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Coagulase-negative Staphylococcus Sepsis in Preterm Infants and Long Term Neurodevelopmental Outcome

by

Belal Alshaikh

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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ABSTRACT

Objective: To examine the effect of *Coagulase-negative Staphylococcus (CoNS)* sepsis in preterm infants on the neonatal and neurodevelopmental outcomes at 36 months corrected age (CA).

Design: A retrospective cohort study.

Subjects: All preterm infants with gestational age ≤ 28 weeks.

Results: A total of 105 eligible infants were exposed to *CoNS* sepsis between 1995 and 2008. Infants exposed to *CoNS* sepsis were less mature (25.9 ± 1.7 vs. 26.2 ± 1.4, p=0.04), had increased risk of retinopathy of prematurity (ROP) (adjusted RR 1.32; 95% CI 1.11 – 1.54), and were more likely to stay longer in the neonatal intensive care unit. Multivariable logistic regression analysis revealed *CoNS* sepsis is an independent predictor for cognitive delay (adjusted RR 1.98; 95% CI 1.01 – 3.63).

Conclusions: *CoNS* sepsis in preterm infants is associated with increased risk for ROP in the neonatal period and for cognitive delay at 36 months CA.

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LIST OF ABBREVIATIONS

BPD: Bronchopulmonary Dysplasia

BSID: Bayley Scale of Infant Development

BW: Birth Weight

CI: Confidence Interval

CoNS: Coagulase negative staphylococcus

CP: Cerebral Palsy

CPAP: Contentious Positive Airway Pressure

CSF: Cerebrospinal Spinal Fluid

CVCs: Central Venous Catheters

DA: Ductus Arteriosus

ELBW: Extremely Low Birth Weight (< 1000 g)

FMC: Foothills Medical Centre

GA: Gestational Age

HAI: Health Associated Infection

HMGB1: High Mobility Group Box 1

IVH: Intraventricular Hemorrhage

IL: Interleukins

IQR: Interquartile Range

LPS : Lipopolysaccharide

MDI: Mental Developmental Index

M-H Test: Mantel Haenszel Test

NEC: Necrotizing Enterocolitis

NICHD: National Institute of Child health and Human Development

NICU: Neonatal Intensive Care Unit

O2: Oxygen

OR: Odds Ratio

PAMPs: Pathogen-Associated Molecular pattern

PDA: Patent Ductus Arteriosus

PDI: Psychomotor Developmental Index

PNFU: Perinatal Follow Up Clinic

PVL: Periventricular Leukomalacia

RDS: Respiratory Distress Syndrome

ROP: Retinopathy of Prematurity

RR: Risk Ratio

SD: Standard Deviation

SGA: Small for Gestational Age

SIRS: Systematic Inflammatory Response Syndrome

TNF: Tumor Necrosis Factor

TPN: Total Parenteral Nutrition

VLBW: Very Low Birth Weight (<1500 g)

WISC-IV: Wechsler Intelligence Scale for Children- IV

WMI: White Matter Injury

WPPSI: Wechsler Preschool and Primary Scale of Intelligence

CHAPTER ONE: INTRODUCTION

Staphylococcus is a family of Gram positive bacteria. They appear under the microscope as round (cocci), and form in grape-like clusters.[1] They are a small component of soil microbial flora. Many of this genus reside normally on the skin of humans.

Coagulase-Negative Staphylococcus (CoNS) is one of 33 known species belonging to the family of *Micrococcaceae*.[2] It is part of human skin and mucous membranes flora. It also can be found in animals. Although *CoNS* is not usually pathogenic, patients with compromised immune systems like preterm infants are at risk for developing an infection.

Friedrich Rosenbach was the first one to distinguish *Staphylococcus epidermidis* (the most common type of *CoNS*) from *Staphylococcus aureus* in 1884.[3] Initially he named *Staphylococcus epidermidis* as *Staphylococcus albus*. He chose the names *aureus* and *albus* because the bacteria formed yellow and white colonies, respectively.[4]

1.1 Introduction to the Research Problem

Coagulase-negative staphylococcus (CoNS) is the most common cause for late onset sepsis (more than 72 hours of life) in infants born weighing less than 1500 g at birth.[5-7] The incidence of *CoNS* infection in neonatal intensive care units (NICUs) varies between 1.3% and 30.9%, depending on gestational age, birth weight and presence of intravascular catheters.[8-10] Overall, *CoNS* is responsible for almost half of bloodstream infections in very low birth weight (VLBW) infants born weighing $\leq$ 1500g.[5]

The infection rate has increased since the 1980s with the increase in survival of VLBW infants and the use of prolonged instrumentation, such as indwelling intravascular lines and endotracheal intubation.[7, 11] Fortunately, *CoNS* infection is generally less severe than infections caused by other pathogens and the mortality rate is less as compared to gram negative infection.[8]

However, many reports in the last few years have raised concerns about the neurodevelopmental outcome in surviving VLBW infants after an episode of *CoNS* sepsis.[12, 13]

1.2 Significance of the Problem

Healthcare associated infection (HAI) is considered a common cause of morbidity in VLBW infants. In Canada, the rate of infection in VLBW infants varies from 6.7% to 74.5%.[14] *CoNS* is the most commonly isolated etiological agent of infection in preterm infants. In general, preterm infants with infection are at increased risk for death, have a longer hospital stay, and utilize more resources than non-affected infants.[6]

Most *CoNS* infections in neonates are catheter-related bloodstream infections, because extremely preterm infants typically require the delivery of nutrients and drugs over long periods via intravenous access.[15] Intravenous access in these infants usually involves the use of central venous catheters (CVCs). Despite the widespread use of aseptic technique in NICUs, the obligatory use for CVCs and the deficient immune system of these infants have maintained relatively high incidence of *CoNS* infection in this population.

Advances in neonatal care in modern NICUs are responsible for increased survival of VLBW infants. However, morbidity and long term neurodevelopmental outcome have minimally changed. Until recently, *CoNS* blood stream infections in preterm infants were considered a “soft infection” due to associated low mortality rate. However, the increased morbidity in these infants has led investigators to study the impact of *CoNS* infection on the premature lung, brain and gut.[12, 13]

Despite the many studies showing a strong association between neonatal sepsis and poor neurodevelopmental (ND) outcome, the specific pathogens associated with poor ND outcome have rarely been reported. Advances in bacterial genetic engineering and human immune

enhancement have opened the door to produce immunoglobulins to protect from *Staphylococcus epidermis* (the most common type of *CoNS*). Recently, investigations using these immunoglobulins have started in preterm infants with limited success.[16, 17]

Finding an association between *CoNS* infection and poorer ND outcome will emphasise the need for more research work to prevent *CoNS* infections. The major weaknesses in the few studies in the neonatal literature that addressed the relationship between *CoNS* sepsis and neurodevelopmental outcomes includes using only one early follow up at 18 to 22 months of age or looking for *CoNS* infection without adjusting for important potential confounding factors such as necrotizing enterocolitis (NEC).[13, 18, 19] Having a better understanding of long term ND outcomes associated with *CoNS* sepsis in preterm infants will improve future management in the neonatal period and help in the prognostication of these preterm infants.

1.3 Literature Review

1.3.1. Definition of *CoNS* Sepsis

Although general definitions of the sepsis continuum have been published for the pediatric population, neonatal health care providers continue to use these definitions interchangeably. The international pediatric consensus conference on pediatric sepsis has defined sepsis as a medical condition characterized by presence of systemic inflammatory response syndrome (SIRS) as a result of infection.[20] Septicemia, another related term, refers to the presence of the pathogenic organism in the bloodstream leading to sepsis. This term has been erratically used by medical professionals in the past to describe the bacteremia which refers to the presence of the organism in the blood in the absence of SIRS.[21]

“Infection” is another term used to describe a proven (by positive culture, tissue stain, or polymerase chain reaction test) infection caused by pathogen or a clinical syndrome associated

with high probability of infection.[20] Evidence of infection in neonate may include findings on clinical exam, imaging, or laboratory tests.

Different definitions have been suggested for the diagnosis of a true *CoNS* sepsis in the neonatal period. The difficulty in diagnosis results from an inability to differentiate infection from contamination with skin commensal flora.[22] Contamination usually occurs during breaking skin integrity for blood collection or intravenous line placement.

The most commonly used definition for *CoNS* sepsis in neonates involves the clinical signs of infection associated with one positive blood culture taken from the peripheral vein or other sterile body fluids and treatment with intravenous antibacterial therapy for at least 5 days after obtaining blood culture or until death (occurring within 5 days after obtaining blood culture).[10, 23-25] A recent study suggested more stringent criteria which include clinical signs of infection and two positive blood cultures from different sites.[26] Using two positive blood cultures for the diagnosis of *CoNS* infection reduced antibiotic usage in 8.2 % and this was speculated to reduce the antibiotic resistance, but at the cost of many vein punctures for blood culture in small preterm infants. Therefore, this definition is less attractive in clinical practice.[27]

Some neonatologists use associations between one positive culture and elevated inflammatory markers such as C reactive protein (CRP) and procalcitonin as an indicator for true *CoNS* sepsis.[28] However, the timing needed for the inflammatory markers to elevate after the *CoNS* sepsis begins often requires multiple blood sampling for these small infants. Polymerase chain reaction (PCR) is a promising rapid method to establish the diagnosis of *CoNS* sepsis by detecting the staphylococcal DNA. PCR has been suggested as an alternative for blood culture. However, most studies recommend doing blood culture in addition to PCR to establish the diagnosis of blood stream infection.[29, 30]

Isolation of *CoNS* from other body fluids (e.g. urine) or the tips of central venous lines is less accurate in determining true infection. *CoNS* have been identified in peritoneal fluid of 30% of infants with NEC that requiring surgery.[31]

1.3.2 Persistent *CoNS* Infection in the Neonatal Population

Several studies suggest that persistent *CoNS* infection is increasing in preterm infants in modern NICUs.[32, 33] The rate of persistent *CoNS* infection ranges between 13% and 48%.[9, 33] Persistent *CoNS* sepsis was defined as three or more consecutive positive blood cultures at least 48 hours apart with the same *CoNS* species during a single septic episode.[33, 34] In a recent study, intubation and presence of central indwelling catheters were important risk factors for persistent *CoNS* infection when assessed separately; however, the biofilm production was the only significant effect when the risk factors were tested jointly (OR 4.69; 95% CI 1.59-13.84).[34] Persistent *CoNS* sepsis has been associated with severe thrombocytopenia and higher C-reactive protein (CRP) during the bacteremia phase, but no study has evaluated whether persistent *CoNS* has a different effect from non-persistent *CoNS* infection on short or long term outcome.

1.3.3. Incidence of *CoNS* Sepsis

The overall incidence of late onset sepsis among VLBW infants ranged from 21% to 24 %.[5, 6] This rate has been fairly consistent over the years in North America.[5, 6] However, at individual NICUs, rates vary from 10% to 38%.[6, 14, 35] *CoNS* is usually the causative pathogen in 40% to 77.6% of episodes of blood stream infection in neonates.[5, 6, 10, 26, 36]

The incidence is inversely related to gestational age and birth weight.[6, 36] Gray et al shows no difference in cumulative incidence of *CoNS* sepsis between inborn and outborn infants.[36] Recurrent bacteremia can affect up to 15% of VLBW during their stay in NICU.[36]

Overall, *CoNS* is responsible for 48% of bloodstream infections in VLBW infants, 29% of eye, ear, nose, and throat infections, 19% of skin and soft-tissue infections, 16% of pneumonias and 10% of infections of the gastrointestinal tract.[6, 11]

1.3.4. Pathogensis of *CoNS*

CoNS colonization of preterm infants begins immediately after birth. Most of these infants are colonized by *CoNS* derived from the body of the mother or other human contacts in NICU. *CoNS* can be isolated from skin, gut, or the respiratory system of most preterm infants during their stay in NICU.[37, 38]

Preterm infants have immature immune systems. Qualitative and quantitative deficiency of complement and IgG factors in these infants and particularly in the VLBW population increases the risk of *CoNS* infection. In addition, preterm infants stay in the NICU for prolonged time. They require long term total parenteral nutrition (TPN) via CVCs. The longer the CVC lines are required for these preterm infants, the greater chance to acquire *CoNS* infection.[39] Chien et al. showed that 22.5% of infants admitted to NICU in Canada require CVCs.[40] The incidence of blood stream infection in this study varies from 2.9 per 1000 noncatheter days, to 7.2 per 1000 umbilical venous catheter days, and 13.1 per 1000 percutaneous catheter days.[40]

In total, 38 species of *CoNS* have been recognized; of these 18 species are known to colonize humans and cause systemic infection.[41, 42] *Staphylococcus epidermidis* accounts for almost 75% of *CoNS* organisms isolated from humans with clinical infection.[43] In comparison to *Staphylococcus aureus*, *CoNS* produces a limited number of virulence factors.[44]These factors

include capsular polysaccharides, surface proteins and plasmids.[45] These virulence factors involve adherence of the organism to the host (e.g. skin) or a foreign body surface (e.g. CVCs) followed by secretion of polysaccharide and formation of a biofilm. This biofilm enables the organism to evade the host defence mechanisms and antibiotics.[46] Formation of biofilm is similar among various microbes and requires a series of discrete steps, however; the exact molecular mechanisms may differ from organism to organism. These steps include attachment of cells to organic or inorganic surfaces, the aggregation of cells into microcolonies, and the maturation and maintenance of architecture [47, 48] Biofilm has been shown to possess hemagglutination activity, provide resistance to human antibacterial peptides, inhibit phagocytosis and killing by polymorphonuclear neutrophils and suppress T-cell function by stimulating production of PGE_2 from monocytes.[49-51] De Silva et al. showed that the quantity of biofilm produced by *Staphylococcus epidermidis* may determine the ability to cause infection in the neonate.[27] Antibiotics resistance is also significantly correlated with biofilm production in *Staphylococcus epidermidis*.[52, 53]

The Plasmids of *CoNS* organisms seem to be an important mechanism for the spread of antibiotic resistance particularly to aminoglycoside and beta-lactam agents.[45] The role of surface proteins in the pathogensis of *CoNS* is not completely established.[45] However, the few toxins produced by *CoNS* are originally the proteins released from its surface. One example of these proteins is the Delta toxin. Investigators showed that Delta toxin produced by *Staphylococcus epidermidis* is frequently associated with NEC in neonates.[54]

***CoNS* Infection and Intravascular Catheters:**

The majority of *CoNS* bloodstream infections are resulted from intravascular catheters. All extremely preterm infants require either peripheral or central intravenous in the first few weeks

of life because of their need for parenteral nutrition and other intravenous drugs. Because peripheral intravenous catheters are used for short period and changed frequently, they are not as commonly infected as CVCs. The pathogenesis of infections in short term peripheral intravenous catheters usually results from *CoNS* stemming from the patient's skin, migrating via the surface of the catheter to gain access to the bloodstream.[55] In contrast, for long term catheters and CVCs, hub colonization (either from the preterm infant's skin flora or from the health care environment) and migration of *CoNS* via the luminal surface becomes very important.[55]

1.3.4 *CoNS* Sepsis and Neonatal Morbidities

1.3.4.1 *CoNS* Sepsis and Bronchopulmonary Dysplasia (BPD)

Sepsis has been implicated as a potential cause of lung injury. Many studies have shown increased risk of lung injury and BPD in preterm infants with pulmonary infection or blood-stream infection. Lung injury was explained by the effect of pro-inflammatory cytokines and the inflammation process. Unexpectedly, *CoNS* was found to play a considerable role. An early animal study showed that the most common primary isolate from lungs at autopsy of baboon models with BPD was *CoNS*; however the collected blood cultures were negative.[56] Beeton et al. have demonstrated that early and late microbial presence in human premature lung fluid is significantly associated with the development of BPD. The microbial presence was identified by amplification of 16S rRNA genes and associated with increase proinflammatory cytokines namely IL 6 and IL 8. *Staphylococcus epidermidis* have been associated with increased levels of these cytokines in preterm infant with BPD.[57] Although *CoNS* isolated from the respiratory tract are often thought to be contaminants, a recent study by Lahra et al. has clarified that bloodstream neonatal sepsis caused by *Staphylococcus epidemidis* has an effect on the

development of BPD. This effect was similar to other more virulent organisms (OR 3.17; 95% CI 2.08-4.83), and completely independent from intrauterine infection and chorioamnionitis.[58]

1.3.4.2 *CoNS* Sepsis and Patent Ductus Areteriosus (PDA)

Another postulated mechanisms for the development of BPD in preterm infants with neonatal sepsis is the PDA. Investigators have described the association between sepsis and increased level of prostaglandins and tumor necrosis factor alpha (TNF- a).[59] These two factors could explain the influences of neonatal infection on late ductal reopening and ductus arteriosus (DA) closure failures.[59] Recently, Chiang et al. showed that preterm infants with PDA had a significantly higher rate of BPD (81.0% vs. 61.0%, p= 0.002) and a relatively higher rate of recurrent sepsis (25.3% vs. 15.2%, p=0.079).[60] *CoNS* was the most common organism in their cohort. Moreover, Gonzales et al. showed that late DA reopening or failure of DA closure is more frequent in preterm infants with infection.[59] In their study, the risk of BPD was increased with PDA (OR 11.7; 95% CI 1.7 - 81) and with infection (OR 3.1; 95% CI 1 - 11). When both factors were temporally associated, they further increased the risk of BPD (OR 29.6; 95% CI 4.5 to >100). Of note, 55% of these infections resulted from *CoNS*.

1.3.4.3 *CoNS* Sepsis and Necrotizing Enterocolitis (NEC)

NEC is a disease of prematurity. The cause for this disorder is not well understood but it is thought to be multifactorial.[54] The rate of NEC varies between NICUs. *CoNS* organisms are the most common isolated pathogen from blood, stool, and peritoneal fluid of preterm infants with NEC.[4, 31] The link and the pathway between *CoNS* organisms and NEC are inconsistent in literature. Scheifelle et al. described the delta toxin of *CoNS* in the stool of preterm infants with NEC as a potential enteropathogic factor for gut injury.[54] In contrast, Rotbart et al.

reported an equal proportions of *CoNS* organism in stool samples of patients with and without NEC.[61] Additionally, they were unable to identify the delta toxin in the stool of infants with NEC.[61] Whether *CoNS* injure the intestinal mucosa and lead to NEC or necrotic intestinal mucosa in infants with NEC facilitates the transport of *CoNS* organisms into the bloodstream is not known.

1.3.4.4 *CoNS* Sepsis and Retinopathy of Prematurity (ROP)

ROP is a multifactorial vasoproliferative retinal disorder. Risk factors for ROP include low gestational age, low birth weight, and supplemental oxygen therapy. The role of neonatal sepsis as a risk for ROP is controversial. Schlapbach et al. showed that neonatal sepsis increases the risk for ROP in preterm infants ≤ 28 weeks' gestation.[13] *CoNS* organism was the most common cause (51%) of sepsis in this study. The proinflammatory cytokines production and the leukocyte adhesion molecules expression by endothelial cells are speculated to alter the development of retinal blood vessels and contribute to the development of ROP.[62]

1.3.4.5 *CoNS* Sepsis and Duration of Hospital Stay

Neonatal infections have a major impact on duration of hospital stay and hospitalization costs.[63] Gray et al. showed an increment of 14 ± 4 days in the length of hospital stay in infants with sepsis after correcting for birth weight, score of neonatal acute physiology (SNAP) at birth, and community retrotransport.[36] Of the 130 infants with sepsis in this study, 101 infants (78%) had *CoNS*. This prolongation of the stay in NICU led to an increase in hospital charges by 25 090 ± 12 051 $ ($p < 0.05$).

