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# Neurodevelopmental outcomes in extremely preterm infants with placental pathologic evidence of fetal inflammatroy response

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**OBJECTIVES:** Neonates born with fetal inflammatory response (FIR) are at increased risk for adverse neonatal outcomes. Our objective was to determine whether FIR and its severity is associated with neurodevelopmental impairment (NDI) at 2 years of age or death among preterm infants.
**METHODS:** A retrospective cohort study of prospectively collected data of all infants born <29 weeks gestational age (GA). FIR and its severity were diagnosed according to the Amsterdam Placental Workshop Group Consensus Statement. Neurodevelopmental outcomes among all participants were quantified according to Bayley III.
**RESULTS:** Mothers of infants with FIR were significantly younger ($P = 0.04$) and had a greater prevalence of antenatal steroid use ($P < 0.01$), infection during pregnancy ($P = 0.01$), PPROM ($P < 0.01$), and clinical chorioamnionitis ($P < 0.01$). Infants with FIR had longer duration of hospitalization ($P < 0.01$), days on oxygen ($P < 0.01$), congenital pneumonia ($P = 0.03$), moderate/severe bronchopulmonary dysplasia (BPD; $P < 0.01$). Notably, infants with FIR were not at increased risk of NDI or death (primary outcome). Those with moderate to severe FIR (≥ stage 2 FIR) were at increased risk of developing motor & language impairment or death ($P < 0.01$).
**CONCLUSION:** This is the first report demonstrating an association between the severity of FIR and subsequent NDI in preterm infants born.

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**IMPACT STATEMENT:**

- Fetal Inflammatory Response (FIR) is not associated Neurodevelopmental Impairement (NDI) or Death in preterm infants
- However, there is significant relationship between moderate to severe FIR and NDI at 2 years of age in preterm infants.
- This is the first study demonstrating the impact of progression and severity of FIR on NDI or Death in preterm infants.
- These observations provide additional insight into understanding the impact of intrauterine exposure to inflammation on the NDI or death in preterm infants.

## INTRODUCTION

Preterm birth is the leading cause of neonatal morbidity and mortality worldwide.[1] Roughly 1 in 10 infants are born preterm, and 2–5 in 1000 infants are born extremely preterm (<29 weeks gestational age), which together confers a financial burden of ~ $26.2 billion annually in the US.[1,2] The greatest risk factor for extremely preterm birth is intrauterine infection.[3–5] Several investigators have reported that the activation of the fetal immune system via intrauterine infection could result in systemic inflammation with consequent injury, particularly to the developing nervous system.[4–10] Interestingly, this injury secondary to intrauterine infection is not caused primarily by the infectious agents, but by the fetal inflammatory response (FIR).[5–10]

Briefly, preserved sequences of pathogen-associated molecular patterns (PAMPS) such as lipopolysaccharides (LPS) or genetic materials are first recognized by fetal T cells through toll-like receptors (TLR) that consequently release pro-inflammatory cytokines to activate the immune system.[9,10] These cytokines then cross the blood-brain barrier and both damage neuronal tissue directly and and activate neuro-immune cells such as microglia as astrocytes, which then secrete cytokines themselves.[9,10] Notably, certain cytokines produced by microglia (TNFα and IFNγ) localize to, and damage the periventricular white matter through excitotoxic, inflammatory, and oxidative mechanisms.[9,10] Ultimately, infants with this brain injury are at a higher risk for a myriad of pathologies such as periventricular leukomalacia (PVL), neonatal encephalopathy, cerebral palsy, and retinal damage resulting in long-term neurodevelopmental and neurobehavioral impairments.[4–10]

As the understanding of FIR and its consequences has grown, investigators have focused on establishing predictive biomarkers.

