Necrotizing Enterocolitis: Using Regulatory Science and Drug Development to Improve Outcomes

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Necrotizing enterocolitis (NEC) is the most common intestinal pathology and cause of death between 2 and 8 weeks of life in neonates born extremely preterm. NEC has an unpredictable and often sudden onset with a rapidly progressive clinical course.[1] The diagnosis is currently made through a constellation of clinical observations and radiographic findings (eg, abdominal distention, bloody stool, pneumatosis intestinalis). NEC is likely triggered by a variety of insults resulting in a final pathway of intestinal dysfunction, inflammation, injury, and necrosis. Although clinical associations (eg, enteral feeding, blood transfusion), predisposing risk factors (eg, prematurity, altered intestinal microbiome, growth restriction), and specific molecular pathway involvement (eg, Toll-like receptor-4 signaling) are well established, the interactions between each of these factors and exposures are not fully understood.[2,3] There are currently no licensed drugs or biologics for the prevention and/or treatment of NEC.

The Critical Path Institute is an independent, nonprofit organization committed to transformational improvement of the drug development process. The Food and Drug Administration working with the Critical Path Institute established the International Neonatal Consortium (INC) in 2015 to advance regulatory science for neonates. A working group for NEC was established to identify challenges associated with the development and licensing of products for the prevention and/or treatment of NEC. In this review, the INC NEC working group addresses key issues that relate to the diagnosis, prevention, and treatment of NEC while suggesting a path forward to evaluate the safety and efficacy of each product. Despite years of clinical investigation, additional key data elements are needed to meet the requirements of regulatory agencies and evidence-based medicine.[4] These include reliable diagnostic criteria, biomarkers predictive of risk and prognosis, and criteria for the design and conduct of clinical trials with consistent and clinically meaningful outcome measures for therapeutic trials.

INC	International Neonatal Consortium
NEC	Necrotizing enterocolitis
RCT	Randomized controlled trial
SIP	Spontaneous or focal intestinal perforation

Diagnosis

Consistent diagnostic criteria are essential to perform epidemiologic studies, to provide a gold standard for biomarker development, and to create appropriate outcome measures for clinical trials. NEC is likely not a single clinical entity. In addition, significant variation in presentation and clinical course has added to the difficulty in arriving at universally accepted diagnostic criteria. The initial system proposed by Bell[5] and modified by Kliegman[6] consisted of 3 stages of NEC (I-suspected; II-definite; III-advanced). Clinical trials and cohort studies have varied in the inclusion of suspected or stage I NEC, increasing the difficulty of estimating disease burden and interpreting meta-analytical syntheses of clinical data.

More recently, 2 important distinctions have prompted the adoption of the diagnostic term "preterm NEC." Spontaneous or focal intestinal perforation (SIP) and NEC are common but distinct clinical entities.[7] SIP generally presents within 10 days of birth, with little or no evidence of bowel wall necrosis. Preterm NEC is more likely to occur 2-8 weeks after birth resulting in focal or widespread intestinal necrosis. Preterm NEC represents a poorly regulated host response to altered microbial colonization/invasion and/or alterations in perfusion. The immunologic and hematologic changes in preterm NEC have been extensively reviewed and are essential to the diagnosis and exclusion of other conditions such as feeding intolerance. Septic ileus is common in preterm neonates and is often associated with inflammation, thrombocytopenia, or coagulopathy. Because there is overlap between SIP, septic ileus, and preterm NEC, consistent criteria are crucial to ensuring that neonates most likely to have preterm NEC are included in clinical research, and those with other

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conditions are excluded.[8] Furthermore, if SIP cases are included as a diagnosis of NEC (as can occur using the Bell criteria) in clinical trials evaluating the efficacy of interventions, then results may lead to skewed or incorrect conclusions,[8] and this may have occurred in several of the probiotic prevention trials. Finally, some patients with abdominal signs and bloody stools are noted to have cow's milk protein allergy. A more aggressive form of food protein-induced enterocolitis syndrome can be confused with NEC.

