European Society for Immunodeficiencies guidelines for the management of patients with congenital athymia

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Congenital athymia is a life-limiting disorder due to rare inborn errors of immunity causing impaired thymus organogenesis or abnormal thymic stromal cell development and function. Athymic infants have a T-lymphocyte–negative, B-lymphocyte–positive, natural killer cell–positive immunophenotype with profound T-lymphocyte deficiency and are susceptible to severe infections and autoimmunity. Patients variably display syndromic features. Expanding access to newborn screening for severe combined immunodeficiency and T lymphocytopenia and broad genetic testing, including next-generation sequencing technologies, increasingly facilitate their timely identification. The recommended first-line treatment is allogeneic thymus transplantation, which is a specialized procedure available in Europe and the United States. Outcomes for athymic patients are best with early diagnosis and thymus transplantation before the development of infectious and inflammatory complications. These guidelines on behalf of the European Society for Immunodeficiencies provide a comprehensive review for clinicians who manage patients with inborn thymic stromal cell defects; they offer clinical practice recommendations focused on the diagnosis, investigation, risk stratification, and management of congenital athymia with the aim of improving patient outcomes. (J Allergy Clin Immunol 2024;154:1391-408.)

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To generate a diverse T-lymphocyte repertoire that is tolerant to self-antigens, bone marrow–derived T-lymphocyte progenitors complete their differentiation and selection in the thymus.[1,2] The thymic stroma, composed of epithelial cells, various vascular cell types, mesenchymal cells, and fibroblasts, provides the intricate and highly specialized 3-dimensional microenvironment critical for this process.[1,2] Thymic epithelial cells (TEC) originate from the endodermal lining of the third pharyngeal pouch, whereas the nonepithelial components of the thymic stroma descend from the surrounding mesoderm and neural crest–derived mesenchyme.[2-6] The correct patterning of the third pharyngeal pouch and early thymus organogenesis are tightly controlled by the action of several transcription factors, including TBX (T-box transcription factor) 1, HOXA3 (homeobox protein A3), PAX (paired box) 1, and PAX9.[7-12] Following a first-fate commitment, differentiation, growth, and function of TEC are dependent on the expression of FOXN1 (forkhead box N1), a member of the forkhead family of transcription factors.[7,13-18] TEC can be subdivided on the basis of anatomic, phenotypic, functional, and more recently transcriptomic characteristics into separate subpopulations and subtypes, defined respectively by their expression of cell-surface markers or by their transcriptomes.[19-26] TEC resident in the outer cortex of the thymus (designated cortical TEC) control the early stages of thymopoiesis, including the attraction of blood-borne lymphoid precursors to the thymus, their commitment to a T-lymphocyte fate, and their expansion and positive selection.[13,23,27-30] The latter constitutes a process that enables

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Abbreviations used

22q11.2DS:	22q11.2 deletion syndrome
CHARGE syndrome:	Syndrome comprising coloboma, heart defects, atresia of nasal choanae, retardation of growth and development, genitourinary anomalies, and ear anomalies
CHD:	Congenital heart disease
CHD7:	Chromodomain helicase DNA binding protein 7
CMV:	Cytomegalovirus
CSA:	Cyclosporine A
DGS:	DiGeorge syndrome
FOXI3:	Forkhead box I3
FOXN1:	Forkhead box N1
GOSH:	Great Ormond Street Hospital
HCT:	Hematopoietic cell transplantation
HLA:	Human leukocyte antigen
HSC:	Hematopoietic stem cell
HSPC:	Hematopoietic stem and progenitor cells
IEI:	Inborn error of immunity
IgRT:	Immunoglobulin replacement therapy
NBS:	Newborn screening
NK:	Natural killer
OFCS2:	Otofaciocervical syndrome type 2
PAX1/9:	Paired box 1/9
SCID:	Severe combined immunodeficiency
TBX1/2:	T-box transcription factor 1/2
TCR:	T-cell antigen receptor
TEC:	Thymic epithelial cells
TREC:	T-cell–receptor excision circle
WES:	Whole exome sequencing
WGS:	Whole genome sequencing

immature T lymphocytes (or thymocytes) that have successfully expressed a T-cell antigen receptor (TCR) with sufficient affinity for a peptide–major histocompatibility complex to receive signals that allow their further survival. Positively selected thymocytes are next subjected to another quality control of the cells' antigen specificity, a process known as central tolerance induction, which encompasses negative selection and regulatory T-lymphocyte development. Negative selection ensures that maturing T lymphocytes with a high affinity for self–peptide–major histocompatibility complex undergo programmed cell death (apoptosis) and are thus deleted from the repertoire because these cells are particularly prone to initiate autoimmunity. Alternatively, thymocytes with an intermediately high affinity for self-antigens may escape deletion and instead be diverted to a regulatory T-cell fate. To efficiently achieve this, TEC collectively express in a promiscuous fashion an almost comprehensive set of tissue-restricted antigens,[24,31-34] some of which are under the control of transcriptional regulators, namely autoimmune regulator (AIRE) and family zinc finger 2 (FEZF2).[24,27,28,31,33] While cortical TEC are singularly responsible for positive selection, negative selection can be mediated by TEC lineages as well as bone marrow–derived antigen presenting cells including dendritic cells and B lymphocytes.[27,28,30,35-43] However, within the thymus, AIRE and FEZF2 are predominantly expressed in a subset of medullary TEC, rendering these cells especially competent to shape the TCR repertoire. Taken together, lymph–stromal interactions within the thymus instruct the commitment and maturation of T lymphocytes and shape a diverse repertoire of TCR specificities that are tolerant to self yet reactive to foreign antigens.

Congenital thymic stromal cell disorders are inborn pathologies caused by abnormalities in thymic organogenesis and stromal cell development and/or function, resulting in deficient and/or dysregulated T-lymphocyte immunity.[44-47] The severity of peripheral T lymphocytopenia correlates with the reduced size of the thymic stromal scaffold.[46] Thymic hypoplasia, depending on its severity, may therefore result in either mild T lymphocytopenia with little or no clinical consequence, or alternatively, it may be characterized by a clinically significant lack of peripheral T lymphocytes, reduced T-lymphocyte proliferative responses, an oligoclonal TCR repertoire, and secondary impaired humoral immunity.[48-54] Complete thymic aplasia is rare and results in a T-lymphocyte–negative, B-lymphocyte–positive, natural killer cell–positive (T-B+NK+) immunophenotype with severe to complete absence of naive T lymphocytes, T-lymphocyte proliferative responses, and T-cell–receptor excision circles (TRECs), which are small circles of DNA created as a by-product of TCR gene rearrangement.[44,50,52-58] A T-B+NK+ immunophenotype may of course also be seen in the context of severe combined immunodeficiency (SCID) due to various hematopoietic cell–intrinsic genetic defects.[45,59]

Aberrant patterning of the third pharyngeal pouch during early embryogenesis may result in congenital athymia associated with defective development of other anatomic structures derived from the adjacent pharyngeal apparatus.[44,60] Defects of thymic organogenesis may therefore variably be associated with additional anomalies affecting multiple organs, including craniofacial structures, the heart, great vessels, and the parathyroids.[61] Such field defects occur in the context of DiGeorge syndrome (DGS), a clinical diagnosis defined by the triad of thymic hypoplasia/aplasia, hypoparathyroidism, and congenital heart disease (CHD), although the immunodeficiency and other features of DGS typically display substantial variability in their clinical penetrance.[50,53,54,62] Multiple genetic and environmental etiologies can cause a DGS phenotype (Table I), with heterozygous chromosomal deletions at 22q11.2 (22q11.2 deletion syndrome, or 22q11.2DS) constituting the most frequent cause, with an estimated incidence of 1:4000 live births.[63] Athymia is uncommon, occurring in ≤1% of individuals with 22q11.2DS, in whom the condition has previously been referred to as complete DGS because of the severity of the ensuing immunodeficiency.[50,56,64] The deletion is typically between 1.5 and 3.0 Mb in size, resulting in the loss of approximately 30 to 100 genes. The size of the deletion does not correlate with the clinical phenotype.[65] Among the deleted genes is *TBX1,* a T-box transcription factor that regulates almost 2000 genes[66] and plays a major role in the pharyngeal patterning defects seen in 22q11.2DS, including immunodeficiency.[10,67,68] The terms "DGS" and "22q11.2DS" are often used interchangeably. However, other genetic and environmental causes of the DGS triad have been identified.[44] Thus, where known, reference should be made to the exact etiology. The second most frequent genetic cause of DGS comprises autosomal-dominant mutations in the *CHD7* (chromodomain helicase DNA binding protein 7) gene, which underlies CHARGE syndrome, or a syndrome with coloboma, heart defects, atresia of nasal choanae, retardation of growth and development, genitourinary anomalies, and ear anomalies.[69,70] While some clinicians consider CHARGE syndrome a separate disease entity, in this guideline, we include CHARGE syndrome as one of the genetic

