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Testing Methodology and Clinical Advantages of a Newborn Screening Protocol for Severe Combined Immunodeficiency

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ABSTRACT

Severe combined immunodeficiency (SCID) is a genetic disorder defined by a disruption in the development of functional T cells and B cells that circulate in the body (1). This immunodeficiency is the most severe form of primary immunodeficiencies, exposing its victims to extreme vulnerability to infectious diseases, resulting in the more common name "the bubble boy disease". Due to the severity of this immunodeficiency disorder, an early diagnosis would be ideal in order to prevent infant fatally in SCID patients. Advanced screening protocols immediately after birth are crucial in providing prompt treatment to infants, determining their quality life and ultimately preventing death. This paper will discuss the etiology of SCID, the clinical presentation and diagnosis of SCID, and will proceed to highlight the advantages and disadvantages of a newborn screening protocol.

Keywords: SCID; Newborn; Screening

1 Introduction

Severe combined immunodeficiency (SCID) is a genetic disorder defined by a disruption in the development of functional T cells and B cells that circulate in the body [(1)]. It is known that SCID involves a defective antibody response that arises either from a direct interaction with B lymphocytes or through a defective activation of B lymphocytes from a non-functional T-helper cell. This immunodeficiency is the most severe form of primary immunodeficiencies, exposing its victims to extreme vulnerability to infectious diseases, resulting in the more common name "the bubble boy disease". In fact, this

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disorder is so severe that it can result in an individual's immune system to be almost entirely absent. SCID is considered a rare genetic disorder with a prevalence said to be around 1 in 100, 000 births [2]. However, many sources believe this number to be underestimated and that a more accurate prevalence of SCID is actually around 1 in 50, 000 births [3]. In addition to its rarity, there are approximately 15 different single gene defects that result in the disruption of the T cells and B cells, each of these mutations giving rise to a different clinical presentation of the disorder [4].

Due to the severity of this immunodeficiency disorder, an early diagnosis would be ideal in order to prevent infant fatally in SCID patients. However, diagnosing SCID early is often problematic as a mother's antibodies often mask the symptoms in a newborn for the first few weeks of life. Without an early diagnosis, most patients will not be diagnosed until about 6 months old, after being treated for opportunistic or otherwise self-limiting infections. The condition is usually fatal within the first or second year of life without an immune restoring therapy such as hematopoietic stem cell transplant. Justifiably, this severe primary immunodeficiency has been classified as a pediatric emergency once diagnosed in infancy or childhood [5]. Fortunately, SCID newborn screening programs are being implemented in many regions around the globe. Advanced screening protocols immediately after birth are crucial in providing prompt treatment to infants, determining their quality life and ultimately preventing death. This paper will discuss the etiology of SCID, the clinical presentation and diagnosis of SCID, and will proceed to highlight the advantages and disadvantages of a newborn screening protocol.

2 Etiology

A primary immunodeficiency refers to any disease that results from an inherited defect of the immune system. SCID is the most severe of these primary immunodeficiencies, comprising a heterogeneous combination of disorders that involve the functioning of cellular T cells and humoral B cell in the adaptive immune system. This disorder is most severe when there is a complete lack of T cell function, resulting in an early death in the 1st year of life due to the inability to fight off recurrent infections [6]. The different gene mutations result in the severity of the disorder inflicting a mild or severe form of the disorder, deepening on whether the gene mutation was a partial (hypomorphic) or a complete (null) defect.

In 2017, The International Union of Immunology Societies released a report categorizing genetically distinct classes of SCID based on the absence or presence of B cells and natural killer (NK) cells, Table 1 highlights the prevalent forms of SCID [7]. The most common case of SCID is a X-linked recessive disorder with mutations arising in the IL2RG gene encoding the common gamma chain, an important protein for many interleukin receptors such as IL-2, IL-4, and IL-7. These particular interleukins are important for the development and differentiation of T cells and B cells. A mutation in the common gamma chain will cause a global defect in the interleukin signaling pathway, leading to an almost complete failure in the development of the immune system [8]. The X-linked recessive form of SCID occurs almost exclusively in males, as males only have one X chromosome and the mutated gene is located on the X chromosome giving rise to the disorder.

Table 1: Classification of different types of severe combined immunodeficiency disorders [7], [8], [10].