1.3.5. *CoNS* Sepsis and Brian Injury

1.3.5.1 White Matter Injury in Preterm Infants with *CoNS* Sepsis

Cerebral white matter injury (WMI) and particularly periventricular leukomalacia (PVL) is the most common manifestation of serious central nervous system injury in premature infants. WMI is well correlated with increased risk of poor ND outcome.[64, 65] Brain MRI studies showed that at least 50% of VLBW infants exhibit some degree of WMI, and this injury is highly correlated with various types of neurodevelopmental deficits.[66, 67] The deficits resulting from WMI include cerebral palsy (5-10%) and cognitive or behavioural deficits (50%).[68-70]

In a prospective cohort study by Silveria et al. on 51 VLBW infants with PVL, neonatal sepsis was an independent risk factor for PVL (OR 11.6; 95% CI 1.42-94.9).[71] In the same study, *CoNS* was the predominant cause of sepsis (69.2%) in preterm infants with PVL, and only one preterm infant without PVL had *CoNS* infection.[71]

1.3.5.2 Mechanism of Brain Injury in Preterm Infants with *CoNS* Sepsis

In general, neonatal sepsis has been postulated to be one of the major initiating pathogenic factors for postnatal white matter injury, in addition to cerebral ischemia and systemic inflammation.[70] These combined factors represent the upstream mechanism to stimulate microglia (brain's resident immune cells) and lead to activation of two critical downstream mechanisms including excitotoxicity and free radical attack by reactive oxygen and nitrogen species.[70] This activation subsequently leads to the death of the vulnerable pre-myelinating oligodendrocytes.[70] The white matter injury resulting from sepsis in premature brain is a multifactorial process involving the production of pro-inflammatory cytokines, increase blood-brain barrier permeability, hypoxic ischemic events resulting from hypotension, impaired autoregulation of cerebral blood flow, and respiratory insufficiency.[64, 71, 72]

Microglia are representatives of the resident mononuclear phagocyte population (specifically monocytes) in the central nervous system (CNS). These cells share several phenotypical and

functional features with other tissue macrophages in addition to peripheral blood monocytes. Recent evidence suggests that microglia contribute in innate immune reactions of the brain. They are known to be rapidly activated in response to any pathology such as infection/inflammation or ischemia. Activation of microglia not only forms the key role in the defence of the brain parenchyma but also explains the neurodegeneration.[73] Many studies have shown that pathologic and chronic activation of microglia leads to tissue damage.[74-76] Monocytes have commonly become an integral part of the pool of parenchymal microglia in the brain during the infection episodes. This newly recruited microglia contributes to increased lysosomal activity, apoptotic cells death, and clearance of damaged tissue.[77]

A recent study by Chau et al. showed that signalling toll-like receptor 2 (TLR2) on monocytes by staphylococcus bacteria, probably in combination with TLR6, leads to massive production of Interleukin 10 (IL-10) followed by apoptosis of these cells.[78] Both the increased lysosomal activity and increased level of IL-10 contribute in the damage to other precious neurons around the microglia. The presence of proinflammatory cytokines in the central nervous system has been shown to activate astrogliosis, suppress proliferation of neuronal precursor cells, and stimulate oligodendrocyte cell death. All these increase the risk of white matter injury.[79, 80]

1.3.5.3 Difference in Pathway of Brain Injury between Gram Negative and Gram Positive Sepsis

Most studies of the link between infection/inflammation and white matter injury have focused on gram-negative infection and lipopolysaccharide (LPS). LPS as an endotoxin, results in the expression of many genes in the brain, specifically those encoding various TLRs.[81, 82] These specific cell-surface receptors particularly on microglia respond to specific molecular motifs called “pathogen-associated molecular patterns” (PAMPs). The LPS motif is recognized by

TLR4 and has a role as a mediator of white matter injury in infection caused by gram-negative organisms.[83]

In contrast, the peptidoglycan motif shared by gram-positive microorganisms like *CoNS* is recognized by TLR2.[84] Peptidoglycan induces IL-6 expression through mainly the TLR2 receptor pathway in microglia.[85] Activation of TLR2 has led to neurodegeneration in mice studies.[86, 87]

Hoffman et al. showed that in gram-positive bacteria, binding of bacterial lipopeptides to TLR2 through intrathecal injection induces meningeal inflammation in rodents results in influx of leukocytes into the cerebrospinal fluid (CSF). This leads to marked increase in regional cerebral blood flow and intracranial pressure followed by increased number of apoptotic neurons in the dentate gyrus.[87] Moreover, stimulation of TLR2 through specific agonist-induced inflammatory responses in microglia of mice results in decreased volume of cerebral gray matter and white matter in the forebrain.[88]

1.3.6. *CoNS* sepsis and Neurodevelopmental Outcome

The studies describing a relationship between *CoNS* infection, brain injury and adverse neurodevelopmental outcome have been evaluated largely under the umbrella of intrauterine or neonatal sepsis.

In the Magnesium and Neurologic Endpoints Trial (MagNET), 30 cases of *CoNS* (out of 107 positive cultures) were isolated from the placental chorioamniotic space of mothers delivering preterm infants.[89] Four of five preterm infants with *CoNS* sepsis were later diagnosed to have cerebral palsy. When multivariate logistic regression was used to control for potential confounders, the association between *CoNS* in the chorioamnion and cerebral palsy remained significant (adjusted OR 37; 95%CI: 3 to + ∞; p=0.003), while the association between culture-

proven *CoNS* in postnatal period (also 4/5) and cerebral palsy became insignificant (adjusted OR 3; 95% CI 0.2 to +∞; p=0.42). Although the sample of the study was small, it raised the concern on impact of *CoNS* on the brain. [89]

Shah et al. looked at 55 preterm infants with *CoNS* sepsis who had a brain MRI at term-equivalent age were followed up at 2 years of age for ND outcome with the Bayley Scales of Infant Development (BSID).[19] White matter abnormalities (WMA) on brain MRI were found in 78% of VLBW infants who had *CoNS* sepsis during their NICU stay. Psychomotor developmental index (PDI) was lower in *CoNS* group than infants with no history of sepsis/NEC (adjusted mean difference=5.8 (0.3, 11.2); p= 0.04).[19] This study had a small sample size and followed infants until 2 years of age only.

A National Institute of Child Health and Human Development (NICHD) study, preterm infants with neonatal sepsis (birth weight ≤ 1250 gm) were evaluated at 18 to 22 months corrected age for neurodevelopmental and growth outcomes.[18] The *CoNS* sepsis group had worse neurodevelopmental outcome (OR 1.3; 95% CI 1.1-1.6), PDI (OR 1.4; 95% CI 1.1-1.9), and visual impairment (OR 1.7; 95% CI 1.2-2.3). Cerebral palsy, mental developmental index (MDI) and hearing impairment did not differ significantly. The outcomes in this study were assessed on only one visit at 18 to 22 months. Assessment of the long term ND outcome of preterm infants needs frequent and longer follow up visits particularly for evaluating mental and psychomotor outcomes. Moreover, the study did not adjust for NEC. NEC is a significant confounder here as it is frequently associated with *CoNS* infection and known to affect the neurodevelopmental outcome.

In a recent study, Schlapbach et al. reported higher incidence of cerebral palsy (OR 5.59; 95% CI 1.87-16.66) in 77 *CoNS* infected extremely premature infants in comparison to uninfected babies

born at less than 28 weeks gestation.[13] The incidence of neurodevelopmental impairment (NDI) was similar in the two groups. Of interest, infants with sepsis caused by other gram-positive and gram-negative bacteria did not show any difference in the incidence of NDI when they compared infected infants with uninfected groups.[13]

There is a paucity of studies on relationships between *CoNS* sepsis and neurodevelopmental outcome particularly beyond 2 years of age and they have many limitations. In addition, the published studies yielded conflicting results regarding the association between the NDI and specifically the type of this impairment and *CoNS* sepsis.

1.4 Prophylactic Measures Against *CoNS* Infection:

As *CoNS* is found habitually on skin of preterm infants, it is no surprise that infection is commonly due to self-contamination during the catheter insertion or from the skin of health care provider during this procedure. Therefore, improved education of healthcare providers in NICU such as hand hygiene and using sterile technique at the time of insertion has led to a reduction in the rate of *CoNS* infection.[90]

Moreover, the use of closed central venous line system and the reduction in the number of connections between the central venous line and the vascular access devices have recently shown to decrease the central line associated blood stream infection in NICU.[91] The use of new technologies such as catheter hub containing an iodinated alcohol solution, short-term chlorhexidine-silver sulfadiazine- impregnated catheters to prevent *CoNS* from adhering onto peripheral and central catheters may help reduce the risk for infection in the future.[92]

At present, there are no anti-staphylococcal vaccines. Active immunization strategies against *CoNS* may be problematic due to the fact that *CoNS* is a ubiquitous human commensal.[93] Passive immunization using monoclonal antibodies against Lipoteichoic acid of the *staphylococcal* cell wall has recently undergone clinical trials in VLBW infants.[17] Unfortunately, these monoclonal antibodies

(Pagibaximab) showed no significant reduction in the rate of *CoNS* sepsis between treated and untreated infants, under the current immunization protocols.[17]

All preventive and therapeutic strategies against *CoNS* infection must omit the complete eradication of *CoNS* as it is an important part of skin flora of preterm infants.[93]

1.5 Research Questions

1.5.1 Primary Research Question

Does *CoNS* sepsis adversely affect the long term neurodevelopmental outcome at 36 months corrected age in preterm infants born ≤ 28 weeks gestation?

1.5.2 Secondary Research Question

- Dose *CoNS* sepsis influence the major neonatal outcomes (PVL, BPD, and NEC) in preterm infants born ≤ 28 weeks?
- Do preterm infants ≤ 28 weeks gestation with persistent *CoNS* sepsis have a different major neonatal outcomes as compared to infants with non-persistent *CoNS*?

1.6 Objectives

The primary objective of this study was

- To examine the effects of *CoNS* on the neurodevelopmental outcome at 36 months corrected age in preterm infants ≤ 28 weeks gestation.

The secondary objectives were

- To compare the neonatal outcome between preterm infants exposed to *CoNS* and those not exposed to *CoNS*.

- To explore the relationship between persistent *CoNS* sepsis and neurodevelopmental outcome at 36 months corrected age in preterm infants.

1.7. Significance of the Study

This study is designed to improve our understanding of the association between *CoNS* sepsis and neurodevelopmental disabilities in preterm infants. We looked at some important neonatal factors related to poor neurodevelopmental outcome in preterm infants. It also explored the association between persistent *CoNS* infection and adverse neurodevelopmental outcome.

Having an understanding of this relationship and identifying the impact of *CoNS* sepsis on neonatal and long term neurodevelopmental outcome will change how *CoNS* is perceived and affect practices toward prevention of *CoNS* and other comorbidites. It also helps physicians in proactively predicting, in combination with other factors, the prognosis for these infants. Lastly, this study will encourage investigators to proceed with immunological trials which currently aim to provide immunoglobulins that protect extremely preterm infants from *CoNS* infection.

CHAPTER TWO: METHODOLOGY

This chapter discusses the methodology that is used in the study. It describes study design, sampling procedure, data collection procedure and the used analysis.

2.1. General Study Design

This is a retrospective cohort study in which the study groups were formed on the basis of exposure and followed up to outcome events of interest that had already occurred by the time the study was initiated. Our current study was conducted on preterm infants with gestational age ≤ 28 weeks born at or transferred to Foothills Medical Centre in Calgary between January 01, 1995 and December 31, 2008.

Preterm infants meeting specified entry criteria for the study were selected. They were sorted on the basis of presence or absence of *CoNS* sepsis during the neonatal period. Infants exposed to *CoNS* sepsis were the exposed group and those unexposed to *CoNS* infection formed the comparison group. Due to the large number of infants in the comparison group and expectation of the need to review many charts for missing data, we restricted the number in the comparison group to only double that in the exposure group. In order to eliminate the risk of selection bias, we selected the next 2 eligible patients who were admitted to NICU after the infant exposed to *CoNS* was selected. Both the exposed and unexposed infants were followed up in our regional Perinatal Follow-Up (PNFU) clinic for immediate neonatal and long term neurodevelopmental outcomes at 36 months corrected age.

The retrospective cohort design was chosen because of the following reasons:

- The design allowed the assessment of the risk of poor neonatal and neurodevelopmental outcome among infants exposed and unexposed to *CoNS* infection.

- The design was suitable in light of the limited time and resources available for the study as a new prospective follow up study would require 15-20 years to complete.

2.2 Study Population

This study was conducted on preterm infants who were born or admitted at the Foothills Medical Centre, Calgary, Alberta between January 1, 1995 and December 31, 2008.

2.2.1 Inclusion Criteria

Infants meeting the following criteria were included:

- Gestational age ≤ 28 weeks.
- Born between January 1, 1995 and December 31, 2008.
- Admitted at the regional neonatal intensive care unit at Foothills Medical Centre, Alberta, Canada.
- All results of blood, CSF, and urine cultures were available.

2.2.2 Exclusion Criteria

Infants with any one of the following problems were excluded:

- Major congenital anomalies.
- Chromosomal anomalies.
- Intrauterine TORCH infections (TORCH stands for *Toxoplasmosis*; Other infections; *Rubella* virus; *Cytomegalovirus (CMV)*; and *Herpes simplex virus (HSV)*. "Other" infections include *Syphilis, Hepatitis B, Coxsackie virus, Epstein-Barr, varicella-zoster virus (VZV), and human parvovirus B-19*).
- Other blood or sterile body fluids infection caused by organisms other than *CoNS*.

2.3 Location of the Study

The Foothills Medical Centre (FMC) is the regional tertiary care facility providing high risk obstetrical care and tertiary care neonatal intensive care to all neonates born in Southern Alberta, Canada. There are 6000 – 7000 deliveries per year at this hospital. Currently, 100 - 120 infants admitted per annum are infants born at ≤ 28 weeks' gestation.

2.4. Sample Size

The large NICHD study reported a difference of 27% in the NDI proportion between preterm infants with any type of bacterial or fungal sepsis and those without sepsis. However, this difference declined to 15% when it compared the NDI outcomes of infants born prematurely who had *CoNS* sepsis in the neonatal period to similar infants without sepsis.[18]

The rate of NDI for our preterm infants ≤ 28 weeks' gestation is 30% based on the perinatal follow up clinic data in Calgary. A sample size of 206 infants (103 in the *CoNS* infected group and 103 in an uninfected group) was required to find a 20% difference between the infants with *CoNS* sepsis and those without *CoNS* sepsis at 80% power and 5% alpha error (Using STATA software 11.1, sample size calculation. College Station, Texas, USA). If we chose the 15% difference from the NICHD study, the total number of subjects required for the study would be 322. Achieving this larger sample size would have required including more infants from the period before 1995. Major advances in neonatal care and long term outcomes happened in that time period including introduction of antenatal steroids and exogenous surfactant. In addition, achieving this sample size was not feasible in the near future as it would require a minimum of 6 extra years to enroll all the subjects for follow up outcomes.

In order to adjust for the effect of various confounders in a multiple logistic regression analysis using the rule of a minimum of 10 events per each variable we decided to enroll 206 infants in

the uninfected group (2:1 ratio). Therefore; the study included a total of 309 infants. From our NICU database, the incidence of *CoNS* infection was 14 - 16 cases per year in 2008 and 2009. Based on that, we estimated to have minimum of 196 infants with *CoNS* infection between 1995 and 2008. We believed that including infants between 1995 and 2008 would achieve our goal of 103 and 206 in the *CONS* and no *CoNS* group respectively.

2.5. Data Collection Procedure

The list of preterm infants admitted between January 01, 1995 and December 31, 2008 at Foothills Medical Centre was obtained from the administrative neonatal database at Foothills Medical Centre after ethics approval from the Conjoint Health Research Ethics Board**.** First, a list of infants born at ≤ 28 weeks gestation who had *CoNS* sepsis during their stay in the NICU was identified. These infants' data were reviewed in order to evaluate the criteria used for inclusion, exclusion, and *CoNS* sepsis definition. The list contained information on the infants FMC identification number, name, date of birth, and gestational age. Central Laboratory Services (CLS) was contacted also to identify the culture results for the preterm infants in our study cohort, but the lack of Accession numbers of the blood, CSF, and urine cultures prohibited the cross reference of neonatal database with CLS to ensure all *CoNS* cases from NICU were included.

The list of infants in the no *CoNS* group were identified using the date of birth of each infant in the *CoNS* group to determine the subsequent two infants born at ≤ 28 weeks who were admitted to NICU and did not have any positive blood, CSF, or urine cultures. The neonatal and neurodevelopmental outcomes of all eligible infants were obtained from the PNFU database. The PNFU database contains pregnancy, delivery and neonatal morbidities abstracted from the hospital charts at death or discharge from the hospital by a trained research assistant. These

include maternal age, maternal education, antenatal risk factors like smoking and alcohol use, gestational age, birth weight, sex, antenatal steroids, mode of delivery, multiple or singleton pregnancy, Apgar scores, umbilical arterial pH, respiratory distress syndrome, use of surfactant, bronchopulmonary dysplasia, intraventricular hemorrhage, periventricular leucomalacia, patent ductus arteriosus, necrotizing enterocolitis, culture proven sepsis, retinopathy of prematurity, duration of ventilation and hospital stay in NICU.

2.5.1 Neurodevelopmental Outcome Data

The neurodevelopmental outcome data were collected from the PNFU database. The criteria to follow up infants in the PNFU clinic include: gestational age ≤ 28 weeks and /or birth weight ≤ 1250 g. However, our study involved only infants born at ≤ 28 weeks' gestation. This was because morbidities in preterm infants correlate with gestational age more than birth weight. In addition, older small for gestational age (SGA) infants behave more like infants born at similar gestation not at similar weight. The frequent use of gestational dating ultrasound when the exact date of last menstrual period of pregnant women is unknown makes gestational age more accurate in predicting the postnatal morbidities in preterm infants. During the study period, preterm infants were prospectively followed up with longitudinal multidisciplinary examinations at adjusted ages of 4, 8, 12, 18 and 36 months in the PNFU clinic at Alberta Children's Hospital. Growth parameters including weight, length and head circumference measurements were determined using standard techniques. Neurodevelopmental assessments were performed independently by neonatologist/developmental pediatrician, physiotherapist, certified psychologist, ophthalmologist and audiologist on every child. The neurological assessment included evaluation of tone, strength, reflexes and posture.

All neurological and neurodevelopmental assessments were performed by individuals who were unaware that a study on association between *CoNS* sepsis in preterm infants and long term neurodevelopmental outcome would be conducted in the population.

2.6 Data Editing and Management

All data in our cohort study were collected by two data analysts. Every subject identified in the neonatal database to be eligible for the study was given a study number. Alberta Health Numbers of these subjects were used temporally to link their information between neonatal and PNFU databases.

Each variable obtained from the data set was entered, coded, and saved in the Microsoft Excel 2007 software package prior to editing. Yes and no variables were coded as 0 = no and 1 = yes. After coding and verification, data in the Excel spreadsheet were imported into the statistical program (Stata 11.0) for the purpose of analysis.

2.7 Study Variables

Information on the following main categories of variables was collected:

- Independent variable
- Dependent variables

Additional information was also collected on:

- Maternal variables
- Neonatal variables

2.7.1 Independent Variable

The independent variable was the "exposure to *CoNS* sepsis". The exposed group consisted of preterm infants who had proven *CoNS* sepsis as defined by positive *CoNS* culture from one of

the sterile body fluids (i.e. blood, CSF or urine), presence of clinical signs of neonatal sepsis and the requirement of treatment by antibiotics for more than 5 days during the initial stay in NICU. The unexposed group consisted of preterm infants who did not have any proven bloodstream, CSF or urine *CoNS* culture.

2.7.2 Dependent Variables

The main outcome variables were divided into neonatal and long term neurodevelomental. Neonatal outcomes included major morbidities identified before discharging home i.e. BPD, NEC, and ROP. Association with IVH and PDA were also studied.

The long term neurodevelopmental outcome variables included cerebral palsy, cognitive delay, blindness, and deafness as determined at 36 months corrected age.

2.7.3 Maternal Variables

Maternal variables included both continuous and discrete variables. These include

- Maternal age (continuous variable)
- Smoking during pregnancy (yes or no)
- Use of alcohol during pregnancy (yes or no)
- Use of recreational drugs (yes or no)
- Completion of high school (yes or no)
- Perinatal infection (yes or no)
- Mode of delivery (vaginal or cesarean section)
- Number of births (singleton or multiple)
- Use of steroid during labour (yes or no)
- Use of antibiotic during the labour and delivery (yes or no)

2.7.4 Neonatal Variables

Information on neonatal variables was collected as continuous and discrete variables**.** Some continuous variables were stratified into categories during the analysis to ease the adjustment for confounding.

2.7.4.1 Neonatal Continuous Variables

- Gestational age at birth (weeks)
- Birth weight (grams)
- Apgar scores at 5 minutes
- Umbilical arterial pH at birth
- Duration of ventilation (days)
- Duration of respiratory support (ventilation or non-invasive ventilation; nasal Continuous Positive Airway Pressure (CPAP)
- Length of stay in the initial hospitalization (days)

2.7.4.2. Neonatal Discrete Variables

- Sex (male or female)
- Respiratory Distress Syndrome (yes or no)
- IVH (yes or no)
- PDA (yes or no)
- BPD (yes or no)
- NEC (yes or no)
- Discharge home on oxygen (yes or no)
- Small for gestational age (SGA) (yes or no)

- Culture proven sepsis as defined for our study (yes or no)
- Mortality (died or survived)

2.8 Data Analysis

Stata version 11.0 software (Stata Corporation, College Station, Texas, USA) was used for all data analyses. Data were analysed at univariate, bivariate and multivariate levels.

2.8.1 Descriptive Statistics

Descriptive statistics were used to describe the study population. The basic features for each variable were described using summary measures including means, medians and standard deviations.

2.8.2 Univariate and Bivariate Analysis

Univariate analysis was used in the first stages to describe the data variables. Appropriate tools were used to describe continuous and discrete variables. The procedures adopted for each analysis are explained separately. Bivariate analysis was used to test the relationship between the dependent and independent variable (association and causality).

In order to compare between continuous variables in *CoNS* and no *CoNS* groups, two sample t tests for independent groups were used on each variable to test the null hypothesis of equal population means**.** For comparing discrete variables, Chi square test (contingency table method) was used. Fisher's exact test was also used when the expected cell frequency was less than five.

