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PENTOXIFYLLINE AS AN ADJUNCT TO ANTIMICROBIAL THERAPY
TO REDUCE LENGTH OF STAY IN INFANTS WITH SEPSIS

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of
Master of Medical Science

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Abstract

Context: Neonatal sepsis is associated with prolonged hospital stay and increased morbidity and mortality despite appropriate antimicrobial therapy. There are discordant results in the existing literature regarding the association between adjunctive pentoxifylline therapy and duration of stay or mortality.

Hypothesis: We hypothesize that among preterm infants with blood culture-confirmed sepsis who are treated with pentoxifylline plus appropriate antimicrobial therapy, as compared to infants treated with placebo plus appropriate antimicrobial therapy, median duration of stay in the neonatal intensive care unit will be significantly reduced.

Methods: We propose a multicenter, double-blind, placebo-controlled, parallel-group randomized controlled trial to investigate the efficacy of adjunctive pentoxifylline therapy to reduce duration of stay in the neonatal intensive care unit.

Significance: Results from this study may identify an agent that can be added to antimicrobial therapy to reduce duration of stay and attenuate the associated morbidity, mortality, and socioeconomic and emotional burdens of neonatal sepsis.

Chapter 1: Introduction

Background

Scope of the Problem

Approximately 4 million infants worldwide die within the first 4 weeks of life each year, with infections (35%), preterm birth (28%), and asphyxia (23%) as the leading causes of death.¹ Bacterial sepsis of the newborn was the seventh leading cause of death in the United States in 2011, responsible for 526 of 23 907 reported deaths (2.2%).² The incidence of neonatal sepsis ranges from 1 to 4 cases per 1000 live births to 6.5 to 38 cases per 1000 live births in the developed and developing world, respectively.³ Some investigators have reported a declining mortality rate from newborn sepsis in recent years.^{2,4} Notably, other investigators have reported a continuing disease burden with associated mortality,⁵ as well as a predominance of virulent pathogens and high sepsis-associated mortality.⁶

Regardless of the true overarching association between neonatal sepsis and mortality, morbidity remains a significant problem. A recently published meta-analysis summarized and analyzed results from 12 studies published between 2000 through 2012 that enrolled a total of 3669 infants of gestational age < 34 weeks and/or birth weight ≤ 1500 grams with sepsis and compared them to a control group of 24 723 infants without sepsis.⁴ There was a significant association between neonatal sepsis and short-term morbidities, including mortality, respiratory distress syndrome, intraventricular hemorrhage (IVH), meningitis, periventricular leukomalacia, and multiple organ failure. There was also a significant association between neonatal sepsis and long-term morbidities, including death after discharge, bronchopulmonary dysplasia (BPD),

cerebral palsy, cognitive delay, psychomotor delay, visual impairment, and auditory impairment.⁴

In addition to morbidity and mortality, neonatal sepsis and the associated neonatal intensive care unit (NICU) stay exert emotional and socioeconomic burdens on infants, parents, families, clinicians, the healthcare system, and society.⁷⁻¹⁴ Parents of an infant being cared for in the NICU often experience stress, feel helpless, and struggle to interact with their child in a developmentally sensitive manner.⁸ Among very low-birth-weight (VLBW) infants, prolonged hospital stay has been shown to influence parent-infant relationships, failure to thrive, child abuse and/or abandonment, and grieving parents.¹⁵ Compounding this burden, NICU costs of care increase by approximately \$16 800 when comparing VLBW infants with NICU-associated bloodstream infection to similar infants without bloodstream infection.¹⁶ Neonatal sepsis is associated with longer duration of hospitalization.^{10,17-19} As prolonged hospital stay is also associated with nosocomial infection¹⁶ and increased exposure to other hospital-acquired morbidities,²⁰ this may potentially exacerbate emotional and socioeconomic strains. In fact, the duration of an infant's initial hospitalization is predictive of his or her long-term medical resource requirements.¹⁸

Definitions

While sepsis is a well-known clinical entity, specific definitions of sepsis and related terminology vary in the literature. That “sepsis” is nebulous is understandable considering the sepsis continuum, which spans systemic inflammatory response syndrome, infection, sepsis, severe sepsis, septic shock, and organ dysfunction,²¹ as well as additional related terms, including bacteremia, septicemia, septic syndrome, sepsis-

induced hypotension, and multiple organ dysfunction syndrome.²² The term “clinical sepsis,” defined as physician-diagnosed sepsis without blood culture confirmation,²³ is not universally applied in the literature. For clarification, clinical signs and hematologic criteria have been proposed and validated for the neonatal population (see Appendix A).²⁴ Validated criteria are crucial in this vulnerable population in which sepsis presents variably and non-specifically.^{14,19,24} In the aforementioned validation study, at least 2 clinical criteria and at least 1 hematologic criterion incited a sepsis evaluation.²⁴ Ultimately, the attending neonatologist uses his or her expert clinical judgment to determine when to evaluate an infant for sepsis.²⁵

Neonatal sepsis is most commonly defined based upon timing of disease onset. Early-onset sepsis is defined as laboratory-confirmed bloodstream infection at ≤ 72 hours of life, while late-onset sepsis is defined as laboratory-confirmed bloodstream infection at > 72 hours of life.^{6,24-26} Risk factors for early-onset sepsis include maternal group B *Streptococcus* colonization, maternal chorioamnionitis, premature rupture of amniotic membranes, prolonged rupture of amniotic membranes (> 18 hours from membrane rupture to delivery), and multiparity.^{25,27,28} Risk factors for late-onset sepsis include delivery by cesarean section, the use of indwelling vascular catheters, invasive procedures, prolonged mechanical ventilation, prolonged antibiotic administration, hyperalimentation, and comorbidities such as patent ductus arteriosus, necrotizing enterocolitis (NEC), and BPD.^{25,26,28} Prematurity^{3,25-27} and its frequently associated lower birth weight^{3,26,27,29} are risk factors for both early-onset^{3,27,29} and late-onset sepsis.^{3,25-27,29}

Microbiology

Just as the semantics of sepsis are dynamic, so too are the isolated microbiological flora. Yale-New Haven Hospital's (YNHH) single-center longitudinal database of neonatal bloodstream infections allows for analysis of the changing microbiological landscape of neonatal sepsis. During the 15-year period from 1989 through 2003, of 755 episodes of sepsis, the most common organisms isolated from blood culture were coagulase-negative staphylococci (29%), *Escherichia coli* (12%), group B *Streptococcus* (10%), and *Staphylococcus aureus* (8%).³⁰ Comparing these results to the previous 60 years, observed trends included: (1) a significant decline in *Streptococcus pneumoniae* and *Streptococcus pyogenes*, with no episodes of resultant sepsis reported from 1989 through 2003; (2) a decline in group B streptococci; (3) a decline in *E coli*; and (4) an increase in commensal species, including coagulase-negative staphylococci, *S aureus*, and *Candida* species.³⁰

More recently at YNHH, of 410 episodes of sepsis during the 10-year period from 2004 through 2013, the most common organisms isolated from blood culture were coagulase-negative staphylococci (29%), *S aureus* (16%), *E coli* (14%), and *Enterococcus faecalis* (12%).⁶ Notable trends during this 10-year study period included: (1) a static rate of group B streptococci, (2) the emergence of *E coli* as the most common etiology of early-onset sepsis, and (3) a decline in coagulase-negative staphylococci as an etiology of late-onset sepsis in conjunction with NICU-wide infection prevention initiatives implemented in 2008 and 2009.⁶

Pathophysiology

The transition from intrauterine to extrauterine life is a hazardous time for infants. Many vehicles, such as the uterus, birth canal, neonatal care unit, invasive procedures and devices, healthcare providers, family, visitors, and the community, can transmit virulent microorganisms.²⁷ In addition to multiple exposures, VLBW and premature infants are vulnerable to sepsis due to relative immunocompromise.^{24,27} Despite myriad research over decades, the exact mechanisms of sepsis remain elusive.³¹ The most basic premise appears dogmatic: sepsis occurs when pathogens invade the body and the ensuing inflammatory response becomes systemic and affects tissues and organ systems at distant sites from the initial infection.³¹ The inflammatory response was initially presumed to be solely pro-inflammatory, with cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, interleukin (IL)-6, and interferon (IFN)- γ playing integral roles. Later hypotheses articulated a subsequent, compensatory anti-inflammatory response with a predominance of IL-10, IL-1 receptor antagonist, and TNF-soluble receptors.³¹ More recent hypotheses support concomitantly produced pro- and anti-inflammatory cytokines that elicit a mixed anti-inflammatory response. No matter the balance between inflammation and immunosuppression, studies employing mathematical modeling of response to sepsis emphasize the necessity and benefit of some degree of inflammation.³¹

Diagnosis and Routine Care

Accurate and timely diagnosis is the first step to ameliorate the morbidity and mortality associated with neonatal sepsis.¹⁹ Peripheral blood culture is the current gold standard of diagnosis.^{25,32,33} Since sepsis is often recognized only when infants begin to clinically deteriorate, blood cultures are obtained for infants with maternal risk factors for

neonatal sepsis as well as for infants with symptoms that suggest infection^{34,35} (see Appendix A). Routine care is supportive care focused on ensuring adequate systemic oxygenation and peripheral perfusion¹⁹ in addition to empirical treatment with parenteral broad-spectrum antimicrobials for 48 to 72 hours while blood culture results are pending.^{19,25,34} Once sepsis is confirmed by blood culture antimicrobial treatment is tailored to the sensitivity pattern of the isolated microorganism.^{19,36,37}

Adjunctive Therapy

Adjunctive therapies for neonatal sepsis mimic the trends in pathophysiological understanding: some attenuate while others augment the immunoinflammatory system. Of many adjunctive therapies investigated among the neonatal population with cohort studies, randomized clinical trials (RCTs), and systematic reviews, those associated with a significant reduction in sepsis or mortality include exchange transfusion, pentoxifylline, selenium, lactoferrin, probiotics, breast milk, and polyclonal intravenous immunoglobulin.³⁸ Adjunctive therapies not shown to significantly reduce sepsis or mortality include granulocyte transfusion, granulocyte- and granulocyte-monocyte colony stimulating factor, activated protein C, melatonin, glutamine, prebiotics, broad-spectrum peripartum antibiotics, immunoglobulin M-enriched intravenous immunoglobulin, monoclonal intravenous immunoglobulin, and antiendotoxin antibodies.^{38,39}

Pentoxifylline

Investigation of pentoxifylline (PTX; 1-[5-oxohexyl]-3,7-dimethylxanthine)⁴⁰ for neonatal sepsis began in the 1990s.⁴¹ Pentoxifylline is a promising adjunctive therapy because it has been shown to modulate the immune response by inhibiting phosphodiesterase enzyme.³⁶ This inhibition in turn activates adenylyl cyclase, increases

cyclic AMP concentrations, and suppresses pro-inflammatory cytokines such as TNF- α ,^{3,10,38} IL-1,^{3,38} IL-6,¹⁰ and IFN- γ .¹⁰ Interestingly, increased concentration of TNF- α is associated with mortality in sepsis.^{3,36,42,43} Tumor necrosis factor- α ^{3,10} and platelet activating factor³ have also been implicated in neonates with NEC. Pentoxifylline has beneficial effects for the cardiovascular system, including decreasing superfluous coagulation, lowering blood viscosity, preventing the hypodynamic state that often accompanies sepsis, improving endothelial cell function, increasing tissue perfusion, and improving renal blood flow.^{10,38} Additionally, PTX improves inflammatory lung injury after chronic endotoxemia.³⁸ Although investigators acknowledge the complexity in seeking treatments for endoplasmic reticulum stress,³¹ PTX mollifies mitochondrial reduction-oxidation stress in a rat model by restoring glutathione, thereby preserving viability of mitochondria showing inhibited respiration.⁷

Pentoxifylline is a modified synthetic theobromine, which is the least toxic of the methylxanthines (ie, as compared to caffeine and theophylline).¹⁰ Eighty mg/kg of PTX is the reported toxic dose, heralded by flushing, fever, hypothermia, agitation, myoclonus, somnolence, loss of consciousness, seizures, hypotension, bradycardia, atrioventricular block, or asystole. At therapeutic doses, PTX does not have significant cardiac and bronchodilating effects as seen with other methylxanthines. In adults, PTX is contraindicated with recent cerebral hemorrhage. Importantly, significant adverse effects, including thrombocytopenia and bleeding, have not been reported among preterm neonates with sepsis or NEC after treatment with PTX.¹⁰

A Cochrane Collaboration meta-analysis of four trials published between 1996 through 2010 showed a beneficial association between adjunctive PTX therapy as

compared to placebo, both in addition to routine care, or as compared to solely routine care and surrogate or clinical outcomes in infants with sepsis.³ There was a statistically significant reduction in all-cause mortality during hospital stay among all infants as well as among subgroups of infants with confirmed sepsis, confirmed Gram-negative sepsis, and preterm infants. On the other hand, there was not a significant association considering the late-onset sepsis subgroup.³ The newest study included in this meta-analysis, a quasi-randomized trial published in 2010, found a statistically significant reduction in duration of hospitalization among neonates with suspected sepsis treated adjunctively with PTX as compared to placebo, both in addition to routine care.³ Conversely, a recent RCT among neonates with clinical and laboratory signs of infection did not reveal a significant association between PTX or IgM-enriched intravenous immunoglobulin or a combination of the two as compared to placebo, each in addition to routine care, and duration of hospitalization or all-cause mortality.⁴⁴

Statement of the Problem

Despite investigation of a plethora of adjunctive treatments for neonatal sepsis, some with statistical significance, none are recommended for routine use.¹⁹ With discordant results regarding the association between PTX as an adjunct to antimicrobial therapy and clinical outcomes in infants with sepsis, why are investigators encouraging further research?^{3,10,19,38} Likely, while hypotheses surmising the benefit of PTX in infants with sepsis are founded on basic science research, many of the studies suffer from selection and information bias as well as presumably being underpowered to detect a difference in treatment effect.

