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ESTABLISHING DIAGNOSTIC CRITERIA FOR SCID, LEAKY SCID, AND OMENN SYNDROME: THE PRIMARY IMMUNE DEFICIENCY TREATMENT CONSORTIUM EXPERIENCE

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Abstract

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Background—The approach to the diagnosis of Severe Combined Immunodeficiency Disease (SCID) and related disorders varies among institutions and countries.

Objectives—The Primary Immune Deficiency Treatment Consortium (PIDTC) attempted to develop a uniform set of criteria for diagnosing SCID and related disorders, and has evaluated the results as part of a retrospective study of SCID in North America.

Methods—Clinical records from 2000 through 2009 at 27 centers in North America were collected on 332 children treated with hematopoietic cell transplant (HCT), enzyme replacement therapy (ERT) or gene therapy (GT) for SCID and related disorders. Eligibility for inclusion in the study and classification into disease groups were established by set criteria and applied by an expert review group.

Result—Two hundred eighty-five (86%) of the patients were determined to be eligible and 47 (14%) were not eligible. Of the 285 eligible patients, 84% were classified as typical SCID; 13% were classified as leaky SCID, Omenn syndrome, or reticular dysgenesis; and 3% had a history of enzyme replacement or gene therapy. Detection of a genotype predicting a SCID phenotype was accepted for eligibility. Reasons for non-eligibility were failure to demonstrate either impaired lymphocyte proliferation or maternal T cell engraftment. Overall (n = 332) rates of testing were: proliferation to PHA 77%, maternal engraftment 35%, and genotype 79% (mutation identified in 62%).

Conclusion—Lack of complete laboratory evaluation of patients prior to HCT presents a significant barrier to definitive diagnosis of SCID and related disorders and prevented inclusion of individuals in our observational HCT study. This lesson is critical for patient care as well as the design of future, prospective treatment studies for such children, since a well-defined and consistent study population is important for precision in outcomes analysis.

Keywords

Allogeneic hematopoietic cell transplantation; gene therapy; primary immunodeficiency; clinical trial

INTRODUCTION

The Primary Immune Deficiency Treatment Consortium (PIDTC) is a group of centers with an interest in hematopoietic cell transplant (HCT) for Severe Combined Immunodeficiency Disease (SCID) [1, 2]. Funded by the National Institutes of Health, the PIDTC has among its missions to assess the outcomes of definitive therapy of SCID and related disorders over the past 5 decades in a retrospective observational and cross-sectional study and to serve as a basis for prospective interventional trials. In addition to typical SCID, the variant forms of severe primary immunodeficiency[3] studied include “leaky SCID”, Omenn syndrome, and reticular dysgenesis. This retrospective and cross-sectional study is designed to identify multiple patient, donor and treatment factors important for survival of children with SCID and SCID-related diseases who have received HCT, gene therapy, and/or enzyme replacement therapy (i.e., polyethylene glycol-conjugated adenosine deaminase, PEG-ADA). Each of these therapies has been life-saving, but among clinical immunologists in North America there is no consensus on how best to diagnose children with these rare disorders and how to deploy available treatments. As part of this retrospective/cross-sectional study, the PIDTC performed a sub-analysis of children who received HCT from January 1, 2000 through December 31, 2009 in order to characterize the current status of care for SCID in North America[3].

HCT can be an effective and permanent cure for children with SCID and related disorders.[4-7] However, prospective trials must be implemented to identify the best

approaches to treatment using HCT and other modalities. The future of the PIDTC clinical trials program critically depends upon uniform definitions of the many forms of SCID and SCID-related conditions with serious T cell deficiencies. Identification of the genotype of patients is becoming a key determinant for the definition of the type of SCID and related disorders.[8, 9] The aim of this retrospective study of patients treated in the most recent decade was to survey what clinical immunologists considered to be sufficient grounds for diagnosis and immune system restorative treatment of children suspected to have the most serious forms of pediatric immunodeficiency diseases (PID) and thus to be unlikely to survive without intervention. [10-24] A review committee was necessary to identify subjects eligible for enrollment in the observational study, and to categorize them into strata encompassing typical SCID patients as well as those with life threatening T cell deficiencies that do not have all of the features of typical SCID. SCID patients who were treated by enzyme replacement or gene therapy were also included.[25-30]

