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The fetal inflammatory response syndrome: the origins of a concept, pathophysiology, diagnosis, and obstetrical implications

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Abstract

The fetus can deploy a local or systemic inflammatory response when exposed to microorganisms or, alternatively, to non-infection-related stimuli (e.g., danger signals or alarmins). The term “Fetal Inflammatory Response Syndrome” (FIRS) was coined to describe a condition characterized by evidence of a systemic inflammatory response, frequently a result of the activation of the

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innate limb of the immune response. FIRS can be diagnosed by an increased concentration of umbilical cord plasma or serum acute phase reactants such as C-reactive protein or cytokines (e.g., interleukin-6). Pathologic evidence of a systemic fetal inflammatory response indicates the presence of funisitis or chorionic vasculitis. FIRS was first described in patients at risk for intraamniotic infection who presented preterm labor with intact membranes or preterm prelabor rupture of the membranes. However, FIRS can also be observed in patients with sterile intra-amniotic inflammation, alloimmunization (e.g., Rh disease), and active autoimmune disorders. Neonates born with FIRS have a higher rate of complications, such as early-onset neonatal sepsis, intraventricular hemorrhage, periventricular leukomalacia, and death, than those born without FIRS. Survivors are at risk for long-term sequelae that may include bronchopulmonary dysplasia, neurodevelopmental disorders, such as cerebral palsy, retinopathy of prematurity, and sensorineuronal hearing loss. Experimental FIRS can be induced by intra-amniotic administration of bacteria, microbial products (such as endotoxin), or inflammatory cytokines (such as interleukin-1), and animal models have provided important insights about the mechanisms responsible for multiple organ involvement and dysfunction. A systemic fetal inflammatory response is thought to be adaptive, but, on occasion, may become dysregulated whereby a fetal cytokine storm ensues and can lead to multiple organ dysfunction and even fetal death if delivery does not occur (“rescued by birth”). Thus, the onset of preterm labor in this context can be considered to have survival value. The evidence so far suggests that FIRS may compound the effects of immaturity and neonatal inflammation, thus increasing the risk of neonatal complications and long-term morbidity. Modulation of a dysregulated fetal inflammatory response by the administration of antimicrobial agents, anti-inflammatory agents, or cell-based therapy holds promise to reduce infant morbidity and mortality.

Keywords

Cerebral palsy; Chorioamnionitis; Congenital dermatitis; Cytokines; Fetal cytokine release syndrome; Fetal cytokine storm; Fetal hematophagocytic syndrome; Fetal macrophage activation-like syndrome; FIRS; Funisitis; Interleukin-6; Intra-amniotic infection; Intra-amniotic inflammation; Neonatal encephalopathy; Neonatal morbidity; Neonatal sepsis; Neuroinflammation perinatal morbidity; Prematurity; Premature birth; Preterm labor; Preterm prelabor rupture of the membranes (preterm PROM); Retinopathy of prematurity; Sensorineuronal hearing loss

1. The term “fetal inflammatory response syndrome” (FIRS)

The human fetus can deploy an inflammatory response when exposed to microbial invasion with bacteria [1], viruses [2,3], fungi [4, 5], and protozoa [6–8], or non-infection related stimuli. The inflammatory process can be localized to an organ (e.g., the lung following fetal aspiration of amniotic fluid) or become systemic when inflammatory mediators enter the circulation.

We coined the term “*Fetal Inflammatory Response Syndrome*” (FIRS) while studying the role of intra-amniotic infection in spontaneous preterm labor to describe the presence of systemic inflammation akin to that observed in adult patients with a systemic inflammatory response syndrome [9].

The term “*Systemic Inflammatory Response Syndrome*” (SIRS) emerged from the consensus conference of the American College of Chest Physicians and the Society for Critical Care Medicine in 1992, as participants recognized that clinical manifestations of sepsis could also be observed in patients without infection (e.g., burns, trauma, pancreatitis, ischemia, immune-mediated injury), and that they resulted from a systemic inflammatory process [10]. The group also proposed the term “*Multiple Organ Dysfunction Syndrome*” (MODS) to refer to the presence of altered organ function in an acutely ill patient, (e.g., homeostasis cannot be maintained without intervention) [10].

SIRS has been diagnosed in adults using the criteria displayed in Table 1. Yet, the definition could not be applied to the human fetus because vital signs (except for the fetal heart rate) and white blood cell counts cannot be readily determined before birth. For this reason, we elected to define FIRS as an elevated concentration of fetal plasma interleukin-6 (IL-6).

In 2001, the American College of Chest Physicians and the Society of Critical Care Medicine reaffirmed the initial criteria for the diagnosis of SIRS. The group of experts noted that an elevation of plasma concentration of certain mediators, such as IL-6, could be associated with SIRS and speculated that this observation could bring about a new definition of the syndrome in adult patients. Concerns at the time were that the clinical and laboratory findings originally proposed to characterize SIRS were non-specific. The terms used to assess patients with suspected sepsis have continued to evolve [11]. Yet, it is clear that the host immune response is key in the recovery of patients as well as in the predisposition to secondary infections and morbidity/mortality.

The immunological response to infection or tissue injury varies over time and involves both pro- and anti-inflammatory responses. The initial concept was that SIRS was followed by compensatory anti-inflammatory response syndrome and that this process led to MODS and predisposed to death. In the early stages of the study of SIRS, it was believed that most patients died because of an excessive inflammatory response. However, subsequent observations uncovered the importance of the counter-inflammatory response that leads to progressive immune suppression and predisposition to secondary infection. It is now recognized that both pro- and anti-inflammatory responses are activated in early sepsis; however, the pro-inflammatory response is predominant [12–14]. As the disorder progresses, the anti-inflammatory limb of the immune response becomes predominant, and patients recovering from sepsis are more susceptible to secondary infections from bacteria, or even reactivation of latent viral infections (e.g., cytomegalovirus, herpes simplex virus) [15, 16]. Indeed, patients who have recovered from sepsis remain at risk of death for approximately one year after the sepsis episode, secondary to the prolonged period of immunosuppression [13,14]. The early pro-inflammatory phase has been attributed predominantly to activity of the innate immune system, while the counter-immune response has been attributed to a dysregulated adaptive immune system: this is probably an oversimplification of the complex nature of the evolution and interaction of different components of the immune system. However, the conceptual framework of an anti-inflammatory response in neonates is important because it may explain why some neonates are born with FIRS [17,18], improve clinically, and then become affected by late-onset neonatal sepsis [19].

2. Why focus on systemic fetal inflammation or FIRS?

In 1998 when the initial work in this field was reported, intra-amniotic infection in patients with preterm labor and intact membranes, as well as preterm prelabor rupture of the membranes (PROM), was known to be associated with impending delivery. However, it was not clear whether labor was associated with an intra-amniotic or a fetal systemic inflammatory response. Moreover, the frequency with which intraamniotic infection led to fetal infection and sepsis was unknown.

We had observed that of preterm neonates born to mothers with intra-amniotic inflammation/infection only a fraction had proven neonatal sepsis; yet, many of these neonates had morbidity which could be attributed at least in part to a systemic inflammatory process, but not necessarily to sepsis. Therefore, an important question emerged: does fetal inflammation predispose to multiple organ dysfunction and result in a higher rate of neonatal morbidity?

The operative definition of FIRS was an elevation in the fetal plasma concentration of IL-6. Since our group was studying pregnancy outcomes using cytokines to define the presence or absence of intra-amniotic inflammation [9], we chose IL-6 because this cytokine could be measured in both amniotic fluid and umbilical cord blood; therefore, we could ascertain the presence and intensity of the intra-amniotic and fetal inflammatory responses based on one analyte. Other cytokines (e.g., TNF- α and IL-1 β) were not consistently detected in peripheral blood with the assays available at the time. We also chose IL-6 as a marker of inflammation because this cytokine is a major mediator of the acute phase response to infection or tissue injury (e.g., IL-6 induces production of C-reactive protein).

We used the term *syndrome* during the course of initial studies given our observation that both intra-amniotic inflammation and fetal systemic inflammation could be caused by infection and non-infection-related etiologies. We predicted that FIRS would: 1) be associated with the spontaneous onset of labor; 2) be associated with a higher rate of neonatal morbidity, since the fetuses were already affected *in utero*; and 3) lead to changes/dysfunction in multiple organ systems.

3. FIRS is followed by the spontaneous onset of preterm labor

Preterm labor in the setting of infection results from the action of pro-inflammatory cytokines secreted by the mother and/or fetus in response to intra-amniotic infection [20]. Delivery would allow the mother to maximize reproductive fitness and the fetus to exit a hostile intrauterine environment. The mechanisms of parturition require cooperation between the mother and the conceptus as the effector organs of parturition are maternal (myometrium, decidua, and cervix), but there is a substantial contribution of the conceptus (chorioamniotic membranes).

In a study of patients with preterm PROM who were not in labor upon admission, we found that FIRS was associated with a higher rate of spontaneous preterm delivery within 48 and 72 h of amniocentesis, compared to those without FIRS (48 h: 88% vs. 29.7%; and 72 h: 88% vs. 35%; p-value <0.05 for both) [20]. Moreover, patients with initiation of labor and

delivery within 48 h of amniocentesis had a higher proportion of fetuses with plasma IL-6 values > 11 pg/mL than patients delivered >48 h [58% (7/12) vs. 8% (1/13), respectively, p-value <0.05] (Fig. 1). Multivariate analysis showed that plasma IL-6 was the only factor associated with pregnancy duration after adjusting for gestational age, amniotic fluid IL-6, and the microbiologic state of the amniotic cavity. The relationship between intra-amniotic inflammation and fetal systemic inflammation and the onset of preterm labor is displayed in Fig. 2. These findings led to the conclusion that FIRS was followed by the onset of preterm parturition in patients with preterm PROM.

4. FIRS is associated with a higher rate of neonatal morbidity and mortality

We then tested the hypothesis of whether fetuses with systemic inflammation would have a higher rate of neonatal morbidity. Just as adult patients with SIRS are critically ill, we reasoned that fetuses with FIRS were more likely to present morbidity after birth. To address this question, we studied patients with preterm labor and intact membranes as well as those with preterm PROM. Severe neonatal morbidity was defined as the presence of respiratory distress syndrome, suspected or proven neonatal sepsis, pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage, or necrotizing enterocolitis. The presence of FIRS (fetal plasma IL-6 >11 pg/mL) conferred a higher rate of neonatal morbidity than in those without FIRS (77.8% vs. 29%; p < 0.001) (Fig. 3) [9]. Multivariate analysis showed that FIRS was an independent predictor of severe neonatal morbidity after adjusting for gestational age, the obstetrical cause of preterm delivery (preterm labor or preterm PROM), clinical chorioamnionitis, presence of microorganisms in the amniotic cavity, and amniotic fluid IL-6 results. Importantly, the determination of the presence/absence of fetal systemic inflammation was done before delivery, thus the findings cannot be attributed to an intrapartum phenomenon.

5. The clinical significance of FIRS

A systematic review and meta-analysis of observational studies including 1116 neonates has shown that FIRS was associated with a higher frequency of adverse outcomes – specifically, early-onset sepsis (RR = 3.1), bronchopulmonary dysplasia (RR = 5.9), intraventricular hemorrhage (RR = 4.9), periventricular leukomalacia (RR = 3.3), respiratory distress syndrome (RR = 2.4), and neonatal death (RR = 7.0), when compared to neonates without FIRS [21]. In preterm neonates, FIRS was significantly and independently associated with an increased risk of retinopathy of prematurity and its progression [22]. Moreover, FIRS is also associated with a neonatal systemic inflammatory response, which manifests as clinically suspected neonatal sepsis with negative blood and cerebrospinal fluid cultures [23–25]. Future studies using a uniform definition of FIRS would strengthen the accuracy of these observations.

