

MSAC Application 1710

Newborn bloodspot screening for X-linked adrenoleukodystrophy

Ratified PICO Confirmation

Summary of PICO/PPICO criteria to define question(s) to be addressed in an Assessment Report to the Medical Services Advisory Committee (MSAC)

Table 1 PICO set 1: Newborn bloodspot screening for X-linked adrenoleukodystrophy (X-ALD)

Component	Description
Population	All newborns in Australia
Prior test	No prior testing.
Intervention	<p>Newborn bloodspot screening (NBS) for assessing risk of X-linked adrenoleukodystrophy (X-ALD), based on:</p> <ul style="list-style-type: none"> • quantification of C26:0-lysophosphatidylcholine (C26:0 LPC) in newborn dried bloodspots, by flow-injection analysis (FIA) tandem mass spectrometry (MS/MS) and/or (high-performance) liquid chromatography ((HPLC)-MS/MS, and • diagnosis confirmed by molecular genetic testing (<i>ABCD1</i> genetic testing). <p>Overseas examples for reference:</p> <ul style="list-style-type: none"> • Three-tier testing: <ul style="list-style-type: none"> ○ 1st tier (screening test): quantification of C26:0-LPC by FIA-MS/MS. ○ 2nd tier (screening test): measurement of C26:0-LPC by HPLC-MS/MS. ○ 3rd tier (confirmatory/diagnostic test): <i>ABCD1</i> genetic testing. • Two-tier testing: <ul style="list-style-type: none"> ○ 1st tier (screening test): FIA-MS/MS or HPLC-MS/MS for C26:0-LPC. ○ 2nd tier (confirmatory/diagnostic test): <i>ABCD1</i> genetic testing. ○ Or, an additional two-tier protocol: 1st tier FIA-MS/MS, then 2nd tier HPLC-MS/MS. <p>For scenario analysis: addition of an X-counter test (detection of number of X chromosomes, to restrict (subsequent) screening tests to newborns with one X chromosome), with location within the testing sequence proposed to be explored in the assessment report.</p> <p>Where a VUS is found, re-evaluation after an appropriate period of time.</p>
Comparator	No screening test (NBS for X-ALD is not currently available in Australia).
Reference standard	Those individuals with clinical disease, including clinical signs and symptoms of X-ALD as well as a pathogenic or likely pathogenic (P/LP) variant in <i>ABCD1</i> .
Outcomes	<p>Outcomes that may change by the addition of X-ALD testing to the NBS, including but not limited to the following:</p> <ul style="list-style-type: none"> • Safety related to tests and treatments (e.g., HSCT), with outcomes stratified by males and females where evidence allows. • Test performance - sensitivity, specificity, positive predictive value, and negative predictive value of (a) screening test(s) and (b) confirmatory/diagnostic test. • Benefits: <ul style="list-style-type: none"> ○ Any differential benefits from diagnostic, prognostic, or predictive clinical utility (e.g., assignment of prognosis, anticipated clinical course, mortality, and morbidity from X-ALD, treatment outcomes or any other patient-relevant outcomes) in individuals diagnosed presymptomatically with P/LP variant in

Component	Description
	<p><i>ABCD1</i> through NBS versus individuals diagnosed through symptomatic presentation.</p> <ul style="list-style-type: none"> ○ Any benefit from the earlier identification of a proband with P/LP variant in <i>ABCD1</i> (e.g., enabling cascade testing, inform pregnancy decision-making). ○ Any benefit of ‘early’ treatment arising from screening and diagnosis, compared to ‘late’ treatment at disease sign/symptom onset. ○ Any differential benefits by sex, by different screening protocols or pathways. ○ Any benefits from (earlier) inadvertent detection of other non-targeted conditions in the screening test (e.g., other peroxisomal disorders). ○ Any beneficial value of knowing (e.g., emotional impact from shortened diagnostic odyssey, benefits to the wider community). <ul style="list-style-type: none"> ● Harms: <ul style="list-style-type: none"> ○ Harms from screening test(s): <ul style="list-style-type: none"> ▪ False positives - e.g., unnecessary subsequent diagnostic tests, and associated physical and/or psychological harms, to the newborn and family. ▪ False negatives - e.g., missed diagnosis or treatment opportunity, early deaths. ○ Harms from confirmatory/diagnostic test: <ul style="list-style-type: none"> ▪ False positives - e.g., unnecessary subsequent monitoring tests (e.g., serial brain MRIs) and/or treatments (e.g., HSCT), to the newborn; unnecessary cascade testing to family members; including any test- or treatment-associated physical and/or psychological harms. ▪ False negatives - e.g., missed diagnosis or treatment opportunity, early deaths. ▪ Identification of a variant of uncertain significance (VUS) in an individual undergoing confirmatory diagnostic testing, and whether these should be reported. If a VUS is reported to the individual, they will also require ongoing monitoring for risk of symptomatic disease, and may require segregation and further testing to classify the pathogenicity of the VUS. If VUSs are not reported, there are potential ethical concerns with withholding information. ○ Any harms from (earlier) detection of variants of uncertain significance in <i>ABCD1</i> or other non-targeted conditions (e.g., other peroxisomal disorders, sex chromosome disorders where the X-counter test is used) (e.g., psychological burden from identified conditions for which there is no treatment available). ○ Any harms from “knowing” or “over-diagnosis” due to incomplete penetrance (e.g., newborns screened positive may not develop clinical manifestations for a few decades, in those with late-onset forms, or may never manifest clinically, owing to poor genetic-phenotype correlation). ● Costs and cost-effectiveness: <ul style="list-style-type: none"> ○ Costs of screening test(s), confirmatory/diagnostic test and further clinical evaluation tests, ongoing monitoring/surveillance tests, specialists care, genetic counselling, and treatments (e.g., HSCT).

Component	Description
	<ul style="list-style-type: none"> ○ Cost of any other relevant healthcare resource use. ○ Cost per positive diagnosis. ○ Cost per quality-adjusted life year. ○ Any differential results by sex and/or by screening/testing protocols (with or without X-counter, 2- or 3-tier testing). ● Financial implications: <ul style="list-style-type: none"> ○ Proportion of eligible population (newborns) likely to accept the screening test and confirmatory/diagnostic test. ○ Number of newborns tested. ○ Prevalence of the disease or biomarker in the whole population and in the test population. ○ Proportion of the eligible population who will proceed to monitoring in the prodromal phase. ○ Number of individuals requiring treatment (report separately for males and females). ○ Total Australian Government health care costs.
Assessment question	What is the comparative safety, effectiveness, and cost-effectiveness of NBS for X-linked adrenoleukodystrophy versus current practice (no screening) in Australia?

C26:0 LPC=C26:0-lysophosphatidylcholine; FIA=Flow-injection analysis; HSCT= haematopoietic stem cell transplantation; LC=liquid chromatography; MRI=magnetic resonance imaging; MS/MS=tandem mass spectrometry; NBS= Newborn Bloodspot Screening program; P/LP=pathogenic or likely pathogenic; VUS=variant of uncertain significance; X-ALD=X-linked adrenoleukodystrophy

Table 2 PICO set 2: Cascade testing of family members of newborns diagnosed with X-ALD via the NBS program

Component	Description
Population	<p>Family members* of newborns diagnosed with a P/LP variant in <i>ABCD1</i> through NBS, and the family members of presenting individuals diagnosed with signs and symptoms of X-ALD (if not already tested following identification through NBS). Scenario analysis: family members* of newborns with one X chromosome and a P/LP variant in <i>ABCD1</i>.</p> <p>* Under universal screening, family members refer to the parents (mother for a male proband, both parents for a female proband) and the siblings** of the proband, and first-degree relatives on the maternal or paternal side, depending on inheritance. Under scenario analysis (male-only screening), family members refer to the mother and siblings** of the proband and first-degree relatives on the maternal side if the mother is a carrier.</p> <p>**The benefits and harms of cascade testing for female siblings under the age of 16 years are to be explored in the assessment stage.</p>
Prior tests	Medical and family history
Intervention	Genetic testing for the <i>ABCD1</i> familial variant (Carrier testing for family members*).
Comparator/s	Cascade testing offered to the family members of presenting individuals diagnosed with signs and symptoms of X-ALD.

Component	Description
Reference standard	None
Outcomes	<ul style="list-style-type: none"> • Safety related to tests and treatments (e.g., HSCT), with outcomes stratified by males and females where evidence allows. • Test performance - sensitivity, specificity, positive predictive value, and negative predictive value of genetic testing for the familial variant. • Benefits: <ul style="list-style-type: none"> ○ Any differential benefits from diagnostic, prognostic, or predictive clinical utility (e.g., assignment of prognosis, prediction of clinical course, mortality, and morbidity from X-ALD, treatment outcomes or any other patient-relevant outcomes) in individuals diagnosed with P/LP variant in <i>ABCD1</i> through family cascade testing versus individuals diagnosed through symptomatic presentation. ○ Any benefit from earlier diagnosis with P/LP variant in <i>ABCD1</i> (e.g., inform pregnancy decision-making). ○ Any benefit of ‘early’ treatment arising from cascade testing, compared to ‘late’ treatment at disease sign/symptom onset. ○ Any beneficial value of knowing (e.g., less anxiety from shortened diagnostic odyssey). • Harms: <ul style="list-style-type: none"> ○ Any harms from cascade testing (from false positives, false negatives; psychological harms). ○ Any harms from “knowing” or “over-diagnosis” due to incomplete penetrance (e.g., individuals test positive from cascade testing may not develop clinical manifestations for a few decades, in those with late-onset forms, or may never manifest clinically, owing to poor genetic-phenotype correlation). ○ Identification of variants of uncertain significance in individuals undergoing cascade testing, and whether these should be reported to individuals. If these are reported, to the individual, they will also require ongoing monitoring for risk of symptomatic disease. • Costs and cost-effectiveness: <ul style="list-style-type: none"> ○ Costs of cascade testing and further clinical evaluation tests, ongoing monitoring/surveillance tests (e.g., MRI), specialists care, genetic counselling, and treatments (e.g., HSCT). ○ Cost of any other relevant healthcare resource use. ○ Cost per positive diagnosis. ○ Cost per quality-adjusted life year. • Financial implications: <ul style="list-style-type: none"> ○ Proportion of eligible population likely to take up cascade testing. ○ Proportion of the eligible population who will proceed to monitoring in the prodromal phase.

Component	Description
	<ul style="list-style-type: none"> ○ Number of individuals requiring treatment (report separately for males and females). ○ Total Australian Government health care costs.
Assessment question	What is the comparative safety, effectiveness, and cost-effectiveness of cascade testing of the family members of newborns diagnosed with P/LP variant in <i>ABCD1</i> at risk for X-ALD via the NBS program, versus current practice of cascade testing of the family members of presenting individuals diagnosed with signs and symptoms of X-ALD?

HSCT= haematopoietic stem cell transplantation; MRI=magnetic resonance imaging; NBS= Newborn Bloodspot Screening program; P/LP=pathogenic or likely pathogenic; X-ALD=X-linked adrenoleukodystrophy.

Purpose of application

An application requesting the addition of X-linked adrenoleukodystrophy (X-ALD) to the National Bloodspot Screening program was received by the Department of Health. Mr [REDACTED] represents [Leukodystrophy Resource & Research Organisation Inc.](#) and Dr [REDACTED], Associate Professor in Paediatric Neurology at the [REDACTED], is a supporting co-applicant.

As noted in the application form, the clinical validity of the screening and diagnosis has not been determined (p6, Application Form), however it was reported that:

Elevated C26:0-lysophosphatidylcholine (C26:0 LPC) is a sensitive biomarker for use as a screening test for X-ALD. However, elevated C26:0 LPC is not pathognomic for X-ALD and may also be seen in individuals with other peroxisomal disorders, such as Zellweger spectrum disorder. There are established measurement methods of C26:0 LPC, e.g., liquid chromatography tandem mass spectrometry (LC-MS/MS), that are highly sensitive, specific, and have low false positive rates (pp5-6, Application Form).

While there is no immediate need for treatment upon diagnosis of P/LP variant in *ABCD1* conferring risk of X-ALD through NBS (i.e., while presymptomatic), about 35% of affected males will develop childhood cerebral ALD (CCALD) for which treatment is available. The effectiveness of treatment according to degree of CCALD requires consideration in the assessment. Post-screening periodic surveillance (e.g., serial brain magnetic resonance imaging or MRI) facilitates the identification of early neurological signs of CCALD and timely referral for potential haematopoietic stem cell transplantation (HSCT). The application form notes that HSCT is the most established therapy for CCALD and can halt disease progression if performed prior to the onset of any neurological symptoms (p3, Application Form). However, for affected males with no known family history of X-ALD, clinical presentation is often too late for HSCT (p4, Application Form). There are case reports from overseas where male infants with X-ALD were identified with adrenal dysfunction through newborn screening, boys aged 3-4 years identified by brain MRI surveillance with progressive brain lesions and were referred for HSCT (p6, Application Form).

Identification of individuals presenting with adrenal insufficiency due to X-ALD is often delayed due to its insidious onset and may lead to significant morbidity or even death (p2, Application Form). Monitoring for adrenal dysfunction offers opportunity for early identification and treatment with adrenal hormone replacement therapy and can be lifesaving from preventing adrenal crisis (p11, Application Form).

In Australia, each State and Territory undertakes its own newborn screening program, underpinned by the NBS National Policy Framework (NBS NPF; provided at Appendix A).

PICO criteria (PICO set 1)

Population

The screening population

The proposed population to be screened for risk of X-ALD is all newborn babies born in Australia.

The application noted that there is an argument for screening only male newborns: that females do not develop adrenomyeloneuropathy (AMN) until in late adulthood, there is no AMN-specific treatment and that less than one percent of females will develop adrenal insufficiency (p13, Application Form). However, it was also noted that heterozygous females (carriers) identified with X-ALD from screening females would also help identify other at-risk individuals (e.g., older male siblings), inform the risk for their future pregnancies, and potentially identify other at-risk family members in the extended pedigree.

