 29: Liu 2016 (question 5)

| Study reference | Liu 2016[77] |
|---|---|
| Study design | Design<br>Retrospective and prospective screening study<br>Objective<br>To measure the gene mutation or deletion of key genes for SMA and to further analyse genotype-phenotype correlation, using DBS samples<br>Dates<br>2011 to July 2014<br>Country<br>South-west China<br>Setting<br>Study was performed at the Children's Hospital of Chongqing Medical University |
| Population characteristics | Patient recruitment<br>**Retrospective study:** 1613 children with limb movement disorders were assessed for eligibility, of which 141 children were confirmed to exhibit the inclusion criteria. These 141 children and an additional 100 normal children were enrolled. Patients were included if they had a confirmed normal nutritional status; disorder on sitting, standing or walking; physical examination showing myasthenia or muscle atrophy; sequencing, multiplex PCR and MLPA detected disorder; and hospitalised<br>**Prospective study:** 2000 DBS samples were randomly selected from 2011 to July 2014 from the Newborn Screening Center at the Children's Hospital of Chongqing Medical University<br>Data collection |

**Study reference** **Liu 2016[77]**

**Retrospective study:** Blood specimens and medical records were collected.

**Prospective study:** 2000 random DBS samples were analysed by multiple RT-PCR and confirmed by DNA sequencing and MLPA from the Newborn Screening Center at the Children's Hospital of Chongqing Medical University

Sample size and demographics

**Retrospective study:** 141 children with limb movement disorders and 100 normal controls. Of these, 75 children were diagnosed with SMA based on the SMA diagnosis guideline.[19]

The general and clinical characteristics of children in different types of SMA:

| | Type I (%) (n=41) | Type II (%) (n=29) | Type III (%) (n=5) | Total (%) (n=75) | p-value* |
|---|---|---|---|---|---|
| **Gender*** | | | | | |
| Boy | 24 (58.54) | 19 (65.52) | 3 (60) | 46 (61.33) | – |
| Girl | 17 (41.46) | 10 (34.48) | 2 (40) | 29 (38.67) | – |
| **Clinical symptoms*** | | | | | |
| Congenital heart disease | 3 (7.32) | 0 | 0 | 3 (4) | 0.5137 |
| Respiratory failure | 6 (14.63) | 2 (6.90) | 0 | 8 (10.67) | 0.7308 |
| Muscular atrophy | 20 (48.78) | 13 (44.83) | 2 (40) | 35 (46.67) | 0.9835 |
| EMG abnormalities | 39 (95.12) | 28 (96.55) | 4 (80) | 71 (94.67) | 0.4405 |
| **Prenatal*** | | | | | |
| Ultrasonography abnormalities | 0 | 0 | 0 | 0 | – |
| Decreased fetal movement | 3 (7.32) | 2 (6.90) | 0 | 5 (6.67) | 1.0000 |
| **CK value*** | | | | | |
| Normal | 6 (14.63) | 8 (27.59) | 2 (40) | 16 (21.33) | 0.3391 |
| Elevation | 35 (85.37) | 21 (72.41) | 3 (60) | 59 (78.67) | – |
| **Molecular detection** | | | | | |
| Homozygous mutation at c.840 C > T in SMN gene | 40 (97.56) | 26 (89.66) | 5 (100) | 71 (94.67) | – |

*Fisher's exact test

**Prospective study:** 2000 dry blood spot samples from newborn screening

There were no inclusion criteria for the prospective study because the samples were selected randomly

**Screening methods**

Screening methods

**Retrospective study:**

*RT-PCR in clinical specimens*

DNA was purified according to the manufacturer's instructions (DP318-03, Tiangen Biotech, Beijing, China). Next, 1μl of DNA template (20 ng/μl) and

| Study reference | Liu 2016[77] |
|---|---|
| | 19µl of a real-time PCR master mix (FP203-02, Tiangen Biotech, Beijing, China), were used to perform RT-PCR. RT-PCR was performed on the Applied Biosystem 7500 RT-PCR system (Applied Biosystems, CA, USA) using the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 55°C for 40 seconds. All standards and samples were performed in triplicate<br>**Prospective study:**<br>RT-PCR was performed on 2000 randomly selected DBS samples. DNA was purified by Chelex-100. Briefly, one DBS (diameter of 3 mm) was clipped and mixed with 500 µl of nuclease-free water to wash and was then centrifuged, and the supernatants were discarded. Next, 5% Chelex-100 was mixed before the addition of 100 µl to each pellet, and the mixture was incubated at 56°C for 10 minutes. The mixture was then mixed and centrifuged to harvest the supernatants. RT-PCR was performed using the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 55°C for 40 seconds. All samples were performed in triplicate<br>Reference standard<br>**Retrospective study:**<br>*Sanger DNA sequencing in clinical specimens*<br>All 141 patients and 100 normal children were analysed using Sanger DNA sequencing for the point mutation at c.840 C>T of the SMN gene. The DNA template was the same template used in RT-PCR. Each PCR reaction was performed in a 50µl volume. PCR was performed on an ABI-Verity Thermal Cycler (Applied Biosystems, Foster City, California, USA) using the following conditions: 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 53°C for 30 seconds and 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. All samples were referred to Sunny (Sunny Biotechnology Co. Ltd., Shanghai, China) for sequencing.<br>**Prospective study:**<br>Positive samples from the RT-PCR methods were reconfirmed by RT-PCR and then DNA sequenced. There was no reference standard for screen-negative patients |
| **Screening results** | **Retrospective study:**<br>*Molecular diagnosis of SMA using novel RT-PCR methods*<br>There were 71 (94.67 %) SMA patients with homozygous deletion on SMN1 among 75 SMA patients<br><br>The results of this novel real-time PCR were compared with those obtained from DNA Sanger sequencing in all 141 patients and 100 controls (n=241):<br>• The accuracy of RT-PCR for the homozygous exon 7 deletion in the SMN gene was 99.6 % (240/241)<br>• The false positive rate of RT-PCR was 0<br>• The false negative rate of RT-PCR was 0.6 % (1/171) |

| Sanger DNA Sequencing/RT-PCR (%) | | | | $\chi^2$ | p-value |
|---|---|---|---|---|---|
| +/+[a] | -/+[b] | +/-[c] | -/-[d] | | |
| 70 (29.1) | 0 | 1 (0.4) | 170 (70.5) | 1.0 | 0.3173 |

[a]There was homozygous mutation on SMN c.840 C>T for both methods, meaning true positive for RT-PCR compared with DNA sequencing

[b]There was no homozygous mutation on SMN c.840 C>T detected by Sanger DNA sequencing but detected by RT-PCR, meaning false positive for RT-PCR compared with DNA sequencing

[c]There was homozygous mutation on SMN c.840 C>T for Sanger DNA sequencing but not detected by RT-PCR, meaning false negative for RT-PCR compared with

| Study reference | Liu 2016[77] |
|---|---|
| | DNA sequencing<br>[d]There were normal and heterozygous mutation on SMN c.840 C>T for both Sanger DNA sequencing and RT-PCR, meaning true negative for RT-PCR compared with DNA sequencing<br><br>**Prospective study:**<br><br>• Initial RT-PCR screen: 23 positive results<br>• After reconfirmation by RT-PCR and then DNA sequenced: 1 true positive, 22 false positives<br>There was no reference standard for screen-negative patients, of the samples ultimately concluded to be negative 1997 had no symptoms related to SMA, and 2 had progressive muscular dystrophy<br><br>Real time PCR for NBS |

| | | | | |
|---|---|---|---|---|
| **DBS screened/Total** | 213/33499 | 637/99841 | 706/110592 | 444/69224 |
| **Positive number** | 0 | 0 | 1 | 0 |

DBS, dried blood spot; DNA, deoxyribonucleic acid; *GTF2H2*, general transcription factor IIH, subunit 2; MLPA, multiplex ligation-dependent probe amplification; *NAIP*, apoptosis inhibitory protein; NBS, newborn screening; NR, not reported; PCR, polymerase chain reaction; RT-PCR, real-time PCR; SMA, spinal muscular atrophy.

Studies relating to the management of SMA are described in Table 30 to Table 33.

## Data extraction – Criterion 10

### Table 30: Bertini 2017 (question 7)

| Study reference | Bertini 2017[20] |
|---|---|
| **Study design** | Design<br>RCT<br>Objective<br>To assess the safety, tolerability, and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 SMA<br>Dates<br>November 18th 2010 to October 9th 2013<br>Countries<br>Belgium, France, Germany, Italy, Netherlands, Poland, and the UK<br>Setting<br>22 neuromuscular care centres, all sites were centres with expertise in treating patients with SMA in line with the published standards of care for SMA |

| **Study reference** | **Bertini 2017[20]** |
|---|---|

**Population characteristics**

Patient recruitment

Patients were recruited mainly via information disseminated through the TREAT-NMD website, patient registries, and in the clinics at each site

Data collection

After screening and baseline visits, follow-up visits were scheduled for week 4 and week 13 after randomisation, after which participants were assessed every 13 weeks for a total of 9 visits over the 24-month treatment period

Sample size and demographics

165 type 2/3 SMA patients enrolled, 160 included in the full analysis set (FAS) (all randomly assigned patients who received at least one dose of olesoxime or placebo and who had at least one post-randomisation assessment of motor function measure [MFM] available) on which efficacy analyses were based

| | Olesoxime (n=103) | Placebo (n=57) | Total (n=160) |
|---|---|---|---|
| Male | 55 (53%) | 25 (44%) | 80 (50%) |
| Female | 48 (47%) | 32 (56%) | 80 (50%) |
| **Age (years)** | | | |
| Mean | 9.1 (5.5) | 11.2 (6.0) | 9.9 (5.7) |
| Median (range) | 7 (3 to 25) | 11 (3 to 27) | 8 (3 to 27) |
| <6 years | 35 (34%) | 13 (23%) | 48 (30%) |
| ≥6 years | 68 (66%) | 44 (78%) | 112 (70%) |
| **SMA type** | | | |
| Type 2 | 74 (72%) | 39 (68%) | 113 (71%) |
| Type 3 | 29 (28) | 18 (32%) | 47 (29%) |

Baseline characteristics and demographics of FAS presented

Inclusion criteria

- Aged 3 to 25 years
- Diagnosis of type 2 or non-ambulatory type 3 SMA
- Weakness and hypotonia consistent with a clinical diagnosis of SMA type 2 or 3
- Genetic diagnosis of SMA with homozygous deletion of *SMN1* exon 7, or a heterozygous deletion accompanied by a point mutation on the other allele
- MFM relative score (percentage of the maximum sum of both dimensions) of 15% or higher (functional domain 1 [D1] plus functional domain 2 [D2] score)
- Hammersmith Functional Motor Scale (HFMS) score at baseline between 3 and 38 (non-ambulatory)
- Onset of symptoms at 3 years of age or younger

| Study reference | Bertini 2017[20] |
|---|---|
| | • The ability to take the study treatment (tested at screening after informed consent)<br>Exclusion criteria<br>• Evidence of renal dysfunction, blood dysplasia, hepatic insufficiency, symptomatic pancreatitis, congenital heart defect<br>• Known history of metabolic acidosis, hypertension, significant central nervous system impairment, or neurodegenerative or neuromuscular disease other than SMA<br>• Any clinically significant electrocardiogram abnormality<br>• Use of medications intended for the treatment of SMA<br>• Inability to meet study visit requirements or cooperate reliably with functional testing<br>• Surgical spinal rod or fixation for scoliosis within the past 6 months or anticipated need of rod or fixation within 6 months of enrolment |
| **Treatments** | Allocation methods<br>Patients were randomly assigned in a 2:1 ratio to receive olesoxime or placebo, with stratification by SMA type and centre. A 2:1 randomisation as a means to limit placebo exposure was deemed more ethically acceptable in a progressive, debilitating disease with no available treatment options. Randomisation lists were generated centrally by an independent statistician using validated randomisation software. To maintain masking, active and placebo treatments were supplied in brown glass bottles, and randomisation details were provided using secure procedures to the clinical research organisation that did the packaging of the treatment units and to the laboratory that did the olesoxime pharmacokinetics bioanalysis assay. All investigators, site personnel, patients, and the sponsor study personnel were masked to treatment assignment until completion of the study<br>An interim efficacy analysis was done by an independent statistician when all patients had been treated for 12 months, to assess the need to continue the study to reach the planned objective. In the event of positive and significant results in favour of olesoxime, the study was to be considered successful and all patients were to be switched to olesoxime to allow assessment of the sustainability of the treatment effect and safety. If the results were significantly in favour of placebo, the study was to be discontinued for failure (futility). The interim efficacy analysis was reviewed by an independent Data Monitoring Committee. The final efficacy and safety analysis was done using data at 24 months<br>Intervention<br>Patients received oral olesoxime 100 mg/mL liquid suspension formulation at a weight-based fixed dose of 10 mg/kg once a day with their main daily meal for 24 months<br>Comparator<br>Patients received matching placebo once a day with their main daily meal for 24 months |
| **Clinical and safety outcomes** | **Efficacy outcomes**<br>MFM Domains 1 and 2, LS Mean CfB to Week 104 |

| Outcome | Intervention and comparator | N | Mean baseline (SD) | Least-squares mean change from baseline to week 104 (SE) [CI] | Estimate of difference from placebo (SE) | 96% CI | p-value |
|---|---|---|---|---|---|---|---|
| MFM D1 + D2 | Olesoxime | 103 | 39.58 (11.701) | 0.18 (0.717) | 2.00 (1.088) | -0.25 to | 0.0676 |

**Study reference** **Bertini 2017[20]**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| score (primary analysis)* | | | | [96% CI -1.30 to 1.66] | | 4.25 | |
| | Placebo | 57 | 38.99 (11.905) | -1.82 (0.901) [96% CI -3.68 to 0.04] | | | |
| MFM D1 + D2 score (sensitivity analysis)^ | Olesoxime | 103 | 39.01 (11.472) | 0.24 (0.696) [95% CI -1.14 to 1.61] | 2.20 (1.050) | 0.12 to 4.27 | 0.0379 |
| | Placebo | 57 | 38.69 (11.689) | -1.96 (0.872) [95% CI -3.68 to -0.24] | | | |

*MMRM; full analysis set; for children aged <6 years who erroneously did the MFM32 assessment, MFM20 score was calculated from MFM32 score; ^data as collected from whichever form of the MFM was used
96%, not 95% Cis are used here.