etiologies of the clinical DGS triad. The incidence of CHARGE syndrome is approximately 1:10,000-17,000 live births;[71] however, the frequency of congenital athymia among these is not known. Other rare genetic causes of DGS include mutations in *TBX1*[72-74] or *TBX2*;[75] 22q11.2 duplications;[76] haploinsufficiency of *FOXI3* (forkhead box I3) due to microdeletions at 2p11.2[77] or heterozygous loss-of-function single gene mutations;[78] and partial deletion of the short arm of chromosome 10.[79-81] Although thymic hypoplasia and other features of DGS have been reported in 22q11.2 duplication, lymphopenia is not commonly present.[82-85] In cases where DGS remains genetically undefined, it is important to consider whether *in utero* exposure in the first trimester to poorly controlled maternal diabetes,[86,87] alcohol,[88] and overexposure[89,90] or underexposure[91] to retinoic acid may have occurred. Retinoic acid metabolism is impaired during pregestational diabetes[92] and has been linked to TBX1 signaling as well as to other transcription factors with a role in thymus organogenesis.[90,93,94]

Congenital athymia has also been identified in other syndromic inborn errors of immunity (IEI) (Table I),[44,95] including otofaciocervical syndrome type 2 (OFCS2) caused by homozygous *PAX1* deficiency and nude "SCID" resulting from homozygous *FOXN1* deficiency.[44,58,70,96-100] PAX1 is an evolutionary conserved transcription factor, expressed in the pharyngeal pouches during embryogenesis, where it is important for patterning and the development of the thymic anlagen. Later, PAX1 expression is maintained in a fraction of cortical TEC in a FOXN1-dependent manner.[9,101] OFCS2 patients have ear anomalies, facial dysmorphism, and skeletal anomalies, and, depending on the degree of residual PAX transcriptional activity conferred by their mutation or mutations, they may present with congenital athymia or with a milder phenotype consisting of combined immunodeficiency with overlapping features with DGS.[99,100,102-105] As the master transcriptional regulator,[14] FOXN1 is essential for TEC differentiation and maintenance, as well as the functional ability to support thymopoiesis by controlling the expression of key genes.[13] The nude SCID phenotype, comprising congenital athymia, alopecia totalis, and nail dystrophy, was first reported in patients with homozygous complete loss-of-function mutations in *FOXN1*. These include early truncating mutations that likely result in nonsense mediated mRNA decay, and missense mutations that alter critical residues within the forkhead domain, abrogating its ability to bind DNA.[44,96,97,106-108] Several studies have recently characterized monoallelic and compound heterozygous mutations, finding a range of functional consequences from complete to partial loss of function, including some mutants that exert a dominant negative effect on the wild-type allele.[109-112] These studies have further revealed the dose dependency for FOXN1 in TEC, such that the severity of the immunodeficiency appears to reflect the degree of residual FOXN1-induced transcriptional activity.[106]

Left untreated, congenital athymia is incompatible with long-term survival. It is therefore crucial to identify and treat affected patients as early as possible. Congenital athymia can be treated by transplanting lymphodepleted thymus tissue, donated by infants undergoing cardiac surgery, into the quadriceps muscles of athymic recipients. This provides a functional stromal environment to generate recipient-derived T lymphocytes. The current tissue culture and implantation process has not changed much since its early medical applications.[113,114] To date, thymus transplantation outcomes have been reported for more than 140 athymic patients treated at 2 centers, Duke University Medical Center in the United States and Great Ormond Street Hospital (GOSH) in the United Kingdom.[115-117] The overall survival of patients with athymia treated with thymus transplantation is approximately 75%, with mortality mainly due to preexisting infections or infections acquired before immune reconstitution is established.[55,58,115,116,118] Although absolute T-lymphocyte counts usually remain below those seen in the normal population, they are sufficient to clear and prevent infections, enabling patients to participate in normal activities and thus improving their quality of life.[115,116,118,119] Autoimmune manifestations, particularly affecting the thyroid gland and blood cells, are relatively common after thymus transplantation,[115,116] suggesting an incomplete establishment of self-tolerance by the transplanted thymic stroma. Overall, thymus transplantation is a lifesaving procedure for congenital athymia, with superior outcomes compared to hematopoietic cell transplantation (HCT).[120] Poorer survival and severe graft-versus-host disease have been reported after HCT, particularly if no matched sibling donor is available.[120,121] Therefore, it is widely agreed that thymus transplantation, as the most appropriate treatment for congenital athymia, should be the standard of care and that every effort should be made to ensure prompt access to treatment, where available.

Here we provide clinical guidelines to help identify, investigate, and manage patients with congenital athymia who should be considered for thymus transplantation. Our expert panel recommendations, summarized in Fig 1, focus on when to suspect a thymic stromal cell defect, how to diagnose and stratify patients according to risk in order to identify those with congenital athymia who might benefit from thymus transplantation, and their initial, supportive clinical management. While we also discuss the role of novel diagnostic approaches, the *status quo* of allogeneic thymus transplantation, its follow-up, and future directions, the emphasis of this guideline is to promote and facilitate early recognition of congenital athymia and prompt referral for specialist treatment to improve clinical outcomes. These guidelines are focused on the management of congenital athymia, but many of the recommendations, particularly those related to diagnosis and investigation, are applicable to congenital thymic hypoplasia. Specific guidance on the management of patients with thymic hypoplasia is available elsewhere.[56]

WHEN TO SUSPECT CONGENITAL ATHYMIA

Infants with congenital athymia can come to medical attention via several different routes (Fig 2). Increasingly, this occurs in the context of newborn screening (NBS) programs for SCID and T lymphocytopenia.[117,122] NBS for SCID started in some parts of the United States in 2008, and since 2018, all 50 states have universal screening programs in place.[123] Universal and pilot NBS programs for SCID are progressively being rolled out in a growing number of countries.[122,124-128] These programs rely on the detection of TRECs in dried blood spot samples routinely taken from newborns shortly after birth. TRECs are stable, circular, episomal DNA excised from genomic sequences during TCR gene rearrangement and are a relative measure of thymic output because they mark newly generated T lymphocytes. Low or undetectable TRECs are a positive finding in SCID NBS, and these TREC-based screening programs also identify infants with thymic aplasia and hypoplasia.[117,122,129,130]

TABLE I. Disorders known to cause congenital thymic aplasia or hypoplasia and associated clinical features

DGS etiology	Condition	Inheritance	Syndromic features
Genetic	22q11.2DS[44]	*De novo* (90-95%); AD (5-10%)	• Variable features of DGS
	CHARGE syndrome (CHD7 haploinsufficiency in majority)[53]	*De novo* (majority)	• Variable features of DGS • Coloboma • Choanal atresia • Growth retardation • Genitourinary abnormalities • Ear anomalies • Cranial nerve dysfunction
	TBX1 deficiency[67]	AD	• Variable features of DGS
	TBX2 deficiency[75]	AD	• Variable features of DGS
	22q11.2 duplication[76]	AD/*de novo*	• Variable features of DGS
	FOXI3 haploinsufficiency, including 2p11.2 microdeletions[77] and heterozygous loss-of-function FOXI3 variants[78]	AD (majority)	• Variable features of DGS
	Partial monosomy 10p[79-81]	*De novo*	• Variable features of DGS • Craniofacial malformation
Environmental	Diabetic embryopathy[86,87]	NA	• Variable features of DGS • Renal agenesis • Vertebral anomalies
	In utero overexposure to alcohol[87]	NA	• Variable features of DGS • Variable features of foetal alcohol syndrome
	In utero overexposure to retinoic acid[89,90]	NA	• Variable features of DGS
	In utero under exposure to retinoic acid[91]	NA	• Variable features of DGS
	Other genetic syndromes associated with defective thymic development		
	Nude SCID (FOXN1 deficiency)[96]	AR	• Congenital alopecia totalis • Nail dystrophy
	Hypomorphic FOXN1 deficiency, including heterozygous variants[110] and compound heterozygote mutations[111]	AD, AR	• Nail dystrophy • Alopecia may be absent, hair thinning • Eczema
	OFCS2 (PAX1 deficiency)[99,100]	AR	• Ear anomalies, preauricular pits, hearing impairment • Branchial cysts/fistulas • Facial dysmorphism • Skeletal anomalies • Intellectual disability • Can have overlapping features with DGS including hypoparathyroidism and congenital heart defects

Variable features of DGS indicates that DGS features include a wide variety of possible anomalies[44] but most commonly the following: congenital cardiac defects; parathyroid hypoplasia; facial dysmorphism; palatal, pharyngolaryngeal, and tracheobronchial defects; and developmental delay.
AD, Autosomal dominant; *AR,* autosomal recessive; *NA,* not applicable.