2.8.3 Multivariate Analysis

Effect modification and possible confounding from various maternal or neonatal variables, on the relationship between neurodevelopmental outcome and exposure to *CoNS* sepsis, were

assessed using classical analysis techniques (Mantel-Haenszel test) and logistic regression modeling.

Initial evaluation of the association between dependent and independent variable ("risk factors" and neonatal morbidities) and *CoNS* sepsis (main exposure) was done using bivariate analysis. Similar analysis was also used to study the association between neurodevelopmental outcomes and *CoNS* sepsis. Covariates (variables in risk factors or neonatal morbidities) with significant association ($P < 0.2$) with *CoNS* sepsis or neurodevelopmental outcome were considered as possible confounders and further analysis was used to assess their eligibility to be in the final model. This further analysis was based on comparison between strata-specific risk ratio (RR) for each variable with potential modification effect, and the difference between crude RR and adjusted RR for each variable with potential confounding effect. In addition, literature review was done to determine the potential confounders or effect modifiers used in the previous studies.

Final model building was performed using backward selection methods. Log likelihood ratio tests were used to evaluate the inclusion of potential confounders (based on bivariate analyses). Any covariate that did not make a statistically significant contribution to the model, when evaluated by the log likelihood ratio test, was dropped from the model. The final model for each outcome was evaluated for Goodness of fit by using Hosmer- Lemeshow test. The final logistic regression model was used to study the effects of *CoNS* infection on neurologic outcome after controlling for variables that might have plausible relationship. Odds Ratio, Risk Ratio and 95% confidence interval were calculated for all outcomes as appropriate.

2.9 Ethical Considerations

This study was approved by the Conjoint Health Research Ethics Board of the University of Calgary. In addition, administrative approval was collected from Calgary Health Region

Division of Neonatology, Alberta Health Services, Calgary Zone. Human subjects were not involved directly in the study; rather a health outcome database of preterm infants admitted at the FMC was accessed retrospectively. A study PIN number was assigned for each patient to allow for examination of accuracy of data. PINs were kept in a separate secure file with access limited to the investigators only. All patient identification was removed from the database and data was stored in a secure computer with password protected data access to ensure patient confidentiality. A separate informed consent was waived by the Conjoint Health Research Ethics Board as the data collected in the study was in keeping with the comprehensive information and consent obtained from the parents at the time of their infants' enrolment in the PNFU clinic. Data was also analysed anonymously and results were compiled without identification of individual infants.

2.10. Operational Definitions

- **True *CoNS* sepsis** is defined when the following 3 criteria are fulfilled: (a) clinical signs of neonatal sepsis, (b) one or more positive blood, CSF, or urine culture for *CoNS*, (c) intravenous antibacterial therapy for at least 5 days after obtaining blood culture.
- **Early onset sepsis** is defined as a disease manifest before 72 hours of life.[94]
- **Late onset sepsis** is defined as a disease manifest after the first 72 hours of life.[94]
- **Meningitis** is defined as positive CSF culture, white blood cell count of more than 30 cells/mm3, or baby treated with therapeutic course of antibiotics based on the other CSF findings.[19]

- **Gestational age** is defined as the best obstetric estimate that is based on early prenatal ultrasound, and obstetric history, unless the postnatal pediatric estimate of gestation age based on Ballard score differed from the obstetric estimate by more than 2 weeks.
- **Respiratory distress syndrome (RDS)** is defined as the presence of respiratory signs including tachypnea, grunting and chest retraction, typical chest x-ray findings, and/or treatment by surfactant with the need for mechanical ventilation for more than 24 hours in the first 3 days of life.
- **Intraventricular hemorrhage (IVH)** is defined according to the criteria of Papile et al. from head ultrasound.[95]
- **Patent ductus arteriosus (PDA)** is defined as clinical diagnosis plus treatment with indomethacin or surgical ligation or both.
- **Bronchopulmonary dysplasia (BPD)** is defined as oxygen dependency at 36 weeks post menstrual age (PMA) for preterm infant who is born at 32 weeks' or less gestation.[96]
- **Periventricular leukomalacia (PVL)** is defined as parenchymal echo densities or lucencies around the ventricles from head ultrasound performed after 32 weeks postmenstrual age.
- **Necrotizing enterocolitis (NEC)** is defined according to the modified criteria of Bell et al. (stage II or higher).[97]
- **Retinopathy of prematurity (ROP)** is defined according to the International Classification for Retinopathy of Prematurity.[98]

Definition of neurodevelopmental outcome variables

- **Cerebral palsy (CP) is** defined as a non-progressive motor impairment characterized by abnormal muscle tone in at least one extremity and decreased range or control of movements (Levine, 1980). The severity of CP is classified into:
    - Mild CP: abnormal tone/reflexes but no functional impairment; very little trouble with activities of daily living (ADL). Minor appliances (if any).
    - Moderate CP: spastic diplegia/hemiplegia, with some function in the most affected limb but needs appliances; can communicate, can undertake ADL.
    - Severe CP: spastic quadriplegia; no functional ability in the most affected limb, totally dependent for ADL.
- **Cognitive delay** is defined as a score of > 2 standard deviation below the mean on standardized assessment (Wechsler Preschool and Primary Scale of Intelligence-Revised, Bayley Scales of Infant Development II or Stanford-Binet IV).
- **Deafness** is defined as sensorineural hearing loss requiring amplification.
- **Blindness** is defined as visual acuity < 20/200 following refractive correction.
- Level of impairment:

Normal: Infants were categorized as normal if no abnormalities were detected on physical and/or neurological examination and developmental testing scores were within 1 SD of mean.

Mild impairment: if the infant had one or more of the following:

isolated muscle tone abnormalities (hypotonia or hypertonia), visual impairment (visual acuity < 20/60 in the best seeing eye), documented hearing loss not requiring amplification or the developmental testing scores between 1 and 2 SDs below mean.

Major impairment: if the infant had at least one of the following:

Moderate to severe cerebral palsy, cognitive delay (>2 SD), blindness or deafness requiring amplification.

CHAPTER THREE: RESULTS

This chapter consists of four parts. The first two parts describe the maternal and neonatal characteristics of infants exposed and unexposed to *CoNS* sepsis in the neonatal period. The third and fourth parts show the differences in the neonatal morbidities and the long term neurodevelopmental outcome for these infants, respectively.

Descriptive Statistics

3.1 Flow of Patient Selection

A total of 1224 preterm infants ≤ 28 weeks gestation were admitted to the regional NICU located at Foothills Medical Centre, Calgary Alberta, between January 01, 1995 and December 31, 2008. One hundred and eighty one infants in the NICU were identified to possibly have *CoNS* sepsis using a total of three 3 different codes for the *Staphylococcus* organisms according to ICD 9 and ICD 10 codes. Of the 181 infants, 136 infants had *CoNS* sepsis fitting the inclusion criteria for this study. The remaining 44 infants were excluded from the analysis due to potential contamination (21), coinfection (12) or other Staphylococcal (i.e. *Staphylococcus aureus*) infection (11). Of the 136 infants, 16 (11.7%) infants were lost to follow up, 5 (3.7%) patients had major congenital anomalies, and 11 (8.0%) infants died as outlined in Figure 1.

For the comparison group, 362 infants with no *CoNS* sepsis were identified from the NICU database as the next 2 admitted infants after each case. Thirty infants were excluded because they had other types of infection (i.e. gram negative organism, fungal or viral isolated from sterile body fluids only). Of the remaining 332 infants in the uninfected group, 36 infants (11.5%) were lost to follow up at 36 months corrected gestation, 9 infants (2.7%) were excluded because they

had major congenital or chromosomal anomalies, and 61 infants (18.3%) died. Figure 1 describes the flow diagram of the study population.

A total of 11 infants died in the *CoNS* group, of which 7 infants died during the initial hospitalization and 4 infants died after discharge from the hospital. In the uninfected group, sixty one infants died, of which 49 infants died during the initial hospitalisation and 12 infants died after discharge.

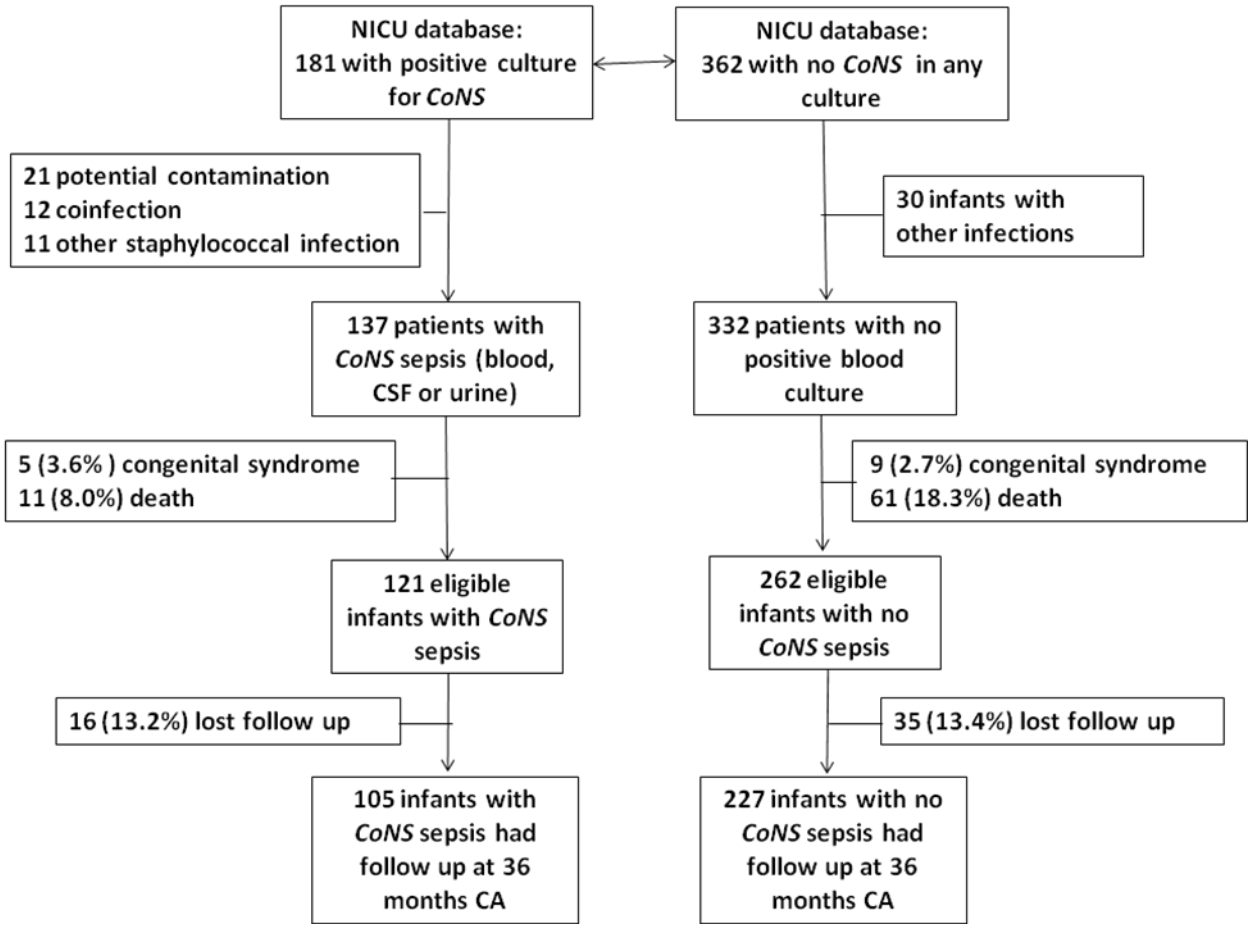

Figure 1. Flow diagram of the study population

Incidence of *CoNS* Sepsis in Live-born Preterm infants by Gestational Age:

The cumulative incidence of *CoNS* sepsis in infants born at ≤ 28 weeks gestation was 13.1%. This incidence was applied after excluding infants with possible contamination only. Table 1

shows the rate of *CoNS* sepsis according to gestational age at birth. The cumulative incidence of *CoNS* is approximately constant between 23 and 26 weeks, but decreased to almost half in the 27 and 28 weeks gestation infants.

Table 1. Cumulative incidence of CoNS sepsis in live-born preterm babies by gestational age

Gestational age (week)	**All preterm infants admitted to NICU (n=1224)**	**Preterm Infants with *CoNS* sepsis (160)**	**(%)**
23	74	13	17.5
24	155	28	18.1
25	188	32	17.0
26	234	40	17.1
27	273	21	7.8
28	300	26	8.6
23 – 28	1224	160	13.1

Site of Confirmed Sepsis:

Table 2 shows the site of sepsis in the *CoNS* group. There were 4 cases of meningitis resulting from *CoNS*. Of the 4, only one infant had positive blood culture. All collected urine samples in infants with *CoNS* sepsis were negative for *CoNS* organisms.

Table 2. Site of CoNS sepsis

Site of *CoNS* sepsis	**Number of infants**
Blood only	101
CSF only	3
Both	1
Total	105

Age at the Time of *CoNS* Sepsis:

Figure 2 shows the distribution of age at the time of first *CoNS* positive blood culture. The median (IQR) age was 15 (9, 21) days. The highest incidence of *CoNS* sepsis was in the third week of life.

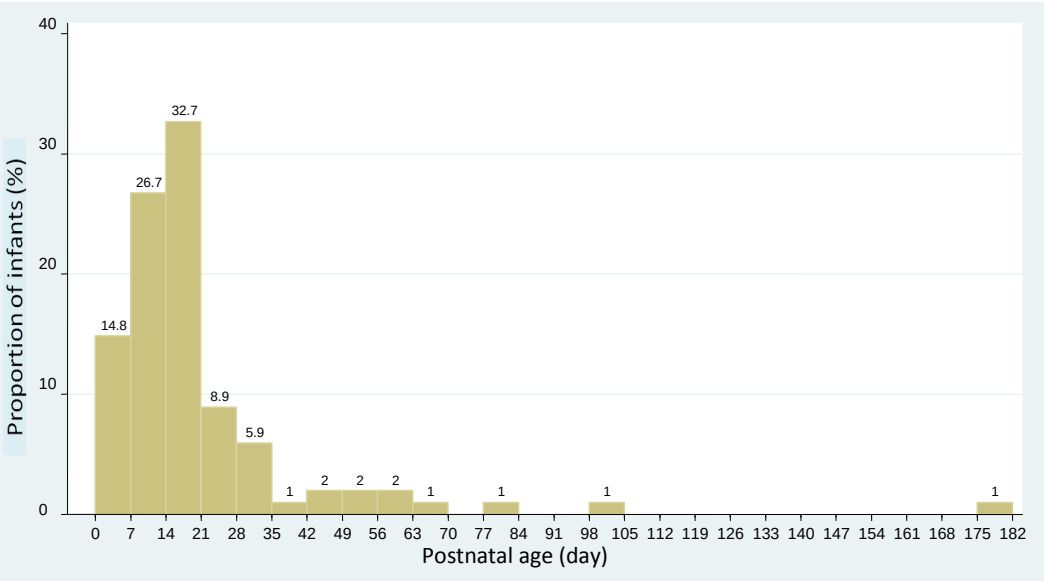

Figure 2. Distribution of the postnatal age (day) at time of first CoNS positive blood culture

3.2 Maternal Characteristics:

This section elaborates on the maternal characteristics of infants included in the two groups. The variables were carefully selected based on the previous literature on neonatal sepsis. The aim was to examine the maternal demographic characteristics as potential risk factors that may be associated with both *CoNS* sepsis and/or long term neurodevelopmental outcome.

3.2.1 Maternal Age at the Time of Birth

The distribution of maternal age at the time of birth for the two groups is shown in Figure 3.

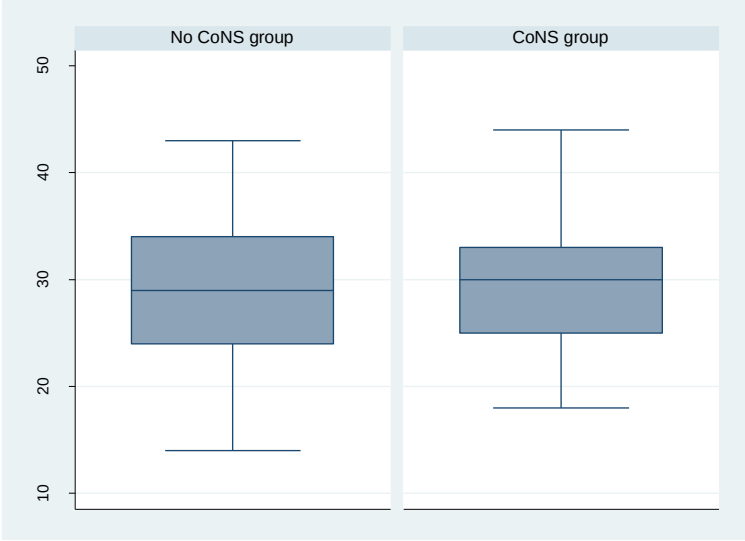

Figure 3. Box plots of maternal age among the CoNS and no CoNS groups. The line in the middle of the box represents the median. The lower and upper borders of the boxes represent the 50% IQR. The lower and the upper whiskers represent the 95%CI. The dot represents the outliers if present. All these will apply to the later boxes as well.

The maternal age distribution was mildly skewed in the *CoNS* group. The median maternal age was 30 (IQR: 25, 33) years in the *CoNS* group and 29 (IQR: 24, 34) in the no *CoNS* group. The mean maternal age was 29.2 ± 5.4 years in the *CoNS* group and 29.2 ± 5.9 years in the no *CoNS*

group. There was no difference between the means of the maternal age between the two groups using t-test (p=0.79) or Mann Whitney test (p=0.85).

3.2.2 Antenatal Steroids

Pregnant women at risk of premature delivery are given antenatal steroid (betamethasone or dexamethasone) to accelerate fetal lung maturation. Using steroids for mothers was coded as 0 for those who did not receive steroid and 1 for those who received steroids. Information on the use of antenatal steroids was available for 324 (97.6%) infants. A Chi-squared test was performed. The result showed no difference in the proportion of the mothers who received steroid or did not receive steroid among the no *CoNS* and *CoNS* groups (82.8% vs. 79.6%; p=0.49).

Table 3. Comparison of use of antenatal steroids between mothers of infants exposed and not exposed to CoNS sepsis

Antenatal steroids	**No *CoNS* group**	***CoNS* group**	**Total**
Not given (%)	38 (17.2)	21 (20.4)	59 (18.2)
Given (%)	183 (82.8)	82 (79.6)	265 (77.8)
Total (%)	221 (100)	103 (100)	324 (100)

3.2.3 Multiple Births

Multiple births were coded as 0 for singleton or 1 for multiple pregnancies. A Chi-squared test was performed. The results are produced in Table 4. The proportions of multiple births were higher in the *CoNS* group compared to the no *CoNS* group, however this was not statistically significant (29.5% vs. 20.3%, p=0.06).

Table 4. Comparison of multiple births between infants exposed and not exposed to CoNS sepsis.

Multiple births	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	181 (79.7)	74 (70.5)	255 (76.8)
Yes (%)	46 (20.3)	31 (29.5)	77 (23.2)
Total (%)	227 (100)	105 (100)	332 (100)

In the no *CoNS* group, 41 infants were one of twins and 5 were one of triplets. Of the multiples in the *CoNS* group, 26 infants were one of twins and 5 infants were one of triplets.

3.2.4 Mode of Delivery

The type of delivery was coded as 0 for vaginal delivery and 1 for delivery by cesarean section. A Chi-squared test was performed. Vaginal delivery was coded as 0 and cesearean section as 1. Information on mode of delivery was available for 331 (99.7%) infants. The results are shown in table 5. The proportion of infants born by cesarean section was not statistically different among the two groups (46.9% vs. 52.4%, p=0.35).

Table 5. Comparison of mode of delivery between infants exposed and not exposed to CoNS sepsis.

Type of delivery	**No *CoNS* group**	***CoNS* group**	**Total**
Vaginal delivery (%)	120 (63.1)	50 (47.6)	170 (51.3)
Cesarean section (%)	106 (46.9)	55 (52.4)	161 (48.7)
Total (%)	226 (100)	105 (100)	331 (100)

3.2.5 Perinatal Infection

Perinatal infection in our database was defined as presence of foul smelling amniotic fluid (or a specific diagnosis of chorioamnionitis from placental pathology), maternal fever > 38° C during labour prior to delivery, and/or a positive maternal culture for *group B streptococcus*. As screening for group B streptococcus is performed after 35 weeks gestation, the perinatal infection in our database, which is a large cohort of preterm infants, represents mainly clinical chorioamnionitis. It was coded as 0 and 1 for no and yes, respectively. Information on perinatal infection was available for 316 (95.2%) infants. Chi-squared test was performed. The proportion of infants born to mothers with possible perinatal infection was statistically not significant between no *CoNS* and *CoNS* groups (18.8% vs. 27.9%, p=0.08).

