Newborn screening for spinal muscular atrophy in Australia: a non-randomised cohort study

Didu S Kariyawasam, Arlene M D'Silva, Hugo Sampaio, Nancy Briggs, Karen Herbert, Veronica Wiley, Michelle A Farrar

Summary

Background In light of a new therapeutic era for spinal muscular atrophy (SMA), newborn screening has been proposed as a gateway to facilitate expedient diagnosis and access to therapeutics. However, there is paucity of evidence on health outcomes outside the homogenous populations in clinical trials to justify broader implementation of newborn screening for SMA. In this real-world study, we aimed to investigate the effectiveness of newborn screening coupled with access to disease-modifying therapeutics, as an intervention for SMA.

Methods In this prospective, non-randomised cohort study done at Sydney Children's Hospital Network (NSW, Australia), we included children younger than 16 years with homozygous exon 7 deletions of survival motor neuron 1 gene (*SMN1*) mutations, non-selectively assigned to a screening group (incident population diagnosed by newborn screening) from Aug 1, 2018, to Aug 1, 2020, or a comparator group (incident population diagnosed by clinical referral) from Aug 1, 2016, to July 31, 2018. We excluded infants with compound heterozygous *SMN1* mutations and those participating in ongoing and unpublished clinical trials. Effectiveness of newborn screening for SMA was compared using motor development milestone attainment defined by WHO Multicentre Growth Reference Study at 2 years post diagnosis. Secondary outcome measures included mortality and change in Hammersmith Infant Neurological Examination-2 (HINE-2) score, ventilation requirements, and enteral requirements 2 years from the time of diagnosis.

Findings 34 children met the study inclusion criteria, but 33 children were included in the study population after one neonate was excluded due to participation in an ongoing unpublished clinical trial. 15 children were included in the screening group (seven [47%] male and eight [53%] female; median age 2·1 weeks [IQR 1·9–2·7]) and 18 children (nine [50%] male and nine [50%] female) were included in the comparator group (median age 47·8 weeks [13·0–99·9]). The 2-year survival rate was 93% (14 of 15 children) in the screening group and 89% (16 of 18) in the comparator group. Among survivors, 11 (79%) of 14 walked independently or with assistance in the screening group, compared with one (6%) of 16 children in the comparator group (χ^2=16·27; p<0·0001). A significantly greater change in motor function was observed in the screening group compared with the comparator group over 2 years (HINE-2 score group difference, 12·32; p<0·0001). The requirement for non-intensive ventilation or feeding support at follow-up was higher in the comparator group than in the screening group (odds ratio 7·1 [95% CI 0·7–70·2]). Significant predictors of functional motor outcomes as determined by HINE-2 score at 2 years post diagnosis were HINE-2 score (p=0·0022), CHOP-INTEND (p=0·0001), compound muscle action potential (CMAP; p=0·0006), and disease status (p=0·023) at diagnosis.

Interpretation Newborn screening for SMA, coupled with early access to disease-modifying therapies, effectively ameliorates the functional burden and associated comorbidities for affected children. For children diagnosed through newborn screening, motor score, CMAP, and disease status at diagnosis has clinical utility to determine functional independence.

Funding Brain Foundation and National Health and Medical Research Council.

Lancet Child Adolesc Health 2023; 7: 159–70

Published Online January 17, 2023 https://doi.org/10.1016/S2352-4642(22)00342-X

See Comment page 146

Department of Neurology (D S Kariyawasam PhD, H Sampaio FRACP, M A Farrar PhD), **and Department of Physiotherapy** (K Herbert GradDipPhys), **Sydney Children's Hospital Network, Sydney, NSW, Australia; Discipline of Paediatrics and Child Health, School of Clinical Medicine, Medicine and Health** (D S Kariyawasam, A M D'Silva PhD, M A Farrar), **and Mark Wainwright Analytical Centre** (N Briggs PhD), **University of New South Wales, Sydney, NSW, Australia; New South Wales Newborn Screening Program, Children's Hospital Westmead, Westmead, NSW, Australia** (V Wiley PhD); **Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia** (V Wiley)

Correspondence to: Dr Didu S Kariyawasam, Department of Neurology, Sydney Children's Hospital Network, Sydney, NSW 2035, Australia **didu.kariyawasam@health.nsw.gov.au**

Introduction

Spinal muscular atrophy (SMA) is an early-onset motor neuron disease characterised by progressive muscle weakness and wasting, leading to substantial disability and reduced survival.[1] The introduction of disease-modifying therapies has rapidly changed the clinical landscape, transforming SMA from a lethal to a treatable disease. The most compelling therapeutic effects of these genetic therapies are observed in individuals treated before the development of clinical symptoms. Pre-symptomatic diagnosis and therapeutic intervention bring the prospect of motor milestone attainment matching the usual childhood developmental trajectories.[2] These therapeutic transformations have driven imperatives to implement newborn screening as a secondary prevention strategy to reduce health burdens for affected children.

The therapeutic pipeline for SMA includes three approved therapies with comparable efficacy when administered in pre-symptomatic children.[2] As newborn screening for SMA facilitates early diagnosis and has the potential for timely proactive management, a comparison of health outcomes against the traditional clinically based

Research in context

Evidence before this study

We completed a literature review from Jan 1, 2016, to Aug 1, 2022, with no language restrictions on PubMed, using the search terms "atrophy", "spinal muscular" AND "newborn screening" AND/OR "outcomes". Nine countries had implemented newborn screening for spinal muscular atrophy (SMA) by 2022, with a paucity of real-world data on the long-term efficacy cited as a rate-limiting factor for wider dissemination. Motor function, survival, and comorbidity outcomes for pre-symptomatic and symptomatic infants were described in the seminal clinical trials for the three disease-modifying agents (nusinersen, risdiplam, and onasemnogene aberparvovec); however, the strict inclusion criteria within these trials precluded generalisability to a real-world incident population of children. Health outcomes for children diagnosed with SMA through newborn screening were based on cumulative results of 77 infants from two prospective real-world studies in New York, USA, and Germany, where motor gains were noted and primarily stratified according to genotype.

Added value of this study

To our knowledge, our study is the first to provide evidence for the clinical effectiveness of newborn screening for SMA as a means to identify patients who could benefit from earlier treatment, directly compared with traditional pathways of diagnosis and management (ie, clinical referral). Our study population represents the heterogenous genotypic and phenotypic spectrum of children with SMA encountered in a real-world setting. This study uniquely incorporates a broad range of outcome measures, including patient-centred endpoints that determine the level of future functional independence for affected children, which are directly relevant to their everyday lives. Our incident, newborn screening population consisted of six children with symptom onset within the newborn period. With only seven children described with phenoconversion within the neonatal period in previous studies, our findings add to a limited pool of data for emergence of a new phenotype. This study is the first to identify clinical, genetic, and electrophysiological measures that influence the prognosis and treatment effect for children with SMA, diagnosed through newborn screening.

Implications of all the available evidence

Newborn screening for SMA coupled with access to disease-modifying therapies is clinically effective as an intervention to promote motor skill attainment, improve functional independence, and reduce comorbidities associated with SMA. Optimisation of health outcomes through this model is observed for a genotypic and phenotypic spectrum of children presenting within real-world practice. This study provides an evidence base and impetus to refigure pre-existing pathways of diagnosis and management, that are currently based on clinical referral. Newborn screening for SMA facilitates a precision medicine approach to prognostication and management, based on a child's individual clinical, genetic, and electrophysiological characteristics.

diagnosis framework (which is used in most of the world) is urgently warranted. An evaluation of the effectiveness of newborn screening for SMA will fill the knowledge gap on whether this pathway is the revolutionary paradigm shift that is required to reduce the substantial burdens associated with this disease, providing the impetus for implementation on a population level.