Term/late preterm NEC (term NEC) is almost exclusively seen in infants with gastroschisis, a hypoxic-ischemic insult, or cyanotic congenital heart disease.

The initial meeting of the INC NEC Workgroup held in conjunction with the NEC Society in April 2017 found consensus that the lack of a robust, universally accepted case-definition for NEC is a significant barrier to progress in investigating pathogenesis and improving efforts to prevent and treat this devastating disease. There was also general consensus that a more rigid case-definition for preterm NEC would improve clinical research but may have less value in clinical decision making, given that the only gold standard in the diagnosis of NEC is the pathologic analysis of resected or autopsied intestinal tissue.

Recent proposals to improve the definition of preterm NEC have included distinguishing between NEC and SIP in all clinical studies and excluding cases of SIP from analyses of studies aimed at either the prevention or treatment of NEC; excluding infants with a postmenstrual age of >36 weeks as this may be a fundamentally different process from preterm NEC; a "2 out of 3 rule" that requires at least 2 of the following diagnostic criteria for preterm NEC: pneumatosis intestinalis or portal venous gas, thrombocytopenia, and/or appropriate timing of onset[8]; a sophisticated point system based on gestational age and clinical and radiologic findings[9]; avoiding use of a fixed loop of bowel, gasless abdomen, and increased or bilious gastric aspirates in diagnostic criteria, as they have a low positive predictive value for NEC.[9]

Developing a Meaningful Definition

With these insights in mind, we propose a diagnostic approach summarized in the **Figure** with an emphasis on timing of onset (x axis) and clinical/radiographic evidence (y axis). Preterm NEC occurs most commonly between 30 and 32 weeks postmenstrual age, regardless of gestational age at birth. For clinical research purposes, a diagnosis of "preterm NEC" may not be appropriate in these situations: preterm neonates (<29 weeks of gestation) with intestinal perforation in the first 10 days after birth that occurs without evidence of pneumatosis intestinalis, portal venous air, or necrotized intestinal tissue at surgery or autopsy; term and late preterm (>36 weeks of gestation) neonates; neonates with isolated feeding intolerance; neonates with congenital cyanotic heart disease; and neonates with gastroschisis.

Neonates with preterm NEC would be further characterized as having either medical or surgical NEC based on the confirmation of necrosis at the time of laparotomy. Neonates with NEC that do not meet the criteria for preterm NEC should be classified as either "atypical NEC" or "term NEC" for reporting in clinical research. Applying this narrower case definition of preterm NEC (as described in the **Figure**) may enrich the potential population of preterm neonates for enrollment in clinical trials, minimize larger sample sizes needed for cohort studies, and generate more robust data. This new case definition of NEC should be evaluated against the Bell classification system prospectively and data evaluated to confirm that the approach will improve study analyses and interpretation.

Practical Recommendations

Given that definitions of NEC will be used across multiple treatment development programs, databases, clinical trials, cohort studies, case-control studies, and case series should capture the individual components of proposed case-definitions, and the results should be published. This would allow consistency in case selection and meta-analyses of any studies. The most efficient way to do this is to define a "data standard" for NEC that comprises the individual components of proposed definitions (**Table I**). These criteria will further improve the impact of clinical research in NEC: operationalizing diagnoses as case definitions, accepting that these are as still imperfect; clearly distinguishing case definitions for research purposes from criteria that are used to guide patient care; engaging editors and reviewers to include clear case definitions in all NEC-related

