SEMMELWEIS EGYETEM

DOKTORI ISKOLA

Ph.D. értekezések

3325.

KOVÁCS KINGA

Asszisztált reprodukciós és magzati diagnosztika

című program

Programvezető: Dr. Urbancsek János, egyetemi tanár

Témavezető: Dr. Gasparics Ákos, egyetemi tanársegéd

PLACENTA – FETAL LUNG IN UTERO

Ph.D. Thesis

Kinga Kovács MD

Translational Medicine Program

Károly Rácz Conservative Medicine Division

SEMMELWEIS UNIVERSITY

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Budapest

2025

"Great things are not done by impulse, but by a series of small things brought together."

Vincent Van Gogh

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1. LIST OF ABBREVIATIONS

AJOG	American Journal of Obstetrics and Gynecology
AOP	Apnea of prematurity
BPD	Bronchopulmonary dysplasia
CAP	Caffeine for apnea of prematurity
CCA	Clinical chorioamnionitis
CONSORT	Consolidated Standards of Reporting Trials
CP	Cerebral palsy
CRP	C-reactive protein
DMC	Data Monitoring Committee
EOS	Early-onset sepsis
FIR	Fetal inflammatory response
FIRS	Fetal Inflammatory Response Syndrome
GA	Gestational age
HCA	Histological chorioamnionitis
IUI	Intrauterine Inflammation
IUGR	Intrauterine growth restriction
IVH	Intraventricular hemorrhage
ICMJE	International Committee of Medical Journal Editors
LOH	Length of hospital stay
LOS	Late-onset sepsis
MIR	Maternal inflammatory response
MTMT	Magyar Tudományos Művek Tára

MV	Mechanical ventilation
NEC	Necrotizing enterocolitis
NICE	National Institute for Health and Care Excellence
NICU	Neonatal intensive care unit
NIPPV	Non-invasive positive pressure ventilation
NIV	Non-invasive respiratory support
OR	Odds ratio
PDA	Patent ductus arteriosus
PEEP	Positive end expiratory pressure
PPROM	Preterm premature rupture of membranes
PVL	Periventricular leukomalacia
QUIPS	Quality in Prognosis Studies
RDS	Respiratory distress syndrome
RoB	Risk of bias
ROP	Retinopathy of prematurity
SGA	Small for gestational age
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials,
SC	Steering Committee
UC	Umbilical cord
WBC	White Blood Cell

2. STUDENT PROFILE

2.1. Vision and mission statement, specific goal

My vision is to prioritize neonatal care that aims to minimize complications associated with prematurity. To achieve this, our mission is to investigate the various risk factors that affect preterm infants during the prenatal, perinatal, and postnatal periods.

I work towards this by seeking evidence-based best practices and deepening the understanding of how different factors influence neonatal outcomes. Through research, collaboration, and continuous learning, I aim to contribute to improved care strategies that support the health and survival of premature infants.

2.2. Scientometrics

Number of all publications:	4
Cumulative IF:	17.6
Av IF/Publication:	4.4
Ranking (SCI Mago):	D1:2, Q1:1, Q2:1
Number of publications related to the subject of the thesis:	2
Cumulative IF:	11
Av IF/Publication:	5.5
Ranking (SCI Mago):	D1:1, Q1:1
Number of citations on Google Scholar:	12
Number of citations on MTMT (independent):	10
H-index:	2

The detailed bibliography of the student can be found on page 80.

2.3. Future plans

As a practicing clinician dedicated to neonatal care, my future goal is to expand my role by actively contributing to research in this field. Building on my clinical experience, I aim to pursue advanced research opportunities that align with my vision of reducing complications associated with prematurity. I am particularly interested in exploring antenatal, perinatal, and postnatal risk factors to generate evidence that informs best practices. By bridging clinical care and research, I hope to drive meaningful improvements in neonatal care. Ultimately, I aspire to be part of a collaborative scientific community that shapes the future of neonatal health through innovation and evidence-based medicine.

3. SUMMARY OF THE THESIS

Preterm birth persists as a principal determinant of neonatal morbidity and mortality on a global scale. A key factor contributing to adverse outcomes in preterm infants is intrauterine inflammation (IUI). My doctoral research aims to improve the understanding, diagnosis, and management of IUI complications in preterm infants by integrating clinical data, histopathology, and prospective intervention strategies.

We conducted a comprehensive meta-analysis published in *American Journal of Obstetrics and Gynecology (AJOG)*, synthesizing data from 50 eligible studies to clarify the relationship between fetal inflammatory response (FIR) and a range of neonatal outcomes. The study identified associations between FIR and early-onset sepsis (EOS), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), and intraventricular hemorrhage (IVH).

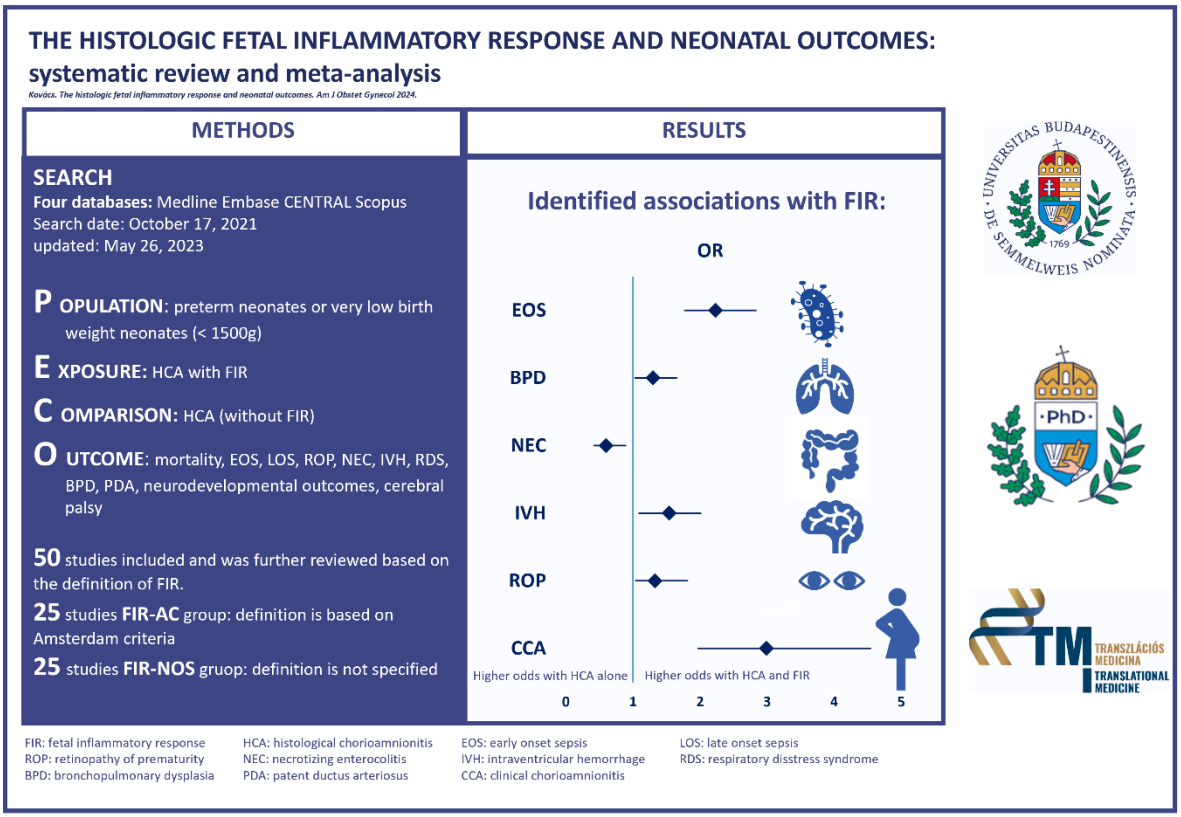
Our retrospective cohort study expands on these findings through a single-centre analysis investigating the topographical direction of FIR in preterm placentas. The results suggest that the inflammation progresses from the placental side to the fetal side, and more extensive inflammatory spread correlates with increased incidence of IVH. These findings complement our meta-analysis by providing anatomical detail to the FIR assessment and suggest that location-specific evaluation of FIR could improve clinical risk stratification in preterm infants. These studies established FIR as a clinically relevant marker of adverse short-term neonatal outcomes.

Not only to investigate but also to mitigate the consequences of prematurity, we launched a randomized controlled trial that addresses a critical therapeutic gap by evaluating the effect of an additional pre-extubational loading dose of caffeine citrate in mechanically ventilated preterm infants. This study aims to determine whether optimizing caffeine dosing can reduce the need for reintubation and improve outcomes.

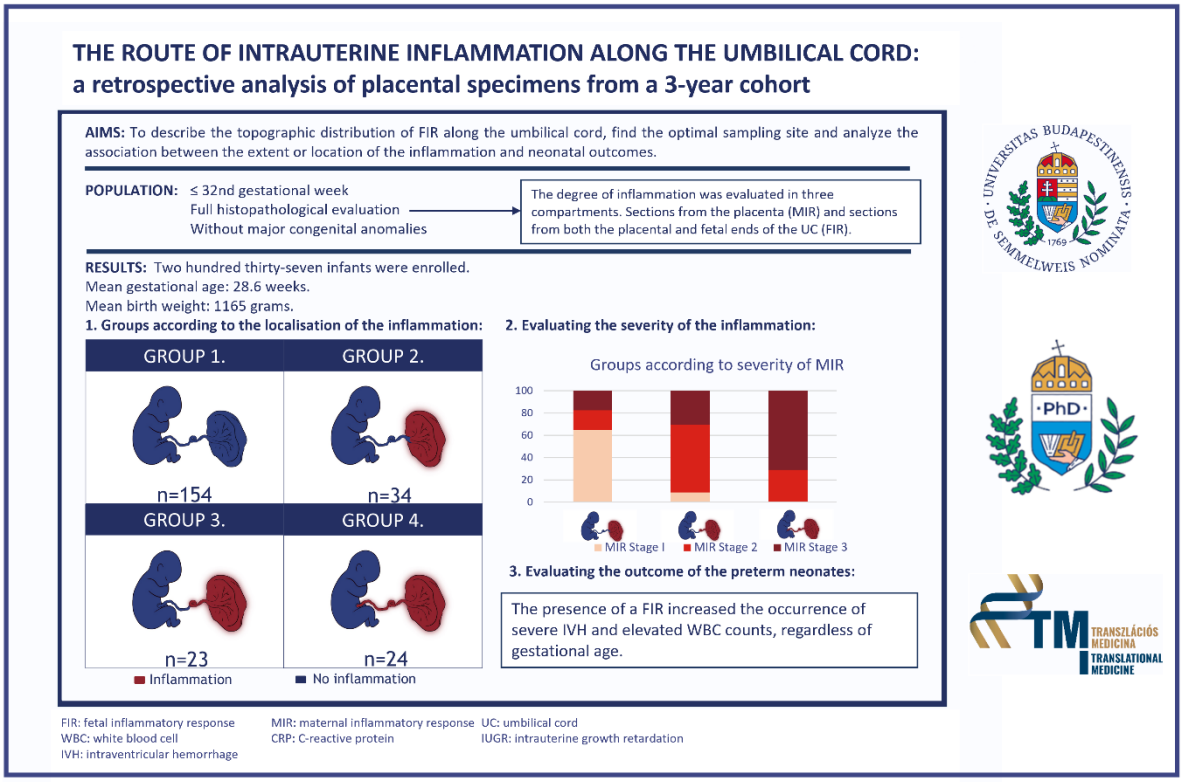
Together, these three studies form a cohesive research trajectory. The primary objective of my PhD work is to move beyond descriptive pathology and advance towards actionable, precision-based neonatal care. This includes linking histological findings with clinical outcomes and applying evidence-based interventions.

4. GRAPHICAL ABSTRACT

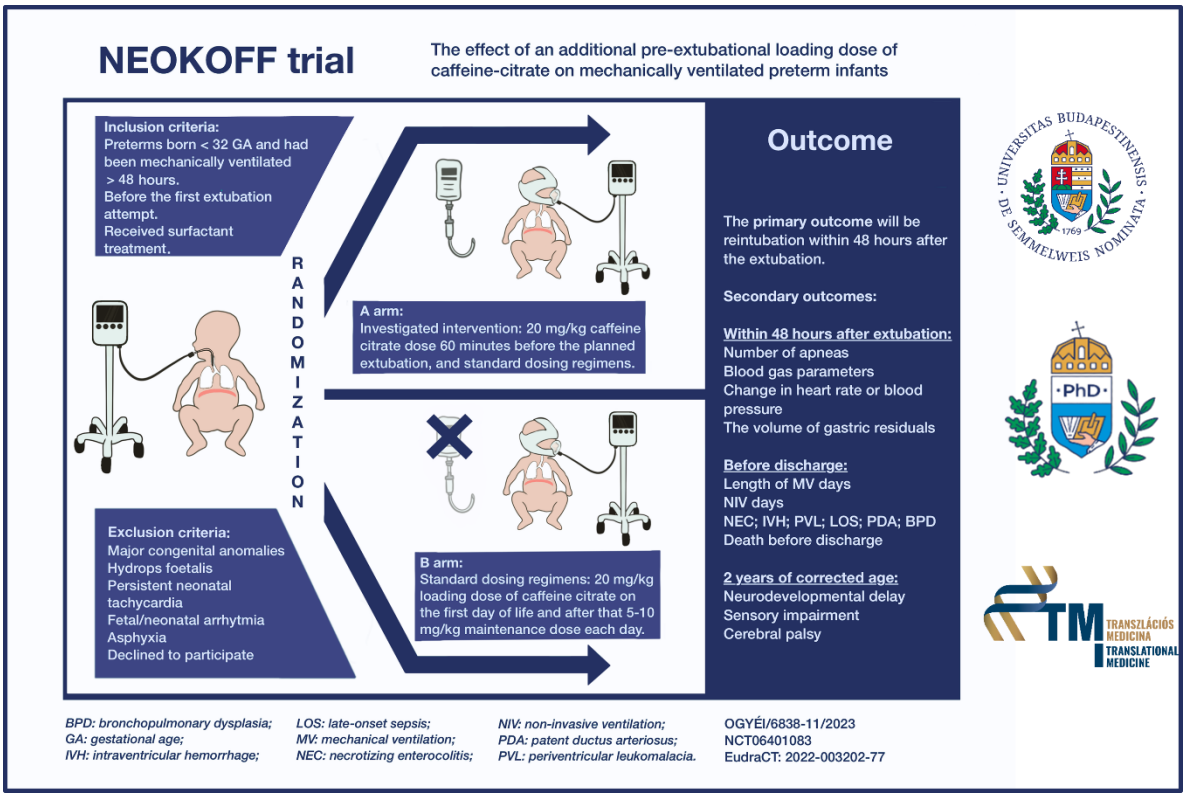
4.1. Study I



4.2. Study II


THE ROUTE OF INTRAUTERINE INFLAMMATION ALONG THE UMBILICAL CORD:
a retrospective analysis of placental specimens from a 3-year cohort
AIMS: To describe the topographic distribution of FIR along the umbilical cord, find the optimal sampling site and analyze the association between the extent or location of the inflammation and neonatal outcomes.
POPULATION: ≤ 32nd gestational week
Full histopathological evaluation
Without major congenital anomalies
The degree of inflammation was evaluated in three compartments. Sections from the placenta (MIR) and sections from both the placental and fetal ends of the UC (FIR).
RESULTS: Two hundred thirty-seven infants were enrolled.
Mean gestational age: 28.6 weeks.
Mean birth weight: 1165 grams.
1. Groups according to the localisation of the inflammation:
GROUP 1.
GROUP 2.
n=154
n=34
GROUP 3.
GROUP 4.
n=23
n=24
Inflammation
No inflammation
2. Evaluating the severity of the inflammation:
Groups according to severity of MIR
100
80
60
40
20
0
MIR Stage I
MIR Stage 2
MIR Stage 3
3. Evaluating the outcome of the preterm neonates:
The presence of a FIR increased the occurrence of severe IVH and elevated WBC counts, regardless of gestational age.
FIR: fetal inflammatory response
WBC: white blood cell
IVH: intraventricular hemorrhage
MIR: maternal inflammatory response
CRP: C-reactive protein
UC: umbilical cord
IUGR: intrauterine growth retardation

4.3. Study III


NEOKOFF trial
The effect of an additional pre-extubational loading dose of caffeine-citrate on mechanically ventilated preterm infants
Inclusion criteria:
Preterms born < 32 GA and had been mechanically ventilated > 48 hours.
Before the first extubation attempt.
Received surfactant treatment.
RANDOMIZATION
A arm:
Investigated intervention: 20 mg/kg caffeine citrate dose 60 minutes before the planned extubation, and standard dosing regimens.
B arm:
Standard dosing regimens: 20 mg/kg loading dose of caffeine citrate on the first day of life and after that 5-10 mg/kg maintenance dose each day.
Exclusion criteria:
Major congenital anomalies
Hydrops foetalis
Persistent neonatal tachycardia
Fetal/neonatal arrhytmia
Asphyxia
Declined to participate
Outcome
The primary outcome will be reintubation within 48 hours after the extubation.
Secondary outcomes:
Within 48 hours after extubation:
Number of apneas
Blood gas parameters
Change in heart rate or blood pressure
The volume of gastric residuals
Before discharge:
Length of MV days
NIV days
NEC; IVH; PVL; LOS; PDA; BPD
Death before discharge
2 years of corrected age:
Neurodevelopmental delay
Sensory impairment
Cerebral palsy
BPD: bronchopulmonary dysplasia;
GA: gestational age;
IVH: intraventricular hemorrhage;
LOS: late-onset sepsis;
MV: mechanical ventilation;
NEC: necrotizing enterocolitis;
NIV: non-invasive ventilation;
PDA: patent ductus arteriosus;
PVL: periventricular leukomalacia.
OGYÉI/6838-11/2023
NCT06401083
EudraCT: 2022-003202-77
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TRANSLATIONAL MEDICINE

5. INTRODUCTION

5.1. Overview of the topic

5.1.1. What is the topic

The primary focus of this doctoral research is the IUI, specifically the FIR, and its clinical implications in preterm infant care, which are examined through a combination of meta-analytic and retrospective approaches. The studies investigate the diagnostic and prognostic dimensions of FIR by analyzing histopathological data and neonatal outcomes. Study I (1)(a meta-analysis) assesses the association between FIR and neonatal morbidity and mortality. Study II investigates the topographical progression of FIR along the placenta and umbilical cord (UC). Study III (a protocol for a randomized clinical trial) (2) evaluates whether modifying periextubation management via an additional caffeine dose can reduce extubation failure among preterm infants.