X-ALD was included on the US Recommended Uniform Screening Panel in February 2016 and implemented in 24 US states and the District of Columbia.¹ Both male and female newborns are included. The Health Council of the Netherlands, however, considered that screening for risk of X-ALD was useful only in male newborns and recommended male-only screening for X-ALD risk.² A male-only screening algorithm including an X-counter test was developed and tested in a pilot study (the SCAN study).³ Screening for X-ALD is expected to be added to the Dutch national NBS program in the coming years.⁴ Expansion of newborn screening for X-ALD and recording results only for male newborns is currently under investigation in a 4-year Japanese pilot study started in April 2021.⁵ Unlike the Dutch study, the Japanese study based sex determination on the shape of external genitalia.

PASC noted the population entering newborn bloodspot screening for X-linked adrenoleukodystrophy in Australia is proposed to be all newborns. PASC considered that the population to be tested would only differ from this if sex on the bloodspot card were used to create separate laboratory workflows based on reported sex (i.e. to only test babies reported to be male). PASC considered that sex reported based on genitalia is unreliable, and any advice to not report results for babies with a number of X chromosomes other than 1 would be based on the results of the X-counter test rather than sex reported on the bloodspot card. PASC also noted that no stakeholder had proposed using sex on the bloodspot card, and that the Victorian NBS laboratory had commented that this would be a departure from current practice that would have significant workflow changes and costs. PASC therefore agreed with the proposed population and advised that a scenario analysis of the population of only phenotypically male newborns is not required, because using sex as per bloodspot card is not proposed, and the assessment's examination of the X-counter will be addressed through scenario analyses of the intervention.

PASC also advised the scenario analysis of the X-counter as a prior test should be removed, because the X-counter test would not be a test conducted prior to newborn bloodspot screening.

The UK National Screening Committee assessed NBS for X-ALD in March 2021 but did not recommend screening. The Committee considered that there was very limited information on the outcome of treatment, and its comparative effectiveness in asymptomatic individuals versus symptomatic individuals. Also, the Committee was uncertain regarding the impact of early diagnosis, especially for individuals who will not develop CCALD and for babies identified with other conditions for which there are no treatments.⁶

In the year ending 31st December 2021, there were 310,012 births in Australia, an increase of 6.2% compared with the previous year and reversing the trend of negative annual growth from 2017 to 2021.⁷

The current uptake rate of the NBS program is around 99%.⁸ Should X-ALD be added to the NBS program, say in 2023, and assuming the same number of births in 2023 as in 2021, 310,012 newborns will be offered NBS for X-ALD. Over 300,000 (306,912) newborns will be screened for risk of X-ALD, assuming an uptake rate of 99%. If screening is provided for male newborns only, assuming 51.4%⁹ of the newborns are males, over 150,000 (157,784) male newborns will be screened for risk of X-ALD. It is anticipated that the Assessment Report will investigate the availability of more accurate estimates in developing utilisation and financial estimates.

The proposed target condition for screening

X-ALD, the most common form of adrenoleukodystrophy (ALD),¹⁰ is a rare X-linked disorder characterised by impaired peroxisomal oxidation of very long-chain fatty acids (VLCFA) due to pathogenic or likely pathogenic variants in the *ABCD1* gene.¹¹ The *ABCD1* gene is located on the X chromosome and encodes adenosine triphosphate (ATP)-binding cassette (ABC), subfamily D, member 1 protein (ABCD1), a peroxisomal transmembrane protein. Failure of the ABCD1 protein to transport very long chain fatty acid (VLCFA)-Coenzyme A (CoA) into peroxisomes for beta-oxidation results in the accumulation of the saturated VLCFAs in all tissues, with manifestations primarily in the adrenal cortex, the myelin of the central nervous system (CNS), and the testicular Leydig cells.^{12,13}

X-ALD is inherited in an X-linked pattern. In an individual with one X chromosome, one altered copy of the *ABCD1* gene is sufficient to cause X-ALD. Additionally, an affected male will pass the variant-containing gene to all of their daughters but none of their sons. In an individual with two copies of the X chromosome, one variant-containing copy of the *ABCD1* gene usually does not cause features of X-ALD that are as severe as those in affected males. Most females with one variant-containing copy of the gene develop some disease signs and symptoms. Additionally, affected females have a 50 percent chance of passing the altered gene to each of their children.

X-ALD has a heterogenous spectrum of clinical presentation, and varying age of onset and severity. Table 3 summarises the clinical presentation of X-ALD phenotypes according to sex.

Table 3 Summary of the clinical presentation of X-ALD phenotypes in males and females

ALD phenotypes	Presentation & pathology	Reported cumulative frequency and age of onset
Phenotypes in males		
Childhood cerebral (CCALD)	Progressive behavioural, cognitive, and neurologic deficit often leading to total disability and death within 4 years of diagnosis. Pathologic hallmark is inflammatory cerebral demyelination.	31%–35% Onset at 3–11 years of age
Adolescent cerebral	Presentation and pathology as in CCALD. Somewhat slower progression than CCALD.	4%–7% Onset 11–21 years of age
Adrenomyeloneuropathy (AMN)	Weakness, spasticity, pain, bladder and bowel dysfunction and impaired movement often resulting in assistive device or wheelchair use. Pathology includes slow progressive distal axonopathy with atrophy of the spinal cord, and peripheral neuropathy.	Most adult males will develop AMN. Onset typically starts in third-fourth decade of life

ALD phenotypes	Presentation & pathology	Reported cumulative frequency and age of onset
Adult cerebral	Dementia, behavioural disturbances, and focal neurologic deficits. Symptom progression may parallel CCALD. Rate of progression is variable with rare self-limiting cerebral demyelination (arrested-cerebral disease).	20% ¹⁴
Addison-only	Primary adrenal involvement without apparent neurologic involvement. Most will continue to develop AMN.	Common in childhood
Asymptomatic	Biochemical and gene abnormality without demonstrable adrenal or neurologic deficit. Detailed studies often show adrenal hypofunction or subtle signs of AMN on examination in adulthood	Common in childhood. 50% of asymptomatic develop AMN within 10 years
Phenotypes in females		
Asymptomatic	No evidence of adrenal or neurologic involvement	
AMN Mild, moderate, and severe	Symptomatology resembles AMN in men, with a slower rate of progression	Increases with age. Estimates of 50% aged >40 years and around 65% by aged 65 years Later onset than men.
Cerebral involvement	Reported in cases with confirmed and suspected X chromosomal inactivation	Rare, few cases reported ¹⁵
Addison's disease	Does not precede AMN phenotype as seen in males	Rare in females (1%)

Source: Adapted from Table 1 in Turk 2020.

AMN=adrenomyeloneuropathy; CCALD=childhood cerebral adrenoleukodystrophy; X-ALD=X-linked adrenoleukodystrophy

The three main phenotypes observed in males with X-ALD are: CCALD, AMN and adrenal insufficiency (Addison's disease).¹⁶

CCALD

CCALD is a rapidly progressive demyelinating condition affecting the cerebral white matter. Babies do not exhibit phenotypes at birth and have unremarkable development. First clinical presentation often occurs between three and ten years of age, with an average age of onset around seven years. Affected boys typically present with learning disabilities and behaviour problems, often initially diagnosed as attention deficit hyperactivity disorder (ADHD), and may respond to stimulant medication. Over time neurologic deterioration follows, with increasing cognitive and behavioural abnormalities, blindness, and quadriparesis. About 20 percent of affected boys may also have seizures.

Spontaneously arrested X-ALD, characterised by absence of symptom progression and lack of lesion growth or enhancement on sequential brain MRI, occurs in approximately 10 to 15 percent of X-ALD cases. These patients may be asymptomatic at diagnosis. A minority of patients with arrested X-ALD eventually convert to progressive X-ALD, so continued vigilance and monitoring is necessary. Younger patients may have a greater risk converting to progressive disease (UpToDate). Most affected individuals have adrenal insufficiency. Some have hyperpigmented skin due to increased adrenocorticotrophic hormone (ACTH) secretion (UpToDate).

Allogeneic haematopoietic stem cell transplant (HSCT) is the current standard therapy for early brain disease.¹⁷ Haematopoietic stem cells may be derived from bone marrow or peripheral blood from related

or unrelated matched donors. The application form notes that HSCT can halt the progression of CCALD provided the intervention was done at an early stage of the disease,¹⁸ with close to 90-95% survival of males with X-ALD identified early and had HSCT at the first MRI sign of progressive brain disease (pp10-11, Application Form). This prodromal period is part of the rationale for screening newborns for X-ALD. HSCT is associated with high morbidity and long-term sequelae because of immunosuppression issue and graft versus host disease. However, HSCT does not correct adrenal dysfunction¹⁹ nor prevent AMN.²⁰ For patients without a suitable donor nor suitable for HSCT, emerging therapies (e.g., gene therapy, targeted small molecules) may offer hope for potential treatment option in the future.²¹

AMN

AMN affects the spinal cord, the primary manifestation of which is spinal cord dysfunction with progressive stiffness and weakness of the legs, abnormal sphincter control, neurogenic bladder, and sexual dysfunction. Numbness and pain from polyneuropathy are also common in males with AMN. Childhood-onset AMN develops in 30-40% of X-ALD males. Adult-onset AMN is less severe and slowly progressive. AMN typically presents in adult males 20-40 years of age (average 28 years) (UpToDate) and in postmenopausal women.²² The majority have adrenal insufficiency, and gonadal dysfunction may precede motor abnormalities. AMN may also present as a progressive cerebellar disorder (UpToDate). Cerebral involvement at the time of diagnosis of AMN is rare (around 6%). However, in long-term follow-up studies, 20-60% of patients with AMN developed symptoms of cerebral involvement and/or cerebral demyelination on brain MRI. Patients with cerebral involvement have more rapidly progressive illness (UpToDate). One-fifth of adult males also develop cerebral disease (rapid progression to disability and death).²³

For adults with AMN, treatment is primarily symptomatic and supportive. HSCT for CCALD does not reverse adrenal insufficiency, nor prevent AMN.²⁴

Adrenal insufficiency (Addison's Disease)

Primary adrenal insufficiency is the initial manifestation of X-ALD in 30-40% of patients and may be the only sign of X-ALD in 8-10% of patients. Clinical diagnosis is challenging, especially in patients without known X-ALD family history. Signs and symptoms may include fatigue, anorexia, nausea, stomach upset, growth failure, and hyperpigmentation owing to increased melanocyte-stimulating hormone, a by-product of adrenocorticotrophic hormone (ACTH) production.²⁵ The Application Form noted that while the treatment of adrenal insufficiency is very effective, the identification of adrenal insufficiency is often delayed and may lead to significant morbidity or even death (p2, Application Form).

Screening newborns for X-ALD, together with subsequent adrenal insufficiency surveillance, may help identify males at high risk of adrenal insufficiency while at the presymptomatic stage, thereby allowing timely initiation of treatment and potentially preventing adrenal crisis.²⁶ Adrenal insufficiency is a major clinical phenotype of X-ALD,²⁷ with a lifetime risk of around 80% in X-ALD males.²⁸

Overall, for males with X-ALD:

About 60% develop a rapidly fatal demyelinating disease (around 35% occurring in childhood 3-10 years of age, 5% in adolescence, before the onset of spinal cord disease, and 20% occurring in adults).²⁹

Most will develop slowly progressive myeloneuropathy around 20-30 years of age.

Most will develop adrenal hormone deficiency (lifetime incidence around 80%: 46.7% age six months to ten years, 28.6% age 10-40 years, and 5.6% after age 40 years).³⁰

For females with X-ALD:

Rarely develop CCALD but often develop symptoms of myelopathy and peripheral neuropathy in adulthood. The age of onset in females is later (after age 35 years) and the clinical course is milder than in affected males (UpToDate).³¹ About 65% will develop symptoms of AMN by 65 years of age. There is therefore, as mentioned in the previous paragraphs, an argument for screening male newborns only. On the other hand, identification of heterozygous females (carriers) from NBS would also help identify other at-risk individuals (e.g., older male siblings), inform future pregnancy decision-making, and potentially identify other at-risk family members in the extended pedigree.

For families with a history of X-ALD, clinical presentation may vary within the family, with male siblings having different forms of the disorder (e.g., a male has the childhood form while a male sibling has the adult form).

Neither the presence of a pathogenic or likely pathogenic genetic variant in *ABCD1* nor the degree of biochemical abnormality in C26:0 LPC accurately predicts the phenotypic presentation in an individual.³²

The Application Form reported that the incidence of X-ALD is around one per 15,000 screened newborns based on overseas studies (p2, Application Form). Clinical experts advised that the Australian estimate would be similar (Pre-PASC teleconference, 2nd June 2022). A retrospective review of all known cases of X-ALD diagnosed in Australia and New Zealand in 1981 to 1996 and their families estimated a minimum annual incidence of 1.6 per 100,000 live births.³³ Assuming that 306,912 newborns undergo screening for X-ALD in Australia per year, there will be 20 newborns diagnosed with X-ALD through the NBS program assuming an annual incidence of 1/15,000 screened newborns, or five individuals diagnosed if assuming an annual incidence of 1.6/100,000.

Regarding the annual incidence of X-ALD and disease burden for Australia, PASC considered that the estimates of five to 20 Australian newborns diagnosed with X-ALD per year through the NBS were likely underestimates, because these estimates were based on the patients in whom X-ALD disease manifested, rather than on probands detected through NBS programs – not all of whom will develop X-ALD. PASC considered that if the annual incidence rates from four US states where X-ALD was included in the NBS programs were applied to Australia, then an estimated 21 to 63 newborns would be detected as being at risk of X-ALD in Australia per year.

Pre-conception carrier testing which includes the *ABCD1* gene is being considered in MSAC application 1637. Should this application be recommended for reimbursement, it is anticipated that over time the incidence of X-ALD may reduce as couples may undergo pre-implantation or prenatal genetic diagnosis to avoid having a potentially affected child. For couples who elect to undergo preimplantation genetic diagnosis to exclude a risk of X-ALD in their future offspring, they would then need to proceed embryo transfer of that selected embryo.

