"UTILITY OF UMBILICAL CORD BLOOD ABSOLUTE LYMPHOCYTE COUNT IN SCREENING FOR SEVERE COMBINED IMMUNODEFICIENCY DISEASE – A ONE YEAR LONGITUDINAL STUDY"

BY

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LIST OF ABBREVIATIONS:

ADA	Adenosine Deaminase
ALC	Absolute Lymphocyte Count
ANC	Absolute Neutrophil Count
APGAR	Appearance, Pulse, Grimace, Activity, Respiration
BCG	Bacillus Calmette-Guérin
BMT	Bone Marrow Transplant
CBC	Complete Blood Count
CD	Cluster Differentiation
CMV	Cytomegalo Virus
CR	Conditioning Regimen
CTLA4	Cytotoxic T-Lymphocyte Associated protein 4
CVID	Common Variable Immune Deficiency
DBS	Dried Blood Spot
DCLRE1C	DNA Cross-Link Repair Enzyme 1C
DNA	Deoxyribonucleic Acid
DOCK8	Dedicator Of Cytokinesis 8
EBV	Epstein Barr Virus
ERT	Enzyme Replacement Therapy
gm	Gram
HCT/HSCT	Hematopoetic Stem Cell Transplant
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
HSV	Herpes Simplex Virus
IL	Interleukin
IPEX	Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked
JAK	Janus Kinase
KREC	Kappa-deleting Recombination Excision Circle
LRBA	Lipopolysaccharide-Responsive & Beige-like Anchor protein

LSSA	Lymphocyte Subset Analysis
MHC	Major Histocompatibility Complex
MMRD	Molecular Minimal Residual Disease
MSD	Matched Sibling Donor
MUD	Matched Unrelated Donor
NBS	Newborn Screening
NGS	Next Generation Sequencing
NK	Natural Killer
OS	Omenn Syndrome
PID	Primary Immunodeficiency Disease
PITDC	Primary Immune Deficiency Treatment Consortium
qPCR	Quantitative Polymerase Chain Reaction
RAC2	Ras-related C3 botulinum toxin substrate 2
RAG	Recombination Activating Gene
RID	Related HLA-Identical Donor
SCID	Severe Combined Immunodeficiency Disease
STAT	Signal Transducer and Activator of Transcription
TCL	T-cell Lymphopenia
TME	Transplacentally Transferred Maternal Cells
TMP-SMX	Trimethoprim Sulfamethoxazole
TREC	T-Cell Receptor Excision Circle
USA	United States of America
VUS	Variants of Unknown Significance
WASP	Wiskott–Aldrich Syndrome Protein
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing
ZAP-70	Zeta-chain Associated Protein kinase-70
μL	Microlitre

ABSTRACT:

"Utility of Umbilical Cord Blood Absolute Lymphocyte Count in Screening for Severe Combined Immunodeficiency Disease – A one year longitudinal study"

Background:

SCID is a severe inherited disorder that affects the function of T-cells and B-cells, making it one of the most critical and fatal types of inherited primary immunodeficiency. Newborns with SCID typically appear healthy at birth but often develop recurring infections in infancy, leading to high mortality within the first year. Most SCID patients have low lymphocyte counts from birth, and regular testing of absolute lymphocyte count (ALC) in umbilical cord blood samples can aid in pre-symptomatic detection and early intervention for neonates with SCID. Measuring cord blood ALC is a simple, rapid and cost-effective method for newborn screening of SCID and related T-cell lymphopenia, especially in resource-limited settings. The true incidence of absolute lymphopenia in newborns in India still remains unknown. We hereby present the first ever SCID screening in India and the fourth worldwide, involving 1550 newborns screened using cord blood ALC. Early diagnosis is indeed-life saving and allows children with SCID the opportunity to lead a healthy life post hematopoetic stem cell transplant therapy.

Objectives:

- **Primary Objective:** To estimate the incidence of absolute lymphopenia in umbilical cord blood samples of newborns and to determine its utility in screening for Severe Combined Immunodeficiency Disease (SCID).
- **Secondary Objective:**

1. To evaluate other causes of lymphopenia in newborns.

2. To study the impact of early detection of SCID on improvement in the patient outcome by prompt referral for curative management

Methodology:

A longitudinal study was conducted on 1550 newborns delivered vaginally or via caesarean section, with a gestational age of over 32 weeks, at a tertiary care center in North Karnataka. Umbilical cord blood (2ml) was collected at delivery in an EDTA bulb and subjected to complete blood count analysis using Sysmex XN 350 - 5 part analyzer. Parameters measured included hemoglobin, WBC count, Absolute Neutrophil Count, Absolute Lymphocyte Count, and Platelet count. Neonates with absolute lymphopenia underwent detailed history-taking, clinical examination, additional investigations, and follow-up according to the study protocol.

Results:

In our study of 1550 newborns in North Karnataka, 6.67% exhibited absolute lymphopenia (ALC < 2500/µl), potentially linked to a higher consanguinity rate in the region. Other causes of lymphopenia included prematurity, sepsis, congenital anomalies, and idiopathic factors. Lymphopenia was more prevalent in males (56.25%) than females (43.75%), and significantly associated with consanguinity (43.75% vs. 10.41% in non-lymphopenic cases). Mode of delivery did not significantly influence lymphopenia incidence. Lymphopenic infants required more active neonatal resuscitation and had lower birth weights and poorer APGAR scores at 1 and 5 minutes compared to non-lymphopenic infants. Dysmorphic features were significantly more common in lymphopenic cases, suggesting a higher suspicion for primary immunodeficiency diseases. Differences in hemoglobin, WBC count, ANC and ALC were statistically significant between lymphopenic and non-lymphopenic

groups. Flow cytometry identified a suspected SCID pattern in some cases, though genetic testing did not confirm mutations in these infants.

Conclusion:

Absolute lymphopenia (ALC<2500/µl) was observed in 6.67% of neonates screened at birth. This is greater than the incidence of absolute lymphopenia in 3 other studies done worldwide which can probably be linked to a higher rate of consanguinity present in the northern Karnataka region. Other causes of lymphopenia included prematurity, sepsis, congenital anomalies, and idiopathic factors. To facilitate early care for newborns with SCID and pre-symptomatic identification, we recommend integrating measurement of the absolute lymphocyte count in umbilical cord blood into screening regimens. Until SCID is definitively ruled out, precautions such as withholding live attenuated vaccines like BCG are advised to mitigate potential risks.

Keywords:

Severe Combined Immunodeficiency Disease, Absolute Lymphocyte Count, Newborn Screening, Lymphopenia, Complete Blood Count, Flow Cytometry

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INTRODUCTION:

SCID is a severe inherited immunodeficiency of T-cell and B-cell function that encompasses a variety of genetic disorders distinguished by a substantial deficit in both cellular and humoral immune capabilities. It is one of the most severe and lethal cases of hereditary primary immunodeficiency. The majority of these newborns are normally asymptomatic at delivery and early infancy, partially due to maternal antibodies' protective effects and minimal exposure to pathogens at birth, but they acquire recurring illnesses during infancy. However, as maternal antibodies decline around 4-6 months of age, these infants may fail to thrive and get severe recurring infections, sometimes resulting in death by 1 to 2 years of age if not treated immediately. The infections associated with SCID can include common bacterial and viral infections as well as rarer opportunistic infections like Pneumocystis jirovecii, fungal infections, and mycobacterial infections. Additionally, SCID infants may present with chronic diarrhoea and failure to thrive. [1]

Severe Combined Immunodeficiency (SCID) has varying prevalence rates across different regions. In parts of the USA where newborn screening (NBS) programs are implemented, the estimated prevalence is 1 in 100,000 live births. [2] However, in the Middle East, the prevalence is higher, at 20 in 100,000 live births. [3] Population-based NBS remains the most effective strategy for early identification of affected newborns before infections and complications set in. Interestingly, the introduction of NBS programs has revealed that SCID is more common than initially thought. [4] For instance, SCID incidence rates have been reported as 1 in 131,485 in Taiwan [5], 1 in 58,000 in the United States [6], and 1 in 11,821 in China. [7] Notably, in countries where consanguineous marriages are common, such as Saudi Arabia, the

incidence is even higher—specifically, 1 in 2,906, which is 20 times higher than the incidence reported from USA NBS programs. [8]

The true incidence of Severe Combined Immunodeficiency Disease in India still remains unknown. Similarly, there are no studies regarding the incidence of SCID from Northern Karnataka belt. We expect higher incidence of this inherited condition in this region as a result of high consanguinity rates. Indeed, a retrospective study done in the paediatric department in the division of Hemato Oncology at our centre found a total of 46 children with inborn errors of immunity over 36 months, out of which 6 were diagnosed with SCID - published in the British Medical Journal (BMJ). [9]

Infants affected by SCID typically succumb to complications arising multiple infections within their first year of life. Furthermore, these infants are inherently susceptible to potential risks such as Vaccine Associated Paralytic Poliomyelitis (VAPP) and BCGosis. Most infants with SCID typically have low levels of lymphocytes at birth. Checking the absolute lymphocyte count (ALC) in umbilical cord blood samples can aid in identifying SCID before symptoms appear, allowing for early intervention. Therefore, a more thorough examination of the complete blood count (CBC), particularly focusing on the absolute lymphocyte count (ALC) prior to administering live vaccines, holds the potential to mitigate vaccine-associated complications and facilitate early detection of SCID. [10]

We took up this study with the aim of estimating the incidence of SCID based on Absolute Lymphocyte Count. This study would also help in the early detection and management of SCID by Hematopoetic Stem Cell Transplant (HSCT) before the infective complications set in; thus improving the patient outcome. Another

prospective advantage of this study would be prevention of SCID as antenatal diagnosis can be offered to the couples who have affected children.

Hematopoietic stem cell transplantation (HSCT) is the definitive therapy for SCID. Infants transplanted before 3.5 months of age have a 94% survival rate at 5 years. In contrast, those transplanted after 3.5 months, especially if they have existing infections, face a reduced survival rate of 50% and higher treatment costs. [11] Early detection of SCID, before the onset of severe infections, significantly enhances the success of HSCT outcomes.

Hence, early diagnosis and timely management of SCID before infections develop are crucial. Newborn screening for SCID is thus highly recommended. SCID also fulfils the internationally-established criteria for a condition to be screened for at birth. Population-based screening has been implemented across all 50 states in the US and several other countries to identify newborns with SCID early, enabling timely diagnosis, infection prevention measures, and prompt management. [1]

The expense of caring for a single infant with SCID, who is not detected via newborn screening may exceed the cost of screening a whole regional population. [11] Screening can be justified for rare illnesses that are not detected by routine clinical examination but are curable and require early detection and treatment to obtain a positive outcome.

Early detection of SCID should be regarded a pediatric emergency since a diagnosis before live immunizations or non-irradiated blood products are administered and before infections arise allows for lifesaving Hematopoetic Stem Cell Transplantation. Many Western countries currently have an extensive SCID screening

program that uses the T-cell receptor excision circle (TREC) assay on a dried blood spot. Even though this test has a higher sensitivity and specificity, it is expensive, requires technical expertise, infrastructure and is not widely available in India. Lymphopenia identified in a CBC warrants careful consideration as a potential marker for SCID. As a precautionary measure, administration of live vaccines should be withheld until the final diagnosis is confirmed. TREC as a screening assay can be considered after our country's transplantation landscape improves and a solid infrastructure for fast and reliable screening and result reporting is in place. [10]

Measuring cord blood ALC is a simple, rapid and cost-effective method for newborn screening of SCID and related T cell lymphopenia, especially in resource-limited settings. Moreover, there are limited studies evaluating utility of this approach in screening of SCID. Most importantly, it is life saving and allows children with SCID the opportunity to live a healthy life. Uniform screening for SCID represents a humanitarian, medical, and economic value proposition that must be advanced. Hence, we would like to undertake study of cord blood ALC in detection of SCID.

OBJECTIVES:

PRIMARY OBJECTIVE:

To estimate the incidence of absolute lymphopenia in Umbilical Cord Blood samples of newborns and to determine its utility in Screening for Severe Combined Immunodeficiency Disease (SCID)

SECONDARY OBJECTIVES:

- To evaluate other causes of lymphopenia (Sepsis, Prematurity, Di George Syndrome, Trisomy 18) in newborns.
- To study the impact of early detection of SCID on improvement in the patient outcome by prompt referral for curative management

REVIEW OF LITERATURE:

A class of hereditary immune system abnormalities known as primary immunodeficiency diseases (PIDs) can result in recurring, potentially fatal infections as well as increased vulnerability to autoimmune, inflammatory, and malignant processes. There are now over 200 different PIDs recognized, most of which are caused by single gene abnormalities. [12] These illnesses have a combined estimated incidence of up to 1 in 1200. [13] It is anticipated that PID prevalence in India would follow worldwide trends. But given the frequency of consanguineous marriages, it is expected that the prevalence of particular PIDs may differ. [14] In India, there are thought to be at least a million PID patients. However, limited diagnostic infrastructure and limited comprehension of these illnesses are the main causes of the lack of available data from India. [14–17] Depending upon the underlying immunological deficiency, the clinical severity of sickle cell disease (SCID) can range from moderate to life-threatening. Based on the inherent immunological deficiency, PIDs are categorized into eight primary groups.[18] To significantly reduce the morbidity and death linked to PIDs, early detection is essential. HSCT is a treatment option for severe cases of PIDs, immunoglobulin supplements are available for antibody shortages, and antimicrobial medication is available to treat and prevent infections. [10] Less than one-third of affected people have a positive family history, and there is a predominance of male patients. SCID is not clinically evident at the time of birth and the infectious problems that bring patients to medical treatment may initially be indistinguishable from regular childhood infections, therefore diagnosis may be delayed substantially. These infants typically die within their first year of life as a result of complications from an array of illnesses. Furthermore, these babies are

at greater risk of developing Vaccine-Associated Paralytic Poliomyelitis (VAPP) & BCGosis. This vulnerability stems from the widespread administration of live vaccinations, such as OPV and BCG, immediately after birth, which is especially prevalent in India. [10]

The greatest prognosis for SCID is attained when HSCT is conducted in the first few months of life, preferably before clinical manifestation with infections and failure to thrive. Screening for SCID at birth not only prevents children from dying before undergoing HSCT, but it also improves the success rate of HSCT. As a result, neonatal screening is the best method for detecting SCID. However, due to its inability to be performed on dried blood spots, this test still lacks popularity in nations where newborn blood spot screening (NBS) is widely used to detect other neonatal diseases. In contrast, the T cell receptor excision circle (TREC) assay, that measures the thymic output of T cells, provides a very sensitive and specific assessment of T cell function. This assay, which is consistent with other NBS assays, has transformed the early diagnosis of SCID. It is being implemented in several states across the US [6] , Europe [19] & various other nations. [20] The full incidence of PIDs in India will not be known until neonatal or population screening for congenital disorders is implemented. The worldwide prevalence of PIDs is projected to be one in every 10,000 live births [21], but this figure is likely to be underestimated due to limited access to diagnostic technologies and the challenges associated with diagnosing patients with atypical clinical symptoms. [22] PIDs are regarded rare on a worldwide scale, although their occurrence is significantly higher in locations with high consanguinity rates, especially in the Middle East, where consanguineous unions account for 65% of marriages. This incidence is significantly higher than that reported in Europe, the Western Pacific region, and Latin America, where consanguinity rates

are lower (5.6%, 2.3%, and 0.96%, respectively). [23, 24] As a result, the incidence of PID in Middle Eastern countries is almost 20 times higher than in North America and Europe.

HISTORY & DEFINITION OF SCID:

Understanding and characterizing Severe Combined Immunodeficiency (SCID) has evolved over time. In the 1950s, clinical observations such as recurring and severe bacterial, viral, and fungal infections, as well as symptoms such as weight loss and diarrhea, were used to make diagnoses. Furthermore, a positive family history, especially in situations of X-chromosome-linked inheritance, was thought to be indicative. [25] In 1972, ADA insufficiency emerged as a metabolic etiology of SCID, which can be diagnosed using biochemical methods. However, the deadly prognosis changed with the introduction of bone marrow transplantation in 1968, providing an opportunity for a functional immune system. [26]

The identification of SCID-related genes began in 1993, starting with IL2RG, which encodes the common γ chain of cytokine receptors. [27, 28] This led to the discovery of numerous genes associated with SCID. [6, 29] Although gene sequencing allows precise diagnosis, its widespread use is limited due to cost and turnaround time. [30]

Newborn screening (NBS) with TRECs has revolutionized early detection, enabling identification shortly after birth, before complications arise. Diagnosis now relies primarily on laboratory findings. [4] Typical SCID patients exhibit fewer than 300 autologous T cells/μL, impaired T cell proliferation, and/or maternal T cell engraftment, alongside deleterious mutations in SCID-associated genes. Leaky SCID

patients have higher T cell counts and lack naïve T cells, with functionally impaired T cells and limited diversity. Some may develop Omenn syndrome, characterized by specific clinical features resulting from dysregulated T cell expansion. [31]

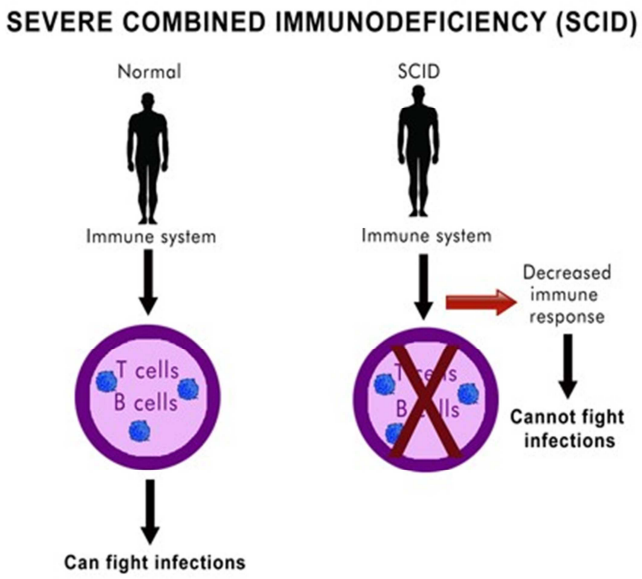


FIGURE 1. Immune system in severe combined immunodeficiency disease

EPIDEMIOLOGY OF SCID:

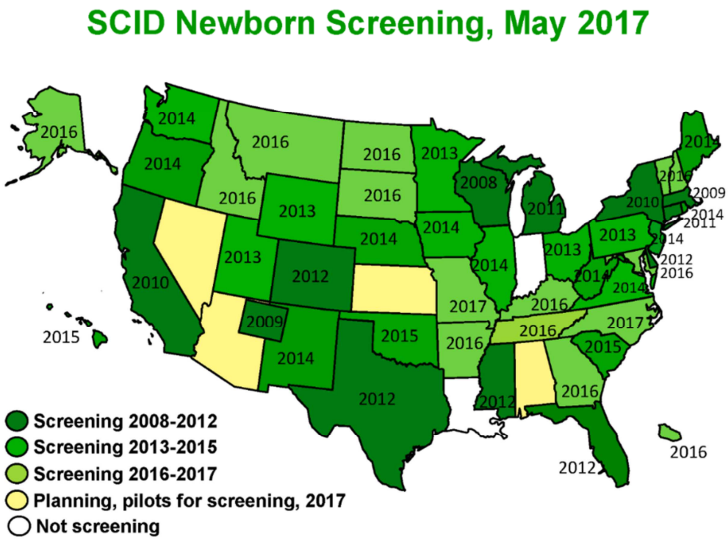


FIGURE 2. Newborn SCID Screening in USA 2017

In 2014, 11 newborn screening (NBS) programs performed TREC testing on over 3,030,083 babies, discovering 52 cases of typical SCID, leaky SCID, and Omenn syndrome, which affects around 1 in 58,000 children. Two years of data from the SCID NBS in California demonstrated a birth prevalence of non-SCID T cell lymphopenia (TCL) of about 1 in 20,000. Wisconsin led the way in implementing SCID NBS in 2008, and there are now more than 46 screening programs countrywide. The other states are expected to soon implement NBS for SCID. In 2016, NBS discovered 90% of SCID cases in the United States. [30]

SCID was formerly predicted to occur in approximately one out of every 100,000 births in the United States. However, improved early detection has indicated a higher actual incidence, closer to one in 50,000-75,000 newborns. There is an increasing interest in utilizing neonatal screening to detect afflicted individuals. Around 50 percent of SCID cases are X-linked (owing to mutations in the common γ chain), whereas the remaining 50 percent are autosomal recessive SCID. Approximately 25% of them are caused by JAK3 deficiency, 40% by ADA deficiency, and the remaining 35% by other illnesses. SCID occurs at a comparable rate worldwide as it does in the US. XL-SCID, like other X-linked illnesses, is more prevalent in populations with a higher degree of consanguinity. Although SCID is frequently underreported, numerous nations now keep registries of primary immunodeficiency illnesses. SCID is projected to affect 0.15 cases per 100,000 live births in Australia, 0.045 in Norway, 0.47 in Switzerland, and 2.43 in Sweden. [32]

Age-based Demographics: The majority of SCID cases occur in patients under the age of three months, with the typical symptom onset at two months and a mean diagnostic age of 6.5 months. Patients with ADA-deficient SCID might carry less

severe mutations that go undetected until maturity. Common γ chain mutations can occur in the second year of life, albeit this is uncommon. Finnish patients with cartilage-hair hypoplasia may live into later childhood or adulthood, when the risk of cancer increases.

Sex-based Demographics: About half of SCID cases are X-linked (affecting males only). Only one-third of males with frequent γ chain mutations had a positive family history, indicating a high rate of de novo mutations. The remaining SCID instances result from autosomal recessive mutations that affect both males and females equally. Family histories of consanguinity or inbred communities should be investigated, as homologous mutations are more likely in these cases. Overall, the male-female ratio is 3:1.

Race-based Demographics: There is no racial bias in the majority of SCID cases. On the other hand, Mennonites are primarily affected by ZAP-70 deficiency and CD3δ deficiency; Navajo and Apache Native Americans are commonly affected by Artemis gene deficiency; JAK3 mutations are more commonly reported in Italy; North Africans are typically affected by MHC II deficiency; RAG1/RAG2-deficient SCID is more common in Europe; and the Finnish population and the Old Order Amish in the United States are affected by cartilage-hair hypoplasia. [32]

A study conducted by Manisha Madkaikar, Jahnavi Aluri, and Sudhir Gupta, focusing on the Guidelines for Screening, Early Diagnosis, and Management of Severe Combined Immunodeficiency (SCID) in India, proposes that SCID is an ideal candidate for newborn screening. They suggest that a basic Complete Blood Count (CBC) analysis of cord blood samples can effectively identify lymphopenic patients. Apart from aiding in the detection of lymphopenia cases, the CBC provides valuable

information on Absolute Lymphocyte Count (ALC), Absolute Neutrophil Count (ANC), and other blood indices such as hemoglobin levels and platelet counts, which are crucial for newborn management. Despite the limitation of potential false negatives and false positives, CBC stands out for its widespread availability, ease of administration, immediate results availability, and routine implementation in most healthcare facilities. Thus, before giving live vaccinations, a thorough assessment of the CBC, especially ALC, may assist avoid vaccine-related side effects and promote an early identification of SCID. As a result, the authors support the routine use of CBC on samples of cord blood. The presence of lymphopenia in CBC appears to be strongly suggestive of SCID and should delay the administration of live vaccines until the definitive diagnosis is made. To confirm SCID, CBC should be performed again a month later and be followed by a flow cytometric analysis. When the transplant facilities in the nation improves and a strong infrastructure is put in place to guarantee prompt and accurate screening and result reporting, the use of TREC as a screening assay may be taken into consideration. [10]

A study conducted by Jessica Quinn, Jordan S. Orange, Vicki Modell, and Fred Modell highlights the critical nature of Severe Combined Immunodeficiency (SCID) and related conditions characterized by T cell lymphopenia in infants. These infants face severe, life-threatening infections and are unlikely to survive beyond their first year without specific interventions to safeguard them from infections and restore their immune function. The TREC assay, a straightforward screening test, can detect SCID and related T cell lymphopenias using the same DBS samples collected for screening other genetic disorders in newborns. Early identification through screening enables infants to undergo hematopoietic stem cell transplantation within the first few months of life, significantly increasing their chances of survival and allowing them to

lead healthy lives. SCID not only poses a fatal threat to infants but also incurs substantial healthcare costs within the first year of life alone. The expenses associated with caring for an undiagnosed infant with SCID could surpass the cost of screening an entire regional population for the disorder. Transplantation within the first 3 months of life boasts a 95–100% success rate in terms of survivorship, although this rate decreases significantly over time. The Jeffrey Modell Foundation played a crucial role in initiating SCID newborn screening in the USA in 2008 and continues to advocate for global SCID screening initiatives. Presently, all 50 states in the USA and Puerto Rico conduct screening for SCID and T cell lymphopenia, with over 27 million newborns screened to date and hundreds successfully diagnosed and treated. T cell lymphopenia includes other conditions such as DiGeorge syndrome, trisomy 21, ataxia telangiectasia (AT), and CHARGE syndrome, among others. In the USA, one in 58,000–65,000 infants are affected by SCID, and one in approximately 15,000 with serious T cell lymphopenia. Since 2003, the Jeffrey Modell Foundation has been actively raising public awareness and educating physicians about primary immunodeficiencies, including severe combined immunodeficiency disease. Through their efforts, they have successfully implemented population-based newborn screening for SCID and T cell lymphopenia, covering 96% of all newborns in the US. The majority of patients (89%) displayed initial symptoms before six months of age, with recurrent pneumonia being the most common (66%), followed by failure to thrive (60%) and chronic diarrhea (35%). According to a US study, 1 in 58,000 live babies had SCID. Furthermore, a greater frequency of autosomal-recessive SCID has been noted in consanguineous connections. Furthermore, screening programs for SCID are being implemented at various phases in about 20 different countries globally. Not only is newborn screening for SCID and associated T cell lymphopenia

economical, but it also saves lives and gives afflicted infants a possibility of a promising healthier future. The advancement and implementation of a uniform global screening approach is crucial from a humanitarian, medical, and economic standpoint. [33]

DNA-based, high-throughput screening has been shown to be feasible and effective in the TREC screening for SCID. This method, like tandem mass spectrometry, has previously demonstrated applicability to other disorders. For example, spinal muscular atrophy was authorized for inclusion in the July 2018 Recommended Uniform Screening Panel. Regardless of the underlying genetics, TRECs have shown to be extremely sensitive and specific indicators for T cell deficiency. As a result, the screening program in California, which employs the TREC test to identify and diagnose SCID in neonates, has made it easier to identify pre-symptomatic infants with known and undiscovered SCID defects early on, allowing for prompt treatment and phenomenal survival rates. Previously, the conclusive diagnosis of SCID was based on laboratory test results rather than clinical characteristics associated with infectious problems, including as failure to thrive, diarrhea, and repeated serious infections. With lentiviral gene therapy becoming more available for known genotypes, particularly mutations in the ADA and X-linked IL2RG genes, and promising protocols emerging for DCLRE1C (Artemis) mutant SCID, approximately half of all infants with SCID may be eligible for gene therapy in the near future. Experimental thymus transplantation has proven to be successful in certain patients of DiGeorge thymic insufficiency, albeit it is not commonly available. Integrating lymphocyte phenotyping by flow cytometry as a follow-up test in California's NBS program for SCID has proven to be both quick and cost-effective. Centralizing performance in a single contract laboratory has reduced costs, assured

thorough result tracking, enabled the adjustment of TREC screening test cutoffs, while preserving quality assurance. This approach has enabled the dismissal of more than 60 percent of cases with abnormal TREC results without the need for a costly and time-consuming immunology specialist review. Recognizing clinically severe non-SCID T cell lymphopenia as an uncommon occurrence (one in per 20,000 births) allows for the identification of newborns who should avoid getting live attenuated rotavirus vaccine, guaranteeing optimal vaccination efficacy as a public health strategy.

The TREC test's specificity for SCID did not cause families to experience undue anxiety or cause primary providers to provide unfavorable feedback. Babies with positive test results are guaranteed an expedited and systematic approach to an accurate diagnosis when screening program personnel and immunologists communicate well and follow up with lymphocyte phenotyping in the NBS program. Persistent idiopathic T cell lymphopenia is rare in this program, occurring once per 100,000. [34]

In a screening study with 500 neonates, El-Sayed SS et al. found that 8 (1.6%) had absolute lymphopenia at delivery, while 492 (98.4%) had an ALC more than 2500/μL. Every patient who was lymphopenic was kept under observation and told not to administer the BCG vaccination. After a month, follow up CBC and ALC were conducted, and on the second examination, all patients had normal ALC levels. 69 (13.8%) had an ALC of 2500-3500/μL, 141 (28.2%) had an ALC of 3500-4500 /μL,129 (25.8%) had an ALC of 4500-5500/μL, 65 (13%) had an ALC of 5500-6500/μL, 43 (8.6%) had an ALC of 6500-7500/μL and 45 (9%) had an ALC of ≥ 7500/μL. In the study population, 222 (44.4%) were primigravida and 278 (55.6%)

were multigravida. Furthermore, 84 (16.8%) of the moms had their membranes rupture prematurely, and 89 (17.8%) had maternal illnesses. Three infants (0.6%) exhibited congenital defects, one (0.2%) had dysmorphic characteristics, and eight (1.6%) had a family history of undetermined death. Neonates with lymphopenia had significantly lower gestational age, birth weights, and APGAR scores at 1 and 5 minutes. Before making a SCID diagnosis, serial monitoring and follow-up are required. [35]

The research carried out by Akyut Poyraz and colleagues comprised 2,000 infants in total. Of them, 1,958 (97.9%) had a lymphocyte count more than 3,000/mm³, and 42 (2.1%) had absolute lymphopenia. At the completion of their first month, two infants still had lymphopenia, which prompted additional testing. The lymphocyte subsets for SCID were examined. The initial baby's lymphocyte subsets revealed a SCID phenotype with T (-), B (-), and natural killer cells (NK) (+), linked to a defective receptor-antigen gene (RAG). A homozygous NM_000448 c.2209C > T (p.R737C) mutation in the RAG1 gene was discovered by Sanger sequencing. According to the lymphocyte subgroups, the second baby had a JAK3 deficiency and a SCID phenotype with T (-), B (+), and NK (-). [36]

In a Turkish study conducted by Seyhan et al, 1,960 DBS samples were examined. Of the 1,856 infants tested, 71 (3.8%) had low T-cell receptor excision circles (TRECs) and/or κ-deleting recombination excision circles (KRECs). The low TREC rate was 1.1%. Preterm neonates had significantly lower levels of TRECs and KRECs compared to term newborns ($p < 0.0001$). Although no severe combined immunodeficiency (SCID) instances were discovered by immunological testing, two neonates with non-SCID T-cell lymphopenia were reported. Despite the lack of total

lymphopenia, these two newborns developed recurrent and severe infectious illnesses or hypogammaglobulinemia across clinical follow-up. It's interesting to note that Turkey seems to have a higher prevalence of non-SCID T-cell lymphopenia than western countries. They intend to include TRECs and KRECs assays in their regular newborn screening (NBS) programs in view of this. [37]

PIDs (primary immunodeficiency diseases) are rare diseases caused by immune system failure to mature at birth. [38] Innate and adaptive immune responses are components of the immune system. First defense against microorganisms comes from the innate response, which is followed by the adaptive immune response, which includes the removal of bacteria and other extracellular microorganisms and the destruction of viruses and other intracellular microorganisms by T-cell-mediated immunity. [39, 40, 41] Of the various PIDs that have been reported, SCID has been investigated most comprehensively. It is considered a pediatric emergency in children. [42] Infants affected by SCID have a severely weakened immune system, leading to their inability to effectively protect against infection, even by the least pathogenic microorganisms. [39, 43] SCID, also known as “the bubble boy disease,” is a rare disorder in which multiple genes involved in the development and function of various immune cells undergo mutation. [44] This condition affects both the adaptive and innate immune systems, often resulting in fatal complications within the first two years of life unless treated with hematopoietic stem cell therapy (HSCT) or gene therapy. [45, 46] In 2010, SCID was incorporated to the Recommended Uniform Screening Panel (RUSP) in the US, and now newborns are screened for this highly fatal disease. [47]

Severe combined immunodeficiency (SCID) is a group of approximately 20 syndromes resulting from genetic defects that cause severe deficiencies in T cell and

B cell function, with abnormally low T cell numbers and function and poor to no B cell function. These conditions are serious and can cause life-threatening infections, although affected infants often appear healthy at birth. Essentially, there are four different types of SCID: variable, atypical/leaky, typical, and Omenn Syndrome. [31, 48, 49] A patient with typical SCID is defined as follows: (a) having a gene mutation associated with a typical SCID phenotype; (b) presenting with severe or opportunistic infections, persistent diarrhea, and failure to thrive; having low (300/μL) or absent CD3+, CD4+, or CD8+ T cells; having reduced naive CD4+ (CD3+CD4+CD45RA+) and/or CD8+ (CD3+CD8+CD45RA+) T cells; elevated γδ T cells; and lacking proliferative responses to mitogens, defined as a proliferative response to phytohemagglutinin (PHA) lower than 10% of the control subject; or (c) having T cells of maternal origin available. The four most prevalent forms of typical SCID are IL7R SCID, X-linked SCID, RAG-1/RAG-2 deficiency, and adenosine deaminase deficiency SCID. [50]

SCID is a complex disorder that involves the interaction of over a dozen genes [51] and is typically transmitted as an X-linked recessive or autosomal recessive trait. [39, 46, 52] While flow cytometry can help with diagnosis, genetic testing is usually necessary for genetic counseling and prognosis. [51] However, early detection and treatment can be difficult since, despite SCID's complicated genetic etiology, more than 80% of cases occur sporadically with no family history of congenital immunodeficiencies. [53, 54] Atypical SCID, or "leaky SCID," is defined by CD3+ counts in excess of 300 cells/μL and a reduced but detectable proliferative response to PHA (10-30% of the control). [50, 55] Variant SCID can be identified in cases where there is no known gene abnormality and T cell numbers remain between 300 and 1500 cells/L with diminished functioning. [56]

CLASSIFICATION:

Severe Combined Immunodeficiency (SCID) is characterized by the following features and classification:

1. **X-Linked SCID**: A marked decrease in T cells and natural killer cells (NK cells) within the immune system is the hallmark of X-Linked SCID. The most common form of SCID is X-linked inheritance, accounting for about 50% of cases. [52] Mutations in the gene encoding the γc chain of the interleukin (IL)-2 receptor are the cause of this disorder. The modulation of T-regulatory homeostasis and suppression, as well as the maturation of thymic T regulatory cells (Tregs), depend extensively on this receptor. People who lack these cells are more susceptible to infections on a regular basis. [57] Especially males are affected with X-linked SCID.
2. **ADA Deficiency**: With 15% of all cases, this is the second most common form of SCID. [58] The adenosine deaminase (ADA) enzyme is deficient in this particular condition, which is characterized by an accumulation of deoxyadenosine within cells. The ADA enzyme is essential in the conversion of adenosine into inosine and then deoxyadenosine into deoxyinosine. [10, 59] One toxic metabolite of deoxyadenosine that is especially detrimental to lymphoid precursors is deoxyadenosine triphosphate (dATP), which can lead to lymphopenia. As a result, neurological symptoms including motor abnormalities, cognitive difficulties, and impairments in vision and hearing are common manifestations of ADA deficiency. [43, 60]
3. **RAG-1 & RAG-2 Deficiency SCID**: This is the third most common kind of SCID, defined by mutations in Recombination activation genes 1 and 2 (RAG-

1 and RAG-2). [46, 61] The RAG genes form a multi-subunit complex that cleaves double-stranded deoxyribonucleic acid (dsDNA) molecules at the intersections of the antigen-receptor-coding region and the flanking recombination signal sequence (RSS). This mechanism initiates V(D)J recombination, which rearranges DNA segments that encode proteins produced on the cell surface in response to specific antigens. [62] In the absence of these enzymes, proper growth of T cell receptors is hampered, leading in the creation of aberrant T cells that predispose patients to a variety of pathogenic complications. [39]

4. **IL-7R Deficiency SCID**: A heterodimer consisting of hepatocyte growth factor and interleukin-7 (IL7) functions as a pre-pro B-cell growth-stimulating factor [63, 64, 65]. Furthermore, this heterodimer has been found to be one of the co-factors in the beta V(D)J rearrangement of T cell receptors, which is essential for T lymphocyte maturation. This is the fourth most prevalent form of SCID. Babies with this illness show a deficiency or lack of both T lymphocytes and B cells. But B cells cannot undergo somatic hypermutation and class switching because of lack of T-lymphocytes. [65, 66]
5. **Leaky SCID**: Leaky SCID has symptoms similar to classical SCID, although T cell levels remain over the threshold for usual SCID classification. This variation gets its name from the phenomena of a large number of T lymphocytes "leaking" into the bloodstream while presenting with an apparently normal count. [67] Despite this, these T cells are ineffective against infections. Leaky SCID may cause autoimmune reactions as overactive T cells target organs and tissues, forcing the body to attack itself. Clinical symptoms include pruritus, alopecia, erythema, weakness, lymphadenopathy,

hepatomegaly, splenomegaly, and diarrhea. Furthermore, it may cause thyroid problems and anemia. [10] Children with leaky SCID may have genetic alterations similar to those found in classical SCID, such as defects in the RAG-1 and RAG-2 genes. Delay in diagnosis is possible, with some cases not being recognized until adulthood. [68] Interestingly, the gene mutation that causes leaky SCID permits normal or higher T cell numbers, which impacts immunological function.

6. **Omenn Syndrome (OS):** It is caused by genetic abnormalities that produce a significant amount of defective T cells but spare B and natural killer (NK) cells from malfunctioning. This T cell dysfunction causes a significant immune system impairment in the infant, which is an extremely serious autosomal recessive T+ or T++ SCID deficiency. [69, 70] OS may manifest as an isolated condition or as SCID. Mutations in the genes encoding DNA ligase 4, RAG-1, RAG-2, Artemis, and adenosine deaminase deficiency are among the genetic causes of OS. The autoimmune illnesses that affect infants with OS are caused by the body mistakenly attacking itself and any weakened immune system components. Early onset seborrheic eruptions, irritable skin eruptions, hair loss, lymphadenopathy, splenomegaly, and hepatomegaly constitute the symptoms. Eosinophilia is common, and serum IgE levels remain high steadily. [59] Initially recognized as a unique form of SCID, OS has a higher mortality rate than typical SCID due to vulnerability to a variety of opportunistic infections. Skin biopsy samples must be microbiologically and histologically examined as soon as possible for diagnosis.
7. **CD3 Complex Component Deficiency SCID:** One of T cells' distinguishing characteristics is the CD3 complex. It plays a critical role in cellular signaling

by enabling communication with the nucleus following antigen binding. Alpha, beta, gamma, delta, epsilon, and zeta transmembrane chains, which cause downstream signaling to the nucleus and promote the production and release of cytokines, are thought to be linked to this activity. Three subtypes of the CD3 complex are present: CD3D, CD3E, and CD247/CD3Z. Mutations in the genes encoding these CD3 components cause diseases that harm T cells by impairing their ability to function. [71]

8. **JAK3 Deficiency SCID:** The Janus kinase 3 (JAK3) gene combines with the interleukin 2 receptor gene (IL2RG) to facilitate the activity of interleukin 2, a cytokine that promotes the proliferation of many immune cells, including T lymphocytes (helper, cytotoxic, and regulatory) and NK cells. Patients with JAK3 deficient SCID show symptoms comparable to those with lymphopenia. JAK3 deficiency affects both male and female newborns, as it is not located on the X chromosome. [72, 73]
9. **Other SCID Variants:** Various forms of SCID include IL-2 α-chain deficiency syndrome, defects in surface receptor/transduction, and defective T cell receptor epsilon chain. Some affected children exclusively present autoimmune symptoms such as vitiligo, autoimmune hemolytic anemia, autoimmune enteropathy, and Hashimoto's thyroiditis. SCID symptoms have also been observed in rare cases of autoimmune hepatitis, Evans syndrome, and nephrotic syndrome. ZAP-70 deficiency, characterized by the absence of CD8 protein, results in SCID characterized by unresponsive CD4+ T cell circulation to TCR-mediated stimuli. Rare instances of CD3 gamma subunit protein deficiencies have been documented. Bare lymphocyte syndrome, defined by the absence of human leukocyte antigens 1 or 2, predisposes

immunodeficient children to opportunistic infections by low-virulence microorganisms, representing a primary immunodeficiency affecting both humoral and cell-mediated immune responses. Short-limbed dwarfism and Nezelof's syndrome are among the varied manifestations of SCID. Nezelof's syndrome presents as a combined immunodeficiency appearing after age five with the presence of immunoglobulins. Griscelli's syndrome is characterized by features like silvery hair, enlarged liver, and lymphadenopathy, and represents another variant of SCID. OKT4 epitope deficiency, identified by the lack of reactivity to CD4+ T cells with the OKT4 monoclonal antibody, is relatively common and exhibits mild susceptibility to infections, with variable occurrences noted across Black, White, and Japanese populations. These diverse forms of SCID underscore the complexity of immune system dysregulation and emphasize the necessity for targeted interventions. [74]

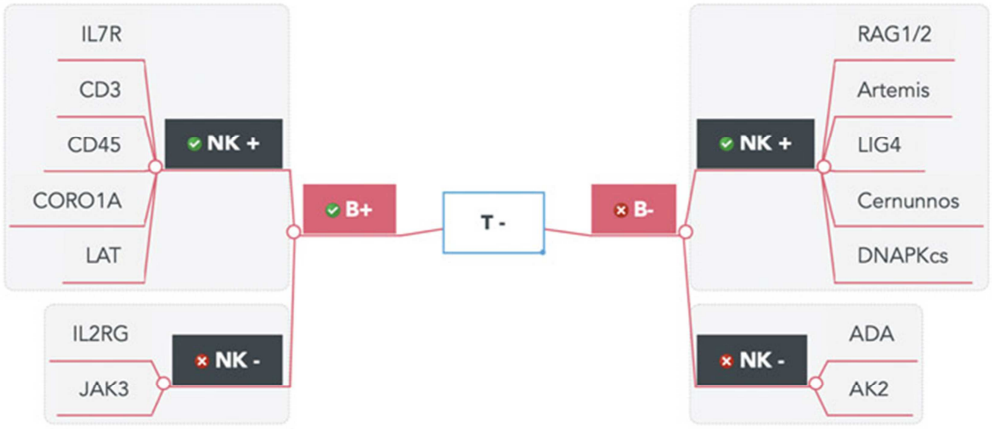


FIGURE 3. Immunophenotypes of major types of severe combined immunodeficiencies. [75]

Disorder (year of definition of molecular basis)	*Chromosomal location*	*Gene*	*Diagnostic tests other than direct mutation analysis*
X linked severe combined immunodeficiency (1993)	Xq13	Common γ chain (γ_c)	γ_c expression by FACS analysis
Adenosine deaminase (ADA) deficiency (1983)	20q12–13	Adenosine deaminase	Red cell ADA levels and metabolites
Purine nucleoside phosphorylase (PNP) deficiency (1987)	14q11	Purine nucleoside phosphorylase	Red cell PNP levels and metabolites
Recombinase activating gene (RAG 1&2) deficiency (1996), Omenn's syndrome (1998)	11p13	RAG1 and RAG2	
T cell receptor deficiencies (1987)	11q23	CD3γ/CD3ε	
Zap70 deficiency (1994)	2q12	ZAP-70	ZAP-70 expression
JAK3 deficiency (1995)	19p13	JAK3	JAK3 expression/signalling
IL-7 receptor deficiency (1998)	5p13	IL-7 receptor α	IL-7 receptor α expression
MHC class II deficiency			
(1993)	16p13	CIITA	HLA-DR expression
(1998)	19p12	RFX-B	
(1995)	1q21	RFX5	
(1997)	13q13	RFXAP	

TABLE 1. MAJOR TYPES OF SCID AND THEIR GENETIC DEFECT. [76]

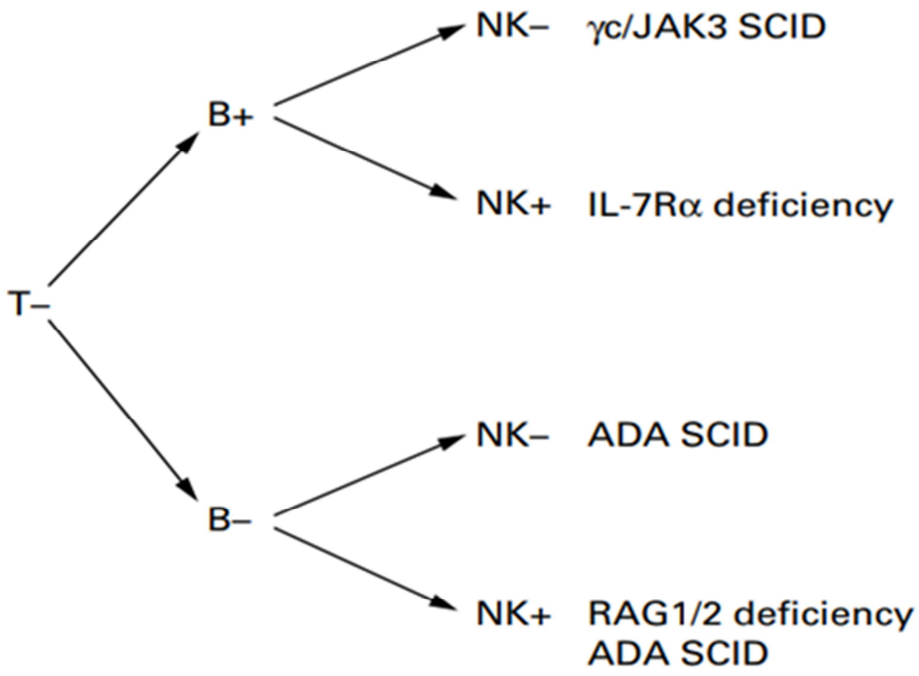


FIGURE 4. IMMUNOPHENOTYPES IN SCID. [76]

TABLE 2. Types of Severe Combined Immunodeficiency:

	Type of Disease	T/B/NK	Gene	Hereditary
	Typical SCID:			
1)	X-linked SCID	T-/B+/NK-	IL2R gamma	X-linked
2)	Adenosine Deaminase Deficiency SCID	T-/B-/NK-	ADA	Autosomal Recessive
3)	RAG-1 AND RAG-2 Deficiency SCID	T-/B-/NK+	RAG-1 and RAG-2	Autosomal Recessive
4)	IL7R Deficiency SCID	T-/B+/NK+	IL7R alpha	Autosomal Recessive
	Other SCID:			
1)	CD3 Complex Component Deficiency SCID	T-/B+/NK+	CD3D CD3E CD247	Autosomal Recessive
2)	CD45 Deficiency SCID	T-/B+/NK+	PTPRC	Autosomal Recessive
3)	Cernunnos-XLF Deficiency SCID	T-/B-/NK+	NHEJ1	Autosomal Recessive
4)	Coronin-1A Deficiency SCID	T-/B+/NK+	CORO1A	Autosomal Recessive
5)	Artemis SCID	T-/B-/NK+	DCLRE1C	Autosomal Recessive
6)	DNA Ligase 4 Deficiency SCID	T-/B-/NK+	LIG4	Autosomal Recessive
7)	DNA-PKCS Deficiency SCID	T-/B-/NK+	PRKDC	Autosomal Recessive
8)	JAK3 Deficiency SCID	T-/B+/NK-	JAK3	Autosomal Recessive
9)	LAT Deficiency SCID	T-/B+/NK+	LAT	Autosomal Recessive
10)	Reticular Dysgenesis SCID	T-/B-/NK-	AK2	Autosomal Recessive
11)	Leaky SCID	T+/B+/NK+	Mainly RAG1 and RAG2 but others are also involved	Autosomal Recessive
12)	Omenn Syndrome	T-/B-/NK+	RAG1 and RAG2	Autosomal Recessive

TABLE 1: Types of Severe Combined Immunodeficiency

SCID: Severe combined immunodeficiency; T: T lymphocytes; B: B lymphocytes; NK: Natural killer cells; IL2R: Interleukin 2 receptor; ADA: Adenosine deaminase; RAG: Recombination activating gene; IL7R: Interleukin 7 receptor; CD: Cluster differentiation; PTPRC: Protein tyrosine phosphatase type C; NHEJ: Non homologous end joining factor; PKCS: Protein kinase catalytic subunit; PRKDC: Protein kinase DNA activated catalytic subunit; JAK: Janus kinase; LAT: Linker for activation of T cells; AK: Adenylate kinase; +: high count; -: low count

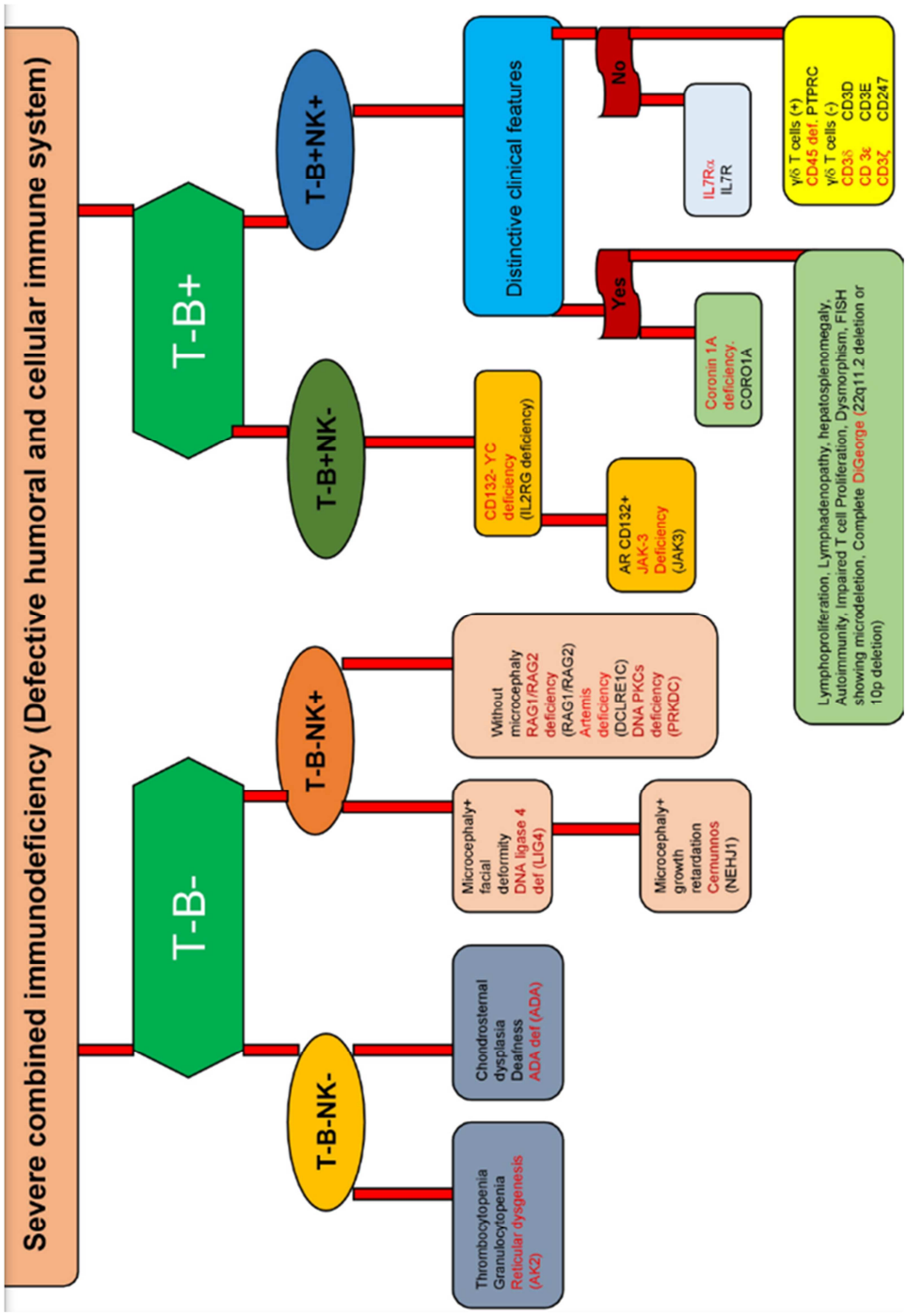


FIGURE 5. GENETICS OF SEVERE COMBINED IMMUNODEFICIENCY. [51]

TABLE 3. SCID classification based on phenotypes of lymphocytes and molecular defects: [1]

LYMPHOCYTE PHENOTYPES	MOLECULAR DEFECTS
T- B+ NK-	*IL2RG*
	JAK-3
T- B+ NK+	*IL7R*
	CD3D
	CD3E
	CD247
	CD45
	CORO1A
	FOXN1
T- B- NK-	*ADA*
	AK2
	MSN
	TTC7A
T- B- NK+	*LIG4*
	NKEJ1
	PRKDC
	RAG1/RAG2
	DCLRE1C

Abbreviations: *ADA*, adenosine deaminase; *AK2*, adenylate kinase; *CD*, cluster of differentiation; *CD3D*, CD3δ; *CD3E*, CD3ε; *CORO1A*, Coronin 1A; *DCLRE1C*, DNA cross-link repair enzyme 1C; *FOXN1*, Forkhead box N1; *IL2RG*, Interleukin 2 receptor common γ chain; *IL7R*, Interleukin 7 receptor; *JAK3*, Janus kinase 3; *LIG4*, DNA ligase IV; *MSN*, Moesin; *NHEJ1*, Nonhomologous end-joining protein 1; *PRKDC*, DNA-dependent protein kinase; *PTPRC*, protein tyrosine phosphatase receptor type C; *RAG*, recombinase activating gene; *TTC7A*, Tetratricopeptide Repeat Domain 7A.

PATHOGENESIS:

X-linked severe combined immunodeficiency (X-SCID), also known as SCID-X1 is the most common form, resulting from defects in a gene on the X chromosome encoding the cytokine receptor subunit gamma-c (the interleukin receptor common gamma chain [IL2RG]) . This receptor subunit is shared by at least six different cytokine receptor complexes, including those for interleukins (IL) 2, 4, 7, 9, 15, and 21 . Thus, leading to impaired T and NK cell development but normal B cell numbers. Cytokine stimulation activates JAK3, initiating signaling pathways crucial for normal immune cell development. In vitro studies indicate the importance of IL-7/IL-7R and IL-15/IL-15R pathways for T and NK cells. Abnormalities in IL-2 and IL-4 signaling may explain B cell defects.

Pathogenic variants in this gene cause profound disruption of the immune system by blocking multiple cytokine pathways critical for lymphocyte development and function. As a consequence, affected individuals exhibit absent T cells and natural killer (NK) cells, along with nonfunctional B cells (T-B+NK- SCID).

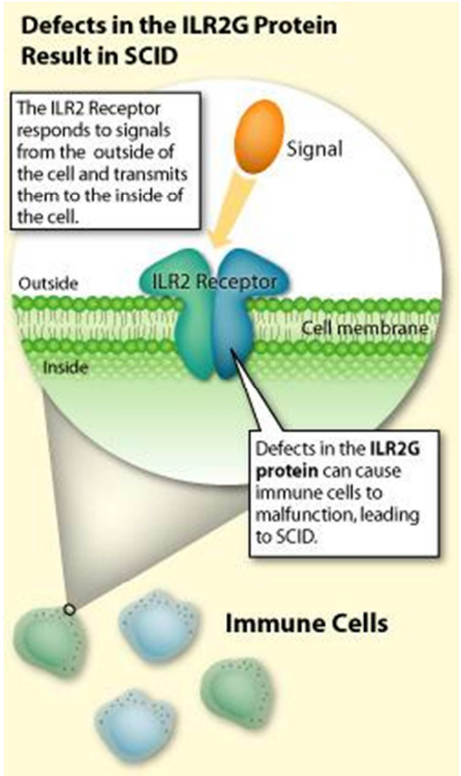


FIGURE 6. X-linked SCID. [77]

Additionally, the gamma-c subunit plays a role in growth hormone receptor signalling. Therefore, growth failure observed in some children with X-SCID may result from both the underlying genetic defect and late effects related to conditioning, recurrent infections, or nutritional deficiencies. This dual influence could explain why certain patients continue to experience growth failure and short stature even after partial correction of the defect through hematopoietic cell transplantation (HCT). [28]

Genetic diagnosis, previously based on family history and clinical profiles, now relies on direct analysis of gamma chain and JAK3 genes. Identification of mutations enables definitive carrier assessment and accurate prenatal diagnosis. Rapid tests examining mutant protein expression aid in diagnosing affected infants. Flow cytometric analysis confirms molecular diagnosis in X-SCID cases. For T−B+NK SCID cases with normal gamma chain expression, further analysis of JAK3 pathway activation using monoclonal antibodies can detect abnormalities before genetic analysis. Identifying molecular defects is crucial for prompt bone marrow transplantation or gene therapy in these forms of SCID.

Stimulation of mononuclear cells by IL-2 leads to the phosphorylation of JAK3 at specific tyrosine-based motifs. Detecting this JAK3 activation using a monoclonal antibody against phosphotyrosine residues allows abnormalities in the signaling pathway to be identified at the protein level before genetic analysis. The clinical presentation variability in SCID forms, particularly in JAK3 deficiency, emphasizes the importance of identifying the molecular defect early for timely bone marrow transplantation. Given the success of somatic gene therapy protocols for X-SCID, rapid molecular diagnosis remains crucial.

