OVERVIEW

All SCID NBS for Puerto Rico is accomplished under the auspices of a PR IRB approved protocol (attached). In brief, the protocol allows SCID NBS to be performed after all routine PR NBS is completed. Because specimens are sent to the NENSP for SCID NBS, each specimen is coded and identifiers are removed prior to shipment to the NENSP. In addition, the "white slip" or the top paper of the specimen collection device, is removed and stored in PR prior to shipping in order to ensure a full record of each of the specimens sent to NENSP. The coded specimens (minus the white slip and devoid of identifiers) from the PR NBS Program are sent weekly to the NENSP, bagged according to PR batching. Upon receipt at the NENSP, each specimen is assigned a NENSP Guthrie number and the PR code is used as the patient name. Due to the absence of a white slip, each PR specimen must be Xeroxed in order to have a clean copy that can be used by data entry personnel.

Validation of specimen transport

In July 2010, Puerto Rico (PR) sent one batch of de-identified newborn blood spot specimens as a test run for the UPS shipping procedure. Notice that a test batch was being sent was given by email from PR NBS to NENSP and a large UPS box containing the specimens was received the following day. The specimens were viewed in preparation for the necessary requirements needed at NENSP(whether we could reliably read the assigned PR codes given the absence of a white slip, whether there was sufficient room on the card for NENSP to assign its Guthrie number) and were stored frozen until return shipping. No testing was performed on these specimens. On September 23rd the test specimens were returned to Puerto Rico (UPS tracking number) to the following mailing address:

Puerto Rico Newborn Screening Program
UPR Pediatric Hospital
Building A 2nd floor - Lab 223
Attention: Sonia I. Ramirez
San Juan, PR 00935

The PR NBS confirmed return receipt of their specimens.

NENSP Procedures for processing Puerto Rico specimens

Each week, the Puerto Rico Newborn Screening Program (PR NBS) informs the laboratory supervisor of the NENSP SCID lab, when PR will ship specimens to NENSP along with an estimate of number of specimens. The SCID lab supervisor informs the NENSP specimen processing supervisor.

Specimens are delivered by UPS to specimen processing and arrive in a cardboard box in a plastic bag inside. Specimen processing staff checks off the receipt on a PR delivery on the PR UPS Delivery check off list, recording date and time of box arrival with initials of person recording this info. We also Xerox the UPS label on the box and attach it to the completed PR UPS Delivery Check off list. Specimen processing staff brings the unopened box of specimens

to the SCID lab for log in there. SCID lab personnel bring the specimens in the plastic bag(s) back to specimen processing after they have logged them in.

Written (with a black sharpie by the PR NBS team) on the outside of each plastic bag is a PR batch number and beginning and ending dates of collection for the specimens contained in that bag. At approximately 2:30pm on day of specimen arrival (when daily record is complete so we know what Guthrie number to start with for PR because PR is last group processed on the day they arrive) specimen processing arranges the bags in order from lower to high batch numbers and then starts with the lowest (batch number) bag, separating specimens in each bag into groups of 80 (one plate). Each batch of 80 is counted again by another staff member. An elastic band is placed around each batch and a post it note with the Guthrie number range for that batch is attached at that time. Supervisor compares the total number of specimens received with the number that PR said they sent. At the same time that this is being done, another member of specimen processing staff is in data entry printing labels containing all the Guthrie numbers for PR specimens. An excel file (PR Number Generator) stored on the F drive (F:\Shared\Newborn\AAA\Subject\SpecimenProcessing) is run to create the labels. Two Guthrie numbers are printed on each label (Avery label #5167, size ½" X 13/4") for a total of 80 Guthrie numbers in each sheet/batch.

When the labels are printed they are brought over to specimen processing and each single label is cut into two parts so each has a Guthrie number on a thin piece of label. Each sheet contains the Guthrie numbers from 1-80 for one batch.