To determine the eligibility of historical patients given HCT, enzyme replacement, or gene therapy in the most recent decade (2000 through 2009), criteria were established to verify suitability for inclusion in the retrospective analysis, and to place each patient into one of 3 strata: A) typical SCID; B) leaky SCID, Omenn syndrome and reticular dysgenesis, and C) SCID treated by enzyme replacement therapy (ERT) or gene therapy (GT), the latter being available for ADA deficient or X-linked SCID. These criteria were designed to rely on universally available quantitative measurements of immunological parameters rather than the more vague and less specific clinical factors such as chronic or recurrent infections, opportunistic infections or failure to thrive. In this report, we discuss determination of eligibility of SCID patients in the period 2000 through 2009 and the distribution of eligible patients among the disease categories.

METHODS

Patient Study Cohort and Informed Consent

Each of the PIDTC centers obtained institutional review board approval by a waiver of written informed consent for chart review of patients, and then collected and compiled subject information for the retrospective part of this protocol, designated as PIDTC Protocol 6902 A Retrospective and Cross-Sectional Analysis of Patients Treated for SCID Since January 1, 1968, (ClinicalTrials.gov Identifier: NCT01346150). The chart reviews included data on patient clinical studies pre-and post-HCT, genotype, HLA type, donor source and HLA type, time to engraftment, immune reconstitution, adverse reactions, growth, development, and survival. A separate report will address the findings of this analysis. The initial step in enrolling a patient into PIDTC Protocol 6902 was to complete the eligibility form that requested key diagnostic information regarding the subject's immune status, genotype, and various other clinical and laboratory data felt to be necessary to either include or exclude a subject and identify the appropriate stratum.

Criteria of Eligibility for Inclusion of Patients into Study and Strata A, B, and C Classification

Detailed criteria for patient eligibility into the study and stratum classification (Table E1) were prepared by a PIDTC Review Committee of 10 experts in the diagnosis and definitive treatment of SCID and other serious T cell deficiencies. Strata A and B included patients treated by (or with an intention to treat by) HCT, whereas Stratum C included patients treated by ERT or GT. Furthermore, Stratum A included typical SCID only, with the exception of reticular dysgenesis, which was placed in Stratum B along with leaky SCID and Omenn syndrome because of its unusual features. Rotating sub-panels of 3 experts reviewed the Patient Eligibility and Stratum assignment forms submitted by participating

sites. (Table E2). A unanimous decision of these 3 experts was required for placement into eligibility status and diagnostic stratum. In case of disputes, all 10 experts evaluated the case and a majority vote determined the outcome (virtually all decisions were unanimous). Enrollment criteria for patients who expired before definitive therapy could be undertaken were the same as for Strata A, B, and C, as summarized in Table I (abbreviated form of Table E1). Failure to thrive and recurrent serious infections were not included because of the age-dependent nature of these variables and the growing efforts to give definitive therapy as early as possible.

References for the eligibility criteria have not been available, as prior studies have at times been inconsistent with each other, and criteria have been vague or qualitative and or derived from very small numbers of patients. In fact, establishing numerical criteria for laboratory results to assist the diagnosis of patients with typical SCID, leaky SCID and Omenn syndrome has been a major aim of this project. The criteria for the PIDTC were arrived at by consensus of experts who have seen substantial numbers of cases over many years. It is anticipated that the criteria may be refined or modified as the project goes forward. Two important aspects of diagnosis that are now changing are (a) the role of genotyping in establishing diagnosis, as in the past gene diagnosis was not available; and (b) little or nothing is known about whether there are differences between pre-infectious vs. post-infectious laboratory values of patients detected by newborn screening or positive family history vs. those who come to medical attention after developing infectious complications.

Data Management and Analysis Plan

Clinical site responses to the patient eligibility questionnaire were submitted to the Rare Diseases Clinical Research Network (RDCRN) Data Management Coordinating Center (DMCC), Bethesda, MD for collation of individual patient information. The data set was summarized with descriptive analysis.