Most neonatal therapies, including adjunctive immunologic interventions, are incompletely evaluated due to issues with statistical power.³⁸ A dual-armed trial would require over 800 patients to reliably detect a 10% risk difference in mortality or disability-free survival.³⁸ Considering the four studies examining PTX and various surrogate and clinical outcomes that are included in the recent Cochrane Review, the primary outcomes were plasma TNF level; plasma TNF, IL-1, and IL-6 levels; all-cause mortality during hospital stay or development of NEC; and coagulation profile and disseminated intravascular coagulation incidence. The respective sample sizes were 40, 100, 50, and 37 infants.^{36,42,43,45} The recent study conducted by Akdag and colleagues examined the association between PTX or IgM-enriched intravenous immunoglobulin or a combination of the two as compared to placebo and all-cause mortality with a sample population of 204 infants.⁴⁴

Another compelling reason for more clinical studies is the ever-changing characteristics of the source population, namely gestational age, birth weight, comorbidities, microbiological flora, and early-onset vs late-onset sepsis; an evolving understanding of sepsis; and novel interventions available in the NICU setting. Advances in technology as well as perinatal care, most notably antenatal corticosteroid and postnatal surfactant therapy, allow many preterm infants born in the United States to survive and contribute to this dynamic population.^{8,46} Hence, robust clinical studies allowing for detailed subgroup analyses will prove indispensable.³¹

Hypothesis

We hypothesize that among preterm infants with blood culture-confirmed sepsis who are treated with pentoxifylline plus appropriate antimicrobial therapy, as compared

to infants treated with placebo plus appropriate antimicrobial therapy, median duration of stay in the neonatal intensive care unit will be significantly reduced.

Goals and Objectives

The purpose of our study is to ascertain the efficacy of PTX as compared to placebo, both in addition to appropriate antimicrobial therapy, in reducing NICU duration of stay among preterm infants with blood culture-confirmed sepsis. As such, this study aims to clarify discordance in the existing body of literature regarding the efficacy of adjunctive PTX therapy among infants with sepsis. We also aim to conduct novel research by investigating adjunctive PTX therapy among preterm infants with blood culture-confirmed sepsis with unique microbiological flora and acuity of care who are cared for using advanced therapeutic interventions, as well as by powering our study to detect a difference in NICU duration of stay. By the end of our study, investigators will determine whether adjunctive PTX therapy as compared to placebo, both in addition to appropriate antimicrobial therapy, significantly reduces NICU duration of stay as well as determine the association between adjunctive PTX therapy and adverse events, morbidity, and mortality.

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Chapter 2: Review of the Literature

Introduction

We reviewed published literature from July 2014 through July 10, 2015 using Ovid MEDLINE and EMBASE electronic journal reference databases. We exploded and combined (1) “infant, newborn” and (2) “sepsis” along with (3) “phosphodiesterase inhibitors” or (4) “pentoxifylline” (1 and 2 and (3 or 4)) to identify articles related to pentoxifylline in infants with sepsis. To identify articles related to NICU length of stay, we exploded and combined “intensive care units, neonatal” and “length of stay.” We limited all searches to those conducted among humans and printed in English. To be comprehensive we did not specify a time limit for articles related to pentoxifylline, but we limited the search for NICU length of stay to articles published within the last 10 years. Our searches returned 51 and 778 articles, respectively. We assessed titles and abstracts for relevance to our hypothesis while concomitantly excluding editorials, commentaries, conference abstracts, conference papers, and letters. We included systematic reviews, meta-analyses, clinical trials, expert reviews, cohort studies, cross-sectional studies, and descriptive studies.

Adjunctive Pentoxifylline Therapy in Infants with Sepsis

Initial Observational Study

Prior to the 1990s, pentoxifylline was an adjunctive immunologic intervention untested in infants with sepsis. Synthesizing prior research showing increased TNF concentrations are associated with mortality rate in children with septic shock, in addition to research showing PTX inhibits TNF production in macrophages, Lauterbach et al retrospectively analyzed a cohort of infants to assess the feasibility of PTX in infants.¹

The sample population included 30 preterm infants (defined as gestational age < 36 weeks) with clinical signs of sepsis confirmed by positive blood culture and without major congenital malformation or grade III or IV IVH. Seventeen infants comprising the exposure group received PTX, 5 mg/kg/h for 6 hours on 3 successive days given concomitantly with antibiotics, in addition to supportive therapy, and were retrospectively compared to a group of 13 infants who had received only antibiotics and supportive therapy. The exposure and comparison groups were statistically similar in birth weight and gestational age at baseline. Mortality rate (12% vs 46%; $P < 0.04$), hypotension (18% vs 69%; $P < 0.006$), and metabolic acidosis (18% vs 61%; $P < 0.01$) were significantly lower in the exposure group than in the comparison group. Survival in the subgroup of infants with Gram-negative sepsis was significantly higher when comparing exposed to unexposed infants (85% vs 25%; $P < 0.01$). There were significantly more infants with neutropenia in the exposed as compared to unexposed group, although the trend was of moderate statistical significance (29% vs 23%; $P = 0.046$). On the other hand, there were no statistically significant differences between exposure and comparison groups in hypoproteinemia (35% vs 38%; $P = 0.057$) or thrombocytopenia (29% vs 53%; $P = 0.16$). There were no reported adverse effects from PTX infusion, defined as hypotension, irritability, and deterioration of vital signs.¹

Since the investigators reported few baseline characteristics there could have been significant differences in vitality as well as other factors between groups at baseline, which represents selection bias and potentially introduces confounding. There may also have been information bias if clinicians observed the exposure group more closely than the comparison group for adverse effects, termed observation bias, not to mention

potentially deficient historical records. Additionally, both groups were given intravenous immunoglobulin according to identical protocols; however, intravenous immunoglobulin is not included in current routine care and therefore reduces external validity.² With these biases and a sample population of 30 infants, the difference in mortality rate between exposure and comparison groups could have been distorted by confounding or resulted from chance alone.

Early Experimental Studies

Following initial observations, Lauterbach and Zembala sought to quantify the association between adjunctive PTX therapy and TNF levels in infants with sepsis as well as further investigate the association between adjunctive PTX therapy and mortality rate and adverse events.³ They designed a single-center, blinded, placebo-controlled RCT and expanded enrollment criteria to include (1) premature infants, defined as gestational age < 36 weeks, (2) with suspected late-onset sepsis based on clinical signs, (3) who, if they exhibited abdominal distension, did not have evidence of NEC on X-ray (according to Bell's criteria) while excluding infants with (1) major congenital malformation, (2) grade III or IV IVH, or (3) congenital infection. Forty infants who met enrollment criteria were assigned using a permuted block randomization scheme to either the intervention group, who received PTX 5 mg/kg/h for 6 hours (infusion initiated 30 minutes before antibiotic administration) on 3 successive days in addition to routine care, or the control group, who received an identical dosage and duration of saline in addition to routine care.³

The groups were statistically similar comparing expanded baseline characteristics, including birth weight, Apgar score, gestational age, symptoms of shock, hypotension, neutropenia, hypoproteinemia, metabolic acidosis, thrombocytopenia, abdominal

distension, and accompanying focal infections.³ In the PTX group, plasma TNF levels were significantly reduced when comparing before vs after PTX infusion on day 1 (671.5 ± 438.0 pg/mL vs 218.4 ± 195.2 pg/mL; $P = 0.001$) and day 3 (160.1 ± 216.2 pg/mL vs 41.0 ± 64.7 pg/mL; $P = 0.01$). In the placebo group, plasma TNF levels were not significantly reduced after placebo infusion. The most striking comparison was between plasma TNF levels before the first infusion of PTX on day 1 as compared to after the last infusion of PTX on day 3 (671.5 pg/mL vs 41.0 pg/mL; $P < 0.000004$). The same comparison within the placebo group was not statistically significant. Therefore, PTX was associated with a significant reduction in plasma TNF level. Although there was a trend toward decreased mortality rate when comparing PTX and placebo groups (0% vs 23%), the investigators omitted hypothesis-testing data. The mortality rate in the subgroup of infants with shock was significantly lower in the PTX group as compared to the placebo group (0% vs 75%; $P < 0.04$). There was a significantly higher incidence of clinical symptoms and radiographic signs of NEC in the placebo vs PTX group (31% vs 6%; $P < 0.004$) as well as a significantly higher incidence of metabolic acidosis ($P < 0.05$) and oliguria or anuria ($P < 0.05$) in the placebo group. The authors omitted treatment effect for the latter two comparisons. Pentoxifylline therapy did not elicit adverse effects, as defined by hypotension, irritability, and deterioration of vital signs.³

This pilot RCT has several limitations. Only infants with suspected late-onset sepsis, but not those with suspected early-onset sepsis, were enrolled in the study, which limits generalizability to the source population. Similarly to the prior study, all infants were given a single dose of intravenous immunoglobulin on the first day of therapy, which also limits external validity. Although the authors reported the method of random

sequence generation and stated that clinicians and laboratory personnel were blinded to treatment allocation, investigators were not explicitly blinded to treatment allocation nor did they describe blinding methods. This could introduce performance bias and overestimate treatment effect. Additionally, 11 of 40 infants enrolled in the study who did not have blood culture-confirmed sepsis were excluded from analysis. Not only does this represent attrition bias, but also per-protocol analysis as compared to intention-to-treat analysis decreases external validity. Last, this study was ostensibly powered to detect a difference in plasma TNF levels, not differences in clinically relevant secondary outcomes.

Moreover, the use of crystalloid as a placebo among preterm infants requires careful analysis. There is a delicate fluid balance in infants, especially those who are preterm and low birth weight.⁴ Fluid balance is even more complex in the first few days of life during the postnatal diuresis. Neonates are prone to disorders of sodium balance due to short renal tubules with limited ability to concentrate urine complicated by avid aldosterone secretion during a sodium load. Fluid boluses, intravenous flushes, and drugs suspended in isotonic fluid have the potential to cause hyperchloremic acidosis, hypernatremia, sodium retention, and edema.⁴ Most notably among extremely low-birth-weight infants, giving excessive sodium and water expands the extracellular compartment and has been shown to be associated with adverse outcomes, including weight gain and increased risk of patent ductus arteriosus and NEC, as well as associated with a trend toward increased risk of BPD, intracranial hemorrhage, and death. Considering very low-birth-weight and extremely low-birth-weight infants, daily fluid requirements during the first 5 days of life are approximately 80-90, 120, 150, 180, and 180 mL/kg, respectively.⁴

In the RCT by Lauterbach and Zembala, the placebo was a volume of normal saline comparable to PTX, so presumably 5 mL/kg/h for 6 hours.³ This equates to a daily 30 mL fluid load with resultant sodium load. Although this dosage and duration of normal saline as placebo was ethical, considering preterm infants' delicate fluid balance a smaller fluid and sodium load from a placebo would be better.