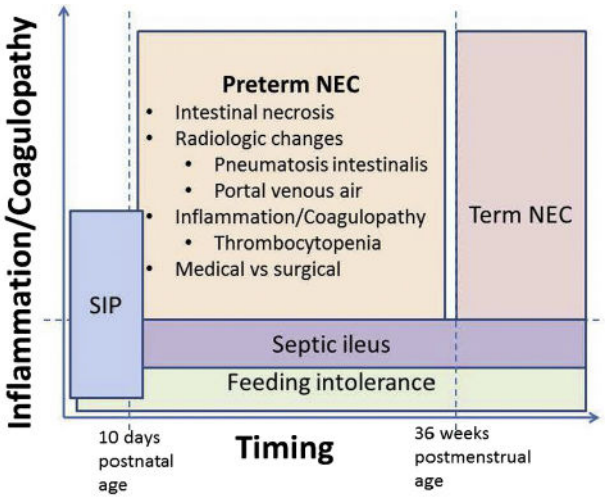

Figure. Proposed diagnosis of preterm NEC requires: (1) signs (abdominal distention and/or hematochezia); (2) timing (between postnatal day 10 and 36 weeks corrected gestational age; most common between 30-32 weeks postmenstrual age); (3) at least 1 of the following: (a) intestinal necrosis at laparotomy or autopsy; (b) either pneumatosis intestinalis or portal venous air (by radiograph or ultrasound); or (c) evidence of vasculitis, coagulopathy, or inflammation in the absence of bacterial, fungal, or viral infection.

Table I. NEC data standards

Inclusion criterion	
Risk factors	Gestational age at birth
	Small for gestational age at birth
	Postmenstrual age at onset
	Feeding type
Clinical signs	Abdominal distention
	Hematochezia
	Abdominal discoloration
Laboratory/surgical evidence	Thrombocytopenia
	Coagulopathy
	Metabolic acidosis
	Tissue necrosis
Radiologic signs (radiograph or ultrasound)	Pneumatosis intestinalis (definite vs possible)
	Portal venous gas
	Pneumoperitoneum
Exclusion criteria	
Diagnoses	Spontaneous intestinal perforation
	Cyanotic congenital heart disease
	Gastroschisis
	Hypoxic ischemic encephalopathy

Modifiable factors that contribute to current case definitions of NEC. Reporting of these key items in future clinical trials, cohort studies, and case series is essential to developing consistent diagnostic criteria for NEC and allowing for severity ratings to be developed.

Table II. Health outcomes associated with NEC

Gastrointestinal	Cholestasis
	Parenteral nutrition at discharge from the neonatal intensive care unit
	Marker of growth (eg, delta z score from birth to neonatal intensive care unit discharge for weight, length, and head circumference)
	Days to full feedings
	Intestinal stricture/obstruction
Pulmonary	Bronchopulmonary dysplasia
	Pulmonary hypertension
	Pulmonary vein stenosis
Central nervous system	Periventricular leukomalacia
	Neurodevelopmental delays

Reporting of these outcomes is valuable in assessing correlations between a given case definition and morbidity.

publications, which may include abandoning the Bell criteria completely or excluding cases with Bell stage 1 (at best inclusion of appropriate data standards; Table I); and including long-term outcomes that are known to be associated with NEC to enhance our understanding of the relationship between case definitions and health outcomes (Table II).

Future developments include promising diagnostic modalities such as abdominal ultrasound and fecal calprotectin. In expert hands, ultrasound may be more sensitive than abdominal radiograph evidence of pneumatosis intestinalis to diagnose NEC, but this approach has not become universal or standard-of-care and should be further investigated.

Biomarkers

Significantly reducing the clinical burden of NEC requires innovative approaches. These include identifying novel biomarkers through comprehensive study of human biologic samples (fluids, tissues), using high content multi-omics technologies (eg, proteomics, metabolomics, genomics), and integrating (clinical and biologic) computational analyses.[10] The *BEST Resource* defines a biomarker as: "A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or response to an exposure or intervention. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers (https://www.ncbi.nlm.nih.gov/books/NBK338448/)." The need to establish a "gold standard" case-definition for NEC is matched by the need for biomarkers, a priori risk assessment (probability of disease), and prediction of outcome. Currently, the means for the early detection of intestinal injury leading to NEC and its progression do not exist.