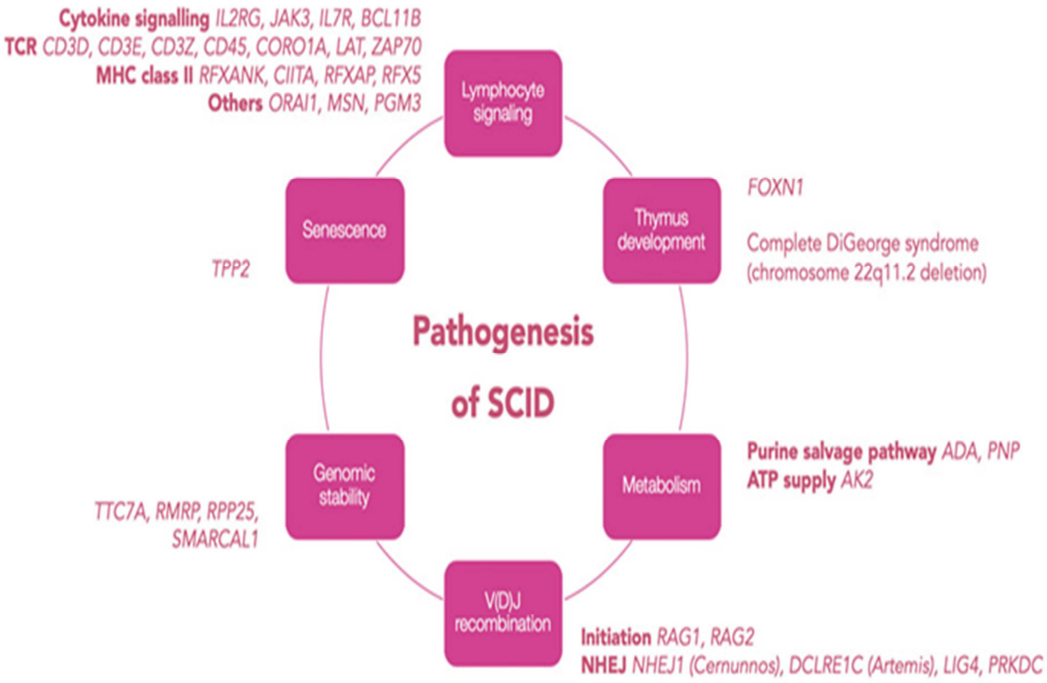


FIGURE 7. PATHOGENIC MUTATIONS IN SCID. [75]

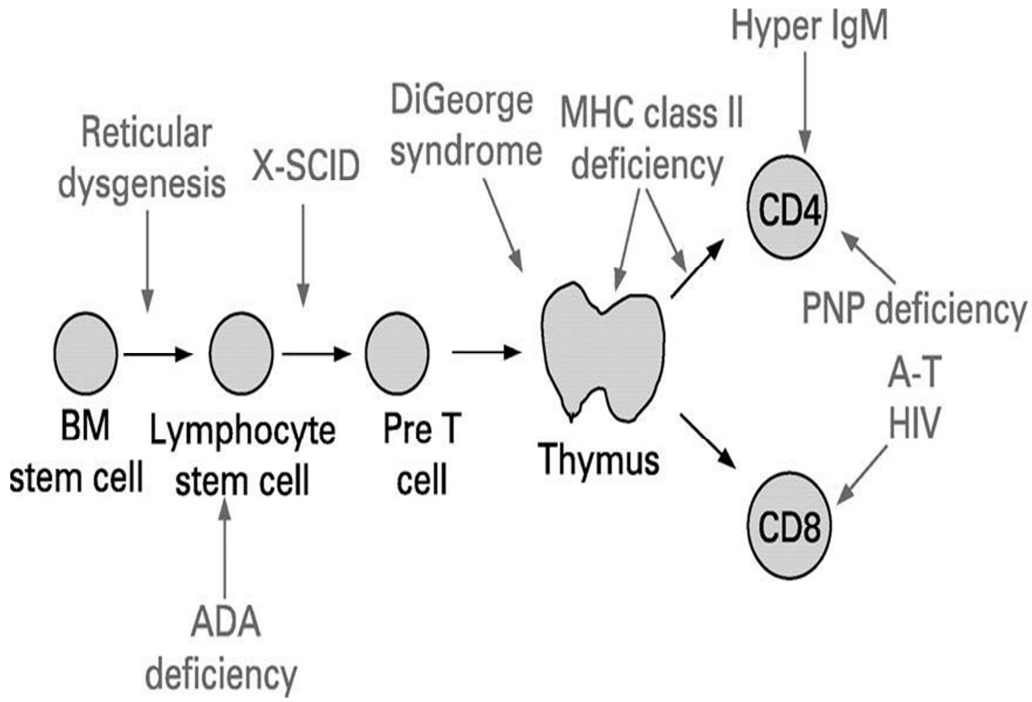


FIGURE 8. PATHOPHYSIOLOGY OF SCID. [78]

Molecular Defects causing SCID:

+----------+

| |

X-linked SCID **Autosomal Recessive JAK3 Deficiency**

(Defect in gamma **(Defect in JAK3 gene)**

chain) |

| |

| |----------|

| | |

Absence of No T and NK cell Gamma chain crucial for

T and NK development, receptor complex signaling

cell dev. normal B cell nos. (IL-4, -7, -9, -15)

Intrinsic defects in |

B cell function |

| |

Diagnosis: |

Direct |

analysis of **Diagnosis:**

gamma Rapid tests

chain and for affected

JAK3 genes infants available

Confirmation: **Confirmation:**

Abnormal gamma Flow cytometric

chain expression analysis of PBMCs

| |

Further Dissection: **Further Dissection:**

Detection of JAK3 JAK3 activation detection

activation after IL-2 stimulation	using monoclonal antibodies
Treatment:	**Treatment:**
Early bone marrow transplantation, successful gene therapy protocols available	Early bone marrow transplantation, successful gene therapy protocols available

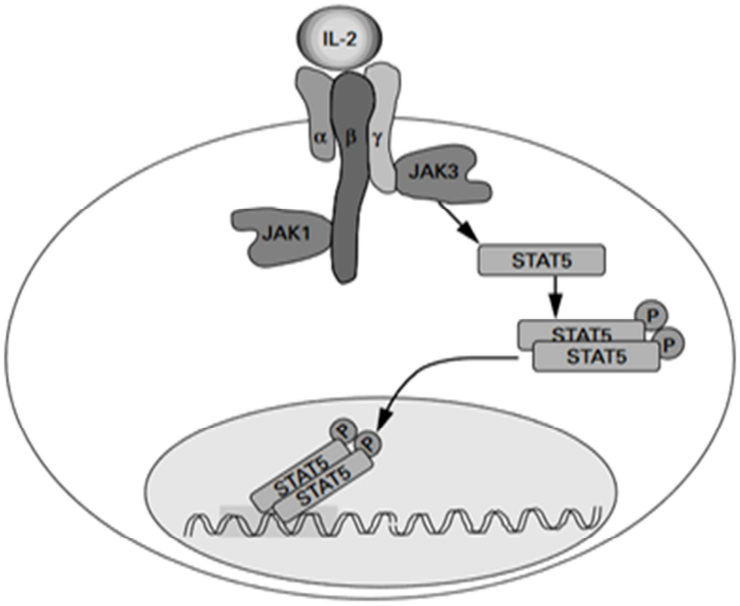


Figure 2 γc/JAK3 signalling pathway. [76]

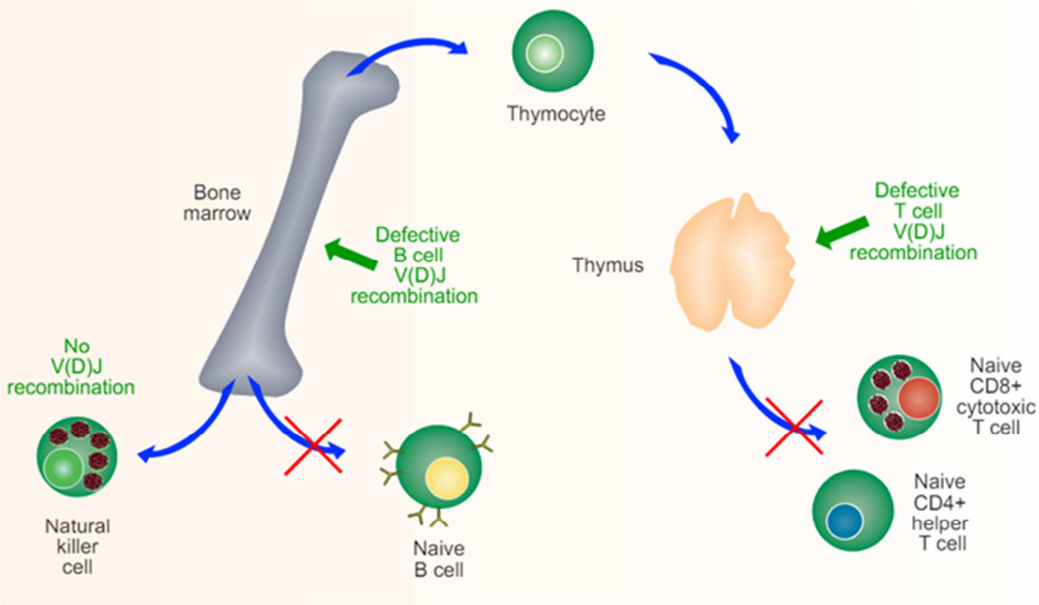


FIGURE 9. SCID: defective T and B cell development, normal NK cells. [79]

SCREENING FOR SCID:

The delayed identification of SCID comes at a high cost, as survival rates fluctuate depending on treatment timing and infection status. Infants who receive an HSCT within the first 3.5 months of life have a better than 90% probability of survival, even without a matched sibling donor. After 3.5 months, the overall survival rate reduces to 70%, [80] with variations ranging from 50% to 90% based on infection status and donor type. [11] Because infants may appear healthy until they develop recurrent infections or fail to thrive, the initial diagnostic window for optimal outcomes is frequently closed by the time the diagnosis is made.

Unfortunately, some infants may die from serious infections before receiving the definitive treatment, or their transplantation outcomes may be worse than those who were detected earlier in life. [81] This knowledge of the need of early diagnosis drove the quest for a biomarker to screen infants for SCID, which led to the

development of the TREC assay. [82] SCID is a rare illness that requires immediate medical attention in children. SCID newborns look to be normal from the outside. However, the disease's manifestation is dependent on both genetic abnormalities and environmental exposure. As a result, the presentation of SCID differs. Infections are frequent in all newborns, including those with SCID. Unfortunately, vital time is frequently lost before SCID is suspected, resulting in high mortality and morbidity.

Furthermore, family history is non-specific and easily neglected. Prompt diagnosis enables immediate lifesaving therapy. It also prohibits the use of live vaccination, which could harm the infant. When HSCT is conducted within the first few months of life, prior to the onset of severe infections, the outcomes are much better than when signs are detected later. Early detection also offers families with genetic information and reproductive risk assessment. Furthermore, it helps to improve understanding of SCID's true incidence and range by educating healthcare providers and the general public about this critical disorder. Newborn screening programs would be appropriate for SCID because of its clinical severity, lack of symptoms at birth, availability of definitive therapy, and potential for considerably enhanced quality of life through early intervention. [10]

SCID is a genetically diverse illness characterized by a variety of genetic abnormalities but a shared clinical presentation. The bulk of these abnormalities, however, impair the formation of T cells in the thymus. [83] T cells in the thymus go through a process called receptor gene splicing and rearrangement, in which T cell antigen receptor (TCR) genes are randomly cut and rejoined, resulting in unique rearrangements in each cell. This process produces circular DNA byproducts known as T cell receptor excision circles (TRECs), which act as persistent identifiers for

recent thymic emigrants. [84, 85] TRECs are expressed only in naïve T cells that have recently left the thymus, making them a surrogate marker for thymic T cell development and a good screening test for SCID. [84, 85] TREC levels in peripheral blood vary dramatically with age. The ratio in normal infants is about 1 TREC per 10 T cells, indicating an abundance of naive T cells that have not experienced considerable proliferation. In contrast, older children and adults have a ratio of about 1 per 100 and 1 per 1000 T cells, showing peripheral expansion. Infants with SCID often have extremely low or undetectable levels of TRECs. [84] Maternal T cells have few TRECs, hence their influence on newborn TREC numbers is minimal. TRECs are quantified using quantitative real-time PCR, which entails creating a standard curve from repeated dilutions of bacterial plasmid-cloned TREC signal junction constructs and extrapolating the TREC value in DBS specimens from the standard curve. [86]

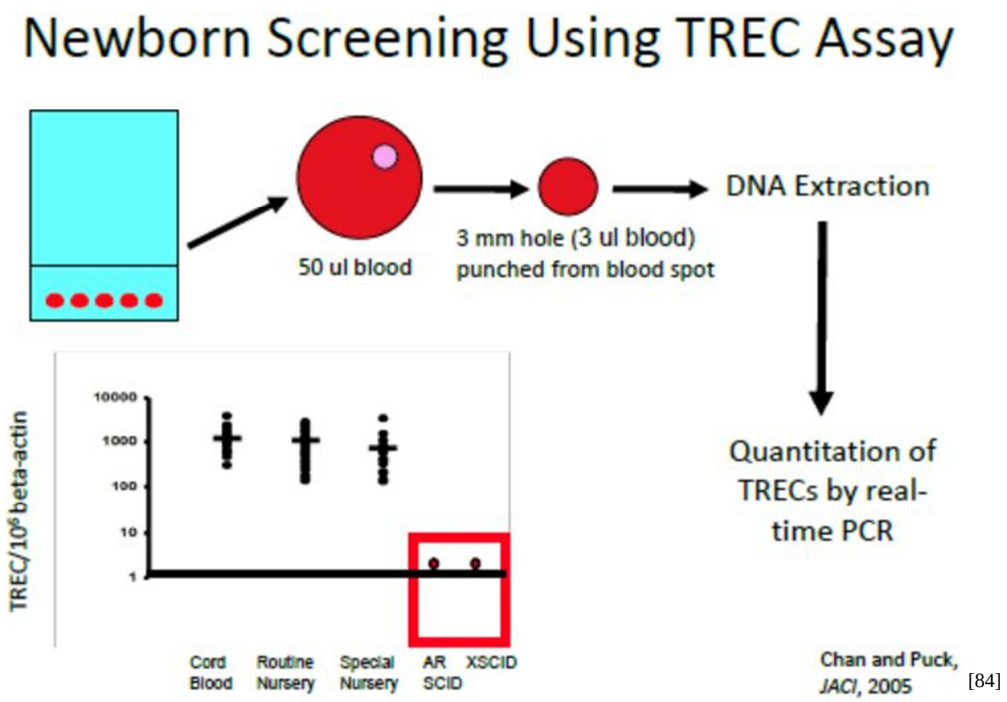


FIGURE 10. Newborn screening using TREC assay

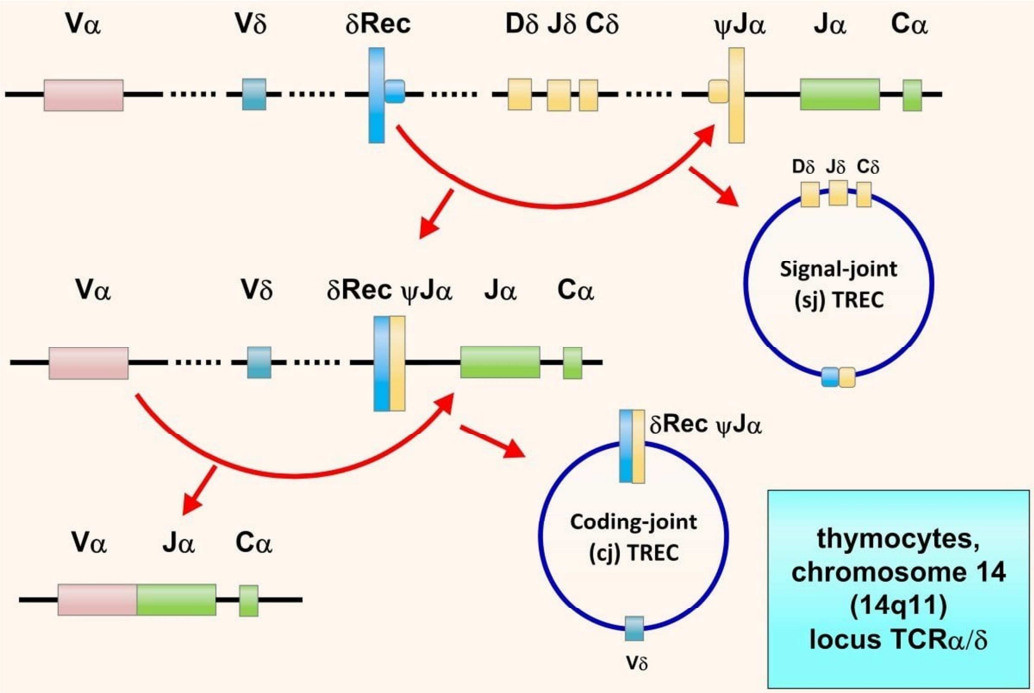


FIGURE 11. T-cell receptor excision circles (TRECs)

T cell Receptor Excision Circles (TRECs). TRECs are episomal DNA circles produced in thymocytes by excisional rearrangements of T cell receptor (TCR) genes; they are stable, not duplicated during mitosis, diluted out with each cell division, and therefore higher in thymocytes, recent thymic emigrants (RTEs) and naïve T cells. Quantitative polymerase chain reaction (PCR) of coding-joint (cj) δRec ψJα TREC, produced at TCRα/δ locus within chromosome 14 (14q11) by > 70% of developing human α:β T cells, counts in the peripheral blood naïve α:β T lymphocytes recently dismetted by thymus: in newborn, values < 25 TRECs/μL indicate SCID. [87]

Conditions identified by TREC screening assay are as follows: [84]

- Typical SCID - These forms of SCID are characterized by fewer than 300 autologous T cells per microliter of blood and less than 10% of normal proliferation in response to mitogens such as phytohemagglutinin (PHA).

- Gene function is partially preserved in leaky SCID or Omenn syndrome, which is caused by mutations in typical SCID genes. The 300–1500 autologous T lymphocytes per microliter are characteristic of these SCID types. In patients with Omenn syndrome, T cell oligoclonality, or limited TCR diversity, is present despite either normal or increased CD3 T cell numbers.

- Variant SCID presents with persistently low T cells but without a defect in a known SCID gene.

- Conditions causing primary T cell lymphopenia (CD3 T cells ≤ 1500 cells/μL) include: Complete DiGeorge syndrome or partial DiGeorge syndrome with low T cells, CHARGE syndrome, Jacobsen syndrome, Trisomy 21, RAC2 dominant interfering mutation, DOCK8 deficient hyper-IgE syndrome, Cartilage hair hypoplasia.

- Secondary T-cell lymphopenia is diagnosed in a group of infants with recognized congenital conditions such as intestinal lymphangiectasia, hydrops, gastroschisis, congenital heart defects, chylothorax, or neonatal leukemia. It can also result from prenatal administration of glucocorticoids or inflammatory conditions (e.g., sepsis).

- Premature infants may present with T cell lymphopenia, defined as T cells ≤1500 cells/μL, which typically resolves with age. [84]

Non-SCID causes of T-cell lymphopenia	Examples
Syndromic disorders and congenital abnormalities	Trisomy 18
	Trisomy 21
	DiGeorge syndrome
	CHARGE syndrome
	Ataxia–Telangiectasia syndrome
Secondary T-cell lymphopenia	Cardiac or gastrointestinal anomalies
	Multiple congenital anomalies
	Neonatal leukemia
	T-cells losses into third space (e.g., hydrops or vascular leakage)
	Prenatal exposure to purine antagonists
Preterm birth	
Idiopathic T-cell lymphopenia	
Combined immunodeficiencies with dysfunctional T cells	DOCK8 deficiency
	Wiskott–Aldrich Syndrome

TABLE 4. CONDITIONS OTHER THAN SCID THAT LEAD TO AN ABNORMAL TREC ASSAY. [82]

Conditions where T-cells undergo thymic development, leading to TREC production, yet exhibit impaired functionality, are not detectable through the TREC assay. This encompasses newborns with Zap70 deficiency, [88] MHC class II deficiency, [89] CD40 ligand deficiency, NEMO deficiency, and individuals experiencing late-onset ADA deficiency.

TREC results interpretation:

Low or nil TREC scores are indicative of T cell lymphopenia. However, because this assay relies on PCR technology, it is possible for flaws like inadequate sample size, subpar DNA elution from the DBS, or the presence of PCR inhibitors such heparin to cause a low result. A reference gene amplified lowers the risk of false positive outcomes. The usual tactics adopted are: [83]

1. Singleplex TREC assay: In samples where the first TREC qPCR reaction produces a result below the predefined TREC cut-off, this technique entails the parallel amplification of a reference gene and TRECs. The test is regarded as inconclusive if the reference gene is not found. The replicate TREC values are used as the final TREC values if they are discovered.

2. Multiplex Real Time qPCR: This method involves amplifying the reference gene and TREC at the same time. The same multiplex assay is used to retest samples that have aberrant multiplex results. The test is deemed inconclusive if the reference gene is not found. The replicate TREC values are used as the final TREC values if they are discovered.

Follow-up algorithm of an abnormal TREC result:

All abnormal screening results necessitate additional testing, including a CBC and lymphocyte subset analysis using flow cytometry to count T, B, and NK cells in whole blood samples. The recommended examination includes:

1. Enumerating ALC: T cells (including total CD3, CD4, CD8 T cells), B cells (CD19), and NK cells (CD16/56).

2. Evaluation of memory and naïve cells using CD45RA and CD45RO markers. Additional markers such as CD62L (L-selectin), CD31, CD27, and CCR7 can be used to further characterize naïve T cells. The whole blood sample specimen should be fresh and processed according to the guidelines of the immunology laboratory. An individual with expertise in interpreting flow cytometry results in children and newborns should assess the results and compare them with age-related normal references. If flow cytometry evaluation confirms lymphopenia, the identified newborn should be promptly referred to a specialized center with expertise in diagnosing and managing PIDs. Clinical evaluation using the checklist (Table 2) is essential, and treatment/interventions should be initiated until further evaluation confirms the diagnosis of SCID or other PIDs [10].

Table 2 Clinical checklist

Physical features s/o SCID (*e.g.*, Skin rash, oral ulcers, midline defects, coarse facial features)
H/o any immunosuppressive to mother
Positive family history in the form of previous siblings with • Infant death • BCGOsis • Severe infections • Diagnosis of PIDs

FIGURE 12 . Clinical checklist for evaluation of SCID

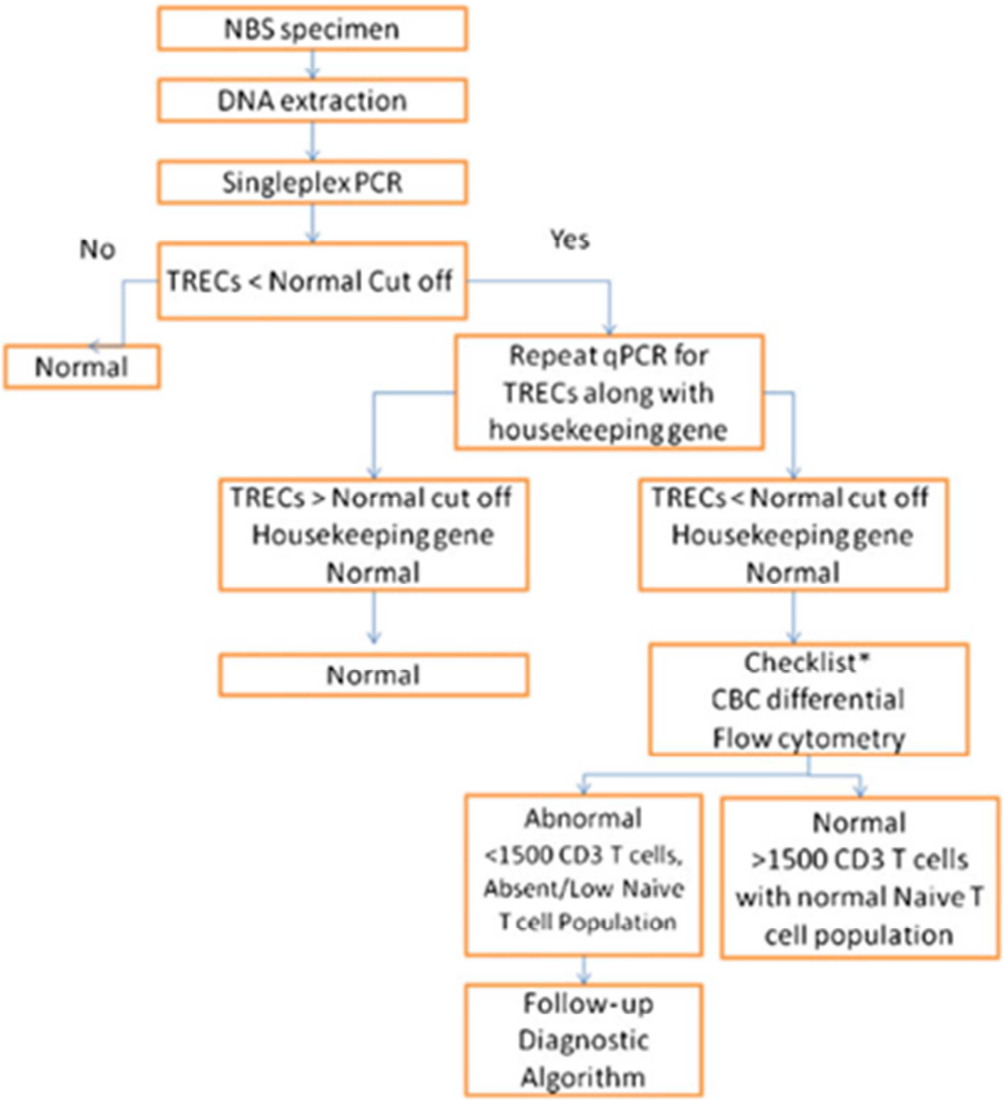


TABLE 5. FLOWCHART FOR SCID SCREENING BY TREC ASSAY. [10]

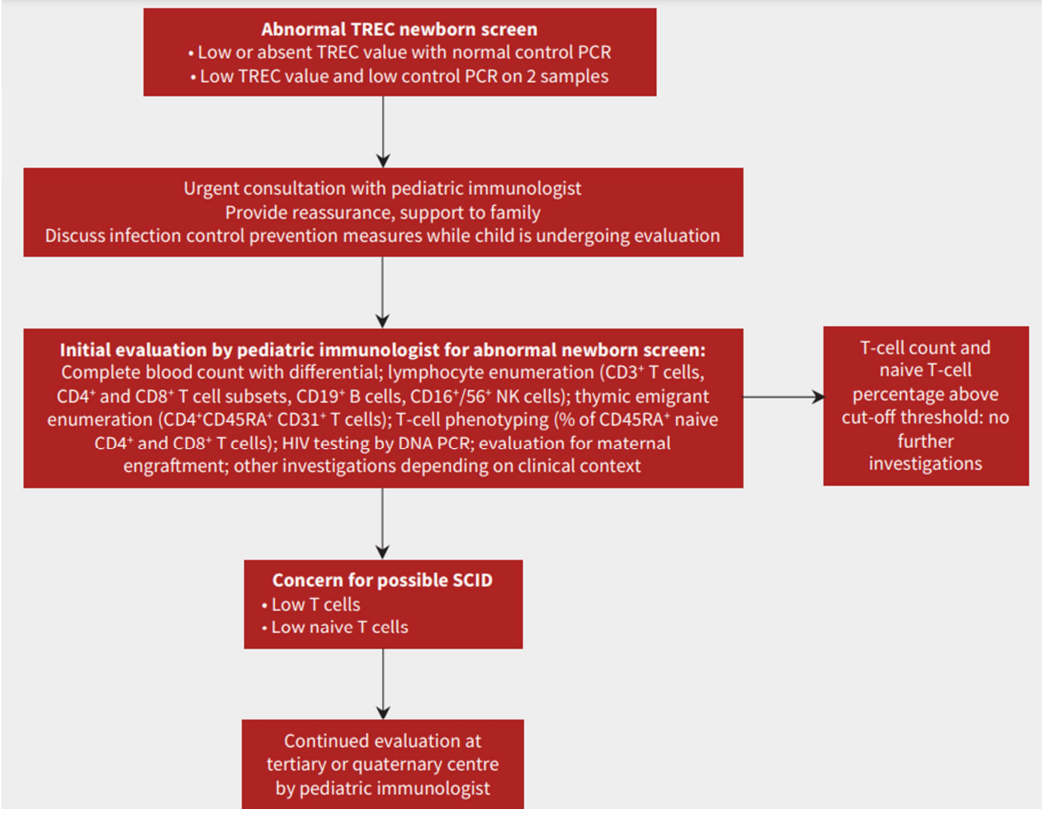


TABLE 6. SUGGESTED INITIAL APPROACH TO A PATIENT WITH AN ABNORMAL NEWBORN SCREEN FOR SCID. [82, 90]

TREC ASSAY:

SCID NBS involves testing all newborns using a heel stick to collect a small blood sample (3μL) applied to a Guthrie filter paper card. The dried blood spot (DBS) is then extracted from the card using a 3.2 mm punch and placed in a DNA extraction buffer. The T-cell receptor excision circles (TRECs), represented as circles, are subsequently amplified using reverse transcriptase quantitative PCR (RT-qPCR) with primers specific to δRecΨJαTREC. Beta-actin serves as an amplification control to verify DNA integrity. In normal newborns, the median TREC count after 40 amplification cycles is 827 copies. Infants with suspected T cell deficiency are flagged for further evaluation if their TREC count falls below 25 copies.

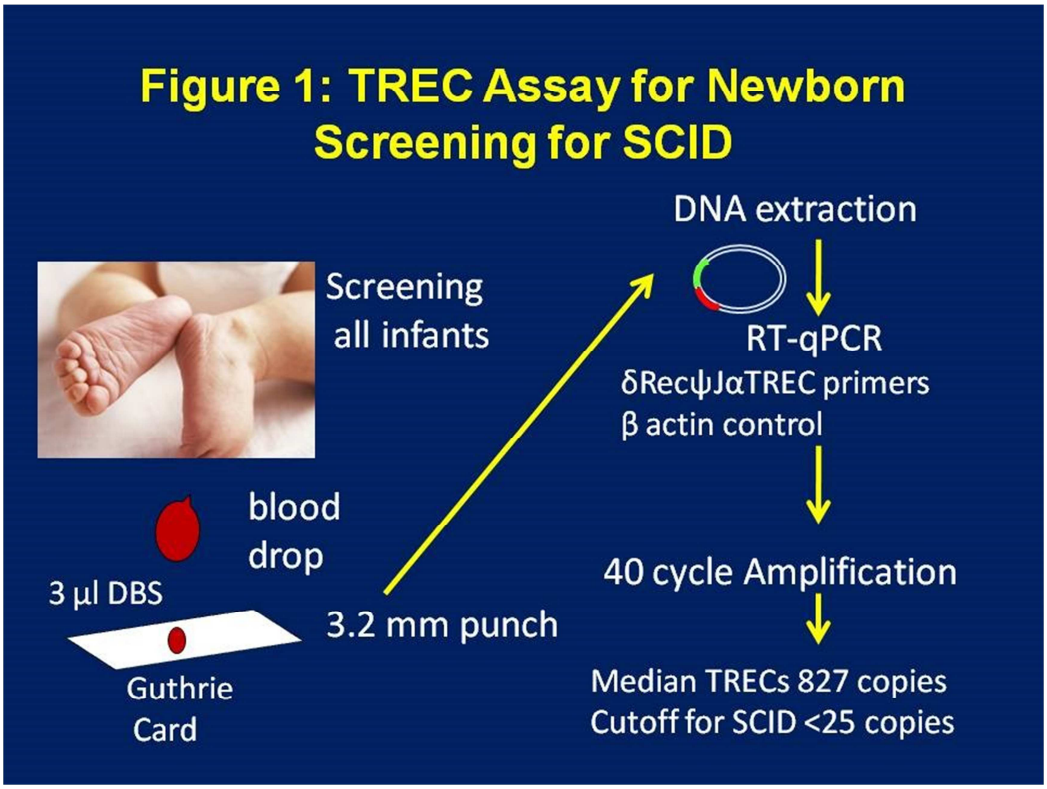


FIGURE 13. TREC ASSAY FOR NEWBORN SCREENING FOR SCID. [91]

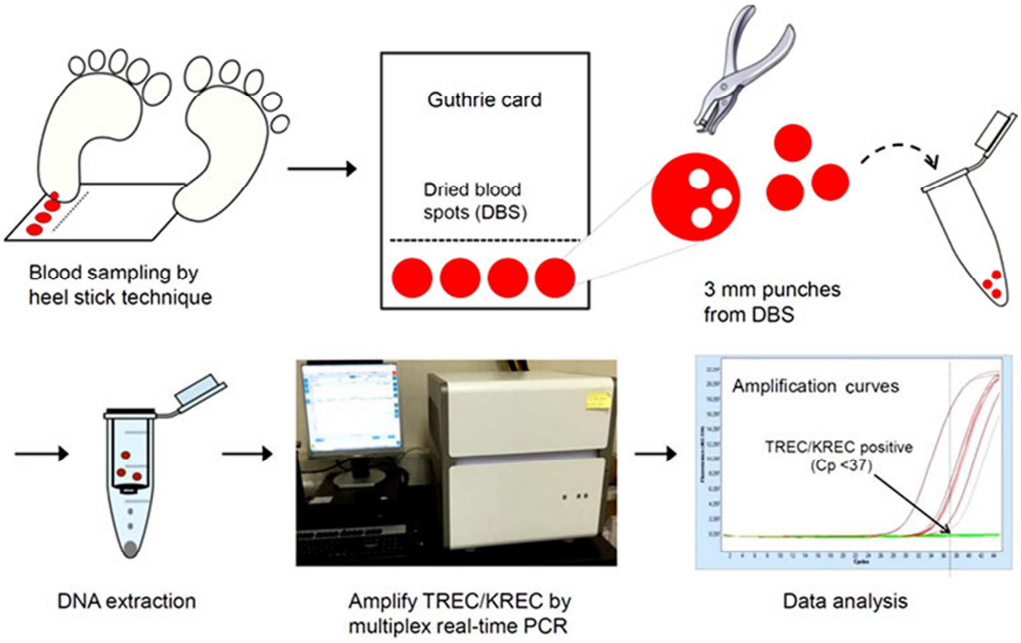
Quantitative polymerase chain reaction (PCR) is the method used in the newborn screening test for SCID to determine the amount of T-cell receptor excision circles (TRECs) in an infant's blood. [84] Circular DNA fragments known as TRECs are produced as a consequence of healthy T-cell maturation in the thymus. [83] DNA taken from dried blood spots collected during standard newborn screening can be used for this experiment. To rule out technical problems as a possible reason for a low number of TRECs, it usually entails PCR analysis of a control gene in addition to TRECs. [83] The number of TRECs is an effective biomarker for T-cell development since it is correlated with the production of distinct T cells.

This screening test is essentially a way to detect T-cell lymphopenia, as it looks for diseases that cause decreased production of thymic T-cells in infants. Few detectable TRECs are seen on the newborn screen in infants who initially appear healthy at birth but are later diagnosed with SCID because of limited or nonexistent T-cell production. [84]

This screening technique was first introduced in Wisconsin in 2008, and it has since spread to the majority of US states as well as many other nations, such as Canada [92, 93]. This screening program was first implemented in Ontario, Canada's pioneer province, and it has now spread to other parts of the nation. [94]

With its remarkable sensitivity and specificity, the SCID newborn screen performs exceptionally well as a diagnostic tool. [93, 95] A number of case series have demonstrated 100% sensitivity in identifying typical cases of SCID, which are defined as newborns with maternal engraftment or a T-cell count <300 cells/μL. [95] Moreover, specificity is remarkably high, with a cohort of infants receiving SCID newborn screening achieving 99.98%. [93] Positive predictive values ranged from 0.8% to 11.2% for diagnosing typical SCID and from 18.3% to 81% for identifying both typical SCID and infants with significantly low T-cell counts that do not meet the severity threshold required for diagnosing SCID, according to a systematic review of cohort studies on SCID newborn screening. [95] However, it is vital to remember the TREC assay's false-positive rate, which is the fraction of infants with poor TREC results who require additional T-cell enumeration but eventually have a normal T-cell count. [92] This rate fluctuates based on the cut-off values used to define normal TREC levels and normal T-cell counts. Because different screening locations use different cutoff values, which are frequently combined with T-cell phenotypic analysis, false-

positive rates range significantly between centers. While low false-positive rates risk missing cases of SCID, greater rates may result in irrational assessments of children who do not have the disease. [92, 96] In order to overcome the difficulties posed by false-positive rates, more research is desperately needed to establish the ideal cutoff values for TRECs and T cells as Canada rolls out the newborn screen for SCID. [94, 95]



GUTHRIE CARD FOR TREC ASSAY NBS

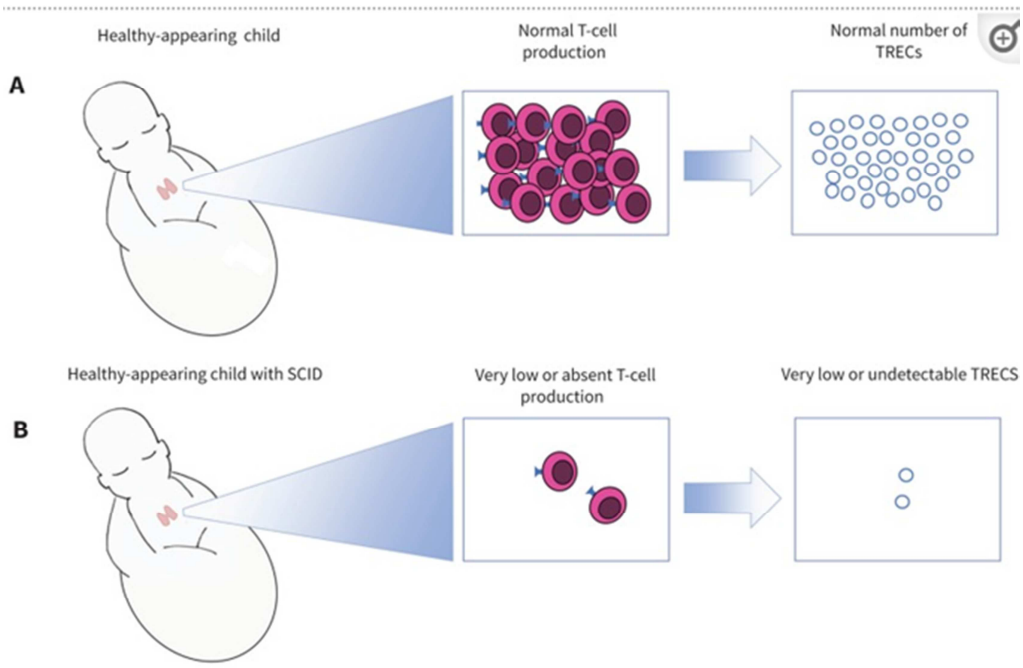


FIGURE 14 . Immune system in healthy child v/s SCID child

In this pictorial representation, there are two scenarios: [82]

1. **Healthy Infant (A)**:

- This infant possesses an intact immune system. The thymus, a critical organ for immune development, produces a normal repertoire of T cells. After these T cells are generated, small DNA circles called TRECs are released into the bloodstream. These TRECs can be quantified from a blood spot obtained during newborn screening.

2. **Infant with SCID (B)**:

- This infant has SCID despite looking apparently healthy. SCID results in very minimal or no T-cell production. Consequently, the newborn screen shows

undetectable levels of TRECs. This early detection of SCID allows for timely intervention and management.

In summary, the TREC-based newborn screen helps identify SCID cases even when outward signs may not be apparent. It plays a crucial role in facilitating early diagnosis and appropriate care for affected infants.

It's important for those caring for infants suspected to have severe combined immunodeficiency to implement some precautions with care and consideration - Prioritizing rigorous hand hygiene and advise against exposure to crowded or unwell environments to reduce the risk of community-acquired infections. Limiting the infant's contact with other young children who may unknowingly spread infections. Ensuring thorough sterilization of feeding bottles and the use of boiled water to safeguard against the transmission of pathogens like cryptosporidium. [93] Refraining from administering live or live-attenuated vaccinations, such as rotavirus, measles, mumps, rubella, varicella, and live-attenuated influenza, to infants or household contacts. Employing only donor irradiated and leukocyte-reduced blood products to minimize potential complications. [90] Acknowledging the impact of cytomegalovirus infection on transplant outcomes for severe combined immunodeficiency patients. [97] If feasible, confirming that blood products received by the infant are free from cytomegalovirus. Recognizing the potential transmission of cytomegalovirus through bodily fluids, including breast milk and oral secretions, also in asymptomatic maternal carriers. And temporarily suspending breastfeeding upon confirmation of low T-cell receptor excision circles until a thorough pediatric immunology evaluation is conducted. Meanwhile, mothers who wish to breastfeed can express and store breast

milk till their cytomegalovirus status is determined through serological testing. If the maternal status is negative, may resume breastfeeding. [90, 98]

LIMITATIONS AND CHALLENGES IN TREC FOR NEONATAL SCREENING:

Despite the widespread implementation of TREC-based neonatal screening programs (NSP), it is imperative for physicians to acknowledge the limitations inherent in this testing method. While the TREC assay effectively detects various causes of T-cell lymphopenia, it may not serve as a comprehensive screening tool for every PID. [99] There exists a subset of Combined Immunodeficiency Disorders (CID) wherein lymphopenia is not a distinguishing feature, yet the immune dysfunction is as severe as in typical SCID cases. These conditions, often labeled as T+ - SCID, CID, and some leaky SCID, pose a challenge for detection using the current TREC cutoff value. [100, 101]

Patients with these conditions may not be identified solely based on their TREC levels: The TREC assay cannot detect diseases in which T-cells grow in the thymus to produce TRECs but have compromised functionality. Also, severe but treatable CIDs like chronic granulomatous disease, congenital neutropenia, and toll-like receptor defects may not exhibit T-cell or B-cell lymphopenia, thus escaping detection by the TREC assay. Additionally, disorders such as ZAP-70, NEMO deficiency & MHC class II deficiency (Bare lymphocyte syndrome) present complexities in diagnosis. [88, 89] While ZAP-70 defects can lead to significant CD8 lymphopenia and a CID phenotype, their TREC levels may remain normal. Similarly, MHC class II deficiency manifests as severe CD4 lymphopenia, resembling SCID, but can evade detection by the TREC assay due to the presence of CD8 cells.

Furthermore, the TREC assay may overlook conditions like ADA deficiency, both in its typical SCID presentation and in cases of late-onset ADA deficiency. Moreover, TREC screening detects less than 50% of cases of ataxia telangiectasia, highlighting its limitations in certain rare disorders. Disorders such as Ora1, Stim1, or CD40 ligand deficiency, which exhibit infectious phenotypes akin to SCID but maintain normal T-cell counts, pose uncertainty regarding their detectability through the TREC assay. [102]

A systematic review of available literature comprehensively assesses the screening efficacy of various algorithms employed in the TREC-NBS for SCID. The synthesized data reveal that nuances in PCR assays and algorithmic features significantly influence the Positive Predictive Value (PPV) of SCID NBS, with the TREC cut-off value emerging as the pivotal determinant. The findings suggest that implementing a TREC cut-off value of up to 25 TRECs/μl and integrating the collection of a repeat Dried Blood Spot (DBS) from Neonatal Intensive Care Unit (NICU) patients with abnormal screening results into the algorithm would optimize the screening efficacy for identifying primary immunodeficiencies characterized by T-cell lymphopenia (TCL). This recommended cut-off value ensures the detection of all typical SCID cases and the majority of other TCL cases; however, lowering the cut-off score may reduce the identification of non-SCID TCL cases while still capturing SCID cases. The inclusion of an additional TREC test for premature infants before referral could enhance the PPV for non-SCID TCL cases. Consequently, new screening facilities are advised to tailor their algorithms based on their objectives regarding the identification of non-SCID TCL cases. Although incorporating KREC-based screening enables the detection of concurrent B-cell lymphopenia, including

cases with delayed-onset ADA-SCID, a comprehensive assessment of the efficacy of supplementary KREC screening is currently lacking. [95]

CBC as a screening test for SCID:

The majority of infants with SCID have low lymphocyte counts at birth. First suggested in 1997 by Buckley and Puck, a complete blood count and differential should be performed on all neonates to ascertain their absolute lymphocyte count (ALC) in blood. [103] A low absolute lymphocyte count is another component of the UK Primary Immunodeficiency Network's SCID evaluation strategy. [35] With a lower normal limit of 2000/μL, the average normal cord blood lymphocyte count is 5500/μL. [104] Although most SCID patients are born with low ALCs (114–2210/μL, based on 25 cases), some may have slightly lower but still normal ALCs because of maternal lymphocytes or B cells resulting from gene defects like IL2RG, JAK3, and IL7R. [35] A normal to high ALC can occasionally be seen in newborns with Omenn syndrome, a condition marked by immunological dysregulation, oligoclonal T cell growth, and defective T cell development. [83] Setting a normal ALC threshold, however, would result in a comparatively large false positive rate for all SCID patients. [83] El-Sayed et al. discovered that 1.6% of 500 neonates showed lymphopenia (ALC < 2500/μL), although none were diagnosed with SCID. [10, 35]

Infants who have tested positive for TREC screening can have their lymphocyte levels assessed using CBC and differential analysis. Since T-cells make up almost two thirds of all lymphocytes in SCID patients, a low absolute lymphocyte count is expected, which inadvertently indicates T-cell lymphopenia. While a normal absolute lymphocyte count can reassure one that SCID is improbable, it is still important to investigate other possible reasons of T-cell lymphopenia that may have

led to the original positive TREC test. It's important to remember that SCID is not the only condition that may result in a low ALC on CBC. Additionally, despite their conditions, patients with leaky SCID presenting as Omenn syndrome or those with maternal T-cell engraftment may show normal or increased lymphocyte counts. [1]

Despite these limitations, utilizing a CBC to determine the ALC as a screening technique at birth provides significant benefits: [10]

a) It is well accessible without appreciably raising the price of infrastructure. b) Compared to TREC testing, the cost per test is about 10 times less. c) The CBC assay is a well-established, technically simple method for carrying out as well as evaluating findings. d) In addition to using the ALC calculation to diagnose SCID, CBC also yields data on absolute neutrophil counts (ANC). A extremely high ANC may indicate leukocyte adhesion deficit (LAD), whereas a very low ANC may indicate severe congenital neutropenia. In addition, CBC provides information on other vital signs such as hemoglobin and platelet count, which are essential for the care of a baby. e) A point-of-care CBC can be performed, and results provided prior to the newborn's discharge allow for prompt intervention to prevent follow-up from being missed. Given these advantages and disadvantages, a screening method with a two-tier ALC threshold is proposed (details below). Lymphopenia detected on CBC and verified by Lymphocyte Subset Analysis (LSSA) requires a clinical assessment utilizing a checklist and suitable treatment/intervention, while additional laboratory tests are performed to confirm the diagnosis. [10]

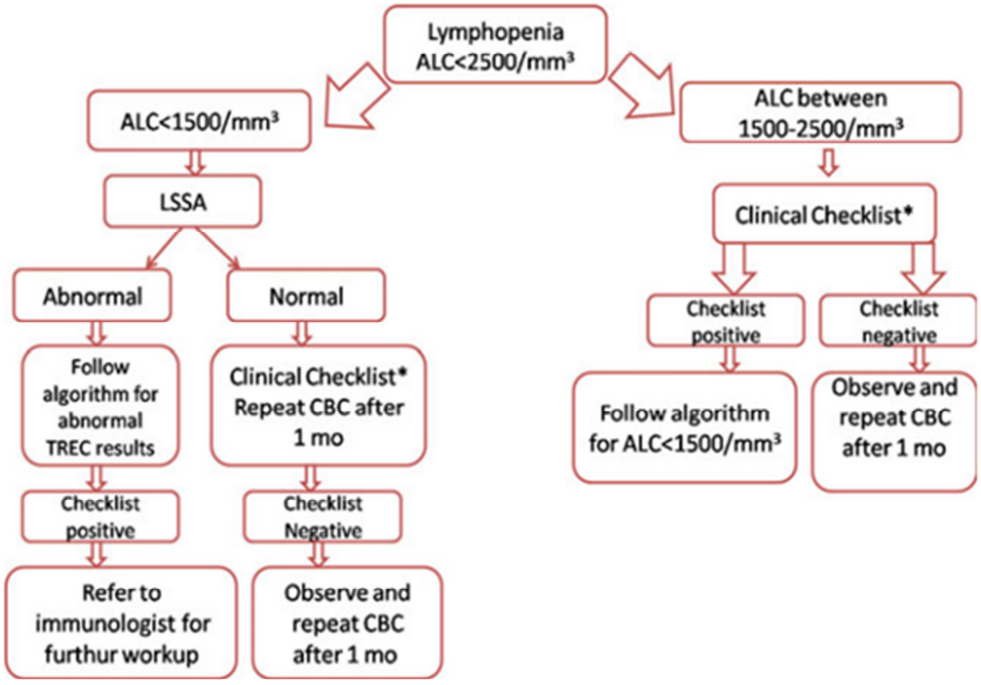


FIGURE 15. Algorithm for SCID screening used in this study [10]

DIAGNOSIS OF SCID:

SUSPECTED SCID: [105]

Patients with abnormal SCID newborn screening (NBS) results or, prior to NBS, a family history of low lymphocyte numbers in a relative with SCID are referred to as suspected SCID patients. Suspected SCID patients exhibit notably low T cell counts. Until a conclusive diagnosis of SCID or a non-SCID condition is made, this classification is often provisional.

Suspected SCID is characterized by:

Suspected SCID is defined as follows:

1. Less than 0.3×10^9/L CD3 T cells, *OR* less than 20% of CD3/CD4 cells with naive cell surface markers (eg, CD3/CD4/CD45RA)

   AND 1 or more of:

   a. Abnormal TRECs on NBS or at presentation
   b. Family history of SCID
   c. Recurrent and/or opportunistic infection(s)

   OR

2. If TRECs not measured or not abnormal and no family history of SCID, then less than 0.3×10^9/L $CD3^+$ cells *AND* less than 20% of naive CD3/CD4 cells.

   OR

3. Features of Omenn syndrome, including

   a. More than 80% of CD3/CD4 cells with memory cell surface markers ($CD45RO^+$). $CD3^+$ cells may be more than 0.3×10^9/L
   b. Generalized skin rash
   c. Eosinophilia **OR** lymphadenopathy **OR** organomegaly

The date of diagnosis of suspected SCID is established as the date of the first lymphocyte phenotyping panel demonstrating the outlined T-cell abnormalities. [105]

CLINICAL AND LABORATORY PARAMETERS TESTING FOR INFANTS WITH SUSPECTED SCID:

To effectively diagnose SCID and rule out other illnesses that may be causing low T-cell counts, a thorough assessment should include the following steps: [106]

1. A thorough medical history with special emphasis on infections, prematurity, other illnesses such as congenital heart disease and lymphatic malformations, maternal comorbidities like immunosuppressive therapy during pregnancy or diabetes, [107] and any history of immunodeficiency or early childhood deaths in the family.

2. Physical examination to look for evidence of Omenn syndrome or maternal graft-versus-host disease (GvHD) or DiGeorge syndrome, or other multisystem disorders, such as a widespread rash, lymphadenopathy, hepatomegaly, and splenomegaly.

3. Complete blood cell count with differential, which comprises eosinophilia assessment as a possible indicator of Omenn syndrome or maternal GvHD.

4. Lymphocyte phenotyping via flow cytometry, which includes assessing T, B, and NK cells, as well as T-cell subsets such as naive and memory CD3/CD4 helper-T cells. Naive CD8 cytotoxic T cells can also be tested, albeit CD4 cells are usually more representative of thymic output. At least one week following the initial assessment, lymphocyte phenotyping should be repeated, or once pathogenic SCID gene mutations have been confirmed through sequencing. If no genetic reason is discovered, repeat phenotyping should be performed at least 8 weeks later to allow transitory T lymphopenia to recover, unless an urgent HSCT is required owing to a clinical urgency. All T-cell measurements should be compared to age-appropriate reference intervals. [108]

5. Quantification of cycle threshold or TRECs, as well as verification of the detection of an appropriate genomic control DNA segment such as actin or RNaseP.

6. Quantitative assessment of immunoglobulins, such as IgE, as a possible Omenn syndrome marker.

7. Further sequencing of the entire exome or genome, ideally with a trio analysis involving the child and parents, may be necessary if the first testing is unable to diagnose the condition. Nowadays, genetic sequencing is a common process that usually begins with a panel of genes linked to immunodeficiency. To arrive at a molecular diagnosis, a variety of genetic testing techniques are employed, including whole exome sequencing, chromosomal microarray analysis, targeted gene panels, and Sanger sequencing. These tests provide a number of services, such as identifying specific lymphocyte dysfunctions, establishing SCID subtypes, supporting the selection of treatment and transplant protocols, and offering genetic counseling to families. It's crucial to remember, nevertheless, that diagnosing SCID does not require the identification of known pathogenic genes.

8. Assessing maternal engraftment (TME) in whole blood or isolated CD3 T cells. Traditionally, fluorescent in situ hybridization was employed to detect a second X chromosome in male patients, which indicated the presence of maternal cells. However, novel methods, such as DNA typing with short tandem repeat markers, are growing in popularity due to their increased sensitivity.

9. If T cells are present, the T-cell receptor diversity is assessed by measuring the T-cell receptor-V beta complementarity determining region 3 by high-throughput sequencing, spectratyping, or flow cytometry. [109]

10. Quantitative immunoglobulin assays can provide interesting information and indirectly reflect how well the adaptive immune system functions.

However, while interpreting these values in newborns, some aspects must be considered. Maternal IgG has a considerable influence on infant IgG levels, making them less useful for diagnosing SCID. IgM levels, on the other hand, reflect the infant's own immune response because they do not cross the placental barrier. SCID is often linked to absent or drastically low IgM levels in babies. While low IgA levels can occur spontaneously in babies, they do not always indicate SCID. In contrast, normal IgM and IgA levels can provide reassurance about immune function.

11. Proliferative testing can be carried out using PHA, anti-CD3, or anti-CD3/CD28 antibodies for mitogen stimulation; however, if the patient satisfies standard SCID criteria, it might not necessarily be indicated to confirm the diagnosis. Reduced proliferation could be a sign of defective or nonfunctional T cells despite normal counts, or low T-cell numbers (which can be verified by routine lymphocyte subset analysis). Conventional radioactive assays identify both low T cells and nonfunctional T cells as abnormal, failing to discriminate between two alternatives. As an alternative, tests based on flow cytometry provide a more accurate means of distinguishing between low and nonfunctional T cells. In the past, if the patient has previously been exposed, T-cell proliferation tests have also included certain antigens like Candida or tetanus; however, these are no longer advised as part of the Revised 2022 Definitions.

12. Two methods are available for HIV testing: protein detection in a patient sample or nucleic acid amplification. Alternatively, confirmation could also

come from records demonstrating consistently negative maternal HIV antibody testing. [105]

FLOW CYTOMETRY IN DIAGNOSIS OF SCID:

Since its establishment fifty years ago, flow cytometry has developed constantly, gaining from notable breakthroughs in apparatus, analytic reagents, and related technologies. Because of its adaptability, it can be used in a variety of cellular sources, such as tissues, blood, bodily fluids, and bone marrow. Flow cytometric assays include a wide range of applications, including functional analysis, phenotyping, and both relative and absolute measurements. Flow cytometry is not limited to measuring the expression of a particular protein; it may also be used to evaluate cell viability, apoptosis, cellular interactions, and cell enrichment. It is an unparalleled tool for primary immunodeficiency screening, diagnosis, and prognosis because of its multitude of applications. [110]

Primary immunodeficiencies (PIDs) encompass a diverse range of inherited immune system disorders. While genetic analysis remains the gold standard for PID diagnosis, it is time-consuming and expensive. Flow cytometry offers a swift and highly sensitive alternative for PID diagnosis. This technique can assess various aspects such as specific cell populations, surface and intracellular proteins, as well as functional immune characteristics associated with particular immune defects. It proves valuable in diagnosing and assessing major forms of PIDs, including severe combined immunodeficiency, X-linked agammaglobulinemia, hyper IgM syndromes, Wiskott-Aldrich syndrome, X-linked lymphoproliferative syndrome, familial hemophagocytic lymphohistiocytosis, autoimmune lymphoproliferative syndrome, IPEX syndrome, CTLA 4 haploinsufficiency, LRBA deficiency, chronic mucocutaneous candidiasis,

and chronic granulomatous disease. While genetic analysis is essential for precise PID diagnosis, flow cytometry offers a cost-effective means of effectively evaluating PID patients. [111]

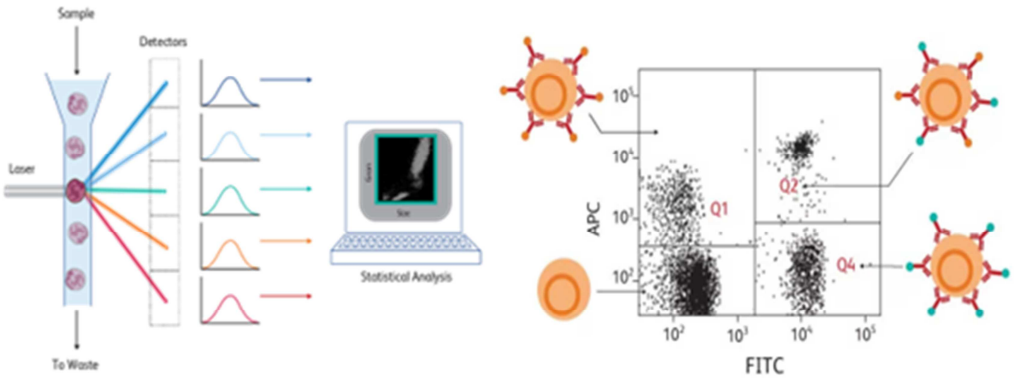


FIGURE 16. WORKING OF FLOW CYTOMETRY. [112]

The primary step in SCID diagnosis is to analyze lymphocyte subsets using flow cytometry to classify SCID into four categories: T-B+NK+, T-B-NK+, T-B+NK-, and T-B-NK- (as indicated in the image below). SCID patients typically have low or no T cell counts (<300 cells/µl). In cases where residual CD3+T cells are detected (>300 cells/µL), further evaluation of subsets reflecting their naive/memory and activation status using markers such CD45RA, CD45RO, and HLA-DR is helpful in diagnosing Leaky SCID and Omenn Syndrome. [111]

TABLE 7. Summary of lymphocyte subsets listing common name, CD/FACs markers, normal range for infants 1 to 4 weeks of age, values for defining SCID and abnormal (T lymphopenia): [113]

Lymphocyte Population	FACs/CD Marker	Normal Range for 1–4 Weeks Percentage Absolute Counts (Cells/μL)	Range for SCID Percentage Absolute Counts (Cells/μL)	Range for Abnormal/T Lymphopaenia Percentage Absolute Counts (Cells/μL)
T cells	CD3+	60–85% 2300–7000	<300	<30% T cells < 1500
B cells	CD19+	4–26% 600–1900		
NK	CD16+/56+	3–23% 200–1400		
CD4 T cells	CD3+CD4+	41–68% 1700–3500		
CD8 T cells	CD3+CD8+	9–23% 400–1700		
Naïve T cells	CD45RA+/CD27+	80–100%	<25%	<70%

SCID disorders are characterized by impaired B-cell function and a total lack of T-cell mediated immunity. Flow cytometry can be used to classify patients with SCID according to their immunophenotypic characteristics. Adenosine deaminase (ADA) deficiency and reticular dysgenesis are two disorders that fall under the T−B−NK− SCID category. Mutations affecting genes including recombination

activating genes (RAG)1 and RAG2, Artemis, DNA ligase IV, and Cernunnos are indicated by T−B−NK+ SCID. While T−B+NK+ SCID encompasses IL-7Rα, CD3δ, CD3ε, and CD3ζ deficits, the T−B+NK− phenotype is typical of X-SCID and JAK3 deficiency. T and NK cell numbers are absent whereas B cell counts are normal or increased in X-SCID, which accounts for over half of all SCID cases. The IL2RG gene is responsible for X-SCID, which codes for the common γ chain (CD132). Therefore, the lack of CD132 identified by flow cytometry strongly suggests X-SCID, despite the possibility that some individuals with IL2RG cytoplasmic domain mutations may express CD132 normally. Common γ chains are involved in the receptors of several cytokines, including IL-4, IL-7, IL-9, IL-15, and IL-21. As a result, these cytokines operate as particular ligands for pathways that require a functional common γ chain. Monoclonal antibodies that only recognize phosphorylated STATs can be used in flow cytometry to measure the phosphorylation of intracellular STATs. Tyrosine phosphorylation of STAT5 and STAT3 is impaired in patients with X-SCID in response to IL-2 and IL-21 activation, respectively. Because JAK3 interacts intracellularly with the common γ chain, this assay also detects JAK3 deficiency. Lack of ZAP70 expression in T cells is a symptom of ZAP70 deficiency, a different form of SCID that is marked by CD8 deficiency. [114]

Because the immunophenotypes of the many SCID categories overlap, genetic diagnosis of SCID can be complicated. Flow cytometric analysis of particular protein expressions can aid in the prompt identification of possible genetic problems in T-B+ SCID instances where a child may have a defect in the IL2RG, IL7RA, or JAK3 gene.

