Australian Government

Department of Health

RATIFIED PICO 1628

Application 1628:

Alpha-1-Antitrypsin Genotyping

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

Intervention 1

Component	Description
Patients	All patients with: a. an abnormally low (<20 µmol/L) serum alpha-1 antitrypsin (AAT) concentration and: • early-onset (<40 years) chronic obstructive pulmonary disease (COPD) (emphysema, persistent airflow obstruction and/or chronic bronchitis) or adult-onset asthma OR • emphysema in the absence of a risk factor (e.g., being a non-smoker), with prominent basilar changes on a chest x-ray, or in the presence of liver or skin disease OR • liver disease at any age, including obstructive jaundice in infancy (perinatal jaundice, cirrhosis, necrotising panniculitis or unexplained liver disease) OR • anti-proteinase 3-positive vasculitis OR b. a family history of AAT deficiency, defined as a relative with an identified pathogenic or likely pathogenic variant in the *SERPINA1* gene
Prior tests	Measurement of serum concentrations of AAT and C-reactive protein (Medicare Benefits Schedule [MBS] items 66635 and 66500)
Intervention	Genotyping of the *SERPINA1* gene for at least 14 of the most common pathogenic variants
Comparator	AAT protein phenotyping with isoelectric focussing (IEF) (MBS item 66638)
Outcomes	Primary effectiveness outcomes: • diagnostic accuracy • clinical utility (for probands and their first-degree relatives) • overall reduction in time to diagnosis and need for other tests Safety outcomes: • psychological, physical or other harms (e.g. inability to qualify for life or disability insurance) from testing/not testing • psychological, physical or other harms related to false-positive, false-negative or inconclusive test results

Component	Description
	Healthcare system outcomes: • number of individuals eligible for genotyping • number of additional tests needed or avoided because of genotyping • cost of genotyping • cost of disease progression in false negatives • cost of monitoring disease progression and ongoing treatment in individuals identified with AAT deficiency or an increased risk of disease • cost savings from illness and care episodes averted in probands and their first-degree relatives • cost of ongoing treatment and care for patients with increased life expectancy as a result of genotyping

Intervention 2

Component	Description
Patients	Patients with a clinical suspicion of AAT deficiency, as described for Intervention 1, and for whom genotyping using Intervention 1 has not identified a pathogenic variant
Prior tests	Measurement of serum concentrations of AAT and C-reactive protein (Medicare Benefits Schedule [MBS] items 66635 and 66500) *Intervention 1 (see previous table)*: Genotyping of the *SERPINA1* gene for at least 14 of the most common pathogenic variants
Intervention	Sequencing of all protein-coding exons of the *SERPINA1* gene
Comparator	Not applicable
Outcomes	Primary effectiveness outcomes: • diagnostic accuracy • clinical utility (for probands and their first-degree relatives) • overall reduction in time to diagnosis and need for other tests Safety outcomes: • psychological, physical or other harms (e.g. inability to qualify for life or disability insurance) from testing/not testing • psychological, physical or other harms related to false-negative test results

Component	Description
	Healthcare system outcomes: • number of patients eligible for gene sequencing • number of additional tests needed or avoided because of gene sequencing • cost of gene sequencing • cost of disease progression in false negatives • cost of monitoring disease progression and ongoing treatment in patients identified with AAT deficiency or an increased risk of disease • cost savings from illness and care episodes averted in probands and their first-degree relatives • cost of ongoing treatment and care for patients with increased life expectancy as a result of gene sequencing

PICO or PPICO rationale for therapeutic and investigative medical services only

Population

Alpha-1-antitrypsin (AAT) deficiency is a rare genetic condition characterised by low serum concentrations of AAT, an acute-phase glycoprotein of the serpin family. AAT is mainly produced in the liver (over 90%), with some local production by macrophages and bronchial epithelial cells. AAT inhibits a variety of serine proteases including trypsin and chymotrypsin, but its main physiological target is the proteolytic enzyme elastase. When the lungs are exposed to inflammatory substances, such as pollutants, germs, dust or tobacco smoke, elastase is released from neutrophils to digest the harmful contaminants. Any surplus elastase is then neutralised by AAT before it acts on lung tissue. AAT provides over 90% of the defence against neutrophil elastase. Serum concentration of AAT ranges from 19 to 47 µmol/L in a healthy individual, but increases three- to five-fold in response to inflammation or tissue injury. The minimum serum concentration of AAT considered necessary to preserve lung function is 11 µmol/L (Brantly et al. 2020; Brode, Ling & Chapman 2012; Franciosi, Carroll & McElvaney 2019; Hazari et al. 2017; Marciniuk et al. 2012; Stoller, JK & Aboussouan 2012).

AAT deficiency arises from variants in the *SERPINA1* gene on chromosome 14 and is inherited in a codominant manner. Approximately one in nine Australians have at least one AAT deficiency allele (de Serres, Blanco & Fernandez-Bustillo 2003). Coding errors can result in a dysfunctional form of AAT or low serum concentrations of functional protein, leading to mild or severe AAT deficiency. Over time, uncontrolled neutrophil elastase activity causes the lung alveoli walls to be digested more quickly than normal, often leading to early-onset (40 to 50 years of age) chronic obstructive pulmonary disease (COPD) and accelerated emphysema progression, particularly in individuals who smoke. In addition, abnormally formed AAT cannot be secreted and accumulates in the liver cells, causing hepatitis, cirrhosis and, sometimes, liver cancer. In severe cases, liver transplantation is required. Other less common complications of AAT deficiency include necrotising panniculitis (approximately 1 per 1,000 AAT-

deficient individuals), granulomatosis with polyangiitis (Wegener's granulomatosis) and vasculitis (Hazari et al. 2017; Lascano & Campos 2017; Lung Foundation Australia 2017; Marciniuk et al. 2012; Newell, Donahue & Hogarth 2019).

The most common cause of death in patients with AAT deficiency is respiratory failure (45–72% of deaths), followed by liver cirrhosis (10–13% of deaths). The overall yearly mortality rate in AAT-deficient individuals ranges from 1.7–3.5% (Stoller, JK & Aboussouan 2012). The development of symptoms, and the age at which they become noticeable, can vary widely and is heavily influenced by environmental factors such as tobacco smoking and exposure to air pollutants. Common early symptoms of AAT deficiency include shortness of breath (84%), wheezing with (76%) or without (65%) upper respiratory tract infection, usual phlegm (46%), chronic cough (42%), decreased exercise tolerance, asthma unresponsive to treatment, abdominal swelling, abnormal liver function tests, enlarged liver or spleen and jaundice (McElvaney et al. 1997; Newell, Donahue & Hogarth 2019; Stoller, JK & Aboussouan 2012). It is estimated that 1–2% of all patients with COPD have severe AAT deficiency, with up to 10% exhibiting some degree of protein deficiency (Lascano & Campos 2017). Among patients with poorly controlled asthma, AAT deficiency is present in at least 2% and 11% are carriers of a deficiency variant (Gramegna et al. 2018).