Motor Function Measure Domains 1 and 2, Adjusted Mean CfB – Difference Between Treatment Groups (Olesoxime [n=103] and Placebo [n=57])

| MFM D1 + D2 score change from baseline | 6 months | 12 months | 18 months | 24 months |
|---|---|---|---|---|
| Difference between treatment groups | 2.41 | 1.77 | 2.74 | 2.00 |
| p value | 0.0039 | 0.0711 | 0.0075 | 0.0676 |

For children younger than 6 years who erroneously did the MFM32 assessment, MFM20 score was calculated from MFM32 score

- Overall treatment difference = **2·23 points** (96% CI 0·50 to 3·96; p=0·0084 in favour of olesoxime)

MFM Domains and HFMS, LS Mean CfB to Month 24 or Month 21

| Outcome | Intervention and comparator | N | Mean baseline (SD) | Least-squares mean change from baseline to week 104 (SE) [95% CI] | Estimate of difference from placebo (SE) | 95% CI | p-value |
|---|---|---|---|---|---|---|---|
| MFM total score | Olesoxime | 103 | 49.32 (10.993) | 0.59 (0.751) [-0.90 to 2.07] | 2.04 (1.138) | -0.21 to 4.28 | 0.0755 |
| | Placebo | 57 | 49.11 (11.432) | -1.45 (0.943) [-3.31 to 0.41] | | | |
| MFM D1 | Olesoxime | 103 | 6.76 (7.933) | 0.07 (0.554) [-1.02 to 1.16] | 0.97 (0.854) | -0.72 to 2.66 | 0.2582 |
| | Placebo | 57 | 7.28 (7.543) | -0.90 (0.706) [-2.29 to 0.49] | | | |
| MFM D2 | Olesoxime | 103 | 74.10 (18.610) | 0.38 (1.217) [-2.02 to 2.78] | 3.16 (1.838) | -0.47 to 6.79 | 0.0873 |
| | Placebo | 57 | 72.64 (18.882) | -2.78 (1.524) [-5.79 to 0.23] | | | |
| MFM D3 | Olesoxime | 103 | 85.41 (13.147) | 2.27 (1.264) [-0.22 to 4.76] | 2.12 (1.945) | -1.72 to 5.96 | 0.2773 |
| | Placebo | 57 | 86.05 (15.412) | 0.15 (1.606) [-3.02 to 3.32] | | | |

**Study reference** **Bertini 2017[20]**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| HFMS (to week 91 **NOT** 104) | Olesoxime | 103 | 16.47 (10.576) | -0.78 (0.416) [-1.60 to 0.04] | 0.94 (0.622) | -0.28 to 2.17 | 0.1309 |
| | Placebo | 57 | 14.86 (10.514) | -1.72 (0.515) [-2.74 to -0.70] | | | |

For children younger than 6 years who erroneously did the MFM32 assessment, MFM20 score was calculated from MFM32 score

MFM and HFMS Responders

| Subgroup | Intervention and comparator | N | Mean MFM D1 + D2 (SD) | p-value for treatment difference |
|---|---|---|---|---|
| Age 3 to <6 | Olesoxime | 35 | 45.89 (10.67) | 0.7459 |
| | Placebo | 13 | 44.07 (10.65) | |
| Age 6 to 15 | Olesoxime | 54 | 37.53 (11.07) | 0.0362 |
| | Placebo | 25 | 36.48 (12.14) | |
| Age >15 | Olesoxime | 14 | 31.71 (9.29) | 0.9602 |
| | Placebo | 19 | 38.81 (11.95) | |

For children younger than 6 years who erroneously did the MFM32 assessment, MFM20 score was calculated from MFM32 score

Adjusted mean change in MFM D1 + D2 scores from baseline to month 24, by age group

| Outcome | Intervention and comparator | N | n (%) | Relative Risk (95% CI) | p-value |
|---|---|---|---|---|---|
| MFM D1 + D2 (to month 24) | Olesoxime | 103 | 56 (54) | 1.43 (0.98 to 2.08) | 0.0609 |
| | Placebo | 57 | 22 (39) | | |
| MFM total score (to month 24) | Olesoxime | 103 | 58 (56) | 1.46 (1.01 to 2.10) | 0.0419 |
| | Placebo | 57 | 22 (39) | | |
| HFMS (to month 21) | Olesoxime | 103 | 51 (50) | 1.82 (1.16 to 2.86) | 0.0091 |
| | Placebo | 57 | 16 (28) | | |

For children younger than 6 years who erroneously did the MFM32 assessment, MFM20 score was calculated from MFM32 score

**Supplementary efficacy data**

Effects of olesoxime on motor function across subgroups

| Effects of olesoxime on motor function | Subgroup | N | Adjusted mean (95% CI) |
|---|---|---|---|
| **Age** | | | |
| <6 years | MFM D1 + D2 | 48 | 0.75 (-3.86 to 5.35) |
| | HFMS | 48 | 1.54 (-1.25 to 4.33) |
| >6 years | MFM D1 + D2 | 112 | 2.21 (-0.21 to 4.62) |
| | HFMS | 112 | 0.68 (-0.71 to 2.06) |
| **SMA type** | | | |
| Type 2 | MFM D1 + D2 | 113 | 2.06 (-0.78 to 4.90) |
| | HFMS | 113 | 0.89 (-0.51 to 2.29) |
| Type 3 | MFM D1 + D2 | 47 | 2.06 (-0.83 to 4.94) |

**Study reference** **Bertini 2017[20]**

| | | | |
|---|---|---|---|
| | HFMS | 47 | 0.72 (-1.72 to 3.16) |
| **Gender** | | | |
| Male | MFM D1 + D2 | 80 | 0.6 (-2.51 to 3.70) |
| | HFMS | 80 | 1.5 (-0.32 to 3.33) |
| Female | MFM D1 + D2 | 80 | 3.05 (-0.11 to 6.21) |
| | HFMS | 80 | 0.72 (-1.02 to 2.47) |
| **Baseline severity** | | | |
| < Median | MFM D1 + D2 | 79 | 2.97 (-0.36 to 6.31) |
| ≥ Median | MFM D1 + D2 | 81 | 1.25 (-1.64 to 4.15) |

Analysis: for children aged <6 years age who erroneously performed the MFM32, MFM20 score was calculated from MFM32 score

Change from baseline to Month 24 for electrophysiology and respiratory function endpoints

| Outcome | Intervention and comparator | N | Baseline (SD) | Month 24, least square mean (95% CI) | p-value |
|---|---|---|---|---|---|
| CMAP, mV | Olesoxime | 70 | 3.74 (2.370) | -0.07 (-0.49 to 0.36) | NR |
| | Placebo | 34 | 4.02 (2.718) | -0.16 (-0.74 to 0.43) | 0.7865 |
| MUNE | Olesoxime | 58 | 39.70 (35.096) | -4.51 (-12.21 to 3.18) | NR |
| | Placebo | 30 | 36.24 (32.149) | -6.69 (-16.86 to 3.48) | 0.7117 |
| FVC/TC % | Olesoxime | 64 | 66.53 (28.321) | 4.28 (-0.32 to 8.88) | NR |
| | Placebo | 38 | 61.32 (21.863) | 6.16 (1.00 to 11.33) | 0.5655 |

Change from baseline to Month 24 in PEDsQL™ Neuromuscular Module

| PEDsQL™ Neuromuscular Module | Intervention and comparator | N | Estimate of difference from placebo* | 95% CI | p-value |
|---|---|---|---|---|---|
| **Patients** | | | | | |
| Young children ≤7 years | Olesoxime | 27 | -7.70 | -20.19 to 4.79 | 0.2163 |
| | Placebo | 8 | | | |
| Children 8 to 12 years | Olesoxime | 25 | -0.48 | -9.90 to 8.94 | 0.9172 |
| | Placebo | 9 | | | |
| Teenagers 13 to 18 years | Olesoxime | 11 | 3.13 | -7.54 to 13.80 | 0.546 |
| | Placebo | 17 | | | |
| Adults >18 years | Olesoxime | 8 | 5.12 | -6.07 to 16.32 | 0.273 |
| | Placebo | 3 | | | |
| All patient ratings (patients >5 years old) | Olesoxime | 71 | 0.25 | -4.58 to 5.08 | 0.9185 |
| | Placebo | 37 | | | |
| **Parents** | | | | | |
| All parent ratings | Olesoxime | 90 | 3.62 | -0.77 to 8.01 | 0.1054 |
| | Placebo | 46 | | | |
| **Subscore: family resources** | | | | | |

**Study reference** **Bertini 2017[20]**

| | | | | | |
|---|---|---|---|---|---|
| Parent reports | Olesoxime | 90 | 6.18 | -0.87 to 13.23 | 0.0851 |
| | Placebo | 46 | | | |
| Patient reports (≥8 years) | Olesoxime | 43 | 4.11 | -4.17 to 12.40 | 0.3427 |
| | Placebo | 29 | | | |
| **Subscore: neuromuscular disease** | | | | | |
| Parent reports | Olesoxime | 90 | 3.75 | -0.79 to 8.29 | 0.1050 |
| | Placebo | 46 | | | |
| Patient reports | Olesoxime | 71 | -0.34 | -5.25 to 4.56 | 0.8899 |
| | Placebo | 37 | | | |
| **Subscore: communication** | | | | | |
| Parent reports | Olesoxime | 88 | -0.66 | -8.44 to 7.13 | 0.8679 |
| | Placebo | 46 | | | |
| Patient reports (≥8 years) | Olesoxime | 43 | 4.83 | -3.11 to 12.78 | 0.2288 |
| | Placebo | 29 | | | |

*Differences between means are estimates of the mean difference between the treatment groups (olesoxime - placebo)

## Safety

Adverse events in all enrolled patients

| Adverse events (AEs); n (%) | Olesoxime (n=108) | Placebo (n=57) | Total (n=165) |
|---|---|---|---|
| **Patients with ≥1 AE** | 103 (95) | 57 (100) | 160 (97) |
| Number of AEs | 1104 | 612 | 1716 |
| Deaths | 1 (1) | 1 (2) | 2 (1) |
| Patients who withdrew from the study due to an AE | 4 (4) | 2 (4) | 6 (4) |
| Patients with ≥1 AE with fatal outcome | 1 (1) | 1 (2) | 2 (1) |
| **Patients with ≥1 SAE** | 34 (31) | 29 (51) | 62 (38) |
| Patients with ≥1 AE leading to withdrawal from the study | 9 (8) | 2 (4) | 11 (7) |
| Patients with ≥1 severe AE | 18 (17) | 14 (25) | 32 (19) |
| **Disease-related AEs (post-hoc)** | | | |
| Lower respiratory tract infections | 13 (12) | 10 (18) | 23 (14) |
| Respiratory failure | 2 (2) | 2 (4) | 4 (2) |
| Reflux disorders | 4 (4) | 4 (7) | 8 (5) |
| Constipation | 5 (5) | 4 (7) | 9 (5) |
| Scoliosis | 14 (13) | 6 (11) | 20 (12) |
| Other joint-related disorders | 13 (12) | 17 (30) | 30 (18) |
| Surgical procedure | 9 (8) | 5 (9) | 14 (8) |

## Supplementary safety data

Adverse events observed in more than 10% of patients (safety-evaluable population)

| AEs in more than 10% patients; n (%) | Olesoxime (n=108) | Placebo (n=57) |
|---|---|---|

| Study reference | Bertini 2017[20] | | |
|---|---|---|---|
| | Nasopharyngitis | 25 (23.1) | 15 (26.3) |
| | Upper respiratory tract infection | 23 (21.3) | 13 (22.8) |
| | Bronchitis | 17 (15.7) | 17 (29.8) |
| | Gastroenteritis | 16 (14.8) | 10 (17.5) |
| | Respiratory tract infection | 17 (15.7) | 6 (10.5) |
| | Pharyngitis | 15 (13.9) | 6 (10.5) |
| | Influenza | 11 (10.2) | 9 (15.8) |
| | Rhinitis | 14 (13.0) | 6 (10.5) |
| | Pneumonia | 6 (5.6) | 6 (10.5) |
| | Vomiting | 25 (23.1) | 16 (28.1) |
| | Abdominal pain | 20 (18.5) | 11 (19.3) |
| | Diarrhoea | 18 (16.7) | 12 (21.1) |
| | Cough | 32 (29.6) | 16 (28.1) |
| | Oropharyngeal pain | 16 (14.8) | 9 (15.8) |
| | Pyrexia | 34 (31.5) | 16 (28.1) |
| | Pain in extremity | 14 (13.0) | 5 (8.8) |
| | Scoliosis | 13 (12.0) | 5 (8.8) |
| | Arthralgia | 2 (1.9) | 7 (12.3) |
| | Fall | 10 (9.3) | 7 (12.3) |
| | Headache | 22 (20.4) | 13 (22.8) |

AE, adverse event; CfB, change from baseline; CI, confidence interval; CMAP, compound muscle action potential; D1, dimension 1 of the MFM (standing and transfers); D2, dimension 2 of the MFM (axial and proximal motor capacity); FAS, full analysis set; FVC, forced vital capacity; HFMS, Hammersmith Functional Motor Scale; LS mean, least square mean; MFM, Motor Function Measure; MUNE, motor unit number estimation; NR, not reported; PEDsQL™, Pediatric Quality of Life Inventory; RCT, randomised controlled trial; SAE, serious adverse event; SD, standard deviation; SE, standard error; SMA, spinal muscular atrophy; *SMN*, survival motor neuron; TC, theoretical capacity; TREAT-NMD, Treatment of Neuromuscular Diseases; UK, United Kingdom.