RESULTS

Eligibility and Stratum Assignment

For 2000 through 2009, 332 Patient Eligibility Forms for PIDTC Protocol 6902 were reviewed. The summary of the sites submitting these forms, the total number of patients reviewed for eligibility, the total number eligible/enrolled, and the total number ruled non-eligible are presented in Table II. A total of 285 (86%) patients qualified for enrollment into the retrospective PIDTC 6902 study, whereas 47 (14%) were judged by the Review Committee to be non-eligible. The reasons for the non-eligibility of these 47 patients are listed in Table III. Lack of PHA (phytohemagglutinin) lymphocyte stimulation testing and lack of assessment of maternal T cell engraftment were the most prominent causes of non-eligibility. The stratum assignment by the Review Committee placed 240 of the 285 eligible patients into Stratum A, 36 into Stratum B, and 9 into Stratum C (Table IV). The typical SCID patients in stratum A had a number of well-recognized SCID-associated genetic defects, including deleterious mutations in *IL2RG* (n=86), *IL7R* (22), *ADA* (14), *DCLER1C* (11), *JAK3* (11), *RAG2* (9), *RAG1* (7), *CD3D* (2), *CD3Z* (1), *CD45* (1), *PNP* (1), and heterozygous *RAG1* and *RAG2* (1). Other patients not tested for genetic defects or in which none were identified (n=74) nonetheless met criteria for Stratum A and were therefore enrolled. The patients in Stratum B had: Leaky SCID (n = 21), Omenn syndrome (13), and reticular dysgenesis (2). There were 9 patients in Stratum C.

Major Barrier to Eligibility: Failure to Perform or Record Laboratory Testing

Barriers to eligibility and stratum assignment are included in Table V. Insufficient information on maternal T cell chimerism was a major shortcoming; testing of maternal

engraftment was not performed in 65% of patients. Furthermore, PHA testing was not performed in 23% of patients or the test report is missing key raw data. Genotyping was performed in 263 patients in the total cohort with a success rate of 62% in identifying deleterious mutations that were concordant with the phenotype. Although not required for eligibility, genotyping was used in determining patient eligibility where other criteria were incomplete or non-informative. From examination of the clinical sites that did not complete these diagnostic tests, it can be seen that certain sites accounted for a large proportion of this non-performance total; 18 sites did maternal engraftment testing on <50% of their patients (of these, 5 did not do maternal chimerism testing on any of their SCID patients), 8 sites did PHA testing on <50% of their patients (of these, 3 did not obtain proliferation testing on any patient), and 3 sites did genotyping evaluation testing on <50% of their patients (of these, 1 did no mutation testing on any patients). Failure to perform these tests led to non-eligibility of many cases. In addition, appropriate HIV testing (nucleic acid based)[31] was performed in only 118 (36%) of the cohort, but none of the inappropriate HIV testing (e.g. HIV antibody of the infant) was used as a criterion for exclusion. Only 5 patients did not have any HIV testing; in 4 of the 5 a SCID genotype was identified and the patients were enrolled.

Dilemmas in Selection of Eligibility and Stratum Assignment: Illustrations

As examples of extended review efforts to determine patient eligibility the following case histories are described. In these challenging cases, the determination of the panel is not an evaluation of the appropriateness or lack of appropriateness of HCT or any other therapy for an individual case. Rather, a determination of eligibility by the review panel means that data sufficient to place the patient into one of the Protocol 6902 categories are available and that the criteria are met successfully. The PIDTC protocol requires strict definitions of SCID for eligibility to the study, in order to make definitive conclusions about the clinical course and outcomes of therapy for particular types of SCID patients.

Case 1

An infant had greater than 300 CD3 cells/μl, PHA, candida and tetanus responses of less than 10% of the lower limit of normal, 99% CD45 RO^+ $CD4^+$ memory phenotype T cells, elevated serum IgE level, pronounced eosinophilia, generalized skin rash, hepatosplenomegaly, lymphadenopathy, and a reported positive test for ADA deficiency. The evaluation for maternal lymphocyte engraftment was not reported. Due to inconsistencies and the lack of maternal engraftment data, the clinical site was contacted for verification and it was discovered that the ADA test result had actually been normal. With this new information, two members of the eligibility review panel voted for Omenn syndrome, and one member voted for ineligibility. A second review was held and a uniform opinion was achieved. The child was rated ineligible for Protocol 6902 because maternal engraftment was not tested. In the absence of an appropriate test for maternal T cell engraftment, it was not possible to distinguish between Omenn syndrome vs. typical SCID with maternal engraftment-associated GvHD. The fact that $CD4^+$ cells were all $CD45RO^+$ did not distinguish between these two possibilities. In communication with the clinical site, the PIDTC Eligibility Review Committee noted that should further analysis of genetic mutations become available, a repeat review of this patient for eligibility to PIDTC Protocol 6902 would be appropriate.