Classification of Severe Combined Immunodeficiency Disorders	
Type	**Overview**
X- linked severe combined immunodeficiency	Most common cause of SCID. Arises from mutations in the IL2RG gene that encodes common gamma chain. This mutation will result in a non-functional common gamma chain and a widespread disruption of interleukin signaling. Ultimately, this leads to almost a complete failure of the immune system to develop and function.
Adenosine deaminase deficiency	The second most common form of SCID. A deficiency in adenosine deaminase (ADA) leads to the accumulation of dATP. This prevents the inhibition of ribonucleotide reductase, ultimately causing inhibition of lymphocyte proliferation and a diminished immune system.
Purine nucleoside phosphorylase deficiency	An autosomal recessive disorder. Mutations arise in the purine nucleoside phosphorylase gene (PNP). A mutation in this gene can cause elevated dGTP leading to T cell deficiency and toxicity.
Omenn syndrome	An autosomal recessive SCID disorder. A hypomorphic missense mutation occurs in T cells (RAG1, RAG2, IL7Ra, etc.). Without treatment, this disorder is fatal.
Reticular dysgenesis	An autosomal recessive immunodeficiency disease. A mutation arises in both copies of the AK2 gene. Absence of AK2 gene disrupts the ability of hematopoietic stem cells to proliferate
JAK3	The enzyme JAK3 mediates transduction downstream of the common gamma chain signal. A mutation in this enzyme can cause SCID

The second most common type of SCID arises when there is a defect in the enzyme that is responsible for the breakdown of the amino acid, adenosine deaminase (ADA). Without the breakdown of adenosine deaminase, the metabolite dATP will begin to accumulate. This subsequent accumulation of dATP results in the inactivity of ribonucleotide reductase, an enzyme that is essential for the reduction of ribonucelotides into deoxyribonucelotides. In order for an individual's immune system to be effective and efficient, lymphocytes require the ability to proliferate. The proliferation process is dependent on deoxyribonucelotide triphosphate (dNTP) synthesis, therefore without functional ribonucleotide reductase the immune system will be compromised [(9)].

3 Diagnosis and Clinical Presentation

Infants with SCID will most often be diagnosed within the first 3 months of life. The infant's symptoms will be masked immediately after birth until approximately 1-3 months after birth. This is due to circulating antibodies passed on to the infant from the mother via the placenta in the last few months of pregnancy. The evolutionary role of the transfer of maternal antibodies to neonates is to protect the infant while their immune system evolves and matures. The antibody IgG is transferred trans-placentally before birth. After birth, the IgG antibodies will be present in the neonates' bloodstream in a finite amount that will decline over time [(11)].

Once the maternal antibodies have depleted from the infant's bloodstream, symptoms of a primary immunodeficiency will usually begin to arise. It is important to note that children who present with symptoms in the first 6 months of life are more likely to have SCID or a severe T cell defect [(12)]. An infant with SCID will have many persistent infections in the first year of life, many of these being life-threatening. Common infections that an infant with SCID may develop include; pneumonia, meningitis, otitis media, hepatitis, candidiasis infection both in the mouth and diaper area, skin infections, diarrhea, and blood infections [(13)]. The patients that suffer from recurrent bronchiolitic illnesses will often exhibit a chronic cough and wheeze that progressively worsens over time. Persistent infections of oral or gastroesophageal candidiasis and/ or viral diarrhea with failure to thrive are also clinical findings that often require a physician to pursue a SCID investigation [(14)].

The first step into investigating a child query for SCID is for the physician to perform a thorough history and clinical examination. A thorough family history is of

extreme importance when there is suspicion that a child may have SCID. If the family history provides evidence that there is a history of affected males, then the potential diagnosis of X-linked SCID should be considered. This is especially true in regard to consanguineous families, when there is an increased risk of genetic disorders due to the expression of autosomal recessive gene mutations from an inherited common ancestor [15]. The absence of lymphoid tissue is regarded as a fundamental sign when suspecting a primary immunodeficiency disorder. However, this is not easy to detect in young infants as lymph nodes and tonsils are normally quite small in this population. A clinical examination of the skin and lymph nodes is imperative to the diagnosing phase of SCID and can often be overlooked. Omenn's syndrome and SCID with maternofetal lymphoid engraftment are categorized by thickened infiltrative skin rash from an expansion of lymphocytic clones and lymphadenopathy [11]. Routine blood tests such as a full blood count are also important to not be overlooked when suspecting a diagnosis of SCID. It is well established that lymphocyte counts are higher in infants compared to lymphocyte counts in adults. Therefore, infants with a lymphocyte count of less than 2.8 x 10^9/liter (2 standard deviations below the mean) have a high probably of being diagnosed with SCID. A lymphocyte count alone does not confirm a SCID diagnosis, though, lymphopenia on more than to two occasions should warrant lymphocyte phenotyping on the infant [16].