5.1.2. What is the problem that needs to be solved

5.1.2.1. Study I and II

Despite decades of research into IUI, the clinical utility of FIR as a prognostic marker remains limited. FIR is frequently assessed in binary terms - present or absent - without considering its anatomical spread or severity. Moreover, although FIR is associated with adverse neonatal outcomes such as sepsis, IVH, and BPD (1), uncertainty persists about how to respond to this information to prevent complications. A clearer understanding of FIR's pathological patterns and their relationship to neonatal outcomes is essential for improving neonatal care.

5.1.2.2. Study III

In neonatal intensive care units (NICUs), clinicians are increasingly favoring the use of non-invasive respiratory (NIV) support whenever feasible (3). However, invasive mechanical ventilation (MV) remains unavoidable in certain clinical situations. In such cases, efforts are made to minimize the duration of MV and to perform extubation as early as is safely possible, due to its well-established associations with adverse outcomes such as necrotizing

enterocolitis (NEC) (4), BPD (5), and impaired neurodevelopmental outcome (6). Despite these efforts, the rate of extubation failure in preterm infants remains high (7). One of the most frequent causes of reintubation in this population is apnea of prematurity (AOP), a common developmental challenge in extremely preterm neonates (8). Methylxanthines such as caffeine and theophylline have long been used to stimulate respiratory drive in these infants, with caffeine emerging as the preferred agent due to its favorable safety profile, longer half-life, and ease of administration (9). Since the landmark Caffeine for Apnea of Prematurity (CAP) trial (10) demonstrated its efficacy in reducing the incidence of AOP and improving neurodevelopmental outcomes, caffeine has become the most widely used methylxanthine in NICUs (11).

5.1.3. What is the importance of the topic?

5.1.3.1. Study I and II

FIR affects up to 25-28% of preterm births (12, 13), and its presence significantly increases the risk of early postnatal complications and long-term neurodevelopmental impairment (14, 15). Yet, current clinical decision-making often lacks input from detailed histopathological insights, and perinatal management protocols rarely integrate FIR status into therapeutic planning. Enhancing the understanding of FIR, particularly in its topographical manifestations and its overall associations with outcomes, could allow for more precise risk stratification and personalized care.

5.1.3.2. Study III

Reducing the duration of mechanical ventilation is critical in preterm infants due to the increased risk of BPD and other ventilation-associated complications (4). Despite efforts to minimize mechanical ventilation, both its usage rate and the frequency of extubation failure remain significantly higher in preterm neonates. This peri-extubation period represents a particularly vulnerable window in neonatal care, during which clinical conditions and practices should be further optimized and standardized (16).

5.1.4. What would be the impact of our research results?

Studies I and II aim to transform the clinical relevance of FIR from a static histological

diagnosis to a dynamic, actionable marker in neonatal medicine. Study I provides high-level evidence that FIR is linked to increased odds of IVH, EOS, and BPD, especially in extremely preterm infants. Study II provides anatomical and mechanistic detail, showing that the direction and extent of FIR correlate with the outcome.

Finally, study III, the NEOKOFF clinical trial protocol (2) proposes a practical intervention - an additional pre-extubational caffeine dose - to reduce extubation failure, potentially improving respiratory outcomes in preterm infants.

Together, these studies support more targeted neonatal monitoring, enhance the diagnostic value of placental pathology, and inform evidence-based interventions to improve outcomes in this high-risk population.

5.2. Intrauterine inflammation

IUI is a common and clinically significant complication of preterm birth, most often caused by ascending bacterial infection leading to an inflammatory response in the fetal membranes and placenta (17). Histologically, IUI is divided into two major components: MIR and FIR, as defined by the Amsterdam Placental Workshop Group Consensus Statement (2016) (18). MIR typically manifests as acute chorioamnionitis (HCA), characterized by infiltration of neutrophils into the chorion and amnion. FIR indicates inflammatory involvement of fetal membranes, such as the UC in funisitis or the chorionic plate vessels in chorionic vasculitis (19, 20).

The progression from MIR to FIR is interpreted as a sign of escalating inflammation (21). FIR is associated with systemic fetal activation of inflammatory pathways, which may have deleterious effects on multiple organ systems (22). FIR is typically staged and graded according to standardized histological criteria based on the depth and intensity of neutrophilic infiltration (18).

5.3. Caffeine therapy

5.3.1. Mechanism of action and clinical utility

Caffeine citrate is a methylxanthine widely used in neonatology, primarily for treating AOP.

Its mechanism involves antagonism of adenosine receptors, resulting in increased respiratory drive**,** enhanced diaphragmatic contractility, and stimulation of central respiratory centers (23). Additionally, caffeine exhibits a mild diuretic effect (24), and it is characterized by a long half-life in preterm infants, often exceeding 100 hours (25).

5.3.2. Evidence-based use and gaps

The pivotal randomized controlled trial - the CAP trial - and observational studies have shown that early caffeine administration reduces the incidence of BPD (10) and improves survival without neurodevelopmental disability at 18 to 21 months of age (26). The standard regimen involves a loading dose of 20 mg/kg**,** followed by maintenance doses of 5–10 mg/kg/day (27). However, timing, dosing strategy, and individualization based on pharmacokinetics remain subjects of ongoing research (11).

Study III (the NEOKOFF trial protocol) addresses one such gap by evaluating the effectiveness of an additional, pre-extubational loading dose of caffeine citrate.

6. OBJECTIVES

6.1. Study I - The histologic fetal inflammatory response and neonatal outcomes: systematic review and meta-analysis

Preterm birth affects roughly 10% of pregnancies worldwide (28), with IUI present in about half of extremely preterm cases (29). While clinical signs aid diagnosis, histological examination of the placenta and UC is the gold standard to diagnose IUI (30). With histological evaluation, MIR or HCA and FIR can be differentiated (20). HCA is strongly associated with adverse neonatal outcomes, including sepsis, BPD, IVH, and long-term neurodevelopmental issues (31-35). In up to 70% of cases, HCA is accompanied by FIR, but its additional impact remains debated (1). To clarify this, we conducted a systematic review and meta-analysis comparing neonatal outcomes in preterm infants with HCA alone versus HCA with FIR.

6.2. Study II - The route of intrauterine inflammation along the umbilical cord: a retrospective analysis of placental specimens from a 3-year cohort

As mentioned above, IUI plays a major role in preterm births and related complications. Based on the inflammatory cells, we can differentiate between MIR and FIR. However, the direction of inflammation remains unclear, as infections may start in the placenta, fetus, or occur randomly. Few studies have examined the directional spread of IUI using histological data, and the patterns are debated (36-38). To address this uncertainty, we conducted a study exploring the topographical progression of FIR from the placenta to the UC. The objective was to determine whether FIR shows a directional progression along the UC and the fetal vessels. This study also examined how these histological findings correlated with maternal and neonatal outcomes. The results aim to improve understanding of inflammation dynamics and reinforce the importance of detailed placental and umbilical cord assessments in preterm deliveries.

6.3. Study III - The effect of an additional pre-extubational loading dose of caffeine citrate on mechanically ventilated preterm infants (NEOKOFF trial): Study protocol for a multicenter randomized clinical trial

Since the CAP trial (10), caffeine has become a standard treatment in neonatal intensive care, but there are still unanswered questions regarding ideal dosing and timing (23, 39). To address this, we designed a randomized clinical trial to evaluate whether a single additional loading dose of caffeine citrate administered one hour before extubation improves extubation success. The study also aims to monitor the frequency and severity of potential side effects.

7. METHODS

7.1. Study I - Systematic review and meta-analysis

The first study was conducted in accordance with the Cochrane Handbook and the updated PRISMA 2020 guidelines for reporting systematic reviews (40). The study protocols were developed using the Population, Exposure, Comparator, and Outcome (PECO) framework and registered on PROSPERO (CRD42021283448) (41).

7.1.1. Literature search and eligibility criteria

A systematic search was conducted in MEDLINE (via PubMed), Embase, CENTRAL, and Scopus on October 17, 2021, and updated on May 26, 2023. No language restrictions were applied. Three reviewers (K.K., D.B., and O.K.) independently screened titles, abstracts, and full texts using EndNote X9 (Clarivate Analytics, Philadelphia, PA, USA). Discrepancies were resolved by consensus. Interrater agreement was measured using Cohen's kappa (42).

The search key was: *(preterm OR premature OR lbw OR "low birth weight") AND (chorioamnionitis OR "intrauterine inflammation" OR "intrauterine infection" OR IUI) AND (funisitis OR "fetal inflammatory response")*

We included observational studies (cohort, case-control, and case series) reporting neonatal outcomes (O) in preterm infants (<37 weeks of gestation or birthweight ≤1500 g) (P) with HCA, with or without (E and C) FIR. Animal studies, case reports, and non-original data were excluded.

7.1.2. Definitions of fetal inflammatory response and subgroups.

To account for variations in the definition of fetal inflammatory response (FIR), we divided the included studies into two groups to reduce heterogeneity. Articles using the definition of FIR based on Amsterdam Placental Workshop Group Consensus Statement (18) - where chorionic vasculitis is a required criterion - were classified as the "FIR Amsterdam Criteria" (FIR-AC) group. Studies that did not specify the FIR definition or used alternative terms formed the "FIR not otherwise specified" (FIR-NOS) group. Accordingly, we conducted two sets of analyses: one including all eligible studies, and a second limited to the FIR-AC subgroup.

7.1.3. Outcomes

Primary outcomes included neonatal mortality, EOS, late-onset sepsis (LOS), NEC, and BPD. Secondary outcomes comprised ROP, IVH, periventricular leukomalacia (PVL), respiratory distress syndrome (RDS), length of hospital stay (LOH), cerebral palsy (CP), neurodevelopmental delay, sensory impairments, small for gestational age (SGA), and clinical chorioamnionitis (CCA).

7.1.4. Data extraction and quality assessment

Two reviewers independently extracted data and assessed risk of bias (RoB) according to the recommendations of the Cochrane Prognosis Methods Group using the Quality in Prognosis Studies tool (43). Disagreements were resolved by consensus.

7.1.5. Statistical analysis

Statistical analyses were conducted using the R software (v4.1.2) with the 'meta' package, following the methodology outlined by Harrer et al. (44). We calculated pooled odds ratios (ORs) with 95% confidence and prediction intervals using a random-effects Mantel-Haenszel model, applying the Paule-Mandel method for between-study variance and the Hartung-Knapp adjustment (45-48).

Heterogeneity was assessed with I^2 statistics and Cochrane's Q test. Subgroup analyses were performed based on mean gestational age (<28 versus ≥28 weeks) when at least three studies per group were available. Publication bias was evaluated using funnel plots and Harbord tests (≥10 studies) (49).

In addition to pooled ORs, we conducted supplemental bivariate meta-analyses to assess the underlying proportions in each group, visualizing the results with 2D scatter plots that included confidence and prediction regions (50). All statistical tests were two-sided, and $p < 0.05$ were considered significant, recognizing the increased risk of false positives due to multiple comparisons.

7.2. Study II – Retrospective cohort study

We conducted a retrospective study of preterm infants born before 32 weeks' gestation at the Semmelweis University Department of Obstetrics and Gynecology (Baross street building) between March 2020 and December 2022. Infants with major congenital anomalies, hydrops

fetalis, or incomplete placental histology were excluded.

7.2.1. Histological Processing

Placental examination followed the triage protocol by Roberts et al. (51), with all preterm births <34 weeks routinely examined microscopically. From 2020, an additional UC sample - taken from the fetal end near the abdominal wall - was also included. Samples were processed according to the Amsterdam Placental Workshop Consensus Statement (18) and evaluated by two experienced perinatal pathologists (E.R. and A.F.). MIR was assessed from full-thickness placental samples, while FIR was assessed from the UC insertion site of the placenta and two UC sections.

7.2.2. Data Collection

Clinical and histological data were extracted from the e-Med Solutions Hospital IT System and compiled in Excel. Information included maternal and perinatal variables (e.g., delivery mode, multiple gestation, steroid use, intrauterine growth restriction (IUGR), preeclampsia, abruption), inflammatory markers (e.g., C-reactive protein (CRP), WBC, fever, microbiology results), and neonatal outcomes (e.g., mortality, RDS, IVH, PVL, PDA, NEC).

7.2.3. Statistical Analysis

Descriptive statistics and 95% confidence intervals were calculated. Fisher's exact test and ANOVA were used to compare proportions and means among groups, followed by pairwise comparisons with Holm correction (52). Logistic regression (adjusted for gestational age) and likelihood-ratio tests assessed associations with clinical outcomes. Linear regression was used for continuous variables (e.g., WBC count). Analyses were performed using R; significance was set at $p < 0.05$.

7.3. Study III - Protocol for a randomized clinical trial

7.3.1. Trial design

This two-arm, open-label, randomized multicenter clinical trial aims to assess whether a pre-extubational loading dose of caffeine citrate improves extubation outcomes in preterm infants. The study plan is to enroll 226 preterm infants treated at the tertiary NICUs of Semmelweis University.

According to institutional protocols, infants born before 32 weeks' gestation routinely

receive caffeine therapy: a 20 mg/kg loading dose on day one, followed by a maintenance dose of 5 mg/kg once or twice daily. Eligible infants who have received mechanical ventilation for ≥48 hours and are facing their first planned extubation are randomly assigned (1:1) to an intervention group (n=113) or control group (n=113), stratified by gestational age and antenatal steroid exposure.

The intervention group receives an additional 20 mg/kg IV caffeine citrate dose 60 minutes before extubation. The control group continues the standard regimen. The study follows SPIRIT guidelines for clinical trial design (53)(Figure 1).