6. Evidence of multi-systemic involvement in FIRS

We had predicted that the fetus with FIRS will have evidence of multisystemic involvement *in utero* or in the immediate neonatal period (Fig. 4). However, there are important

limitations to the study of this hypothesis in humans. Improved understanding of FIRS comes from studies in animal models—in particular, the pioneering contributions of the laboratories of Kallapur, Jobe, Adams, and Waldorf in the United States, Newnham in Australia, Kramer in the Netherlands, and Hallman in Finland.

6.1. Hematopoietic system

Neutrophils, a major component of the innate immune system, play a critical role in the host response against infection and other insults. During human fetal development, neutrophils first appear in the clavicular bone marrow at 12–13 weeks of gestation [26]. However, lymphocytes are the predominant circulating white blood cells in the preterm fetus [27]. After 32 weeks of gestation, the proportion of neutrophils increases in fetal blood to become the predominant leukocyte at term [27,28].

The initial study of the hematologic profile in FIRS reported that affected fetuses had a higher median corrected white blood cell count and corrected neutrophil count than unaffected fetuses. Neutrophilia (neutrophil count >95th percentile for gestational age) was found in 71% (30/42) of cases, while neutropenia (neutrophil count <5th percentile for gestational age) was present in 4.8% (2/42) [29]. No detectable changes in lymphocyte, monocyte, basophil or eosinophil counts were observed in the initial report.

In a subsequent study of preterm neonates born after spontaneous preterm labor and preterm PROM, Kim et al. reported the hematologic profiles in umbilical cord blood. FIRS was defined as the presence of funisitis, and the authors reported that preterm neonates with funisitis had significantly higher rates of neutrophilia (>95th percentile for gestational age) [93.2% (41/44) vs. 77.8% (119/153); $p = 0.027$] and monocytosis (>95th percentile for gestational age) [81.8% (36/44) vs. 64.1% (98/153); $p = 0.026$] than those without funisitis [30].

In addition to the change in number, FIRS (defined by the presence of funisitis) has also been associated with phenotypic evidence of monocyte and granulocyte activation [31]. Indeed, the umbilical cord blood of neonates with acute funisitis had a higher mean channel brightness for CD14 and CD64 on granulocytes and of CD64 on monocytes and a higher basal level of intracellular reactive oxygen species and oxidative burst in monocytes [32].

The mechanisms responsible for the quantitative and qualitative changes in neutrophils in the presence of FIRS have not been elucidated. However, the concentration of granulocyte colony stimulating factor (G-CSF), a cytokine and major physiologic regulator of neutrophil production released during stressful conditions, as well as the activation of neutrophils [33–35], is higher in fetuses with FIRS compared to those without FIRS [36].

Changes in the red blood cell lineage in FIRS (defined by an elevated fetal plasma IL-6 concentration) have also been observed. Affected fetuses have a slightly higher median nucleated red blood cell count (adjusted for gestational age) compared to those without FIRS [29]. An elevated nucleated red blood cell count has also been observed in neonates delivered from patients with prolonged (>24 h) rupture of the fetal membranes [37], histologic chorioamnionitis [38], a high acute placental inflammation score [39], and early-

onset neonatal sepsis [40]. Since changes in nucleated red blood cells in FIRS are not associated with a lower pH or PO₂ [41], metabolic acidemia/hypoxemia is unlikely to be the cause of the observed changes [42]. Given that the IL-6 concentration in umbilical cord blood correlates with the nucleated red blood cell count [43], IL-6 may mediate these changes [44,45], either directly or indirectly, through erythropoietin. The latter possibility must be considered since the serum erythropoietin concentration is higher in newborns with funisitis than in those without funisitis [46].

Experimental fetal systemic inflammation induced by the administration of IL-1 α , an alarmin, into the amniotic cavity of pregnant sheep has been associated with a decreased number of circulating neutrophils within 24 h (early response), followed by an increase in neutrophil numbers at 3 and 7 days (late response) [47]. Fetal systemic inflammation induced by exposure to bacterial endotoxin in ewes is associated with an increased number of circulating neutrophils 7 days after exposure [48]. Consistent with these findings, preterm pigs exposed to intra-amniotic bacterial endotoxin for 3 days before delivery also demonstrated increased blood neutrophil counts at birth [49]. It is possible that the frequency of neutrophilia in human umbilical cord blood reflects at least, in part, the duration of exposure to an inflammatory stimulus.

6.2. Thymus

Thymic involution occurs after infection in the fetus and neonate [50–55]. Subclinical chorioamnionitis has been associated with a small thymus in very-low-birth-weight infants [50], possibly from acute fetal [51] and neonatal [51,52] involution. In neonates, thymic involution correlates with the duration of acute illness and with the percentage of lymphocytes in peripheral blood [52]. The proposed mechanism for thymic involution is thought to be stress (via glucocorticoid production) and the effects of proinflammatory cytokines [56–58].

A sonographically small thymus for gestational age has been reported by Di Naro et al. [59] and other investigators [53–55,60] in the presence of intra-amniotic infection and inflammation (preterm labor and intact membranes or preterm PROM). Another study reported that infants born preterm (<28 weeks of gestation) with sonographic signs of cerebral white matter damage have thymic involution more frequently than a control group matched for gestational age [61]. This has been interpreted as indicating that neurologic injury and thymic involution may have a common origin, namely fetal systemic inflammation.

Intra-amniotic administration of bacterial endotoxin in sheep results in both decreased thymic weight and corticomedullary ratio. The effects can be observed as early as 5 h after exposure to endotoxin [62,63]. Increased expression of IL-6 and type I interferons, as well as glucocorticoids, has been implicated in thymic involution [63–67]. In addition, an increase in the percentage of CD3⁺ cells, but a decrease in the percentage of CD8⁺ (cytotoxic lymphocytes) and FOXP3⁺ cells, occurs after exposure to endotoxin [62,63,68]. Such findings suggest that endotoxin exposure has an effect not only on the innate but also on the adaptive immune response. Taken together, FIRS can induce structural, functional, and immunologic changes in the thymus.

Activation of T cells (belonging to the adaptive limb of the immune response) has been observed in term and preterm infants born to mothers with clinical chorioamnionitis. Such infants demonstrate overexpression of CD25, HLA-DR, or CD69 markers in T cells [69], a higher number of memory T cells (CD45RO⁺), and a decreased number of naive T cells [69–72]. Further studies are required to comprehensively describe changes in cells involved in adaptive immunity in the presence of fetal systemic inflammation from different etiologies (e.g., bacterial and viral infections, non-infection related causes) and at varying gestational ages (i.e., preterm and term).

It is noteworthy that studies of fetal rhesus macaques report that the number of T regulatory cells (FOXP3⁺) is decreased, while there is an increase in the number of IL-17 (pro-inflammatory cytokine) producing cells in lymphoid tissues after intra-amniotic administration of IL-1 β [73]. However, these changes were not observed in fetal blood [73].

6.3. Spleen

The spleen is the largest secondary lymphoid organ, and plays an important role in immunological processes, hematopoiesis, and red blood cell clearance [74]. The unique physical organization of the organ is thought to facilitate filtering of microorganisms and abnormal cells [74,75]. In adults affected by sepsis, there is a loss of B and CD4 T cells in the spleen, and similar findings have been reported in fetuses and neonates. In a study of 20 fetuses exposed to intra-amniotic infection/inflammation (diagnosed by acute histologic chorioamnionitis or funisitis), 10 neonates died of proven sepsis and showed splenic depletion [76]. Indeed, the percentage of red and white pulp areas occupied by CD45RA⁺, CD45RO⁺, and CD20⁺ cells was reduced [76].

Changes in the fetal splenic circulation have also been observed in the context of intra-amniotic infection/inflammation [77,78]. Under normal circumstances, the fetal splenic vein has a non-pulsatile Doppler waveform. However, Musilova et al. reported a pulsatile flow pattern in the fetal splenic vein in 84% (16/19) of cases with funisitis but in only 15% (9/60) of cases without fetal inflammation [79]. In addition, fetuses with a pulsatile splenic vein flow pattern exhibited a higher concentration of umbilical cord blood IL-6 than those with continuous flow [median 56.7 pg/mL versus 5.6 pg/mL; $p < 0.0001$] [77]. Collectively, this suggests that non-invasive interrogation of the fetal circulation may help to identify the fetus with systemic inflammation. The mechanism whereby pulsatile flow in the splenic vein is generated is thought to result from increased intrasplenic pressure secondary to microbial product-induced impairment of splenic drainage into the lymphatic circulation.

Several experimental studies have examined the effect of cytokines and bacterial endotoxin on the fetal spleen [48,80]. Kramer et al. demonstrated a two-fold increase in TNF- α expression in the fetal spleen 24 h after intra-amniotic injection of LPS [48]. A subsequent study showed that intra-amniotic LPS-induced changes in the splenic cytokine profile and increased CD3 expression, a marker for T cells [80]. Cytokine changes in the fetal spleen have been detected as early as 5 h after the injection of LPS and for up to 15 days after the onset of inflammation, indicating that intrauterine exposure to an inflammatory stimulus caused a rapid and sustained response in the fetal spleen [80]. Taken together, the available evidence suggests that intra-amniotic infection or intra-amniotic inflammation can induce

changes in the developing fetal spleen. The long-term consequences of these changes in the spleen require investigation.

6.4. Adrenal glands

The adrenal glands are involved in the response to sepsis and systemic inflammation. Adult patients admitted to the intensive care unit with sterile inflammatory processes, such as burns or pancreatitis, present endocrine evidence of stress, as indicated by an elevation in the cortisol and dehydroepiandrosterone sulfate ratio [81]. The same has been reported in the human fetus [81] and has been implicated in the mechanisms for preterm labor. Specifically, Yoon et al. reported a significant association between the fetal plasma cortisol and dehydroepiandrosterone sulfate ratio and pregnancy duration in patients with preterm PROM (hazard ratio 2.9; 95% CI: 1 to 8.4) [81]. Patients with preterm PROM who went into spontaneous labor and delivered within 7 days of sampling had a significantly higher median fetal plasma concentration of cortisol than those who delivered after 7 days ($p < 0.0001$). Indeed, a fetal plasma cortisol, but not a maternal cortisol, concentration was an independent predictor of pregnancy duration after adjusting for both gestational age and the results of amniotic fluid cultures (hazard ratio 2.9; 95% CI 1.3 to 6.7). Interestingly, there is a significant correlation between fetal plasma cortisol and IL-6 concentrations ($r = 0.3$; $p < 0.05$) [81]. Such endocrine changes may have short- and long-term implications given observations about the effects of glucocorticoids in fetal programming of several metabolic functions [82–86].

6.5. Thyroid

Transient hypothyroxinemia of prematurity occurs in 30–80% of preterm infants (<30 weeks of gestation) and is characterized by a transient reduction in the serum concentrations of T4 and T3 as well as a low or normal TSH [87]. De Felice et al. reported that preterm neonates with acute histologic chorioamnionitis had a significantly lower T4 concentration on post-delivery day 4 in comparison to those without this placental lesion [88]. Low T4 concentrations in preterm infants have been linked to persistent neurodevelopmental deficits in cognitive and motor function at 2 years of age [89]. The mechanism responsible for these findings has not been established. Sepsis in adults has been associated with lower concentrations of T3 (“low T3 syndrome”), and this is thought to result from apoptosis of thyroid cells [90,91].