Diagnostic imaging is also a key determinant when investigating for SCID. Chest radiographs may show clues such as an absence of a thymic shadow, interstitial pneumonitis, hyperinflation of the lungs, and in the case of ADA deficient SCID, there may be evidence of cupping and flaring in the costochondral junction region. Gathering information from the parents via a thorough history is also a critical aspect of the diagnosis process. The details about each prior infection will provide necessary infective clues in order to ensure the appropriate microbiological investigations are carried out.

Since infants with SCID are highly vulnerable to infections, it is common for opportunistic pathogens such as Pneumocystis carinii, cytomegalovirus (CMV) and atypical mycoplasma infections to be present. Pneumocystis carinii is the most common cause of respiratory infections in SCID patients and often co-presents with respiratory viruses such as respiratory syncytial virus (RSV) or parainfluenza virus. In order to make a diagnosis based on these opportunistic pathogens, a good specimen from respiratory

secretions with abundant cellular material is crucial. Rotavirus and adenovirus are often the culprits for causing gastrointestinal infections and failure to thrive in SCID. Bacterial infections from staphylococci, streptococci, enterococci and Pseudomonas spp. in SCID are less common but can cause serious life-threatening complications such as skin sepsis [17].

In addition to respiratory secretions, a physician may require further samples from stool, urine, bronchoalveolar washings, cerebrospinal fluid and specific tissue biopsy in order to classify the extent of the infection. If there is a high suspicion of SCID with the presence of an undiagnosed infection, a physician will be obligated to perform extensive and invasive investigations in collaboration with microbiology specialists.

4 Treatment

SCID is usually fatal within the first or second year of life without an immune restoring therapy. Currently, the standardized treatment for a patient with SCID is an allogenic hematopoietic stem cell transplantation [18]. Evidence has shown that the transplantation should be performed in the first 3.5 months of the infant's life in order to be effective. A delay in making the diagnosis of SCID can reduce the success of a curative hematopoietic stem cell treatment and often leads to death [19]. This particular procedure is peculiar, in that it does not require myeloablation or immunosuppression to achieve the engraftment. It is ideal for the patient to obtain an identical HLA bone marrow donor. The transplant will cause a rapid T cell reconstruction after an expansion of donor memory T lymphocyte pool. Newly generated T cells can be identified in a patient approximately 3-4 months after a bone marrow transplant. In the absence of graft versus host disease (GVHD), the likelihood of success has been >90% [20].

The ADA deficient form of SCID can be also be treated with enzymatic substitution by coupling ADA to polyethylene glycol (PEG-ADA). This treatment involves the patient receiving weekly intramuscular injection, resulting in normalizing dATP levels in the blood. Within a few weeks of this treatment the patient's T cell levels will also rise, leading to specific T cell immune responses. The PEG-ADA treatment is successful in 90% of patients [21], with complications arising in patients with the most severe phenotypes and in those who develop antibodies to ADA.

5 Screening Protocols

The diagnosis of SCID is considered a pediatric emergency. Screening protocols for this immunodeficiency disorder have started to be implemented in several regions around the world. The main advantage of screening tests for SCID is to discover the immunodeficiency early before the infant develops an infection. There is currently very scant data on the advantages and disadvantages of newborn screening for SCID in combination of treatment initiation. However, The Institute for Quality and Efficiency in Heath Care of Germany (IQWiG) found that there was a much lower death rate in newborns who were given antibiotics prior to acquiring their first infection [22]. Research suggests that routinely screening for SCID in newborns does have advantages. The main advantage of screening newborns for SCID is that it will help detect the disorder earlier meaning that preventative treatment can be introduced sooner. Overall, screening can increase a child's chance of survival and normal development.