AAT deficiency-related liver disease displays a biphasic pattern, with a first peak in early childhood (≤5 years) and a second peak at age 50–65 years. Clinically significant liver fibrosis occurs in 20–36% of severely AAT-deficient individuals. In children with AAT deficiency-related liver disease, 7% will develop liver cirrhosis and 17% will require a liver transplant. Of those surviving to adulthood, 11% will have liver cirrhosis and 15% will require transplantation. Risk factors for developing advanced liver fibrosis in the presence of AAT deficiency include male sex, age over 50 years and presence of metabolic syndromes (diabetes, hypertension) or obesity (Bouchecareilh 2020; Strnad, McElvaney & Lomas 2020; Townsend et al. 2018).

The proposed genetic variant panel test and gene sequencing are designed to identify AAT deficiency variants in the DNA of patients who have symptoms suggestive of AAT deficiency and abnormally low (<20 µmol/L) AAT serum concentrations or a demonstrated family history of AAT deficiency (cascade testing).

PASC advised that cascade testing should not be restricted to first-degree family members, but rather should be explicitly extended to include testing of other family members in recognition that clinical utility is expected to be greater for individuals identified through predictive genetic (cascade) testing than for affected individuals confirmed through diagnostic genetic testing.

PASC also advised that the cascade testing populations be extended to include reproductive partners of relatives who test positive, and testing of a fetus, particularly in families with paediatric-age disease onset where liver transplantation is a potential management. PASC asked the Department to further develop options out of session for additional proposed MBS items as might be considered necessary for these additional cascade populations. The applicant noted that although they did not consider these

additional populations to be a priority for testing, they would consider any proposed item descriptors put forward by the Department.

Rationale

The common AAT variants are designated M (medium), S (slow) and Z (very slow), which correlate with the phenotype of the expressed proteins (labelled PI* for protease inhibitor) and their migration speed on an electrophoresis gel. M is the most common allele, present in 80-90% of individuals and encoding a normal AAT protein (PI*MM). The most common deficiency variants—S and Z—result in proteins with structural and functional abnormalities. Thus, the most common genotypes resulting from the combination of these allelic forms are as follows: homozygous wild type PI*MM; heterozygous PI*MZ and PI*MS forms; and semi-deficient and deficient phenotypes PI*SZ, PI*SS and PI*ZZ. In addition to these variants, there are about 50 other infrequent allelic forms that occur in up to 0.7% of the population, including null alleles that produce no AAT and alleles that produce normal quantities of dysfunctional protein (Table 1) (Ferrarotti et al. 2012; Gramegna et al. 2018; Marciniuk et al. 2012; Sandhaus et al. 2016).

The MM, MS and MZ genotypes produce adequate quantities of AAT to protect the lungs, and S alleles are usually only of clinical significance when combined with another pathogenic allele (e.g. SZ) and the serum AAT concentration is below the protective threshold (<30% of normal). In contrast, protein expression in the ZZ genotype, which is the cause of 95% of severe AAT deficiency, is generally 10–20% of that produced by the MM genotype (Table 1) (Siri, Farah & Hogarth 2013; Strnad, McElvaney & Lomas 2020).

Severe AAT deficiency is undiagnosed in approximately 90% of cases because the symptoms are often indistinguishable from asthma and nonhereditary emphysema. The interval from symptom onset to diagnosis ranges from 5 to 7 years, and the average age at diagnosis is 46 years; 30% of patients are diagnosed after 50 years of age. Consequently, severe AAT is often diagnosed at a point when the patient has already developed significant loss of respiratory function and irreversible lung damage (Miravitlles et al. 2017; Siri, Farah & Hogarth 2013; Strnad, McElvaney & Lomas 2020).

Table 1: Serum AAT concentrations in relation to genotype and disease risk

Genotype (% Australian population)	% of normal genotype AAT concentration	Median serum AAT concentration in µmol/L (5^{th}–95^{th} percentile)	Risk of lung disease	Risk of liver disease
MM	100% Normal	27 (19–47)	No increased risk	No increased risk
MS (8%)	85%	23 (16–40)	No increased risk	No increased risk
MZ (2.5%)	61% Low to normal	17 (11–28)	Risk only increased in smokers or those with environmental exposure	Slight increased risk

Genotype (% Australian population)	% of normal genotype AAT concentration	Median serum AAT concentration in µmol/L (5^{th}–95^{th} percentile)	Risk of lung disease	Risk of liver disease
SS (0.2%)	65% Borderline normal to low	18 (8–28)	No increased risk	No increased risk
SZ (0.1%)	42% Low	11 (6–20)	20%–50% Increased risk in the 11% with serum concentration 11 µmol/L[a] who smoke	Slight increased risk
ZZ (0.02%)	<20% Very low	≤5 (≤5–10)	High risk 80%–100%	High risk
Null-null (rare)	0%	0	High risk 100%	No increased risk

[a]A serum AAT concentration ≤11 µmol/L is associated with increased risk for emphysema

AAT: alpha-1 antitrypsin

Shading represents AAT deficiency of clinical significance

Source: Craig & Henao 2018; de Serres, Blanco & Fernandez-Bustillo 2003; Kueppers & Sanders 2017; Stoller, Hupertz & Aboussouan 2020

PASC advised that the units of concentration used should be consistently presented in SI units throughout the document (i.e. µmol/L only, and not also mg/dL). These updates were made throughout the document for consistency.

Although 60–80% of patients with the ZZ genotype develop early-onset emphysema, those who smoke have significantly lower survival rates than their non-smoking counterparts and generally die up to 20 years sooner. In contrast, never-smoking asymptomatic ZZ homozygotes identified through probands have a normal life expectancy, as do never-smoking MZ and SZ heterozygotes. The risk of death from liver disease among ZZ genotypes is 2–3% in childhood, while clinically significant liver fibrosis is present in 20–36% of adults (Siri, Farah & Hogarth 2013; Stoller, JK & Aboussouan 2012; Strnad, McElvaney & Lomas 2020).