6.6. Lung

Amniotic fluid is inhaled by the fetus and reaches the airways, as demonstrated by color Doppler ultrasound imaging [92–95]. Congenital pneumonia, which is frequently diagnosed in cases of fetal death, is thought to be secondary to fetal aspiration of microorganisms. Meconium may be detected in the alveoli in stillbirths, indicating that amniotic fluid can reach the alveoli [96,97]. Tracheobronchial fluid obtained from neonates via an endotracheal tube placed shortly after birth often shows the presence of white blood cells and microorganisms in patients affected by intra-amniotic infection [98]. Interestingly, fetuses with intra-amniotic infection often have a dramatic decrease in fetal “breathing movements,” which may decrease the likelihood of bacterial entry into the lung [99–102].

The relationship between intra-amniotic inflammation/infection and the development of respiratory complications, such as respiratory distress syndrome (RDS) and chronic lung disease/bronchopulmonary dysplasia (BPD) has been the subject of intensive investigation [103–105]. The *Watterberg hypothesis* proposes that intra-amniotic inflammation/infection is associated with a decreased rate of RDS (early protective effect) but an increased rate of BPD [106]. A systematic review and meta-analysis, which included 158 studies and 244,096 preterm infants, showed that there is a significant association between clinical and histologic chorioamnionitis and subsequent development of BPD [107]. However, chorioamnionitis was not a risk factor for the development of RDS. A multivariate meta-regression revealed that a model combining the difference in gestational age and the odds of RDS explained 64% of the variance in the association between chorioamnionitis and BPD (36 weeks' post-menstrual age) across studies [107].

Watterberg et al. reported that low-birth-weight infants exposed to chorioamnionitis had higher concentrations of IL-1 β in tracheal lavage samples and a lower incidence of RDS, but a higher rate of BPD in comparison to the control group [106]. Ghezzi et al. reported that IL-8 concentrations in amniotic fluid were higher in women who presented with spontaneous preterm labor (intact or ruptured membranes) and delivered infants at 24–28 weeks of gestation who later developed BPD [108]. Indeed, preterm neonates born at 33 weeks of gestation or earlier who developed BPD had median amniotic fluid concentrations of IL-1 β and IL-8 that were significantly higher than those in whom BPD did not develop [109]. Collectively, the evidence suggests that exposure to intra-amniotic inflammation is a risk factor for BPD.

Subsequently, Yoon et al. reported that neonates born between 25 and 34 weeks of gestation and in whom BPD developed had a significantly higher median umbilical cord plasma IL-6 concentration in comparison to matched preterm infants without BPD. This indicates that the risk for BPD is higher in the offspring of pregnancies in which there is intra-amniotic inflammation and fetal systemic inflammation [110].

This set of clinical observations led to systematic investigation of the effect of microbial products and pro-inflammatory cytokines on fetal lung development. The models include intra-amniotic administration of endotoxin, administration of IL-1 β , or the inoculation of bacteria in the amniotic cavity. The major findings have been that inflammation stimulates surfactant protein production but alters lung development [111–113].

Bry et al. first reported that intra-amniotic administration of IL-1 α to pregnant rabbits increased mRNA and protein expression of surfactant proteins A and B, as well as surfactant lipids [114]. This was accompanied by improved neonatal lung function. Injection of IL-1 α also induced preterm labor and delivery; therefore, the effect of inflammation on surfactant production could be interpreted as promoting lung maturation in anticipation of preterm delivery.

The laboratories of Jobe and Newnham have systematically studied endotoxin-induced fetal lung injury in sheep and rhesus macaques [112, 115–128]. Intra-amniotic administration of LPS induces fetal lung inflammation, and this is accompanied by a dramatic increase

in the number of mononuclear cells and granulocytes in bronchoalveolar lavage fluid and mRNA expression for IL-1, IL-8, and IL-6 in the lung tissue [112]. An increase in surfactant production, and structural changes in the developing fetal lungs, such as decreased alveolar number, thinning of alveolar septae, and increased alveolar size has been consistently reported [115]. In addition, there is down-regulation of the expression of elastin [127] and several genes involved in vascular development (vascular endothelial growth factor A, vascular endothelial growth factor receptor 2, and endothelial nitric oxide synthase) [119, 120]. The latter is thought to predispose to pulmonary hypertension [125]. The structural changes reported after endotoxin administration have also been observed after intra-amniotic inoculation with *U. urealyticum* in sheep and rhesus macaques [113,129–132].

Fetal lung inflammation is characterized by robust expression of proinflammatory mediators such as IL-1 β , IL-8, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein 1, and serum amyloid A3 in both sheep and monkeys [73,112,122,124]. It is noteworthy that IL-1 β is the major cytokine involved in fetal lung injury, as TNF- α and interferon- γ do not elicit the same degree of inflammation [73,133]. Moreover, pre-treatment with intra-amniotic injection of an IL-1 α antagonist before the administration of bacterial endotoxin prevents lung inflammation and maturation [121].

In summary, exposure to microbial products and intra-amniotic inflammation induces fetal lung maturity, which favors survival in the context of preterm delivery [134]. However, acceleration of lung maturity is accompanied by dramatic changes in the anatomy of the lung (e.g., reduction in the number of alveoli, impaired microvascular development, and thickening of the arteriolar walls, which collectively resemble changes observed in infants with bronchopulmonary dysplasia) [135]. Therefore, the short-term gain in lung maturity appears to predispose to the development of chronic lung disease.

6.7. Heart

Cardiac dysfunction can occur during the course of a systemic inflammatory response and sepsis in adults [136–140]. Impaired cardiac performance results from a combination of systolic and diastolic dysfunction, which was originally attributed to the presence of circulating myocardial depressant factors and, more recently, to direct effects of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 [141]. The pattern of myocardial depression is characterized by left ventricular dilation, decreased left ventricular ejection fraction, and a normal or increased cardiac index [136]. Acute ventricular dilation within the first days of septic shock is more frequently observed among survivors, and this is believed to represent compensatory cardiac dilation to maintain stroke volume despite the loss of myocardial contractility [136].

In preterm PROM and intra-amniotic infection, human fetal echocardiographic studies have also reported evidence of cardiac diastolic dysfunction consistent with increased left ventricular compliance, when compared to those with uncomplicated pregnancies [142]. It is thought that these changes reflect a compensatory mechanism similar to that observed in adults with sepsis.

Another investigation has shown impairment in both fetal diastolic and systolic cardiac performance of the right ventricle through tissue Doppler and strain rate imaging in fetuses with preterm PROM and proven intra-amniotic infection [143]. These observations are consistent with the study of Yanowitz et al., who found that umbilical cord IL-6 concentrations inversely correlated with systolic, mean, and diastolic blood pressure, and that the presence of funisitis was also associated with changes in right ventricular cardiac output, consistent with lower systemic vascular resistance [144]. These hemodynamic abnormalities are thought to predispose infants to brain injury, thereby buttressing the case for the importance of multisystem organ involvement in the pathophysiology of neonatal complications in the context of FIRS.

Cardiotocography of fetuses exposed to intra-amniotic inflammation (defined as histologic chorioamnionitis) has shown an increased fetal heart baseline, a higher number of low variation episodes, and a higher number of short-term variations [145].

Several experimental studies conducted in mice, pregnant sheep, and rhesus macaques have shown that intra-amniotic administration of endotoxin or *Candida albicans* leads to functional abnormalities in the fetal heart and a change in the gene regulatory networks that program cardiac development [146–151]. Specifically, in non-human primates, intra-amniotic administration of group B streptococcus or *E. coli* led to increased concentrations of IL-6 and IL-8 in the fetal myocardium without significant evidence of histopathologic inflammation of the heart [151]. This was associated with changes in the gene set involving cardiac morphogenesis and vasculogenesis. This observation is important given that epidemiologic studies have shown a 17-fold increased risk of heart failure after preterm birth. Moreover, adults who were born prematurely have exhibited substantial changes in myocardial structure and function [152]. Thus, exposure to intra-amniotic inflammation can explain, at least in part, the predisposition of preterm neonates to develop long-term cardiovascular disorders and, specifically, heart failure [153–156].

6.8. Gut

The fetal intestines can be exposed to microorganisms and inflammatory mediators after amniotic fluid is swallowed. FIRS is a risk factor for necrotizing enterocolitis [23,157]. A systematic review and meta-analysis, inclusive of 22,601 patients from 12 studies, demonstrated that clinical chorioamnionitis (OR 1.24; 95% CI: 1.01–1.52) and histological chorioamnionitis with acute funisitis (OR 3.29; 95% CI: 1.87–5.78) were significantly associated with necrotizing enterocolitis [157]. The mechanism for these associations is thought to involve impaired gut barrier function and immune dysfunction [158].

Experimental evidence suggests that immaturity and prenatal inflammation alters maturation of the fetal intestine [49,159–162]. In a sheep model, Wolfs et al. reported that administration of intra-amniotic endotoxin prevented maturation of tight junctions in intestinal epithelial cells [159]. Abnormal tight junctional distribution predisposes to easy access of microorganisms and toxins to the mucosa and inner layers of the gut pre- and post-natally.

In a recent study, intraperitoneal administration of bacterial endotoxin to pregnant rats increased the frequency of necrotizing enterocolitis and neonatal mortality [163]. The proposed mechanism for this observation was fetal TNF- α -mediated impairment of intestinal microvasculature, which was associated with a decrease in vascular endothelial growth factor (VEGF) and VEGF receptor 2 protein expression [163]. This was ameliorated by a hypoxia-inducible factor-1 α (HIF-1 α) stabilizing agent (HIF-1 α is a master regulator of VEGF function), and was abrogated by neutralizing TNF- α activity. This body of work indicates that prenatal inflammation increases the risk for necrotizing enterocolitis and that therapeutic approaches to maintain VEGF function may help prevent this complication.

Meconium-stained amniotic fluid, a common phenomenon (1 in 7 pregnancies), has traditionally been attributed to hypoxia [164]. Interestingly, such fluid frequently contains bacteria [165–167], endotoxin [167,168], and higher concentrations of inflammatory mediators, such as IL-1 β , TNF- α [169], IL-8 [169,170], and phospholipase-A2. We have proposed that meconium passage *in utero*, in some cases, may represent accelerated bowel transit (i.e., fetal diarrhea). Most of the time, meconium-stained amniotic fluid is a benign finding and is not associated with adverse neonatal outcome. However, meconium aspiration syndrome is a serious neonatal complication that develops in 5% of cases having meconium-stained amniotic fluid.

To explore why some infants with meconium-stained amniotic fluid develop this syndrome and others do not, we examined the role of fetal systemic inflammation. We reported that the combination of meconium-stained amniotic fluid and FIRS had a higher frequency of meconium aspiration syndrome than those without FIRS (Fig. 5) [164]. Thus, we proposed a chain of events in that meconium (with its proinflammatory properties), when aspirated before birth and combined with a fetal systemic inflammatory response involving the fetal lungs, can predispose to meconium aspiration syndrome [164,171,172]. In addition, elevated CRP concentrations, and low white blood cell and neutrophil counts in neonatal blood are associated with the severity of meconium aspiration syndrome during the early phases of the disease [171].