If not captured by NBS programs, infants with congenital athymia may present clinically with complications consequent to their severe T-lymphocyte deficiency,[115-117] typically within the first few months of life, with failure to thrive as well as unusually persistent, severe, or opportunistic infections such as *Pneumocystis jirovecii* pneumonia, CMV pneumonitis, or disseminated bacillus Calmette-Guérin infection. Persistent respiratory tract infections—for example, those caused by respiratory syncytial virus, parainfluenza virus, or adenovirus—or persistent candidiasis should also prompt further immunologic investigation. Gastrointestinal infection and chronic diarrhea are common features; causes include viruses such as adenovirus, norovirus, or rotavirus (wild type or vaccine strain). Immune dysregulation may occur, which most commonly manifests with Omenn syndrome–like features, including erythroderma, diarrhea, hepatosplenomegaly, lymphadenopathy, elevated IgE, and eosinophilia.[57,99,106,131] More rarely, patients present with symptoms of autoimmunity, particularly hematologic cytopenias.

In addition, clinical features related to an underlying syndrome are often present (Table I). Because athymia is most frequently encountered in the context of DGS, field defects affecting structures derived from the adjacent pharyngeal apparatus, including the parathyroids, aortic arch, cardiac outflow tract, thyroid, maxilla, mandible, and external/middle ear, are variably present (Table I). Parathyroid hypoplasia can manifest with hypocalcemia and neonatal seizures, and it may be the presenting feature of DGS. Cardiac conotruncal outflow tract defects lead to particular CHD, including tetralogy of Fallot, truncus arteriosus, interrupted aortic arch, double outlet right or left ventricle, transposition of the great arteries, and aortopulmonary septal defects.[44] In addition to the classical DGS triad, other clinical features may be present, including facial dysmorphism, palatal and/or pharyngolaryngeal defects, tracheobronchomalacia, gastrointestinal problems (ie, feeding difficulties, constipation, and gastroesophageal reflux disease), renal anomalies, skeletal problems (ie, scoliosis and talipes equinovarus), and developmental

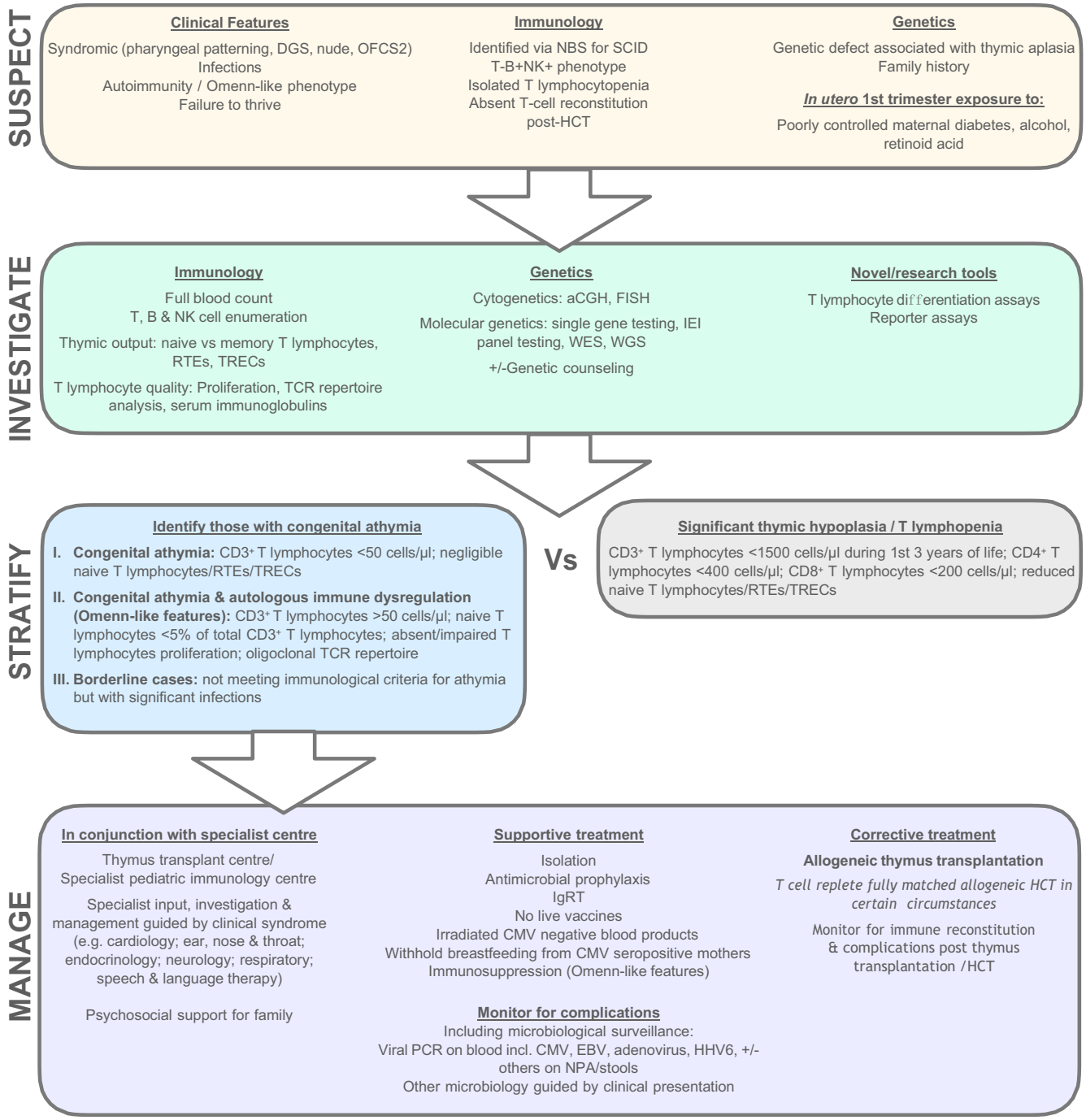

FIG 1. Flowchart summarizing clinical guidelines for recognition, investigation, and management of patients with congenital athymia. *aCGH,* Array comparative genomic hybridization; *EBV,* Epstein-Barr virus; *FISH,* fluorescence *in situ* hybridization; *HHV6,* human herpesvirus 6; *NPA,* nasopharyngeal aspirate; *RTE,* recent thymic emigrant.

delay.[49,132] In addition to DGS with underlying 22q11.2DS, other genetic and environmental etiologies of DGS have distinct clinical features that may be evident (Table I).[44] Patients with CHARGE syndrome may have coloboma, atresia choanae, retardation of growth and/or development, genitourinary and/or ear anomalies, and cranial nerve dysfunction.[133,134] Infants born to diabetic mothers may have features of caudal dysplasia sequence, in particular renal agenesis and vertebral anomalies.[86,135] Clinical features of homozygous FOXN1 deficiency include congenital alopecia totalis and nail dystrophy.[96,97] PAX1 deficiency underlies OFCS2 with preauricular pits and hearing impairment, facial dysmorphism, skeletal anomalies, and intellectual disability.[99,100,102,103] While the aforementioned clinical features should alert clinicians to the possibility of immunodeficiency resulting from impaired thymic stromal cell development, the severity of the immunologic phenotype in an individual patient

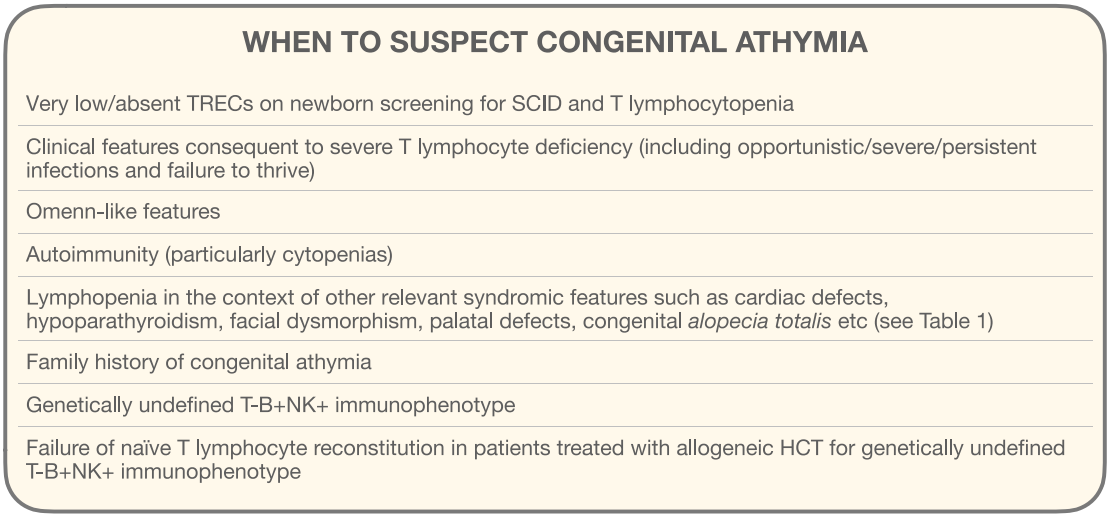

FIG 2. When to suspect congenital athymia.

cannot be predicted by the severity of the other features. Therefore, all children with suggestive clinical features should undergo immunologic and genetic investigations to exclude immunodeficiency—and, crucially, to identify those with life-threatening athymia who require corrective treatment.