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Romero et al. described elevated interleukin-6 levels in the umbilical cord blood of neonates with FIR and coined the term fetal inflammatory response syndrome (FIRS) to describe systemic fetal inflammation in response to infectious stimuli.[5,6] Since then, several studies have demonstrated that fetal plasma IL-6 concentration of > 11 pg/L independently predict severe neonatal morbidity after adjusting for relevant confounders.[5–7] The placental pathologic hallmark of this syndrome is fetal neutrophilic infiltration of umbilical cord vessels and the chorionic plate vessels as seen in placental histopathologic analyses, and is graded based upon severity.[5–7]

There is a paucity of work focused on the association between placental pathologic findings of FIR and neurodevelopmental impairment and/or death among extremely preterm infants.[8] Since umbilical cord IL-6 levels are not as readily available as placental tissue in the clinical setting, the correlation of placental evidence of FIR with neonatal outcomes is highly relevant to patients and may contribute to prognostication of their outcomes. Therefore, the primary objective of this study was to determine whether FIR is associated with neurodevelopmental impairment (NDI) or death at 2 years of age among preterm infants born <29 weeks gestational age (GA). As prior research suggests a direct relationship between the levels of inflammatory markers and subsequent neuroinflammation and fetal injury, we hypothesized that FIR would be significantly associated with NDI or death at 2 years of age among extremely preterm infants in a dose-dependent manner, with higher severity and progression of FIR portending a worse outcome. To our knowledge, this is the first study investigating the relationship between the severity and progression of placental evidence of FIR and NDI or death in extremely preterm infants.

Briefly, it is important to note another precipitant of the fetal inflammatory response syndrome that several studies have demonstrated to be entirely distinct from FIR. More specifically, FIR is characterized by upregulation of the host immune response and downregulation of T cell processes with a consequent cytokine storm, usually because of intrauterine infection.[11] This phenomenon, on the other hand, is hallmarked by maternal anti-fetal rejection during the first trimester, usually because of HLA mismatch, that resembles graft-versus-host disease (GVHD).[11,12] Like FIR, this phenomenon has been linked to NDD in some studies.[11] On placental pathology, however, it remains poorly defined, although some studies have reported an association with chronic inflammatory lesions of placenta including villitis of unknown etiology, chronic deciduitis and chronic chorioamnionitis.[11,12] While FIR is the primary focus of this study, we will assess chronic placental inflammatory lesions as well to elucidate its relationship with NDD and/or death in comparison to FIR. Additionally, we will assess whether there is an additive effect of FIR and chronic placental inflammatory lesions with respect to our primary outcome of NDD and/or death.

## METHODS

### Study design

A retrospective cohort study of prospectively collected data was conducted at Parkland Hospital, Dallas, TX between January 2010 and January 2021 in preterm infants <29 weeks GA. This study was approved by the University of Texas Southwestern Medical Center and Parkland Hospital and Health Systems Institutional Review Boards. All preterm neonates born 23 0/7 to 28 6/7 weeks GA during the study period were identified in the Parkland Neonatal Intensive Care Unit (NICU) Registry. Infants receiving planned comfort care in the delivery room and those born with major congenital anomalies or chromosomal abnormalities were excluded from the analysis. Specifically, major chromosomal abnormalities included trisomies and monosomies. Congenital anomalies included any major malformations in one or more organ systems. Infants were divided into two groups: (1) placental pathologic evidence of FIR; and (2) no placental evidence of FIR. Pathology reports for all participants were manually reviewed and classified. FIR and its severity and progression were diagnosed according to the Amsterdam Placental Workshop Group Consensus Statement guidelines (13). Neurodevelopmental outcomes among all participants were quantified according to the Bayley Scale of Infant and Toddler Development–Third Edition (Bayley III) (12).

### Placental pathology

All available placentas associated with infants at a gestational age less than or equal to 34 weeks are routinely collected for pathologic examination at Parkland Hospital. This examination is performed by a pediatric pathologist according to a standardized protocol as described in our prior published work.[13–16] Initial gross examination includes removal of the umbilical cord, all fetal membranes, and any nonadherent blood clots; this is followed by measuring placental weight. The disc of the placenta is serially sectioned at 1- to 2-cm intervals for examination of parenchyma for any gross lesions. Representative sections of umbilical cord, normal placental parenchyma, fetal membranes, and any lesions identified on gross exam are submitted for histological analysis. A standardized classification system of placental lesions and strict adherence to diagnostic terminology for major placental findings is used, as described.[13–16]