The initial clinical evaluation of patients diagnosed with AAT deficiency focuses on early detection and follow-up of associated conditions such as emphysema, bronchiectasis and liver disease. Treatment for AAT deficiency-related lung disease is the same as treatment for COPD. There is no specific treatment for AAT deficiency-associated liver disease other than preventing further liver injury. A liver transplant may be required for severe AAT deficiency-related liver disease (Bouchecareilh 2020; Newell, Donahue & Hogarth 2019).

Since averting irreversible lung or liver damage is the cornerstone of managing AAT deficiency, early diagnosis is essential. Smoking cessation is central for all forms of AAT deficiency, in addition to other lifestyle changes such as avoidance of environmental exposure to air pollutants, seeking appropriate immunisation against influenza, hepatitis and pneumonia, limiting alcohol intake and maintaining a normal body mass index. Early diagnosis also provides the opportunity for genetic counselling on

reproductive choices, optimising current therapies and monitoring disease progression (Miravitlles et al. 2017; Newell, Donahue & Hogarth 2019; Strnad, McElvaney & Lomas 2020). Identification of AAT deficiency at birth is associated with a lower rate of smoking initiation, and smokers who are aware of their AAT deficiency and the risk factors for progressive disease are more likely to attempt and succeed in smoking cessation than are those who are not AAT deficient (Carpenter et al. 2007; Sandhaus et al. 2016).

The application proposed the following criteria for selecting patients, which appear to be based on recommendations by the American Thoracic Society and the European Respiratory Society (American Thoracic & European Respiratory 2003; Miravitlles et al. 2017):

- early-onset (<40 years) COPD (emphysema, persistent airflow obstruction and/or chronic bronchitis) or adult-onset asthma
- emphysema in the absence of a risk factor (e.g., being a non-smoker)
- emphysema with prominent basilar changes on a chest x-ray
- a family history of AAT deficiency, COPD, emphysema, bronchiectasis, liver disease or panniculitis
- liver disease at any age, including obstructive jaundice in infancy (perinatal jaundice, cirrhosis, necrotising panniculitis or unexplained liver disease)
- anti-proteinase 3-positive vasculitis.

These recommendations generally align with a recently published position statement from the Thoracic Society of Australia and New Zealand (Dummer et al. 2020), which recommends testing for AAT deficiency in the following groups:

- all patients with chronic airflow obstruction (the Australian COPD-X Plan recommends testing in all patients with COPD younger than 40 years of age (Yang et al. 2020))
- patients with asthma and persistent airflow limitation
- patients with emphysema disproportionate to their smoking history or in the presence of liver or skin disease.

The applicant suggested that the number of services provided by MBS item 66638 (qualitative phenotyping with IEF) may be indicative of the number of patients likely to access AAT genotyping. A relatively steady number of services were provided from 2014 to 2019, averaging 1,655 tests annually (Australian Government 2020), although it is not possible to distinguish the proportion of services attributed to repeat or cascade testing—first-degree relatives of AAT-deficient probands also require testing.

PASC noted that the number of patients in the main population proposed for targeted genotype testing who would likely need to go on to gene sequencing is difficult to determine due to the variation reported across different studies, but considered that the numbers are likely to be low in any case because the main predictor of high diagnostic yield in this population is a low serum concentration of AAT. The applicant agreed with the PASC, noting the numbers are likely to be very low.

PASC confirmed this proposed population for targeted genotype testing, which encompasses patients of all ages and not solely adults, but noted that it needs to be identified fully and matched to the proposed MBS item descriptor(s), including:

a. *as the main aspect of defining this population, setting the serum AAT concentration threshold for eligibility at <20 µmol/L rather than 11 µmol/L (without any need to adjust this, e.g. in the presence of abnormal CRP);*
b. *then also including other sources of relevant AAT deficiency signs and symptoms used to trigger the decision to test serum AAT beyond the respiratory symptoms already identified, including those relating to the liver, skin and vascular system (see details in PASC Outcome under 'Proposed item descriptor' below)*

Prior test

AAT deficiency is usually diagnosed via a multistep testing process, although there is no single universally accepted laboratory algorithm. According to current recommendations, quantitative serum AAT measurement, usually by nephelometry, is the initial step (MBS item 66635) (Torres-Durán et al. 2018). This is accompanied by measurement of serum C-reactive protein (MBS item 66500), since concentrations of serum AAT can be transiently elevated during infection or inflammation (Strnad, McElvaney & Lomas 2020). Serum AAT concentration above 19 µmol/L is considered to indicate absence of the ZZ genotype (99.8% negative predictive value) (Greulich et al. 2017). However, quantitative AAT measurement alone is not recommended because it does not fully characterise disease risk, particularly when AAT is present but dysfunctional. Complementary phenotyping or targeted genotyping is recommended for individuals with low serum concentrations of AAT in the presence of normal C-reactive protein concentrations or other cases where AAT variation is suspected (Dummer et al. 2020; Torres-Durán et al. 2018).

Protein phenotype characterisation uses IEF to determine the type of AAT protein present in the blood (MBS item 66638). IEF separates molecules based on their migration pattern (isoelectric point) across a polyacrylamide gel in the presence of an electric field and a pH gradient (Pergande & Cologna 2017). *SERPINA1* is a polymorphic gene with more than 150 variants, many of which lead to amino acid substitutions affecting the protein's mobility in gel electrophoresis. The AAT protein variants are designated with letters A (faster) to Z (slower) depending on their mobility relative to the most common (normal) variant, M (Bouchecareilh 2020; Marciniuk et al. 2012; Miravitlles et al. 2017).

Intervention

There are two interventions relevant to this application:

1. Gene panel test for identifying 14 specific variants of the *SERPINA1* gene
2. Gene sequencing of the *SERPINA1* protein-coding exons.

PASC queried whether it would be more appropriate for the targeted genotyping intervention via item AAAA to test for only the S and Z variants (anticipated to identify 95% of patients with a pathogenic variant), rather than 14 variants (anticipated to identify 99% of patients with a pathogenic variant),

noting that it would be cheaper to test for two variants, but a greater proportion of patients would then require gene sequencing via item BBBB. Comparison between two- and 14-variant options for intervention 1 is to be included in the DCAR to enable a more formal assessment of the more cost-effective alternative, also based on the different fee options for item AAAA ($100 for the 14-variant option and $78 for only the S and Z variants).

The applicant advised that their strong preference is for the more comprehensive 14-gene panel, which would identify up to 99% of AATD patients without the need for the more expensive gene sequencing option (BBBB). However, if the results of sensitivity analyses in the cost-effectiveness analysis supports testing for only the S and Z variants, with negative patients undergoing gene sequencing of SERPINA1, then the applicant would find this an acceptable option.