Intervention

Several aspects of the proposed intervention are not yet established:

- how many tiers of testing should be assessed (both two-tier and three-tier testing algorithms are options), and
- it is proposed that the assessment also includes a scenario analysis of the addition of an X-counter test, which would result in only newborns with one X chromosome proceeding to subsequent test/s (see paragraph X-counter below). As the optimal location of the X-counter test within the test sequence appears uncertain, it is also proposed that the assessment report explore the

appropriate location of the X-counter test, i.e., before/during/after MS/MS testing, within the testing sequence.

The screening strategy for NBS for X-ALD described in the Application Form appeared to be as follows, based on:

- the quantification of C26:0-lysophosphatidylcholine (C26:0 LPC) in newborn dried bloodspots, by flow-injection analysis (FIA) tandem mass spectrometry (MS/MS) and/or (high-performance) liquid chromatography ((HP)LC)-MS/MS; and
- confirmed by molecular genetic testing (*ABCD1*, single-gene sequencing).

Elevated C26:0-LPC is an established biomarker for assessing risk of X-ALD. It was stated that there are several established methods for C26:0-LPC measurement and the screening laboratories in the US use either three- or two-tier testing (pp5-6, Application).

Overseas examples for reference:

Three-tier testing (e.g., New York³⁴, California³⁵) (p5, Application Form):

- 1st tier: quantification of C26:0 LPC by FIA-MS/MS (some positives from the first tier may contain isobaric contaminants).
- 2nd tier: reanalysis by HP LC-MS/MS (to remove the false positives from the first tier; some positives from the second tier may be from disorders¹ also with elevated C26:0-LPC² in the newborn dried bloodspots).
- 3rd tier: *ABCD1* gene sequencing (to separate from other disorders that would also have elevated C26:0-LPC in the dried bloodspot).

Two-tier testing (US states with lower birth rates than New York or California, e.g., North Carolina³⁶) (p5, Application Form):

- 1st tier: MS/MS for C26:0-LPC in either positive or negative mode.
- 2nd tier: *ABCD1* gene sequencing or plasma VLCFA biochemical studies plus referral to a geneticist.

Depending on the number of newborn samples screened, and what tests are multiplexed with X-ALD screening, the screening laboratories in the USA have chosen either three or two-tier testing program. The larger states in the USA such as New York and California have a three-tier X-ALD newborn screen. States with a lower birth rate than New York or California are using a two-tier testing program (p5, Application Form). This implies that the birth rate can be used to determine whether a two-tier or three-tier program is more appropriate.

¹ E.g., Zellweger spectrum disorders (ZSD) and the single enzyme defects of peroxisomal fatty acid oxidation (acyl-CoA oxidase, D-bifunctional enzyme), *ABCD5* and the contiguous *ABCD1* *DXS* gene deficiencies (p5, Application Form). The applicant reported that there is no effective treatment for these disorders, although identification at birth would shorten the diagnostic odyssey and facilitate earlier genetic counselling to the affected families.

² The increase in saturated VLCFA, especially hexacosanoic acid (C26:0), is diagnostic, but not specific for many peroxisomal disorders (Moser 2022).

In general, both C26:0-LPC and sequencing of the *ABCD1* gene can be done on the same bloodspot. In some cases where the C26:0-LPC value is at the cut off value for X-ALD newborn screening, a second dried bloodspot is used for confirmation before sequencing the *ABCD1* gene (p6, Application Form). It was suggested that if *ABCD1* gene sequencing is not performed by the screening laboratory, then plasma VLCFA could be an alternative confirmatory test after detection of elevated C26:0-LPC levels (p9, Application Form) – however the potential inadequacy of plasma VLCFA as a confirmatory test should be considered, given the VLCFA test would not provide a genetic diagnosis so could not lead to cascade testing, nor could a categorical diagnosis be given based on VLCFA alone. The Assessment Report is advised to explore and evaluate the comparative benefits and harms of three- as versus two-tier screening protocols.

In symptomatic patients with no family history of X-ALD, the diagnostic algorithm includes clinical, neuroimaging, biochemical and genetic evaluation. The first step is to check for elevated VLCFA (males) or C26:0 LPC levels (females), followed by confirmatory *ABCD1* gene sequencing.

PASC noted that X-ALD newborn bloodspot screening is comprised of multiple tests, and that there are several possible sequences of tests that could be used. PASC considered that if the FIA-MS/MS and HPLC-MS/MS have low false negative rates, then all potential test sequences using ABCD1 genetic testing for confirmation would then ultimately have the same accuracy. PASC noted that one test sequence proposed by the Victorian NBS laboratory used (HP)LC-MS/MS as the confirmatory test instead of ABCD1 genetic testing. PASC commented that ABCD1 genetic testing would likely be the most costly and time-consuming component.

PASC noted that feedback had been received from New South Wales (NSW), Victorian and South Australian NBS laboratories, and that these state NBS laboratories have different proposals/preferences regarding the test sequence options. PASC noted the Victorian laboratory proposed multiplexing the FIA-MS/MS with the existing metabolic panel, and considered that this also aligned with the South Australian laboratory's preference for the Dutch algorithm,³⁷ which also uses FIA-MS/MS first. PASC noted the NSW laboratory preferred the first test to be (HP)LC-MS/MS rather than FIA-MS/MS, though that it had also commented if it cannot validate this test it then would support the Dutch algorithm.

PASC considered it was unclear how the identification of an ABCD1 variant of uncertain significance (VUS) in a newborn was intended to be reported and/or followed up.

PASC noted that a small proportion (approximately 3%) of X-ALD probands are reported to have an ABCD1 variant that is a large deletion/duplication not detectable by gene sequencing.³⁸ For negative ABCD1 gene sequencing results, other methods such as [quantitative PCR](#), long-range PCR, multiplex ligation-dependent probe amplification (MLPA), or [gene-targeted microarray](#) may be used to detect these variants. PASC advised the assessment should also explore the method or methods to be used for confirmatory ABCD1 genetic testing, including the potential addition (after gene sequencing) of other genetic test methods to detect probands with variants that would be missed by ABCD1 gene sequencing alone. PASC noted this would relate to the appropriate ABCD1 genetic testing method description to include for confirmatory diagnosis as per NBS NPF Criterion 4.7.

X-counter test

Sex determination is a complex screening algorithm challenge for the following reasons: inconsistency between chromosomal sex and phenotypic sex, not classifiable male/female phenotype, administrative errors.³⁹ The X-counter is the core of the design of a males-only screening algorithm, developed and tested in a Dutch pilot study (the SCAN study).⁴⁰ A key objective of the study was to identify males with X-ALD but

without unsolicited findings (e.g., other chromosome disorders), and that can be integrated into the Dutch newborn screening program. The X-counter (various commercial kits available) determines the number of X chromosomes without assessing the presence of a Y chromosome and was integrated as the second tier in the pilot Dutch 4-tier screening algorithm:

- Tier 1: Quantification of C26:0-LPC by FIA-MS/MS.
- Tier 2: X-counter.
- Tier 3: Quantification of C26:0-LPC by HPLC-MS/MS.
- Tier 4: *ABCD1* gene sequencing.

Various potential locations of the X-counter test are proposed (Figure 4, Figure 5).

The Assessment Report is advised to explore the impact of the various tiers of screening/testing sequence, as well as the integration of further evaluation/confirmatory tests (*ABCD1* gene sequencing), including scenario analysis for the intervention with integrated X counter test. Note that poor genotype-phenotype correlation renders the prediction of clinical course and health outcomes difficult, and potentially increasing the risk of unnecessary treatment.⁴¹ Also, as other genetic disorders with no treatment may also give rise to elevated C26:0 levels, genetic testing consideration is to limit to single gene assessment, as advised in the pre-PASC teleconference.

PASC noted consultation feedback from the NBS laboratories, Australian Genomics and others suggesting it may be inappropriate under the NBS NPF to report results for female newborns. PASC considered that while all newborn babies will enter NBS testing for X-ALD, it remains to be determined whether it is ethically acceptable and appropriate to report results for female newborns under the NBS NPF. PASC considered that the assessment should examine the ethical acceptability of reporting results for females, noting that carrier testing for late onset disorders is generally discouraged in individuals aged under 18 years as it is unclear that they can participate in informed decision-making. PASC advised that the assessment should consider the evidence of the benefits and harms of also reporting the results of female newborns against all NBS NPF Decision-Making Criteria, to enable MSAC to advise on whether results for X-ALD should also be reported for female newborns, in terms of ethical acceptability and alignment with the NBS NPF. This consideration would be best supported by assessment of the benefits and harms of screening separately for male and female newborns.

PASC considered that MSAC's position on the reporting of results for females will inform its advice as to whether the X-counter test is to be used (i.e. if it is unacceptable to report results for female newborns then the X-counter will be required), so the assessment would need to inform MSAC on the economic and financial consequences of test sequences using and not using the X-counter, and advised the X-counter test will need to be considered by the assessment. PASC advised that the assessment should use scenario analyses to examine the cost-effectiveness of each relevant test sequence, including potential positions of the X-counter within the sequence of tests. PASC considered that MSAC may decline to advise that a specific test sequence must be used, and advised the economic and financial analyses for the assessment should use the most cost-effective test sequence (with and without the X-counter). PASC considered that the assessment report's assessment of the cost-effectiveness of multiple test sequence options would permit informed decision-making on the test sequence to be used by laboratories.

PASC noted that the X-counter testing uses molecular methodology, which is relatively expensive and also would not permit multiplexing with existing initial testing as per feedback from the NBS laboratories. PASC considered the X-counter test is therefore unsuitable for Tier 1 screening for a rare disorder, and advised that the assessment did not need to examine test sequences in which the X-counter is the first test. PASC

also noted that the Victorian NBS laboratory had proposed additional test sequences not already included in the PICO: FIA-MS/MS, followed by confirmation using either (HP)LC-MS/MS or ABCD1 genetic testing, with the X-counter being located between the two if it is supported. PASC advised these additional test sequences needed to also be examined by the assessment. PASC noted that the Department was seeking input from the two NBS laboratories who had not provided pre-PASC comment, and considered that any further additional test sequences proposed by those laboratories should also be included in the assessment.

PASC considered it was unclear how the identification of an ABCD1 variant of uncertain significance (VUS) in a newborn was intended to be reported and/or followed up. PASC considered that the long-term monitoring of an individual with a VUS would be hard to justify as there is insufficient evidence for a classification of P/LP, and presumably the absence of segregation studies. However, PASC considered that the re-evaluation of a VUS after an appropriate period of time may be useful in this scenario and more generally, and advised an MBS item for this should be added to the intervention.

Cascade screening/testing (c.f. PICO criteria (PICO set 2) below)

As X-ALD is X-linked recessive, family members (see PICO set 2 below) of the proband are offered testing (plasma VLCFAs for males, C26:0-LPC for females or targeted ABCD1 analysis for the familial variant found for the proband) (p4, Application Form).

Comparator

The appropriate comparator is no screening test (NBS for X-ALD is not currently available in Australia). PASC advised the proposed comparator was appropriate.

At present, identification of cases of X-ALD relies on the symptomatic presentation of individuals with CCALD, adrenal insufficiency or AMN prior to formal diagnosis.

Reference standard

The reference standard used to determine the accuracy of the proposed newborn bloodspot screening for X-ALD (including screening test/s and confirmatory ABCD1 gene sequencing) is those individuals with clinical disease (i.e., clinical signs and symptoms of X-ALD, including radiology, pathology, histology) as well as a P/LP variant in ABCD1 gene. PASC advised the proposed reference standard was appropriate.

Outcomes

Outcomes that may change by the addition of X-ALD to the NBS, include but not limited to the following:

Safety

- Safety related to tests and treatments (e.g., HSCT), with outcomes stratified by treatments reported separately for males and females where evidence allows).

Test performance

- Sensitivity, specificity, positive predictive value, negative predictive value of (a) screening test(s) and (b) confirmatory/diagnostic test(s).

Benefits:

- Any differential benefits (e.g., assignment of prognosis, prediction of clinical course, clinical utility, mortality and morbidity from X-ALD, treatment outcomes or any other patient-relevant outcomes) in individuals diagnosed presymptomatically with P/LP variant in *ABCD1* through NBS versus individuals diagnosed through symptomatic presentation.
- Any benefit from the earlier identification of a proband with P/LP variant in *ABCD1* (e.g., enabling cascade testing and inform pregnancy decision-making).
- Any benefit of ‘early’ treatment arising from screening and diagnosis, compared to ‘late’ treatment at disease sign/symptom onset.
- Any differential benefits by sex, by different screening protocols or pathways.
- Any benefits from (earlier) detection of other non-targeted conditions in the screening test (e.g., other peroxisomal disorders).
- Any beneficial value of knowing (e.g., emotional impact from shortened diagnostic odyssey, benefits to the wider community).

Harms:

- Harms from screening test(s)
 - False positives - e.g., unnecessary subsequent diagnostic tests, and associated physical and/or psychological harms, to the newborn and family. Data on the harms of screening and presymptomatic identification are limited⁴² and quantification of psychological harms in terms of utilities is like to be challenging.
 - False negatives - e.g., missed diagnosis or treatment opportunity, early deaths).
 - Identification of variants of uncertain significance in individuals undergoing confirmatory diagnostic testing, and whether these should be reported to individuals. If these are reported, to the individual, they will also require ongoing monitoring for risk of symptomatic disease.
- Harms from confirmatory/diagnostic test:
 - False positives – e.g., unnecessary subsequent monitoring tests (e.g., serial brain MRIs) and/or treatments (e.g., HSCT), to the newborn; unnecessary cascade testing to family members; including any associated physical and/or psychological harms.
 - False negatives – e.g., missed diagnosis or treatment opportunity, early deaths.
- Any harms from (earlier) inadvertent detection of variants of uncertain significance in *ABCD1* or other non-targeted conditions (e.g., other peroxisomal disorders, sex chromosome disorders where the X-counter test is used) (e.g., psychological burden from identified conditions for which there is no treatment available).
- Any harms from “knowing” or “over-diagnosis” due to incomplete penetrance (e.g., newborns screened positive may not develop clinical manifestations for a few decades, in those with late-onset forms, or may never manifest clinically, owing to poor genetic-phenotype correlation).