Case 2

Upon initial review by the PIDTC Review Panel, the patient appeared ineligible for inclusion in the study due to a paucity of laboratory data submitted by the site, despite a clinical picture suggestive of reticular dysgenesis. The patient had less than 200 neutrophils/μL, a very low CD3 cell count (22 cells/μl), a “negative” HIV antibody test, and a bone marrow examination demonstrating “a serious defect in myelopoiesis.” Testing for maternal

T cell engraftment, PHA testing, and genotype were not performed. The panel requested additional information from the site, including a review of the bone marrow pathology. Sensorineural deafness was indicated as not recorded. Unfortunately, the patient had expired post-transplant, and further data were not available. Review of the bone marrow biopsies demonstrated mature granulocytes present although reduced in number, dysplasia of megakaryocytes and hemophagocytosis, all inconsistent with reticular dysgenesis in which a block in maturation of granulocytes at the promyelocyte stage is seen. The review panel determined this patient to be ineligible for PIDTC Protocol 6902 due to insufficient availability of data.

DISCUSSION

The implementation of prospective studies to determine the optimal therapeutic approaches to reconstitution of immune function in patients with SCID and related disorders has been a long-term goal of clinical immunologists in North America and elsewhere.[1-3] Until the formation of the PIDTC, there has not been a North American collaborative study of SCID. Instead there have been reports from individual centers regarding their approaches to immune reconstitution of SCID children.[32-38] European clinical immunologists have been more successful in collaborative retrospective SCID-HCT studies, resulting in larger numbers of patients entered into a curated immunodeficiency registry and generation of definitive statements of method, survival and outcomes.[39-41] Formulation within the PIDTC of a network of investigators who treat patients with SCID may help critically assess the outcome of HCT for SCID and improve management of this condition. However, in North America, Europe and elsewhere, a uniform diagnosis of SCID and related disorders has been difficult to develop, and this may affect not only retrospective analyses, but also prospective interventional studies.

One of the purposes of the retrospective PIDTC protocol 6902 is to learn what investigators have accomplished in the past 45 years with SCID transplants. The most recent decade of experience was chosen for the present study to take advantage of the latest improvements in techniques that impact understanding of the pathogenesis of SCID and related disorders and to increase the awareness of SCID and timeliness of referral of SCID patients to major transplant centers for prompt and effective definitive therapy.[41] Using the eligibility criteria developed for PIDTC Protocol 6902, the review committee was able to assign 86% of patients with SCID or SCID-related conditions to one of the established strata. There was considerable difficulty in dealing with 14% of the patients because of the lack of critical laboratory information, despite the committee's attempt to place all patients into an appropriate stratum. It is essential that all clinicians diagnosing and caring for patients with SCID and SCID-related disorders obtain a minimal but critical amount of laboratory information before implementing definitive therapy. This is important not only for the appropriate care of these affected children, but also for the evaluation of outcomes as a result of the various therapeutic approaches. In future prospective studies, the PIDTC will certainly review this lesson of inadequate laboratory information and require participating centers to obtain the studies that will appropriately diagnose and permit as many patients as possible to be eligible. It must be acknowledged, however, that eligibility criteria may evolve over time. For example, when the entry/exclusion criteria for PIDTC Protocol 6902 were set up, very few infants had newborn screening with TRECs, but now 15 states and >50% of infants in the US will be screened, and undetectable or very low TRECs may be appropriate to add for study eligibility. Moreover, a recent TREC study of nearly 1 million newborn infants revealed a SCID incidence of 1/66,000, nearly double that previously estimated.[42]

There were mitigating factors for some failures to provide adequate laboratory information. We learned that at some centers large amounts of blood were required from small infants, such as 20 mL for proliferation studies in some clinical laboratories. Also, some sites were not aware that chimerism could be studied and maternal cells detected successfully when the absolute number of T cells by flow cytometry was very low or 'undetectable.' Gene sequencing was sometimes omitted because it was expensive and took so long that HCT would often already be done by the time results were back. Better information for HCT centers and access to clinical laboratories that serve babies with SCID will be key to improved rigor for the diagnosis of SCID and related disorders. An important factor to keep in mind in coming to a decision on diagnosis is the belief of PIDTC experts that the inability to eliminate maternal cells is found only with the most profound T cell defects and always indicates a diagnosis of typical SCID.