Targeted gene panel test

The applicant proposed a 14-variant panel test similar to the AAT Genotyping Test manufactured by Progenika Biopharma, S.A. (Spain). The AAT Genotyping Test, which is based on polymerase chain reaction (PCR) methods, uses DNA extracted from blood (whole or dried blood spot) or saliva samples to detect 14 of the most common clinically significant variants (including S and Z) in the *SERPINA1* gene. Target DNA sequences are amplified and biotinylated using real-time PCR and then hybridised to oligonucleotide primers. The hybridised DNA is then labelled with fluorescent probes to allow detection and quantification of the PCR product and determination of the genotype.

The various genotypes that include PI*S and PI*Z alleles (PI*M/PI*S, PI*M/PI*Z, PI*S/PI*Z, PI*S/PI*S and PI*Z/PI*Z) cover more than 95% of variant alleles identified in the *SERPINA1* gene (Ottaviani S et al. 2017; United States Food and Drug Administration 2017, 2019; Veith et al. 2019). The applicant claims that a gene panel test identifying the 14 most prevalent known AAT deficiency variants will identify approximately 99% of affected individuals (Table 2).

Table 2: Clinical relevance of the allelic variants and their associated alleles included in the proposed gene panel test

Allelic variant	Most common associated allele	AAT protein activity	Frequency among individuals with AAT
c.187C>T	PI*I	Impaired secretion and mild plasma deficiency	<0.001% (heterozygous)
c.194T>C	PI*M procida	Impaired secretion (degradation) and severe plasma deficiency	<0.001% (heterozygous)
c.226_228delTTC	PI*M malton	Impaired secretion (polymerization) and severe plasma deficiency	<0.0001% (PI*M malton carriers)
	PI*M palermo		
	PI*M nichinan		
c.230C>T	PI*S iiyama	Impaired secretion (polymerization) and severe plasma deficiency	Unknown
c.552delC	PI*Q0 granite falls	None (no protein)	<0.001% (carriers)

Allelic variant	Most common associated allele	AAT protein activity	Frequency among individuals with AAT
c.646+1G>T	PI* Q0 west	Truncated protein/intracellular degradation	Unknown
c.721A>T	PI*Q0 bellingham	None (no protein)	<0.001% (heterozygous)
c.739C>T	PI*F	Impaired secretion and mild plasma deficiency	<0.001% (heterozygous)
c.839A>T	PI*P lowell	Impaired secretion (degradation) and mild plasma deficiency	<0.001% (PI*P lowell heterozygous)
	PI*P duarte		
	PI*Q0 cardiff	Truncated protein/intracellular degradation	
	PI*Y barcelona	Impaired secretion (degradation) and mild plasma deficiency	
c.863A>T	PI*S	Impaired secretion (degradation) and mild plasma deficiency	5-10% (carriers)
c.1096G>A	PI*Z	Impaired secretion (polymerization) and severe plasma deficiency	1-3% (carriers)
c.1130dupT	PI*Q0 mattawa	Truncated protein/intracellular degradation	<0.001% (PI*Q0 mattawa carriers)
	PI*Q ourem		
c.1158dupC	PI*Q0 clayton	Truncated protein/intracellular degradation	<0.001% (PI*Q0 clayton heterozygous)
	PI*Q0 saarbruecken		
c.1178C>T	PI*M heerlen	Impaired secretion (degradation) and severe plasma deficiency	<0.001% (heterozygous)

AAT: alpha-1 antitrypsin

Source: United States Food and Drug Administration 2017; Veith et al. 2019

The AAT 14-variant panel is a once per lifetime test, the results of which will be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of individuals with AAT deficiency. Required blood or saliva samples are drawn by a registered medical practitioner (respiratory specialist or consultant physician) via a finger stick or a buccal swab (Lascano & Campos 2017). Samples are tested and interpreted by approved practising pathologists or medical scientists in pathology laboratories accredited by the National Association of Testing Authorities (NATA).

The applicant advised that while non-commercial in vitro diagnostic tests must be regulated, they do not need to be listed on the ARTG. The proposed gene panel test falls into this category so does not require ARTG listing. In Australia, the Royal College of Pathologists Australasia (RCPA) and NATA oversee the regulation of genetic testing for clinical purposes and accreditation of pathology laboratories. Any RCPA/NATA-accredited laboratory can provide equivalent variant analysis services to a minimum standard and is not required to use the reagents, equipment or analysis pipelines of a specific manufacturer.

PASC noted that the 14-variant 'gene panel' manufactured by Progenika is not available in Australia, and that Australian laboratories will need to develop their own panels for testing.

PASC noted that, if laboratories developed their own panels, they would need a positive control for each variant, some of which are very rare; otherwise, all positive samples would require subsequent sequencing to confirm the presence and identity of the variant.

Following a positive diagnosis, it is recommended that parents, children and siblings of the proband be offered genetic testing (Table 3). In the reproductive setting, testing should also be discussed with partners of individuals who are homozygous or heterozygous for AAT deficiency (Marciniuk et al. 2012; Miravitlles et al. 2017). The results of genetic tests may require genetic counselling, although there is currently no MBS rebate for this service. Extensive follow-up of lung or liver function may be required depending on the patient's AAT genotype profile.

Table 3: Chances of individuals passing on AAT deficiency to their children

Genotype	Partner Normal	Partner Carrier
Patient with AAT deficiency	All children will be carriers No children will have AAT deficiency	For each child there is a 1 in 2 chance they will be a carrier or have AAT deficiency
Patient carrier	For each child there is a 1 in 2 chance they will be a carrier. No children will have AAT deficiency.	For each child there is a 1 in 2 chance they will be a carrier. For each child there is a 1 in 4 chance they will have AAT deficiency.

AAT: alpha-1 antitrypsin

Source: American Thoracic & European Respiratory 2003; Miravitlles et al. 2017; Service c2020

Gene sequencing

The applicant stated that if the results from AAT genotyping are inconclusive, sequencing of the *SERPINA1* gene should be conducted. This involves sequencing the protein coding exons of the *SERPINA1* gene (exons II, III, IV and V) using PCR amplification followed by cycle sequencing of the PCR products.