Evaluating Biomarkers

Biomarker development requires a discovery phase including identification of the specific molecular mechanisms and pathways involved in preterm NEC and an evaluation phase to determine predictive value. These phases should be completed before any biomarkers are used in clinical trials to evaluate any potential novel therapies. To fully understand the utility of a biomarker, a clear understanding of disease prevalence (prior probability) is needed in the test population. This depends on a shared, reliable definition of the disease process. The predictive value (diagnostic accuracy) of a test is highly dependent on disease prevalence, which for a rare disease like NEC (eg, 2016 Vermont Oxford Network data IQR 3.4%-9.4%) is likely to be confounded by a high false positive rate needed to achieve high sensitivity. Biomarkers used in studies of prevention strategies will require a different approach than biomarkers used to identify effectiveness of various treatment approaches. Multivariate models with standard measures along with Bayesian models that provide conditional probabilities may together provide key differential insights.

Food and Drug Administration guidance regarding the development of biomarkers used in multiple drug development programs is currently being revised (https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM230597). European Medicines Agency guidance includes the following key concepts (http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004201.pdf)[11]: context of use: "Full, clear and concise description of the way a novel methodology is to be used and the medicine development related purpose of the use. The Context of Use is the critical reference point for the regulatory assessment of any qualification application."; endpoints, including sensitivity, specificity, predictive values, and likelihood ratios as well as relationship to the clinical state of the neonate; clinical utility, including impact on patient management and outcome; standard of truth, as defined or a surrogate identified; analytic platform, including full validation of any analytical method

Table III. Assessment of diagnostic biomarkers NEC

Biomarkers	Sample source	Sensitivity	Specificity	Strengths (S) and limitations (L)	References Authors, Year
Abdominal ultrasound	Ultrasound	Low	Low to high	L: High specificity may be limited to more advanced disease L: Low sensitivity	Janssen Lok et al[12], 2018
Calprotectin	Stool	Low-high	Low-medium	S: Levels are usually elevated in patients with NEC and correlate with disease severity. L: Can be elevated in preterm infants for reasons besides NEC, limiting its specificity. L: Sensitivity not consistently high across studies to warrant use as a biomarker to accurately "rule-out" NEC.	MacQueen et al[13], 2016 Pergialiotis et al[14], 2016
CBC	Serum			L: Elements of CBC are not sensitive or specific for NEC.	Gordon et al[15], 2016
Claudins	Urine	Low	Low	L: Associated with NEC, but sensitivity and specificity are low.	Thuijls et al[16], 2010
CRP	Serum	Low-high	Low	S: Unlikely normal in the setting of NEC, though sensitivities among studies varies. L: Relatively slow rise, rendering it impractical for early diagnosis. L: Low specificity.	Cetinkaya et al[17], 2011 Pourcyrous et al[18], 2005 Yakut et al[32], 2014
Cytokines	Serum			S: IL-6, IL-8, and IL-10 are associated with NEC, and IL-8 appears to have high sensitivity and specificity. L: IL-8 has only been studied to a limited extent.	Benke et al[19], 2013 Benke et al[20], 2014
C5a	Serum			L: Strongly associated with NEC though has only been studied to a limited extent.	Tayman et al[21], 2011
EGF	Serum			L: Limited investigation. No reported validity testing.	Nair et al[22], 2008 Shin et al[23], 2000
Genomics	Serum			S: Variants found to be associated with NEC L: No studies assess accuracy of variants in NEC diagnosis or prognostication. L: Expensive	Chan et al[24], 2014 Hartel et al[25], 2016 Sampath et al[26], 2017
Hydrogen extraction	Exhaled breath	Medium	Medium	S: Noninvasive collection and moderate sensitivity and specificity. L: Limited investigation.	Cheu et al[27], 1989
I-FABP	Serum or urine	Low	Medium	S: Moderate specificity (91%) for serum I-FABP in meta-analysis of 7 studies L: Low sensitivity and specificity for urinary I-FABP	Yang et al[28], 2016
IAIP	Serum	High	Medium	S: High sensitivity and NPV. May be very useful for ruling-out NEC in suspected cases. L: Limited investigation. L: Unclear whether Ialp can distinguish NEC from sepsis.	Al-Hamad et al[29], 2017
Metabolomics	Serum or urine			S: Population based assessment of metabolic screen utility for identifying high risk of NEC (Sylvester) S: Metabolites linked to possible NEC linked dysbiosis (Morrow) L: poor PPV	Morrow et al[30], 2013 Sylvester et al[31], 2017
IMA	Serum	Medium	Low	S: High sensitivity than CRP in one study. L: Limited investigation.	Yakut et al[32], 2014
Microbiota analysis	Stool	Low-high	Low-high	S: Very high sensitivity and specificity with different models reported in one study. L: Inconsistency in microbiome profiles across studies. L: Limited sample size for models with high sensitivity and specificity.	Morrow et al[30], 2013 Pammi et al[33], 2017
NIRS	Skin lead			S: Possible use for monitoring course after initial NEC diagnosis to detect complications	Shah et al[34], 2017
PAF	Serum and Stool			S: Associated with NEC. L: Low specificity. L: Limited investigation.	Amer et al[35], 2004 Rabinowitz et al[36], 2001
Procalcitonin	Serum	Low-medium	High	S: High specificity if limited to patients with NEC and sepsis. L: Unable to identify patients with NEC without sepsis.	Cetinkaya et al[17], 2011 Turner et al[37], 2007