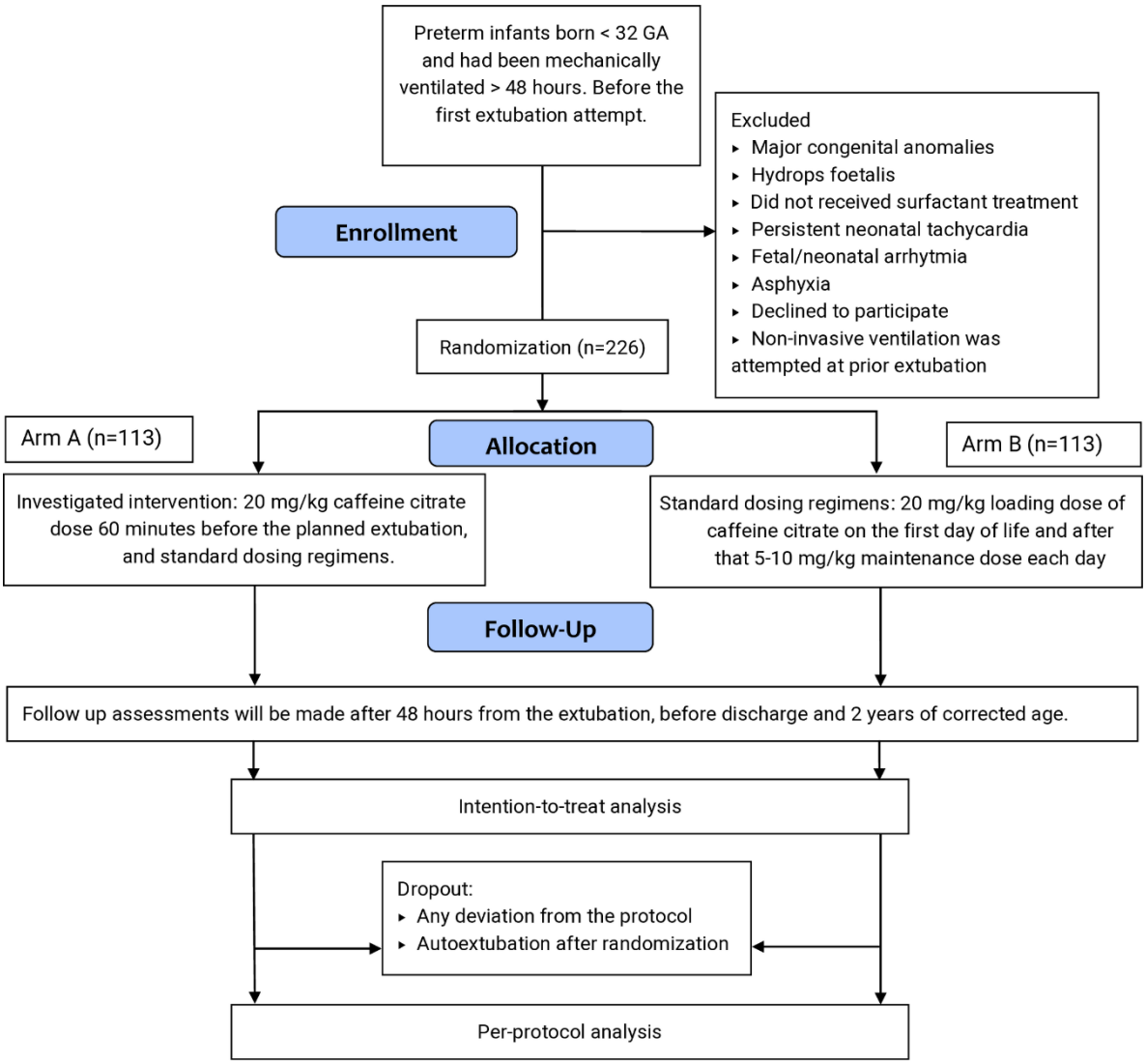


Figure 1. *SPIRIT flow chart. (53)*
SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials, GA, Gestational age

7.3.2. Eligibility

Inclusion criteria:

- Born before 32 weeks' gestation
- On mechanical ventilation ≥48 hours
- Undergoing the first planned extubation
- Received surfactant therapy.

Exclusion criteria:

- Lack of parental consent
- Major congenital anomaly or hydrops fetalis
- Persistent tachycardia or arrhythmia prior to extubation
- History of asphyxia
- Prior unplanned extubation followed by a non-invasive ventilation attempt.

Eligibility will be determined at the time of extubation. Informed consent will be obtained by a trained clinician during the ventilation period. No biological samples will be collected during the trial.

7.3.3. Intervention

This study is structured as a two-arm, randomized, open-label clinical trial. The intervention consists of administering an additional intravenous loading dose of caffeine citrate (CITRATE DE CAFEINE COOPER; Coopération Pharmaceutique Française, Melun, France) 60 minutes prior to planned extubation. The 60-minute timing aligns with the pharmacodynamic profile of caffeine, given its 20-minute infusion time and peak diaphragmatic contractility approximately 25–30 minutes post-administration (54).

Participants in Arm A (intervention group) receive a standard 20 mg/kg IV loading dose of caffeine citrate on the first day of life, followed by a 5 mg/kg IV maintenance dose administered once or twice daily, as is the standard of care at participating units. Prior to planned extubation, this group receives an additional 20 mg/kg IV dose of caffeine citrate. The dosing regimen is weight-adjusted: for infants <1000 g, the solution is diluted to 1 mL and infused at 3 mL/hr; for those between 1000–2000 g, dilution is 2 mL and infused at 6 mL/hr; and for infants >2000 g, the dilution is 3 mL with a 9 mL/hr infusion rate. Dilution is performed with maintenance fluids or 5% dextrose in fully enterally fed infants.

Arm B (control group) receives the same standard loading and maintenance doses, including on the day of extubation, without an additional loading dose. Any deviation from the prescribed protocol (e.g., early or higher dosing) is documented by the Steering Committee (SC) and reported to the Data Monitoring Committee (DMC) as a protocol deviation/dropout. Extubation is conducted when a preterm infant is considered ready, at the discretion of a senior clinician. Post-extubation respiratory support includes non-invasive positive pressure ventilation (NIPPV) in accordance with local guidelines, with a minimum positive end expiratory pressure (PEEP) of 7 H_2Ocm.

7.3.4. Sample size

Sample size estimation was based on anticipated rates of extubation failure. It is hypothesized that Arm A will have a reintubation rate of 20%, based on institutional data where an additional loading dose was administered. For Arm B, a rate of 36.8% is expected (55). To detect this difference with 80% power and a significance level of 0.05, a total of 226 infants (113 per arm) will be required. Given the short timeframe between enrollment, intervention, and outcome measurement, a low dropout rate is anticipated.

7.3.5. Statistical analysis

Both intention-to-treat (primary analysis) and per-protocol analyses will be conducted. Descriptive statistics (counts and percentages) will summarize baseline characteristics. Continuous variables will be compared using t-tests or Mann–Whitney U tests, and categorical data will be analyzed using chi-squared or Fisher's exact tests, as appropriate (56). Significance will be determined at $p < 0.05$. All analyses will be conducted using R software.

7.3.6. Randomization and data collection

Eligible infants are randomized post-consent with a 1:1 allocation ratio. Stratification is based on gestational age and administration of antenatal corticosteroids. The randomization sequence si generated via the Big-Stick Design algorithm in R and implemented through REDCap software (Research Electronic Data Capture), which also facilitates data collection.

7.3.7. Outcomes

7.3.7.1. Primary outcome

Reintubation within 48 hours post-extubation. This timeframe aligns with the observed peak in reintubation events and pharmacokinetics of caffeine (25, 57, 58). In the absence of universally accepted reintubation criteria (59), the decision will be made by a senior clinician.

7.3.7.2. Secondary outcomes

Data are collected via structured forms:

Form A: Perinatal and baseline demographics.

Form B: Post-extubation (first 48 hours) events, including apnea, tachycardia, hypertension, and gastric residuals.

Form C: Complications prior to discharge, such as IVH, PVL, NEC, and BPD at 36 weeks' postmenstrual age.

Form D: Neurodevelopmental status at two years corrected age. Scheduling of follow-up visits is included in the discharge documentation.

7.3.8. Interim analysis and trial termination

An interim analysis will be conducted upon enrollment and discharge of 50% of the study population. The SC may halt the trial early for safety or efficacy reasons.

7.3.9. Participating centers

Initially conducted at tertiary NICUs at Semmelweis University, the study will be extended to other eligible centers. Participating sites must meet three criteria: tertiary NICU designation, assigned local trial coordinators, and team participation in a preliminary training session. Centres must submit a formal letter of intent to the corresponding author.

7.3.10. Monitoring and safety

Caffeine toxicity is rare below serum levels of 50 mg/L, with therapeutic ranges between 5–25 mg/L (60, 61). Despite occasional supratherapeutic levels reported under standard regimens (62), routine drug-level monitoring is not implemented, in accordance with NICE guidelines (63).

The SC will oversee implementation and safety. Adverse events and protocol deviations will be reported to the DMC and ethics committees (Hungarian Medical Research Council, National Institute of Pharmacy and Nutrition).

7.3.11. Ethical approval and dissemination

This clinical trial has been registered on ClinicalTrials.gov under the identifier NCT06401083 and has received ethical approval from the Hungarian Ethics Committee for Clinical Pharmacology of the Medical Research Council and the National Institute of Pharmacy and Nutrition, reference number OGYÉI/6838-11/2023. In 2025 it had been transitioned to the Clinical Trials Information System (2024-519041-29-02).

Upon completion of the study, findings will be disseminated to the scientific and clinical community through peer-reviewed publication and conference presentations. Authorship will be determined in accordance with the International Committee of Medical Journal Editors (ICMJE) (53) authorship guidelines. The reporting of study outcomes will adhere to the CONSORT (Consolidated Standards of Reporting Trials) statement to ensure transparency and methodological rigor (64).

8. RESULTS

8.1. Study I - Systematic review and meta-analysis

8.1.1. Study selection

A total of 7,881 records were screened during the initial selection process. Following title, abstract, and full-text review, 47 studies (32, 65-110) met the criteria for qualitative synthesis, with 46 included in the quantitative analysis. An updated search yielded 3 additional eligible studies(111-113). Of the included publications, 25 defined FIR based on the Amsterdam Placental Workshop Group Statement and were categorized under the FIR-AC group (Table 1). No further eligible studies were identified through reference screening. The selection process is detailed in the PRISMA flow diagram (Figure 2).

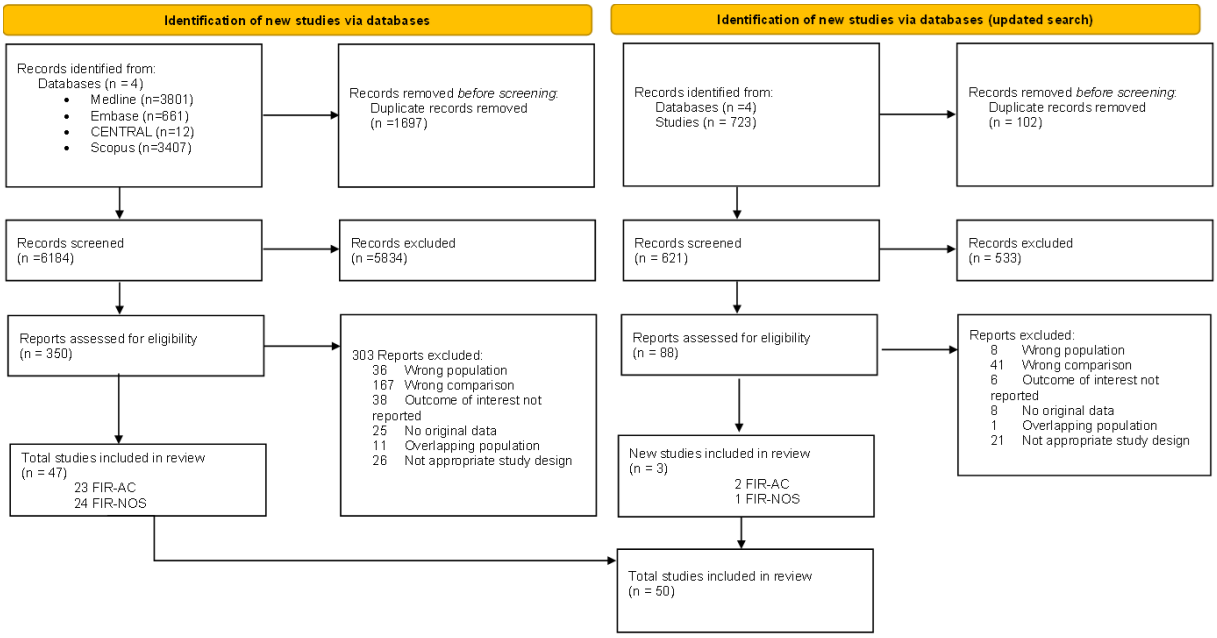


Figure 2. *Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart(40)*

CENTRAL, Cochrane Central Register of Controlled Trials; FIR-AC, fetal inflammatory response Amsterdam criteria; FIR-NOS, fetal inflammatory response not otherwise specified.

Table 1. Characteristics of included studies in the meta-analysis and systematic review

Studies	Country	Design	Sample size	No. of cases in exposure /control group	FIR definition	Inclusion criteria	Mean GA ± SD	Outcomes
Al-Mulaabed et al., 2017(72) ‡	USA	Retrospective	85	11/29	FIR-NOS	BW < 1500 g	28.4 ± 3	NEC
Babnik et al., 2006(91)	Slovenia	Prospective	125	26/21	FIR-NOS	GA< 30 weeks	27.4 ± 2	IVH
Been et al., 2009(68)	The Netherlands	Prospective	301	68/53	FIR-AC	GA ≤ 32 weeks	28.31 ± 2.01	CCA; SGA; Mortality; RDS; EOS; LOS; IVH; Cystic PVL; NEC; PDA; BPD
Budal et al., 2023(111)	Norway	Prospective	71	51/20	FIR-AC	GA ≤ 28 weeks	25,73	CCA; SGA Mortality; EOS; NEC; ROP; BPD
Burgner et al., 2017(96)	Australia	Retrospective	1218	406/171	FIR-AC	GA<30 weeks	27	Mortality
Dessardo et al., 2019(101)	Croatia	Prospective	262	45/60	FIR-AC	GA ≤ 32 weeks	29.2 ± 2.3	PDA; BPD
Durrmeyer et al., 2012(102)	France	Prospective	384	85/93	FIR-NOS	GA< 28 weeks	NA	Mortality; BPD
Gantar et al., 2011(104)	Slovenia	Retrospective	115	28/9	FIR-NOS	GA< 30 weeks	27.26 ± 1.45	BPD
Gisslen et al., 2016(84)	USA	Prospective	477	31/79	FIR-NOS	GA> 32 weeks and GA < 37 weeks	34.71 ± 1.52ł	CCA; RDS; LOHlo

Hendson et al., 2011(99)	Canada	Prospective	628	134/95	FIR-NOS	GA ≤ 32 weeks and BW≤ 1250 g	26.1 ± 0.1	CP, Visual impairment, Hearing impairment; Mental delay
Holcroft et al., 2004(109)	USA	Retrospective	354	87/59	FIR-NOS	GA< 34 weeks	28.0 ± 3.06	CCA; Mortality; RDS; NEC
Horvath et al., 2012(87)	Hungary	NA	141	14/29	FIR-NOS	BW < 1500 g	29.92 ± 6.88	CP
Ikeda et al., 2014(94)	Japan	Retrospective	294	180/50	FIR-NOS	GA< 32 weeks	27.71 ± 1.63 ł	BPD
Kelly et al., 2022 ‡v(113)	USA	Retrospective	152	36/24	FIR-NOS	GA< 30 weeks	NA	RDS; IVH; PVL; NEC; ROP; BPD
Kent et al., 2004(77)	Australia	Retrospective	241	40/40	FIR-NOS	GA< 30 weeks	27.1 ± 1.95	BPD
Kent et al., 2005(110)	Australia	Retrospective	220	33/39	FIR-NOS	GA< 30 weeks	27.03 ± 1.89	Mortality; IVH; CP
Kim et al., 2015(100)	Korea	Retrospective	258	34/65	FIR-NOS	BW < 1500 g	28.14 ± 2.71	CCA; SGA; Mortality; RDS; EOS; LOS; IVH; Cystic PVL; NEC; ROP; BPD
Kim et al., 2016(95)	Korea	Retrospective	267	54/74	FIR-NOS	GA ≤ 32 weeks	28.32 ± 2.47	Hearing impairment
Lahra et al., 2008(92)	Australia	Prospective	724	219/138	FIR-NOS	GA< 30 weeks	27.1 ± 1.6	SGA; RDS
Lahra et al., 2009(97)	Australia	Prospective	761	208/140	FIR-NOS	GA< 30 weeks	27.4 ± 1.5	PDA

Lee et al., 2015(93)	Korea	Retrospective	339	189/29	FIR-AC	GA< 34 weeks	29.2 ± 2.99	Mortality; CCA; RDS; IVH; PVL; NEC; ROP; BPD
Liu et al., 2012(66)	China	Prospective	216	51/53	FIR-AC	GA< 34 weeks	31.46 ± 1.64	Mortality; RDS; EOS; LOS; IVH; Cystic PVL; NEC; PDA; ROP; BPD
Lynch et al., 2017(107)	USA	Retrospective	1217	305/82	FIR-AC	GA ≤ 30 weeks or BW ≤ 1500g	29 ± 2.5	ROP
Maisonneuve et al., 2020(73)	France	Prospective	1470	217/211	FIR-AC	GA< 32 weeks	NA	CP
Matulova et al., 2022(112)	Czech Republic	Retrospective	818	343/151	FIR-AC	GA< 37 weeks	ŧ	Mortality; RDS; EOS; LOS; IVH; NEC; ROP; BPD; SGA
Mestan et al., 2010(71)	USA	Prospective	256	54/40	FIR-AC	GA< 37 weeks	28.75 ± 2.7	CCA; IVH; NEC; BPD
Mir et al., 2019(90)	USA	Retrospective	241	75/42	FIR-AC	GA< 29 weeks	26 ± 1.53	BPD
Park et al., 2015(98)	Korea	NA	378	125/94	FIR-NOS	GA ≤ 34 weeks	30.2 ± 2.29ŧ	RDS
Cortelyou et al., 2020(105)	USA	Retrospective	255	61/21	FIR-AC	GA< 33 weeks	28.6 ± 2	IVH
Perniciaro et al., 2020(79)	Italy	Retrospective	162	17/31	FIR-NOS	BW < 1500 g	28.7 ± 3.1	SGA; PDA; BPD
Pietrasanta et al., 2019(70)	Italy	Prospective	807	61/73	FIR-AC	GA<35 weeks, and/or BW ≤ 1500 g	30.14 ± 3.32	CCA; SGA; Mortality; RDS; EOS; LOS; IVH; PDA; ROP; BPD;
Plakkal et al., 2013(69)	Canada	Retrospective	529	186/84	FIR-AC	GA< 29 weeks	25.7 ± 1.6	CCA; SGA; Mortality; RDS; NEC; PDA; BPD; LOH