Screening tests are offered to all parents with newborn babies. A breakthrough in newborn SCID screening occurred with the introduction of the T cell receptor excision circle quantification assay (TREC test) [23]. This assay can be performed using the dried blood spot via the heel prick method that is already collected from newborns to screen for other conditions. During maturation, 70% of thymocytes express $\alpha\beta$- T cell receptors that will have circular TREC DNA. The TREC circles becomes diluted as T cells proliferate, due to the fact that the circles do not increases after cell division. By performing a quantitative polymerase chain reaction (PCR) across the joint of circular DNA will provide a TREC copy number, a marker for newly formed, antigenically naïve thymic emigrant T cells [24]. Evidence showed that infants with SCID, who were sampled both at diagnosed and during the neonatal dried blood spot, had very low or even undetectable TRECs [24]. More importantly, maternal T cells present in newborns with SCID do not falsely raise the TREC count. Consequently, as long as a sample obtained proved adequate DNA for PCR, the number of TRECs can be used as an exceptional biomarker for autologous T cell production.

Although most would argue in favour of newborn screening for SCID, there are still some limitations to the newborn screening protocol that are worthy of addressing. The TREC assay has been a great breakthrough, however, it is not able to identify all infants with serious defects in T cell functioning. Individuals with MHC II deficiency or

ZAP70 deficiency have their T cell receptor rearrangement intact, with maturation and function pathways being deficient. These disorders are not expected to have their TREC number decreased. Therefore, screening for depleted TREC counts will only detect SCID diseases that have an impaired T cell production and do not out rule the possibility of other primary immunodeficiencies. In addition to the limitations of TREC assays, there is also some controversy regarding collection, storage, testing and utilization of dried blood spots from infants. Another disadvantage to the issue of newborn screening is the lack of public awareness regarding rare disorders such as primary immunodeficiencies. Public health initiatives to educate people on the importance of screening and early diagnosis is one approach in combating this disadvantage. It is possible that linking prenatal obstetrical care and counseling to postnatal newborn screening as DNA based testing would also be an effective way of educating

6 Conclusion

In conclusion, SCID is defined by poor T cell production and the failure of B cells to generate protective antibodies, leaving individuals with a non-functioning immune system. The most common form of SCID to be diagnosed is the X-linked recessive form, occurring almost exclusively in males. SCID is the most severe form of the primary immunodeficiencies, leading to a pediatric emergency if a diagnosis is made, and is usually fatal unless the patient receives immediate treatment. Currently, the most standard treatment for a patient with SCID is to receive a functional adaptive immune system through an allogenic hematopoietic stem cell transplantation or adenosine deaminase enzyme replacement therapy. That being said, early diagnosis of SCID is imperative to a successful course of treatment. The main advantage to implementing newborn screening is to diagnosis the disorder before the child develops multiple severe infections.

There is currently limited data in this area, however, the current research suggests that routinely screening for SCID in newborns will help to detect the disorder and initiate treatment to restore a functional immune system to the infant as soon as possible. Newborn screening for SCID is being adopted by many regions across the globe and being offered to many parents of newborn babies. The TREC test has been imperative to the screening protocol and diagnosing method of SCID. This assay can be performed using the dried blood spot that is already carried out in the newborn heel prick test for

many other conditions such as Cystic Fibrosis. Although the newborn screening protocol increases a child's chance of survival and normal growth, there are still some limitations that are important to address. The main limitation to screening is that newborns are protected by their mother's IgG antibodies in the first few months of life, so they will appear to have a functioning immune system. In addition, SCID is a very rare disorder with approximately 80% of cases being sporadic with no known family history.

Overall, SCID is a curable disorder and after successful hematopoietic stem cell transplantation, the patients go on to live normal, healthy lives with fully functioning immune systems. Survival is improving with newborn screening protocols becoming more prevalent in Europe and across the globe. There is still little data in this field, however, continued research and collaboration among pediatricians, microbiologists and immunologists is crucial to facilitate screening and diagnosing strategies and to ultimately promote public health initiatives in order to raise awareness about SCID.

7 Competing Interests

The author certifies that they have no affiliations with or involvement in any organization with any financial or non-financial interest in the subject matter discussed in this manuscript.

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