6.9. Liver

The liver is exposed to exogenous agents that reach the fetus either directly from the placenta (via the umbilical vein) or from the amniotic fluid, which is swallowed and resorbed in the intestine (via the portal vessels). Kupffer cells are macrophages lining the hepatic sinusoids, which are capable of recognition and phagocytosis of a wide range of bacteria as well as immune complexes [173]. Kupffer cells are able to produce cytokines (IL-6, IL-1 β , TNF- α) [174,175], which can modify fetal hematopoiesis. During fetal development, hematopoiesis occurs mainly in the fetal liver, followed by localization to the bone marrow. Fetuses with funisitis exhibit a significant increase in fetal hepatic hematopoiesis and myelopoiesis during the second trimester [176–178], indicating that the fetal response to intra-amniotic infection involves the liver.

Experimental intra-amniotic inflammation induced by bacterial endotoxin in sheep fetuses led to hepatic inflammation and liver dysfunction in the neonatal period. Specifically, there was an increase in the number of CD3⁺ T lymphocytes as well as in IL-1 β and TNF α .

mRNA in the fetal liver [179]. In addition, fetal hepatic inflammation was associated with metabolic disturbances, including increased umbilical cord plasma concentrations of total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides [179]. Two weeks after endotoxin injection, inflammation was still detected in the fetal liver, and there were higher glucose concentrations in the blood [179]. In a subsequent study focusing on the effects of bacterial endotoxin exposure during fetal life, Vlassaks et al. reported that liver triglycerides and plasma cholesterol concentrations were increased in LPS-exposed lambs compared to controls even though hepatic inflammation had resolved [180]. There was also evidence of increased lipid peroxidation, leptin receptor mRNA expression, and expression of cytochrome c oxidase subunit 4. This represents the first evidence that fetal inflammation has an effect on the liver and lipid metabolism. Whether this has long-term consequences by increasing the susceptibility to hepatic inflammation and insulin resistance is yet to be determined.

6.10. Kidneys

The fetal kidney is a potential target organ during FIRS [181–183]. Children and adult patients with sepsis can experience oliguria secondary to pre-renal failure (i.e., hypoperfusion) [184]. Since fetal urine is the main source of amniotic fluid, several investigators have examined the relationship between amniotic fluid volume and intra-amniotic and fetal inflammation. Yoon et al. reported that oligohydramnios in women with preterm PROM was associated with higher IL-6 concentrations in umbilical cord plasma at birth, higher concentrations of amniotic fluid proinflammatory cytokines (IL-6, IL-1 β , and TNF- α), and a higher frequency of clinical and acute histologic chorioamnionitis compared to those without oligohydramnios [182]. Similarly, Lee et al. reported that patients with intra-amniotic infection and inflammation had a higher frequency of oligohydramnios than those with a negative amniotic fluid culture [183]. Carroll et al. reported that fetuses with bacteremia diagnosed by cordocentesis had a higher frequency of oligohydramnios than in those with a negative fetal blood culture [181]. Taken together, the data suggest that oligohydramnios is associated with intra-amniotic inflammation and FIRS.

In the context of preterm PROM, reduced amniotic fluid volume may be partly attributed to the loss of amniotic fluid through the site of membrane rupture. However, it is possible that fetal urine production is altered in FIRS, and this can result from redistribution of blood flow away from the kidneys to ensure adequate blood flow to critical organs and increase efficiency of oxygen utilization [185,186]. It is interesting that neonates delivered to mothers with intra-amniotic inflammation have higher serum blood urea nitrogen concentrations and that such concentrations correlate with amniotic fluid IL-6 [187].

In a murine model, maternal intraperitoneal endotoxin injection induced fetal leukocyte activation, recruitment, and infiltration of the fetal renal parenchyma [188]. Moreover, fetal sheep exposed to intra-amniotic endotoxin have a reduction in nephron numbers by 23% as well as low glomerular density [189]. Evidence of renal injury has also been reported in preterm pigs after intra-amniotic injection of endotoxin, which induced an altered postnatal renal biochemical profile (i.e., increased concentration of urinary microalbumin, microalbumin: creatinine ratio, and sodium) [49]. In summary, microbial products and

inflammatory mediators in the amniotic fluid may predispose preterm fetuses to impaired renal function during the postnatal period and increased risk of hypertension and renal dysfunction in later life [190, 191].

6.11. Brain

A considerable body of evidence supports the concept that fetal systemic inflammation and often infection is causally related to fetal/neonatal neuroinflammation, brain injury, and neurodevelopmental disorders, including mental illness [192–197].

In 1955, Eastman and DeLeon reported that intrapartum maternal fever (often from intraamniotic infection with bacteria) conferred a seven-fold increased risk for cerebral palsy [198]. Subsequently, a solid body of evidence has accumulated over the last six decades showing that inflammation and infection play a role in the genesis of brain injury. In 1971, Leviton and Gilles [199] reported on the characteristics of *perinatal telencephalic leucoencephalopathy* (white matter abnormalities in the fetal brain after midgestation) and that there was a potential link to neonatal bacteremia and endotoxin exposure [200]. This original concept has gained considerable support and its evolution is described in another article in this issue [201]. Other articles in this issue describe the etiology, mechanisms of brain injury, short- and long-term neurologic outcome after fetal exposure to inflammation, and potential interventions to downregulate the inflammatory response [202–205]. The work of Nelson has supported the concept that maternal infection plays a role in the genesis of cerebral palsy in infants born at or close to term [206–208]. Other articles in this issue of *Seminars in Fetal and Neonatal Medicine* describe FIRS and its manifestations in term or late preterm gestation. Therefore, the role of intra-amniotic neuroinflammation does not seem to be limited to preterm neonates, a concept that is important given that most cases of cerebral palsy derive from infants born at term.

Briefly, the evidence in support of this concept indicates that 1) proven intra-amniotic infection is present in at least 25% of all preterm deliveries [209–215] and in 61% of patients with clinical chorioamnionitis at term [216]; 2) a fetal inflammatory response (diagnosed by the presence of funisitis, elevated concentrations of IL-6 or C-reactive protein in umbilical cord blood) is present in a large fraction of patients with intra-amniotic infection [9,217,218]; 3) there is a strong association between intra-amniotic infection/inflammation and the subsequent development of white matter lesions of the neonatal brain [219–237]; 4) a fetal inflammatory response (diagnosed by funisitis) increases the risk of both periventricular leukomalacia and cerebral palsy [9,20,192–195,238–262]; 5) intrauterine infection with bacteria or intra-amniotic administration of LPS can induce a fetal systemic inflammatory response, neuroinflammation, and lesions resembling periventricular leukomalacia with gliosis and neuronal injury [193, 223–225,228,263–265], and this neuroinflammatory process can be observed in animals delivered preterm as well as in those who delivered at term [233,266]; 6) intrauterine administration of bacterial endotoxin has been used to generate an animal model of cerebral palsy, in which there is neuroinflammation [267,268]; and 7) down-regulation of fetal neuroinflammation with the administration of anti-inflammatory agents (e.g. N-acetyl-cysteine) or stem cell-related

products can reverse the neuroinflammatory process and the phenotype in animals [269–278].

The relative contributions of fetal inflammation and postnatal inflammation have been the subject of investigation. All available evidence suggests that both play a role and that sustained inflammation is particularly important.

Frontiers for clinical and experimental research are the non-invasive detection of fetal neuroinflammation in the early postnatal period, followed by treatment to prevent the sequelae. There are reasons to believe that both goals can be met with the use of molecular imaging and nanotechnology and cell-based therapies. A comprehensive discussion of the subject of neuroinflammation is available in other articles in this special issue.

6.12. Eyes

Approximately 2% of preterm birth survivors are diagnosed with blindness, and visual acuity is impaired compared to those born at term [279–282]. Moreover, among adolescents who were born preterm, 50% have vision-related problems [283]. These disorders have been traditionally attributed to either macular sequelae from severe retinopathy of prematurity (ROP) or cerebral visual impairment. Recent evidence suggests that ROP may be part of a spectrum that includes both disorders of the retina and the cerebrovascular interface [284]. ROP is considered to result from an arrest of neural and vascular development. Indeed, children affected with ROP are more likely to have cognitive and psychomotor development disorders at two years of age [284].

ROP is more frequent in infants born to mothers with histologic or clinical chorioamnionitis than in those without exposure to antenatal inflammation [229,285–290]. A recent systematic review and meta-analysis, which included 50 studies (38,986 infants and 9258 chorioamnionitis cases), showed a significant association between the presence of chorioamnionitis and any ROP stage (OR 1.39; 95% CI: 1.11 to 1.74), as well as severe ROP (stage 3) (OR 1.63; 95% CI: 1.41 to 1.89) [291]. The risk of severe ROP was substantially increased when histologic chorioamnionitis co-occurred with birth <29 weeks [287]. Of particular interest, infants who had a fetal inflammatory response (diagnosed by funisitis) had a higher risk of ROP than those with acute histologic chorioamnionitis in the absence of funisitis [291].

Further evidence of an association between perinatal inflammation and ROP derives from studies examining cytokine concentrations in neonatal and umbilical cord blood. For example, Sood et al. studied 877 infants and reported an association between ROP and cytokine concentrations measured serially in the first three weeks of life. Preterm neonates with ROP had higher concentrations of IL-6 and lower concentrations of IL-17 and IL-18 in the first three days of life [292]. Subsequently, such findings were confirmed, as serum umbilical cord concentrations of cytokines (IL-7, monocyte chemoattractant protein-1, macrophage inflammatory protein 1 α , and macrophage inflammatory protein 1 β) were significantly higher in preterm neonates who developed ROP vs. healthy preterm neonates without ROP [293]. Recently, Park et al. reported that an elevated umbilical cord plasma

concentration of IL-6 (>6.5 pg/mL) was an independent risk factor of severe ROP in a cohort of 110 preterm neonates [22].

There is also an association between ROP and neonatal sepsis. A meta-analysis including 16 studies (12,466 premature infants and 2494 cases of ROP) showed that neonatal sepsis was closely related to any stage of ROP (OR 1.57; 95% CI: 1.31 to 1.89) and severe stages of ROP (OR 2.33; 95% CI: 1.21 to 4.51) in premature infants [294]. Collectively, these results indicate that fetal systemic inflammation is associated with an increased risk of ROP.

6.13. Ears

Sensorineuronal hearing loss is more frequent in infants born preterm vs. term, and the earlier the gestational age at birth, the greater the risk. The proposed mechanisms for the increased risk of hearing loss have largely focused on postnatal factors, such as immaturity, administration of ototoxic medications (e.g., aminoglycosides, diuretics), environmental noise, hyperbilirubinemia, and hypoxia [295–298]. However, the role of congenital factors has been known since 1897 when Aschoff described the concept of *otitis media neonatorum*, which was thought to be a sterile middle ear inflammation related to aspiration of amniotic fluid cellular content based on microscopic study of the temporal bone in fetuses and neonates [299]. Subsequent studies have shown that the middle ear may contain both meconium and purulent material in cases of fetal death in patients with histologic chorioamnionitis [300]. Thus, intra-amniotic inflammation and infection has been implicated as a cause of otitis media for decades. This has been invoked as a cause of chronic otitis media and hearing loss. In addition, otitis media has been implicated as a cause of meningitis in the neonatal period [301,302].

An interest in this subject has been rekindled in recent years. Microbial invasion or inflammation of the middle ear in patients with intra-amniotic inflammation/infection, neuroinflammation, or a combination of both may lead to hearing loss. Experimental studies in guinea pigs have also shown that fluorescent bacterial endotoxin injected into the middle ear can reach the cochlea of the inner ear [303].