## Table 31. Finkel 2017 (question 7)

| Study reference | Finkel 2017 |
|---|---|
| Study design | Design<br>RCT with open-label extension<br>Objective<br>To assess the clinical efficacy and safety of nusinersen in infants who had received a genetic diagnosis of SMA<br>Dates<br>21st August 2014 (first treatment) to 21st November 2016 (last visit)<br>Countries |

| **Study reference** | **Finkel 2017** |
|---|---|
| | Australia, Belgium, Canada, France, Germany, Italy, Japan, Republic of Korea, Spain, Sweden, Turkey, UK, US (from clinicaltrials.gov)<br><br>Setting<br>31 centres |
| **Population characteristics** | Patient recruitment<br>Infants at 31 centres were enrolled in the trial<br><br>Data collection<br>Efficacy end points were assessed on days 64, 183, 302, and 394 (±7 days for each visit). Safety monitoring visits occurred on days 16, 30, 65, 184, and 303. Follow-up after the procedure consisted of weekly assessments by telephone and a visit to the study centre on day 394 (±7 days)<br><br>A prespecified interim analysis was performed by the sponsor and the data and safety monitoring board when approximately 80 infants had been enrolled for at least 6 months, which showed a benefit–risk assessment in favour of nusinersen and resulted in early termination of the trial. At that time, infants were invited to complete an end-of-trial visit at least 2 weeks after they had received their most recent dose of nusinersen or undergone their most recent sham procedure. The assessments that were scheduled to be performed on day 394 were performed at the end of trial visit. Infants who completed the trial were invited to enrol in the open-label extension study<br><br>Analysis groups<br>For the final analysis, the 121 infants (80 in the nusinersen group and 41 in the control group) who had undergone randomisation and the assigned procedure at least one time were included in time-to-event analyses, and the 110 infants (73 in the nusinersen group and 37 in the control group) who had been enrolled at least 6 months before the last infant's last visit were included in all other analyses<br><br>Sample size and demographics<br>A total of 149 infants were screened, and 122 underwent randomisation<br>• 81 were assigned to the nusinersen group, and 41 to the control group<br>• One infant in the nusinersen group was withdrawn from the trial before treatment; 121 infants underwent the assigned procedure<br><br>Baseline characteristics |

| **Characteristic** | **Nusinersen group (N=80)** | **Control Group (N=41)** |
|---|---|---|
| Female, n (%) | 43 (54) | 24 (59) |
| **Age at first dose, days** | | |
| Mean | 163 | 181 |
| Range | 52 to 242 | 30 to 262 |
| **Age at symptom onset, weeks** | | |
| Mean | 7.9 | 9.6 |
| Range | 2 to 18 | 1 to 20 |
| **Age at diagnosis of SMA, weeks** | | |
| Mean | 12.6 | 17.5 |

| Study reference | Finkel 2017 | | |
|---|---|---|---|
| | Range | 0 to 29 | 2 to 30 |
| | **Disease duration at screening, weeks** | | |
| | Mean | 13.2 | 13.9 |
| | Range | 0 to 25.9 | 0 to 23.1 |
| | **Symptoms of SMA, n (%)** | | |
| | Hypotonia | 80 (100) | 41 (100) |
| | Developmental delay of motor function | 71 (89) | 39 (95) |
| | Paradoxical breathing | 71 (89) | 27 (66) |
| | Pneumonia or respiratory symptoms | 28 (35) | 9 (22) |
| | Limb weakness | 79 (99) | 41 (100) |
| | Swallowing or feeding difficulties | 41 (51) | 12 (29) |
| | Other | 20 (25) | 14 (34) |
| | Use of ventilatory support, n (%) | 21 (26) | 6 (15) |
| | Use of gastrointestinal tube, n (%) | 7 (9) | 5 (12) |
| | **Total HINE-2 score, mean (SD)** | 1.29 (1.07) | 1.54 (1.29) |
| | Head control | | |
| | Unable to maintain head upright | 66 (82) | 32 (78) |
| | Wobbles | 14 (18) | 7 (17) |
| | All the time maintained upright | 0 | 2 (5) |
| | Sitting | | |
| | Cannot sit | 77 (96) | 40 (98) |
| | Sits with support at hips | 2 (2) | 1 (2) |
| | Props | 1 (1) | 0 |
| | Stable sit | 0 | 0 |
| | Pivots (rotates) | 0 | 0 |
| | Voluntary grasp | | |
| | No grasp | 26 (32) | 9 (22) |
| | Uses whole hand | 50 (62) | 30 (73) |
| | Index finger and thumb but immature grasp | 3 (4) | 1 (2) |
| | Pincer grasp | 1 (1) | 1 (2) |

**Study reference** **Finkel 2017**

| | | |
|---|---|---|
| Ability to kick | | |
| No kicking | 56 (70) | 32 (78) |
| Kick horizontally, legs do not lift | 23 (29) | 8 (20) |
| Upward (vertically) | 1 (1) | 0 |
| Touches leg | 0 | 1 (2) |
| Touches toes | 0 | 0 |
| Rolling | | |
| No rolling | 79 (99) | 36 (88) |
| Rolling to side | 1 (1) | 5 (12) |
| Prone to supine | 0 | 0 |
| Supine to prone | 0 | 0 |
| Crawling | | |
| Does not lift head | 80 (100) | 41 (100) |
| On elbow | 0 | 0 |
| On outstretched hand | 0 | 0 |
| Crawling flat on abdomen | 0 | 0 |
| Crawling on hands and knees | 0 | 0 |
| Standing | | |
| Does not support weight | 80 (100) | 41 (100) |
| Supports weight | 0 | 0 |
| Stands with support | 0 | 0 |
| Stands unaided | 0 | 0 |
| Walking | | |
| No walking | 80 (100) | 41 (100) |
| Bouncing | 0 | 0 |
| Cruising (walks holding on) | 0 | 0 |
| Walking independently | 0 | 0 |
| **CHOP INTEND score, mean (SD)** | 26.63 (8.13) | 28.43 (7.56) |
| **CMAP amplitude, mV** | | |
| Peroneal, mean (SD) | 0.371 (0.31) | 0.317 (0.29) |
| Ulnar, mean (SD) | 0.226 (0.19) | 0.225 (0.12) |

Scores on Section 2 of the HINE-2 range from 0 to 26, with higher scores indicating better motor function. Scores on the CHOP INTEND range from 0 to 64, with higher scores indicating better motor function

| **Study reference** | **Finkel 2017** |
|---|---|
| | Key inclusion criteria (from clinicaltrials.gov)<br>• Gestational age between 37 and 42 weeks<br>  ○ 7 months of age or younger at screening<br>• Medical diagnosis of SMA<br>  ○ Genetic documentation of a homozygous deletion or mutation in the *SMN1* gene<br>  ○ Onset of clinical symptoms that were consistent with SMA at 6 months of age or younger<br>• *SMN2* copy number = 2<br>• Body weight equal to or greater than 3rd percentile for age using appropriate country-specific guidelines<br>• Ability to follow all study procedures<br>• Reside within approximately 9 hours ground-travel distance from a participating study centre, for the duration of the study<br>Key exclusion criteria (from clinicaltrials.gov)<br>• Hypoxemia (oxygen [$O_2$] saturation awake less than 96% or $O_2$ saturation asleep less than 96%, without ventilation support) during screening evaluation<br>• Clinically significant abnormalities in haematology or clinical chemistry parameters or ECG, as assessed by the Site Investigator, at the screening visit that would render the participant unsuitable for participation in the study<br>• Participant's parent or legal guardian not willing to meet standard of care guidelines (including vaccinations and respiratory syncytial virus prophylaxis if available), nor provide nutritional and respiratory support throughout the study |
| **Treatments** | Allocation methods<br>After a screening period of up to 21 days, eligible infants were randomly assigned, in a 2:1 ratio, to undergo intrathecal administration of nusinersen (nusinersen group) or a sham procedure (control group)<br>Randomisation was stratified according to disease duration at screening, which was the age at screening minus the age at symptom onset (≤12 weeks or >12 weeks)<br>Intervention<br>The nusinersen dose was adjusted according to the estimated volume of CSF for the infant's age on the day of dosing, such that the infant received a dose that was equivalent to a 12mg dose in a person 2 years of age or older; thus, younger infants were injected with smaller volumes that contained lower doses of the drug<br>Doses were administered on days 1, 15, 29, and 64 and maintenance doses on days 183 and 302<br>To maintain blinding, nusinersen was administered by dedicated trial personnel who were aware of the group assignments, whereas the infant's parents and key trial personnel who were responsible for assessments were unaware of the group assignments and were not present for the procedure<br>Comparator<br>The sham procedure consisted of a small needle prick to the skin over the lumbar spine, which was covered with a bandage to simulate the appearance of a lumbar-puncture injection<br>In the control group, sham procedures were performed on the same days as the nusinersen group (1, 15, 29, and 64 and maintenance doses on days |

| Study reference | Finkel 2017 |
|---|---|

183 and 302)

To maintain blinding, the sham procedure was performed by dedicated trial personnel who were aware of the group assignments, whereas the infant's parents and key trial personnel who were responsible for assessments were unaware of the group assignments and were not present for the procedure

**Clinical and safety outcomes**

**Efficacy outcomes**

Primary and Secondary Endpoints

| Outcome | Intervention and comparator | N/total number (%) | HR | 95% CI | p-value |
|---|---|---|---|---|---|
| **Primary endpoints** | | | | | |
| Motor-milestone response | | | | | |
| Interim analysis | Nusinersen | 21/51 (41) | - | - | <0.001 |
| | Control | 0/27 | | | |
| Final analysis | Nusinersen | 37/73 (51) | - | - | - |
| | Control | 0/37 | | | |
| No death or use of permanent assisted ventilation | Nusinersen | 49/80 (61) | 0.53 | 0.32 to 0.89 | 0.005 |
| | Control | 13/41 (32) | | | |

Motor-milestone response was defined according to scores on the HINE-2, which assesses the development of motor function through the achievement of motor milestones; in this trial, the scores accounted for seven of the eight motor milestone categories, excluding voluntary grasp. Infants were considered to have a motor-milestone response if they met the following two criteria: improvement in at least one category (i.e., an increase in the score for head control, rolling, sitting, crawling, standing, or walking of ≥1 point, an increase in the score for kicking of ≥2 points, or achievement of the maximal score for kicking) and more categories with improvement than categories with worsening (i.e., a decrease in the score for head control, rolling, sitting, crawling, standing, or walking of ≥1 point or a decrease in the score for kicking of ≥2 points).
Permanent assisted ventilation was defined as tracheostomy or ventilatory support for at least 16 hours per day for more than 21 continuous days in the absence of an acute reversible event, as determined by an independent end-point adjudication committee.
A CHOP INTEND response was defined as an increase of at least 4 points from baseline in CHOP INTEND score at the end-of-trial visit (day 183, 302, or 394). A CMAP response was defined as an increase in the peroneal CMAP amplitude to at least 1 mV (or maintenance of an amplitude of ≥1 mV) at the end-of-trial visit (day 183, 302, or 394).

- In the nusinersen group, 22% of the infants achieved full head control, 10% were able to roll over, 8% were able to sit independently, and 1% were able to stand; in the control group, no infants achieved these milestones

| Outcome | Intervention and comparator | N/total number (%) | HR | 95% CI | p-value |
|---|---|---|---|---|---|
| **Secondary endpoints** | | | | | |
| CHOP INTEND response | Nusinersen | 52/73 (71) | - | - | <0.001 |
| | Control | 1/37 (3) | | | |
| No death | Nusinersen | 67/80 (84) | 0.37 | 0.18 | 0.004 |

**Study reference** **Finkel 2017**

| | | | | | |
|---|---|---|---|---|---|
| | Control | 25/41 (61) | | to 0.77 | |
| No use of permanent assisted ventilation | Nusinersen | 62/80 (78) | 0.66 | 0.32 to 1.37 | 0.13 |
| | Control | 28/41 (68) | | | |
| CMAP response | Nusinersen | 26/73 (36) | - | - | - |
| | Control | 2/37 (5) | | | |
| No death or use of permanent assisted ventilation among those with disease duration ≤13.1 weeks at screening | Nusinersen | 30/39 (77) | 0.24 | 0.10 to 0.58 | - |
| | Control | 7/21 (33) | | | |
| No death or use of permanent assisted ventilation among those with disease duration >13.1 weeks at screening | Nusinersen | 19/41 (46) | 0.84 | 0.43 to 1.67 | - |
| | Control | 6/20 (30) | | | |

Permanent assisted ventilation was defined as tracheostomy or ventilatory support for at least 16 hours per day for more than 21 continuous days in the absence of an acute reversible event, as determined by an independent end-point adjudication committee.
A CHOP INTEND response was defined as an increase of at least 4 points from baseline in CHOP INTEND score at the end-of-trial visit (day 183, 302, or 394). A CMAP response was defined as an increase in the peroneal CMAP amplitude to at least 1 mV (or maintenance of an amplitude of ≥1 mV) at the end-of-trial visit (day 183, 302, or 394).

- Among infants who were alive at the end of the trial and had been enrolled for at least 6 months, most of the infants in the nusinersen group had an increase from baseline in the HINE-2 score; 16 of the 58 infants in the nusinersen group (28%) had an increase of 5 points or more, whereas only 1 of the 20 infants in the control group (5%) had any increase. Of the 41 infants who had any increase in the HINE-2 score, 28 (68%) had a shorter disease duration at screening (≤12 weeks). In addition, 6 of the 18 infants (33%) in the nusinersen group who received permanent assisted ventilation during the trial had an increase in the HINE-2 score
- An increase of at least 1 point from baseline in the CHOP INTEND score was observed in 73% of the infants in the nusinersen group versus 3% in the control group; a decrease in the score was observed in 7% versus 49%
- By the cut-off date for the final analysis, 39% of the infants in the nusinersen group and 68% in the control group had died or had received permanent assisted ventilation

**Study reference** **Finkel 2017**

- The median time to death or the use of permanent assisted ventilation was 22.6 weeks in the control group and was not reached in the nusinersen group
- Overall, the risk of death or the use of permanent assisted ventilation was 47% lower in the nusinersen group than in the control group (HR: 0.53; 95% confidence interval [CI], 0.32 to 0.89; p=0.005)
- The risk of death was 63% lower in the nusinersen group than in the control group (HR: 0.37; 95% CI, 0.18 to 0.77; p=0.004)

Subgroups

- In the subgroup analyses, the likelihood of event-free survival was higher among infants who had a shorter disease duration at screening (≤13.1 weeks) than among those who had a longer disease duration

**Safety**

| AEs, n (%) | Nusinersen (N=80) | Control (N=41) |
|---|---|---|
| Any AE | 77 (96) | 40 (98) |
| AE leading to discontinuation | 13 (16) | 16 (39) |
| Severe AE* | 45 (56) | 33 (80) |
| SAE** | 61 (76) | 39 (95) |
| **SAE with fatal outcome**** | 13 (16) | 16 (39) |
| Respiratory disorder† | 7 (9) | 12 (29) |
| Cardiac disorder | 2 (2) | 3 (7) |
| General disorder | 2 (2) | 1 (2) |
| Nervous-system disorder | 2 (2) | 0 |
| **AE that occurred in ≥20% of infants in either group†** | | |
| Pyrexia | 45 (56) | 24 (59) |
| Constipation | 28 (35) | 9 (22) |
| Upper respiratory tract infection | 24 (30) | 9 (22) |
| Pneumonia | 23 (29) | 7 (17) |
| Respiratory distress | 21 (26) | 12 (29) |
| Respiratory failure | 20 (25) | 16 (39) |
| Atelectasis | 18 (22) | 12 (29) |
| Vomiting | 14 (18) | 8 (20) |
| Acute respiratory failure | 11 (14) | 10 (24) |
| Gastroesophageal reflux disease | 10 (12) | 8 (20) |
| Decreased oxygen saturation | 10 (12) | 10 (24) |
| Cough | 9 (11) | 8 (20) |
| Dysphagia | 9 (11) | 9 (22) |
| **SAE that occurred in ≥10% of infants in either group**†** | | |
| Respiratory distress | 21 (26) | 8 (20) |
| Respiratory failure | 20 (25) | 16 (39) |

**Study reference** **Finkel 2017**

| | | |
|---|---|---|
| Pneumonia | 19 (24) | 5 (12) |
| Atelectasis | 14 (18) | 4 (10) |
| Acute respiratory failure | 11 (14) | 9 (22) |
| Pneumonia aspiration | 8 (10) | 5 (12) |
| Cardiorespiratory arrest | 5 (6) | 5 (12) |
| Respiratory arrest | 5 (6) | 4 (10) |
| Viral upper respiratory tract infection | 3 (4) | 6 (15) |
| Bronchial secretion retention | 1 (1) | 5 (12) |

For infants who had more than one adverse event, only the event of the highest severity was counted.
*Severe AEs were defined as symptoms that caused severe discomfort, incapacitation, or substantial effect on daily life
**SAEs were defined as any untoward medical occurrence that resulted in death or a risk of death, hospitalisation or prolonged hospitalisation, persistent or substantial disability or incapacity, or a congenital anomaly or birth defect.
†These events could plausibly be linked to SMA

AEs leading to treatment discontinuation

| AEs, n (%)<br>MedDRA SOC and PT | Nusinersen (N=80) | Control (N=41) |
|---|---|---|
| AE leading to discontinuation | 13 (16) | 16 (39) |
| Respiratory, thoracic, and mediastinal disorders | 7 (9) | 12 (29) |
| Respiratory failure | 4 (5) | 8 (20) |
| Acute respiratory failure | 1 (1) | 1 (2) |
| Respiratory arrest | 1 (1) | 0 |
| Respiratory distress | 1 (1) | 2 (5) |
| Aspiration | 0 | 1 (2) |
| Cardiac disorders | 2 (2) | 3 (7) |
| Cardiorespiratory arrest | 2 (2) | 3 (7) |
| General disorders and administration site conditions | 2 (2) | 1 (2) |
| Death | 1 (1) | 1 (2) |
| General physical health deterioration | 1 (1) | 0 |
| Nervous system disorders | 2 (2) | 0 |
| Brain injury | 1 (1) | 0 |
| Hypoxic-ischemic encephalopathy | 1 (1) | 0 |

An infant was counted only once within each system organ class and preferred term. Preferred terms are presented by decreasing incidence in the nusinersen column within each system organ class. MedDRA denotes Medical Dictionary for Regulatory Activities. In accordance with journal policy, percentages ending in exactly .5 have been rounded up to the higher integer if even and down to the lower integer if odd.