Table 6. Comparison of perinatal infection between infants exposed and not exposed to CoNS sepsis

Perinatal infection	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	155 (72.1)	82 (81.2)	237 (75)
Yes (%)	60 (27.9)	19 (18.8)	79 (25)
Total (%)	215 (100)	101 (100)	316 (100)

3.2.6 Maternal Educational Status

The information on maternal educational status was available for 312 (94%) infants. Proportion of mothers who completed a high school degree was similar in *CoNS* and no *CoNS* groups (81.4 % vs. 81.4%, p = 0.51). The results are shown in Table 7.

Table 7. Comparison of mothers who completed high school between infants exposed and not exposed to CoNS sepsis

Mothers completed high school	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	33 (15.7)	19 (18.6)	52 (16.7)
Yes (%)	77 (84.3)	83 (81.4)	260 (83.3)
Total (%)	210 (100)	102 (100)	312 (100)

3.2.7 Use of Antibiotics during Labour

The information on the use of antibiotics during labour was available for 322 (97%) infants. As shown in Table 8, the proportion of mothers who received antibiotics during the labour period was similar in *CoNS* and no *CoNS* groups (63.5% vs. 67.9%, p=0.43).

Table 8. Comparison of mother's use of antibiotics between infants exposed and not exposed to CoNS sepsis

Use of antibiotics during labour	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	70 (32.1)	38 (35.5)	108 (33.6)
Yes (%)	148 (67.9)	66 (63.5)	214 (66.4)
Total (%)	218 (100)	104 (100)	322 (100)

3.2.8 Smoking during Pregnancy

The information on maternal smoking during pregnancy was available for 324 (97.6) infants. The proportion of mothers who smoked and did not quit during pregnancy was not different between *CoNS* and no *CoNS* groups (15.8% vs. 15.5%, p = 0.94). Including mothers who quit smoking during pregnancy did not alter our study results (25.2% vs. 23.1%; p=0.94). Table 9 shows the distribution of mothers who smoked and did not quit smoking during pregnancy in the *CoNS* and no *CoNS* groups.

Table 9. Comparison of mother's smoking between infants exposed and not exposed to CoNS sepsis

Smoking during pregnancy	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	186 (84.2)	87 (84.5)	273 (84.3)
Yes (%)	35 (15.8)	16 (15.5)	51 (15.7)
Total (%)	221 (100)	103 (100)	324 (100)

3.2.9 Use of Alcohol during Pregnancy

The information on Alcohol use during pregnancy was available for 321 (96.7%) infants. A total of 15 mothers used alcohol during pregnancy in our study. The distribution of these mothers in the two groups is shown in Table 10. Fisher's exact test was performed. The proportion of alcohol use during pregnancy was not significantly different between mothers in *CoNS* and no *CoNS* groups (2.9% vs. 4.6%, p= 0.56).

Table 10. Comparison of use of alcohol in mothers of infants exposed and not exposed to CoNS sepsis

Use of Alcohol during pregnancy	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	208 (95.4)	100 (97.1)	308 (96)
Yes (%)	10 (4.6)	3 (2.9)	13 (4)
Total (%)	218 (100)	103 (100)	321 (100)

3.2.10 Use of Recreational Drugs during Pregnancy

The information on the use of recreational drugs was available for 321 (96.7%) infants. There was no significant difference in the proportion of mothers who admitted using recreational drug during pregnancy between the *CoNS* and no *CoNS* groups (2.9% vs. 5.5 %, p= 0.40). Table 11 shows the distribution of mothers used recreational drugs in the *CoNS* and no *CoNS* groups.

Table 11. Comparison of use of recreational drugs between mothers of infants exposed and not exposed to CoNS sepsis

Use of recreational drugs	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	206 (94.5)	100 (97.1)	306 (95.2)
Yes (%)	12 (5.5)	3 (2.9)	15 (4.8)
Total (%)	218 (100)	103 (100)	321 (100)

3.2.11 Summary of Maternal Characteristics

The following table shows a summary of maternal characteristics of infants exposed and not exposed to *CoNS* sepsis.

Table 12. Summary of maternal characteristics of infants exposed and not exposed to CoNS sepsis

Maternal characteristics	**No *CoNS* group**	***CoNS* group**	**P value**
Age, mean (SD)	29.2 (5.9)	29.4 (5.4)	0.79
Antenatal steroids (%)	183 (82.8)	82 (79.6)	0.49
Multiple births (%)	46 (20.3)	31 (29.5)	0.06
Perinatal infection (%)	60 (27.9)	19 (18.8)	0.08
Cesearean section (%)	106 (46.9)	55 (52.4)	0.35
Use of antibiotics during labour (%)	148 (67.9)	66 (63.5)	0.43
Mothers completed high school (%)	177 (84.3)	83 (81.3)	0.51
Smoking (%)	35 (15.8)	16 (15.5)	0.94

Alcohol use (%)	10 (4.6)	3 (2.9)	0.56
Recreational drugs (%)	12 (5.5)	3 (2.9)	0.40

3.3 Neonatal Characteristics

3.3.1 Gestational Age

Figure 4 describes the distribution of gestational age in the two groups. The distribution is skewed towards the left side in the no *CoNS* group as lower gestational age categorises had fewer infants. In general, the number of preterm infants with *CoNS* sepsis increases simultaneously with the increase of the number of births in the higher gestational age until 26 weeks when it starts to decline again.

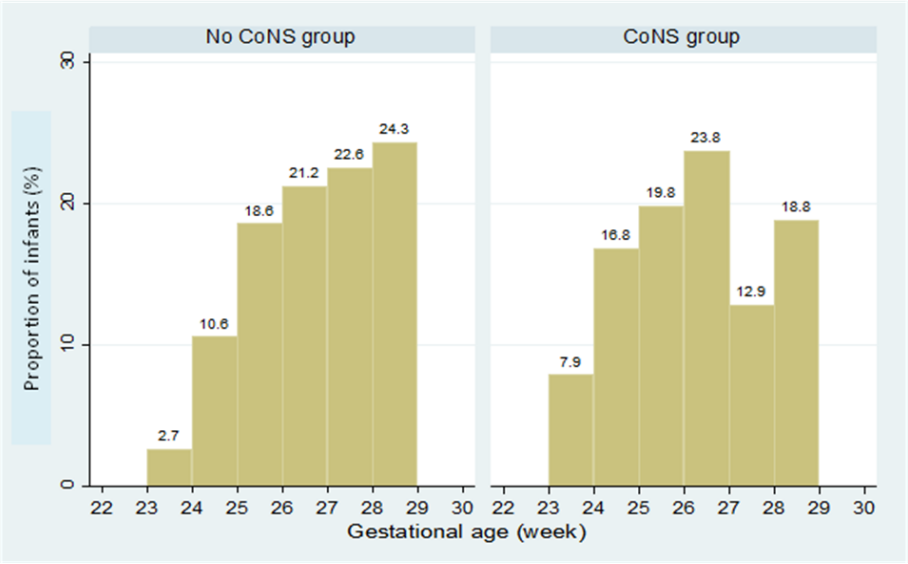

Figure 4. Histogram showing comparison of gestational age (week) by CoNS sepsis status

Table 13 shows the mean gestational age as measured by completed weeks. Using Mann Whitney test, infants in the *CoNS* group were younger compared to infants in the no *CoNS* groups (p=0.02). Since, the SD was similar between the 2 groups; t-test can also be used. The t-test showed a statistically significant difference (p=0.04).

Table 13. Comparison of gestational age (weeks) between infants exposed and not exposed to CoNS sepsis

	No *CoNS* group	***CoNS* group**	**P-value**
Gestational age (weeks) (SD)	26.2 (1.4)	25.9 (1.7)	0.04

3.3.2 Birth Weight

The graphic and numeric comparison of the birth weight is shown in figure 5 and table 14, respectively. The distribution of birth weight was not skewed. The t-test was performed. Infants in the *CoNS* group weighed less, as compared to subjects who were in the no *CoNS* group (p=0.01).

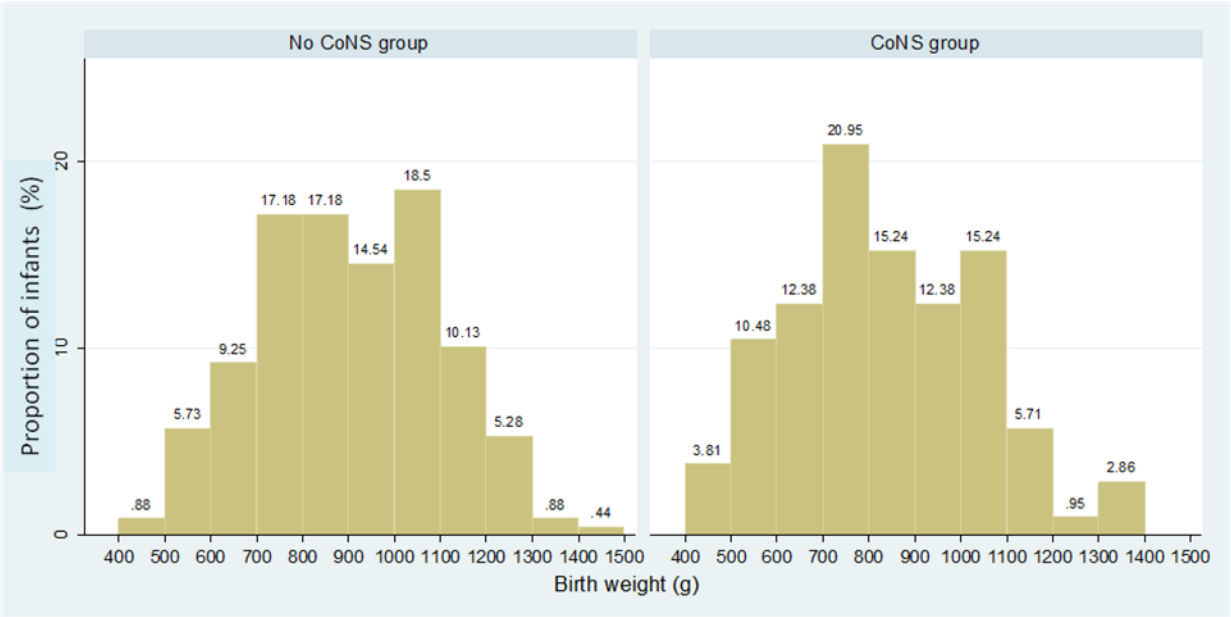

Figure 5. Histogram showing distribution of birth weight by CoNS sepsis status

Table 14. Comparison of birth weight (grams) between infants exposed and not exposed to CoNS sepsis

	No *CoNS* group	***CoNS* group**	**P-value**
Birth weight, mean (g) (SD)	900 (197)	834 (211)	0.01

3.3.3 Sex of the Neonate

Table 15 shows the comparison of neonatal sex between the *CoNS* and no *CoNS* groups. A Chi-squared test was performed to compare the distribution of the sex between the two groups. The proportion of male neonates in the *CoNS* group was not statistically different from that in the no *CoNS* group (60% versus 51.1%, p=0.13).

Table 15. Comparison of neonatal sex between infants in CoNS sepsis and no CoNS groups

Sex	**No *CoNS* group**	***CoNS* group**	**Total**
Female (%)	111 (49.9)	42 (40)	153 (46.1)
Male (%)	116 (51.1)	63 (60)	179 (53.9)
Total (%)	227 (100)	105 (100)	332 (100)

3.3.4 Small for Gestational Age:

The information on small for gestational age was available for 331 (99.7%) infants. Infants in the *CoNS* group were more likely to be small for gestational age (birth weight < 10 percentile), when compared with the infants in the no *CoNS* group (13.4% vs. 5.7%, p=0.02).

Table 16. Comparison of SGA status between infants in CoNS and no CoNS groups

Small for gestation	**No *CoNS* group**	***CoNS* group**	**Total**
Not SGA (%)	214 (94.3)	90 (86.6))	304 (92.2)
SGA (%)	13 (5.7)	14 (13.4)	27 (11.2)
Total (%)	227 (100)	104 (100)	331 (100)

3.3.5 Apgar Score at 5 minutes

Apgar score is one of the measures of the newborn's well being at the time of birth and also indicates the effectiveness of resuscitation, if needed. It is assigned at 1 and 5 minutes of life. The distribution of Apgar score at 5 minutes in the two groups is shown in figure 6.

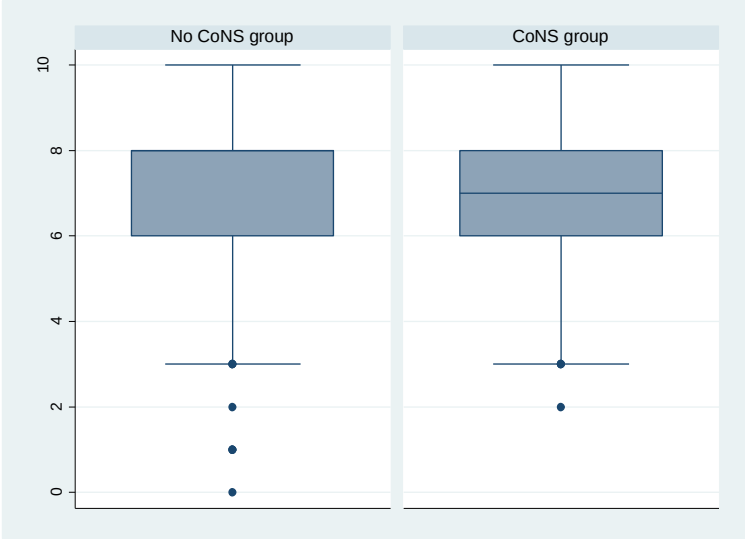

Figure 6. Box plots of Apgar score at 5 minutes in the tow groups

Apgar score is considered as an ordinal variable. Mann-Whitney test was performed. The median (IQR) Apgar score at 5 minutes was 8 (6, 8) and 7(6, 8) in the no *CoNS* and *CoNS* group respectively (p=0.03). There was a significant statistical difference in the median of 5 minute Apgar score between the two groups but this difference is not thought to be clinically significant. Moreover, there was no significant difference between the infants with Apgar score less than 7 in the *CoNS* and no *CoNS* groups (46.1% vs. 34.8%; p=0.08).

3.3.6. Umbilical Arterial pH at Birth

The mean umbilical arterial pH was lower in *CoNS* group compared to no *CoNS* group. (7.28 ± 0.09 versus 7.30 ± 0.09, p=0.04). However, this difference is also not thought to be clinically significant.

3.3.7 Summary of Neonatal Characteristics

A summary of the demographic characteristics of infants who had neurodevelopmental evaluation at 36 months corrected age is shown in Table 17.

Table 17. Summary of neonatal demographic characteristics in infants exposed and not exposed to CoNS sepsis

Demographic characteristics	**No *CoNS* group (n=227)**	***CoNS* group (n=105)**	**P value**
Gestational age, mean (wk) ± SD	26.2 ± 1.4	25.9 ± 1.7	0.04
Birth weight, mean (g) ± SD	900 ± 197	834 ± 211	0.01
Male (%)	51.1	60	0.13
SGA (%)	5.7	13.4	0.02
Apgar score at 5 min, median (IQR)	8 (6, 8)	7 (6, 8)	0.03
Cord PH, mean ± SD	7.30 ± 0.09	7.28 ± 0.09	0.02

Preterm infants in the *CoNS* group were lower gestational age at birth, had lower birth weight, and the proportion of SGA infants was higher. The 5 minute Apgar score and cord PH were lower in the *CoNS* group, though these differences are thought not to be clinically significant.

3.4 Neonatal Outcomes

3.4.1 Intraventricular Hemorrhage (IVH)

The information on IVH was available for 331 (99.7%) infants. IVH is classified into 4 grades as identified on the head ultrasonography. A total of 104 (31.4%) infants had IVH in the study

cohort. Chi-squared test was performed. Table 18 shows the proportions of IVH, all grades, in no *CoNS* and *CoNS* groups.

Table 18. Association between IVH and CoNS sepsis

IVH	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	164 (72.6)	63 (60)	227 (69.6)
Yes (%)	62 (27.4)	42 (40)	104 (31.4)
Total (%)	226 (100)	105 (100)	331 (100)

The proportion of infants who had IVH in the *CoNS* sepsis group were significantly higher than the proportion of infants with IVH in the no *CoNS* group (40 % vs. 27.4%, p=0.02).

Risk Ratio:

The unadjusted risk ratio of total IVH is 1.57 (95% CI; 1.16 – 2.12). This suggests that preterm infants exposed to *CoNS* were 1.57 times more likely to have IVH, either before or after the *CoNS* sepsis episode. This association was statistically significant, as 95% CI (1.16 – 2.12) does not cross one and has an associated p value of 0.02. Of interest, the association between IVH and *CoNS* sepsis continue to be significant after adjusting for gestational age (RR 1.4, 95% CI 1.0 – 1.85).

The proportion of severe IVH (Grade 3 or Grade 4 IVH) in the *CoNS* group was statistically not different from that in the no *CoNS* group (12.3% vs. 9.6%, p=0.46). Table 19 shows the distribution of severe IVH among preterm infants in the *CoNS* and no *CoNS* groups.

Table 19. Association between severe IVH (grade 3 and 4) and CoNS infection

Severe IVH	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	204 (90.4)	92 (87.7)	296 (90.3)
Yes (%)	22 (9.6)	13 (12.3)	35 (9.7)
Total (%)	226 (100)	105 (100)	331 (100)

3.4.2 Respiratory Outcome

Respiratory Distress Syndrome (RDS):

The information on the RDS was available for 329 (99.1%) infants. Table 20 shows the distribution of RDS in the *CoNS* and no *CoNS* groups. Chi-squared test was used to compare between the two groups. The proportion of infants diagnosed to have RDS in the *CoNS* group was larger than the same proportion in the no *CoNS* group (89.5% vs. 80.8%; p=0.047).

Table 20. Association between CoNS sepsis and RDS

RDS	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	43 (19.2)	11 (10.5)	54 (16.4)
Yes (%)	181 (80.8)	94 (89.5)	275 (83.6)
Total (%)	224 (100)	105 (100)	329 (100)

Duration of respiratory support:

Respiratory support was defined as having any type of invasive ventilation modes (Conventional and High Frequency ventilation) or non-invasive modes (Continuous Positive Airway Pressure and Biphasic Positive Airway Pressure). Figure 7 shows the distribution of the total days of respiratory support during the initial hospital stay. Mann-Whitney test was performed because of the skewed distribution. The median (IQR) duration of respiratory support was longer in *CoNS* group 38 (20, 59) days compared to no *CoNS* group 24 (7, 46) days ($p < 0.001$). The mean duration of respiratory support was 40.4 ± 26.2 days in the *CoNS* group compared to 28.8 ± 23.6 days.

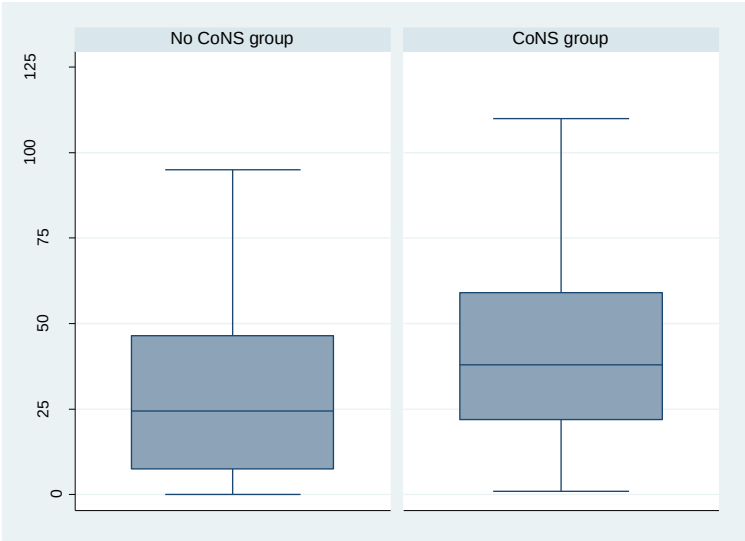

Figure 7. Box plots of duration of respiratory support in the CoNS and no CoNS group

Bronchopulmonary Dysplasia:

Data on BPD at 36 weeks gestational age was available in (91.8%) of surviving infants. There was no statistically significant difference in the proportion of BPD between the *CoNS* and no *CoNS* groups (82.3 % vs. 75.1%, p = 0.16).The risk ratio was 1.09 (95% CI 0.97-1.24). The distribution of BPD in *CoNS* and no *CoNS* group is shown in table 21.

Table 21. Association between CoNS sepsis and BPD

BPD	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	52 (24.9)	17 (17.7)	69 (22.7)
Yes (%)	157 (75.1)	79 (82.3)	236 (77.3)
Total (%)	209 (100)	96 (100)	305 (100)

Use of Postnatal Steroids:

Postnatal steroids and particularly dexamethasone were commonly used in preterm infants to facilitate extubation or treat severe BPD before 2003. Increased risk of poor neurodevelopmental outcome of infants exposed to dexamethasone has alerted neonatologists to largely restrict its use. A total of 80 (24.2%) infants in the study cohort received postnatal steroids. Postnatal steroids in our database included use of dexamethasone mainly between 1995 and 2008. However, hydrocortisone was included in the coding of postnatal steroids late in 2008. Table 22 shows the distribution of steroid use in the *CoNS* and no *CoNS* groups. The proportion of use of postnatal steroids in *CoNS* group was higher than no *CoNS* group (36.2% vs. 18.6%; p=0.001).