FIR progression and its severity was defined according to the Amsterdam Placental Workshop Group Consensus Statement.[13] The progression of FIR was divided as in the following: (1) stage 1: involvement of chorionic plate vessels, and/or umbilical vein; (2) stage 2: one or both umbilical arteries; (3) stage 3: presence of fetal neutrophils and/or cellular debris in a concentric ring around at least one of the umbilical vessels (also called necrotizing funisitis). FIR severity was graded as: (1) Grade 1 (i.e., not severe, as defined) and (2) Grade 2 (severe: near confluent intramural polymorphonuclear leukocytes with attenuation of vascular smooth muscle). Other placental lesions assessed based upon Amsterdam criteria included:

1. Acute histologic chorioamnionitis without FIR (i.e., only maternal inflammatory response or MIR).
2. Villitis of unknown etiology (VUE): classified as low grade, high grade, or villitis with avascular villi.
3. Maternal vascular malperfusion (MVM): diagnosis includes maternal vascular lesions as well as infarcts, hemorrhage or hematoma, thrombi (>5% of parenchyma); villous changes, and placental hypoplasia.
4. Fetal vascular malperfusion (FVM).
5. Other lesions: includes inflammatory lesions associated with chronic deciduitis with plasma cells, perivillous fibrin, massive chronic intervillositis, and histiocytic intervillositis, as well as delayed villous maturation, and villous edema.
6. Abnormalities in size of placentas were categorized as either small for gestational age (<10th percentile, SGA) or large for gestational age (>90th percentile, LGA).

With reference to the literature, chronic placental inflammatory lesions were defined as chronic villitis, chronic deciduitis, chronic chorioamnionitis, and/or villitis of unknown etiology.[11,12] All placental pathology reports were reviewed by both a researcher and neonatologist (I.N.M and Y.J.A). Study patients were divided into two groups based on placental pathology: (1) No evidence of FIR, (2) Evidence of Fetal Inflammatory Respose (FIR). Pariticants classified as having evidence of FIR were then subcategorized by severity: Stage 1 FIR (mild FIR) or ≥ Stage 2 FIR (moderate to severe FIR).

*Other variables*. Demographic information of both the mothers and neonates was taken from three well established and validated prospective Parkland Hospital databases, the Obstetrics Database, the Neonatal Resuscitation Database and the NICU Database. Small for GA (SGA) was defined as birthweight <10th percentile for sex and GA using Olsen's curves (39, 40).

### Neurodevelopmental assessment

Neurodevelopmental impairment was diagnosed based on the Bayley Scales of Infant and Toddler Development - Third edition (Bayley-III) at 22–26 months. Neonates born <29 weeks GA routinely undergo standardized neurologic assessments including the Bayley Scales of Infant and Toddler Development—Third edition (Bayley III) at 22–26 months