Timely recognition of congenital athymia and early referral for treatment are the result of several factors. Awareness among clinicians has increased to recognize the syndromic features associated with athymia and is matched by expanding access to TREC-based NBS and next-generation sequencing to facilitate diagnosis of cases with a genetic etiology. Nonetheless, incomplete clinical penetrance, together with clinical and genetic heterogeneity, can still make timely recognition of athymia challenging. It may only be recognized after failure of naive T-lymphocyte reconstitution despite adequate donor engraftment and reconstitution of other blood lineages in patients treated empirically with allogeneic HCT for genetically undefined suspected SCID, highlighting the importance of extensively investigating patients with a molecularly undefined T-B+NK+ immunophenotype to distinguish between hematopoietic cell–intrinsic SCID and congenital athymia before considering allogeneic HCT as a treatment strategy.[99,136]

HOW TO INVESTIGATE INFANTS WITH SUSPECTED CONGENITAL ATHYMIA

Children with possible congenital athymia should undergo a series of investigations aimed at rapidly securing the diagnosis and etiology, as well as characterizing the severity of their underlying immune defect (Fig 3). The latter will guide immunologic management and, importantly, will identify those likely to derive benefit from allogeneic thymus transplantation.

Immunology

A finding of lymphocytopenia based on a full blood count with differential is often the initial finding in congenital athymia. Lymphocyte subsets enumerating T, B, and NK cells typically demonstrate a low $CD3^+$ T-lymphocyte count with normal B- and NK-cell counts, corresponding to a T-B+NK+ immunophenotype. Athymic patients with Omenn syndrome–like clinical features (previously also referred to as athymia with atypical features[56]) have higher, or even normal, lymphocyte counts due to oligoclonal expansion of dysregulated T lymphocytes of memory phenotype ($CD45RA^-CD27^+$).[57,131] More definitive immunologic assessment involves quantitative evaluation of thymic output. Thymic output can be assessed by enumeration of TRECs using real-time quantitative PCR on isolated peripheral blood mononuclear cells and provides a practical and accepted indicator of thymic output, with low or absent TRECs indicating lack of thymic naive T-lymphocyte production.[137] This is distinct from the NBS assay, which measures TRECs in a dried blood spot sample.[49,138] Although cutoff values vary in different NBS programs, a TREC value of <20 copies/μL will successfully identify SCID cases regardless of the underlying cause.[139,140] However, because this assay is not routinely available in many clinical laboratories, naive T lymphocytes ($CD45RA^+CD27^+$) or recent thymic emigrants ($CD45RA^+CD31^+$) can alternatively be measured by flow cytometry. Their frequencies strongly correlate with TREC levels, suggesting either can be used as a marker for thymic output.[43] In congenital athymia, naive T lymphocytes are profoundly reduced (<50 cells/μL or <5% of the total T lymphocytes).[56,141] Flow cytometric immunophenotyping is a fast and widely available technique but can be associated with significant variability among different centers in sample processing, immunostaining, instrument setup, and data analysis. Standardized protocols have been developed to generate reproducible and reliable results and have been shown to be highly useful in the diagnosis of conditions including SCID, alongside immunologic functional and genetic testing.[142]

If the above investigations are consistent with athymia, qualitative T-lymphocyte tests are of limited value and are not routinely necessary. In patients who have measurable peripheral T lymphocytes, qualitative assessment can be performed by measuring diversity of the TCR repertoire and by assessing T-lymphocyte function. Because the diversity of the TCR repertoire is almost completely reflective of the naive T-lymphocyte compartment, patients with reduced thymic output have a restricted TCR repertoire.[138,143] In cases with an Omenn syndrome–like phenotype, oligoclonality is seen.[116,131] Assessment of TCR diversity can be performed using flow cytometry to quantify TCR Vβ usage, or more reliably using spectratyping,

INVESTIGATION OF PATIENTS WITH SUSPECTED CONGENITAL ATHYMIA

Test	Congenital athymia
Basic immunological assessment	
Full blood count	Lymphocytopenia (variable)
Lymphocyte subsets	Low $CD3^+$ T lymphocytes, normal B and NK cells. Normal /high $CD3^+$ T lymphocytes if Omenn-like features or maternal engraftment present
TRECs	Very low / absent
Naïve T lymphocytes / RTEs	Profoundly reduced (<50 cells/μL or <5% of the total T lymphocytes)
Immunoglobulins	IgM and IgA very low, IgG may be normal depending on patient age and presence of maternal IgG IgE may be elevated if Omenn-like features present
Qualitative T lymphocytes tests (if measurable T lymphocytes present)	
TCR repertoire diversity	Restricted TCR repertoire, oligoclonality if Omenn-like features
T lymphocyte proliferation	Absent/very low responses to mitogens Patients with Omenn-like features can have partial or normal proliferative mitogen responses but impaired responses to specific antigens
Genetic	
Cytogenetic studies for chromosomal abnormalities (aCGH or FISH)	Del22q11.2, Dup22q11.2, Del10p13-14, Del2p11.2
WGS or WES (typically trio or proband only)	
IEI gene panel testing	Including *CHD7, TBX1, TBX2, FOXI3, FOXN1, PAX1*
Single gene sequencing	For highly suggestive phenotypes or positive family history
Research Diagnostic assays	
Reporter assays to asses pathogenicity of novel genetic variants in transcription factors	
Ex vivo T lymphocyte differentiation assay to differentiate thymic stromal cell defects from primary hematopoietic defects	

FIG 3. How to investigate infants with suspected congenital athymia. *aCGH,* Array comparative genomic hybridization; *Del,* deletion; *Dup,* duplication; *FISH,* fluorescence *in situ* hybridization; *RTE,* recent thymic emigrant.

a molecular technique that measures the length distribution of complementarity determining region 3 (aka CDR3).[144] T-lymphocyte function can be evaluated by measuring *in vitro* proliferative capacity to mitogenic stimulants such as phytohemagglutinin or anti-CD3/28. While patients with congenital athymia normally have absent or very low responses to mitogens, athymic patients with oligoclonal T-lymphocyte expansion may demonstrate normal proliferative mitogen responses but lack response to specific antigens. However, these assays are usually only performed in specialized laboratories and can be unreliable in lymphopenic patients, reflecting the reduced number of proliferating T lymphocytes rather than intrinsic T-lymphocyte dysfunction.[56] IgM and IgA are usually quite low, whereas, depending on the age of the patient, IgG may be normal as a result of maternal transplacental IgG transfer. Further investigation of humoral immunity by assessing specific antibody production is time-consuming and unnecessary in the diagnosis of athymia but is recommended in milder cases.