Richardson et al., 2006(106)	UK	Prospective	660	178/114	FIR-NOS	GA< 34 weeks	29.44 ± 2.64	Mortality; CCA; RDS; IVH; Cystic PVL; BPD
Rocha et al., 2007(76)	Portugal	Retrospective	452	81/44	FIR-AC	GA< 34 weeks	29.8 ± 1.73 ł	IVH; Cystic PVL
Rovira et al., 2011(67)	Spain	Prospective	177	45/42	FIR-NOS	GA< 32 weeks or BW < 1500 g	28.3 ± 2.5	Mortality; IVH; Cystic PVL; CP; Visual impairment, Hearing impairment; Mental delay
Salas et al., 2013(85)	USA	Retrospective	347	110/38	FIR-AC	GA< 19 weeks	25.35 ± 2.31 ł	CCA; RDS; CP, Visual impairment, Hearing impairment; Mental delay
Sharma et al., 2021(78)	USA	Retrospective	246	127/34	FIR-AC	GA< 28 weeks	25.23 ± 1.22	BPD
Smit et al., 2015(75)	The Netherlands	Retrospective	300	55/80	FIR-AC	GA ≤ 32 weeks	28.84 ± 2.08	RDS; IVH; Cystic PVL; NEC; PDA; BPD
Soraisham et al., 2013(32)	Canada	Retrospective	384	140/57	FIR-NOS	GA< 29 weeks	26.6 ± 1.3	CP; Visual impairment, Hearing impairment; Mental delay
Strunk et al., 2018(86)	Australia	Retrospective	1089	396/131	FIR-AC	GA< 30 weeks	26.52 ± 1.94	EOS; LOS; NEC; PDA; ROP; BPD;
Torchin et al., 2017(83)	France	Prospective	1731	269/250	FIR-AC	GA< 30 weeks	29.1 ± 2.52 ł	BPD; Mortality
Trevisanuto et al., 2013(65)	Italy	Prospective, case-control	320	44/27	FIR-AC	GA< 32 weeks	27.76 ± 2.73	CCA; SGA; EOS; LOS; Mortality; IVH; Cystic PVL; NEC; BPD; LOH;
Tsiartas et al., 2013(74)	Czech Republic	NA	231	45/97	FIR-NOS	GA< 37 weeks	32.00 *	PDA

van Doorn et al., 2021(81)	The Netherlands	Retrospective	1014	168/72	FIR-NOS	GA ≤ 32 weeks or BW < 1500 g	29.0 ± 2.1	LOS
Vergani et al., 2000	Italy	Retrospective	88	5/37	FIR-NOS	GA< 32 weeks	28.03 ± 2.22	IVH
Woo et al., 2012(80)	Korea	Retrospective	246	35/52	FIR-NOS	GA ≤ 32 weeks	29.2 ± 1.9	ROP
Yamada et al., 2015(89)	Japan	NA	272	93/19	FIR-AC	GA< 34 weeks	25	Mortality; IVH; NEC; BPD
Ykema et al. 2018(103)	The Netherlands	Retrospective	88	12/3	FIR-AC	GA< 32 weeks	28.4 ± 5.43 ŧ	EOS
Zanardo et al., 2008(88)	Italy	Prospective	287	16/52	FIR-AC	GA< 32 weeks	31.7 ± 5.1	IVH
Zanardo et al., 2011(82)	Italy	Prospective	234	71/46	FIR-NOS	GA< 32 weeks	27.67 ± 3 ŧ	RDS

FIR-AC, fetal inflammatory response - Amsterdam Criteria; FIR-NOS, fetal inflammatory response - not otherwise specified; GA, gestational age; BW, birth weight; CCA, clinical chorioamnionitis; SGA, small for gestational age; RDS, respiratory distress syndrome; EOS, early onset sepsis; LOS, late-onset sepsis; IVH, intraventricular hemorrhage; PVL, periventricular leukomalacia; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; BPD, bronchopulmonary dysplasia; CP, cerebral paresis; ROP, retinopathy of prematurity; LOH, length of hospital stay.
ŧ Estimated from the median
**Median*
NA: not applicable
‡ Conference abstract

8.1.2. Study characteristics

An overview of the characteristics of the included studies is presented in Table 1. Most of the studies were observational cohort studies, and 1 study was a retrospective case-control study. Moreover, 25 articles included chorionic vasculitis as part of FIR. The remaining 25 studies either did not report on its status or separated it as a different entity. We included two conference abstracts(72, 113), and because of the high RoB, we also performed sensitivity analyses, omitting these articles from the analysis.

8.1.3. Risk of bias and quality assessment

Studies with moderate or high RoB were included for all outcomes, mainly due to the observational nature of cohort studies and the fact that most were not specifically designed to compare the two groups of interest. As a result, many lacked detailed information on confounding variables and group differences. Additionally, inconsistent or unclear outcome definitions contributed to the higher RoB. Publication bias was assessed for 11 outcomes, and evidence of bias was found for PVL based on funnel plot asymmetry and the Harbord test.

8.1.4. Synthesis of the results

8.1.4.1. Quantitative synthesis

Seventeen studies involving 3,547 preterm infants were analyzed for mortality. Among them, 62.4% had HCA with FIR. No significant association was found between FIR and mortality (OR: 1.18; 95% CI: 0.91–1.52), a result consistent in the FIR-AC subgroup (OR: 1.19; 95% CI: 0.72–1.97) (Figure 3).

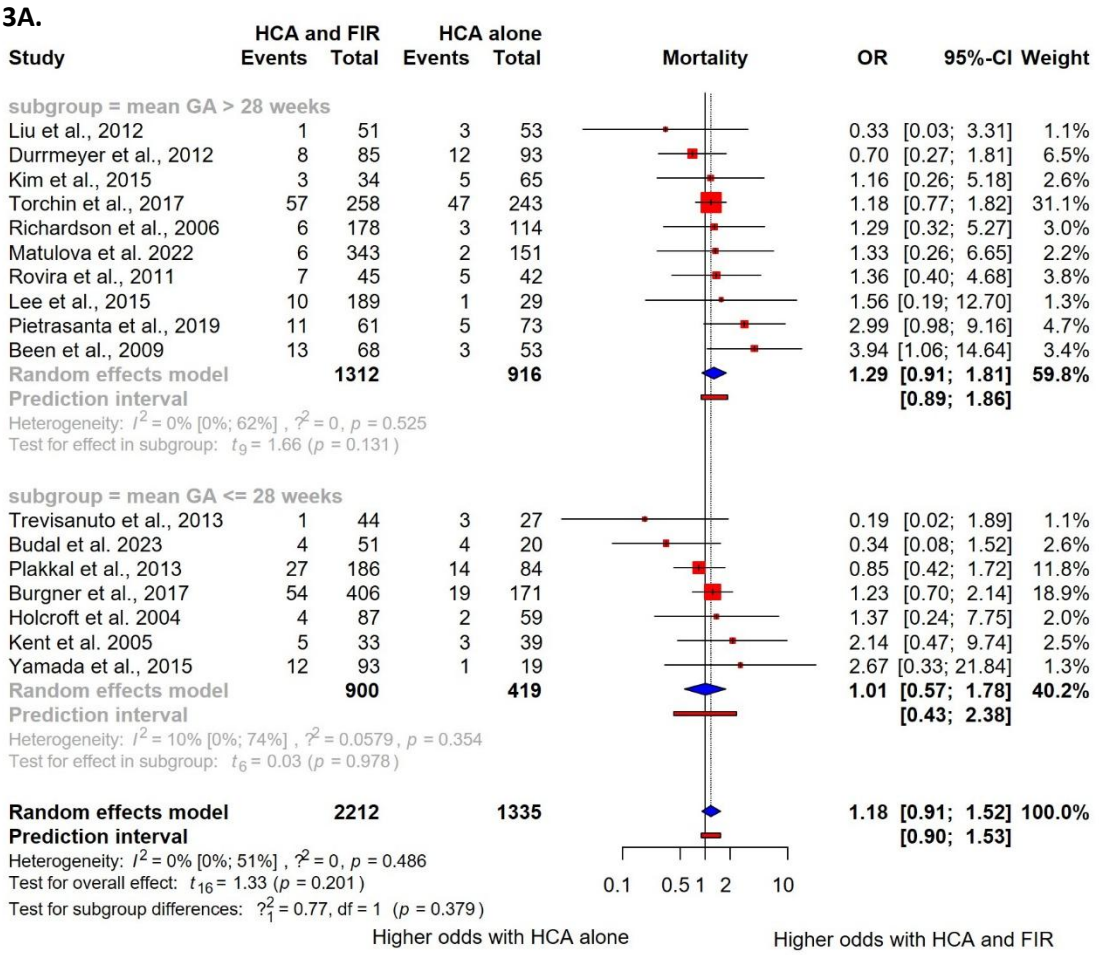


Figure 3*. No change in case of mortality. Forest plot with pooled odds ratio, representing the odds of mortality among preterm neonates with HCA and FIR and with HCA alone. 3A: FIR-NOS Group All studies included; 3B: FIR-AC Group*
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age

3B. (continued)

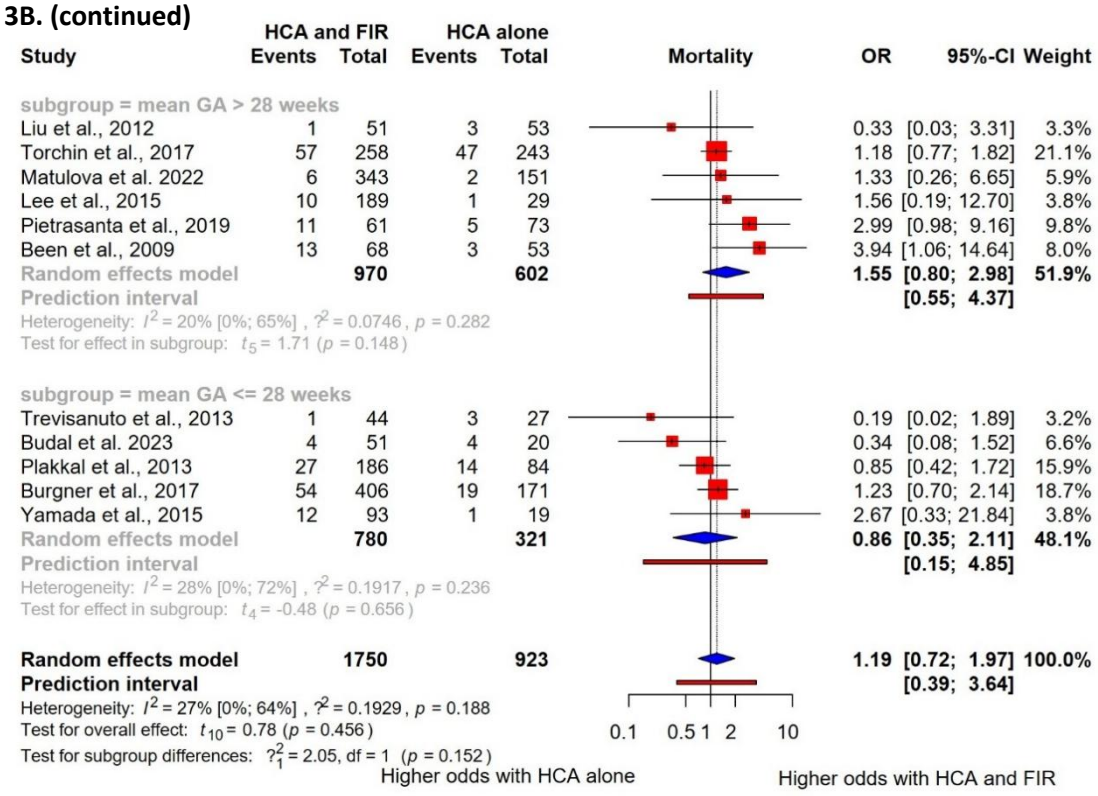


Figure 3*. No change in case of mortality. Forest plot with pooled odds ratio, representing the odds of mortality among preterm neonates with HCA and FIR and with HCA alone. 3A: FIR-NOS Group All studies included; 3B: FIR-AC Group*
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age

The odds of EOS was significantly higher in neonates with FIR (OR: 1.30; 95% CI: 1.02–1.66), especially in infants of lower gestational age. However, no difference was observed in the rates of LOS between the FIR and control groups (Figures 4 and 5).

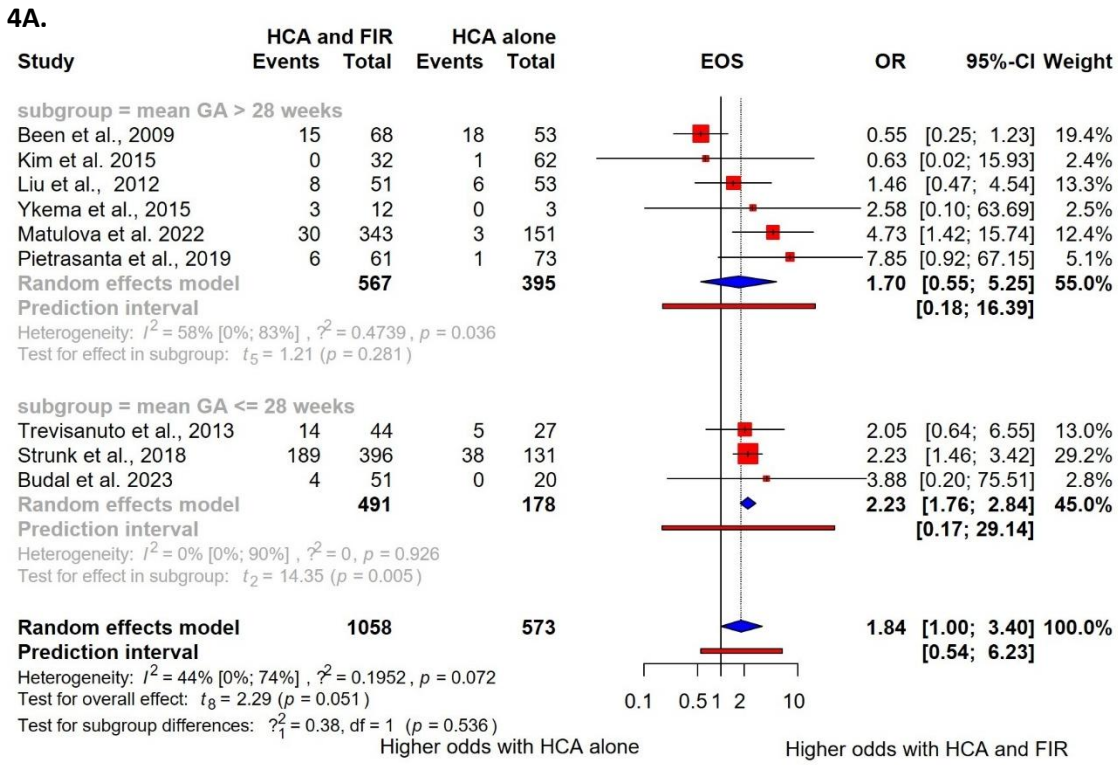


Figure 4. *The odds of early-onset sepsis. Forest plot with pooled odds ratio, representing the odds of early-onset sepsis among preterm neonates with HCA and FIR and with HCA alone.*
4A: FIR-NOS Group All studies included; 4B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, EOS: early-onset sepsis