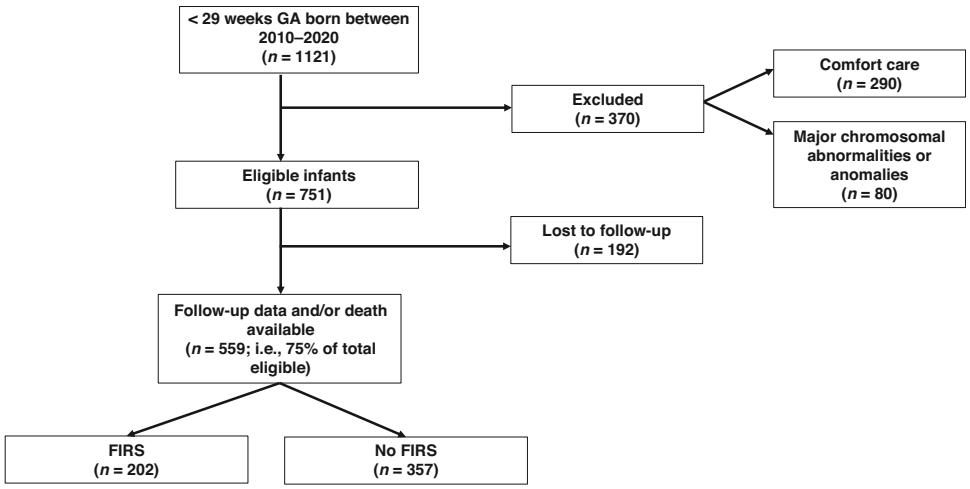


**Fig. 1** Flow Chart showing the patient population for the study.

**Table 1.** Relationship between Fetal Inflammatory Response (FIR) and Maternal and Infant characteristics.

| *n* (%) | No FIR (*n* = 357) | FIR (*n* = 202) | *P* value |
|---|---|---|---|
| Maternal characteristics | | | |
| Age, yr (median, IQR) | 30 (23, 34) | 28 (22, 33) | **0.04** |
| Race/Ethnicity | | | 0.17 |
| White | 16 (5) | 10 (5) | |
| Black | 97 (27) | 47 (23) | |
| Hispanic | 219 (61) | 120 (59) | |
| Other | 25 (7) | 25 (12) | |
| Antenatal Steroids | 203 (57) | 155 (77) | **<0.01** |
| Maternal diabetes | 55 (15) | 18 (9) | 0.03 |
| Illicit drug use | 19 (5) | 10 (5) | 0.85 |
| Preeclampsia (PIH) | 175 (49) | 8 (4) | **<0.01** |
| Infections during pregnancy | 78 (22) | 58 (29) | 0.01 |
| Antenatal Magnesium | 236 (66) | 123 (61) | 0.22 |
| PPROM | 43 (12) | 93 (46) | **<0.01** |
| Clinical Chorioamnionitis | 7 (2) | 37 (18) | **<0.01** |
| Cesarean section | 282 (79) | 96 (48) | **<0.01** |
| Infant characteristics | | | |
| Female | 160 (45) | 115 (57) | **<0.01** |
| Obstetrical EGA, wks | 27 (25, 28) | 26 (25, 27) | **<0.01** |
| Birth weight, g | 920 (760, 1150) | 910 (780, 1120) | 0.97 |
| Multiple pregnancies | 67 (19) | 28 (14) | 0.14 |
| Apgar scores | | | |
| 1 min | 4 (2, 6) | 4 (2, 6) | 0.59 |
| 5 min | 7 (5, 8) | 6 (5, 8) | **<0.01** |

Data are presented as Median (25th, 75th centile). Numbers in parenthesis are percentages. Data is analyzed using Chi-square or Fischer's exact test *FIR* Fetal Inflammatory Response, *PPROM* Premature and Prolonged Rupture of Membranes, *EGA* Estimated Gestational Age.
Bold values are variables with statistically significant *P*-values.

corrected age at our outpatient follow-up clinic.[17,18] NDI was defined by the presence of Bayley III motor, cognitive or language score <85, and severe NDI was defined as the presence of Bayley III cognitive, language or motor score <70. A composite of either death or NDI was the primary outcome of this study. Pre-specified secondary outcomes included death, IVH, BPD, pulmonary HTN, pulmonary hemorrhage, and the individual component scores of the neurodevelopmental assessment. This set of clinical outcome measures were chosen for our analysis since placental pathology has previously been associated with these morbidities.

## Statistical analysis

We used descriptive statistics including percentages, means, medians, and measures of variability to describe demographic data. Primary analysis of our outcomes used a Chi-square analysis or Fisher exact test for categorical data and a Mann-Whitney rank sum test for continuous data. The significance level for all tests was set at $p < 0.05$. A priori power analysis was conducted to test differences between two independent group proportions using a two-tailed test, a medium effect size ($d = 0.10$), and an alpha of 0.05. We used SPSS (version 25) to perform all statistical analyses.

A multiple logistic regression model was used to assess the relationship between FIR diagnosed on placental pathology and moderate NDI ± death controlling for birth weight, gestational age, and antenatal steroid use.

## RESULTS

### Study subjects

Between January 2010 and January 2021, a total of 1121 infants < 29 weeks gestational age were born at Parkland Hospital. Of these, 370 patients were excluded (290 received comfort care and 80 had a major chromosomal abnormality or congenital anomaly). Of the remaining 751 eligible infants, 192 were lost to follow up, resulting in 559 infants included in the analysis (Fig. 1). Among the study population, 202 (36%) of infants had evidence of FIR on placental histopathologic analysis.