Rationale

The current stepwise approach to diagnosing AAT deficiency is time consuming, and each step has its own intrinsic limitations. Quantitative measurement of serum AAT concentrations cannot discriminate between different genotypes, and values may be falsely elevated in the presence of infection, cancer, thyroid disorders and other inflammation, as well as by oral contraceptives, vaccinations, pregnancy and stress (Marciniuk et al. 2012; Sandhaus et al. 2016; Siri, Farah & Hogarth 2013). Thus, serum AAT concentrations can be used to separate severely deficient subtypes (ZZ, Z-null, Null-null and most SZ) from intermediate subtypes (MZ, MS and M-null) and wild types (MM), but are insufficient to definitively diagnose intermediate deficiency due to M heterozygosity. Serum AAT concentrations must be supplemented by qualitative tests to identify the genetic cause of the AAT deficiency (Miravitlles et al. 2017).

IEF is a time-consuming and difficult technique that requires specific expertise, and it cannot be batch processed like genotype tests. Also, IEF can only identify a limited number of protein phenotypes. It is unable to detect genetic variants that result in the complete absence of AAT, some of the rarer M-like variants and heterozygous carriers with normal or near normal serum concentrations of AAT (Lascano & Campos 2017; Maltais et al. 2018; Marciniuk et al. 2012).

Targeted genotyping identifies previously defined *SERPINA1* variants by PCR using primers specific to known genetic variants. Although highly specific and easy to interpret, commercially available targeted genotyping tests can only only screen for a small number of common variants, such as those giving rise to the S and Z alleles, because a separate set of primers is required for each variant. This is a major limitation given that more than 250 gene variants have been reported (Kueppers & Sanders 2017; Lascano & Campos 2017; Maltais et al. 2018; Marciniuk et al. 2012). Definitive AAT gene sequencing may be used for selected patients where a null or deficient variant other than S or Z is suspected or when the phenotype and genotype results are incongruous (Dummer et al. 2020; Franciosi, Carroll & McElvaney 2019).

The applicant advised that at least two laboratories in Australia offer AAT genotyping: Pathology NSW Health and Pathology Queensland. However, the test detects only the two most common variants (those producing the S and Z alleles). Neither AAT genotyping nor SERPINA1 gene sequencing are listed on the MBS.

The applicant noted that in 2019, Pathology Queensland conducted 2,269 tests for AAT genotyping (two variants) and seven SERPINA1 gene sequencing tests. With the introduction of a broader genotyping service encompassing 14 variants it is expected that the need for SERPINA1 sequencing tests will be reduced by up to 63% (Veith et al. 2019). Based on the numbers provided by Pathology Queensland, this would equate to a reduction from seven patients to approximately four per year requiring gene sequencing once the gene panel test is introduced. The applicant predicted that the number of tests (both index and cascade) will decrease in years two and three, once the prevalent population has been tested in the first year of MBS listing. There may also be some leakage, with physicians testing all patients with symptoms of COPD in order to identify the underlying cause (reducing the pre-test probability would reduce the diagnostic yield and thus require a greater proportion of patients to receive gene sequencing to confirm a negative result from the gene panel test) and potentially testing second-degree relatives of probands.

Comparator

Intervention 1

The comparator for the gene panel test is IEF (as defined in the Prior Test section of this document; MBS item 66638). The proposed items will be offered in place of IEF phenotyping for patients with suspected AAT deficiency.

PASC confirmed that the comparator for targeted genotyping is IEF.

PASC noted that IEF is an outdated technology that many laboratories are not investing in. PASC noted that some laboratories are abandoning IEF testing altogether, whereas some are already switching to targeted genotyping. The applicant agreed with the PASC, adding that few laboratories have the capability to conduct IEF, whereas all Australian pathology laboratories conduct genotyping.

Intervention 2

Since gene sequencing is the criterion standard for detecting allelic variants, there is no comparator for this test.

PASC confirmed that the subsequent gene sequencing intervention has no direct comparator.

Rationale

Genetic testing can reveal the cause of the AAT deficit, provide a confirmatory diagnosis for low AAT serum concentrations, and is essential for investigating family members of probands. The proposed gene panel test can detect 14 rare variants simultaneously, instead of only the two variants (S and Z) detected by current targeted genotyping, leading to faster clinical diagnosis (Table 2). Avoiding the use of intermediate tests, such as AAT protein phenotyping with IEF, simplifies the testing algorithm and can reduce the need for *SERPINA1* gene sequencing by up to 63% (Dummer et al. 2020; Maltais et al. 2018; Miravitlles et al. 2017; Veith et al. 2019). However, there are over 70 known sequence variants in the *SERPINA1* gene that are clinically significant (Kueppers et al. 2019). Although rare, there will be cases where the 14-variant panel test will return an inconclusive result and further investigation with *SERPINA1* gene sequencing will be required (Figure 1). However, where the gene panel test is negative due to poor diagnostic yield, there could be greater than expected uptake of gene sequencing.

The criterion standard for detecting rare or novel variants is *SERPINA1* protein-coding exon sequencing in conjunction with serum AAT measurement. Although highly accurate, gene sequencing is more expensive than genotyping and used when targeted genotyping is unable to conclusively identify the variant, which is commonly the case for null variants (Dummer et al. 2020; Kueppers & Sanders 2017).

Reference standard

The reference standard for assessing the analytical validity of the gene panel test would be sequencing of the *SERPINA1* gene, since sequencing is able to identify specific rare and null variants that are not detected by IEF. The 510(k) submission to the United States Food and Drug Administration by Progenika Biopharma, S.A. for a similar 14-variant panel test used bi-directional Sanger sequencing as the reference standard (United States Food and Drug Administration 2017).

Outcomes

Patient relevant

The clinical claim is for superior effectiveness in identifying AAT deficient alleles, compared with IEF. It is also claimed that the 14-genetic variant panel test will obviate the need for IEF phenotyping. *SERPINA1* gene sequencing is proposed for identifying the very rare variants not represented by the 14-genetic variant panel test. However, the requirement for this service is expected to be reduced by the introduction of a 14-variant panel test. The following outcomes apply to both proposed interventions.