Costs and cost-effectiveness:

- Costs of screening test(s), confirmatory/diagnostic test, further clinical evaluation tests, ongoing monitoring/surveillance tests, specialists care, genetic counselling, and treatments (e.g., HSCT). The Application form reported that an FDA-approved newborn screening kit for X-ALD is available for USD\$5 per patient and that the costs of plasma VLCFA measurement and *ABCD1* sequencing are AUD\$80 and around AUD\$1,000 respectively (pp8-9, Application Form). An MRI costs AUD\$555 when performed under general anaesthesia (\$405 without anaesthesia), and HSCT costs around AUD\$150,000 for a related donor (\$250,000 for an unrelated donor) (p12, Application Form).
- Cost of any other relevant healthcare resource use.
- Cost per positive diagnosis.
- Cost per quality-adjusted life year.
- Any differential results by sex and/or by screening protocols (with or without X counter, 2- or 3-tier testing).

Financial implications:

- Proportion of eligible population (newborns) likely to accept the screening test and confirmatory/diagnostic test.
- Number of newborns tested per year.
- Prevalence of the disease or biomarker in the whole population and in the test population.
- Proportion of the eligible population who will proceed to monitoring in the prodromal phase.
- Number of individuals requiring treatment (report separately for males and females).
- Total Australian Government health care costs.

PASC agreed with the outcomes proposed, though noted that a new guideline⁴³ published in 2021 recommends brain MRI surveillance twice per year for males aged 3-12 years (compared with surveillance twice a year for males aged 3-10 years in page 3 of the Application Form).

PASC considered that a key issue for this assessment and consideration by MSAC will be whether results for female newborns should also be reported. PASC considered that evidence of the benefits and harms of early detection and reporting of results for newborns with positive screening results are likely to differ for males and females, so advised outcomes should be examined separately for males and females where the evidence allows. PASC considered this stratification would facilitate assessment against the NBS NPF, and MSAC's advice on the appropriateness of reporting results for female newborns under the NBS NPF. PASC noted that females cannot receive HSCT for X-ALD, though considered that a gene therapy may be developed by the time these female babies reach adulthood.

PASC noted policy advice that there is no specific funding source for follow-on medical services arising out of NBS (including treatments and monitoring), and advised that the assessment would need to take into account the appropriate funding sources.

*PASC considered it was unclear how the identification of an *ABCD1* variant of uncertain significance (VUS) in a newborn was intended to be reported and/or followed up. PASC considered that segregation studies of*

the VUS with elevated VLCFA levels in the family would provide evidence towards the classification of pathogenicity, and recalled that MSAC had previously supported the segregation testing of relatives for classification of VUSs in Application 1585 Genetic testing for the diagnosis of early-onset or familial neuromuscular disorders. PASC considered that segregation testing should be added to the medical services required for follow-up after identification of a VUS. If familial testing is not possible, or if the variant remains classified as a VUS after segregation studies, PASC advised functional screening on fibroblasts for ALD protein expression may help with classification of pathogenicity, or other follow-up over time. If VUSs are to be reported, then follow-up would need to be the same as for newborns with P/LP variants.

PASC noted that the applicant stated that there are several international guidelines regarding indications for treatment and a review of pre-treatment evaluations should be considered in line with the statement that “that poor genotype-phenotype correlation renders the prediction of clinical course and health outcomes difficult, and potentially increasing the risk of unnecessary treatment”.

PASC noted that the applicant stated in its pre-PASC response that the US FDA is due to decide whether to endorse elivaldogene autotemcel (eli-cel) in September 2022. PASC noted that eli-cel had been withdrawn in Europe, and considered that Eli-cel is not registered or publicly funded in Australia, and therefore advised it is only a potential future therapy so should not be assessed as part of this application.

PASC noted that the applicant stated that “childhood AMN” does not exist.

PASC noted that the applicant stated that arrested X-ALD needs more scientific study, and that there is little evidence it is permanent.

PICO criteria (PICO set 2)

Population

Family members* of newborns diagnosed with P/LP variant in *ABCD1* through NBS.

Scenario analysis: family members* of newborns with one X chromosome and a P/LP variant in *ABCD1*.

* Under universal screening, family members refer to the parents (mother for a male proband, both parents for a female proband) and the siblings** of the proband, and first-degree relatives on the maternal side or paternal side, depending on inheritance. Under scenario analysis (male-only screening), family members refer to the mother and siblings** of the proband and first-degree relatives on the maternal side if the mother is a carrier.

**The benefits and harms of cascade testing for female siblings under the age of 16 years are to be explored in the assessment stage.

For the purposes of this document the term male is used to reflect the majority of those with one X chromosome, noting that females with Turner syndrome (XO) may also rarely be identified as at risk in screening.

PASC considered that the population for cascade testing was the relatives of probands who were identified specifically through NBS.

PASC advised that if the proband is male, then the mother is an obligate carrier (unless de novo mutation) and cascade testing should be offered to the male siblings and other maternal relatives as proposed.

However, if the proband is female (carrier female), then testing of the father should also be considered. PASC also noted that at least 4.1%⁴⁴ of individuals with X-ALD have a pathogenic de novo ABCD1 mutation.

PASC considered that if NBS identified carrier females, then the variant could also have been inherited from the father. PASC advised the assessment should also consider whether testing the father is appropriate.

Regarding testing for carrier females in those under 18 years of age, PASC noted that the NBS NPF recommends that the test protocol should, on balance, be socially and ethically acceptable to health professionals and the public (NBS NPF Criterion 5). PASC considered that the assessment should examine the ethical acceptability of cascade testing (for example, of a proband's female siblings), noting that carrier testing for late onset disorders is generally discouraged in individuals aged under 18 years as it is unclear that they can participate in informed decision-making.

PASC considered that complex ethical issues are involved with testing beyond the proband, and that the ethical complexities of cascade testing will also form part of MSAC's consideration even though the NBS NPF focuses on the newborn.

Intervention

Genetic testing for the ABCD1 familial variant.

PASC agreed that the appropriate intervention for cascade testing was variant-specific testing for the known familial variant. PASC advised that cascade testing should be conducted in a stepped manner, with step 1 being testing of the mother of the proband to determine maternal carrier status, and step 2 being, where the mother is a carrier, to then offer testing to the male siblings of the proband and other maternal relatives.

PASC considered that cascade testing of family members is generally not performed where the proband has a variant of uncertain significance (VUS), and advised that to conduct cascade testing for ABCD1 VUSs would be inappropriate.

PASC noted policy advice that there is no specific funding source for cascade testing arising out of NBS, and advised that the assessment would need to take into account the appropriate funding source for this testing.

Regarding the testing for carrier females, the PASC noted that carrier testing in individuals aged under 18 years of age is generally discouraged as it is unclear that they can participate in informed decision-making, and noted the NBS NPF's criterion that the screening test protocol should, on balance, be socially and ethically acceptable to health professionals and the public (Criterion 5).

Comparator

Cascade testing offered to the family members of presenting individuals diagnosed with X-ALD.

PASC advised the proposed comparator was appropriate.

Outcomes

Safety related to tests and treatments (e.g., HSCT), with outcomes stratified by males and females where evidence allows.

Test performance — sensitivity, specificity, positive predictive value, and negative predictive value of genetic testing for the family variant/s.

Benefits:

- Any differential benefits (e.g., assignment of prognosis, prediction of clinical course, clinical utility, mortality and morbidity from X-ALD, treatment outcomes or any other patient-relevant outcomes) in individuals diagnosed with P/LP variant in *ABCD1* through family cascade testing versus individuals diagnosed through symptomatic presentation.
- Any benefit from earlier diagnosis with P/LP variant in *ABCD1* (e.g., inform pregnancy decision-making).
- Any benefit of ‘early’ treatment arising from cascade testing, compared to ‘late’ treatment at disease sign/symptom onset.
- Any beneficial value of knowing (e.g., less anxiety from shortened diagnostic odyssey).

Harms:

- Any harms from cascade testing (from false positives, false negatives; psychological harms).
- Any harms from “knowing” or “over-diagnosis” due to incomplete penetrance (e.g., individuals test positive from cascade testing may not develop clinical manifestations for a few decades, in those with late-onset forms, or may never manifest clinically, owing to poor genetic-phenotype correlation).
- Identification of variants of uncertain significance in individuals undergoing cascade testing, and whether these should be reported to individuals. If these are reported, to the individual, they will also require ongoing monitoring for risk of symptomatic disease.

Costs and cost-effectiveness:

- Costs of cascade testing and further clinical evaluation tests, ongoing monitoring/surveillance tests (e.g., MRI), specialists care, genetic counselling, and treatments (e.g., HSCT).
- Cost of any other relevant healthcare resource use.
- Cost per positive diagnosis.
- Cost per quality-adjusted life year.

Financial implications:

- Proportion of eligible population likely to take up cascade testing.
- Proportion of the eligible population who will proceed to monitoring in the prodromal phase.
- Number of individuals requiring treatment (report separately for males and females).
- Total Australian Government health care costs.

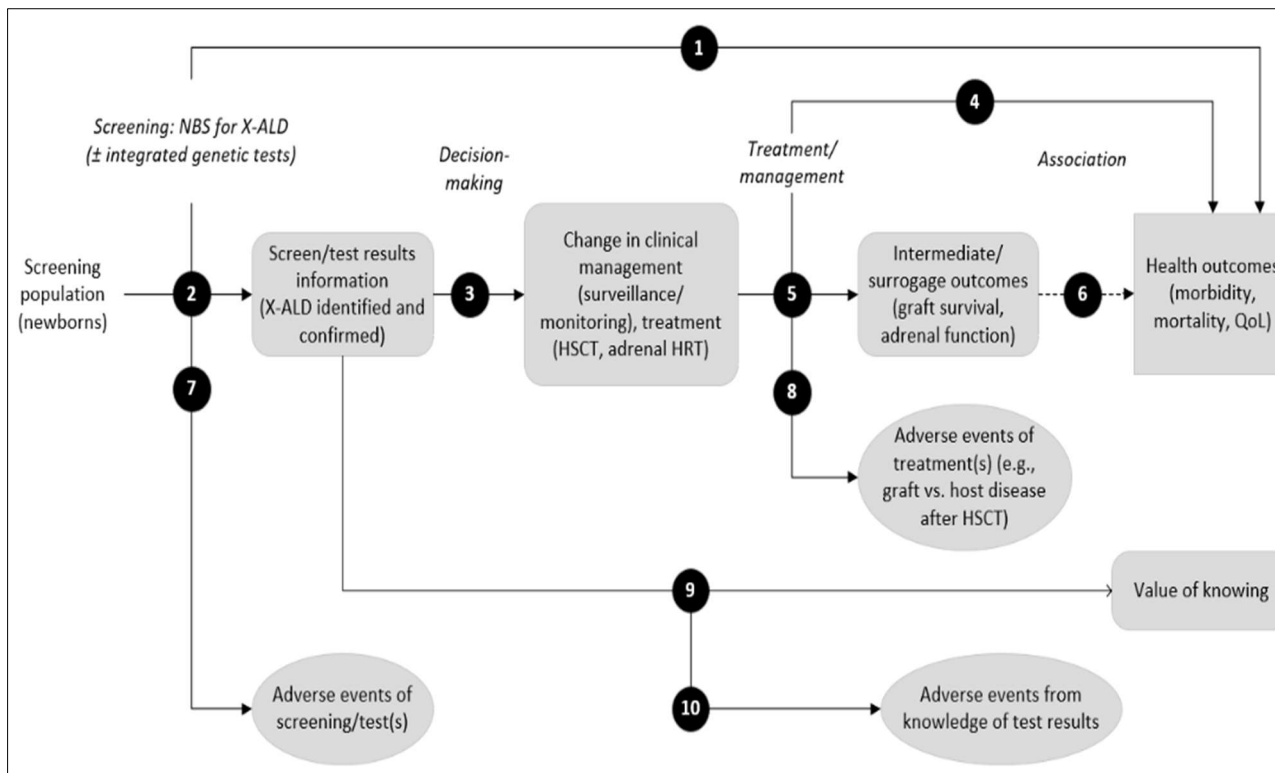
It is understood that new models of genomic newborn screening are currently under development by the Genomics Health Futures Mission, funded by the Medical Research Future Fund.

PASC advised the proposed outcomes were appropriate.

Assessment framework (for investigative technologies)

Figure 1 provides the assessment framework to show how the link between the test and relevant outcomes will be achieved.

Figure 1 Assessment framework showing the links from the test population to health outcomes



HSCT= haematopoietic stem cell transplant; HRT=hormone replacement therapy; NBS=newborn bloodspot screening; QoL=quality of life; X-ALD=X-linked adrenoleukodystrophy

Notes: 1: direct from test to health outcomes evidence; 2: test accuracy; 3: change in diagnosis/treatment/management; 4: influence of the change in management on health outcomes; 5: influence of the change in management on intermediate outcomes; 6: association of intermediate outcomes with health outcomes; 7: adverse events due to screening/testing; 8: adverse events due to treatment; 9: value of knowing; 10: adverse events from knowledge of screen/test results

The assessment questions related to the HTA assessment framework are:

- 1) Is there direct from screening to health outcomes evidence to support the claim that screening leads to improved health outcomes?
- 2) Test accuracy: When compared with VLCFAs analysis or sequencing of the *ABCD1* gene as reference standard, how does the information from FIA-MS/MS or LC-MS/MS analysis of C26:0 LPC differ from the information from VLCFAs analysis and/or genetic sequencing? What are the implications of discordances among the test results?
- 3) Do the screen/test results (diagnosis of P/LP variant in *ABCD1* at birth) impact, either immediately or in future, the clinical management of the individual?