Currently, the benefits of early diagnosis and intervention are based on evidence of health outcomes derived from clinical trials, applicable to highly homogenous populations. Notably, in pre-symptomatic studies, 14 of 45 children with genetically confirmed SMA younger than 6 weeks did not reach the eligibility criteria for clinical trial enrolment due to symptom onset.[3] Clinical trial enrollment and inclusion criteria do not reflect the heterogenity of newborns with SMA that are identified and managed in real-world clinical practice.[4] Unique subgroups of newborns and infants with SMA have emerged, including those with evolving signs and symptoms of disease, and being treated within the newborn period, which is typically defined as the first 6 weeks of life.[5] For this population, health outcomes and evolution of phenotype might be markedly different from children with classical infantile-onset SMA (SMA type 1) and pre-symptomatic SMA, and accordingly, have not been characterised or included within clinical trials.

The inability to predict outcomes across the genotypic and phenotypic spectrum of SMA led to clinical uncertainties.[6] At the patient–clinician interface, health-care professionals are unable to accurately and on an individual basis establish the risk–benefit of disease-modifying treatment, set realistic therapeutic expectations, and target the correct therapeutic window within which to optimise health outcomes.[7] Although clinical, electrophysiological, and molecular measures are being investigated for their prognostic potential, substantial data gaps exist as to the interplay of these factors and associations with individual outcomes for children entering early diagnostic and treatment pathways.[8,9] Thus, evidence rooted in real-world outcomes is required to inform clinical expectations, develop and standardise the best models of health practice, and inform a precision medicine model of care for all children identified through newborn screening for SMA. We aimed to investigate the effectiveness of newborn screening coupled with access to disease-modifying treatment as an intervention for SMA, and to identify and evaluate genetic, electrophysiological, disease-related, and phenotypic measures that could

modify health outcomes for children with SMA identified by newborn screening.

Methods

Study design and participants

In this prospective, single-centre, non-randomised interventional cohort study, the study population included incident newborns (between birth and 6 weeks of age), infants (between 6 weeks and 12 months of age), and children (between 12 months and 16 years) with homozygous exon 7 deletions of survival motor neuron 1 gene (*SMN1),* based on a multiplex PCR assay (appendix p 1). Children were referred to and clinically managed within the tertiary neuromuscular service at Sydney Children's Hospital Network (SCHN), NSW, Australia. Children were included independent of survival motor neurone 2 (*SMN2*) copy number, disease status, functional ability, and presence of comorbidities including need for support in ventilation and feeding at diagnosis. Infants with compound heterozygous *SMN1* mutations were excluded. Newborns and infants participating in ongoing and unpublished clinical trials were also excluded. Gender and sex data were obtained retrospectively through medical records.

During the pilot newborn screening programme for SMA that ran from Aug 1, 2018, all newborns born in the states of New South Wales and the Australian Capital Territory, Australia, were genetically screened for SMA from dried blood spots taken within the first few days of birth, unless parents opted out of newborn screening for their child.[5] The screening group included newborns diagnosed with SMA following a positive newborn screening result, recruited during the first 2 years of the Australian pilot newborn screening for SMA programme (Aug 1, 2018–Aug 1, 2020). For children within this group, individual treatment plans were instigated after diagnostic confirmation, with potential to access therapies through approved, reimbursable pathways for nusinersen and managed access programmes or clinical trials for the other disease-modifying agents. Decision making on the therapeutic avenue was complex and predicated on age, clinical status, and genotype, within an evolving therapeutic and regulatory landscape.

The comparator group included infants and children with SMA diagnosed following clinical referral with signs and symptoms of disease, presenting consecutively in the 2 years before the introduction of newborn screening for SMA (Aug 1, 2016–July 31, 2018). Without newborn screening, a diagnosis of SMA required clinical suspicion, appropriate referral, and investigations. The comparator group also had the same genetic mutation as those in the screening group. Genetic testing was only undertaken after symptom onset in this group, as an investigation based on clinical suspicion. Although the study commenced in 2016, children in the comparator group would have been born outside of this timepoint. Symptom onset is variable and many people with SMA experience a delay in diagnosis. The upper age limit of 16 years for the study population reflects the upper age for referrals to the statewide paediatric neuromuscular service based on current, local clinical diagnostic paradigm. Treatment for the comparator group was initiated through a managed access programme for nusinersen, and subsequently government subsidised reimbursement (Pharmaceutical Benefit Scheme), for this therapeutic agent. The clinical diagnostic route continues to be the main pathway for referral in SMA across Australia, outside the New South Wales and Australian Capital Territory SMA newborn screening programme.

The study was approved by the Sydney Children's Hospital Network Human Research Ethics Committee (HREC LNR/18/SCHN/307) and South-Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee. Written informed, voluntary consent for participation in the study was given by the parent or legal guardian, or young person, according to the principles set out in the Declaration of Helsinki.

See Online for appendix

Procedures and outcomes

Sociodemographic, clinical, and genetic (*SMN* genotype) data for study participants were collated from electronic medical records prospectively by authors DSK and AMD, from Aug 1, 2018, to Aug 1, 2020. For the screening group, children were dichotomised as newborn screening and pre-symptomatic, or newborn screening and symptomatic, determined by clinical and electrophysiological assessments at the time of implementation of individualised treatment plans. All children underwent careful neurological examination by experienced paediatric neurologists with expertise in SMA. A symptomatic status was defined by a constellation of SMA manifestations, including but not limited to hypotonia, areflexia, tongue fasciculations and swallowing or feeding problems.[10] Birthweight percentile was derived from the Maternal and Child Family Health record and denoted using Fenton's weight charts, aligning with gestational age and sex, for children within the screening group.

All outcome measures were assessed in survivors at 2 years post diagnosis by trained allied health professionals within the context of a multidisciplinary clinical assessment framework. The primary outcome was highest motor milestone attainment, measured using the WHO Multicentre Growth Reference Study (WHO-MRGS) criteria. The assessment includes six scaling items: (1) sitting without support, (2) hands and knees crawling, (3) standing with assistance, (4) walking with assistance, (5) standing alone, and (6) walking alone.[11] This is an observational assessment, evaluating a typical developmental hierarchy which assesses the quality of progression of motor skills. The lowest attainable item is (1) sitting without support and the highest attainable item is (6) walking alone.

Secondary outcomes were survival and change in ventilatory and feeding requirements; and changes in the

Hammersmith Infant Neurological Examination-2 (HINE-2) score, Functional Independence Measure for Children (WeeFim), and The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND).

HINE-2 is a standardised tool for assessing motor milestone achievement.[12] Scores range from 0 to 26, consisting of structured, developmentally appropriate items that assess incremental changes in head control, sitting, voluntary grasp, ability to grasp, ability to kick, rolling, crawling, standing, and walking. Higher scores indicate better motor function. Change in HINE-2 score from diagnosis to 2 years post diagnosis were collated to evaluate change in function.

For more on the **R Core Team** see https://www.R-project.org/

To measure maximal functional independence across self-care, mobility, and cognition, WeeFIM was recorded 2 years post diagnosis.[13] This tool monitors functional gains in children aged between 6 months and 7 years with disability. Comprised of 18 items, each is assessed against a 7-point ordinal scale, where the higher the score for an item, the more independently the patient performs the tasks. A composite score is generated from summation of the three domains.