Primary effectiveness outcomes for affected individuals include the following:

- diagnostic accuracy
- clinical utility
    - change in clinical management (e.g. patient education, earlier initiation of preventive therapy or symptom surveillance) or behaviour (e.g. avoidance of risk factors)
    - change in patient outcomes (mortality, morbidity, quality of life, number and length of hospital stays)
    - change in confidence of diagnosis (physician and patient)
    - change in perspective toward genetic counselling, risk modification and reproductive choices
- reduction in time to diagnosis and need for other tests

Primary effectiveness outcomes for cascade testing of first-degree relatives of probands include the following:

- change in clinical management (e.g. patient education, earlier initiation of preventive therapy or symptom surveillance) or behaviour (e.g. avoidance of risk factors)
- change in patient outcomes (avoidance of illness)
- change in perspective toward genetic counselling, risk modification and reproductive choices

Safety outcomes include the following:

- psychological, physical or other harms (e.g. inability to qualify for life or disability insurance) from testing or not testing
- psychological, physical or other harms related to false-positive, false-negative or inconclusive test results

Healthcare system

Outcomes related to the healthcare system include the following:

- numbers of individuals eligible for genotyping or gene sequencing, and for cascade testing
- number of additional tests needed or averted because of genotyping or gene sequencing
- cost of genotyping and gene sequencing
- cost of disease progression in false negatives

- cost of monitoring disease progression and ongoing treatment in individuals identified with AAT deficiency or an increased risk of developing this disease
- cost savings from illness and care episodes averted through early risk modification and treatment adherence among patients identified with AAT deficiency or an increased risk of developing this disease
- cost of ongoing treatment and care for patients who live longer because of genotyping or gene sequencing or cascade testing

Rationale

In this context, diagnostic performance is a patient relevant outcome because efficient and accurate diagnosis of AAT deficiency is essential for averting potentially reversible lung and liver damage, slowing or avoiding disease progression, and alerting asymptomatic carriers of deficiency variants. In fact, the main benefits are likely to accrue from cascade testing and subsequent preventive management. Safety outcomes related to obtaining blood or saliva samples for testing are unlikely to be of high importance in this population, whereas potential psychological, physical or other harms related to undergoing or not undergoing genetic testing need to be addressed.

PASC confirmed the outcomes listed in this PICO document, and suggested that the PICO Confirmation should indicate that the main benefits are likely to accrue from cascade testing and subsequent preventive management.

The main economic effects on the healthcare system will centre on the ongoing costs of monitoring and treating individuals with AAT deficiency or an increased risk of disease and providing genetic counselling to probands and their family members, as well as the cost savings accrued from illnesses averted by early detection and risk modification in asymptomatic individuals. Given the high degree of accuracy associated with genetic testing, it is likely that outcomes related to false-negative, and particularly false-positive, results will be less important. The applicant noted that, unlike phenotyping by IEF, genotyping allows for batch processing of large runs of patient samples. With the decreasing cost of genotyping it is expected that, over time, the proposed gene panel testing will accrue significant cost savings to the health system.

Current and proposed clinical management algorithms

Current clinical management algorithm for identified population

The current clinical management algorithm for patients with suspected AAT deficiency is presented in Figure 1. For completeness, this includes services that are currently available within the health system but are not listed on the MBS.

PASC advised that "user pays" testing should be removed from Figure 1. This was removed from the clinical management algorithm below.

Proposed clinical management algorithm for identified population

The proposed clinical management algorithm for patients with suspected AAT deficiency is presented in Figure 2. It is noted that gene sequencing (Intervention 2) is only required when the genotyping test (Intervention 1) provides an inconclusive or negative result. If the diagnostic yield of Intervention 1 is low, then this will offset the claimed benefit of reducing the need for gene sequencing. The diagnostic yield will be influenced by the pre-test probability, which in turn will be influenced by the eligibility criteria of the affected individual population.

The applicant advised that the turnaround time for genotype testing is typically one week, but this depends on the number of samples tested and the prioritisation of testing. Gene sequencing may take 2 to 6 weeks, depending on laboratory workload, although a mean turnaround time of 11 days has been reported (Maltais et al. 2018).

PASC noted that the proposed clinical management algorithm has two boxes for serum AAT concentration on the left-hand side that do not match the proposed item descriptors. PASC agreed that the algorithms should be simplified and have a single AAT threshold (<20 µmol/L), below which patients would proceed to intervention 1, and then intervention 2 if no pathogenic variant was identified by intervention 1.

The applicant agreed with PASC's advice, however considered the clinical algorithm must still reflect that some patients will have normal levels of AAT but still have a clinical suspicion of AATD. The applicant considered the addition of another box that states "Low serum AAT (<20 µmol/L) OR Serum AAT (>20 µmol/L) but moderate to high clinical suspicion of AAT deficiency", was appropriate.

PASC advised that the proposed requirement for confirming the serum AAT concentration on the left-hand side of the proposed algorithm should be removed. PASC considered that this would not prevent the accepted good practice of repeating the serum AAT test when considered clinically appropriate – for example, if interpretation of the initial serum AAT test was affected by a concurrent C-reactive protein (CRP) measurement.

Proposed economic evaluation

The gene panel test is expected to be superior to IEF in identifying AAT-deficient alleles. It is expected that test accuracy and minimisation of false negative results will be valued more highly than the cost of testing given that the service will be required only once per lifetime and is needed by relatively few patients. Therefore, the most appropriate economic evaluation is a cost-effectiveness/cost-utility analysis to determine costs relative to the test's effectiveness in averting illness and increasing quality of life.

PASC confirmed that the appropriate economic evaluation is a cost-effectiveness analysis or cost-utility analysis.

PASC queried if the true cost of IEF needs to be established before it can be considered a true cost comparator. Recalling that normal MSAC practice is to accept the MBS fee as the unit cost of a

healthcare resource in the base case of an economic evaluation if it is listed as an MBS item, PASC suggested that a sensitivity analysis of the economic evaluation could examine the consequence varying the unit cost of this service within an appropriate range.

For gene sequencing, a budget impact analysis would be sufficient given the extremely low number of patients that would likely require this test if the proposed algorithm is adopted.

Proposed item descriptor

The applicant advised that at least two laboratories in Australia offer AAT genotyping: Pathology NSW Health and Pathology Queensland (Queensland Health). Pathology Queensland currently uses Taqman QT-PCR to detect only the two most common variants (S and Z alleles) at a cost of $78. The cost of genotyping in NSW is $80 (personal communication Pathology Queensland).

The applicant stated that the proposed in-house gene panel test will be developed by RCPA/NATA-accredited laboratories using technology amenable to multiplexing to identify the 14 most common variants identified in the application (Table 2). The cost of the new service will be approximately $100 per test.

The applicant noted that *SERPINA1* gene sequencing is not currently listed on the MBS, but it is offered by Queensland Health for A$260 (personal communication Pathology Queensland).

Testing of individuals who have a demonstrated family history of AAT deficiency is currently incorporated in the item descriptor for genotypic testing (item descriptor AAAA). For cascade testing, it is unclear whether a genotyping test for a single variant would more or less cost-effective than the 14-variant panel test. If PASC and the applicant consider it appropriate to add a separate item for single variant genotyping in first-degree relatives of a proband, the fee should be no more than $100 and potentially less, given that only a single variant needs to be identified.