- 4) Does the change in the clinical management (monitoring and treatment, e.g., HSCT, early/timely initiation of adrenal hormone replacement therapy) of the identified individual improve health outcomes (morbidity, mortality, QoL)?
- 5) Does the change in the clinical management (monitoring and treatment) of the identified individual result in an improvement in intermediate/surrogate outcomes (e.g., graft survival in individuals receiving HSCT, adrenal function in individuals on adrenal hormone replacement therapy)?
- 6) Is the observed change in intermediate/surrogate outcomes associated with an improvement in health outcomes (morbidity, mortality, QoL), and how strong is the association?
- 7) What are the adverse events associated with newborn bloodspot screening for risk of X-ALD, when compared to the current practice of not screening for X-ALD on NBS programs?
- 8) What are the adverse events associated with the monitoring and treatment of individuals diagnosed with P/LP variant in *ABCD1* through NBS who are at risk for symptomatic X-ALD?

Other relevant considerations

Proposals considered by MSAC can have aspects that are unique to the proposed technology, circumstances of use or funding arrangement, such that MSAC is unlikely to have considered the factors previously in the same context. Other relevant considerations should be explored in section 5 of the assessment report.

Additional information relevant to decision-making that is not captured elsewhere in the assessment is anticipated to include:

- Whether the proposed testing meets the criteria of the NBS National Policy Framework (see Appendix A).
- Potential impact of the addition of screening for risk of X-ALD on NBS participation.
- Reporting of VUS should be consistent with the current approach in NBS programs and other hereditary disease test settings
- Is there equitable access to the intervention and health care services for families, including from rural and remote areas?
- Are there any additional costs, such as the purchasing of new technology or performing training, that are associated with screening for this condition?

The PASC considered that there are ethical complexities for this application (e.g., should results be reported for females, and the ethical acceptability of withholding results), though noted that ethical issues are encompassed by MSAC's remit. PASC therefore advised that the assessment needs to include a comprehensive ethical analysis to support MSAC's decision-making on ethical aspects. PASC advised it would require consultation with/input from geneticists and bioethicists to make an informed recommendation.

The PASC also considered that consideration of fulfilment of the NBS NPF criteria should be central to the assessment, as this is a key aspect for MSAC's consideration. PASC advised the DCAR should recommend an approach based on the benefits and harms of reporting the results of female newborns against all NBS NPF criteria.

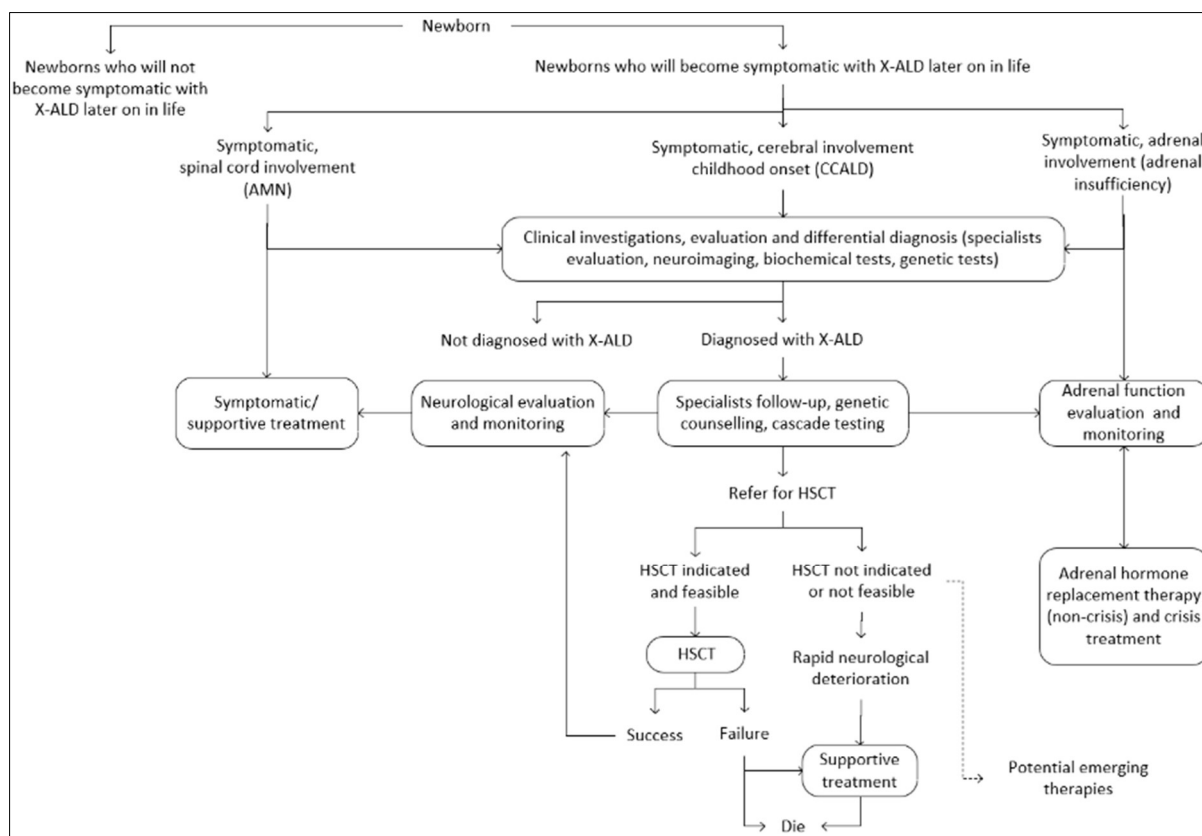
PASC noted that the policy area intends to continue further consultation with the state NBS laboratories.

Clinical management algorithms

PICO set 1

The Application Form did not present a clinical management algorithm for current practice or given the proposed screening for risk of X-ALD. Figure 2 was constructed during the PICO development stage based on literature and clinical advice, and revised following PASC. It provides the current clinical management algorithm for patients presenting with symptomatic X-ALD (PICO set 1). *Regarding the current clinical management algorithm, PASC recommended shifting symptomatic AMN up to be at the same level as symptomatic CCALD and symptomatic, adrenal involvement (adrenal insufficiency) as they are the three major clinical presentations. AMN should then lead to clinical investigations, and the later in life and subsequent asymptomatic parts of that branch of the algorithm removed. PASC also advised that the asymptomatic term on the same level as the clinical presentations of symptoms should be removed, as this is not a symptomatic presentation.*

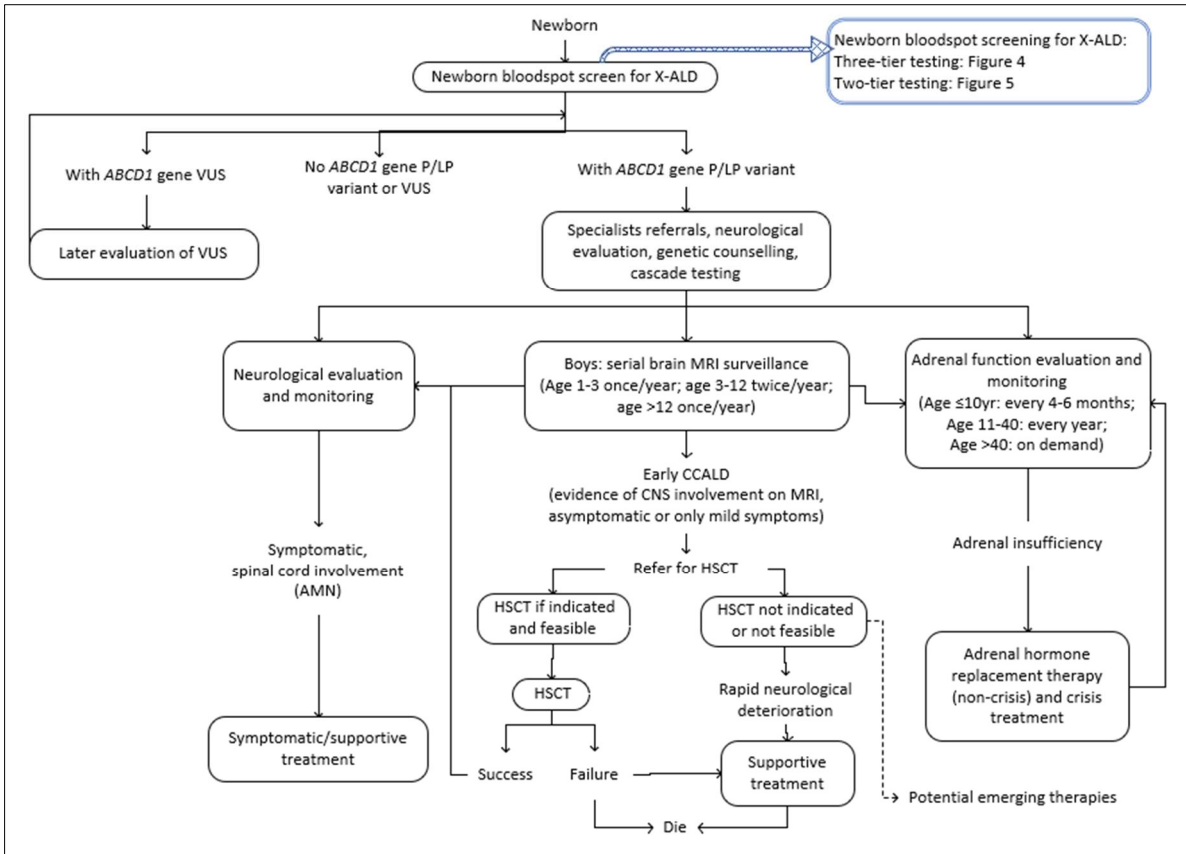
Figure 2 Current clinical management algorithm (PICO set 1)



Source: diagram constructed during the PICO development stage based on information presented on pp3-5 of the Application Form
 AMN=adrenomyeloneuropathy; C26:0-LPC=C26:0-lysophosphatidylcholine; CCALD=childhood cerebral ALD; HSCT=haematopoietic stem cell transplant; X-ALD=X-linked adrenoleukodystrophy.

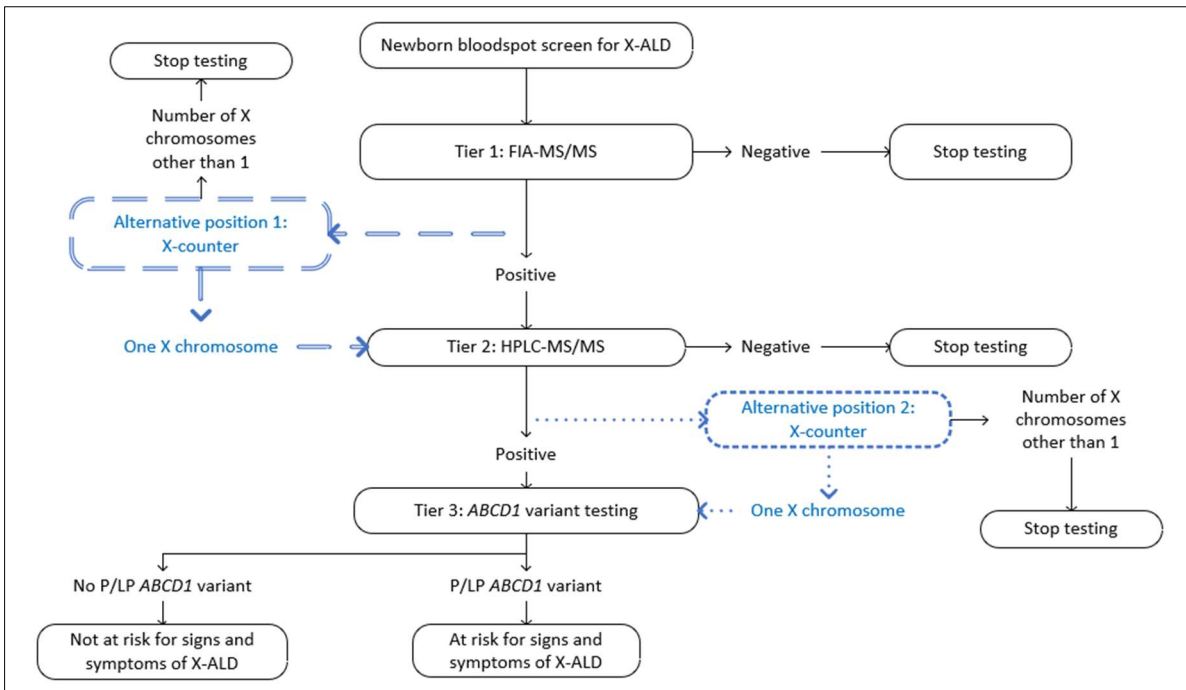
Figure 3 provides the clinical management algorithm for the proposed addition of X-ALD to the NBS program (PICO set 1). *Regarding the proposed clinical management algorithm, PASC advised that a new guideline⁴⁵ published in 2021 recommends brain MRI surveillance twice per year for boys aged 3-12 years (compared with surveillance twice a year for boys aged 3-10 years in page 3 of the Application Form).*

Figure 3 Proposed clinical management algorithm (PICO set 1)



Source: diagram constructed during the PICO development stage based on information presented on pp3-5 of the Application Form
 ALD=adrenoleukodystrophy; AMN=adrenomyeloneuropathy; C26:0 LPC=C26:0-lysophosphatidylcholine; CNS=central nervous system; FIA=Flow-injection analysis; HSCT=haematopoietic stem cell transplant; LC=liquid chromatography; MRI=Magnetic Resonance Imaging; MS/MS=tandem mass spectrometry; X-ALD=X-linked adrenoleukodystrophy; VLCFA=very long chain fatty acid; VUS=variant of uncertain significance.