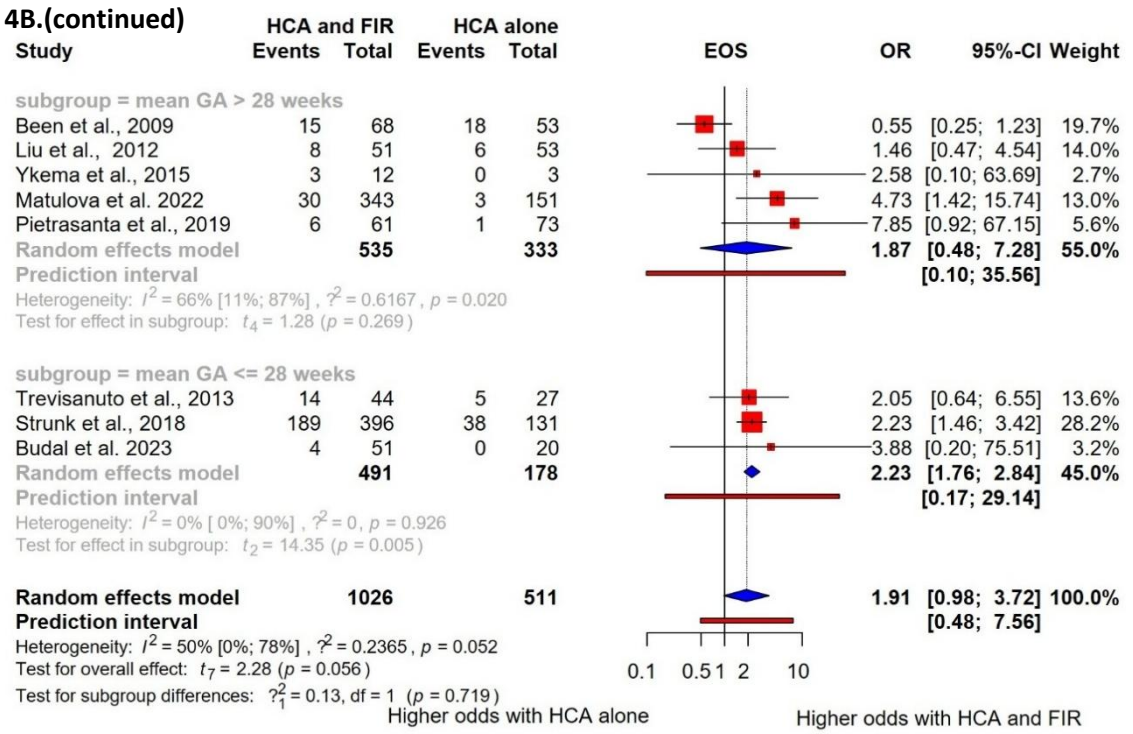


Figure 4. *The odds of early-onset sepsis. Forest plot with pooled odds ratio, representing the odds of early-onset sepsis among preterm neonates with HCA and FIR and with HCA alone.*

4A: FIR-NOS Group All studies included; 4B: FIR-AC Group

FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, EOS: early-onset sepsis

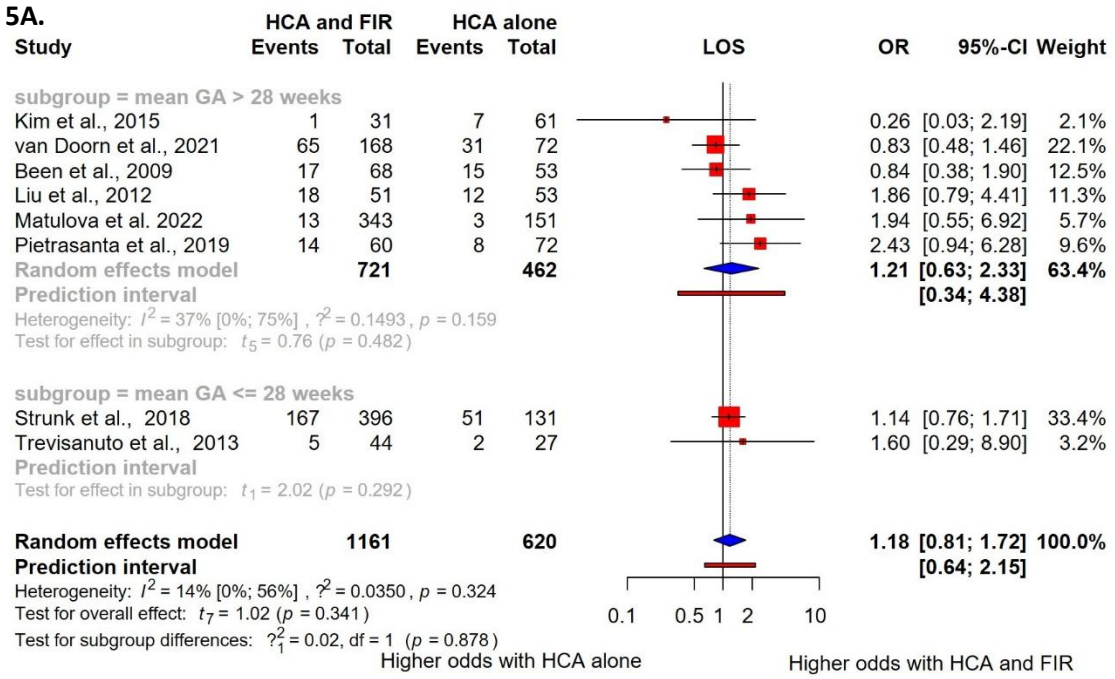


Figure 5. *No change in case of late-onset sepsis. Forest plot with pooled odds ratio, representing the odds of late-onset sepsis among preterm neonates with HCA and FIR and with HCA alone.*
5A: FIR-NOS Group All studies included; 5B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, LOS: late-onset sepsis

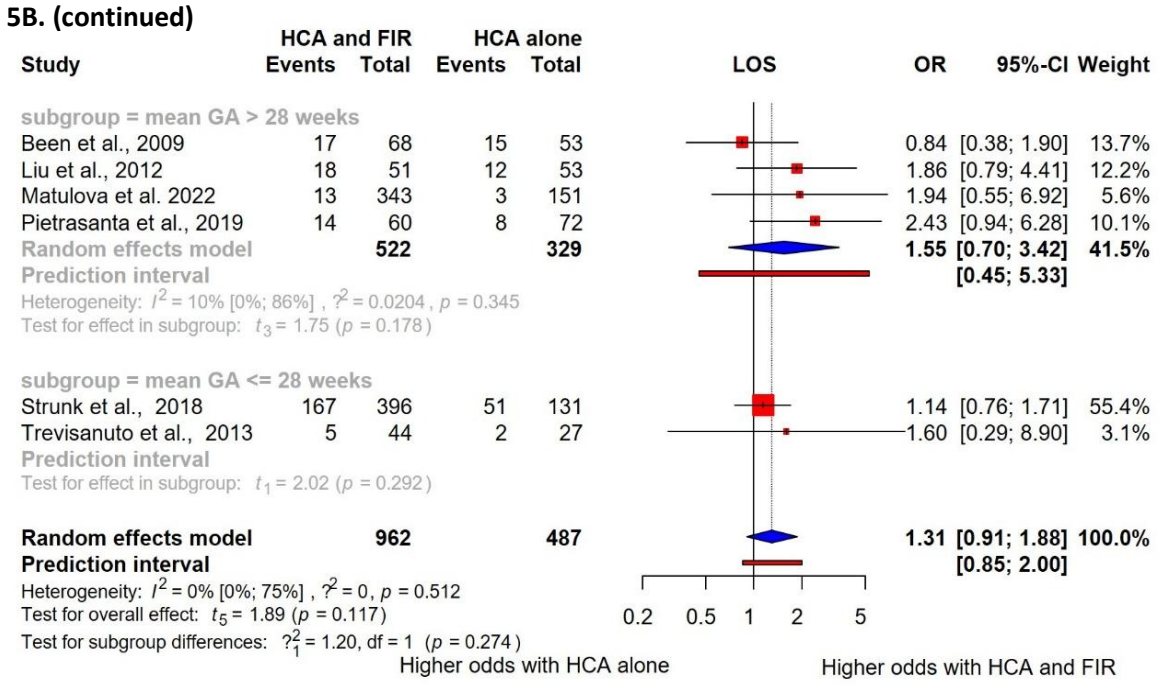


Figure 5. *No change in case of late-onset sepsis. Forest plot with pooled odds ratio, representing the odds of late-onset sepsis among preterm neonates with HCA and FIR and with HCA alone.*
5A: FIR-NOS Group All studies included; 5B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, LOS: late-onset sepsis

NEC was not significantly associated with FIR overall; however, in the subgroup of infants with lower gestational age, FIR was linked to a reduced odds of NEC (OR: 0.60; 95% CI: 0.41–0.90) (Figure 6).

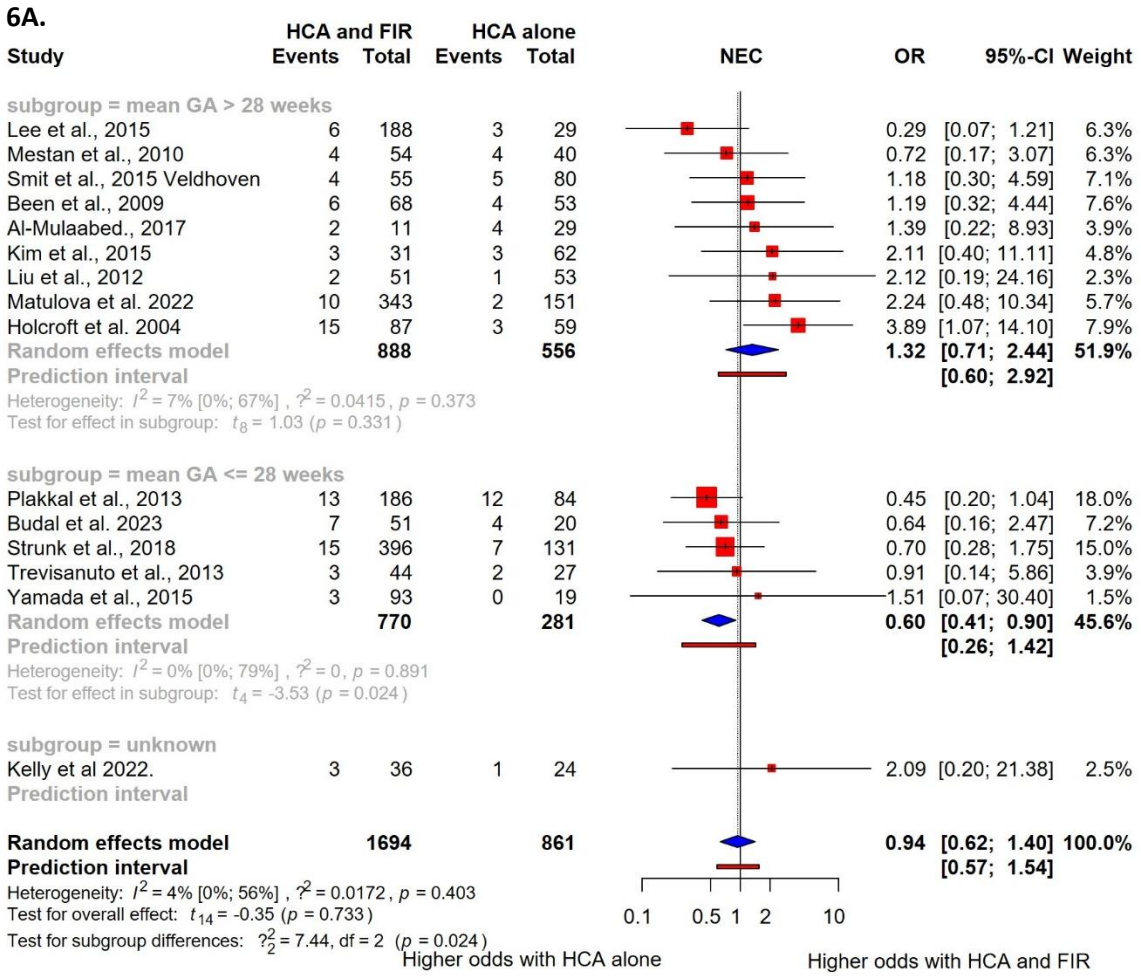


Figure 6. The odds of necrotizing enterocolitis. Forest plot with pooled odds ratio, representing the odds of NEC among preterm neonates with HCA and FIR and with HCA alone.
6A: FIR-NOS Group All studies included; 6B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, NEC: necrotizing enterocolitis

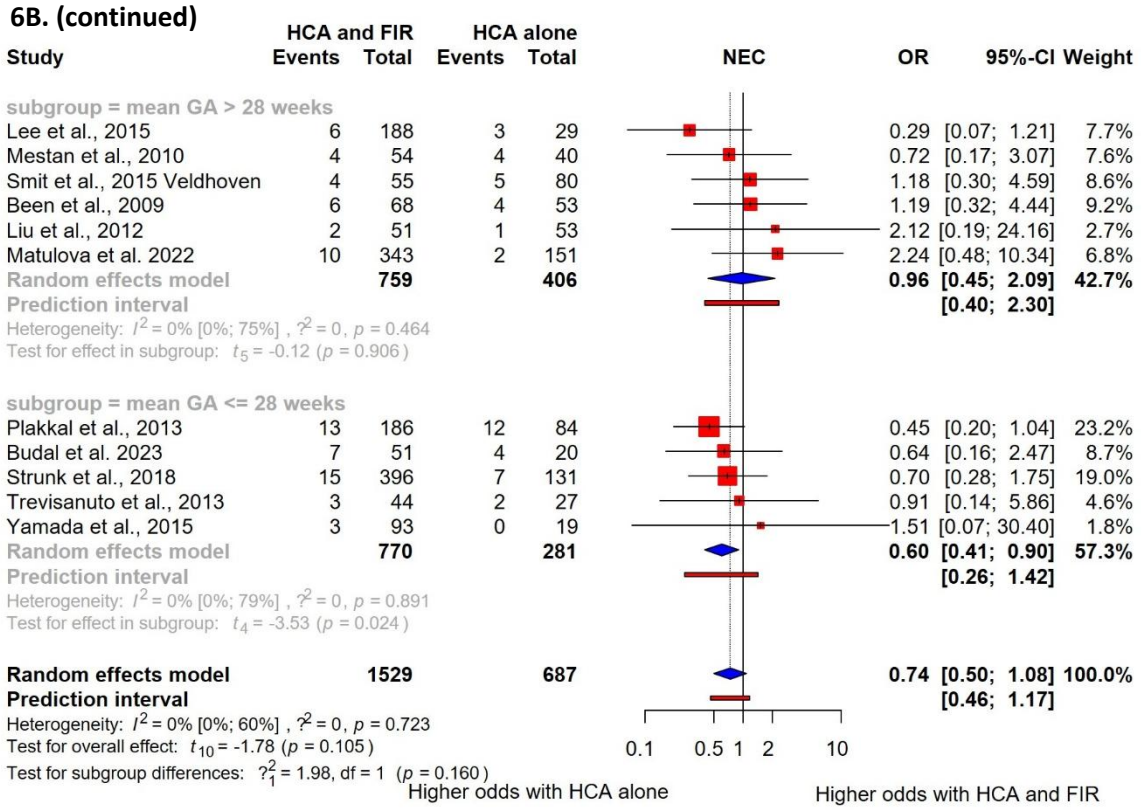


Figure 6. The odds of necrotizing enterocolitis. Forest plot with pooled odds ratio, representing the odds of NEC among preterm neonates with HCA and FIR and with HCA alone.
6A: FIR-NOS Group All studies included; 6B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, NEC: necrotizing enterocolitis

Although no overall association between FIR and BPD was found (OR: 1.32; 95% CI: 0.95–1.82), infants with lower mean gestational age showed significantly higher odds of BPD in the presence of FIR (Figure 7).