- All AEs that led to treatment discontinuation had fatal outcomes

AE, adverse event; CI, confidence interval; CHOP INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CMAP, compound muscle action potential; CSF, cerebrospinal fluid; ECG, electrocardiogram; HINE-2, section 2 of the Hammersmith Infant Neurological Examination (development of motor function through the achievement of motor milestones); HR, hazard ratio; PT, preferred term; RCT, randomised controlled trial; SAE, serious adverse event; SD, standard deviation; SMA, spinal muscular atrophy; *SMN,* survival motor neuron; SOC, system organ class; UK, United Kingdom; US, United States.

## Table 32: Kirschner 2014 (question 7)

<table>
<tr><td><u>Study reference</u></td><td>Kirschner 2014[81]</td></tr>
<tr><td>Study design</td><td><u>Design</u><br>RCT (crossover)<br><u>Objective</u><br>To determine whether daily administration of subcutaneous growth hormone (GH) can improve muscle strength and function in type 2/3 SMA patients<br><u>Dates</u><br>December 2007 to February 2010<br><u>Countries</u><br>Germany<br><u>Setting</u><br>Five university hospitals in Germany (Berlin, Essen, Freiburg, Göttingen and Munich), approved trial sites of the muscular dystrophy network and hold longstanding experience in the care of patients with SMA</td></tr>
<tr><td>Population characteristics</td><td><u>Patient recruitment</u><br>NR<br><u>Data collection</u><br>Primary and secondary outcome assessments were performed at baseline (weeks 0 and 20), during the first treatment block (weeks 4 and 12), after the washout period and during the crossover treatment block (weeks 24 and 32)<br>Possible adverse effects of the medication were assessed in the clinical examination of the patients at each visit<br><u>Sample size and demographics</u><br>20 type 2/3 SMA patients<br>• mITT population: n=19 (one patient dropped out after 34 days of somatropin injections in the first treatment block due to patient-reported weakness of the dominant upper limb)<br>• PP population: n=17 (2 additional patients dropped out during the second treatment block)
<table>
<tr><th></th><th colspan="3">Modified intention-to-treat analysis (n=19)</th></tr>
<tr><th></th><th>SMA type 2 (n=4)</th><th>SMA type 3 (not ambulatory) (n=5)</th><th>SMA type 3 (ambulatory) (n=10)</th></tr>
<tr><td>Male</td><td colspan="3">12</td></tr>
<tr><td>Female</td><td colspan="3">7</td></tr>
</table></td></tr>
</table>

**Study reference** **Kirschner 2014[81]**

| | | | |
|---|---|---|---|
| Mean (SD) age, years [range] | 14.7 (6.75) [6 to 36] | | |
| | 9.0 (NR) | 19.6 (NR) | 14.6 (NR) |
| *SMN2* copies | 2 (n=1)<br>3 (n=3) | 3 (n=3)<br>4 (n=2) | 2 (n=1)<br>3 (n=1)<br>4 (n=8) |

Baseline demographics of mITT analysis set

| | PP analysis (n=17) | | |
|---|---|---|---|
| | **SMA type 2 (n=4)** | **SMA type 3 (not ambulatory) (n=5)** | **SMA type 3 (ambulatory) (n=8)** |
| Mean age, years | 9.0 | 19.6 | 14.5 |
| *SMN2* copies | 2 (n=1)<br>3 (n=3) | 3 (n=3)<br>4 (n=2) | 2 (n=1)<br>4 (n=7) |

Baseline demographic of per-protocol analysis set

Baseline characteristics

| | Somatropin | | | Placebo | | |
|---|---|---|---|---|---|---|
| **Outcome** | **Mean/median (SD)** | **Min/25%** | **Max/75%** | **Mean/median (SD)** | **Min/25%** | **Max/75%** |
| Arm megascore [N] | 37.26 (28.96) | 5.5 | 130.5 | 37.39 (27.92) | 6 | 115.5 |
| Elbow flexion [N] | 49.16 (40.14) | 8 | 166 | 49.68 (37.36) | 10 | 146 |
| Hand grip [N] | 25.37 (19.5) | 3 | 80 | 25.11 (19.74) | 2 | 85 |
| Leg megascore [N] | 26.63 (16.65) | 3 | 74 | 28.39 (16.88) | 4 | 66 |
| Knee flexion [N] | 36.72 (23.7) | 4 | 86 | 39.11 (24.33) | 6 | 80 |
| Knee extension [N] | 18.05 (14.03) | 1 | 51 | 19.37 (13.22) | 2 | 48 |
| %MRC | 53.87 (16.9) | 21 | 76.7 | 53.83 (18.8) | 22.7 | 80.7 |
| HFMS (expanded version) | 46 | 24 | 53.5 | 45 | 28.5 | 56 |
| 10 m walking time (n=10) [s] | 8.3 (4.1) | 4.4 | 17.1 | 7.7 (3.8) | 4.1 | 16.1 |
| Gowers time (n=8) [s] | 21.5 (23.5) | 3 | 70 | 17.9 (19.2) | 2.8 | 61 |
| FVC (n=19) [l] | 2.83 (1.44) | 0.5 | 6.6 | 3.0 (1.47) | 0.4 | 6.2 |
| Peak cough flow (n=12) [l/min] | 388.25 (177.9) | 150 | 653 | 391.15 (178.45) | 150 | 680 |

Mean and min/max values are displayed for normally distributed variables (myometric measurements, %MRC score, 10 m walking time, Gowers time, pulmonary function tests), median and interquartile range values are displayed for not normally distributed variables (HFMS).

Inclusion criteria

- 6 to 36 years of age

| Study reference | Kirschner 2014[81] |
|---|---|
| | • Genetically confirmed diagnosis of SMA verifying *SMN1* deletion or mutation<br>• Type 2/3 SMA (independent sitting is or was possible)<br>• The physical ability to cooperate on assessment of at least the primary outcome measure<br>Exclusion criteria<br>• Diagnosis of GHD<br>• Treatment with any medication that could potentially affect muscle strength within 8 weeks prior to trial onset<br>• Pregnancy, lactation or if the woman was of child-bearing age and sexually active without verified contraception<br>• Participation in another clinical trial within 3 months of trial begin<br>Any contraindication for GH treatment |
| **Treatments** | Allocation methods<br>Patients (n=19) with type 2/3 spinal muscular atrophy were randomised to receive either somatropin or placebo for 3 months, followed by a 2-month wash-out phase before 3 months of treatment with the contrary remedy<br>Computer-generated randomisation lists and trial medication was provided by NovoNordisk and distributed to the central pharmacy (Freiburg) for this trial. The trial medication for each patient was delivered to the participating trial site after the patient was randomly allocated to a test group by the central pharmacy. Somatropin and placebo did not differ in appearance to ensure that patients, physicians and physiotherapists involved in this trial were unaware which drug regimen was being administered. Unblinding was performed only after the last patient had finished study participation and the statistical analysis of the primary outcome measure was completed<br>Intervention<br>Patients who fulfilled inclusion criteria and who were randomly selected for the treatment group received 0.015 mg recombinant somatropin per kg body weight daily for 1 week, following by 0.03 mg/kg somatropin daily in weeks 2 through 12. Somatropin was administered using an injection pen by the patients or their parents, who were adequately trained in its use. After a washout period of 2 months, patients in the treatment group received placebo for an additional 3 months at the same dosage.<br>Comparator<br>The other group received daily placebo injections for 3 months. Placebo was administered using an injection pen by the patients or their parents, who were adequately trained in its use. After a washout period of 2 months, patients in the placebo group received somatropin for an additional 3 months at the same dosage. |
| **Clinical and safety outcomes** | **Efficacy outcomes**<br>Changes in efficacy measures in the mITT and per-protocol analysis sets |

| Outcome | mITT analysis | | | | | | PP analysis |
|---|---|---|---|---|---|---|---|
| | Intervention and | N | Mean/median change from | Estimate of difference | | p-value (2-tailed) | |
| | | | | Point | 95% CI | | p-value (2- |

**Study reference** **Kirschner 2014[81]**

| | comparator | | baseline (SD) | estimate mean | | | tailed) |
|---|---|---|---|---|---|---|---|
| Arm megascore [N] | Somatropin | NR | -1.05 (6.42) | 0.08 | -3.79 to 3.95 | 0.965 | 0.629 |
| | Placebo | NR | 0.30 (10.60) | | | | |
| Elbow flexion [N] | Somatropin | NR | -2.22 (11.31) | 0.98 | -5.16 to 7.11 | 0.741 | 0.654 |
| | Placebo | NR | -3.28 (13.98) | | | | |
| Hand grip [N] | Somatropin | NR | -0.16 (6.09) | -0.82 | -5.00 to 3.37 | 0.686 | 0.843 |
| | Placebo | NR | 0.94 (6.7) | | | | |
| Leg megascore [N] | Somatropin | NR | 2.96 (7.64) | 2.23 | -2.19 to 6.63 | 0.302 | 0.491 |
| | Placebo | NR | 0.95 (9.93) | | | | |
| Knee flexion [N] | Somatropin | NR | 2.16 (12.71) | 2.53 | -4.36 to 9.41) | 0.448 | 0.668 |
| | Placebo | NR | 0.27 (13.81) | | | | |
| Knee extension [N] | Somatropin | NR | 2.61 (6.72) | 2.82 | -0.83 to 6.48 | 0.121 | 0.200 |
| | Placebo | NR | -0.77 (6.29) | | | | |
| HFMS (expanded version) | Somatropin | NR | 0.05 | 0.25 | -1 to 2.5 | 0.58 | 0.62 |
| | Placebo | NR | -1.05 | | | | |
| %MRC | Somatropin | NR | -2.31 (5.1) | -2.76 | -7.0 to 1.5 | 0.191 | 0.239 |
| | Placebo | NR | 0.43 (7.0) | | | | |
| 10 m walking time (block 1 data only) [sec] | Somatropin | NR | -0.18 (0.6) | -0.32 | -1.07 to 0.44 | 0.366 (n=10) | 0.467 (n=8) |
| | Placebo | NR | 0.09 (0.63) | | | | |
| Gowers time [sec] | Somatropin | NR | -1.79 (6.71) | -1.77 | -8.09 to 4.54 | 0.518 (n=8) | 0.798 (n=6) |
| | Placebo | NR | 0.12 (2.79) | | | | |
| Gowers scale | Somatropin | NR | 0 | 0 | -∞ to +∞ | 0.794 (n=9) | 0.429 (n=7) |
| | Placebo | NR | 0 | | | | |
| FVC [l] | Somatropin | NR | 0.12 (0.25) | 0.22 | -0.02 to 0.4 | 0.075 (n=19) | 0.098 (n=17) |
| | Placebo | NR | -0.11 (0.40) | | | | |
| Peak cough flow [l/min] | Somatropin | NR | -0.51 (63.2) | -16.2 | -43.1 to 10.7 | 0.210 (n=12) | 0.201 (n=11) |
| | Placebo | NR | 3.94 (48.15) | | | | |

Mean and min/max values are displayed for normally distributed variables (myometric measurements, %MRC score, 10 m walking time, Gowers time, pulmonary function tests), median and interquartile range values are displayed for not normally distributed variables (HFMSE, Gowers scale)

## Safety Outcomes

Adverse events and withdrawals

Somatropin group:

- 11 patients (55%) experienced 14 adverse events, of which 7 corresponded with known side effects listed in the Norditropin SPC
- Five of these were of moderate intensity (headache, arthralgia, myalgia, peripheral oedema, elevated serum TSH), while the other 2

| Study reference | Kirschner 2014[81] |
|---|---|
| | (myalgia and progressive headache) were more severe and led to early termination of trial participation in both patients.<br>• In the patient with headache idiopathic intracranial hypertension was suspected. Evaluation was only possible 2 weeks after discontinuation of somatropin, when headache had already disappeared, and did not reveal papilledema or raised intracranial pressure<br>• The third drop out patient was clearly attributed to non-compliance and not associated with occurrence of AEs<br><br>Placebo group:<br>• Total of 9 adverse events, which did not correspond with typical GH side effects, in 7 patients (35%)<br><br>Serum concentrations<br>• Serum concentrations of IGF1 and IGFBP3 were elevated in all patients during somatropin compared to placebo treatment, indicating that patients' compliance to the injection protocol was probably good<br>• The increase to these serum values became highly significant after 4 weeks (IGF1: p=0.0004; IGFBP3: p=0.010)<br>• Values were no longer significant after 12 weeks (IGF1: p=0.060; IGFBP3: p=0.343) |

AE, adverse event; CI, confidence interval; FVC, forced vital capacity; GH, growth hormone; GHD, growth hormone deficiency; HFMS, Hammersmith Functional Motor Scale; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; mITT, modified intention-to-treat; %MRC, Medical Research Council scale of muscle strength; [N], Newtons; NR, not reported; PP, per-protocol; RCT, randomised controlled trial; SD, standard deviation; SMA, spinal muscular atrophy; *SMN*, survival motor neuron; SPC, summary of product characteristics; TSH, thyroid stimulating hormone.