Genetics

Attempts should be made to genetically define suspected congenital thymic aplasia/hypoplasia; selection of the most appropriate genetic investigations may be guided by the patient's clinical phenotype (Table I). Chromosomal abnormalities underlie the majority of DGS cases but are routinely missed by whole exome sequencing (WES). Cytogenetic studies should therefore be performed, particularly in patients with a DGS phenotype. In this context, array comparative genomic hybridization is

preferable to fluorescence *in situ* hybridization or karyotyping because it allows for high-resolution genome-wide screening for genetic copy number variation and has been shown to increase diagnostic yield in the context of suspected DGS.[145,146] Where a monogenic disorder is suspected, the choice of molecular genetic tests is likely to be influenced by what is locally available. Broad next-generation sequencing approaches are preferred, including gene panel testing, where it is imperative to ensure that candidate genes (Table I) are included in the panel being used, and WES or whole genome sequencing (WGS). With the latter 2 modalities, a patient's genetic variants are usually first filtered against a list of genes known to cause IEI before more agnostic approaches are applied that have the potential to identify novel genetic causes.[147,148] For WES and WGS, although only sequencing of the proband can be performed, the inclusion of unaffected (typically parents) and, where available, affected, family members strengthens diagnostic power. WGS offers several advantages over WES because of its ability to detect noncoding and structural variants, as well as superior coverage of coding regions.[149,150] Single gene sequencing might suffice where the clinical phenotype is highly suggestive of a specific genetic diagnosis or if there is a positive family history, such as in nude SCID *(FOXN1)* or OFCS2 *(PAX1),* particularly if it is the most accessible and most quickly available diagnostic modality. When a genetic cause is identified, the family should be referred for genetic counseling and, if indicated, further genetic and clinical assessments. With regard specifically to 22q11.2DS, although most cases occur *de novo,* 5% to 10% of patients inherit the microdeletion from a parent.[50,151] Therefore, the risk of recurrence should be considered, with genetic testing extended to relatives and future progeny as appropriate.

Increasing access to next-generation sequencing and NBS for SCID has the potential to uncover genetic variation of uncertain clinical significance as well as to broaden the spectrum of disease associated with known IEI genes. The latter is exemplified by the recent description of hypomorphic *FOXN1* variants and consequent attenuated clinical phenotypes.[106,109-112] In contrast to nude "SCID"–causing null mutations, these hypomorphic variants may lead to delayed presentation, atypical, and/or milder clinical and immunologic phenotypes, which may improve with age and are unlikely to necessitate thymus transplantation.[106,110,111] Despite increasing access to comprehensive genetic testing, approximately 10% of all SCID patients remain genetically undefined.[152,153] Similarly, 13% of athymic patients who were recently referred to GOSH for thymus transplantation did not have a genetic diagnosis.[117,130] Patients with a T-B+NK+ immunophenotype in whom the underlying cause could either be a primary hematopoietic defect or congenital athymia require additional investigations, including an evolving array of research assays to assist their clinical management.

Other diagnostic investigations, including research assays

In genetically undefined patients, it is not possible to distinguish between hematopoietic cell intrinsic SCID and congenital athymia solely on the bases of immunophenotyping and proliferation assays. Imaging for thymus tissue does not differentiate between the 2 conditions, as an absent or greatly reduced thymic shadow can be seen in both and may be misleading when thymus tissue is ectopically positioned.[154]

Research assays are increasingly used to assist in the diagnosis of thymic stromal cell defects and in therapeutic decision-making. For example, to functionally assess the pathogenicity of novel genetic variants in transcription factors, reporter assays can be used to test the ability of the mutant transcription factor to bind to its target promoter DNA sequence and activate expression of a reporter protein. In the context of thymic stromal cell defects, such assays have been utilized to assess the pathogenicity of novel genetic variants in *FOXN1, PAX1, TBX1,* and *FOXI3*.[78,99,100,106,110,112,155] For genetically undefined T lymphocytopenia, the use of *ex vivo* T-lymphocyte differentiation research assays has been proposed to distinguish patients with primary hematopoietic defects from those with thymic stromal cell defects and thus direct the most appropriate form of corrective treatment —HCT versus thymus transplantation, respectively.[55,156,157] Patient $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) are cocultured with stromal cell lines engineered to express the human Notch ligands DLL-1 or DLL-4 (delta-like ligand 1/4) in the presence of growth factors to promote T-lymphocyte lineage commitment and differentiation, either in 2-dimensional monolayer cultures[158] or in 3-dimensional artificial thymic organoids or reaggregate thymus organ cultures.[159] Feeder cell–free systems are also available for *ex vivo* T lymphopoiesis.[160] On the one hand, in principle, successful production *ex vivo* of $CD4^+$ and $CD8^+$ double-positive and TCR^+ $CD3^+$ stages from patient-derived HSPCs after 6 to 8 weeks of coculture argues against a hematopoietic defect and is instead suggestive of a possible thymic stromal cell defect. On the other hand, HSPCs from patients with primary hematopoietic defects are expected to be intrinsically impaired in their ability to differentiate to these stages.[156,157,160] There are, however, exceptions to this, and HSPCs from patients with a number of hematopoietic stem cell (HSC)-intrinsic defects, such as hypomorphic variants in *RAG* genes or *IL2RG,* have been shown to differentiate *ex vivo* beyond their expected developmental block into double-positive and $CD3^+TCR\ \alpha\beta^+$ stages; and those with adenosine deaminase deficiency defy expectations because *in vitro* T-lymphocyte development is normal.[156,157] The sensitivity and specificity of these assays are not well established, and there is a lack of standardization across different research laboratories. Despite these limitations, *ex vivo* T-lymphocyte differentiation assays are helpful when facing the therapeutic dilemma of HCT versus thymus transplantation in patients with molecularly undefined, selective T-lymphocyte deficiency.[161] To avoid treatment delays, research assays to assist clinical decision-making are best arranged in coordination with a thymus transplantation team with experience in their clinical interpretation.

HOW TO IDENTIFY PATIENTS WHO NEED CORRECTIVE TREATMENT

Patients with congenital thymic stromal cell defects can have variable degrees of thymic hypoplasia and consequently thymic output. This means that the overall immunologic consequences can range from normal T-lymphocyte immunity to a T-B+NK+ immunophenotype. It is therefore important to stratify patients according to the extent of their immunodeficiency to distinguish those with complete athymia requiring thymus transplantation from those with thymic hypoplasia and residual thymic function, requiring just supportive care. This stratification is mainly based on immunophenotyping with quantification of thymic output and on clinical presentation (Fig 4).

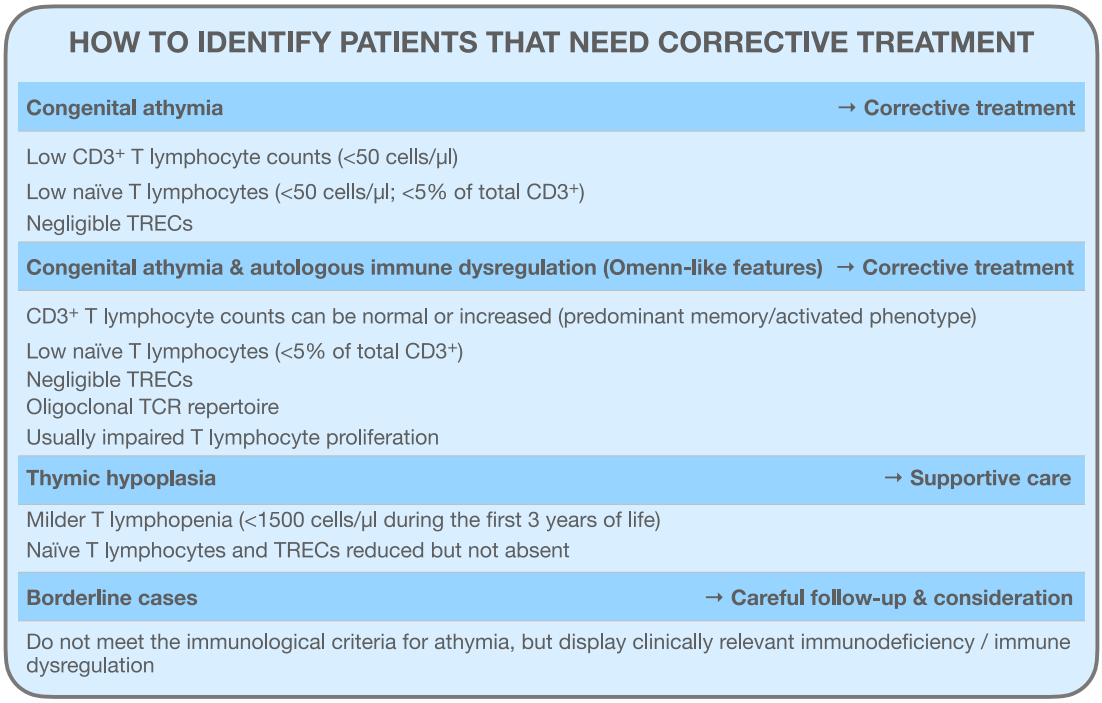

FIG 4. How to identify patients who need corrective treatment.