### Maternal and infant characteristics

Mothers of infants with FIR on placental pathology were significantly younger with a greater prevalence of antenatal steroid use, infection during pregnancy, preterm premature rupture of membranes (PPROM), and clinical chorioamnionitis; further, they had a lower prevalence of cesarean section, maternal diabetes, and pre-eclampsia (Table 1). Infants with FIR were significantly younger in gestational age at birth, more commonly female sex, and had lower Apgar scores at 5 min (Table 1).

### Association of FIR with secondary outcomes

Infants with evidence of FIR on placental pathology had increased prevalence of congenital pneumonia and pulmonary hemorrhage (Table 2). They had longer duration of hospitalization, days on oxygen & CPAP and a higher prevalence of moderate or severe BPD (Table 2).

### Association of FIR with neurodevelopmental impairment or death

There was no association between FIR and the primary composite outcome of neurodevelopmental impairment or death (Table 3A). However, infants with FIR were noted to have significantly more motor impairment alone (Table 3B). The severity and progression of FIR were associated with NDI or death, specifically motor impairment or death, and language impairment or death (Table 4). A forward step model revealed that FIR diagnosed via placental pathology was the only risk factor for NDI or death (OR > 1.0) among other potential confounding variables (Table 5). The other variables included- gestational age, race/ethnicity, antenatal steroids, female sex, APGAR at 5 min, and max FiO2- were found to be protective factors for NDI or death (Table 5).

### Association of chronic placental inflammatory lesions with NDD and/or death

There was no association between FIR 2 and the primary composite outcome of neurodevelopmental impairment or death (Table 6). Additionally, there was no association between FIR I in combination with chronic inflammatory lesions of the placenta and the primary outcome of neurodevelopmental impairment or death, regardless of FIR I stage/severity.

## DISCUSSION

The main finding of this study is that FIR was not significantly associated with the composite primary outcome of NDI or death in extremely preterm infants born <29 weeks GA. However, when stratifying for FIR stage, we found that moderate to severe FIR (i.e., ≥ stage 2 FIR) was significantly associated with NDI or death by 2 years of age. Within the domains of NDI, we found a significant

**Table 2.** Relationship between FIR and Short-Term Outcomes in Preterm Infants born <29 weeks GA.

| *n* (%) | No FIR (*n* = 357) | FIR (*n* = 202) | *P* value |
|---|---|---|---|
| Short term Respiratory outcomes | | | |
| Respiratory Distress Syndrome | 334 (94) | 186 (92) | 0.51 |
| Received Surfactant | 285 (80) | 138 (68) | **<0.01** |
| Congenital pneumonia | 12 (3) | 15 (7) | 0.03 |
| Pulmonary Artery HTN | 19 (5) | 15 (7) | 0.32 |
| Pulmonary Hemorrhage | 37 (10) | 11 (5) | 0.046 |
| PIE | 102 (29) | 55 (27) | 0.73 |
| Pneumothorax | 30 (8) | 18 (9) | 0.84 |
| BPD<br>$O_2$ at 36 wks (Physiologic Definition) | 144 (40) | 108 (54) | **<0.01** |
| Moderate/Severe BPD | 76 (21) | 64 (32) | **<0.01** |
| Death or BPD | 191 (54) | 125 (62) | 0.06 |
| IV steroids for BPD | 22 (6) | 15 (7) | 0.56 |
| Days on Oxygen | 34 (9, 71) | 52 (23, 88) | **<0.01** |
| Days on mechanical ventilation | 5 (1, 15) | 7 (1, 22) | 0.07 |
| Days on CPAP | 24 (10, 38) | 31 (15, 48) | **<0.01** |
| Max $FiO_2$ | 100 (55, 100) | 100 (50, 100) | 0.40 |
| Other outcomes | | | |
| Sepsis proven (early and late) | 64 (18) | 42 (21) | 0.41 |
| IVH Grade III or IV | 42 (12) | 35 (17) | 0.07 |
| Symptomatic PDA | 89 (25) | 56 (28) | 0.47 |
| Days of hospitalization, | 83 (66, 110) | 98 (82, 115) | **<0.01** |
| Death | 52 (15) | 21 (10) | 0.16 |

Data are presented as Median (25th, 75th centile). Numbers in parenthesis are percentages. Data is analyzed using Chi-square or Fischer's exact test.
*FIR* Fetal Inflammatory Response, *BPD* Bronchopulmonary Dysplasia, *IVH* Intraventricular Hemorrhage, *PDA* Patent Ductus Arteriosus; *GA* Gestational Age.
Bold values are variables with statistically significant *P*-values.