Investigators have examined whether exposure to antenatal inflammation is associated with an increased risk of hearing loss. When FIRS is defined as the presence of fetal vasculitis based on placental examination and/or umbilical cord serum IL-6 (>18.2 pg/mL), it was associated with an increased risk for hearing screen failure in preterm neonates (OR 3.6; 95% CI: 1.38 to 9.5) [297]. Moreover, Shim et al. reported that elevated umbilical cord plasma IL-6 and high CRP concentrations in the immediate neonatal period were significantly associated with neonatal hearing screening test failure in a cohort of 127 premature infants [304]. These results suggest that a fetal inflammatory response may increase the risk of hearing loss.

6.14. Skin

In the setting of intra-amniotic inflammation/infection, microorganisms and their inflammatory mediators are in direct contact with the fetal skin, an important part of the innate immune system. A newly described entity, *congenital fetal dermatitis*, has been observed in the context of intra-amniotic infection [305]. We found that the skin

of fetuses exposed to intra-amniotic infection had the typical findings of dermatitis: an inflammatory infiltrate composed mainly of CD15⁺ neutrophils, CD3⁺ T lymphocytes, and CD68⁺ histiocytes; the overexpression of cytokines (such as IL-1 α , TNF- α , and IL-8) and antimicrobial peptides (human defensins 2 and 3); and the overexpression of pattern recognition receptors, such as TLR-2, in the epidermis [305].

Experimental studies in an ovine pregnancy model have shown that exposure of the fetal skin to bacterial endotoxin results in the increased expression of IL-1 β , IL-6, IL-8, and TNF- α [306]. On the other hand, intra-amniotic administration of *U. parvum* serovar 3 in an ovine pregnancy induced the increased expression of MCP-1, but not IL-1 β , IL-6, IL-8, or TNF- α , in fetal skin [307]. Dermatitis is a local fetal response to microbial products or inflammatory mediators. The long-term effects of congenital dermatitis on the skin's health and disease require further study.

7. Diagnosis of FIRS

The initial description of FIRS was based on an elevated concentration of IL-6 in the fetal blood obtained by cordocentesis. A cut-off value of IL-6 >11 pg/mL was used, as this was associated with severe perinatal morbidity [9]. The population subject to study included patients with preterm labor and preterm PROM, which are conditions frequently associated with intra-amniotic infection and inflammation. Nevertheless, funisitis is the histologic counterpart of elevated IL-6 levels [323–325].

Several factors need to be considered in defining FIRS when using the initial observations of other studies. IL-6 was quantitated using an enzyme-linked immunoassay developed for research purposes. At the time of our report, an international standard to calibrate immunoassays was not widely available. However, such a standard is now available from the National Institute of Biological Standards and Control of the U. K., called the WHO First International Standard for IL-6; details of this standard are available at <https://www.nibsc.org> [308]. Investigators should be aware of the need to calibrate the assays to be able to compare results among laboratories and studies.

The concentration of IL-6 in fetal blood appears to change with advancing gestational age [309,310] and with the presence or absence of labor [311–316]. Therefore, consideration should be given to the original findings that were based on preterm gestations and that the results and cutoff values may differ if sampling occurs after labor (i.e., umbilical cord blood obtained after spontaneous labor).

Many studies have used multiplex assays to measure several cytokines simultaneously. However, these are not the typical platforms used to quantitate analytes in clinical medicine. Most assays are targeted toward a particular protein. Therefore, it is important that studies report performance characteristics of assays, including sensitivity, cross-reactivity, inter- and intra-assay coefficients of variation, and the method of calibration. This will aid in meaningful comparison of results. Commercially available assays for clinical testing are now available.

The field of cytokine measurements has matured over the last several decades, and the time is now appropriate to undertake systematic determination to define normal ranges, not only for IL-6 but for other analytes that could be used to assess the cytokine profile at the time of birth.

C-reactive protein (CRP), an acute phase reactant widely used to detect the presence of systemic inflammation, is synthesized by the liver in response to IL-6. Given that assays for IL-6 have not been widely available, several investigators have used umbilical cord concentrations of CRP to identify FIRS because such assays are largely available in most hospitals [217,317]. CRP can be measured by ELISA (enzyme-linked immunosorbent assay), immunoturbidimetry, or antibody-based nephelometric assays. High-sensitivity assays for CRP that allow detection in the range of 0.5–10 mg/L are now available and have been used to assess the risk of cardiovascular disease. An international standard for human CRP is available (i.e. WHO 1st International Standard). A strong correlation exists between the umbilical cord plasma concentrations of IL-6 and CRP [217], and CRP determinations from umbilical cord blood (obtained for the purposes of blood and Rh typing) could allow an assessment of fetal systemic inflammation without venipuncture of the neonate. The diagnostic performance of umbilical cord plasma concentrations of IL-6 and CRP in the identification of funisitis, neonatal sepsis, and intra-amniotic infection are displayed in Table 2 [217]. The relative diagnostic performance of CRP, IL-6, and other analytes (e.g., procalcitonin) to identify neonatal sepsis has been the subject of multiple studies [318–320].

It is now clear that neonates can evince elevated concentrations of IL-6 and CRP yet show no evidence of funisitis, and the reverse is also true.

An elevation in proinflammatory cytokines or acute phase reactants may result from fetal inflammation unrelated to intra-amniotic inflammation, such as in cases of fetuses with anemia from alloimmunization [321] or maternal autoimmune disorders [322]. Given that such pathologic processes are unrelated to intra-amniotic inflammation, there is no chemotactic gradient in the amniotic cavity that would bring neutrophils from the fetal blood into the walls of the umbilical arteries and vein. The explanation for the absence of an elevated IL-6 or CRP in cases of funisitis is less clear at this time.

8. Funisitis and chorionic vasculitis: pathologic hallmarks of FIRS

Inflammation of the umbilical vessels (*acute funisitis*) and/or inflammation of the chorionic vessels (*chorionic vasculitis*) strongly correlate with a fetal plasma IL-6 concentration >11 pg/mL [323–325] and, therefore, are considered the histological counterparts of FIRS. Funisitis is characterized by the presence of fetal neutrophils infiltrating into the umbilical vein wall (*stage 1*) and one or both umbilical artery walls (*stage 2*) with or without subsequent invasion into the Wharton's jelly [325]. Necrotizing funisitis (*stage 3*) is distinguished by the presence of fetal neutrophils and/or cellular debris in a concentric ring around at least one of the umbilical vessels [325,326]. Chorionic vasculitis represents inflammation of the vessels on the surface of the chorionic plate of the placenta [325]. More advanced stages of funisitis are associated with a higher concentration of IL-6 or CRP in umbilical cord blood [327] (Fig. 6).

The term “acute placental inflammation” is often used in discussions about FIRS. It is noteworthy that acute histologic chorioamnionitis is a maternal inflammatory response while funisitis and chorionic vasculitis are fetal inflammatory responses. Therefore, neutrophil inflammation of the extraplacental membranes or the chorionic plate, which is of maternal origin, should not be misconstrued to represent evidence of fetal inflammation.

The availability of a placenta to make a histologic diagnosis of FIRS is unique, as there is no equivalent for children and adults. Studies in which the umbilical cord has been serially sectioned at 1 mm intervals suggest that the process begins as multiple discrete foci which then merge as the inflammatory process progresses (Fig. 7) [328].

9. FIRS, cytokine storm, cytokine release syndrome, and the macrophage-like activation syndrome

The current literature in sepsis uses the terms *cytokine storm*, *cytokine release syndrome*, *macrophage-like activation syndrome*, and *hemophagocytic syndrome* to refer to conditions in which hyperinflammation is responsible for morbidity and mortality in the context of infection or tissue injury. These terms denote an enhanced response of the innate immune system, generally diagnosed by increased concentrations of inflammatory mediators such as IL-6, IL-1 β , TNF- α , and chemokines (e.g. MCP-1).

The term *cytokine storm* was originally introduced in the context of graft vs. host disease [329]; *cytokine release syndrome* was first used to describe changes in the concentration of cytokines in response to the administration of an anti-CD3 antibody (CD3 is a molecule expressed by T cells) [330,331]. The term *hemophagocytic lymphohistiocytosis* was employed to describe a hyperinflammatory state which could be familial (from mutations in the genes encoding for porphyrin or proteins required for the docking and fusion of granules in NK cells). The term *macrophage-like activation syndrome* overlaps with secondary hemophagocytic lymphohistiocytosis. It is interesting that these syndromes were identified often in the context of non-infectious conditions, such as cancer. However, these syndromes overlap with SIRS.

The phrase *cytokine storm* is often used to imply that there is dysregulation of the inflammatory response and that the inflammatory mediators could injure host cells and cause multi-organ dysfunction [332]. However, as of this writing, there is not a good way to differentiate an appropriate from a dysregulated inflammatory response. We envision a need to characterize the cellular and soluble profiles of pro- and anti-inflammatory mediators in umbilical cord blood to determine the level of the immune system at the time of birth, and to examine the clinical significance of these findings in terms of short- and long-term morbidity.

10. Transcriptome and proteome analyses in FIRS

The transcriptome of umbilical cord leukocytes obtained from patients with FIRS (elevated umbilical cord IL-6 concentrations and funisitis) showed a dramatic upregulation of 296 genes and downregulation of 252 genes based on microarray analysis (Fig. 8) [333].

Differential gene expression in cord blood RNA with and without FIRS is displayed in Table 3 and Fig. 9. Ontological and pathway analyses showed the significant enrichment of biological processes, including antigen processing and presentation, immune response, and process critical to cellular metabolism. We compared the transcriptomes of FIRS, those of patients with pediatric sepsis, and volunteers with experimental endotoxin administration. The main observation was the remarkable similarity between FIRS and SIRS, despite the differences in maturity of the immune response. Our findings showed that in addition to the pro-inflammatory response, there was activation of the anti-inflammatory response (increased concentration of IL-10). Increased concentrations of IL-6, IL-8, IL-10, TNF- α , sIL-2Ra, CXCL-10, and MCP-1 (also known as CCL2) were observed in the serum of neonates born with FIRS [333].

11. Heterogeneity of FIRS: types I and II

In the course of studying the mechanisms responsible for preterm labor, we identified a cluster of patients who had chronic inflammatory lesions of the placenta (i.e., chronic chorioamnionitis, villitis of unknown etiology, chronic deciduitis) rather than acute inflammatory lesions (e.g., acute chorioamnionitis, funisitis). These lesions were associated with a unique fetal systemic inflammatory response, which was characterized by the increased concentration of the cytokine CXCL-10 (a T-cell chemokine) rather than IL-6. We proposed that such placental lesions were related to *maternal anti-fetal rejection* rather than intra-amniotic infection/inflammation [334–341]. The umbilical cord transcriptome of these fetuses differed from that previously described in fetuses born to mothers with intra-amniotic infection/inflammation (*FIRS Type I*), consistent with the presence of an alloimmune reaction (Fig. 10). The serum proteome of umbilical cord blood was also different [340]. These findings suggest that FIRS may be present in patients without intra-amniotic infection/inflammation; hence, we proposed the term *FIRS Type II* [340]. Future investigation will determine the short- and long-term significance of this novel form of fetal systemic inflammation.

12. Strategies for the management of FIRS

Several approaches can be used to manage the course of FIRS in the context of intra-amniotic inflammation: delivery of the fetus, antibiotic therapy, and immunomodulation of the inflammatory response.

Delivery of the fetus provides a rapid exit from a hostile environment. However, preterm delivery places the unborn child at risk for complications of prematurity. Therefore, the risks of prematurity and intra-amniotic infection/inflammation must be balanced. Antibiotic treatment can reduce the rate of intra-amniotic infection/inflammation, as well as funisitis in a subset of patients with spontaneous preterm delivery with intact membranes [342], cervical insufficiency [343], short cervix [344], and preterm PROM [345–347].