Recognizing the association between PTX therapy and decreased levels of TNF, researchers next looked for additional pro-inflammatory cytokines that might be reduced by PTX therapy and the respective association with clinical outcomes in infants with sepsis.⁵ Lauterbach and colleagues conducted a dual-center, double-blind, placebo-controlled RCT among preterm infants with suspected late-onset sepsis (as determined by clinical signs and hematologic criteria) to examine the association between adjunctive PTX therapy in addition to routine care, as compared to placebo and routine care, and plasma levels of TNF, IL-1, and IL-6; adverse effects, including metabolic acidosis, hypotension, disordered peripheral perfusion, oliguria or anuria, hepatic failure, disseminated intravascular coagulation, and NEC; and mortality rate. One hundred infants were randomly allocated to either the intervention group (PTX, 5 mg/kg/h for 6 hours, on 6 successive days) or placebo group (0.9% saline at the same dosage and for the same duration as the PTX group). Identical bottles of PTX or placebo that were coded using computer-generated random numbers ensured blinding to treatment allocation and intervention or placebo group. Based on research that showed disturbed perfusion potentially diminishes penetration of antibiotics at the site of infection, along with research that showed PTX potentially improves tissue perfusion, in those infants with disordered peripheral circulation antibiotic administration was postponed by 30 minutes

for PTX or placebo initiation. Otherwise, medical staff administered PTX or placebo concomitantly with antibiotics.⁵

In the PTX group, plasma TNF levels declined steadily over 6 days of follow-up. There was a statistically significant reduction in plasma TNF levels when comparing levels before PTX infusion on the first day to levels after PTX infusion on the sixth day (347.2 ± 452.3 pg/mL vs 68.1 ± 127.5 pg/mL; $P = 0.009$).⁵ Tumor necrosis factor trended downward on day 3 and then upward on day 6 in the placebo group, but not in a statistically significant manner. When measured after administration of PTX or placebo on the sixth day, plasma IL-6 levels were significantly lower in the PTX group as compared to the placebo group (13.6 ± 16.6 pg/mL vs 197.5 ± 280.2 pg/mL; $P = 0.04$). Interleukin-1 trends did not differ significantly within the intervention or placebo group. Sepsis complicated by shock occurred in both groups, but only among the subgroup of infants with Gram-negative microorganisms isolated from blood culture. One of 6 infants in the PTX group and 4 of 8 infants in the placebo group who had symptoms of shock at enrollment died during follow-up; however, the difference between groups was not statistically significant (17% vs 50%; hypothesis-testing data omitted). Also of note, the mortality rate in the PTX group was significantly lower than in the placebo group, albeit marginally (2.5% vs 15.8%; $P = 0.046$). Of the other secondary outcomes, the only significant difference was a lower incidence of NEC in the PTX group as compared to the placebo group (5% vs 21%; $P = 0.01$).⁵

This study used similar enrollment criteria and statistical methods to the previous study: infants with early-onset sepsis were not included in the sample population and 10 infants in the intervention group and 12 infants in the placebo group who did not have

blood culture-confirmed sepsis were excluded from analysis. One advantage of this study is that randomization was adequately described and investigators, medical personnel, and parents were blinded to treatment allocation and intervention or placebo group. Adequate blinding allows for a more accurate analysis of mortality differences between groups. Unfortunately, the investigators did not report duration of stay as an outcome. It is imperative to note that this study, as well as the previously discussed RCT, is ostensibly powered to detect a difference in the primary outcome. Therefore, differences in morbidity and mortality between groups must be interpreted with caution.

Other investigators aimed to confirm the association between adjunctive PTX therapy in addition to routine care and plasma TNF and IL-6 levels.⁶ Thirteen infants with suspected sepsis who were successively admitted to the study center comprised the exposure group while the next 7 successively admitted infants with suspected sepsis comprised the comparison group. Infants in the exposure group were adjunctively treated with PTX, 0.5 mg/kg/h for 24 hours, initiated 30 minutes prior to antibiotic therapy. The comparison group received solely routine care. The exposure and comparison groups were statistically similar comparing age at admission, gestational age, and weight at baseline ($P > 0.05$ for each comparison). Investigators report no statistically significant differences between exposure and comparison groups in any of the reported outcomes, including C-reactive protein, TNF, IL-6, leukocyte count, and mortality (0% vs 23%; $P > 0.05$).⁶ This study potentially suffers from sampling bias, although the few reported baseline characteristics were similar between groups. This study is also limited by selection bias, performance bias, and detection bias since infants were not truly

randomized and the investigators were not blinded to treatment allocation or exposure or comparison group. As such, these results are of limited value.

Recent Experimental Studies

To fill the void of studies with clinically relevant primary outcomes, Ali and colleagues enrolled 50 preterm infants with blood culture-confirmed sepsis but without IVH or congenital infection and assigned them (the authors omitted treatment allocation details) to either intervention (PTX, 5 mg/kg/h for 6 hours on 3 successive days given concomitantly with antimicrobial therapy) or control (routine care) groups.⁷ The intervention and control groups were ostensibly similar at baseline. Infants were followed for the duration of their hospital stay. The mortality rate in the control group was significantly higher as compared to the intervention group (40% vs 16%; $P < 0.02$). Similarly, the mortality rate in the subgroup of infants with Gram-negative sepsis was significantly higher in the control group as compared to the intervention group (53% vs 17%; $P < 0.04$). There was a significantly lower incidence of NEC in the PTX group as compared to the control group (8% vs 28%; $P < 0.01$). Although there was a trend toward reduced duration of hospital stay (15 days vs 30 days) and reduced duration of mechanical ventilation (72 hours vs 120 hours) among infants treated adjunctively with PTX as compared to those receiving routine care, the investigators omitted hypothesis-testing data. In agreement with all prior studies, there was no evidence of adverse effects from PTX, defined as hypotension, irritability, or deterioration of vital signs.⁷

While the investigators correctly identified the paucity of studies powered to detect a difference in mortality, their study has several limitations. First, the authors omitted details of randomization. Hence, there is possibly selection bias. Although

baseline characteristics were reportedly similar between groups, hypothesis-testing data were omitted so the null hypothesis cannot be reliably accepted. Timing of disease onset was not reported, so subgroup analysis between early-onset and late-onset sepsis could not be conducted. The authors also omitted whether parents, medical staff, and investigators were blinded to treatment allocation or outcome assessment, potentially introducing performance bias and detection bias as well as overestimating treatment effect. One advantage of this study is that all infants were included in statistical analysis, minimizing attrition bias and allowing for intention-to-treat analysis. Importantly, this study adds to the existing body of literature by reporting duration of hospital stay and duration of mechanical ventilation as secondary outcomes. Unfortunately, the authors did not include standard deviation (for parametrically distributed data), range (for nonparametrically distributed data), or hypothesis-testing data for either outcome.

Following the example set by Ali and colleagues to focus on clinically relevant primary and secondary outcomes, Adel et al designed a single-center, blinded, placebo-controlled clinical trial with the goal of examining the efficacy of adjunctive PTX therapy as compared to placebo, both in addition to routine care, to attenuate microcirculatory derangement in neonates with sepsis.⁸ Infants were eligible for enrollment with suspected sepsis, but were excluded by any of the following criteria: (1) major congenital malformations, (2) IVH, (3) symptoms of congenital infection, or (4) treatment with steroids, another phosphodiesterase inhibitor, anticoagulants, or antiplatelet agents. Thirty seven neonates were assigned by quasi-randomization (neonates admitted to the study center on Tuesday or Thursday were assigned to the intervention group, while neonates admitted to the study center on Monday or Wednesday were assigned to the placebo

group) to the intervention (PTX, 5 mg/kg/h (daily required volume of PTX removed from ampule, diluted up to 12 mL with normal saline, and then infused at 2 mL/h) for 6 hours on 6 successive days given concomitantly with antimicrobials in addition to routine care) or placebo (normal saline, 2 mL/h for 6 hours on 6 successive days given concomitantly with antimicrobials in addition to routine care) group.⁸ The groups were ostensibly similar at baseline.

Primary outcomes were coagulation parameters, as measured by prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer; C-reactive protein (CRP); white blood cell count; and platelet count.⁸ The authors reported a moderately significant reduction in CRP between PTX and placebo groups when comparing levels before infusion of PTX or placebo on day 1 to after infusion on day 3 ($P = 0.045$) and after infusion on day 6 ($P = 0.04$). Conversely, there was not a statistically significant difference between PTX and placebo groups considering fibrinogen, D-dimer, PT, aPTT, platelets, or leukocytes. Secondary outcomes were organ dysfunction, as measured by clinical bleeding, fresh frozen plasma requirement, disseminated intravascular coagulation, disordered peripheral circulation, requirement of inotropic agent, shock, NEC, liver failure, convulsions, or oliguria; multiple organ dysfunction syndrome; duration of hospitalization; and mortality. Comparing groups adjunctively treated with PTX vs placebo, there was a significant decrease in clinical bleeding, (23.5% vs 60%; $P = 0.0128$), fresh frozen plasma requirement (36% vs 80%; $P = 0.003$), incidence of multiple organ dysfunction syndrome (23.5% vs 55%; $P = 0.037$), and duration of hospitalization (22.6 ± 13.2 days vs 33.8 ± 16.2 days; $P = 0.044$).⁸ Importantly, 5 infants with sepsis required adjunctive PTX therapy in order for 1 infant to

have a significant reduction in mean duration of hospital stay (risk difference = -0.19; 95% CI, -0.29 to -0.08; NNT 5; 95% CI, 3-12; $P = 0.04$).⁹ There were no other statistically significant differences in secondary outcomes, most notably mortality, between intervention and placebo groups. Additionally, there were no adverse effects associated with PTX infusion, as measured by thrombocytopenia, cholestatic jaundice requiring therapy, irritability, and gastrointestinal disturbances.⁸

The first limitation of this study is that neonates were assigned to intervention or placebo groups by quasi-randomization as opposed to truly random treatment allocation, which potentially introduces selection bias. Also, while the investigators stated that baseline characteristics were similar between groups, hypothesis-testing data were omitted. Although PTX and normal saline were filled in identical syringes, performance bias and detection bias are concerns because the study was quasi-randomized and therefore blinding of medical staff and investigators cannot be reliably assessed. While the authors report a difference in duration of stay between PTX and placebo groups, application of these results is limited by the investigators' choice to operationalize duration of stay as a parametric outcome measure. Operationalizing duration of stay as a parametric outcome measure as compared to a nonparametric outcome measure increases type II error and decreases explanatory power of multivariate models when raw data are not normally distributed, which is commonly the case in healthcare settings.¹⁰ Two strengths of this study are intention-to-treat analysis despite 6 of 37 infants without blood culture-confirmed sepsis as well as expansion of clinically relevant outcomes to include coagulopathy and organ dysfunction.

Most Current Empirical Studies

To inform evidence-based pharmacotherapeutic decisions for neonates with sepsis, the Cochrane Collaboration updated a systematic review and meta-analysis⁹ in 2011 to synthesize results from selected aforementioned clinical trials. Using Mantel-Haenszel multivariate modeling, the authors reported a statistically significant reduction in all-cause mortality during hospital stay among all infants with suspected or confirmed sepsis treated adjunctively with PTX as compared to placebo, both in addition to routine care, or as compared to solely routine care (pooled RR = 0.3966; 95% CI, 0.2030-0.7749; $P = 0.007$) as well as among subgroups of infants with confirmed sepsis (pooled RR = 0.2714; 95% CI, 0.1141-0.6458; $P = 0.003$), confirmed Gram-negative sepsis (pooled RR = 0.2735; 95% CI, 0.1158-0.6462; $P = 0.003$), and preterm infants (pooled RR = 0.2714; 95% CI, 0.1141-0.6458; $P = 0.003$).⁹ Conversely, there was not a significant association between adjunctive PTX therapy and mortality considering the late-onset sepsis subgroup (pooled RR = 0.1919; 95% CI, 0.0361-1.0190; $P = 0.05$). Duration of hospital stay was significantly reduced when comparing infants with suspected or confirmed sepsis treated adjunctively with PTX to infants given placebo, both in addition to routine care (mean risk difference = -11.2; 95% CI, -22.0948 to -0.3052; $P = 0.04$). Inspection of forest plots and analysis of I^2 statistics did not uncover significant heterogeneity among trials for any comparison.⁹

Despite these statistically significant associations and statistical homogeneity among trials, meta-analysis cannot control for source bias, previously described in detail for each study. Meta-analyses are also subject to publication bias. Furthermore, each of the trials included in this meta-analysis enrolled relatively small sample populations.

Since investigators did not justify sample sizes, statistical power to detect a difference in treatment effect cannot be reliably assessed. Overall, if bias, confounding, and lack of statistical power misconstrued results, then there may not be a beneficial association between adjunctive PTX therapy and clinical outcomes among infants with sepsis.