Immunology in patients with complete athymia

Patients with complete athymia typically have low total lymphocyte counts, although they can be normal as a result of increased B- and/or NK-cell numbers or as a result of oligoclonal T-lymphocyte expansions. More rarely, elevated T-lymphocyte counts can also be found as a result of maternal T-lymphocyte engraftment. Here, the engrafted T lymphocytes are usually predominantly $CD4^+$ or $CD8^+$ and have an activated or memory phenotype. All athymic patients, regardless of T-lymphocyte counts, have negligible TRECs and less than 5% of T lymphocytes displaying a naive phenotype, reflecting their absent thymic output.[56,141] Their immunodeficiency is life-limiting, and they need to be referred for corrective treatment without delay.

Immunology in patients with thymic hypoplasia

After exclusion of congenital athymia, certain patients are diagnosed with significant thymic hypoplasia, which is associated with milder T lymphocytopenia, defined as a $CD3^+$ T-lymphocyte count of <1500 cells/μL during the first 3 years of life.[49] Typical findings include reduced $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes (respectively, <400 and <200 cells/μL), naive T lymphocytes, and TRECs reflecting reduced but not absent thymic output.[49,56] T-lymphocyte numbers improve with age, reaching levels similar to healthy adult controls as a result of homeostatic proliferation with accumulation of memory cells and resultant skewing of the TCR repertoire.[49,51] Naive T lymphocytes and TRECs, however, remain reduced at all ages compared to age-matched controls, indicating persistently reduced thymic function.[51] T-lymphocyte function in patients with thymic hypoplasia is largely intact with normal mitogen responses. In patients with marked T lymphocytopenia, responses to specific antigens may be reduced, although this is likely solely due to low numbers rather than an intrinsic functional defect.[51] Regulatory T cells are reduced in number and frequency with defective suppressive capacity, which may contribute to the increased incidence of autoimmunity.[64,162] Abnormalities in humoral immunity may include low immunoglobulins, most commonly IgM or IgA deficiency, poor specific antibody responses, and occasionally hypogammaglobulinemia requiring immunoglobulin replacement therapy (IgRT).[163,164] The panel does not recommend administration of live vaccines in patients with $CD4^+$ T lymphocytes <400/μL and $CD8^+$ T lymphocytes <200/μL, or in patients with nonprotective IgG titers after tetanus immunization.[56] These patients may require supportive treatment, but they are not eligible for corrective treatment with thymus transplantation.

Borderline cases

Some cases do not meet the immunologic criteria for athymia but nevertheless display clinically relevant immunodeficiency with a history of significant infections and/or immune dysregulation. These patients require careful clinical and immunologic follow-up because their phenotype may evolve over time. For patients falling into this category case-by-case consideration regarding the most appropriate therapeutic strategy is best achieved in conjunction with a thymus transplantation center. This is particularly relevant when considering treatment options for patients with novel or ultrarare defects.[105,117,119] Moreover, if patients develop Omenn syndrome–like features, this should be considered as a marker of athymia, indicating the need for corrective treatment.

HOW TO MANAGE PATIENTS WITH CONGENITAL ATHYMIA

These guidelines are focused on the management of infants with congenital athymia (Fig 5). Guidance for the management of patients with thymic hypoplasia is available elsewhere.[56,165-167]

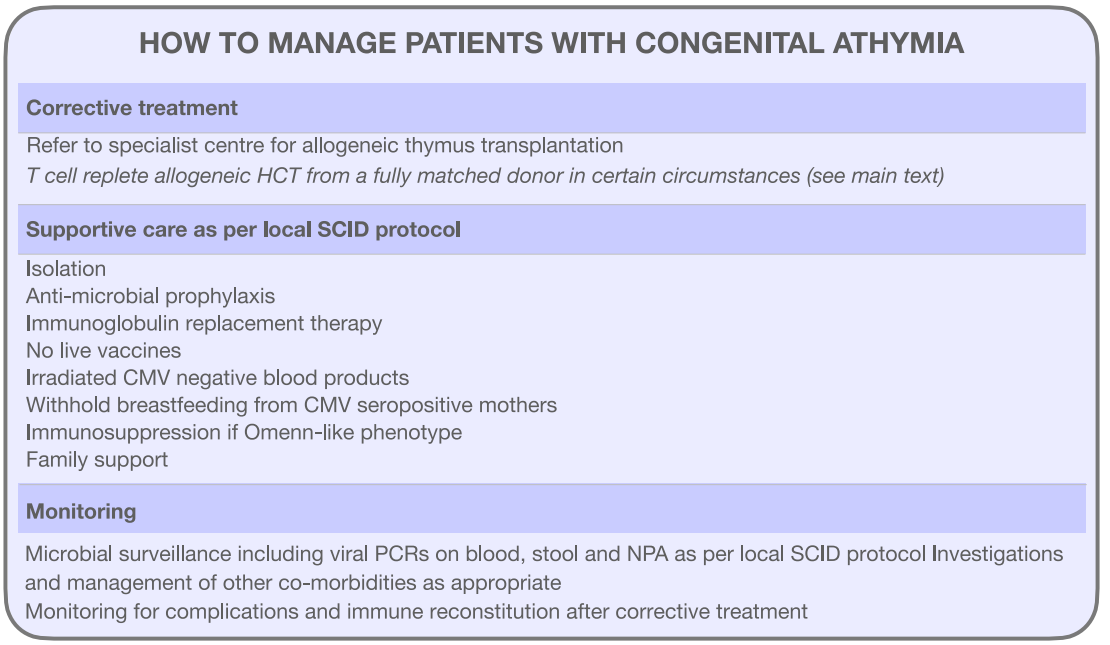

FIG 5. How to manage patients with congenital athymia. *NPA,* Nasopharyngeal aspirate.

Ideally, thymus transplantation should be considered as the first-line treatment in patients with congenital athymia, although geographical and financial constraints might limit access. Akin to outcomes after HCT for hematopoietic cell–intrinsic SCID,[168] outcomes after thymus transplantation are better when patients are treated early,[117] before they acquire infections.[115-117] Treatment at a younger age additionally seems to be associated with better initial immune reconstitution as a result of higher thymic output earlier after thymus transplantation.[117] Therefore, there should be no delay in referring patients to a thymus transplantation center while instituting comprehensive supportive measures locally.

Supportive measures and monitoring for complications

Patients are best managed by a local specialist pediatric immunology unit in conjunction with a thymus transplantation center. While awaiting corrective treatment, all efforts should be made to ensure that the patient remains free from infection and in the best possible overall clinical state because these factors influence outcome. Preventative care should be promptly instigated once the diagnosis of athymia is suspected, and SCID management protocols can be co-opted to guide comprehensive supportive care as well as to monitor for and treat complications.[56,169] Although there is variability among centers in such protocols, all athymic patients should be subject to reverse isolation measures, avoidance of ill contacts, and restricted non-staff caregivers.[169] Antimicrobial prophylaxis therapy should be initiated in line with local SCID protocols, including *P jirovecii* pneumonia prophylaxis (ie, trimethoprim–sulfamethoxazole), an azole antifungal (preferably fluconazole or itraconazole; itraconazole requires regular therapeutic drug monitoring), IgRT, and seasonal anti–respiratory syncytial virus prophylaxis using passive immunization with monoclonal antibody. Antimycobacterial prophylaxis with azithromycin should be considered in at-risk patients, particularly if there is likely to be a lengthy wait for thymus transplantation.[170] Live immunizations, such as bacillus Calmette-Guérin, rotavirus, and oral polio vaccines, are contraindicated, and patients should only receive irradiated, CMV-negative blood products. Breast-feeding should be withheld until maternal CMV status is known and discontinued if the mother is found to be CMV seropositive. Monitoring for viral infections is recommended, including regular PCRs on blood (for CMV, Epstein-Barr virus, adenovirus, and human herpesvirus 6), stool, and nasopharyngeal aspirates as per local SCID protocols. Additional microbiologic investigations may be required, guided by clinical presentation.

If a patient develops Omenn syndrome–like features, it is important to confirm this complication by documenting the occurrence of oligo-clonal T-lymphocyte expansions by immunophenotyping and/or spectratyping, and, if applicable, by showing spongiosis with T-lymphocyte infiltration on analysis of skin biopsy samples.[131] Patients developing an Omenn syndrome–like phenotype should be treated with cyclosporine A (CSA), with careful therapeutic monitoring of drug levels, typically aiming for trough levels of 150 to 200 μg/L. They also require careful skin management with emollients and, for troublesome skin symptoms, additional topical corticosteroids. Expert nutritional support is also essential. In patients with more severe clinical features, it may be necessary to temporarily treat with systemic steroids before immunosuppression while awaiting therapeutic CSA levels to be established. Systemic steroids should be reduced and stopped when the inflammation is under control and CSA levels optimal. In severe cases, immunosuppression with antithymocyte globulin may be considered. Alemtuzumab should be avoided before thymus transplantation because of its potential for depleting dendritic cells.[171] CSA treatment should be continued until after thymus transplantation.

