UK National Screening Committee

Duchenne Muscular Dystrophy

An evidence map to outline the volume and type of evidence related to screening for Duchenne Muscular Dystrophy for the UK National Screening Committee

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The UK National Screening Committee secretariat is hosted by the Department of Health and Social Care.

About the UK National Screening Committee (UK NSC)

The UK NSC advises ministers and the NHS in the 4 UK countries about all aspects of population screening and supports implementation of screening programmes. Conditions are reviewed against evidence review criteria according to the UK NSC's evidence review process.

Read a complete list of UK NSC recommendations.

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Summary

This document discusses the findings of the evidence map on screening for Duchenne Muscular Dystrophy (DMD).

Evidence maps are a way of scanning published literature to look at the volume and type of evidence in relation to a specific topic. They inform whether the evidence is sufficient to commission a more sustained analysis on the topic under consideration.

Based on the findings of this evidence map, no further work on screening for DMD should be commissioned at the present time.

The UK National Screening Committee (UK NSC) will return to screening for DMD in 3 years' time.

Introduction and approach

Background and objectives

The UK National Screening Committee (UK NSC) external reviews (also known as evidence summaries or evidence reviews) are developed in keeping with the UK NSC evidence review process to ensure that each topic is addressed in the most appropriate and proportionate manner. Further information on the evidence review process can be accessed online.

Screening for DMD is a topic currently due for an updated external review.

DMD is a childhood form of muscular dystrophy, which primarily affects males at a rate of between 1 in 3,600 to 6,000 live male births.[1] DMD is caused by genetic mutations in the DMD gene, leading to the absence of or alteration in a protein called dystrophin that helps keep muscles working properly. Loss of dystrophin leads to chronic inflammation and muscle damage, resulting in deteriorating muscle strength, as well as circulatory and breathing complications. As a consequence, patients require complex care such as cardiac and respiratory management alongside physiotherapy, monitoring of bone health and wheelchair assistance.[1, 2] Ultimately, DMD leads to wheelchair dependence at adolescence and eventually death.[1, 3, 4] The life expectancy for patients with DMD has improved during recent decades, however, it is still poor, with a 2020 systematic review and meta-analysis of studies in Organisation for Economic Co-operation and Development (OECD) countries finding a median life expectancy between 21.0 and 39.6 years, provided patients received ventilatory support.[5] Similarly, a 2016 chart review of all deaths in the DMD population in North East England found that the mean age of death caused by underlying cardiac or respiratory failure was 23.9 years.[6]

Symptoms and diagnosis

The primary symptom of DMD is abnormal proximal muscle function, first presenting as delays in walking, a waddling gait, toe walking, difficulty in running or climbing, and frequent falling. Presence of the characteristic Gowers' sign (observed when arising from the ground) will usually trigger suspicions of DMD and initiate diagnostic investigations.[1] Whilst most children with DMD are diagnosed at around 5 years of age, the condition may be initially suspected earlier, due to the delays in developmental milestones — it has been reported that approximately half of children with DMD present with delayed motor milestones.[7] As the condition progresses, children with DMD may develop scoliosis.[1]

The specific path to a confirmed diagnosis of DMD can vary, but guidelines emphasise a timely diagnosis by a neuromuscular specialist as of utmost importance to the care

pathway.[1] A confirmed diagnosis of DMD typically requires blood sample analysis for a deletion or duplication mutation in the DMD gene, followed by sequencing to identify small insertions/deletions, point mutations and other rare mutations.[1, 8] A muscle biopsy may also be performed to evaluate the level of dystrophin protein expression (absence indicative of DMD), although it is not required if a genetic diagnosis has already been confirmed. Additional genetic testing is beneficial to allow for genetic counselling and to facilitate selection of future targeted treatment options.[1]

Treatment options

Current treatment options for DMD focus on treating the symptoms of DMD, including physiotherapy, supportive respiratory care and high-dose corticosteroids.[2] Physiotherapy is recommended as a continuous preventative therapy, helping to preserve muscle function, control pain and minimise contractures.[9] Patients who receive corticosteroids are able to walk for longer, require ventilatory support later in life, and have a lower incidence of cardiomyopathy.[10] Across 7 European countries in Eastern (Bulgaria, Czech Republic, Hungary and Poland) and Western Europe (Denmark, Germany and the UK), surveyed patients from the UK reported the highest levels of current or past steroid use (83.6%) and lowest use of professional physiotherapy (48.4%), although this was supplemented with the highest levels of instructions for at-home physiotherapy (70.6%).[9] Additionally, lower than expected receipt of guideline-recommended lung function testing was identified in both ambulatory (62.8%) and non-ambulatory patients (30.5%) across all surveyed countries. Multidisciplinary care involving regular lung function testing is important to allow for timely use of assisted breathing (such as lung volume recruitment, assisted coughing, nocturnally assisted or daytime ventilation), if required.[2]

For children aged 2 years and over with a nonsense mutation in the dystrophin gene (which is the cause of approximately 10–15% of cases)[11] who can walk 10 steps unaided, a further treatment option is ataluren (Translarna™). Ataluren has been approved by the National Institute for Health and Care Excellence (NICE) since 2016 under a managed access agreement. Re-evaluation of the NICE decision will occur when the managed access agreement ends (extended to January 2023 from July 2021 due to COVID-19).[10] While not beneficial to all children with DMD, the latest data analysing the efficacy of ataluren showed that the drug delays loss of ambulation by up to 5 years in boys and slows decline in pulmonary function compared to matched control groups receiving the current standard of care.[12, 13]

