REVIEW ARTICLE

Diagnostic Approach and Treatment of Severe Combined Immunodeficiency

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ABSTRACT

Severe combined immunodeficiency (SCID) is an inherited primary immunodeficiency disorder caused by a developmental defect of T lymphocytes or inadequate B-cell activation due to insufficient T lymphocyte activity or direct involvement of B lymphocytes that causes a defective antibody response. It is one of the most severe forms of primary immunodeficiency diseases, resulting in early death. Affected patients usually manifest in early infancy with lymphopenia, recurrent infections, opportunistic infections and may be fatal if untreated. The management and diagnosis of SCID depends on the country expertise and availability of comprehensive immunodiagnostic laboratory capacity. Early detection of SCID has aided in improving outcomes for those with the disease. The epidemiology, diagnosis, and treatment approaches of the disease are discussed in this study.

Keywords: Severe Combined Immunodeficiency, Inborn Error of Immunity, Immunological screening, Malaysia, Children healthcare

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INTRODUCTION

Primary immunodeficiency diseases (PID), also known as inborn errors of immunity, are inherited disorders of heterogeneous group of genetic disorders manifests as increased susceptibility to infections, immune dysregulation, autoimmunity, inflammation, allergy and malignancy (1, 2). The most severe form of PID is severe combined immunodeficiency (SCID); is a heterogenous group of diseases characterized by impaired T lymphocyte and/or B lymphocyte formation or differentiation, leading to impaired lymphocyte functions and presence with severe abnormalities of the immune system (3-5).

Early diagnosis and curative intervention of the disease is vital, because otherwise patients will rapidly succumb to infections leading to death within the first year of life or later (6, 7). Early intervention through newborn screening and transplantation within the first three and half months of life has been found to have better outcomes (8). Prompt intervention by interprofessional team such as paediatrician, clinical immunologist, geneticist, nurses and researcher could minimize complications and save lives by administering the required treatment for the affected patients (1). Despite various data supporting favorable outcome in those diagnosed and received curative therapy early, diagnosing SCID still remains as a challenge. A continuous effort in improving awareness among the healthcare professionals is important as part of improving early diagnosis of SCID in affected patients. This review outlines the epidemiology, diagnostic and management approach to SCID.

DEFINITIONS OF SCID

According to the consensus proposed by the Primary Immune Deficiency Treatment Consortium, SCID are further defined as typical SCID and leaky SCID (5). Typical SCID is characterised by absent or very low numbers of T cells (CD3+ T cells <300/uL) and no or very low T cell function (<10% lower range of normal), as measured by proliferation to phytohaemagglutinin (PHA) or detectable transplacental maternal engraftment (TME) when T cell proliferation do not available (9).

Atypical or leaky SCID is characterised by a reduced number of CD3+ T cells for age, less than 30% of lower limit of normal T cell function as measured by proliferation with PHA, reduced or absent naıve T cells and absence of TME (10). Leaky SCID is a less severe

form of SCID, occurs when a patient has symptoms similar to typical SCID, but with low T cell count which can also be detected by newborn screening (11). T cell proliferation assay usually shows a weak T cell response to PHA (less than 30% of lower limit of normal T cell function) with absence of TME (9).

SCID EPIDEMIOLOGY IN MALAYSIA

The first systematic review that have been conducted in Malaysia showed the prevalence of PIDs in Malaysia, which was 0.37 per 100,000 population from 1979 until 2019 (1). This retrospective data was systematically reviewed to provide information for the medical community, the health authorities, the patients and their families and add to the body of evidence on the epidemiology of PID in view of the absence of Malaysian national PID registry. The data showed that the commonest reported PID diagnosis was SCID, affecting 22 patients, with 10 genetically diagnosed SCID patients which includes common gamma chain cytokine receptor (*IL2RG*), adenosine deaminase (*ADA*), purine nucleoside phosphorylase (*PNP),* tetratricopeptide repeat domain-7A (*TTC7A*) and zeta-associated protein 70 (*ZAP70*).