The use of immunomodulatory approaches in FIRS needs to be considered given recent successful approaches with the treatment of SARS-CoV-2, the virus responsible for COVID-19 (Coronavirus disease-19) [348–354]. The success of IL-1 blockade with high-

dose anakinra and the blockade of the IL-6 pathway with a recombinant humanized monoclonal antibody suggests that these agents might be useful to manage hyperinflammation in the perinatal period. The subject has been reviewed by Xiong and Wintermark [355] in this issue, and recently by Schuller [356]. A frontier is whether these interventions can be extended to the prenatal period. Recent studies suggest (1) that an anti-IL-6 receptor antibody inhibits preterm labor and delivery in a bacterial endotoxin model [357] and (2) that a similar intervention targeting IL-1 may reduce inflammation-induced fetal injury and improve neonatal and developmental outcomes in mice after exposure to bacterial endotoxin during pregnancy [358].

A combination of antibiotics and immunomodulatory agents (dexamethasone and indomethacin) has been shown to be effective in nonhuman pregnant primates to eradicate intra-amniotic infection, suppress the local inflammatory response, and prolong gestation in experimental premature labor induced by intra-amniotic inoculation with group B streptococci [359]. In addition, we recently showed that clarithromycin prevents preterm birth and reduces neonatal mortality in mice intra-amniotically injected with *Ureaplasma parvum* [360]. Collectively, this evidence indicates that immunomodulation may be an effective intervention in preventing fetal injury and prolonging gestation among patients with inflammation/infection-induced preterm labor.

Given that cells of the immune system are derived from pluripotent hematopoietic stem cells, stem cells have emerged as a promising potential neuroprotective and neuro-reparative treatment in the context of the fetal inflammatory response. Many immunomodulatory cells are present in umbilical cord blood, including mesenchymal stem cells and endothelial progenitor cells. In a mouse model of bacterial endotoxin-induced chorioamnionitis, maternal administration of mesenchymal stem cells resulted in reduced IL-6 concentration in the fetal brain, and improved neurobehavioral outcomes [361]. Umbilical cord blood cells have some benefits that support their therapeutic use for infants after birth who are at high risk for brain injury following antenatal or birth complications [362–368].

12.1. Practice points

- Obstetricians should consider placental examination to determine whether preterm and term neonates have deployed a systemic inflammatory response, which can be identified by the presence of funisitis or chorionic vasculitis
- An inflammatory process in the chorioamniotic membranes (i.e., acute chorioamnionitis) in the absence of funisitis or chorionic vasculitis is a maternal inflammatory response and should not be misconstrued as evidence of a fetal immune response
- FIRS (defined as an elevation of cytokines in umbilical cord blood, presence of acute phase reactants, or severe funisitis) in preterm neonates is a risk factor for early neonatal sepsis, neuroinflammation, and chronic lung disease
- FIRS can result not only from intra-amniotic infection but also sterile intra-amniotic inflammation. Efforts should be directed to identify the causes of fetal

systemic inflammation before birth. This may require examination of amniotic fluid samples to ascertain the presence of microorganisms.

- Evidence from clinical and experimental studies suggest that antimicrobial agents can eradicate intra-amniotic inflammation and intra-amniotic infection. Early preliminary evidence indicates that this approach may also reduce the rate of fetal systemic inflammation.

12.2. Research agenda

- Determine the frequency and clinical significance of FIRS in each obstetrical syndrome leading to preterm birth, such as preterm labor with intact membranes, preterm PROM, pre-eclampsia, and fetal growth restriction
- Develop biomarkers for rapid and practical diagnosis of FIRS that would allow ascertainment of this condition at the time of birth in high, as well as medium and low resource settings (e.g., dipstick to identify if a newborn has systemic inflammation by examining umbilical cord blood)
- Test whether down-regulation of the fetal/neonatal inflammatory response with antimicrobial agents, anti-inflammatory agents, and cell-based therapies can improve the outcome of neonates and infants affected by FIRS

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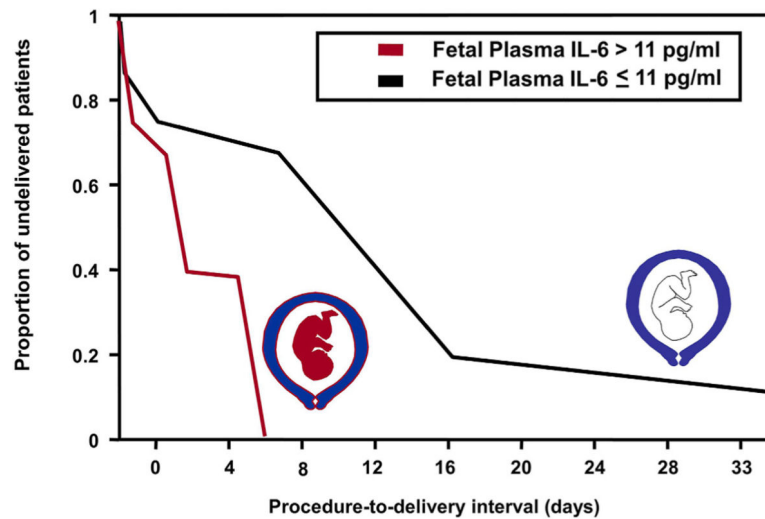


Fig. 1.

Fetuses with fetal inflammatory response syndrome (FIRS) have a shorter intrauterine stay than those without FIRS. Mothers were admitted with preterm premature rupture of membranes and patients were not in labor at admission. The interval between the procedure and delivery reflects duration of pregnancy and spontaneous onset of labor. Fetuses with fetal plasma IL-6 concentrations greater than 11 pg/mL have a shorter procedure-to-delivery interval than those with plasma IL-6 concentrations of 11 pg/mL or less (median 0.8 days [range 0.1–5 days] vs. median 6 days [range 0.2–33.6 days]; respectively; $P < 0.05$). (Modified with permission from Romero R, Gomez R, Ghezzi F et al.: A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. *Am J Obstet Gynecol* 1998; 179:186–193.).





		n	Procedure-to-Delivery Interval (median, range, days)
I	AF IL-6 \leq 7.9 ng/ml FP IL-6 \leq 11 pg/ml		14 5 (0.2-33.6)
II	AF IL-6 > 7.9 ng/ml FP IL-6 \leq 11 pg/ml		5 7 (1.5-32)
III	AF IL-6 \leq 7.9 ng/ml FP IL-6 > 11 pg/ml		6 1.2 (0.25-2)
IV	AF IL-6 > 7.9 ng/ml FP IL-6 > 11 pg/ml		5 0.75 (0.13-10)

Fig. 2.

Duration of pregnancy according to whether or not there is intraamniotic inflammation and fetal systemic inflammation (FIRS). The key determinant of pregnancy duration is fetal systemic inflammation regardless of the inflammatory status of the amniotic cavity, as reflected by the procedure-to-delivery interval. The inflammatory status of the amniotic cavity and the fetus was assessed with interleukin-6 (IL-6) concentrations. A white color fetus represents no inflammation [defined as fetal plasma (FP) IL-6 less than 11 pg/mL]. A red color fetus represents fetal systemic inflammation (FP IL-6 greater than 11 pg/mL). The amniotic fluid compartment is either white (no intraamniotic inflammation) or yellow in color (intraamniotic inflammation present). The cut-off value of 7.9 ng/ml was used to define intra-amniotic inflammation. The number of patients in each group is depicted (n). (Reproduced with permission from Romero R, Gomez R, Ghezzi F et al: A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. *Am J Obstet Gynecol* 179:186–193, 1998.).

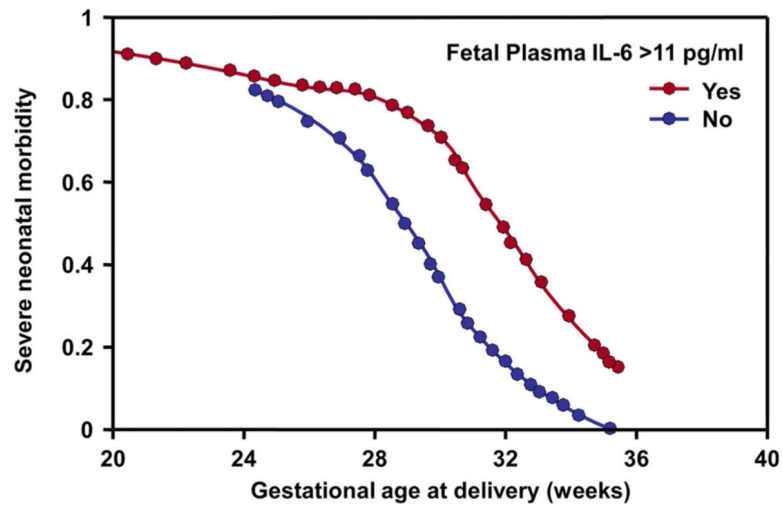


Fig. 3. Fetal inflammatory response syndrome (FIRS) was associated with a higher severe neonatal morbidity than the absence of FIRS. FIRS was defined as a fetal plasma IL-6 >11 pg/mL. This was calculated using logistic regression to adjust for gestational age and other covariates. (Reproduced and modified with permission from Gomez R, Romero R, Ghezzi F et al.: The fetal inflammatory response syndrome. *Am J Obstet Gynecol* 179:194–202, 1998.).