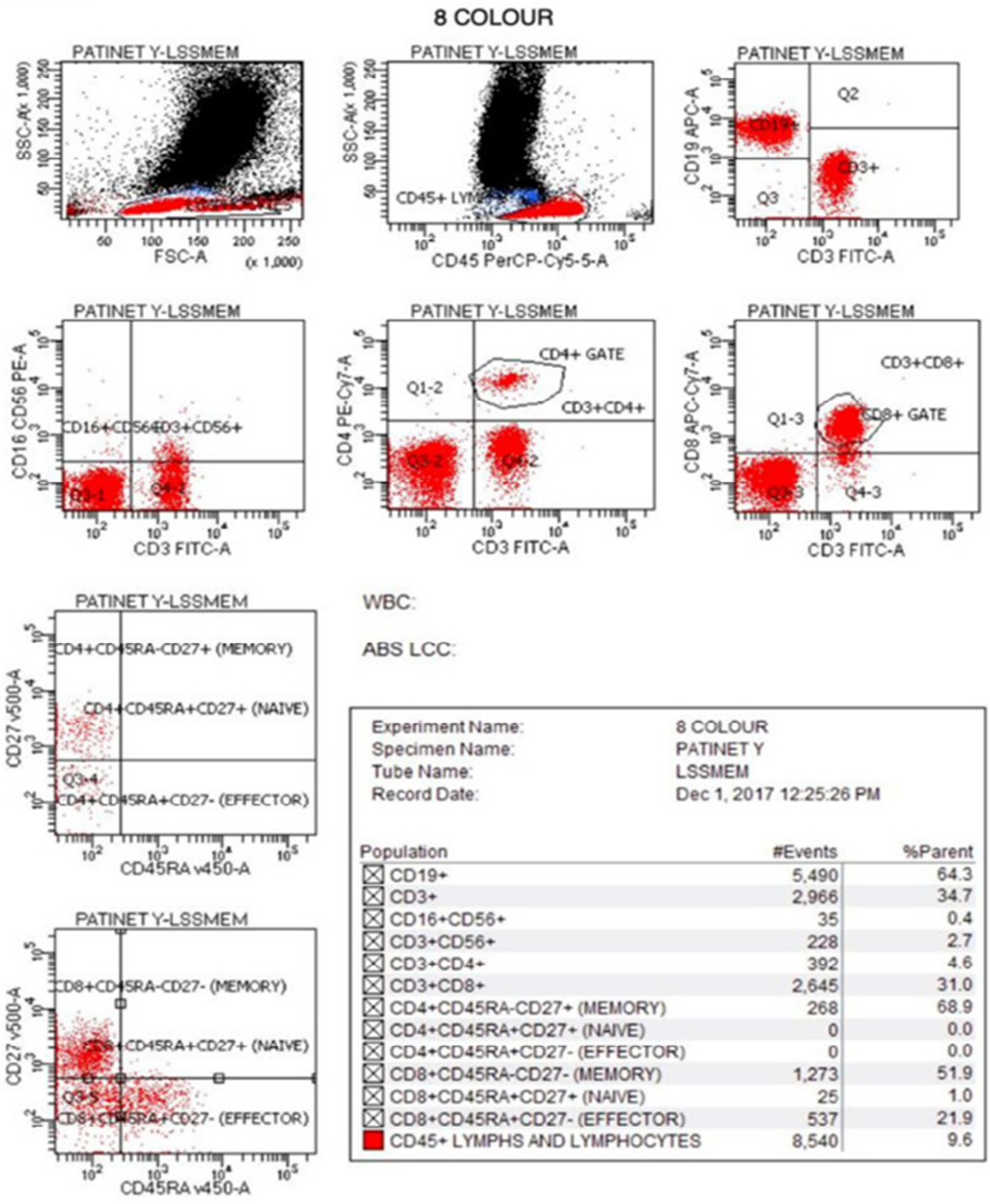
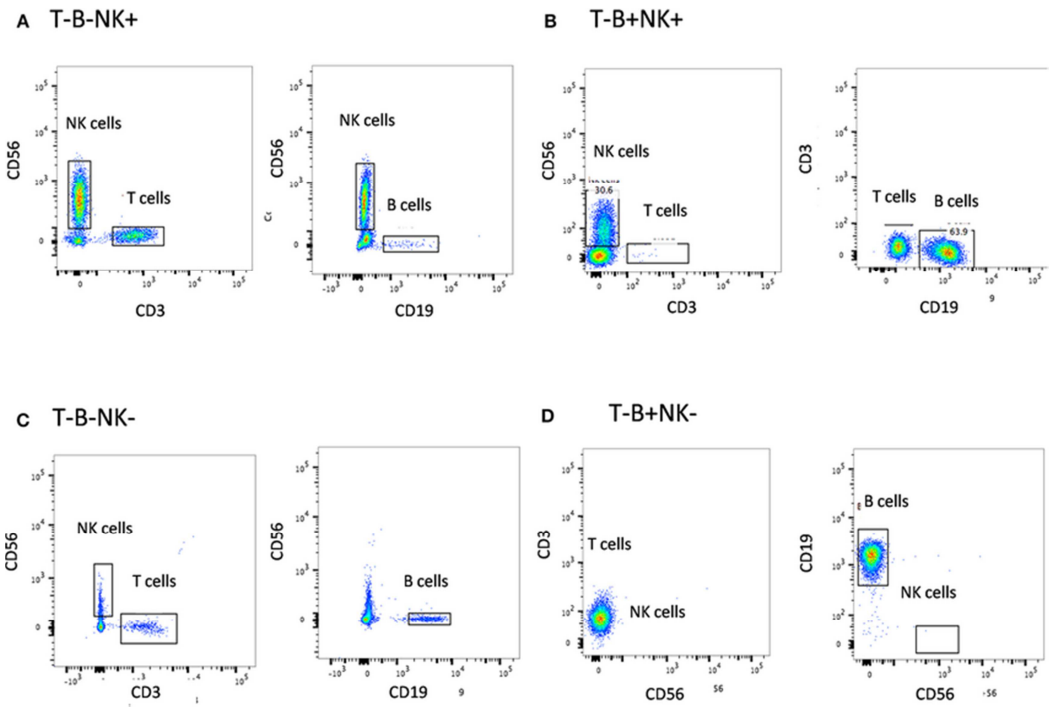


FIGURE 17. CD PLOTS FROM A SCID AFFECTED INFANT. [113]



Lymphocyte subset analysis for SCID. Samples were analyzed by flow cytometry, gating on lymphocytes by forward/side scatter and gating T cells (CD3+), B cells (CD19+), and NK cells (CD56+CD3-) (A) T-B-NK+ (B) T-B-NK+ (C) T-B-NK- (D) T-B+NK- SCID, respectively. [111]

FIGURE 18. Lymphocyte subset analysis in different types of SCID

One quick method for finding, classifying, and measuring different aspects of a single cell is flow cytometry. It may evaluate cells extracted from a variety of biological samples, such as urine, bone marrow, tumor and lymph node biopsies, peripheral blood, and cerebrospinal fluid (CSF). Flow cytometry's fundamentals are simple, but they result in a powerful analytical instrument. [115] First, fluorescently tagged antibodies are introduced to the cells, where they attach to particular antigens on the cell surface. The cells are then placed into the flow cytometer for examination after being incubated for a while and suspended in a neutral solution. The fluid containing the cells travels through a chamber called a flow cell inside the flow cytometer, where each cell passes separately and in a controlled manner. As each cell

moves through the flow cell, a laser beam activates the fluorophores bound to the antibodies, causing fluorescence. This event, known as an event, enables for the collection of light scatter from the cell as well as fluorescence emitted by the antibody-fluorophore complex. These signals are subsequently recorded by optical detectors and translated to a digital format for analysis, which is commonly shown as dot plots or histograms. [115]

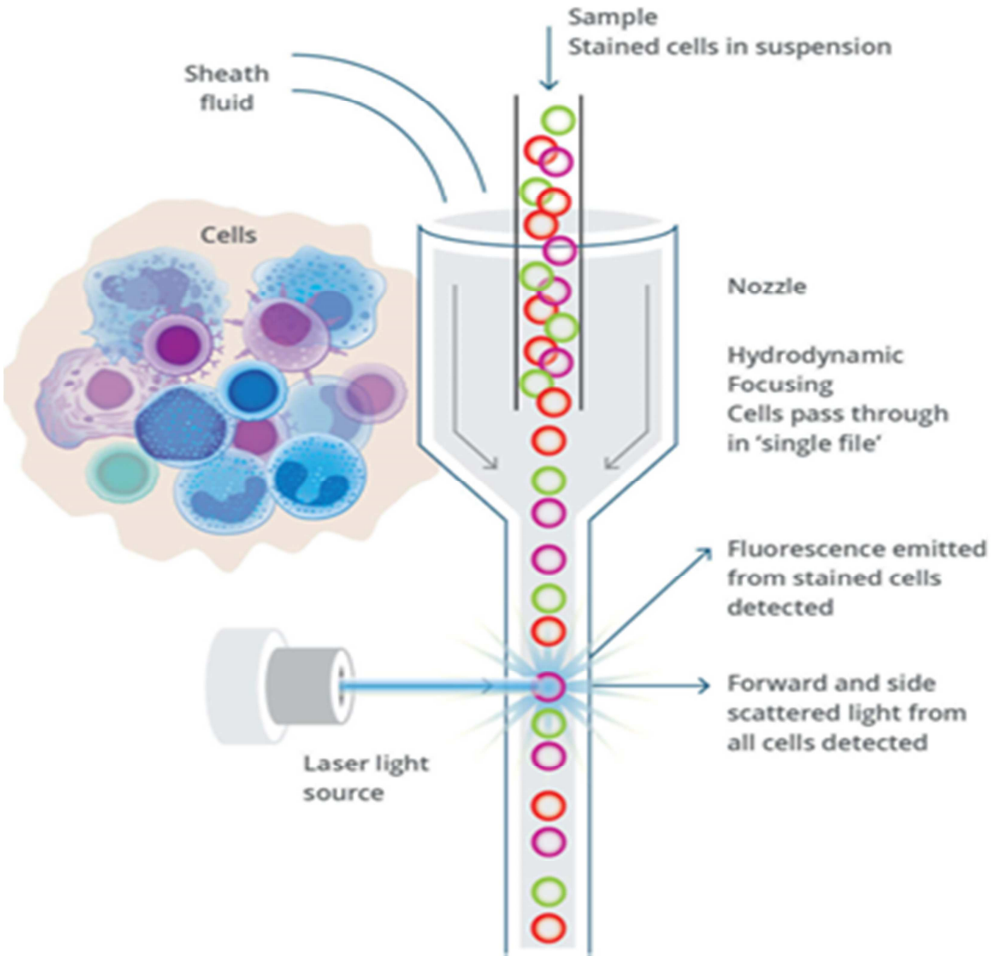


FIGURE 19. PRINCIPLE OF FLOW CYTOMETRY [115]

The patterns formed by the light scatter gathered by side scatter (SSC) and forward scatter (FCS) reflect the internal complexity and size of the cell. This

identifies the types of cells, such as lymphocytes, monocytes, granulocytes, and platelets. The cytoplasmic and surface antigens of the cell, which are frequently referred to as clusters of differentiation (CD) markers, are recognized by the fluorescently labeled antibodies. Clinicians look into the source of issue when they observe aberrant expression of the CD markers. Testing with flow cytometry is done in both clinical and research settings. For the purpose of diagnosing and monitoring diseases, the data gathered by "interrogating" cells can be highly beneficial. [115] Despite advances in our knowledge of the pathophysiology of primary immunodeficiency diseases (PIDs), treating these illnesses is still challenging. Hematopoietic stem cell transplantation (HSCT) is essential for the long-term survival of the majority of individuals with PIDs, which are genetic illnesses. However, the scarcity of HLA-matched donors or the high expense of treatment can make transplantation impractical in nations like India. Genetic counseling becomes crucial in these situations, providing affected families with choices for prenatal diagnostics and carrier discovery. Phenotypic prenatal diagnosis using cordocentesis offers a useful substitute for molecular diagnosis, even though it may not always be available. This is especially true for families that have an index case with well-characterized PID. Flow cytometry, a simple and fast method, can supplement or replace molecular characterization. [111] Overall, flow cytometry plays an important role in studying SCID immunology. It allows for the counting of various types of lymphocytes, the assessment of thymus function and TCR diversity, the measurement of particular receptor expression, the examination of molecules later in the process, and the evaluation of T cell performance. It also serves as a useful tool for determining the most likely genetic issue leading to SCID development.

WHOLE EXOME SEQUENCING (WES) / NEXT GENERATION SEQUENCING (NGS) IN SCID:

The understanding of primary immunodeficiency disorders (PIDs) has been completely transformed by the development of Next-Generation DNA Sequencing (NGS). These diseases are no longer only thought to be monogenic conditions controlled by conventional Mendelian inheritance patterns. Through the ability to sequence the whole exome or genome, NGS has made it possible to discover the wide range of atypical phenotypes caused by mutations in genes that were previously linked to PID. For example, patients presenting a phenotype similar to common variable immunodeficiency (CVID) have been found to have hypomorphic mutations in genes including RAG1, DCLRE1C, and JAK3, which are generally associated with SCID. Moreover, multigenic PID identification has been made easier by the objective methodology of whole exome sequencing (WES) and whole genome sequencing (WGS). [116]

Next-generation sequencing (NGS) holds promise for enhancing the diagnostic and prognostic capabilities of newborn screening (NBS) initiatives. It serves as a potent tool for detecting various genetic abnormalities in newborns, revolutionizing genetics by enabling the sequencing of numerous genes rapidly and simultaneously. The expanding utility of NGS, coupled with significant cost reductions and increased community acceptance, suggests its potential for diagnosing inherited disorders. NGS can be applied to dried blood spot (DBS) samples from neonates, where DNA isolation allows for improved investigative and prognostic efficiency in NBS programs. The primary objective of NGS in NBS is to conduct targeted investigations and differentiate gene variants, particularly for conditions that are preventable or treatable. [117]

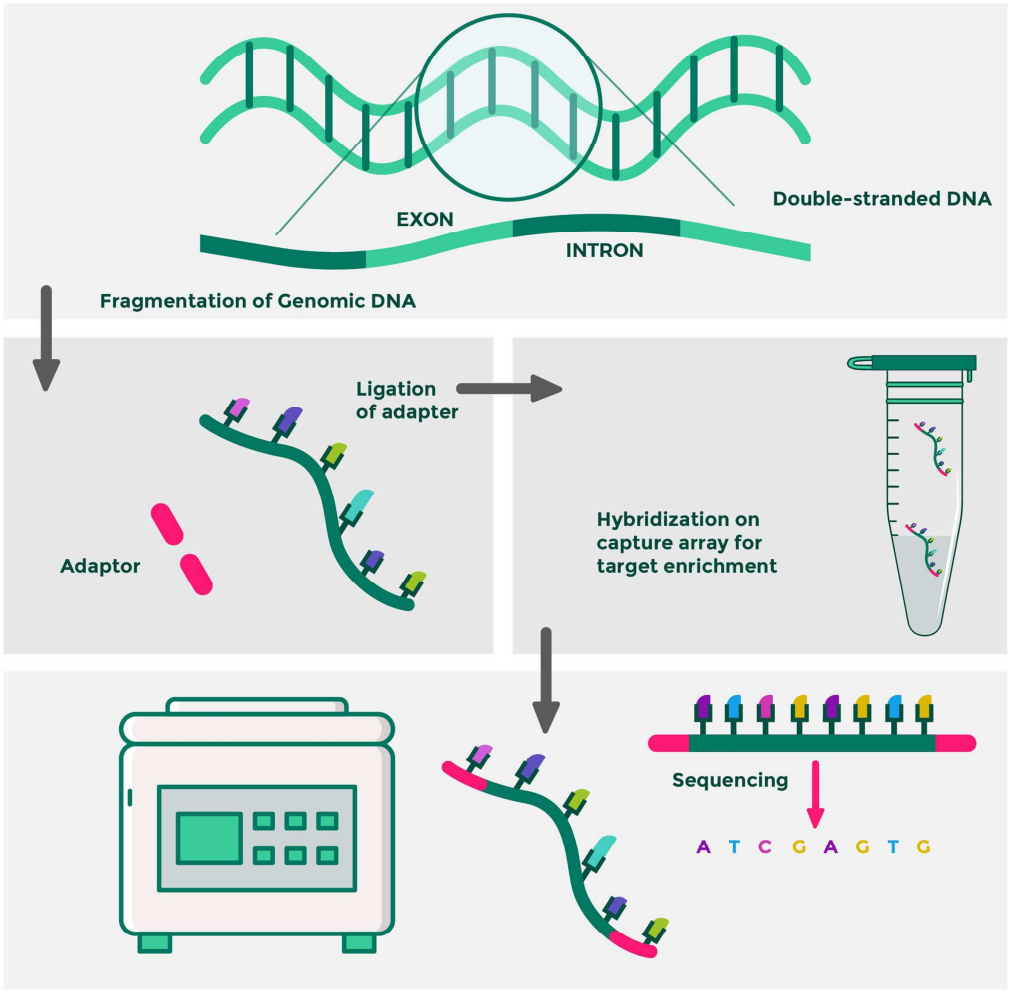


FIGURE 20. UNDERSTANDING WHOLE EXOME SEQUENCING [118]

The current newborn screening (NBS) method for SCID relies on quantifying T cell receptor excision circles (TRECs) to assess the production of naïve T-cells & kappa light chain-deleting recombination excision circles (KRECs) assays for evaluating the generation of naïve B cells. These assays are relatively cost-effective, but they fail to offer a molecular diagnosis and are restricted to identifying defects related to the production of naïve T and B cells. Although NGS currently incurs higher costs compared to TRECs/KRECs assays, the declining expenses associated with sequencing technology may facilitate the development of targeted panels for

early diagnosis, as evidenced by various feasibility studies that, indicate that NGS has the potential to enhance the sensitivity of NBS. [116] The effectiveness of NGS technology hinges on the inherent limitations of each method. Whole Exome Sequencing (WES) and Targeted Gene Panels (TGP) require the creation of libraries containing fragments of patient DNA that correspond to either the exome or a specific set of genes, respectively. Typically, NGS methods necessitate PCR amplification of these libraries to attain sufficient DNA quantities for high-throughput sequencing. Incomplete library preparation can produce sequencing gaps, potentially leading to the missing of pathogenic variants. Furthermore, because to the non-contiguous nature of target regions, WES or TGP make it even more challenging to detect structural abnormalities such as significant insertions or deletions, translocations, inversions, and copy-number variants than Whole Genome Sequencing (WGS). The detection of structural differences is crucial for PID diagnosis, as pathogenic variants are typically discovered in large, repetitive genes such as DOCK8 and LRBA. [116]

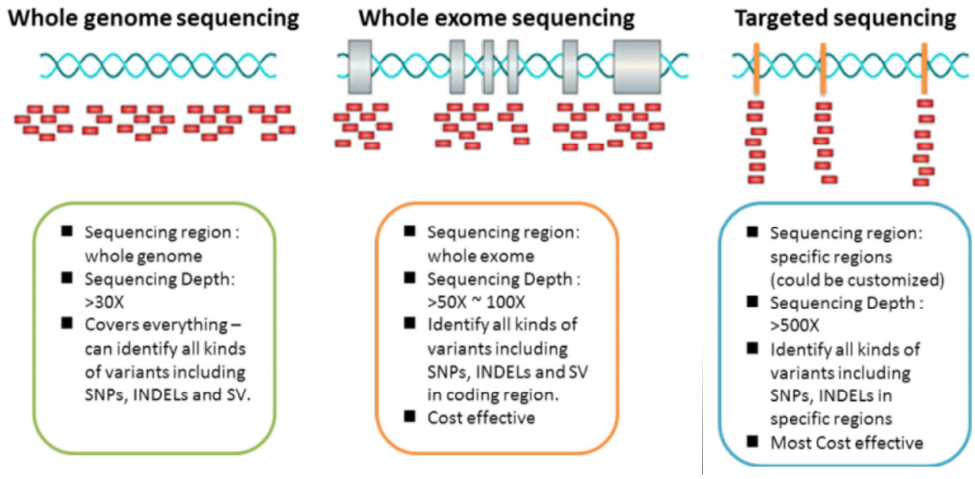


FIGURE 21. Difference between Whole Genome Sequencing, Whole Exome Sequencing and Targeted Sequencing

Research studies involving PID patients have shown that there is an extensive amount of variation in the diagnostic performance of NGS techniques. In six patient studies, a direct comparison of immunodeficiency patients' WES and WGS revealed that WGS found 656 more coding variations than WES. Moreover, copy-number variations that corresponded to non-coding areas in this small sample set were not reliably detected by WES. A 40% diagnostic yield was obtained from WES analysis of 278 PID families' patients, which resulted in updated clinical diagnoses for half of the patients and changes to their treatment plans for 25% of them. [116]

LIMITATIONS/PITFALLS OF NGS:

For individuals with Primary Immunodeficiency Disorders (PIDs), prior research using Whole Exome Sequencing (WES) or Targeted Gene Panels (TGPs) as diagnostic modalities has shown that at least 60% of patients do not have a conclusive diagnosis. On the other hand, only 15% of patients receive a diagnosis from conventional genetic testing techniques such chromosomal microarrays, karyotyping, and Sanger sequencing for particular genes. This means that 85% of patients remain undiagnosed. Even though Next-Generation Sequencing (NGS) has a better potential for diagnosis than traditional genetic testing, a sizable percentage of patients remain unidentified, indicating shortcomings in the technology, data processing, or our understanding of PIDs. Candidate mutation lists generally do not include synonymous mutations since they are generally regarded as benign variants because they do not affect the amino acid sequence of the resultant protein. In fact, PIDs can result from synonymous mutations, according to current research.

Clinical criteria have been developed to standardize the interpretation of genetic variants, taking into account both genetic and biological factors to define

variants as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance (VUS). VUS are variants that do not fit the criteria for clear classification and may include mutations in genes whose clinical significance is not well recognized. This is especially true in PIDs, where advances in the field are rapid. The fundamental problem in genomic diagnostics is the paucity of functional evidence obtained just by sequencing. Functional assays are required to demonstrate the biological significance of a variant. Given the rarity of PIDs, novel defects frequently arise in solitary cases, with no evidence of several unrelated individuals sharing the same genotype-phenotype link. As a result, criteria have been established to determine the causal relationship between a patient's genotype and phenotype. [116]

The expanding availability of public databases facilitates researchers and clinicians in determining mutation prevalence in the general population. While Next-Generation Sequencing (NGS) serves as a valuable screening tool, its full diagnostic potential relies on multidisciplinary expertise for variant interpretation and validation, especially for previously unreported mutations or poorly characterized genes. This poses a significant challenge in resource-limited regions where comprehensive molecular and cellular biology, biochemistry, and immunology expertise may be scarce. [116]

Severe combined immunodeficiency is found in about 30% of patients with abnormal newborn screening (NBS) results (SCID). Four main subtypes of SCID were identified by the PIDTC 2014 Criteria: conventional SCID, leaky SCID, Omenn syndrome, and reticular dysgenesis (due to mutations in AK2). While patients with reticular dysgenesis exhibit unique clinical presentations, such as severe neutropenia

and sensorineural deafness, that set them apart from other SCID presentations, patients with AK2 pathogenic variations should be categorized into one of three major subtypes, according to the revised PIDTC Definitions of 2022: typical SCID with significantly lower T cells, leaky/atypical SCID with fewer T cells, or Omenn syndrome. This classification recognizes the necessity for tailored planning of hematopoietic cell transplantation (HCT) to address both lymphoid and myeloid differentiation deficiencies in patients with AK2 pathogenic variations. While the terms "typical" and "leaky/atypical" SCID have historically influenced decisions regarding allogeneic HCT with or without conditioning, the 2022 Definitions solely describe presenting clinical features and do not imply a preferred treatment approach. [105]

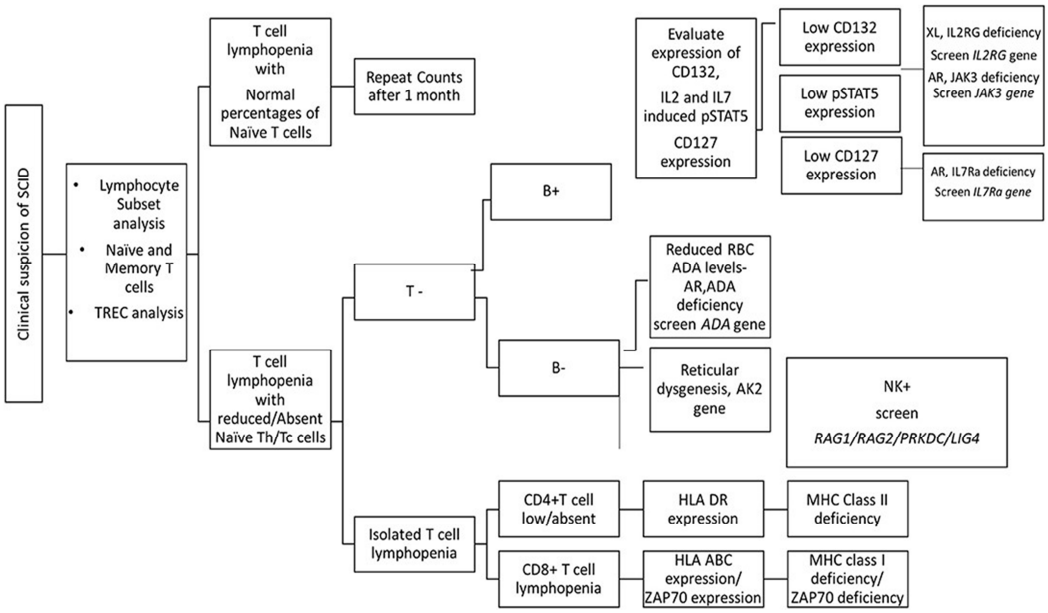


TABLE 8. ALGORITHM FOR DIAGNOSIS OF SCID PATIENTS USING FLOW CYTOMETRY-BASED ASSAY [111]

TABLE 9. PIDTC Criteria and Definitions for SCID 2022: [105]

SCID subtype	Diagnosis requires	Criterion 1	Criterion 2	Criterion 3	Criterion 4
Typical SCID (very low autologous T cells)	Criteria 1 & 2 **OR** Criteria 1 & 3 **OR** Criterion 4	Very low T cells ($<0.05 \times 10^9$/L)*	Pathogenic gene variant(s)†	No alternate explanation for low T-cell count‡ **AND, EITHER:** Undetectable or low TRECs§ **OR** <20% of $CD4^+$ T cells have naive cell surface markers||	Presence of TME¶
Leaky/atypical SCID (low T cells)	Criteria 1 & 2 & 4 **OR** Criteria 1 & 3 & 4	Two or more of: ● Low T-cell number for age (0.05-1.0 $\times 10^9$/L)# ● Oligoclonal T cells** ● Abnormal TRECs **OR** <20% of $CD4^+$ T cells are naive	Pathogenic gene variant(s)	Reduced proliferation††	Does not have: ● Other SCID subtype ● CID with known genotype ● Thymic disorder ● Other disorder with low T-cell numbers‡‡
Omenn syndrome	All 4 Criteria	>80% of $CD4^+$ T cells have $CD45RO^+$ memory phenotype	Pathogenic gene variant(s)	Generalized rash **AND** Absence of TME	Two or more of: ● Eosinophilia (>0.8 $\times 10^9$/L) ● Elevated IgE ● Abnormal TRECs ● Lymphadenopathy ● Hepatomegaly and/or splenomegaly ● Oligoclonal T cells

*T-cell subset determination (with naive/memory phenotyping) should be repeated at least once, with the second test used as the criterion value. In patients with an identified pathogenic variant, the interval between tests must be at least 1 wk; however, in patients without an identified pathogenic gene variant, the T-cell number must remain $<0.05 \times 10^9$/L for at least 8 wk to qualify as typical SCID due to the potential for spontaneous improvement, with a shorter interval only if urgent hematopoietic cell transplant is required before 8 wk.
†Pathogenic variant(s) identified in a gene whose product is known to be essential for T-cell development (examples in Table III).
‡Alternate explanations for low T-cell counts include those listed in Criterion 4 of leaky/atypical SCID.
§Number of TRECs below the normal cutoff, or cycle threshold value above the normal cutoff defined as consistent with SCID by performing laboratory.
||Naive T cells should be measured via CD3/CD4/CD45RA, or with additional naive markers.
¶Best performed by DNA analysis, such as with short tandem repeats, from whole blood or CD3-seperated cells, with any level of detection considered positive. Documented TME classifies patients as typical SCID; TME testing is strongly recommended for patients considered to possibly have leaky/atypical SCID.
#Low T-cell numbers for age defined as $<0.6 \times 10^9$/L (any age), $<0.8 \times 10^9$/L if aged 2-4 y, or $<1.0 \times 10^9$/L if younger than 2 y.
**Oligoclonal T cells as defined by laboratory performing testing, eg, <5 peaks in ≥4 T-cell receptor (TCR) Vbeta families on spectratyping, evidence of expansion of ≥2 TCR Vbeta families to >2 × the upper limit of normal for those families, or low Shannon [H] entropy index on high-throughput sequencing of TCR Vbeta variable regions.
††Reduced proliferation is defined as a proliferative response to PHA, anti-CD3, or anti-CD3/CD28 <50% lower limit of reference range for laboratory.

1) TYPICAL SCID:

Patients classified as typical SCID have the most severe deficiencies in T-cell numbers; these patients are usually caused by null pathogenic variants in genes critical for T-cell development; mutations in more than 15 genes are known, but mutations in 7 genes (IL2RG, RAG1, RAG2, ADA, DCLRE1C, IL7R, and JAK3) account for at least 80% of cases of SCID; novel sequence changes in known SCID genes require expert variant interpretation to determine pathogenicity based on available data; a common finding in many typical SCID genotypes is the presence of

maternal T cells in peripheral blood, which is the result of the failure to reject transplacentally transferred cells. Though it is present in approximately 50% of typical SCID patients, the threshold for considering Transplacentally Transferred Maternal Cells (TME) positive has not been definitively established. It is less common in genetic subtypes like ADA, RAG1, RAG2, and DCLRE1C, potentially because of the elimination of maternal cells by NK cells or residual host T cells. Additionally, the proliferation of transferred maternal T cells over time may result in delayed detection, where a blood sample obtained early in life that shows no TME could later test positive upon repetition, particularly if T-cell numbers increase significantly.

In a typical SCID, TME can increase the total number of T cells. The T-cell threshold for typical SCID in the absence of TME was set at less than 0.3 x 10^9/L in the original PIDTC 2014 Criteria. However, in the revised PIDTC 2022 Definitions, this was lowered to less than 0.05 x 10^9/L CD3 T cells to better capture patients with profound T lymphopenia and limited proliferative capacity. Before immune-restoring therapy is started, T-cell enumeration and phenotyping must also be done at least once to rule out non-SCID conditions where T-cell levels may be low initially but eventually rise. Patients who have pathogenic variants identified must wait at least one week between tests; in the case of patients without a genetic defect, T-cell counts must stay below 0.05 x 10^9/L for a minimum of eight weeks in order to be considered typical SCID, unless an earlier urgent hematopoietic cell transplant is required. [105]

2) ATYPICAL/LEAKY SCID:

Patients with partial impairments in T-cell numbers, variety, and maturity—characterized by a decrease in naive T cells—are classified as having leaky/atypical SCID. This disorder is caused by either unknown defects (called "atypical SCID") or hypomorphic or "leaky" pathogenic variations in the same genes that cause typical SCID (called "leaky SCID").

A diagnosis of leaky/atypical SCID is made when two out of the following criteria are satisfied: (1) oligoclonal T-cell population; (2) low percentages of naive T cells and/or low or undetectable TRECs; (3) low T-cell numbers based on age-specific thresholds (<0.6 x 10^9/L for any age, <0.8 x 10^9/L for ages 2-4 years, or <1.0 x 10^9/L for children <2 years). The second or final pretreatment value is used in subsequent T-cell enumeration tests to identify the SCID subtype.

The majority of patients with leaky/atypical SCID—nearly 90% of them—have an identified pathogenic gene variant, which classifies them as leaky SCID. Common gene defects in leaky SCID include RAG1, RAG2, ADA, and RMRP. If no pathogenic variant is found, testing for Transplacentally Transferred Maternal Cells (TME) is important because the presence of maternal T cells would instead indicate typical SCID. If TME testing is not available, atypical SCID can be diagnosed if the impaired proliferation in response to specific stimuli—PHA, anti-CD3, or anti-CD28—is less than 50% of the lower limit of the reference range. It's crucial to remember that many laboratory results associated with atypical SCID can also arise in specific types of CID brought on by thymic abnormalities, syndromes, or non-SCID gene deficiencies such as WASP or CD40L. In order to rule out known non-SCID

disorders, patients lacking an identified pathogenic variant in a known SCID gene, particularly those with a B1NK1 lymphocyte profile, should be tested. [105]

3) OMENN SYNDROME:

Omenn syndrome is a form of leaky SCID characterized by an increase in host-derived memory T lymphocytes entering the skin and other organs, resulting in a unique widespread erythematous rash that is frequently accompanied by lymphadenopathy, hepatosplenomegaly, and other clinical symptoms. The rash in Omenn syndrome can be comparable to that seen in Graft-versus-Host Disease (GvHD), emphasizing the significance of ruling out Transplacentally Transferred Maternal Cells (TME) and maternal GvHD around the rash's commencement to precisely establish the diagnosis. The PIDTC 2014 Criteria required Omenn syndrome patients to have more than 0.3 x 10^9/L T cells. However, the amended 2022 Definitions recognize that any T-cell count is feasible in peripheral blood. Furthermore, identifying Omenn syndrome now requires meeting particular criteria: a generalized rash, no TME, and more than 80% of CD4 T cells expressing the memory marker CD45RO. Omenn syndrome has occurred in people who were first diagnosed as having conventional or leaky SCID, mandating continued monitoring for its progression.

Previously, some Omenn syndrome cases lacked a recognized pathogenic SCID gene variant, but current standards require genotype confirmation for diagnosis, with RAG1 or RAG2 mutations being common, but alternative genotypes do exist. This allows for distinction from other forms of neonatal erythroderma, such as Netherton and DiGeorge syndromes.

Furthermore, Omenn syndrome patients must demonstrate at least two additional supportive features: Symptoms may include abnormal TRECs, elevated eosinophil counts (0.8-1 x 10^9/L), elevated IgE levels, lymphadenopathy, organomegaly (hepatosplenomegaly), and oligoclonal T cells. [105]

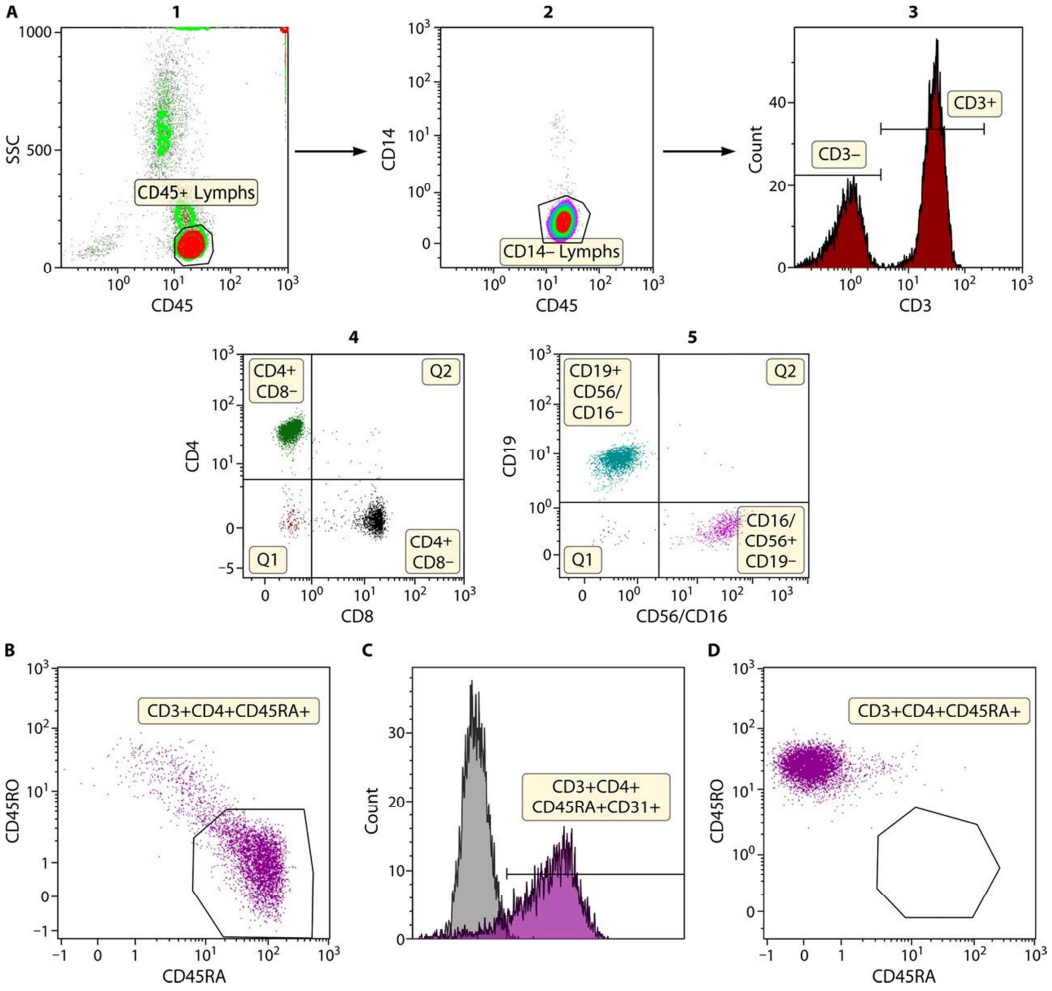


FIGURE 22. IMMUNOPHENOTYPING OF LYMPHOCYTE SUBSETS [110]

Immunophenotyping of lymphocyte subsets involves several methodologies as follows: [110]

(A) Quantitation of the lymphocyte subpopulation. Flow cytometry can be used to quantify T, B, and NK cells. The top panels (panels 1 through 3) demonstrate side scatter (SSC) and CD45-based lymphocyte identification. By using CD14, it is possible to distinguish between monocytes that can unintentionally belong to the lymphocyte population. CD3− lymphocytes and CD3+ T cells are classified further into CD45+ lymphocytes. Panel 4 of the analysis of CD3+ T cells looks for CD4+ and CD8+ T cells, whereas Panel 5 looks for B and NK cells among the CD3− lymphocytes. Fluorescent beads can be used for absolute quantitation (counting the number of cells per microliter).

(B) Identifying naive and memory T cells using flow cytometry: CD3+ T cells can be further separated into naive and memory subsets based on certain cell markers (usually CD45RA and CD45RO, though other markers may be employed). In the CD4+ T cell compartment of a healthy newborn, memory CD45RO+ T cells are rare and the majority of the cells are naive CD45RA+ T cells.

(C) Using flow cytometry to identify recent naive thymic emigrants: Since they are freshly generated from thymic output, CD4+ T cells usually do not exhibit antigen-induced peripheral cell expansion and express CD31, a marker for recent thymic emigration. In a healthy neonate, CD31 is expressed by a significant fraction of naive CD45RA+ CD4+ T cells.

(D) Flow cytometry-based identification of memory and naive T cells in an Omenn syndrome patient: There are typically no naive CD45RA+ CD4+ T cells in newborns

with leaky SCID or Omenn syndrome; instead, the bulk of T cells are oligoclonally expanded and express CD45RO, a memory marker. Due to the lack of thymic secretion, they also have no recent thymic emigrants. In this patient, CD45RO expression is seen in over 99% of CD4+ T cells in the blood, whereas CD45RA+ expression is absent. In addition to the clinical traits linked to Omenn syndrome, this patient also showed significantly skewed T cell receptor repertoire diversity and missing thymic function. A RAG1 gene mutation linked to a hypomorphic type of SCID was seen in this female patient. [110]

PREVENTION:

1. Isolation measures: The relationship between infection and adverse outcomes in HSCT patients with SCID is widely established. [11,60] Parents must prioritize placing their infant in protective isolation, avoiding visitors and social groups, especially with other small children who may unintentionally carry diseases from school. To reduce illness risks, education of household members about universal measures and proper hand sanitation is critical. Given the COVID-19 era, parents should also steer clear of social events to avoid bringing home transmittable diseases. If any family member exhibits signs of infection, isolating them from the SCID patient becomes imperative. Minimizing clinic visits is recommended to reduce sick contacts; however, if a clinic visit is necessary, thorough disinfection of the room, limited staff interaction, and the use of protective equipment are essential precautions.

2. Dietary consideration: The avoidance of well water is crucial due to its potential for waterborne infections like Cryptosporidium, Norovirus, Giardia

lamblia, Campylobacter, or rotavirus. [119] One should use bottled water or bolied tap water as a safer alternative.

3. Vaccination: Vaccination plays a crucial role, but it's essential to understand the types of vaccines suitable for patients with SCID. Live attenuated vaccines like mumps/measles/rubella, varicella, BCG (Bacilli Calmette-Guerin), oral polio, and rotavirus vaccines are not recommended due to the risk of life-threatening infections. Inactivated vaccines are safer since they do not cause infection, although they may be less effective. [120] For household members, using live viral polio vaccine is not advised as it could lead to viral shedding and active infections in immunocompromised individuals. [120] Similar caution is advised for other live vaccines like varicella, measles, and Rotavirus vaccines in siblings. [121, 122] However, standard vaccines, including the COVID-19 vaccine and annual influenza vaccine, are crucial for maintaining herd immunity in the family, including extended family members who frequent the household.

4. Breastfeeding: Breastfeeding practices should be carefully managed in the context of maternal cytomegalovirus (CMV) status. It's advised to suspend breastfeeding until CMV status is known because CMV viral shedding is common in CMV-positive mothers. Even in cases of inactive CMV infection (CMV IgM-negative and CMV IgG-positive), breastfeeding should be avoided due to the potential risk of viral excretion and reactivation in SCID infants, [123] which can lead to life-threatening disseminated infection and increased mortality after hematopoietic cell transplant. [121] While research has shown similar CMV infection rates in SCID infants regardless of their mothers' CMV

status, further investigation is necessary to assess the risk-benefit balance of breastfeeding in this patient group. During this waiting period for CMV status confirmation, mothers may opt to continue pumping breast milk to maintain milk production. However, if the mother tests positive for CMV IgG and/or IgM, breastfeeding should be avoided, and ready-to-feed infant formula is recommended. [60] Conversely, if the mother is CMV seronegative, breastfeeding can be resumed, [122] although there remains a potential risk of CMV transmission if the mother acquires CMV infection after serology screening.

5. Blood products: Regarding blood products, precautions should be taken to prevent CMV transmission and graft-versus-host disease (GVHD) from donors' viable lymphocytes, including irradiating blood products, leukocyte reduction with filters, and ensuring they are CMV-negative. [124]

6. Antibiotic Prophylaxis: Furthermore, prophylactic steps against particular infections are essential as soon as an infant's diagnosis of SCID is confirmed. This includes the use of oral trimethoprim-sulfamethoxazole or intravenous pentamidine as chemoprophylaxis against Pneumocystis jirovecii pneumonia (PJP), fluconazole as a fungal prophylaxis, azithromycin as a MAC prophylaxis, and acyclovir as a viral prophylaxis against the Herpesviridae family of viruses. During the respiratory syncytial virus (RSV) season, which runs from November to March, palivizumab may also be taken into consideration. [122] It is imperative to rapidly commence immunoglobulin replacement therapy in order to boost the immunological function of the newborn. [90]

TREATMENT APPROACHES IN SCID:

It's recommended to isolate infants suspected of having SCID right away and to avoid contact with sick people. Healthcare professionals should refrain from giving live vaccinations, such as the rotavirus vaccine, and suggest to moms refrain from breastfeeding while their mother's cytomegalovirus (CMV) IgG level is being assessed for prior exposure. An immunologist's consultation is an essential next step. Detailed family history, including consanguinity, and a comprehensive physical examination to screen for congenital abnormalities, infections, rashes, and respiratory condition should be part of infant care. Repeating the measurement of lymphocyte subsets, such as T-cell CD45RA/RO using flow cytometry, and getting quantitative serum immunoglobulins are necessary for the confirmation of lymphopenia. Lymphocyte function should be tested via PHA stimulation. For newborns who fit the SCID criteria, IgG replacement therapy is commenced. Additional tests include blood chemistry, albumin and liver function tests. Infection tests should include PCR or antigen (rather than antibody) for adenovirus, CMV, EBV, Hepatitis B, HIV, HSV, and parvovirus B19. To avoid iatrogenic anemia, it is recommended to restrict the amount of blood drawn. Fluconazole and acyclovir are administered sequentially during the first two weeks of prophylaxis, followed by TMP-SMX after four weeks. [90]

The newborn child suffering from severe combined immunodeficiency would require frequent hospitalization with many tests and severe painful examinations. The patient is completely isolated, like being in a bubble. This is because there are high chances of getting an infection from the surroundings through sources such as the parents, relatives or the hospital setting itself. The cytomegalovirus is one of the most

easily infecting viruses in patients with severe combined immunodeficiency. This virus could lead to years of difficulties in the child leading to disabilities and long-duration symptoms. The child needs to be isolated from overcrowded areas like playgrounds, group child care, shops and many other public places. There is no major role of nutrition and diet in this disease, but it is important for the proper growth and development of the child. The newborn with severe combined immunodeficiency is not able to sustain and absorb the given diet due to chronic diarrhoea resulting in malnutritional diseases. For maintaining nutrition intravenous diet is provided as the child is in a weak condition for any oral feed. [125] The symptomatic treatment is the first line of treatment for the disease. The child needs to be stabilized and cleared of all the secondary or opportunistic infections from the body by treatment with various classes of drugs like anti-bacterial, anti-viral, anti-fungal, anti-helminthic and other related drugs. The specific treatment for the gene mutation cause is done by the following therapy: hematopoietic stem cell transplantation (HSCT), gene therapy, enzyme replacement therapy (ERT), and chemotherapy. [126]

1) Hematopoietic Stem Cell Therapy (HSCT):

The recommended presumably curative treatment for SCID is hematopoietic stem cell transplantation (HSCT). [127, 128] Although HSCT saves lives, it only partially restores immunity since recovery is a dynamic process. [129] SCID patients that underwent transplants before the age of 3.5 months had the best survival rates. [130] Early transplantation provides the greatest outcomes for SCID neonates. [131] Adenosine deaminase conjugated with polyethylene glycol (PEG-ADA), an enzyme replacement drug, has been used to treat SCID in infants with ADA deficiency with modest efficacy, but it is not curative. [43]

Infants diagnosed to have SCID are treated with HSCT, also known as bone marrow transplant. This is a complicated medical procedure that necessitates significant time and planning. [39, 43] After selecting a suitable donor, hematopoietic stem cells are harvested and infused into SCID newborns. These immature stem cells eventually mature into red blood cells, white blood cells, and platelets. These cells multiply over time, resulting in a restoration of immunity. The survival rate for this kind of procedure is between 70 and 95 percent. [132]

1) Factors affecting the outcome of HSCT: Early transplantation (1-4 months of age) lowers the risk of opportunistic infections and failure to thrive. [131]

2) Finding HLA-compatible donors for hematopoietic stem cell transplantation can be difficult due to the infant's lack of HLA-matched siblings. This search may prolong treatment, raising the risk of additional infections or diseases.

3) Pre-treatment conditioning: Prior to HSCT, children may be offered chemotherapy to prepare their body for the new stem cells. This pre-treatment frequently leads to defective B cell reconstitution, requiring lifetime immunoglobulin replacement therapy. [39, 43, 74]

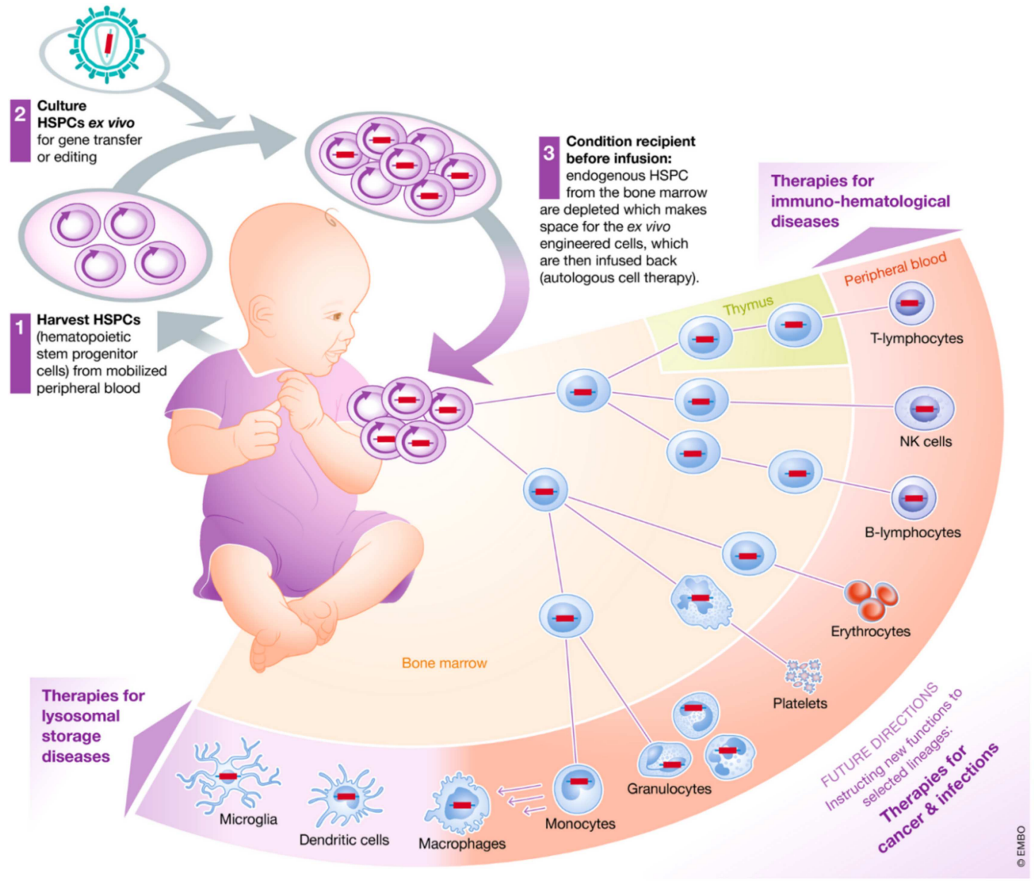


FIGURE 23. HSC GENE THERAPY [133]

Hematopoietic cell transplantation (HCT) has varying mortality rates among patients with this disease, as seen in different studies. Retrospective studies from multiple centers show an overall survival rate of 65% to 70%, whereas recent prospective studies report higher rates, ranging from 85% to 90%. [134] The most significant factor impacting mortality is the use of matched sibling donors (MSD), which yields better outcomes than other donor categories. An in-depth analysis of 571 non-MSD HSCT patients revealed several factors influencing survival, including age, infection status at the time of HSCT, genotype, fetal thymus transplantation (FTT), and race/society. [11] Skipping a conditioning regimen (CR) has potential benefits, such as avoiding chemotherapy-related side effects and reducing the risk of graft-

versus-host disease (GvHD) due to less stress on recipient epithelial cells. However, it limits donor myeloid engraftment, leading primarily to donor T-lymphocyte progenitor engraftment. [135] On the other hand, using CR with myeloablative agents generally promotes stem cell engraftment, resulting in better reconstitution of T- and B-lymphocytes.

Retrospective analyses found no significant difference in survival between no-CR/immunosuppression-only approaches and reduced intensity conditioning (RIC) or myeloablative conditioning (MAC) containing busulfan. However, in cases of active infection during transplantation, using CR was associated with poorer survival outcomes. In such instances, a two-step procedure was proposed: an initial HCT without CR, followed by a second HCT with CR once the infection clears, facilitating stem cell engraftment.

For patients with RAG and DCLRE1C deficiencies, using CR makes sense due to residual NK cell function and the need to clear thymic and marrow niches for donor progenitor engraftment. [136] However, it becomes complicated in cases of DCLRE1C deficiency and other DNA repair defects, as CR has been linked to severe long-term toxicity. Consequently, alternative strategies, like a chemotherapy-free myeloablative approach with anti-stem cell monoclonal antibodies, are being explored. [137]

Typically, HSCT with MSDs is done without CR and achieves survival rates exceeding 90%. Some centers are now exploring reduced toxicity CR approaches for MSDs to enhance donor B-lymphocyte engraftment and avoid extended immunoglobulin replacement therapy.

Severe combined immunodeficiency is a life-threatening condition unless treated with stem cells capable of rebuilding a functional immune system. The most successful outcomes have been observed when using donors from within the family who have identical HLA types to the recipient. In one study, a 92.3% long-term survival rate was found among patients who underwent RID (Related HLA-Identical Donor) BMT. [138]

In one of the studies, for the majority of SCID patients (>85% of cases), perfectly matched donors are unavailable, necessitating consideration of alternative options like MMRD (Molecular Minimal Residual Disease) or MUD (Matched Unrelated Donor) BMT. Survival rates for patients undergoing MMRD BMT varied from 45% to 78%. [135, 139, 140] It was concluded that MUD BMT not only significantly enhances SCID patient survival but also leads to robust long-term immune reconstitution. [138] The ultimate goal of BMT for SCID patients is to fully restore immune function and enable them to lead normal, unrestricted lives indefinitely. Over 80% of patients who received a MUD transplant exhibited robust immune reconstitution, with near-complete restoration of humoral immunity in most patients. [138] Critically, none of the long-term survivors showed increased susceptibility to infections or malignancies. Conversely, almost one-third of MMRD BMT recipients required a second transplant due to graft failure, and 38.9% of long-term survivors exhibited abnormal T-cell receptor expression. These findings align with recent reports outlining immune dysfunction post-MMRD BMT for SCID. [141, 142] Rigorous donor T-cell depletion is necessary with MMRD BMT to prevent severe graft-versus-host disease, yet this depletion may contribute to delayed and aberrant engraftment, leading to heightened infection risk or immune dysregulation. [138]

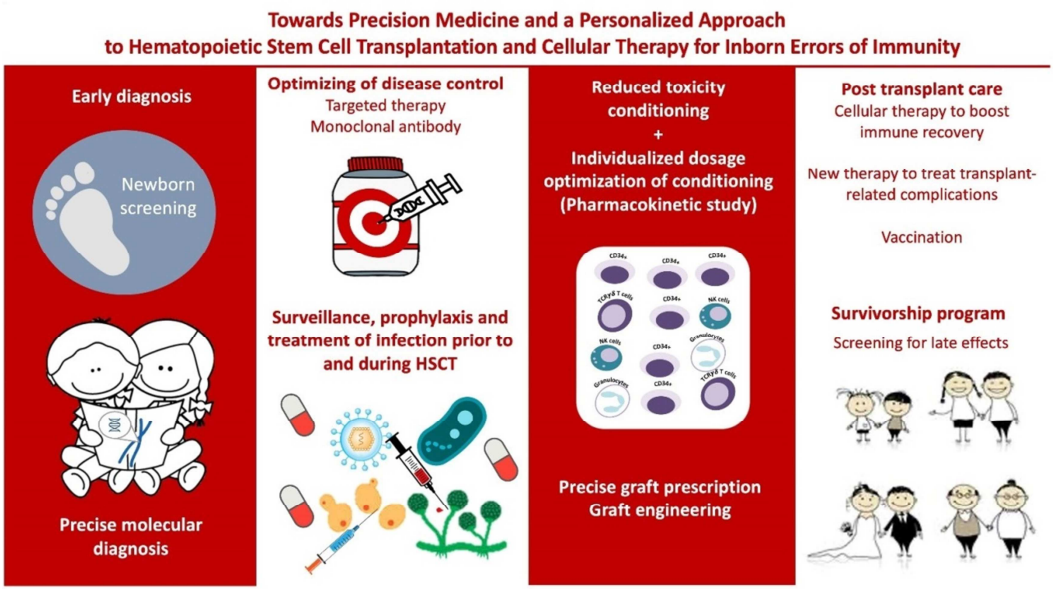


FIGURE 24. APPROACH TO HSCT FOR INBORN ERRORS OF IMMUNITY [143]

2) GENE THERAPY:

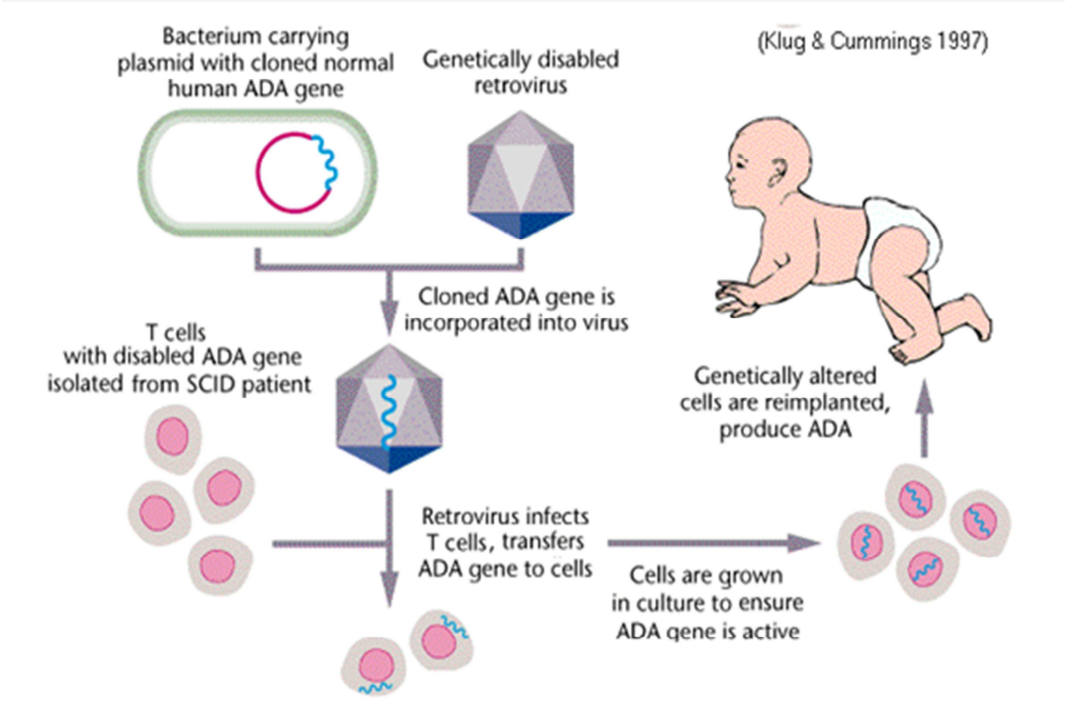


FIGURE 25. GENE THERAPY FOR SCID [144]

SCID results from a faulty Adenosine Deaminase (ADA) gene. To address this, a retrovirus is genetically engineered to carry the normal human ADA gene and is capable of transferring its DNA into normal eukaryotic cells (transfection). In cell culture, isolated T-cell stem line cells from the patient are exposed to this retrovirus, absorbing the ADA gene. By reintroducing these transgenic cells into the patient's bone marrow, a lineage of cells with functional ADA is established, effectively treating SCID. Gene therapy has shown promise as an effective treatment, notably for X-linked SCID. [145] In this procedure, stem cells are extracted from the patient's bone marrow and the normal gene is introduced via a carrier known as a vector before the repaired cells are returned to the patient. [39] Initial attempts at gene therapy to treat X-linked SCID in children were successful in restoring T cell function. [52] However, approximately 25% of these infants got leukemia 2 to 5 years later. This outcome was linked to the vectors used in gene therapy, which were suspected of improperly activating genes involved in cell formation, resulting in leukemia. Modern gene therapy procedures now use modified vectors, which are more efficient and have fewer potential hazards. [74, 146]

Artemis SCID gene therapy is now attainable for infants diagnosed with X-linked SCID. This process entails extracting hematopoietic stem cells with the mutant gene from bone marrow or blood. The extracted gene is then duplicated in a lab to create "corrected" copies. These copies are then inserted into a deactivated virus, enabling for effective entry into hematopoietic stem cells. Once within the cell, the regular hematopoietic stem cell fuses with the new copy, incorporating it into the hematopoietic stem cell pool. This repaired cell is then permitted to divide and preserved in a cryogenic environment. The newborn is conditioned, [43, 147] either

with chemotherapy or immunosuppressive drugs, before the corrected hematopoietic stem cells are injected via IV and disseminated throughout the body. [74, 131]

Gene therapy has proven to be quite effective in treating SCID patients. This involves incorporating a viral vector containing the corrected gene into the patient's hematopoietic stem cells (HSCs), integrating it into their genetic structure, and then reintroducing the transformed cells into the body. [148] It has successfully treated people with both X-linked and ADA-deficient SCID. However, early trials experienced difficulties such as graft failure, and in certain cases, vector insertion resulted in mutations, causing lymphoproliferation and leukemia, notably in X-linked SCID cases. [149] Retroviral vector designs have been modified in order alleviate these potential risks. This includes using self-inactivating gamma-retroviral vectors with modified U3 sections, as well as lentiviral vectors, to reduce the likelihood of mutations during integration. Emerging gene editing techniques use particular DNA cleavage enzymes to delete the defective gene and replace it with the corrected form using the cell's natural DNA repair mechanisms. This level of precision enables greater control over gene expression, making it a more biologically accurate technique of genetic modification. [150] While promising in cell lines, attaining complete repair of genetic abnormalities in primary HSCs has been complicated. Current gene therapy techniques do not reliably cure all genetic abnormalities. Some studies used low-dose chemotherapy to improve the engraftment of treated stem cells, giving them a competitive advantage over untreated cells. With the potential widespread use of SCID screening, a chemotherapy-free alternative to standard HSCT or gene-targeted therapy may become viable. This method would alleviate worries about long-term adverse effects while also ensuring a long-term and effective immunological recovery. [126,151]

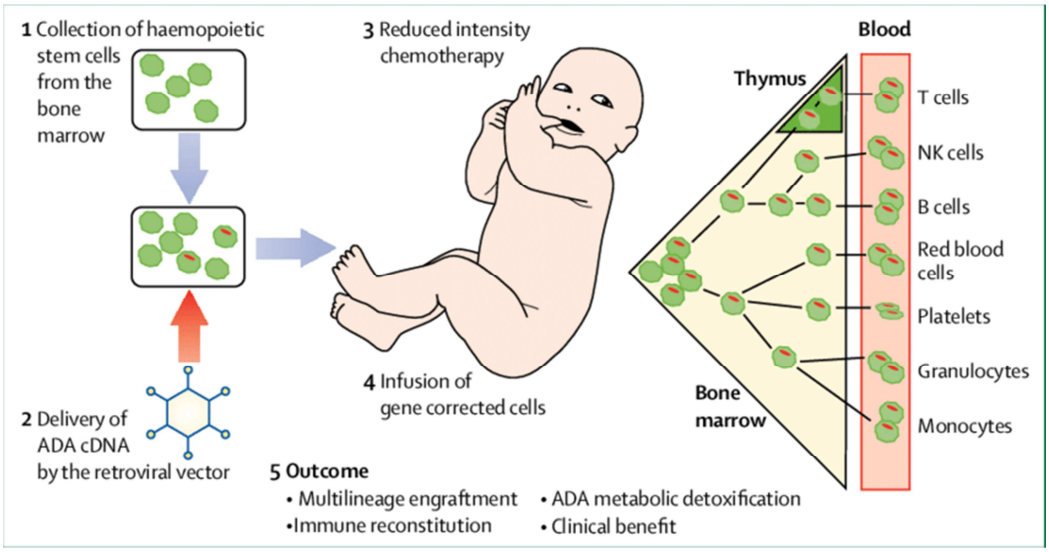


FIGURE 26. Gene therapy clinical trial for adenosine deaminase-severe combined immunodeficiency disorder CD34+ cells are extracted from the child's marrow, transduced with a viral vector expressing ADA, and, after mild myeloablation, are reinfused into the same patient. [152]

3) ENZYME REPACEMENT THERAPY:

The enzyme polyethylene glycol-conjugated adenosine deaminase (PEG-ADA), also known as Revcovi/ Elapegademase, utilized in Enzyme Replacement Therapy (ERT), is accessible for both children and adults diagnosed with ADA-SCID. ADA-SCID individuals lack the essential enzyme adenosine deaminase, crucial for proper immune system functionality. [126] In ERT, children with ADA-SCID receive weekly intramuscular injections of Revcovi / Elapegademase [74] containing the deficient enzyme, aiding in the restoration of normal immune function. Following initial guidance, parents can administer these injections at home, eliminating the need for medical assistance. ERT serves as a significant interim solution for ADA-SCID patients, even if they are considering more permanent interventions such as HSCT.