**Table 3.** Relationship between FIR and Neurodevelopmental Impairment or Death in Preterm Infants born <29 weeks GA.

| *n* (%) | No FIR (*n* = 357) | FIR (*n* = 202) | *P* value |
|---|---|---|---|
| Moderate NDI (motor and/or cognitive <85) or Death | 262 (73) | 154 (76) | 0.46 |
| Severe NDI (<70) or Death | 119 (34) | 65 (33) | 0.77 |
| Moderate Cognitive(<85) or Death | 174 (49) | 90 (45) | 0.34 |
| Severe cognitive (<70) or Death | 70 (20) | 33 (16) | 0.34 |
| Moderate Motor (<85) or Death | 116 (33) | 80 (40) | 0.10 |
| Severe Motor (<70) or Death | 72 (21) | 35 (18) | 0.39 |
| Moderate Language (<85) or Death | 229 (64) | 138 (69) | 0.30 |
| Severe Language (<70) or Death | 113 (32) | 61 (30) | 0.73 |
| ***n* (%)** | **No FIR (*n* = 305)** | **FIR (*n* = 181)** | ***P* value** |
| Moderate NDI (motor and/or cognitive <85) | 210 (69) | 133 (74) | 0.28 |
| Severe NDI (<70) | 67 (22) | 44 (25) | 0.56 |
| Moderate Cognitive(<85) | 122 (40) | 69 (38) | 0.68 |
| Severe cognitive (<70) | 18 (6) | 12 (7) | 0.75 |
| Moderate Motor (<85) | 64 (22) | 59 (33) | **<0.01** |
| Severe Motor (<70) | 20 (7) | 14 (8) | 0.64 |
| Moderate Language (<85) | 177 (58) | 117 (65) | 0.14 |
| Severe Language (<70) | 61 (20) | 40 (22) | 0.57 |

Data are presented as Median (25th, 75th centile). Numbers in parenthesis are percentages within a column. Data is analyzed using Chi-square or Fischer's exact test.
*FIR* Fetal Inflammatory Response; *NDI* Neurodevelopmental Impairment.
A: FIR and NDI or Death. B: FIR and NDI
Bold values are variables with statistically significant *P*-values.

**Table 4.** Relationship between the severity and progression of FIR and NDI or Death Preterm Infants born <29 weeks GA.

| *n* (%) | No FIR (*n* = 357) | Stage 1 FIR (*n* = 74) | ≥ stage 2 FIR (*n* = 128) | *P* value |
|---|---|---|---|---|
| Moderate NDI (any category< 85) or Death | 262 (73)[a] | 43 (58)[b] | 111 (87)[c] | **<0.01** |
| Severe NDI (<70) or Death | 119 (34) | 22 (30) | 43 (34) | 0.81 |
| Moderate Cognitive (<85) or Death | 174 (49) | 30 (41) | 60 (47) | 0.44 |
| Severe cognitive (<70) or Death | 70 (20) | 14 (19) | 19 (15) | 0.49 |
| Moderate Motor (<85) or Death | 116 (33)[a] | 22 (30)[a,b] | 58 (46)[b] | **0.02** |
| Severe Motor (<70) or Death | 72 (21) | 15 (21) | 20 (16) | 0.50 |
| Moderate Language (<85) or Death | 229 (64)[a,b] | 41 (56)[b] | 97 (76)[a] | **0.01** |
| Severe Language (<70) or Death | 113 (32) | 20 (27) | 41 (32) | 0.75 |

Each subscript letter denotes a subset of categories whose column proportions do not differ significantly from each other at the 0.05 level.
Bold values are variables with statistically significant *P*-values.

association between increasing severity and progression of FIR and motor as well as language impairment at 2 years of age. Together, these data suggest a possible relationship between increasing severity and progression of fetal inflammation and subsequent brain injury, with the most pronounced effects on motor and language development. Our results support the growing body of evidence demonstrating an association between placental inflammation and long-term NDI.[4–10,19–30]