CHOP-INTEND, a validated SMA-specific motor scale for weak infants or children younger than 2 years, was administered at diagnosis and used to provide a measure of baseline motor function.[14] The CHOP-INTEND has been used across all clinical trials independent of disease status as it remains the only scale that is sensitive enough to compare children at diagnosis who are young and weak. It was thus used in our study to reflect changes and identify the more severe phenotypes in both the comparator and screening groups.

Ventilatory status was categorised into those with and without need for non-invasive ventilation, for any proportion of a 24-h period, as determined by respiratory and sleep specialist input.

Bulbar dysfunction and the requirement for nutritional support in participants were concomitantly evaluated. The child's nutritional status and safety of swallow were assessed by the neuromuscular multidisciplinary team. Children were categorised into those able to feed orally and those requiring supplemental enteral tube feeding. The Oral and Swallowing Abilities Tool (OrSAT) was applied through retrospective analysis of patient records to provide an objective measure of bulbar function at 2 years from diagnosis.[15]

Compound muscle action potential (CMAP) was obtained from the abductor digiti mini (ADM) muscle by stimulating the ulnar nerve below the elbow for the screening group at the time of diagnosis, with a minimum of three G1 electrode positionings to ensure measurement of maximum amplitude.

Statistical analysis

Assuming equal variances, a minimum of 15 children in each group was required to achieve 80% power with a population effect size of 1·06 and a significance level of 0·050. Descriptive statistics were calculated for demographic, clinical, and genetic data using IBM Statistical Package for the Social Sciences, Statistics 25 software. For categorical variables, frequencies and percentages were reported and for continuous, normally distributed variables, medians and IQRs were reported.

χ^2 testing on highest motor milestone achieved (as per WHO-MRGS) at 2 years post diagnosis was completed to evaluate group differences. To understand group differences in HINE scores and WHO motor milestones (with each item being assigned 1 point), change scores were modelled in a multiple linear regression using R Core Team, with baseline value as covariate and group as predictor. Moderators of the group effect were also examined (age at diagnosis, age at treatment, CHOP-INTEND score at diagnosis). WeeFIM at 2 years post diagnosis was examined with an independent samples *t* test.

Change in binary outcomes (non-invasive ventilation and need for supplemental enteral tube feeding) was tested using McNemar's test, and odds ratios (ORs) were calculated. Unpaired *t* tests were completed to analyse mean differences in functional scores and electrophysiological measures between screening and comparator groups.

For the screening group, possible predictors of HINE scores and WHO motor milestones at follow-up were examined in simple linear regression models. Variables tested were birthweight centile, age at treatment, *SMN2* copy number, CHOP-INTEND, CMAP, and HINE at diagnosis.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Of 34 children who met the study inclusion criteria, 33 formed the study population with one neonate excluded due to participation in an unpublished ongoing clinical trial. Of the total cohort, 15 children formed the screening group, with 18 children included in the comparator group (table). In the screening group, seven (47%) children were male and eight (53%) were female and in the comparator group, nine (50%) children were male and nine (50%) were female. The screening group were significantly younger than the comparator group at diagnosis, therapeutic intervention, and, by extension, follow-up (table).

Age of treatment differed between the newborn screening and symptomatic subgroup compared with the newborn screening and pre-symptomatic subgroup due to differences in clinical pathways to access to disease-modifying therapy, dependent on disease status and *SMN2* copy number.

Within the screening group, six (40%) of 15 children were symptomatic within the first weeks of life (defined as newborn screening and symptomatic; appendix p 2). They presented with signs and symptoms of disease by a median of 2·9 (IQR 1·9–3·7) weeks, equivalent to 20 days after birth, with five (83%) of six having a two *SMN2* copy genotype and one (17%) of six having a three *SMN2* copy genotype. Within the screening group, CMAP amplitude was significantly lower in children with two *SMN2* copies (two *SMN2* copies, mean CMAP 2·2 [SD 1·5] *vs* three *SMN2* copies, mean CMAP 4·6 [1·2]; p=0·021).

For symptomatic children with newborn screening, CMAP and CHOP-INTEND score were significantly lower than newborns who remained pre-symptomatic at the time of diagnosis (newborn screening and symptomatic, mean CMAP 1·7 mV [SD 1·4] *vs* newborn screening and pre-symptomatic, mean CMAP 4·2 mV [1·2]; p=0·0023; newborn screening and symptomatic, mean CHOP-INTEND score 41·2 [SD 12·7] *vs* newborn screening and pre-symptomatic mean CHOP-INTEND score 57·8 [5·4]; p=0·0070). Children within the comparator group were symptomatic at a median age of 21·4 weeks (IQR 7·9–48·2), presenting with a range of motor milestones at baseline (table).

Of the study population three (9%) of 33 children, all with two *SMN2* copy numbers, went through a palliative pathway. One child in the screening group had a dual diagnosis of SMA and a severe blood disorder. Two children in the comparator group had profound quadriparesis and devastating comorbidities, with treatment burden and quality of life together informing shared decision making by caregivers and health professionals. In the screening group, 13 (87%) of 15 children underwent disease-modifying therapy following diagnosis. One child with four *SMN2* copies underwent active surveillance for phenoconversion (defined as the point at which an individual clinically manifests disease from a pre-symptomatic stage), based on shared decision making between the health-care professional and parents, continuing to be pre-symptomatic at age 2 years. In the comparator group, 16 (89%) of 18 children initiated and continued treatment.

At 2 years post diagnosis, 14 (93%) of 15 children in the screening group and 16 (89%) of 18 children in the comparator group were alive. Of the screening group, 11 (79%) of 14 children were walking independently or with assistance compared with one (6%) of 16 children in the comparator group (χ^2=16·27; p<0·0001). In this comparator group, at a median chronological age of 2·84 years, the highest motor milestone attained by most children was sitting ability, observed in nine (56%) children (figure 1).

Over 2 years, there were significant differences in the magnitude of motor milestone attainment between the screening and comparator groups. The screening group showed a significantly greater change in HINE-2 score (group difference, 12·3 [95% CI 9·5–16·2]) when adjusting for baseline value.

In the screening group, an early and steep rise in HINE-2 score over the first 6 months from diagnosis was observed, with an ongoing albeit slower trajectory of motor gains noted after the first 10 months from diagnosis. By contrast, the trajectory of motor acquisition in the comparator group gradually progressed over the 2 years following diagnosis (appendix p 3). At follow-up, children in the comparator group showed a lower mean HINE-2 score, but with a wider variability than observed in their younger counterparts in the screening group (mean HINE-2 score at 2 years post diagnosis in screening group, 23·0 [SD 4·2]; comparator group 15·1 [6·7]; p=0·0013).

