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Development of the Necrotizing Enterocolitis Society Registry and Biorepository

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Abstract

Necrotizing enterocolitis (NEC) is a devastating disease affecting premature infants. New advances in diagnostic and treatment options are desperately needed. Accordingly, the NEC Society initiated a research collaborative with a group of investigators dedicated to advancing the state of NEC-associated knowledge. Recent advances in high-content molecular interrogation and bio-computation (*e.g.* genomics, transcriptomics, proteomics, metabolomics) can provide new insights from afflicted infants with NEC, however, individual centers do not have sufficient cases to conduct these studies independently. The development of a NEC Society Biorepository (NSB) has emerged to advance collaboration among institutions through the shared use of biologic samples in the dedicated pursuit of molecular indicators of disease and to gain greater pathophysiologic insights through research. The NSB will provide key infrastructure across several centers to harness the potential for new discoveries, while ensuring specimens are processed consistently, appropriate clinical data is collected, and privacy is maintained. The NSB will provide a comprehensive framework for sharing biological samples and clinical data through a robust and secure system that supports the investigation of research studies on NEC.

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Keywords

Biorepository; registry; necrotizing enterocolitis; specimen; intestine

INTRODUCTION

Necrotizing enterocolitis (NEC) has been and continues to be a leading cause of morbidity and mortality in premature infants[1]. Mortality rates are contingent on multiple factors including gestational age, birthweight, and disease severity[2]. Survivors are often left with ongoing medical needs leading to a financial, emotional and social strain on their families. Despite research focused on associated clinical factors leading to NEC[3], detection methods[4, 5] and treatment regimens[6, 7], there has been minimal change in overall outcomes and new disease insights have been limited.

In order to develop a broader knowledge base on the scope of the disease and to coordinate research efforts, the NEC Society was established and became a non-profit organization in 2014. A multidisciplinary group of clinicians, researchers, and families affected by NEC held an inaugural NEC Society meeting at UC Davis on April 5–7, 2017. During the meeting, a focus group was convened to consider the merits and challenges of establishing a national biorepository and registry. The NEC Society Biorepository (NSB) will focus on collection, processing, and storage of biologic materials from infants with NEC and control infants via standard operating procedures (SOP) across all centers. These specimens will be linked to demographic data in support of ongoing and future investigation by researchers dedicated to understanding the pathogenesis of NEC. To minimize logistical complexity and expense while maximizing specimen integrity through decreased handling, the NSB group elected to adopt a federated biorepository strategy where each center maintains its onsite inventory, while utilizing multi-center institutional review board (IRB) approvals and SOPs.

There are multiple examples in which a biorepository has proven beneficial to improve disease understanding. The DHREAMS (Diaphragmatic Hernia Research & Exploration; Advancing Molecular Science) study collects tissue and demographic data from infants born with Congenital Diaphragmatic Hernia (CDH) in an effort to improve the understanding of the molecular and genetic basis of CDH. These data have resulted in multiple publications, including several that have resulted in practice changes in the care of sick neonates[8–11]. The Children's Oncology Group (COG) is an additional example of an organization that maintains a robust biorepository that has produced great translational utility as documented through numerous publications[12]. Similarly, the NEC Society consists of a unique group of individuals with a shared vision to advance the state of the science to combat NEC. An accessible national biorepository and research collaborative focused on NEC investigation is both appropriate and needed to address the unmet needs identified by the NEC Society and its constituents.

The mission of the NSB is to aid scientists and clinicians in their quest to study and eradicate NEC. The objective of this article is to describe the aims and the development of the NSB. We will first discuss the standard operating procedures for obtaining specimens,

we will then highlight the infrastructure required for the biorepository, database and sharing capabilities, followed by potential funding opportunities for this endeavor.

DEVELOPMENT OF A BIOREPOSITORY FOR NEC

Specimen Procurement

We aim to provide a NEC specimen biorepository to promote, facilitate and accelerate basic and clinical/translational studies of NEC in human infants. To accomplish this objective, the NSB provides an opportunity to expand the number of available samples with their corresponding clinical data in order to facilitate more impactful studies on NEC in humans that would be difficult at any one institution (Table 1). Several US and international centers are currently collecting biological samples from infants with NEC. This is done without standardization of sample procurement protocols between centers and with little avenue for sharing data. The NSB will be a multi-center federated or “virtual” biorepository where each participating center procures their samples, maintains their own inventory and updates a shared database as detailed below. The biorepository leadership will provide guidelines to ensure the quality, as well as manage the accessibility and distribution of the samples for studies. This leadership will consist of an executive committee and a scientific advisory board made up of experts in the field to evaluate requests. A formal application process for specimen allocation will be available to researchers via a website (https://necsociety.org/nec-society-research-collaborative) with requests reviewed by the scientific advisory board and resources shared in an equitable fashion. The NEC Society members considered the most desired biologic material balancing scientific yield with feasibility of obtainment and concluded that bowel (large and small intestine) and stool were the highest priority. Additional tissues with high value to the team of investigators included blood, serum, and urine. As the NSB is established and capacity realized, additional specimens of high interest may include gastric and tracheal aspirates, saliva, and maternal breast milk. Specimens considered to have great potential, but receiving lower priority scores due to perceived difficulties in procurement include collections from family members (*e.g.* blood for vertical genotype-phenotype determinations).