It is encouraging that genotyping of patients has become a preferred laboratory evaluation since it has a high yield of contributing to definitive patient diagnosis and may prove to have implications regarding selection of treatment, not only for enzyme replacement or gene therapy, but also for genotype-specific HCT protocols in the future. Thus, the power of genetics to assist clinical immunologists to come to a correct diagnosis for SCID and related disorders cannot be over-stated, from T-cell receptor excision (TREC) testing to targeted mutation analysis to whole exome/whole genome sequencing. Genotype information provides substantial and accurate information regarding current eligibility of patients for study databanks and stratum assignment.[43] However, maternal chimerism and lymphocyte function (proliferation to PHA) tests in addition to the absolute numbers of T, B and NK cells, remain essential; and appropriate therapy should not be delayed if genotyping cannot be completed in a timely fashion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations for Manuscript

ADA-SCID	Adenosine Deaminase-Deficient SCID
ERT	Enzyme Replacement Therapy
GT	Gene Therapy
HCT	Hematopoietic Stem Cell Transplantation
PEG-ADA	Polyethylene Glycol-Conjugated Adenosine Deaminase
PID	Primary Immunodeficiency Disease
PIDTC	Primary Immune Deficiency Treatment Consortium
SCID	Severe Combined Immunodeficiency Disease
TREC	T-cell Receptor Excision Circle

CLINICAL IMPLICATIONS

A more complete laboratory evaluation of patients with SCID and related disorders will permit more accurate diagnosis and placement of patients into appropriate prospective therapeutic protocols designed to optimize immune correction and survival outcomes.

Table I

Outline of Inclusion and Exclusion Criteria for Patient Eligibility and Assignment to Strata

Stratum A, Typical SCID

Patients who meet the following inclusion criteria and who received HCT as initial treatment are eligible for enrollment into Stratum A (Classic SCID) of the study:

- Absence or very low number of T cells (CD3 T cells < 300/microliter), **AND** no or very low T cell function (< 10% of lower limit of normal) as measured by response to phytohemagglutinin (PHA)

Or

- T cells of maternal origin present

Stratum B, Leaky SCID, Omenn Syndrome, Reticular Dysgenesis

Patients who were treated initially with HCT and who meet the following criteria are eligible for enrollment into Stratum B of the study:

Leaky SCID

- Reduced number of CD3 T cells
  - for age up to 2 years < 1000/microliter
  - for > 2 years up to 4 years < 800/microliter
  - for > 4 years < 600/microliter
- Absence of maternal engraftment
- < 30% of lower limit of normal T cell function (as measured by response to PHA)

Omenn Syndrome

- Generalized Skin Rash
- Absence of maternal engraftment.
- Detectable CD3 T cells, ≥ 300/microliter
- Absent or low (up to 30% of normal) T cell proliferation to antigens to which the patient has been exposed

If the proliferation to antigen was not performed, but at least 4 of the following 10 supportive criteria, at least one of which must be among those marked with an asterisk (*) below are present, the patient is eligible:

- Hepatomegaly
- Splenomegaly
- Lymphadenopathy
- Elevated IgE
- Elevated absolute eosinophil count
- *Oligoclonal T cells measured by CDR3 length or flow cytometry
- * >80% of CD3+ or CD4+ T cells are CD45RO+
- *Proliferation to PHA is reduced <30% of lower limit of normal
- *Proliferative response in mixed leukocyte reaction is reduced <30% of lower limit of normal
- *Mutation in SCID-causing gene

Reticular Dysgenesis (RD)

- Absence or very low number of T cells (CD3 T cells <300/microliter)
- No or very low (<10% of lower limit of normal) T cell function (as measured by response to phytohemagglutinin (PHA)
- Severe neutropenias (absolute neutrophil count <200/microliter)
- Sensorineural deafness and/or absence of granulopoiesis at bone marrow examination and/or a deleterious AK2 mutation

Stratum C, SCID with Non-HCT Treatments

Patients who met the above criteria for Stratum A or B and were initially treated with PEG-ADA ERT or gene therapy with autologous modified cells are eligible for enrollment into Stratum C (SCID with non-HCT treatments) of the study.