The mean composite WeeFIM score at follow-up was higher in the screening group, despite these children being of a younger age than the comparator cohort (screening group, 70·1 [SD 23·1]; comparator group,

	Screening group (n=15)	Comparator group (n=18)	p value
Age, weeks			
Age at diagnosis	2·1 (1·9–2·7)	47·8 (13·0–99·9)	0·0003
Age at symptom onset*	2·9 (1·9–3·7)	21·4 (7·9–48·2)	0·0086
Age at 2-year follow-up	104·0 (64·0–115·0)	147·7 (105·8–178·4)	0·0052
Age at treatment	3·9 (2·7–5·1)	49·9 (123·9–145·6)	0·0015
Sex			
Male	7 (47%)	9 (50%)	..
Female	8 (53%)	9 (50%)	..
***SMN2* copy number**			
Two copies	9 (60%)	9 (50%)	..
Three copies	5 (33%)	8 (44%)	..
Four copies	1 (7%)	1 (6%)	..
Motor skill at diagnosis†			
Non-sitter	..	9 (50%)	..
Sitter	..	8 (44%)	..
Walker	..	1 (6%)	..
Access to disease-modifying interventions			
Yes	13 (87%)	16 (89%)	..
No	2 (13%)	2 (11%)	..
Management approach at diagnosis			
Nusinersen	8 (53%)	16 (89%)	..
Risdiplam	0	0	..
Onasemnogene abeparvovec	5 (33%)	0	..
Palliative care pathway	1 (7%)	2 (11%)	..
Active surveillance for phenoconversion	1 (7%)	NA	..
Scores at diagnosis			
HINE-2	2·0 (1·0–4·0)	4·0 (1·8–11·0)	0·0016
CHOP-INTEND	57·0 (42·0–60·5)	34·0 (30·0–47·0)	0·0056

Data are median (IQR) or n (%), unless otherwise specified. NA=Not applicable as all children were classified as symptomatic at diagnosis. HINE-2=Hammersmith Infant Neurological Examination-2 motor score. CHOP-INTEND=The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders. *Represents the median age of treatment for six (40%) of 15 symptomatic children in the screening group and all 18 symptomatic children in the comparator group. †As evaluated by the highest motor skill achieved against the WHO Multicentre Growth Reference Study, at diagnosis.

Table: **Clinical, genetic, and functional characteristics of the study population**

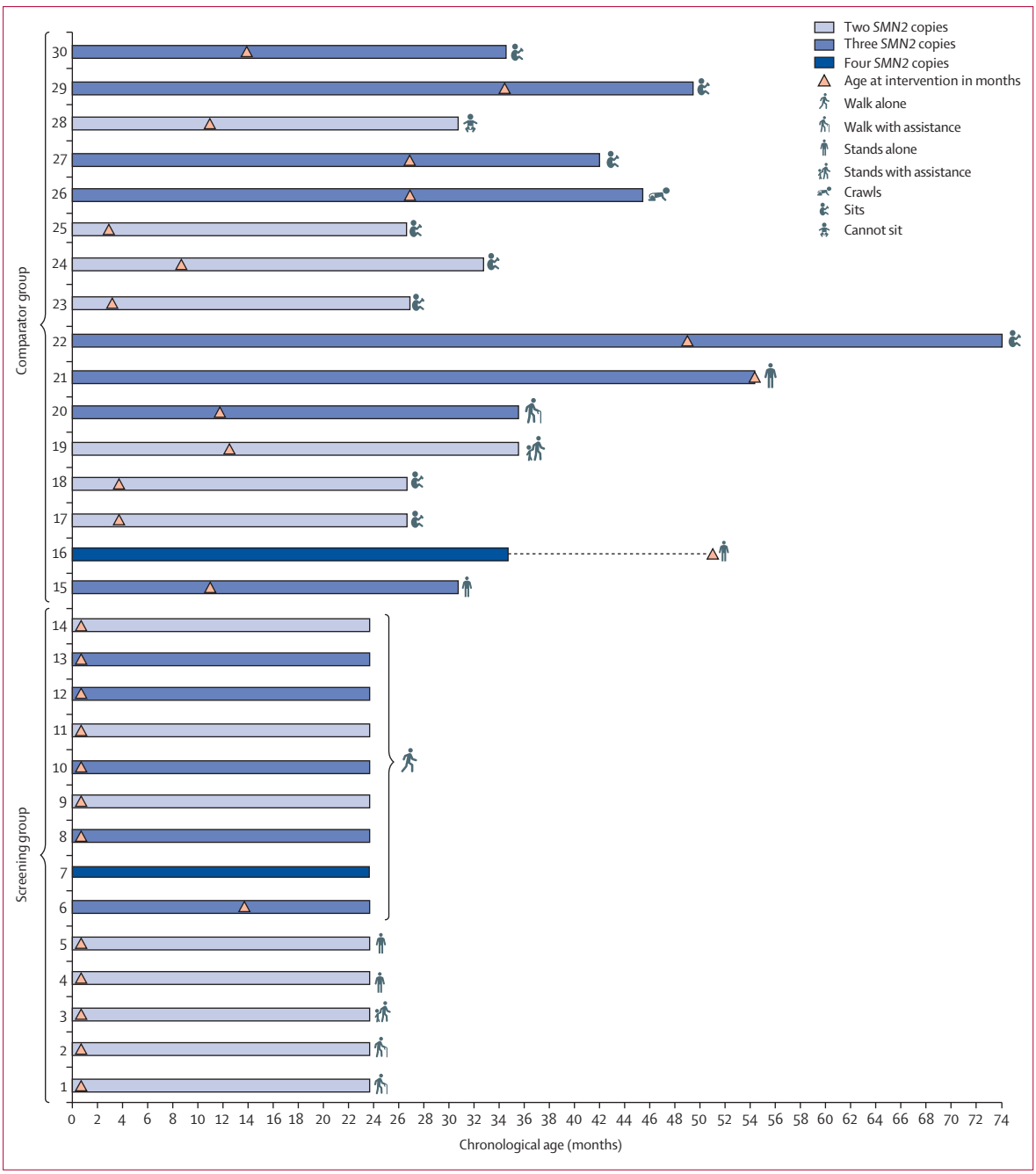

Figure 1: **Maximal WHO Multicentre Growth Reference Study motor milestone achieved at 2 years post diagnosis for each surviving child in the screening and comparator groups**

Age at intervention (triangle) and *SMN2* copy number are depicted for each participant. The dotted line for child 16 in the comparator group denotes treatment beyond the 2 year follow-up period based on shared decision making by caregivers.

60·6 [31·8]; p=0·38) with similar mean self-care skills between the groups (screening cohort, 21·4 [9·4]; comparator cohort, 25·1 [16·1]; p=0·44).

Newborn screening for SMA (represented by the variable of age at diagnosis) significantly changed the correlation effects of motor function gain between the screening and comparator groups. With increasing age at diagnosis, children in the screening group showed increases in HINE-2 scores (r=0·08, p=0·79) and WHO motor milestones (r=0·25, p=0·63). By contrast, negative correlation effects were noted with increasing age of diagnosis in the comparator group across both HINE-2 (r=–0·37, p=0·18) and WHO motor milestone measures (r=–0·22, p=0·41; figure 2).