## Table 33: Kissel 2014 (question 7)

| Study reference | Kissel 2014[82] |
|---|---|
| **Study design** | Design<br>RCT (crossover)<br>Objective<br>To investigate the use of valproic acid (VPA) in the treatment of ambulatory SMA adults<br>Dates<br>NR<br>Countries<br>NR (author affiliations all from USA)<br>Setting<br>Single-centre |
| **Population characteristics** | Patient recruitment<br>The study was designed to recruit 36 patients with an anticipated 20% dropout rate to yield at least 28 analysable patients, a number sufficient to provide |

**Study reference** **Kissel 2014[82]**

90% power to detect a 9.0-N change in MVICT

59 patients were screened to enrol 33 patients (because of a higher retention rate than anticipated recruitment was curtailed after 33 patients were enrolled)

Data collection

All subjects completed 2 baseline visits within a 6-week period

Treatment assessments were performed at 3 (V1), 6 (V2), and 12 (V3) months. Safety laboratory studies were performed at baseline, 2 to 3 weeks after initiation, at each treatment visit, and midway between V2 and V3. They included a basic chemistry profile, complete blood and platelet count, transaminases, carnitine profile, amylase, lipase, and trough VPA levels

Sample size and demographics

33 SMA patients with adult ambulatory disease

| | VPA (n=16) | Placebo (n=17) | Total (n=33) |
|---|---|---|---|
| Mean age (SD) | 35.93 (8.72) | 38.30 (9.48) | 37.15 (9.06) |
| Male, n (%) | 11 (68.8) | 9 (52.9) | 20 (60.6) |
| Female, n (%) | 5 (31.3) | 8 (47.1) | 13 (39.4) |
| ***SMN2, n (%)*** | | | |
| 2 | 1 (6.7) | 2 (12.5) | 3 (9.7) |
| 3 | 3 (20.0) | 2 (12.5) | 5 (16.1) |
| 4 | 8 (53.3) | 11 (68.8) | 19 (61.3) |
| 5 | 3 (20.0) | 1 (6.3) | 4 (12.9) |
| Mean weight (SD) | 85.70 (25.33) | 82.03 (27.25) | 83.81 (25.99) |
| **Height** | | | |
| N | 16 | 16 | 32 |
| Mean height (SD) | 171.55 (10.41) | 163.46 (32.73) | 167.50 (24.24) |
| **BMI** | | | |
| N | 16 | 16 | 32 |
| Mean BMI (SD) | 28.76 (6.90) | 26.07 (5.50) | 27.41 (6.29) |
| Mean SIP score (SD) | 1.26 (2.31) | 3.98 (4.74) | 2.66 (3.96) |

Inclusion criteria (taken from clinicaltrials.gov as NR in the published paper)

- Ambulatory adults with type 3 SMA, ages 18 to 60. The diagnosis of SMA must be documented by the homozygous deletion of both *SMN1* genes on standard genetic tests for the disorder. Patients must be able to walk thirty feet without assistance (ie. no canes, walkers)
- Interest in participating and the ability to meet the study requirements

| Study reference | Kissel 2014[82] |
|---|---|
| | • Women of child bearing age are required to be on birth control or abstain while participating in the study<br>Exclusion criteria<br>• Coexisting medical conditions that precluded travel, testing, or study medications<br>• Participation in a treatment trial for SMA in the 3 months prior to this trial, or plan to enrol in any other treatment trial during this study<br>• Requirement for any mechanical respiratory support >12 hours per day<br>• Inability to meet visit requirements or cooperate reliably with functional testing<br>• Mental or legal incapacitation from giving informed consent, or inability to read and understand written material including in the consent form<br>• Abnormalities in baseline blood testing beyond established values<br>Use of medications or supplements which interfere with VPA metabolism, or are hypothesized to have a beneficial effect in SMA animal models or human neuromuscular disorders within 3 months of study enrolment, including riluzole, creatine, butyrate derivatives, growth hormone, anabolic steroids, albuterol, anticonvulsants, or other HDAC inhibitors |
| **Treatments** | Allocation methods<br>All subjects completed 2 baseline visits within a 6-week period to assure that the methodologies were reliable and that subjects enrolled in the study exhibited test–retest stability. After the second visit, subjects were randomised to receive either VPA or an identical placebo<br>After the 6-month visit, subjects were switched to the other treatment group (ie. VPA or placebo) in a blinded fashion<br>Intervention<br>VPA was provided as 250 mg divalproex sodium capsules and administered in divided doses 2 or 3 times daily at a starting dose of 10 to 20 mg/kg orally to maintain trough levels of 50 to 100 mg/dl<br>VPA levels at each visit with dosing adjusted for both VPA and placebo groups by an unblinded investigator<br>Comparator<br>Placebo was provided as 250 mg divalproex sodium capsules and administered in divided doses 2 or 3 times daily at a starting dose of 10 to 20 mg/kg orally to maintain trough levels of 50 to 100 mg/dl<br>VPA levels at each visit with dosing adjusted for both VPA and placebo groups by an unblinded investigator |
| **Clinical and safety outcomes** | **Efficacy Outcomes**<br>MVICT CfB at 6 months |

| Outcome | Intervention and comparator | N | Mean change from baseline at 6 months (SD) | Median change from baseline at 6 months | p-value |
|---|---|---|---|---|---|
| Elbow extensors | VPA | 12 | -0.01 (0.49) | -0.01 | 0.8100 |

**Study reference** **Kissel 2014[82]**

| | | | | | |
|---|---|---|---|---|---|
| | Placebo | 14 | -0.09 (1.04) | 0.21 | |
| Elbow flexors | VPA | 14 | -0.20 (1.31) | 0.24 | 0.5396 |
| | Placebo | 16 | 0.09 (1.21) | -0.04 | |
| Upper extremity | VPA | 14 | -0.24 (1.17) | -0.20 | 0.5704 |
| | Placebo | 16 | -0.01 (1.05) | 0.05 | |
| Knee extensors | VPA | 11 | 0.12 (0.77) | 0.06 | 0.7114 |
| | Placebo | 13 | 0.01 (0.64) | -0.18 | |
| Knee flexors | VPA | 12 | -0.09 (1.34) | -0.43 | 0.8508 |
| | Placebo | 16 | -0.19 (1.58) | 0.06 | |
| Lower extremity | VPA | 13 | -0.02 (0.65) | -0.04 | 0.3674 |
| | Placebo | 16 | 0.35 (1.30) | 0.10 | |
| Grip | VPA | 14 | 1.45 (4.77) | 0.17 | 0.2462 |
| | Placebo | 16 | -0.34 (3.47) | 0.01 | |
| Total MVICT | VPA | 14 | -0.46 (2.99) | 0.10 | 0.5708 |
| | Placebo | 16 | 0.03 (1.55) | 0.01 | |

LS means for MVICT over 12 months

| Outcome | Intervention and comparator | N | Least- squares means for MVICT over 12 months (SE) | 95% CI |
|---|---|---|---|---|
| Upper extremity | VPA | NR | 8.3836 (0.1944) | 7.9860 to 8.7813 |
| | Placebo | NR | 8.8093 (0.1976) | 8.4050 to 9.2135 |
| Lower extremity | VPA | NR | 4.8155 (0.2532) | 4.2968 to 5.3343 |
| | Placebo | NR | 5.0016 (0.2576) | 4.4740 to 5.5292 |
| Grip | VPA | NR | 16.3168 (0.8080) | 14.6643 to 17.9693 |
| | Placebo | NR | 16.8072 (0.8109) | 15.1488 to 18.4656 |
| Total MVICT | VPA | NR | 8.7514 (0.4066) | 7.9198 to 9.5831 |
| | Placebo | NR | 8.9608 (0.4082) | 8.1260 to 9.7956 |

**Safety Outcomes**

Adverse events

- ≥1 AE occurred at some time during the study in 88% of subjects, with a total of 96 AEs reported
- Two AEs led to study withdrawal: 1 patient developed fatigue and 1 noted increased arm weakness, which the subject attributed to VPA
- There were 2 SAEs (cardiac failure related to chronic aortic valve disease and an episode of recreational drug overdose), neither related to VPA use
- Headache (21%), fatigue (12%), and nausea (12%) were the most common AEs

AE, adverse event; BMI, body mass index; CfB, change from baseline; CI, confidence interval; LS mean, least square mean; MVICT, maximum voluntary isometric contraction testing; NR, not reported; RCT, randomised controlled trial; SD, standard deviation; SIP, sickness impact profile; SMA, spinal muscular atrophy; *SMN*, survival motor neuron; SAE, serious adverse event; USA, United States of America; VPA, valproic acid.

## Table 34. Mercuri 2018 (question 7)

| Study reference | Mercuri 2018 |
|---|---|
| **Study design** | Design<br>RCT with open-label extension<br>Objective<br>To evaluate the efficacy and safety of nusinersen in children with later-onset SMA (symptom onset after 6 months of age)<br>Dates<br>24th November 2014 (first procedure) to 20th February 2017 (last visit)<br>Countries<br>Canada, France, Germany, Hong Kong, Italy, Japan, Republic of Korea, Spain, Sweden, US (from clinicaltrials.gov)<br>Setting<br>24 centres |
| **Population characteristics** | Patient recruitment<br>Children at 24 centres were enrolled in the trial<br>Data collection<br>Trained clinical evaluators assessed the HFMSE score (primary endpoint) twice during the screening period and at 3, 6, 9, 12, and 15 months<br>Safety was evaluated throughout the trial<br>Data analysis<br>The prespecified interim analysis of the primary end point was performed in the intention-to-treat population, which included patients who were randomly assigned to a group and underwent at least one assigned procedure; this analysis was conducted when all the children had been enrolled for at least 6 months and at least 39 children had completed their 15-month assessment. In the interim analysis, nusinersen showed efficacy superior to that of the sham procedure. Therefore, all the children who had not had a 15-month assessment were invited to attend a visit that represented the end of the double-blind period; at this visit, all assessments that had been scheduled for the 15-month assessment were performed. Children who completed the trial were invited to enrol in the open-label extension study in which all children were to receive nusinersen.<br>Sample size and demographics<br>A total of 179 children were screened; 126 were enrolled in the trial, were randomly assigned to a group, and underwent the assigned procedure |

**Study reference** **Mercuri 2018**

- 84 were assigned to the nusinersen group, and 42 to the control group
- At the time of the final analysis, no child had been withdrawn from the trial. 66 children (79%) in the nusinersen group and 34 (81%) in the control group had completed the 15-month assessment; 26 children were enrolled in the open-label extension study early

| Characteristic | Nusinersen group (N=84) | Control Group (N=42) |
|---|---|---|
| Female, n (%) | 46 (55) | 21 (50) |
| **Race, n (%)** | | |
| White | 64 (76) | 30 (71) |
| Asian | 16 (19) | 7 (17) |
| Black | 1 (1) | 1 (2) |
| Multiple | 3 (4) | 4 (10) |
| **Age at screening, years** | | |
| Median | 4.0 | 3.0 |
| Range | 2 to 9 | 2 to 7 |
| <6 years, n (%) | 70 (83) | 36 (86) |
| ≥6 years, n (%) | 14 (17) | 6 (14) |
| **Age at symptom onset, months** | | |
| Median | 10.0 | 11.0 |
| Range | 6 to 20 | 6 to 20 |
| **Age at diagnosis of SMA, months** | | |
| Median | 18.0 | 18.0 |
| Range | 0 to 48 | 0 to 46 |
| **Disease duration, months*** | | |
| Median | 39.3 | 30.2 |
| Range | 8 to 94 | 10 to 80 |
| ***SMN2* copy number, n (%)** | | |
| 2 | 6 (7) | 4 (10) |
| 3 | 74 (88) | 37 (88) |
| 4 | 2 (2) | 1 (2) |
| Unknown | 2 (2) | 0 |
| **Geographic region** | | |
| North America | 47 (56) | 23 (55) |
| Europe | 28 (33) | 14 (33) |

**Study reference** **Mercuri 2018**

| | | |
|---|---|---|
| Asia-Pacific | 9 (11) | 5 (12) |
| **Motor milestones ever achieved, n (%)**** | | |
| Ability to sit without support | 84 (100) | 42 (100) |
| Ability to walk without support | 20 (24) | 14 (33) |
| Ability to walk independently, ≥15 ft | 0 | 0 |
| Ability to stand without support | 11 (13) | 12 (29) |
| Attended physical therapy, n (%) | 78 (93) | 38 (90) |
| HFMSE score, mean (SD)† | 22.4 (8.3) | 19.9 (7.2) |
| WHO motor milestones achieved, mean (SD)†† | 1.4 (1.0) | 1.5 (1.0) |
| WHO motor milestones, n (%) | | |
| Sitting without support | 84 (100) | 42 (100) |
| Hands and knees crawling | 16 (19) | 8 (19) |
| Standing with assistance | 9 (11) | 8 (19) |
| Walking with assistance | 7 (8) | 3 (7) |
| Standing alone | 3 (4) | 3 (7) |
| Walking alone | 0 | 0 |
| WHO motor milestone total score, n (%) | | |
| 1 | 66 (79) | 29 (69) |
| 2 | 10 (12) | 9 (21) |
| 3 | 1 (1) | 1 (2) |
| 4 | 5 (6) | 1 (2) |
| 5 | 2 (2) | 2 (5) |
| Median (IQR) total score | 1 (1 to 1) | 1 (1 to 2) |
| RULM score, mean (SD)††† | 19.4 (6.2) | 18.4 (5.7) |

No formal statistical testing was performed to assess differences between trial groups in baseline characteristics. Percentages may not total 100 because of rounding
*Disease duration is a child's age at screening minus the age at symptom onset
**These data do not reflect the maximal milestone achieved
†HFMSE scores range from 0 to 66, with higher scores indicating better motor function
††The six WHO motor milestones are sitting without support, standing with assistance, hands and knees crawling, walking with assistance, standing alone, and walking alone
†††RULM scores range from 0 to 37, with higher scores indicating better function

Key inclusion criteria (from clinicaltrials.gov)

- Parent or guardian has signed informed consent and, if indicated per participant's age and institutional guidelines, participant has signed

| **Study reference** | **Mercuri 2018** |
|---|---|
| | informed assent<br>• Aged 2 to 12 years at Screening<br>• Medical diagnosis of SMA<br>  o Genetic documentation of 5q SMA (a homozygous deletion, mutation, or compound heterozygote in *SMN1*<br>  o Onset of clinical signs and symptoms consistent with SMA at greater than 6 months of age<br>• Ability to sit independently, but never the ability to walk independently<br>• HFMSE score greater than or equal to 10 and less than or equal to 54 at Screening<br>• Ability to complete all study procedures, measurements and visits and parent or guardian and subject has adequately supportive psychosocial circumstances, in the opinion of the Investigator<br>• Estimated life expectancy of greater than 2 years from Screening, in the opinion of the Investigator<br>• Meets the age-appropriate institutional criteria for use of anaesthesia and sedation, if use is planned for study procedures<br>• Satisfies study contraceptive requirements<br><br>Key exclusion criteria (from clinicaltrials.gov)<br>• Respiratory insufficiency, defined by the medical necessity for invasive or non-invasive ventilation for greater than 6 hours during a 24 hour period, at Screening<br>• Medical necessity for a gastric feeding tube, where the majority of feeds are given by this route, as assessed by the Site Investigator<br>• Severe contractures or severe scoliosis evident on X-ray examination at Screening<br>• Hospitalisation for surgery (i.e., scoliosis surgery, other surgery), pulmonary event, or nutritional support within 2 months of Screening or planned during the duration of the study<br>• Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the screening period<br>• History of brain or spinal cord disease, including tumours, or abnormalities by MRI or CT that would interfere with the LP procedures or CSF circulation<br>• Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter<br>• History of bacterial meningitis<br>• Dosing with IONIS-SMN Rx (nusinersen) in any previous clinical study<br>• Prior injury (e.g., upper or lower limb fracture) or surgical procedure which impacts the subject's ability to perform any of the outcome measure testing required in the protocol and from which the subject has not fully recovered or achieved a stable baseline<br>• Clinically significant abnormalities in haematology or clinical chemistry parameters or ECG, as assessed by the Site Investigator, at the Screening visit that would render the subject unsuitable for inclusion<br>• Treatment with another investigational drug (e.g., oral albuterol or salbutamol, riluzole, carnitine, creatine, sodium phenylbutyrate), biological agent, or device within one month of Screening or 5 half-lives of study agent, whichever is longer. Treatment with valproate or hydroxyurea within 3-months of Screening. Any history of gene therapy, antisense oligonucleotide therapy, or cell transplantation.<br>• Ongoing medical condition that according to the Site Investigator would interfere with the conduct and assessments of the study. Examples are medical disability (e.g., wasting or cachexia, severe anaemia) that would interfere with the assessment of safety or would compromise the ability of the subject to undergo study procedures |
| **Treatments** | Allocation methods<br>To ensure balance across the trial groups, the children were stratified according to age at screening (<6 years vs. 6 years) and then were randomly assigned, in a 2:1 ratio, to undergo intrathecal administration of nusinersen at a dose of 12 mg (nusinersen group) or a sham procedure (control group) |

| Study reference | Mercuri 2018 |
|---|---|

Randomisation was performed with the use of an interactive Web response system

Intervention

Nusinersen was administered at a dose of 12 mg by dedicated personnel who were aware of the group assignments; the child's parents and key trial personnel who performed assessments were unaware of the group assignments until trial completion and were not present for the procedure. Participants were sedated to avoid any awareness of the procedure