This temporary approach rapidly reduces the buildup of specific toxins caused by the absence of the ADA enzyme, which leads to the destruction of white blood cells, including T cells, the underlying cause of SCID. Administering ERT to infants before HSCT increases T cell counts, enhancing resistance to infections until definitive treatment is obtained. In certain cases, ERT may be extended over months to years. [153] However, HSCT or gene therapy are generally preferred long-term solutions. The duration of ERT and the optimal stopping point vary based on the child's choice of treatment, which physicians discuss with parents to determine the most appropriate course of action. Additionally, although not definitively proven, elevated toxin levels are suspected to contribute to secondary complications in ADA-SCID cases. While many patients may not experience significant secondary issues, some may encounter challenges such as liver problems, skeletal issues, skin tumors, or neurological disorders. Reducing toxin exposure early on may potentially mitigate the risk or severity of these complications. Nonetheless, one drawback of ERT is the requirement for regular muscle injections, typically once or twice weekly. Furthermore, lifelong commitment to this treatment is necessary and is associated with side effects such as coughing, vomiting, anemia, skin concerns, and a risk of immune system-related cancers. [126, 154]

4) CHEMOTHERAPY:

Following a confirmed diagnosis, the focus shifts to determining the most effective treatment strategy. One approach is to introduce unfractionated donor hematopoietic stem cells (HSC) without preparative chemotherapy, aiding in T-lymphocyte immune recovery. [139] However, the success of establishing thymopoiesis and the durability of T-lymphocyte function vary based on the patient's phenotype and

genotype. Infants with NK cell-negative SCID generally have better survival rates compared to those with recipient NK cells, as they typically possess a substantial presence of donor T-lymphocytes, ensuring sustained CD4+ T-lymphocyte immunity without the necessity for prior chemotherapy. Conversely, the presence of recipient NK cells strongly suggests the need for preparative chemotherapy to facilitate the engraftment of T-lymphocyte precursors, promoting robust and enduring T-lymphocyte reconstitution. [155] However, the choice of donor also influences outcomes; utilizing an unrelated HLA-matched donor significantly increases the risk of graft-versus-host disease (GvHD) compared to using a matched sibling donor.

There are various concerns associated with chemotherapy conditioning. Acute toxicities are frequently observed, and if an active infection is present, mortality rates rise unless a matched sibling donor is available. While durability and sustainability of thymopoiesis and B-lymphocyte function are more likely in most SCID forms following chemotherapy, especially in those with recipient NK cells, concerns arise about the effects of chemotherapy, even in short durations, on young infants. [156] Currently, comprehensive multicenter studies evaluating the long-term (over 20 years) immunological, overall health, or quality-of-life outcomes of HSCT in SCID, including chemotherapy preconditioning and direct donor inoculum infusion, are lacking. As most infants are now diagnosed during the neonatal period through newborn screening initiatives, the challenges associated with administering chemotherapy are receiving increased attention. [157] While the risk of mortality from chemotherapy, particularly in healthy patients with no other health issues, is low, it is not negligible, prompting exploration of alternative strategies. [126] Chemotherapy conditioning enhances immunoreconstitution but comes with short- and long-term toxicities, as well as increased mortality rates. The implementation of newborn

screening for SCID will drive efforts to discover safe and efficient approaches to achieving donor cell engraftment and complete immunoreconstitution without harmful consequences. [158]

Minimally intensive regimens utilizing monoclonal antibodies have effectively treated SCID, even in the presence of significant health issues. [159] In animal models, the administration of an anti-c-Kit receptor antibody, which disrupts a vital signaling pathway involved in HSC homing, adhesion, maintenance, and survival within the hematopoietic niche, has shown promising results when given in utero, followed by transplantation on the first day of life. However, further research is required to confirm actual patient benefits. Nonetheless, clinical trials utilizing therapeutic-grade antibodies are currently in the planning stages. [126]

5) TREATMENT OF INFECTIONS:

Reverse isolation, which includes maintaining the patient in a protected environment, avoiding live immunizations, employing therapeutic immunoglobulins, and starting early preventive antibiotic treatment, can help manage infections even if stem cell therapy (SCT) is the ultimate treatment for SCID. In the treatment of SCID, early prophylactic antibiotic medicine is frequently utilized to lower the incidence and severity of infections, especially bacterial sinopulmonary infections. Preventive antifungal and antiviral drugs are also thought to be crucial in SCID. The past twenty years have seen the development of novel antifungal medications, such as liposomal amphotericin B, voriconazole, posaconazole, and isavuconazole, as well as echinocandins like micafungin, caspofungin, and anidulafungin. [74]

6) ANTIBIOTIC PROPHYLAXIS:

Prophylactic administration of Sulfamethoxazole-trimethoprim prior to stem cell therapy aims to combat pneumocystic pneumonia (PCP), predominantly caused by Pneumocystis jirovecii. Fluconazole is commonly administered to prevent mucocutaneous candidiasis, while acyclovir is used to prevent HSV infection. Antifungal medicines have been used to treat invasive pulmonary aspergillosis in persons with SCID. Oral valganciclovir is being prescribed as an alternative to ganciclovir in immunocompromised children with CMV infection, particularly those with SCID. Valacyclovir has been used in investigations to investigate the tropism of herpes simplex virus (HSV-1) for human sensory neurons infected in vivo, utilizing dorsal root ganglion xenografts from SCID mice. [74, 160]

MATERIALS AND METHODOLOGY:

Over the course of a year (January 2023 to December 2023), 1550 newborns delivered vaginally or via cesarean section at the KLE's Dr. Prabhakar Kore Hospital & MRC in Belagavi, Karnataka, India, were included in this prospective longitudinal study. The study covered all babies born with gestational age more than 32 weeks. There were 709 females and 841 males. Their mean gestational age was 37.44 ± 1.74 weeks, with a range of 32–41 weeks. Their weight (mean 2.71 ± 0.49 kg) spanned from 1.3 to 4.3 kg. Merely five of the 1550 newborns showed congenital abnormalities, whereas seventeen had a family history of unexplainable sibling deaths. Of the 1550 newborns, 670 were delivered by vaginal delivery and 880 via cesarean section. Out of 1550 neonates, 108 needed bag and mask resuscitation. Before enrollment, the parents or other caregivers of each child provided written consent in their language. The institutional ethics committee gave its approval to the study protocol.

SOURCE OF DATA: Umbilical cord blood obtained from newborns born to mothers more than 32 weeks period of gestation who gave consent for the study delivered in labour rooms and operation theatres of a tertiary care hospital in Belagavi between January 2023 to December 2023.

INCLUSION CRITERIA: Newborns more than 32 weeks of gestation

EXCLUSION CRITERIA:

1. Newborns less than 32 weeks of gestation
2. Antenatal use of steroids or immunosuppressive medications for more than 5 days.
3. Trisomy 21
4. Hydrops
5. Parents not consenting for the study.

SAMPLE SIZE:

1. Sample size was calculated assuming the proportion of Incidence of lymphopenia as 1.61% as per the study by El-Sayed SS et al. [35]

2. Sample size calculation were based on 0.54% absolute precision and 95% confidence level. The sample size was calculated using the following formula.

3. Based on previous hospital records, 4000 possible eligible individuals were estimated to attend the study throughout the data collection period. As a result, a finite population adjustment was done for 4000. According to Daniel WW et al.'s study, the sample size was calculated using the following formula.

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1) + Z^2P(1-P)}$$

Where n' = Sample size

N= Size of population= 4000

Z= Z statistic for a confidence level= 1.960

P = Expected prevalence/proportion of outcome= 0.0161

d = Precision= 0.0054

According to the calculations above, the required sample size was 1372. To account for a non-participation/loss to follow-up rate of around 5%, an additional 69 subjects were added to the sample. As a result, 1441 was the final sample size.

STUDY MEASUREMENTS:

An in-depth antenatal and perinatal history was elicited from the mother. A family history of previously diagnosed PID sibling or death, whether from infections or an unknown reason, was also considered. Apgar scores were recorded for all babies after one and five minutes. Furthermore, all neonates underwent a thorough general and systemic clinical evaluation.

Each infant's umbilical cord blood was obtained (at the time of delivery) under strict aseptic conditions and placed in a vacutainer tube containing ethylene diamine tetraacetic acid (EDTA). Complete blood count was processed using Sysmex XN 350 – 5 part analyzer including hemoglobin (HB), white blood cells (WBC), platelets, red blood cells (RBCs), absolute lymphocyte count (ALC) and absolute neutrophil count (ANC). Manual differential was done. Parents of newborn infants with lymphopenia were advised against administering live attenuated vaccines until further investigations were conducted.

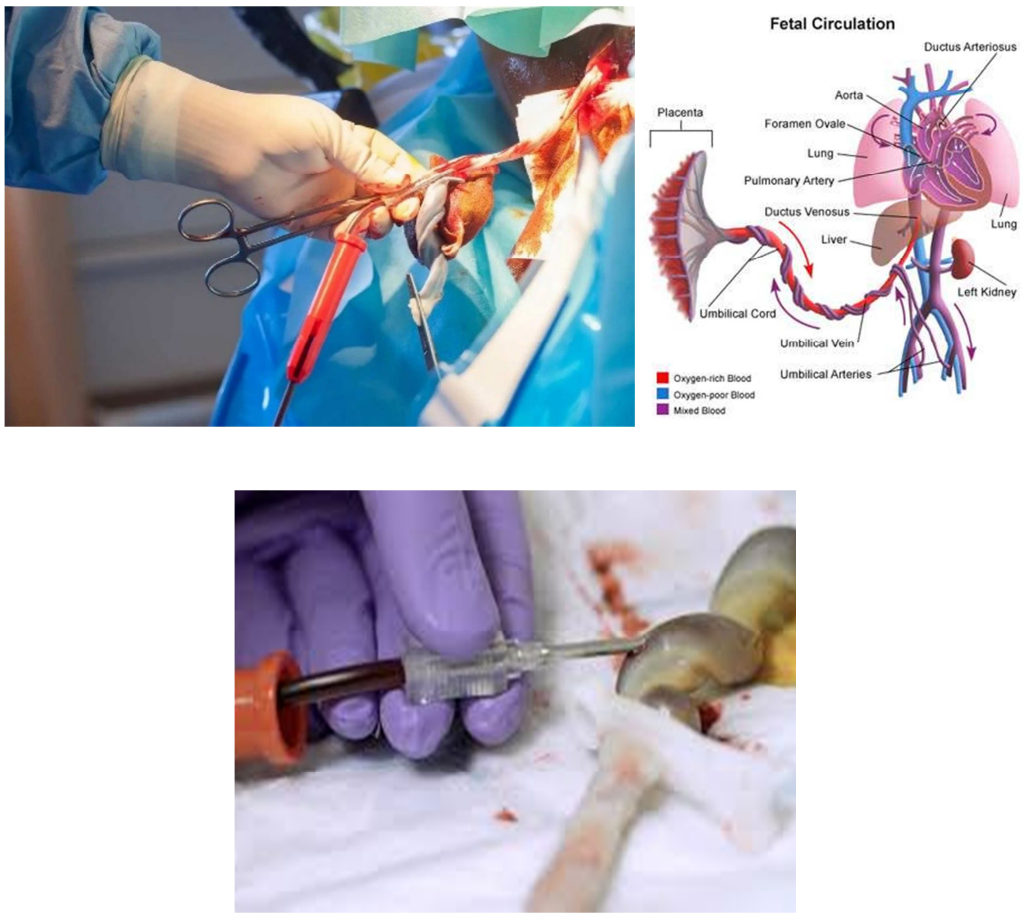


FIGURE 27. METHOD OF UMBILICAL CORD SAMPLING

FIGURE 28. EDTA VACCUTAINER **SYSMEX – XN 350: 5 PART ANALYSER**

STUDY PROTOCOL:

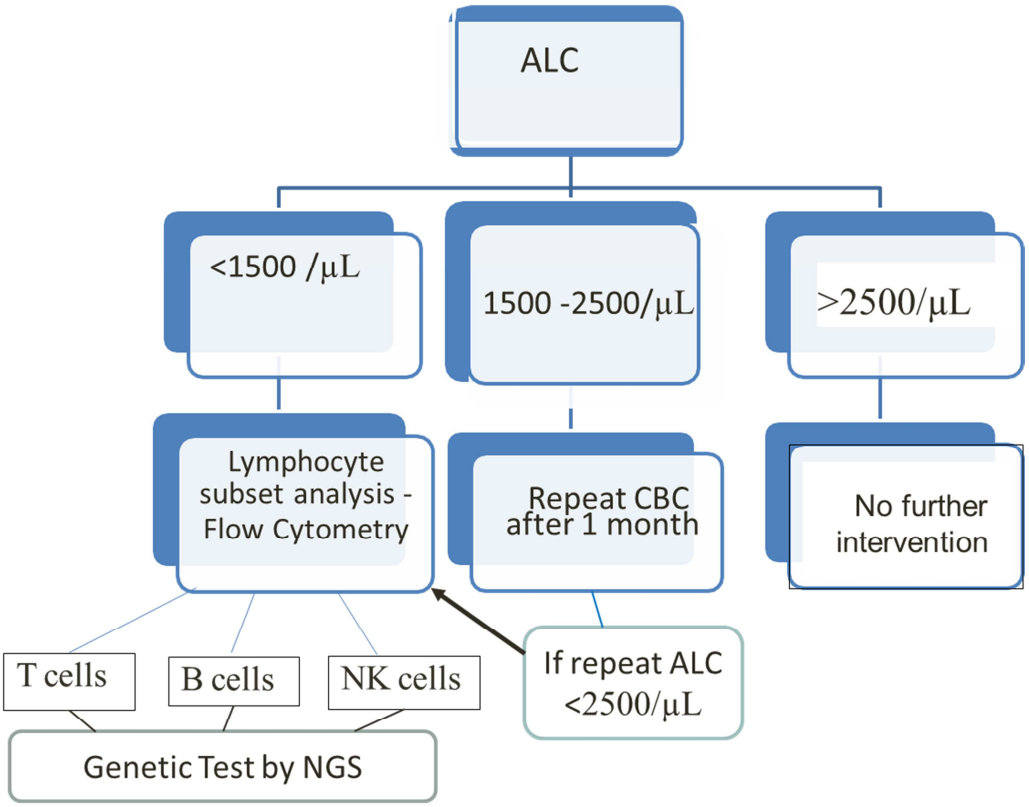


FIGURE 29: STUDY PROTOCOL

- Absolute lymphocyte count >2500/µL in the cord blood sample was considered as normal and no further intervention was done.
- Absolute lymphopenia in any cord blood sample was defined as absolute lymphocytic count<2500/µL.
- If the Absolute Lymphocyte Count (ALC) was between 1500-2500/µL and mother had received steroids for less than 5 days, then follow-up CBC was scheduled after one month to check ALC. On one month follow up, newborns with ALC <2500/µL were subjected to Lymphocyte Subset Analysis (LSSA) by Flow Cytometry.

- Lymphocyte Subset Analysis (LSSA) by Flow Cytometry was performed in newborns with ALC less than 1500/µL in cord blood sample.
- If Flow Cytometry was suggestive of SCID, then those patients were subjected to a genetic test – Whole Exome Sequencing (WES)/Next Generation Sequencing (NGS) for confirmation.
- Parents of newborns suspected to have SCID were counselled regarding the condition, including the need for antenatal diagnosis in the subsequent pregnancy.

FLOW CYTOMETRY FOR LYMPHOCYTE SUBSET ANALYSIS:

Flow cytometric analysis was performed on EDTA-blood samples from the cord blood of newborns to determine the absolute numbers of T cell subsets (CD3, CD4, CD8), B cells (CD19), and NK cells (CD16/CD56). Quantification was carried out using Multitest 6-color TBNK and Truecount beads in accordance with the manufacturer's instructions (Bioscience BD, San Jose, CA). T cell subpopulations were further characterized by identifying recent thymic emigrants (RTE), naive and memory CD4+ T cells, and, if plausible, other T and B cell subsets.

Following a 15-minute room temperature incubation period of EDTA samples with suitably titrated antibodies, erythrocytes were lysed using BD FACS Lysing Solution (BD Beckman Dickinson, San Jose, CA) to facilitate T cell analysis on EDTA blood samples. Before being incubated with antibodies, samples were subjected to two washes for B cell analysis. A Gallios Flow Cytometer (Beckman Coulter, San Diego, CA) was used for data acquisition. The cell subset identification process involved the use a variety of antibodies from manufacturers such as Becton Dickinson, Beckman Coulter, R&D Systems, eBioscience, Invitrogen, and Dako. T

cells were gated as CD3+, and further classified as follows: naive CD4+ (CD4+, CD45RA+), recent thymic emigrants (CD4+, CD45RA+, CD31+), CD4+ memory (CD4+, CD45RO+), follicular-like CD4+ (CD4+, CD45RO+, CCR5+), regulatory T cells (CD4+, CD25++, CD127–), naive CD8+ (CD8+, CD27+, CD28+), early effector memory CD8+ (CD8+, CD27+, CD28–), and late effector memory CD8+ (CD8+, CD27–, CD28–). CD19+ B cells were gated and then further categorized as IgM memory (CD27+, IgD+, IgM+), class-switched (CD27+, IgM–, IgD–), naive (IgD+, IgM+, CD27–), and transitional (IgM++, CD38++, CD24+). [161, 162]

FIGURE 30. BD FACS™ FLOW CYTOMETRY SYSTEM. [162]

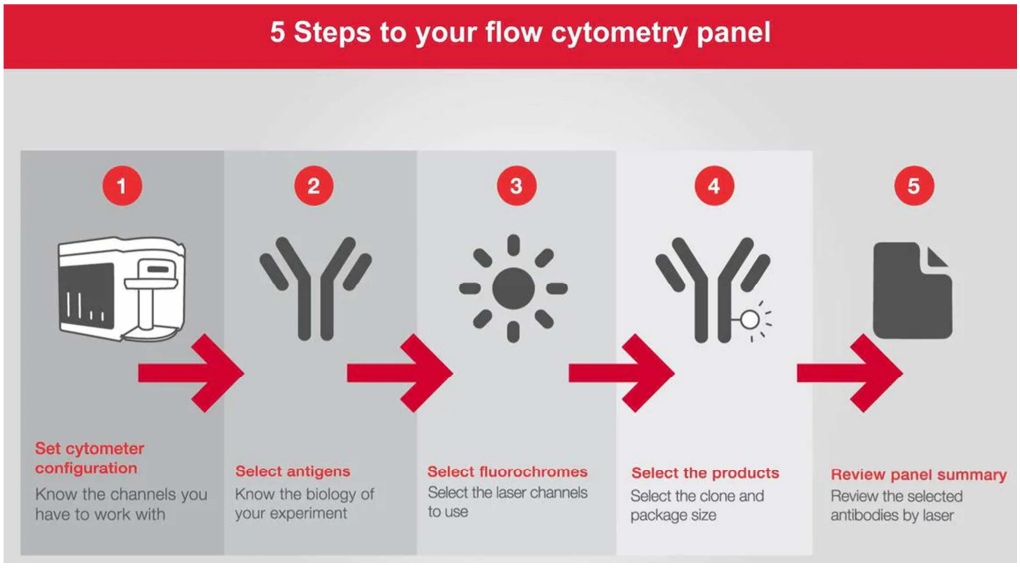


FIGURE 31. WORKFLOW OF FLOW CYTOMETRY. [163]

Innate tube														
Fluorochrome	**BV421**	**Pac Orange**	**BV605**	**BV711**	**FITC**	**PE**	**PE-DyLight 594**		**PerCP-Cy5.5**	**PE-Cy™7**	**APC**	**Alexa Fl. 700**	**APC-Cy™7**	
Target	CD127	CD45	CRTH2	CD56	CD117	tested CD	CD3	CD19	CD14	CD11c	CD123	HLA-DR	CD16	
clone	A019D5	2D1	BM16	HCD56	104D2		UCHT1	LT19	MEM-15	BU15	6H6	L243	3G8	
Volume	1.25 µl	5 µl	2 µl	1.25 µl	2.5 µl		2.5 µl	2.5 µl	2.5 µl	1.25 µl	1.25 µl	2.5 µl	2.5 µl	
Adaptive tube Fluorochrome	**Pac Blue**	**Pac Orange**	**BV605**	**FITC**	**PE**	**PE-Dazzle594**	**PerCP-Cy5.5**		**PE-Cy™7**		**APC**	**Alexa Fl. 700**	**APC-Cy™7**	
Target	CD45RA	CD45	CXCR5	CD27	tested CD	CD127	CD4	IgD	TCRgd	CD19	CD25	CD3	CD8	
clone	MEM-56	2D1	J252D4	LT27		A019D5	MEM-241	IA6-2	B1	LT19	MEM-181	UCHT1	MEM-31	
Volume	5 µl	5 µl	0.625 µl	2.5 µl		0.625 µl	2.5 µl	2.5 µl	5 µl	1.25 µl	1.25 µl	2.5 µl	2.5 µl	dried reagents

Dried reagents shaded.

TABLE 10. SELECTION OF FLUOROCHROMES FOR LYMPHOCYTE SUBSET ANALYSIS.[164]

GENETIC ANALYSIS BY NEXT GENERATION SEQUENCING (NGS)/WHOLE EXOME SEQUENCING (WES):

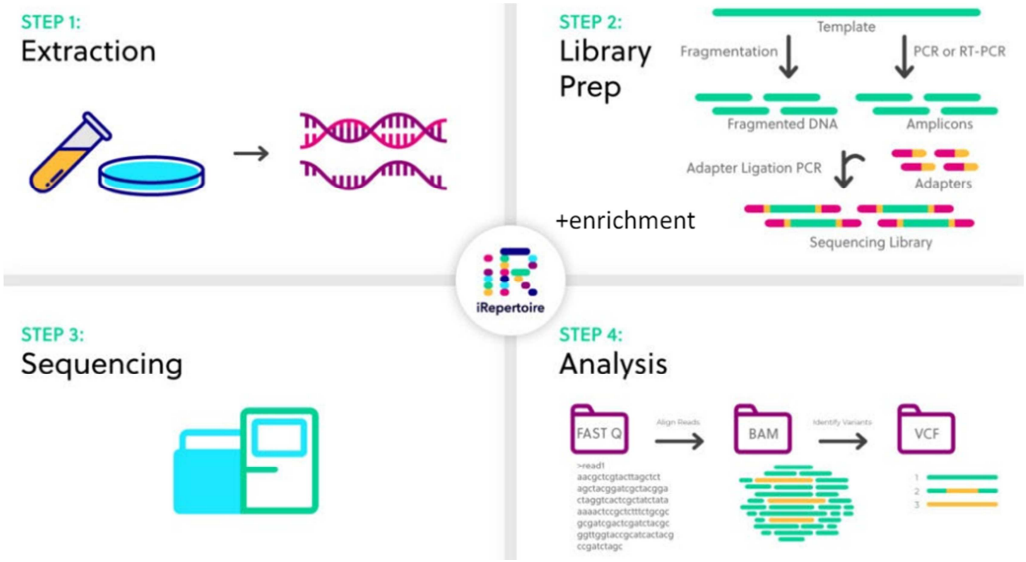


FIGURE 32. NGS WORKFLOW [166]

Next Generation Sequencing (NGS)/Whole Exome Sequencing (WES) was done on Illumina NovaSeq 6000. In this study, DNA extracted from the 3ml EDTA blood was analyzed using the AmpliSeq for Illumina Immune Response Kit v2, which includes a pre-designed gene panel consisting of 52 primary immunodeficiency disease genes. The sequencing was conducted on a benchtop platform from Ampliseq Illumina. Variant calling was performed using Ion ReporterTM Software, with filtering to highlight variants specifically within genes relevant to SCID and severe T cell deficiencies. Using Ion Reporter's Confident CNVs-CNVs Only filter in conjunction with manual gene inspection of certain target genes, copy number variants (CNVs) were found in the next-generation sequencing (NGS) data. IGV (Integrated Genomics Viewer) was used to display BAM files. The American College of Medical Genetics and Genomics (ACMG) recommendations were adhered to for interpreting variants. Furthermore, a tailored gene panel intended for newborn screening (NBS) was created utilizing the AmpliSeq Illumina on-demand workflow. Genes linked to congenital bone marrow failure, severe T cell deficits, metabolic abnormalities, and SCID are included in this panel.

FIGURE 33. ILLUMINA NOVASEQ 6000 – KIT [165].

ILLUMINA NOVASEQ 6000 [165]
NEXT GENERATION SEQUENCING MACHINE

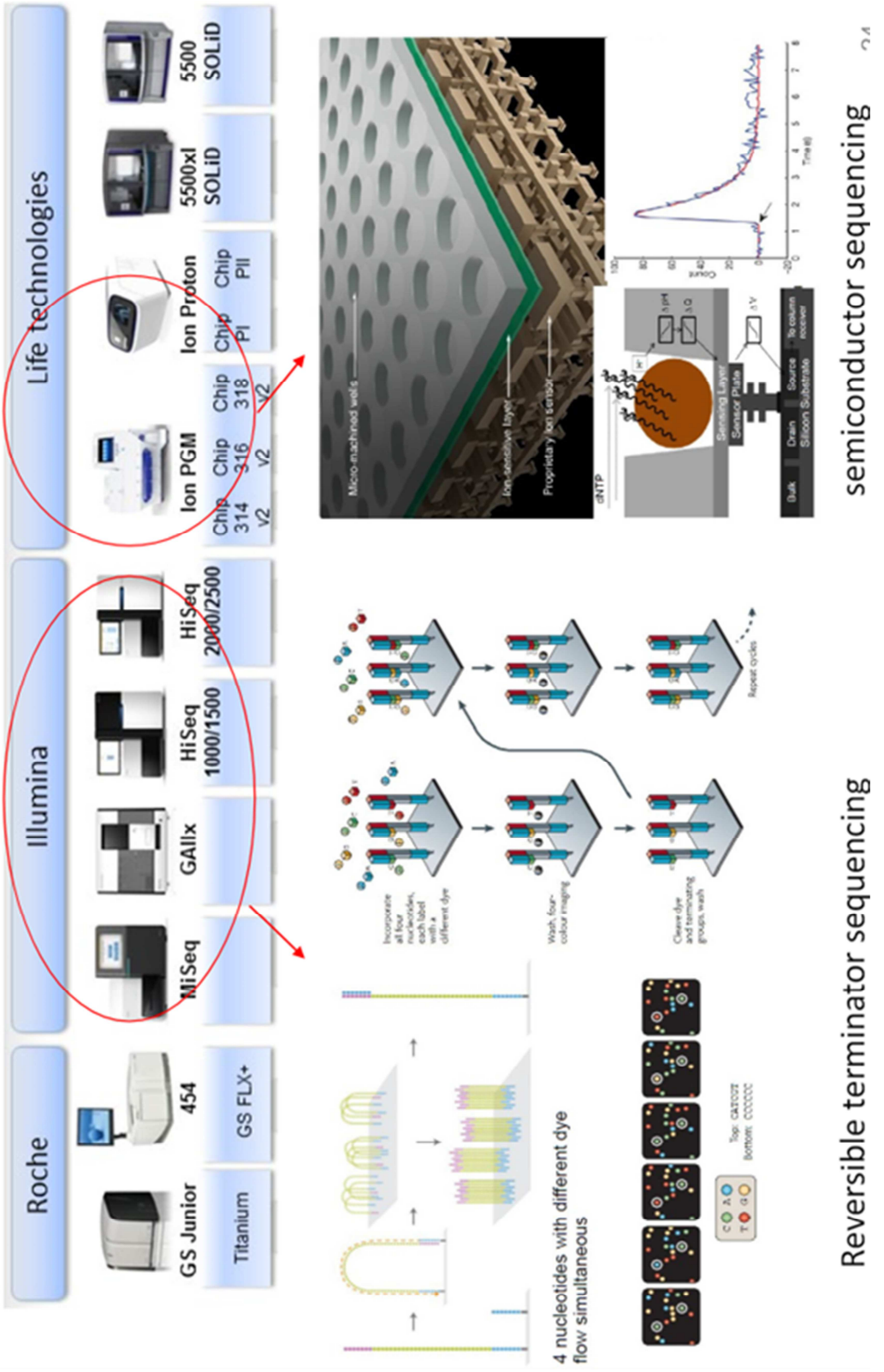


FIGURE 34. ILLUMINA SEQUENCING [167]

STATISTICAL ANALYSIS:

For quantitative data, descriptive analysis was performed using mean and standard deviation; for categorical variables, frequency and proportion were used. Additionally, data was shown using suitable graphics, such as bar graphs.

Cross tabulation and percentage comparison were used to evaluate the relationship between explanatory variables and categorical outcomes. To evaluate statistical significance, the chi square test was utilized.

The association between quantitative explanatory variables and categorical outcomes was assessed by independent sample t-test (2 groups) will be used to assess statistical significance.

P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis [168]

The sample size of the study was calculated to be 1441. However, since 106 samples were clotted and 3 newborn babies expired shortly after birth, we have accounted for these 109 missed samples and added those to the original sample size. Thus, total of 1550 (1441+109) cases have been considered for descriptive analysis, while laboratory evaluation has carried out done for 1441 cases.

RESULTS:

Table 11: Descriptive analysis of gender in the study population (N=1550)

Gender	Frequency	Percentages
Male	841	54.26%
Female	709	45.74%

Graph 1: Pie chart of gender in the study population (N=1550)

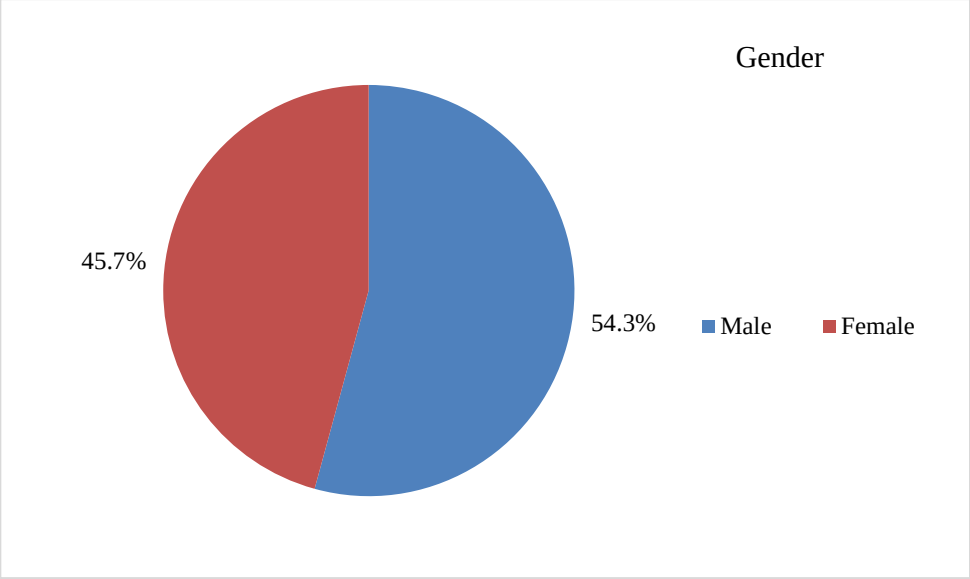


Table 12: Descriptive analysis showing consanguinity in the study population (N=1550)

Consanguinity	**Frequency**	**Percentages**
Present	191	12.32%
Absent	1359	87.68%

Graph 2: Pie chart showing consanguinity in the study population (N=1550)

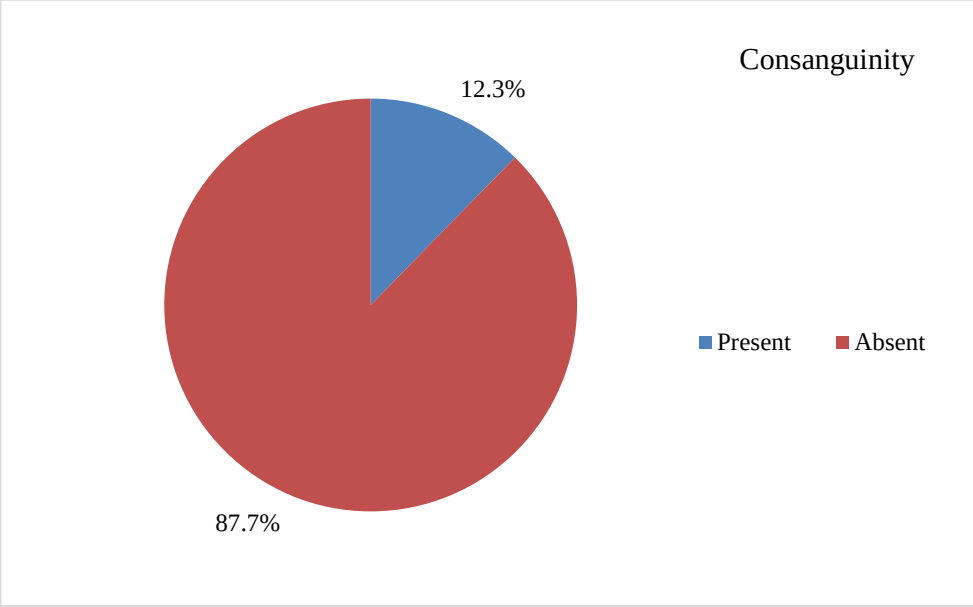


Table 13: Descriptive analysis of mode of delivery in the study population (N=1550)

Mode Of Delivery	Frequency	Percentages
Normal Vaginal Delivery	670	43.23%
LSCS	880	56.77%

Graph 3: Pie chart of mode of delivery in the study population (N=1550)

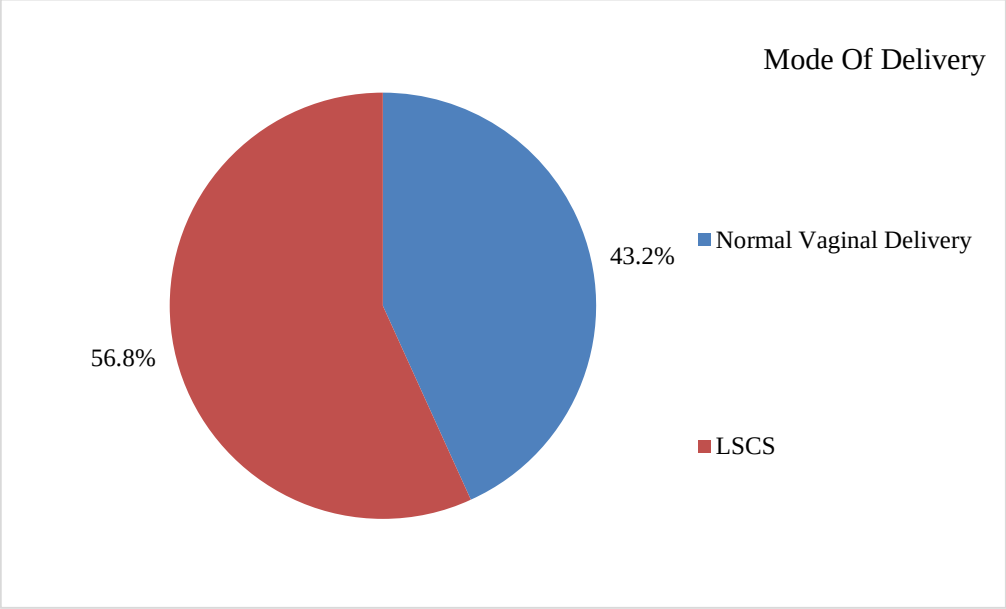


Table 14: Descriptive analysis of requirement of neonatal resuscitation (bag & mask) in the study population (N=1550)

Neonatal Resuscitation required - Bag & Mask	Frequency	Percentages
Yes	108	6.97%
No	1442	93.03%

Graph 4: Pie chart of requirement of neonatal resuscitation (bag & mask) in the study population (N=1550)

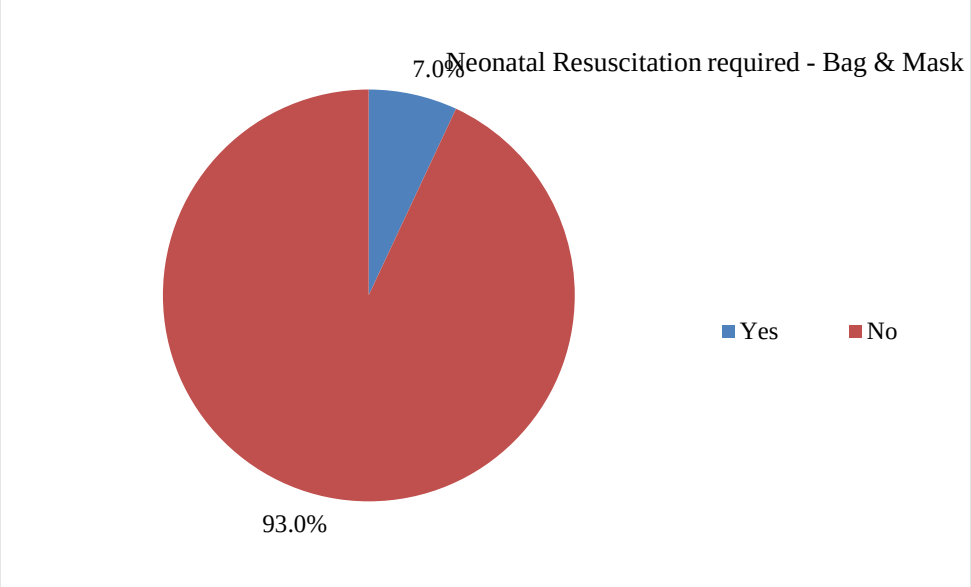


Table 15: Descriptive analysis of gestational age and birth weight (kg) in the study population (N=1550)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Gestational Age (In Weeks)	37.44 ± 1.74	38.0	32.0	41.0	37.4	37.5
Birth Weight (Kg)	2.71 ± 0.49	2.7	1.3	4.3	2.7	2.7

Table 16: Descriptive analysis of other associated conditions contributing to lymphopenia in the study population (N=96)

Other Associated Conditions Contributing to Lymphopenia	Frequency	Percentages
Congenital Anomaly	5	5.21%
Idiopathic	23	23.96%
Preterm	48	50.00%
Sepsis	5	5.21%
Preterm, Sepsis	15	15.63%

Graph 5: Bar chart of other associated conditions contributing to lymphopenia in the study population (N=96)

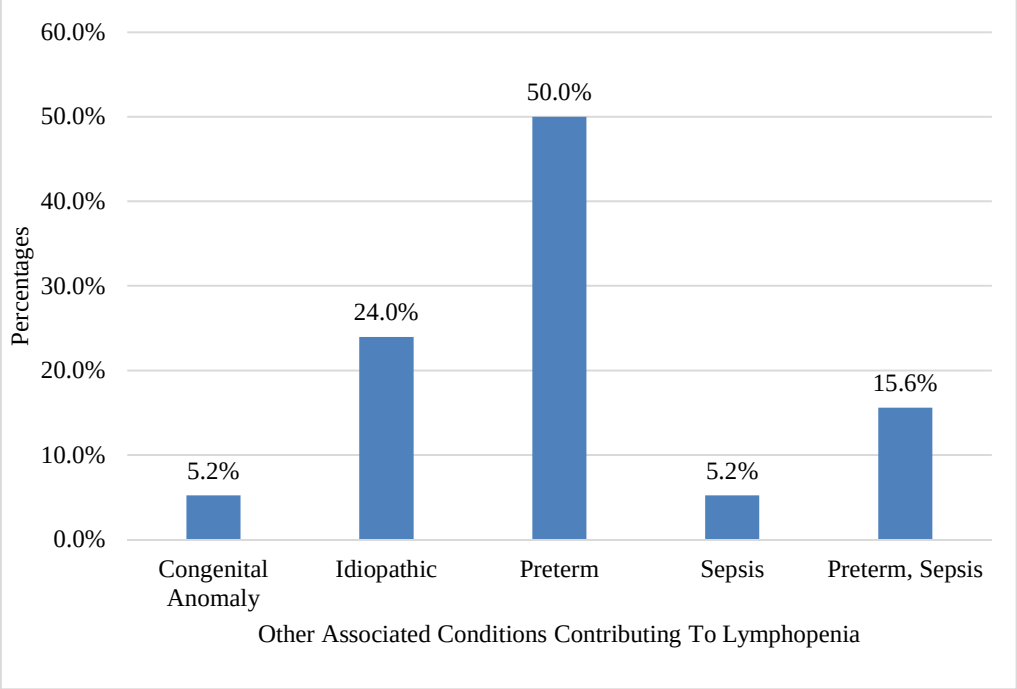


Table 17: Descriptive analysis of Apgar Score in the study population (N=1550)

Apgar Score (Out of 10)	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
At 1 Min	6.67 ± 1.11	7.0	2.0	9.0	6.6	6.7
At 5 Min	8.72 ± 0.81	9.0	5.0	10.0	8.7	8.8

Table 18: Descriptive analysis of dysmorphic features in the study population (N=1550)

Dysmorphic Features	Frequency	Percentages
Present	30	1.94%
Absent	1520	98.06%

Graph 6: Pie chart of dysmorphic features in the study population (N=1550)

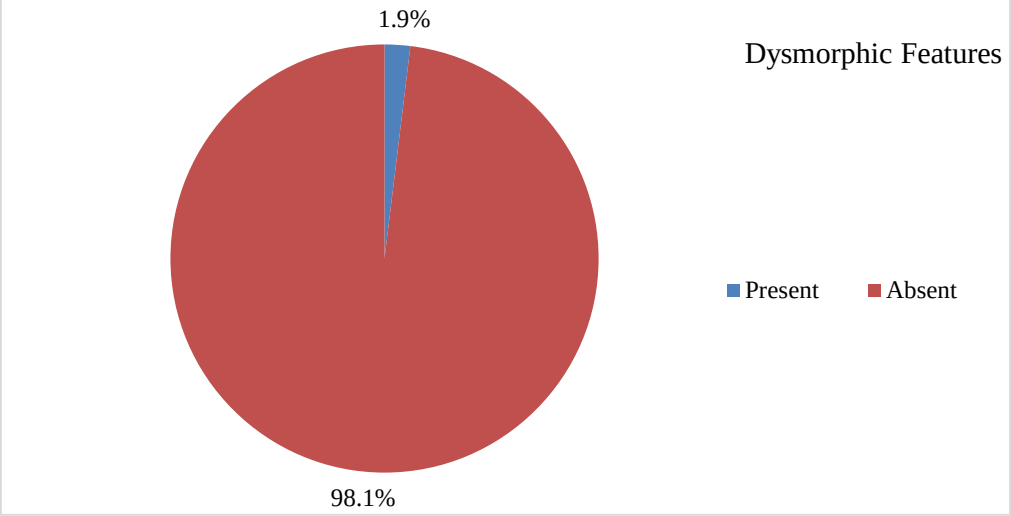


Table 19: Descriptive analysis of family history / sibling death in the study population (N=1550)

Positive Family History / Sibling Death	Frequency	Percentages
Yes	17	1.10%
No	1533	98.90%

Graph 7: Pie chart of family history / sibling death in the study population (N=1550)

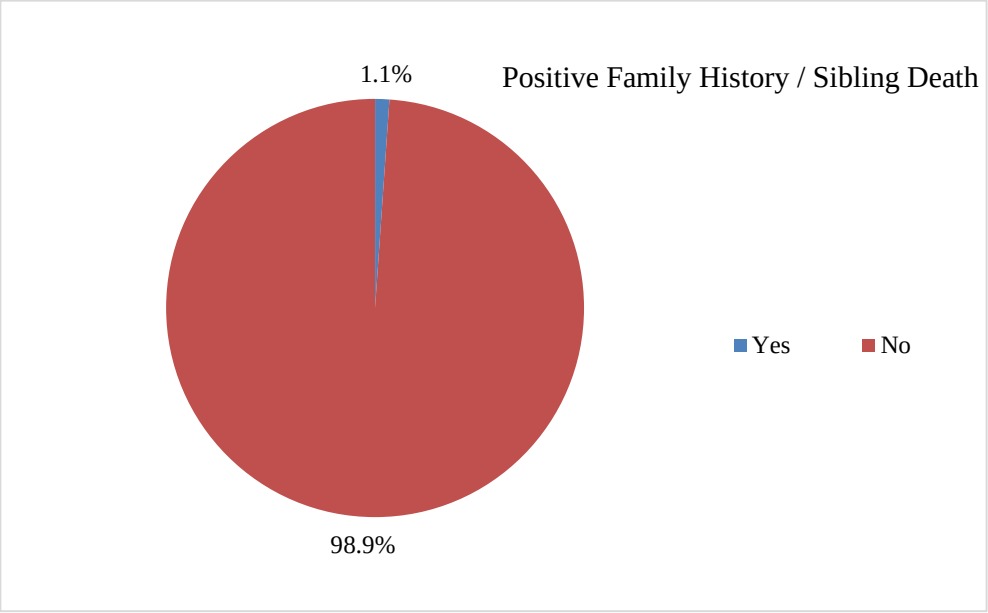


Table 20: Descriptive analysis of mean laboratory parameters in the study (N=1441):

Lab Parameters	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Haemoglobin (Gm%)	14.9 ± 2.06	14.9	9.4	21.5	14.8	15.0
WBC Count ($X10^3/\mu l$)	14.94 ± 3.62	14.7	5.4	28.9	14.8	15.1
ANC ($X10^3/\mu l$)	9.58 ± 2.95	9.6	1.1	20.7	9.4	9.7
ALC ($X10^3/\mu l$)	4.46 ± 1.84	4.2	0.9	14.5	4.4	4.6

Table 21: Descriptive analysis of absolute lymphopenia in the study population (N=1441)

Absolute Lymphopenia (ALC<2500/µl)	Frequency	Percentages
Yes	96	6.66%
No	1345	93.34%

Graph 8: Pie chart showing incidence of absolute lymphopenia in the study (N=1441)

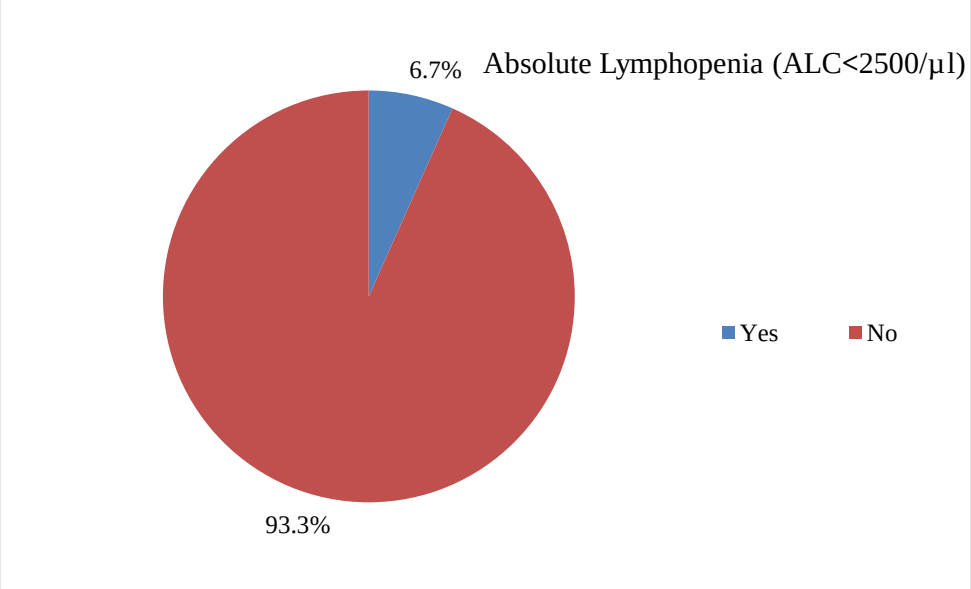
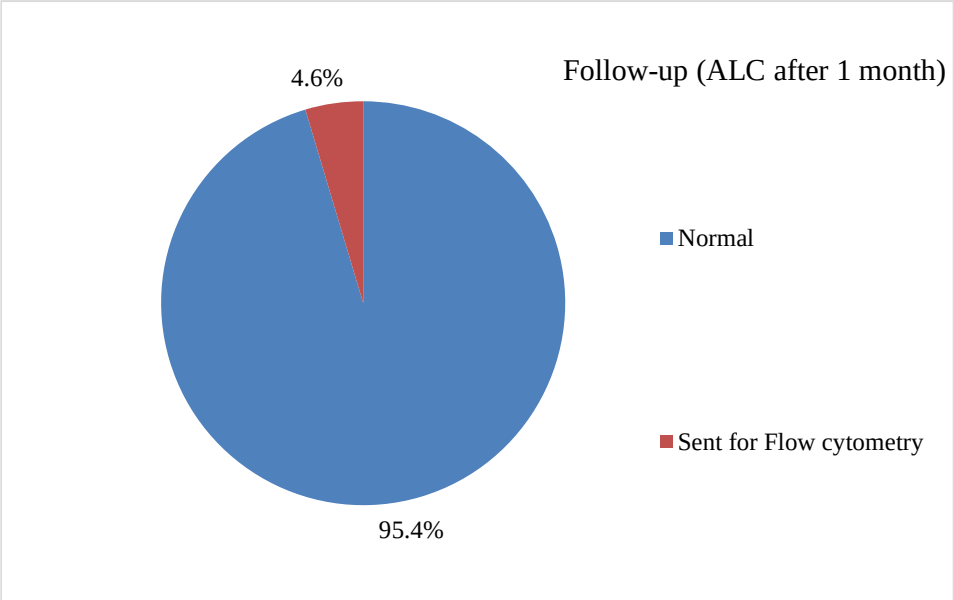


Table 22: Descriptive analysis of babies with Absolute Lymphopenia (ALC<2500/µl) in the study population (N=96):

Parameter	Frequency	Percentages
Flow cytometry (LSSA) (N=12)		
Done	9	75.00%
Lost to follow-up / Death	3	25.00%
If Flow Cytometry (LSSA) done, (n=9)		
Normal Pattern	5	55.56%
Suspected SCID Pattern	4	44.44%
Follow-up (ALC after 1 month) (N=84)		
Done	65	77.38%
Lost to follow-up / Death	19	22.62%
If Follow-up (ALC after 1 month) done, (N=65)		
Sent for Flow cytometry (ALC<2500)	3	4.62%
Normal (ALC≥2500)	62	95.38%
Sent for Flow cytometry (LSSA) (N=3)		
Normal Pattern	3	100.00%
Overall Absolute Lymphopenia (ALC<2500/µl) (N=96)		
Sent for Flow cytometry (LSSA) (N=12)		
Normal Pattern	8	66.67%
Suspected SCID Pattern	4	33.33%
NGS/WES in patients with Suspected SCID on flow cytometry (N=4)		
Not suggestive of SCID	4	100.00%

Graph 9: Pie chart of follow-up for Absolute Lymphopenia (ALC<2500/µl) cases (N=65)



Graph 10: Pie chart of Flow cytometry (lymphocyte subset analysis) reports in the Absolute Lymphopenia (ALC<2500/µl) population (N=12)

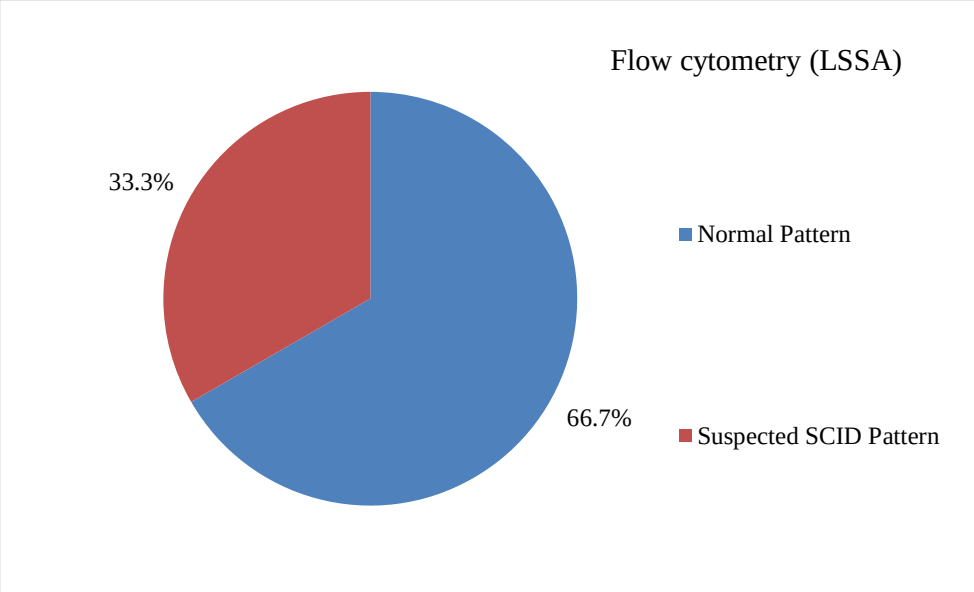


Table 23: Comparison of gender between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

Gender	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	Present (N=96)	Absent (N=1345)		
Male	54 (56.25%)	724 (53.83%)	0.211	0.646
Female	42 (43.75%)	621 (46.17%)		

Graph 11: Cluster bar chart of comparison of gender between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

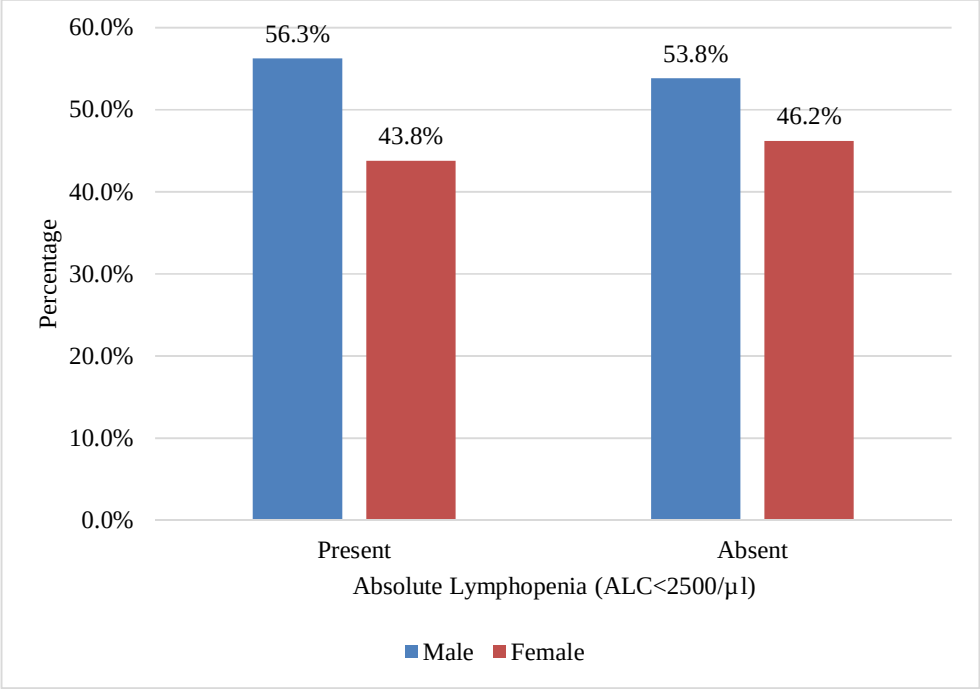


Table 24: Comparison of Consanguinity between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

Consanguinity	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	Present (N=96)	Present (N=1345)		
Present	42 (43.75%)	140 (10.41%)	90.265	<0.001
Absent	54 (56.25%)	1205 (89.59%)		

Graph 12: Cluster bar graph of comparison of consanguinity between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

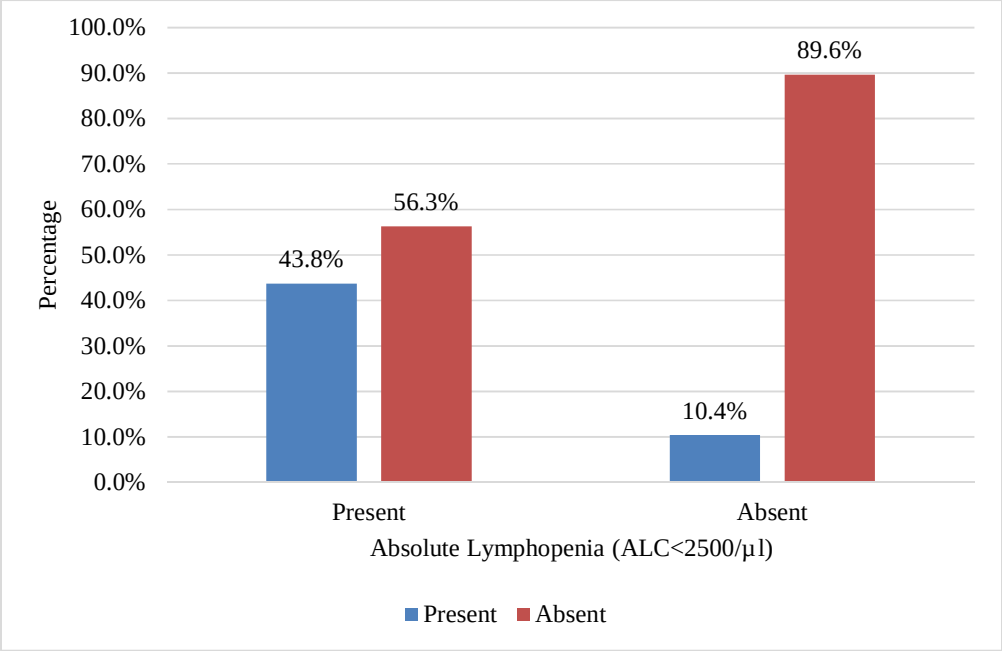


Table 25: Comparison of mode of delivery between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

Mode Of Delivery	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	Present (N=96)	Absent (N=96)		
Normal Vaginal Delivery	36 (37.5%)	585 (43.49%)	1.313	0.252
LSCS	60 (62.5%)	760 (56.51%)		

Graph 13: Cluster bar graph of comparison of mode of delivery between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

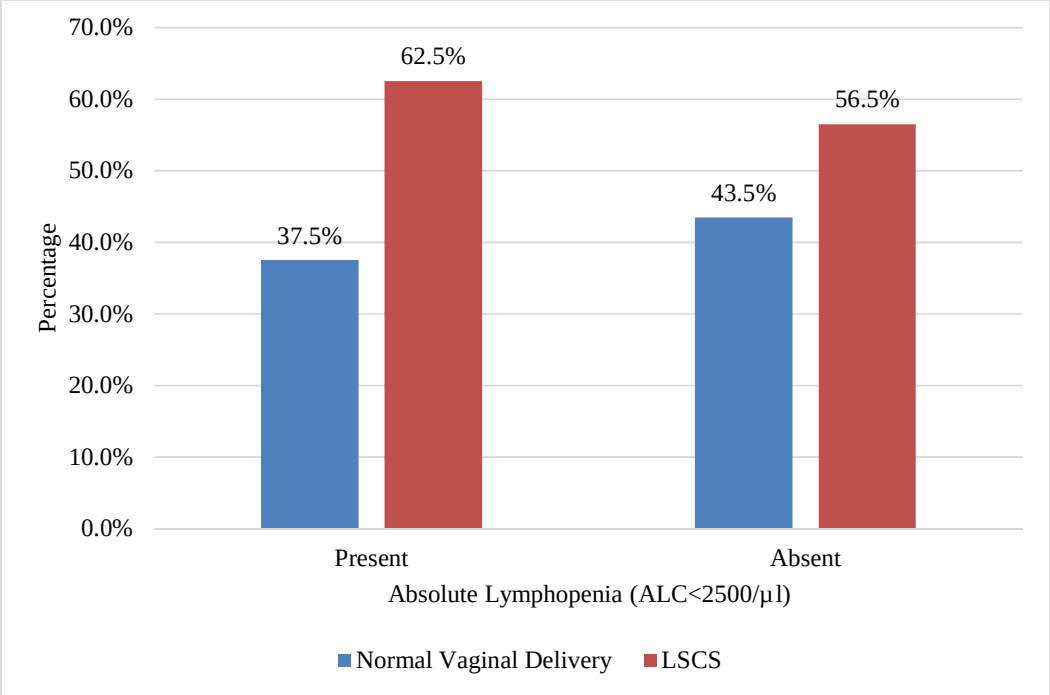


Table 26: Comparison of requirement of neonatal resuscitation (bag & mask) between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