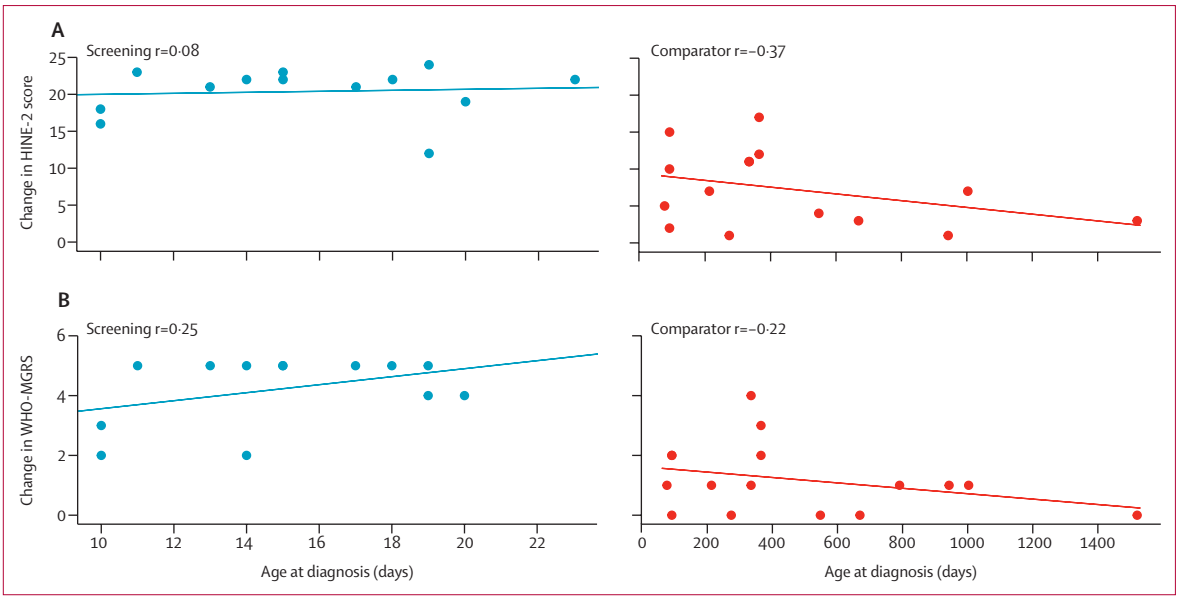

Figure 2: **Correlation effects of age at diagnosis (days) against change in functional motor score from diagnosis (baseline) to 2 years post diagnosis in the screening and comparator groups**

(A) Change in HINE-2 score. (B) Change in motor milestones using WHO-MGRS criteria. HINE-2=Hammersmith Infant Neurological Examination-2 score. WHO-MGRS=WHO Multicentre Growth Reference Study criteria.

Initiation of disease-modifying therapy following diagnosis of SMA after a positive newborn screening (represented by the age of treatment) was also an important and significant influencer of motor function, changing the correlation effects between the screening and comparator group. For children within the comparator group, an increasing age of treatment correlated with decreasing motor attainment in HINE-2 scores (r=–0·41, p=0·13) and WHO motor milestones (r=–0·17, p=0·54). In comparison, early treatment in the screening group was strongly and significantly associated with a positive change in motor function (HINE-2 score, r=0·74, p=0·009; WHO motor milestones, r=0·67, p=0·02; figure 3A). Age of treatment was particularly influential within the screening group, with newborn screening and symptomatic children showing smaller gains in motor function with delays in therapeutic intervention, even over a matter of days, than children in the newborn screening and pre-symptomatic group. (figure 3B). The statistical differences in HINE-2 score are noted between groups: newborn screening and symptomatic mean HINE-2 score, 17·0 (SD 3·7) versus newborn screening and pre-symptomatic mean HINE-2 score, 21·7 (1·9; p=0·020; figure 3).

Baseline motor function as determined by the CHOP-INTEND score at diagnosis, significantly influenced the level of motor attainment at 2 years post diagnosis. A higher score at diagnosis correlated with greater achievement of functional motor scores with time. This finding was replicated across both screening and comparator cohorts, with the strongest associations noted for children in the screening cohort (HINE-2, r=0·88, p<0·05) and WHO motor milestones (r=0·67, p=0·01; appendix p 4).

Non-invasive ventilation was required at baseline in one (7%) of 14 in the screening group and three (19%) of 16 in the comparator group. Although non-invasive ventilation requirements remained stable at 2-year follow-up in the screening group at one (7%) in 14, over this interval six (38%) of 16 children in the comparator group required ventilatory support (p=0·25). Furthermore, despite the proportion of children requiring supplemental feeding remaining stable in the screening group (baseline, one [7%] of 14; 2-year follow-up, one [7%] of 14), a higher proportion of children developed supplemental feeding requirements in the comparator group (baseline, two [13%] of 16; 2-year follow up, six [38%] of 16; p=0·13). For the the comparator group, four (67%) of six were classified as having severe impairment on the OrSAT score, necessitating full supplemental enteral feeding, while one (17%) of six required some form of supplemental feeding to sustain their nutritional input. The requirement for non-intensive ventilation or feeding support at follow-up was higher in the comparator group than in the screening group (OR 7·1 [95% CI 0·74–70·20]). All children who had evidence of either respiratory or bulbar comorbidities at diagnosis or over the 2-year follow-up period had a two *SMN2* genotype.

Significant predictors of functional motor outcomes as determined by HINE-2 score at 2 years post diagnosis were HINE-2 (p=0·0022), CHOP-INTEND (p=0·0001), CMAP (p=0·0006), and disease status (p=0·023) at diagnosis (appendix p 5). Age of treatment was a significant predictor of HINE-2 score and achievement of WHO motor

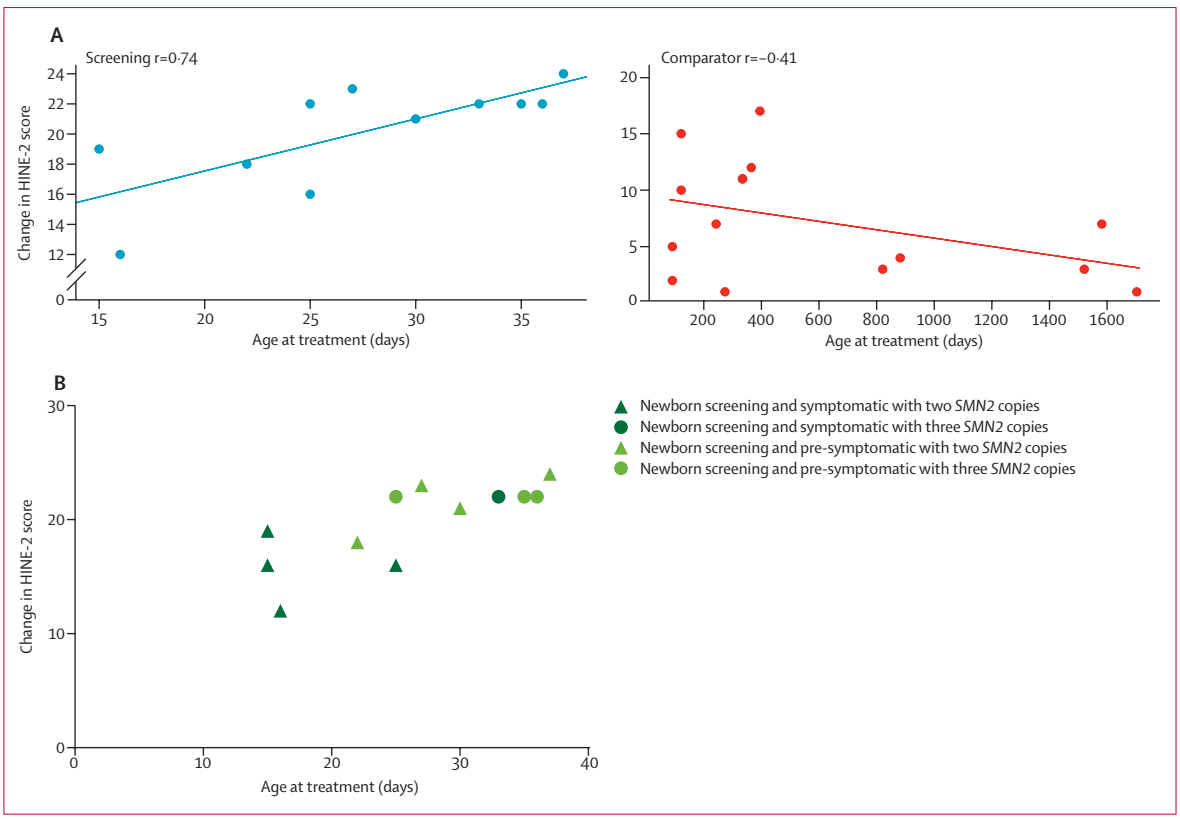

Figure 3: **Correlation effects of age at treatment against change in functional HINE-2 score from diagnosis (baseline) to 2 years post diagnosis in the screening and comparator groups (A) and scatter plots to illustrate how change in HINE-2 score at 2 years from diagnosis is stratified by disease status and genotype in the screening population (B)**