Nusinersen was administered intrathecally on days 1, 29, and 85, and a maintenance dose was on day 274. Children were observed at the trial site for at least 24 hours after the first procedure was performed and for at least 6 hours after each procedure thereafter

Treatments that were considered to be necessary to manage AEs or provide supportive care were permitted, in accordance with standard of care guidelines

Comparator

The sham procedure consisted of a small needle prick to the lower back, which was covered with a bandage to simulate the appearance of a lumbar puncture and was performed by dedicated personnel who were aware of the group assignments; the child's parents and key trial personnel who performed assessments were unaware of the group assignments until trial completion and were not present for the procedure. Participants were sedated to avoid any awareness of the procedure

In the control group, sham procedures were performed on the same days as the nusinersen group (days 1, 29, and 85, and a maintenance dose on day 274). Children were observed at the trial site for at least 24 hours after the first procedure was performed and for at least 6 hours after each procedure thereafter

Treatments that were considered to be necessary to manage AEs or provide supportive care were permitted, in accordance with standard of care guidelines

**Clinical and safety outcomes**

## Efficacy outcomes

Primary and Secondary Endpoints

| Outcome | Nusinersen (N=84) | Control (N=42) | Difference | p-value |
|---|---|---|---|---|
| **Primary endpoint: change from baseline in HFMSE score, least-squares mean (95% CI)*** | | | | |
| Interim analysis** | 4.0 (2.9 to 5.1) | –1.9 (–3.8 to 0) | 5.9 (3.7 to 8.1) | <0.001 |
| Final analysis^ | 3.9 (3.0 to 4.9) | –1.0 (–2.5 to 0.5) | 4.9 (3.1 to 6.7) | - |

*The least-squares mean change and least-squares mean difference in change between groups were based on an analysis of covariance, with group assignment as a fixed effect and with adjustment for each child's age at screening and the value at baseline

**The interim analysis of the primary endpoint was conducted when all the children had been enrolled for at least 6 months and at least 39 children had completed the 15-month assessment. The analysis was performed with the use of a multiple-imputation method. The number of children with observed data for the 15-month assessment was 35 in the nusinersen group and 19 in the control group, and the number of children with imputed data was 49 in the nusinersen group and 23 in the control group

^In the final analysis, the endpoints were analysed with the use of a multiple-imputation method: change from baseline in the HFMSE score, percentage of children with a change in HFMSE score of at least 3 points, and change from baseline in the RULM score. Only children with observed data were included in the other analyses. The number of children with observed data for the 15-month assessment was 66 in the nusinersen group and 34 in the control group, and the number of children with imputed data was 18 in the nusinersen group and 8 in the control group

**Study reference** **Mercuri 2018**

| Outcome | Nusinersen (N=84) | Control (N=42) | Difference | p-value |
|---|---|---|---|---|
| **Secondary endpoint: final analysis*** | | | | |
| **Children with change in HFMSE score of ≥3 points^** | | | | |
| % (95% CI)** | 57 (46 to 68) | 26 (12 to 40) | 30.5 (12.7 to 48.3) | |
| Odds ratio (95% CI) | - | - | 6 (2 to 15) | <0.001 |
| **Children who achieved ≥1 new WHO motor milestone** | | | | |
| Number of patients | 13 | 2 | - | - |
| % (95% CI)† | 20 (11 to 31) | 6 (1 to 20) | 14 (−7 to 34) | 0.08 |
| **Change from baseline in number of WHO motor milestones achieved, least-squares mean (95% CI)††** | 0.2 (0.1 to 0.3) | −0.2 (−0.4 to 0) | 0.4 (0.2 to 0.7) | - |
| **Change from baseline in RULM score, least-squares mean (95% CI)††** | 4.2 (3.4 to 5.0) | 0.5 (−0.6 to 1.6) | 3.7 (2.3 to 5.0) | - |
| **Children who achieved ability to stand alone** | | | | |
| Number of patients | 1 | 1 | - | - |
| % (95% CI)† | 2 (0 to 8) | 3 (0 to 15) | −1 (−22 to 19) | - |
| **Children who achieved ability to walk with assistance** | | | | |
| Number of patients | 1 | 0 | - | - |
| % (95% CI)† | 2 (0 to 8) | 0 (0 to 10) | 2 (−19 to 22) | - |

*In the final analysis, the endpoints were analysed with the use of a multiple-imputation method: change from baseline in the HFMSE score, percentage of children with a change in HFMSE score of at least 3 points, and change from baseline in the RULM score. Only children with observed data were included in the other analyses. The number of children with observed data for the 15-month assessment was 66 in the nusinersen group and 34 in the control group, and the number of children with imputed data was 18 in the nusinersen group and 8 in the control group

**Study reference** **Mercuri 2018**

^ A change in the HFMSE score of at least 3 points is considered to be clinically meaningful
**The percentages and difference (in percentage points) were based on binomial proportions
†The percentages were based on an exact confidence interval, and the differences (in percentage points) on an exact unconditional confidence interval
††The least-squares mean change and least-squares mean difference in change between groups were based on an analysis of covariance, with group assignment as a fixed effect and with adjustment for each child's age at screening and the value at baseline

Subgroups

- Similar results favouring nusinersen were observed in all sensitivity analyses for the primary end point and across subgroups defined according to *SMN2* copy number
- Analyses of the change from baseline to month 15 in the HFMSE score according to age and disease duration revealed greater improvements in younger children and in those who received treatment earlier in their disease course, respectively

## Supplementary efficacy data

Change from baseline in HFMSE score to Month 15 by *SMN2* copy number

| ***SMN2* gene copy number** | **Intervention** | **Number of patients** | **Mean (SD) change from baseline** |
|---|---|---|---|
| 2 | Nusinersen | 6 | 3.3 (5.9) |
| | Control | 3 | -2.3 (4.5) |
| 3 | Nusinersen | 57 | 4.1 (4.9) |
| | Control | 30 | -0.3 (4.5) |
| 4 | Nusinersen | 1 | 5.0 |
| | Control | 1 | -10.0 |
| Unknown | Nusinersen | 2 | 2.0 (1.4) |
| | Control | 0 | - |

Analyses are based on observed values.

## Safety

| **AEs, n (%)** | **Nusinersen (N=84)** | **Control (N=42)** |
|---|---|---|
| Any AE | 78 (93) | 42 (100) |
| Any moderate or severe AE | 39 (46) | 23 (55) |
| Any severe AE | 4 (5) | 3 (7) |
| Any SAE | 14 (17) | 12 (29) |
| Any AE leading to treatment discontinuation | 0 | 0 |
| Any AE leading to withdrawal from the trial | 0 | 0 |
| **AEs with the highest incidence*** | | |
| Pyrexia | 36 (43) | 15 (36) |
| Upper respiratory tract infection** | 25 (30) | 19 (45) |
| Headache | 24 (29) | 3 (7) |
| Vomiting | 24 (29) | 5 (12) |

**Study reference** **Mercuri 2018**

| | | |
|---|---|---|
| Back pain | 21 (25) | 0 |
| Cough** | 21 (25) | 9 (21) |
| Nasopharyngitis** | 20 (24) | 15 (36) |
| **SAEs with the highest incidence†** | | |
| Pneumonia** | 2 (2) | 6 (14) |
| Influenza** | 0 | 2 (5) |
| Respiratory distress** | 2 (2) | 2 (5) |
| Fecaloma | 0 | 2 (5) |
| Dehydration | 0 | 2 (5) |
| **AEs with an incidence ≥5 percentage points higher in the nusinersen group than in the control group††** | | |
| Pyrexia | 36 (43) | 15 (36) |
| Headache | 24 (29) | 3 (7) |
| Vomiting | 24 (29) | 5 (12) |
| Back pain | 21 (25) | 0 |
| Epistaxis | 6 (7) | 0 |

Investigators rated the severity of each AE (mild, moderate, or severe). Moderate AEs were defined as events that caused discomfort and interrupted the child's usual daily activities. Severe AEs were defined as events that caused severe discomfort or incapacitation or had a substantial effect on daily life. Investigators reported an AE as an SAE if it met the following criterion: any untoward medical occurrence that resulted in death or a risk of death, hospitalisation or prolonged hospitalisation, persistent or substantial disability or incapacitation, or a congenital anomaly or birth defect. Reporting of SAEs and rating of the severity of each AE were conducted separately, on the basis of the criteria for each type of AE. For participants who reported more than one AE, only one event of the highest severity was counted in the total incidence

*The events, classified according to MedDRA preferred terms, occurred in at least 20% of children in either trial group

**The events could plausibly be linked to SMA

†The events, classified according to MedDRA preferred terms, occurred in at least 5% of children in either trial group

††The events were classified according to MedDRA preferred terms. A child was counted only once within each category

- The overall rate of events associated with lumbar puncture (i.e., back pain, cerebrospinal fluid leakage, headache, nausea, the post–lumbar puncture syndrome, procedural pain, procedural nausea, procedural headache, and vomiting) within 24, 72, 120, and 168 hours after the assigned procedure was 9%, 14%, 15%, and 15%, respectively, in the nusinersen group and 3% for each time period in the control group

AE, adverse event; CI, confidence interval; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; ECG, electrocardiogram; HFMSE, Hammersmith Functional Motor Scale Expanded; MedDRA, Medical Dictionary for Regulatory Activities; MRI, magnetic resonance imaging; RCT, randomised controlled trial; RULM, Revised Upper Limb Module; SAE, serious adverse event; SD, standard deviation; SMA, spinal muscular atrophy; *SMN,* survival motor neuron; US, United States; WHO, World Health Organization.

## Appraisal for quality and risk of bias – Criterion 1

A quality assessment for the study included for criterion 1 is reported in Table 35 below.

### Table 35: Quality assessments of studies relating to question 1

| Question | Verhaart 2017[71] |
|---|---|
| Was the sample frame appropriate to address the target population? | **Yes**: general population |
| Were study participants sampled in an appropriate way? | **Unclear**: Genetic laboratories were recruited, but it is unclear how each laboratory received samples from the population. The UK lab also tests samples from abroad. |
| Was the sample size adequate? | **Yes** |
| Were the study subjects and the setting described in detail? | **No**: A range of sources are listed, but unclear which sources were used for the UK data specifically |
| Was the data analysis conducted with sufficient coverage of the identified sample? | **Yes**: Responses were received from laboratories responsible for >80% of all SMA tests, and from 11 of 12 laboratories in the UK specifically |
| Were valid methods used for the identification of the condition? | **Unclear**: Genetic testing carried out by various laboratories that could have taken different approaches |
| Was the condition measured in a standard, reliable way for all participants? | **Unclear**: Genetic testing carried out by various laboratories that could have taken different approaches |
| Was there appropriate statistical analysis? | **Yes**: numerator and denominator clearly reported and percentages given with confidence intervals (calculated using Poisson distribution) |
| Was the response rate adequate, and if not, was the low response rate managed appropriately? | **Yes**: 11 of 12 UK laboratories responded to the survey |

## Appraisal for quality and risk of bias – Criterion 4

Quality assessments of included studies for criterion 4 are reported in Table 36 and Table 37 below.

### Table 36: Quality assessments of studies relating to question 2

| Question | Feng 2017[73] | Wang 2015[74] |
|---|---|---|
| **PATIENT SELECTION** | | |
| Was a consecutive or random sample of patients enrolled? | **Unclear:** no information on the source of the samples | **Unclear:** NR |

| Question | Feng 2017[73] | Wang 2015[74] |
|---|---|---|
| Was a case-control design avoided? | **Yes:** prior to testing, it was not known whether the samples were patients, controls or carriers | **Yes**: SMA patients were excluded from the study, so therefore only potential carriers and controls were included, thus avoiding a case-control design |
| Did the study avoid inappropriate exclusions? | **Unclear:** patient flow is reported in very little detail and so exclusions are not mentioned | **Yes:** SMA patients were excluded. However, this is not inappropriate for a carrier screening study |
| Could the selection of patients have introduced bias? | **Unclear:** the selection of patients is reported in very little detail and so the level of bias is not clear. The demographics of the population are NR | **Unclear:** the selection of patients is reported in very little detail and it is unclear if bias has been introduced. It is unclear if patients were randomly selected, or if they suspected that they were SMA carriers. The demographics of the population are NR |
| Is there concern that the included patients do not match the review question? | **Unclear:** patient details are NR and so this cannot be determined | **Unclear:** individuals under the age of 18 were included despite the question specifying that these studies should be in adults |
| **INDEX TESTS** | | |
| Were the index test results interpreted without knowledge of the reference standard? | **Unclear:** the order of events is not clearly stated | **Unclear** |
| If a threshold was used, was it pre-specified? | **Unclear:** Although the script for detection of *SMN1* copy numbers is freely available online, it is unclear whether the thresholds for converting the raw copy number ratios into integer copy number ratios were pre-specified. For example, for samples with a real-life *SMN1*:*SMN2* copy number ratio of 1:1, the actual ratios detected by the algorithm had a mean value of 1.17 (SD 0.091) | **Unclear:** use of threshold NR |
| Could the conduct or interpretation of the index test have introduced bias? | **Unclear:** the index test was well-designed and was performed robustly; however, the study was designed to optimise the performance of the index test and from the details reported it is unclear whether this could have introduced bias | **Unclear:** all samples were screened and the methods seem standardised for all samples, reducing the likelihood of bias, however the order in which the index test and reference standard were conducted is unclear, and it is not known whether a prespecified threshold value was used |
| Is there concern that the index test, its conduct, or interpretation differ from the review question? | **High:** Within the full study population, the test was only used to identify copy numbers of *SMN1* rather than all of the genetic changes associated with carrier status | **High:** the index test can be used to detect SMA carriers in an adult population as per the review question. Tests were only used to identify copy numbers of *SMN1* rather than all of the genetic changes associated with carrier status |
| **REFERENCE STANDARD** | | |
| Is the reference standard likely to correctly classify the test condition? | **Yes:** commonly used reference standards for the detection of SMA carrier status, but not the standard specified in the eligibility criteria | **Yes:** commonly used reference standard for the detection of SMA carrier status, but not the standard specified in the eligibility criteria |
| Were the reference standard results | **Unclear:** the order of events is not clearly stated | **Unclear:** the order of events is not clearly stated |