Table 22. Association between CoNS sepsis and use of postnatal steroids

Postnatal Steroids	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	183 (81.4)	67 (63.8)	250 (75.8)
Yes (%)	42 (18.6)	38 (36.2)	80 (24.2)
Total (%)	225 (100)	105 (100)	330 (100)

The use of postnatal steroids was highly correlated with the duration of respiratory support ($p<0.001$). The odds of receiving postnatal steroids increased by 0.03 (95% CI 0.02 – 0.04) with each day increase in the duration of respiratory support.

Discharge Home on O2:

Discharge home on O2 represents another marker of severe chronic lung disease in preterm infants. Table 23 shows the distribution of infants who went home on O2 in the *CoNS* and no *CoNS* groups. There was no difference in the proportions of infants who were discharged home on O2 in the two groups (53.2% vs. 52%; $p=0.82$).

Table 23. Association between going home on oxygen and CoNS sepsis

Discharge home on O2	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	108 (48)	49 (46.8)	157 (47.6)
Yes (%)	117 (52)	56 (53.2)	173 (52.4)
Total (%)	225 (100)	105 (100)	330 (100)

3.4.3 Patent Ductus Arteriosus (PDA)

Two hundred thirty (67.8%) infants were diagnosed with PDA in the study cohort. Table 24 shows distribution of infants with PDA in the two groups.

Table 24. Association between PDA and CoNS sepsis

PDA	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	86 (47.9)	21 (16)	107 (32.3)
Yes (%)	141 (62.1)	84 (84)	225 (67.7)
Total (%)	227 (100)	105 (100)	332 (100)

There was a statistically significant difference in the proportion of PDA between *CoNS* and no *CoNS* groups (80% vs. 62.1%, p = 0.001). This association remained significant after adjusting for gestational age [RR 1.78 (95% CI 1.19 – 2.49); p=0.007].

Surgical ligation:

Of the 84 infants who had PDA in the *CoNS* group, 46 (54.8%) infants had surgical ligation of the PDA, compared to 63 (44.7%) infants of the 141 with PDA in the no *CoNS* group (p=0.004).

3.4.4 Necrotizing Enterocolitis (NEC)

NEC is usually categorized into several stages as per modified Bell's criteria depending on the severity of the disease. The presence of stage II or more (confirmed) is considered a significant morbidity. Chi-squared test was performed. The proportion of infant who had confirmed NEC in the *CoNS* group was higher than that in the no *CoNS* group (21.9% vs. 13.6%) as shown in table 25. However; this was not statistically significant (p=0.06). The risk ratio of the association between NEC and *CoNS* sepsis was 1.6 (95% CI 0.98 - 2.61).

Table 25. Association between NEC and CoNS sepsis

NEC	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	196 (86.4)	82 (79.9)	278 (83.7)
Yes (%)	31 (13.6)	23 (21.9)	56 (16.3)
Total (%)	227 (100)	105 (100)	332 (100)

3.4.5 Periventricular Leukomalacia

Seven infants had PVL in the study cohort. Fisher's exact test was used for the comparison. Table 26 shows the proportions of PVL in the *CoNS* and no *CoNS* groups. There was no significant difference between the groups (2.8% vs. 1.8%; p=0.68).

Table 26. Association between PVL and CoNS sepsis

PVL	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	219 (98.2)	102 (97.2)	321 (96.9)
Yes (%)	4 (1.8)	3 (2.8)	7 (3.1)
Total (%)	223 (100)	105 (100)	228 (100)

3.4.6 Retinopathy of Prematurity (ROP)

The information on ROP outcome in the NICU was available for 248 (74.7%) infants only. The proportion of ROP (all stages) in the *CoNS* group was significantly higher than that in the no *CoNS* group (77.8% **v**s. 58.8%, p= 0.002). The risk ratio of retinopathy of prematurity was 1.32 (95% CI 1.12 - 1.46). Risk difference was 0.18 (95% CI 0.07-0.30). Table 27 shows the distribution of ROP between the *CoNS* and no *CoNS* groups.

Table 27. Association between ROP and CoNS sepsis

All ROP before discharge	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	65 (41.2)	20 (22.2)	85 (34.3)
Yes (%)	93 (58.8)	70 (77.8)	163 (67.7)
Total (%)	158 (100)	90 (100)	248 (100)

The risk of all stages of ROP continued to be important in infants exposed to *CoNS* sepsis after adjusting for gestational age and duration of oxygen requirement as potential confounders (RR 1.25; 95% CI 1.01 – 1.43).

Severe ROP was defined if the infant ever had stage 3 or greater or had plus disease at 4 months corrected gestational age. Data was available for 324 (95.8%) infants. The proportion of available data on severe ROP was higher than the proportion of data on all stages ROP. This difference may be caused by the bias to report severe ROP more than the milder form in the list of diagnosis at time of discharge, or the fact that some infants may have their ROP progressed to severe stages after their discharge from the hospital. Table 28 shows the proportion of severe ROP in the two groups.

Table 28. Association between severe ROP and CoNS sepsis

Severe ROP	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	170 (77.3)	61 (62.3)	231 (72.7)
Yes (%)	50 (22.7)	37 (37.7)	87 (27.3)
Total (%)	220 (100)	98 (100)	318 (100)

The proportion of severe ROP was significantly higher in the *CoNS* group compared to the no *CoNS* group (37.7% vs. 22.7%; p=0.005). The risk ratio of the association between CoNS sepsis and severe ROP was 1.66 (95% CI 1.16-2.36) and the risk difference was 0.15 (95% CI 0.04 – 0.26). After correcting for gestational age and duration of oxygen requirement, there was no difference in the proportion of severe ROP in infants exposed or not exposed to *CoNS* sepsis (RR 1.26; 95% CI 0.79 – 1.85).

The variable "Eye surgery" indicates if any type of ROP surgery (i.e. cryotherapy, laser, or photocoagulation) was performed to the time of NICU discharge. Data was available for 292 (86.4%) infants. Forty-nine (16.8%) infants in the study cohort required eye surgery. Table 29 shows the proportions of infants who required eye surgery in both groups before NICU discharge.

Table 29. Association between ROP requiring eye surgery and CoNS sepsis

Eye surgery	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	180 (89.1)	63 (70)	243 (83.2)
Yes (%)	22 (10.9)	25 (30)	43 (16.8)
Total (%)	202 (100)	90 (100)	292 (100)

More infants in the *CoNS* group required eye surgery compared to no *CoNS* group (27.2% vs. 10.8%, p<0.001). Risk ratio of eye surgery was 2.51 (95% CI 1.48-4.24) and the risk difference was 0.16 (95% CI 0.06-0.26). After correcting for gestational age and duration of oxygen requirement, there was no association between ROP requiring surgery and *CoNS* sepsis (RR 1.53; 95% CI 0.91 – 2.25).

3.4.7 Length of Hospital Stay

The Median (IQR) length of hospital stay was 81 (70, 100) days in the no *CoNS* group and 98 (79, 124) days in the *CoNS* group. The mean length of hospital stay was 87.3 ± 30.6 days in the no *CoNS* group compared with 104.7 ± 40 in the *CoNS* group. Figure 8 shows the distribution of length of hospital stay both groups. The mildly skewed distribution of data, the presence of outliers, and the difference in standard deviation (SD) between the two groups suggest the better use of non-parametric statistics.[99] Parametric statistics assume that data has a normal

distribution, therefore; using parametric statistics in skewed distribution data may give misleading results. In addition, non-parametric statistics is less sensitive to outliers.

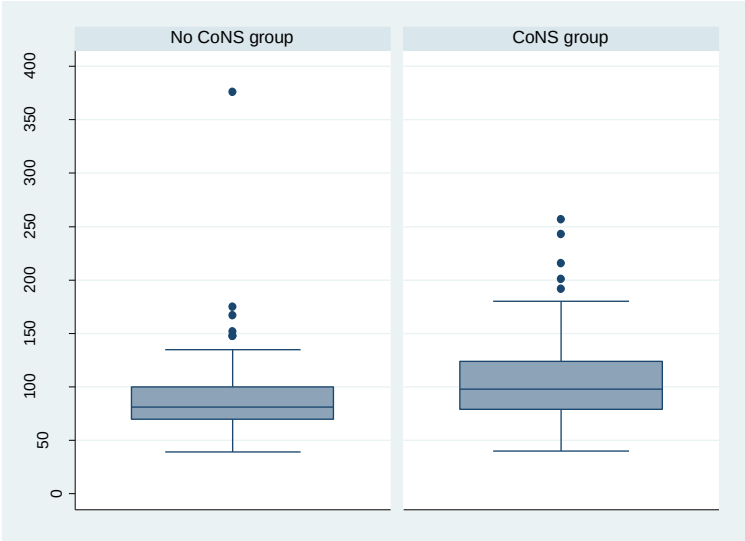

Figure 8. Length of hospital stay (day) in CoNS and no CoNS groups before adjusting for gestational age

Mann Whitney test was performed for the comparison. The hospital stay in the *CoNS* group was significantly longer than no *CoNS* group ($p<0.001$). Gestational age is thought to be a potential confounder for the relationship between the length of stay in the hospital and *CoNS* infection. Multivariable median linear regression was performed to adjust for gestational age as the outliers may heavily affect the results of the mean. The regression model showed that gestational age is a strong confounder for the relationship between *CoNS* sepsis and the length of hospital stay. The difference of median of length of hospital stay between the 2 groups decreases from 17 days to 12 (95% CI 7.1-16.9) days (variable that make more than 15 % difference when included in the model is highly considered as confounder).[100] However, this new difference between the two groups is still clinically and statistically significant ($p<0.001$).

Because the outliers are always a source of concern when present, the analysis was repeated after removing all of them from both groups. The difference between the length of stay between the no *CoNS* and the *CoNS* groups was 16.5 days using the median for measuring the central tendency [80 (IQR: 69, 99) vs. 96.5 (IQR: 77, 120)] and 14.5 days using the mean (84.3 ± 21.2 vs. 98.8 ± 30.2). These differences remained significant using t-test and Mann Whitney test ($p<0.001$). In addition, adjustment for gestational age as a potential confounder yielded a difference of 11.75 days (7.1 – 16.4) between the two groups ($p<0.001$). Moreover, this difference continued to be important even after including other morbidities in the analysis such as severe IVH, BPD, NEC and severe ROP [difference of 8.5 days (3.1- 13.9); $p=0.002$)].

3.4.8 Mortality

Eleven (8%) infants of the 137 infants with *CoNS* sepsis died compared with 61 (18.3%) infants of the 332 in the no *CoNS* group died. Table 30 shows the distribution of age of death in the two groups. Death at 0-7 days represents early neonatal death while death at 8-28 days represents the late neonatal death.

Table 30. Age at death in the CoNS and no CoNS groups

Age at death (day)	**No *CoNS* group**	***CoNS* group**	**Total**
0-7 (%)	28 (45.9)	0 (0)	28 (38.8)
8-28 (%)	17 (27.8)	5 (45.5)	22 (30.6)
29-365 (%)	15 (24.7)	5 (45.5)	20 (27.8)
>365 (%)	1 (1.6)	1 (9)	2 (2.8)
Total (%)	61 (100)	11 (100)	72 (100)

The mortality difference between the 2 groups was statistically significant (p=0.005). Of the total 72 dead infants, 21 (29.2%) infants died in the first 3 days of life. Due to the fact that *CoNS* infection is a late onset sepsis (occurs usually after day 3 of life), infants who died in the first week of life could have *CoNS* infection during their initial stay in the NICU if they had lived longer. No difference was found between the two groups after excluding the infants who died in the first week of life (p= 0.36).

3.4.9 Summary of the demographic characteristics and neonatal outcomes

A summary of the neonatal outcomes of infants who had neurodevelopmental evaluation at 36 months corrected age is shown in Table 31.

Table 31. Summary of neonatal outcomes in infants exposed and not exposed to CoNS sepsis

Neonatal outcome	**No *CoNS* group (n=227)**	***CoNS* group (n=105)**	**P value**
RDS (%)	80.8	89.5	0.047
Length of respiratory support day, median (IQR)	24 (7, 46)	38 (20, 59)	<0.001
Postnatal steroid (%)	18.6	36.2	0.001
BPD (%)	75.1	82.3	0.16
IVH (%)	27.4	40	0.02
Severe IVH (%)	9.6	12.3	0.46
PDA (%)	62.1	80	0.001
Surgical PDA (%)	27.7	43.8	0.004
NEC (%)	13.6	21.9	0.06
ROP (%)	58.8	77.8	0.002
Severe ROP ≥ stage 3 (%)	22.7	37.7	0.005

ROP requiring surgery (%)	10.8	27.2	<0.001
Length of stay day, median (IQR)	81 (70, 100)	98 (79, 124)	<0.001

Using bivariate analysis, the proportions of all RDS, use of surfactant, use of postnatal steroids, all grades IVH, PDA, and ROP were higher in the CoNS group. Moreover, infants in the *CoNS* groups required respiratory support for longer period and their stay in the NICU was significantly prolonged compared to no *CoNS* group.

3.5 Neurodevelopmental Outcome

3.5.1 Assessment of Lost to Follow-up Patients

The corrected age at follow up was 37.1 ± 2.1 months in the *CoNS* group and 36.9 ± 1.85 months in the no *CoNS* group (p=0.4). Of the 51 infants who were lost to follow up at 36 months, two had missing data in the neonatal period. Of the 51 infants, 46 initially attended the follow up clinic (anytime from 4 to 28 months CA). There was no difference in the proportion of infants who were lost to follow up at 36 months CA between *CoNS* and no *CoNS* groups (13.2% vs. 13.4%; p=0.97).

Table 32 shows comparison of the baseline demographic and neonatal morbidity between those with and without neurodevelopmental assessment at 36 months corrected age

Table 32. Baseline characteristics of infants who were lost to follow up and infants who had neurodevelopmental (ND) evaluation at 36 months corrected gestational age

Demographic characteristics	**Infants who had ND evaluation (n=332)**	**Infants who were lost to follow up (n=49)**	**p-value**
Gestational age, mean (wk) ± SD	26.1 ± 1.5	26.5 ± 1.8	0.13
Birth weight, mean (g) ± SD	879 ± 203	901 ± 215	0.48
Male (%)	53.9	59.2	0.49
SGA (%)	8.1	6.1	0.62
Apgar score at 5 min, median (IQR)	7 (6, 8)	8 (7, 8)	0.44
Antenatal steroid (%)	81.8	83.6	0.75
Perinatal infection (%)	25	13.3	0.08
Cesearean section (%)	48.5	57.1	0.26
Multiple births (%)	23.2	14.3	0.16

Table 33. Neonatal morbidity of infants who were lost to follow up and infants who had neurodevelopmental evaluation at 36 months corrected gestational age and infants

Neonatal outcome	**Infants who had ND evaluation (n=332)**	**Infants who were lost to follow up (n=49)**	**p-value**
IVH (%)	31.3	16.3	0.03
Severe IVH (%)	10.6	2.0	0.07
BPD (%)	77.3	76.1	0.84
PDA (%)	68.4	75.5	0.31
Surgical PDA (%)	32.8	34.7	0.38
NEC, n (%)	16.2	16.3	0.99
ROP (%)	65.7	64.3	0.87
Severe ROP ≥ stage 3(%)	27.3	13.3	0.04
ROP requiring surgery (%)	16.1	8.8	0.26
Length of stay, median (day)	87 (72, 106)	81 (71, 100)	0.30
Length of respiratory support, median (day)	29 (10, 49)	28 (7, 45)	0.36

There were no differences in the baseline demographic and neonatal morbidity except for the IVH (all grades) and severe ROP. Infants who remained in the study had higher proportion of IVH and also revealed a trend to have higher proportion of severe IVH. However, in the lost to follow up group, there were only 2 (15.4%) infants with IVH in the *CoNS* group compared to 6 (16.7%) in the no *CoNS* group (p=1.0). Of the 6, only one had severe IVH. No infant in the *CoNS* group had severe IVH.

In the lost to follow up group also, there were 6 (50%) infants in the *CoNS* group with ROP compared to 12 (75%) in the no *CoNS* group (p=0.17). One (7.6%) infant had severe ROP in the *CoNS* group compared to 5 (15.6%) of the infants in the no *CoNS* group (p=0.65).

3.5.2 Cerebral Palsy

A total of 35 out of 332 (10.5%) infants developed cerebral palsy. Table 34 describes the association between cerebral palsy and *CoNS* sepsis.

Table 34. Association between cerebral palsy and CoNS sepsis

Cerebral palsy	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	203 (89.4)	94 (89.5)	297 (89.5)
Yes (%)	24 (10.6)	11 (10.5)	35 (10.5)
Total (%)	227 (100)	105 (100)	332 (100)

The proportion of infants with all grades of CP in the *CoNS* group was similar to that in the no *CoNS* group (10.5% vs. 10.6%, p=0.98). Risk ratio of CP in children exposed to *CoNS* in the neonatal period was 0.99 (95% CI 0.50 – 1.94). The potential confounders for the association between CP and *CoNS* sepsis are severe IVH and gestational age. Including both in the logistic regression model yield RR 0.75 (95% CI 0.33 – 1.68) and p=0.53.

Table 35 shows the comparison of the severity of cerebral palsy between the *CoNS* and the no *CoNS* groups. Of the 11 children who developed cerebral palsy in the *CoNS* group, 8 (72.7%) had moderate to severe cerebral palsy and 3 (27.3 %) had mild form. Of the 22 children with cerebral palsy in no *CoNS* group, 15 (62.5%) had moderate to severe cerebral palsy and 9 (37.5%) had mild cerebral palsy. The proportion of infants with moderate to severe CP in the *CoNS* group was similar to that in the no *CoNS* group (8 % vs. 7 %; p=0.75).

Table 35. Comparison of the severity of cerebral palsy between CoNS and no CoNS groups

Severity of CP	**No *CoNS* group**	***CoNS* group**	**Total**
Mild (%)	9 (37.5)	3 (27.3)	12 (34.3)
Moderate (%)	9 (37.5)	3 (27.3)	12 (34.3)
Severe (%)	6 (25)	5 (54.5)	10 (28.6)
Total (%)	24 (100)	11 (100)	35 (100)

3.5.3 Cognitive Outcome

Of the 332 infants, 304 (91.5%) infants had a report on the type of the standardised test used to evaluate the cognitive outcome. The most commonly used tests were Wechsler Preschool and Primary Scale of Intelligence II (WPPSI-II) in 65.8% of children, Stanford Binet-IV in 14.5% and Bayley Scale of Infant Development (BSID-II) in 10.8% respectively. BSID-III, Leiter Scale, and McCarthy were used in 8.1% of children.

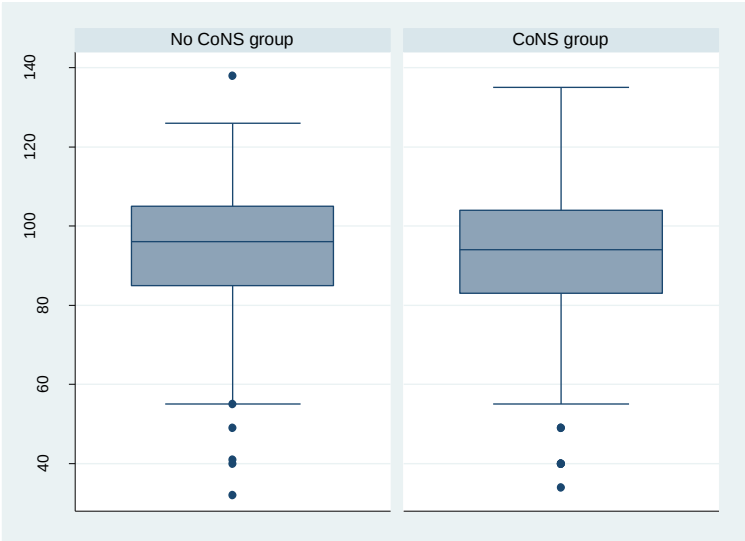

Figure 9. The distribution of cognitive index in the two groups

Table 36 shows the comparison of cognitive outcome between the two groups. The mean cognitive index was 90.8 ± 21.0 in the *CoNS* group and 94.7 ± 16.3 in infants not exposed to *CoNS* group. The t-test demonstrated a p value of 0.08 when comparing between the two groups. As the figure shows, the distributions of the subjects were mildly skewed. The result of Mann Whitney test demonstrated a p value = 0.22.