There are also a number of therapies in the drug development pipeline for DMD. These include treatments that restore or replace dystrophin, such as novel gene-based therapies; exon skipping therapies; utrophin modulators; and secondary therapies that target DMD symptoms, including myostatin inhibitors to reduce inhibition of muscle growth; stem cells for producing healthy muscle fibre and reducing inflammation; repurposed drugs (tamoxifen, rimeporide, and metformin); alternatives to existing

steroids (aiming to minimise side-effects) and nutraceuticals.[14, 15] In the United States, multiple exon skipping drugs have been approved, targeting exons of the dystrophy gene. These include eteplirsen (EXONDYS 51),[16] golodirsen (VYONDYS 53),[17] viltolarsen (VILTEPSO™)[18] and casimersen (AMONDYS 45).[19] Confirmatory trials for these therapies are ongoing, and are expected to provide evidence in support of marketing authorisation applications in the UK and European Union. As such, several novel treatments for DMD may be available in the near future. Key ongoing trials are summarised in Table 1.

Table 1. Key current clinical studies in DMD

Company	Trial name	Therapy	Phase	Expected study completion	Participant criteria
NS Pharma, Inc.	RACER53 (NCT04060199)	Viltolarsen	III	December 2024	Males 4−7 years Ambulant Mutations amenable to exon 53 skipping
Pfizer	CIFFREO (NCT04281485)	PF-06939926	III	January 2028	Males 4−7 years Ambulant
Italfarmaco	EPIDYS (NCT02851797)	Givinostat	III	March 2022	Males 6−17 years Ambulant
Italfarmaco	GIVINOSTAT extension (NCT03373968)	Givinostat	II/III	December 2023	Males 7+ years Ambulant or non-ambulant
Sarepta Therapeutics, Inc.	MOMENTUM (NCT04004065)	SRP-5051	II	May 2022	Males 4−21 years Ambulant or non-ambulant Mutations amenable to exon 51 skipping
Sarepta Therapeutics, Inc.	NCT03532542	Casimersen or Golodirsen	III	August 2026	Males 7−23 Ambulant or non-ambulant Exon 45 or 53 mutations
ReveraGen BioPharma, Inc.	VISION-DMD (NCT03439670)	Vamorolone	IIb	August 2021	Males 4−7 Ambulant
PTC Therapeutics	Registry of Translarna (NCT02369731)	Ataluren	IV	May 2025	Males 2+ years Ambulant or non-ambulant Nonsense mutation

Benefits of early diagnosis

An early diagnosis and earlier initiation of treatment benefits the duration and quality of life,[2] with evidence that boys who are treated earlier with corticosteroids show better motor function acquisition and maintaining ambulation for longer.[20-22] Furthermore, an earlier diagnosis may allow for informed family planning decisions (which is particularly relevant given that approximately 1/5 families in an Australian survey had more than one child living with Duchenne or Becker muscular dystrophy),[23] time to move to an adaptable/adapted house and participation in potential clinical trials with novel investigational agents.[24] However, earlier diagnosis may also have disadvantages. For example, some parents of children diagnosed in screening programmes have reported

feeling that they are robbed of the “blissful unknown” and that they bonded differently to their child, without seeing benefits of the earlier diagnosis. These issues may be compounded if genetic counselling is not available to support testing programmes or communication to families about initial screening results is managed poorly.[25]

A further benefit of early diagnosis is a corresponding earlier treatment with interventions such as ataluren (Translarna™), which is only available to ambulant patients. While there is currently no evidence showing additional benefit of starting treatment with ataluren earlier, it is generally accepted that muscle wasting commences before symptoms present and that early treatment with agents that restore dystrophin may be beneficial. There are delays of up to 2 years between the appearance of first symptoms and diagnosis, with a case note review for boys who were diagnosed at the MRC Centre for neuromuscular diseases in Newcastle finding that while symptoms first appeared at a mean age of 2.7 years, the mean age at diagnosis was 4.3 years.[24] Similarly, data collected from the international Duchenne Registry from 2007 to 2019 showed that the mean age at diagnosis was 4.43 years,[26] and an Australian study surveying parents found that while the median age of first symptoms was 2 years 9 months, the median age at diagnosis was 3 years 9 months.[27] Since ataluren is only available to ambulant patients in the UK, a 2 year diagnostic delay potentially represents a significant reduction in the time during which patients can receive the drug.[10]
As such, there is considerable interest in screening for increased DMD risk in newborns, as this would allow earlier diagnosis and intervention.[8]

Screening for DMD

While diagnosis of DMD is usually only possible via genetic testing, other markers can be used to determine if a newborn is at an increased risk for DMD. This can then be followed by confirmatory genetic testing. Male infants with DMD have elevated serum levels of creatine kinase (CK-MM), a biomarker of membrane fragility and muscle degeneration. Increased CK is a secondary marker for the dystrophic process and may represent the most frequent finding leading to a suspicion of DMD.

The CK test can lead to both false-positive and false-negative results. The former is often due to birth trauma or other muscular dystrophies, while the latter may be due to the insensitivity of the analytical tests. To overcome false positives, there is a need for confirmation of the results by a second CK test. A two-tiered approach of CK screening followed by DNA testing could overcome this, and is proposed as the test of choice to screen for and diagnose DMD.[20, 28]

Dried Blood Spots test for CK in newborn male infants via the heel prick test is being considered as a screening test for DMD. Newborn dried blood spot screening via the heel prick test is already established in clinical practice in the UK, with nine conditions being screened for at present. This means that screening for DMD could be incorporated into the newborn screening (NBS) programme in England.[29, 30] However, it is unclear

whether dried blood spot screening for DMD is sufficiently accurate for affected children to benefit from a national programme (see below).