In the conclusion of the aforesaid Cochrane Review, the authors implore future researchers to continue studying PTX as adjunctive therapy in neonates with sepsis, and perhaps to investigate it in conjunction with other promising adjunctive therapies such as colony stimulating factors or intravenous immunoglobulin.⁹ Akdag et al heeded this call with a single-center, blinded, placebo-controlled RCT.¹¹ They hypothesized that among infants with sepsis, adjunctive therapy with PTX, immunoglobulin M (IgM)-enriched intravenous immunoglobulin (IVIG), or a combination of the two, as compared to placebo, would decrease mortality rate. To test this hypothesis, they enrolled 204 infants with suspected sepsis but without (1) major congenital abnormalities, (2) grade III or IV IVH, (3) symptoms of congenital infection, (4) inborn errors of metabolism, or (5) prior treatment with PTX or IgM-enriched IVIG. Enrolled infants were assigned to receive routine care in addition to PTX (6 mg/kg/h over 4 hours for 3 successive days), IgM-enriched IVIG (250 mg/kg over 4 hours for 3 successive days), PTX plus IgM-enriched IVIG (PTX 6 mg/kg/h over 4 hours given first, followed by IgM-enriched IVIG 250 mg/kg over 4 hours, for 3 successive days) or placebo (normal saline 5 mL/kg over 4 hours for 3 successive days).

While groups were statistically similar at baseline in prematurity, recurrent apnea, feeding intolerance, temperature instability, and glucose instability, the PTX, IVIG, and placebo groups were comprised of significantly more infants with lethargy than the PTX

plus IVIG group ($P < 0.004$).¹¹ There was not a statistically significant difference in mortality rate between the 4 groups (PTX: 9.8% vs IVIG: 7.8% vs PTX+IVIG: 13.7% vs placebo: 3.9%; $P > 0.05$). The groups were also ostensibly similar regarding incidence of morbidities, including oliguria/anuria, hepatic failure, NEC, disseminated intravascular coagulation, and pulmonary hemorrhage, but hypothesis-testing data were omitted. Although there was a trend toward reduced duration of hospital stay in the PTX and placebo groups, the authors omitted hypothesis-testing data (PTX: median 28 days; range 5-246 days vs IVIG: median 32 days; range 5-136 days vs PTX+IVIG: median 38 days; range 5-119 days vs placebo: median 27 days; range 1-168 days). Additional secondary outcomes included WBC count, neutrophil CD64, and plasma CRP, IL-6, and TNF levels measured before initiation of the study protocol on day 1 and repeated on days 2 and 4. There was a trend toward reduced neutrophil CD64 levels in the IVIG and PTX plus IVIG groups as compared to PTX and placebo groups, but the investigators did not clearly report treatment effect and omitted hypothesis-testing data.¹¹

Although the investigators reported using cards in sealed, opaque, sequentially numbered envelopes to blind treatment allocation, random allocation is incompletely described because they did not describe the method of random sequence generation. The method of blinding is adequately described. Regarding sepsis diagnosis, the authors reported positive blood, urinary tract, cerebrospinal fluid, and catheter cultures.¹¹ However, sampling blood via indwelling catheters could increase false-positive cultures, which represent contamination, as opposed to true-positive cultures, which represent bloodstream infection.¹² The investigators also did not specify which antimicrobials were administered, which limits internal and external validity.¹¹ Three additional limitations

include: (1) the sample size was calculated using an estimated 50% mortality rate, based on 12% mortality rate from NEC, presumably to decrease sample size; (2) moderate statistical power (70%) to detect a difference in treatment effect; and (3) the majority of infants in the sample population had Gram-positive infections, while research has shown the most significant benefit from adjunctive IgM-enriched IVIG therapy among patients with Gram-negative septic shock.¹¹ This study adds to the existing body of literature by investigating combined adjunctive immunologic interventions.

Duration of Stay in Infants with Sepsis

Economic Burden

Although mortality is an irreplaceable outcome,¹³ with notable statistical power difficulty¹⁴ and mortality from newborn sepsis declining^{15,16} in combination with survival from prematurity increasing,^{17,18} duration of stay should be considered for primary outcome in clinical studies.^{19,20} Reducing excess duration of stay is a pragmatic goal.¹⁹⁻²¹ Investigators explored the economic burden of bloodstream infection by retrospectively comparing 900 VLBW infants with or without NICU-associated late-onset bloodstream infection admitted to any of three NICUs in the greater Cincinnati region from 2005-2007.²¹ The primary outcomes were mean, per-patient, adjusted in-hospital costs of care and duration of NICU stay. Among the subgroup of infants without major anomaly or major surgery who survived to NICU discharge, infants with NICU-associated bloodstream infection as compared to infants without bloodstream infection had significantly greater cost of care by \$19 272 (mean risk-adjusted overall cost of care = \$116 305; 95% CI, \$95 336-\$137 274 vs \$97 033; 95% CI, \$81 056-\$113 010; $P = 0.01$) and significantly longer duration of NICU stay by 7 days (mean = 54 days; 95% CI, 46-

61 days vs 46 days; 95% CI, 41-52 days; $P = 0.006$). Among the subgroup of infants without major anomaly or major surgery who died prior to NICU discharge, infants with NICU-associated bloodstream infection as compared to infants without bloodstream infection had significantly greater cost of care by \$58 515 (mean risk-adjusted overall cost of care = \$142 673; 95% CI, \$102 116-\$183 230 vs \$84 158; 95% CI, \$52 860-\$115 457; $P = 0.004$) and significantly longer duration of NICU stay by 28 days (mean = 53 days; 95% CI, 35-72 days vs 25 days; 95% CI, 14-43 days; $P = 0.008$). The investigators acknowledged that NICU duration of stay is only one of many factors contributing to the increased costs of NICU-associated bloodstream infection.²¹

Quantification in the United States

One objective of Yale-New Haven Hospital's single-center longitudinal database of neonatal bloodstream infections is to examine changes in outcomes among infants with sepsis.¹² Infants were included in the database from 2004 through 2013 if they were (1) admitted to the NICU of YNHH Children's Hospital, (2) had a traditional neonatal pathogen isolated from blood culture, or (3) had a commensal neonatal pathogen isolated from at least 1 blood culture after which appropriate antimicrobial therapy was administered. Infants were excluded from the database if blood cultures did not meet the aforementioned criteria or if isolated pathogens were believed to be contaminants (eg, *Corynebacterium* and nonspecified Gram-positive bacilli). Additionally, infants were considered to have only 1 sepsis episode if multiple blood cultures drawn from the same infant within 7 days yielded the same species of microorganism with identical antibiotic susceptibility patterns. Over the 10 years spanning 2004-2013, 452 microorganisms were isolated during 410 sepsis episodes among 340 infants. Bizzarro and colleagues

interrogated the database and reported that 10% of sepsis episodes (42 of 410 sepsis episodes) were classified as early-onset sepsis, whereas 90% of sepsis episodes (368 of 410 sepsis episodes) were classified as late-onset sepsis.¹² Among the subgroup of infants with early-onset sepsis, the mean gestational age was 30 ± 6 weeks and the mean birth weight was 1601 ± 1108 grams, with 60% of infants (25 of 42 infants) classified as VLBW. Furthermore, duration of stay in the early-onset subgroup was 45.4 ± 49.9 days and 31% of infants (13 of 42 infants) died. Among infants with late-onset sepsis, the mean gestational age was 29 ± 5 weeks and the mean birth weight was 1283 ± 869 grams, with 72% of infants (215 of 298 infants) classified as VLBW. Duration of stay in the late-onset subgroup was 81.8 ± 58.1 days and 21% of infants (64 of 298 infants) died.¹²

This study has several limitations. First, the Centers for Disease Control and Prevention (CDC) updated its surveillance definition in 2008 to require 2 blood cultures yielding commensal pathogens, as compared to 1 blood culture after which appropriate antimicrobial therapy was administered, to be defined as a positive blood culture.¹² Investigators chose to use the older definition of commensal-related positive blood culture throughout the study period to enhance comparisons over time, but this could also have introduced sampling bias. Second, NICU-wide infection prevention initiatives were implemented in 2008 and 2009. Since these initiatives presumably contributed to a reduction in both true-positive and false-positive blood cultures, especially blood cultures isolating coagulase-negative staphylococci, there could have been a predominance of virulent pathogens since that time that contributed to an inflated mortality rate.¹² Third, the database consists of information gathered from a single center as compared to multiple centers, which decreases external validity.

Other investigators conducted a retrospective analysis of VLBW infants with blood culture-confirmed late-onset sepsis admitted to the NICU of YNHH Children's Hospital between 1989-2007 to characterize clinical and laboratory features associated with mortality.²² The investigators analyzed extensive demographic, antepartum, intrapartum, clinical, and laboratory data from 424 VLBW infants with at least 1 episode of monomicrobial late-onset sepsis, defined as preliminary isolation of a Gram-positive, Gram-negative, or fungal microorganism from blood culture. Infants with episodes of polymicrobial sepsis were excluded, and in infants with multiple episodes of late-onset sepsis during their NICU stay only the first sepsis episode was included in analysis. Among infants who survived sepsis, those infants with fungal sepsis had a longer duration of hospital stay as compared to infants with Gram-positive or Gram-negative sepsis (fungal: median 97 days; range 58-155 days vs Gram-positive: median 63 days; range 4-273 days vs Gram-negative: median 54 days; range 3-351 days; $P < 0.0001$).²² The investigators also reported using logistic regression that sepsis-related mortality was higher among infants with Gram-negative sepsis as compared to Gram-positive or fungal sepsis ($P < 0.0001$). In total, 98 of 424 infants (23.1%) died from sepsis-related causes. Also of note, clinical features, laboratory features, and comorbidities associated with increased mortality risk included endotracheal intubation, prior antibiotic exposure, need for pressors, H₂ blocker exposure, hypoglycemia, neutropenia, thrombocytopenia, leukocytosis, and concurrent NEC.²² Since this study was conducted prior to the change in CDC surveillance definition of commensal species-related bloodstream infection, this study could also have overestimated the burden of sepsis compared to the current source population. By applying current CDC definitions, some infants with coagulase-negative

staphylococci would presumably be excluded and thereby more infants with more virulent bloodstream infections would be included, which could prolong duration of stay and increase mortality rate.

Robinson and colleagues aimed to examine the association between persistent candidemia as compared to non-persistent candidemia and duration of hospitalization or mortality.²³ The investigators retrospectively examined charts from a single NICU and included infants with a single blood culture that yielded any *Candida* species; infants with recurrent candidemia, defined as 2 negative peripheral blood cultures between incidents of *Candida* isolated from blood culture; and infants with persistent candidemia, defined as a *Candida*-related blood culture that remained positive for > 5 days. As revealed by the *k*-sample equality of medians test, there was a trend toward prolonged median duration of hospitalization among infants without persistent candidemia (27 of 37 infants) as compared to infants with persistent candidemia (9 of 37 infants), but not of statistical significance (median 70 days; IQR, 65-110 days vs median 90 days; IQR, 60-90 days; $P = 0.74$). Five of 37 infants with candidemia (14%) died before hospital discharge.²³ This study is limited by its retrospective design and small sample population, for which the authors omitted justification. On the other hand, an advantage of this study is that the investigators appropriately compared duration of stay between groups using nonparametric analysis.

Sohn and colleagues have also reported using a point prevalence survey of 29 NICUs within the Pediatric Prevention Network that infants in the NICU who have nosocomial bloodstream infections, or nosocomial infections of the ear, nose, and throat, lower respiratory tract, or urinary tract, have a significantly prolonged median duration of

stay as compared to infants without infection (median 88 days; range 8-279 days vs median 32 days; range 1-483 days; $P < 0.001$).²⁴ There was also a trend toward increased mortality among infants with nosocomial infection as compared to infants without nosocomial infection, but not of statistical significance (treatment effect and hypothesis-testing data omitted).²⁴ Since a point prevalence study represents only a snapshot in time, these results should be interpreted with caution.