In comparison to other parts of the world, a study in Switzerland reported a higher incidence of SCID, 24.3 cases per 100,000 live births, whereas the incidence of SCID in the United States of America (USA) was estimated to be 1 in 100,000 births (12, 13). After newborn screening was implemented in the USA, the most striking finding was that the frequency of SCID observed was 1 in 58,000 livebirths, with 52 cases of typical SCID, leaky SCID, and Omenn syndrome, implying that SCID may not be as rare as we believe (12). The reported SCID prevalence worldwide ranged from 1 in 10,000 to 1 in 50,000 livebirths, with most identified mutation was *IL2RG* (13, 14). Due to X-linked inheritance in the most common SCID genotype, males in an outbred population are more affected than females (10).

The lack of a national PID registry database, insufficient immunodiagnostic capacity, a national newborn screening programme, and a lack of awareness among healthcare providers all contribute to the difficulty in determining the prevalence of SCID in Malaysia (15). From the evidence stated above, SCID was under-diagnosed and under-reported in Malaysia. To address these concerns, a coordinated effort from multiple authorities such as the Ministry of Health, Malaysia, and the universities is required.

GENETIC OF SCID

There are 1800–2000 genes in the human genome that are believed to be involved in immune responses (16). Inborn errors of immunity revealed that single genes and their products have non-redundant roles in immune function (2). The study of the molecular aetiologies of SCID has yielded valuable insights into immune cell development and regulation. SCID is caused by a variety of genetic mutations that affect T and B cell functions, including those in the *IL2RG, JAK3, IL7R, RAG1* and *RAG2, DCLRE1C*, and *ADA* genes (4, 17, 18). In some instances, SCID may be present with only T cell defect but since B cells rely on T cells signaling to generate sufficient antibodies, T-cell abnormalities may prevent B cells from functioning normally (19).

The general classification of SCID phenotypes includes T-B- SCID or T-B+ SCID phenotypes. This is based on B cell status, with further sub-categories based on natural killer cell status (20). T-B+ SCID are characterised by the absence of mature T and NK lymphocytes, while B cells are present in increased number (21). This X-linked SCID is due to mutations in the *IL2RG* gene, which encodes the common gamma chain and associates with very low T cells, normal to high B cells with low immunoglobulins levels, and low NK cells (2). Autosomal recessive inheritance for T-B+ SCID includes *JAK3* deficiency, *IL7Rα* deficiency, *CD45* deficiency, *CD3δ* deficiency, *CD3ε* deficiency, *CD3ξ* deficiency, *Coronin-1A* deficiency and *LAT deficiency* (2). T-B- SCID is typically characterised by a defect in both T and B cells with normal to low functional of NK cells, which includes *RAG* deficiency, *Artemis* deficiency, *DNA PKcs* deficiency, *Cernunnos/XLF* deficiency, *DNA ligase IV* deficiency, *ADA* deficiency, *AK2* defect and activated *RAC2* defect (2).

The genotype-phenotype correlations based on common SCID gene mutation are described according to The International Union of Immunological Societies (IUIS) Expert Committee Table I. The International Union of Immunological Societies (IUIS) Expert Committee reported the updated classification of Inborn Errors of Immunity/Primary Immunodeficiencies (22). The report includes main clinical and laboratory features of 456 inborn immunity errors, including 26 gene defects that were recently discovered. The growth and increasing complexity of the field have been remarkable, encompassing an increasing variety of conditions, and the IUIS classification serve as a critical reference for immunologists and researchers worldwide with the updated genetic cause of immune deficiency (23).