Despite NBS programmes being well established in many countries, with countries such as Italy screening for more than 40 diseases in some regions,[4] no national screening programmes for DMD have been established so far.[4, 31]

There are some ongoing pilot programmes (Table 2), such as an NBS programme in New York State, which was first launched in October 2019 and had screened nearly 14,000 newborns by the end of August 2020.[32] [32] Fourteen newborns were referred for follow-up testing, due to having elevated levels of the muscle isoform of creatine kinase (CK-MM).[32] Another recent NBS pilot programme has been conducted in the Zhejiang province in China since 2015, where over 40,000 newborns have been screened for elevated CK-MM levels indicative of DMD, leading to 11 confirmed DMD diagnoses by February 2019.[33]

A further pilot programme in Italy, funded by the pharmaceutical company PTC Therapeutics as part of their Innovative Research Funding programme, PRIORITY, has been ongoing since 2019 in the Italian provinces of Messina and Catania, aiming to screen 30,000 male infants over a period of 1.5 years, between the ages of 6 and 42 months.[4]
Over the last 45 years, other newborn screening programmes and pilot studies for DMD, which have since ended, have been conducted in multiple countries, including New Zealand,[34] Germany,[35] Canada,[36] France,[37] Belgium,[38] Cyprus,[39] Scotland, [40] Wales[41], the United States[42] and Australia.[43] A summary of the screening programmes is presented in Table 2.

The longest-running pilot study for DMD in the UK took place in Wales over a period of 21 years between 1990 and 2011, where over the course of the programme over 300,000 male infants were tested for DMD by measuring CK levels in dried blood spots. Out of all tested infants, 145 had elevated CK levels, 66 of which continued to have elevated levels at a follow-up at 6 to 8 weeks of age. Ultimately, 56 boys were diagnosed with DMD following bloodspot CK analysis (confirmed by DMD genotyping/muscle biopsy studies) and 13 false-negative cases were identified as of 2013. Despite a high screening uptake, the screening programme was terminated in December 2011 due to the external quality assurance programme being withdrawn, leading to the UK Clinical Pathology Accreditation Service being unable to accredit the testing service.[41] No screening programme for DMD in the UK has been established since.[31]

An appropriate threshold for the level of CK that indicates DMD risk has not been consistently defined. Studies conducted in maternity hospitals in Columbus and Cincinnati (Ohio, US) have recommended the following procedure:[42]

1. Dried Blood Spot testing for CK in newborn males
    a. Second test of venous blood in newborn males with CK ≥600 U/L
    b. DNA testing for DBS in newborn males with CK ≥750 U/L

However, further studies are needed to confirm these findings.

Table 2. Summary of pilot DMD screening programmes in different countries

Country	Programme or region	Dates	Population	Screening protocol	Notable findings
Ongoing screening programmes					
United States	New York State Pilot programme[32, 44] Similar methodology to the Ohio pilot study[45]	2019 to present	14,000 newborns in first year Aiming to screen 100,000 overall	**Index test** DBS CK-analysis using the GSP® Neonatal CK-MM kit	14 screen-positive cases were identified in the first year of the pilot and 2 were confirmed to have Duchenne/Becker muscular dystrophy. Further details were not reported
China	NBS DMD Pilot programme in Hangzhou, Zhejiang[33, 46]	2015 to present	42,862 newborns	**Index test** Blood samples were drawn at 3 to 7 days after birth and testing for elevated levels of CK-MM, with a cut-off of 700 ng/mL	11 cases of DMD had been diagnosed by 2019. An application has been submitted to the Chinese Ministry of Health to extend DMD screening to the whole country
Italy	Pilot programme in Catania and Messina[4]	2019 to present	Aim: 30,000 male newborns	**Index test** Blood samples collected between 6 months and 42 months of age are screened using LC-MS. Samples with CK levels between 250 U/L and 1,000 U/L will be re-tested Screen-positive cut-off: CK ≥1,000 U/L **Reference standard** DNA testing	NR
Past screening programmes					
Belgium	Antwerp[38]	1979 to 2003	281,214 male newborns	**Index test** DBS collected at day 5 of life were screened for elevated CK levels Detection limit: 150 U/L Screen-positive threshold: 500 U/L All screen-positive results are re-screened at 4 to 6 weeks of life **Reference standard** Muscle biopsy and/or genetic testing	False positive: 0.02% Positive predictive value: 54.8% Negative predictive value: 99.99%