| Question | Feng 2017[73] | Wang 2015[74] |
|---|---|---|
| interpreted without knowledge of the results of the index test? | | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Unclear:** it is unclear what proportion of samples were tested using one or both reference standards, but otherwise conduct and interpretation unlikely to introduce bias | **Unclear:** all samples were screened and the methods seem standardised for all samples, reducing the likelihood of bias |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | **High:** the reference standards (MLPA and qPCR) are able to determine *SMN1* copy number, but will not identify all SMA carriers | **High:** the reference standard (DHPLC) is able to determine *SMN1* copy number, but will not identify all SMA carriers |
| **PARTICIPANT FLOW** | | |
| Was there an appropriate interval between the index test(s) and the reference standard? | **Yes:** tests done on same blood sample | **Unclear:** NR |
| Did all participants receive a reference standard? | **Yes:** but It is unclear what proportion of samples were tested using one or both reference standards | **Yes**: all of the samples were assessed by the HRMA carrier-screening test and the DHPLC assay to validate the copy numbers of *SMN1* and *SMN2*<br>Therefore, samples from each patient were screened by the reference standard |
| Did participants receive the same reference standard? | **No:** both qPCR (Fluidigm) and MLPA were used but NR which samples underwent each screening test or if both were used in all cases | **Yes:** see above |
| Were all participants included in the analysis? | **Yes** | **Yes** |
| Could the participant flow have introduced bias? | **Unclear:** patient (sample) flow NR and so bias cannot be determined | **Low:** all patients were screened using HRMA and the reference standard and no inappropriate exclusions occurred |

DHPLC, denaturing high performance liquid chromatography; HRMA, high-resolution melting analysis; MLPA, multiplex ligation-dependent probe amplification; NR, not reported; PGCNARS, paralogous gene copy-number analysis by ratio and sum; qPCR, quantitative polymerase chain reaction; SMA, spinal muscular atrophy; *SMN*, survival motor neuron.

## Table 37: Quality assessment of studies relevant to question 5

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[77] |
|---|---|---|---|---|
| **PATIENT SELECTION** | | | | |
| Was a consecutive or random sample of patients enrolled? | **Unclear:** a total of 88 patients, carriers and controls were recruited into the study but it is unclear as to if these | **Yes:** a consecutive sample of newborns born in the centre were tested in a consecutive series during a defined time | **Unclear:** DNA samples from SMA patients, carriers and normal individuals were obtained from Chung-Ho | Retrospective study:<br>**No:** 141 patients with suspected limb movement disorders were enrolled. It is |

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[77] |
|---|---|---|---|---|
| | patients were randomly or consecutively enrolled | period | Memorial Hospital, Kaohsiung Medical University, but information about how these samples were selected is NR | not clear how the 100 controls were recruited<br><br>Prospective study<br>**Yes:** 2000 randomly selected samples from the Newborn Screening Center at the Children's Hospital of Chongqing Medical University |
| Was a case-control design avoided? | **No:** case-control design utilised here with the number of patients, carriers and controls specified at the outset of the study | **Yes** | **No:** case-control design is used in this study (30 patients and 30 controls) | Retrospective study:<br>**No:** case-control design utilised here with the number of suspected patients and controls specified at the outset of the study<br><br>Prospective study<br>**Yes** |
| Did the study avoid inappropriate exclusions? | **Unclear:** 88 individuals enrolled with 88 DBS samples analysed, but in the specificity and sensitivity calculations the total sample number is 77, details of this discrepancy NR. No details of patient exclusion are provided | **Yes:** patients were only excluded due to no consent (n=21,607) and infants from parents who had undergone SMA carrier testing were not rejected from the study | **Unclear:** patient flow NR and so exclusions are not mentioned. For the sensitivity and specificity analyses comparing HRM analysis to DHPLC, a total of 80 samples were examined not 60. It is unclear as to what the additional 20 samples where | Retrospective study:<br>**No:** only patients with suspected limb movement disorders were enrolled. As the study was looking at test clinical performance measurements, this may bias the results given the population is not representative of the general population<br><br>Prospective study<br>**Yes:** all 2000 random samples selected underwent real time PCR. As samples were randomly selected no inappropriate exclusions occurred |
| Could the selection of patients have introduced bias? | **High:** as the number of study participants who were patients, carriers and controls | **Low:** a consecutive and random stream of patients born at the hospital were | **High:** case-control design therefore there are as many SMA patients as healthy | Retrospective study:<br>**High:** only patients with suspected limb movement |

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[77] |
|---|---|---|---|---|
| | was known at the onset of the study, this may have influenced sensitivity/specificity values as the study population was not randomly recruited | screened and so it is unlikely that those samples from which results are taken represent a bias population | controls, this may introduce bias as sensitivity and specificity are determined in a population not representative of the incidence of SMA in the general population which would ultimately be screened for SMA | disorders were enrolled and the number of study participants who were suspected patients and controls was known prior to the study. This may have influenced test clinical performance measurements as the study population was not randomly recruited and therefore introduced bias<br><br>Prospective study<br>**Low:** patients' samples were randomly selected |
| Is there concern that the included patients do not match the review question? | **High:** enrolled individuals include SMA patients, carriers and controls but it is unclear whether these patients are adults or are under the age of 18, which could represent deviation from the review question if a neonatal population is not used, newborn screening is referred to throughout but it is not explicitly stated that individuals are a newborn population. Additionally, further patient details are NR so this cannot be determined | **Low:** only newborns were screened which aligns with the neonate population | **High:** review question specifies that included patients should be neonates, however, this is unclear in this study as participants' ages are NR and the study employed a case-control design which is not representative of a screening study | Retrospective study:<br>**High:** included patients are specified as children, with suspected limb movement disorders. The review's population are specified as neonates (irrespective of suspected limb movement disorders), therefore the population in this study does not match the population specified in the review<br><br>Prospective study:<br>**Low:** patients are newborns and so likely fit the population criteria as set out in the review question |
| **INDEX TESTS** | | | | |
| Were the index test results interpreted without knowledge of the reference standard? | **No:** index test was conducted with knowledge of a previously conducted reference standard | **Yes:** index test was conducted first and then confirmed using the reference standards | **No:** reference test conducted before index test | Retrospective study:<br>**Unclear:** it is unclear if the RT-PCR results were interpreted without the knowledge of the Sanger DNA sequencing results |

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[77] |
|---|---|---|---|---|
| | | | | Prospective study:<br>**Yes:** reference standard only conducted after a positive screening result with the index test |
| If a threshold was used, was it pre-specified? | **Unclear:** used the threshold (cut-off) quantification cycle (Cq) value of 12 in the mCOP-PCR methodology. The cycle number of 12 was enough to confirm the specific amplification with matched primers, and to avoid non-specific amplification with mismatched primers. It is unclear if this threshold was pre-specified | **Yes:** pre-specified threshold: the cut-off for screening was set arbitrarily at *SMN1* ΔRn (normalised reporter fluorescence intensity) <1, based on values of known patients and normal newborns. It is unclear how many patients and normal newborns were used to determine the threshold value | **Unclear:** In the normalised and temperature-shifted difference plots used in the HRM analysis, *SMN1;SMN2* gene ratio of 0:2 was chosen as the horizontal baseline and the relative differences in the melting profiles of all other samples were plotted relative to this baseline. It is unclear if this baseline was pre-specified | Retrospective study:<br>**Unclear:** for RT-PCR, a threshold of relative fluorescent units (RFU) was set as $10^2$. It is unclear if this threshold was pre-specified<br><br>Prospective study:<br>**Unclear:** NR |
| Could the conduct or interpretation of the index test have introduced bias? | **Unclear:** methodology of the index test appears robust and *SMN2* presence/absence was used as a screening process confirmation of the ability of the test to detect SMN1 deletion. However, index test was conducted with knowledge of a previously conducted reference standard which could have introduced bias | **Low:** the screening method was validated by testing 2,937 anonymous newborn DBS samples and 9 DNA samples with known *SMN1* and *SMN2* copy numbers. Seventy-seven additional DNA samples with a known SMA affected status were tested using this method, and the results were perfectly matched, making the sensitivity and specificity both 100%. All methods seem standardised for all samples, reducing the likelihood of bias | **Unclear:** Interpretation could have introduced bias as the index test was conducted with knowledge of a previously conducted reference standard | Retrospective study:<br>**Unclear: i**nterpretation could have introduced bias as it is not clear if the index test was conducted with knowledge of a previously conducted reference standard, however test conduct appears unlikely to have introduced bias as all samples were treated identically and validated through repetition<br><br>Prospective study:<br>**Low:** conduct of the index test unlikely to introduce bias |
| Is there concern that the index test, its conduct, or interpretation differ from the review question? | **Low:** the test is in line with the review question and is sensitive for detecting the loss of *SMN1* exon 7 | **Low:** the index test in combination with ddPCR testing, tests for SMA using DBS methodologies, as per the review question | **Low:** HRMA is used to test for SMA by detecting the substitution of a single nucleotide in *SMN1* exon 7 (c.840 C>T), as per the review question | Retrospective and prospective study:<br>**Low:** RT-PCR from clinical specimens (retrospective study) and DBS samples (prospective study) are used |

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[77] |
|---|---|---|---|---|
| | | | | to test for SMA by detecting the substitution of a single nucleotide in *SMN1* exon 7 (c.840 C>T), as per the review question |
| **REFERENCE STANDARD** | | | | |
| Is the reference standard likely to correctly classify the test condition? | **Yes:** commonly used reference standard, but not the standard specified in the eligibility criteria (prior to the post hoc modifications) | **Yes:** MLPA has been shown to be a versatile and fast technique for determining different nucleic acid sequences in a single reaction, so likely to correctly classify the positive/false positive results | **Yes:** commonly used reference standard | Retrospective study:<br>**Yes:** DNA sequencing, that was used as the reference standard in this study, is described as the 'gold standard' to detect a single nucleotide difference (however, MLPA is described as the gold standard for detecting exon loss though, which is not used here and would be useful to determine exon 7 loss in SMA patients)<br><br>Prospective study:<br>**Yes:** reconfirmation occurred through another RT-PCR and DNA sequencing. However, only samples testing positive at the first round of RT-PCR underwent re-confirmation RT-PCR testing and DNA sequencing |
| Were the reference standard results interpreted without knowledge of the results of the index test? | **Yes:** reference standard testing was conducted before the index test | **No:** RT-PCR and second-tier ddPCR was used and then positive results confirmed using MLPA | **Yes:** reference conducted before index text (results previously confirmed by DHPLC) | Retrospective study:<br>**Unclear:** it is unclear if the Sanger DNA sequencing results were interpreted without the knowledge of the RT-PCR results<br><br>Prospective study:<br>**No:** reference standard only conducted after index test, and only in screen positive |

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[77] |
|---|---|---|---|---|
| | | | | patients |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Unclear:** the methodology is NR in detail in the publication | **High:** only positive results underwent ddPCR/MLPA and therefore the interpretation may be biased, false negatives cannot be determined and so this will affect sensitivity/specificity calculations | **Unclear:** the methodology is NR in detail in the publication | Retrospective study: **Unclear:** test conduct appears unlikely to have introduced bias as all samples were treated identically and validated through repetition, but it is unclear if the Sanger DNA sequencing results were interpreted without the knowledge of the RT-PCR results<br><br>Prospective study: **High:** only positive screening tests were reconfirmed using a reference standard; negative results did not undergo the reference standard |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | **Low:** the reference standard was used to evaluate *SMN1* exon 7 deletion | **Low:** MLPA should detect SMA as defined by the review question | **Low:** DHPLC should screen for SMA as defined in the review question | Retrospective and prospective study: **Unclear:** DNA sequencing is described as the 'gold standard' to detect a single nucleotide difference, which indicates a diagnosis of SMA (however, MLPA is described as the gold standard for detecting exon loss though, which is not used here and would be useful to determine exon 7 loss in SMA patients) |
| **PARTICIPANT FLOW** | | | | |
| Was there an appropriate interval between the index | **Unclear:** (although it is stated that DBS samples | **Unclear:** NR | **Unclear:** The reference standard was conducted first, | Retrospective and prospective study: |

| **Question** | **Ar Rochmah 2017**[75] | **Chien 2017**[63] | **Er 2012**[76] | **Liu 2016**[77] |
|---|---|---|---|---|
| test(s) and the reference standard? | were kept from between 1 week to 5 years) | | but the interval between the reference standard and index test being conducted was not reported | **Unclear:** NR, but in the prospective study the index test was conducted before the reference standard although time interval was not specified |
| Did all participants receive a reference standard? | **Yes**: all participants had previously been screened for *SMN* genes by PCR-RFLP using DNA extracted from freshly collected blood | **No:** only positive results from RT-PCR testing underwent ddPCR/MLPA | **Yes:** all results were confirmed by DHPLC | Retrospective study:<br>**Yes:** all results were confirmed by DNA Sanger sequencing<br><br>Prospective study:<br>**No:** reference standard only conducted after a positive screening result with the index test; negative results did not undergo the reference standard |
| Did participants receive the same reference standard? | **Yes:** all participants had previously been screened for *SMN* genes by PCR-RFLP using DNA extracted from freshly collected blood | **No:** only positive results from RT-PCR testing underwent ddPCR/MLPA, the DBS ddPCR assay excluded 8 false-positives, and the other 7 patients were confirmed by the MLPA assay | **Yes:** all patients' samples underwent DHPLC | Retrospective and prospective study:<br>**Yes:** all samples which received a reference standard received the same reference standard |
| Were all participants included in the analysis? | **Unclear:** 88 samples from 88 study participants are said to have undergone analysis i.e. received the index test, however, the tables indicating sensitivity and specificity only reports 77 total, the discrepancy here is not explained | **Yes:** all study participants with a satisfactory test result were included in the analyses | **Yes:** all patients included in the sensitivity and specificity analysis. However, 80 samples were used in the sensitivity and specificity analyses, rather than the 60 samples that were stated in the methodology; it is unclear what the additional 20 samples were | Retrospective study:<br>**Yes:** all study participants were included in the analyses<br><br>Prospective study:<br>**No:** analysis of test occurred only for patients who received the reference standard i.e. those with a positive screening result with the index test |
| Could the participant flow have introduced bias? | **Unclear:** if the aforementioned exclusions are inappropriate then this could introduce bias, however, it is not clear if this | **High:** the flow itself is unlikely to have introduced bias but the analysis/use of reference standards only in the positively screened | **Unclear:** It is unclear where the additional 20 samples used in the sensitivity and specificity analyses, but not reported in the methodology, | Retrospective study:<br>**Unclear:** all patients appear to have been included in the analyses but it is unclear if the index test was interpreted |

| Question | Ar Rochmah 2017[75] | Chien 2017[63] | Er 2012[76] | Liu 2016[77] |
|---|---|---|---|---|
| | is the case | individuals may introduce bias | where obtained from, and whether this may have introduced bias | without knowledge of the reference standard (and vice versa). If the interpretation had occurred with knowledge of the other test results, this may have introduced bias<br><br>Prospective study:<br>**High:** patient flow unlikely to have introduced bias but only tests that were positive from the index test (RT-PCR), were then re-tested using RT-PCR as well as DNA Sanger sequencing, which may have introduced bias |

DBS, dried blood spot; ddPCR, droplet digital PCR; DHPLC, denaturing high performance liquid chromatography; DNA, deoxyribonucleic acid; HRMA, high-resolution melting analysis; mCOP-PCR, modified competitive oligonucleotide priming-polymerase chain reaction; MLPA, multiplex ligation-dependent probe amplification; NBS, newborn screening; NR, not reported; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SMA, spinal muscular atrophy; *SMN*, survival motor neuron.