- ADA Deficient SCID treated with PEG-ADA ERT.
- Any SCID treated with gene therapy.

Exclusion Criteria (see Table E1)

Table II

Eligibility of Patients for Enrollment into the Retrospective PIDTC 6902 Protocol Study 2000 to 2009

Site rank number	Total patients reviewed for eligibility	Total eligible/enrolled	Total not eligible
1	64	64	0
2	28	27	1
3	24	23	1
4	21	19	2
5	19	16	3
6	17	15	2
7	17	6	11
8	16	16	0
9	14	13	1
10	13	11	2
11	11	7	4
12	10	10	0
13	10	8	2
14	10	8	2
15	9	3	6
16	7	7	0
17	6	5	1
18	6	2	4
19	5	5	0
20	4	4	0
21	4	4	0
22	3	3	0
23	3	3	0
24	3	2	1
25	3	1	2
26	3	2	1
27	2	1	1
Totals	**332**	**285 (86%)**	**47 (14%)**

Note: 27 total sites are participating in the 6902 2000-2009 study (there are currently **33 sites** participating in the PIDTC)

Table III

Reason for Non-Eligibility of 47 Patients for Enrollment into PIDTC Retrospective Protocol Study 2000 – 2009

No PHA; no mutation	16 (34%)
Maternal Engraftment Not Tested No Mutation (and does not meet criteria for Stratum A)	19 (40%)
Maternal Engraftment, PHA Not Tested No Mutation (and does not meet criteria for Stratum A)	3 (6%)
Otherwise Does not Meet Any Criteria	6 (13%)
Mutation Indicates Not SCID	2 (4%) 1 CD40 1 XLP
No HIV Testing; No Mutation	1 (2%)

CD40L, CD40 Ligand deficiency

XLP, X-linked lymphoproliferative syndrome

Table IV

Stratum Assignment of 285 Patients Eligible for PIDTC Protocol 6902

A Typical SCID	B Leaky SCID, Omenn	C Enzyme, Gene Rx
240	36	9
84%	13%	3%

Table V

Performance of Diagnostic Laboratory Tests for SCID in 322 Patients*

Maternal Engraftment – Testing Not Done			
For All Sites: Number of Patients ME Testing Not Done N = 215 of 332 = 65%	Number of Sites that did ME Testing on <50% of their Patients N = 18 of 27 = 67%	For 18 Sites: Number of Patients ME Testing Not Done N = 193 of 228 = 85%	For 18 sites: Number of Patients ME Testing Not Done that are Not Eligible N = 35 of 193 = 18% (lack of ME testing accounts at least in part for Not Eligible)
PHA – Testing Not Done (or incomplete record, descriptive only)			
For All Sites: Number of Patients PHA Testing Not Done N = 76 of 332 = 23%	Number of Sites that did PHA Testing on <50% of their Patients N = 8 of 27 = 30%	For 8 Sites: Number of Patients PHA Testing Not Done N = 49 of 68 = 72%	For 8 sites: Number of Patients PHA Testing Not Done that are Not Eligible N = 20 of 49 = 41% (lack of PHA testing accounts at least in part for Not Eligible)
Genotype – Testing Not Done (note: genotype not a requirement for eligibility)			
For All Sites: Number of Patients Genetic Testing Not Done N = 69 of 332 = 21%	Number of Sites that did Genetic Testing on <50% of their Patients N = 3 of 27 = 11%	For 3 Sites: Number of Patients Genetic Testing Not Done N = 21 of 31 = 85%	For 3 sites: Number of Patients Genetic Testing Not Done that are Not Eligible N = 10 of 21 = 48% (lack of genetic testing accounts at least in part for Not Eligible)

ME, maternal engraftment

* The lack of performing certain laboratory testing is multifactoral: near zero CD3 counts/μL, lack of key raw data, concern for blood volume in small infants, and unfamiliarity of some sites with certain assays