Country	Programme or region	Dates	Population	Screening protocol	Notable findings
Canada	Manitoba, Canada[20, 36]	1986 to 2007	172,860 male newborns	Voluntary "opt-out" pilot **Index test** CK-levels from blood spots	18 boys with DMD were identified The programme was withdrawn in 2007 due to insufficient funds
Australia	New South Wales[43]	2013 to NR	5,661 newborn males and 5,445 females (Cohort 1); 82 newborn males and 61 newborn females (Cohort 2) 65 newborn males and 56 newborn females (Cohort 3)	**Index test** Samples were collected 48 to 72 hours after birth (Cohort 1), 6 to 7 days after birth (Cohort 2) or 6 to 12 weeks after birth (Cohort 3) and screened for elevated CK levels **Reference standard** Mutational analysis of DMD gene	This study was designed to identify the best practice for DMD NBS in Australia Goals of the programme included to determine the feasibility of using DBS to screen CK-MM levels as a biomarker for DMD, as well as to establish a "normal" level of CK-MM in newborns as compared to newborns with DMD CK levels were found to decrease with the age of newborns, however no results in relation to diagnostic accuracy of the screening have been identified
Cyprus	Pilot programme[39]	1992 to NR	30,014 newborns (1992 to 1996)	**Index test** DBS were obtained and screened for CK levels using the bioluminescence method **Reference standard** DNA analysis or dystrophin analysis	Screen-positive cases: 43 5 boys were diagnosed with DMD or BMD False-positive rate:0.10%
Germany	Private DMD screening programme[20, 35]	1974 to 2011	537,000 boys	**Index test** Blood samples were obtained 4 to 6 weeks after birth and screened using a luciferase test Screen-positive threshold: 200 U/L CK **Reference standard** Genetic testing	False positive rate (1983/1984): 0.016% for 300 U/L cut-off and 0.061% for 180 U/L cut-off 155 boys were diagnosed with DMD and 35 with BMD
New Zealand	Auckland and Northland area[34]	1979	10,000 newborn males	**Index test** Creatine phosphokinase levels were measured on using blood samples taken on days 1 and 4 of life Screen-positive threshold ≥20 SDs above batch mean **Reference standard**	2 cases of DMD were diagnosed

Country	Programme or region	Dates	Population	Screening protocol	Notable findings
				Muscle biopsy	
Scotland	Eastern General Hospital, Edinburgh[40]	1976 to 1980	2,703 newborns (2336 male, 367 female)	**Index test** DBS were collected as part of the routine test for PKU on day 5 of life and CK levels were screened using a luciferase assay Specimens were re-assayed if CK levels were ≥400 U/L	False positive rate: 0.78% 16 newborns required a second test due to high CK levels No false-positive were found following the introduction of more sensitive reagents in mid-1979
United States	Cincinnati and Columbus, Ohio[42]	2007 to 2011	37,649 male newborns	**Index test** DBS were used to measure CK levels using a fluorometric assay **Reference standard** DNA analysis	False positive rate: 600 U/L cut-off: 1.6% 750 U/L cut-off: 0.52% DMD mutations were found in 6 boys, all had CK levels ≥2000 U/L
Wales	Wales Newborn Bloodspot DMD Screening[41]	1990 to 2011	343,170 male infants	"Opt-in" screening programme **Index test** DBS CK enzyme activity analysis; bloodspots were collected between days 5 and 8 of life Screen-positive threshold ≥250 U/L **Reference standard** Elevated serum CK and genotyping/muscle biopsy	False-positive rate: 0.023% False-negative rate: NR, but 13 false-negative cases were identified as of 2013 The screening programme was terminated in December 2011 due to withdrawal of the external quality assurance programme. The external quality assurance programme was withdrawn because of a lack of sufficient participants to support a viable scheme

Abbreviations: BMD, Becker muscular dystrophy; CK, creatinine kinase; CK-MM, creatine kinase muscle isoform; CPK, creatinine phosphokinase; DBS, dried blood spot; DMD, Duchenne muscular dystrophy; LC-MS, liquid chromatography – mass spectrometry; PKU, phenylketonuria; NR, not reported; SD, standard deviation

Previous review on screening for DMD

The UK NSC currently recommends against screening for DMD. The Committee based this recommendation on the evidence provided by the 2016 review carried out by Bazian Ltd.[47] The 2016 review aimed to identify evidence on whether there is a reliable, high throughput screening strategy; any additional benefits from early treatment following screen detection or an optimum age for treatment initiation; and demonstration of wider effects or benefits from screening for DMD, such as on reproductive choices. Insufficient evidence to recommend the introduction of a systemic neonatal population screening programme for DMD was found by the 2016 review, specifically:[47]

- there was insufficient high quality evidence of a suitable population screening test in newborns, or a reliable and appropriate screening strategy, based on evidence from 3 studies. One study was a published report of the Welsh DMD screening programme with data collected between 1990 and 2011 using CK enzyme activity screening. The test was reported to have a poor performance with a sensitivity of 81.6% and a high false negative rate of 18.4%.[41] One study was a pilot screening programme in Ohio, USA, which used a 2-tiered approach of CK screening followed by DNA testing. However, diagnosis was only confirmed for screen-positive samples and not screen-negative samples, meaning that the true number of false negatives and specificity, sensitivity, positive predictive value and negative predictive value could not be determined.[42] The third study evaluated the performance of muscle-specific micro-RNAs to differentiate between cases of DMD and controls, but was not performed in a population that was generalisable to newborn screening.[48]
- there was a lack of evidence for any additional benefit for early treatment when newborns with DMD are identified during screening. While 17 studies assessing the impact of treatment on symptoms or function in DMD were identified, no studies that assessed outcomes of treatment after screen-detection of DMD were found. All of the included trials were in males aged 4–38, therefore none of the treatments were started in the newborn period, which is when those identified by screening would be able to start treatment.
- there was a lack of evidence to demonstrate wider effects or benefits from screening for DMD, such as on reproductive choices. No comparative studies or systematic reviews assessing wider benefits of screening for DMD, such as allowing the newborn's parents to make informed reproductive choices, were identified.