Only survivors and those accessing treatments at 2 years post diagnosis are represented. HINE-2=Hammersmith Infant Neurological Examination-2 score.

milestones at 2 years post diagnosis, while *SMN2* copy number was a significant predictor of HINE-2 score only. With a baseline CHOP-INTEND score of 48 or higher, HINE-2 score of 2 or higher, or a CMAP from the ADM of 2·5 mV or higher, there was a higher probability of achieving walking status at 2 years post diagnosis (figure 4).

Discussion

In this study, we compare an emerging paradigm of early diagnosis and prompt access to disease-modifying therapy for SMA against the traditional and widely prevalent symptom-based pathways of diagnostic confirmation and management on a broad range of clinical outcomes. We bridged the evidence gaps left by clinical trials that show efficacy of early intervention within narrow populations, defined by rigid inclusion criteria. Our study instead reflects the real-world impact of newborn screening for SMA in children within a spectrum of clinical, genetic, and sociodemographic factors, which will inform clinical practice and broaden health policy.

We showed that newborn screening for SMA ameliorates diagnostic and therapeutic delays and is clinically effective for most children across the genetic and phenotypic spectrum of the condition. Newborn screening for SMA reversed correlation effects of motor function when compared with children diagnosed through traditional pathways. The 12-point increase in HINE-2 score 2 years from diagnosis translated into significant functional differences between groups, with 79% of children achieving walking status in the screening group. By comparison, walking status was rarely achieved in those diagnosed through a traditional pathway, noted in only 6% of the cohort. For most of this group, the highest motor milestone achieved was the ability to sit. Scores for functional independence (forming part of the WeeFIM) assessment were comparable between groups, emphasising that those in the comparator group, despite being older, were as functionally dependent on their caregivers as the 2-year-old children in the newborn screening cohort.

The unequivocal gains in motor function for children diagnosed through a newborn screening pathway independent of treatment modality replicate improvements in neurodevelopmental outcomes from other international newborn screening for SMA pilot programmes,[16,17] which underpin the concept that early postnatal SMA diagnosis is essential to trigger emergency access to disease-modifying therapy.[18] Cumulatively, these studies highlight

the necessity for a rapid newborn screening pipeline, including configuring pathways for efficient diagnosis, treatment, and quality assurance. With the majority of children achieving walking status in the screening group, the sequelae of newborn screening for SMA feed directly into the perspectives of families affected by SMA, where functional autonomy and the ability to mobilise independently are highly valued by caregivers.[19] Although this overall trajectory of newborn screening for SMA effectiveness has been replicated across other real-world studies, the outcome measures used are different to the present study,[16] or collated retrospectively,[17] thus precluding direct comparisons between findings. This emphasises the need to develop consensus on an international toolkit of clinically relevant measures to evaluate health outcomes.

Baseline motor function has been noted in previous studies to be a predictor of survival in treated children with symptomatic SMA,[20,21] and a prognostic feature of improved motor function in pre-symptomatic clinical trials.[22,23] Our study determines that motor function at diagnosis is a significant predictor of motor function outcomes across the incident population and spectrum of children with SMA. If motor function is the clinical representation of the number and functional capacity of the underlying motor neuron pool,[24] these results give credence to the hypothesis that SMN repletion facilitates developmental recovery of the motor unit while also reducing neurodegeneration.[8] Our findings also determine that the evolution of comorbidities, including requirements for supportive ventilation and feeding, were reduced 7-fold by access to newborn screening for SMA pathway.

In our study, a significant proportion of children with two *SMN2* copies had symptoms of SMA following a positive newborn screening, highlighting the rapid neurodegeneration that can occur in utero and neonatally and emphasising the urgency to confirm diagnosis and initiate disease-modifying therapy in this genotype. Alongside clinical assessment, CMAP and CHOP-INTEND score at diagnosis were useful in differentiating symptomatic children from those who remained pre-symptomatic, as has been noted in other newborn screening for SMA studies.[17] In our newborn screening cohort, all children with a CMAP of more than 2 mV and CHOP-INTEND score of more than 47 were pre-symptomatic before therapeutic intervention.

Children with SMA, who were diagnosed through newborn screening and symptomatic at diagnosis had lower functional motor scores than those who were pre-symptomatic, but still significantly higher than children with long disease durations and no newborn screening. These findings emphasise that while having two copies of *SMN2* might represent a less than normative developmental trajectory for children diagnosed through newborn screening, intervening at the earliest opportunity improves motor function gains over the longer term,

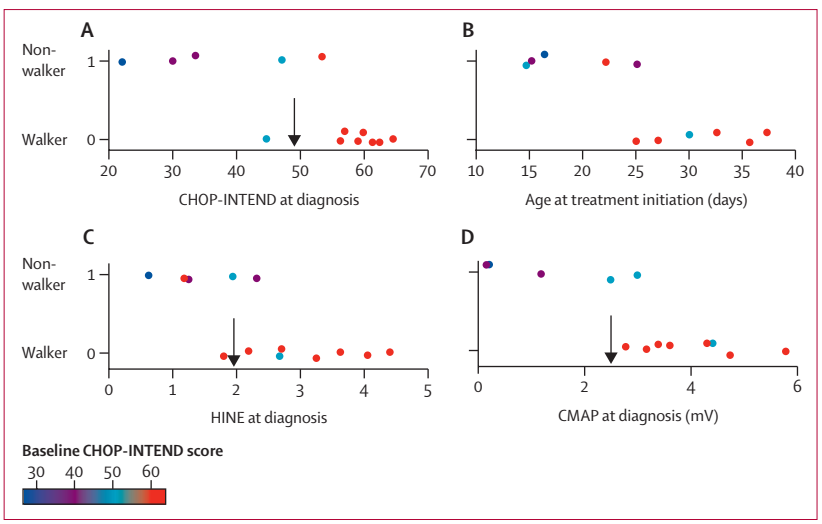

Figure 4: **Predictors of walking status at 2 years post diagnosis for children diagnosed with spinal muscular atrophy through newborn screening**
(A) CHOP-INTEND score at diagnosis. (B) Age of treatment. (C) HINE-2 score at diagnosis. (D) CMAP at diagnosis. The arrow represents an observational point where a higher probability of achieving walking status at 2 years post diagnosis is noted, based on segregation of values for each parameter between walker and non-walker status. CHOP-INTEND=The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders. CMAP=Compound muscle action potential. HINE-2=Hammersmith Infant Neurological Examination-2 score.

despite emergent motor neuron loss (or phenoconversion). In an evolving therapeutic landscape, these results could inform the evidence base for bridging therapeutics to expedite a state of rapid SMN repletion in children with two *SMN2* copies.