When all batches have been double counted and batched with a post it note identifying Guthrie number beginning and end, specimen processing staff each take one batch and the corresponding sheet of prepared labels and place one label on each specimen in the batch. The label is usually placed above the PR patient ID number in the upper left hand corner of the card. Sometimes there is not enough room there, perhaps because there is a note written there, and in those cases the label is place under the Patient ID number or in any available space on that side of card.

When all labels have been attached, each batch is rechecked to make sure that each specimen has a label containing our Guthrie number.

The Daily Record is updated after this is done to reflect the addition of these last batches this day.

We immediately start to Xerox each specimen batch by batch using the dedicated copy machine in specimen processing. We must copy each specimen so that data entry has a copy for use to enter the data (the blood specimen itself never goes to data entry). Since SCID lab does not run these specimens on the day arrive, we copy as much as we can as quickly as we can over the next few days. The next morning we create PR batches in the NENSP NBS application for all batches that have been copied and then forward the copies to data. From then on, every time we can copy more PR batches, we do that until all are copied. We create the corresponding batches in the NBS application as we go, giving the Xerox copies to data each time.

On day of arrival when we done dealing with the PR specimens the specimens are placed in a separate bin (labeled PR specimens and arrival date) which is stored in the west wing refrigerator on a shelf to the left of entry. They are removed from there and returned as necessary until all copying is complete. SCID lab personnel remove them as needed to test,

etc. Once the specimens are copied and those copies given to data entry, the SCID lab is responsible for storage and disposition of all specimens.

After all processing of these is done for the day, the specimen processing supervisor prepares and send an email to PR NBS (copy to NENSP personnel) stating how many specimens were received, repeating what they said they sent us (their batch numbers with dates of collection range for each).

Processing PR specimens

PR specimens typically arrive on a Friday and are processed through the week, in the order of batches numbered by PR NBS. The UPS delivery that arrives on one Friday will have had testing completed by the time of the next UPS delivery that arrives on the next Friday. Other than the "batching" aspect, PR specimens are subjected to the same algorithms as Massachusetts specimens.

Reporting SCID NBS results for PR specimens.

PR specimens are subjected to the same testing, interpretation and data algorithms as Massachusetts specimens; each specimen's results are in the NBS core, from which formal individual reports can be issued.

Out of range results or SCID-specific Unsats. Out of range SCID NBS results are reported as soon as they are generated (no batching). For each out-of-range SCID NBS result, an email is sent to the PR NBS program with a formal individualized report issued by the NENSP and the NENSP tracks for confirmation of receipt of email (same day). The PR NBS contacts the provider and issues reports and recommended next steps using interpretation and follow up algorithms designed in Massachusetts to determine whether to send a repeat NBS sample or to send blood for flow cytometry or to recommend a clinical consultation.

In range results. In-range SCID NBS results are sent in batches. NENSP staff query the NBS core to generate an excel worksheet that lists all specimens with in-range results (as well as the already-reported out-of-range results and any program unsats). The excel worksheets are sent to the PR NBS program.

Title: Pilot Studies for new test in the Newborn Screening Program

Investigators

Dr. Pedro J. Santiago Borrero – Professor of Pediatrics, Head of the Pediatric Hematology and Oncology Section, Director, PR Newborn Screening Program

Dr. Anne Marie Comeau, Ph.D. – Deputy Director, New England Newborn Screening Program; Associate Professor, Department of Pediatrics, University of Massachusetts Medical School

Dr. Carmen L. Cadilla – Professor of Biochemistry, Director of the RCMI Human Molecular Genetics Unit

Dr. Jhon Guerra – MD, FAAP, Assistant Professor of Pediatrics, Director Pediatric Oncology Section, University of Puerto Rico.

Mrs. Sonia I. Ramírez Morales – Medical Technologist, Supervisor, DNA Analysis Laboratory, PR Newborn Screening Program

Description

The Puerto Rico Newborn Screening Program (NBS) at the University Pediatric Hospital started operations in 1987 by mandate of Law 84 of July 2, 1987, under the direction of Dr. Pedro J. Santiago Borrero, Pediatric Hematologist-Oncologist and founder of the Puerto Rico Inherited Diseases Program.