Aims of the evidence map

Evidence maps are rapid evidence products which aim to gauge the volume and type of evidence relating to a specific topic.

This evidence map has been developed to assess whether a more sustained review on screening for DMD should be commissioned at this time, and to evaluate the volume and type of evidence on key issues related to screening for DMD.

The aim was to address the following question:

> **Q1:** What is the volume and type of evidence on suitable screening tests using dried blood spots to detect DMD?

This evidence map will focus on studies reporting outcomes relating to the diagnostic accuracy of dried blood spot screening for DMD compared to a reference standard of genetic analysis of the DMD gene.

The findings of this evidence map will provide the basis for discussion to support decision making on whether there is sufficient evidence to justify commissioning a more sustained review of the evidence on DMD.

The aim of this document is to present the information necessary for the UK NSC to decide this.

Search methods and results

The searches were conducted on 20 April 2021 in 3 databases: MEDLINE, Embase and the Cochrane Library. The search period was restricted to 1 January 2015 to 20 April 2021. MEDLINE (including MEDLINE In-Process, MEDLINE Daily and Epub Ahead of Print) and Embase were searched simultaneously via the Ovid SP platform. The Cochrane Library databases (Cochrane Database of Systematic Reviews and Cochrane Central Register of Controlled Trials) were searched via the Wiley Online platform.

The detailed search strategies, including exclusion and inclusion criteria are available in Appendix 1. One reviewer screened all titles and abstracts. All references were reviewed at abstract level, though in some cases full texts were reviewed to clarify uncertain pieces of information. A formal quality appraisal of the evidence was not required, given the remit of the evidence map.

The search returned 906 results across Medline, Embase and the Cochrane Library databases. After automatic and manual de-duplication, 886 unique references were assessed for relevance to the review question. Six studies were deemed potentially eligible for inclusion and the full texts were reviewed to ascertain their relevance. Of the 6 studies checked, 5 were excluded. Ultimately, only one reference was included in the evidence map. A flow diagram summarising the number of studies included and excluded is presented in Figure 1. The abstract reporting table is available in **SOURCES SEARCHED**: Ovid MEDLINE® In-Process & Other Non-Indexed Citations, Daily and Epub Ahead of Print, Ovid MEDLINE® and Versions 1946 to 19 April 2021, Embase® 1974 to 19 April 2021, and the Cochrane Library (Cochrane Database of Systematic Reviews and Protocols, Issue 4 of 12, April 2021; Cochrane Trials, Issue 3 of 12, March 2021)

DATES OF SEARCH: 1 January 2015 to 20 April 2021 for all databases. Searches were run on 20 April 2021.

SEARCH STRATEGIES:

MEDLINE and Embase (searched simultaneously via the Ovid SP platform)
1. Muscular Dystrophy, Duchenne/ or Duchenne muscular dystrophy/ 2. (Duchenne or DMD or pseudohypertrophic progressive or muscular dystrophy).ti,ab,kw,kf. 3. 1 or 2 4. Infant, Newborn/ 5. (newborn$ or neonatal$ or infant$).ti,ab,kw,kf. 6. 4 or 5 7. 3 and 6 8. dried blood spot testing/ 9. dried blood spot.ti,ab,kw,kf. 10. (detect$ or predict$ or identif$ or diagnos$ or test$).ti.

11. mass screening/ or screen.ab. /freq=3
12. or/8-11
13. 3 and 12
14. 7 or 13
15. ("Conference Abstract" or "Conference Review" or comment or editorial or note or case reports or news or news release).pt.
16. (case stud$ or case report$).ti,ab.
17. historical article/ or case study/
18. exp animals/ not exp humans/
19. or/15-18
20. 14 not 19
21. limit 20 to yr="2015-current"
22. remove duplicates from 21

Cochrane Library (searched via the Wiley Online platform)

1. [mh ^"Muscular Dystrophy, Duchenne"]
2. (Duchenne or DMD or "pseudohypertrophic progressive" or "muscular dystrophy"):ti,ab,kw
3. #1 or #2
4. [mh ^"Infant, Newborn"]
5. (newborn* or neonatal* or infant*):ti,ab,kw
6. 4 or #5
7. #3 and #6
8. [mh ^"dried blood spot testing"]
9. "dried blood spot":ti,ab,kw
10. (detect* or predict* or identif* or diagnos* or test*):ti
11. [mh ^"mass screening"] or screen:ab
12. {or #8-#11}
13. #3 and #12
14. #7 or #13
15. #14 with Cochrane Library publication date Between Mar 2015 and May 2021, in Cochrane Reviews, Cochrane Protocols
16. #14 with Cochrane Library publication date Between Mar 2015 and May 2021, in Trials

Results by database

MEDLINE and Embase	854
Cochrane Library	52
Total	906

Inclusions and exclusions

Studies were included based on the eligibility criteria listed in Table 3.