In addition to the specimens that are obtained from infants afflicted with NEC, it is of critical importance to obtain biological samples from appropriate control infants who do not have NEC for comparison. Inclusion of specimens from a large number of control infants will allow matching by weight, gestational and postconceptional age to affected NEC counterparts. Each NEC Society Biorepository site primary investigator (PI) will determine the team of individuals responsible for collecting and maintaining the biorepository at her/his center.

Standard Operating Procedures (SOP)

To ensure comprehensive collections that are triggered by clinically meaningful events and to move beyond the short-comings of convenience sampling, NSB leadership is advocating that centers establish 24 hour capabilities for study enrollment and sample acquisition. The NSB Working Group agreed that standard operating procedures for biospecimen handling must be made to optimize handling of the biospecimens in order to ensure specimen

integrity and to minimize molecular changes in an *ex vivo* environment. To ensure optimal quality of the intestinal specimen in particular, useful approaches currently in use by NEC Society investigators include minimizing cold ischemic time and aliquoting samples for alternative purposes at the time of collection. This includes a piece of specimen placed in fixative, an RNA stabilization reagent and snap frozen pieces for microbiota 16S analysis plus a banked back up sample for any ongoing collaborations or *ex vivo* studies. All samples can be stored at −80 degrees Celsius after processing[13] (with the exception of the histologic sample), as it is critical to avoid freeze/thaw cycles on the samples until the assays are being performed. In addition, all other biological fluids can be aliquoted with a unique identifier and stored at −80 degrees Celsius. Consideration will be given to the storage location of the human specimens to allow for efficient retrieval by specimen type, and a barcoding and sample tracking system is essential. Importantly, the NSB Working Group agreed that all biospecimens will be stored in secure freezers with alarms and procedures in place for loss of electrical power, with access limited to authorized study personnel. NSB SOPs will be agreed upon and quality improvement initiatives will be undertaken to ensure specimen quality across all contributing centers including RNA integrity analysis and histological review.

Infrastructure

The NSB infrastructure will consist of an executive committee with NSB leadership and family advocates, a scientific advisory board with expertise in NEC as well as general membership, which consists of centers involved in contributing biological samples. One of the important factors in the biorepository framework is that all specimens and data must be handled uniformly under a rigorous quality management system[14]. The NSB Working Group decided that this would start with IRB approvals that were similar at each institution (Central IRBs) and a standardized informed consent would be provided to each participating center. NEC diagnostic criteria must be strictly adhered to as in other studies[15] and will be adopted utilizing consensus definitions resulting from ongoing work from collaborative groups such as the Critical Path Institute's International Neonatal Consortium[16], which seeks to advance regulatory science for neonates. Strict adherence to the NEC disease definitions will be critical to ensure that cases of non-NEC diagnoses including spontaneous intestinal perforations or other ischemic gut processes are excluded. While documentation is crucial, the NSB will rely on a designated research coordinator and/or site PI of the NSB Working Group who will oversee all work related to the local repository. In addition to the site PI, ideally, a surgeon and pathologist invested in the program will aid in sample collection.

Consenting

Approaches to NSB consenting requires further consideration as the timing of specimen processing is crucial and any increase in time from removal to processing, or cold ischemic time, can have significant impact on end target analysis[17, 18]. A team of individuals trained on the standardized, multi-center IRB informed consent, procuring, storing, capturing the clinical data of the specimens, as well as specimen shipping to a collaborating center in accordance with the NSB SOP helps maximize the efficiency of data collection, tissue storage, and can inhibit deterioration of samples during transport. Furthermore, infants with

NEC can often deteriorate quickly and require surgical intervention prior to arrival of their family. Thus, it is important for the IRB protocol to allow for critical sample procurement such as the bloody stool at the time of NEC diagnosis as well as the resected intestinal specimen and then obtain consent after parents arrive within a specified amount of time. If the parents elect not to consent to the study, then the specimens are disposed of. This essential component to the consent process can also be obtained by maintaining a separate IRB protocol whereby intestinal samples are able to be collected in a de-identified fashion without consent, therefore maximizing the ability to obtain samples when parents are unavailable. It is important to note, that with this type of IRB, no additional clinical information would be able to be obtained/recorded, however it does provide a safety net of sample procurement. Since NEC is unpredictable, another alternative approach to consenting might be to obtain pre-emptive consent in high risk premature infants which would allow for immediate collection of specimens at the time of NEC development.