Although prognostication within clinical practice currently depends predominantly on *SMN2* copy number,[25] our study emphasises that a constellation of factors are relevant when predicting end phenotype in children diagnosed through newborn screening. We ascertained that for every 3-point increase in CHOP-INTEND score and 0·5 mV increase in CMAP at diagnosis, the final predicted HINE-2 score milestone would increase by 1 point. Children diagnosed through newborn screening for SMA with three or more *SMN2* copies showed on average a higher HINE-2 score than did those with two *SMN2* copies by 5 points.

Although clinicians value the HINE-2 score and WHO criteria as a measure of functional progress, for families, the ability to predict walking status remains one of the foremost issues, so that functional independence and social inclusion for their child can be preserved.[26] We observed that those who had a higher probability of walking by age 2 years had a CHOP-INTEND score of 48 or higher, HINE-2 score of 2 or higher, or a CMAP from the ADM of at least 2·5 mV; however, complete or near-complete separation precluded calculation of odds ratios. Taking these individual clinical predictors into account sets the foundation for an algorithm of care based on a child's unique clinical characteristics, paving the way for a more personalised approach to setting therapeutic expectations and informed decision making for families.[7]

Even with the high cost of these therapeutics, economic analyses suggests that newborn screening for SMA provides good value for money from the long-term economic perspective, especially for individuals with two and three copies of *SMN2*, providing a substantial economic impetus to individualise intervention.[27] The clinical variables identified might therefore inform and broaden policy and practice decisions regarding access to therapeutics for children with SMA who would most benefit from them.

Our findings emphasise the heterogeneity of presentation and the unmet needs of neonates who are positive for SMA via newborn screening, and symptomatic at the time of diagnosis. Optimising their outcomes might depend on the amalgamation of real-world data, designed with mutual endpoints and including patient-centred outcomes as a focus. Within clinical trials, there is an urgent need to broaden the stratification and selection of patients beyond that of *SMN2* copy number and age at diagnosis, with inclusion of modifying variables as seen in this study including CMAP, and motor and functional ability. Furthermore, placebo-controlled randomised trials might now be considered unethical given the substantial therapeutic effectiveness noted in children who underwent newborn screening and had access to disease-modifying therapy.

Health-care services will also need to anticipate and adapt to a realm of changing unmet needs for the incident population of SMA, as a new phenotypic spectrum is emerging. For children with symptomatic SMA and no newborn screening, the ability to tolerate prolonged sitting, while still having weakness, will change musculoskeletal dynamics, prompting a proactive approach to truncal support and strengthening.[28] Similarly, children treated symptomatically continue to have severe respiratory and bulbar health needs despite intervention, with a growing proportion evolving to require support in either one or both domains at 2 year follow-up. Ongoing surveillance and rehabilitation in these domains should thus be prioritised. For children diagnosed through newborn screening and symptomatic at time of diagnosis, unmet health needs continue, with multidisciplinary care and access to early intervention forming the backbone of management to optimise outcomes. By contrast, children treated pre-symptomatically through the newborn screening model had remarkable motor skill acquisition and a low burden of comorbidities. For these children, management might shift towards a focus on higher functional gross motor skills and monitoring of progress against a normative peer group. Although the non-randomised methodology used in this study could be prone to selection bias, this was mitigated by the chronological (non-selective) enrolment of children as they were referred into the state-wide tertiary neuromuscular service. The risk of observer bias, inherent to an unblinded study design, was mitigated by evaluation of outcome measures within a multidisciplinary framework. Of note, an age-matched comparator group was not possible, due to the inherent diagnostic delays associated with a clinically based diagnosis. This is important when we consider SMA as a disease that is unique in that neurodegeneration occurs through a period of neurodevelopment.[8] However, the results of the study appear even more profound, considering that despite children in the newborn screening group being chronologically younger, their developmental and functional motor gains far exceeded that of their older, clinically diagnosed counterparts.

The study was done amidst an evolving therapeutic and regulatory landscape and within a configuration of screening and clinical care services that was inherently designed to expedite and enable equitable access to diagnosis and treatment. Therefore, our findings might not be generalisable to jurisdictions where there are delays in these aspects of management, including biases in accessing appropriate health services and treatment, underpinning the need to develop global consensus guidelines for children to have equity of access to best practice. Although recent newborn screening programmes are beginning to report effectiveness, and limited comparisons are currently possible, our study design is the first to do so in such a rapidly changing landscape while also considering predictors of outcomes.

Although the study identified several individual factors that held clinical utility in predicting long-term health outcomes, future work will involve extending the evaluation of outcomes for more children and longer follow-up. This particularly pertains to children with three *SMN2* copies who encompass a phenotypic range as seen in our study. This includes severe phenotypes in 10% of the population,[6] highlighting the importance of clinical assessment and follow-up. Our study included one child in the screening group with four *SMN2* copies, making up 6% of our newborn screening for SMA cohort, comparable to other large epidemiological studies of population genotype and data from pilot newborn screening for SMA programmes.[6,17] This child underwent proactive surveillance and had not developed signs or symptoms of disease by the age of 2 years, replicating outcomes from other pilot newborn screening for SMA programmes where a so-called watchful waiting paradigm has been similarly adopted.[16] Differences in clinical practice pertaining to the need and timing of treatment for children with four or more *SMN2* copies necessitates a broader evidence base for outcomes within this genotype to facilitate standardisation of care.[16]

Variances in the *SMN2* genotype noted across studies might be attributable to epidemiological differences or techniques of genetic confirmatory testing, and population studies are required to interrogate this further. Future research includes investigation of structural variants within the *SMN* gene loci and evaluating neurofilaments as clinically useful prognostic

biomarkers, with substantive work warranted at a basic science level before effective clinical translation.

We are at an inflexion point in the history of SMA. Although rapid advances in therapeutics and genetic screening technologies have driven recommendations for pilot programmes and routine adoption of newborn screening for SMA in select jurisdictions, the implementation of this type of screening continues to lag, globally. As of 2021, only nine countries had implemented newborn screening for SMA, with fewer than 2% of newborns across the world screened for the condition.[29] This study has bridged the data gap, providing evidence for the effectiveness of screening for SMA and impetus for its wider dissemination.[30] On a broader scale, this study also determines the clinical variables at diagnosis that hold prognostic value to inform best practice, health-care provision, regulatory decisions on access to therapeutics, and clinical trial design. Optimisation of health outcomes are predicated on developing efficient newborn screening pathways that allow expedient access to disease-modifying therapies, particularly for those with two *SMN2* copies who are more likely to develop symptoms within the first weeks of life.

Contributors

DSK and MAF conceived and planned the study, and completed Ethics and Governance submissions. DSK, MAF, AMD, KH, and HS conducted the study. DSK, AMD, and NB analysed the data. DSK interpreted the data and drafted the initial manuscript. DSK, AMD, MAF, VW, KH, NB, and HS contributed to manuscript revisions. DSK, MAF, and AMD accessed and verified the data. All authors approved the final text. All authors confirm they had access to all the data in the manuscript and take responsibility for the decision to submit for publication.

Declaration of interests

MAF reports honoraria for scientific advisory boards from Novartis Gene Therapies, Biogen, and Roche; and research grants from Biogen. DSK and AMD report honoraria from Biogen and Novartis. All other authors declare no competing interests. Industry sponsors of disease-modifying treatment for spinal muscular atrophy include Biogen (nusinersen), Roche (risdiplam), and Novartis (onsamnogene aberparvovec).