Table 3: Eligibility criteria for the review question

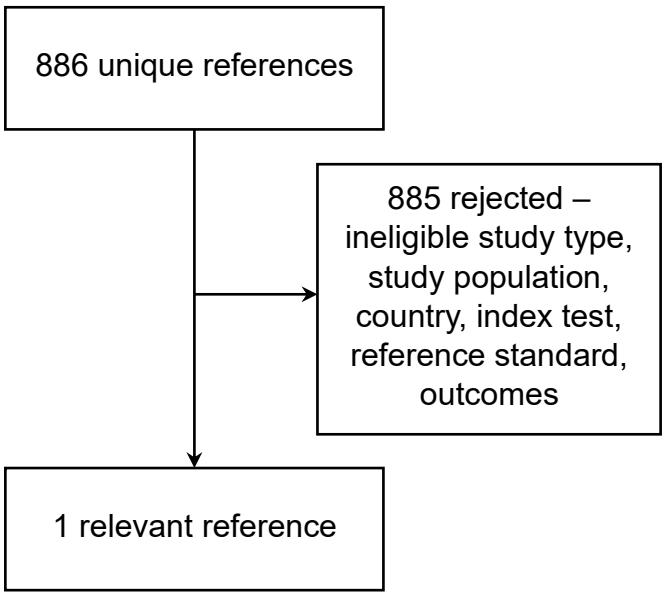
PICOS domain	Inclusion Criteria	Exclusion Criteria
Patient population	Newborns, defined as <12 months of age	• Children who are not newborns • Adults
Intervention	**Index test:** • Any tests used to detect DMD using dried blood spots **Reference standard:** • Mutation analysis of DMD gene	**Index test:** • Any other index test **Reference standard:** • N/A
Comparator	• Any or none	• N/A
Outcomes	Outcomes relating to diagnostic accuracy, including but not limited to: • Sensitivity • Specificity • PPV • NPV • LR • AUC	Outcomes not relevant to diagnostic accuracy
Study design	**Tier 1:** • RCTs • Non-randomised studies with consecutively enrolled populations (e.g. prospective and retrospective cohort studies) • SLR/(N)MAs of these study designs **Tier 2:** • Case-control studies • Cross-sectional studies • Case series • SLR/(N)MAs of these study designs	Any other study design, including: • Case reports • Narrative reviews • Editorials • Commentaries • Conference abstracts • Other publication types that have not been peer-reviewed

PICOS domain	Inclusion Criteria	Exclusion Criteria
Setting	**Tier 1:** • Studies conducted in the UK **Tier 2:** • Studies conducted in high-income countries where the population, screening methods and technology are expected to be similar to that of the UK (OECD and EEA countries excluding South Korea and Mexico)	• Studies in ineligible countries, or international studies where outcomes for eligible countries are not presented separately to outcomes from ineligible countries
Other considerations	• Articles published in the English language • Articles published since March 2015	• Studies with abstract not in the English language • Articles published before March 2015

Abbreviations: AUC, area under the curve; DMD, Duchenne muscular dystrophy; EEA, European Economic Area; LR, likelihood ratio; N/A, not applicable; (N)MA, (network) meta-analysis; NPV, negative predictive value; OECD, Organisation for Economic Co-ordination and Development; PPV, positive predictive value; RCT, randomised controlled trial; SLR, systematic literature review

Appendix 2.

Figure 1. Summary of included and excluded publications



Summary of findings

Question 1: What is the volume and type of evidence on suitable screening tests using dried blood spots to detect DMD?

One study was identified as relevant to the evaluation of the suitability of screening tests using dried blood spots for the detection of DMD. Timonen *et al.* (2019) was a retrospective study that investigated the value of a novel CK-MM immunoassay (GSP® Neonatal CK-MM kit) for the diagnosis of DMD from dried blood spots, and compared this with CK enzyme activity determination by fluorescence measurement.[49] The analyses were conducted on dried blood spot specimens that had been stored for up to 15 years prior to the study. The specimens came from two different populations, one from the US (the California Biobank Program [analysed in Finland]; n=719) and one from Denmark (the Danish Neonatal Screening Biobank [analysed in Denmark]; n=1,424).

The key outcomes of relevance to the evidence map question related to (a) the evaluation of an appropriate cut-off threshold for CK-MM concentration to be used for the categorisation of samples as screen-positive or -negative and (b) the diagnostic accuracy of these threshold values. The reference standard used to evaluate the diagnostic accuracy of the test was confirmation of DMD diagnosis by treating physicians and by molecular genetic testing in the newborn from whom the specimen was taken. Using samples from the US population, the study also compared the results from the CK-MM immunoassay to those gained from fluorescence measurement of CK enzyme activity and investigated the long-term stability of CK-MM in specimens. The impact of gestational age and the age of the newborn at sampling on CK-MM concentrations was investigated in both populations.

For the Danish population, the overall percent agreement between confirmed DMD diagnosis and the CK-MM assay at a 99.5th percentile cut-off value (675 ng/mL) was reported at 99.6% (95% confidence interval [CI] 99.2 to 99.9%). The total number of false positive, true positive, false negative and true negative results were also reported, at 4, 15, 1 and 1,404 cases respectively, out of the 1,424 tested. **Sensitivity** (true positives/[true positives + false negatives]*100) was **93.8% (95% CI 69.8 to 99.8%)** and **specificity** (true negative/[true negatives + false positives]*100) was **99.7% (95% CI 99.3 to 99.9%)**.† These data suggest that the CK-MM assay misses 6.2% of true DMD cases and incorrectly classifies only 0.3% of healthy babies as having DMD. The

† The values for sensitivity and specificity were reported as "positive percent agreement" (PPA) and "negative percent agreement" (NPA), respectively in the publication. However, PPA and NPA are intended to be used when a test is compared to another test, rather than a reference standard. As the comparison in the paper appears to be between the CK-MM assay result and confirmed cases of DMD, it has been assumed that the reported results are sensitivity and specificity.

numbers of true and false positives and negatives have been used to calculate positive and negative predictive values, but these figures were not directly reported in the text. The calculated **positive predictive value** (true positives/[true positives + false positives]*100) was **78.9%** and the calculated **negative predictive value** (true negatives/[true negatives + false negatives]*100) was **99.9%**.