Discharge Complexity

Although reducing NICU duration of stay has numerous benefits, preterm infants of low birth weight who require NICU care as compared to healthy term infants have an increased risk of readmission or death after hospital discharge.²⁵ Among extremely low-birth-weight infants < 27 weeks gestational age, prolonged NICU duration of stay is a risk factor for mortality after discharge from the NICU.²⁶ Preterm infants are 1 of 4 high-risk categories of infants who have complex needs regarding NICU discharge.²⁵ This requires individualized planning and the attending neonatologist's expertise to weigh the risks, benefits, and alternatives of expedited discharge from the NICU to ensure the infant's best medical interests and the family's preparedness to care for their infant at home.²⁵

Additional Potential Confounding Variables

Many studies explore myriad variables associated with duration of stay, morbidity, or mortality among premature and low-birth-weight infants. Lee et al interrogated data from the California Perinatal Quality Care Collaborative, comprising 129 NICUs, to model NICU duration of stay among extremely low-birth-weight infants weighing 401-1000 grams.²⁷ The investigators examined diverse variables, including

maternal characteristics and intrapartum variables (maternal age, race/ethnicity, prenatal care, location of birth, antenatal steroid use, multiple gestation, delivery mode, antenatal conditions), delivery room variables (Apgar score), and infant characteristics (gender, birth weight, gestational age, and small for gestational age). Of many significant associations, birth weight ($P < 0.0001$), gender ($P < 0.0001$), small for gestational age ($P < 0.0001$), black race/ethnicity ($P = 0.0007$), antenatal steroid use ($P = 0.0098$), fetal distress ($P < 0.0001$), maternal hypertension ($P < 0.0001$), 5-minute Apgar score ≤ 3 ($P = 0.0003$), and 5-minute Apgar score 4-7 ($P < 0.0001$) most closely predicted length of stay in the final risk-adjusted model. There was also significant variation in length of stay between hospitals.²⁷ Another study among VLBW infants (defined as 501-1500 grams) compared data gathered from 1995-1996 to that of 1997-2002 with the aim of investigating trends in morbidity and mortality.¹⁷ Comparing the later to the earlier period, survival increased for multiple births (26% vs 22%; $P < 0.05$), antenatal corticosteroid use (79% vs 71%; $P < 0.05$), and maternal antibiotics (70% vs 62%; $P < 0.05$).¹⁷ Inguinal hernia and patent ductus arteriosus are also associated with longer duration of stay among VLBW infants.²⁸

Multiple studies designed to investigate duration of hospital stay or mortality among premature infants 23-36 weeks gestational age confirm the importance of variables reported in studies among low-birth-weight infants, including maternal age, race/ethnicity, prenatal care, location of birth (inborn/outborn), antenatal steroid use, multiple gestation, delivery mode (vaginal vertex/vaginal breech/cesarean section), Apgar score, gender, birth weight, gestational age, small for gestational age, BPD, IVH, NEC, and specific hospital.^{20,29-32} These studies also uncover novel variables related to duration

of stay. Hintz and colleagues reported using a generalized linear model for predicting time to hospital discharge that among infants 25-26 weeks gestational age the odds of early hospital discharge were significantly lower without maternal insurance coverage (OR = 0.40; 95% CI, 0.29-0.54; $P < 0.012$), with chest compression and/or drugs for resuscitation (OR = 0.47; 95% CI, 0.25-0.86; $P < 0.014$), and with diagnosis of patent ductus arteriosus (OR = 0.65; 95% CI, 0.46-0.93; $P < 0.018$).²⁰ Additionally, late-onset sepsis (OR = 1.45; 95% CI, 1.04-2.01; $P = 0.028$), BPD (OR = 4.17; 95% CI, 2.84-6.13; $P < 0.001$), postnatal steroids (OR = 1.67; 95% CI, 1.06-2.62; $P = 0.026$), stage III retinopathy of prematurity (OR = 1.69; 95% CI, 1.10-2.56; $P = 0.014$), and surgery for retinopathy of prematurity, NEC, or patent ductus arteriosus (OR = 2.73; 95% CI, 1.85-4.03; $P < 0.001$) were positively associated with late discharge.²⁰ Among neonates ≤ 28 weeks gestational age, the odds of prolonged hospital stay were significantly higher with 2 or more doses of surfactant (OR = 1.50; 95% CI, 1.17-1.93) and significantly lower with birth in 1999 vs 1998 (OR = 0.63; 95% CI, 0.46-0.87).³¹

Altman and colleagues focused their investigation on moderately preterm infants 30-34 weeks gestational age and found that infants with hyperbilirubinemia ($P < 0.01$) or respiratory distress syndrome ($P < 0.001$) had a significantly higher postmenstrual age (gestational age plus postnatal age)³³ when discharged from the hospital.²⁹ Conversely, infants who received breast milk exclusively or in part as compared to those who did not receive breast milk were discharged from the hospital with a postmenstrual age 2.7 days lower ($P < 0.001$). However, the difference between postmenstrual age at discharge between infants who received breast milk as compared to infants who did not receive breast milk decreased to 1.9 days when controlling for maternal risk factors and neonatal

morbidity in multivariate analysis.²⁹ Other variables among infants 32-36 weeks gestational age reported to influence duration of stay or morbidity include antepartum hemorrhage, including placenta previa and placental abruption,^{34,35} oligohydramnios, intrauterine growth restriction, and maternal hypertension, including chronic hypertension and hypertensive disease of pregnancy.³⁵ Considering both preterm and term infants, hospital discharge rates were significantly lower on the weekends as compared to the weekdays, which also influences duration of stay.³⁶

Transfer from an outside facility,^{37,38} variations in neonatal medical practice,³⁹ even as simple as varying definitions for apnea,⁴⁰ or uncharacterized differences between NICUs^{20,27,32} confound duration of stay^{20,27,32,37,38,40} and mortality.³⁷ Lead-time bias, in which appropriate interventions are initiated earlier in a patient transferred from an outside medical facility, as compared to infants admitted directly or from the emergency department, could explain differences in outcomes.³⁷ Moreover, some centers refer only the sickest infants to tertiary care centers while others refer only vigorous infants.³¹

Conclusion

Three studies investigating the efficacy of adjunctive PTX therapy as compared to placebo, both in addition to routine care, or as compared to solely routine care in infants with sepsis have revealed varying differences in duration of hospital stay between groups. Some investigators have reported a significant reduction in duration of hospital stay when comparing adjunctive PTX and placebo groups⁸ (mean 22.6 days \pm 13.2 days vs mean 33.8 days \pm 16.2 days; mean difference = -11.2; 95% CI, -22.0948 to -0.3052; $P = 0.04$),⁹ while other investigators have reported a trend toward reduced duration of hospital stay in the adjunctive PTX group as compared to the routine care group (15 days vs 30 days)⁷

or a similar duration of hospital stay between groups (PTX: median 28 days; range 5-246 days vs placebo: median 27 days; range 1-168 days; $P > 0.05$).¹¹ Considering the former two studies, when comparing PTX and control groups duration of hospital stay was reduced by 33% and 50%, respectively. Unfortunately, these studies bear substantial sampling, selection, and information bias from nonrandom sampling of participants, improper randomization techniques, inadequate blinding to intervention and control groups, improper statistical operationalization of duration of stay, and incompletely reported hypothesis-testing data. There was also presumably limited ability to detect a difference in treatment effect since duration of stay was a secondary outcome, not to mention sample sizes were rarely justified. Without appropriate sampling, true randomization, adequate blinding, assessment of many confounding variables, and justified, appropriate sample sizes these clinical trials had limited internal and external validity and overestimated differences in duration of stay, morbidity, and mortality between intervention and control groups. Inferences about the efficacy of adjunctive PTX therapy to the source population are tenuous. The question remains unanswered as to whether a clinical trial with improved internal and external validity and sufficient power to detect a difference in treatment effect will confirm or refute the reduction in duration of stay associated with adjunctive PTX therapy among infants with sepsis.

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Chapter 3: Study Methods

Study Design

We propose a multicenter, double-blind, placebo-controlled, parallel-group randomized controlled trial among preterm infants with blood culture-confirmed sepsis to investigate the efficacy of PTX plus appropriate antimicrobial therapy, as compared to placebo plus appropriate antimicrobial therapy, to reduce median duration of stay in the neonatal intensive care unit (see Appendix B).

Study Population and Sampling

The source population will consist of preterm infants with blood culture-confirmed sepsis. The study population will consist of preterm infants with blood culture-confirmed sepsis being cared for in the NICU of Yale-New Haven Hospital Children's Hospital, Bridgeport Hospital, Lawrence and Memorial Hospital, or one of two campuses of Connecticut Children's Medical Center, including Hartford Hospital and University of Connecticut Health Center. We will consecutively sample infants from this study population who fulfill eligibility criteria.

Infants will be empirically evaluated for sepsis when born preterm, if born to a mother with chorioamnionitis, or with clinical and hematologic criteria indicating neonatal sepsis (see Appendix A), each at the discretion of the attending neonatologist. A sepsis evaluation will consist of (1) a complete blood count, (2) two peripheral blood cultures (each with a minimum of 0.5 mL) collected using sterile technique, and (3) urine and cerebrospinal fluid cultures and an abdominal X-ray to investigate urinary tract infection, meningitis, and NEC (defined as pneumatosis intestinalis on X-ray), respectively, as determined by the attending neonatologist. Each sepsis evaluation will be

considered a separate event, so that an individual infant will be eligible for enrollment at each sepsis evaluation during his or her NICU stay.

Inclusion criteria will be: (1) viable infant, defined as the ability, after delivery, to survive (given the benefit of available medical therapy) to the point of independently maintaining heartbeat and respiration; (2) who is preterm (< 37 weeks gestational age); (3) with blood culture-confirmed sepsis (see below); and (4) with legally effective parental informed consent (see Appendix C). Blood cultures will be assessed for carbon dioxide with a fluorescent-detection system. Blood culture-confirmed sepsis will be defined as at least 1 positive preliminary blood culture, defined as isolation of a traditional Gram-positive, Gram-negative, or fungal microorganism. Diagnosis of bloodstream infection with commensal species (eg, coagulase-negative staphylococci) will comply with the updated CDC surveillance definition, which requires commensal species to be isolated from at minimum 2 blood cultures to qualify as a positive blood culture.

Infants will be excluded from enrollment by any of the following: (1) admitted to the NICU for preterminal comfort care (defined as neither intubation nor cardio-respiratory resuscitation); (2) suspected or proven congenital or chromosomal anomalies (defined as anomalies detected at birth involving at least a single organ or organ system); functional states, such as patent ductus arteriosus, will not be defined as anomalies; (3) prior surgery, defined as procedures performed in an operating room, not to include bedside procedures or circumcision; (4) transferred from an outside medical facility; (5) grade III (defined as blood in the ventricles with ventriculomegaly) or grade IV (defined as blood/echodensity in the parenchyma) IVH; (6) prior treatment with PTX; (7) enrolled

in a competing study; or (8) parental refusal. Infants who fulfill all inclusion criteria and no additional exclusion criteria will be enrolled in the proposed study.

Subject Protection and Confidentiality

The proposed study protocol will first be reviewed and approved by the Pediatric Protocol Review Committee and then by the Institutional Review Board (IRB) at each of the 5 study centers prior to initiating the study. Legally effective informed consent (see Appendix C) will be obtained from the newborn's parent(s), or if neither parent is able to consent because of unavailability, incompetence, or temporary incapacity, then from her or his legally authorized representative. If the pregnancy resulted from rape or incest then legally effective informed consent from the father or his legally authorized representative is unnecessary. The informed consent document will be signed and dated by one of the aforementioned individuals as well as by the individual obtaining informed consent. Informed consent documentation will be written in a language that is understandable to the parent or her or his legally authorized representative and will not contain exculpatory language. If the parent or legally authorized representative does not speak English, a certified bilingual translator will be present to facilitate the informed consent process. Furthermore, if the participant is of limited reading ability then an impartial witness will be present during the informed consent process and will also sign and date the informed consent document. Participants will be given a copy of the signed and dated consent document.

An independent data and safety monitoring board (DSMB), consisting of at minimum a neonatologist, pediatric infectious disease specialist, and statistician, will analyze unblinded data as it accrues once 58 infants (one-third of the predetermined

sample population) and again when 116 infants (two-thirds of the predetermined sample population) are recruited for the study to assess benefit or harm to participants. If the DSMB uncovers new information that may impact parents' willingness to allow their infants to continue involvement in the proposed study, then revised consent will be reviewed and approved by the IRB at each study center and signed by participants' parent(s). Consent documentation will also be modified for future participants. The study will be discontinued if the DSMB determines that significant benefit or harm exists in either the intervention or control group such that participants' parent(s) can no longer be reasonably assured equipoise and safety.

A unique study identification number, as opposed to personally identifiable information, will identify study participants. In accordance with Health Insurance Portability and Accountability Act regulations, personally identifiable information and protected health information will be securely stored in a locked file cabinet or in a password-protected file on a password-protected computer and will only be accessible by the principal investigator, predetermined research staff, and the DSMB.

Recruitment

Recruiters will screen all infants admitted for any reason to the NICU of each study center. Recruiters will approach the mothers (or fathers, as previously described) of infants who are viable, preterm, and who do not fulfill any additional exclusion criteria. After legally effective informed consent is obtained, when the infant is confirmed to have sepsis by positive blood culture then the infant will be enrolled in the study.

Methodology Considerations

Allocation and Blinding

After enrollment each participant will be assigned a unique study identification number through a random number generator. Next, a computer program will be used to randomly select identifiers for the PTX and placebo groups. The principal investigator will hold the key linking study participants to their study identification number.