Neonatal Resuscitation Required - Bag & Mask	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	Present (N=96)	Absent (N=1345)		
Yes	27 (28.13%)	67 (4.98%)	78.709	<0.001
No	69 (71.88%)	1278 (95.02%)		

Graph 14: Cluster bar graph of comparison of requirement of neonatal resuscitation (bag & mask) between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

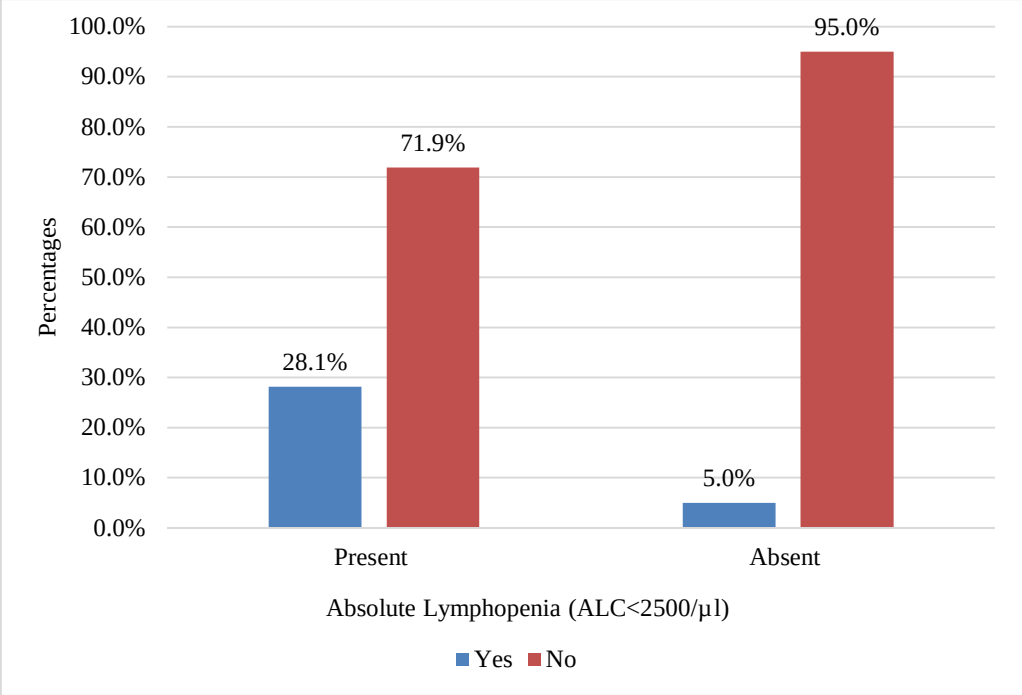


Table 27: Comparison of mean gestational age (in weeks) and birth weight (kg) between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

Parameter	Absolute Lymphopenia (ALC<2500/µl) (Mean± SD)		P value
	Present (N=96)	Absent (N=1345)	
Gestational Age (in weeks)	35.78 ± 2.3	37.54 ± 1.62	<0.001
Birth Weight (kg)	2.3 ± 0.49	2.73 ± 0.48	<0.001

Graph 15: Line chart for comparison of mean of gestational age (in weeks) and Birth Weight (kg) between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

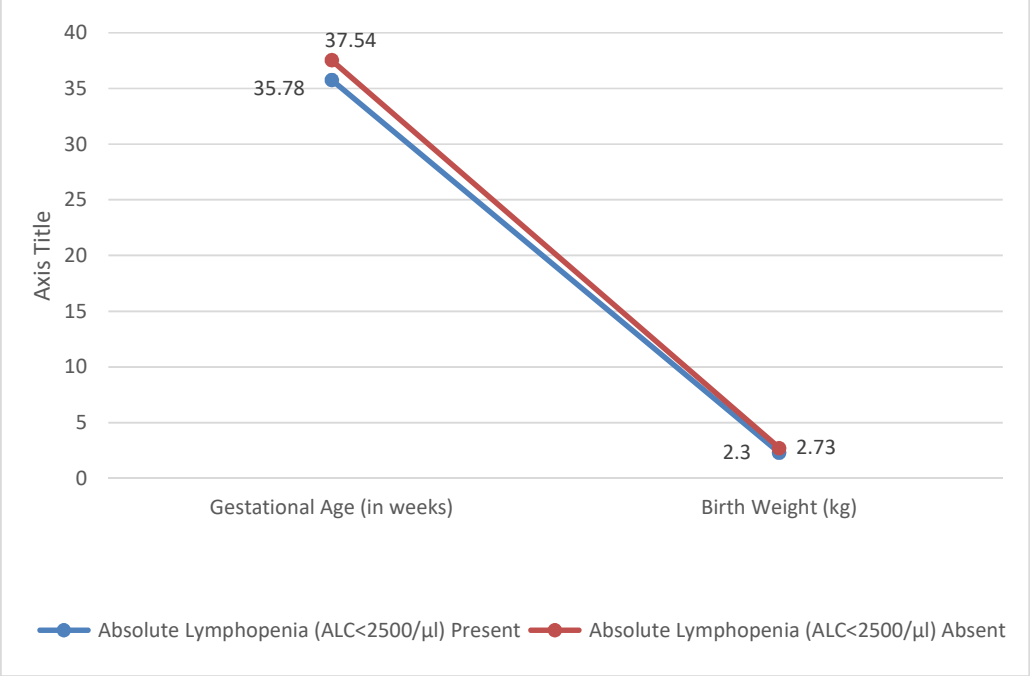


Table 28: Comparison of mean APGAR Score between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

APGAR Score	Absolute Lymphopenia (ALC<2500/µl) (Mean± SD)		P value
	Present (N=96)	Absent (N=1345)	
At 1 min	5.68 ± 1.39	6.73 ± 1.07	<0.001
At 5 min	8.22 ± 1.08	8.76 ± 0.79	<0.001

Graph 16: Line chart for comparison of mean of APGAR Score between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

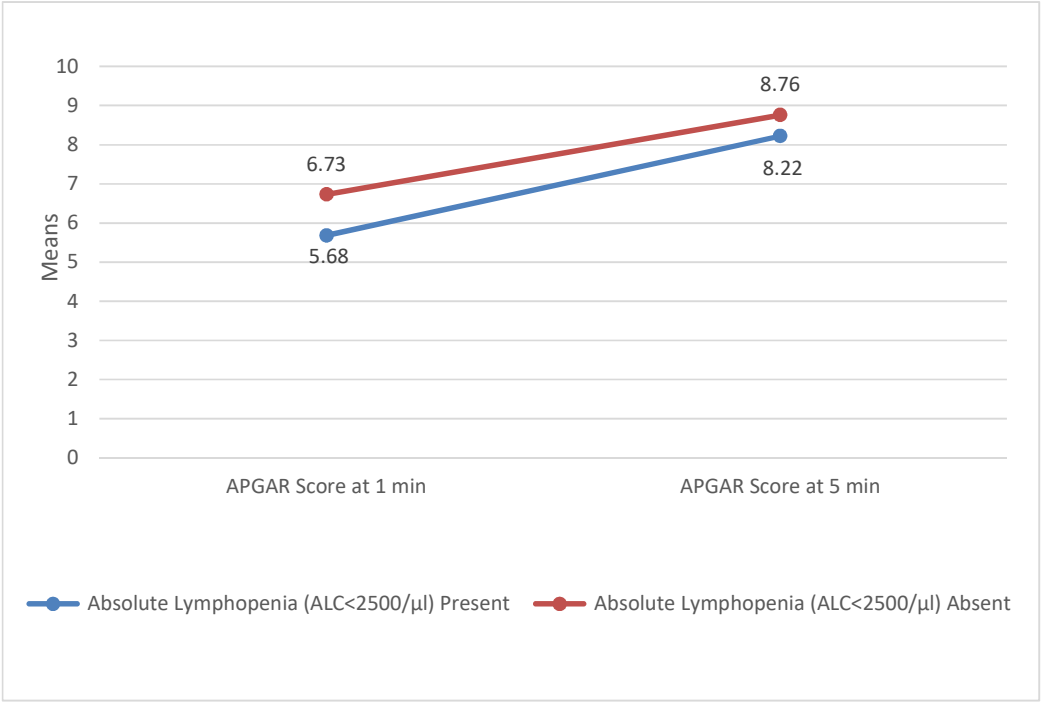


Table 29: Comparison of dysmorphic features between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

Dysmorphic Features	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	Present (N=96)	Absent (N=1345)		
Present	17 (17.71%)	13 (0.97%)	123.201	<0.001
Absent	79 (82.29%)	1332 (99.03%)		

Graph 17: Cluster bar graph of comparison of dysmorphic features between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

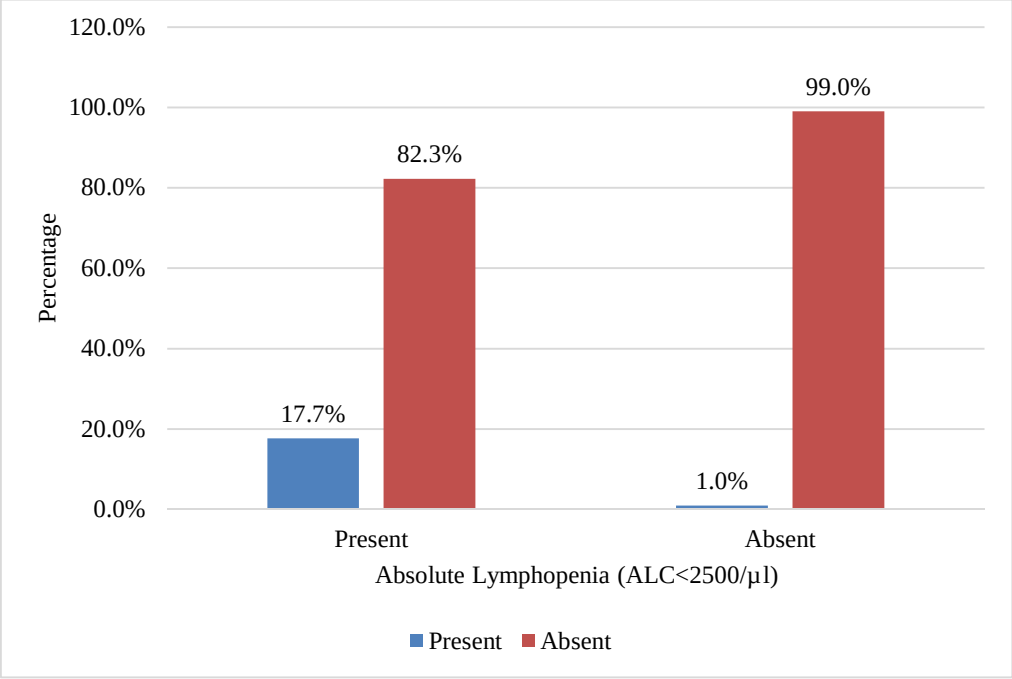


Table 30: Comparison of positive family history / sibling death between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

Positive Family History / Sibling Death	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	Present (N=96)	Absent (N=1345)		
Yes	10 (10.42%)	7 (0.52%)	75.273	<0.001
No	86 (89.58%)	1338 (99.48%)		

Graph 18: Cluster bar chart of comparison of positive family history / sibling death between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

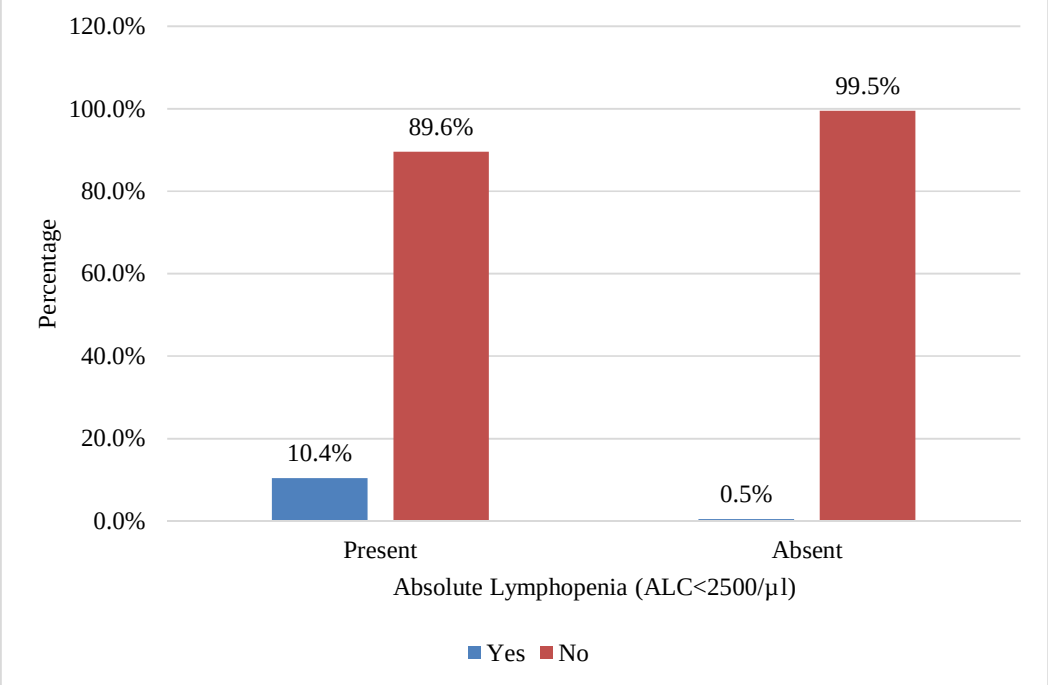


Table 31: Comparison of mean laboratory parameters between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

Lab Parameter	Absolute Lymphopenia (ALC<2500/µl) (Mean± SD)		P value
	Present (N=96)	Absent (N=1345)	
Haemoglobin (gm%)	13.75 ± 2.28	14.98 ± 2.02	<0.001
WBC count (x10³/µl)	10.94 ± 3.38	15.22 ± 3.47	<0.001
ANC (x10³/µl)	8.16 ± 2.95	9.68 ± 2.93	<0.001
ALC (x10³/µl)	1.88 ± 0.37	4.64 ± 1.76	<0.001

Graph 19: Line chart for comparison of mean laboratory parameters between lymphopenic (N=96) and non-lymphopenic cases (N=1345)

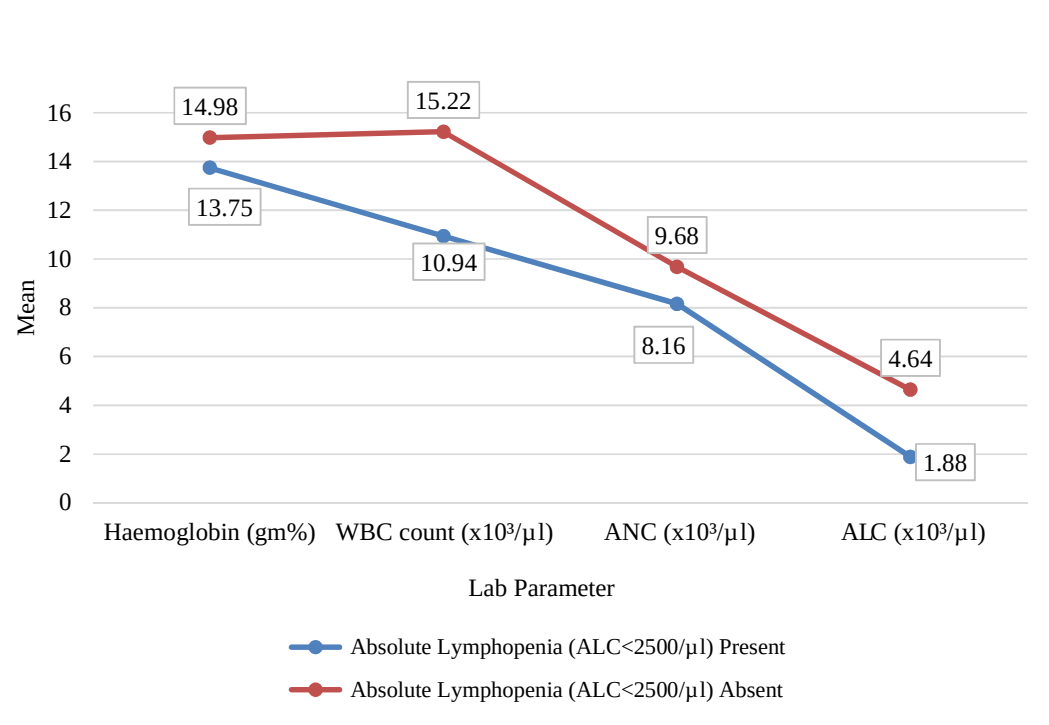


Table 32: Comparison of mean parameters in flow cytometry reports (Lymphocyte subset analysis) (N=12)

Parameter	Flow cytometry (LSSA) (Mean± SD)		P value
	Suspected SCID Pattern (N=4)	Normal Pattern (N=8)	
WBC count x10³/µl	698 ± 160.75	2040.38 ± 600.92	0.002
Absolute Lymphocyte Count (ALC) x10³/µl	698 ± 160.75	2040.38 ± 600.92	0.002
Lymphocyte%	24 ± 7.53	33.25 ± 6.36	0.049
B cells (CD19+)	61 ± 47.38	160.63 ± 125.19	0.163
T Cells (CD3+)	543.5 ± 257.37	1579.75 ± 457.8	0.002
T cells (CD4+)	380.5 ± 175.3	1056.25 ± 305.98	0.002
T cells (CD8+)	141.5 ± 103.42	453.5 ± 166.11	0.007
NK cells (CD3- CD16/56+)	76 ± 78.56	225 ± 189.59	0.170

Graph 20: Line chart for comparison of mean of parameters of lymphocyte subset analysis among babies that have undergone flow cytometry (N=12)

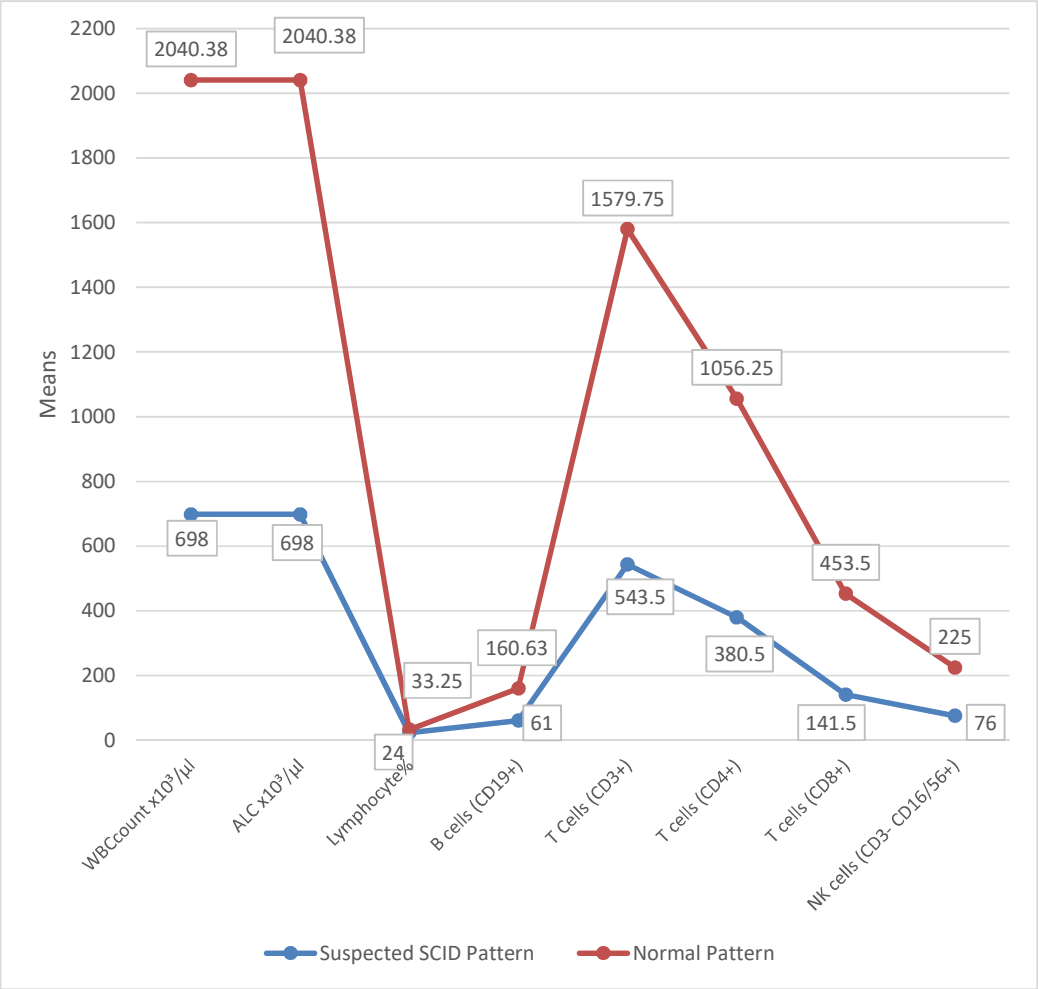


Table 33: Descriptive analysis of level of ALC (x10³/µl) in the study population (N=1441)

ALC (x10³/µl)	Frequency	Percentages
<1.5	16	1.11%
1.5-2.5	125	8.67%
2.5-5	872	60.51%
5-10	412	28.59%
>10	16	1.11%

Graph 21: Bar chart of ALC (x10³/µl) in the study (N=1441):

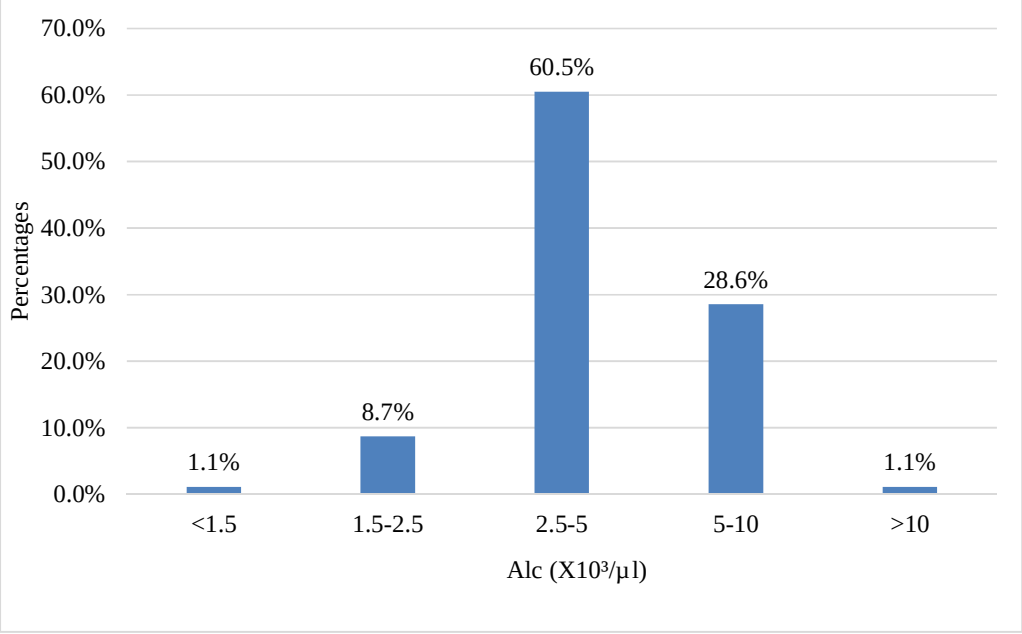


Table 34: Comparison of gender in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Gender	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)		
Male	46 (54.76%)	8 (66.67%)	0.605	0.437
Female	38 (45.24%)	4 (33.33%)		

Graph 22: Cluster bar chart of comparison of gender in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

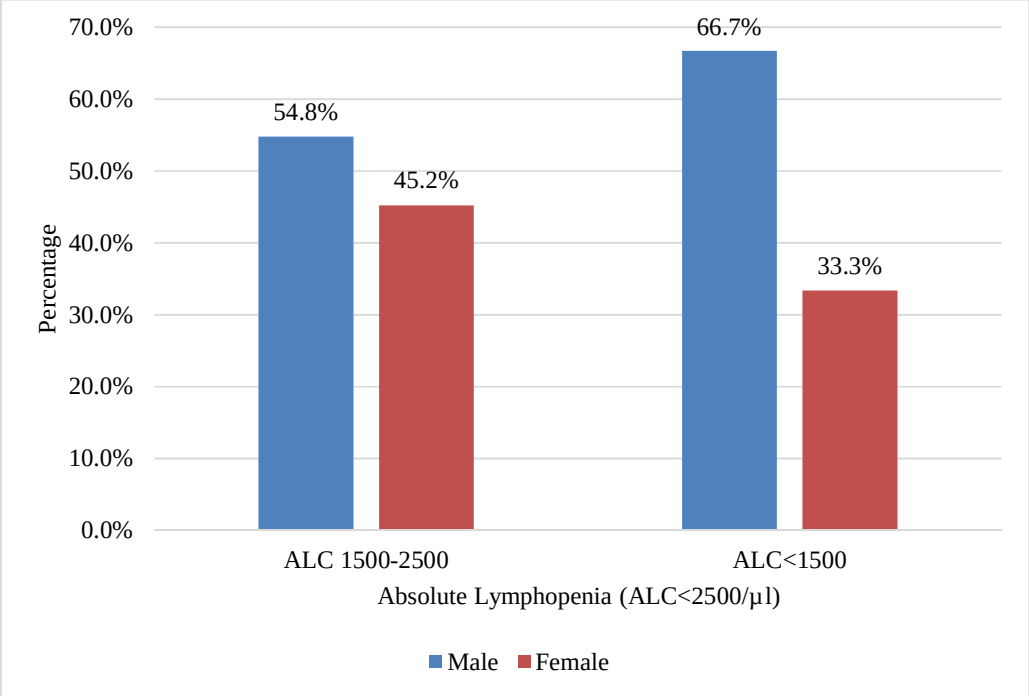


Table 35: Comparison of consanguinity in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Consanguinity	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)		
Present	35 (41.67%)	7 (58.33%)	1.185	0.276
Absent	49 (58.33%)	5 (41.67%)		

Graph 23: Cluster bar chart of comparison of consanguinity in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

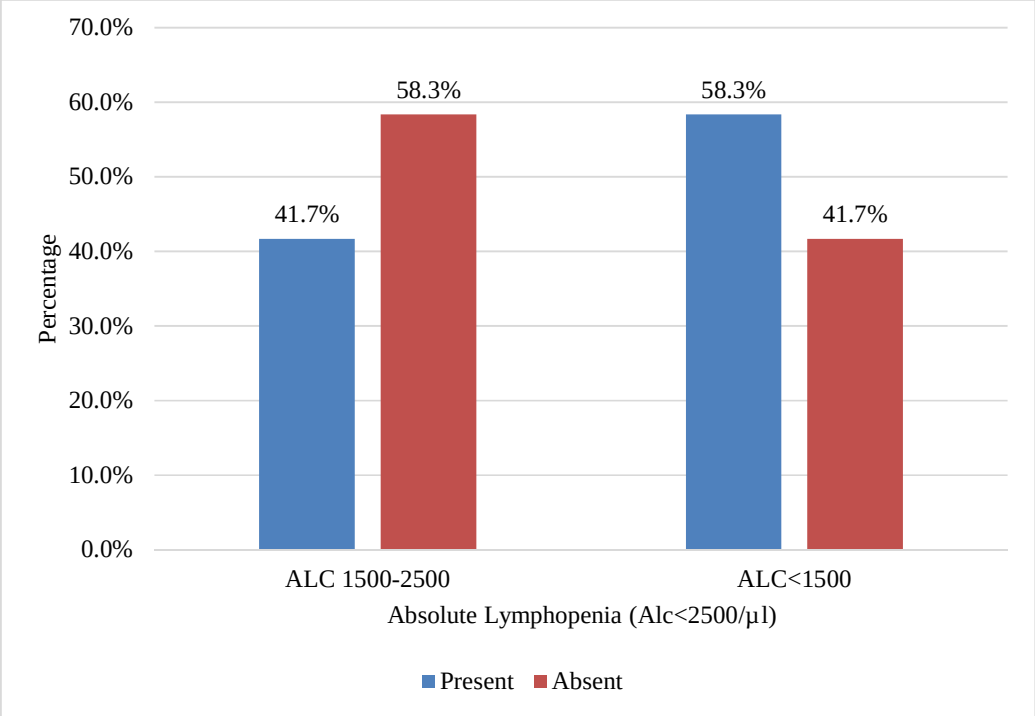


Table 36: Comparison of mode of delivery in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Mode Of Delivery	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)		
Normal Vaginal Delivery	34 (40.48%)	2 (16.67%)	2.540	0.201
LSCS	50 (59.52%)	10 (83.33%)		

Graph 24: Cluster bar chart of comparison of mode of delivery in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

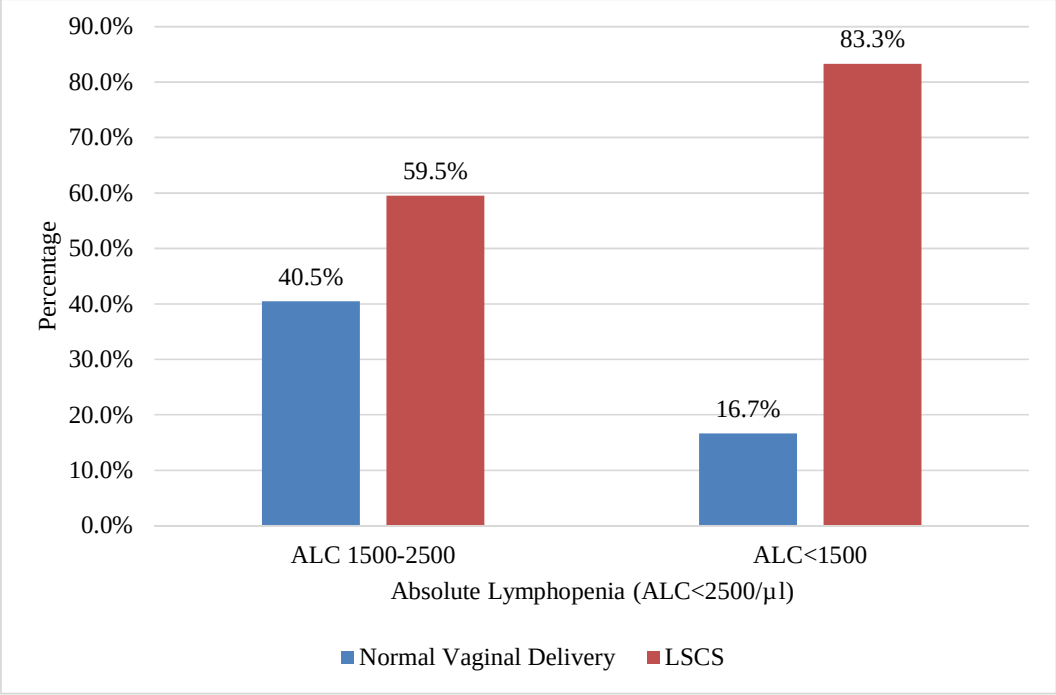


Table 37: Comparison of requirement of neonatal resuscitation (bag & mask) in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Neonatal Resuscitation Required - Bag & Mask	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)		
Yes	18 (21.43%)	9 (75%)	14.907	<0.001
No	66 (78.57%)	3 (25%)		

Graph 25: Cluster bar chart of comparison of neonatal resuscitation required - bag & mask in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

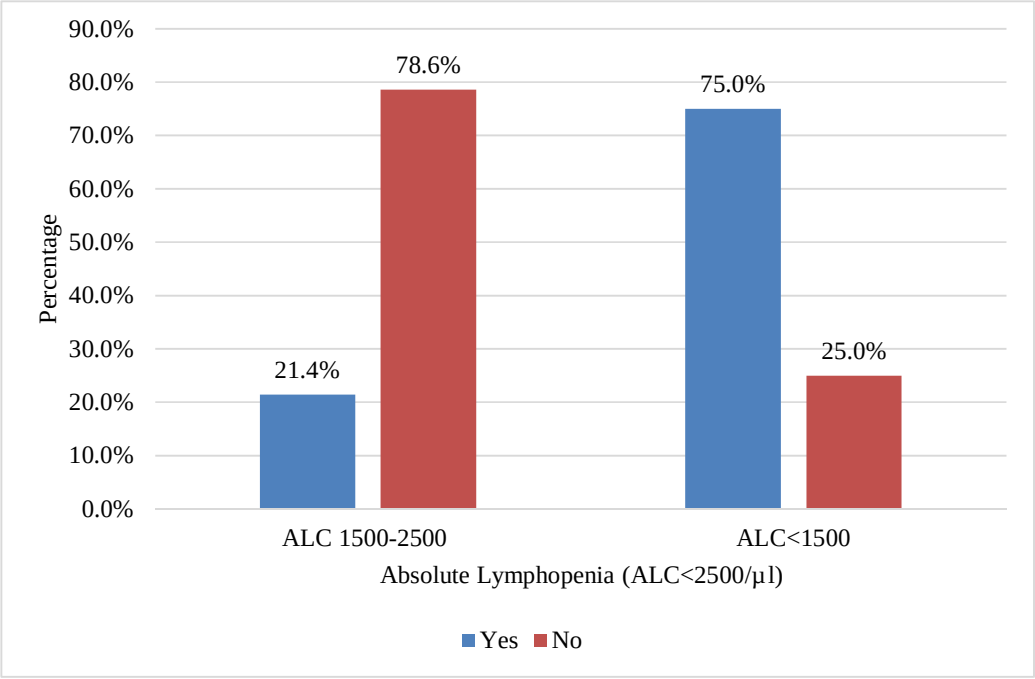


Table 38: Comparison of mean gestational age (in weeks) and Birth Weight (kg) in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Parameter	Absolute Lymphopenia (ALC<2500/µl) (Mean± SD)		P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)	
Gestational Age (in weeks)	36.02 ± 2.28	34.08 ± 1.78	0.006
Birth Weight (kg)	2.36 ± 0.49	1.9 ± 0.25	0.002

Graph 26: Line chart for comparison of mean gestational age (in weeks) and Birth Weight (kg) in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

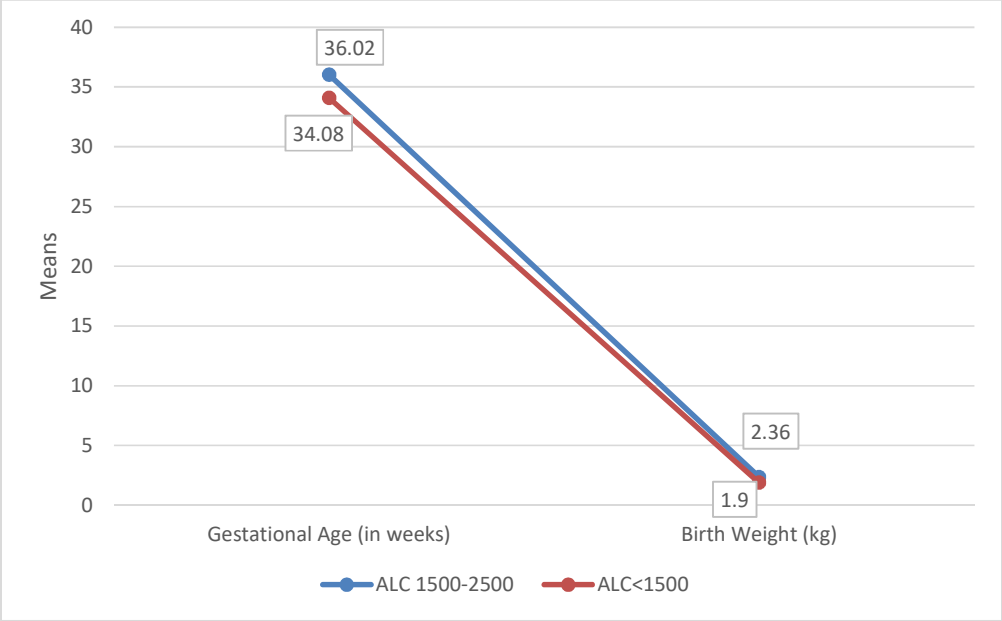


Table 39: Comparison of mean APGAR Score between absolute lymphopenia cases - ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Apgar Score	Absolute Lymphopenia (ALC<2500/µl) (Mean± SD)		P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)	
At 1 min	5.85 ± 1.34	4.5 ± 1.17	0.001
At 5 min	8.27 ± 1.1	7.83 ± 0.83	0.187

Graph 27: Line chart for comparison of mean APGAR Score in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

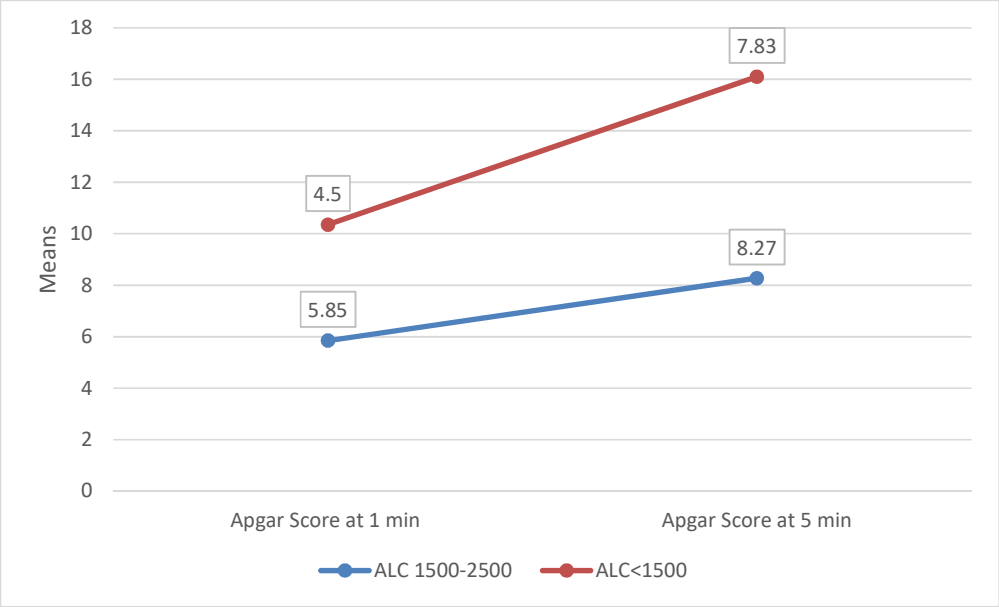


Table 40: Comparison of dysmorphic features in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Dysmorphic Features	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)		
Present	12 (14.29%)	5 (41.67%)	5.402	0.035
Absent	72 (85.71%)	7 (58.33%)		

Graph 28: Cluster bar chart of comparison of dysmorphic features between absolute lymphopenia (ALC<2500/µl) (N=96)

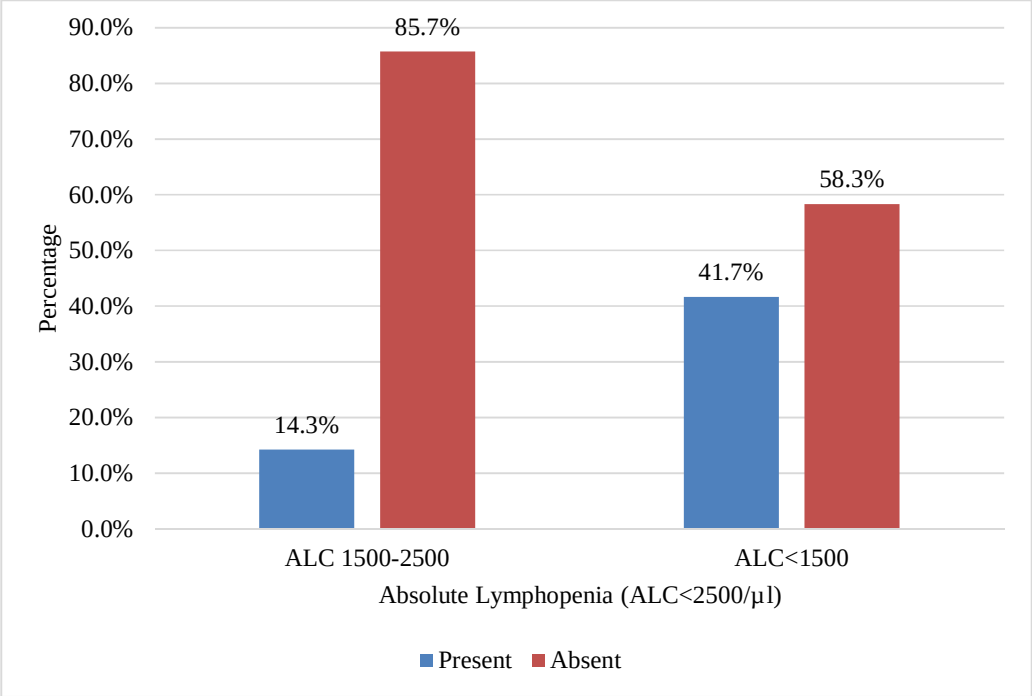


Table 41: Comparison of positive family history / sibling death in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Positive Family History / Sibling Death	Absolute Lymphopenia (ALC<2500/µl)		Chi square	P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)		
Yes	4 (4.76%)	6 (50%)	23.027	<0.001
No	80 (95.24%)	6 (50%)		

Graph 29: Cluster bar chart of comparison of positive family history / sibling death in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

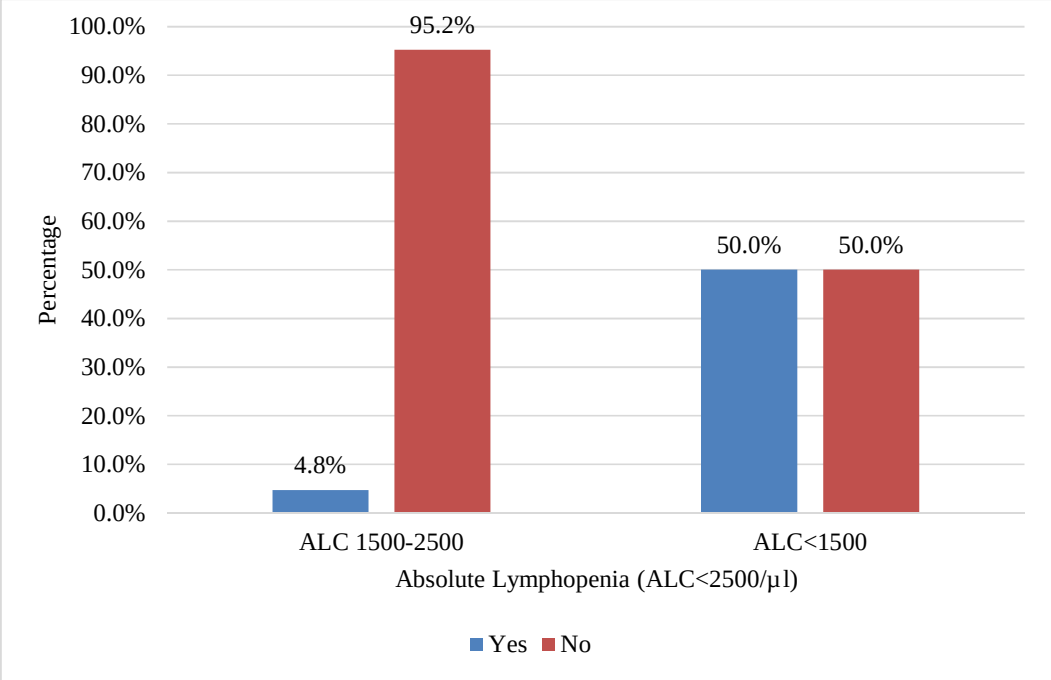


Table 42: Comparison of mean laboratory parameters in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

Parameter	Absolute Lymphopenia (ALC<2500/µl) (Mean± SD)		P value
	ALC 1500-2500 (N=84)	ALC<1500 (N=12)	
Haemoglobin (gm%)	13.96 ± 2.28	12.27 ± 1.74	0.015
WBC count (x10³/µl)	11.17 ± 3.31	9.36 ± 3.61	0.082
ANC (x10³/µl)	8.26 ± 2.91	7.48 ± 3.34	0.399
ALC (x10³/µl)	1.98 ± 0.26	1.14 ± 0.16	<0.001

Graph 30: Line chart for comparison of mean laboratory parameters in absolute lymphopenia cases – ALC 1500-2500/µl (N=84) v/s ALC<1500/µl (N=12)

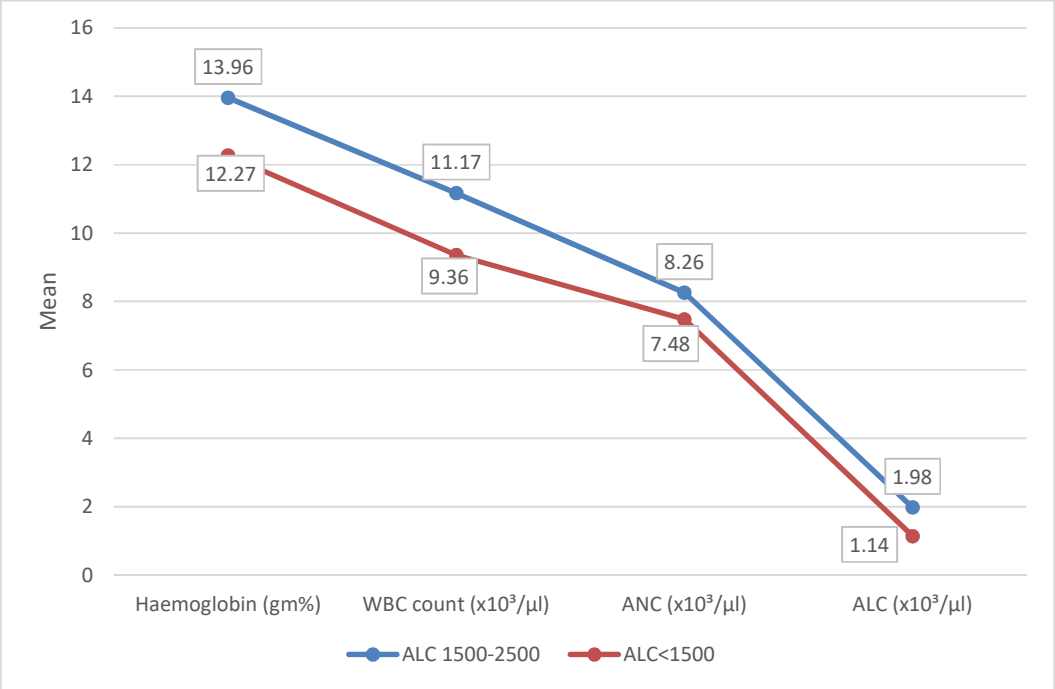
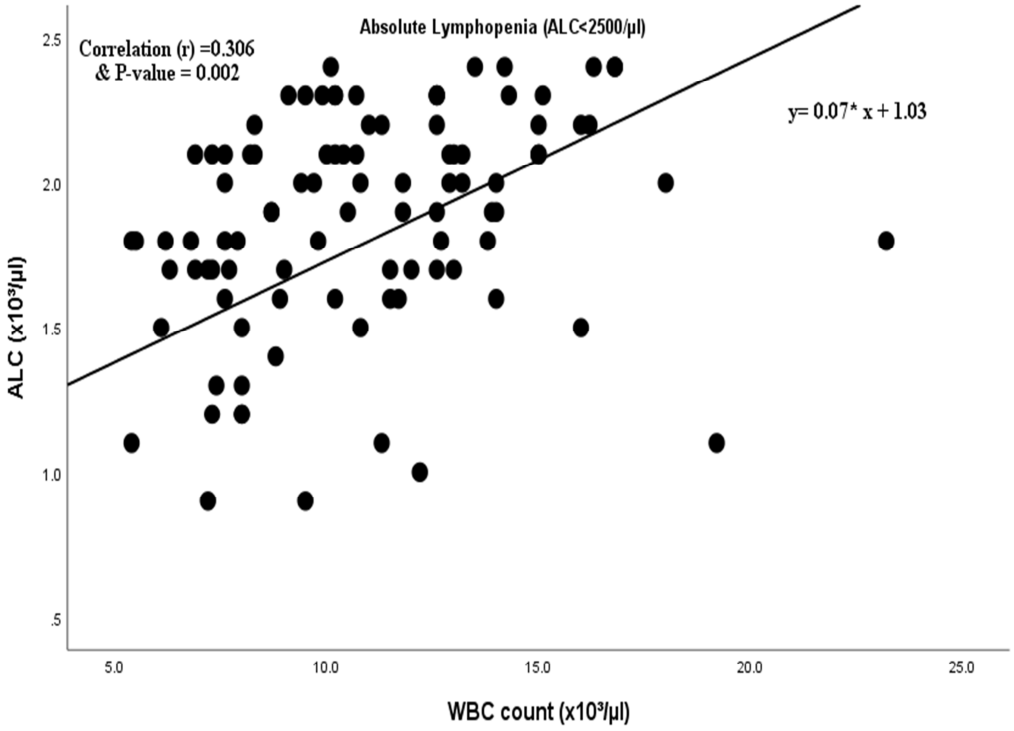


Table 43: Correlation between ALC with WBC, birth weight, gestational age and APGAR score in lymphopenic v/s non- lymphopenic population:

Pairs	Pearson's Correlation (r)	P-value
Absolute Lymphopenia (ALC<2500/µl) (N=96)		
ALC with WBC	0.306	0.002
ALC with Birth weight	0.330	0.001
ALC with APGAR score at 1 min	0.414	<0.001
ALC with APGAR score at 5 min	0.318	0.002
ALC with Gestational Age	0.311	0.002
Non-Lymphopenia (ALC<2500/µl) (N=1345)		
ALC with WBC	0.487	<0.001
ALC with Birth weight	0.040	0.145
ALC with APGAR score at 1 min	-0.026	0.343
ALC with APGAR score at 5 min	0.021	0.440
ALC with Gestational Age	0.047	0.084

Graph 31: Scatterplot for Correlation between ALC with WBC in absolute lymphopenia (ALC<2500/µl) population (N=96)



In babies with Absolute Lymphopenia (ALC < 2500/µl, N=96), there are statistically significant moderate positive correlations between ALC and various clinical parameters. Specifically, ALC shows a correlation of 0.306 with WBC (P=0.002), 0.330 with birth weight (P=0.001), 0.414 with APGAR score at 1 minute (P<0.001), 0.318 with APGAR score at 5 minutes (P=0.002), and 0.311 with gestational age (P=0.002). These correlations indicate that higher ALC levels are associated with higher WBC counts, greater birth weights, better initial health status (as measured by APGAR scores), and older gestational age in this group. The significance of these correlations suggests that ALC might be an important marker for assessing the overall health and development in infants with lymphopenia.

In contrast, in babies without Lymphopenia (ALC ≥ 2500/µl, N=1345), the only significant correlation observed is between ALC and WBC, with a correlation of 0.487 (P<0.001), showing a moderate to strong positive relationship. The correlations of ALC with birth weight (r=0.040, P=0.145), APGAR score at 1 minute (r=-0.026, P=0.343), APGAR score at 5 minutes (r=0.021, P=0.440), and gestational age (r=0.047, P=0.084) are very weak and statistically insignificant. This suggests that, in the absence of lymphopenia, ALC does not appear to be a strong indicator of these clinical outcomes. The significant correlation with WBC indicates a consistent relationship between lymphocyte count and overall white blood cell count regardless of lymphopenia status**.**

DISCUSSION:

Severe Combined Immunodeficiency Disease (SCID) is an ideal disorder which qualifies for newborn screening. Many nations have already adopted the TREC test for SCID screening. The TREC assay has a high sensitivity and specificity for identifying newborns with SCID. However, adopting the TREC test in India presents concerns in terms of cost-effectiveness, intervention expenses, and availability of transplantation facilities. Furthermore, running the assay centrally on Guthrie's cards would delay results by 4-6 weeks, risking missed follow-up and inadequate care of live vaccine-related complications.

An alternate method is to do a complete blood count (CBC) on cord blood samples. According to a 1990 study, the absolute lymphocyte count was the most effective screening diagnostic test since lymphopenia occurs in almost all patients with SCID from birth. [169] The CBC identifies lymphopenic patients and offers additional information such as the Absolute Lymphocyte Count (ALC), Hemoglobin (Hb), and platelet counts. Although CBC has limitations (false negatives and positives), it is widely available, simple to use, and yields quick results. We can avoid vaccination-related complications and contribute in the early diagnosis of SCID by carefully reviewing CBC findings, particularly the ALC, prior to administering live vaccine. Therefore, we recommend routine CBC testing on cord blood samples. Lymphopenia (ALC<2500/µl) on CBC should prompt further evaluation for SCID, with a cautious approach to live vaccine administration until the final diagnosis is confirmed. In India, the management of Severe Combined Immunodeficiency (SCID) remains challenging due to very limited transplant centres, ultimately leading to unsatisfactory treatment outcomes with substantial financial and emotional burdens

for affected families and society. Consequently, genetic counselling becomes crucial for families impacted by SCID.

Research conducted by Buckley et al at Duke University compared lymphocyte counts in newborns with SCID to those in healthy infants at birth. The findings revealed that a majority of SCID patients exhibited low absolute lymphocyte counts, with counts ranging from 114 to 2210 lymphocytes/mm3, compared to 1670 to 8910 lymphocytes/mm3 in healthy newborns. Notably, many SCID patients had previous blood tests indicating lymphopenia, although healthcare providers often did not recognize its significance. It's important to note that while lymphopenia is common in SCID, not all lymphopenic children have SCID, and some SCID patients may present with normal absolute lymphocyte counts due to the presence of B cells (IL2RG, JAK3 and IL7R gene defects) or maternal lymphocytes. [170, 171]

Notably, pilot trials in Wisconsin (44 months), Massachusetts (31 months), and New York (11 months) examined neonates for SCID and T-cell lymphopenia. Wisconsin detected four SCID cases and seven unrelated T-cell lymphopenia cases among 243,707 neonates. Among 161,707 neonates, Massachusetts detected one SCID case and 14 unrelated T-cell lymphopenia cases. In New York, four SCID instances and twelve unrelated T-cell lymphopenia cases were discovered among 136,635 neonates. Treatment, such as hematopoietic stem cell transplantation or enzyme replacement therapy, was administered to the diagnosed cases, resulting in survival in most of these cases. [171, 172, 173] In California, newborn screening for severe combined immunodeficiency (SCID) has been ongoing since 2010. During this span of time, 358,000 babies were screened, yielding five cases of SCID, six cases of variant SCID, and three cases of T-cell lymphopenia unrelated to SCID. All infants

identified with SCID received adequate treatment and are currently thriving. [172] Furthermore, pilot researches in Puerto Rico, Louisiana, and the Navajo Nation have found no SCID instances, but four cases of T-cell lymphopenia unrelated to SCID among a total of slightly more than 60,000 examined neonates. Notably, the TREC test employed for SCID screening appears to be very sensitive, with no further SCID patients overlooked during the pilot studies. This screening strategy identified several other problems associated with T-cell lymphopenia, such as DiGeorge syndrome, idiopathic T-cell lymphopenia and trisomy 21. [172]

Absolute lymphopenia in cord blood was defined as absolute lymphocytic count of less than 2500/µL. [10, 174] **In the present study, absolute lymphopenia (ALC<2500/µl) was observed in 6.67% (96/1441) of neonates screened from the cord blood at delivery, while 93.33% (1345/1441) of them had ALC>2500/µl.** All lymphopenic cases were monitored and instructed not to administer the BCG vaccine.

In a study done by Akyut Poyrat et al where 2000 newborns were screened, absolute lymphopenia was detected in 2.1% (42/2000) newborns which is lower as compared to our study. [175] In a study done in Birmingham by Krishna MT et al, 3% of infants under 3 months of age were found to have absolute lymphopenia. [176] A study done in 2018 by Can Ceren et al in Turkey on 2945 term neonates observed absolute lymphopenia in only 9 (0.3%) neonates. [177] In an Egyptian study done by El-Sayed et al, absolute lymphopenia was found in 1.6% (8/500) neonates at delivery. [35] Multiple other studies in the western world have used the TREC assay for screening of SCID in newborns.

In our study, absolute lymphopenia (ALC<2500/µl) was observed in 6.67% (96/1441) of newborns screened for SCID from the cord blood. This is greater than the incidence of absolute lymphopenia in most other studies which can probably be linked to a higher rate of consanguinity present in the northern Karnataka region.

The present screening study with a sample size of 1550 newborns showed that 54.26% (841) of cases were males and 45.74% (709) were females. In an Egyptian study done by El-Sayed et al on 500 newborns, there were 248 (49.6%) males and 252 (50.4%) were females. [35] Can Ceren et al's study from Turkey had 55% (1614) female and 45% (1322) male babies. [177] In the current study, out of a total of 96 cases, absolute lymphopenia was seen in 54 (56.25%) male and 42 (43.75%) female babies, indicating a male predominance. In a study done by El-Sayed et al, absolute lymphopenia was found to be seven times higher in female babies as compared to male babies. [35] Can Ceren et al's study from Turkey also revealed a higher female predominance as 77.7% (7/9) females and 22.3% (2/9) were found to be lymphopenic. [177] This was contradictory to our study since more male babies were affected, owing to an overall greater number of male births. However, a study based on SCID genotypic and phenotypic diversity by Rebecca et al in Maryland, USA discovered that 82.4 % (89/108) males and 17.6% (19/108) females were affected with SCID [103] which was in concordance with our study. A study on 100 SCID patients by Jennifer et al revealed 61% males and 39% females, indicating a male predominance which is consistent with our study. [178]

Consanguineous marriages, which involve union between close relatives, can elevate the risk of inheriting autosomal recessive disorders and multifactorial

diseases. Most PIDs are inherited in an autosomal recessive pattern; thus, they are more common in areas with high rates of consanguineous marriage. [179] This heightened risk of both physical and mental health conditions in offspring underscores the significant public health implications associated with consanguinity. The prevalence of consanguineous marriages within a population is influenced by various factors including demographics, social dynamics, cultural norms, and religious practices, thereby leading to fluctuations in its frequency worldwide. While consanguineous marriages are rare in the United States and many European nations, they remain a prevalent practice within family systems in the Middle East and certain Asian regions. [180] Research indicates a wide-ranging prevalence of consanguinity among Muslim populations in the Middle East, ranging from 20% to over 70 percent. [180, 181] Consanguineous marriages are prevalent among the Omani population, with a reported rate of nearly 49%. This practice has contributed to a high frequency of congenital genetic diseases in the country. Specifically, autosomal recessive conditions causing inborn error of immunity (IEI) have become increasingly burdensome. In 2016, AL Tamimi et al. estimated the prevalence of primary immunodeficiency (PID) in the Omani population at 7.0 cases per 100,000 live births. However, their study identified only 10 out of 140 children with SCID, suggesting the possibility of suboptimal ascertainment. [182] In an Iranian study done by Rezaei et al, it was found that consanguinity is most commonly seen in patients with autosomal recessive inheritance such as Chediak-Higashi syndrome (100%), SCID (85.7%), ataxia-telangiectasia (60%), Shwachman-Diamond syndrome (71.4%), chronic granulomatous disease (76.4%) and chronic mucocutaneous candidiasis (61.5%). [183] A study from India done by Aluri et al report that 36% of diagnosed SCID patients belonged to consanguineous parents. [57] In this study, consanguinity was found to be

present in 191 (12.32%) cases and absent in the remaining 1359 (87.68%) cases. Consanguinity was present in a staggering 43.75% (42/96) of babies with absolute lymphopenia (ALC<2500 µl) which was in concordance to other similar studies. While it was present in only 10.41% (140/1345) of non-lymphopenic babies (p<0.001).