Data sharing

The data central to this study are available to suitably qualified researchers through any reasonable requests. Data that underlie the results reported in this Article can be made available on request, after de-identification. Applicants willing to receive the data should apply between 1 and 12 months after the manuscript has been published in print and should demonstrate that the proposed use of the data has been approved by an independent review committee identified for this purpose. The data request should then be sent to the corresponding author, and de-identified data will be shared with a signed data access agreement.

Acknowledgments

This is an investigator-initiated study. The New South Wales (NSW) pilot newborn screening study was funded by Luminesce Alliance, a not-for-profit cooperative joint venture across the Sydney Children's Hospital Network, Children's Medical Research Institute, and Children's Cancer Institute, established with the support of the NSW Government. Luminesce Alliance is also affiliated with University of New South Wales, Sydney and The University of Sydney. DSK acknowledges funding from the Brain Foundation Grant 2022. MAF received grant support from the National Health and Medical Research Council of Australia: investigator grant (APP1194940). The co-authors acknowledge philanthropic giving from the "Move 4 Bella and Ride 4 Bella Challenge" to raise awareness and support for spinal muscular atrophy and rare diseases, which supported this work.

References

1 Zerres K, Rudnik-Schöneborn S. Natural history in proximal spinal muscular atrophy: clinical analysis of 445 patients and suggestions for a modification of existing classifications. *JAMA Neurol* 1995; **52:** 518–23.

2 Mercuri E, Pera MC, Scoto M, Finkel R, Muntoni F. Spinal muscular atrophy—insights and challenges in the treatment era. *Nat Reviews Neurol* 2020; **16:** 706–15.

3 Strauss KA, Farrar MA, Muntoni F, et al. Onasemnogene abeparvovec for presymptomatic infants with three copies of SMN2 at risk for spinal muscular atrophy: the Phase III SPR1NT trial. *Nat Med* 2022; **28:** 1390–97.

4 D'Silva AM, Kariyawasam DS, Best S, et al. Integrating newborn screening for spinal muscular atrophy into health care systems: an Australian pilot programme. *Dev Med Child Neurol* 2022; **64:** 625–32.

5 Kariyawasam DST, Russell JS, Wiley V, Alexander IE, Farrar MA. The implementation of newborn screening for spinal muscular atrophy: the Australian experience. *Genet Med* 2020; **22:** 557–65.

6 Calucho M, Bernal S, Alias L, et al. Correlation between SMA type and SMN2 copy number revisited: an analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. *Neuromuscul Disord* 2018; **28:** 208–15.

7 D'Silva A, Farrar MA. Personalised medicine for children with spinal muscular atrophy: towards the holy grail. *Muscle Nerve* 2020; **62:** 425–26.

8 Kariyawasam DST, D'Silva AM, Herbert K, et al. Axonal excitability changes in children with spinal muscular atrophy treated with nusinersen. *J Physiol* 2022; **600:** 95–109.

9 Kariyawasam D, D'Silva A, Howells J, et al. Motor unit changes in children with symptomatic spinal muscular atrophy treated with nusinersen. *Neurol Neurosurg Psychiatry* 2020; **92:** 78–85.

10 Tizzano EF. Treating neonatal spinal muscular atrophy: a 21st century success story? Early Hum Dev 2019; **138:** 104851.

11 Bhan MK, Norum K. The WHO multicentre growth reference study (MGRS): rationale, planning, and implementation. *Food Nutr Bull* 2004; **25** (suppl 1)**:** S15–26.

12 Bishop KM, Montes J, Finkel RS. Motor milestone assessment of infants with spinal muscular atrophy using the hammersmith infant neurological exam—part 2: experience from a nusinersen clinical study. *Muscle Nerve* 2018; **57:** 142–46.

13 Msall ME, DiGaudio K, Rogers BT, et al. The Functional Independence Measure for Children (WeeFIM) conceptual basis and pilot use in children with developmental disabilities. *Clinical Pediatr* 1994; **33:** 421–30.

14 Glanzman AM, Mazzone E, Main M, et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): test development and reliability. *Neuromuscul Disord* 2010; **20:** 155–61.

15 Berti B, Fanelli L, de Sanctis R, et al. Oral and Swallowing Abilities Tool (OrSAT) for type 1 SMA patients: development of a new module. *Neuromuscul Disord* 2021; **8:** 589–601.

16 Vill K, Schwartz O, Blaschek A, et al. Newborn screening for spinal muscular atrophy in Germany: clinical results after 2 years. *Orphanet J Rare Dis* 2021; **16:** 153.

17 Lee BH, Deng S, Chiriboga CA, et al. Newborn Screening for spinal muscular atrophy in New York State: clinical outcomes from the first 3 years. *Neurology* 2022; **99:** e1527–37.

18 Sumner CJ, Crawford TO. Early treatment is a lifeline for infants with SMA. *Nat Med* 2022; **28:** 1348–49.

19 Farrar MA, Carey KA, Paguinto S-G, Kasparian NA, De Abreu Lourenço R. "The whole game is changing and you've got hope": Australian perspectives on treatment decision making in spinal muscular atrophy. *Patient* 2020; **13:** 389–400.

20 Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *N Engl J Med* 2017; **377:** 1723–32.

21 Mercuri E, Darras BT, Chiriboga CA, et al. Nusinersen versus sham control in later-onset spinal muscular atrophy. *N Engl J Med* 2018; **378:** 625–35.

22 Darras BT, De Vivo DC, Farrar MA, et al. safety profile of nusinersen in presymptomatic and infantile-onset spinal muscular atrophy (SMA): interim results from the NURTURE and ENDEAR-SHINE studies (1659). *AAN Enterprises* 2020; **94** (suppl 15)**:** 1659.

23 Mendell JR, Al-Zaidy SA, Lehman KJ, et al. Five-year extension results of the phase 1 START trial of onasemnogene abeparvovec in spinal muscular atrophy. *JAMA Neurol* 2021; **78:** 834–41.

24 Bromberg MB, Brownell AA. Motor unit number estimation in the assessment of performance and function in motor neuron disease. *Phys Med Rehabil Clin N Am* 2008; **19:** 509–32.

25 Kirschner J, Butoianu N, Goemans N, et al. European ad-hoc consensus statement on gene replacement therapy for spinal muscular atrophy. *Eur J Paediatr Neurol* 2020; **28:** 38–43.

26 Farrar MA, Carey KA, Paguinto S-G, Chambers G, Kasparian NA. Financial, opportunity and psychosocial costs of spinal muscular atrophy: an exploratory qualitative analysis of Australian carer perspectives. *BMJ Open* 2018; **8:** e020907.

27 Shih ST, Farrar MA, Wiley V, Chambers G. Newborn screening for spinal muscular atrophy with disease-modifying therapies: a cost-effectiveness analysis. *Neurol Neurosurg Psychiatry* 2021; **92:** 1296–304.

28 Schorling DC, Pechmann A, Kirschner J. Advances in treatment of spinal muscular atrophy - new phenotypes, new challenges, new implications for care. *Neuromuscul Disord* 2020; **7:** 1–13.

29 Dangouloff T, Servais L. Clinical evidence supporting early treatment of patients with spinal muscular atrophy: current perspectives. *Ther Clin Risk Manag* 2019; **15:** 1153–61.

30 Dangouloff T, Vrščaj E, Servais L, Osredkar D. Newborn screening programs for spinal muscular atrophy worldwide: where we stand and where to go. *Neuromuscul Disord* 2021; **31:** 574–82.