The Board of Inherited Diseases, whose members were appointed by the Secretary of Health and the Governor of Puerto Rico, determined the need to begin doing newborn screening for early detection of three disorders (Hypothyroidism, PKU and sickle cell disease). These conditions can be controlled well by early detection and treatment with relatively inexpensive therapy, thus avoiding incapacity or death.

State funding was provided with a base assignment of $200,000 annually, to cover for infrastructure costs and medically indigent patients. The economic support was decreased to 180,000 two years ago, while multiple other test has been added to our screening program. For a disorder to be included in the routine screening panel list three conditions must be met: 1) the disorder must be fairly common, 2) there is a good relatively inexpensive test available, 3) early medical intervention would benefit affected infants.

During the last twenty years, new technology and methods have become available to identify many other conditions and to prevent death, disability, or complications such as mental retardation. Almost all states now screen for at least 28 medical disorders. The Puerto Rico Newborn Screening Program is working hard to keep up with the standard of care provided by USA leading states in newborn screening.

The goal of the Newborn Screening Program (PRNSP) is to be testing all newborns in Puerto Rico for early signs of a large number of treatable disorders (~ 28) in the year 2010. The PRNSP is planning to conduct research on several disorders affecting our population but needing more evidence to be included in routine testing.

At present we are screening for 7 disorders in practically 100% of all newborns in this island and we also are testing 80% of all infants for 19 metabolic disorders on pilot studies. The next goals include testing of newborns for two other new diseases (Biotinidase deficiency and IRT testing for cystic fibrosis) in 2010-11, thus screening 100% of newborns for 28 disorders.

As a standard procedure during the last seven years, we have been keeping all the dried blood samples for two years and thereafter a 10% random sample of each year's births is being kept for epidemiological and hereditary conditions research and as an educational resource for fellows and students. We conduct population-based studies making sure the sample is demographically representative of the births in the entire island.

During the month of April, 2010, the New England Newborn Screening Program of University of Massachusetts Medical School (NENSP UMMS), a leader in Newborn Screening, invited us to participate in a collaboration project to screen for markers of Severe Combined Immunodeficiency (SCID). The proposed study will include an additional 46,000 infants who will be screened for SCID by the NENSP SCID NBS protocol, will complement an existing statewide newborn screening program for SCID in Massachusetts (>80,000 infants screened to date; sponsored in part by the Center for Disease Control (CDC) Grant 1U01EH000362) The proposed study will be funded by a subcontract to UMMS from the NICHD contract awarded to Dr. Ken Pass at the Wadsworth Institute, NY.

SCID is a Primary Immune Deficiency disorder that has been under consideration for population-based newborn screening. The Federal Advisory Committee on Heritable Disorders in Newborns and Children voted to recommend SCID to the national uniform panel of newborn screening disorders and Secretary Sebelius recently adopted the recommendation of the Advisory Committee (May 21 letter to Dr. Howell, Chairperson of the Committee). Untreated, SCID has a high morbidity and mortality. Infants who are diagnosed early and transplanted in the course of the first year, have the best chance of survival and fewer medical complications than those diagnosed later.

SCID describes a clinical state that has been associated with more than 13 independent genetic conditions and a multiplicity of alleles. Thus, a test for typical Mendelian markers of disease would be challenging at best. However, all infants who carry a diagnosis of SCID have extremely low or absent T cells. A test that can count the number of infant T cells in a blood sample using a molecular marker has been developed (Chan and Puck,2005) and optimized (Gerstel-Thompson,…Comeau, in press) for use in newborn screening. The optimized test is in use in Massachusetts.

The specific aims of the PRNSP research activities are: 1) To continue to make available to our faculty, graduate students and residents/fellows the research infrastructure necessary for epidemiological and hereditary conditions, 2) To provide research and technical support in the UPR Medical Sciences Campus for epidemiological and hereditary conditions. 3) To address important quality improvement and pilot research questions for new test inclusion in newborn screening: a) what is the extent of benefit from SCID newborn screening in terms of saving lives, serious outcomes prevention and treatment effectiveness? b) How often does SCID occur in Puerto Rico? c) How good are the laboratory tests used to screen for SCID? The answers to some of these questions will be answered directly from implementation of SCID NBS in Puerto Rico. The answers to other questions will more easily be answered by combining aggregate data from multiple population-based newborn screening programs.