A total of 670 (43.23%) babies were born via normal vaginal delivery and 880 (56.77%) babies were delivered by caesarean section. In this study, 36 (37.5%) babies were delivered normally and 60 (62.5%) were delivered via a caesarean section, owing to an overall higher number of caesarean deliveries (p=0.252). A screening study by El-Sayed et al reports that 264 (52.8%) neonates were delivered normally and 236 (47.2%) were delivered via an LSCS. [35] Can Ceren et al's study states that 1685 (57.3%) babies were delivered vaginally and 1251 (42.7%) by caesarean section. In the lymphopenic group, 55.5% babies were delivered vaginally and 44.5% by caesarean section. [177]

Active neonatal resuscitation (bag and mask ventilation) was required by 108 (6.97%) newborns in this study. There was a significant increase in the requirement of active neonatal resuscitation (with bag and mask) in the lymphopenic group with a whopping 28.13% (27/96) cases requiring the same, as against only 4.98% (67/1345) babies requiring it in the non-lymphopenic group (p<0.001). Can Ceren et al's study on term newborns reports 3.46% of total newborns requiring an NICU admission and one out of 9 lymphopenic babies requiring an NICU admission. [177]

The mean gestational age observed in our study population was 37.44 ± 1.74 weeks with a median of 38 weeks. The minimum and maximum gestational age being 32 and 41 weeks respectively. Babies born with a gestational age of less than 32

weeks were dropped from the study as per our exclusion criteria. In lymphopenic and non-lymphopenic groups, the mean gestational age (weeks) observed was 35.78 ± 2.3 and 37.54 ± 1.62 in the respectively ($p<0.001$). Thus, the mean gestational age of babies with lymphopenia was significantly lower than the gestational age of non-lymphopenic babies justifying prematurity as one of the causes of absolute lymphopenia. In a study by El-Sayed et al, the mean gestational age (weeks) of lymphopenic newborns was 34.3 ± 3.8 and of non-lymphopenic babies was 37.9 ± 2.6 which is in concordance with our study. [35] In the study by Can Ceren et al, the gestational age (weeks) of lymphopenic babies (38.07 ± 1.22) was also lower than that of babies not having lymphopenia (38.21 ± 1.61). The difference noted in this study was minor as it only included term babies with >37 weeks of gestational age. [177]

In our study, the overall mean birth weight was 2.71 ± 0.49kg and median was 2.7kg. While the minimum and maximum birth weight was found to be 1.3kg and 4.3kg respectively. Upon comparing the birth weight (Kg) of the babies in the two groups, we realised that the mean birth weight in the lymphopenic group was significantly lower 2.3 ± 0.49kg, i.e. “Low Birth Weight (LBW)” while the mean birth weight in the non-lymphopenic group was 2.73 ± 0.48kg (Normal weight) ($p<0.001$). A study by El-Sayed et al reports a mean birth weight of 2.1±1.0kg in lymphopenic babies and 3.1 ± 0.6kg in non-lymphopenic babies which is consistent with our study. [35] While Can Ceren et al’s study also reports a lower mean birth weight 3.06 ± 0.2kg in lymphopenic babies as against 3.25 ± 0.44kg in non-lymphopenic babies [177] which is also in harmony with our study.

The mean APGAR score (out of 10) in our study population was observed to be 6.61 ± 1.11 and 8.72 ± 0.81 at 1 and 5 minutes respectively. The median score being 7 and 9 at 1 and 5 minutes respectively. The minimum APGAR score was 2 and 5 at 1 and 5 minutes respectively, whereas the maximum score was 9 and 10 at 1 and 5 minutes respectively. The comparison in the mean APGAR scores at 1 and 5 minutes revealed a statistically significant correlation between lymphopenic and non-lymphopenic groups as the cases having lymphopenia were found to have lower APGAR scores at both 1 and 5 minutes. The mean APGAR score at 1 minute was 5.68 ± 1.39 and 6.73 ± 1.07 in the lymphopenic and non-lymphopenic groups respectively ($p<0.001$). While the mean APGAR score at 5 minutes was 8.22 ± 1.08 and 8.76 ± 0.79 in the lymphopenic and non-lymphopenic groups respectively ($p<0.001$). In the study conducted by El-Sayed et al, the mean APGAR score at 1 minute in lymphopenic and non-lymphopenic babies was 4.4 ± 1.0 and 5.4 ± 0.9 respectively. The mean APGAR score at 5 minutes in lymphopenic and non-lymphopenic babies was 7.6 ± 0.7 and 8.6 ± 0.6 respectively which is in alignment with our study. [35] However, the Apgar scores at one and five minutes in lymphopenic and non-lymphopenic babies did not differ significantly in Can Ceren et al's study which is incongruent to our study. [177]

Dermatologists frequently play a significant role in the early diagnosis of primary immunodeficiency disorders. Skin manifestations, including eczematous dermatitis, warts, recurrent nonhealing ulcers, skin abscesses, erythroderma, mucocutaneous candidiasis, petechiae, cutaneous viral infections such as disseminated molluscum contagiosum and nail changes may serve as initial or predominant clinical indicators of PIDs. [184] Eczema, a prevalent skin disorder affecting approximately 10–20% of children, is commonly encountered in pediatric dermatology outpatient

departments. Moreover, eczema often emerges as a primary clinical manifestation in many PIDs. SCID typically manifests within the initial six months of life with significant bacterial, viral, and fungal infections. Common clinical presentations include pneumonia, otitis media, recurrent ulcers, persistent diarrhoea, sepsis, oral thrush, and meningitis. Notably, in the event of receiving an irradiated blood transfusion, affected children may develop a diffuse erythematous maculopapular rash across the body, often accompanied by frequent scaling and alopecia. It is imperative to consider a diagnosis of SCID in infants experiencing such a reaction post-blood product transfusion. A common clinical presentation of Omenn syndrome, a subtype of SCID includes erythroderma, skin peeling with hair loss. [185] In this study, dysmorphic features in the form of facial abnormalities, short limbs, microcephaly, recurrent ulcers or skin infections were seen in 30 (1.94%) out of the 1550 screened newborns. A massive 17.71% (17/96) of lymphopenic cases showed dysmorphic features, while only 0.97% (13/1345) of non-lymphopenic babies had them ($p<0.001$), thus indicating a higher index of suspicion of PIDs in babies born with dysmorphic features. A study done by El-Sayed et al states that the presence of dysmorphic features did not show a significant variation among the lymphopenic and non-lymphopenic groups which is in contrast with our study. [35]

Family history of sibling death was present in 17 (1.10%) and absent in the remaining 1533 cases in our study. After eliciting a detailed history, it was found that a family history of sibling death was present in a total of 10.42% (10/96) lymphopenic cases and only 0.52% (7/1345) of the non-lymphopenic cases, revealing a significant difference ($p<0.001$), thus unmasking the importance of good history taking. A study from Maryland, USA done by Rebecca et al on 108 SCID infants showed a positive family history of previously affected family members in 9 cases (8.3%) along with

multiple sets of twins being affected. [103] While the study by El-Sayed et al did not show a significant variation in the family history of lymphopenic and non-lymphopenic cases. [35]

The laboratory parameters in CBC that were compared in our study (1441 babies) include the haemoglobin level, WBC count, Absolute Neutrophil Count (ANC) and Absolute Lymphocyte Count (ALC). The mean haemoglobin level (gm%) in our study population was 14.9 ± 2.06 and the median being 14.9. The maximum and minimum haemoglobin level observed was 9.4 and 21.5. The mean haemoglobin level (gm%) calculated was 13.75 ± 2.28 and 14.98 ± 2.02 in the lymphopenic and non-lymphopenic group respectively ($p<0.001$). The mean WBC count ($x10^3/\mu l$) in our study population was 14.94 ± 3.62 and the median was found to be 14.7. The minimum and maximum WBC count encountered was 5.4 and 28.9. The mean WBC count ($x10^3/\mu l$) was 10.94 ± 3.38 and 15.22 ± 3.47 in the lymphopenic and non-lymphopenic group respectively. The mean absolute neutrophil count ($x10^3/\mu l$) was 9.58 ± 2.95 with the median being 9.6. The minimum and maximum ANC was 1.1 and 20.7 respectively. The mean ANC ($x10^3/\mu l$) in our study was 8.16 ± 2.95 and 9.68 ± 2.93 in the lymphopenic and non-lymphopenic group respectively. The mean absolute lymphocyte count ($x10^3/\mu l$) was 4.46 ± 1.84 and the median ALC was 4.2. The minimum and maximum ALC was found to be 0.9 and 14.5 respectively. The mean ALC ($x10^3/\mu l$) in our study was 1.88 ± 0.37 and 4.64 ± 1.76 in the lymphopenic and non-lymphopenic group respectively. Thus, all the laboratory parameters in CBC, i.e. haemoglobin level, WBC count, Absolute Neutrophil Count (ANC) and Absolute Lymphocyte Count (ALC) that were compared in our study (between lymphopenic v/s non-lymphopenic group) turned out to be statistically significant ($p<0.001$). In Can Ceren et al's study, the mean haemoglobin (gm%) in lymphopenic cases was

18.75 ± 2.06 and in non-lymphopenic cases was 18.70 ± 2.14. This study did not show a statistically significant difference in the levels of hemoglobin, red blood cell count, hematocrit, and platelets between the lymphopenic and non-lymphopenic groups. [177] However, the mean WBC count, absolute lymphocyte count as well as the absolute neutrophil count recorded in the lymphopenic cases was significantly lower than that of non-lymphopenic cases ($p<0.05$) which is in harmony with our study. The mean WBC count, ALC and ANC in lymphopenic cases was 12.09 ± 7.30, 2.09 ± 0.35 and 8.72 ± 3.27 respectively as against 22.25 ± 5.67, 4.32 ± 0.21 and 13.37 ± 4.50 respectively in the non-lymphopenic cases. [177] In the study done by El-Sayed et al, there was a positive correlation seen in the ALC and WBC count in the lymphopenic v/s non-lymphopenic groups ($p<0.05$) which was in concordance with our study. [35]

In this study, in babies with absolute lymphopenia (ALC < 2500/µl, N=96), it was observed that there are statistically significant moderate positive correlations between ALC and various clinical parameters. Specifically, ALC shows a correlation of 0.306 with WBC (P=0.002), 0.330 with birth weight (P=0.001), 0.414 with APGAR score at 1 minute (P<0.001), 0.318 with APGAR score at 5 minutes (P=0.002), and 0.311 with gestational age (P=0.002). These correlations indicate that higher ALC levels are associated with higher WBC counts, greater birth weights, better initial health status (as measured by APGAR scores), and older gestational age in this group. The significance of these correlations suggests that ALC might be an important marker for assessing the overall health and development in infants with lymphopenia. A significant positive correlation between ALC and WBC count was also elicited in two other studies done by El-Sayed et al and by Can Ceren-et al which is consistent with our study. [35, 177]

Other conditions contributing to absolute lymphopenia amongst the 96 cases are as follows. A total of 48 (50%) babies with absolute lymphopenia were found to preterm (gestational age <37 weeks). An additional 15 (15.63%) babies who were preterm also developed sepsis, whereas 5 (5.21%) term babies (gestational age >37 weeks) with absolute lymphopenia had sepsis. Congenital anomaly was associated with 5 (5.21%) babies. And no specific cause could be found for 23 (23.96%) lymphopenic babies which were labelled as 'idiopathic'. A statewide newborn screening study done in Wisconsin by John Routes et al on 71 thousand infants by TREC assay demonstrated 17 neonates born with <37 weeks gestation with atleast one abnormal TREC assay (25/µL). A total of 23 preterm infants had an abnormal TREC assay initially. The causes of T-cell lymphopenia were found to be idiopathic in 2 cases, disorders of lymphocyte extravasation in 3 cases and DiGeorge syndrome in 2 cases. [173] In the study conducted by Can Ceren et al, lymphopenia at birth was associated with sepsis in 11.1% (1/9) cases, respiratory distress in 11.1% (1/9) and congenital anomaly (spina bifida) in 11.1% (1/9) cases. Preterm babies were excluded in this study. [177] In El-Sayed et al's study, congenital anomaly was found to be present in 0.6% (3/500) cases screened, but did not show a positive correlation with absolute lymphopenia. [35]

The lymphopenic cases were further divided into two groups – those with ALC between 1500/µl - 2500/µl and those with ALC<1500/µl. The absolute lymphocyte count of 87.5% (84/96) lymphopenic cases was between 1500/µl to 2500/µl and the ALC of remaining 12.5% (12/96) cases was <1500/µl. These 84 cases whose ALC was between 1500/µl to 2500/µl were supposed to be followed up with a CBC to check their ALC after one month. However, 19 (22.62%) of these were either lost to follow up or expired or did not give consent for the same. The remaining 65

(77.38%) cases were followed up after one month with CBC to mainly check their ALC. Out of which 62 (95.39%) cases presented with a normal ALC (>2500/µl) on follow up, which was normal and no further intervention was done. This is line with a study done by El-Sayed et al where all the neonates having lymphopenia at birth had a normal ALC on one month follow up. [35] Can Ceren et al's study also did not show any lymphopenic baby having a low ALC count on one month follow up. [177] In a study by Poyraz et al in 2023, 2 out of 42 (4.76%) infants had persistent lymphopenia at the end of first month. [175] However, in our study 3 (4.61%) out of 65 cases had an ALC<2500/µl on follow up and were subjected to further testing with Flow Cytometry for Lymphocyte Subset Analysis as per our study protocol. The 12 cases whose ALC was <1500/µl at birth were directly subjected to further testing by Flow Cytometry for Lymphocyte Subset Analysis. But 3 (25%) out of these 12 cases were either lost to follow up or expired. Hence, we have presented the Flow Cytometry data of a total of 12 cases, i.e. 3 follow up cases (ALC<2500/µl after one month) plus 9 cases whose ALC was <1500/µl at birth. Out of these 12 cases who underwent flow cytometry for Lymphocyte subset analysis (LSSA), 8 (66.67%) cases were found to have a normal pattern while 4 (33.33%) cases showed a Suspected SCID pattern. For confirmation, Next Generation Sequencing (NGS)/ Whole Exome Sequencing (WES) was done for all these 4 cases whose flow cytometry showed a suspected SCID pattern. But none of them were finally proven to be diagnostic of SCID, probably due to the limitation of this test as described in the review of literature. In a study by Poyraz et al in 2023 published in the American Journal of Perinatology, two infants who exhibited persistent lymphopenia by the end of the first month, underwent lymphocyte subset analysis SCID. In the first baby, lymphocyte subgroup analysis revealed a phenotype consistent with T (−), B (−), and NK (+) SCID caused by a

RAG deficiency. Sanger sequencing detected a homozygous mutation (NM_000448 c.2209C > T, p.R737C) in the RAG1 gene. The second infant's lymphocyte subgroups showed a phenotype similar to T (-), B (+), and NK (-) SCID due to a JAK3 deficiency. Both patients received hematopoietic stem cell transplants from human leukocyte antigen-matched family members. [175] Jennifer Puck et al. used the TREC assay to screen 500,000 babies in California, and they found 6 cases of SCID with known genetic mutations, 3 cases of SCID without any known gene defect, 1 case of Omenn syndrome, and 3 cases of DiGeorge syndrome. [83]

CONCLUSION:

Absolute lymphopenia (ALC<2500/µl) was observed in 6.67% (96/1441) of neonates screened at birth. This is greater than the incidence of absolute lymphopenia in 3 other studies done worldwide which can probably be linked to a higher rate of consanguinity present in the northern Karnataka region. Other causes of lymphopenia included prematurity, sepsis, congenital anomalies, and idiopathic factors. Lymphopenia showed a male predominance (56.25%), with consanguinity significantly more prevalent among affected infants (43.75% vs. 10.41% in non-lymphopenic cases). Neonates with lymphopenia had lower birth weights (mean 2.3kg vs. 2.73kg), lower APGAR scores at 1 and 5 minutes, and a higher incidence of dysmorphic features (17.71% vs. 0.97%) and family history of sibling death (10.42% vs. 0.52%). Significant differences were observed in hemoglobin levels, WBC counts, ANC, and ALC between lymphopenic and non-lymphopenic groups (all $p<0.001$). Flow cytometry suggested a suspected SCID pattern in some cases, but genetic testing did not confirm mutations in those tested. Lymphopenic neonates also required active neonatal resuscitation more frequently (28.13% vs. 4.98%). Overall, the study underscores the complex etiology and clinical implications of neonatal absolute lymphopenia, advocating for comprehensive evaluation and management strategies.

Early identification of severe combined immunodeficiency (SCID) holds tremendous potential to dramatically improve outcomes for affected individuals, transforming what was once a fatal condition. Integrating a straightforward test such as cord blood CBC into newborn screening initiatives has the dual benefit of saving lives and alleviating suffering for patients and their families. Prompt diagnosis enables early intervention, which not only improves clinical outcomes but also

reduces overall healthcare costs compared to delayed detection and treatment. Ongoing research into optimal cutoff levels for absolute lymphocyte count and T-cell receptor excision circles (TRECs) will refine screening protocols, ensuring best practices are upheld. Future research aim to expand screening methods to include other primary immunodeficiencies, facilitating early recognition and treatment for a broader range of conditions.

The implementation of newborn screening programs in many countries has proven pivotal in identifying SCID before symptoms manifest. In the early diagnosis and treatment of people with SCID, primary care doctors are essential. Educating parents on infection prevention strategies is essential prior to definitive diagnosis, as infections significantly impact survival rates in SCID patients. Further diagnostic evaluations, including flow cytometry and genetic testing, are typically coordinated through pediatric centers with specialized immunological expertise. Upon confirmation of diagnosis, patients should receive appropriate prophylactic measures against infections and be referred to a transplant center for potential stem cell transplantation. Primary care physicians should be aware about the causes of T-cell lymphopenia other than SCID that can yield positive results in newborn screening.

Despite its severity, SCID can be effectively managed with early diagnosis through newborn screening and comprehensive multidisciplinary care, leading to favorable clinical outcomes. To facilitate early care for newborns with SCID and pre-symptomatic identification, we recommend integrating measurement of the absolute lymphocyte count in umbilical cord blood into screening regimens. Until SCID is definitively ruled out, precautions such as withholding live attenuated vaccines like BCG are advised to mitigate potential risks.

LIMITATIONS:

1. This was a single centre-based study.

2. Some neonates having absolute lymphopenia were lost to follow up and a few did not give consent for further investigation which may have led to missed cases.

3. Whole genome sequencing could not be done in view of financial constraints.

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ANNEXURE-I

INFORMED CONSENT FORM

KAHERS JNMC BELAGAVI

"UTILITY OF UMBILICAL CORD BLOOD ABSOLUTE LYMPHOCYTE COUNT IN SCREENING FOR SEVERE COMBINED IMMUNODEFICIENCY DISEASE – A ONE YEAR LONGITUDINAL STUDY"

Reg No. BM0121007

- **Objective:** To estimate the incidence of absolute lymphopenia in Umbilical Cord Blood samples of newborns and its utility in Screening for Severe Combined Immunodeficiency Disease (SCID).

- **Introduction:** You are being invited to participate in this study to find out utility of Umbilical Cord Blood Absolute Lymphocyte Count in Screening for Severe Combined Immunodeficiency Disease. Participation of your baby will help us to know the Utility of Umbilical Cord Blood Absolute Lymphocyte Count in Screening for Severe Combined Immunodeficiency Disease and the incidence of lymphopenia. SCID is one of the most severe and fatal forms of inherited primary immunodeficiency. Most of these newborns are normal at birth but develop recurrent infection during infancy. Many of them succumb to infections in the first year of life. The above- mentioned study will therefore help us know if your baby has lymphopenia at birth, and we will be able to detect and offer treatment for any potentially life-threatening immunodeficiency disorders early.

- **Explanation of procedure:** 2 ml of blood is to be taken from the umbilical cord immediately after the delivery of the baby. The complete blood counts will be processed, and if required you will be asked for a follow-up after one month. Few basic demographic and birth history will be asked to you as a part of the proforma.

- **Withdrawal from participation in the study:** Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

- **Possible benefits from participating in the study:** The early detection of lymphopenia in your baby can be lifesaving since Severe Combined Immunodeficiency and other causes of lymphopenia can be potentially fatal within the 1 st year of life. The data gathered will help the population at large.

- **Possible risks from participating in the study:** There are no risks involved in participating in this study.

- **Privacy and confidentiality:** The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

- **Financial incentives:** You will not receive any payment for participating in this study.

- **Authorization for publication of aggregated data:** Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

- **Questions:** In case of any questions with regard to this study, you are free to contact the pediatrics postgraduate student with registration number – BM0121007. If you have any question or complaints with regard to your right as study participant you may contact the Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

- **Legal rights:** By signing this consent form, we are not waving any of your legal rights.

CONSENT STATEMENT:

I am making a voluntary decision to participate in the study "**UTILITY OF UMBILICAL CORD BLOOD ABSOLUTE LYMPHOCYTE COUNT IN SCREENING FOR SEVERE COMBINED IMMUNODEFICIENCY DISEASE**". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE II – PROFORMA

PATIENT'S PROFORMA

Contact Number-

Name of Mother:

Name of Father:

Address:

Mode Of Delivery: Normal Vaginal Delivery OR LSCS In View Of

Neonatal Resuscitation Required: Yes / No
If yes, Mode:

Birth Weight (In Kgs):

APGAR Score:

AT 1 MIN	AT 5 MINS

Findings On General Physical Examination: Any Dysmorphic features/ congenital anomaly/ oral ulcers?

Positive Family: Yes / No

Any History Of Sibling Death: Yes / No If Yes, Cause: _____

Pedigree Chart:

Investigation: Umbilical Cord Blood CBC –

Date	Hemoglobin	WBC Count	Platelet Count	ALC	ANC	Peripheral Smear finding

Absolute Lymphocyte Count (ALC):

- If ALC Count <1500/µL – Send Lymphocyte Subset Analysis
- If ALC Count Between 1500 – 2500/µL: Repeat CBC After 1 Month (Follow Up)
- If ALC Count >2500/µL – No Further Intervention

ANNEXURE III –MASTER CHART

Sl No	Gender(M/F)	Consanguinity (Present/Absent)	Mode of Delivery (N/L)	Neonatal Resuscitation reqd - Bag & Mask (Y/N)	Gestational Age (in weeks)	Birth Weight (kg)	Other associated conditions contributing to lymphopenia (Nil - N, Idiopathic -I, Sepsis -S, Congenital Anomaly -A, Preterm -P)	APGAR SCORE at 1 min (out of 10)	APGAR SCORE at 5 min (out of 10)	Dysmorphic Features(P/A)	Family History / sibling death (Y/N)	Hemoglobin (gm%)	WBC count (x10^3/µl)	ANC (x10^3/µl)	ALC (x10^3/µl)	Lymphopenia (Y/N)	1month Follow up ALC	Flow cytometry (LSSA) - detailed reports in separate file	NGS/WES in patients with Suspected SCID on flow cytometry
1	F	A	N	N	37	2.6	N	7	8	A	N	16.2	12.2	7.1	3.4	N			
2	M	A	L	N	40	3.1	N	6	9	A	N	17.6	14.6	8	5.1	N			
3	M	A	L	N	39	3.3	N	6	7	A	N	18	13.2	6.9	4.9	N			
4	F	A	N	N	36	2.1	N	7	9	A	N	15.4	17.8	10.3	5.2	N			
5	F	A	L	N	35	2.6	N	6	9	A	N	14	18	11.4	5	N			
6	F	A	L	N	37	2.4	N	9	10	A	N	17.1	15.1	7.4	4.1	N			
7	M	A	L	N	36	2.8	N	7	8	A	N	19.5	14.2	3.5	9.1	N			
8	M	A	L	N	39	2.9	N	7	9	A	N	20.1	16	10.6	4.5	N			
9	F	A	N	N	36	2.6	N	6	8	A	N	17	21.7	14	4.9	N			
10	M	A	L	N	40	3.3	N	6	9	A	N	14.9	17.8	8.9	6.2	N			
11	M	P	N	N	32	1.6	P, S	3	5	A	Y	12.2	10.2	7.5	1.6	Y	3.2	-	
12	M	A	N	N	38	3	N	6	9	A	N	17.5	19.5	12.5	6.4	N			
13	F	A	L	N	39	3.9	N	7	8	A	N								
14	F	A	N	N	38	4.1	N	6	10	A	N	12.7	13.1	7.9	4	N			
15	F	A	L	N	36	2.4	N	8	9	A	N	11.9	16.5	12.2	3.7	N			
16	M	P	N	N	35	2	N	6	8	A	N	15.6	12	6.6	3.9	N			
17	F	A	L	Y	36	1.6	N	5	9	A	N	16.6	13.9	7	6.5	N			
18	M	A	N	N	36	2.5	N	8	9	A	N	16	22.6	13.1	7.6	N			
19	F	A	L	N	35	2.3	N	7	9	A	N	14.2	14.4	9.6	2.5	N			
20	M	A	L	N	34	2.2	N	7	8	A	N	13.8	10.5	6.2	3	N			
21	F	A	N	N	36	2.7	N	6	7	A	N	17.5	13.8	7.9	3.9	N			
22	M	P	L	N	38	3.1	N	5	8	A	N	15.3	16.6	11.1	4.2	N			
23	M	A	N	N	39	3.7	N	8	10	A	N	15	9.5	5	3.3	N			
24	F	A	N	N	36	2.6	N	8	9	A	N	14.8	16.7	8.6	6.5	N			
25	F	A	N	N	37	2.8	N	7	8	A	N								
26	M	A	L	N	39	3	N	6	9	A	N	16.1	12.3	6.3	5.4	N			
27	F	A	L	N	38	2.4	N	6	9	A	N	15.8	14.1	9	4.1	N			
28	M	A	N	N	39	3.5	N	8	10	A	N	12.9	16.8	12.3	3.8	N			
29	M	A	L	N	40	3.6	N	8	9	A	N	17.5	20.4	11	8.2	N			
30	F	A	N	N	39	2.4	N	7	10	A	N	19.8	13.5	7.7	5.2	N			
31	F	P	L	N	38	2.4	N	6	8	A	N	13.7	10.3	6	2.7	N			
32	M	A	L	N	39	2.7	N	7	9	A	N	14.5	14.7	8.1	4.2	N			
33	F	A	L	N	38	2.6	N	7	8	A	N	15.2	12.2	7.3	4.4	N			
34	M	A	N	N	38	2.9	N	8	9	A	N	16.1	9.9	6.4	2.9	N			
35	M	A	L	Y	33	1.8	P	5	8	A	Y	10.9	8.2	5.8	2.1	Y	3.1	-	
36	F	A	L	Y	34	2.2	P	4	7	P	N	11.5	7.2	5.1	1.7	Y	Missed		

37	F	A	N	N	38	2.8	N	8	9	A	N	12.9	8	4.5	3.1	N			
38	M	A	L	Y	35	2.1	N	4	8	A	N	14	15.3	11.8	3.3	N			
39	M	A	L	N	34	1.7	N	6	7	A	N	12.8	6.8	3.9	2.5	N			
40	M	A	N	N	38	3.4	N	8	9	A	N								
41	F	A	L	N	38	3	N	6	10	A	N	17.7	14.4	10.6	2.6	N			
42	M	A	L	N	39	2.9	N	7	8	A	N	16.5	13.5	9.7	2.8	N			
43	M	P	N	N	38	2.8	I	7	9	A	N	15.1	9.5	5.8	2.3	Y	3	-	
44	M	A	L	N	38	2.4	N	7	10	A	N	16.2	11.4	5.5	4.1	N			
45	F	P	L	N	37	2.7	N	6	9	A	N	15.5	12.2	8.3	3.1	N			
46	M	A	L	N	38	2.5	N	5	7	A	N	14.7	15.3	9.3	4.8	N			
47	F	A	N	N	37	2.9	N	6	8	A	N	15	16.8	10.7	4.2	N			
48	M	A	L	N	39	3.4	N	8	10	A	N	15.9	12	6.2	4.6	N			
49	M	P	L	N	38	3.1	N	6	9	A	N	16.5	17.9	11.4	5.9	N			
50	M	A	N	N	37	3	N	7	9	A	N	18	11	5.8	3.4	N			
51	F	A	N	N	35	2	N	7	10	A	N	16.2	16.1	11.5	4.7	N			
52	M	A	L	N	36	2.4	N	7	8	A	N	17	13.3	8.6	4.2	N			
53	F	A	N	N	39	2.9	N	9	10	A	N	16.7	12.7	10.1	2.2	N			
54	M	A	L	N	33	1.5	N	7	10	A	N	15.4	14	9.6	3.1	N			
55	F	A	L	N	38	2.6	N	6	8	A	N	14.9	13.5	6.7	5.7	N			
56	M	A	L	N	36	2.9	N	6	9	A	N	17	18.8	12.2	5.5	N			
57	F	A	N	Y	39	3.1	N	5	8	A	N	16.5	28.9	14.7	11.2	N			
58	M	A	N	N	40	3	N	6	8	A	N								
59	M	A	L	N	38	2.5	N	7	9	A	N	18.3	14.2	10.3	3.1	N			
60	F	A	N	N	37	2.1	N	8	9	A	N	17.5	15	11.4	3.1	N			
61	M	A	L	N	36	2.3	N	7	8	A	N	14.9	16.8	9.4	6.2	N			
62	F	P	L	N	39	3.1	N	8	10	A	N	14.3	18.9	14.1	4.5	N			
63	M	A	N	N	40	3.2	N	7	9	A	N	15.1	15.4	10.1	4.9	N			
64	F	P	L	N	40	3.2	N	6	9	A	N	19.8	12	8.7	3.1	N			
65	M	A	N	N	41	4.2	N	6	8	A	N	20.3	11.6	6.6	4.3	N			
66	F	A	N	N	34	1.9	P	7	8	A	N	13.8	7.6	5.4	1.8	Y	4.7	-	
67	M	A	L	N	38	3.1	N	8	9	A	N	14.6	15.6	11.5	3.5	N			
68	F	A	L	N	38	3	N	7	8	A	N	15.7	14.5	8.8	5	N			
69	M	A	N	N	39	2.8	N	6	9	A	N								
70	F	A	L	N	37	2.7	N	7	8	A	N								
71	M	A	N	Y	38	2.6	N	5	7	A	N	17	14.7	10.3	3.6	N			
72	F	A	L	N	37	2.6	N	6	9	A	N	14.2	12.3	7.3	4.5	N			
73	F	A	L	N	40	3.3	N	7	10	A	N	11.2	12.1	7.7	4.1	N			
74	M	A	N	N	37	2.3	N	6	9	A	N	13	14.8	10.5	2.9	N			
75	M	P	L	N	36	2.4	N	6	8	A	N	11.9	22.8	14.9	7.7	N			
76	F	A	L	N	38	2.6	N	8	10	A	N	17.4	17.6	10	6.4	N			
77	M	A	N	N	39	3	N	8	9	A	N	16.5	13.5	8.8	4.3	N			
78	F	P	L	N	40	2.9	N	6	8	A	N	16.8	26.6	16.2	9	N			
79	M	A	N	N	40	3.1	N	7	9	A	N	17.2	16.4	12	4.2	N			
80	F	A	N	N	38	3	N	7	8	A	N	15.2	20.3	12.4	7.1	N			
81	F	A	L	N	38	2.4	N	8	9	A	N	15.6	10.7	6.7	3.9	N			
82	M	A	L	N	37	2.6	N	7	10	A	N								
83	F	A	N	N	38	3.8	N	6	7	A	N	19.3	10	5.3	4.2	N			
84	M	A	L	N	39	3.5	N	7	9	A	N	20.7	9.3	6.4	2.6	N			
85	M	P	L	N	37	2.5	N	8	9	A	N	14.6	8.7	5.8	2.8	N			
86	F	A	N	N	36	2.6	N	9	10	A	N	17.6	11.3	7.5	3.3	N			
87	F	P	L	Y	40	3.2	S	5	8	A	N	14.4	9.7	7.4	2	Y	Missed		
88	M	A	L	N	37	2.7	N	7	9	A	N	15.6	15	9.3	5.6	N			

89	M	A	N	N	38	2.8	N	8	9	A	N	14.9	12.4	8.1	3.8	N			
90	F	A	N	N	38	3	N	6	8	A	N	14.4	13.7	9.5	3.9	N			
91	M	A	L	N	36	2.1	N	7	9	A	N	13.9	14.3	10	4.1	N			
92	F	P	L	N	38	2.6	N	6	9	A	N	16.6	15.9	11.1	4.4	N			
93	M	A	L	N	36	2.4	N	8	10	A	N	15.2	20.1	14.8	5	N			
94	M	A	L	N	39	3	N	7	8	A	N	14.3	18.3	12.7	4.9	N			
95	F	A	N	N	40	3.2	N	7	9	A	N	17	16.4	8.4	7.6	N			
96	M	A	L	N	38	2.8	N	8	10	A	N	15.1	12.1	9.1	2.7	N			
97	F	A	N	N	40	2.7	N	6	9	A	N	14.9	13.2	8.5	4.4	N			
98	M	A	N	N	38	2.5	N	5	8	A	N	10.7	10	7	2.9	N			
99	F	A	L	N	39	3.2	N	5	10	A	N	9.8	8.8	4.7	3.1	N			
100	M	A	L	N	36	2.4	P	6	8	A	N	11.7	7.7	5.7	1.7	Y	3.3	-	
101	M	A	L	N	38	2.9	N	7	9	A	N	18.5	13.2	9.8	3.1	N			
102	F	P	N	N	38	3	N	8	9	A	N	14.6	12.6	9	3.3	N			
103	M	A	L	N	33	1.5	P	7	8	P	N	13.9	6.1	4.4	1.5	Y	2.9	-	
104	F	A	L	N	37	3.1	N	6	9	A	N	15.5	10.9	8.2	2.5	N			
105	M	A	N	N	38	3	N	6	8	A	N	15	8.9	4.1	4.5	N			
106	M	A	L	N	36	2.5	N	7	9	A	N	12.2	6.8	3.6	2.9	N			
107	F	A	L	N	35	2.4	N	8	9	A	N	14.2	14.5	11	3.1	N			
108	M	A	L	N	39	2.9	N	7	9	A	N								
109	F	A	N	N	37	2.8	N	6	8	A	N	12.9	11.7	7.2	4.1	N			
110	M	A	L	N	38	2.6	N	7	10	A	N	14.2	16.8	13.1	3	N			
111	F	A	L	Y	34	2	N	5	9	P	N	17.6	14.2	8.7	4.8	N			
112	M	A	N	N	36	2.8	N	7	9	A	N	18	15	6.5	8.2	N			
113	F	P	L	N	38	2.7	N	8	10	A	N	13.2	13.1	9.2	3.4	N			
114	M	P	N	N	36	2.2	N	5	7	A	N	15.3	17.3	12.5	4.2	N			
115	F	A	L	N	39	3.4	N	7	9	A	N	14.9	14.5	9.1	4.8	N			
116	F	A	N	N	38	3	N	8	9	A	N	15	11	6.7	4.2	N			
117	M	A	L	N	35	2.3	P	6	8	A	N	11.7	7.9	5.5	1.8	Y	4.1	-	
118	M	A	N	N	38	2.6	N	6	10	A	N	11.3	11.3	10.3	2.6	N			
119	F	A	L	N	37	2.6	N	8	9	A	N	17.6	12.2	8.6	3.3	N			
120	M	A	N	N	38	2.9	N	8	9	A	N								
121	F	A	N	N	40	3	N	5	9	A	N	14.8	19.8	14.1	5.5	N			
122	M	A	N	N	38	3.7	N	6	9	A	N	16.1	22.1	10	11.2	N			
123	F	A	N	N	35	1.8	N	6	8	A	N	15.7	19.3	14.1	4.4	N			
124	M	A	L	N	34	2	N	7	9	A	N	16.2	18	14.3	3.1	N			
125	M	A	N	Y	35	1.8	P	2	7	A	N	12.7	6.9	4.5	2.1	Y	3.8	-	
126	F	A	L	N	34	1.9	N	6	9	A	N	15	14.1	10.6	3.1	N			
127	M	A	L	N	34	2	N	7	9	A	N	14.7	14	10.7	3.2	N			
128	M	A	N	N	38	2.5	N	7	8	A	N	16.1	17	12.1	4.4	N			
129	F	A	L	N	36	2.6	N	7	9	A	N	15	12.9	7.2	4.9	N			
130	M	P	N	N	36	2.4	N	7	8	A	N	18.1	14.7	10.3	3.1	N			
131	M	A	N	N	37	2.7	N	6	9	A	N	17	16	11.4	4.3	N			
132	F	P	L	N	32	1.4	P, S	3	5	A	N	16.2	6.2	4	1.8				
133	F	P	L	N	36	2.3	P	7	9	A	N	16.7	8.3	5.8	2.1	Y	5.7	-	
134	F	A	N	N	37	2.7	N	5	8	A	N	15	15.2	11.3	3.5	N			
135	F	A	L	N	39	2.7	N	8	9	A	N	14.7	12.3	7.1	5	N			
136	F	P	N	N	40	3	N	7	8	A	N	13.8	18	11.9	5.7	N			
137	M	A	L	N	37	3.6	N	7	9	A	N								
138	F	A	N	N	38	3.2	N	9	10	A	N	15.7	14.1	10.2	3.5	N			
139	M	A	L	N	38	3	N	8	9	A	N	16.1	9.7	4.8	4.5	N			

140	F	A	N	N	38	2.7	N	5	7	A	N	19	12.7	8.2	4.1	N			
141	F	A	L	N	37	2.8	N	8	9	A	N	17.3	16.3	13	2.9	N			
142	M	A	L	N	37	2.5	N	6	9	A	N	18.1	12.9	5.1	7.7	N			
143	M	P	L	N	40	3.4	N	7	9	A	N	15.4	18.7	12.1	6.4	N			
144	M	A	L	N	37	3	N	5	8	A	N	16.3	14.2	9.6	4.3	N			
145	F	A	N	N	38	2.7	N	8	9	A	N	15	13.1	3.6	9	N			
146	F	A	N	N	38	2.6	N	9	10	A	N	14.1	15.9	11.5	4.2	N			
147	F	A	L	N	37	3.1	N	6	7	A	N								
148	M	A	L	N	38	3.8	N	5	9	P	N	15.9	11.4	7	3.9	N			
149	M	A	N	N	39	3	N	6	8	A	N	17.5	15.2	9.8	5.1	N			
150	F	A	L	Y	33	1.7	N	7	8	A	N	17	13.5	8.8	4.2	N			
151	F	A	N	N	38	3	N	7	9	A	N	16.1	12.4	8.7	3.3	N			
152	M	A	L	N	35	2.3	N	7	8	A	N	12.5	16.7	11.8	4	N			
153	M	A	L	N	36	2.7	N	7	9	A	N	14.1	15	8.5	5.6	N			
154	F	A	N	N	38	2.4	N	8	9	A	N	13.8	14.2	10.1	3.8	N			
155	M	A	L	N	39	3	N	9	10	A	N	14.6	13.7	8.4	3.9	N			
156	M	A	N	N	39	3.1	N	7	9	A	N								
157	M	A	N	N	39	3	N	6	9	A	N	11.1	16.6	11.7	4.4	N			
158	M	A	L	N	35	2.1	N	5	8	A	N	11.8	14.5	9.1	5	N			
159	M	A	L	N	39	2.8	N	8	9	A	N	10.8	17	11.2	4.9	N			
160	M	A	L	N	39	2.8	N	8	9	A	N	17.2	11.4	5.8	5	N			
161	M	P	N	N	38	2.6	I	8	9	A	N	11.8	7.3	5	2.1	Y	Missed		
162	F	A	L	N	40	2.6	N	7	8	A	N	17.4	10.2	6.5	3.4	N			
163	F	A	N	N	39	2.8	N	8	10	A	N	16.1	7.5	6.3	2.7	N			
164	M	A	L	N	40	3	N	7	9	A	N	15	20.8	13	7.6	N			
165	F	A	L	N	38	3.5	N	7	9	A	N	14.2	16.9	11.1	5.6	N			
166	M	P	L	N	40	2.9	N	7	10	A	N	12.4	17.7	10.4	6.7	N			
167	F	P	L	Y	35	2.4	P	4	6	A	N	13.4	10.8	9.2	1.5	Y	2.7	-	
168	M	A	N	N	37	2.5	N	6	8	A	N	15.9	15	11.1	3.7	N			
169	F	P	N	N	37	2.5	N	9	10	A	N	14	14.6	10.5	3.7	N			
170	M	A	L	N	39	2.8	N	8	9	A	N	10.8	13.2	7.9	5.1	N			
171	F	A	L	N	39	3	N	7	9	A	N	18.1	22.4	14.8	7.1	N			
172	F	A	N	N	38	3.4	N	7	9	A	N	15	18.6	11.7	6.3	N			
173	M	A	L	N	38	3	N	6	9	A	N	14.4	14.1	10.8	3	N			
174	M	A	N	N	36	2.4	N	8	10	A	N	14.3	17	8.9	7.7	N			
175	F	A	L	N	37	2.8	N	8	9	A	N	17.4	14.7	9.1	4.9	N			
176	M	A	L	Y	37	2.5	N	4	7	A	N	16.1	13.8	8.5	4.5	N			
177	M	A	N	N	36	2.3	N	7	8	A	N	16.5	16.8	10.6	5.6	N			
178	F	A	N	N	38	3.2	N	8	9	A	N	12.8	18.1	11.5	6	N			
179	F	P	L	N	37	3	N	7	9	A	N	13	17.5	11.4	5.5	N			
180	F	P	L	N	34	2.3	N	8	10	A	N								
181	M	P	N	N	36	2.8	N	7	9	A	N	13.8	10.4	7.5	2.7	N			
182	M	A	L	N	38	3.2	N	6	8	A	N	14.2	16.5	10.3	5.5	N			
183	M	A	N	N	39	3.4	N	7	9	A	N	17	14.1	5.2	8.4	N			
184	F	A	N	N	34	2.1	P	6	8	P	N	17.1	6.9	4.4	1.7	Y	4	-	
185	F	A	L	N	39	2.6	N	8	9	A	N	11.8	17.3	13.2	3.6	N			
186	M	A	L	N	40	2.7	N	6	10	A	N	16	13	6	6.8	N			
187	F	A	N	N	38	2	N	8	10	A	N	12.5	17.2	10.1	6.7	N			
188	F	A	L	N	38	3	N	9	10	A	N	13.8	18.1	9.8	7.5	N			
189	M	A	N	N	38	3.7	N	6	8	A	N	16.1	13.5	5.4	7	N			
190	F	A	L	N	37	2.7	N	8	9	A	N	14	16.5	11.7	4.6	N			
191	M	A	L	N	33	1.9	N	7	8	A	N								

192	F	A	L	N	38	2.6	N	7	10	A	N	16.9	14.4	11	3.5	N			
193	F	A	N	N	34	1.7	N	6	9	A	N	15.4	10.7	7.3	3.2	N			
194	M	P	L	N	38	2.9	N	7	9	A	N	15	12.3	8.8	3.4	N			
195	M	A	N	N	37	2.7	N	6	8	A	N	14.9	16	12.1	3.3	N			
196	F	A	L	N	38	3.4	N	5	9	P	N	13.2	18.4	10.8	7	N			
197	M	A	L	N	39	3.1	N	7	9	A	N	13.1	11.3	7.4	3.6	N			
198	F	A	N	N	36	2.3	N	5	8	A	N	12.5	12	6.6	4.9	N			
199	M	A	N	N	34	2	P	6	9	A	N	14.6	11.5	9.7	1.6	Y	5.5	-	
200	F	A	N	N	37	2.8	N	8	9	A	N	11.7	16	10.5	5	N			
201	M	A	L	N	37	2.6	N	7	9	A	N	11.9	13.2	8	4.4	N			
202	F	A	L	N	38	3	N	7	10	A	N	14.7	14.1	7.7	5.9	N			
203	M	A	N	N	38	2.5	N	8	9	A	N	15.5	13.6	7.8	5	N			
204	M	A	L	N	37	2.4	N	7	8	A	N	13.8	17.9	10.2	7	N			
205	F	A	L	N	37	3.6	N	9	10	A	N	14.1	23.4	14.7	8.4	N			
206	F	P	L	Y	34	1.9	N	4	8	A	N								
207	F	A	N	N	34	2	N	5	9	A	N								
208	M	A	L	N	38	2.8	N	6	9	A	N	17.5	11.7	5.9	3.6	N			
209	F	A	N	N	38	2.5	N	7	9	A	N	12.4	16.3	11.5	4.1	N			
210	M	A	L	Y	37	3	N	5	8	P	N	11.8	27.2	15.2	9.1	N			
211	M	A	L	N	38	2.8	N	8	9	A	N	10.7	12	8.1	3.6	N			
212	M	A	N	N	40	2.7	N	7	8	A	N	14.8	11.2	8.3	2.5	N			
213	M	A	L	N	38	2.5	N	8	10	A	N	13.1	10.3	6.2	3.4	N			
214	M	A	L	N	39	3	N	7	9	A	N	14.5	9.5	5	4.3	N			
215	F	A	N	N	37	3.2	N	7	8	A	N	12.7	6.8	3.9	2	N			
216	F	A	L	N	38	3.5	N	8	10	A	N	17.8	14.5	11	3.4	N			
217	F	A	L	N	35	1.8	N	7	8	A	N	16	27.3	18.7	7.6	N			
218	M	A	N	N	33	1.5	N	8	9	A	N	14.3	14.3	10.1	3.6	N			
219	F	A	L	N	38	3	N	9	10	A	N	15.1	15.8	10.5	4	N			
220	M	P	N	N	39	4.3	N	8	9	A	N	12.8	13	8.1	4.4	N			
221	F	A	L	N	39	3.2	N	7	8	A	N	13.6	19.4	15	3.9	N			
222	M	P	N	N	37	3	N	8	9	A	N	14.7	17.1	14.1	2.8	N			
223	M	A	L	N	35	2.4	I	7	9	A	N	15.6	14.2	11.3	2.4	Y	5.2	-	
224	F	P	L	N	38	3.1	N	8	10	A	N	19.1	15.3	11.3	3.2	N			
225	M	P	L	N	37	2.8	N	7	9	A	N	11.5	16.4	10.9	3.3	N			
226	F	A	N	N	38	2.9	N	7	9	A	N	10.3	14.9	9.5	4.6	N			
227	M	A	L	Y	35	2.1	N	7	9	A	N	10.9	18.4	15.2	2.6	N			
228	F	A	N	N	36	2.6	N	7	8	A	N	12.6	17.2	13.2	3.6	N			
229	M	A	L	N	38	3.7	N	6	9	A	N	12.9	13.8	9.2	3.8	N			
230	F	A	L	N	39	3	N	5	9	A	N	13.7	14.4	6.7	7.6	N			
231	F	A	N	N	38	2.8	N	6	9	A	N	13.4	22.7	16	5.5	N			
232	F	A	L	N	39	3.8	N	7	9	A	N	14.2	26.4	20.1	5.5	N			
233	M	A	L	N	40	2.6	N	9	10	A	N	16	12.2	6.3	5.4	N			
234	F	P	N	N	36	2.2	N	7	9	A	N	15.1	16.4	11.7	2.9	N			
235	M	A	N	N	34	1.8	N	7	8	A	N	16.8	15.8	11.5	3.7	N			
236	F	A	L	N	34	1.9	N	8	10	A	N	18.7	17.3	10.6	6.3	N			
237	M	A	L	N	35	2.2	N	8	9	A	N	11.4	18.4	13.3	4.5	N			
238	F	A	N	N	35	2	N	9	10	A	N	12.8	18.2	12	4.7	N			
239	M	A	L	N	38	2.7	N	7	8	A	N	16.3	13.5	9.1	3.8	N			
240	F	P	L	N	39	2.6	N	6	8	A	N	15.8	14.4	8.7	5.6	N			
241	M	A	L	N	40	3.1	N	5	8	A	N	17	16.1	10.4	4.2	N			
242	M	A	N	N	38	2.8	N	6	9	A	N	14.7	18.7	11.2	6.9	N			
243	F	A	N	N	37	2.6	N	8	9	A	N								

244	M	A	N	N	39	2.4	N	7	9	A	N	13.2	20.5	12.8	6.5	N			
245	F	A	L	N	37	4.1	N	8	9	A	N	15.8	19.3	13	5.8	N			
246	M	P	N	N	38	2.8	N	7	8	A	N	14	18.8	11.2	7	N			
247	M	A	L	Y	36	1.8	N	5	7	A	N	11.8	17.2	12.3	4.5	N			
248	F	A	N	N	38	2.5	N	6	9	A	N	12.6	14.1	9	4.9	N			
249	M	A	N	N	38	2.6	N	5	8	A	N	16.5	16	12.5	2.7	N			
250	M	A	L	N	37	2.5	N	8	9	A	N	21.5	25.5	13.4	11.1	N			
251	F	P	L	N	38	3	N	9	10	A	N	19.7	18.3	10	7.2	N			
252	M	A	N	N	39	2.8	N	7	9	A	N	12.3	15.7	9.2	5.4	N			
253	M	A	L	N	40	2.7	N	6	8	A	N	14.5	21.7	11.7	8.8	N			
254	F	A	L	N	40	3.6	N	6	8	A	N	13.9	24.8	14.7	8.5	N			
255	M	A	N	N	40	3	N	8	9	A	N	15.7	22.4	15.3	6.2	N			
256	F	A	L	N	38	2.8	N	7	9	A	N	16	16.3	11.8	4.1	N			
257																			
258	M	A	L	N	36	2	N	7	9	A	N	18.6	18.3	13.8	4.2	N			
259	M	A	L	N	38	3.2	N	8	10	A	N	15.7	16.5	12.2	4.1	N			
260	F	A	N	N	36	2.2	N	7	8	A	N								
261	F	A	L	N	37	2.7	N	7	9	A	N	14	20.4	14.5	4.8	N			
262	F	A	L	N	37	2.4	N	8	9	A	N	14.7	14.8	7.9	5.8	N			
263	M	A	N	N	37	2.8	N	7	8	A	N	15.2	15.9	11.4	3.3	N			
264	F	A	N	N	38	2.9	N	7	9	A	N	12.8	16	10.7	3.7	N			
265	M	A	L	N	37	2.6	N	6	9	A	N								
266	M	A	L	N	38	2.4	N	7	9	A	N	14.3	17.4	12.8	3.9	N			
267	M	A	N	N	37	3	N	8	10	A	N	15.1	14.9	12	2.6	N			
268	M	A	L	N	34	1.8	N	7	8	A	N	16	17.3	13.3	3.6	N			
269	F	A	N	N	35	2.2	N	6	9	A	N	17.8	15	11.2	3.3	N			
270	F	A	L	N	37	3.5	N	7	9	A	N	14.9	18.5	12.8	4.5	N			
271	M	P	N	N	38	2.6	N	6	8	A	N	12.9	13.7	7.7	5.1	N			
272	F	A	L	N	38	2.7	N	9	10	A	N	14.8	12.5	4.1	7.8	N			
273																		-	
274	M	A	N	N	39	3	N	6	8	A	N	14.3	12.6	7.7	4.2	N			
275	F	P	L	N	40	3	N	7	9	A	N	17	13.2	7.9	4.5	N			
276	M	A	L	N	38	2.8	N	8	9	A	N	16.3	15.8	12.3	2.7	N			
277	F	A	L	N	37	2.7	N	8	10	A	N	16.5	13.7	10.1	2.8	N			
278	M	A	N	N	37	2.6	N	7	8	A	N	14.7	14	9.6	3.2	N			
279	M	A	N	N	35	2.4	N	6	9	A	N	15.6	15.1	10.2	4	N			
280	F	A	L	N	38	3	N	7	9	A	N	11.8	18.2	12.3	5.1	N			
281	F	A	N	N	38	3.6	N	8	10	A	N	12	16	9.3	6.2	N			
282	M	A	L	N	38	2.8	N	7	9	A	N	19.3	15.3	8.4	5.9	N			
283	F	A	L	N	35	2	N	5	8	A	N	16.5	12.9	4.4	7.1	N			
284	M	A	N	Y	39	2.7	N	4	7	P	N	15	14.4	7.5	5.9	N			
285	F	A	L	N	40	2.6	N	5	8	A	N	14.8	17	8.9	6.3	N			
286	M	A	L	N	38	2.7	N	8	9	A	N								
287	F	A	N	N	37	2.8	N	6	8	A	N	15.1	17.3	13	2.9	N			
288	M	A	L	N	36	1.9	N	7	9	A	N	12.8	13.8	7.8	3.8	N			
289	M	A	N	N	37	3	N	8	9	A	N								
290	M	A	N	N	38	3.4	N	7	8	A	N	14.5	21.3	15.5	4.6	N			
291	F	A	L	N	39	3.1	N	6	10	A	N	17.1	22.7	12.6	7.8	N			
292	F	P	L	N	33	1.7	N	8	9	A	N	16.3	26.4	18.9	5.8	N			
293	F	A	N	N	38	3.4	N	6	9	A	N	18	20.5	13.6	5	N			
294	M	P	L	N	34	2.1	P	5	7	A	N	11	6.3	4.3	1.7	Y	3	-	

295	F	A	L	N	38	3	N	6	7	A	N	14.1	16.7	9.6	6.4	N			
296	M	A	L	N	38	2.8	N	6	8	A	N	14.3	17.8	11.1	5	N			
297	F	A	N	N	39	2.8	N	7	9	A	N	15	13.2	1.1	11.9	N			
298	M	A	N	N	32	1.4	N	7	8	A	N								
299	F	A	L	N	39	3	N	8	9	A	N	19.5	17.6	2.3	14.5	N			
300	M	A	N	N	38	2.6	N	9	10	A	N	17.8	15.1	7.5	6.9	N			
301	F	A	L	N	38	2.8	N	8	10	A	N	10.9	13.2	4.4	8.5	N			
302	M	A	N	N	38	3	N	7	8	A	N	16.2	11.4	6.7	4	N			
303	F	A	L	N	37	2.7	N	6	8	A	N	15.1	14	8.8	4.9	N			
304	M	A	L	N	39	2.3	N	7	9	A	N	14.8	16.3	9.4	5.8	N			
305	F	A	L	N	39	2.8	N	8	9	A	N	14.7	14.5	7.6	6.4	N			
306	F	A	N	N	37	2.5	I	6	8	A	N	14.3	5.5	3	1.8	Y	Missed	-	
307	M	A	L	N	38	2.8	N	7	9	A	N	13.6	17.1	13.5	2.9	N			
308	F	A	N	N	36	2.5	N	8	10	A	N	18.3	16.3	10.7	4.9	N			
309	M	A	L	N	37	2.5	N	6	9	A	N	17.5	14.8	9.2	5.2	N			
310	M	A	L	N	36	2.2	N	8	10	A	N	17.9	15.5	9.9	5	N			
311	F	A	L	N	39	4.2	N	8	9	A	N								
312	M	A	N	N	37	2.4	N	7	8	A	N	12.3	18.2	8.5	9.1	N			
313	M	A	N	N	39	3.3	N	7	10	A	N	14.2	13.7	6.4	6	N			
314	F	A	L	N	38	2.6	N	7	9	A	N	17.7	14.1	11.2	2.5	N			
315	M	P	N	N	37	2.7	N	6	8	A	N	16	24.5	17.7	4.9	N			
316	F	A	L	N	39	3.5	N	7	9	A	N	15.8	20	12.7	6.4	N			
317	M	A	L	N	40	3	N	5	9	A	N	15.2	18.4	11.3	5.9	N			
318	F	A	N	N	38	2.6	N	8	10	A	N	14.6	17.4	12.6	4.5	N			
319	M	A	N	N	38	2.7	N	6	8	A	N	13.8	11.8	6.5	4.2	N			
320	M	A	L	N	39	2.9	N	7	9	A	N	13.6	17.6	10.4	6.6	N			
321																			
322	F	A	N	N	38	3.1	N	8	9	A	N	15	21.5	16.8	3.6	N			
323	M	A	N	N	40	3.5	N	8	10	A	N	16.3	16.3	9.3	6.8	N			
324	F	P	L	N	35	2	N	7	9	A	N	17.1	17.1	13.5	3.2	N			
325	F	P	N	N	38	2.7	N	6	8	A	N	18.6	14.9	8.3	6	N			
326	M	A	N	N	37	2.6	N	7	10	A	N	18.1	13.8	6.2	5.3	N			
327	M	A	L	N	39	3.6	N	7	9	A	N	13.3	17.4	13.8	3.3	N			
328	M	A	N	N	38	3	N	7	9	A	N	15.8	16.5	8	7.5	N			
329	M	A	L	Y	33	1.3	N	8	9	A	N	14.6	14.7	9.4	4.7	N			
330	F	A	L	Y	33	1.6	N	5	7	A	N	10.8	17.2	8.1	8.7	N			
331	M	A	L	N	38	3	N	6	10	A	N	9.4	12.3	8.3	3.2	N			
332	F	A	N	N	38	2.6	N	7	9	A	N								
333	M	A	L	N	37	2.4	N	6	8	A	N	17.4	18.7	14.1	3.6	N			
334	M	A	N	N	37	2.8	N	9	10	A	N	15	15.4	12.8	2.7	N			
335	F	A	L	N	36	2.7	N	8	9	A	N	15.2	12.7	8.5	2.5	N			
336	M	A	L	N	39	3	N	7	9	A	N	16.8	13.6	7.2	4.9	N			
337	F	A	N	N	39	2.9	N	6	9	A	N	14.9	16.4	5.5	9.7	N			
338	M	A	L	N	39	2.8	N	7	10	A	N	13.6	15.8	10.1	5.2	N			
339	M	P	N	N	38	2.6	I	6	9	A	N	14.5	9.9	7.1	2.3	Y	4.4	-	
340	F	A	L	N	37	2.7	N	7	10	A	N	9.7	10.6	5.1	5.2	N			
341	M	A	L	N	38	3.4	N	7	8	A	N	18.2	9.2	4.7	4.1	N			
342	M	P	N	N	39	3.1	N	6	9	A	N	15.3	15.2	9.5	5.3	N			
343	F	A	L	N	40	3	N	7	9	A	N	17.3	16.1	10.3	4.7	N			
344	F	A	L	N	40	2.8	N	8	10	A	N	16	15	10.8	3.7	N			
345	F	A	N	N	38	2.9	N	6	8	A	N	13.8	12.5	8.9	2.6	N			
346	M	A	L	N	40	3.2	N	7	9	A	N	14.3	14.1	9.2	3.5	N			

347	F	A	N	Y	38	3	N	5	7	A	N	12.5	10.8	3	6.3	N			
348	F	A	L	Y	37	2.4	N	3	6	A	N	11.7	14.3	7.4	5.8	N			
349	M	A	N	N	36	2.3	P	6	8	A	N	15	11.8	8.7	2	Y	2.8	-	
350	F	A	N	N	37	3	N	7	8	A	N	13.5	12.7	7.8	4.1	N			
351	M	A	N	N	38	3.1	N	9	9	A	N	16.1	13.5	9.1	3.5	N			
352	M	A	L	N	38	3.1	N	8	9	A	N	14.2	17.9	14.5	3	N			
353	M	P	L	Y	39	2.8	N	5	9	A	N	16.8	20.4	16.6	2.7	N			
354	F	A	L	N	35	2.5	N	9	10	A	N	11.8	22	14.3	6.7	N			
355	M	A	N	N	38	2.6	N	8	9	A	N	12.9	16.9	10.2	6	N			
356	M	A	L	N	40	2.8	N	6	8	A	N	14	17.2	13.8	2.6	N			
357	F	A	N	N	37	2	N	8	9	A	N	13.6	12.6	8.8	2.8	N			
358	M	A	L	N	37	2.4	N	7	10	A	N	17.5	8.5	2.3	5.8	N			
359	F	A	N	N	38	3	N	7	9	A	N								
360	M	A	L	N	39	3.8	N	6	8	A	N	15.4	20.3	16.2	3.8	N			
361	F	A	L	N	36	1.8	N	7	9	A	N	14	21.7	13	6.7	N			
362	M	P	N	N	35	1.9	N	8	9	A	N	14.8	13.4	5.7	7.2	N			
363	F	A	L	N	35	2	N	6	8	A	N	13.6	17.3	11.9	5.2	N			
364	F	A	L	N	38	2.6	N	7	8	A	N	12.9	13.1	5.3	6.4	N			
365	F	A	N	N	36	2.7	N	6	9	A	N	16.8	14.4	10.1	4	N			
366	F	A	N	N	35	2	N	6	9	A	N	18.2	15.2	9.8	4.3	N			
367	F	A	L	N	37	2.4	N	7	9	A	N	17	18.7	12.3	4.9	N			
368	M	A	L	N	36	2.5	N	7	8	A	N	17.4	16.9	11.4	4.7	N			
369	F	A	N	N	39	2.9	N	5	8	A	N	16.9	19.5	12.5	5.8	N			
370	F	A	L	Y	38	2.8	N	5	9	A	N								
371	M	A	L	N	34	1.8	N	6	10	A	N	16.4	14.3	7.1	6	N			
372	M	P	N	N	38	3	N	5	10	A	N	15	18	9.8	7	N			
373	F	P	L	N	35	2	N	6	8	A	N	15.1	16.5	13.2	2.5	N			
374	M	A	L	N	36	2.1	N	7	9	A	N	14.8	12.1	9	2.7	N			
375	M	A	N	N	36	2.2	N	7	8	A	N	16.3	16.6	11.2	4.5	N			
376	F	A	L	N	38	2.8	N	6	9	A	N	16	17.2	12.3	4.1	N			
377	M	A	N	N	39	3.4	N	7	9	A	N								
378	M	P	L	N	38	3	N	6	8	A	N	11.6	16.5	8.1	7.4	N			
379	M	A	L	N	40	2.6	N	6	10	A	N	16.7	14	9.2	4	N			
380	M	A	L	Y	38	3.8	N	5	7	A	N	15	12.8	9.6	2.7	N			
381	F	A	N	N	36	2	N	6	9	A	N	14.7	20.5	13.4	6.3	N			
382	F	A	N	N	38	2.7	N	7	8	A	N	17.3	18.6	12.9	5	N			
383	M	A	L	N	40	2.9	N	7	9	A	N								
384	F	P	N	N	40	2.8	N	7	9	A	N	13	15.4	11	3.6	N			
385	M	A	L	N	38	2.5	N	6	8	A	N	16.2	14.9	10.4	3	N			
386	F	P	L	Y	36	2	P	7	9	A	N	11.2	12.6	9.7	1.9	Y	Missed	-	
387	M	A	L	N	38	2.7	N	6	8	A	N	10.9	17.8	12.3	4.8	N			
388	M	P	L	N	37	2.6	N	7	8	A	N	19.5	14.6	9.7	4	N			
389	F	A	N	N	38	3	N	8	10	A	N	16.3	17.5	10.4	6.2	N			
390	M	A	L	N	36	2.1	N	7	9	A	N	15	20.7	14.3	5	N			
391	F	A	L	N	35	1.8	N	7	9	A	N	13.4	12	6.6	5.1	N			
392	M	A	N	N	35	1.6	N	6	7	A	N	14.3	14.8	6.9	7.6	N			
393	M	A	L	N	37	3.3	N	6	9	A	N	15	27.3	20.7	4.5	N			
394	M	A	L	N	39	3	N	7	8	A	N	15.8	15.4	8.5	5.3	N			
395	F	A	N	N	40	3.1	N	6	9	A	N	19.3	16.7	9.1	7	N			
396	M	A	N	N	39	2.5	N	7	8	A	N	12.4	13	9.3	2.6	N			
397	M	A	L	N	40	2.9	N	6	9	A	N								
398	M	A	N	N	38	2.6	N	8	10	A	N	15.5	11.9	6.8	4.1	N			