For the US population, the authors reported only true positive and false negative results at the 99th percentile for both the CK-MM assay (1,190 ng/mL) and CK enzyme activity fluorescence measurement (1,980 U/L). Using the CK-MM assay, there were 19 true-positive cases of DMD detected by screening. There were no false-negatives and the number of false-positive cases was not reported. In comparison, using the CK enzyme activity method, there was one false-negative result, as one of the 19 DMD-affected samples was below the 99th percentile cut-off. Incomplete reporting of the number of true and false positives and negatives means screening performance metrics cannot be calculated for the US population.

The key conclusion reported by the study authors was that the CK-MM assay is better than the CK enzyme activity fluorescence method at discriminating between newborns with and without DMD. This conclusion is supported by comparing the results of this study to the findings from the Welsh DMD CK screening programme as reported in the previous 2016 evidence review. Sensitivity of CK enzyme activity screening in the Welsh programme was lower than CK-MM screening in this study (81.6% compared with 93.8%) and false negative rate was higher for CK screening than CK-MM screening (18.4% compared with 6.2%). However, caution should be used in making this comparison between different studies because differences in the study populations (confounding factors) may contribute to the apparent difference in results.

Despite the promising findings for the CK-MM assay, so far they have only been reported in one published study with a relatively small sample size (n=1,424) and the cut-off values for the CK-MM assay were defined relevant to separate reference sets of samples for the US and Danish populations.

The authors did not define a consistent cut-off value for the assay, which may limit the generalisability of these results to other contexts. Updates from the ongoing pilot studies in the US (New York)[32, 44] and China[33, 46] that are using the CK-MM assay, including much larger numbers of newborns will be beneficial to see if this study's findings are replicated. However, details of the dates of expected updates from these pilot studies are not readily available.

In summary, as only one study was identified over the search period covered in this evidence map there is an insufficient volume of evidence in this key area to justify commissioning an evidence summary as it is unlikely that further evidence would be identified at the present time.

Furthermore, the limited evidence identified from this evidence map is unlikely to lead to a change in the UK NSC's current position because the findings have not yet been replicated in more than one or larger studies. However, updates from the ongoing pilot studies in the US and China will warrant reconsideration of this topic.

Conclusions

The findings of this evidence map are unlikely to impact on current recommendations on screening for DMD as limited new evidence was identified.

Recommendations

On the basis of this evidence map, the volume and type of evidence related to screening for DMD is currently insufficient to justify an update review at this stage. It is recommended that the topic be reconsidered upon updates from the ongoing pilot screening studies in the US and China or in 3 years' time.

Appendix 1 — Search strategy for the evidence map

SOURCES SEARCHED: Ovid MEDLINE® In-Process & Other Non-Indexed Citations, Daily and Epub Ahead of Print, Ovid MEDLINE® and Versions 1946 to 19 April 2021, Embase® 1974 to 19 April 2021, and the Cochrane Library (Cochrane Database of Systematic Reviews and Protocols, Issue 4 of 12, April 2021; Cochrane Trials, Issue 3 of 12, March 2021)

DATES OF SEARCH: 1 January 2015 to 20 April 2021 for all databases. Searches were run on 20 April 2021.

SEARCH STRATEGIES:

MEDLINE and Embase (searched simultaneously via the Ovid SP platform)
23. Muscular Dystrophy, Duchenne/ or Duchenne muscular dystrophy/ 24. (Duchenne or DMD or pseudohypertrophic progressive or muscular dystrophy).ti,ab,kw,kf. 25. 1 or 2 26. Infant, Newborn/ 27. (newborn$ or neonatal$ or infant$).ti,ab,kw,kf. 28. 4 or 5 29. 3 and 6 30. dried blood spot testing/ 31. dried blood spot.ti,ab,kw,kf. 32. (detect$ or predict$ or identif$ or diagnos$ or test$).ti. 33. mass screening/ or screen.ab. /freq=3 34. or/8-11 35. 3 and 12 36. 7 or 13 37. ("Conference Abstract" or "Conference Review" or comment or editorial or note or case reports or news or news release).pt. 38. (case stud$ or case report$).ti,ab. 39. historical article/ or case study/ 40. exp animals/ not exp humans/ 41. or/15-18 42. 14 not 19 43. limit 20 to yr="2015-current" 44. remove duplicates from 21
Cochrane Library (searched via the Wiley Online platform)
17. [mh ^"Muscular Dystrophy, Duchenne"] 18. (Duchenne or DMD or "pseudohypertrophic progressive" or "muscular dystrophy"):ti,ab,kw

19. #1 or #2
20. [mh ^"Infant, Newborn"]
21. (newborn* or neonatal* or infant*):ti,ab,kw
22. 4 or #5
23. #3 and #6
24. [mh ^"dried blood spot testing"]
25. "dried blood spot":ti,ab,kw
26. (detect* or predict* or identif* or diagnos* or test*):ti
27. [mh ^"mass screening"] or screen:ab
28. {or #8-#11}
29. #3 and #12
30. #7 or #13
31. #14 with Cochrane Library publication date Between Mar 2015 and May 2021, in Cochrane Reviews, Cochrane Protocols
32. #14 with Cochrane Library publication date Between Mar 2015 and May 2021, in Trials

Results by database

MEDLINE and Embase	854
Cochrane Library	52
Total	906

Inclusions and exclusions

Studies were included based on the eligibility criteria listed in Table 3.