Table 36. Comparison of children with cognitive delay between the CoNS and no CoNS groups

Cognitive delay (> 2 SD)	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	208 (92.5)	87 (82.4)	295 (89.4)
Yes (%)	17 (7.5)	18 (17.6)	35 (10.6)
Total (%)	225 (100)	105 (100)	330 (100)

In contrast, the proportion of infants with cognitive delay > 2 SD below the mean was higher in the *CoNS* group compared with the no *CoNS* group (17.6% vs. 7.5%, p=0.01). The risk ratio of cognitive delay in children who had *CoNS* sepsis in the neonatal period was 2.27 (95% CI 1.22-3.94). Gestational age and severe IVH were thought to confound the relationship between *CoNS* sepsis and cognitive delay as both were associated in our study with *CoNS* sepsis and have been identified in the literature to affect cognitive outcome. Adjusting for both factors in a logistic regression model yielded RR 1.98 (95% CI 1.01 – 3.63) and p=0.04. Therefore, the risk of cognitive delay in children born at ≤ 28 weeks gestation who had *CoNS* sepsis in the neonatal period is approximately two times the risk of having this outcome in children with no *CoNS* sepsis.

A total of 82 children had any grade of cognitive delay including the mild form (cognitive delay between 1 and 2 SD below the mean). The proportion of having any grade of cognitive delay was

higher in infants exposed to *CoNS* sepsis compared with unexposed infants, however; this difference was not statistically significant (30.5% vs. 22.4%; p=0.12).

3.5.4 Hearing Outcome

Deafness in the study was defined as sensory neural hearing loss requiring hearing aid. Table 37 shows the distribution of deafness between infants in the two groups.

Table 37. Comparison of deafness between infants in the CoNS and no CoNS groups

Deafness	**No *CoNS* group**	***CoNS* group**	**Total**
Normal (%)	221 (97.8)	98 (94.3)	319 (96.7)
Deafness (%)	5 (2.2)	6 (5.7)	11 (3.3)
Total (%)	226 (100)	104 (100)	330 (100)

Six infants (5.7 %) in the *CoNS* group developed sensory neural hearing loss requiring amplification compared with five infants (2.2%) in the no *CoNS* group. Chi-squared test was performed to compare between the 2 groups. There was no statistically significant difference in the proportion of deafness between children exposed and not exposed to *CoNS* sepsis in the neonatal period (p=0.09) (Fisher's test p=0.1). The risk ratio was 2.61 (95% CI 0.81 - 8.35). The risk difference was 0.03 (-0.01 to 0.08).

Milder neurosensory hearing loss was defined in our perinatal follow-up program when loss does not require amplification or implants, or require amplification in one ear. Six children in our study cohort had this type of hearing loss. Fisher's test was performed for the comparison. The proportion of children with this milder hearing loss in the *CoNS* group was relatively higher but this was not statistically significant (4.1% vs. 0.9%; p=0.07).

The overall neurosensory hearing impairment was identified in 17 children. The proportion of children with any degree of this impairment was higher in the group exposed to *CoNS* sepsis compared to those not exposed to *CoNS* sepsis (9.6% vs. 3.1%; p=0.01).

3.5.5 Vision Outcome

Blindness was defined as visual acuity < 20/200 following refractive correction in the better eye. One infant (1%) exposed to *CoNS* sepsis developed blindness compared with 4 infants (1.7%) in the no *CoNS* group. Fisher's test was performed for the comparison. No statistical difference was found between the two groups (p=1.0). Table 38 shows the distribution of blindness in the two groups.

Table 38. Comparison of blindness rate between infants with CoNS and no CoNS groups

Blindness	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	220 (98.3)	101 (99)	321 (98.5)
Yes (%)	4 (1.7)	1 (1)	5 (1.5)
Total (%)	224 (100)	102 (100)	326 (100)

Milder visual impairment was defined when corrected acuity < 20/60 but > 20/200 in the better eye, significant refractive errors such as severe myopia or significant hyperopia, or unilateral blindness. Twenty-seven children in the study cohort had this type of visual impairment. There was no significant difference in the milder visual impairment between children who exposed or not exposed to *CoNS* sepsis in the neonatal period (9.9% vs. 7.8%; p=0.53). Moreover, the overall visual impairment including bilateral blindness and the milder form was also not statistically different between the two groups (10.8% vs. 9.4%; p=0.71).

3.5.6 Major Neurodevelopmental Outcome

The primary outcome of our study was the incidence of major neurodevelopmental disability at 36 months CA. [i.e. cerebral palsy, cognitive delay (cognitive score 2 SD below the mean on standardized psychological testing), vision loss and deafness].

Nine children had one or two missing data items in one or two of the major neurodevelopmental categories. No one in the CP category was lost to follow up at 36 months, while 2 children in the cognitive delay category, 6 in the blind category and 2 in the deafness group were lost to follow up at the equivalent corrected month. Only one child had missing data in two categories. No child had missing data for more than 2 items. If the child had any positive outcome (yes) in one of the 4 categories, he/she was considered to have major neurodevelopmental disability. In contrast, if the child had a missing value for one or two categories of the major neurodevelopmental outcome, he/she was considered to have missing data for the major neurodevelopmental outcome. As a result, 7 children had missing values on neurodevelopmental disability. Of the 7, 3 children were in the *CoNS* group and 4 children were in the no *CoNS* group. These 7 children were excluded prior to conducting the analysis of the major neurdevelopmental impairment (the primary outcome).

Table 49 describes the number of children with major neurodevelopmental impairment in the *CoNS* and the no *CoNS* group. The proportion of children who had severe neurodevelopmental impairment in the *CoNS* group was significantly higher than that in the no *CoNS* group (25.4 % Vs 15.2%, p=0.03).

Table 49. Proportion of major NDI in the CoNS and no CoNS group

Major NDI	**No *CoNS* group**	***CoNS* group**	**Total**
No (%)	189 (84.8)	76 (74.6)	265 (81.6)
Yes (%)	34 (15.2)	26 (25.4)	60 (18.4)
Total (%)	223 (100)	102 (100)	325 (100)

Mild and Overall neurodevelopmental Outcome:

Definition of mild neurodevelopmental impairment includes mild cerebral palsy, mild cognitive delay (defined as cognitive score between 1 and 2 standard deviations below mean i.e. 70-85), hearing impairment not requiring amplification or visual impairment (not blind). There was no statistical difference between the proportions of mild neurodevelopmental impairment (NDI) between infants exposed and not exposed to *CoNS* (26.6 % vs. 25.6%, p=0.87).

The overall NDI includes all children with any form of neurodevelopmental impairment. A total of 117 children in the study cohort had any NDI. The infants in the *CoNS* group were more likely to have NDI but this difference was not statistically significant (43.1% vs. 33.0%, p=0.07)

3.5.6.1 Classical analysis

Effect modification and confounding:

Effect modification and confounding caused by various maternal and infants variables on the association between *CoNS* sepsis and severe NDI were investigated by stratified analysis. The risk ratios between exposure to *CoNS* sepsis and severe NDI were calculated in strata of the third variable. The null hypothesis underlying equal risk ratios in the strata was examined through a test of homogeneity. A p value of less than 0.05 in the Wald statistics supports the third variable as an effect modifier. If the test was not significant, then crude and Mantel-Haenszel (M-H) relative risks were compared to study if the variable confounds the association between the *CoNS* infection and severe NDI. The study used a 15 % change in the adjusted risk ratio (M-H combined) in relation to the crude risk ratio as a quantitative criterion to assess the confounding [101] in addition to the clinical significance of this change.

For the measured data, box plots of the variable were plotted against *CoNS* sepsis status (yes or no) as a major category and severe NDI as subcategory. If the box plots are similar among groups then it is assumed that there is no effect modification. A presence of either effect modification or confounding is considered if the differences in the box plots among the groups exist.

Table 40 demonstrates the effect of various maternal and neonatal characteristics on the association between *CoNS* infection and severe NDI using the stratified analysis.

Table 40. Effect of various maternal and neonatal characteristics on the association between CoNS sepsis and major NDI using the stratified analysis

Variable	**Absent RR (95% CI)**	**Present RR (95% CI)**	**Test of homogeneity (M-H) p-value**
Maternal and Neonatal characteristics			
Perinatal infection	2.2 (1.26 – 3.81)	0.5 (0.12 – 2.03)	0.05
Antenatal steroid	1.62 (0.79 -3.36)	1.51 (0.85 – 2.68)	0.87
Multiple births	1.34 (0.77 – 2.31)	3.17 (1.20 – 8.35)	0.13
Cesearean section	1.87 (1.01 – 3.46)	1.57 (0.79 – 3.11)	0.71
Male	2.14 (1.06 – 4.32)	1.38 (0.76 – 2.49)	0.34
SGA	1.48 (0.89 – 2.48)	1.71 (0.54 – 5.42)	0.82
Neonatal morbidities			
IVH	2.14 (1.07 - 4.31)	1.07 (0.61 - 1.89)	0.12
Severe IVH	2.03 (1.13 – 3.65)	0.97 (0.56 – 1.64)	0.05
Postnatal steroids	1.77 (0.91 – 3.54)	1.01 (0.56 – 1.79)	0.20

BPD	0.53 (0.07 – 4.09)	1.18 (1.10 – 2.86)	0.25
PDA	2.02 (0.39 – 10.31)	1.36 (0.86 – 2.16)	0.64
ROP	2.16 (0.68 – 6.92)	1.31 (7.8 – 2.21)	0.44
ROP≥3	1.64 (0.75 – 3.56)	1.23 (0.73 – 2.07)	0.54

There was no significant effect modification from all variables included in the study. However, perinatal infection and severe IVH showed a trend toward modifying the relationship between the major neurodevelopmental impairment and the exposure to *CoNS* sepsis in the neonatal period as the exact p values were 0.0512 and 0.0508, respectively. Children of mothers without exposure to perinatal infection tend to have higher association between *CoNS* sepsis and major developmental impairment when compared with children born to mothers with no history of perinatal infection, however; this effect was not statistically significant because of the overlap between the 95% CI and the p-value of more than the exact 0.0500. The same also applied for severe IVH. Table 41 describes the comparison between the crude risk ratio and the adjusted (M-H combined) risk ratio as a process to identify potential confounding factors.

Table 41. Comparison between crude and adjusted risk ratio of major neurodevelopmental outcome in infants exposed to CoNS sepsis using stratified analysis

Variable	**Crude RR (95% CI)**	**M-H Combined (95%CI)**	**Difference between crude and adjusted risk (%)**
Maternal characteristics			
Perinatal infection	1.67 (1.06 – 2.63)	1.66 (1.01 – 2.73)	1.9
Antenatal steroid	1.67 (1.06 – 2.63)	1.55 (0.98 – 2.43)	7.2
Multiple births	1.67 (1.06 – 2.63)	1.67 (1.05 – 2.68)	0
Cesearean section	1.67 (1.06 – 2.63)	1.72 (1.09 – 2.72)	3
Male	1.67 (1.06 – 2.63)	1.64 (1.05 – 2.58)	1.8
SGA	1.67 (1.06 – 2.63)	1.52 (0.95 – 2.43)	9
Postnatal steroids	1.67 (1.06 – 2.63)	1.32 (0.86 – 2.03)	19
Neonatal characteristics			
IVH	1.67 (1.06 – 2.63)	1.44 (0.93 – 2.22)	13.8
Severe IVH	1.67 (1.06 – 2.63)	1.54 (1.02 – 2.33)	7.8
BPD	1.67 (1.06 – 2.63)	1.60 (1.01 – 2.54)	4.2

PDA	1.67 (1.06 – 2.63)	1.41 (0.90 – 2.19)	15
ROP	1.67 (1.06 – 2.63)	1.43 (0.89 – 2.29)	14.4
ROP ≥ stage 3	1.67 (1.06 – 2.63)	1.37 (0.89 – 2.12)	18

The values of adjusted RR of postnatal steroids and severe ROP were 19% and 18 % less than their crude RR, respectively. Therefore, postnatal steroids and severe ROP were considered as potential confounders.

In order to assess the possibility that maternal age, gestational age and birth weight could modify the association between exposure to *CoNS* in the neonatal period and the major neurodevelopmental outcome at 36 months corrected age, comparison was made between the subgroups using graphics. The following figures illustrate the distribution of measured continuous variables across the *CoNS* sepsis group and severe NDI.

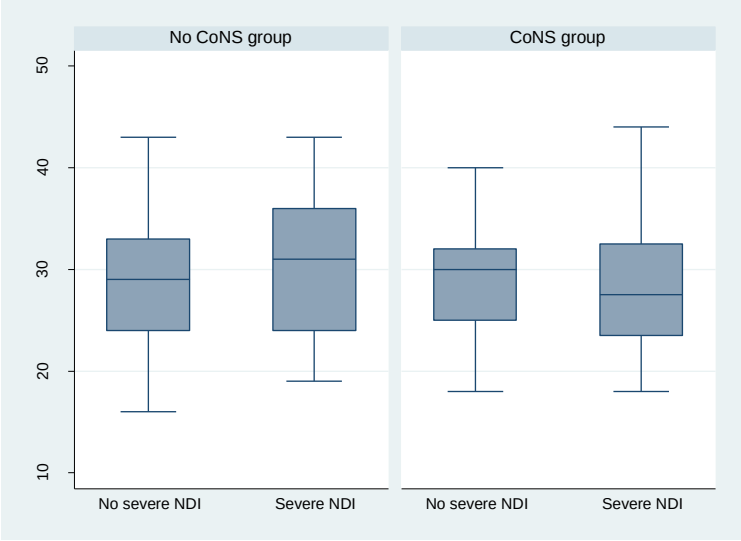

Figure 10. Comparison of box plots of maternal age in severe NDI among CoNS groups

Figure 10 shows the distributions of maternal age across *CoNS* and severe NDI status. Maternal age distribution appeared similar across the groups hence was not included into further analysis.

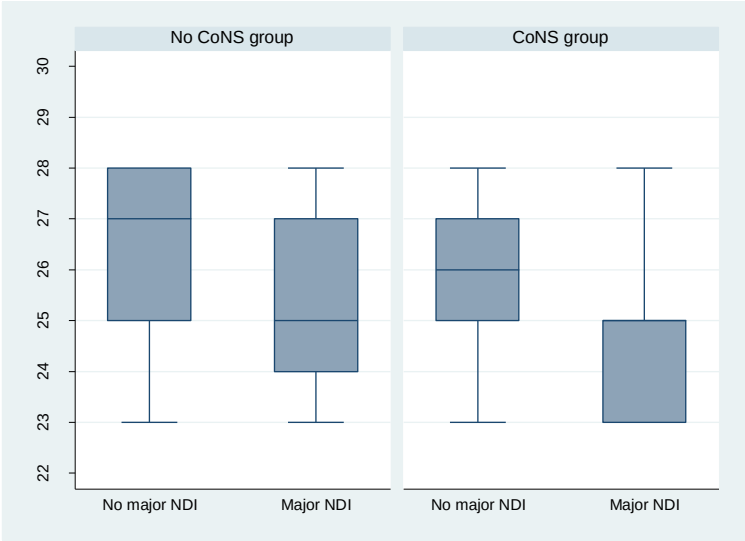

Figure 11. Comparison of box plots of gestational age in severe NDI among CoNS groups

Figure 11 shows that children with lower gestational age were more likely to have major NDI compared to those without major NDI in both *CoNS* group and no *CoNS* group, thus no effect modification was observed. The same observation is applied to the birth weight.

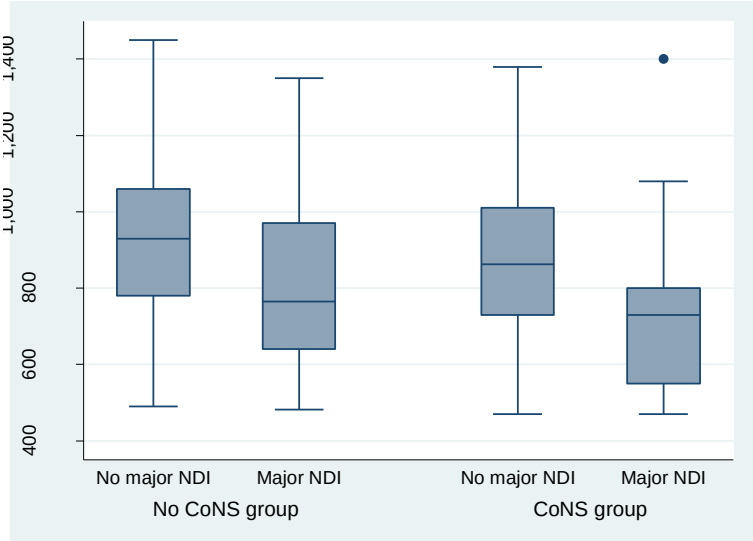

Figure 12.Comparison of box plots of birth weight in severe NDI among CoNS groups

For further evaluation of possible effect modification or confounding of the measured neonatal variables (gestational age and birth weight) on the association between *CoNS* infection and severe NDI, both variables were dichotomized. Gestational age was dichotomized into to <26 weeks (i.e. $25^{6/7}$ and less) and ≥ 26 weeks (i.e. $26^{0/7}$ and more) as babies born at less than 26 weeks represent the limits of viability of premature infants and historically have poorer neurodevelopmental outcome compared with infants born at ≥ 26 weeks. In addition, the mean of gestational age in our sample is 26.2 weeks (median is 26 weeks). Birth weight was dichotomized into < 878 g and ≥ 878 g and this cut-off represents the mean birth weight as well (median is 870 g). Table 42 illustrates that there was no effect modification by centred gestational age or centred birth weight.

Table 42. Assessment of confounding factors using stratified analysis for birth weight and gestational age

Variable	**Absent RR (95%CI)**	**Present (95%CI)**	**Test of homogeneity (M-H) p-value**
Gestation (≥26 weeks)	1.59 (0.93-2.71)	1.49 (0.89 - 2.49)	0.70
Birth weight (≥ 878 g)	1.57 (0.89 - 2.49)	1.57 (0.62 – 3.97)	0.93

Table 43 illustrates no confounding when centred gestational age or centred birth weight used in the stratified analysis.

Table 43. Assessment of confounding factors using stratified analysis for birth weight and gestational age

Variable	**Crude RR (95%CI)**	**M-H Combined RR(95%CI)**	**Difference between crude and adjusted risk (%)**
Gestation (≥ 26 weeks)	1.67 (1.06 – 2.63)	1.48 (0.95 – 2.31)	11.4
Birth weight (≥ 878 g)	1.67 (1.06 – 2.63)	1.51 (0.97 – 2.37)	9.6

As the stratified analysis has its own limitation in handling measured variables, further investigation into the potential confounding effect by birth weight and gestational age was done using the linear prediction logistic regression with lines (Modification also was studied but was not significant as the p value did not suggest any difference between the 2 groups).

Figure 13 illustrates the effect of gestational age on the association between major neurodevelopmental impairment and exposure to *CoNS* sepsis in preterm infants born at less than 28 weeks using linear prediction.

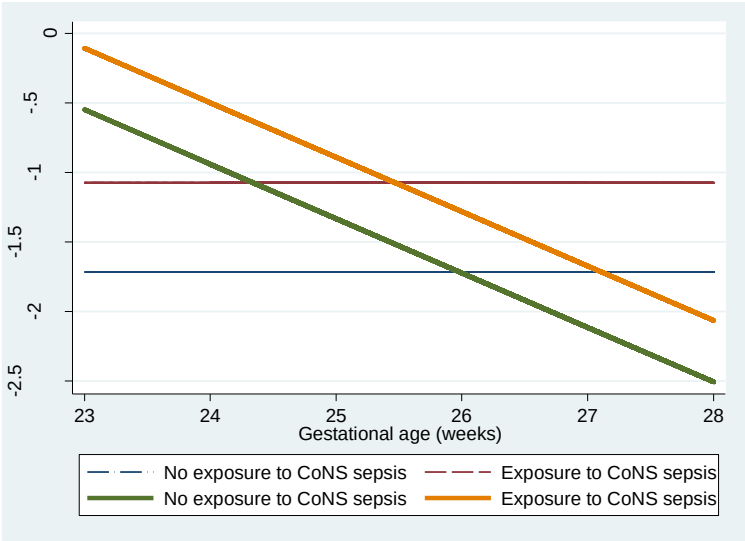

Figure13. The effect of gestational age on the association between major NDI and exposure to CoNS sepsis in the neonatal period. The distance between the thin lines represents the log odds ratio of major NDI in children exposed to CoNS infection in the neonatal period before adjusting for the gestational age. The distance between the thick lines represents the log of odds ratio of major NDI in children exposed to CoNS after adjusting for gestational age.

The log odds ratio of major NDI in children exposed to *CoNS* sepsis after adjusting for gestational age (the distance between the thick lines) is smaller than the log of same odds ratio, but before adjusting for *CoNS* sepsis (the distance between the thin lines). The figure also shows that the log odds of major NDI in both *CoNS* and no *CoNS* group decreases as the gestational age increases.