399	F	P	L	N	40	3	N	8	9	A	N	12.7	14.5	11.5	2.6	N			
400	F	A	N	N	40	2.8	N	8	10	A	N	12	17.8	11	6.3	N			
401	M	A	L	N	40	3.2	N	5	9	A	N	13.4	10.2	7.1	2.5	N			
402	F	A	N	N	37	2	N	7	8	A	N	14	8.8	5.4	2.8	N			
403	M	A	L	N	39	3	N	6	9	A	N	13.8	14.3	10.2	2.9	N			
404	F	A	L	N	38	3.1	N	5	8	A	N	14.7	15.2	11.2	3.7	N			
405	M	A	N	N	37	2.8	N	7	9	A	N	14.6	13.7	8.5	4.6	N			
406	M	A	N	N	36	2.6	N	7	9	A	N	14.5	14	8.3	5.1	N			
407	M	A	L	N	34	2	N	6	8	A	N	13	17.1	11.6	5	N			
408	M	A	N	N	38	3.5	N	6	9	A	N	18.7	22.1	13.3	6.8	N			
409	F	A	L	N	39	3.4	N	8	9	A	N								
410	M	A	N	N	38	3	N	5	8	A	N	13	12.6	6.9	4.7	N			
411	F	A	N	Y	34	1.9	N	5	7	A	N	12.7	19.4	14.2	4.2	N			
412	M	P	L	N	40	2.8	N	8	9	A	N	13	15.3	11.3	2.7	N			
413	M	P	N	N	35	2.1	P	6	8	A	N	14.9	10.2	7	2.3	Y	2.6	-	
414	F	A	L	N	38	2.9	N	8	10	A	N	18.2	16.2	12.4	3.5				
415	M	A	N	N	37	2.5	N	9	10	A	N	14.6	17.5	12.6	3.9				
416	M	A	L	N	37	2.7	N	7	9	A	N	15.3	14.2	8.3	4.4				
417	F	A	N	N	37	2.6	N	6	9	A	N	12.4	16.3	10.8	4.6				
418	M	A	N	N	35	2.3	P	5	8	A	N	11.7	16.2	11.9	2.2	Y	2.7	-	
419	F	A	N	N	36	2.4	N	7	8	A	N	15.6	17.9	11.3	5.7	N			
420	M	A	L	N	39	2.8	N	6	9	A	N	13.4	14	10.1	3	N			
421	F	A	L	N	38	2.6	N	6	8	A	N	17.6	13.2	10	2.6	N			
422	M	A	L	N	35	1.6	N	8	9	A	N	16	14.6	8.7	5.4	N			
423	M	P	L	N	37	3	N	7	8	A	N	16.4	9.9	5	4.1	N			
424	M	A	N	N	40	3.2	N	7	9	A	N	15.8	10.3	6.3	3	N			
425	F	A	L	N	40	3	N	5	9	A	N	17.3	17	12.1	3.8	N			
426	F	A	L	N	38	3.2	N	6	9	A	N	16.3	16.4	11.2	4.6	N			
427	F	A	N	N	39	2.5	N	6	9	A	N	16	14.5	9.4	4.5	N			
428	M	A	L	N	38	2.5	N	6	8	A	N	14.7	16.7	10.3	5.4	N			
429	M	A	L	N	37	2.6	N	7	9	A	N	14.1	17	12.5	4.2	N			
430	F	A	L	N	38	3	N	6	8	A	N	12.9	17.3	12.8	2.6	N			
431	F	A	N	N	39	3.5	N	7	9	A	N	15.8	14.3	9.7	3.2	N			
432	M	P	L	Y	34	1.7	N	4	8	A	N	16.5	24.1	9.9	11.5	N			
433	M	A	N	N	40	2.7	N	5	9	A	N								
434	M	A	L	N	39	2.5	N	7	10	A	N	15.3	12.3	8.4	3.7	N			
435	F	A	N	N	38	2.3	N	7	9	A	N	16.5	10.8	6	4.1	N			
436	M	A	N	N	38	2.1	N	6	8	A	N	14.3	14.6	7.3	6.4	N			
437	M	A	L	N	40	3.1	N	8	10	A	N	12.8	19.2	15.1	2.5	N			
438	M	A	L	N	38	3	N	6	8	A	N	15	14.2	10	3.6	N			
439	M	A	L	N	38	2.4	N	5	8	A	N	13.9	16	12.2	3	N			
440	M	A	N	N	37	2.8	N	7	9	A	N	14.7	16.6	11.7	4.2	N			
441	M	A	L	N	38	3	N	6	9	A	N	20.3	17.3	12.9	4	N			
442	F	A	N	Y	40	3.4	N	5	8	A	N	14.6	12.2	7.8	3.8	N			
443	F	P	L	Y	39	2.9	N	5	7	A	N	15.5	19.9	9.7	8.4	N			
444	M	A	N	N	37	2.6	N	7	9	A	N	13	18.6	12.5	5.6	N			
445	F	A	L	N	38	2.8	N	7	8	A	N	17.6	20.4	12.2	7.1	N			
446	F	A	L	N	37	2.3	N	6	9	A	N	10.8	26.3	18.2	7	N			
447	M	A	N	N	38	3.2	N	6	8	A	N	14.8	14.2	11.2	2.7	N			
448	F	A	N	N	41	4	N	7	9	A	N	14.5	17	11.6	5.2	N			
449	M	A	L	N	38	2.6	N	6	8	A	N	12.6	14.6	8.6	4.7	N			
450	F	A	L	N	36	2.4	N	7	10	A	N	16.2	15.5	12.3	2.8	N			

451	F	A	L	N	37	2.8	N	5	8	A	N	12.5	16.1	8.3	6.5	N			
452	F	A	N	N	34	1.9	N	5	7	P	N	14.8	16.3	9.4	6.1	N			
453	M	A	N	N	35	2.2	N	6	9	A	N	13.7	18.6	14.8	3.6	N			
454	F	A	L	N	36	2.9	N	7	9	A	N	14.4	14.5	7.7	4.7	N			
455	M	A	L	N	38	3.1	N	8	9	A	N	15.8	17.2	14.2	2.6	N			
456	F	P	L	N	37	2.2	N	7	9	A	N	16.2	20	16	3.1	N			
457	M	A	N	N	37	2.4	N	8	9	A	N	16.1	16.6	7.2	8.3	N			
458	M	A	L	N	38	3	N	6	8	A	N	14.9	19.2	13.1	5.4	N			
459	M	P	N	N	37	2.7	N	6	9	A	N	15.2	15.7	10.8	4.5	N			
460	F	A	L	N	37	2.6	N	5	8	A	N	14.7	14.4	9.2	4	N			
461	M	P	L	N	40	2.8	N	5	9	A	N	14.2	18.3	9.5	7.8	N			
462	F	A	L	N	35	1.8	N	6	9	A	N	15.8	15.2	11.4	2.9	N			
463	M	A	N	Y	39	3.4	N	5	8	A	N	14	17	12.9	3.2	N			
464	M	A	N	N	40	3.2	N	7	8	A	N	13.3	19.3	12	6.1	N			
465	F	A	L	N	40	3	N	6	9	A	N	13.5	17.7	9.8	7.6	N			
466	F	A	L	N	33	1.8	P, S	6	8	A	N	12.3	6.8	4.7	1.8	Y	3.7	-	
467	M	A	N	N	33	1.9	N	7	8	A	N	18.7	15.9	10.1	5.2	N			
468	M	A	L	N	38	2.9	N	8	9	A	N	15.2	14.5	9.7	3.5	N			
469	M	A	L	N	38	2.7	N	7	10	A	N	12.3	14.2	9	4.6	N			
470	M	A	N	N	38	2.6	N	6	9	A	N	16.3	23.1	14.2	8	N			
471	F	A	L	N	39	3.5	N	7	9	A	N	13.2	14.6	6.5	7.5	N			
472	F	A	N	N	37	3	N	6	8	A	N	13.1	11.3	6.8	3.9	N			
473																			
474	F	P	N	N	40	3	N	7	9	A	N	14.8	17.5	11.4	5.3	N			
475	M	A	N	N	40	3.5	N	5	8	A	N	16.8	12.3	5.9	6	N			
476	F	A	L	N	38	3	N	6	8	A	N	14.3	16.9	11.3	5.1	N			
477	M	A	L	N	39	3.1	N	7	9	A	N	15.6	14.5	9.3	4.5	N			
478	M	A	N	N	34	2.3	N	6	10	A	N	14.7	18.6	10.3	7.4	N			
479	M	A	N	N	37	2.4	N	6	9	A	N	14	22.7	13	8.4	N			
480	F	A	L	Y	38	2.6	N	5	9	A	N								
481	F	A	N	N	35	2	N	7	8	A	N								
482	M	A	L	N	35	2.5	N	6	8	A	N	17.6	24.2	12.8	8.6	N			
483	F	A	N	N	39	2.9	N	7	9	A	N	17	14	8.6	4.7	N			
484	M	P	L	N	40	2.9	N	8	10	A	N	15.6	15.4	9.8	4.6	N			
485	F	A	N	N	40	3	N	6	8	A	N	14.9	18.3	12.1	5.4	N			
486	F	A	N	N	39	3.4	N	7	9	A	N	12.7	14.5	6.9	6.1	N			
487	M	A	L	N	40	3.2	N	7	10	A	N	18.7	13.2	10.4	2.6	N			
488	M	P	L	N	34	1.9	P	6	8	A	N	11.2	9.4	7.1	2	Y	3.2	-	
489	M	P	L	Y	34	2.1	P	4	7	A	N	10.3	7.6	5.2	1.6				
490	F	A	N	N	39	2.8	N	7	9	A	N	18.1	13.2	9.6	2.8	N			
491	M	A	N	N	38	2.6	N	6	8	A	N	11.6	11.4	6.9	2.6	N			
492	M	P	L	N	36	2.4	N	7	9	A	N	13.3	10.5	7	3.2	N			
493	M	A	L	N	37	2.6	N	6	9	A	N	15.3	18.9	11.3	7	N			
494	M	A	L	N	37	3	N	7	10	A	N	16	14.3	8.6	4.7	N			
495	M	A	N	N	35	2	N	6	9	A	N	14.5	18.8	12.5	5.4	N			
496	F	A	L	N	37	2.7	N	7	9	A	N	15	16.6	10.1	5.1	N			
497	F	A	N	N	33	1.8	N	6	9	A	N	15.8	13.9	7.8	4	N			
498	M	A	L	N	33	1.5	N	4	7	A	N	16.9	20.2	15.5	4.3	N			
499	F	A	N	N	38	2.9	N	8	9	A	N	14.7	13.7	7.8	4.5	N			
500	F	A	L	N	37	2.6	N	7	9	A	N	15.6	15.3	9.1	4.8	N			
501	F	A	N	N	38	2.8	N	7	9	A	N	15	18.2	14.1	3.5	N			

502	M	A	N	N	35	2.4	N	6	8	A	N	12.4	14.1	10.2	3.6	N			
503	F	A	N	N	38	3	N	5	8	A	N	12	11	7.7	3.2	N			
504	M	A	L	N	37	3.2	N	6	9	A	N	16.2	27.6	15.3	10.5	N			
505	F	A	L	N	39	3.6	N	6	9	A	N	12.7	14.8	7.3	7.1	N			
506	M	A	L	N	36	2.5	N	5	8	A	N	14.2	15.4	11.2	3.7	N			
507	F	A	N	Y	36	2	N	5	9	A	N								
508	M	A	L	N	36	2.2	N	7	9	A	N	15.2	9.6	6.4	2.5	N			
509	F	A	L	N	37	2.8	N	7	9	A	N	15.3	8.5	5.3	2.9	N			
510	F	A	L	N	39	2.5	N	7	9	A	N	14	21.3	12.7	7.8	N			
511	M	A	N	N	35	2	N	7	8	A	N	17.8	16.9	10.5	5.7	N			
512	F	A	N	N	38	3.9	N	8	10	A	N	17.2	17.2	12.9	3.6	N			
513	M	A	L	N	40	3.6	N	5	7	A	N	11.3	14.7	9.7	3.4	N			
514	F	A	N	N	40	3	N	7	9	A	N	16.6	10.8	7.2	3	N			
515	M	P	L	N	39	2.8	N	6	8	A	N	12.3	9.7	5.4	3.4	N			
516	F	A	L	N	40	3	N	5	7	A	N	17.5	8.3	5.5	2.5	N			
517	M	A	N	N	38	2.7	N	6	8	A	N	12.5	13.2	10.1	2.5	N			
518	F	A	L	N	35	2.3	P	3	5	P	N	11.9	11.8	9.3	1.9	Y	2.9	-	
519	F	A	L	N	40	3	N	7	9	A	N	12.8	14.1	7.7	6	N			
520	M	A	N	N	39	2.6	N	7	9	A	N	13.6	16.3	9.3	5.7	N			
521	F	A	N	N	39	3	N	8	9	A	N	14	12.2	7	4.6	N			
522	M	A	L	N	38	2.8	N	5	8	A	N	12.8	9.3	5.1	4	N			
523	F	A	N	N	37	2.4	N	6	9	A	N	19.3	18.3	8.3	9.4	N			
524	M	A	L	N	37	2.6	N	7	8	A	N	18.5	15.2	12	2.8	N			
525	M	A	N	N	35	2	P	7	9	A	N	12.5	16.8	12.7	2.4	Y	3.3	-	
526	F	A	N	Y	37	3.2	N	5	8	A	N	14	12.3	6.6	5.3	N			
527	M	A	L	N	38	3.6	N	7	9	A	N	15.4	14.4	10.3	3.2	N			
528	F	A	N	N	39	3	N	7	9	A	N	14.6	8.7	4.8	2.7	N			
529	M	A	L	N	37	2	N	6	8	A	N	12	9.8	4.5	4.3	N			
530	F	A	L	N	36	2.1	N	6	9	A	N	12.6	10.2	5.6	4.1	N			
531	F	A	N	N	36	1.9	N	4	7	A	N	14.7	18.3	15.1	2.5	N			
532	M	P	L	N	36	2.5	N	7	9	A	N	14	15.6	11.4	2.9	N			
533	F	A	L	N	33	1.6	N	7	8	A	N								
534	F	A	N	N	34	2	N	6	9	A	N	14.1	12.8	5.3	6.1	N			
535	M	A	N	N	38	3	N	7	8	A	N	11.8	17.6	11.3	5.1	N			
536	F	A	L	N	36	1.9	N	8	9	A	N	17.5	12.4	8.5	2.8	N			
537	M	A	N	N	39	3	N	6	8	A	N	16.7	15.3	10.4	4.6	N			
538	F	A	L	N	38	3.2	N	7	9	A	N	13.7	12.7	7.1	4.4	N			
539	M	A	L	N	39	3.4	N	6	9	A	N	15.2	14.1	6.6	5.7	N			
540	F	A	N	N	40	3	N	6	9	A	N	12.6	12.9	8.2	2.6	N			
541	M	A	L	N	35	1.8	N	7	8	A	N	11.5	16.8	9.3	6.1	N			
542	F	A	N	N	39	2.8	N	7	9	A	N	18.6	14.2	8	3.8	N			
543	M	A	L	N	40	2.6	N	6	8	A	N	10.8	12.4	7.4	3.7	N			
544	M	A	N	N	40	2.6	N	7	9	A	N								
545	F	A	L	N	38	3	N	7	8	A	N	16.5	16.8	11.7	4	N			
546	F	A	L	N	38	2.4	N	8	10	A	N	14.8	9.4	4.9	4.2	N			
547	M	A	N	Y	36	2.2	N	6	8	A	N	13.8	13	7.8	4.8	N			
548	M	A	N	N	37	3	N	7	9	A	N	12.7	14.3	4.1	8.8	N			
549	F	A	L	N	38	3.1	N	8	10	A	N	13.4	17.6	8.2	7.3	N			
550	M	A	N	N	39	3.2	N	6	8	A	N								
551	F	A	L	N	36	2.3	N	8	9	A	N	12.8	14.3	10.7	2.6	N			
552	M	A	N	Y	39	2.6	N	4	7	A	N	13.3	12.3	8.5	2.6	N			
553	F	P	L	N	37	3	N	7	8	A	N	15.1	16	11.8	3.7	N			

554	F	A	N	N	40	3.6	N	6	8	A	N	16.2	11.2	7.9	3.1	N			
555	M	A	L	Y	40	2.7	N	4	9	A	N	13.1	13.5	7.2	4.8	N			
556	M	A	L	N	37	2.9	N	9	10	A	N	14.9	10.5	5.5	4.5	N			
557	F	A	L	N	38	2.5	N	6	9	A	N	12.7	14.8	8.3	5.8	N			
558	M	P	N	Y	36	2.3	S	5	9	A	N	11.5	11.3	8.8	2.2	Y	3.2	-	
559	M	A	L	N	37	2.7	N	8	9	A	N	16.3	16.2	12.3	2.5	N			
560	F	A	L	N	37	2.6	N	4	7	A	N	15.5	15.2	9.8	2.9	N			
561	M	A	N	N	34	1.8	N	6	8	A	N	16.8	17.3	13.2	2.6	N			
562	F	A	L	N	35	2.1	N	7	9	A	N	14.2	12.5	8.4	3.2	N			
563	M	A	N	N	35	2.2	N	5	8	A	N	16.2	16	11.7	3.8	N			
564	F	P	L	N	36	2	N	6	9	A	N	12.8	15.6	10.8	4	N			
565	M	A	N	N	38	2.5	N	7	8	A	N								
566	F	A	L	N	37	3	N	6	9	A	N	17.6	22.1	14	7.6	N			
567	M	A	N	N	38	2.5	N	7	8	A	N	15.9	13.8	5.4	5.1	N			
568	F	P	L	N	38	3	N	7	9	A	N	18.4	13.1	6.8	6	N			
569	M	A	L	N	38	3.1	N	6	9	A	N	16	16.4	12	3.4	N			
570	F	P	N	Y	39	3.8	N	3	8	A	N	12.7	18.2	14.2	3	N			
571	F	P	N	N	34	2	P	4	7	A	N	14.1	13.8	11.1	1.8	Y	2.8	-	
572	M	A	L	N	37	2.4	N	5	8	A	N	15	14.3	8.7	4.4	N			
573	M	P	N	N	37	2.5	N	7	9	A	N	16.2	17.4	9.9	6.7	N			
574	M	A	L	N	36	2	N	7	10	A	N	14.2	25.6	15.6	8.1	N			
575	M	A	N	N	38	3.2	N	6	9	A	N	12.7	18.3	14.6	2.9	N			
576	M	A	L	N	39	3	N	7	8	A	N	16.5	10.4	6.7	3	N			
577	F	A	N	N	40	2.8	N	8	10	A	N	13.3	17	13.1	3.3	N			
578	M	A	N	N	40	2.6	N	7	8	A	N	14.7	15.3	7.3	7	N			
579	F	A	N	N	35	1.7	P, S	6	8	A	N	12.9	16.3	12.5	2.4	Y	4.1	-	
580	M	A	N	N	38	2.8	N	7	8	A	N	11.7	14.3	7	4.5	N			
581	M	A	L	Y	39	2.4	N	4	8	A	N	15.6	16	10.2	4.9	N			
582	F	A	L	N	37	2.6	N	7	9	A	N								
583	M	A	N	N	37	3	N	8	9	A	N	15	6.8	3.7	2.7	N			
584	M	A	N	N	38	3.1	N	6	8	A	N	13.6	8.9	4.3	3.6	N			
585	F	A	N	N	37	3.2	N	9	10	A	N	13.8	17.1	11.5	5.7	N			
586	M	A	L	N	34	1.8	N	7	8	A	N	14.1	16.5	10.8	4.1	N			
587	M	A	N	N	38	2.6	N	8	9	A	N	16.9	16.4	8.5	6.1	N			
588	M	A	N	N	37	2.8	N	7	9	A	N	18.2	17.2	12.7	2.8	N			
589	F	P	L	N	39	2.6	N	8	9	A	N	13.8	15.9	8.3	6.7	N			
590	F	A	L	N	35	2	N	5	8	A	N	11.6	10.7	5.9	3.9	N			
591	M	A	N	N	37	3	N	6	8	A	N	15.4	9.5	5.4	2.6	N			
592	F	P	L	N	38	2.6	I	6	9	A	N	16.7	13.2	10.5	2.1	Y	Missed	-	
593	M	A	L	N	39	3.2	N	7	8	A	N	17.1	20.3	12.3	7	N			
594	F	A	N	N	38	3	N	7	9	A	N	17	8.7	4.9	3.2	N			
595	M	A	N	N	38	3.4	N	7	8	A	N	12	10.2	6.8	2.7	N			
596	M	A	L	N	37	2.8	N	5	7	A	N	16.3	14.4	8.4	5	N			
597	F	A	N	N	36	2.3	N	7	9	A	N	12.5	14.1	8.8	4.2	N			
598	M	A	L	N	38	2.6	N	6	10	A	N	15.6	15.2	10.1	4.7	N			
599	M	A	L	N	38	2.5	N	8	9	A	N	13.5	15.6	9.3	5	N			
600	F	A	N	N	37	2.7	N	7	10	A	N	14	14.2	7.2	6.3	N			
601	M	A	L	N	38	3	N	7	9	A	N	14.3	16.3	12.2	3.6	N			
602	M	A	N	N	39	3.1	N	6	9	A	N	12.9	17.5	9.7	6	N			
603	M	P	L	N	36	2.3	N	6	8	A	N								
604	M	A	L	N	37	3.3	N	7	9	A	N	13.5	14.1	8.9	4.1	N			
605	F	A	N	N	39	3.2	N	5	8	A	N	15.8	13.6	7.4	4.3	N			

606	M	A	N	N	40	2.7	N	6	9	A	N	16.1	16.3	11.3	4	N			
607	F	A	L	N	39	2.9	N	6	8	A	N	12.8	24.1	16.7	6.1	N			
608	M	A	N	N	38	3	N	8	9	A	N	18.2	20.3	13.5	6	N			
609	M	A	L	N	37	2	S	7	9	P	Y	17	8.9	7	1.6	Y	2.5	-	
610	M	A	L	N	38	2.4	N	7	10	A	N	12.4	12.8	4.7	7.6	N			
611	F	A	N	Y	38	2.6	N	5	7	A	N	14.3	11.3	5.1	5.8	N			
612	M	A	L	N	37	2.4	N	7	8	A	N	14.6	17.2	11	5.1	N			
613	M	A	N	N	37	2.9	N	6	9	A	N								
614	F	A	L	N	38	3.3	N	7	8	A	N	16.1	12.5	5.9	3.6	N			
615	M	A	N	N	38	3.1	N	7	10	A	N	16.7	12	7.3	4.2	N			
616	M	A	L	N	38	3	N	7	9	A	N	15.5	14.1	11.1	2.8	N			
617	F	A	L	N	39	2.4	N	8	9	A	N	12.6	13.5	9.2	3.5	N			
618	M	P	L	N	37	2.8	N	7	8	A	N	14.2	14.3	10.5	2.6	N			
619	M	A	L	N	36	2.2	P	7	9	A	N	12.2	15.1	12.4	2.3	Y	3.8	-	
620	M	A	N	N	37	2.7	N	6	8	A	N	13.5	12.5	7.3	4.5	N			
621	M	A	L	N	37	3.1	N	7	9	A	N	16.5	11.8	6.9	4	N			
622	F	A	L	N	39	3	N	8	9	A	N	15.8	11.1	7.3	3.2	N			
623	F	A	L	N	40	3.5	N	6	8	A	N	14.7	12.3	7.2	3.5	N			
624	M	A	N	N	38	2.5	N	5	9	A	N	14	10.8	6.6	2.9	N			
625	F	A	L	N	37	2.4	N	6	8	A	N	14.9	14	11	2.6	N			
626	M	A	N	N	39	3	N	8	10	A	N	13.6	15.6	7.9	7	N			
627	F	A	N	N	40	3.1	N	5	9	P	N	16.5	17.4	10.8	6.1	N			
628																			
629	M	A	L	Y	35	2.2	N	5	7	A	N	11.5	12.2	7.2	4.3	N			
630	F	P	N	N	39	3.2	N	7	8	A	N								
631	M	A	L	N	38	2.7	N	6	9	A	N	13.6	15.1	11.3	2.5	N			
632	F	A	N	N	37	2.4	N	7	9	A	N	13.1	14.2	10.4	2.7	N			
633	M	A	L	N	36	2.6	N	8	10	A	N	14.7	16.4	9	6	N			
634	F	A	L	N	35	1.9	N	6	9	A	N	17.6	13	8.7	3.5	N			
635	M	A	N	N	39	3.7	N	6	8	A	N	15.4	12.4	7.3	4.1	N			
636	M	A	L	N	38	3	N	6	9	A	N	12.8	18.3	12.9	5	N			
637	M	A	N	N	39	2.8	N	7	9	A	N	11.6	14.6	8.5	4.6	N			
638	F	A	L	N	37	2.4	N	8	9	A	N	19.6	9.5	5.1	4.2	N			
639	F	A	L	N	37	2.5	N	5	8	A	N	12.5	16.8	13.1	2.7	N			
640	F	A	N	N	36	2.3	N	6	9	A	N	16.3	12.8	7	4.2	N			
641	F	A	L	N	36	2.4	N	8	9	A	N								
642	F	P	N	N	37	2.9	N	7	10	A	N	14.8	14.7	10.9	2.7	N			
643	M	A	L	N	37	2.5	N	6	9	A	N	14	16	12.1	2.6	N			
644	F	A	N	N	39	2.6	N	7	8	A	N	14.5	14.2	10.2	3.7	N			
645	M	A	N	N	38	3.3	N	8	9	A	N								
646	M	A	L	N	37	2.4	N	7	9	A	N	12.9	15.4	9.4	2.9	N			
647	F	A	N	N	36	2.6	N	7	10	A	N	16.3	16.1	10.5	4.1	N			
648	F	A	L	N	37	2.8	N	6	9	A	N	12.4	17.1	11.3	3.6	N			
649	F	A	L	N	33	1.9	N	7	8	A	N	13.8	10.3	7.1	2.8	N			
650	M	A	N	N	37	2.7	N	8	10	A	N	14.7	11.2	6	4.9	N			
651	M	A	L	N	38	2.5	N	7	9	A	N	13.8	10	4.9	4.6	N			
652	F	A	N	N	37	2.5	N	7	9	A	N	12.6	18	14.2	3	N			
653	F	A	N	N	38	2.9	N	6	9	A	N	13.6	14.2	10.1	3.2	N			
654	M	A	L	N	37	3.6	N	7	8	A	N	14.4	15.3	10.2	3.6	N			
655	M	A	L	N	40	2.8	N	7	8	A	N	15	14.7	9.7	4	N			
656	F	A	N	N	37	2.4	N	8	9	A	N	16	15.9	7.8	6.2	N			
657	F	A	L	N	37	3.1	N	7	9	A	N	12.5	16	10.4	2.8	N			

658	M	A	N	N	39	3	N	7	8	A	N	18.7	7.9	3	4.2	N			
659	F	A	L	N	38	3.2	N	9	10	A	N	17.3	15	10.4	3.3	N			
660	F	A	L	N	40	3	N	6	8	A	N	13.6	16.5	12.3	2.7	N			
661	M	A	L	N	40	3	N	7	9	A	N	12.7	12.3	8.1	3.2	N			
662	M	P	N	N	35	2	N	7	9	A	N								
663	M	A	N	N	35	2.1	N	6	8	A	N	14.4	16.1	11.2	4.5	N			
664	M	A	L	N	35	2.4	N	7	8	A	N	15.6	13.3	9.7	3.2	N			
665	F	A	N	N	38	3.4	N	7	9	A	N	16	15.3	10.8	3.6	N			
666	M	A	L	N	39	3.1	N	8	9	A	N	16.8	16.2	9.6	4.5	N			
667	F	A	N	N	35	2.2	N	8	9	A	N	19.1	11.4	8	2.8	N			
668	F	A	L	N	36	2.6	N	8	10	A	N	10.2	19.4	15	2.7	N			
669	M	A	L	N	37	2.7	N	7	9	A	N	12.6	16.8	11.3	2.9	N			
670	F	A	N	N	40	3	A	6	8	A	N	16.9	14	10.1	2	Y	Missed	-	
671	M	A	N	N	37	2.6	N	7	9	A	N	16.5	15.8	9.4	3.1	N			
672	M	A	L	N	37	2.5	N	8	10	A	N	15.2	14.6	10.5	2.7	N			
673	F	A	N	N	34	1.7	N	7	8	A	N	15.3	16.3	13.1	2.5	N			
674	F	P	L	N	38	3.3	N	7	9	A	N	14	16	12	3.2	N			
675	M	A	N	N	39	3.4	N	7	9	A	N	14.8	14.7	8.4	5	N			
676	M	P	L	N	35	1.8	N	6	8	A	N	12.8	14.4	7.5	4.8	N			
677	F	A	L	N	35	2	N	7	9	A	N	13.3	15.1	10.3	2.5	N			
678	F	A	N	N	38	2.7	N	7	8	A	N								
679	M	A	L	N	40	3.1	N	8	9	A	N	15.7	16.4	10.2	4.7	N			
680	F	A	N	N	40	3	N	6	8	A	N	14.7	14	9.9	2.6	N			
681	M	A	L	N	38	2.6	N	5	8	A	N	16.2	10.3	6.8	3.6	N			
682	F	A	N	Y	40	3	N	5	7	A	N	15.2	8.2	4.7	2.5	N			
683	F	P	L	N	35	2.2	N	8	9	A	N	14.7	18	13.5	3	N			
684	F	A	L	N	40	2.7	N	7	8	A	N	16.6	16.8	12.2	3.8	N			
685	M	A	L	Y	38	2.8	N	5	7	A	N	15	17.2	11.3	5	N			
686	M	A	N	N	39	3.5	N	8	9	A	N	12.6	16.3	13.1	2.6	N			
687	M	P	L	N	39	3.1	N	7	8	A	N								
688	F	A	L	N	35	2.4	N	7	9	A	N	11.6	15.1	10.6	2.7	N			
689	M	A	N	N	38	2.9	N	9	10	A	N	16.5	12.1	7.7	4.2	N			
690	F	A	N	N	37	2.7	N	8	9	A	N	14.6	13.2	8.7	4	N			
691	M	A	L	N	35	2.3	N	8	9	A	N	12.8	14.8	7.4	6.3	N			
692	M	P	L	Y	36	2.3	P	5	9	P	N	11	10.7	7.8	2.3	Y	Missed	-	
693	F	A	N	N	37	3.1	N	6	8	A	N	11.6	19.3	11.5	6.8	N			
694	F	A	L	N	37	2.6	N	8	10	A	N	16.3	12.3	7.1	4.7	N			
695	M	A	L	N	38	3	N	6	8	A	N	16.5	11	6	4.6	N			
696	F	A	N	Y	40	3.4	N	5	7	A	N	17	12.4	5.5	5.4	N			
697	F	A	L	N	40	3.4	I	7	8	A	N	14.7	7.6	5.1	2	Y	3.2	-	
698	M	A	L	N	40	2.8	N	7	9	A	N	16.4	17	9.7	5.8	N			
699	F	A	N	N	38	2.5	N	7	8	A	N	14	16.3	10.3	4.5	N			
700	M	A	N	N	39	3.1	N	6	8	A	N	12.5	14.8	9.6	3.4	N			
701	F	P	N	N	40	3	N	7	9	A	N	14.8	12.8	8.4	3.1	N			
702	F	P	N	N	38	2.6	N	7	8	A	N	16.2	12.2	7.1	3.4	N			
703	M	A	L	N	37	3.1	N	6	9	A	N	17.6	14.6	8	5.1	N			
704	M	A	L	N	40	3.3	N	6	7	A	N	18	13.2	6.9	4.9	N			
705	F	A	N	N	36	2.1	N	7	9	A	N	15.4	17.8	10.3	5.2	N			
706	F	A	L	N	36	2.6	N	6	9	A	N	14	18	11.4	5	N			
707	M	A	N	N	38	3	N	6	9	A	N	17.5	19.5	12.5	6.4	N			
708	F	A	L	N	39	3.9	N	7	8	A	N								
709	F	A	N	N	40	4.1	N	6	10	A	N	12.7	13.1	7.9	4	N			

710	F	P	L	N	37	2.4	N	8	9	A	N	11.9	16.5	12.2	3.7	N			
711	M	A	N	N	36	2	N	6	8	A	N	15.6	12	6.6	3.9	N			
712	F	A	L	Y	34	1.6	N	5	9	A	N	16.6	13.9	7	6.5	N			
713	M	A	N	N	37	2.5	N	8	9	A	N	16	22.6	13.1	7.6	N			
714	F	A	L	N	35	2.3	N	7	9	A	N	14.2	14.4	9.6	2.5	N			
715	M	A	L	N	35	2.2	N	7	8	A	N	13.8	10.5	6.2	3	N			
716	F	A	N	N	37	2.7	N	6	7	A	N	17.5	13.8	7.9	3.9	N			
717	M	P	L	N	37	3.1	N	5	8	A	N	15.3	16.6	11.1	4.2	N			
718	M	A	N	N	38	3.7	N	8	10	A	N	15	9.5	5	3.3	N			
719	F	A	N	N	39	2.6	N	8	9	A	N	14.8	16.7	8.6	6.5	N			
720	F	A	N	N	39	2.8	N	7	8	A	N	14.2	24.9	14.2	8	N			
721	M	A	L	N	39	3	N	6	9	A	N	16.1	12.3	6.3	5.4	N			
722	F	A	L	N	37	2.4	N	6	9	A	N	15.8	14.1	9	4.1	N			
723	M	P	N	N	39	3.5	N	8	10	A	N	12.9	16.8	12.3	3.8	N			
724	M	A	L	N	38	3.6	N	8	9	A	N								
725	F	A	N	N	36	2.4	N	7	10	A	N	19.8	13.5	7.7	5.2	N			
726	F	A	L	N	36	2.4	N	6	8	A	N	13.7	10.3	6	2.7	N			
727	M	A	L	N	37	2.7	N	7	9	A	N								
728	F	A	L	N	40	2.6	N	7	8	A	N	15.2	12.2	7.3	4.4	N			
729	M	A	N	N	39	2.9	N	8	9	A	N	16.1	9.9	6.4	2.9	N			
730	F	A	L	N	40	3	N	7	10	A	Y	10.9	11.2	7.3	2.9	N			
731																			
732	F	A	N	N	40	2.8	N	8	9	A	N	12.9	8	4.5	3.1	N			
733	M	A	L	Y	36	2.1	N	4	8	A	N	14	15.3	11.8	3.3	N			
734	M	P	L	N	36	1.7	N	6	7	A	N	12.8	6.8	3.9	2.5	N			
735	M	A	N	N	38	3.4	N	8	9	A	N	18.6	11.9	6.7	4.5	N			
736	F	A	L	N	38	3	N	6	10	A	N	17.7	14.4	10.6	2.6	N			
737	M	A	N	N	39	2.9	N	7	8	A	N	16.5	13.5	9.7	2.8	N			
738	M	P	L	N	38	2.7	I	6	9	A	N	13	13	8.9	2.1	Y	3	-	
739	M	A	L	N	38	2.4	N	7	10	A	N	16.2	11.4	5.5	4.1	N			
740	F	A	L	N	38	2.7	N	6	9	A	N	15.5	12.2	8.3	3.1	N			
741	M	A	N	N	37	2.5	N	5	7	A	N	14.7	15.3	9.3	4.8	N			
742	F	A	N	N	38	2.9	N	6	8	A	N	15	16.8	10.7	4.2	N			
743	M	A	N	N	38	3.4	N	8	10	A	N	15.9	12	6.2	4.6	N			
744	M	A	L	N	39	3.1	N	6	9	A	N								
745	M	A	N	N	38	3	N	7	9	A	N	18	11	5.8	3.4	N			
746	F	A	N	N	36	2	N	7	10	A	N	16.2	16.1	11.5	4.7	N			
747	M	A	L	N	37	2.4	N	7	8	A	N	17	13.3	8.6	4.2	N			
748	F	A	L	N	37	2.4	N	9	10	A	N	17.1	15.1	7.4	4.1	N			
749	M	A	L	N	38	2.8	N	7	8	A	N	19.5	14.2	3.5	9.1	N			
750	M	P	L	N	39	2.9	N	7	9	A	N	20.1	16	10.6	4.5	N			
751	F	A	N	Y	41	3.5	I	4	9	A	N	14.7	10	7.5	2.1	Y	3.9	-	
752	F	A	N	N	40	2.6	N	6	8	A	N	17	21.7	14	4.9	N			
753	M	A	L	N	40	3.3	N	6	9	A	N	15.9	17.8	8.9	6.2	N			
754	F	A	N	Y	35	2.1	P	3	5	A	N	12.2	10.2	6.5	2.3	Y	2.9	-	
755	F	A	N	N	39	2.9	N	9	10	A	N	16.7	12.7	10.1	2.2	N			
756	M	A	L	N	34	1.5	N	7	10	A	N	15.4	14	9.6	3.1	N			
757	F	A	L	N	37	2.6	N	6	8	A	N	14.9	13.5	6.7	5.7	N			
758	M	A	L	N	38	2.9	N	6	9	A	N	17	18.8	12.2	5.5	N			
759	F	A	N	Y	38	3.1	N	5	8	A	N	16.5	28.9	14.7	11.2	N			
760	M	A	N	N	39	3	N	6	8	A	N								

761	M	A	L	N	39	2.5	N	7	9	A	N	18.3	14.2	10.3	3.1	N			
762	F	A	N	N	35	2.1	N	8	9	A	N	17.5	15	11.4	3.1	N			
763	M	A	L	N	36	2.3	N	7	8	A	N	14.9	16.8	9.4	6.2	N			
764	F	A	L	N	38	3.1	N	8	10	A	N	14.3	18.9	14.1	4.5	N			
765	M	P	N	N	37	3.2	N	7	9	A	N	15.1	15.4	10.1	4.9	N			
766	F	A	L	N	37	3.2	N	6	9	A	N	19.8	12	8.7	3.1	N			
767	M	A	N	N	40	4.2	N	6	8	A	N	20.3	11.6	6.6	4.3	N			
768	F	A	N	Y	33	1.9	N	7	8	A	N	13.8	7.6	2.4	5	N			
769	M	A	L	N	38	3.1	N	8	9	A	N	14.6	15.6	11.5	3.5	N			
770	F	A	L	N	39	3	N	7	8	A	N								
771	M	A	N	N	40	2.8	N	6	9	A	N	16.6	17.7	10.8	5.7	N			
772	F	A	L	N	38	2.7	N	7	8	A	N	18.2	16.1	12.1	3.4	N			
773	M	A	N	Y	38	2.6	N	5	7	A	N	17	14.7	10.3	3.6	N			
774	F	A	L	N	37	2.6	N	6	9	A	N								
775	M	A	L	N	38	3.3	N	7	10	A	N	11.2	12.1	7.7	4.1	N			
776	M	A	N	N	35	2.3	N	6	9	A	N	13	14.8	10.5	2.9	N			
777	M	A	L	N	37	2.4	N	6	8	A	N	11.9	22.8	14.9	7.7	N			
778	F	A	L	N	37	2.6	N	8	10	A	N	17.4	17.6	10	6.4	N			
779	M	P	N	N	38	3	N	8	9	A	N	16.5	13.5	8.8	4.3	N			
780																			
781	F	A	N	N	40	3.1	N	7	9	A	N	17.2	16.4	12	4.2	N			
782	F	A	L	N	37	2.4	N	8	9	A	N	15.6	10.7	6.7	3.9	N			
783	M	A	N	N	38	3	N	7	8	A	N	15.2	20.3	12.4	7.1	N			
784	M	A	L	N	40	3.2	I	5	9	A	N	12.3	10.1	8	2.4	Y	4.2	-	
785	M	A	L	N	39	3.5	N	7	9	A	N	20.7	9.3	6.4	2.6	N			
786	M	A	L	N	37	2.5	N	8	9	A	N	14.6	8.7	5.8	2.8	N			
787	M	A	L	N	37	2.6	N	7	10	A	N	15	17.5	11.8	5.1	N			
788	F	P	N	N	38	3.8	N	6	7	A	N	19.3	10	5.3	4.2	N			
789	F	A	N	N	38	2.6	N	9	10	A	N	17.6	11.3	7.5	3.3	N			
790	M	A	L	N	36	2.1	N	7	9	A	N	13.9	14.3	10	4.1	N			
791	F	A	L	N	37	2.6	N	6	9	A	N	16.6	15.9	11.1	4.4	N			
792	M	A	L	N	38	2.4	N	8	10	A	N	15.2	20.1	14.8	5	N			
793	M	A	L	N	39	3	N	7	8	A	N								
794	F	A	N	N	36	3.2	N	7	9	A	N	17	16.4	8.4	7.6	N			
795	M	A	L	N	37	2.8	N	8	10	A	N	15.1	12.1	9.1	2.7	N			
796	F	A	N	N	37	2.7	N	6	9	A	N	14.9	13.2	8.5	4.4	N			
797	M	A	N	N	36	2.5	N	5	8	A	N	10.7	10	7	2.9	N			
798	F	A	L	N	37	3.2	N	5	10	A	N	9.8	8.8	4.7	3.1	N			
799	M	A	L	N	36	2.4	P	6	8	A	N	20.2	9	6.7	1.7	Y	Missed	-	
800	M	A	L	N	37	2.9	N	7	9	A	N	18.5	13.2	9.8	3.1	N			
801	F	P	N	N	39	3	N	8	9	A	N	14.6	12.6	9	3.3	N			
802	F	A	L	N	40	3.1	N	6	9	A	N	15.5	10.9	8.2	2.5	N			
803	M	A	N	N	40	3	N	6	8	A	N	15	8.9	4.1	4.5	N			
804	M	A	L	N	38	2.5	N	7	9	A	N	12.2	6.8	3.6	2.9	N			
805	F	A	L	N	37	2.4	N	8	9	A	N	14.2	14.5	11	3.1	N			
806	M	A	L	N	37	2.9	N	7	9	A	N	16.3	13.2	10.7	2.3	N			
807	F	A	N	N	37	2.8	N	6	8	A	N	12.9	11.7	7.2	4.1	N			
808	M	P	L	N	36	2.7	N	7	9	A	N	15.6	15	9.3	5.6	N			
809	M	P	N	N	37	2.8	N	8	9	A	N								
810	F	A	N	N	39	3	N	6	8	A	N	14.4	13.7	9.5	3.9	N			
811	M	A	L	N	40	2.6	N	7	10	A	N	14.2	16.8	13.1	3	N			
812	F	A	L	Y	38	2	N	5	9	A	N	17.6	14.2	8.7	4.8	N			

813	M	A	N	N	39	2.8	N	7	9	A	N	18	15	6.5	8.2	N			
814	F	A	L	N	38	2.7	N	8	10	A	N	13.2	13.1	9.2	3.4	N			
815	M	A	N	N	36	2.2	N	5	7	A	N	15.3	17.3	12.5	4.2	N			
816	F	P	L	N	36	2.1	P	6	9	A	N	14	10.8	7.7	2	Y	2.7	-	
817	F	A	L	N	38	3.4	N	7	9	A	N	14.9	14.5	9.1	4.8	N			
818	F	A	N	N	39	3	N	8	9	A	N	15	11	6.7	4.2	N			
819	M	A	N	N	38	2.6	N	6	10	A	N	11.3	11.3	10.3	2.6	N			
820	F	A	L	N	37	2.6	N	8	9	A	N	17.6	12.2	8.6	3.3	N			
821	M	A	N	N	38	2.9	N	8	9	A	N								
822	F	P	N	N	39	3	N	5	9	A	N	14.8	19.8	14.1	5.5	N			
823	M	A	N	N	39	3.7	N	6	9	A	N	16.1	22.1	10	11.2	N			
824	M	A	L	N	35	2.2	P	7	8	P	N	14.9	9.1	6	2.3	Y	Missed	-	
825	F	A	N	N	35	1.8	N	6	8	A	N	15.7	19.3	14.1	4.4	N			
826	M	A	L	N	35	2	N	7	9	A	N	16.2	18	14.3	3.1	N			
827	F	A	L	N	34	1.9	N	6	9	A	N	15	14.1	10.6	3.1	N			
828	M	A	L	N	38	2.8	N	8	9	A	N	10.8	17	11.2	4.9	N			
829	M	A	L	N	39	2.8	N	8	9	A	N	17.2	11.4	5.8	5	N			
830	M	A	L	N	40	2.7	N	7	9	A	N	14.1	15	8.5	5.6	N			
831	F	A	L	N	38	2.6	N	7	8	A	N	17.4	10.2	6.5	3.4	N			
832	M	A	L	N	34	2	N	7	9	A	N	14.7	14	10.7	3.2	N			
833	M	A	N	N	38	2.5	N	7	8	A	N	16.1	17	12.1	4.4	N			
834	F	A	L	N	37	2.6	N	7	9	A	N								
835	M	A	N	N	37	2.4	N	7	8	A	N	18.1	14.7	10.3	3.1	N			
836	M	A	N	N	36	2.7	N	6	9	A	N	17	16	11.4	4.3	N			
837	F	A	N	N	37	2.7	N	5	8	A	N	15	15.2	11.3	3.5	N			
838	F	A	L	N	37	2.7	N	8	9	A	N	14.7	12.3	7.1	5	N			
839	F	A	N	N	39	3	N	7	8	A	N	13.8	18	11.9	5.7	N			
840	M	A	L	N	40	3.6	N	7	9	A	N	14.9	13.2	9.5	3.4	N			
841	F	A	N	N	40	3.2	N	9	10	A	N	15.7	14.1	10.2	3.5	N			
842	M	A	L	N	39	3	N	8	9	A	N	16.1	9.7	4.8	4.5	N			
843	F	A	N	N	40	2.7	N	5	7	A	N	19	12.7	8.2	4.1	N			
844	F	A	L	N	38	2.8	N	8	9	A	N	17.3	16.3	13	2.9	N			
845	M	A	L	N	37	2.5	N	6	9	A	N								
846	M	A	L	N	37	3.4	N	7	9	A	N	15.4	18.7	12.1	6.4	N			
847	M	P	L	N	38	3	N	5	8	A	N	16.3	14.2	9.6	4.3	N			
848	F	A	N	N	40	2.7	N	8	9	A	N	15	13.1	3.6	9	N			
849	F	A	N	N	38	2.6	N	9	10	A	N	14.1	15.9	11.5	4.2	N			
850	F	A	L	N	38	3.1	N	6	7	A	N	13.7	24.3	14.7	9.1	N			
851	M	A	L	N	39	3.8	N	5	9	A	N								
852	F	A	L	N	38	2.6	N	8	10	A	N	10.2	19.4	15	2.7	N			
853	F	P	L	N	36	2.3	P	7	9	A	N	16.1	15	12.2	2.1	Y	4.9	-	
854	M	A	L	N	37	2.7	N	7	9	A	N	12.6	16.8	11.3	2.9	N			
855	F	A	L	N	38	3	N	6	8	A	N	17.6	17.5	10.1	5.3	N			
856	M	A	N	N	39	2.6	N	7	9	A	N	16.5	15.8	9.4	3.1	N			
857	M	P	N	N	39	3	N	6	8	A	N	17.5	15.2	9.8	5.1	N			
858	F	A	L	N	35	1.7	N	7	8	A	N	17	13.5	8.8	4.2	N			
859	F	A	N	N	38	3	N	7	9	A	N	16.1	12.4	8.7	3.3	N			
860	M	A	L	N	35	2.3	N	7	8	A	N	12.5	16.7	11.8	4	N			
861	F	A	N	N	36	2.4	N	8	9	A	N	13.8	14.2	10.1	3.8	N			
862	F	A	N	N	37	2.8	N	8	10	A	N	16.1	7.5	6.3	2.7	N			
863	M	A	L	N	38	3	N	7	9	A	N	15	20.8	13	7.6	N			
864	F	A	L	N	40	3.5	N	7	9	A	N	14.2	16.9	11.1	5.6	N			

865	M	A	L	Y	37	2.6	S	4	7	A	N	10.8	15	12.1	2.1	Y	Missed	-	
866	M	A	L	N	39	2.9	N	7	10	A	N	12.4	17.7	10.4	6.7	N			
867	F	P	N	N	36	2.4	P	6	9	A	N	13.3	11.7	9.2	1.6	Y	5	-	
868	M	A	N	N	37	2.5	N	6	8	A	N	15.9	15	11.1	3.7	N			
869	F	A	N	N	37	2.5	N	9	10	A	N	14	14.6	10.5	3.7	N			
870	M	A	L	N	38	2.8	N	8	9	A	N	10.8	13.2	7.9	5.1	N			
871	F	A	L	N	39	3	N	7	10	A	N								
872	F	A	N	N	39	3.4	N	7	9	A	N	15	18.6	11.7	6.3	N			
873	M	A	L	N	40	3	N	6	9	A	N	14.4	14.1	10.8	3	N			
874	M	A	N	N	38	2.4	N	8	10	A	N	14.3	17	8.9	7.7	N			
875	M	A	N	N	39	2.7	I	7	9	A	N	16	14.3	11	2.3	Y	3.5	-	
876	F	A	L	N	38	2.8	N	8	9	A	N	17.4	14.7	9.1	4.9	N			
877	M	A	L	Y	38	2.5	N	5	8	A	N	16.1	13.8	8.5	4.5	N			
878	M	A	N	N	37	2.3	N	7	8	A	N	16.5	16.8	10.6	5.6	N			
879	F	A	N	N	39	3	N	8	9	A	N	12.8	18.1	11.5	6	N			
880	F	A	L	N	39	3.2	N	7	10	A	N	13	17.5	11.4	5.5	N			
881	F	A	L	N	36	2.3	N	8	9	A	N	16.9	13.5	7.2	6	N			
882	M	A	N	N	36	2.8	N	7	9	A	N	13.8	10.4	7.5	2.7	N			
883	M	A	L	N	37	3.2	N	6	8	A	N	14.2	16.5	10.3	5.5	N			
884	M	A	N	N	39	3.4	N	7	9	A	N	17	14.1	5.2	8.4	N			
885	F	A	L	N	39	2.6	N	8	9	A	N	11.8	17.3	13.2	3.6	N			
886	M	P	L	N	38	2.7	N	6	9	A	N	16	13	6	6.8	N			
887	F	A	N	N	35	2	N	8	9	A	N	12.5	17.2	10.1	6.7	N			
888	F	A	L	N	38	3	N	9	10	A	N	13.8	18.1	9.8	7.5	N			
889	M	A	N	N	39	3.7	N	6	8	A	N								
890	F	A	L	N	39	2.7	N	8	9	A	N	14	16.5	11.7	4.6	N			
891	M	A	L	N	34	1.9	N	7	8	A	N	17.2	13.8	10	3.5	N			
892	M	P	L	Y	36	2.2	P	6	9	A	N	15.6	12	9.9	1.7	Y	2.8	-	
893	F	A	L	N	37	2.6	N	7	10	A	N	16.9	14.4	11	3.5	N			
894	F	P	N	N	35	1.7	N	6	9	A	N	15.4	10.7	7.3	3.2	N			
895	M	A	L	N	37	2.9	N	7	9	A	N	15	12.3	8.8	3.4	N			
896	M	A	N	N	37	2.7	N	6	8	A	N	14.9	16	12.1	3.3	N			
897	F	A	L	N	37	3.4	N	5	9	A	N	13.2	18.4	10.8	7	N			
898	M	A	L	N	38	3.1	N	7	9	A	N	13.1	11.3	7.4	3.6	N			
899	F	A	N	N	35	2.3	N	5	8	A	N	12.5	12	6.6	4.9	N			
900	F	A	N	N	40	2.8	N	8	9	A	N	11.7	16	10.5	5	N			
901	M	A	L	N	39	2.6	N	7	9	A	N	11.9	13.2	8	4.4	N			
902	F	P	L	N	38	3	N	7	10	A	N	14.7	14.1	7.7	5.9	N			
903	M	A	L	N	38	2.6	I	7	9	A	N	17	12.6	7.8	2.3	Y	3	-	
904	M	A	L	N	37	2.4	N	7	8	A	N	13.8	17.9	10.2	7	N			
905	F	A	N	N	38	3.6	N	9	10	A	N	14.1	23.4	14.7	8.4	N			
906	F	A	L	Y	35	1.9	N	4	8	A	N	17	18.3	10.5	7.2	N			
907	F	A	N	N	35	2	N	5	9	A	N								
908	M	A	L	N	38	2.8	N	6	9	A	N	17.5	11.7	5.9	3.6	N			
909	F	A	N	N	37	2.5	N	7	9	A	N	12.4	16.3	11.5	4.1	N			
910	M	A	L	Y	39	3	N	5	8	A	N	11.8	27.2	15.2	9.1	N			
911	M	A	L	N	40	2.8	N	8	9	A	N								
912	M	A	N	N	40	2.7	N	7	8	A	N	14.8	11.2	8.3	2.5	N			
913	M	A	L	N	38	2.5	N	8	10	A	N	13.1	10.3	6.2	3.4	N			
914	M	A	L	N	37	3	N	9	10	A	N	14.6	13.7	8.4	3.9	N			
915	M	P	N	N	38	3.1	N	7	9	A	N	16	13	8.7	4.1	N			
916	M	A	N	N	39	3	N	6	9	A	N	11.1	16.6	11.7	4.4	N			

917	M	A	L	N	36	2.1	N	5	8	A	N	11.8	14.5	9.1	5	N			
918	M	A	L	N	38	3	N	7	9	A	N	14.5	9.5	5	4.3	N			
919	F	A	N	N	38	3.2	N	7	8	A	N	12.7	6.8	3.9	2	N			
920	F	A	L	N	39	3.5	N	8	10	A	N	17.8	14.5	11	3.4	N			
921	F	A	L	N	34	1.8	N	7	8	A	N	16	27.3	18.7	7.6	N			
922	M	A	N	N	33	1.5	N	8	9	A	N	14.3	14.3	10.1	3.6	N			
923	F	A	N	N	38	2.9	N	7	9	A	N								
924	M	A	L	N	35	2.1	N	7	9	A	N	10.9	18.4	15.2	2.6	N			
925	F	A	N	N	39	2.6	N	7	8	A	N	12.6	17.2	13.2	3.6	N			
926	M	P	L	N	39	3.7	N	6	9	A	N	12.9	13.8	9.2	3.8	N			
927	F	A	L	N	40	3	N	5	9	A	N	13.7	14.4	6.7	7.6	N			
928	F	P	N	N	40	2.8	N	6	9	A	N	13.4	22.7	16	5.5	N			
929	F	A	L	N	38	3.8	N	7	9	A	N	14.2	26.4	20.1	5.5	N			
930	M	A	L	N	37	2.6	N	9	10	A	N	16	12.2	6.3	5.4	N			
931	F	A	N	N	35	2.2	N	7	9	A	N	15.1	16.4	11.7	2.9	N			
932	M	A	N	N	35	1.8	N	7	8	A	N	16.8	15.8	11.5	3.7	N			
933	F	A	L	Y	35	1.9	N	8	10	A	N	18.7	17.3	10.6	6.3	N			
934	M	P	L	N	37	2.2	N	8	9	A	N	11.4	18.4	13.3	4.5	N			
935	F	A	N	N	37	2	N	9	10	A	N	12.8	18.2	12	4.7	N			
936	M	A	L	N	38	2.7	N	7	8	A	N	16.3	13.5	9.1	3.8	N			
937	F	A	L	N	38	2.6	N	6	8	A	N	15.8	14.4	8.7	5.6	N			
938	M	A	L	N	39	3.1	N	5	8	A	N								
939	F	A	L	N	40	2.8	N	6	9	A	N								
940	F	A	N	N	40	2.6	N	8	9	A	N	16.3	14	8.7	4.5	N			
941	M	A	N	N	38	2.4	N	7	9	A	N	13.2	20.5	12.8	6.5	N			
942	F	A	L	N	40	4.1	N	8	9	A	N	15.8	19.3	13	5.8	N			
943	M	A	N	N	38	2.8	N	7	8	A	N	14	18.8	11.2	7	N			
944	M	A	L	N	40	4	A	6	9	P	N	14.3	10.4	7.1	2.1	Y	Missed	-	
945	M	A	L	Y	34	1.8	N	5	7	A	N	11.8	17.2	12.3	4.5	N			
946	F	A	N	N	38	2.5	N	6	9	A	N	12.6	14.1	9	4.9	N			
947	M	A	N	N	37	2.6	N	5	8	A	N	16.5	16	12.5	2.7	N			
948	F	A	L	N	37	2.5	N	8	9	A	N								
949	F	A	L	N	38	3	N	9	10	A	N	19.7	18.3	10	7.2	N			
950	M	A	N	N	38	2.8	N	7	9	A	N	12.3	15.7	9.2	5.4	N			
951	M	A	N	N	38	2.7	N	6	8	A	N	14.5	21.7	11.7	8.8	N			
952	F	A	L	N	37	3.6	N	6	8	A	N								
953	M	P	N	N	38	3	N	8	9	A	N	15.7	22.4	15.3	6.2	N			
954	F	A	L	N	39	2.8	N	7	9	A	N	16	16.3	11.8	4.1	N			
955	M	A	L	N	35	2	N	7	9	A	N	18.6	18.3	13.8	4.2	N			
956	M	A	L	N	37	3.2	N	8	10	A	N	15.7	16.5	12.2	4.1	N			
957	F	A	N	N	36	2.2	N	7	8	A	N	16	17	7.8	8.1	N			
958	F	A	L	N	37	2.7	N	7	9	A	N	14	20.4	14.5	4.8	N			
959	F	A	L	N	37	2.4	N	8	9	A	N	14.7	14.8	7.9	5.8	N			
960	M	A	N	N	37	2.8	N	7	8	A	N	15.2	15.9	11.4	3.3	N			
961	F	A	N	N	38	2.9	N	7	9	A	N	12.8	16	10.7	3.7	N			
962	M	A	L	N	39	2.6	N	6	9	A	N	17.1	18.2	11.6	5.8	N			
963	M	A	L	N	38	2.4	N	7	9	A	N								
964	M	P	N	N	38	3	N	8	10	A	N	15.1	14.9	12	2.6	N			
965	M	A	L	N	34	1.8	N	7	8	A	N	16	17.3	13.3	3.6	N			
966	F	A	N	N	34	2.2	N	6	9	A	N	17.8	15	11.2	3.3	N			
967	F	A	L	N	38	3.5	N	7	9	A	N	14.9	18.5	12.8	4.5	N			
968	M	A	N	N	39	2.6	N	6	8	A	N	12.9	13.7	7.7	5.1	N			