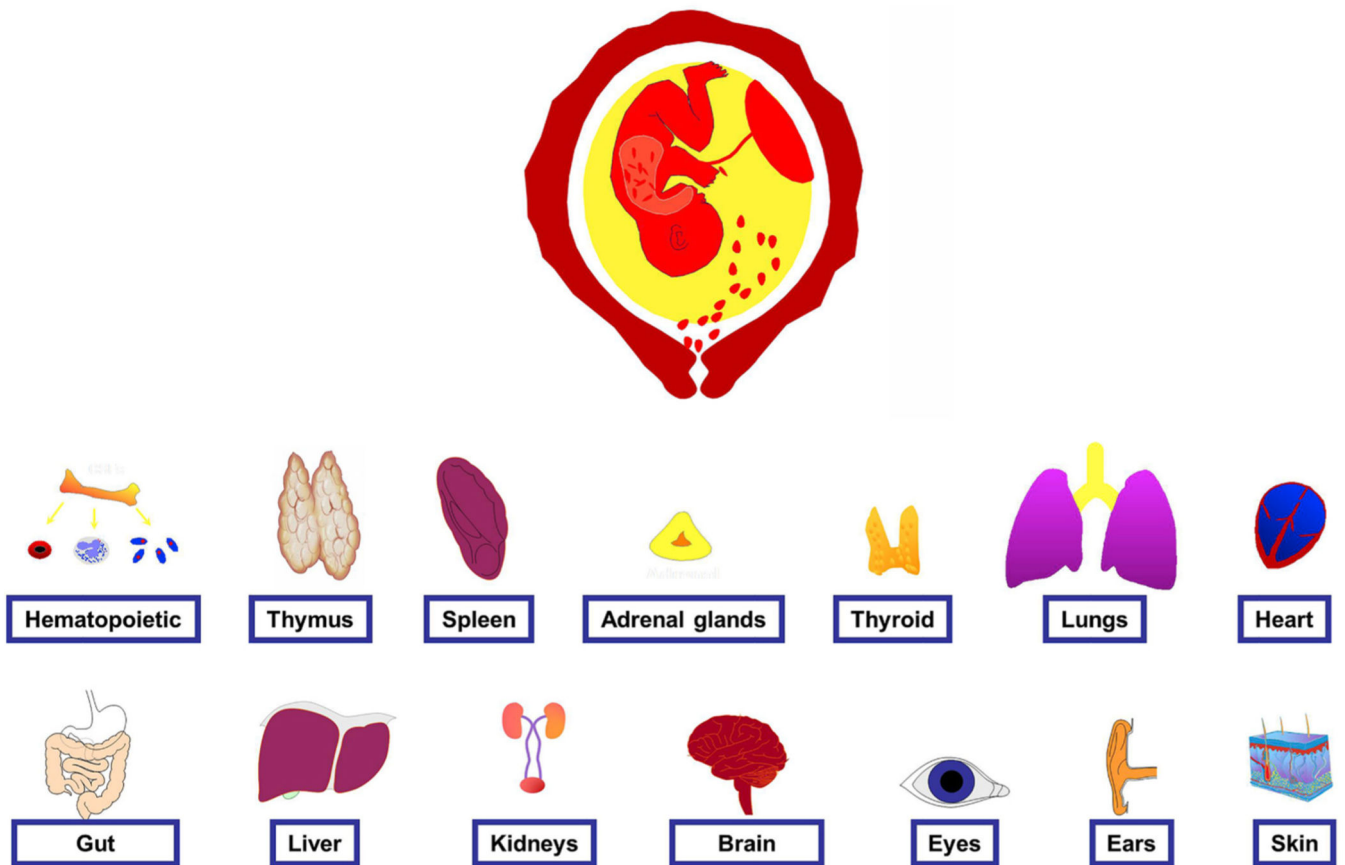


Fig. 4. The fetal inflammatory response syndrome (FIRS) is associated with multi-systemic involvement. Clinical and/or experimental evidence suggests that there is involvement of the organs displayed in the figure. The data to support this conclusion is reviewed in the article. (Modified with permission from Gotsch F, Romero R, Kusanovic JP, Mazaki-Tovi S, Pineles B, Erez O, Espinoza J, Hassan SS. Fetal Inflammatory Response Syndrome. *Clinical Obstetrics and Gynecology* 50:652–683; 2007).

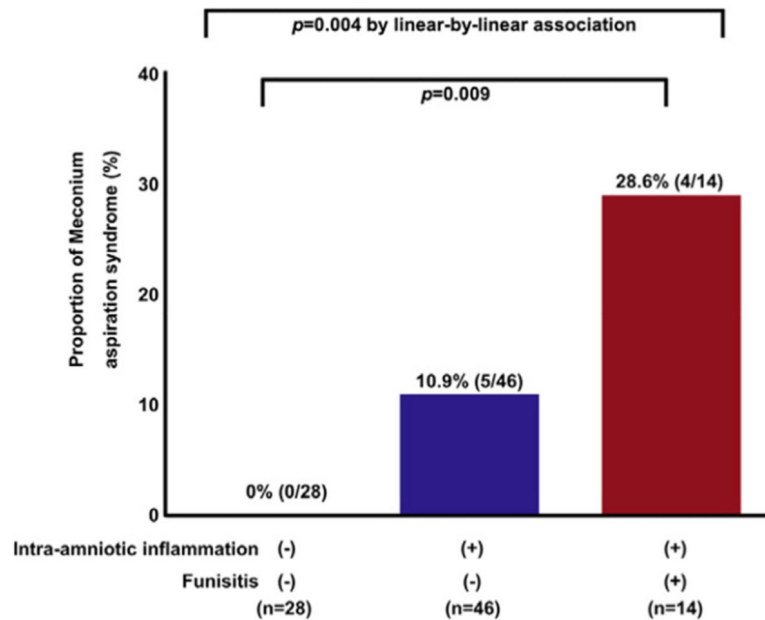


Fig. 5.

Meconium aspiration syndrome (MAS) is more likely to occur in patients with meconium-stained amniotic fluid if intraamniotic inflammation and fetal inflammatory response syndrome ascertained by funisitis are present. Frequency of MAS in the context of intraamniotic inflammation and funisitis. Neonates exposed to both intraamniotic inflammation and funisitis were at significantly greater risk of MAS than newborns exposed to neither of these 2 conditions [28.6% (4/14) vs 0% (0/28), $P = 0.009$]. In contrast, newborns exposed to only intraamniotic inflammation without funisitis were not at greater risk of MAS than newborns exposed to neither of these 2 conditions [10.9% (5/46) vs 0% (0/28); $P = 0.15$]. MAS did not occur in the absence of intraamniotic inflammation. (Reproduced with permission from Lee J, Romero R, Lee KA et al. Meconium aspiration syndrome: a role for fetal systemic inflammation. *Am J Obstet Gynecol* 214:366.e1–9, 2016.).

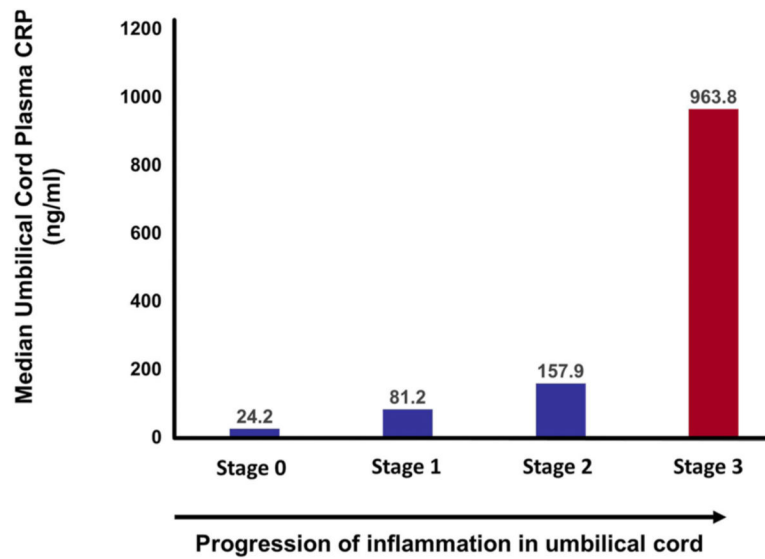


Fig. 6. The higher the umbilical cord plasma concentration of C-reactive protein (CRP), the more severe the inflammatory process in the umbilical cord, assessed by the severity of funisitis by stage. (Reproduced with permission from Oh JW, Park CW, Moon KC et al. The relationship among the progression of inflammation in umbilical cord, fetal inflammatory response, early-onset neonatal sepsis, and chorioamnionitis. *PLoS One* 14:e0225328, 2019.).

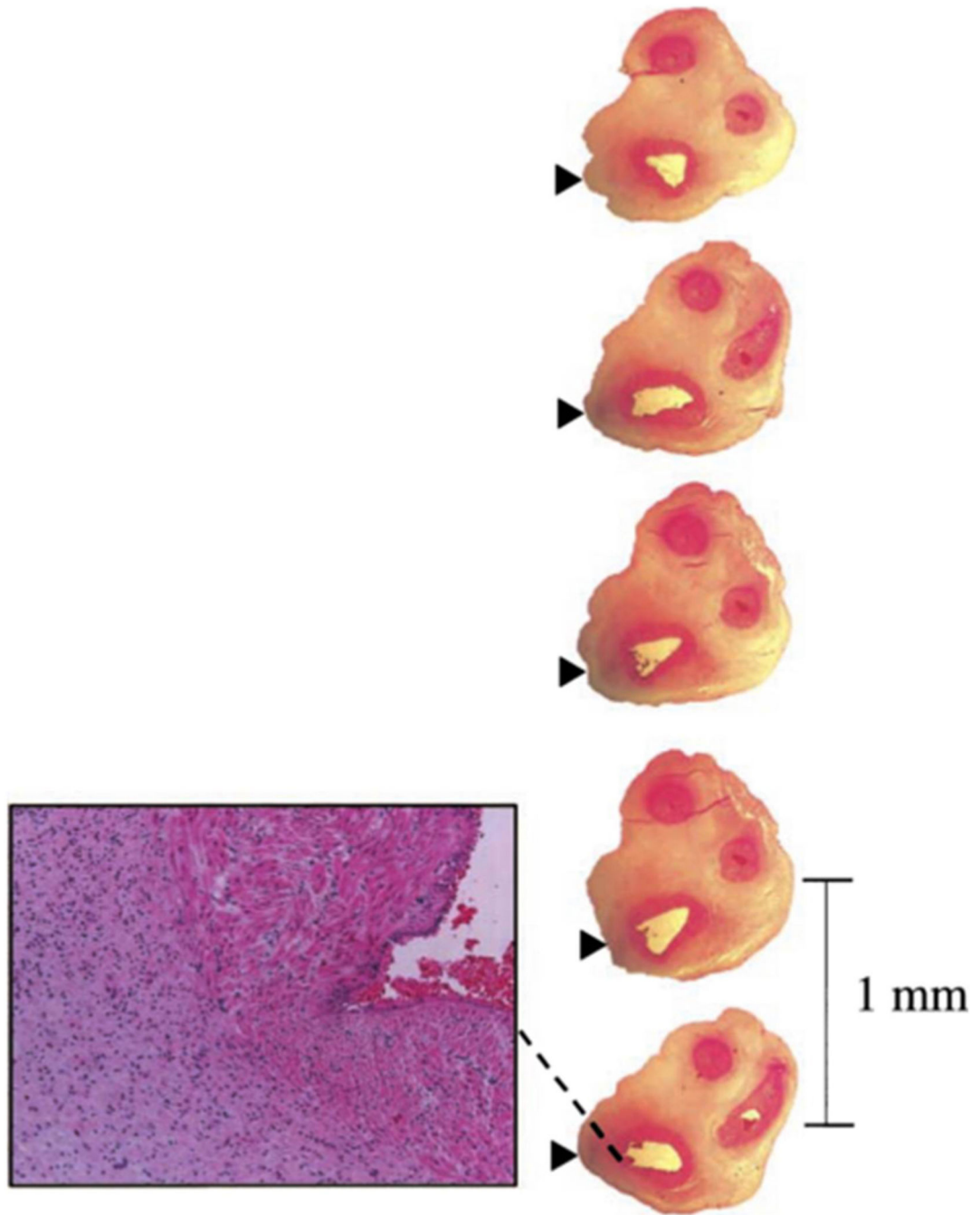


Fig. 7.

Funisitis is inflammation of the umbilical vein, either or both umbilical arteries, and the Wharton's jelly. Serial sections of the umbilical cord were taken at 1 mm intervals and showed neutrophil infiltration of the umbilical vein (dashed line). (Reproduced with permission from Kim CJ, Yoon BH, Kim M et al. Histo-topographic distribution of acute inflammation of the human umbilical cord. *Pathol Int* 51:861–5, 2001.).