PASC noted that the proposed item descriptors did not align with the proposed clinical management algorithm, and advised changing the algorithm accordingly (see Proposed clinical management algorithm section).

The MBS descriptors as agreed to by the PASC are presented below.

Category 6 (Pathology Services) – GROUP P7 GENETICS
Proposed item descriptor: AAAA Genotypic testing to identify the 14 most common pathogenic variants in the *SERPINA1* gene where the patient has abnormally low (<20 µmol/L) AAT levels, as determined by item number 66635, and: – emphysema without exposure to tobacco smoke or air pollutants OR – emphysema at a young age OR – basal emphysema OR – panniculitis OR – cirrhosis or liver function abnormalities (including neonatal hepatitis) without other risk factors OR – anti-proteinase 3-positive-positive vasculitis OR demonstrated family history of AAT deficiency, defined as a relative with an identified pathogenic or likely pathogenic variant in the *SERPINA1* gene, requested by a specialist or consultant physician. Maximum one test per lifetime. Fee: $100 Practice Note: The genotype test should have sufficient diagnostic range and sensitivity to detect at least 95% [or 99%] of pathogenic SERPINA1 variants likely to be present in the patient. For the two-variant option, these should be the S (p.Glu288Val) and Z (p.Glu366Lys) variants; for the 14-variant option, these should be the 14 most common pathogenic variants.

PASC advised that if item AAAA were to be limited to two pathogenic variants, then these should be the S (p.Glu288Val) and Z (p.Glu366Lys) variants; if it were to be for 14 pathogenic variants, then these should be "14 of the most common pathogenic variants; and also noted the Department had proposed to add a Practice Note, which could be along the lines of "sufficient diagnostic range and sensitivity to detect at least 95% [or 99%] of pathogenic SERPINA1 variants likely to be present in the patient".

PASC advised that a single variant test would not be appropriate for siblings of a proband with two different pathogenic variants (compound heterozygote). In the context of high-throughput genotype testing it may not be cheaper to have a specific test just for one variant. PASC therefore advised that cascade testing of such siblings would use the same test as item AAAA, be that a 2 or 14 panel test.

PASC noted that placement of commas in the descriptor for item AAAA caused confusion about the criteria for testing, and advised that the order should be the serum AAT threshold, followed by the symptoms and family history which indicate that serum AAT measurement would be appropriate. PASC noted that, for the very rare cases where the serum AAT concentration are normal but the protein is non-functional, this sequence would not be appropriate and such patients would miss out on genetic testing.

Category 6 (Pathology Services) – GROUP P7 GENETICS
Proposed item descriptor: BBBB Sequencing of the *SERPINA1* gene to identify an alpha-1-antitrypsin (AAT) pathogenic variant where the result after genotyping using item number AAAA is inconclusive, requested by a pathologist, specialist or consultant physician. Maximum one test per lifetime. Fee: $260

If deemed necessary by PASC and the applicant, an additional MBS item for cascade testing of first-degree relatives of a proband is suggested below.

PASC considered that the need for a separate cascade item CCCC rather than subsuming cascade testing in item AAAA under "demonstrated family history of AAT deficiency" would also depend on the consequences for the possibility of a different intervention at a lower cost, and therefore a lower fee (due to the single variant to be tested, see PASC Outcome under 'Intervention', above).

PASC confirmed the gene sequencing intervention as a separate and subsequent intervention (item BBBB) to the targeted genotype testing.

PASC advised that item BBBB should be made pathologist determinable as well as being able to be requested by a specialist or consultant physician.

PASC advised that depending on which fee is established for item AAAA, the need for a separate item CCCC at lower cost and fee for cascade testing could then be determined.

The applicant agreed that as advised by PASC, a single variant test would not be appropriate for siblings of a proband with two different pathogenic variants (compound heterozygote). Therefore, the applicant queried whether a separate item descriptor for cascade testing was required. The applicant considered item AAAA should encompass testing of both the index population and "cascade testing", and would not restrict testing to first-degree relatives.

Category 6 (Pathology Services) – GROUP P7 GENETICS
Proposed item descriptor: CCCC Characterisation of a pathogenic variant in the *SERPINA1* gene in an individual who is a first-degree relative of a patient who has had a pathogenic or likely pathogenic variant identified in this gene, and has not previously received a service under items AAAA or BBBB, requested by a specialist or consultant physician. Maximum one test per lifetime. Fee: [$78] / [$100]*

* Note: the specification of "the S and Z" or "14" variants in the item descriptor and the fee of $78 or $100 are proposed to follow the decision made for proposed item AAAA (panel test for affected individuals).