CLINICAL MANIFESTATION OF SCID

Most of SCID patients presented normally at birth within six months of age and initial symptoms such as recurrent pneumonia (66%), followed by failure to thrive (60%) and chronic diarrhoea (35%) (24). These clinical manifestations of SCID patients presented with bacterial, viral, fungal, and protozoal infections usually begins at 6 months of age or earlier (19). Children with SCID are exposed to community acquired infection before diagnosis which may induce end organ damage,

Table I: List of SCID genetic defects according to IUIS Classification (2)

Disease	Genetic defect		Associated feature
T-B+ SCID	*IL2RG*	IL-2R common gamma chain	absent IL receptor for a range of cytokines due to lack of common gamma chain
	JAK3	Janus kinase 3	lack of Jak 3 kinase to follow signal via IL-R binding.
	IL7R	IL-7Rα chain	no IL-7α chains that lead to the failure of T cell differentiation
	PTPRC	CD45 deficiency	Normal γ/δ T cells
	CD3D	CD3δ deficiency	Normal NK, no γ/δ T cells
	CD3E	CD3ε deficiency	Normal NK, no γ/δ T cells
	CD3Z	CD3ζ deficiency	Normal NK, no γ/δ T cells
	CORO1A	Coronin-1A deficiency	Detectable thymus
	LAT	LAT deficiency	Typical SCID or combined immunodeficiency, the latter with adenopathy, splenomegaly, recurrent infections, autoimmunity
T-B- SCID	*RAG 1/RAG2*	Recombinase activating genes 1 and 2	RAG1/2 enzymes to snip DNA for VDJ rearrangement for TCR and BCR.
	ADA	Adenosine Deaminase	leads to toxic metabolites
	DCLRE1C	DNA cross link repair enzyme 1C (Artemis)	failure to repair DNA after RAG1/2 snips
	LIG4	DNA Ligase IV	Radiation sensitivity
	PNP	Purine nucleoside phosphorylase	Neurological impairment
	NHEJ1	Cernunnos/XLF deficiency	Normal NK, radiation sensitivity, microcephaly
	AK2	AK2 defect	Reticular dysgenesis with neutropenia; deafness
	RAC2	Activated RAC2 defect	Recurrent bacterial and viral infections, lymphoproliferation; neutropenia

especially to the lungs and liver, and are linked to a higher rate of morbidity and mortality (25). Infants with SCID usually develop a bronchiolitic type illness such as chronic cough and wheezing. The symptoms worsened over time with increase suspicion of *Pneumocystis carinii pneumonia*, *cytomegalovirus* (*CMV*), or *aspergillus* infection (6) and failure to thrive once the all the complication started. A chest radiograph of a SCID patient shows an absence of the normal widening of the mediastinum suggesting an absence or decrease volume of the thymus (26). Gastrointestinal infection that may cause failure to thrive in SCID is usually from viral infection such as rotavirus.

Live vaccines are generally contraindicated in immunodeficiency for example rotavirus vaccination in SCID patients and therefore the undiagnosed SCID patients are at higher risk of developing untreatable diarrhoea (27). *Omenn* syndrome is a form of combined immunodeficiency marked by a generalised erythematous rash, swollen lymph nodes, hepatosplenomegaly, extreme infection sensitivity, eosinophilia, and hyper-immunoglobulinemia (28). SCID infants vaccinated with *Bacille Calmette-Guérin* (BCG) vaccine at birth are also at increased risk of disseminated BCG infection (29). SCID are vulnerable to candidiasis and frequently colonised the skin, oropharynx, and gut in SCID patient (6, 30).

DIAGNOSIS

A paediatric immunologist should be consulted right away if a newborn is suspected of having SCID (10). Suspected SCID cases should be assessed according to the standard protocol that are available in the institution. A thorough assessment of the immune system, which involves evaluating medical and family history, physical examination, blood or vaccine testing, are among the first steps in diagnosing SCID (31). The diagnostic investigation of a patient with SCID in Malaysia includes the assessment of blood lymphocyte subpopulations, quantitative serum immunoglobulins, lymphocyte stimulation assays, metabolic investigations, and genetic testing. These investigations are outlined in Table II.