Newborn screening helps to find the babies who have these metabolic disorders, sometimes before symptoms appear. With rapid diagnosis, these disorders can be managed with treatment, and counseling. Hence, early

diagnosis and disease awareness is critical to reduce the risk of diseases with potentially fatal outcomes.

Materials and Methods

Newborn samples received at the Puerto Rico Newborn Screening Program will be sent to the New England Newborn Screening Program to be tested for markers of SCID after all routine newborn screening services provided by PRNSP are complete. An optimized new molecular assay for the quantitation of DNA structures that indicate autologous T-cell function (a multiplexed TREC assay) will be carried out by the NENSP. The utility of a Luminex-based antibody assay for quantitation of T cells and decision-making on the optimal algorithm for early identification of SCID may also be performed by NENSP if it is incorporated into the NENSP SCID NBS algorithm.

Each PRNSP specimen that NENSP tests will be identifiable by PRNSP (coded to NENSP) and infants whose specimens meet criteria for an “out of range” SCID NBS will be tracked and followed to evaluation and treatment by PRNSP. The code number that PRNSP assigns to every PRNSP sample (printed on the Guthrie card) will be the identifier that NENSP records in the NENSP database. The only other demographic variables that will reside in the NENSP database are the gender, weight, age, and if the baby was in NICU (neonatal intensive care unit) will be provided. Confidentiality of all records and materials will be guarded to the fullest extent possible. The NENSP laboratory and data systems are in a secure environment meeting the privacy standards for the Departments of Health of Massachusetts, Maine, Rhode Island, New Hampshire, Vermont, Pennsylvania and other contractors.

NENSP will send results to PRNSP when the results are available with the same level of urgency applied to Massachusetts NBS samples. Results will be sent to us as soon as they are ready. However, in coded (unidentified) cases found to have a potentially fatal disease or likely to result in severe incapacitating disease, the Newborn Screening Program Director (Dr. Santiago or his designee) is expected to open the code. The follow up algorithm in general consists of obtaining a subsequent NBS specimen and if that is also out of range, obtaining a special liquid blood sample for flow cytometry, which will be performed at the University of Puerto Rico, Medical Sciences Campus, Pathology Department. If the flow cytometry is also out of range, then the recommendation is to obtain another liquid sample for mitogen testing at the same time that there is an immunology consultation. We will follow the NENSP recommendations and exceptions to general rules.

NENSP has provided a confidential copy of a manuscript showing an extremely low false positive rate in well term infants and a reasonable false positive rate in NICU infants. Only PRNSP program staff will contact the parents of the presumptive positive newborn about the need to do confirmatory testing on their child due to a positive result for SCID on the infant's coded sample that was sent to NENSP. They will be invited to participate in the study that will consist of getting a blood sample from the infant to do confirmatory testing for SCID. If repeated test confirms a positive finding, the parents will be informed and counseled regarding the urgent need of treatment to the child.

They will be invited to an initial information visit. On that first visit an informed consent will be given to them to read or to take home if they want to take more time to make the decision.

Data Analysis and Reporting

Information obtained from this study may be published in the medical and public health literature. However, no personal information will be disclosed that might result in identification of the subject.

It is the Puerto Rico Newborn Screening Program's responsibility to re-contact the parents of newborns if result is found to be positive, to assure prompt treatment that could greatly reduce the risk of a potentially fatal outcome.

References:

1. Comeau AM, Hale JE, Pai S-Y, Bonilla FA, Notarangelo LD, Pasternack MS, Meissner HC, Cooper, ER, DeMaria A, Sahai I, Eaton RB. Guidelines for Implementation of Population-based newborn screening for severe combined immunodeficiency. J Inherit Metab Dis. In Press.