An increasing number of studies support the role of intrauterine infection in neonatal white matter injury with consequent NDI. Interestingly, a majority of human studies report a causal relationship between the maternal inflammatory response (hallmarked by chorioamnionitis on placental pathology) and cerebral palsy in particular.[19–30] However, this is the first study involving human patients that demonstrates the relationship between NDI and activation and progression of systemic fetal inflammation as evidenced by FIR on placental pathology. The mechanism by which inflammation from FIRS targets brain regions involved in motor function is not well delineated. One theory is that lipopolysaccharides (LPS) and other genetic materials released by offending pathogens are recognized by fetal T cells, which then activate the fetal immune cascade via pro-inflammatory cytokines.[9,10] Due to a combination of prematurity and immune dysregulation, the blood brain barrer is compromised in these neonates, allowing cytokines to enter the central nervous system with deleterious consequences.[4–10] Specifically, neuroinflammation leads to oligodendrocyte and neuronal injury, as well as microglia activation, the latter of which is specifically implicated in white matter damage within the periventricular region.[9,10] The importance of periventricular white matter integrity in motor development is well known, as this region is rich in motor pathways and damage leads to spasticity, tonicity, and weakness.[4,8,19–23] Together, this suspected pathophysiology best explains our finding that motor impairment at 2 years of age is significantly associated with increasing severity and progression of FIR.

In this study, mothers of infants with FIR had a higher rate of antenatal steroid use, contrary to prior literature that has repeatedly demonstrated lower rates of neonatal mortality and morbidity with antenatal steroid use in cases of histologic

chorioamnionitis and/or intrauterine infection.[27–29,31] Until 2016, pregnant individuals with pre-eclampsia or diabetes during pregnancy did not routinely receive antenatal steroids per Parkland Hospital protocol and often underwent cesarean sections due to their underlying conditions. Accordingly, we found significantly higher rates of cesarean section, diabetes, and pre-eclampsia among mothers of infants without FIR. Thus, the lower antenatal steroid use among mothers of infants without FIR observed may be ascribed to other concomitant conditions in conjunction with Parkland protocol in place at the time. The remaining maternal characteristics we found to be significantly associated with FIR support results from prior investigations. This includes PPROM- even after controlling for other potential causes of preterm birth- and clinical chorioamnionitis, which was expected due to its functional component in the development of FIRS.[5–7]

As severity and prevalence of neonatal morbidity and mortality is inversely related to gestational age[4,5] and FIR is an established risk factor of prematurity,[5–8] the significant association between younger gestational age and FIR on pathology, with and without stratification by severity, was not surprising. Our analysis also demonstrated significantly lower Apgar scores at 5 min among infants with FIR on placental pathology which is in agreement with prior studies that have demonstrated a significant association between lower Apgar scores and neurodevelopmental impairment (an expected consequence of severe FIRS).[28,31]

**Table 5.** Severity of FIR and Relationship with Neurodevelopmental Outcome or Death at 24 months of age.

| | Odds ratio (95% CI) | *P*-value |
|---|---|---|
| Step 1 | | |
| FIR ≥ 2 | 2.7 (1.5, 4.8) | 0.001 |
| OBEGA | 0.88 (0.78, 0.99) | 0.04 |
| Step 2 | | |
| FIR ≥ 2 | 2.9 (1.7, 5.2) | <0.001 |
| OBEGA | 0.88 (0.78, 0.99) | 0.03 |
| Female | 0.51 (0.35, 0.77) | 0.001 |
| Step 3 | | |
| FIR ≥ 2 | 3.2 (1.8, 5.7) | <0.001 |
| OBEGA | 0.87 (0.77, 0.99) | 0.03 |
| Female | 0.53 (0.35, 0.79) | 0.002 |
| Antenatal steroids | 0.57 (0.37, 0.88) | 0.03 |
| Step 4 | | |
| FIR ≥ 2 | 3.1 (1.7, 5.6) | <0.001 |
| OBEGA | 0.92 (0.80, 1.04) | 0.18 |
| Female | 0.54 (0.36, 0.81) | 0.003 |
| Antenatal steroids | 0.59 (0.38, 0.92) | 0.02 |
| APGAR 5 min | 0.87 (0.77, 0.98) | 0.02 |