There are several common pitfalls in diagnosing SCID. A full blood count is the most commonly requested but the absolute lymphocyte count is often ignored (6). A study had showed that absolute lymphocyte count is the most important screening tool for T-cell defect, however, normal lymphocyte count does not exclude SCID, but further immunologic workup should be done (32). When infants have an absent or very low numbers of T cells (CD3+ T cells < 300/μL) it is very likely that they suffer from SCID (10). If a low absolute lymphocyte count observed, lymphocyte phenotyping with monoclonal antibodies and fluorescence-activated cell sorting (FACS)

Table II: Proposed investigations for diagnosis of Severe Combined Immunodeficiencies

Line of investigation	Test	Finding
First line	Full Blood Count	Lymphopenia, absolute lymphocyte count should be compared to correct age of reference
	Lymphocyte subsets (TBNK measurement)	Determination of SCID phenotype
	Serum Immunoglobulin level (IgG, IgA, IgM)	Normal or low
Second line	Lymphocyte proliferation test to PHA mitogens	Functional assay to assess the proliferation of lymphocyte towards stimuli
	Narıve/memory T cells	Absence in SCID
	ADA and PNP enzyme assays	For suspected ADA and PNP SCID
Third line	Genetic testing	For confirmatory diagnosis and genetic counseling

analysis is the most important confirmatory examination to be done where absolute numbers of lymphocyte subtypes are more useful than percentages, and each phenotype pattern indicates a particular diagnosis (6). Immunoglobulin is more commonly used as a screening test, but it is less effective and can be misleading in the early months of life due to transplacental transfer of maternal IgG and physiologically low levels IgA and IgM in early infancy and the value must be compared with age-specific references (6, 33).

The lymphocyte proliferation assay of SCID patients is a critical diagnostic work up that evaluate an absent or low responses to the mitogen phytohemagglutinin (PHA) (34). Absence or low response of T cell to the PHA indicate defective T cell functions. Lymphocyte proliferation studies revealed that lymphocytes are anergic, and thus T cell activation defects may be observed even if the lymphocytes are anergic to specific mitogens (6). Raised erythrocyte deoxy ATP levels in *ADA* deficiency, or decreased erythrocyte purine nucleoside phosphorylase (PNP) activity in *PNP* deficiency can be detected through metabolic investigation (6).

In the diagnosis of many primary immunodeficiency disorders, genetic testing is very crucial and constitutes standard of care (35, 36). Suspected diagnosis for SCID and in cases of unexplained SCID-like presentation, genetic analysis can be expanded *in silico*. According to a recent study, Malaysia needs to improve its role in the field of genetic testing as Malaysians are ethnically diverse, with a high frequency of genetic diseases (37). In Malaysia, genetic testing for SCID is not generally available locally, and most of them are paid for by patients and their families (1, 15). Development of genetic test specifically for SCID in Malaysia are important and essential as some genetic studies are expensive and not readily accessible by the healthcare professional. Main challenge of immunologists and geneticists in determining a genetic diagnosis for SCID patients is related to the wide genetic heterogeneity of SCID (38). Awareness of the disease among medical practitioners and referral to a regional immunology center for further investigation of SCID is very important for early intervention (39). A study conducted on the awareness of community among Malaysians in Klang Valley regarding the potential providers of genetic testing has shown strong evidence (64.6%) of people believe that genetic testing should only be done in hospitals with a doctor's prescription (40).

Next generation sequencing (NGS) technologies are DNA sequencing technology that used in clinical practice which enable the sequencing whole human genome has led to an unprecedented increase in the number of successful SCID variants being identified in PID patients (41). One of the technologies include the use of targeted gene panels in which the clinical suspicion is directed toward a narrow set of reported SCID genes, allowing higher sequences coverage and an easier first-line evaluation of results (42, 43). While gene panels have been implemented for distinct groups of SCID genes, clinical exome sequencing has been proposed to analyse hundreds of PID genes simultaneously (44). To date, the available clinical exome data has recorded nearly 400 genes defect identified in patients with distinct PID (45). In cases with no obvious genetic explanation for the disease, whole genome sequencing can be preferred although it requires complicated analysis given the large amount of data (44). Further, exome and genome sequencing are able to re-analyse data in the light of newly discovered disease-related genes in patients without a genetic diagnosis (43). However, several drawbacks can hinder the interpretation of genetic results. First, exome sequencing may miss structural defects and some genes may be poorly covered in NGS. Lastly, genetic studies may yield lots of data of uncertain significance, requiring new immunologic studies for a definite confirmation (46).