Table 3: Eligibility criteria for the review question

PICOS domain	Inclusion Criteria	Exclusion Criteria
Patient population	Newborns, defined as <12 months of age	• Children who are not newborns • Adults
Intervention	**Index test:** • Any tests used to detect DMD using dried blood spots **Reference standard:** • Mutation analysis of DMD gene	**Index test:** • Any other index test **Reference standard:** • N/A
Comparator	• Any or none	• N/A
Outcomes	Outcomes relating to diagnostic accuracy, including but not limited to: • Sensitivity • Specificity • PPV • NPV • LR • AUC	Outcomes not relevant to diagnostic accuracy

PICOS domain	Inclusion Criteria	Exclusion Criteria
Study design	**Tier 1:** • RCTs • Non-randomised studies with consecutively enrolled populations (e.g. prospective and retrospective cohort studies) • SLR/(N)MAs of these study designs **Tier 2:** • Case-control studies • Cross-sectional studies • Case series • SLR/(N)MAs of these study designs	Any other study design, including: • Case reports • Narrative reviews • Editorials • Commentaries • Conference abstracts • Other publication types that have not been peer-reviewed
Setting	**Tier 1:** • Studies conducted in the UK **Tier 2:** • Studies conducted in high-income countries where the population, screening methods and technology are expected to be similar to that of the UK (OECD and EEA countries excluding South Korea and Mexico)	• Studies in ineligible countries, or international studies where outcomes for eligible countries are not presented separately to outcomes from ineligible countries
Other considerations	• Articles published in the English language • Articles published since March 2015	• Studies with abstract not in the English language • Articles published before March 2015

Abbreviations: AUC, area under the curve; DMD, Duchenne muscular dystrophy; EEA, European Economic Area; LR, likelihood ratio; N/A, not applicable; (N)MA, (network) meta-analysis; NPV, negative predictive value; OECD, Organisation for Economic Co-ordination and Development; PPV, positive predictive value; RCT, randomised controlled trial; SLR, systematic literature review

Appendix 2 – Abstract reporting tables

Question 1: What is the volume and type of evidence on suitable screening tests using dried blood spots to detect DMD?

TITLE	
Citation	*Timonen* et al*. (2019), Duchenne Muscular Dystrophy Newborn Screening: Evaluation of a New GSP® Neonatal Creatine Kinase-MM Kit in a US and Danish Population, International Journal of Neonatal Screening 5(3):27.*[49]
BACKGROUND	
Study type	*Retrospective analysis of historical specimens.* *[Full text consulted]*
Objectives	*To explore screening of newborns for DMD using a novel immunoassay for CK-MM isoform and compare with CK activity determination by fluorescence measurement. The study also evaluated how stable CK-MM concentrations were over time, and the effect of the age of the newborn at the time of sampling and gestational age on CK-MM concentrations, and how stable the CK-MM was over time.*
Components of the study	***Population:*** *DBS samples from newborns in two populations (Denmark [n=1,424] and US [n=719)* ***Index test:*** *GSP® Neonatal CK-MM kit* ***Comparator:*** *CK activity determination by fluorescence measurement* ***Reference standard:*** *DMD diagnosis confirmed by treating physicians and by molecular testing* ***Outcomes:*** *diagnostic accuracy at different screen-positive cut-off percentiles (95%, 99% and 99.5%)* *The study also reports:* • *relationship between CK-MM concentration and CK activity* • *long-term sample stability* • *quality control performance data* • *impact of age of the newborn at the time of sampling on CK-MM concentration* • *impact of gestational age of the newborn on CK-MM concentration* *[Full text consulted]*

RESULTS	
Results	*Outcomes relevant to question 1:* *US population:* • *With the GSP® CK-MM kit using the 99th percentile cut-off value (1,190 ng/mL):* ○ *All 19 DMD-affected specimens were classified as screening positive* • *With the CK enzyme activity method using the 99th percentile cut-off value (1,980 U/L):* ○ *18 out of the 19 DMD-affected specimens were classified as screening positive and 1 specimen as screening negative* *Danish population:* • *With the GSP® CK-MM kit using the 99.5th percentile cut-off value (675 ng/mL):* ○ *15 of the DMD-affected specimens were classified as screening positive and 1 specimen as screening negative* ○ *4 of the DMD-unaffected specimens were classified as screening positive and 1,404 as screening negative* ○ *Overall percent agreement ([true positives + true negatives)/total]: 99.6% (95% CI 99.2 to 99.9%)* ○ *Positive percent agreement (true positives/[true positives + false negatives]): 93.8% (95% CI 69.8 to 99.8%)* ○ *Negative percent agreement (true negatives/[true negatives + false positives]): 99.7% (95% CI 99.3 to 99.9%)* *[Outcomes as specified by the commissioning document were not reported in the abstract; full text consulted]*
Conclusions	*The novel GSP® CK-MM assay discriminates between DMD-unaffected and DMD-affected populations better than the CK enzymatic activity fluorescence method.*

Abbreviations: CI, confidence interval; CK, creatine kinase; DBS, dried blood spot; DMD, Duchenne muscular dystrophy; MM, muscle type.

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