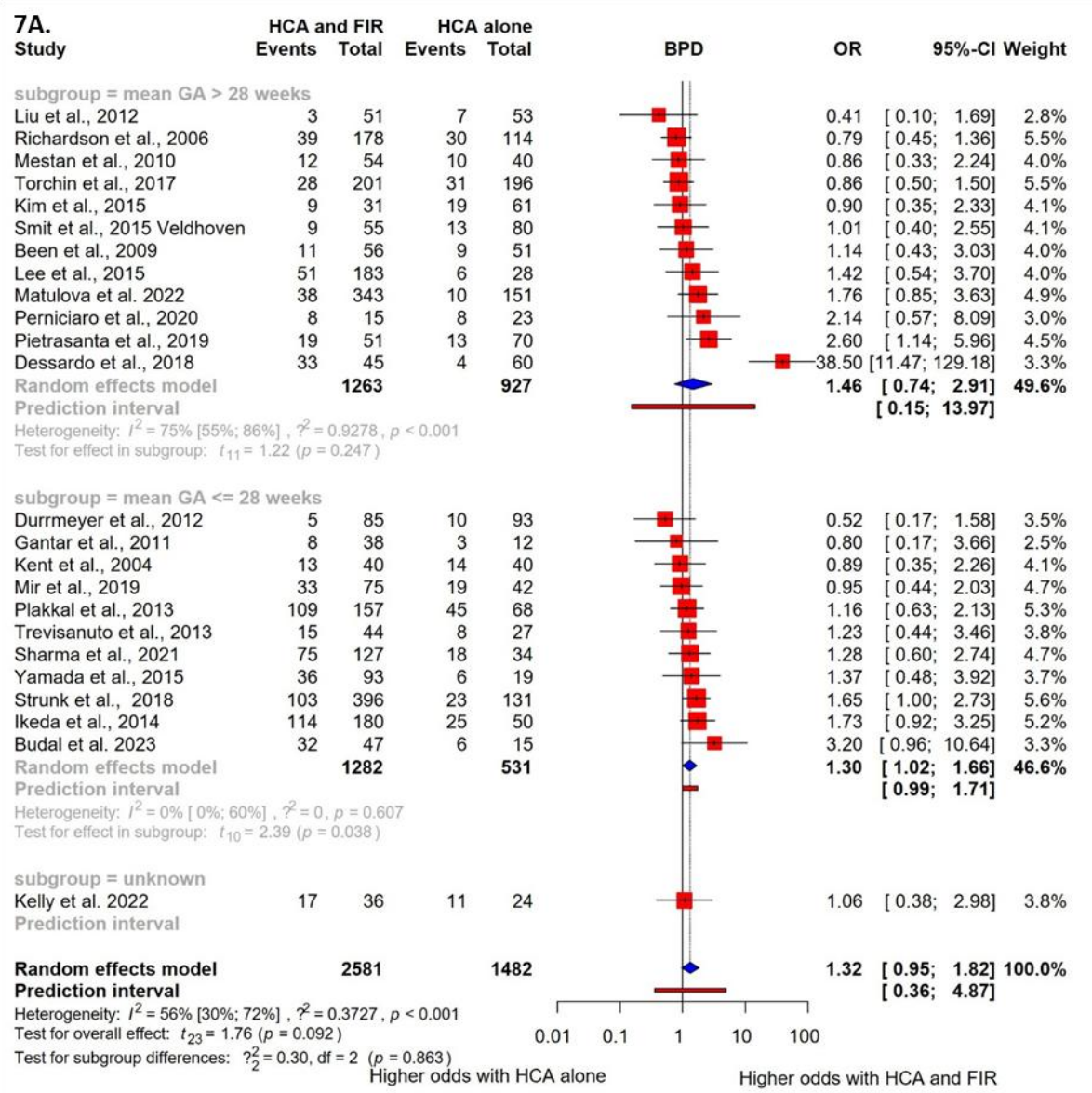


Figure 7. *The odds of bronchopulmonary dysplasia. Forest plot with pooled odds ratio, representing the odds of BPD among preterm neonates with HCA and FIR and with HCA alone.*
7A: FIR-NOS Group All studies included; 7B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, BPD: bronchopulmonary dysplasia

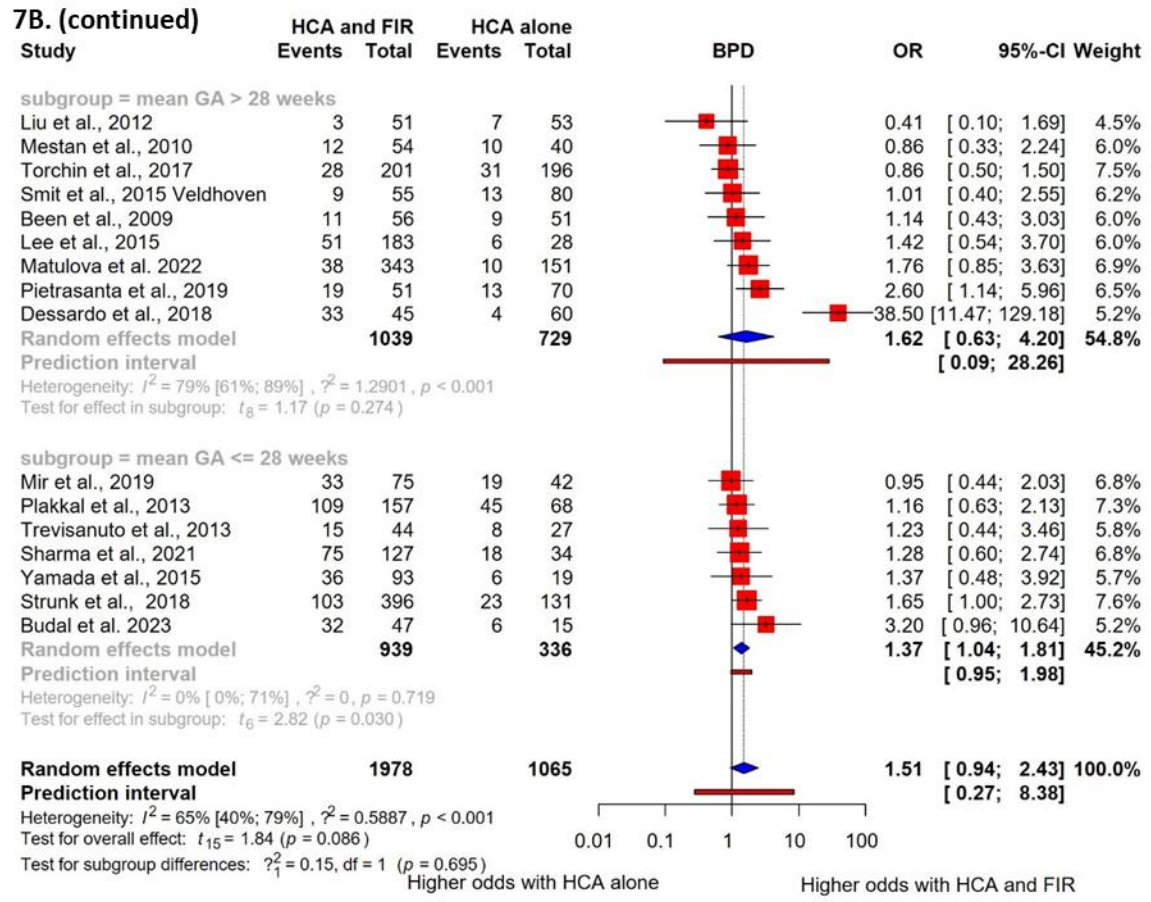


Figure 7. *The odds of bronchopulmonary dysplasia. Forest plot with pooled odds ratio, representing the odds of BPD among preterm neonates with HCA and FIR and with HCA alone.*
7A: FIR-NOS Group All studies included; 7B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, BPD: bronchopulmonary dysplasia

FIR was significantly associated with an increased risk of IVH (OR: 1.54; 95% CI: 1.18–2.02) (Figure 8), although this was not seen for severe IVH. ROP also showed higher odds with FIR (OR: 1.37; 95% CI: 1.03–1.82). No significant associations were found for PVL or CP.

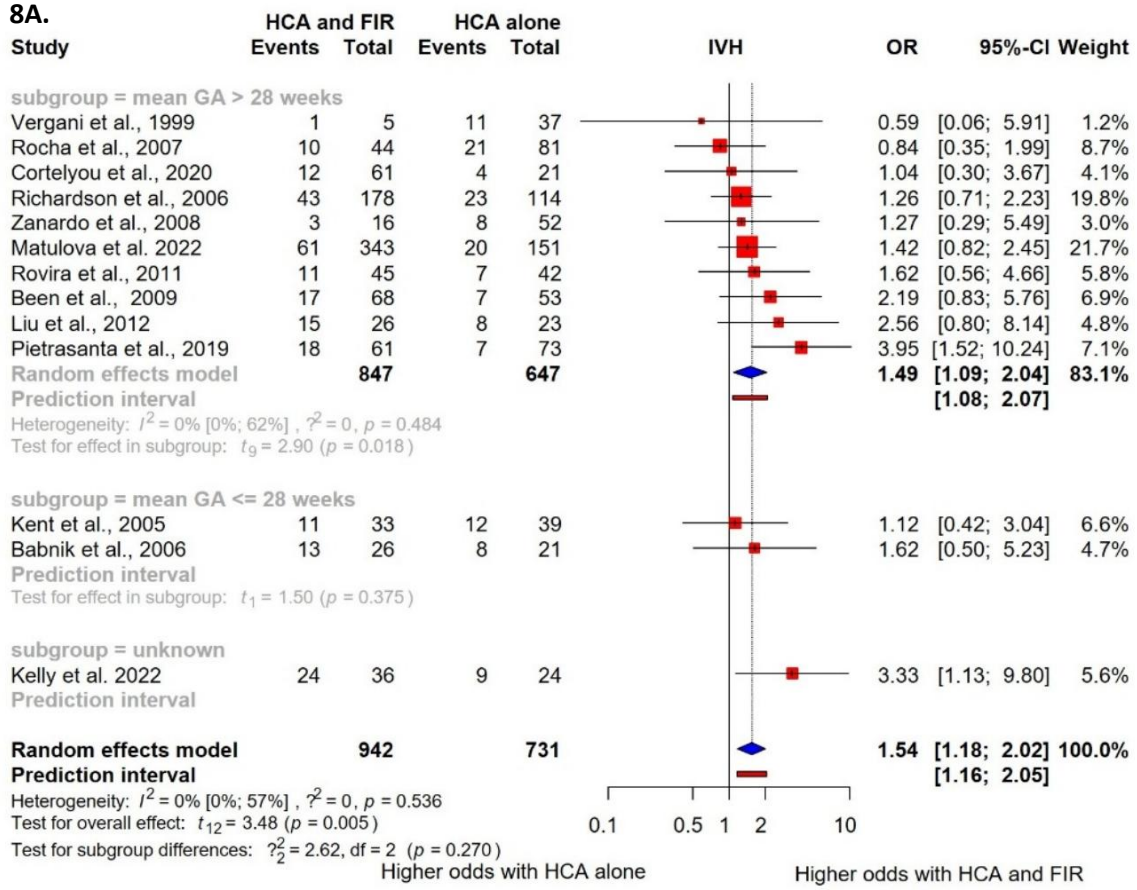


Figure 8. *Higher incidence of intraventricular hemorrhage in the case of FIR. Forest plot with pooled odds ratio, representing the odds of intraventricular hemorrhage among preterm neonates with HCA and FIR and with HCA alone.*
8A: FIR-NOS Group All studies included; 8B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, IVH: intraventricular hemorrhage

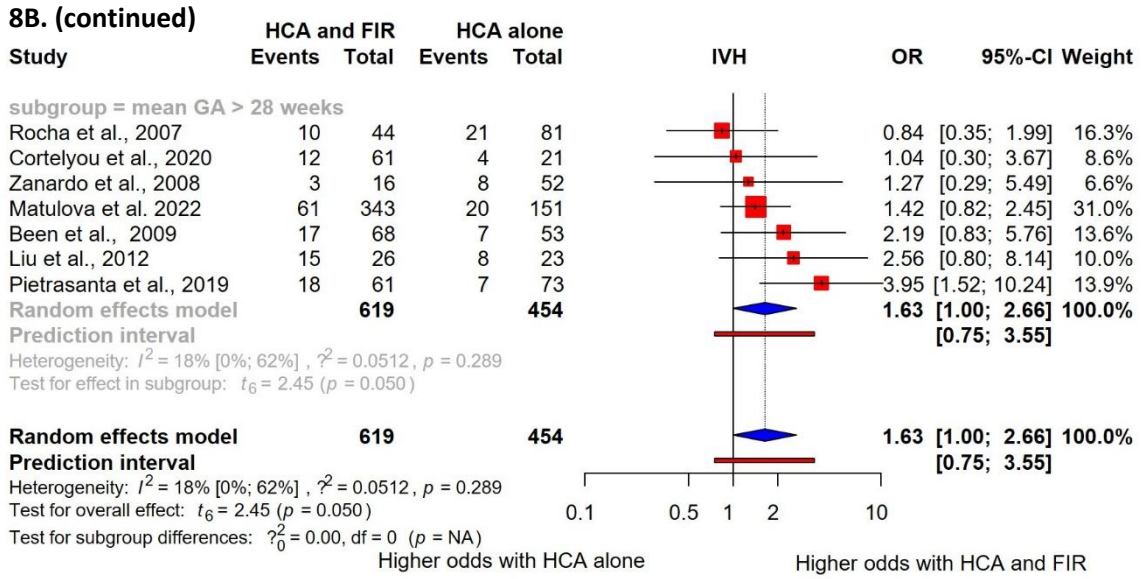


Figure 8. *Higher incidence of intraventricular hemorrhage in the case of FIR. Forest plot with pooled odds ratio, representing the odds of intraventricular hemorrhage among preterm neonates with HCA and FIR and with HCA alone.*
8A: FIR-NOS Group All studies included; 8B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, IVH: intraventricular hemorrhage

Clinical chorioamnionitis was nearly three times more likely in the presence of FIR (OR: 2.99; 95% CI: 1.96–4.55), although this association diminished in infants with lower mean gestational age (Figure 9).

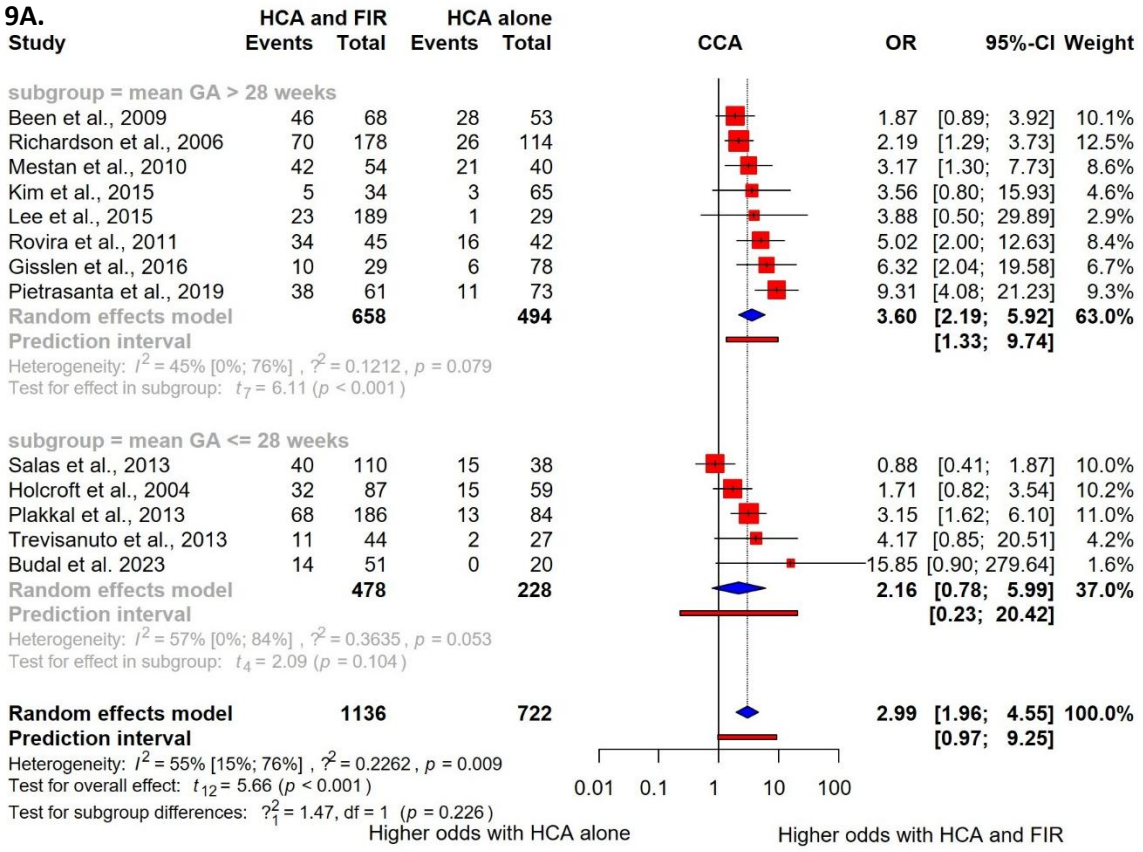


Figure 9. *Higher incidence of clinical chorioamnionitis in case of FIR. Forest plot with pooled odds ratio, representing the odds of clinical chorioamnionitis among preterm neonates with HCA and FIR and with HCA alone.*
9A: FIR-NOS Group All studies included;
9B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, CCA: clinical chorioamnionitis