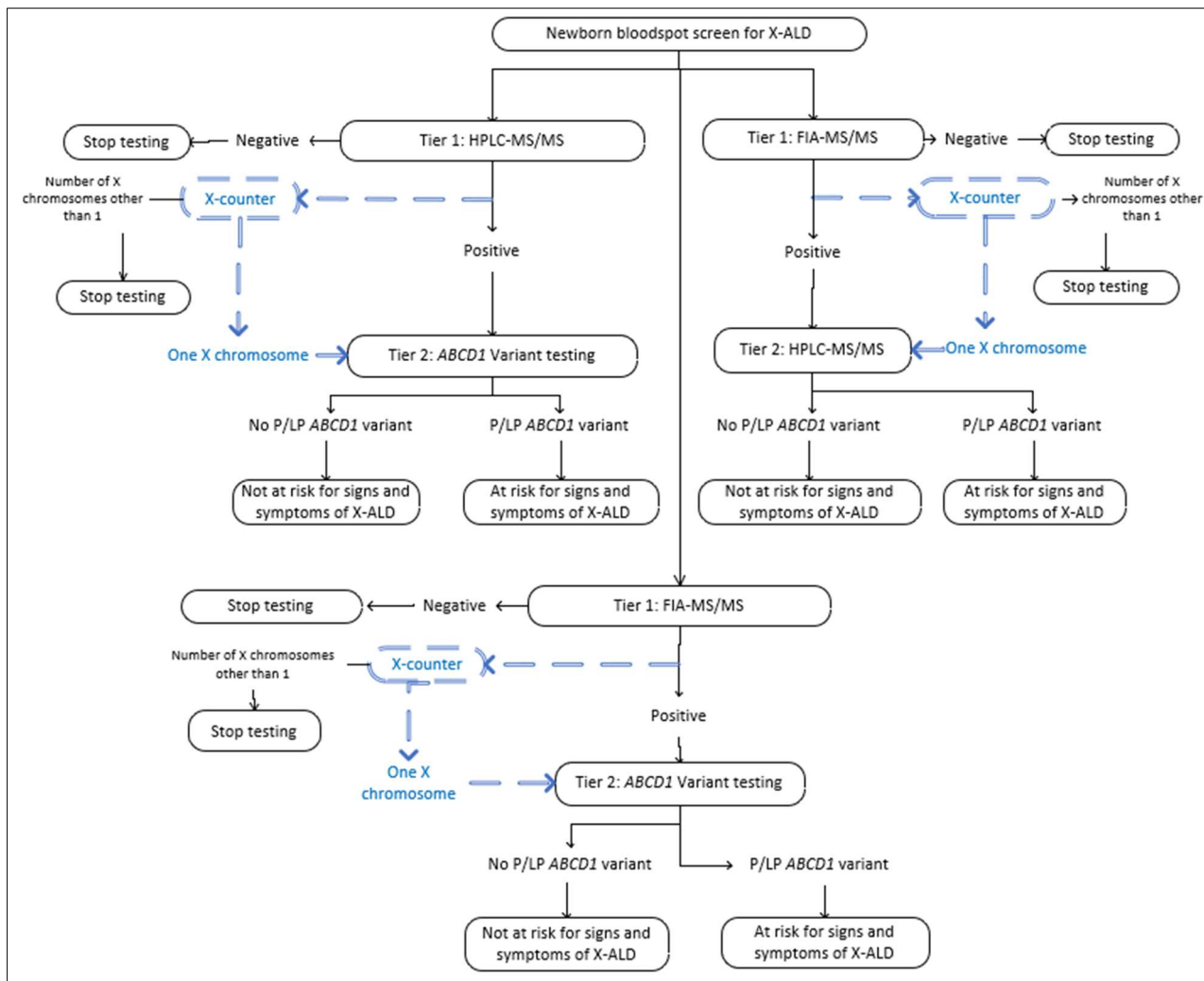
Figure 4 Proposed NBS for X-ALD (three-tier protocol) (no X-counter in black, X-counter if included in blue)



Source: diagram constructed during the PICO development stage based on information presented in the Application Form
 FIA=Flow-injection analysis; HPLC=high performance liquid chromatography; MS/MS=tandem mass spectrometry; P/LP=pathogenic/likely pathogenic; X-ALD=X-linked adrenoleukodystrophy.

X-counter depicted in blue dotted line represent the alternative positionings of the X-counter test for the purpose of scenario analysis.

Figure 5 Proposed NBS for X-ALD (alternative two-tier protocol) (no X-counter in black, X-counter if included in blue)



Source: diagram constructed during the PICO development stage based on information presented in the Application Form and consultation feedback from the NBS laboratories received prior to PASC.

FIA=Flow-injection analysis; HPLC=high performance liquid chromatography; MS/MS=tandem mass spectrometry; P/LP=pathogenic/likely pathogenic; X-ALD=X-linked adrenoleukodystrophy.

X-counter depicted in blue dotted line represent the alternative positionings of the X-counter test for the purpose of scenario analysis.

A fundamental difference between the current and the proposed clinical management algorithms is the identification of asymptomatic newborns with risk for X-ALD with the proposed X-ALD newborn screening. As a result, there are periodic surveillance and monitoring tests for these identified young infants and children (mainly male probands), as well as subsequent earlier treatments (e.g., HSCT, adrenal hormone replacement therapy) when indicated.

Confirmation is required as to whether a two-tier or three-tier testing program would be implemented in Australia. Figures 4 and 5 are simple representations of overseas examples of three- and two-tier testing programs. Note that the X-counter test depicted in blue dotted lines represent alternative positioning of the X-counter test for the purpose of scenario analysis (screening newborns with one X chromosome only). In the Dutch pilot SCAN study, the X-counter test was added between FIA-MS/MS and HPLC-MS/MS. The relative merits of three- versus two-tier screening protocols, including the optimal positioning of the X-counter test, are proposed to be explored and evaluated by the assessment report.

Males with risk for X-ALD identified at birth will be referred to a paediatric neurologist or metabolic physician for monitoring of early brain demyelination. Cerebral involvement is assessed by neuroimaging with brain MRI⁴⁶ at diagnosis and monitored by brain MRIs at periodic follow-ups (once per year between

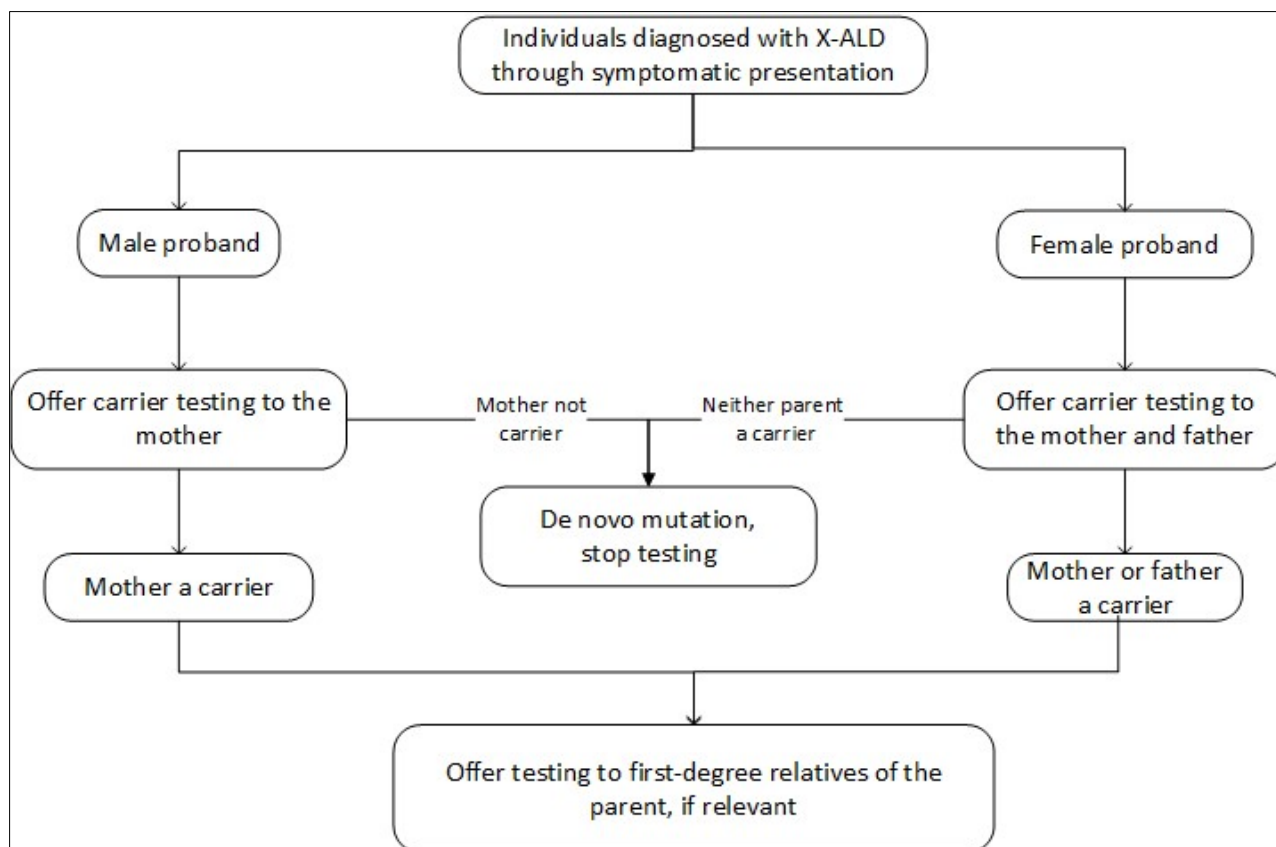
age one to three years, then every six months to age ten years and annually after age ten) (p3, Application Form). At the first sign of a progressive brain lesion, the boy will be referred for HSCT. The proposed MRI surveillance frequency is broadly similar to a neuroimaging surveillance consensus guideline⁴⁷ to detect early-stage brain lesions in neurologically asymptomatic boys with risk for X-ALD: a baseline MRI between age 12-18 months, a second MRI a year later, a contrast-enhanced MRI every six months between age 3-12 years old, and annual MRI after age 12 years. The guideline was based on a literature review of 123 studies published between 1970 and 2019 and expert panel consensus.

To detect adrenal insufficiency, the Application Form recommended adrenal testing every four to six months for boys with risk for X-ALD identified at birth aged ten years or less, once per year for those aged 11-40 years and on-demand testing for those over 40 years of age. The US Pediatric Endocrine Society developed an algorithm for adrenal insufficiency surveillance in males with risk for X-ALD.⁴⁸ There are no recommendations for screening females with risk for X-ALD for adrenal insufficiency.⁴⁹ Life-saving adrenal hormone replacement therapy will be offered when adrenal insufficiency is detected (p3, Application Form). Hydrocortisone is the preferred glucocorticoid.⁵⁰ Screening females with X-ALD for adrenal insufficiency is not recommended.⁵¹

PICO set 2

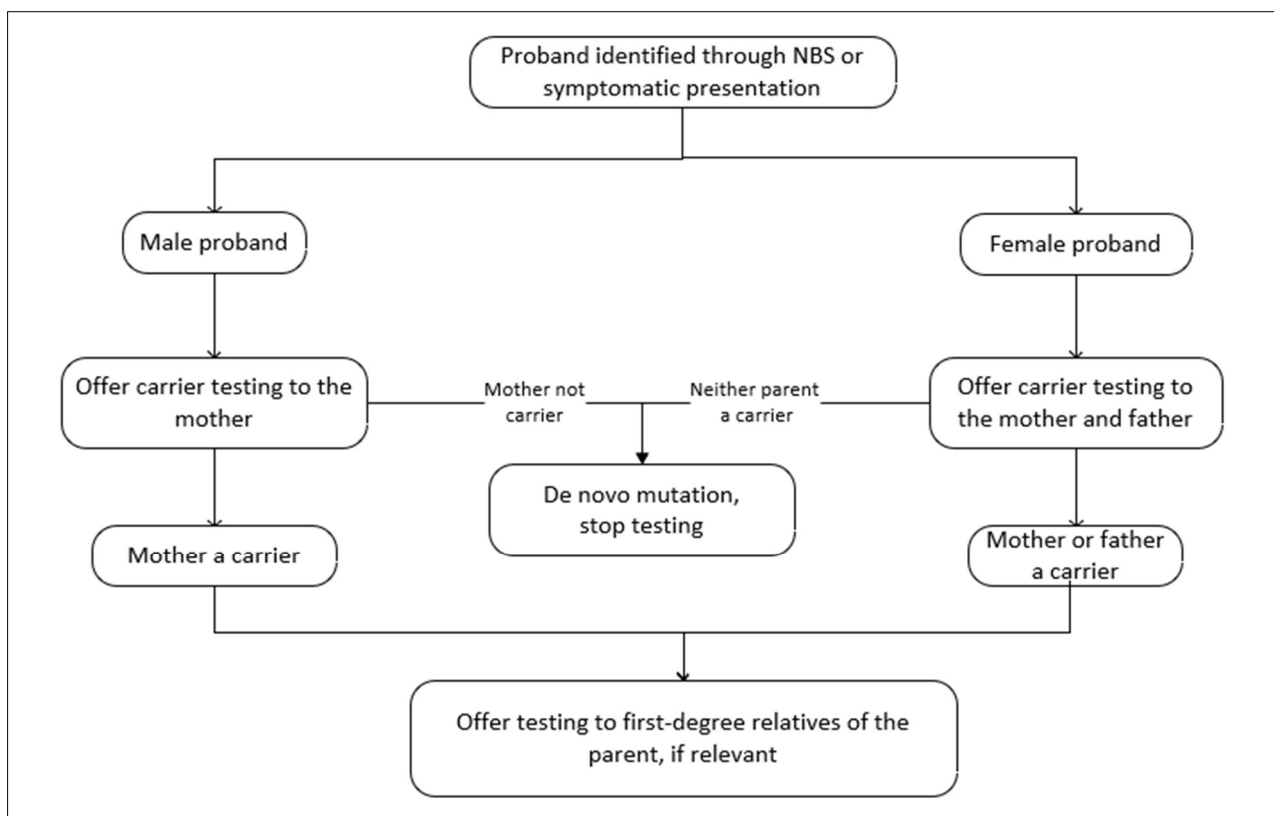
Figure 6 and Figure 7 present the current and proposed cascade testing protocols.