Because athymia is often part of a wider congenital syndrome, patients may have comorbidities that require acute medical

attention, and some of these will require stabilization before treatment with thymus transplantation can be contemplated.[117,119,172] These include patients with CHD, who may need cardiac surgery before thymus transplantation; airway stabilization with positive airway pressure or tracheostomy due to underlying anatomic anomalies; and correction of hypocalcemia due to hypoparathyroidism. In patients with very wasted quadriceps muscles resulting from failure to thrive, implantation of thymus tissue may be difficult, and a period of nutritional support may be required to achieve muscle gain before the procedure. In some patients with life-limiting comorbidities, in particular cardiac and/or neurologic, palliative care is considered in discussion with the parents and multidisciplinary teams.[117,119] For all patients and their families, provision of adequate psychosocial support is essential.[119]

Overall, athymic patients often require complex clinical care, which benefits from multispecialty coordination and early involvement of the thymus transplantation team.[119,172]

Thymus transplantation

Thymus transplantation programs have been established in the United States at Duke University Medical Center since 1993[115] and in the United Kingdom at GOSH since 2009.[116] In the United States, cultured thymus tissue implantation, was approved as a regenerative medicinal product (Rethymic by Enzyvant, now Sumitomo Pharma) by the US Food and Drug Administration in 2021 for the treatment of congenital athymia at Duke Medical Center. Conversely, in the United Kingdom, thymus transplantation is not considered a medicinal product but is offered as a transplantation procedure as part of a nationally commissioned transplantation service regulated by the Human Tissue Authority. Both transplantation services are available to patients outside of the United States or the United Kingdom, although the service offered by GOSH is significantly less expensive than the commercialized treatment in the United States. At GOSH, treatment of patients from the European Union is currently still funded through reciprocal health care agreements with the United Kingdom, and those outside of the European Union can access thymus transplantation through the International and Private Patients service.[173]

Although the treatment access pathways are different, tissue preparation and implantation are broadly similar across both centers. Thymus tissue is donated by immunocompetent infants undergoing median sternotomy for cardiac surgery if tissue needs to be removed to improve access to the surgical field.[55,141] The tissue is processed into slices, which are then cultured for 13 to 19 days to deplete donor thymocytes while preserving the thymic stroma.[113] Once thymus tissue is in culture, rapid transfer of the recipient to the thymus transplantation center needs to be arranged. Donors and recipients do not need to be tissue type matched for human leukocyte antigens (HLAs), given the ability of donor thymus to induce tolerance,[174,175] but screening for anti-HLA antibodies in the recipient is recommended before proceeding with implantation of donor tissue. After microbiologic and histopathologic assessment to confirm safety and suitability,[176] cultured thymus tissue is implanted bilaterally into the quadriceps muscles of the athymic recipient.

Immunosuppression before transplantation is only required in patients with Omenn syndrome–like features and oligoclonal T-lymphocyte expansions. This is achieved with antithymocyte globulin serotherapy (Genzyme, 2 mg/kg once daily, 3 doses) in the days just before allograft implantation, in addition to previously established CSA treatment (trough levels of 150-200 μg/L). CSA is subsequently continued after transplantation until initial thymic output is evidenced by a frequency of naive $CD4^+$ T lymphocytes of >10% within total peripheral $CD4^+$ T lymphocytes, after which it is slowly decreased over 8 weeks.

Patients are usually transferred back to the referring center 2 to 4 weeks after the thymus transplantation procedure. Immune reconstitution after thymus transplantation is slow,[115,116] typically taking 5 or 6 months before naive T lymphocytes can be found in the peripheral blood—and longer in particular in patients with certain risk factors, such as systemic viral infections or ongoing cardiorespiratory instability. Therefore, patients initially remain severely immunocompromised after transplantation and require continued isolation, monitoring, and unchanged antimicrobial prophylaxis until satisfactory immune reconstitution is achieved. Avoidance of procedures requiring (prolonged) general anaesthesia and invasive ventilation in the weeks after thymus transplantation is recommended, if possible, because these may compromise revascularization of the allograft.[177] In the posttransplantation period, care is also needed in avoiding potential adverse consequences of treatments for comorbidities, such as receipt of corticosteroid therapy for airway issues and receipt of testosterone, which has negative trophic effects on the thymus,[178,179] for treatment of micropenis when present in CHARGE syndrome.

On revascularization of the implanted thymus tissue, the allograft will be repopulated with recipient-derived T-lymphocyte precursors, which then undergo stepwise maturation into functional T lymphocytes before egressing into the peripheral circulation. While this takes several months, thymopoiesis can typically be detected within the thymic grafts by 2 to 3 months after the procedure.[180] The panel recommends histopathologic assessment of thymic graft biopsy samples approximately 3 months after transplantation. This is best done by specialist surgeons, requiring a short readmission to the thymus transplantation center. Knowing the status of thymopoiesis in the thymic graft allows more informed decisions when managing potential complications such as autoimmune or inflammatory disease before initial recovery of peripheral T-lymphocyte immunity.

MANAGEMENT OF EARLY COMPLICATIONS AFTER THYMUS TRANSPLANTATION (BEFORE IMMUNE RECONSTITUTION)

The majority of deaths occur in the first year after thymus transplantation.[115,116] Infection is the most frequent cause of mortality, including preexisting infections and posttransplantation infections acquired before immune reconstitution has been achieved. Systemic viral infections are particularly challenging to manage in these patients because recovery of T-lymphocyte immunity after thymus transplantation is slow.

In the first months after thymus transplantation, patients should be carefully monitored for inflammatory complications. Before immune reconstitution, patients may still develop Omenn syndrome–like features as a result of their underlying condition, necessitating immunosuppression with CSA (targeting trough levels of 150-200 μg/L). At the time of immune reconstitution, preexisting infections or previously administered bacillus Calmette-Guérin can provoke an immune reconstitution inflammatory

response.[116] Life-threatening immune reconstitution inflammatory response requires treatment with high-dose steroids, which inhibits thymopoiesis and thus delays immune reconstitution. Early inflammatory complications are often transient, and if mild, steroid-sparing strategies should be explored to protect early thymopoiesis in the thymic allografts. A significant proportion of patients develop transient autoimmunity early after thymus transplantation, including autoimmune cytopenias and nephropathy.[115,116] Steroid-sparing strategies such as immunomodulation with high-dose intravenous immunoglobulin and/or B-lymphocyte depletion with rituximab are preferable. When faced with these various inflammatory and autoimmune complications, prior documentation of the status of thymopoiesis on thymic graft biopsy samples is helpful to aid therapeutic decision making, allowing the risks of these complications to be balanced against those posed by steroid-related toxicity within the developing allograft.[119] In a small number of patients, early donor T-lymphocyte engraftment has been found,[115] but without any adverse effects such as graft-versus-host disease. The level of this engraftment diminishes over time.

LONG-TERM FOLLOW-UP AFTER THYMUS TRANSPLANTATION AND LATE COMPLICATIONS

After the first emergence of naive T lymphocytes to the periphery, T-lymphocyte counts progressively increase over time, peaking 1 to 2 years after thymus transplantation.[115,116] In most patients, the absolute numbers of total T lymphocytes, naive T lymphocytes, and TRECs (quantified on peripheral blood T lymphocytes) remain below the 10th percentile for age. Nevertheless, thymic output is sustained with at least 10% of T lymphocytes continuing to have a naive phenotype, the TCR repertoire becomes diverse, and T-lymphocyte proliferative responses to mitogens and antigens normalize. All this, taken together, suggests that despite suboptimal counts, thymic output is sufficient for satisfactory T-lymphocyte immunity, allowing clearance of existing and new infections. Antimicrobial prophylaxis and IgRT can usually be discontinued.

Continuation of IgRT for 1 or 2 years after thymus transplantation until there is evidence of increasing and sustained recovery of T-lymphocyte immunity is recommended. The choice of home therapy with subcutaneous immunoglobulin infusions should be provided to families, if available, via the child's primary hospital with appropriate training and support for parents.[181] Immunizations, following national immunization schedules, should be commenced 3 months after discontinuation of IgRT if IgG levels are maintained within normal ranges and the patient remains clinically well. Once immunizations begin, it is important to document protective antibody titers against inactivated vaccines before proceeding with administration of live attenuated vaccines. The latter have been administered to most transplanted patients without any adverse events despite low $CD8^+$ T-lymphocyte counts in some patients. Long-term clinical and immunologic outcomes at >2 years after thymus transplantation have not been published for most patients, and multicenter data collection is limited by the absence of congenital athymia as an entity in IEI registries.[119] A standardized immunophenotyping protocol for monitoring is recommended, including assessment of naive T lymphocytes and/or recent thymic emigrants at regular intervals after thymus transplantation. The long-term monitoring of thymic output also benefits from TREC analysis on sorted T lymphocytes and thymus donor engraftment studies.