969	F	A	L	N	39	2.7	N	9	10	A	N	14.8	12.5	4.1	7.8	N			
970	M	A	N	N	38	3	N	6	8	A	N	14.3	12.6	7.7	4.2	N			
971	F	A	L	N	40	3	N	7	9	A	N	17	13.2	7.9	4.5	N			
972	M	A	L	N	39	2.8	N	8	9	A	N	16.3	15.8	12.3	2.7	N			
973	F	A	L	N	40	2.7	N	8	10	A	N								
974	M	A	N	N	38	2.6	N	7	8	A	N	14.7	14	9.6	3.2	N			
975	M	A	N	N	36	2.4	N	6	9	A	N	15.6	15.1	10.2	4	N			
976	F	A	L	N	38	3	N	7	9	A	N	11.8	18.2	12.3	5.1	N			
977	F	A	N	N	39	3.6	N	8	10	A	N	12	16	9.3	6.2	N			
978	M	A	L	N	35	2.3	N	8	9	A	N	12.8	14.8	7.4	6.3	N			
979	M	A	N	N	37	3.1	N	6	8	A	N	11.6	19.3	11.5	6.8	N			
980	F	P	L	N	38	3.4	I	7	8	A	N	14.7	7.6	5.1	2.1	Y	3	-	
981																		-	
982	F	A	L	N	38	2.6	N	8	10	A	N	16.3	12.3	7.1	4.7	N			
983	M	A	L	N	37	3	N	6	8	A	N	16.5	11	6	4.6	N			
984	F	A	N	Y	37	3.4	N	5	7	A	N	17	12.4	5.5	5.4	N			
985	M	A	L	N	38	2.8	N	7	9	A	N	16.4	17	9.7	5.8	N			
986	F	A	N	N	36	2.5	N	7	8	A	N	14	16.3	10.3	4.5	N			
987	M	A	N	N	37	3.1	N	6	8	A	N	12.5	14.8	9.6	3.4	N			
988	M	A	L	N	38	2.8	N	7	9	A	N	19.3	15.3	8.4	5.9	N			
989	F	A	L	N	36	2	N	5	8	A	N	16.5	12.9	4.4	7.1	N			
990	M	P	N	Y	37	2.7	N	4	7	A	N	15	14.4	7.5	5.9	N			
991	F	A	L	N	37	2.6	N	5	8	A	N	14.8	17	8.9	6.3	N			
992	M	A	L	N	39	2.7	N	8	9	A	N	16.3	16.2	9.8	5	N			
993	F	A	N	N	39	2.8	N	6	8	A	N	15.1	17.3	13	2.9	N			
994	M	A	L	N	34	1.9	N	7	9	A	N								
995	M	A	N	N	38	3	N	8	9	A	N								
996	M	A	N	N	39	3.4	N	7	8	A	N	14.5	21.3	15.5	4.6	N			
997	F	A	L	N	40	3.1	N	6	10	A	N	17.1	22.7	12.6	7.8	N			
998	F	A	L	N	35	1.7	N	8	9	A	N	16.3	26.4	18.9	5.8	N			
999	F	A	N	N	39	3.4	N	6	9	A	N	18	20.5	13.6	5	N			
1000	F	A	L	N	40	3	N	6	7	A	N	14.1	16.7	9.6	6.4	N			
1001	M	A	L	N	40	2.8	N	6	8	A	N	14.3	17.8	11.1	5	N			
1002	F	A	N	N	38	2.8	N	7	9	A	N	15	13.2	1.1	11.9	N			
1003	M	A	N	N	32	1.4	N	7	8	A	N	12.9	14.3	9.8	4.1	N			
1004	F	P	L	N	38	3	N	8	9	A	N	19.5	17.6	2.3	14.5	N			
1005	M		N	N	39	2.6	N	9	10	A	N	17.8	15.1	7.5	6.9	N			
1006	F	P	L	N	38	2.8	N	8	10	A	N	10.9	13.2	4.4	8.5	N			
1007	M	A	N	N	34	2.1	P	5	9	A	N	16.3	7.3	5	1.7	Y	5.6	-	
1008	M	A	N	N	38	3	N	7	8	A	N	16.2	11.4	6.7	4	N			
1009	F	P	L	N	39	3.3	I	6	8	A	N	17	12.6	9.3	1.7	Y	Missed	-	
1010	F	A	L	N	38	2.7	N	6	8	A	N	15.1	14	8.8	4.9	N			
1011	M	A	L	N	35	2.3	N	7	9	A	N	14.8	16.3	9.4	5.8	N			
1012	F	A	L	N	38	2.8	N	8	9	A	N	14.7	14.5	7.6	6.4	N			
1013	F	A	L	N	39	2.8	N	7	9	A	N	13.6	17.1	13.5	2.9	N			
1014	M	A	N	N	40	2.5	N	8	10	A	N								
1015	M	A	L	N	38	2.5	N	6	9	A	N	17.5	14.8	9.2	5.2	N			
1016	F	A	L	N	37	2.2	N	8	10	A	N	17.9	15.5	9.9	5	N			
1017	F	A	L	N	39	4.3	N	8	9	A	N	16	14.9	6.9	7.2	N			
1018	M	A	N	N	37	2.5	N	7	8	A	N	12.3	18.2	8.5	9.1	N			
1019	F	A	N	N	37	3.3	N	7	10	A	N	14.2	13.7	6.4	6	N			
1020	M	A	L	N	38	2.6	N	7	9	A	N	17.7	14.1	11.2	2.5	N			

1021	M	A	N	N	38	2.7	N	6	8	A	N	16	24.5	17.7	4.9	N			
1022	F	A	L	N	39	3.5	N	7	9	A	N								
1023	M	A	L	N	40	3	N	5	9	A	N	15.2	18.4	11.3	5.9	N			
1024	F	A	N	N	37	2.6	N	8	10	A	N	14.6	17.4	12.6	4.5	N			
1025	M	A	N	N	38	2.7	N	6	8	A	N	13.8	11.8	6.5	4.2	N			
1026	M	A	L	N	37	2.9	N	7	9	A	N	13.6	17.6	10.4	6.6	N			
1027																			
1028	F	A	N	N	37	3.1	N	8	9	A	N	15	21.5	16.8	3.6	N			
1029	M	P	N	N	38	3.5	N	8	10	A	N	16.3	16.3	9.3	6.8	N			
1030	F	A	L	N	35	2	N	7	9	A	N	17.1	17.1	13.5	3.2	N			
1031	F	A	N	N	37	2.7	N	6	8	A	N	18.6	14.9	8.3	6	N			
1032	M	A	N	N	38	2.6	N	7	10	A	N	18.1	13.8	6.2	5.3	N			
1033	M	A	L	N	39	3.6	N	7	9	A	N	13.3	17.4	13.8	3.3	N			
1034	M	A	N	N	37	3	N	7	9	A	N								
1035	M	A	L	N	33	1.3	N	8	9	P	N	14.6	14.7	9.4	4.7	N			
1036	F	A	L	Y	33	1.6	N	5	7	A	N	10.8	17.2	8.1	8.7	N			
1037	M	A	L	N	39	3	N	6	10	A	N	9.4	12.3	8.3	3.2	N			
1038	F	A	N	N	37	2.6	N	7	9	A	N	16.3	12	9	2.8	N			
1039	M	A	L	N	37	2.4	N	6	8	A	N	17.4	18.7	14.1	3.6	N			
1040	M	A	N	N	38	2.8	N	9	10	A	N	15	15.4	12.8	2.7	N			
1041	F	A	L	N	38	2.7	N	8	9	A	N	15.2	12.7	8.5	2.5	N			
1042	M	A	N	N	33	1.8	P	6	9	A	N	17	8.3	5.1	2.2	Y	2.9	-	
1043	M	A	L	N	39	3	N	7	9	A	N	16.8	13.6	7.2	4.9	N			
1044	F	A	N	N	40	2.9	N	6	9	A	N	14.9	16.4	5.5	9.7	N			
1045	M	A	L	N	40	2.8	N	7	10	A	N	13.6	15.8	10.1	5.2	N			
1046	F	A	L	N	38	2.7	N	7	10	A	N	9.7	10.6	5.1	5.2	N			
1047	M	A	L	N	40	3.4	N	7	8	A	N								
1048	M	A	N	N	37	3.1	N	6	9	A	N	15.3	15.2	9.5	5.3	N			
1049	F	A	L	N	38	3	N	7	9	A	N	17.3	16.1	10.3	4.7	N			
1050	F	P	L	N	40	2.8	N	8	10	A	N	16	15	10.8	3.7	N			
1051	F	A	N	N	39	2.9	N	6	8	A	N	13.8	12.5	8.9	2.6	N			
1052	M	A	L	N	38	3.2	N	7	9	A	N	14.3	14.1	9.2	3.5	N			
1053	F	A	N	Y	40	3	N	5	7	A	N	12.5	10.8	3	6.3	N			
1054	F	A	L	Y	37	2.4	N	3	6	A	N	11.7	14.3	7.4	5.8	N			
1055	M	A	N	N	34	2.1	P	7	9	A	N	15	13.5	9.7	2.4	Y	5.2	-	
1056	F	A	N	N	37	3	N	7	8	A	N	13.5	12.7	7.8	4.1	N			
1057	M	P	N	N	38	3.1	N	9	9	A	N	16.1	13.5	9.1	3.5	N			
1058	M	A	L	N	37	3.1	N	8	9	A	N	14.2	17.9	14.5	3	N			
1059	M	A	L	Y	37	2.8	N	5	9	A	N	16.8	20.4	16.6	2.7	N			
1060	F	A	L	N	38	2.5	N	9	10	A	N								
1061	M	A	N	N	37	2.6	N	8	9	A	N	12.9	16.9	10.2	6	N			
1062	M	A	L	N	38	2.8	N	6	8	A	N	14	17.2	13.8	2.6	N			
1063	F	A	N	N	35	2	N	8	9	A	N	13.6	12.6	8.8	2.8	N			
1064	M	A	L	N	37	2.4	N	7	10	A	N	17.5	8.5	2.3	5.8	N			
1065	F	A	N	N	37	3	N	7	9	A	N	16.6	9.4	4.9	4	N			
1066	M	A	L	N	37	3.8	N	6	8	A	N	15.4	20.3	16.2	3.8	N			
1067	F	A	L	N	33	1.8	N	7	9	A	N	14	21.7	13	6.7	N			
1068	M	A	N	N	35	1.9	N	8	9	A	N	14.8	13.4	5.7	7.2	N			
1069	F	A	L	N	35	2	N	6	8	A	N	13.6	17.3	11.9	5.2	N			
1070	F	A	L	N	37	2.6	N	7	8	A	N	12.9	13.1	5.3	6.4	N			
1071	F	P	N	N	37	2.7	N	6	9	A	N	16.8	14.4	10.1	4	N			
1072	F	A	N	N	35	2	N	6	9	A	N	18.2	15.2	9.8	4.3	N			

1073	F	A	L	N	37	2.4	N	7	9	A	N	17	18.7	12.3	4.9	N			
1074	M	A	L	N	36	2.5	N	7	8	A	N	17.4	17	11.4	4.7	N			
1075	F	A	N	N	37	2.9	N	5	8	A	N	16.9	19.5	12.5	5.8	N			
1076	F	A	L	Y	38	2.8	N	5	9	A	N	11.7	13.3	7.3	5.1	N			
1077	M	A	L	N	34	1.8	N	6	10	A	N	16.4	14.3	7.1	6	N			
1078	F	A	L	N	37	3	N	9	10	A	N								
1079	M	A	N	Y	38	4.3	N	8	9	A	N	12.8	13	8.1	4.4	N			
1080	F	A	L	N	39	3.2	N	7	8	A	N	13.6	19.4	15	3.9	N			
1081	M	A	N	N	40	3	N	8	9	A	N	14.7	17.1	14.1	2.8	N			
1082	F	A	L	N	37	3.1	N	8	10	A	N	19.1	15.3	11.3	3.2	N			
1083	M	A	L	N	37	2.8	N	7	9	A	N	11.5	16.4	10.9	3.3	N			
1084	M	A	L	N	36	2.1	N	7	9	A	N	14.8	12.1	9	2.7	N			
1085	M	A	N	N	36	2.2	N	7	8	A	N	16.3	16.6	11.2	4.5	N			
1086	F	A	L	N	40	2.7	I	6	9	A	N	15.4	16	12.2	1.5	Y	2.6	-	
1087	M	P	N	N	37	3.4	N	7	9	A	N	15.2	17.3	10.1	6.7	N			
1088	M	A	L	N	40	3	N	6	8	A	N	11.6	16.5	8.1	7.4	N			
1089	M	A	L	N	37	2.6	N	6	10	A	N	16.7	14	9.2	4	N			
1090	M	A	L	Y	39	3.8	N	5	7	A	N	15	12.8	9.6	2.7	N			
1091	F	P	N	N	34	2	N	6	9	A	N	14.7	20.5	13.4	6.3	N			
1092	F	A	N	N	37	2.7	N	7	8	A	N								
1093	M	A	N	N	37	3	N	5	10	A	N	15	18	9.8	7	N			
1094	F	A	L	N	36	2	N	6	8	A	N	15.1	16.5	13.2	2.5	N			
1095	M	A	L	N	37	2.9	N	7	9	A	N	12.5	17.6	14.5	2.8	N			
1096	F	A	L	N	35	1.8	N	7	9	A	N	13.4	12	6.6	5.1	N			
1097	F	A	N	N	37	2.8	N	7	9	A	N	13	15.4	11	3.6	N			
1098	M	A	N	N	34	1.9	P, S	6	9	A	N	14.1	12.9	8.1	2	Y	4.8	-	
1099	M	A	L	N	36	2.5	N	6	8	A	N	16.2	14.9	10.4	3	N			
1100	M	A	L	N	37	2.7	N	6	9	A	N	10.9	17.8	12.3	4.8	N			
1101	M	A	L	N	37	2.6	N	7	8	A	N	19.5	14.6	9.7	4	N			
1102	F	A	N	N	38	3	N	8	10	A	N	16.3	17.5	10.4	6.2	N			
1103	M	A	L	N	35	2.1	N	7	9	A	N	15	20.7	14.3	5	N			
1104	M	P	N	N	33	1.6	N	6	7	A	N								
1105	M	A	L	N	38	3.3	N	6	9	A	N	15	27.3	20.7	4.5	N			
1106	M	A	L	N	37	3	N	7	8	A	N	15.8	15.4	8.5	5.3	N			
1107	F	A	N	N	39	3.1	N	6	9	A	N	19.3	16.7	9.1	7	N			
1108	M	A	N	N	38	2.5	N	7	8	A	N	12.4	13.1	9.3	2.6	N			
1109	M	A	L	N	40	2.9	N	6	9	A	N	16.6	14.2	10.1	3.4	N			
1110	M	A	N	N	39	2.6	N	8	10	A	N	15.5	11.9	6.8	4.1	N			
1111	M	A	L	N	36	2.1	P	7	9	A	N	15.1	10.2	7	2.1	Y	3.6	-	
1112	F	A	L	N	37	3	N	8	9	A	N	12.7	14.5	11.5	2.6	N			
1113	F	A	N	N	38	2.8	N	8	10	A	N	12	18	11	6.3	N			
1114	M	A	L	N	38	3.2	N	5	9	A	N	13.4	10.2	7.1	2.5	N			
1115	F	A	N	N	35	2	N	7	8	A	N	14	8.8	5.4	2.8	N			
1116	M	A	L	N	39	3	N	6	9	A	N	13.8	14	10.2	2.9	N			
1117	F	P	L	N	39	3.1	N	5	8	A	N								
1118	M	A	N	N	38	2.8	N	7	9	A	N	14.6	13.7	8.5	4.6	N			
1119	F	A	N	N	39	2.9	I	7	9	A	N	19	12.9	9.3	2.1	Y	3.9	-	
1120	M	A	L	N	34	2	N	6	8	A	N	13	17.1	11.6	5	N			
1121	M	A	N	N	39	3.5	N	6	9	A	N	18.7	22.1	13.3	6.8	N			
1122	F	A	L	N	40	3.4	N	8	9	A	N	17.4	18.4	7.9	8.6	N			
1123	M	A	N	N	40	3	N	5	8	A	N	13	12.6	6.9	4.7	N			
1124	F	A	N	Y	34	1.9	N	5	7	A	N	12.7	19.4	14.2	4.2	N			

1125	M	A	L	N	37	2.8	N	8	9	A	N	13	15.3	11.3	2.7	N			
1126	F	A	L	N	38	2.9	N	8	10	A	N	18.2	16.2	12.4	3.5	N			
1127	M	A	N	N	37	2.5	N	9	10	A	N	14.6	17.5	12.6	3.9	N			
1128	M	A	L	N	37	2.7	N	7	9	A	N	15.3	14.2	8.3	4.4	N			
1129	F	A	N	N	38	2.6	N	6	9	A	N								
1130	F	P	N	N	38	3	N	7	8	A	N	15.6	17.9	11.3	5.7	N			
1131	M	A	L	N	39	2.8	N	6	9	A	N	13.4	14	10.1	3	N			
1132	F	A	L	N	37	2.6	N	6	8	A	N	17.6	13.2	10	2.6	N			
1133	M	A	L	N	34	1.6	N	8	9	A	N	16	14.6	8.7	5.4	N			
1134	M	A	L	N	39	3	N	7	8	A	N	16.4	9.9	5	4.1	N			
1135	M	A	N	N	39	3.2	N	7	9	A	N	15.8	10.3	6.3	3	N			
1136	F	A	L	N	38	3	N	5	9	A	N	17.3	17	12.1	3.8	N			
1137	F	A	L	N	39	3.2	N	6	8	A	N	16.3	16.4	11.2	4.6	N			
1138	M	A	N	N	37	3	I	7	9	A	N	11.8	16	11.5	2.2	Y	4.3	-	
1139	M	A	N	N	38	2.5	N	6	9	A	N	16	14.5	9.4	4.5	N			
1140	F	A	L	N	35	2.1	N	6	8	A	N	14.7	16.7	10.3	5.4	N			
1141	M	A	L	N	36	2.6	N	7	9	A	N	14.1	17	12.5	4.2	N			
1142	F	P	L	N	39	3	N	6	8	A	N	12.9	17.3	12.8	2.6	N			
1143	F	A	N	N	39	3.5	N	7	9	A	N	15.8	14.3	9.7	3.2	N			
1144	M	A	L	Y	33	1.7	N	4	8	A	N	16.5	24.1	9.9	11.5	N			
1145	M	A	N	N	39	2.7	N	5	9	A	N								
1146	M	A	L	N	37	2.5	N	7	10	A	N	15.3	12.3	8.4	3.7	N			
1147	F	A	N	N	37	2.3	N	7	9	A	N	16.5	10.8	6	4.1	N			
1148	M	A	N	N	36	2.1	N	6	8	A	N	14.3	14.6	7.3	6.4	N			
1149	M	A	L	N	39	3.1	N	8	10	A	N	12.8	19.2	15.1	2.5	N			
1150	M	A	L	N	39	3	N	6	8	A	N	15	14.2	10	3.6	N			
1151	M	A	L	N	38	2.4	N	5	8	A	N	13.9	16	12.2	3	N			
1152	M	A	N	N	37	2.8	N	7	9	A	N								
1153	M	A	L	N	39	3	N	6	9	A	N	20.3	17.3	12.9	4	N			
1154	F	A	N	Y	39	3.4	N	5	8	A	N	14.6	12.2	7.8	3.8	N			
1155	F	A	L	Y	40	2.9	N	5	7	A	N	15.5	19.9	9.7	8.4	N			
1156	M	A	N	N	39	2.6	N	7	9	A	N	13	18.6	12.5	5.6	N			
1157	F	A	L	N	38	2.8	N	7	8	A	N	17.6	20.4	12.2	7.1	N			
1158	F	P	L	N	35	2.3	P	6	9	A	N	10.8	23.2	18.2	1.8	Y	2.8	-	
1159	M	A	N	N	37	3.2	N	6	8	A	N	14.8	14.2	11.2	2.7	N			
1160	F	A	N	N	39	4	N	7	9	A	N	14.5	17	11.6	5.2	N			
1161	M	A	L	N	40	2.6	N	6	8	A	N	12.6	14.6	8.6	4.7	N			
1162	F	A	L	N	37	2.4	N	7	10	A	N	16.2	15.5	12.3	2.8	N			
1163	F	A	L	N	37	2.8	N	5	8	A	N	12.5	16.1	8.3	6.5	N			
1164	F	A	N	N	36	1.9	N	5	7	A	N	14.8	16.3	9.4	6.1	N			
1165	M	A	N	N	36	2.2	N	6	9	A	N	13.7	18.6	14.8	3.6	N			
1166	F	A	L	N	38	2.9	N	7	9	A	N								
1167	M	A	L	N	39	3.1	N	8	9	A	N	15.8	17.2	14.2	2.6	N			
1168	F	A	L	N	35	2.2	N	7	9	A	N	16.2	20	16	3.1	N			
1169	M	P	N	N	38	2.4	N	8	9	A	N	16.1	16.6	7.2	8.3	N			
1170	M	P	L	N	37	3	N	6	8	A	N	14.9	19.2	13.1	5.4	N			
1171	M	A	N	N	37	2.7	N	6	9	A	N	15.2	15.7	10.8	4.5	N			
1172	F	A	L	N	37	2.6	N	5	8	A	N	14.7	14.4	9.2	4	N			
1173	M	A	L	N	39	2.8	N	5	9	A	N	14.2	18.3	9.5	7.8	N			
1174	F	A	L	N	34	1.8	N	6	9	A	N	15.8	15.2	11.4	2.9	N			
1175	M	A	N	Y	38	3.4	N	5	8	A	N	14	17	12.9	3.2	N			
1176	M	A	N	N	39	3.2	N	7	8	A	N	13.3	19.3	12	6.1	N			

1177	F	A	L	N	40	3	N	6	9	A	N	13.5	17.7	9.8	7.6	N			
1178	F	P	L	N	35	2.1	P	6	9	A	N	13.2	9.8	7	1.8	Y	Missed	-	
1179	M	A	N	N	35	1.9	N	7	8	A	N	18.7	15.9	10.1	5.2	N			
1180	M	A	L	N	39	2.9	N	8	9	A	N	15.2	14.5	9.7	3.5	N			
1181	M	A	L	N	39	2.7	N	7	10	A	N	12.3	14.2	9	4.6	N			
1182	M	A	N	N	37	2.6	N	6	9	A	N	16.3	23.1	14.2	8	N			
1183	F	A	L	N	38	3.5	N	7	9	A	N	13.2	14.6	6.5	7.5	N			
1184	F	A	N	N	40	3	N	6	8	A	N	13.1	11.3	6.8	3.9	N			
1185	F	A	N	N	38	3	N	7	9	A	N	14.8	17.5	11.4	5.3	N			
1186	M	A	N	N	39	3.1	N	5	8	A	N	16.8	12.3	5.9	6	N			
1187	F	A	L	N	40	3	N	6	8	A	N								
1188	M	P	L	N	38	3.1	N	7	9	A	N	15.6	14.5	9.3	4.5	N			
1189	M	A	N	N	40	2.9	N	6	10	A	N	14.7	18.6	10.3	7.4	N			
1190	M	A	N	N	37	2.4	N	6	9	A	N	14	22.7	13	8.4	N			
1191	F	A	L	Y	38	2.7	N	5	9	A	N	15.2	13.8	9.7	2.9	N			
1192	F	A	N	N	34	2	N	7	8	A	N	15.2	20.5	13	6.9	N			
1193	M	A	L	N	37	2.5	N	6	8	A	N	17.6	24.2	12.8	8.6	N			
1194	F	A	N	N	37	2.9	N	7	9	A	N	17	14	8.6	4.7	N			
1195	M	A	L	N	38	2.9	N	8	10	A	N	15.6	15.4	9.8	4.6	N			
1196	M	P	L	Y	32	1.6	P	4	7	P	N	9.7	5.4	3.8	1.8	Y	Missed	-	
1197	F	P	N	N	37	3	N	6	8	A	N	14.9	18.3	12.1	5.4	N			
1198	F	A	N	N	37	3.4	N	7	9	A	N	12.7	14.5	6.9	6.1	N			
1199	M	A	L	N	39	3.2	N	7	10	A	N	18.7	13.2	10.4	2.6	N			
1200	M	P	L	N	37	2.6	I	5	8	A	N	10.3	8	5.3	1.5	Y	2.9	-	
1201	F	A	N	N	38	2.8	N	7	9	A	N	18.1	13.2	9.6	2.8	N			
1202	M	A	N	N	39	2.6	N	6	8	A	N	11.6	11.4	6.9	2.6	N			
1203	M	A	L	N	36	2.4	N	7	9	A	N	13.3	10.5	7	3.2	N			
1204																		-	
1205	M	A	L	N	38	2.6	N	6	9	A	N	15.3	18.9	11.3	7	N			
1206	M	P	L	N	37	3	N	7	10	A	N	16	14.3	8.6	4.7	N			
1207	M	A	N	N	35	2	N	6	9	A	N	14.5	18.8	12.5	5.4	N			
1208	F	A	L	N	37	2.7	N	7	9	A	N	15	16.6	10.1	5.1	N			
1209	F	A	N	N	33	1.8	N	6	9	A	N	15.8	13.9	7.8	4	N			
1210	M	A	L	N	33	1.5	N	4	7	A	N	16.9	20.2	15.5	4.3	N			
1211	F	A	N	N	38	2.9	N	8	9	A	N								
1212	F	A	L	N	39	2.6	N	7	9	A	N	15.6	15.3	9.1	4.8	N			
1213	F	A	N	N	40	2.8	N	7	9	A	N	15	18.2	14.1	3.5	N			
1214	M	A	N	N	37	2.4	N	6	8	A	N	12.4	14.1	10.2	3.6	N			
1215	F	A	N	N	38	3	N	5	8	A	N	12	11	7.7	3.2	N			
1216	M	A	L	N	38	3.2	N	6	9	A	N	16.2	27.6	15.3	10.5	N			
1217	F	A	L	N	39	3.6	N	6	9	A	N								
1218	M	A	L	N	37	2.5	N	5	8	A	N	14.2	15.4	11.2	3.7	N			
1219	F	A	N	Y	34	2	N	5	9	A	N	14.5	17.2	12.8	3.6	N			
1220	M	A	L	N	35	2.2	N	7	9	A	N	15.2	9.6	6.4	2.5	N			
1221	F	A	L	N	37	2.8	N	7	9	A	N	15.3	8.5	5.3	2.9	N			
1222	F	A	L	N	37	2.5	N	7	9	A	N	14	21.3	12.7	7.8	N			
1223	M	A	N	N	34	2	N	7	8	A	N	17.8	16.9	10.5	5.7	N			
1224	F	A	N	N	39	3.9	N	8	10	A	N	17.2	17.2	12.9	3.6	N			
1225	M	A	L	Y	39	2.9	I	3	5	P	Y	12	11.5	9	1.7				
1226	M	A	L	N	40	3.6	N	5	7	A	N	11.3	14.7	9.7	3.4	N			
1227	F	A	N	N	40	3	N	7	9	A	N	16.6	10.8	7.2	3	N			

1228	M	P	L	N	37	2.8	N	6	8	A	N	12.3	9.7	5.4	3.4	N			
1229	F	A	L	N	38	3	N	5	7	A	N	17.5	8.3	5.5	2.5	N			
1230	M	A	N	N	37	2.7	N	6	8	A	N	12.5	13.2	10.1	2.5	N			
1231	F	A	L	N	38	3	N	7	9	A	N	12.8	14.1	7.7	6	N			
1232	M	A	L	N	37	2.4	N	7	9	A	N	13.6	16.8	9.3	5.7	N			
1233	F	A	N	N	37	3	N	8	9	A	N	14	12.2	7	4.6	N			
1234	M	A	L	N	37	2.8	N	5	8	A	N	12.8	9.3	5.1	4	N			
1235	F	A	N	N	36	2.4	N	6	9	A	N	19.3	18.3	8.3	9.4	N			
1236	M	A	L	N	37	2.6	N	7	8	A	N	18.5	15.2	12	2.8	N			
1237	M	P	N	N	36	2.1	P	7	9	A	N	12.7	13.9	11	1.9	Y	6	-	
1238	F	A	N	Y	39	3.2	N	5	8	A	N	14	12.3	6.6	5.3	N			
1239	M	P	L	N	37	3.6	N	7	9	A	N	15.4	14.4	10.3	3.2	N			
1240	F	A	N	N	39	3	N	7	9	A	N	14.6	8.7	4.8	2.7	N			
1241	M	A	L	N	36	2	N	6	8	A	N	12	9.8	4.5	4.3	N			
1242	F	A	L	N	36	2.1	N	6	9	A	N	12.6	10.2	5.6	4.1	N			
1243	M	A	N	N	37	3.5	N	8	9	A	N	12.6	16.3	13.1	2.6	N			
1244	F	A	N	N	39	2.4	N	7	9	A	N	14.2	15.6	11.4	2.9	N			
1245	M	A	L	N	33	1.6	N	7	8	A	N	15	14.4	10.7	3.3	N			
1246	M	A	L	N	36	2.3	N	6	9	A	N								
1247	F	A	N	N	37	3	N	7	8	A	N	11.8	17.6	11.3	5.1	N			
1248	M	A	L	N	39	3.1	N	7	8	A	N	11.7	15.6	9.8	4.4	N			
1249	F	A	L	N	38	2.4	N	7	9	A	N	11.6	15.1	10.6	2.7	N			
1250	M	P	N	N	38	2.9	N	9	10	A	N	16.5	12.1	7.7	4.2	N			
1251	F	A	N	N	37	2.7	N	8	9	A	N	14.6	13.2	8.7	4	N			
1252	F	A	N	N	37	3	N	7	9	A	N	14.8	12.8	8.4	3.1	N			
1253	F	A	N	N	35	1.9	N	4	7	A	N	14.7	18.3	15.1	2.5	N			
1254	F	A	L	N	35	1.9	N	8	9	A	N	17.5	12.4	8.5	2.8	N			
1255	M	A	N	N	37	3	N	6	8	A	N								
1256	F	A	L	N	39	3.2	N	7	9	A	N	13.7	12.7	7.1	4.4	N			
1257	F	A	L	N	40	2.6	I	7	10	A	N	16.5	13.2	10.7	2	Y	4.8	-	
1258	M	A	L	N	40	3.4	N	6	9	A	N	15.2	14.1	6.6	5.7	N			
1259	F	A	N	N	39	3	N	6	9	A	N	12.6	12.9	8.2	2.6	N			
1260	M	A	L	N	34	1.8	N	7	8	A	N	11.5	16.8	9.3	6.1	N			
1261	F	A	N	N	39	2.8	N	7	9	A	N	18.6	14.2	8	3.8	N			
1262	M	A	L	N	39	2.6	N	6	8	A	N	10.8	12.4	7.4	3.7	N			
1263	M	A	N	N	37	2.6	N	7	9	A	N	12.7	16.3	11	4.5	N			
1264	F	A	L	N	37	2.4	N	8	10	A	N	14.8	9.4	4.9	4.2	N			
1265	M	A	N	N	36	2.2	N	6	8	A	N	13.8	13	7.8	4.8	N			
1266	M	A	N	N	38	3	N	7	9	A	N								
1267	F	A	L	N	38	3.1	N	8	10	A	N	13.4	17.6	8.2	7.3	N			
1268	M	A	N	N	39	3.2	N	6	8	A	N	14.6	19	12	6.9	N			
1269	F	A	L	N	36	2.3	N	8	9	A	N	12.8	14.3	10.7	2.6	N			
1270	M	A	N	Y	38	2.6	N	4	7	A	N	13.3	12.3	8.5	2.6	N			
1271	F	P	L	N	37	3	N	7	8	A	N	15.1	16	11.8	3.7	N			
1272	F	A	N	N	38	3.6	N	6	8	A	N	16.2	11.2	7.9	3.1	N			
1273	M	A	L	Y	39	2.7	N	4	9	A	N	13.1	13.5	7.2	4.8	N			
1274	M	A	L	N	40	2.9	N	9	10	A	N	14.9	10.5	5.5	4.5	N			
1275	F	P	L	N	37	2.5	N	6	9	A	N	12.7	14.8	8.3	5.8	N			
1276	M	A	N	N	38	2.5	N	7	8	A	N	15.9	13.8	5.4	5.1	N			
1277	F	A	L	N	40	3	N	7	9	A	N	18.4	13.1	6.8	6	N			
1278	M	A	L	N	39	3.1	N	6	9	A	N	16	16.4	12	3.4	N			
1279	F	A	N	Y	37	3.8	N	3	8	A	N	12.7	18.2	14.2	3	N			

1280	M	A	L	N	36	2.4	N	5	8	A	N	15	14.3	8.7	4.4	N			
1281	M	A	N	N	37	2.5	N	7	9	A	N	16.2	17.4	9.9	6.7	N			
1282	M	A	L	N	35	2	N	7	10	A	N	14.2	25.6	15.6	8.1	N			
1283	F	P	N	N	34	2	P, S	4	7	A	N	18.1	14	11	1.9	Y	4.6	-	
1284	M	A	N	N	39	3.2	N	6	9	A	N	12.7	18.3	14.6	2.9	N			
1285	M	A	L	N	36	3	N	7	8	P	N	16.5	10.4	6.7	3	N			
1286	F	A	N	N	37	2.8	N	8	10	A	N	13.3	17	13.1	3.3	N			
1287	M	A	N	N	37	2.6	N	7	8	A	N								
1288	F	A	N	N	33	1.9	P, S	6	9	A	N	13.1	16.8	12	2.4	Y	Missed	-	
1289	M	A	N	N	40	2.8	N	7	8	A	N	11.7	14.3	7	4.5	N			
1290	M	A	L	Y	36	2.4	N	4	8	A	N	15.6	16	10.2	4.9	N			
1291	F	A	L	N	37	2.6	N	7	9	A	N	14.4	13.2	7.6	3.9	N			
1292	M	P	N	N	39	3	N	8	9	A	N	15	6.8	3.7	2.7	N			
1293	M	A	N	N	40	3.1	N	6	8	A	N	13.6	8.9	4.3	3.6	N			
1294	F	A	L	Y	35	2.2	N	9	10	A	N	13.8	17.1	11.5	5.7	N			
1295	M	A	L	N	37	1.8	N	7	8	A	N	14.1	16.5	10.8	4.1	N			
1296	M	A	N	N	37	2.6	N	8	9	A	N								
1297	M	A	N	N	37	2.8	N	7	9	A	N	18.2	17.2	12.7	2.8	N			
1298	F	A	L	N	38	2.6	N	8	9	A	N	13.8	15.9	8.3	6.7	N			
1299	F	A	L	N	35	2	N	5	8	A	N	11.6	10.7	5.9	3.9	N			
1300	M	A	N	N	37	3	N	6	8	A	N	15.4	9.5	5.4	2.6	N			
1301	F	P	L	N	38	2.5	S	7	9	A	N	14	12.6	10	2.3	Y	3.5	-	
1302	M	P	L	N	40	3.2	N	7	8	A	N	17.1	20.3	12.3	7	N			
1303	M	A	N	N	40	3.4	N	7	8	A	N	12	10.2	6.8	2.7	N			
1304	M	A	L	N	38	2.8	N	5	7	A	N	16.3	14.4	8.4	5	N			
1305	F	A	N	N	37	3	N	7	9	A	N	12.5	14.1	8.8	4.2	N			
1306	M	A	L	N	39	2.6	N	6	10	A	N	15.6	15.2	10.1	4.7	N			
1307	M	A	L	N	37	2.5	N	8	9	A	N	13.5	15.6	9.3	5	N			
1308	F	A	N	N	37	2.7	N	7	10	A	N	14	14.2	7.2	6.3	N			
1309	M	A	L	N	38	3	N	7	9	A	N	14.3	16.3	12.2	3.6	N			
1310	M	A	N	N	38	3.1	N	6	9	A	N	12.9	17.5	9.7	6	N			
1311	M	A	L	N	37	2.3	N	6	8	A	N	13.7	13.6	8.5	4.9	N			
1312	M	A	L	N	38	3.3	N	7	9	A	N	13.5	14.1	8.9	4.1	N			
1313	F	P	N	N	40	3	A	7	9	A	N	17	8.7	4.9	1.9	Y	3.7	-	
1314	F	A	N	N	37	3.2	N	5	8	A	N	15.8	13.6	7.4	4.3	N			
1315	F	P	L	N	36	2	N	7	9	A	N	13.3	15.1	10.3	2.5	N			
1316	F	A	N	N	37	2.7	N	7	8	A	N	13.1	16.3	12	4	N			
1317	M	A	L	N	37	3.1	N	8	9	A	N								
1318	F	A	N	N	38	3	N	6	8	A	N	14.7	14	9.9	2.6	N			
1319	M	A	L	N	38	2.6	N	5	8	A	N	16.2	10.3	6.8	3.6	N			
1320	F	A	N	Y	38	3	N	5	7	A	N	15.2	8.2	4.7	2.5	N			
1321	F	A	L	N	36	2.2	N	8	9	A	N	14.7	18	13.5	3	N			
1322	M	A	N	N	37	2.7	N	6	9	A	N	16.1	16.3	11.3	4	N			
1323	F	A	L	N	37	2.9	N	6	8	A	N	12.8	24.1	16.7	6.1	N			
1324	M	A	N	N	39	3	N	8	9	A	N	18.2	20.3	13.5	6	N			
1325	M	A	L	N	38	2.4	N	7	10	A	N								
1326	F	A	N	Y	38	2.6	N	5	7	A	N	14.3	11.3	5.1	5.8	N			
1327	M	A	L	N	37	2.4	N	7	8	A	N	14.6	17.2	11	5.1	N			
1328	M	A	N	N	37	2.9	N	6	9	A	N	15.3	17.8	10.7	6	N			
1329	F	A	L	N	37	3.3	N	7	8	A	N	16.1	12.5	5.9	3.6	N			
1330	M	A	N	N	40	3.1	N	7	10	A	N	16.7	12	7.3	4.2	N			
1331	M	A	L	N	37	3	N	7	9	A	N	15.5	14.1	11.1	2.8	N			

1332	F	A	L	N	37	2.4	N	8	9	A	N	12.6	13.5	9.2	3.5	N			
1333	M	A	L	N	38	2.8	N	7	8	A	N	14.2	14.3	10.5	2.6	N			
1334	M	A	N	N	37	2.7	N	6	8	A	N	13.5	12.5	7.3	4.5	N			
1335	M	A	L	N	39	3.1	N	7	9	A	N	16.5	11.8	6.9	4	N			
1336																			
1337	F	A	L	N	38	3	N	8	9	A	N	15.8	11.1	7.3	3.2	N			
1338	F	A	L	N	39	3.5	N	6	8	A	N	14.7	12.3	7.2	3.5	N			
1339	M	A	N	N	38	2.5	N	5	9	A	N	14	10.8	6.6	2.9	N			
1340	F	A	L	N	37	2.4	N	6	8	A	N	14.9	14	11	2.6	N			
1341	M	P	N	N	39	3	N	8	10	A	N	13.6	15.6	7.9	7	N			
1342	F	A	N	N	40	3.1	N	5	9	A	N	16.5	17.4	10.8	6.1	N			
1343	M	A	L	Y	35	2.2	N	5	7	A	N	11.5	12.2	7.2	4.3	N			
1344	F	A	N	N	38	3.2	N	7	8	A	N								
1345	M	A	L	N	37	2.7	N	6	9	A	N	13.6	15.1	11.3	2.5	N			
1346	F	A	N	N	36	2.4	N	7	9	A	N	13.1	14.2	10.4	2.7	N			
1347	M	A	L	N	36	2.6	N	8	10	A	N	14.7	16.4	9	6	N			
1348	F	P	L	N	36	1.9	N	6	9	A	N	17.6	13	8.7	3.5	N			
1349	F	A	N	N	34	2	P	8	9	A	N	12	12.7	9	1.8	Y	3	-	
1350	M	A	N	N	39	3.7	N	6	8	A	N	15.4	12.4	7.3	4.1	N			
1351	M	A	L	N	40	3	N	6	9	A	N	12.8	18.3	12.9	5	N			
1352	M	A	N	N	38	2.8	N	7	9	A	N	11.6	14.6	8.5	4.6	N			
1353	F	A	L	N	39	2.4	N	8	9	A	N	19.6	9.5	5.1	4.2	N			
1354	F	A	L	N	37	2.5	N	5	8	A	N	12.5	16.8	13.1	2.7	N			
1355	F	A	N	N	38	2.3	N	6	9	A	N	16.3	12.8	7	4.2	N			
1356	F	A	L	N	38	2.4	N	8	9	A	N	12.7	16.7	11.6	4	N			
1357	F	P	N	N	39	2.9	N	7	10	A	N	14.8	14.7	10.9	2.7	N			
1358	M	A	L	N	36	2.5	N	6	9	A	N	14	16	12.1	2.6	N			
1359	F	A	N	N	37	2.6	N	7	8	A	N	14.5	14.2	10.2	3.7	N			
1360	M	A	L	N	38	2.7	N	8	9	A	N	16.3	16.2	12.3	2.5	N			
1361	F	A	L	N	37	2.6	N	4	7	A	N	15.5	15.2	9.8	2.9	N			
1362	M	A	N	N	34	1.8	N	6	8	A	N	16.8	17.3	13.2	2.6	N			
1363	F	A	L	Y	34	2.1	N	7	9	A	N								
1364	M	P	N	N	35	2.2	N	5	8	A	N	16.2	16	11.7	3.8	N			
1365	F	A	L	N	35	2	N	6	9	P	N	12.8	15.6	10.8	4	N			
1366	M	A	N	N	37	2.5	N	7	8	A	N	13.1	20.9	10.6	8.2	N			
1367	F	A	L	N	39	3	N	6	9	A	N	17.6	22.1	14	7.6	N			
1368	M	A	N	N	40	3.3	N	8	9	A	N	15.4	16.5	9.2	6	N			
1369	M	A	L	N	38	2.4	N	7	9	A	N	12.9	15.4	9.4	2.9	N			
1370	M	A	N	N	37	2.6	N	7	10	A	N	16.3	16.1	10.5	4.1	N			
1371	F	A	L	N	37	2.8	N	6	9	A	N	12.4	17.1	11.3	3.6	N			
1372	F	A	L	N	34	1.9	N	7	8	A	N	13.8	10.3	7.1	2.8	N			
1373	M	A	N	N	39	2.7	N	8	10	A	N	14.7	11.2	6	4.9	N			
1374	M	A	L	N	38	2.5	N	7	9	A	N	13.8	10	4.9	4.6	N			
1375	M	P	L	Y	33	2.3	P	5	9	P	N	11	10.7	7.5	2.1	Y	Missed		
1376	F	A	N	N	37	2.5	N	7	9	A	N	12.6	18	14.2	3	N			
1377	F	A	N	N	37	2.9	N	6	9	A	N	13.6	14.2	10.1	3.2	N			
1378	M	A	L	N	39	3.6	N	7	8	A	N	14.4	15.3	10.2	3.6	N			
1379	M	A	L	N	38	2.8	N	7	8	A	N	15	14.7	9.7	4	N			
1380	F	A	N	N	36	2.4	N	8	9	A	N	16	15.9	7.8	6.2	N			
1381	F	A	L	N	37	3.1	N	7	9	A	N	12.5	16	10.4	2.8	N			
1382	M	P	N	N	37	3	N	7	8	A	N	18.7	7.9	3	4.2	N			
1383	F	A	L	N	39	3.2	N	9	10	A	N	17.3	15	10.4	3.3	N			

1384	F	A	L	N	40	3	N	6	8	A	N	13.6	16.5	12.3	2.7	N			
1385	M	A	L	N	37	3	N	7	9	A	N								
1386	M	A	N	N	35	2	N	7	9	A	N	16.3	14.5	6.5	7	N			
1387	M	A	N	N	37	2.1	N	6	8	A	N	14.4	16.1	11.2	4.5	N			
1388	M	A	L	N	37	2.4	N	7	8	P	N	15.6	13.3	9.7	3.2	N			
1389	F	A	N	N	39	3.4	N	7	9	A	N	16	15.3	10.8	3.6	N			
1390	M	P	L	N	38	3.1	N	8	9	A	N	16.8	16.2	9.6	4.5	N			
1391	F	A	N	N	35	2.2	N	8	9	A	N	19.1	11.4	8	2.8	N			
1392																			
1393	M	A	L	N	37	2.5	N	8	10	A	N	15.2	14.6	10.5	2.7	N			
1394	F	A	N	N	33	1.7	N	7	8	A	N	15.3	16.3	13.1	2.5	N			
1395	F	A	L	N	38	3.3	N	7	9	A	N	14	16	12	3.2	N			
1396	M	A	N	N	39	3.4	N	7	9	A	N	14.8	14.7	8.4	5	N			
1397	M	A	L	N	33	1.8	N	6	8	A	N	12.8	14.4	7.5	4.8	N			
1398	F	A	L	N	39	2.7	N	7	8	A	N	16.6	16.8	12.2	3.8	N			
1399	M	A	L	Y	39	2.8	N	5	7	A	N	15	17.2	11.3	5	N			
1400	F	A	N	N	38	3.1	I	8	9	A	N	12.7	14	11.5	1.6	Y	3.3	-	
1401	F	A	L	N	36	2.2	N	9	10	A	N	13.8	17.1	11.5	5.7	N			
1402	M	A	L	N	36	1.8	N	7	8	A	N	14.1	16.5	10.8	4.1	N			
1403	M	A	N	N	39	2.6	N	8	9	A	N								
1404	M	A	N	N	40	2.8	N	7	9	A	N	18.2	17.2	12.7	2.8	N			
1405	F	A	L	N	37	2.6	N	8	9	A	N	13.8	15.9	8.3	6.7	N			
1406	F	P	L	N	37	2	N	5	8	A	N	11.6	10.7	5.9	3.9	N			
1407	M	A	N	N	39	3	N	6	8	A	N	15.4	9.5	5.4	2.6	N			
1408	M	A	L	N	36	2.3	P	8	9	A	N	17.6	12.6	10	2.2	Y	Missed	-	
1409	M	A	L	N	38	3.2	N	7	8	A	N	17.1	20.3	12.3	7	N			
1410	M	A	N	N	39	3.4	N	7	8	A	N	12	10.2	6.8	2.7	N			
1411	M	P	L	N	37	2.8	N	5	7	A	N	16.3	14.4	8.4	5	N			
1412	F	A	N	N	37	3	N	7	9	A	N	12.5	14.1	8.8	4.2	N			
1413	M	A	L	N	36	2.6	N	6	10	A	N	15.6	15.2	10.1	4.7	N			
1414	M	A	L	N	36	2.5	N	8	9	A	N	13.5	15.6	9.3	5	N			
1415	F	A	N	N	38	2.7	N	7	10	A	N	14	14.2	7.2	6.3	N			
1416	M	A	L	N	39	3	N	7	9	A	N								
1417	M	P	N	N	38	3.1	N	6	9	A	N	12.9	17.5	9.7	6	N			
1418	M	P	L	N	37	2.3	N	6	8	A	N	13.7	13.6	8.5	4.9	N			
1419	M	A	L	N	40	3.3	N	7	9	A	N	13.5	14.1	8.9	4.1	N			
1420	F	A	N	N	38	3	I	7	9	A	N	17	8.7	4.9	1.9	Y	3.5	-	
1421	F	A	N	N	38	3.2	N	5	8	A	N	15.8	13.6	7.4	4.3	N			
1422	F	A	L	N	38	2.9	N	7	9	A	N	13.3	15.1	10.3	2.5	N			
1423	F	A	N	N	39	2.7	N	7	8	A	N	13.1	16.3	12	4	N			
1424	M	A	L	N	37	3.1	N	8	9	A	N	15.7	16.4	10.2	4.7	N			
1425	F	A	N	N	39	3	N	6	8	A	N	14.7	14	9.9	2.6	N			
1426	M	A	L	N	38	2.6	N	5	8	A	N	16.2	10.3	6.8	3.6	N			
1427	M	P	L	Y	36	2	P, S	5	8	A	N	15	18	15.3	2	Y	2.6	-	
1428	F	A	L	N	37	2.2	N	8	9	A	N	14.7	18	13.5	3	N			
1429	M	A	N	N	38	3.5	N	6	9	A	N	16.1	16.3	11.3	4	N			
1430	F	A	L	N	40	2.9	N	6	8	A	N	12.8	24.1	16.7	6.1	N			
1431	M	A	N	N	38	3	N	8	9	A	N	18.2	20.3	13.5	6	N			
1432	M	A	L	N	36	2.4	N	7	10	A	N	12.4	12.8	4.7	7.6	N			
1433	F	A	N	Y	36	2.6	N	5	7	A	N	14.3	11.3	5.1	5.8	N			
1434	M	A	L	N	39	3.4	N	7	8	A	N	14.6	17.2	11	5.1	N			

1435	M	A	N	N	37	2.9	N	6	9	A	N	15.3	17.8	10.7	6	N			
1436	F	A	L	N	38	3.3	N	7	8	A	N	16.1	12.5	5.9	3.6	N			
1437	M	A	N	N	40	3.1	N	7	10	A	N	16.7	12	7.3	4.2	N			
1438	M	A	L	N	39	3	N	7	9	A	N								
1439	F	P	L	N	36	2.4	N	8	9	A	N	12.6	13.5	9.2	3.5	N			
1440	M	P	L	N	37	2.8	N	7	8	A	N	14.2	14.3	10.5	2.6	N			
1441	F	A	N	N	39	3.1	N	8	9	A	N	15	21.5	16.8	3.6	N			
1442	M	A	N	N	40	3.5	N	8	10	A	N	16.3	16.3	9.3	6.8	N			
1443	F	A	L	N	35	2	N	7	9	A	N	17.1	17.1	13.5	3.2	N			
1444	F	A	N	N	39	2.7	N	6	8	A	N	18.6	14.9	8.3	6	N			
1445	M	A	N	N	37	2.6	N	7	10	A	N	18.1	13.8	6.2	5.3	N			
1446	M	A	L	N	39	3.6	N	7	9	A	N	13.3	17.4	13.8	3.3	N			
1447	M	P	N	N	38	3	N	7	9	A	N	15.8	16.5	8	7.5	N			
1448	M	A	L	N	34	1.7	N	8	9	P	N	14.6	14.7	9.4	4.7	N			
1449	F	A	L	Y	34	1.6	N	5	7	A	N	10.8	17.2	8.1	8.7	N			
1450	M	A	L	N	39	3	N	6	10	A	N	9.4	12.3	8.3	3.2	N			
1451	F	A	N	N	40	2.9	N	6	9	A	N	14.9	16.4	5.5	9.7	N			
1452	M	A	L	N	38	2.8	N	7	10	A	N	13.6	15.8	10.1	5.2	N			
1453	M	P	L	N	34	2.4	P	7	9	A	N	14	11	8.1	2.2	Y	4.1	-	
1454	M	A	L	N	39	3.4	N	7	8	A	N	18.2	9.2	4.7	4.1	N			
1455	F	A	N	N	38	3.1	N	6	9	A	N	15.3	15.2	9.5	5.3	N			
1456	F	A	N	N	37	2.6	N	7	9	A	N	16.3	12	9	2.8	N			
1457	M	A	L	N	37	2.4	N	6	8	A	N								
1458	M	A	N	N	38	2.8	N	9	10	A	N	15	15.4	12.8	2.7	N			
1459	F	A	L	N	38	2.7	N	8	9	A	N	15.2	12.7	8.5	2.5	N			
1460	M	A	L	N	34	1.8	N	7	9	P	N	11	9.1	4	3.4	N			
1461	M	A	L	N	39	3	N	7	9	A	N	16.8	13.6	7.2	4.9	N			
1462	F	P	L	N	40	3	N	7	9	A	N	17.3	16.1	10.3	4.7	N			
1463	F	A	L	N	38	2.8	N	8	10	A	N	16	15	10.8	3.7	N			
1464	F	A	N	N	37	2.9	N	6	8	A	N	13.8	12.5	8.9	2.6	N			
1465	M	A	L	N	37	3.6	N	8	9	A	N	17.5	20.4	11	8.2	N			
1466	F	A	N	N	35	2.4	N	7	10	A	N	19.8	13.5	7.7	5.2	N			
1467	F	P	L	N	37	2.4	N	6	8	A	N	13.7	10.3	6	2.7	N			
1468	M	A	L	N	37	2.7	N	7	9	A	N	14.5	14.7	8.1	4.2	N			
1469	F	A	L	N	36	2.6	N	7	8	A	N	15.2	12.2	7.3	4.4	N			
1470	M	A	N	N	39	2.9	N	8	9	A	N	16.1	9.9	6.4	2.9	N			
1471	M	A	L	Y	37	3.3	N	5	9	A	Y	11.9	9	5.8	2.9	N			
1472	M	A	L	Y	34	2.1	P	4	8	P	N	16.6	13	9.8	1.7	Y	2.6	-	
1473	F	A	N	N	39	2.8	N	8	9	A	N	12.9	8	4.5	3.1	N			
1474	F	P	L	Y	36	2.1	N	4	8	A	N	14	15.3	11.8	3.3	N			
1475	M	A	L	N	36	1.7	N	6	7	A	N	12.8	6.8	3.9	2.5	N			
1476	M	P	N	N	39	3.4	N	8	9	A	N	18.6	11.9	6.7	4.5	N			
1477	F	A	L	N	40	3	N	6	10	A	N	17.7	14.4	10.6	2.6	N			
1478	M	A	L	N	39	2.9	N	7	8	A	N	16.5	13.5	9.7	2.8	N			
1479	F	A	L	N	34	1.9	P, S	6	9	A	N	11.7	10.5	7	1.9	Y	Missed	-	
1480	M	A	N	N	38	3.9	N	7	10	A	N	16.2	11.4	5.5	4.1	N			
1481	F	A	L	N	38	2.7	N	6	9	A	N	15.5	12.2	8.3	3.1	N			
1482	M	A	L	N	36	2.5	N	5	7	A	N	14.7	15.3	9.3	4.8	N			
1483	F	A	N	N	38	2.9	N	6	8	A	N	15	16.8	10.7	4.2	N			
1484	M	A	L	N	37	3.4	N	8	10	A	N	15.9	12	6.2	4.6	N			
1485	M	A	L	N	37	3.1	N	6	9	A	N	16.5	17.9	11.4	5.9	N			
1486	M	A	N	N	40	3	N	7	9	A	N	18	11	5.8	3.4	N			

1487	F	A	N	N	34	2	N	7	10	A	N								
1488	M	A	L	N	38	2.4	N	7	8	A	N	17	13.3	8.6	4.2	N			
1489	F	A	N	N	38	2.9	N	9	10	A	N	16.7	12.7	10.1	2.2	N			
1490	M	P	L	N	32	1.5	N	7	10	A	N	15.4	14	9.6	3.1	N			
1491	F	P	L	N	38	2.6	N	6	8	A	N	14.9	13.5	6.7	5.7	N			
1492	M	A	L	N	37	2.9	N	6	9	A	N	17	18.8	12.2	5.5	N			
1493	F	A	N	Y	38	3.1	N	5	8	A	N	16.5	28.9	14.7	11.2	N			
1494	M	A	N	N	37	3	N	6	8	A	N	16.2	19.1	13.5	4.4	N			
1495	M	A	L	N	36	2.5	N	7	9	A	N	18.3	14.2	10.3	3.1	N			
1496	F	A	N	N	37	2.1	N	8	9	A	N	17.5	15	11.4	3.1	N			
1497	M	A	L	N	37	2.3	N	7	8	A	N	14.9	16.8	9.4	6.2	N			
1498	F	A	L	N	39	3.1	N	8	10	A	N	14.3	18.9	14.1	4.5	N			
1499	F	A	L	N	40	2.8	N	6	9	A	N	12.4	17.1	11.3	3.6	N			
1500	F	A	L	N	34	1.9	N	7	8	A	N	13.8	10.3	7.1	2.8	N			
1501	M	A	N	N	38	2.7	N	8	10	A	N	14.7	11.2	6	4.9	N			
1502	M	A	L	N	38	2.5	N	7	9	A	N	13.8	10	4.9	4.6	N			
1503	F	A	N	N	37	2.5	N	7	9	A	N	12.6	18	14.2	3	N			
1504	F	A	N	N	37	2.9	N	6	9	A	N	13.6	14.2	10.1	3.2	N			
1505	M	A	L	N	38	3.6	N	7	8	A	N	14.4	15.3	10.2	3.6	N			
1506	M	P	L	N	37	2.8	N	7	8	A	N	15	14.7	9.7	4	N			
1507	F	A	N	N	36	2.4	N	8	9	A	N	16	15.9	7.8	6.2	N			
1508	F	A	L	N	39	3.1	N	7	9	A	N	12.5	16	10.4	2.8	N			
1509	M	A	N	N	40	3	N	7	8	A	N								
1510	F	A	L	N	40	3.2	N	9	10	A	N	17.3	15	10.4	3.3	N			
1511	M	A	N	N	40	3	N	6	8	A	N	15.4	9.5	5.4	2.6	N			
1512	F	A	L	N	35	2.5	P	7	9	A	N	14	12.6	10	2.3	Y	2.7	-	
1513	M	A	L	N	37	3.2	N	7	8	A	N	17.1	20.3	12.3	7	N			
1514	M	A	N	N	39	3.4	N	7	8	A	N	12	10.2	6.8	2.7	N			
1515	M	A	L	N	39	2.8	N	5	7	A	N	16.3	14.4	8.4	5	N			
1516	F	A	N	N	38	3	N	7	9	A	N	12.5	14.1	8.8	4.2	N			
1517	M	A	L	N	37	2.6	N	6	10	A	N	15.6	15.2	10.1	4.7	N			
1518	M	A	L	N	37	2.5	N	8	9	A	N	13.5	15.6	9.3	5	N			
1519	F	A	N	N	39	2.7	N	7	10	A	N	14	14.2	7.2	6.3	N			
1520	M	A	L	N	40	3	N	7	9	A	N	14.3	16.3	12.2	3.6	N			
1521	M	A	N	N	37	3.1	N	6	9	A	N	12.9	17.5	9.7	6	N			
1522	M	A	L	N	38	2.3	N	6	8	A	N	13.7	13.6	8.5	4.9	N			
1523	M	A	L	N	39	3.3	N	7	9	A	N								
1524	M	A	N	N	37	2.6	N	8	9	A	N	16.9	16.4	8.5	5.9	N			
1525	M	A	N	N	38	2.8	N	7	9	A	N	18.2	17.2	12.7	2.8	N			
1526	F	P	L	N	36	2.6	N	8	9	A	N	13.8	15.9	8.3	6	N			
1527	F	A	L	N	35	2	N	5	8	A	N	18	10.7	5.9	3.9	N			
1528	M	A	N	N	38	3	N	6	8	A	N	15.4	9.5	5.4	2.6	N			
1529	F	A	L	N	40	2.7	I	7	9	A	N	12.9	15	12.2	2.2	Y	4.3	-	
1530	M	A	L	N	37	3.2	N	7	8	A	N	17.1	20.3	12.3	7	N			
1531	F	A	N	N	38	3	N	7	9	A	N	17	8.7	4.9	3.2	N			
1532	M	A	N	N	37	3.4	N	7	8	A	N	12	10.2	6.8	2.7	N			
1533	M	A	L	N	38	2.8	N	5	7	A	N								
1534	F	A	N	N	36	2.1	N	7	9	A	N	12.5	14.1	8.8	4.2	N			
1535	M	A	L	N	40	2.6	N	6	10	A	N	15.6	15.2	10.1	4.7	N			
1536	M	P	L	N	40	3	N	8	9	A	N	13.5	15.6	9.3	5	N			
1537	F	P	N	N	39	2.7	N	7	10	A	N	14	14.2	7.2	6.1	N			
1538	M	A	L	N	38	3	N	7	9	A	N	20.8	16.3	12.2	3.6	N			

1539	M	A	N	N	38	3	N	8	9	A	N	13.9	14.9	3.3	10	N			
1540	M	A	N	N	37	3.4	N	7	8	A	N								
1541	F	A	L	N	39	3	N	6	10	A	N	17.1	22.7	12.6	7.8	N			
1542	F	A	L	Y	35	1.7	N	8	8	A	N	16.3	26.4	18.9	5.7	N			
1543	F	A	N	N	36	2.4	N	6	8	A	N	18	20.5	13.6	5	N			
1544	F	A	L	N	38	3	N	6	10	A	N	10.9	16.7	9.6	6.4	N			
1545	M	A	L	N	40	3.4	N	6	9	A	N	14.3	17.8	11.1	5	N			
1546	F	A	N	N	38	2.8	N	7	8	A	N	15	13.2	1.1	11.6	N			
1547	M	A	N	N	35	1.7	N	7	9	A	N	13	14.3	9.8	4.1	N			
1548	F	A	L	N	38	3	N	8	9	A	N	19.5	17.6	2.3	13.8	N			
1549	M	P	N	N	38	2.9	N	9	10	A	N	17.8	15.1	7.5	6.9	N			
1550	M	A	L	N	39	2.3	N	7	9	A	N								