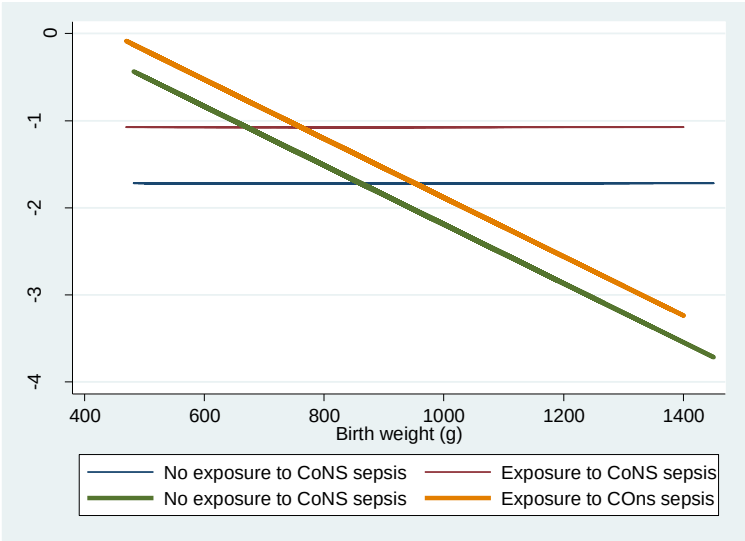

Figure 14. The effect of birth on the association between major NDI in children born ≤ 28 weeks and exposure to CoNS infection in the neonatal period

Figure 14 shows similar results to what was found previously when examining the gestational age as a potential measured confounder for the association between exposure to CoNS and major NDI but with birth weight this time. The difference between the log Odds Ratios before and after adjusting for birth weight is even more important compared with the gestational age. Both figures showed that gestational age and birth weight are potential significant confounders to the association between *CoNS* sepsis and major NDI.

3.5.6.2 Logistic Regression

Model Building:

The goal of multivariable logistic regression analysis is to find the best fitting model to describe the relationship between the outcome of interest (dependent variable) and a set of predictors or explanatory variables (covariates).

Multicollinearity Assessment:

Collinearity between the independent variables was examined using the matrix of correlation. Correlation matrix is a table indicating the correlations between each pair of explanatory variables using the square root of R^2. Values close to 1 or -1 indicates that variables are strongly associated with each other and multicollinearity may be a problem. A correlation coefficient (r) of more than 0.6 was considered significant. Birth weight and gestational age were found to be highly correlated ($r=0.67$, $p < 0.001$).

Choosing Variables in the Model:

Logistic regression was performed to explore the association between the major neurodevelopmental impairment (dependent outcome) and *CoNS* sepsis (main exposure). Other possible predictor variables (covariates) were examined for possible modification and confounding effect.

Given that the goal of our multivariable model was to assess the effect of *CoNS* sepsis on neurodevelopmental outcome, while controlling for supposed confounding variables, variable selection using change-in-estimate method (between crude and adjusted RR or OR) is preferred over the plain statistical significant of p-value. A potential confounder in this method is included in the model if it changes the effect estimate (RR or OR) of the primary exposure by 15%.[100] This method has been proven to produce more reliable models compared to the methods using just a statistical significant ($p <0.05$ and even stricter criteria like $p <0.2$).[102] Methods using only statistical significant to include variables in the multivariable analyses has shown to produce models with substantial residual confounding.[101]

In order to include all significant covariates, a more cautious approach was used to guard against confounding. This approach considers adding covariate in the model if its p value is less than

0.20 or if it's inclusion resulted in change of 15 % or more in the estimate of the main effect of *CoNS* sepsis exposure. In addition, any variable identified as confounder in the literature is highly considered for inclusion in the final model.

Primarily, covariates with significant association ($p < 0.2$) with *CoNS* sepsis or neurodevelopmental outcome were considered as potential confounders.[103, 104] However, these covariates must follow the criteria of confounding to be considered as confounder factors. A confounding variable must be correlated with exposure, be a direct cause of outcome or a surrogate for a direct cause of the outcome, must not be an intermediate step on the causal pathway between exposure and outcome. Recently, researchers have declared that a confounding variable cannot be a variable which is affected by the exposure.[105]

Multiple births, perinatal infection, gestational age, birth weight, sex, small for gestational age, postnatal steroids, PDA, NEC, and ROP were identified as potential confounders based on statistical significance ($p < 0.2$). Cord pH and Apgar score were not thought to be clinically different in measuring the sickness at birth.

Using the 15% change-in-estimate rule (table 41): postnatal steroids, PDA, IVH and ROP (all types or severe only) were considered potential confounders as a discrete variables, whereas gestational age and birth weight behave like a potential confounders as measured variables.

In the literature, studies on neonatal sepsis and neurodevelopmental delay have adjusted for gestational age, birth weight, sex, SGA, postnatal steroids, severe IVH, severe ROP, NEC, BPD, and chorioamnionitis.[13, 18, 19, 106, 107] Other covariates were also used in these studies including maternal age, prolonged rupture of membranes, PDA, and cesearean delivery, but all these variables do not have an impact on neurodevelopmental outcome, therefore were eliminated from the analysis at the beginning.[108, 109]

In this cohort only 60 infants had major NDI, limiting the study to 5 variables in addition to the exposure to *CoNS* sepsis under the rule of 10 events for each variable in the multivariable logistic regression analysis.[110, 111] **Of all the covariates found in our selection approach, gestational age, birth weight, postnatal steroids, chorioamnionitis, severe IVH, and NEC fit the definition of confounding.**

Gestational age and birth weight are well known predictors of neurodevelopmental outcome of infants born prematurely and at the same time have been associated with increased risk of sepsis. As mentioned previously, birth weight and gestational age were collinear to each other. Gestational age was selected in favour of birth weight because most neurodevelopmental studies have used gestational age as a predictor for long term outcome, in addition to the fact that infants with heaver weight usually behave like other infants with similar gestation not with similar weight.

Despite that the difference between the crude and adjusted RR analysis showed that IVH is the main confounder rather than severe IVH, severe IVH was selected for the multivariable analyses because of its better prediction of poor neurodevelopmental outcome. In addition all the literature used severe IVH as a confounder for the association between sepsis and NDI.[13, 18, 109]

ROP (and severe ROP) and BPD are factors known to increase the risk for NDI, However both may be affected, to some extent, by the *CoNS* sepsis and may also be an intermediate step on the causal pathway between *CoNS* infection in these premature infants. The model was run initially without severe ROP and BPD then repeated later on to determine whether entering these factors in the model would make any difference in the results.

Analysis of the final logistic regression model was performed using backward selection method including all covariates that met the definition of confounding. Log likelihood ratio tests were

used to compare between the models after removing each variable. Covariates that did not make statistically significant contribution to the model, as evaluated by the log likelihood test, were removed. The final regression model for each neurologic outcome was evaluated for Goodness of fit by using the Hosmer-Lemeshow test. The Hosmer-Lemeshow test suggested that there was no difference in the expected frequencies and observed frequencies of severe neurodevelopmental outcome. The final model was a good fit (p = 0.84).

Table 45 demonstrates the adjusted major neurodevelopmental impairment as a function of exposure to *CoNS* infection. On controlling confounding variables, exposure to *CoNS* sepsis did not associate with major neurodevelopmental impairment (adjusted OR 1.44; 95% CI 0.72 – 2.86, p=0.29).

Table 44. Adjusted neurodevelopmental outcome at 36 months corrected age as a function of CoNS sepsis

Major NDI	**OR**	**Std.Err**	**Z**	**P> |Z|**	**95%CI**
CoNS sepsis	1.15	0.42	0.39	0.69	0.56 – 2.36
Gestational age	0.81	0.09	-1.77	0.08	0.64 – 1.02
Postnatal steroids	3.30	1.21	3.26	0.001	1.61 – 6.78
Chorioamnionitis	1.10	0.42	0.26	0.79	0.52 – 2.35
Severe IVH	9.81	4.46	5.01	0.000	4.01 – 23.96
NEC	2.06	0.84	1.76	0.08	0.92 – 4.60

Log likelihood= - 114, LR chi2 (6) =60, Prob > chi2: <0.001

Chorioamnionitis made only a very small contribution to the model as the p value was 0.79. Moreover, the likelihood ratio test after deleting chorioamnionitis did not show statistical significant difference between the involved models, P= 0.82.

To complete the assessment of the final model, we checked graphically how the model predicts the log odds of severe NDI as the continuous variable (gestational age) change. Figure 15 shows relationship between the fractional polynomial fit of predicted log odds of major NDI and the gestational age. Increasing gestational age was associated with lower log of odds of major NDI. The 95% CI of the log odds of major NDI was wider for infants less than 24 weeks because of the small number of infants who were born at this gestational age. Also, it was wider around the 28 weeks as the number of infants with major NDI was also less. The log odds and its 95 %CI of major NDI levels off between 27 and 28 weeks indicates that the probability of having major NDI reaches its usual population incidence by 27 to 28 weeks regardless of the associated morbidity in our model.

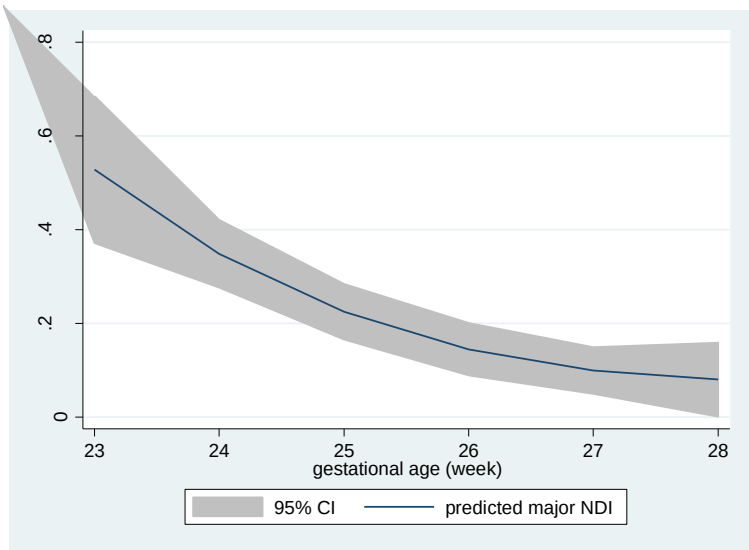

Figure 15. Fractional polynomial fit graph to predict the log of odds of major NDI as a change of gestational age

3.7 Persistent *CoNS* and Neurodevelopmental Outcome

Table 45 shows the subgroup analysis comparing the infants with persistent *CoNS* sepsis and non-persistent *CoNS* sepsis with the group of infants with no *CoNS* sepsis. There were 12 infants with persistent *CoNS* infection. Two of them were excluded because they had other gram negative sepsis and one was missed at follow up at 36 months corrected gestation. Fisher's test and Chi-squared tests were performed as appropriate to compare between the 2 groups. Infants with persistent *CoNS* sepsis had significantly higher risk of major NDI (44.4% vs. 17.9%, P=0.03) but not cognitive delay (22.2% vs. 7.5%, p=0.16). However, the group of children with persistent *CoNS* sepsis was very small. There were no differences in the proportions of cerebral palsy, deafness and blindness among the two groups.

Table 45. Neurodevelopmental outcomes infants with persistent CoNS, non-persistent CoNS and no CoNS sepsis

Neurodevelopmental outcome	**No *CoNS* Group (n=227)**	**Non Persistent *CoNS* group (n=96)**	**P value**	**Persistent *CoNS* group (n=9)**	**P value***
Cerebral palsy, n (%)	24 (10.6)	11 (11.5)	0.97	1 (11.1)	1.0
Blindness, n (%)	4 (1.7)	1 (1.1)	1	0 (0)	1.0
Deafness, n (%)	5 (2.2)	5 (5.2)	0.16	1 (11.1)	0.21
Cognitive delay, n (%)	17 (7.5)	16 (16.7)	0.01	2 (22.2)	0.16
Major NDI, n (%)	34 (15)	22 (22.9)	0.08	4 (44.4)	0.04

* Comparison between infants in the persistent *CoNS* and others in the no *CoNS* group

Of note, we were interested to see whether children who born prematurely and exposed to non-persistent *CoNS* sepsis in the neonatal period had any major NDI. The bivariate analysis comparing between major NDI of non-persistent *CoNS* group and no *CoNS* group showed that children in the non-persistent group were more likely to have cognitive delay (16.7% vs. 7.5%, p=0.01). There were 34 infant with a cognitive delay more than 2 SD after excluding the children who had persistent *CoNS* sepsis. Based on the rule of 10 events for each variable in the logistic regression model we could adjust for gestational age and severe IVH. The OR of cognitive delay more than 2 SD in children exposed to *CoNS* sepsis was 2.44 (95% CI 1.13 – 5.24; p=0.02).

CHAPTER FOUR: DISCUSSION

Summary of Results:

A total of 1224 preterm infants ≤ 28 weeks were admitted to the regional NICU in Calgary between January 1995 and December 2008, of which 13.1% infants were diagnosed with *CoNS* sepsis. The majority of infants were followed up at 36 months corrected age. The proportion of infants who were lost to follow up at 36 months corrected age were approximately equal in infants exposed and not exposed to *CoNS* sepsis (13.2% vs. 13.4%). The maternal characteristics were similar between preterm infants exposed and not exposed to *CoNS* sepsis. The incidence of *CoNS* sepsis was higher in infants with lower gestational age, lower birth weight, and SGA infants. Although the Apgar score at 5 minute of age was lower in the *CoNS* group, the proportion of infants with Apgar score less than 7 was not statistically different from the infants in the no *CoNS* group. The proportions of RDS, IVH, PDA, and use of postnatal steroids were higher in preterm infants with *CoNS* sepsis. All these conditions except postnatal steroids were thought to be a predictive of the *CoNS* sepsis; however, some cases of *CoNS* sepsis occurred before the diagnosis of these morbidities.

4.1 Primary Research Objective

Neurodevelopmental Outcome:

- Three hundred and thirty two preterm infants (86.6% of the total eligible survivors) underwent neurodevelopmental evaluation at 36 months corrected age. The rate of follow up for eligible infants in the *CoNS* survivors and the no *CoNS* survivors was similar (86.7% vs. 86.6%).

- Cerebral palsy was diagnosed in 10.5% of children in our study cohort. The rate of cerebral palsy was 10.5% in the *CoNS* group compared with 10.6% in the no *CoNS* group.
- The rate of cognitive delay was significantly higher in children exposed to *CoNS* sepsis in the neonatal period (17.6%) when compared with other children (7.8%). The association between *CoNS* sepsis and cognitive delay (more than 2 SD below the mean) remained significant after adjusting for potential confounders included gestational age and severe IVH (OR 2.16; 95% CI 1.01 – 4.62).
- There was no significant difference between the two groups in the rate of neurosensory deafness required hearing aids (5.7% vs. 2.2%). However; the proportion of any degree of neurosensory hearing impairment was higher in the *CoNS* group (9.6%) compared with infants in the no *CoNS* group (3.1%).
- There was no significant difference between the two groups in the proportion of blindness (1% vs. 1.7%) or overall visual impairment (9.9% vs. 7.8%).
- Infants exposed to *CoNS* sepsis were relatively more likely to have any degree of NDI at 36 months corrected age compared with unexposed infants (43.1% vs. 33.0%; p = 0.07).
- The rate of major NDI in the bivariate analysis was higher in children exposed to *CoNS* sepsis in the neonatal period than unexposed children (25.4% vs. 15.2; p=0.03). However, this association between major NDI and *CoNS* sepsis disappeared after adjusting for potential confounders such as gestational age, chorioamnionitis, postnatal steroids exposure, severe IVH and NEC (OR 1.15; 95% CI 0.56 – 2.36).

4.2 Secondary objectives

4.2.1 Neonatal characteristics and morbidities

- Preterm infants in the *CoNS* group were more premature at birth (25.9 weeks ± 1.7 vs. 26.2 weeks ± 1.4; p = 0.04) and had lower birth weight (834 g ± 211 vs. 900 ± 197; p = 0.01). Small for gestational age infants were more likely to acquire *CoNS* sepsis during their stay in NICU than other preterm infants who were appropriate for gestational age (13.4% vs. 5.7%; p=0.02) at birth.
- There was a trend towards an increased rate of NEC in preterm infants exposed to *CoNS* sepsis (21.9% vs. 13.6%; p=0.06). The risk ratio of this association was 1.6 (95% CI 0.98 – 2.61). However, there was no temporal association between *CoNS* sepsis and NEC after excluding infants who did not have simultaneous *CoNS* sepsis and NEC (RR 1.25; 95% CI 0.73 – 2.16).
- The risk of total ROP was significantly higher among preterm infants exposed to *CoNS* sepsis as compared with unexposed infants (77.8% vs. 58.8%; p= 0.002). The risk ratio of the association between *CoNS* sepsis and total ROP was 1.32 (95% CI 1.11 – 1.54). Correcting for gestational age and duration of oxygen requirement did not alter this result. In contrast, the risk of severe ROP and ROP requiring surgery (i.e. stage III or more) was similar in infants exposed and not exposed to *CoNS* sepsis after correcting for gestational age and duration of oxygen requirement.
- The rate of BPD and PVL was similar between infants exposed and not exposed to *CoNS* sepsis.
- Infants exposed to *CoNS* sepsis were more likely to require respiratory support for a longer period (38 days; IQR: 20, 59) when compared with unexposed infants (24 days; IQR: 7, 46) (p<0.001). The difference of this duration between infants exposed and

unexposed to *CoNS* sepsis remained significant after accounting for potential confounders (difference 11 days; 95% CI 5.9 – 16.1) ($p < 0.001$).

- Infants exposed to *CoNS* sepsis were more likely to stay longer in NICU (98 days; IQR: 79, 124) when compared with unexposed infants (81 days; IQR: 70, 100) ($p < 0.001$). This difference between the two groups continued to be significant after adjusting for gestational age, severe IVH, BPD, NEC, and severe ROP (difference of 8.5 days; 95% CI 3.1 – 13.9).

4.2.2 Persistent *CoNS* Sepsis and Major Neurodevelopmental Impairment

- Only 9 preterm infants had persistent *CoNS* sepsis in the study.
- Compared with infants who were not exposed to *CoNS* sepsis, infants with persistent *CoNS* sepsis had significant increased risk of major NDI ($p = 0.03$).
- There was no association between persistent *CoNS* sepsis in preterm infants and cerebral palsy, blindness, deafness, or cognitive delay.
- Infants with non-persistent *CoNS* sepsis were more likely to have cognitive delay when compared with infants not exposed to *CoNS* sepsis ($p = 0.01$).

4.3 Comparison with Previous Studies

4.3.1 Comparison of Neonatal Characteristics and Outcomes

- Preterm infants born at ≤ 28 weeks gestation are at higher risk for sepsis during their initial stay in NICU because of their immature immune system and frequent exposure to invasive diagnostic and therapeutic procedures. *CoNS* organism is the most common pathogen for late onset sepsis in this population. The incidence of *CoNS* sepsis in the current study was 13.1%. This was similar to the rates reported by other investigators.

For example, Stoll et al. reported an incidence of 10.1% for *CoNS* sepsis in VLBW infants.[6] Gestational age of many VLBW infants in the former study was more than 28 weeks, and this could explain the higher incidence of *CoNS* sepsis in our study which was limited to infants ≤ 28 weeks' gestation, as the younger infants are more vulnerable to *CoNS* sepsis. Another study by Freeman et al. reported a rate of sepsis between 5% and 30%, depending on birth weight.[112] The incidence of *CoNS* sepsis in the current study was higher in preterm infant with lower gestational age and lower birth weight. This observation is consistent with the previous studies related to *CoNS* sepsis in the neonatal period.[6, 10, 113, 114] Extremely preterm infants are more likely to require prolonged use of central venous catheters for parenteral nutrition and medication in addition to their prolonged need for mechanical ventilation. All these factors may explain the inverse relationship between gestational age and incidence of *CoNS* sepsis. The current study did not look at the risk factors for *CoNS* sepsis such as the number of days these infants required central venous catheters or parenteral nutrition. The duration of respiratory support using invasive or non-invasive ventilation were prolonged in preterm infants with *CoNS* sepsis. This could be because preterm infants who required respiratory support for longer period were at higher risk for *CoNS* sepsis, or that these infants needed additional respiratory support at time of *CoNS* sepsis episode.

- The highest incidence of *CoNS* sepsis was observed in the third week of life (median 15 days; IQR 9, 21). This observation is slightly different from the reported highest incidence of *CoNS* sepsis in the previous studies. Jean-Baptiste et al. showed that rate of *CoNS* sepsis is highest between postnatal age of 7 and 14 days.[113] Isaacs et al. showed that the mode of postnatal age for *CoNS* sepsis is 10 days. [10] These two former studies

included preterm and term infants admitted to NICU compared with only extremely preterm infants in our study. In addition, the diverse practice in inserting and maintaining central venous catheters between NICUs may explain this difference.