CRP, c-reactive protein; *cbc*, complete blood count; *IFABP*, intestinal fatty acid binding protein; *IAIP*, inter-alpha inhibitor protein; *IMA*, inferior mesenteric artery; *NIRS*, near infrared spectroscopy; *PPV*, positive predictive value; *IL*, interleukin; *PAF*, platelet activating factor.

(selectivity, carry- over, lower limit of quantification, calibration curve, accuracy, precision, dilution integrity, matrix effect, stability).

To avoid inconclusive clinical studies and waste of resources, an operational case definition of NEC must be prespecified and the candidate biomarker must then be tested for predictive value. Only then can a validated biomarker be used as either a primary (surrogate) or key prespecified secondary outcome in clinical trials.

Biomarkers in NEC

Molecular and imaging biomarkers have been extensively studied to identify early signs of NEC or to predict prognosis, and these are summarized in Table III. Comparator groups (case and control definitions), study size, and location (single or multicenter) can vary widely. Statistical approaches and study design also vary between studies. These limitations, along with a failure to prospectively validate candidate biomarkers in multicenter studies,

render most biomarkers published to date of limited utility for drug development. In addition, utility of biomarkers is limited if costs are excessive or use in clinical practice is difficult.

Of the candidate biomarkers reviewed in Table III, it may be that no single marker possesses sufficient test performance characteristics (eg, specificity, sensitivity, positive predictive value) to anticipate a clear picture of clinical validity and utility will be gained with further study. However, it may be possible to combine disparate features that capture different aspects of either the gold standard definition being championed herein or reflect the current understanding of NEC pathophysiology. Accordingly, a composite approach of abdominal ultrasound for the presence of pneumatosis (high specificity), with a highly sensitive biomarker to capture enteric inflammation (stool calprotectin) or mucosal injury (intestinal fatty acid binding protein [IFABP]) may be sufficiently complementary. Given the well-documented emergence of proteobacteria enteric blooms preceding NEC in human patients, further study of the pathophysiologic mechanism by which widespread dysbiosis in neonatal intensive care unit newborns produces NEC is likely justified to gain greater biologic insight and discover clinically useful biomarkers.

Practical Recommendations

(1) Develop appropriate biomarkers before performing confirmatory trials.
(2) Limit validation studies to definitive, established cases of preterm NEC.
(3) Conduct and report research that gives comprehensive information about each biomarker by defining its context of use, validating it across multiple centers, and developing predictive values or likelihood ratios with sensitivity and specificity. Studies should determine the effects of employing the biomarker in clinical practice.
(4) Refine and enhance a priori risk assessment through subcohorting.
(5) Identify unique biology, develop novel insight to causality, and capture both with well- characterized biomarkers.

INC Consortium activities could develop shared ways of working or undertake the development of biomarkers to be used in multiple drug development programs.