Thymus transplantation is aimed as a one-off therapy, and only one patient has been reported to require a second thymus transplantation procedure after the first graft failed in the context of sepsis shortly after implantation.[116] To date, few late deaths have been reported after thymus transplantation[115,117] and no late complications have occurred, such as significant infections due to hypothetical thymic graft exhaustion or malignancies, although overall follow-up is still relatively short.

Ongoing autoimmunity is relatively common after thymus transplantation,[55,115,116] suggesting that the allografts may generate suboptimal recipient-specific central tolerance. While chronic cytopenias have been reported in a tiny number of patients, autoimmunity after thymus transplantation mainly seems to be restricted against the thyroid, with autoimmune thyroiditis observed at significantly higher rates than in patients with thymic hypoplasia.[49] It is therefore important to regularly monitor thyroid function in patients and, if impaired, to test for antithyroid autoantibodies.

The panel recommends multidisciplinary long-term follow-up. Syndromic comorbidities do not resolve after thymus transplantation, but their management becomes easier after satisfactory immune reconstitution.[172] A degree of predisposition to recurrent infections may persist in patients with coexisting anatomic or functional abnormalities, particularly of the airways. However, infection risk overall decreases, and patients (and their families) can proceed with socialization, thus improving their overall quality of life with better access to health, education, and social services.[119] Access to ongoing familial support can be provided through immunology clinical nurse specialists and dedicated clinical nurse specialists at the thymus transplantation center.[119] This support encompasses emotional and psychological support, education and training, and signposting. There are no established patient advocacy groups for congenital athymia, but clinical teams may be able to offer peer support from previously treated families.

ROLE FOR HCT IN CONGENITAL ATHYMIA

Although thymus transplantation is recognized as the most appropriate treatment for congenital athymia, T-cell–replete HCT from a fully HLA-matched donor can be attempted under certain circumstances.[74,120,182] These include situations where geographic and/or financial constraints prevent access to thymus transplantation, and in the context of preexisting systemic viral infection (ie, disseminated CMV disease and adenofibroma). In such situations, HCT can confer some degree of T-lymphocyte immunity through the transfer of postthymic mature T lymphocytes. Overall, HCT in congenital athymia yields poor outcomes, with a survival rate below 50% and a high risk of graft-versus-host disease.[99,120] One study reported improved outcomes if a matched sibling donor is available, with an initial survival rate of 60% (8/13), and 25% (2/8) for those who received HCT from HLA-matched unrelated donors.[120,121] The quality of immune reconstitution after HCT is also inferior to that achieved with thymus transplantation, as it relies on homeostatic expansion of transferred mature T lymphocytes rather than generation of new T lymphocytes. Naive T-lymphocyte counts are therefore lower and the TCR repertoire remains restricted after HCT.[117] If HCT is from a matched family donor, it is recommended that no

conditioning be provided, and this should allow immune reconstitution to a degree capable of controlling viral infections significantly more rapidly compared to after thymus transplantation.[74,182] It may be possible to subsequently proceed with a thymus transplantation procedure to improve immunity, provided there have been no severe complications after HCT, as has been reported in 5 patients.[105,115] Thymus transplantation after HCT requires careful selection of donor thymus tissue with partial tissue type matching with the HSC donor at any alleles that were mismatched with the recipient.[105,115] The development of more effective antiviral medications and advances in adoptive virus-specific T-lymphocyte therapies may also provide temporary solutions for patients before thymus transplantation.[183,184]

TRANSLATIONAL RESEARCH AND FUTURE DIRECTIONS FOR NOVEL TREATMENT STRATEGIES

We have already emphasized the growing use of research assays, in particular transcription factor reporter assays and *ex vivo* T-lymphocyte differentiation assays in the clinical diagnosis and therapeutic management of patients with a genetically undefined T-B+NK+ immunophenotype. These *in vitro* assays also play a role in disease modeling of novel defects associated with impaired T lymphopoiesis, including novel thymic stromal cell defects, by delineating their likely HSC-intrinsic or HSC-extrinsic nature.[78,100,156,157,160,185,186] Additionally, induced pluripotent stem cells can be differentiated into thymic epithelial progenitor cells,[187-189] making it possible to specifically investigate the impact of novel variants in known disease-causing genes or in candidate genes on TEC development and function, when starting from patient-derived and/or gene-edited induced pluripotent stem cell lines.[99,185,190] Together, these approaches facilitate the characterization of novel defects, contributing to strengthening the diagnostic pathway on validation of more disease-causing genes.

Research is also focused on optimizing the current treatment approach using cultured postnatal thymus tissue, and on developing novel treatment strategies.[55] How HLA-mismatched thymic allografts are able to support thymopoiesis remains poorly understood, yet the incomplete recovery of T-lymphocyte immunity and relatively common autoimmune manifestations after thymus transplantation suggest that T-lymphocyte development and induction of central tolerance are suboptimal. To date, no beneficial effect has been reported for fortuitous partial HLA matching between thymus donor and athymic recipient.[116,191] This needs to be reassessed in the growing cohorts of transplanted patients, but in theory at least, partial tissue type matching may contribute to improving outcomes. Generation of a thymus tissue bank would be required for partial tissue type matching to be feasible, and encouragingly, preclinical data suggest that cryopreserved thymus tissue can support T-lymphocyte development after culture and transplantation into athymic mice.[192,193]

Considerable progress has been made recently toward producing engineered thymic stroma suitable for clinical applications.[55] Natural decellularized extracellular matrix from human thymus can be obtained and seeded with human thymus stromal progenitor cells that can be expanded *in vitro*.[20,26] Larger amounts of human thymus stroma could be produced with this approach, and because stromal progenitors can be banked, partial tissue type matching would also be feasible.

CONCLUSIONS

Congenital athymia is a life-limiting disorder, requiring corrective treatment, ideally by transplanting lymphodepleted donor thymus tissue. We have provided expert guidance on the diagnosis, investigation, and management of patients with congenital athymia, with the aim of improving their outcomes. Our panel recommendations are summarized in Fig 1.

Outcomes are best with early diagnosis and institution of both supportive and definitive management, before infectious and inflammatory complications have developed. We have therefore emphasized the scenarios in which congenital athymia can present. Namely, patients might present clinically with syndromic features, infections, or inflammatory complications; increasingly, patients are identified in the context of TREC-based NBS programs for SCID; and finally, failed T-lymphocyte reconstitution after allogeneic HCT for genetically undefined suspected SCID should alert to the possibility of an absent thymic niche. These scenarios should prompt the completion of the set of investigations recommended herein.

Increasing access to NBS and comprehensive genetic testing are promoting early diagnosis and treatment, resulting in better outcomes after thymus transplantation, but have also revealed a particular challenge with respect to the diagnosis of athymia versus hematopoietic cell–intrinsic defects in molecularly undefined congenital T-lymphocyte deficiency, where knowing the cellular etiology influences treatment choice between thymus transplantation and HCT. With higher morbidity and mortality rates after HCT in athymic patients, clinical translation of research assays to assist in diagnosis and therapeutic decision-making is essential.

Thymus transplantation, the recommended first-line treatment for congenital athymia, is a highly specialized treatment, currently only available in 2 centers worldwide. Patients treated with thymus transplantation have good outcomes overall, with durable T-lymphocyte immunity and improved quality of life. Mortality is mostly due to infections occurring before treatment or before successful immune reconstitution, which is typically slow after thymus transplantation. Autoimmune complications are relatively common, in particular transient autoimmune cytopenias and persistent autoimmune thyroiditis. The panel recommends centralized recording of long-term clinical and immunologic outcomes after thymus transplantation to adequately prioritize strategies aimed at improving outcomes for athymic patients yet is complicated by the geographical spread of the patients and the lack of national and international registries for congenital athymia. Cost and availability of lifesaving novel gene and cell therapies for rare conditions are a concern even in high-income countries, and while in Europe accessible and timely treatment with thymus transplantation is available, continued efforts are necessary to overcome geographic and economic challenges and to promote equitable and timely access to treatment. In the future, the further expansion of NBS programs, the creation of dedicated registries, the development of new approaches for thymus replacement therapy, and the support of initiatives promoting sustained accessibility to novel therapies may address some of these challenges.

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