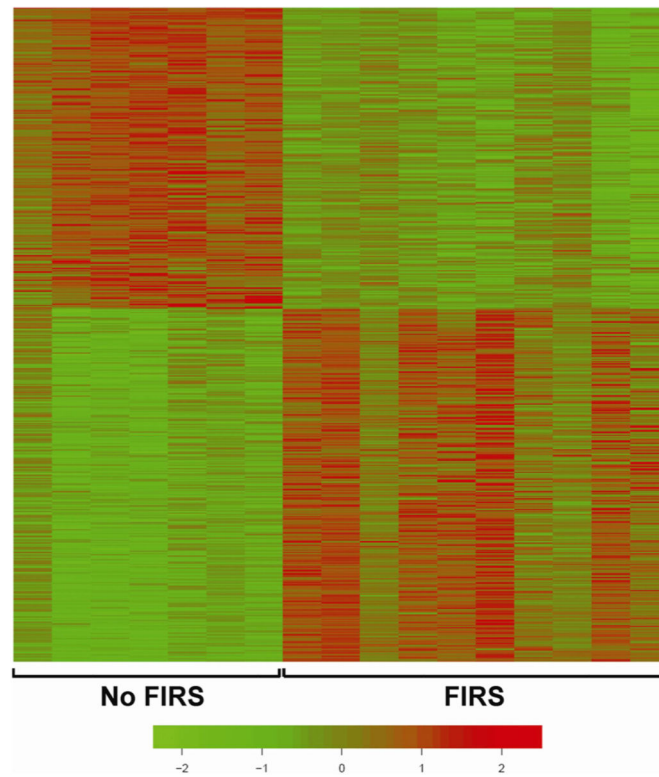


Fig. 8. The peripheral blood transcriptome of fetuses with fetal inflammatory response syndrome (FIRS) is dramatically different from that of fetuses with-outlammatory response syndrome (FIRS). Similar results have been reported in patients with pediatric sepsis and after volunteers have been given bacterial endotoxin. Transcriptome analysis of white blood cells collected from the umbilical cord in patients with preterm labor and preterm prelabor rupture of membranes was performed. Red color depicts upregulation, while green color depicts downregulation. Displayed are the 296 genes that were differentially upregulated in leukocytes from neonates with FIRS and the 252 that were decreased (False discovery rate <0.05). (Reproduced with permission from Madsen-Bouterse SA, Romero R, Tarca AL et al. The transcriptome of the fetal inflammatory response syndrome. *Am J Reprod Immunol* 63:73–92, 2010.).

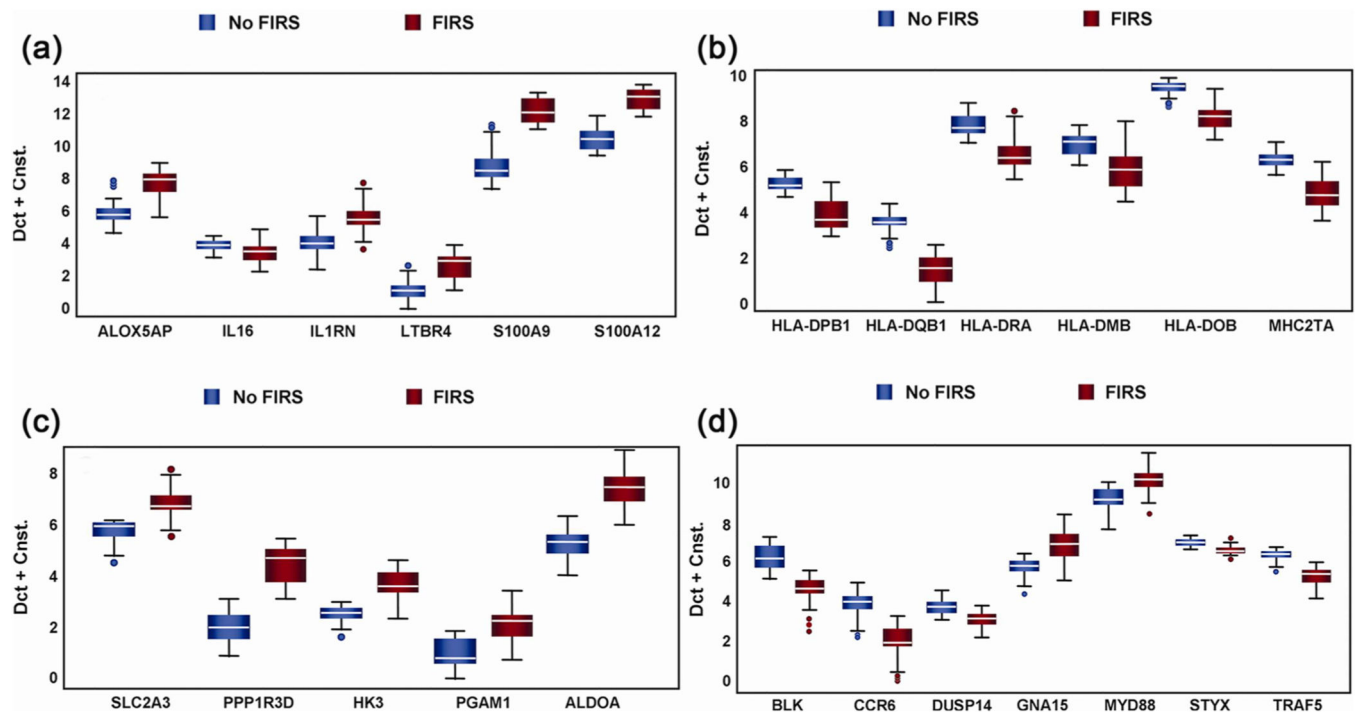


Fig. 9.

Differentially expressed genes in umbilical cord blood according to the presence or absence of fetal inflammatory response syndrome (FIRS). The data were generated by qRT-PCR. Genes were selected after the results of microarray analysis. Altered abundance of genes within ontological categories of immune response and inflammation (a), MHC II receptor activity (b), carbohydrate metabolism (c) and signal transduction (d) was confirmed using qRT-PCR. Box-plots include 50% of the data with the middle line showing the median value; $P < 0.05$ for all genes is shown when modeled using GEE. Depending on the statistical method used, 93–95% of the genes tested confirmed the change observed by microarray analysis. (Reproduced with permission from Madsen-Bouterse SA, Romero R, Tarca AL et al. The transcriptome of the fetal inflammatory response syndrome. *Am J Reprod Immunol* 63:73–92, 2010.).

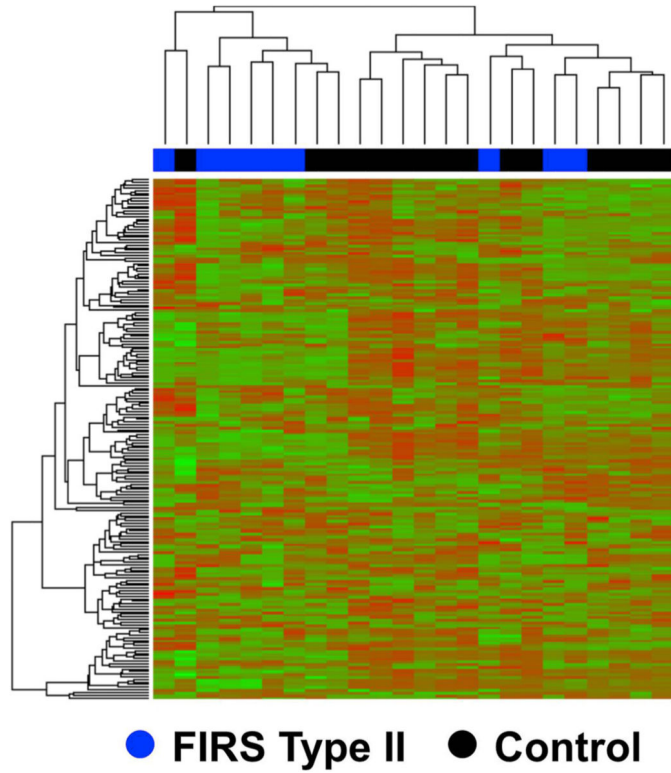


Fig. 10. Transcriptome analysis of fetal blood using whole genome DASL[®] assay according to the presence or absence of fetal inflammatory response syndrome (FIRS) type II that is associated with maternal anti-fetal rejection. A clustered heat map based on the top 200 differentially expressed genes displays two main clusters: one dominated by samples of the fetal inflammatory response associated with maternal anti-fetal rejection group (FIRS II) (left) and one dominated by samples in the control group (right). (Reproduced with permission from Lee J, Romero R, Chaiworapongsa T et al. Characterization of the fetal blood transcriptome and proteome in maternal anti-fetal rejection: evidence of a distinct and novel type of human fetal systemic inflammatory response. *Am J Reprod Immunol* 70:265–84, 2013.).

Table 1Criteria for systemic inflammatory response syndrome^a.

Two or more of the following criteria should be met:

- Temperature >38 °C or <36 °C
- Heart rate >90 beats/min
- Respiratory rate >20 breaths/min or PaCO₂ <32 mm Hg
- White blood cell count >12,000/mm³ or <4000/mm³ or >10% immature bands

^aModified with permission from American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med. 1992; 20:864–74.

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Table 2

Diagnostic indices of cord plasma C-reactive protein (CRP) and interleukin-6 (IL-6) in the identification of a positive amniotic fluid culture, neonatal sepsis and funisitis^a.

Parameters	Sensitivity	Specificity
Amniotic fluid infection		
CRP	52% (23/44)	76% (82/108)
IL-6	57% (25/44)	73% (79/108)
Neonatal sepsis		
CRP	82% (9/11)	74% (209/281)
IL-6	82% (9/11)	69% (192/279)
Funisitis		
CRP	62% (47/76)	83% (191/231)
IL-6	67% (51/76)	76% (174/229)

^aModified with permission from Yoon BH, Romero R, Shim JY, Shim SS, Kim CJ, Jun JK. C-reactive protein in umbilical cord blood: a simple and widely available clinical method to assess the risk of amniotic fluid infection and funisitis. *J Matern Fetal Neonatal Med.* 2003; 14:85–90.

Table 3

Genes differentially expressed in fetal inflammatory response syndrome, pediatric sepsis, and endotoxin challenge models of sepsis.

Gene Symbol	Fetal inflammatory response ^a	Systemic inflammatory response in humans ^b	Endotoxin challenge ^c	Pediatric septic shock ^d
ADM	↑	↑	↑	↑
ALOX5AP	↑	↑	↑	↑
ALPL	↑	↓	↑	↑
ANXA3	↑	↑	↑	↑
BAZ1A	↑	↑	↑	↑
CEACAM1	↑	↑	↑	↑
CKAP4	↑	↑	↑	↑
EMR1	↑	↓	↑	↑
FGR	↑	↓	↑	↑
FLOT1	↑	↑	↑	↑
HLA-DPA1	↓	↓	↓	↓
HLA-DPB1	↓	↓	↓	↓
HLA-DQB1	↓	↓	↓	↓
HP	↑	↑	↑	↑
IL1RN	↑	↑	↑	↑
LIMK2	↑	↑	↑	↑
MAPK14	↑	↑	↑	↑
PGD	↑	↑	↑	↑
PGLYRP1	↑	↑	↑	↑
S100A12	↑	↑	↑	↑
SERPINB1	↑	↑	↑	↑
SLC2A3	↑	↑	↑	↑
SPOCK2	↓	↓	↓	↓
UGCG	↑	↑	↑	↑
UPP1	↑	↑	↑	↑

Arrows indicate direction of change relative to healthy controls.

^aModified with permission from Madsen-Bouterse SA, Romero R, Tarca AL et al. The transcriptome of the fetal inflammatory response syndrome. *Am J Reprod Immunol.* 2010 Jan; 63(1):73–92.

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^bCalvano SE, Xiao W, Richards DR et al. A network-based analysis of systemic inflammation in humans. *Nature* 2005; 437:1032–1037.

^cTalwar S, Munson PJ, Barb J et al. Gene expression profiles of peripheral blood leukocytes after endotoxin challenge in humans. *Physiol Genomics* 2006; 25:203–215.

^dWong HR, Shanley TP, Sakthivel B et al. Genome-level expression profiles in pediatric septic shock indicate a role for altered zinc homeostasis in poor outcome. *Physiol Genomics* 2007; 30:146–155.