## Appraisal for quality and risk of bias – Criterion 10

Quality assessments of included studies for criterion 10 are reported in Table 38 below.

### Table 38: Quality assessment of studies relevant to question 7

| Question | Bertini 2017[20] | Finkel 2017[9] | Kirschner 2014[81] | Kissel 2014[82] | Mercuri 2018[10] |
|---|---|---|---|---|---|
| **ARE RESULTS OF THE TRIAL VALID? SCREENING QUESTIONS** | | | | | |
| Did the trial address a clearly focused issue? | **Yes**: they investigated the safety and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 SMA | **Yes:** the trial aimed to assess the efficacy and safety of nusinersen in infants with SMA | **Yes:** evaluated the efficacy and safety of growth hormone treatment for type 2/3 SMA | **Yes:** investigated efficacy and safety of VPA in the treatment of adults with ambulatory SMA | **Yes:** the trial aimed to assess the efficacy and safety of nusinersen in children with later onset SMA |
| Was the assignment of | **Yes:** patients were | **Yes:** infants were | **Yes:** patients were | **Yes:** subjects were | **Yes:** children were |

| Question | Bertini 2017[20] | Finkel 2017[9] | Kirschner 2014[81] | Kissel 2014[82] | Mercuri 2018[10] |
|---|---|---|---|---|---|
| patients to treatment treatments randomised? | randomised 2:1 to olesoxime and placebo with stratification by SMA type and centre | randomly assigned, in a 2:1 ratio, to undergo intrathecal administration of nusinersen (nusinersen group) or a sham procedure (control group), although method of randomisation not detailed, so appropriateness of methodology cannot be assessed | randomised to receive either somatropin followed by placebo or placebo followed by somatropin (crossover) | randomised to receive either VPA followed by placebo or placebo followed by VPA (crossover) | randomly assigned, in a 2:1 ratio, to undergo intrathecal administration of nusinersen at a dose of 12 mg (nusinersen group) or a sham procedure (control group) |
| Were all of the patients who entered the trial properly accounted for at its conclusion? | **Yes:** trial profile provided in figure 1 highlighting the flow of patients through the trial and the reasons for any exclusions | **Yes:** a total of 149 infants were screened, and 122 underwent randomisation (81 were assigned to the nusinersen group, and 41 to the control group). One infant in the nusinersen group was withdrawn from the trial before treatment; 121 infants underwent the assigned procedure and all are included in the primary efficacy and safety analyses | **Yes:** figure 1 provides a flow diagram of the trial design where patient numbers and exclusions are explained | **Yes:** CONSORT diagram provided to show the flow of patients through the study and exclusions are explained | **Yes:** a total of 179 children were screened; 126 were enrolled in the trial, were randomly assigned to a group, and underwent the assigned procedure (84 in the nusinersen group, and 42 in the control group. All 126 patients are analysed in the efficacy and safety analyses |
| **ARE RESULTS OF THE TRIAL VALID? DETAILED QUESTIONS** | | | | | |
| Were patients, health workers and study personnel 'blind' to treatment? | **Yes:** all investigators, site personnel, patients, and the sponsor study personnel were masked to treatment assignment until completion of the study | **Yes:** this is a double-blind trial; to maintain blinding, nusinersen was administered or the sham procedure was performed by dedicated trial personnel who were aware of the group assignments, whereas the infant's parents and key trial | **Yes:** patients, physicians and physiotherapists involved in this trial were unaware which drug regimen was being administered; blinding of study personnel unclear | **No:** patients were blind to treatment; a medical monitor who reviewed all subjects' blood tests and adverse events, performed dose adjustments and conducted additional testing where necessary was unblinded; NR as to | **Yes:** this is a double-blind trial; administration of nusinersen or performance of the sham procedure was performed by dedicated personnel who were aware of groups assignments, however, parents and key trial |

| Question | Bertini 2017[20] | Finkel 2017[9] | Kirschner 2014[81] | Kissel 2014[82] | Mercuri 2018[10] |
|---|---|---|---|---|---|
| | | personnel who were responsible for assessments were unaware of the group assignments and were not present for the procedure | | whether health workers were blinded, but assumed based on double blind classification of trial | personnel were unaware of group assignments until trial completion and were not present for the procedure. Participants were sedated to avoid any awareness of the procedure |
| Were the groups similar at the start of the trial? | **Yes:** patient demographic and baseline characteristics were mainly well balanced between the treatment groups, including proportions of patients with type 2 or type 3 SMA. However, both mean and median ages were lower in the olesoxime group than in the placebo group, with a difference of 2·1 years in mean ages and a difference of 4 years in median ages across treatment groups. Additionally, there were slight differences in the proportion of males and females between groups. | **Unclear:** baseline characteristics were generally balanced between the two groups, except for age at the time of diagnosis of SMA, use of ventilatory support, and the presence of symptoms specific to SMA; the infants in the nusinersen group had earlier onset of symptoms and greater burden of disease than the infants in the control group (however, formal statistical testing was not performed) | **Yes:** baseline data from outcome measures did not differ between groups, however, baseline demographics are not compared | **Yes:** there was no significant difference between these groups in any demographic or baseline assessment | **Yes:** the demographic characteristics of the children at baseline were similar in the two trial groups; there were slight differences in age, sex, race, disease duration, and motor milestones achieved, but no formal statistical testing was performed |
| Aside from the experimental intervention, were the groups treated equally? | **Yes:** patients received either oral olesoxime 100 mg/mL liquid suspension formulation once a day or matching placebo with their main daily meal for 24 months and following screening and baseline visits, follow-up visits were scheduled for | **Yes:** nusinersen and sham procedures were given on the same days (days 1, 15, 29, and 64 and maintenance doses on days 183 and 302) and efficacy and safety were assessed on the same days (days 64, 183, 302, and 394 (±7 days for each visit) and | **Yes:** patients who fulfilled inclusion criteria and who were randomly selected for the treatment group received 0.015 mg recombinant somatropin per kg body weight daily for 1 week, following by 0.03 mg/kg somatropin daily in | **Yes:** VPA and placebo were provided by Abbott Pharmaceuticals as 250 mg divalproex sodium capsules and administered in divided doses 2 or 3 times daily at a starting dose of 10 to 20 mg/kg orally, treatment assessments were performed at 3 | **Unclear** nusinersen and sham procedures were given on the same days (days 1, 29, 85, and 274) and efficacy analysis was assessed at the same timepoints (twice during the screening period and at 3, 6, 9, 12, and 15 months). Other efficacy |

| Question | Bertini 2017[20] | Finkel 2017[9] | Kirschner 2014[81] | Kissel 2014[82] | Mercuri 2018[10] |
|---|---|---|---|---|---|
| | week 4 and week 13 after randomisation, after which participants were assessed every 13 weeks for a total of 9 visits over the 24-month treatment period | days 16, 30, 65, 184, and 303, respectively), follow-up for both groups took the form of weekly assessments by telephone and a visit to the study centre on day 394 (±7 days) | weeks 2 through 12. The other group received daily placebo injections for 3 months. Somatropin or placebo was administered using an injection pen by the patients or their parents, who were adequately trained in its use. After a washout period of 2 months, patients in the treatment group received placebo and vice versa for an additional 3 months at the same dosage | (V1), 6 (V2), and 12 (V3) months. Safety laboratory studies were performed at baseline, 2 to 3 weeks after initiation, at each treatment visit, and midway between V2 and V3 | and safety follow-up is not clear and it is therefore unclear whether the two groups were treated equally |
| **RELEVANCE TO THE RAPID REVIEW** | | | | | |
| Can the results be applied to a UK population? | **Yes:** the study included patients from 3 sites in the UK (8 received placebo and 10 received Olesoxime) | **Yes:** two UK centres in the trial (of 31 centres); UCL Institute of Child Health/Great Ormond Street Hospital and the Centre for Neuromuscular Diseases at Newcastle, Institute of Genetic Medicine Newcastle University. However, the remaining 29 centres were not in the UK | **Yes:** the study was a multicentre trial in 5 German hospitals and did not treat any UK patients, however, it can be assumed that treatments would be similarly effective in UK patients | **Yes:** single-centre in the US, did not treat any UK patients, however, it can be assumed that treatments would be similarly effective in UK patients | **Yes:** no UK centres were included in this trial and there was no ethnicity data, but study centres were in generally similar high-income countries |
| Were outcomes of importance to the rapid review considered? | **Yes:** question 7 requires studies informing the effectiveness of pharmacological treatments for SMA (with a sub-question regarding whether treatments are more successful for particular | **Yes:** both clinical and safety outcomes were assessed for a pharmacological therapy in a population of individuals with SMA, and compared to a sham procedure | **Yes:** clinical and safety outcomes of a pharmacotherapy for SMA are reported and therefore this study is relevant to question 7 | **Yes:** clinical and safety outcomes of a pharmacotherapy for SMA are reported and therefore this study is relevant to question 7 | **Yes:** both clinical and safety outcomes were assessed for a pharmacological therapy in a population of individuals with SMA, and compared to a sham procedure |

| Question | Bertini 2017[20] | Finkel 2017[9] | Kirschner 2014[81] | Kissel 2014[82] | Mercuri 2018[10] |
|---|---|---|---|---|---|
| | types); therefore, this paper provides relevant outcomes concerning the effectiveness of olesoxime versus placebo for the treatment of SMA and also compares efficacy outcomes for type 2 and type 3 SMA | | | | |

CONSORT, Consolidated Standards of Reporting Trials; MSA, spinal muscular atrophy; UK, United Kingdom; US, United States; VPA, valproic acid.

# Appendix 4 – UK NSC reporting checklist for evidence summaries

All items on the UK NSC Reporting Checklist for Evidence Summaries have been addressed in this report. A summary of the checklist, along with the page or pages where each item can be found in this report, is presented in Table 39.

**Table 39: UK NSC reporting checklist for evidence summaries**

| | Section | Item | Page no. |
|---|---|---|---|
| **1.** | **TITLE AND SUMMARIES** | | |
| **1.1** | Title sheet | Identify the review as a UK NSC evidence summary. | Title page |
| **1.2** | Plain English summary | Plain English description of the executive summary. | 4 |
| **1.3** | Executive summary | Structured overview of the whole report. To include: the purpose/aim of the review; background; previous recommendations; findings and gaps in the evidence; recommendations on the screening that can or cannot be made on the basis of the review. | 5 |
| **2.** | **INTRODUCTION AND APPROACH** | | |
| **2.1** | Background and objectives | Background – Current policy context and rationale for the current review – for example, reference to details of previous reviews, basis for current recommendation, recommendations made, gaps identified, drivers for new reviews | 11 |
| | | Objectives – What are the questions the current evidence summary intends to answer? – statement of the key questions for the current evidence summary, criteria they address, and number of studies included per question, description of the overall results of the literature search. | 21 |
| | | Method – briefly outline the rapid review methods used. | 22 |
| **2.2** | Eligibility for inclusion in the review | State all criteria for inclusion and exclusion of studies to the review clearly (PICO, dates, language, study type, publication type, publication status etc.) To be decided *a priori*. | 22 |
| **2.3** | Appraisal for quality/risk of bias tool | Details of tool/checklist used to assess quality, e.g. QUADAS 2, CASP, SIGN, AMSTAR. | 33 |
| **3.** | **SEARCH STRATEGY AND STUDY SELECTION (FOR EACH KEY QUESTION)** | | |
| **3.1** | Databases/ | Give details of all databases searched (including platform/interface and coverage dates) and date of final search. | 33 |

| | | | |
|---|---|---|---|
| | sources searched | | |
| **3.2** | Search strategy and results | Present the full search strategy for at least one database (usually a version of Medline), including limits and search filters if used. Provide details of the total number of (results from each database searched), number of duplicates removed, and the final number of unique records to consider for inclusion. | 60 |
| **3.3** | Study selection | State the process for selecting studies – inclusion and exclusion criteria, number of studies screened by title/abstract and full text, number of reviewers, any cross checking carried out. | 22 |
| **4.** | **STUDY LEVEL REPORTING OF RESULTS (FOR EACH KEY QUESTION)** | | |
| **4.1** | Study level reporting, results and risk of bias assessment | For each study, produce a table that includes the full citation and a summary of the data relevant to the question (for example, study size, PICO, follow-up period, outcomes reported, statistical analyses etc.). Provide a simple summary of key measures, effect estimates and confidence intervals for each study where available. For each study, present the results of any assessment of quality/risk of bias. | Study level reporting: 68<br>Quality assessment: 5 |
| **4.2** | Additional analyses | Describe additional analyses (for example, sensitivity, specificity, PPV, etc.) carried out by the reviewer. | Not performed |
| **5.** | **QUESTION LEVEL SYNTHESIS** | | |
| **5.1** | Description of the evidence | For each question, give numbers of studies screened, assessed for eligibility, and included in the review, with summary reasons for exclusion. | 33 to 56 |
| **5.2** | Combining and presenting the findings | Provide a balanced discussion of the body of evidence which avoids over reliance on one study or set of studies. Consideration of 4 components should inform the reviewer's judgement on whether the criterion is 'met', 'not met' or 'uncertain': quantity; quality; applicability and consistency. | 33 to 56 |
| **5.3** | Summary of findings | Provide a description of the evidence reviewed and included for each question, with reference to their eligibility for inclusion. Summarise the main findings including the quality/risk of bias issues for each question. Have the criteria addressed been 'met', 'not met' or 'uncertain'? | 33 to 56 |
| **6.** | **REVIEW SUMMARY** | | |
| **6.1** | Conclusions and implications for policy | Do findings indicate whether screening should be recommended? Is further work warranted? Are there gaps in the evidence highlighted by the review? | 57 |
| **6.2** | Limitations | Discuss limitations of the available evidence and of the review methodology if relevant. | 58 |

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