Investigators will be blind to treatment allocation. An independent pharmacist who is neither part of the investigation team nor part of participants' treatment teams will apply an adhesive label bearing the participant's name and medical record number to an identical, opaque, unmarked infusion of PTX or placebo, as selected by the computer program. A research assistant will then hand deliver the infusion to the participant's assigned nurse and remain in the NICU until the nurse confirms that the infusion was initiated. As such, all parties involved in the proposed RCT, including investigators, parents, and medical personnel (such as phlebotomists, nurses, and clinicians) will be blind to group assignment. Investigators, parents, and medical personnel cannot be blind to primary or secondary outcome assessment.

Adherence

If at any time during the study the attending neonatologist in charge of the participant's care decides that it is in a participant's best interests to be removed from the study, then the infant will be removed. Attending neonatologists will make this decision based on adverse events and morbidity (see Secondary Outcomes) as well as overall clinical judgment. Since the intervention and placebo will confer a daily 12 mL fluid load and resultant sodium load for 6 successive days, the attending neonatologist might need

to remove participants, especially those who are extremely low birth weight, from the study based on electrolyte imbalance (eg, hypernatremia, hyperchloremia, etc). Dedicated research assistants will monitor adherence by recording the administration site, dosage, and duration by which study participants receive either PTX or placebo.

Routine Care

Goals of routine care will be to maintain adequate systematic oxygenation and peripheral perfusion and eradicate the bloodstream infection. As with any neonatal emergency, the first step will be to secure the airway, ensure breathing, and facilitate circulation. Supplemental oxygen or noninvasive or invasive mechanical ventilation will be used to maintain systemic oxygenation. Extracorporeal membrane oxygenation is an alternative therapeutic intervention that may be considered in some patients to maintain systematic oxygenation after maximal medical therapy fails. Intravenous fluid resuscitation with isotonic saline or other crystalloids in 10-mL/kg bolus(es) in conjunction with inotropes and steroids, as needed, will be used to maintain peripheral perfusion. Inotropes (with dopamine as a typical first-line agent and dobutamine, epinephrine, or vasopressin as second-line agents) will be given preferentially by central venous access as opposed to peripheral intravenous or, much less commonly, via intraosseous access.

Empirical treatment with parenteral broad-spectrum antimicrobials will be initiated when an infant is suspected of having sepsis. Antimicrobial choice will be guided by the anticipated pathogen, local susceptibility data, and presumed nidus of infection. Antimicrobial dosing is dependent upon an infant's postmenstrual age.

References will be available to aid clinicians with dosing. Once preliminary blood

cultures are positive, parenteral antimicrobial therapy will be adjusted according to the sensitivity pattern of the isolated microorganism. Antimicrobial therapy is typically given for a minimum of 10 to 14 days after blood cultures become negative, but complications such as meningitis may require a longer course.

Study Variables and Measures

Intervention

The intervention will be an intravenous infusion of pentoxifylline, 5 mg/kg/h (daily required volume of PTX will be diluted up to 12 mL using 0.9% sodium chloride and infused at a rate of 2 mL/h), initiated when sepsis is confirmed by positive blood culture and continued for 6 hours on 6 successive days, given concomitantly with appropriate antimicrobial therapy.

Placebo

The placebo will be an intravenous infusion of 0.9% sodium chloride, infused at a rate of 2 mL/h, initiated when sepsis is confirmed by positive blood culture and continued for 6 hours on 6 successive days, given concomitantly with appropriate antimicrobial therapy.

Primary Outcome

The primary outcome will be NICU duration of stay, defined as the number of days of NICU care with day 1 beginning the day of admission to the NICU, incrementing at midnight, and ending with discharge from the NICU or death. Neonatal intensive care unit duration of stay will be the sum of days of care within the study center NICU plus days of convalescent care in a community NICU, if applicable. The attending physician in charge of the participant's care will determine the participant's suitability for discharge

from the NICU. Death will be defined as in-hospital mortality from any cause before discharge from the NICU.

Secondary Outcomes

Adverse events, morbidity, and mortality will be secondary outcomes used to quantify the safety and efficacy of the intervention. Adverse events will include allergic reaction (defined as urticarial or morbilliform rash, toxic epidermal necrolysis, or anaphylaxis), feeding intolerance (defined as tenderness, distension, or increased or absent bowel sounds on abdominal examination; emesis; any change in frequency of stools, frank blood in stools, or guaiac positive stools), hypotension (defined as decrease in blood pressure < fifth percentile for age, or systolic blood pressure > 2 standard deviations less than normal for age, or mean arterial pressure < 30 mm Hg with capillary refill time > 4 seconds), neutropenia (defined as absolute neutrophil count < 2000/mm³), thrombocytopenia (defined as platelet count < 80 000/mm³), hypoproteinemia (defined as total plasma protein level < 5 g/l), metabolic acidosis (defined as pH < 7.25), renal insufficiency (defined as serum creatinine > 2 times upper limit of normal for age, 2-fold increase in baseline creatinine, or urine output < 1 mL/kg/h), disordered peripheral circulation (defined as mottled skin with capillary refill > 4 seconds), hepatic failure (defined as 50% increase in alanine aminotransferase over baseline value or international normalized ratio > 2), cholestatic jaundice requiring phototherapy, duration of supplemental oxygen (days), and duration of assisted ventilation (days).

Morbidity will include disseminated intravascular coagulation (defined as generalized hemorrhagic diathesis with bleeding from venipuncture sites, hypofibrinogenemia < 150 mg/dl, elevated fibrin split products > 10 µg/mL, platelets <

100 x 10³/mm³, and prolonged aPTT or PT for age), multiple organ dysfunction syndrome (defined as organ dysfunction in ≥ 2 organ systems), any grade (I-IV) IVH, periventricular leukomalacia (defined as characteristic cranial ultrasound, CT, or MRI), Bell's stage II or greater NEC, respiratory distress syndrome (defined as respiratory distress and characteristic chest radiograph), BPD (defined as oxygen requirement at 36 weeks postmenstrual age), any stage (I-V) retinopathy of prematurity, patent ductus arteriosus (defined as required treatment for patent ductus arteriosus), and mortality (defined as in-hospital mortality from any cause).

Data Collection

First, dedicated research assistants will be trained in clinical definitions for all data to be collected. These trained research assistants will then abstract and de-identify data concerning baseline variables (see Appendix D) and primary and secondary outcomes by retrospectively reviewing the mothers' medical records and prospectively reviewing the participants' medical records. Research assistants will gather data from participants' entire hospital course. If an infant is transferred to a community NICU for convalescent care then research assistants will record both the transfer date from the study center NICU and the admission and discharge date from the community NICU. For those infants who die during follow-up, research assistants will record the presumed cause of death or the cause of death as noted on autopsy, if available. To improve validity of outcome assessment, an independent committee that is blind to group assignment will adjudicate the primary and secondary outcomes.

Sample Size Calculation

The proposed study will be powered to detect a difference in median NICU duration of stay between PTX and placebo groups. Based on the most recently published study conducted in the NICU at YNHH, infants with a mean gestational age of 29.1 weeks and a mean birth weight of 1314.8 grams who have blood culture-confirmed early-onset or late-onset sepsis are treated with routine care in the NICU for an average of 78 days. The intervention is estimated to reduce NICU duration of stay by 10%. Assuming a 2-tailed α error of 5%, the proposed study will have 80% power to detect an 8 ± 14 -day reduction in NICU duration of stay with a crude sample population of 100 infants, 50 in each group. Power and Precision statistical software was used to calculate this sample size (see Appendix E). The final sample size will be 15% greater than the crude sample size to detect a difference in median, as compared to mean, NICU duration of stay. We are planning for a priori 5% drop out. In addition, we plan to have 80% power for secondary analysis of surviving infants while excluding an estimated 22% of the sample population who may die during follow-up, which is a liberal estimate of mortality rate based on recent data. Multiplying the crude sample size of 100 infants by the inverse of one minus 0.42 yields the final sample size of 174 infants, 87 in each group.

Analysis

Primary analysis will be performed according to the intention-to-treat principle using the most current version of Statistical Analysis System. For univariate analysis of baseline characteristics, we will test for differences between groups in categorical variables using the χ^2 test or Fisher Exact test, as appropriate, differences in continuous variables using the unpaired t test, and differences in ordinal variables using the

Wilcoxon rank sum test. Central tendency of NICU duration of stay will be reported as a median and interquartile range. We will perform a test of normality on raw NICU duration of stay data before performing bivariate analysis. Next, we will use the Wilcoxon rank sum test for unadjusted analysis of median NICU duration of stay between intervention and placebo groups. We will log transform raw NICU duration of stay data prior to multivariate analysis. We plan to use multiple linear regression to control for potential confounding variables (as determined by univariate comparisons with $P \geq 0.05$) when comparing NICU duration of stay between groups. If data is highly skewed and linear regression cannot accurately model NICU duration of stay, we will improve goodness-of-fit using negative binomial regression or Poisson regression. Since it is difficult to quantify differences in care between centers, we predetermine to stratify multivariate analysis by study center. We will perform subgroup analysis of NICU duration of stay between infants with early-onset vs late-onset sepsis; between infants with Gram-positive vs Gram-negative vs fungal sepsis; between cohorts of birth weight; between cohorts of prematurity; as well as analysis of only those infants who remain in the NICU for their entire duration of stay while excluding infants who die during follow-up. Since adverse events, morbidity, and mortality are dichotomous outcome measures, we will test for unadjusted differences between intervention and placebo groups using the χ^2 test and use multiple logistic regression for multivariate models that control for potential confounding variables (as determined by univariate comparisons with $P \geq 0.05$).

Timeline and Resources

To adhere to the allocated 2-year timeframe for recruitment through follow-up, we will utilize NICUs housed within 5 local study centers. There were 410 episodes of

blood culture-confirmed sepsis at YNHH Children's Hospital 54-bed NICU between 2004-2013, which equates to 68 opportunities for recruitment over 20 months if sepsis episodes are evenly distributed. Each of the other 4 study centers has approximately half as many NICU beds as YNHH. We will assume an equivalent incidence of blood culture-confirmed sepsis at each center, providing a pool of 204 infants for recruitment over 20 months. Based on recent trials of therapeutic interventions in term and preterm infants in the NICU, we estimate 10% parental refusal rate. As such, we can feasibly complete rolling recruitment of 174 participants over the course of 20 months, with the last enrolled participants completing their NICU stay within the allocated timeframe.

To complete our study, we will require dedicated research assistants, certified bilingual translators to assist with the informed consent process, an independent DSMB, an independent pharmacist who is neither part of the investigation team nor part of participants' treatment teams, and an independent committee to adjudicate primary and secondary outcomes.

Chapter 4: Conclusion

Advantages

The first advantage of the proposed study is its novelty in being powered to detect a difference in NICU duration of stay among preterm infants with blood culture-confirmed sepsis who are unique in their microbiological flora and acuity of care and who are cared for using advanced therapeutic interventions. Unlike prior studies, enrollment criteria will not be restricted by timing of disease onset, which helps control for inclusion bias,¹ allows for more detailed subgroup analysis, and improves external validity. The proposed study further improves upon similar studies by ensuring true randomization and successful blinding to intervention and placebo groups, log transforming raw NICU duration of stay data for statistical analysis, and justifying an appropriate sample size based on available evidence. The proposed study thereby improves internal validity and augments generalizability to the source population. We also systematically assessed potential confounding variables to improve the validity of multivariate comparisons of NICU duration of stay, adverse events, morbidity, and mortality between PTX and placebo groups. Another advantage of the proposed study is that infants will be followed for the duration of their hospital course until discharge from either the study center NICU or a community NICU. This helps to prevent potential bias toward an underestimation of treatment effect. If infants who are transferred to community NICUs were not included in follow-up, this would potentially increase the number of acutely ill infants who require longer NICU duration of stay within study center NICUs.²