MANAGEMENT OF SCID PATIENTS

The focus of treatment for SCID patients is to early identification, prevention and management of active infection followed by preparing them for hematopoietic stem cell transplantation. All patients with SCID should be considered for haematopoietic stem cell transplantation (HSCT) as it offers a chance of curative treatment. Parents should be counselled for transplant and urgent donor search and HLA typing of patient, siblings and parent should be arranged immediately. Delayed diagnosis, pneumonia requiring assisted ventilation, graft versus host disease are amongst the adverse prognostic features reported in the previous study which emphasized on early diagnosis of SCID is

very important to reduce postponement of bone marrow transplant in Malaysia (39) .

Supportive care and prophylaxis medication

Prophylaxis for infections is warranted for SCID patients in view of their underlying immune defects. Several prophylaxis medications are as follow: Co-trimoxazole for *Pneumocystis jirovecii* infection, Fluconazole as antifungal prophylaxis and acyclovir as *Herpes simplex* virus prophylaxis (Table III) (7). Infections should be treated aggressively according to the culture and sensitivity results. A high index of suspicion for fungal or viral infections are warranted especially if patient did not respond to standard antibiotics therapy. SCID patients should receive irradiated, leuko-depleted and cytomegalovirus (CMV) negative blood products if needed blood transfusions prior to transplantation.

Live vaccines should be avoided in SCID patients as they may progress into active overwhelming infections. The serological status of mothers for cytomegalovirus should be checked before considering for breast feeding. Breast feeding are usually discouraged if mother is CMV seropositive and the patient is CMV PCR negative (13). Viral PCR investigations should be performed for viral screening in SCID patients instead of serological method.

The value of *ADA* enzyme replacement (polyethylene glycol modified adenosine deaminase) in *ADA* SCID is debatable especially in situation where HLA-matched donors are available in view of inferior long-term immune reconstitution after transplant. However, most may benefit from *ADA* enzyme replacement therapy as temporary measure before transplant or gene therapy rather than as a long-term treatment (4, 7).

Immunoglobulin replacement therapy

Immunoglobulin replacement treatment should be considered upon diagnosis, irrespective of immunoglobulin levels. The dosage suggested is at 0.4g/kg every 3-4 weeks for intravenous route (3,13). However, the regimen may change during and after transplantation period as per local protocol.

Haematopoietic stem cell transplantation (HSCT)

Allogeneic HSCT offers the option of curative therapy to SCID patients. It should be planned and performed immediately and requires thorough explanation to affected parent and patients about the risks and benefits of the procedure. The best survival outcome of transplantation has been showed in those receiving it before the age of 3.5 months old and no active infection at the time of transplantation (8).

The best donor is HLA-matched sibling donor. However, it may not be available to all SCID patients and families. Availability of better T cell depletion techniques such as TCR αβ and CD19+ depletion has makes haploidentical donor options more attractive as the survival outcome was improved to 83.9% - 96.7% (47, 48). The availability of effective conditioning regimen with lesser toxicity such as Treosulfan compared to Busulfan are promising (49). Usage of conditioning chemotherapy were associated with better long-term thymopoiesis and

Table III: Recommended infection prophylaxis for SCID patients as per guideline by the Australasian Society of Clinical Immunology and Allergy Transplantation and Primary Immunodeficiency (TAPID) group (7).