- The vast majority of *CoNS* organisms in the current study were isolated from blood. Only a small fraction was attributed to CSF culture, and no cases of urinary tract infection were reported in our study. Though the diagnosis of *CoNS* sepsis was primarily through cultures of blood and CSF, it is possible that urinary tract infection was underestimated as urine samples were not always collected or collected after starting antibiotics in a large number of preterm infants.
- The current study showed that infants with *CoNS* sepsis had lower mortality rates than infants not exposed to any type of sepsis (8% vs. 18.3%; p=0.005). This finding may be attributable to an increase number of infants who died in the first week of life in the no *CoNS* group. As *CoNS* sepsis is a late onset sepsis and commonly occurs after the first week of life, infants who died in the first week of life could potentially have had *CoNS* sepsis during their NICU stay if they had lived longer.
- In the current study, *CoNS* sepsis was associated with a higher rate of total IVH. Infants who developed *CoNS* sepsis were less mature than unexposed infants. This difference in gestational age may be explained in part by the increased risk of total IVH in infants with *CoNS* sepsis as this association continued to be significant after adjusting for gestational age. The fact that 90 % of IVH occurs in the first 3 days of life [115], and *CoNS* sepsis is a late-onset infection (occurs after 3 days of life) makes IVH more likely to be a risk factor for *CoNS* sepsis. Careful interpretation of this finding is necessary given that the pathway between IVH and increased risk of *CoNS* sepsis is unclear and the need for more

studies to confirm this association. In addition, the proportion of severe IVH (IVH grade 3 or 4) was similar between infants exposed and not exposed to *CoNS* sepsis. Overall, this observation of no association between severe IVH and *CoNS* sepsis is consistent with the results of other studies on neonatal sepsis in preterm infants. Studies by Shah et al. (2008) and Schlapbach et al. (2011) showed no difference in the severe IVH in preterm infants with or without sepsis.[13, 19] In these studies infection was caused by any organism while the sepsis in the current study was limited to *CoNS*. Nevertheless, *CoNS* organism was the most common cause of sepsis in both former studies.

- NEC is a serious disease in preterm infants. The exact cause is unknown.[54] The rate of NEC varies greatly between NICUs. It frequently occurs in endemic and epidemic patterns.[116] *CoNS* organisms are the most common isolated pathogen from blood and stool of preterm infants with NEC.[4] *CoNS* organisms have been isolated from blood in 4.2% of preterm infants with NEC, and from peritoneal fluids in 29.6% of infants required surgery for NEC.[31] However, the rate of NEC in infants exposed to *CoNS* sepsis is lacking in the literature. The rate of simultaneous NEC in infants exposed to *CoNS* sepsis in our study was 16.2%. This was not significantly different from the rate of NEC in the infants without *CoNS* sepsis (p=0.41). The link and the pathway between NEC and *CoNS* infection are inconsistent in the literature. Scheifele et al. described the delta toxin of *CoNS* bacteria in the stool of preterm infants with NEC as a potential enteropathologic factor for gut injury. [54] In contrast, Rotbart et al. reported an equal incidence of *CoNS* organism in stool samples of patients with and without NEC.[61] They were also unable to identify the delta toxin in the stool of infants with NEC.[61]

- ROP is a multifactorial vasoproliferative retinal disorder. Several risk factors have been extensively studied in the last 3 decades, including low gestational age, low birth weight, and supplemental oxygen therapy. The role of neonatal sepsis as a risk for ROP is controversial. The current study showed that *CoNS* sepsis is associated with increased risk for total ROP. This finding is consistent with the results of Schlapbach et al. who identified a correlation between neonatal sepsis and ROP in extremely preterm infants.[13] This study did not look specifically at type of bacterial infection, however; *CoNS* organism was the most common cause (51%) of sepsis. Manzoni et al. showed that fungal (but not bacterial) sepsis is independently associated with ROP in only ELBW infants and only with threshold ROP.[117] However, this study looked at the link between the bacterial sepsis as a whole and the incidence of ROP, but not *CoNS* organisms specifically. Tadesse et al. reported an association between systemic fungal sepsis (particularly *Candida Albicans*) and ROP.[118] Sepsis caused by *Candida* species has been shown to stimulate the proinflammatory cytokines production and the leukocyte adhesion molecules expression by endothelial cells, both are speculated to alter the development of retinal blood vessels.[62] Whether *CoNS* sepsis has similar or different pathogenesis to affect the retinal blood vessels requires more investigation.
- BPD is a common morbidity in extremely preterm infants and it is multifactorial in origin. [119] Several studies have focused on sepsis as a possible cause of BPD in preterm infants, although few of them correlated BPD with a specific bacterial organism.[120-122] The current study showed no effect of *CoNS* sepsis on the rate of BPD in preterm infants. In contradistinction, Liljedahl et al. showed that BPD is significantly increased in preterm infants with *CoNS* sepsis compared with similar infants

with other bacterial sepsis, and compared with preterm infants without sepsis.[123] The number of infants with *CoNS* sepsis in the former study was limited (22 only), and they were more mature than the infants in our cohort study (the median gestational age was 28 week). Postnatal steroids have been widely used to prevent or treat preterm infants with BPD. Reports in the early 2000s raised concerns about increased risk of NDI in preterm infants exposed to steroids in the neonatal period.[124] In the current study, the rate of use of postnatal steroids in infants exposed to *CoNS* sepsis was higher than that in unexposed infants. This was a different observation from the Liljedahl et al. study. Moreover, the use of postnatal steroids was highly correlated with the duration of respiratory support in our study. The higher use of postnatal steroids to prevent or treat BPD in infants exposed to *CoNS* sepsis in the current study may explain the similarity in the rate of BPD between preterm infants exposed and not exposed to *CoNS* sepsis.

- Neonatal infections have a major impact on duration of hospital stay and hospitalization costs.[63] Our study showed an increase in the length of hospital stay by 8.5 days in infants exposed to *CoNS* sepsis after adjusting for gestational age, severe IVH, BPD, NEC and severe ROP. Gray et al. found an increment of 14 ± 4 days in the length of hospital stay in infants with bacteremia after adjusting for birth weight, score of neonatal acute physiology (SNAP) at birth, and community retrotransport.[36] Of the 130 infants with bacteremia in the former study, 101 infants (78%) had *CoNS* bacteremia. The difference in length of hospital stay between Gray's study and our study could be because of inclusion infants with sepsis caused by other organisms and the lack of adjustment for neonatal morbidities in Gray's study.

4.3.2 Comparison of Neurodevelopmental Outcome

Clinical Studies:

In the current study, *CoNS* sepsis has no association with overall major NDI in extremely preterm infants. However, the assessment of the type specific NDI in these infants showed that *CoNS* sepsis is independently associated with a 2.1 fold increase risk for cognitive delay in this population.

Our finding on major NDI is similar to that reported by Schlapbach et al.[13] The Schlapbach study revealed that exposure to CoNS sepsis in 77 preterm infants born between $24^{0/7}$ and $27^{6/7}$ weeks was not associated with major NDI at two years corrected age (OR 1.58; 95% CI 0.82 – 3.04); p=0.17). However, infants exposed to *CoNS* sepsis in the former study had higher risk for developing cerebral palsy (OR 5.59; 95%CI: 1.87 – 16.66); (p=0.002). Nonetheless, only 10 (13%) infants with *CoNS* sepsis in this study developed cerebral palsy compared to 4 (3%) infants in the unexposed group. Our study revealed similar proportion of CP in infants exposed and unexposed to *CoNS* (10.5% vs. 10.6%). The findings on association between *CoNS* sepsis and CP require cautious interpretation because of the small number of infants with CP in both studies. The Sclapbach et al. study did not report on the other components of the neurodevelopmental outcome.

In contrast to our study, Stoll et al. reported increased risk of major NDI in infants exposed to *CoNS* sepsis in the neonatal period (OR 1.3; 95% CI 1.1 – 1.6).[18] The risk of cerebral palsy was also higher in those infants (OR 1.4; 95% CI 1.0 – 1.9). The crude analysis in our study showed that *CoNS* sepsis is associated with major NDI in infants born ≤ 28 weeks gestation (25.4% vs. 15.2%; p=0.03), but this association disappeared after adjusting for potential confounders including NEC. The study by Stoll et al. did not correct for NEC as a confounder.

NEC is known to correlate with *CoNS* sepsis and affect the neurodevelopmetnal outcome of preterm infants.[31, 125-127] In addition, this study reported no difference in cognitive delay between infants exposed and unexposed to *CoNS* sepsis. The cognitive outcome of this study as well as the other outcomes was a result of a single point follow up at 18 to 22 months corrected age. Assessment of cognitive outcome at 18 to 22 months corrected age may encounter many difficulties as the rate of acquiring new skills in these infants differs largely during this period.[68] Moreover, consistency of cognitive outcome findings over frequent visits to PNFU clinic provides more reliable diagnosis of cognitive delay, and this was lacking in Stoll et al.'s study.[18]

Our study supports the findings reported by Shah et al. who found a significant difference in the psychomotor developmental index (PDI) at 2 years of age in infants exposed to *CoNS* sepsis when compared with unexposed infants.[19] The mean difference after correcting for potential confounders was 5.8 points (95% CI 0.3 – 11.2; p=0.04). The difference in mental developmental index (MDI) was not statistically significant between *CoNS* sepsis exposed and unexposed infants. The number of infants with cerebral palsy was very small (only 6) to be compared between the groups. This study did not assess the impact of *CoNS* sepsis on major NDI, vision, and hearing.[19]

A recent systematic review and meta-analysis on neonatal sepsis in VLBW infants and long term neurodevelomental outcomes showed that neonatal sepsis increases the risk of any type neurodevelopmental impairment by 2.09 fold.[128] The analysis of NDI in infants exposed to *CoNS* sepsis included the 3 studies mentioned earlier (Stoll, Shah, and Schlabpach) [13, 18, 19] showed similar findings to the overall sepsis, but the magnitude was smaller (OR 1.3; 95% CI 1.09 – 1.57). The analysis of the type-specific impairment showed increased risk of CP (OR 1.7;

95% CI 1.02 – 2.87) in infants exposed to *CoNS* sepsis. Overall, the studies included in this meta-analysis were heterogeneous, and the number of studies reporting the NDI in *CoNS* sepsis group was limited.

Only 5 children in the current study were blind despite the fact that severe ROP requiring surgery was significantly higher in the *CoNS* group (27.2% vs. 10.8%; $p < 0.001$). The incidence of blindness was similar in children exposed and not exposed to *CoNS* in the neonatal period. We found a small number of children with blindness in this study cohort. The prompt interventions by ophthalmologists are thought to be the key for low rate of blindness.

Eleven children had neurosensory deafness requiring hearing amplification in our study. There was no difference in the incidence of the deafness between children exposed and not exposed to *CoNS* sepsis. However, when we include the milder form of neurosensory deafness in the analysis, children exposed to *CoNS* sepsis were at risk for this outcome (9.6% vs. 3.1%; $p = 0.01$). Use of antibiotics such as vancomycin and gentamicin in preterm infants is shown to increase the risk of neurosensory hearing impairment.[129, 130] These antibiotics are commonly used to treat *CoNS* sepsis and is proposed to be a possible cause of hearing impairment in this population.

The current study showed an association between persistent *CoNS* sepsis and major NDI (50% vs. 17.9%; $p = 0.03$). Due to the small number of infants with persistent *CoNS* sepsis (only 9 cases), we were not able to adjust for potential confounders, however; the difference in proportions between the two groups is striking. To our knowledge, and despite the small number of infants with persistent *CoNS* sepsis, this is the first study to report the long term neurodevelopmental outcome of preterm infants with persistent *CoNS* sepsis.

Studies on Etiology of Brain Injury:

Extreme prematurity is one of the major causes of CP in children.[131] RDS, IVH, NEC, male sex, chorioamnionitis, mechanical ventilation, and major congenital anomalies have shown to increase the risk of CP in this population.[131, 132] Beano et al. showed that cerebral lesion and particularly periventricular leukomalacia are the most important predictors of CP in very preterm infants.[133] Neonatal sepsis was an independent risk factor for PVL in VLBW infants as shown by Silveira et al.[71] Of the 13 infants with culture-proven sepsis in the Silveira et al. study, eight infants were diagnosed to have *CoNS* sepsis. Careful interpretation of this result is necessary given the small number of infants exposed to *CoNS* sepsis in the study. The number of infants with PVL in Silveira et al. study is very large (57.8%) compared with 7 infants only (3%) with PVL in our study cohort.

Recent studies have shown that 40 – 50% of very preterm infants have a degree of cognitive delay, and 15-20% of this population are at risk for severe neurodevelopmental delay.[134, 135] Cognitive delay is highly correlated with white matter injury.[136] The study by Shah et al. showed that lower PDI in infants with sepsis in the neonatal period is mediated by white matter injury.[19] The most common causative pathogen in this study was *CoNS*. Diagnosis of white matter injury needs a brain MRI at term equivalent gestation. Yet, there is no agreement on routine use of brain MRI for extremely preterm infants. Brain MRI at NICU in Calgary was rarely done before 2008, and for different purposes than looking for white matter injury. Cranial ultrasound is a screening tool for PVL, but its ability to detect other white matter abnormalities is limited.[137]

The pathogenesis of white matter injury resulting from *CoNS* sepsis is thought to be as with other postnatal sepsis in preterm infants. It has two related mechanisms (upstream and downstream).[70] Production of pro-inflammatory cytokines, increase blood-brain barrier

permeability, hypoxic ischemic events resulting from hypotension, and impaired autoregulation of cerebral blood flow represent the upstream mechanisms to stimulate the brain's microglia.[64, 71, 72, 138] This leads to activation of downstream mechanisms which include excitotoxicity and free radical attack caused by reactive oxygen and nitrogen species.[70] This activation subsequently leads to the death of the vulnerable pre-myelinating oligodendrocytes.[70]

Evidence from animal studies showed that signaling TLR2 and TLR6, presented on microglia and other monocytes in the brain, by *staphylococcus* bacteria leads to increase in lysosomal activity and massive production of IL-10 followed by apoptosis of these cells.[78] Subsequently, this participates in the damage to other precious neurons around the microglia.

Critical care studies in adult patients have demonstrated a permanent cognitive impairment in sepsis survivors.[139] This impairment is correlated with hippocampus atrophy.[139, 140] In addition, a recent study on mice with sepsis revealed increased serum levels of high mobility group box 1 (HMGB1), a critical mediator of acute sepsis pathophysiology.[141] This increase in HMGB1 was correlated with anatomic changes in the hippocampus associated with a loss of synaptic plasticity.[141] The study demonstrated an association between increased HMGB1, and persistent impairments in learning and memory.[141] In addition, early administration of neutralizing anti-HMGB1 antibody to mice with sepsis survivors improved their memory and learning impairments.[141] Despite the fact that the adult and the mice studies did not specify the type of organisms affecting the cognitive outcome; they provided direction to possible pathways that *CoNS* sepsis may affect the brain.

4.4 Impact of Bias in the Results

Like any other retrospective cohort study, the current study is subject to various types of bias. Selection and information biases are potential risks for any cohort study.[142] All achievable efforts were taken to minimise these types of biases during the various phases of the study.

Selection bias in the current cohort study may be introduced by choosing subjects in the comparison group. For example, including preterm infants with an uncomplicated course in NICU with predicted good neurodevelopmental outcome may show that infants with *CoNS* sepsis have poorer neurodevelopmental outcome. This risk was minimized by choosing, for each preterm infant in the *CoNS* group, the following two preterm infants ≤ 28 weeks' gestation admitted in the NICU and did not develop any culture-proven sepsis. These infants comprised the comparison group.

Information bias can occur when the methods of obtaining information about the subjects in the study are inadequate. As a result, some information on the exposure or outcome is incorrect.[143] Misclassifying infants with no *CoNS* sepsis as having *CoNS* sepsis is a probable source for information bias. This misclassification may occur in the *CoNS* exposed group because of labelling infants with *CoNS*-contaminated culture to have *CoNS* sepsis while indeed they should have been in the no *CoNS* group. Including the requirement for treatment for more than 5 days in the definition criteria of *CoNS* sepsis as a surrogate for the presence of clinical signs, in addition to the blood culture, helped to exclude many infants with possible contamination. However, some infants with *CoNS* contamination may still have been included in the *CoNS* group. This potential bias would result in diluting the association between the *CoNS* sepsis and the major NDI towards the null. Nonetheless, this possibility is unlikely to have happened as the 95% CI for the risk ratio included wide spread values around one (95% CI 0.72

– 2.84). In contrast, the current study suggests a possible association between *CoNS* sepsis and cognitive delay. This positive relationship may also have been diluted, and it may have been actually stronger than what we reported. The lost to follow up or attrition bias was unlikely to affect the results of the study as the follow up rate of surviving infants was high (86%) and similar between the two groups. Moreover, measurement biases resulted from different measures of outcomes were avoided by defining objective outcome criteria (i.e. definitions of NDI, cerebral palsy, cognitive delay, etc). Relying on Neonatal and PNFU databases assumes these data sources to be complete and valid. However, we could not confirm if the information in these two databases reflects what is in the charts.

In addition, to minimize the impact of known confounding factors, all the variables that may act as confounder factors to the relationship between *CoNS* sepsis and neurodevelopmental outcome were identified at the design stage of the study. At the analysis phase, the potential confounding factors were examined using the multivariate logistic regression model.

4.5 Implications for Clinical Practice

Having an understanding of the association between *CoNS* sepsis and increased risk for cognitive delay and for ROP in preterm infants, will change the perception of *CoNS* sepsis from "soft" infection towards an infection with significant morbidities. This will help optimise the management of *CoNS* sepsis in form of preventative strategies, treatment, and follow up. Apart from the tertiary prevention, yet there have been no specific evidence based strategies to primary or secondary prevention of cognitive delay in preterm infants.

From a practical point of view, this study alerts health care providers to actively use infection control strategies to protect all preterm infants in NICU from *CoNS* sepsis. It also emphasizes the

importance of aggressive treatment and long term follow-up of preterm infants with *CoNS* sepsis. In addition, it also helps clinicians to counselling parents of preterm infants with *CoNS* sepsis. Health care providers should be looking proactively for early signs of cognitive delay in infants exposed to *CoNS* sepsis and refer them to professional learning program which can improve their functional outcome.

4.6 Strengths

- The strength of the current study comprises comprehensive multidisciplinary follow up. The follow up rate of 86% in the study is good. Fewtrell et al. suggested that follow up rate of more than 80% is considered good for studies on long term neurodevelopmental outcome.[144] In general, higher follow up rate would have been ideal, but was not feasible.
- All neurodevelopmental assessments were performed by individuals who were unaware that this current study would be conducted in the perinatal follow up clinic.
- The neurodevelopmental assessment at 36 months corrected age provided more accurate diagnosis than all assessments of outcome at less than 24 months of age. This is particularly important for assessment of cognitive outcome as all earlier tests are not highly predictive. In addition, this will avoid including transient neurological abnormalities seen before 24 months of age in the analysis.
- The study took many steps to minimize bias and confounding in the design and the analysis phases. Use of a priori operational definitions provided an accurate tool to protect the study from measurement bias.

- To our knowledge, this is the first study that reported the relationship between *CoNS* sepsis and ROP in preterm infants.

4.7. Limitations

- This study was a report from the only perinatal follow up clinic of southern Alberta, thus it reflected the unique diagnostic and follow up experience of this "single" centre.
- The study used the clinical signs, positive blood culture for *CoNS*, and use of antibiotics for duration longer than 5 days to define *CoNS* sepsis and exclude infants with *CoNS* contamination. Use of additional criteria to the definition such as changes in infection laboratory indicators (complete white blood cells, CRP, etc) was thought to be helpful in lowering the risk of *CoNS* contamination. However, this was not feasible as the data on CBC and CRP were not available or not ordered for a large number of patients. In addition, the fact that many preterm infants have a decreased inflammatory response to various types of *CoNS* organism which limits the benefit of using these markers.[52]
- The sample size was small for examining the impact of persistent *CoNS* sepsis on the neurodevelopmental outcome.
- Reliance on Neonatal database and PNFU database is also a limitation of our study as the information in these two databases has not been validated yet.
- The retrospective design limits the ability to investigate the biological pathways of the association between the *CoNS* sepsis and ROP or cognitive impairment.

4.8 Recommendation for Future Studies

- The findings of the current study will open the door for researchers to explore the association between *CoNS* sepsis and ROP in preterm infants, and the association between *CoNS* sepsis and cognitive delay. Future studies should investigate the pathogenesis of how *CoNS* sepsis may harm the developing retina and brain.
- Although the infection control strategies remain the gold standard to prevent *CoNS* sepsis in extremely preterm infants, it is unlikely to stop it completely because of the deficient immune system of these patients and the almost obligatory need for CVCs. Adult studies have confirmed the role of specific immunoglobulin in preventing *CoNS* in immune deficient children. Similarly, specific immunoglobulins have been successful in preventing respiratory syncytial virus (RSV) in preterm infants and approved by Health Canada. More studies are strongly recommended to possibly find immunoglobulin that could protect preterm infants from *CoNS* sepsis.
- Studies on mice have shown promising results of using anti-cytokines agents such as anti-HMGB1 to protect the brain against injury caused by sepsis. Future studies should be focus on how to protect the developing brain from the *CoNS* sepsis.
- Future studies should evaluate effect of persistent *CoNS* sepsis on neurodevelopmental outcome. Multicenter, prospective, cohort study with appropriate sample size is recommended for such studies.
- Studies on neurodevelopmental outcome for preterm infants with *CoNS* sepsis should be carried out up to school age in order to explore whether cognitive delay continues beyond 36 months corrected age.

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