Our study also demonstrated a significant association between evidence of FIR on placental pathology and a higher prevalence of congenital pneumonia and moderate or severe bronchopulmonary dysplasia. Prior research suggests that inflammation from swallowed pathogens in the amniotic fluid increases fetal lung maturity, which compromises the long-term development of normal, functional lung anatomy.[8,32] Consequently, fetuses with FIRS are predisposed to a variety of lung pathologies with a higher risk for chronic lung disease.[8,17,32,33] Accordingly, infants with FIR on placental pathology also required a significantly longer duration of supportive lng therapies including supplemental oxygen, CPAP, and surfactant. Some of this may also be explained by the increased risk for lung disease associated with pre-maturity itself.

### Strengths and limitations

To our knowledge, this is the first study to demonstrate a significant relationship between severity and progression FIR on placental pathology and neurodevelopmental impairment. Furthermore, ours is the largest cohort of placentas to undergo a rigorous and thorough pathologic examination to stage FIR. Despite these strengths, there are limitations that confound our interpretation of the data. Specifically, this study is limited by inherent biases of a retrospective analysis including missing, incomplete, or inaccurate data. We were limited to placenta pathology reports available to us, and relied on histologic documentation from several different pathologists, none of whom were blinded to maternal infection status, which is a potential source of bias. Furthermore, we were without long-term data on cerebral palsy and blindness, which would have provided additional clinical relevance to the significant impairments observed. Additionally, this study does not include brain MRI which- if showing damage to the periventricular white matter- would strengthen evidence of a connection between FIR and NDD through neuroinflammatory injury.

Another notable consideration is the role of the exposome in development in NDD pathogenesis. The maternal immune activation (MIA) hypothesis describes the effect of environmental factors on programming of the immune and developmental epigenetic code, conferring increased vulnerability to NDD and other chronic diseases.[18] More specifically, this includes factors like socioeconomic status, the built environment (i.e. exposure to pollutants, availability of green spaces, etc.), stress, poverty, and comorbidities (i.e. diabetes, obesity) of the birthing parent. In the current study, relevant risk factors including diabetes and maternal drug use were not significantly associated with FIR on placental pathology. With that said, we did not robustly evaluate risk factors such as income, mental illness, or other comorbidities- to name a few- that may have deleterious epigenetic effects, which remains a limitation of this study.

## CONCLUSION

Our study demonstrates a significant association between moderate to severe FIR on placental pathology and

**Table 6.** Relationship between FIR and Chronic Placental Inflammation with NDI or Death.

| | NDI or Death (*N* = 143) | NDI or Death (*N* = 416) | *P* value |
|---|---|---|---|
| FIR | 48 (34) | 154 (37) | 0.46 |
| Chronic Placental Inflammatory Lesions | 15 (11) | 41 (10) | 0.83 |
| FIR + Chronic Placental Inflammatory Lesions | 7 (5) | 12 (3) | 0.29 |
| FIR (≥ stage 2) and Chronic Placental Inflammatory Lesions | 3 (2) | 7 (2) | 0.72 |

Numbers in parenthesis are percentages within the column; Data is analyzed using Chi Qquare Test or Fischer's Exact Test; *FIR* Fetal Inflammatory Response, *NDI* Neurodevelopmental Impairment.

neurodevelopmental impairment or death at 2 years of age. Within the different domains of NDI, the severity and progression of FIR was significantly associated with motor and language impairment. Our dataset contributes to the growing body of literature linking intrauterine infection, fetal inflammation, and neurologic injury that leads to long-term adverse neurodevelopmental outcomes. Finally, this study underscores the importance of standardized placental pathologic examination in the care of critically ill neonates.[34–39]

## DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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## AUTHOR CONTRIBUTIONS

Yasmeen Alayli wrote the first version of the manuscript. Steven Brown performed statistical analyses. All authors participated in the study design, data collection, data interpretation, and revision and approval of the final version of the manuscript.

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## COMPETING INTERESTS

There are no conflicts of interest, including relevant financial interests, activities, relationships, and affiliations.

## CONSENT STATEMENT

The Institutional Review Board of University of Texas Southwestern Medical Center and Parkland Health approved the study (STU 09-2015-013) and waived the need for individual consent.

## ADDITIONAL INFORMATION

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