Medication	Indication	Dosing	Alternatives	Notes
Co-trimoxazole	Pneumocystis jirovecii prophylaxis	As per local protocol OR 2.5 mg/kg trimethoprim component BD on 3 days of the week from 4 weeks of age	Intravenous pentamidine 4 mg/ kg every 4 weeks or oral atovaquone as per Australian Medicines Handbook (AMH) dosing (age dependent)	-
Fluconazole	Fungal prophylaxis	As per local protocol OR AMH guidelines suggested range 3–6 mg/kg Every 72 h if neonate <2 weeks of age Every 48 h if neonate 2–4 weeks of age Daily if >4 weeks of age	Consider itraconazole or alternative mould active antifungal agent in neutropenic patients	Commence at diagnosis
Aciclovir	Herpes simplex virus prophylaxis	Patients <6 months; 300 mg/m2 three times a day orally Patients 6–24 months old; 100 mg three times a day orally	-	Consider if any carer with direct patient contact has history of recurrent cold sores
Palivizumab	Respiratory syncytial virus prophylaxis	As per local protocol	-	-
Immunoglobulin replacement therapy		Intravenous or subcutaneous 0.4–0.5 g/kg/month starting dose	-	Commence at diagnosis irrespective of IgG level

immune reconstitution in SCID patients (50, 51). SCID is curable with allogeneic HSCT or, in some genotypes, enzyme replacement therapy (ERT) or gene therapy, despite the fact that it is deadly without treatment (52).

Recent cohort report showed good success and survival outcome of hematopoietic stem cell transplantation performed in local institution (53). However, several main challenges of the success HSCT have been identified such as delayed in diagnosis, delayed in initiating treatment, financial support for procedures and HSCT center experience for highly technical procedures for stem cell manipulation.

NEWBORN SCREENING FOR SCID

Newborn screening (NBS) test for SCID detects the presence or absence of adequate levels of T cell receptor excision circles (TREC) which will have tremendously reduce numbers of thymically derived T cells, and therefore very low or absent TREC levels (52, 54, 55). In the absence of a national newborn screening programme, SCID patients typically present with natural progression of the disease and manifest with severe, recurring, or persistent infections, as well as opportunistic infections, within the first few months (56). This newborn screening has been implemented in several countries such as United States of America, New Zealand, Sweden and Germany. Newborn screening for SCID offers early diagnosis and is a cost-effective method for early diagnosis of the disease. Apart from SCID, other causes of CD4 lymphopenia were also detected from the newborn screening program such as Di George Syndrome, Down Syndrome, Ataxia telangiectasia, CHARGE Syndrome and secondary causes of CD4 lymphopenia (57).

The availability of NBS is still absence in many developing countries including Malaysia (58). An abnormal NBS necessitates an urgent subspecialty immunology examination at a facility specialising in the diagnosis of SCID and its genetic causes (59). In order to encourage health officials to incorporate screening in priority health spending, local incidence and outcome statistics are employed (58). Diagnosing infants at birth may protect them from infection and transplanted earlier will enhance their chances of survival since the cost-effectiveness of the established NBS from many research dramatically improves the outcomes of SCID (60).

GENE THERAPY

Autologous haematopoietic stem cell gene therapy provides the ability to address genetic abnormalities across haematopoietic lineages without the drawbacks of allogeneic HSCT therapy. This technology has been applied for *ADA* SCID and *IL2RG* SCID via clinical trials, however was hampered with leukemia/myelodysplasia in patients due to insertional mutagenesis from gamma-retroviral gene addition approaches (61). Lentiviral vectors have since been created to overcome this problem. Ex-vivo lentiviral gene therapy resulted in high overall and event-free survival with sustained *ADA* expression, metabolic correction, and functional immune reconstitution in *ADA*-SCID patients (62). However, this therapy is still not available in Malaysia.

CONCLUSION

It is crucial for early diagnosis and early intervention for SCID as best curative outcome can be achieved. The advancement in diagnostic, safer transplant technique and conditioning options and progress in newborn screening program has made possible for SCID patients to survive and lead an optimal life. Collaborative work among all the healthcare professionals and scientists are important in improving healthcare for SCID patients in Malaysia.

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