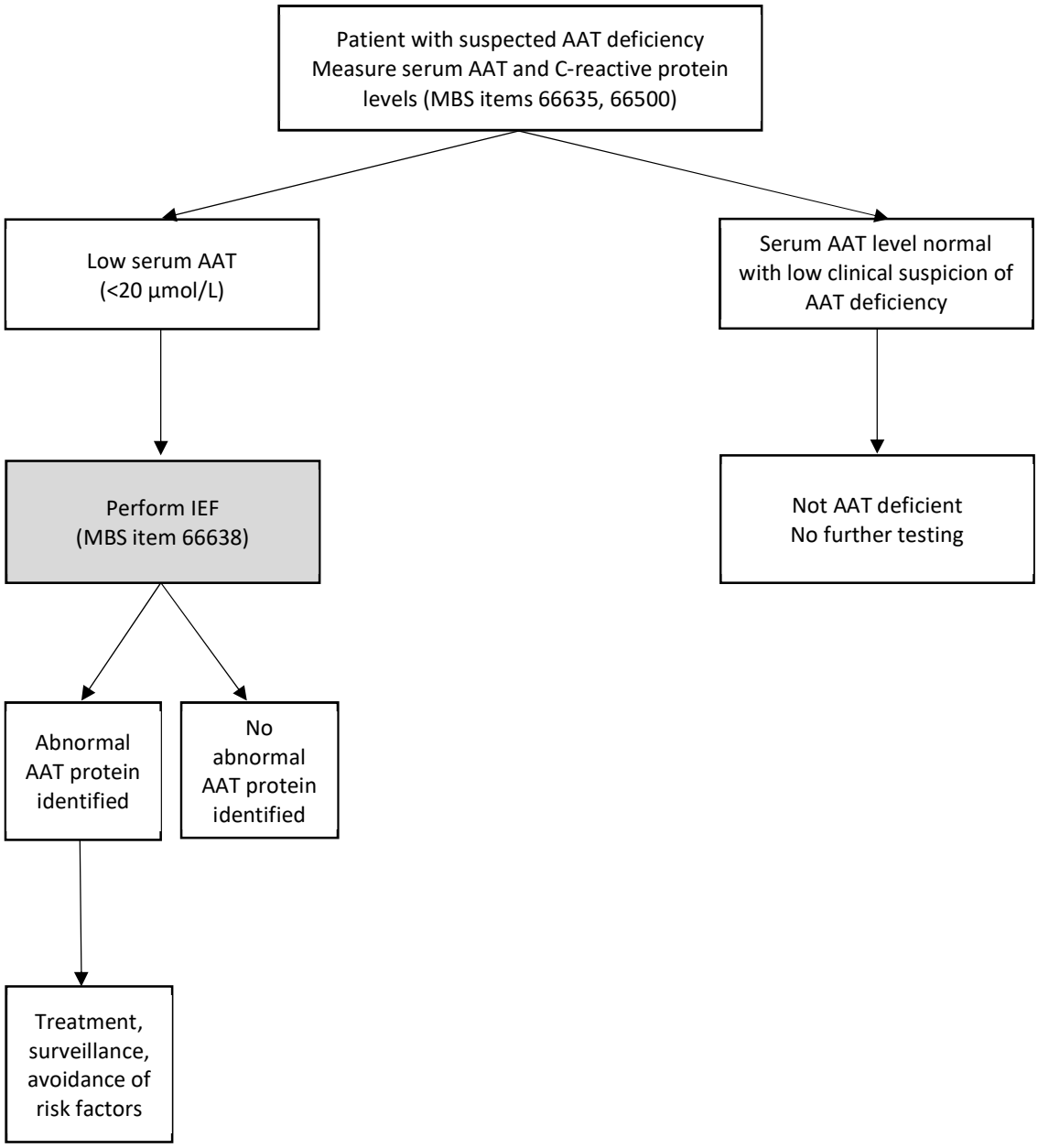

Figure 1: Current clinical management algorithm for patients with suspected AAT deficiency

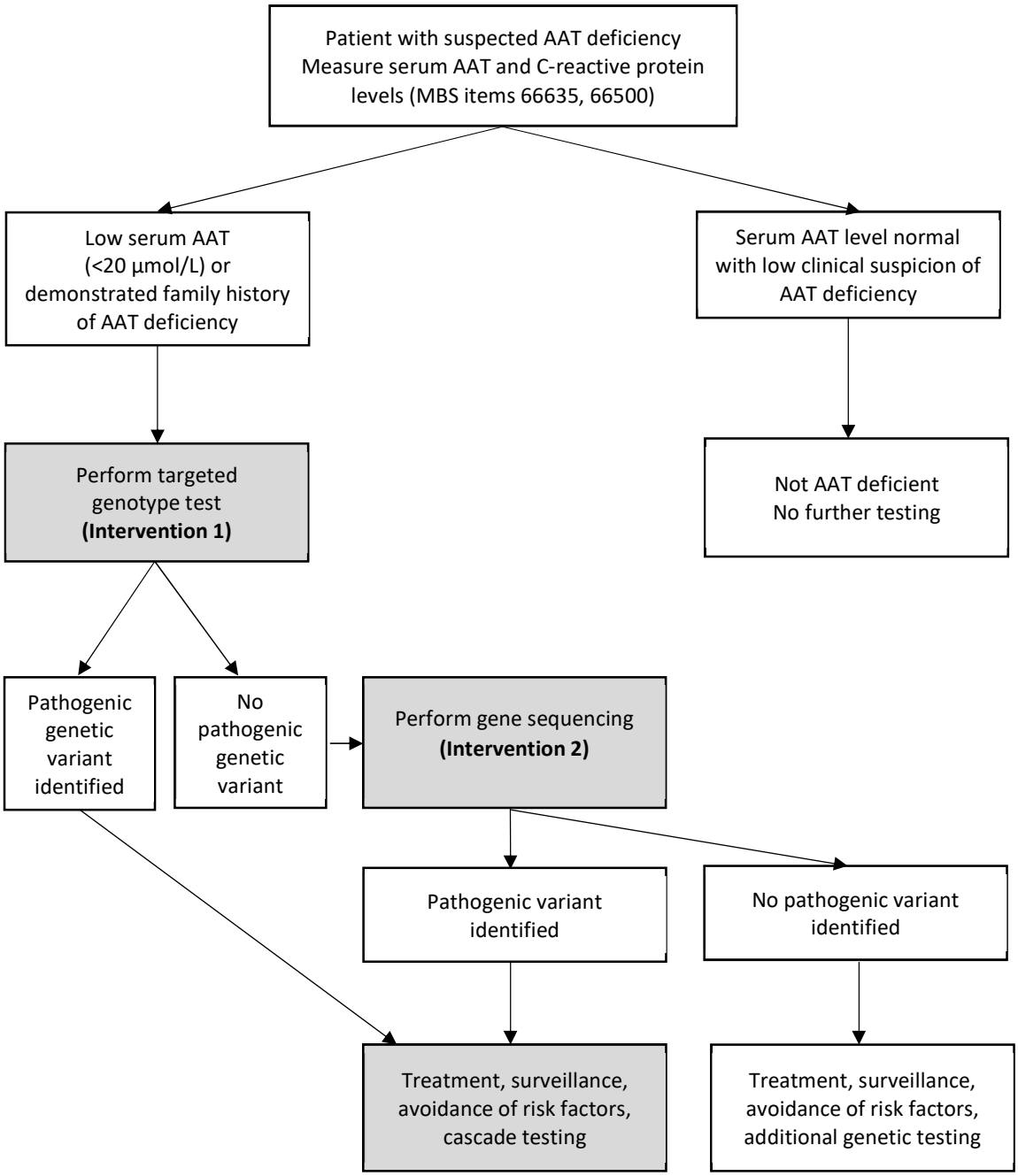

Figure 2: Proposed clinical management algorithm for patients with suspected AAT deficiency

Consultation feedback

PASC noted that the consultation feedback was supportive from many groups.

PASC noted that one group suggested that the term 'gene panel' was misleading.

Next steps

PASC advised that, upon ratification of the post-PASC PICO, the application can proceed to the Evaluation Sub-Committee (ESC) stage of the MSAC process.

PASC noted the applicant has elected to progress its application as a DCAR (Department-contracted assessment report).

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