Database capabilities/maintenance

Success of a biorepository hinges on its capabilities to maintain and store a sustainable database that can be easily searched and retrieved in an efficient manner[19]. The cohesive infrastructure required to achieve a successful biorepository is based on material collected, maintenance, and distribution of resources for a variety of research questions. Database maintenance of the NSB will allow for reliability in the studies done with the biomaterials. The sharing and distribution of resources used to develop the NSB creates a new standard, as individual centers serve as a part of the whole nationwide repository. By creating this distribution of resources across various centers, the individual burden and costs of shipment and maintenance to any one center decreases, while linkage of clinical data is optimized and accessible. When an individual research study calls for biologic material, the scientific review board can review the impact and feasibility of performing the study without compromising the precious sample resource. Then, the individual repositories will send the stored materials to the requesting center, ideally using funding that has been obtained by the NSB and shared across the centers. The clinical data will be housed in a REDCap (Research Electronic Data Capture) database, de-identified to all centers except the procuring center, and linked to the material sent to the requesting center.

Material/Data Sharing

Investigators from any institution including those that are at participating centers will have the ability to request collaboration with the NSB. A standard application will be available online and will include information from the principal investigator, including a detailed outline of the study, institutional IRB approval, funding source and specimen type requested. Reviewers from the NSB scientific advisory board will screen applications and after pre-screening for appropriateness, the application will come under final review by the NSB executive committee. Researchers at noncontributing centers to the NSB will require sponsorship/partnership from an investigator at a contributing center.

Funding opportunities

NSB funding opportunities discussed can be categorized into those for housing a repository and those for potential discovery: genetic susceptibility, biomarkers, preventative strategies,

and potential treatment opportunities. In terms of housing a repository, the largest potential funder exists in the Biorepositories and Biospecimen Research Branch of the National Cancer Institute. Additionally, the National Institute of Child Health and Human Development supports the Neonatal Research Network and the Cochrane Neonatal Review Group. Private groups who may be interested in funding the development of a biorepository include biospecimen providers and pharmaceutical companies[20]. Funding opportunities involving potential discovery can be obtained by individual centers for a specific study rather than provide support for a repository, which may yield low return. Centers that have agreed to participate in the NSB have existing infrastructure for human sample studies and therefore, in order to get the NSB launched, the initial costs will be handled by the participating centers. After the NSB has been established and collaborations demonstrated, the NSB will seek external funding for a targeted set of questions involving genomic, transcriptomic, proteomic, and metabolomic approaches.

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Summary

In summary, a group of dedicated NEC Society investigators are committed to the development of a national repository of biological samples from infants afflicted with NEC. This biorepository aims to improve human specimen studies by individual NEC investigators and foster collaborations across multiple centers. A particular focus of the NEC Society group with this biorepository will be biomarker development utilizing a multifaceted approach. It is the hope of all involved in the NEC Society Biorepository that we can improve, facilitate, and accelerate basic and clinical/translational studies of NEC.

Table 1

Limitations of Existing Specimen Collections and the Ideal NEC Society Biorepository

Limitations	Ideal NEC Society Biorepository
Variation in sample collection, processing, storage techniques, and difficulty obtaining adequate samples for large-scale studies	Multi-center biorepository of NEC, healed NEC and premature intestine resected for other indications employing standardized operating procedures for storage, distribution, and collection of associated clinical data
Incomplete data collection due to limitations of resources and research coordinators	Established infrastructure across several centers with online access to available specimens and their de-identified clinical data
Restricted access to researchers outside institution where specimens are collected	Access to a large number of specimens across multiple centers
Reluctance to share precious and limited samples	Collecting as much data as necessary from specimens so as to not duplicate efforts and balance utility and futility
Variable consenting practices that may be insufficient for genomics research	Standardized consent for all specimens which includes capability for genomic studies

The optimized NEC Society Biorepository will be a multi-center specimen collection effort with particular standard operating procedures for each type of specimen from the time of collection through aliquoting and processing to storage. Clinical metadata will be captured at the same time and recorded in a database that is de-identified to investigators outside the procuring institution.