Disadvantages

The proposed study is subject to sampling bias because attending neonatologists, despite having validated clinical signs and hematologic criteria to guide decisions about which infants to evaluate for sepsis, must often rely on their clinical discretion to guide these decisions. The known high false-negative rate of blood cultures is another source of sampling bias.^{3,4} Both sources of sampling bias could exclude infants who have a bloodstream infection from enrollment, thereby decreasing external validity. There is potential bias toward an overestimation of treatment effect when infants die during follow-up. This could decrease the number of acutely ill infants who require longer NICU duration of stay.² Moreover, including only infants with blood culture-confirmed sepsis, as compared to also including infants with suspected sepsis, underestimates the true burden of neonatal sepsis within the NICU and could potentially overestimate treatment effect.⁵ To investigate potential overestimation of treatment effect resulting from in-hospital mortality, we predetermine to increase the sample size by 22% for subgroup analysis with 80% power to detect a difference in treatment effect while excluding infants who die during follow-up. Even within 1 study center, let alone between multiple study centers, duration of stay is potentially confounded by heterogeneous medical care, prognosis, resource allocation, ethical approaches, and clinicians' and parents' attitudes about intensive care among premature and VLBW infants born at the threshold of viability.^{1,2,6-9} Heterogeneity between centers limits generalizability to the source population.¹⁰ Nurse staffing levels^{1,11} and unit census¹¹ also influence patient outcomes but will not be measured in the proposed study. Despite our best effort to recognize potential confounding variables and sources of bias, duration of stay is a composite

outcome influenced by many variables, even those unforeseen. Additionally, this study will not be powered to detect a difference between groups in important secondary outcomes, including adverse events, morbidity, and mortality. As a result of the 2-year timeframe for recruitment through follow-up, we cannot gather data on long-term neurological outcomes.¹²

Clinical and Public Health Significance

Assuming a conservative estimate of daily NICU costs of care (\$1250 per day)¹⁰ and a potentially 8-day shorter NICU duration of stay among infants adjunctively treated with PTX yields a cost savings of \$10 000 per infant. With the most conservative estimate of neonatal sepsis incidence in developed countries (1 in 1000 live births)¹² and 3 953 593 annual live births in the United States in 2011,¹³ approximately 3954 infants suffer from sepsis each year. If each of these infants required prolonged NICU care, the proposed intervention could attenuate socioeconomic burden by approximately \$40 million annually. With more liberal estimates of 0.004% neonatal sepsis incidence¹² and \$2000 daily NICU costs of care,¹⁰ 8 fewer days of care in the NICU among infants adjunctively treated with PTX could reduce healthcare system costs by approximately \$253 million each year. In addition to socioeconomic impact, reducing duration of stay could also ease parental anxiety and promote bonding between infants and their parents,^{10,14} potentially enhancing critical parenting skills.¹⁰ Finally, clinicians may be able to answer more confidently and hopefully when parents frequently ask, “When will my child go home?”^{8,15}

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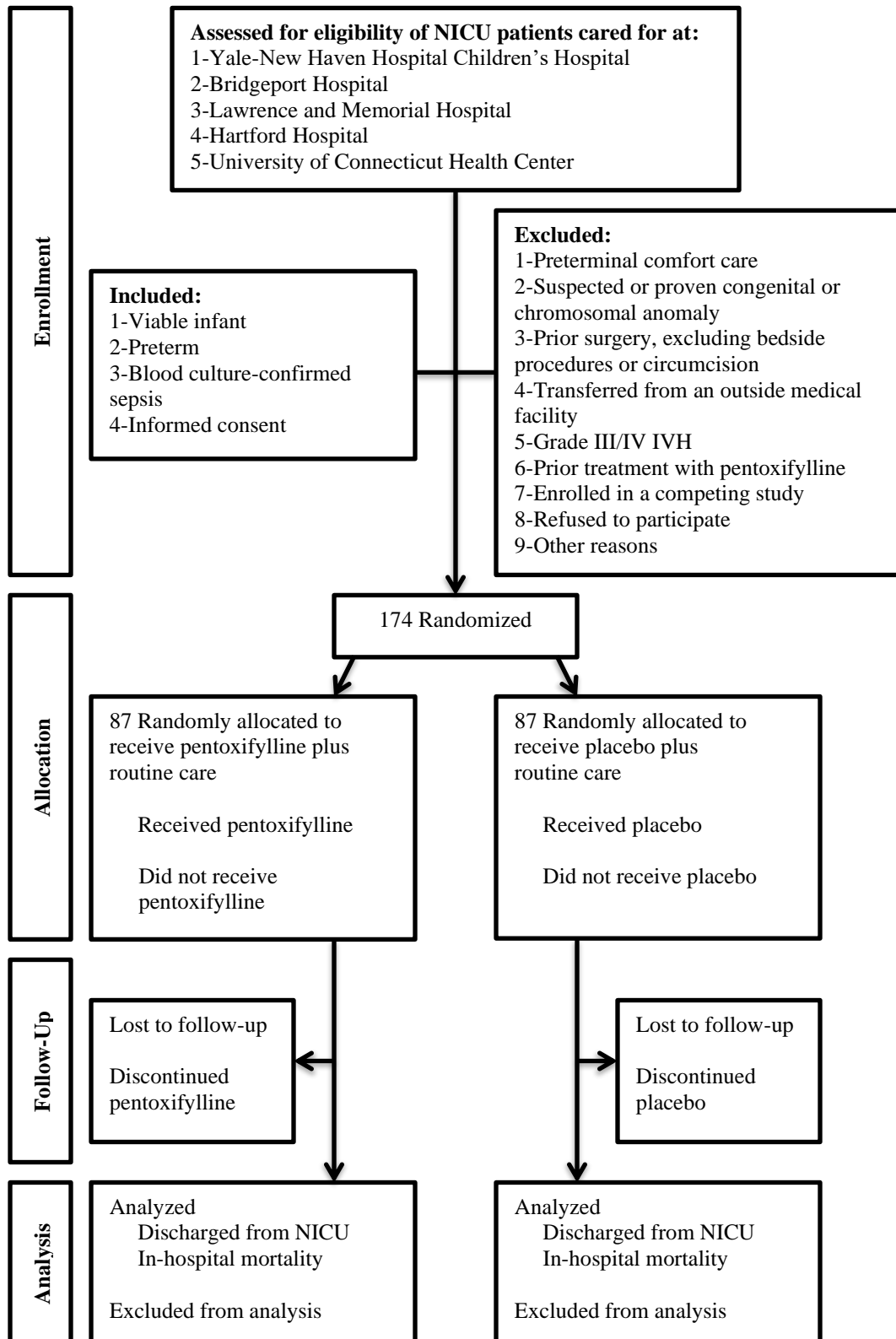
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Appendices

Appendix A: Clinical Signs and Hematologic Criteria of Neonatal Sepsis

Clinical Signs	Hematologic Criteria
<p>Respiratory compromise</p> <ul style="list-style-type: none"> • Tachypnea (respiratory rate of > 60/min) • Increased apneas (cessation of respiration \geq 20 seconds occurring at a rate of \geq 2/h) • Severe apneas (any single episode requiring positive pressure ventilation) • Increased ventilatory support (with no other obvious cause, eg, pneumothorax) • Increased desaturations (pulse oximetry readings \leq 85%) <p>Cardiovascular compromise</p> <ul style="list-style-type: none"> • Bradycardia (heart rate < 100 beats/min) • Pallor • Decreased perfusion (capillary refill \geq 3 seconds or cold extremities) • Hypotension (defined by age-specific percentiles for neonates) <p>Metabolic changes</p> <ul style="list-style-type: none"> • Hypothermia (rectal temperature < 36°C) • Hyperthermia (rectal temperature > 38°C) • Feeding intolerance (increased gastric residuals of > 30% of the volume of food in at least 2 feedings in 24 hours) • Glucose instability (blood sugar < 45 mg/dL or > 125 mg/dL) • Metabolic acidosis (pH < 7.25) <p>Neurologic changes</p> <ul style="list-style-type: none"> • Lethargy • Hypotonia • Decreased activity 	<ul style="list-style-type: none"> • Absolute neutrophil count (ANC) < 7500/mm³ OR > 14 500/mm³ • Absolute band count (ABC) > 1500/mm³ • Immature to total neutrophil ratio (I/T ratio) > 0.16 • Platelet count < 150 000/mm³

Appendix B: Flow Diagram of Study Participants



Appendix C: Informed Consent

COMPOUND AUTHORIZATION AND PARENTAL PERMISSION FOR PARTICIPATION IN A RESEARCH PROJECT

YALE UNIVERSITY SCHOOL OF MEDICINE
YALE-NEW HAVEN HOSPITAL
BRIDGEPORT HOSPITAL
LAWRENCE AND MEMORIAL HOSPITAL
HARTFORD HOSPITAL
UNIVERSITY OF CONNECTICUT HEALTH CENTER

Study Title: Pentoxifylline as an Adjunct to Antimicrobial Therapy to Reduce Length of Stay in Infants with Sepsis

Principal Investigators: Philip J. Yinger, PA-SII; Lindsay C. Johnston, MD

Funding Source: Yale University School of Medicine Physician Associate Program

Parental Permission - Invitation to Have Your Child Participate in a Research Project and a Description of the Project

The purpose of this consent form is to provide you with the information you need to consider in deciding whether to allow your child to participate in this research study.

We are inviting your child to participate in a research study designed to reduce the number of days your child needs to be cared for in the neonatal intensive care unit should your child develop an infection called sepsis, which can occur when there are harmful substances (bacteria or fungi) in the blood. You have been asked to participate because your child is being cared for in the neonatal intensive care unit (NICU) and might develop sepsis. You are under no obligation to participate and, if you decide not to participate, it will not affect your child's care in any way.

In order to decide whether you wish for your child to be a part of this research study you should know enough about its risks and benefits to make an informed decision. This permission form gives you detailed information about the research study, which a member of the research team will discuss with you. This discussion should go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures, possible benefits, and possible alternative treatments. Once you understand the study, you will be asked if you wish for your child to participate. If so, you will be asked to sign this form and will be given a copy to keep as a record.

Description of Procedures

Your child's participation in this study involves obtaining a complete medical history, including maternal health records such as demographic and pregnancy/delivery details. We will review your child's and the mother's medical records to look for factors other than the medicine we are studying that could influence the number of days your child is

cared for in the NICU. The study will include a total of 174 patients admitted to the NICU at Yale-New Haven Hospital Children's Hospital, Bridgeport Hospital, Lawrence and Memorial Hospital, Hartford Hospital, or University of Connecticut Health Center. If your child's medical team is concerned that your child could have an infection in his/her blood, they will draw blood to test for the presence of an infection. Your child's medical team will also give your child antibiotics as per routine care. If the clinical laboratory finds harmful substances (bacteria or fungi) in your child's blood, then your child will continue to be given antibiotics for approximately 10 to 14 days.

If you decide to have your child participate in this study, when the clinical laboratory confirms that your child has an infection in his/her blood then your child will continue to receive antibiotics as per routine care. At the same time, we will use a computer to randomize (like flipping a coin) your child to be given either pentoxifylline (PTX) or placebo, both for 6 days, through an intravenous (inside the vein) site. The research staff and medical team will be blind to which substance your child is given, meaning they will not know whether your child receives PTX or placebo. The entire time your child is being cared for in the NICU neither the research staff nor the medical team will know which substance your child is given.

Pentoxifylline is a medicine that has been shown to decrease the body's response to infection. Specifically, PTX reduces the level of substances (proteins) that allow cells that help to fight infection to communicate with each other. These cells that help to fight infection, called the inflammatory system, can also be harmful to the body when they are over-active. Pentoxifylline can help balance the helpful and harmful aspects of the inflammatory response. Your child could also be randomly selected (like flipping a coin) to receive placebo (like a "sugar pill" or "sugar water"). The placebo we will give is normal saline, which is one of the possible fluids given to your child when his/her blood pressure is low. Normal saline is not currently known to influence the way your child's body responds to infection. The study will not require you or your child to do anything else after 6 days of receiving either PTX or placebo in addition to antibiotics.

Since there is not conclusive evidence about whether PTX can help children with sepsis recover and be discharged from the NICU more quickly than children with sepsis not given PTX, there is the same chance of benefit or harm whether your child is given PTX or placebo. The purpose of this study is to determine whether PTX can help children with sepsis be discharged from the NICU more quickly. The doctor in charge of your child's medical team will determine when your child is ready to be discharged from the NICU. The research staff will have no influence over the timing of your child's discharge from the NICU. We will look at your child's medical chart to determine the number of days he/she is cared for in the NICU, and also to determine whether he/she experiences medical problems that other children in the NICU sometimes experience.

A group of experts who are not part of the research team will monitor medical information about all children involved in the study. You will be told of any significant new findings that develop during the course of your child's participation in this study that may affect your willingness to continue to participate.

Risks and Inconveniences

We estimate that the risks of the present protocol to the patient are minimal. Prior studies among children given PTX did not report problems with irritability or vital signs, such as temperature, heart rate, breathing rate, and blood pressure. However, participation in this study may involve risks that are currently not known. Your child will be monitored per standard practice in the NICU. The medical team will be in charge of your child's care and will treat any problems, such as with your child's brain, eyes, heart, lungs, kidneys, and stomach or intestines, as they would if your child was not involved in the study. The study protocol allows your child to receive antibiotics for the same duration of time your child would if he/she was not in the study, and also allows your child to be transferred or discharged from the NICU according to the advise of the physician in charge of the medical team.