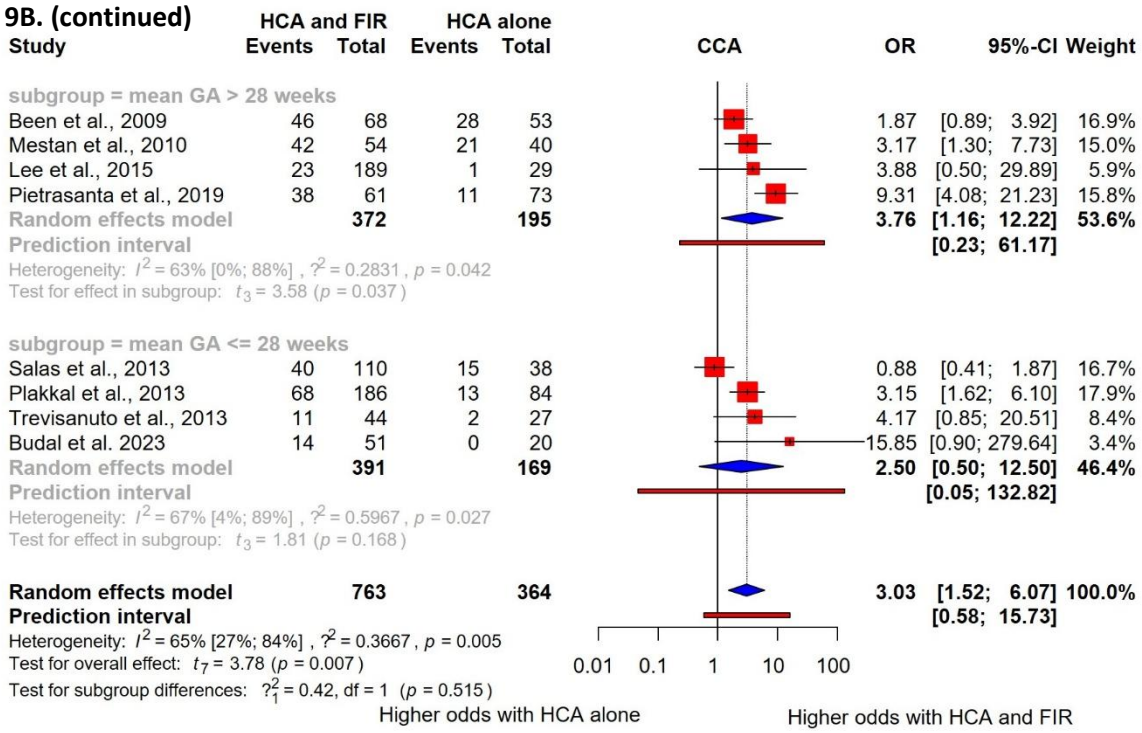


Figure 9. *Higher incidence of clinical chorioamnionitis in case of FIR. Forest plot with pooled odds ratio, representing the odds of clinical chorioamnionitis among preterm neonates with HCA and FIR and with HCA alone.*
9A: FIR-NOS Group All studies included;
9B: FIR-AC Group
FIR-NOS: Fetal inflammatory response – not otherwise specified, FIR-AC: Fetal inflammatory response – Amsterdam Criteria, HCA: Histological chorioamnionitis, FIR: Fetal inflammatory response, Mean GA: Mean gestational age, CCA: clinical chorioamnionitis

No differences were found between FIR and non-FIR groups in terms of RDS, PDA, SGA.

8.1.4.2. Qualitative synthesis

Four studies examined neurodevelopmental outcomes (32, 67, 85, 99); however, none demonstrated statistically significant differences between neonates exposed to FIR and those not exposed. Auditory outcomes, including hearing impairment or deafness, were evaluated in five studies(32, 67, 85, 95, 99), and visual impairment in four(32, 67, 85, 99) - none of which reported a significant association with FIR. Due to substantial methodological and reporting heterogeneity, these endpoints were excluded from quantitative analysis. Although the LOH was prespecified as an outcome, comparative analysis was not feasible due to variability in institutional discharge criteria and care capacities across the three reporting studies (Table 2.).

Table 2. Summary of the outcomes investigated in the systematic review

Outcome	**OR (CI 95%)**	**Subgroup analysis OR (CI 95%)**
Mortality	1.18 (0.91; 1.52)	H: 1.29 (0.91; 1.81) L:1.01 (0.57; 1.78)
Early-onset sepsis	1.84 (1.00; 3.40)	H: 1.7 (0.55; 5.25) L:2.23 (1.76; 2.84)
Late-onset sepsis	1.18 (0.81; 1.72)	
Necrotizing enterocolitis	0.94 (0.62; 1.40)	H: 1.32 (0.71; 2.44) L:0.6 (0.41; 0.90)
Bronchopulmonary dysplasia	1.3 (1.02; 1.66)	H: 1.46 (0.74;2.91) L:1.30 (1.02; 1.66)
Intraventricular hemorrhage	1.54 (1.18; 2.02)	
Severe intraventricular hemorrhage	1.22 (0.66; 2.24)	H: 1.16 (0.53; 2.58) L:1.75 (0.40; 7.78)
Retinopathy of prematurity	1.37 (1.03; 1.82)	
Periventricular leukomalacia	1.05 (0.54; 2.05)	
Cerebral palsy	1.22 (0.47; 3.18)	
Patent ductus arteriosus	1.32 (0.76; 2.28)	H: 1.78 (0.84; 3,75) L: 0.82 (0.44; 1.54)
Respiratory distress syndrome	0.93 (0.61; 1.44)	H: 1.14 (0.73; 1.79) L: 0.59 (0.18; 1.93)
Clinical chorioamnionitis	2.99 (1.96; 4.55)	H:9.60 (2.19; 5.92) L: 2.16 (0.78; 5.99)
Small for gestational age	0.91 (0.54; 1.52)	H: 0.68 (0.23; 1.96 L: 1.28 (0.57; 2.85)
Sensoric impairment	Narrative review	
Neurodevelopmental delay	Narrative review	
Length of hospital stay	Narrative review	

H: Pooled odds ratio in the subgroup of articles with higher mean gestational age. L: Pooled odds ratio in the subgroup of articles with lower mean gestational age.

Study II – Retrospective cohort study

Over a three-year period, a total of 256 placental and UC samples from preterm births were examined at the 1st Department of Pathology and Experimental Cancer Research, Semmelweis University. After excluding 19 cases, 237 preterm neonates were included in the final analysis (Figure 10). The cohort had a mean GA of 28.6 (± 2.8) weeks and an average birth weight of 1165 (± 420.4) grams.

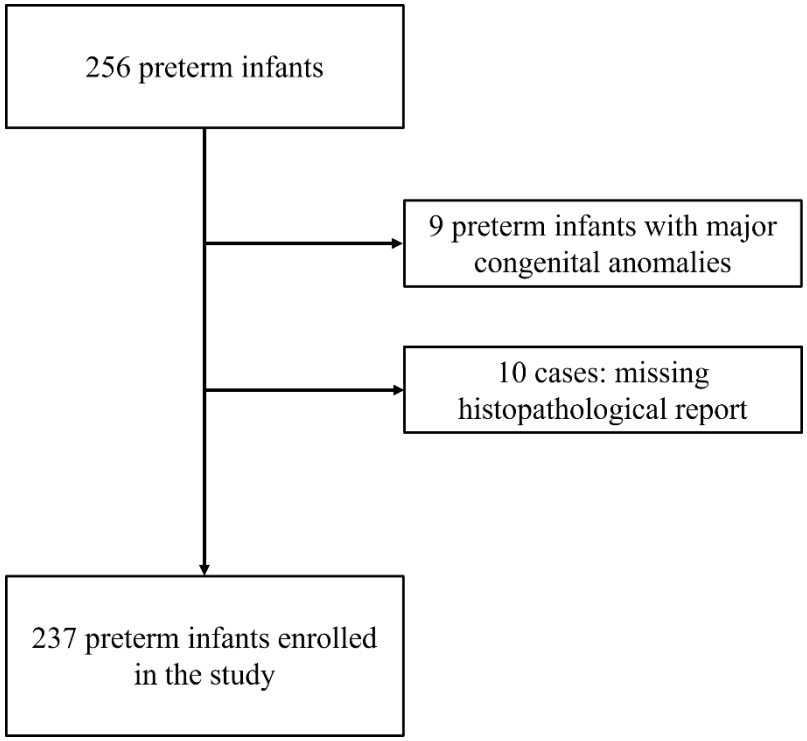


Figure 10. *Flowchart of the preterm infants included in the retrospective study*

8.1.5. Histological analysis

Based on histopathological findings, neonates were stratified into four groups (Figure 11.):

- Group 1 (n=154; 65%)**:** No histological signs of IUI
- Group 2 (n=34; 14.3%)**:** Presence of MIR only
- Group 3 (n=23; 9.7%)**:** MIR with FIR localized only on the placental side
- Group 4 (n=24; 10.1%)**:** MIR with FIR at both placental and fetal sides of the UC

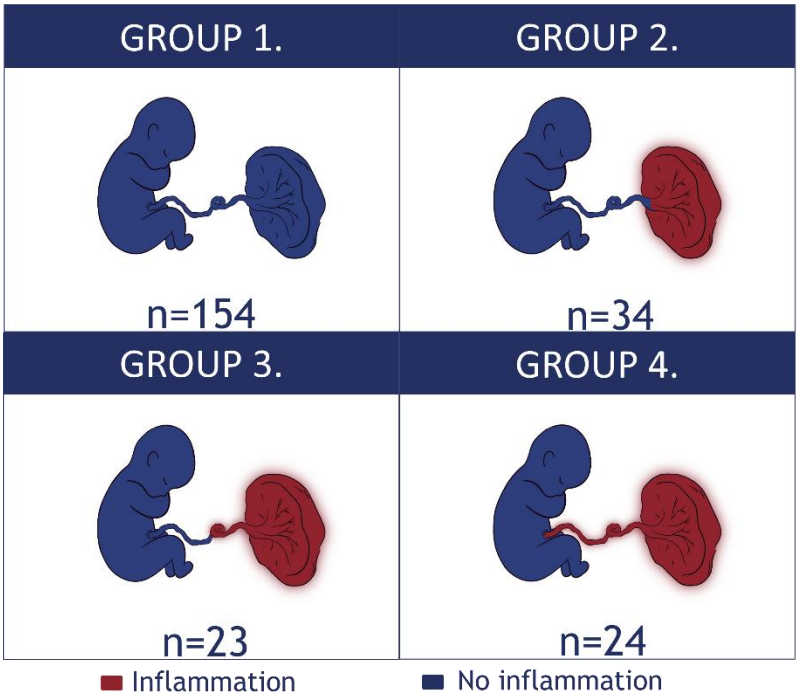


Figure 11. *Groups were created based on histological examination. Group 1: No histological signs of intrauterine inflammation. Group 2: Presence of maternal inflammatory response only. Group 3: Presence of maternal inflammatory response and fetal inflammatory response on the placental side. Group 4: Presence of maternal inflammatory response and fetal inflammatory response on both sides of the umbilical cord.*

Two additional cases (0.8%) presented atypical patterns and were excluded from group-wise comparisons: one with FIR limited to the fetal end of the UC and another with FIR on the fetal UC and placenta but not the placental UC.

Key observations regarding the location and severity of the inflammation:

- Predominance of inflammation in the placental side: FIR was more frequently identified on the placental side (UC or fetal chorioamnionitis) when MIR was present ($p < 0.001$, Table 3A).
- Correlation between FIR at different sites: The presence of FIR on the fetal UC end was strongly associated with concurrent FIR on the placental side ($p < 0.001$, Table 3B).
- Asymmetric inflammation: Unilateral inflammation was more common on the placental UC side than on the fetal side ($p = 0.003$, Table 3C).
- Severity of MIR by group (Figure 12): The distribution of stage 3 MIR across study groups 2, 3, and 4 was assessed. In cases where inflammation was limited to the placenta (Group 2), stage 3 MIR was observed in 18% of samples. This proportion increased to 30% when FIR on the placental side was present, and further rose to 71% when both the placental and fetal ends of the cord were involved (Group 4). Conversely, stage 1 MIR was identified in 66% of cases in Group 2, but was entirely absent in Group 4, indicating a correlation between broader inflammatory spread and increased severity of MIR.

Table 3. Findings of the topographical distribution of inflammation.

A.		**MIR and placental FIR**	**MIR and fetal FIR**	**p-value**
	MIR and FIR (m=48)	47	25	<0.001
B.		**Without placental FIR**	**With placental FIR**	**p-value**
	Fetal FIR (n=26)	2	24	<0.001
C.		**Isolated placental FIR**	**Isolated fetal FIR**	**p-value**
	Unilateral FIR (n=25)	23	2	0.003

FIR: fetal inflammatory response; MIR: maternal inflammatory response

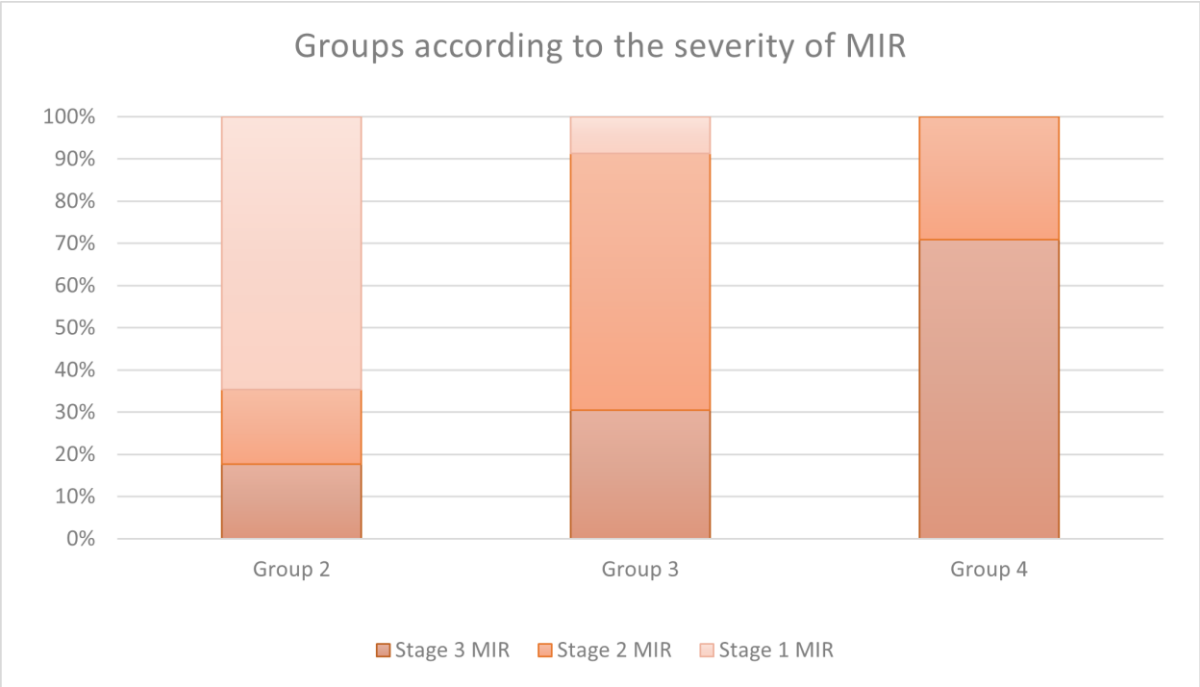
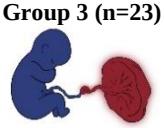


Figure 12. *Groups according to the severity of MIR. The rate of stage 1 MIR was higher in Group 2. The rate of stage 3 MIR was higher in Group 4.*
MIR: maternal inflammatory response

8.1.6. Maternal and perinatal characteristics

Analysis of perinatal variables revealed significant differences among the groups in cesarean section rate ($p = 0.001$), multiple gestation rate ($p = 0.004$), mean GA ($p < 0.001$), birth weight ($p = 0.031$), and incidence of IUGR ($p < 0.001$) (Table 4). Pairwise comparisons showed higher cesarean delivery rates and GA in Group 1, increased multiple pregnancy rates in Group 1 compared to Group 2, and lower birth weight in Group 2 compared to Group 1. No statistically significant groupwise differences in IUGR were observed, despite overall group variation.

Table 4. Analysis of perinatal factors based on the extent of inflammation.