Figure 6 Current cascade testing protocol (PICO set 2)



Source: diagram constructed based on information presented in the Application Form and clinical advice. X-ALD=X-linked adrenoleukodystrophy.

Figure 7 Proposed cascade testing protocol (PICO set 2)



Source: diagram constructed based on information presented in the Application Form and clinical advice.
NBS = Newborn Bloodspot Screening

PASC considered that segregation studies of a VUS with elevated VLCFA levels in the family would provide evidence towards the classification of pathogenicity, and recalled that MSAC had previously supported the segregation testing of relatives for classification of VUSs in Application 1585 Genetic testing for the diagnosis of early-onset or familial neuromuscular disorders. PASC considered that segregation testing should be added to the medical services required for follow-up after identification of a VUS. If familial testing is not possible, or if the variant remains classified as a VUS after segregation studies, PASC advised functional screening on fibroblasts for ALD protein expression may help with classification of pathogenicity, or other follow-up over time.

PASC advised that the assessment did not need to examine test sequences in which the X-counter is the first test, given its advice that the X-counter is not appropriate as the first-tier test.

PASC also considered that the algorithms proposed by the Victorian NBS laboratory were different again to those proposed in the PICO, so should also be added to the test sequences to be assessed.

Proposed economic evaluation

A preliminary search of the literature suggests that:

- The sensitivity and specificity of newborn bloodspot screening for risk for X-ALD (via HPLC-MS/MS for C26:0-LPC), especially with genetic confirmatory test, is very high.
- Also, it appears that early HSCT before neurological deterioration is effective, and timely initiation of adrenal hormone replacement therapy. Therefore, provisional research supports the claim of superior effectiveness.

Table 4 provides a guide for determining which type of economic evaluation is appropriate.

Table 4 Classification of the comparative effectiveness and safety of the proposed newborn bloodspot screening for X-ALD versus the current practice of no screening, and guide to the suitable type of economic evaluation

Comparative safety	Comparative effectiveness			
	Inferior	Uncertain ^a	Noninferior ^b	Superior
Inferior	Health forgone: need other supportive factors	Health forgone possible: need other supportive factors	Health forgone: need other supportive factors	? Likely CUA
Uncertain ^a	Health forgone possible: need other supportive factors	?	?	? Likely CEA/CUA
Noninferior ^b	Health forgone: need other supportive factors	?	CMA	CEA/CUA
Superior	? Likely CUA	? Likely CEA/CUA	CEA/CUA	CEA/CUA

CEA=cost-effectiveness analysis; CMA=cost-minimisation analysis; CUA=cost-utility analysis

? = reflect uncertainties and any identified health trade-offs in the economic evaluation, as a minimum in a cost-consequences analysis

^a 'Uncertainty' covers concepts such as inadequate minimisation of important sources of bias, lack of statistical significance in an underpowered trial, detecting clinically unimportant therapeutic differences, inconsistent results across trials, and trade-offs within the comparative effectiveness and/or the comparative safety considerations

^b An adequate assessment of 'noninferiority' is the preferred basis for demonstrating equivalence

The type of economic evaluation proposed is cost-effectiveness or cost-utility analysis, although there may be limited data on psychological harms as well as difficult to quantify in terms of disutilities.

PASC noted that MSAC advises on public funding from the perspective of the healthcare system, so the economic evaluation should use this perspective. Although the costs of the testing proposed in this application will primarily arise from NBS testing itself, other costs to the healthcare system including the costs of cascade testing and follow-up testing including monitoring and treatment must also be considered.

PASC advised that the economic assessment should be considered against the NBS NPF criteria. PASC advised that the assessment should use scenario analyses to examine the cost-effectiveness of each potential test sequence for X-ALD NBS, including potential positions of the X-counter within the sequence of tests. PASC considered that MSAC may decline to support a specific test sequence, and advised the economic and financial analyses for the assessment should assume the most cost-effective sequence (with and without the X-counter) is used. PASC considered that the assessment report's assessment of the cost-effectiveness of multiple test sequence options would permit informed decision-making on the test sequence to be used by laboratories.

PASC considered that the addition of X-ALD to NBS is likely to increase resource use (monitoring, surveillance, treatments and the management of associated adverse events). PASC noted policy advice that

there is no specific funding source for cascade testing or follow-on medical services arising out of NBS (including treatments and monitoring), and advised that the assessment would need to take into account the appropriate funding source for this. This would be reflected in financial costs to various funding sources (e.g. MBS, NBA, States/Territories) as appropriate.

Proposal for public funding

The application proposes the addition of screening for risk for X-ALD to the NBS program.

While public funding is not sought through the MBS, should X-ALD be included in the Newborn Bloodspot Screening program, there will be flow-on impact on resource use downstream including but not limited to:

- relating to likely increased resource use in the:
 - monitoring and surveillance of asymptomatic individuals identified as at risk for X-ALD from birth (e.g., specialists care, serial brain MRIs, genetic counselling services).
 - treatments (e.g., HSCT) and the management of associated adverse events.

PASC noted the proposal did not seek public funding through the MBS, though considered that while NBS itself is funded under the NHRA, follow-up testing using MRIs, and potentially also other medical services as part of monitoring and treatment may be fundable through the MBS. PASC considered that MSAC provides public funding advice from the perspective of the healthcare system therefore relevant funding sources for medical services beyond NBS testing itself need to also be considered by the assessment. PASC advised clarification should be sought from the relevant policy area(s) of the Department required regarding eligibility for relevant MBS items, and if new MBS items are required to be proposed then these should be included in the DCAR.

Summary of public consultation input

PASC noted and welcomed consultation input from 5 professional organisations, 4 consumer organisations and 1 health professional. The 9 organisations that submitted input were:

- Australian Pompe Association (APA)
- Human Genetics Society of Australasia (HGSA)
- Australian Genomics
- Victorian Clinical Genetic Services (VCGS) (Victorian NBS laboratory)
- Better Access Australia
- Genetic Undiagnosed and Rare Disease Collaborative Australia (GUaRD)
- Rare Voices Australia (RVA)
- Western Sydney Genetics Program (WSGP) (NSW NBS laboratory)
- SA Pathology (SA NBS laboratory)

The consultation feedback received was largely supportive of public funding for Newborn Bloodspot Screening for X-ALD. The consultation feedback raised a number of concerns, predominately in relation to the population.

Clinical need and public health significance

- The main benefits of public funding received in the consultation feedback included:
 - Timely diagnosis
 - Earlier detection and treatment of affected individuals
 - Allows for cascade testing and identification of further affected family members
 - Improved survival outcomes
 - Facilitate referrals to appropriate services
 - Help inform future reproductive decision making
 - Reduced primary financial and psychosocial impact on families affected by cerebral forms and adrenal insufficiency
 - Reduced financial burden
 - Gives families opportunity to plan lifestyle and set goals for treatment
 - Allows for further genetic characterisation and phenotyping
 - Wilson and Jungner criteria for screening of male babies clearly met
 - PEX disorders may also be detected
 - Value of knowing for affected females
 - Symptom awareness
 - Lack of publicly funded screening means many tests are not even available to be privately purchased, publicly funding the proposed service may improve this.
 - Increased innovation in screening and testing technology
 - Reduced need for higher cost genetic sequencing
 - Reduced diagnostic odyssey
 - Reduced trauma from misdiagnosis and protracted medical assessments
 - Reduces presentations of adrenal crisis associated with X-ALD

- The main disadvantages of public funding received in the consultation feedback included:
 - Inability to predict phenotype from lab tests
 - Potential for a prolonged period of medical follow up
 - Not enough data on risk of screen detected babies to develop classical disease, raising questions on whether screening results overall benefit or harm families
 - Poor family compliance with prolonged medical follow up could lead to poorer than expected outcomes in screen-detected cases
 - False-positives and associated harms
 - No preventative treatment for affected females
 - Additional resources needed for longer term follow up in adult onset disease
 - Uncertainty on how to manage novel variants with uncertain significance in *ABCD1*
 - Currently no treatments for AMN apart from steroid replacement therapy in affected females
 - Potential harms from the knowledge of a rare disease developing later in life
 - Ambiguity around clinical outcomes
 - Transplant does not protect against the manifestation of adrenal insufficiency adult-onset AMN

- Other services identified in the consultation feedback as being needed to be delivered before or after the intervention included:
 - If boys-only model selected, laboratory IT and data entry capacity will need updating
 - Metabolic dietician services

- Inter-jurisdictional policies on process required for paediatric haematopoietic stem cell transplant as this service is not available in all states and territories and Tasmania, ACT and NT do not have metabolic units
- Neurological surveillance may need to be coordinated by neurology teams with periodic input from metabolic teams from other states if no service is available
- Counselling
- Endocrine services
- Haematology services
- Oncology services
- Imaging services
- Neurometabolic services
- Neuropsychologists
- Availability of matched donors for HSCT
- HSCT transplant centres
- Transplant centres
- Potentially gene therapy centres
- Population health program
- Genetic counselling
- Reproductive interventions
- Community Services for out of home care children
- Genetic pathology services
- Scientific curators
- Bioinformatics
- Peer support groups
- Social workers
- Palliative care
- NDIS
- Centrelink support for carers
- Educational material

PASC considered that the consultation feedback provided broad agreement that the main benefit is early detection and treatment. PASC also noted comments about the benefits of genetic knowledge to the families of carrier girls, and for their own knowledge when older.

Indication(s) for the proposed medical service and clinical claim

- The consultation feedback ranged from disagreeing to strongly agreeing with the proposed population(s).

HGSA stated that there is no evidence that the current situation for ALD is variable between states. VCGS agreed, stating that there is no evidence indicating a differing incidence between NBS jurisdictions in Australia. HGSA went on to state that it is undesirable that the current 'post-code lottery' of inequity between state newborn screening panels be perpetuated. Better Access Australia agreed stating that the treatments and support services for these diseases are federally universal, so too must their screening be. GUaRD further echoed this stating that research has shown greater infant mortality in the least privileged in our community.

Australian Genomics stated that if proposing to also screen females, the balance of universal screening and intrusion into the autonomy of an asymptomatic female carrier needs to be considered. VCGS added that if screening is approved for both male and female babies, a controversy is raised around the identification of adult-onset disorders as part of NBS programs and the individual's right to know vs the potential harm caused for patients in waiting.

WSGP agreed with the view that NBS is not warranted in females stating that they are at very low risk for presenting with the forms, CCALD or adrenal insufficiency. They added that diagnosis of the disorder in newborns facilitates cascade testing and identification of affected family members but goes beyond currently accepted criteria for introduction into the NBS program.

Australian Genomics further stated that the benefits of screening need to be carefully balanced with the problem that most people diagnosed on NBS will not develop cerebral disease, and the anxiety that this causes. Better Access Australia stated that they wished to remind the committee that not all cystic fibrosis is early onset, and yet Australia has confidently included this disease in newborn bloodspot screening since the 1980s.

VCGS stated that the reported incidence suggests that the Victorian NBS should detect 2-3 male babies per year. They added that *ABCD1* is now part of most expanded carrier screening panels which is increasingly being taken up by women/couples and expect that incidence will decrease.

WSGP were of the view that there is merit in the Dutch NBS programme for X-ALD where abnormal screening results are only reported in male newborns. They stated that this is because females with ALD-causing *ABCD1* gene lesions are at very low risk for developing the treatable components of CCALD or adrenal insufficiency. They added that there is no specific treatment for adult onset AMN and that screening females introduces the risk of false positives and associated anxiety for families. SA pathology agreed stating that they would only report findings in male newborns.

- The consultation feedback all strongly agreed with the proposed comparator(s).

Cost information for the proposed medical service

- The consultation feedback agreed with the proposed service fee.

Australian Genomics stated that the costs suggested for VLCFA (\$80 AUD) and *ABCD1* sequencing (\$1000 AUD) is an appropriate estimate.

HGSA stated that cost-effectiveness needs to be evaluated specifically for the Australian context and intended screening model. They went on to say that the usual methodology weighs only easily measurable costs and lists other costs.

VCGS stated that cost of screening needs to be reviewed in terms of decision to offer screening to only male babies compared to screening all babies. They added that if only male babies are screened, there will be significant costs for NBS laboratory related process management, staffing, equipment, and IT costs. They went on to say that, if female babies are included, the work will fit into many of the current processes.

WSGP agreed that it is important that the infrastructure required within the NBS programs is adjusted as required to accommodate the increased workload. SA Pathology also agreed.

VCGS added that the cost of the burden for female carriers needs to be assessed by a health economist.

Better Access Australia cautioned that relying on a diagnosis to arrive too late to adequately treat and sustain a life in the long term should not be the basis of the costing of a health intervention.

GUaRD stated that the cost effectiveness for this service is for communities with less physical distance, so may not apply to the Australian context. They suggested a more accurate description would be of high and low estimates to provide an expected range of savings.

WSGP stated that the price of the kit run on instruments in use within the NSW laboratory is pending but is not expected to significantly impact the current newborn screening programme. They added that preventing the associated morbidity and mortality of the condition averts the load. They went on to say that the kit used in the NSW laboratory is also anticipated to facilitate multiplexing with other disorders not currently on the NSW NBS as well as those currently being screened.

Additional comments

Additional comments on the proposed service include:

HGSA stated that benefits and harms from screening will be model-dependent. They also stated that it must be ensured that diagnostic services and treatment monitoring services have capacity to absorb additional workload. Australian Genomics echoed this, stating that, as more individuals with ALD are identified at birth, there will be increasing need for various clinical services. They added that published guidelines recommend expedited referral to an endocrinologist and a schedule for MRI monitoring. They went on to say that international programs have suggested that the initial encounter with the family should be provided by a program with appropriate experience and a multidisciplinary team that can coordinate care.

HGSA stated that there is controversy about the use of a low fat diet in association with Lorenzo's oil in delaying the onset or prevention of potential childhood onset CCALD and that practice varies internationally.