Clinical Trial Design: Probiotics

This section uses the specific example of probiotics to illustrate how existing clinical trials of NEC have (or have not) used state-of-the-art trial design and informed regulatory requirements. The large number of randomized controlled trials (RCTs) and cohort studies of probiotic administration is commendable and has moved the field forward. However, many sites no longer maintain equipoise to conduct unbiased studies, reinforcing the need to blind treatment assignment because there is already extensive use of many of these biologic agents worldwide. This also provides an opportunity to consider the quality of studies performed to date and how future studies of NEC prevention can improve upon currently available data and adapt consistency and excellence of methodology into practice. Many of the concerns outlined below also apply to published clinical research of dietary interventions (eg human milk fortifier, lactoferrin, and donor milk) and other treatments (antibiotic prophylaxis, preventive steroids, immunoglobulin A, and several pharmaceutical products in development) to prevent NEC. Systematic reviews to identify strengths and weaknesses of the academic literature through evaluation of selected elements of clinical trials are useful exercises. To evaluate studies on probiotics as an example, RCTs were identified by performing a PubMed search for recent publications and drawing on several recent systematic reviews.[38,39] All RCTs that evaluated probiotics in neonates and reported on NEC were included. Of 117 records screened, a total of 46 RCTs enrolling 12 185 infants were identified for inclusion in an analysis of key components of clinical research (**Supplemental Table and Supplemental References**; available at www.jpeds.com). A clinical development program must involve multiple clinical components discussed below.

Pharmaceutical Quality Trial reports did not consistently provide information on the probiotics used, often reporting only genus and species (but not specific strains or explicit product data). Concerns about probiotic quality control were highlighted by a recent study that found the content of most commercially available probiotic products do not match the label.[40] The Quality Control of probiotic products is inconsistent between countries and health systems. Specific regulation by the European Food Standards Agency promotes quality in Europe but does not meet the needs of pharmaceutical regulators. Data on strain-specific actions remain very sparse and few studies report validation of purity and number of viable organisms administered.[41] Several trials included potential confounders such as lactoferrin or a prebiotic administered in conjunction with probiotic supplementation.[42] High-quality studies involving pharmaceutical grade products with large sample sizes are lacking and in urgent need of completion.

Dosage Regimen

None of the studies offered a justification for the dose used. Common dosing regimens ranged from 10^8 to 10^9 colony forming units per day (a fairly small range for replicating organisms), with most studies initiating probiotic supplementation in the first week of postnatal life and continuing for at least one month. This represents a significant gap in knowledge that must be addressed.

Study Population

Baseline NEC rates varied considerably, ranging from 0% to 18%. This may reflect differences between centers and countries in the incidence and definition of NEC, differences in

standard care and treatments, and extent of exposure to human milk. The reporting of a specific case definition, data standard (Table I), and concomitant treatments in all clinical trials, cohort studies, and case series of NEC is essential.

Outcome Measures (Surrogate and Clinically Meaningful)

Most NEC trials evaluate NEC and/or death as a primary outcome, though many do not include a comprehensive assessment of additional related outcomes associated with the disease that significantly impact the health of the patient. Table II includes several measurable outcomes that influence potential effectiveness of NEC prevention or treatment trials. In our example of probiotics, no RCT reported a pre-planned assessment of longer-term clinical outcomes. RCTs did not identify whether they were contributing to a surrogate, functional, or other clinically relevant outcome (or a combination). This occurred despite the essential need to document longer-term safety and efficacy of any medicinal products in this population. We hypothesize that the inclusion of additional outcome measures to future clinical trials will provide additional support for intervention effectiveness and allow clinicians, regulators, and pharmaceutical companies to reach appropriate conclusions from the study results. In our meta- analysis, it is important to note that varying definitions of NEC were used reflecting a lack of consistency among studies, which makes the data more difficult to analyze and interpret (see 'Diagnosis').

Safety Reporting

Concerns about safety include the development of microbial resistance, risk of probiotic-associated sepsis, administration of a contaminated product, the presence of any additives not included on the package label, and cross-contamination between supplemented and unsupplemented neonates.[43] None of the trials included assessment of the effects of early probiotic administration on the development and stability of the intestinal microbiome and any related functional consequences. Despite these concerns, the use of *Lactobacillus rhamnosus* GG was associated with septicaemia only once during treatment of many preterm neonates over prolonged periods of time.[44,45] No RCT reported whether individual adverse events were analyzed with respect to causality or severity, or the results of these assessments.