Potential Benefits

We hope that the knowledge gained by your child's participation in this study may eventually lead to a better treatment of sepsis in infants, as well as a shorter required NICU stay for infants with sepsis. Your child's participation in this study may help physicians manage patients with infection better in the future.

Alternatives

Pentoxifylline is not part of routine care by which infants with sepsis are currently managed. You have the option of deciding whether your child may or may not participate in the study. If you choose not to participate in the study, it will not affect your child's care at Yale-New Haven Hospital Children's Hospital, Bridgeport Hospital, Lawrence and Memorial Hospital, Hartford Hospital, or University of Connecticut Health Center, either now or in the future.

Economic Considerations

No payment will be provided for participation in this study. All costs associated with your child's hospitalization and treatment for his/her neonatal condition will be billed to you or your insurance company, as would be done if your child were not participating in this study.

Confidentiality and Privacy

All identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. When the results of the research are published or discussed in conferences, no information will be included that would reveal your child's identity unless your specific permission for this activity is obtained. No public disclosure of this information will be made.

We understand that information about your child obtained in connection with their health is personal, and we are committed to protecting the privacy of that information. If you decide to allow your child to be in this study, the researcher will get information that

identifies your child and his or her personal health information. This may include information that might directly identify your child, such as his or her name, date of birth, and medical record number. This information will be de-identified at the earliest reasonable time after we receive it, meaning we will replace your child's identifying information with a code that does not directly identify him or her. The principal investigator will keep a link that identifies your child to his or her coded information, and this link will be kept secure and available only to the principal investigator or selected members of the research team. Any information that can identify your child will remain confidential. This information will be maintained in a password-protected file on a password-protected computer. All copies of this consent form will be maintained in a locked file cabinet. There will be nothing in your child's hospital record that shows his/her participation in this study. The research team will only give this coded information to others to carry out this research study. The link to your child's personal information will be kept until the study data is analyzed, after which time the link will be destroyed and the data will become anonymous. The data will be kept in this anonymous form indefinitely. The information about your child's health that will be collected in this study includes:

- Research study records.
- Medical and laboratory records of only those services provided in connection with this Study.
- The entire research record and any medical records held by Yale-New Haven Hospital Children's Hospital, Bridgeport Hospital, Lawrence and Memorial Hospital, Hartford Hospital, or University of Connecticut Health Center created from his/her birth through participation in this study.
- Maternal health records including demographic and pregnancy/delivery details will be collected.

Information about your child and your child's health which might identify your child may be used by or given to:

- The U.S. Department of Health and Human Services (DHHS) agencies.
- Representatives from Yale University, the Yale Human Research Protection Program and the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential.
- Those providers who are participants in the Electronic Medical Record (EMR) system.
- Those individuals at Yale who are responsible for the financial oversight of research including billings and payments.
- The Principal Investigators (Philip J. Yinger and Dr. Lindsay C. Johnston).
- Governmental agencies to which certain diseases (reportable diseases) must be reported.
- Health care providers who provide services to your child in connection with this study.
- Laboratories and other individuals and organizations that analyze your child's health information in connection with this study, according to the study plan.

- Co-Investigators and other investigators.
- Study Coordinator and Members of the Research Team.
- Data and Safety Monitoring Boards and others authorized to monitor the conduct of the Study.
- Others as noted: Independent adjudication committee to confirm the duration your child spent in the NICU and to confirm other health problems sometimes experienced by children in the NICU; independent pharmacist to dispense either PTX or placebo.

By signing this form, you authorize the use and/or disclosure of the information described above for this research study. The purpose for the uses and disclosures you are authorizing is to ensure that the information relating to this research is available to all parties who may need it for research purposes.

All health care providers subject to HIPAA (Health Insurance Portability and Accountability Act) are required to protect the privacy of your and your child's information. The research staff at the Yale School of Medicine, Yale-New Haven Hospital, Bridgeport Hospital, Lawrence and Memorial Hospital, Hartford Hospital, and University of Connecticut Health Center are required to comply with HIPAA and to ensure the confidentiality of your and your child's information in ways not mentioned in this form. However, to better protect your child's health information, agreements are in place with these individuals and/or companies that require that they keep your child's information confidential.

You have the right to review and copy your child's health information in your child's medical record in accordance with institutional medical records policies. There will be nothing in your child's hospital record that shows his/her participation in this study. We will keep a copy of this parental consent form, and we will provide you with a copy for your records. If in the future you desire any information about the outcome of this research, you can contact us and we will be more than willing to provide you with a summary of the results.

The results of the research will not be recorded in the infant's medical record, nor will the results be reported back to the parents or the infant's physician and will have no impact on care.

In Case of Injury

If your child is injured as a result of his or her participation in this study, treatment will be provided. Yale School of Medicine, Yale-New Haven Hospital, Bridgeport Hospital, Lawrence and Memorial Hospital, Hartford Hospital, and University of Connecticut Health Center do not provide funds for the treatment of research-related injury. You or your insurance carrier will be expected to pay for the costs of this treatment. No additional financial compensation for injury or lost wages is available. You and your child do not give up any of your legal rights by signing this form.

Voluntary Participation and Withdrawal

Your child's participation in this study is completely voluntary. You can decide not to participate or withdraw from the study at any time during its course. If you choose not to participate or withdraw from the study, it will not affect your child's medical care, the payment for your child's health care, or your child's health care benefits at Yale-New Haven Hospital, Bridgeport Hospital, Lawrence and Memorial Hospital, Hartford Hospital, or University of Connecticut Health Center either now or in the future. However, you will not be able to enroll your child in this research study and your child will not receive study procedures as a study participant if you do not allow use of your child's information as part of this study.

If you do allow your child to become a participant, you are free to stop and withdraw from this study at any time during its course. To withdraw from the study, you can call a member of the research team at any time and tell them that your child no longer wants to take part. When you withdraw your child's permission, no new health information identifying your child will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to insure the integrity of the study and/or study oversight.

The authorization to use and disclose your and your child's information will never expire unless and until you change your mind and revoke it.

The study doctors may decide to remove your child from the study if they believe that it is in his/her best interests. If you choose not to allow your child to participate or if you withdraw your child, it will not harm your relationship with your own doctors or with Yale-New Haven Hospital, Bridgeport Hospital, Lawrence and Memorial Hospital, Hartford Hospital, or University of Connecticut Health Center. Withdrawing from the study will involve no penalty or loss of benefits to which your child is otherwise entitled.

Questions

We have used some technical terms in this form. Please feel free to ask about anything you don't understand and to consider this research and the permission form carefully – for as long as you feel is necessary – before you make a decision. If you have any questions, please do not hesitate to ask and we will do our best to answer them.

Authorization and Permission

I have read this form (or someone has read it to me) and have decided to allow my child to participate in the project described above. Its general purposes, the particulars of my child’s involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this permission form. I understand that my participation is voluntary and that I can withdraw my child from the study at any time without prejudice. I have read the above and agree to enter this research study. Signing this form does not waive any of my legal rights. By signing this form, I give permission to the researchers to use and give out information about my child for the purposes described in this form. By refusing to give permission, I understand that my child will not be able to be in this research.

Name of Subject: _____ Name of Subject: _____

Relationship: _____ Relationship: _____

Signature: _____ Signature: _____

Date: _____ Date: _____

Signature of Person Obtaining Permission Date

If you have further questions about this project or if you have a research-related problem, you may contact the Principal Investigator [Philip J. Yinger, PA-SII at (xxx) xxx-xxxx].

If after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at (203) 432-5919.

If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.

***THIS FORM IS NOT VALID UNLESS THE FOLLOWING BOX
HAS BEEN COMPLETED IN THE HIC OFFICE***

THIS FORM IS VALID ONLY THROUGH:

INITIALED:

Appendix D: Baseline Characteristics

Baseline Characteristics
Maternal characteristics and intrapartum variables <ul style="list-style-type: none">• Maternal age (years)• Race/ethnicity (free text)• Marital status (single/married)• Level of education (less than high school, high school graduate, post-secondary graduate)• Mother's primary health insurance (public, private, unknown)• Prenatal care (Y/N)• Location of birth (inborn/outborn)• Multiple gestation (singleton/multiple)• Chronic hypertension (Y/N)• Hypertensive disease of pregnancy (Y/N)• Established diabetes (Y/N)• Gestational diabetes (Y/N)• Unspecified fever > 38.3°C (Y/N)• Chorioamnionitis (Y/N)• Group B <i>Streptococcus</i> colonization status (colonized/not colonized)• Antenatal antibiotic use (Y/N)• Antenatal steroid use (Y/N)• Oligohydramnios (Y/N)• Antenatal hemorrhage (placenta previa or placental abruption) (Y/N)• Prolonged rupture of membranes (> 18 hours from membrane rupture to delivery) (Y/N)• Antenatal fetal distress (Y/N)
Delivery room variables <ul style="list-style-type: none">• Delivery mode (Vaginal vertex, vaginal breech, cesarean section)• 1-minute Apgar score (0-10)• 5-minute Apgar score (0-10)• 10-minute Apgar score (0-10)• Positive pressure ventilation (Y/N)• Endotracheal intubation (Y/N)

<ul style="list-style-type: none"> • Chest compressions (Y/N) • Drugs for resuscitation (Y/N)
<p>Infant characteristics</p> <ul style="list-style-type: none"> • Birth year (free text) • Gender (M/F) • Gender-specific birth weight percentile (percentile) • Gestational age (completed weeks) • Birth weight (grams) • Small for gestational age (birth weight < 10th percentile for gestational age) (Y/N)
<p>Comorbidities</p> <ul style="list-style-type: none"> • Accompanying focal infections (lungs, urinary tract, skin, other) • Intraventricular hemorrhage (grade I/II) (Y/N) • Periventricular leukomalacia (Y/N) • Necrotizing enterocolitis (Y/N) • Respiratory distress syndrome (Y/N) • Therapeutic surfactant (number of doses) • Bronchopulmonary dysplasia (Y/N) • Retinopathy of prematurity (Y/N) • Hydrocephalus (Y/N) • Meconium aspiration syndrome (Y/N) • Pneumothorax (Y/N) • Required treatment for patent ductus arteriosus (Y/N) • Inborn errors of metabolism (Y/N) • Inguinal hernia (Y/N)
<p>Clinical variables</p> <ul style="list-style-type: none"> • Mental status change (lethargy, hypotonia) (Y/N) • Severe apnea (any single episode requiring positive pressure ventilation)/bradycardia (heart rate < 100 beats/min) (Y/N) • Abdominal distension/feeding intolerance (Y/N) • Hypotension/symptoms of shock (Y/N) • Indwelling Foley catheter (Y/N) • Central venous catheter (Y/N) • Total parenteral nutrition/intralipid solution (Y/N) • H₂ blockers (Y/N) • Postnatal steroids (Y/N) • Endotracheal intubation (Y/N) • Duration of any respiratory support (supplemental oxygen from 6 hours to at least 24 hours of age; mechanical ventilation and/or continuous positive airway pressure at 24 hours of age; conventional or high-frequency ventilation at any time during hospitalization) (days) • Cardiovascular support (volume resuscitation or inotropes) (Y/N) • Prior treatment with another phosphodiesterase inhibitor or anticoagulants (Y/N) • Received breast milk at any time during NICU stay (Y/N) • Time to reach full feeds (days)

Laboratory variables

- Hypoproteinemia (Y/N)
- Glucose instability (Y/N)
- Leukocytosis/leukopenia (Y/N)
- Direct hyperbilirubinemia (Y/N)
- Indirect hyperbilirubinemia (Y/N)
- Metabolic acidosis (Y/N)

Sepsis-related variables

- Timing of disease onset (early-onset/late-onset)
- Temperature instability in the 24 hours preceding sepsis evaluation (Y/N)
- Glucose instability in the 24 hours preceding sepsis evaluation (Y/N)
- Neutrophil abnormalities (neutropenia) at the time the blood culture was obtained (Y/N)
- Platelet abnormalities (thrombocytopenia) at the time the blood culture was obtained (Y/N)
- Duration of antibiotic exposure prior to positive blood culture (days)
- Age at sepsis evaluation (days)
- Corrected gestational age at sepsis evaluation (weeks)
- Isolated microorganism (Gram-positive, Gram-negative, fungal; identified species)

Appendix E: Sample Size Calculation

Group	Population Mean	Standard Deviation	N Per Group	Standard Error	95% Lower	95% Upper
Pentoxifylline	70.0	14.0	50			
Placebo	78.0	14.0	50			
Mean Difference	-8.0	14.0	100	2.80	-13.54	-2.46

Alpha= 0.050, Tails= 2, Power = 0.808

Computational option: Variance is estimated (t-test)

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