HGSA stated that if any part of the screening algorithm includes differential testing or reporting by gender, then significant IT system changes and improved demographic data entry resource will be required by all 5 screening laboratories. VCGS echoed this, stating that if screening is approved for male babies only, there are significant logistical considerations for the NBS laboratory including a totally separate workflow for samples identified as male. They added that resourcing for testing should be established prior to introduction of NBS.

HGSA stated that there is some concern from the Educational Ethics and Social Issues Committee that X-ALD does not fit the 'screenable condition' criteria; it isn't early severe onset, its time of onset is unpredictable, treatment success is variable, definitive treatment does not really exist and one of the major issues is how to treat heterozygotes.

Australian Genomics stated that collection of long-term outcome data will be essential to demonstrate and improve quality of care of individuals affected by adrenoleukodystrophy. GUaRD agreed stating that research into women's health, social and, psychological experiences is needed.

VCGS stated that their preferred method of testing is to use the two-tier method. They added that they do not support the three-tier process option, stating that they believe it does not add value and is redundant. Better Access Australia agreed that second tier testing will be required, but stated that it would be limited to the very small subset of positive screening tests.

WSGP stated that they plan to use a 3-tier process, incorporating FIA-MS/MS as first tier quantification of the biomarker C26:0-LPC, followed by a second tier 'X-counter' sex determination with male identified newborns proceeding to have *ABCD1* gene sequencing. However, they stated that, should the kit they intend to use not be validated for use with HPLC-MS/MS, they will revert to the 4-tier screening algorithm as used by the Dutch programme.

VCGS recommended that a voice be given to known female carriers of X-ALD in forming the final decision of whether to include screening for female infants. GUARD agreed with this stating that research into lived symptom experience of female carriers needs to be undertaken regardless of age.

GUARD stated that consultation is needed with CALD population groups and First Nations Peoples for their requirements of the NBS process that will align with their values, belief systems and culture.

Better Access Australia stated that there may be benefit in ensuring any positive recommendation made by the committee is fed into the current consultations and design of the new national program for newborn bloodspot screening.

WSGP pointed out that there are risks associated with HSCT such as graft vs host disease (\geq grade 2) and that the long-term follow up mortality rate is ~5% in early treated cases presenting asymptotically. They added that HSCT stabilisation of cerebral disease doesn't commence until 3-24 months after infusion, and that symptoms can progress during this lag interval.

Health Professionals

The comments from the health professional were in support of the public funding for newborn bloodspot screening for X-linked adrenoleukodystrophy. The health professional was a medical professional with experience in metabolic disorders including NBS.

They stated that NBS would allow males with X-ALD to be monitored by MRI and, for those developing the cerebral form, to have HSCT early. They went on to state that NBS diagnosis allows monitoring for, and early recognition of, adrenal insufficiency and cerebral X-ALD, which are potentially fatal. They stated that some males affected with cerebral X-ALD have been diagnosed too late for successful HSCT.

They added that a further advantage to the proposed service is the genetic knowledge for the family. They went on to say that cascade testing of the family would allow males with the gene to be appropriately followed and would allow both males and females with the gene to make informed decisions about reproduction. Further, they added that for females identified by the NBS, genetic knowledge is beneficial for them and their families as females have a high risk of adrenomyeloneuropathy (AMN).

Further benefits to public funding of the proposed service identified by the health professional are that hemizygous males who do not develop the cerebral form and heterozygous females are at high risk of developing AMN and that knowing they have the gene may enable earlier treatment and better outcomes. They added that by the time these individuals reach adult age there is a reasonable chance that therapy may be available for AMN.

The health professional did state that some disadvantages to the proposed service, mainly that the majority of males with X-ALD detected by NBS will not develop the cerebral form, and therefore will not need HSCT. They went on to say that it is not possible at birth to determine which affected males develop the cerebral form, and that, in some families, one male may develop the cerebral form and another the late onset form. They added that biochemical testing is also currently not helpful with predicting clinical

course, and that the psychological impact of prolonged testing on the male and their family needs to be considered.

Further disadvantages to the proposed service identified by the health professional are that males who have presymptomatic HSCT for the cerebral form of X-ALD have about a 5% risk of dying from the procedure and that there are associated morbidities with HSCT. They also stated that females detected by NBS are not at risk of the cerebral form, Addison's disease is rare in females, and they are at high risk for developing AMN later in life, so would likely have decades of follow up before this develops.

The health professional stated that they agreed with the population and believed all babies should be screened. They added that males would have a greater benefit as they are at risk of the cerebral form of X-ALD, however, they added that the genetic knowledge to the family and the patient would be beneficial to female babies. They agreed that the comparator for the proposed service is no screening.

They stated that not all associated interventions had been adequately captured in the nomination form.

They noted that follow up brain MRI should be performed for affected males. Current

recommendations are to start at 12 -18 months with 6 monthly MRI until 12 years of age and then yearly after that. A new protocol for MRI surveillance of boys with X-ALD detected by NBS was published in 2021³. It recommends starting at 12 or 18 months with a second MRI 12 months later, then 6th monthly from 3-12 years and then yearly. They noted that MRI scans in young children need a general anaesthetic which limits how often they are performed. Current protocols recommend MRI every 6 months which means that changes may have progressed for up to 5 months before they are detected.

They also noted the following associated interventions:

- There is a potential improvement to surveillance using biomarkers to detect neuroinflammation of cerebral X-ALD

Other services identified by the health professional were:

- Psychological counselling for patients and families
- Genetic counselling for patients and families
- Psychological support for those with the gene
- Cascade genetic testing of families

Jurisdictional experts

Two jurisdictional X-ALD experts were approached for targeted consultation feedback.

When asked about the feasibility of implementing either 2-tier or 3-tier testing the first representative stated that a 3-tier screening strategy would be the preferred method as it affords 1st tier high through-put analysis by FIA-MS/MS followed by a 2nd tier LC-MS/MS for confirmatory to exclude false positives from the 1st tier. They further stated that using an FIA-MS/MS allows incorporating into existing routine methods used for inborn errors of metabolism (IEM) by amino acids and acylcarnitines and that this can be

³ Mallack, E., Turk, B., Yan, H., *et al.*, 2021. MRI surveillance of boys with X-linked adrenoleukodystrophy identified by newborn screening: Meta-analysis and consensus guidelines. *Journal of Inherited Metabolic Disease*, 44(3), pp.728-739.

done with minimal effort nor disruption of existing methods. They also added that using a 1st tier LC-MS/MS method will consume time on a single MS/MS instrument as analysis time is ~5-10 minutes as sample compared to FIA that takes ~1 minute per sample and can be combined with the determination of both AA & ACP in a single analysis event.

They further stated that this FIA-MS/MS followed by LC-MS/MS method is utilised for the screening of other conditions such as congenital adrenal hyperplasia (CAH) measuring 17OHP by an immunoassay (IA) followed by LC-MS/MS on those identified above the IA cut-off. Finally, they clarified that a 3rd tier of reflexing to *ABCD1* variant analysis using the initial blood-spot will be required.

The second Jurisdictional expert agreed stating that using LC-MS/MS as second tier test has been currently applied in NBS for some analytes like for screening of methylmalonic acidaemia, the high C3-carnitine in FIA-MS/MS will be followed by MMA measurement in LC-MS/MS.

In response to if there is consensus on the feasibility and desirability of including an 'X-counter' test, the first jurisdictional expert stated that the consensus is that an 'X-Chromosome' counter to vet out females is highly preferred and would be what they would implement. They added that this is based on the X-ALD disease course in females, although adding an additional tier at a cost, but only needs to be applied to those that have an elevated C26:0-LPC in the 1st tier.

When asked about the timeframe for implementation if X-ALD screening was recommended, the first jurisdictional expert stated that, given the technology is already in place for other newborn screening, the addition of the measurement of C26:0-LPC and the stable isotope of d4-C26:0-LPC into the existing FIA method isn't going to be difficult and relatively straight forward. They added, though, that the LC-MS/MS method will need work-up but should not be difficult for any of the newborn bloodspot screening labs in Australia.

When asked what the appropriate confirmatory diagnostic test is following screen positivity, the first jurisdictional expert stated that the diagnostic workup once the baby has been recalled will follow standard metabolic clinic procedures that will include plasma VLCFA by GC-FID, the current standard for the diagnosis of PD, whole exome sequencing and if required whole genome sequencing.

The second jurisdictional expert did not provide comment in regard to the questions concerning the inclusion of an 'X-Counter' or the timeframes for implementation of X-ALD screening should it be recommended. However, they did state that the mainstay of treatment for XALD is targeting on the childhood cerebral ALD (CCALD) and adrenal insufficiency. They added that HSCT has shown to significantly reduce the progression of CCALD if performed prior to the onset of neurodegeneration of myelination but noted that paediatric HSCT is not available in all states.

They went on to state that currently, paediatric patients in SA need to go interstate for HSCT, so, the resources implication on the treatment will be across the states.

The second jurisdictional expert also stated that treatment of the adrenal insufficiency is easily performed with administration of oral glucocorticoid.

Appendix A NBS National Policy Framework Criteria

NBS National Policy Framework Criteria

The condition

1. The condition should be a serious health problem that leads to significant morbidity or mortality.

1.1 What data are there on the incidence of the condition, including in the Australian population? How is this incidence determined—through screening studies, international programs, cases identified clinically, modelled estimates based on data from variant databases or some other means? Are there any known differences in incidence in Australian sub-populations?

1.2 What is the burden of disease associated with the condition, including morbidity and mortality? Does the burden of disease vary between individuals?

2. There should be a benefit to conducting screening in the newborn period.

- While the benefit to the baby must always be the first consideration, for some conditions a benefit for the family and/or community, as well as the benefit to the baby, may also be important and warrant consideration. This might include benefits to the family for conditions where there is currently no intervention and which will be likely to lead to early mortality but where a definitive diagnosis might be aided by a screening test.

2.1 What are the known health benefits from early detection that exist, or can be achieved, through screening for the condition? This may include early intervention, prevention of symptoms or reduction in condition severity.

2.2 Why is screening for this condition during the newborn period the most beneficial method of early detection?

2.3 Does detection of this condition provide families with actionable information that assists them in making informed choices about reproduction in the future?

2.4 What emotional or social benefits does early detection provide?

2.5 What harms may arise from screening for the condition in the newborn period?

3. The natural history of the condition, including development from latent to declared disease, should be adequately understood.

- 3.1 What information is known on the natural history of the condition in Australia or comparable international populations?
- 3.2 When would the condition usually be detected clinically?
- 3.3 Explore the current knowledge of penetrance of the condition. Are there known benign or milder late-onset forms?

4. There should be a suitable test protocol to identify the presence of the condition.

- 4.1 What test protocols could be used to identify the presence of the condition? Is there consensus on the most appropriate test protocol?
- 4.2 When considering the test protocol, what is the clinical and analytic validity based on a consideration of:
 - Sensitivity;
 - Specificity;
 - False positive rate;
 - False negative rate;
 - Positive predictive value;
 - Negative predicative value.
- 4.3 Is the test protocol simple and reliable?
- 4.4 Can the test protocol be performed on the available dried bloodspot?
- 4.5 Can the test be multiplexed within existing newborn bloodspot screening panels?
- 4.6 What is the cost of the test protocol?
- 4.7 Will genetic testing be used as part of the test protocol? If genetic testing is needed:
 - Will this be by common mutations or sequencing?
 - Which mutations would be tested?
 - What is the penetrance of the mutations?
 - Are there variants of uncertain significance?

5. The test protocol should, on balance, be socially and ethically acceptable to health professionals and the public.

5.1 Can the test protocol detect other conditions of clinical or unknown significance and/or carriers and, if so, what are the implications?

5.2 What are the potential benefits and harms associated with the preferred test protocol(s)?

The Intervention

6. Health care services for diagnosis and management should be available so that these services can be offered if there is an abnormal screening result.

6.1 What health care services are currently involved in the diagnosis and ongoing management of the condition?

6.2 What impact would screening for the condition have on the health care services that would be required to support diagnosis and management following an abnormal screening result?

6.3 Is diagnostic testing readily available and reliable?

6.4 Do current health care services have capacity to support the diagnosis and ongoing management of the condition?

6.5 Are current health care services of sufficient quality to support the diagnosis and ongoing management of this condition?

6.6 Is there equitable access to these health care services for families, including those from rural and remote areas?

7. There should be an accepted intervention for those diagnosed with the condition.

7.1 What accepted intervention(s) is (are) available for newborns that receive an early diagnosis through screening?

7.2 How well is the intervention and treatment pathway understood? Is there agreement on when intervention is required?

7.3 How effective is the intervention? Does it alleviate the symptoms of the condition or slow or halt its progression? What influence does the intervention have on quality and length of life?

7.4 How urgent is the intervention? Does the intervention need to be initiated before symptoms of the condition present?

7.5 Is the intervention readily available and accessible?

NBS National Policy Framework Criteria

7.6 What are the potential harms associated with the intervention, and to what extent can these harms be mitigated or managed?

7.7 What is the cost of the intervention? What costs will be incurred for the diagnosis, management, and treatment of conditions, including the costs for false positives?

7.8 Is there equitable access to the intervention for families, including those from rural and remote areas?

Additional considerations

8. The benefit of screening a condition must be weighed against its impact on the program as a whole.

8.1 Can screening for this condition be achieved within the current screening pathway?

8.2 Is the addition of this condition likely to require ethical considerations that may warrant a separate consent process?

8.3 Would it be likely that screening for the condition would impact negatively upon other elements of the program? For example, could it be anticipated that participation rates might fall?

8.4 Are there any additional costs, such as the purchasing of new technology or training, which are associated with screening for this condition?

8.5 What is the economic impact of excluding/including the condition? Do benefits exceed costs? Is it cost-effective to screen? It may be necessary for a detailed economic evaluation to consider these questions and other relevant economic issues.

9. What other information relevant to decision making should be considered that has not been captured elsewhere?

References

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