Data Quality

Several of the RCTs lacked description of trial registration. In addition, there was significant uncertainty regarding blinding, concealment of treatment allocation, randomization procedures, duration of follow-up, selective reporting, and exclusion of neonates after randomization. RCTs also generally lacked statistical power with only 2 recruiting more than 1000 infants.[46,47] The publications provided no information about measures taken to assure the quality of the data collected including extent of monitoring of data quality. In summary, although several studies demonstrated good quality and design, there were limitations with others, and potential conclusions from the meta-analyses should reflect this balance. Furthermore, it is possible that the discrepancy in the results from a large, randomized trial (Costeloe et al, no difference) compared with several meta-analyses reflects considerations in trial design as noted above.

Additional Challenges of Probiotic Trials

A meta-analysis of several underpowered clinical trials is not a substitute for data derived from well-designed, randomized trials of sufficient power that will be the standard required for licensing products that target NEC. A statistically significant effect found from a meta-analysis does not address many questions central to patient safety such as dosing, selection of the strain, and balance between efficacy and safety outcomes acting on diverse timescales. The "pragmatic" approach of replicating whatever dosage was used in a statistically significant clinical trial is highly problematic if the dosage regimen has no scientific foundation or if statistical significance is found in multiple studies that each use different doses and/or species of probiotic.

The challenges of determining appropriate sample size in dose-finding studies with preterm NEC as the primary outcome must be acknowledged. To compare 2 doses of a single probiotic strain with NEC as the primary outcome would require hundreds or thousands of infants depending on the baseline incidence of the disease. The only phase 1 dose escalation probiotic study in preterm neonates reported to date used the published range of probiotic doses then available (5 doses from 5×10^7 to 4×10^9 organisms) for 2 strains of validated purity and viability, with the composition of the fecal microbiota as the primary outcome.[48] Such an approach is feasible, but may not represent a reasonable surrogate outcome, as fecal microbiota may not correlate well with the specific outcome of NEC.

Finally, it should be noted that there is disagreement globally as to whether probiotics should be used to prevent NEC in preterm babies, and routine usage is standard of care practice in several countries. Lack of equipoise on probiotic effectiveness may preclude future international trials and influence subsequent clinical trial enrollment.

Practical Recommendations

If clinical research is to contribute to the development of therapies to prevent or treat NEC, a number of trial design issues must be addressed including the following: Core datasets comprising individual discrete data elements are required for case-definitions (Table I), outcomes (Table II), and concomitant treatments; reliable whole-population incidence figures, adjusted for major confounding variables such as gestational age and exposure to human milk; long-term cohort studies to better elucidate the natural history of the disease and validate candidate biomarkers of disease risk, progression, and outcome; formulations of appropriate pharmaceutical grade products with quality control measures; dose-finding studies;

consideration of innovative methodologies for randomized and nonrandomized clinical studies (eg, Bayesian methods, adaptive designs, quasi-randomized observational studies, comparative effectiveness opt-out trials); within and between country collaborations; and strong parent-public support for research targeted upon NEC.

Conclusions

There is an urgent need to develop effective and safe approaches to prevent preterm NEC. Progress with the prevention and treatment of NEC requires consistent criteria for the diagnosis of the condition. This is also an essential prerequisite for the development of biomarkers and a gold-standard case-definition. The existing clinical research using probiotics for the prevention of NEC exemplifies the challenges inherent in designing adequately powered and high quality clinical trials of treatments for prevention of this disease, and clear, comprehensive outcome measures are not always described. An internationally agreed upon consensus case-definition and validated biomarkers for NEC would be invaluable in facilitating regulatory ready treatment development programs. Agreement among key stakeholders that include clinicians, investigators, parents, regulators, and industry representatives will further facilitate the development of this urgently needed therapy to significantly improve outcome of preterm neonates. ■

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