Perinatal data	Group 1 (n=154) 	Group 2 (n=34) 	Group 3 (n=23) 	Group 4 (n=24) 	p-value (Fisher-test)
Caesarean sections	150 (97.4%; 0.94; 0.99)	25 (73.5%; 0.56; 0.87)	10 (43.5%; 0.23; 0.66)	17 (70.8%; 0.49; 0.87)	0.001
Multiple pregnancies	56 (36.4%; 0.29; 0.45)	10 (29.4%; 0.15; 0.48)	4 (17.4%; 0.05; 0.39)	1 (4.2%; 0.001;0.21)	0.004
Complete steroid prophylaxis	92 (59.7%; 0.52; 0.68)	16 (47.1%; 0.3; 0.65)	10 (43.5%; 0.23; 0.66)	14 (58.3%; 0.37; 0.8)	0.3
Any steroid prophylaxis	140 (90.9%; 0.85; 0.95)	32 (94.1%; 0.8; 0.99)	21 (91.3%; 0.72; 0.99)	24 0.86-1.00	0.33
Gender (female)	74 (48.1%; 0.4; 0.56)	16 (47.1; 0.3; 0.65)	8 (34.8%; 0.16; 0.57)	9 (37.5%; 0.19; 0.6)	0.575
Birthweight (grams)	1217.73 (1149,34; 1286.11)	1016.18 (898.79; 1133.57)	1079.57 (883; 1275.45)	1131.25 (960.39; 1302.11)	0.031
Gestational age (weeks)	29.33 (28.92; 29.75)	27.35 (26.45; 28.26)	26.7 (25.38; 28.02)	27.33 (26.29; 28.37)	<0.001
IUGR	32 (20.8%; 0.15; 0.28)	1 (2,9%; 0.001; 0.15)	0 0.00-0.15	0 0.00-0.14	<0.001

Data are presented as n (%; 95%CI) or the median (%; 95%CI). P values were assessed using Fisher's exact test. IUGR: intrauterine growth retardation

Regarding maternal factors (Table 5), significant differences between groups were observed in rates of preeclampsia ($p < 0.001$), maternal fever ($p < 0.001$), leukocytosis ($p < 0.001$), elevated CRP ($p = 0.005$), and positive cervical swab cultures ($p = 0.004$). Preeclampsia was most common in Group 1. Elevated maternal CRP and WBC were more frequent in FIR-associated groups, and Group 4 exhibited the highest proportion of positive cervical microbiological findings.

Table 5. Analysis of maternal factors based on the extent of inflammation

Maternal data	Group 1 (n=154) 	Group 2 (n=34) 	Group 3 (n=23) 	Group 4 (n=24) 	p-value (Fisher-test)
PPROM	27 (17.5%; 0.12; 0.25)	6 (17.6%; 0.07; 0.35)	5 (21.7%; 0.08; 0.44)	6 (25%; 0.1; 0.47)	0.76
Preeclampsia	39 (25.3%; 0.19; 0.33)	1 (2.9%; 0.001; 0.15)	0 (0.00-0.15)	0 (0.00-0.14)	< 0.001
Placental abruption	17 (11%; 0.07; 0.17)	6 (17.6%; 0.07; 0.35)	5 (21.7%; 0.08; 0.44)	0 (0.000-0.14)	0.06
Maternal fever	0 (0.000-0.024)	0 (0.000-0.103)	4 (17.4%; 0.05; 0.39)	2 (8.3%; 0.01;0.27)	< 0.001
Maternal WBC count (G/L)	12.25 (11.54; 12.96)	13.3 (11.68; 14.92)	17.49 (14.63; 20.35)	17.16 (14.85; 19.46)	< 0.001
Elevated maternal CRP	18/107 (16.8%, 0.10-0.25)	10/26 (38.5%, 0.20-0.59)	11/19 (57.9%, 0.34-0.8)	13/23 (56.5%, 0.35-0.77)	0.005
Positive cervical swab culture	37/92 (40.2%; 0.3; 0.51)	10/18 (55.6%; 0.30; 0.79)	8/12 (66.7%; 0.349; 0.901)	17/21 (81%;0.581;0.946)	0.004
Positive urine culture	5/22 (22.7%; 0.08; 0.45)	5/8 (62.5%; 0.25; 0.92)	1/4 (25%; 0.01; 0.81)	2/7 (28.6%; 0.04; 0.71)	0.238

Data are presented as n (%; 95%CI) or the median (%; 95%CI). P values were assessed using Fisher's exact test. PPROM: preterm premature rupture of membranes; WBC: white blood cell.

8.1.7. Neonatal outcomes

Clinical and laboratory data of the neonates revealed significant differences in the incidence of IVH ($p = 0.001$), severe IVH ($p = 0.001$), and postnatal WBC count ($p < 0.001$) (Table 6). Pairwise tests indicated increased IVH rates in Groups 3 and 4 compared to Group 1, a greater incidence of severe IVH in Group 3 relative to Group 1, and elevated WBC counts in Groups 3 and 4 compared to Group 1.

Table 6. Analysis of clinical data of preterm infants based on the extent of inflammation

Clinical data of preterm infants	Group 1 (n=154) 	Group 2 (n=34) 	Group 3 (n=23) 	Group 4 (n=24) 	p-value (Fisher-test)
WBC	7.25 (6.78; 7.72)	8.69 (6.59; 10.8)	12.16 (8.49; 15.82)	18.03 (12.9; 23.16)	<0.001
CRP	24/152 (13.7%; 0.09;0.2)	4/33 (10.8; 0.03; 0,25)	6/22 (27.3%; 0.11; 0.5)	3/24 (12.5%; 0.03; 0.33)	0.482
IVH	32/132 (24%; 0.17; 0.33)	13/32 (40.6%; 0.24; 0.59)	13/22 (59.1%; 0.36; 0.79)	13/23 (56.5%; 0.35; 0.77)	0.001
Severe IVH	4/132 (3.03%; 0.01; 0.08)	3/32 (9.4%; 0.02; 0.25)	8/22 (36.4%; 0.17; 0.59)	1/23 (4.35%; 0.001; 0.22)	0.001
PVL	6/132 (4.5%; 0.02; 0.1)	3/32 (9.4%; 0.02; 0.25)	2/22 (9.1%; 0.01; 0.3)	4/23 (17.4%; 0.05; 0.39)	0.086
RDS	92/154 (59.7%; 0.51; 0.68)	22/34 (64.7%; 0.47; 0.8)	18/23 (78.3%; 0.56; 0.93)	18/24 (75%; 0.53; 0.9)	0.238
PDA	34/154 (22.1%; 0.16; 0.3)	7/34 (20.6%; 0.09; 0.38)	9/23 (39.1%; 0.2; 0.62)	3/24 (12.5%; 0.03; 0.32)	0.204
NEC	9/154 (5.8%; 0.03; 0.11)	4/34 (11.8%; 0.03; 0.28)	2/23 (8.7%; 0.01; 0.28)	2/24 (8.3%; 0.01; 0.25)	0.487
Neonatal mortality	14/154 (9.1%; 0.05; 0.15)	6/34 (17.6%; 0.07; 0.35)	5/23 (21.7%; 0.08; 0.44)	1/24 (4.2%; 0.001; 0.21)	0.113

Data are presented as n/N (%; 95%CI) or the median (%; 95%CI). P-values were assessed using Fisher's exact test. WBC white blood cell count; IVH, intraventricular haemorrhage; PVL, periventricular leukomalacia; RDS, respiratory disstress syndrome; PDA, patent ductus arteriosus; NEC, necrotizing enterocolitis

Adjusted regression analyses confirmed that FIR presence was significantly associated with increased neonatal WBC and a higher risk of severe IVH (particularly in Group 3). Group 4 was associated with a reduced incidence of PDA, independent of GA.

9. DISCUSSION

9.1. Summary of findings, international comparisons

9.1.1. Study I and II

Studies I and II together offer a comprehensive investigation into the role of FIR in preterm infants' outcomes, combining extensive epidemiological evidence with detailed anatomical findings. Study I, a systematic review and meta-analysis, is the most extensive synthesis to date examining FIR in relation to short- and long-term neonatal outcomes. Our results indicate that FIR is significantly linked to higher odds of EOS, IVH, and ROP. The odds of BPD were also increased in the subgroup of infants with lower GA. Study II, a retrospective cohort study conducted at Semmelweis University, provides additional insight by mapping the spatial progression of IUI from the placenta through the UC.

Several prior meta-analyses (31, 33, 35, 114-116) have examined the relationship between HCA and adverse neonatal outcomes, some of which included FIR as part of the fetal inflammatory response syndrome (FIRS). Our review focused strictly on histologically confirmed FIR and its specific association with neonatal morbidity.

In infants with a mean gestational age below 28 weeks, we observed an increased incidence of BPD, suggesting a GA-dependent vulnerability to inflammation-related pulmonary injury. The literature shows similar inconsistencies - while BPD is often linked to IUI, its role as an independent risk factor remains unproven (31). Experimental studies suggest that IUI can promote fetal lung maturation through exposure to microbial components; however, this accelerated maturation may result in structural changes that predispose the neonate to impaired lung function(117, 118).

We also observed a significant association between FIR and EOS, contrasting with earlier meta-analysis - likely due to the inclusion of more recent studies or differing population criteria (119).

In contrast, our analysis revealed a lower incidence of NEC in this subgroup. Since NEC is multifactorial and heavily influenced by local feeding and antibiotic protocols, this may explain the variation across studies (120).

No significant associations were identified with LOS or PDA, which aligns with previous

studies (33, 116, 119). However, consistent with prior work by Villamor-Martinez et al., we confirmed a higher risk of ROP in neonates with FIR when analyzing a larger dataset (115). We also showed an increased risk of IVH in infants with FIR and HCA, although this did not apply to severe IVH. This finding did not align with Villamor-Martinez et al. (32); however, there were significant differences in the design and the number of studies included. Regarding the comparison between the analysis of IVH and severe IVH, the variability in grading systems and the multifactorial origin of parenchymal hemorrhage may explain this discrepancy (121-123).

Our analysis found a threefold increase in CCA among infants exposed to both HCA and histological FIR. This indicates that HCA alone is rather clinically silent, and FIR may develop with prolonged or more intense inflammatory exposure. Over 90% of CCA cases result in delivery within 12 hours (124), suggesting a potential chronological association in which FIR might represent a later stage in the inflammatory process. However, due to the inherent limitations of the current study and the research question itself, the chronological progression of chorioamnionitis cannot be definitively confirmed.

The findings from Study II showed that inflammation tends to progress from the placenta toward the fetal side of the UC, and that this progression is associated with increasing severity of MIR (125, 126).

This supports prior observations that FIR correlates with more severe MIR and elevated inflammatory markers, reinforcing its role as a marker of advanced inflammation (21).

Although limited, previous literature on the topographical progression of FIR presents mixed interpretations. While Kim et al.(100) suggested a random onset along the UC, Damman et al.(38) proposed a fetal origin, and Katzman et al.(36) reported no frequency difference between UC ends but found higher FIR stages at the placental side - aligning with our findings. Variations in sampling, definitions, and gestational age likely explain the variability among studies.

The anatomical distribution of FIR may also be influenced by the dynamics of fetal circulation. Slower venous flow in the UC favors neutrophil infiltration, while higher arterial flow may delay inflammatory cell extravasation, except where anatomical structures (e.g., Hyrtl’s anastomosis) reduce flow velocity (127, 128).

Significant differences in cesarean section rates, multiple gestation, preeclampsia, and IUGR reflect the distinct etiologies of preterm birth. Spontaneous preterm birth is frequently associated with IUI, which triggers the upregulation of pro-inflammatory mediators, leading to elevated prostaglandin levels. These changes promote uterine contractility, enzymatic degradation of the chorioamniotic extracellular matrix, and cervical ripening through the stimulation of matrix metalloproteinases (15, 129). On the other hand, preeclampsia and IUGR are common triggers for medically indicated preterm delivery, typically via cesarean section. Furthermore, multiple gestations remain a well-established risk factor for preterm delivery (130-132).

Consistent with previous studies, our findings also demonstrated lower gestational age and birthweight in preterm infants affected by IUI (70). This observation raises the question of whether IUI acts as an independent factor driving adverse perinatal outcomes or simply reflects the underlying processes linked to prematurity.

Maternal fever, leukocytosis, and positive cervical cultures were more frequent in groups with FIR, further supporting the link between histological severity and clinical chorioamnionitis, as described in previous studies.

FIR was associated with elevated neonatal WBC counts and increased risk of IVH, even after adjusting for gestational age. These findings support earlier research identifying histological chorioamnionitis as a risk factor for IVH (1, 133). This association may be explained by both the direct and indirect effects of pro-inflammatory mediators. Certain cytokines can act directly on vascular smooth muscle, causing vasodilation and hypotension, or indirectly by stimulating endothelial cells to release vasoactive substances. Additionally, pro-inflammatory cytokines can initiate a neuronal inflammatory cascade, promoting the activation and adhesion of platelets and neutrophils via the upregulation of chemoattractants and adhesion molecules. These mechanisms may contribute to endothelial injury, altered blood rheology, and impaired cerebral perfusion - factors that collectively increase the vulnerability of preterm infants to IVH (134).

Interestingly, the incidence of PDA was significantly lower in the most severe FIR group. Although the relationship between IUI and PDA is not fully understood, it has been

hypothesized that inflammation may promote early ductal closure, similar to its effect on lung maturation (135).

By integrating these two studies, several critical insights emerge. First, FIR is not a binary phenomenon but a progressive, spatially dynamic process that can extend from maternal to fetal tissues. Second, the anatomical location and extent of FIR provide important prognostic information.

9.1.2. Study III

This thesis presents an open-label, two-armed, randomized clinical trial designed to evaluate the impact of an additional pre-extubational loading dose of caffeine citrate in preterm infants. The central hypothesis is that a single, timely administered extra dose - given in the critical peri-extubation period - may enhance extubation success by achieving higher plasma caffeine levels when respiratory stability is most vulnerable.

Reintubation in preterm neonates has been associated with a range of complications, including increased mortality, ventilator-associated pneumonia, pneumothorax, and neurological injury (136). Previous studies have suggested that higher maintenance doses of caffeine may reduce the likelihood of extubation failure (55, 137, 138). However, findings across trials remain inconsistent, particularly regarding long-term benefits and optimal dosing strategies (139). Unlike these studies, our trial specifically examines the effect of a one-time, higher loading dose administered immediately prior to extubation - targeting a window where pharmacological support may be most effective.

Participant enrollment began in December 2023, with study completion anticipated by 2027. The finalized first version of the protocol was completed on December 14, 2023.

9.2. Strengths

The overarching strength of this thesis lies in its comprehensive, multidisciplinary approach to addressing inflammation-related morbidity in preterm infants. By integrating meta-analytic evidence, original histopathological investigation, and a forward-looking clinical trial protocol, this work contributes novel insights and practical tools to both the scientific and clinical communities.

A key strength of the meta-analysis is its methodological rigor and adherence to PRISMA guidelines, including pre-registration, risk of bias assessment using the QUIPS tool, and sensitivity analyses. It synthesizes data from 50 studies, making it the largest quantitative assessment to date of the impact of histological FIR on neonatal outcomes. Importantly, the meta-analysis distinguishes between studies using the definitions of the Amsterdam Placental Workshop Group Consensus Statement and those with less specific definitions, allowing for higher-resolution subgroup analyses. The use of a bivariate model to calculate proportions and heterogeneity-adjusted estimates further strengthens its statistical reliability.

The retrospective cohort analysis introduces a novel histopathological framework for understanding the topography and severity of intrauterine inflammation. Unlike earlier studies, this study utilized the Amsterdam Workshop Group Consensus Statement definitions to consistently classify both maternal and fetal inflammatory responses. The inclusion of histological samples from three distinct locations (placenta, placental end of the UC, and fetal end of the UC) allowed for unprecedented spatial mapping of IUI progression. By correlating these patterns with clinical and laboratory outcomes, the study highlights the importance of inflammation distribution in predicting neonatal outcomes.

The NEOKOFF randomized controlled trial protocol is a major strength, as unlike prior studies that investigated daily high-dose caffeine regimens with mixed results, NEOKOFF focuses on targeted, time-sensitive administration. The protocol adheres to SPIRIT guidelines, incorporates stratified randomization, and includes intention-to-treat analysis, ensuring future data will be robust. Ethical approvals have been secured, and recruitment is underway, indicating feasibility and institutional support.

9.3. Limitations

The studies also have several limitations. The meta-analysis is affected by the inherent heterogeneity in the included studies, particularly regarding definitions of FIR, outcome measures, and study populations. Most of the included studies had a moderate to high risk of bias, largely due to their retrospective designs and the presence of unadjusted confounders.

Regarding Study I, although our cohort study is innovative, it is a single-centre retrospective study, and its generalizability is limited by sample size and the relatively low incidence of

severe FIR cases. Furthermore, as with any histological study, sampling error and variability in tissue processing cannot be completely ruled out.

The limitations of the NEOKOFF trial stem from the open-label design that could introduce performance bias, and the strict eligibility criteria may prolong recruitment or limit generalizability across NICUs with differing patient demographics or extubation practices. The decision to exclude therapeutic drug monitoring, although justified, may also be seen as a trade-off between feasibility and pharmacokinetic rigor.

Across studies, a central challenge lies in the translational gap between histopathological findings and clinical implementation. Placental diagnostics often occur postnatally, while clinical interventions such as extubation occur within hours of birth, limiting the immediate applicability of placental histology in real-time decision-making. However, this thesis attempts to bridge that gap by proposing routine UC sampling and leveraging known inflammatory patterns to inform prospective care strategies.

10. CONCLUSION

This thesis explored the impact of IUI and respiratory support strategies on the health of preterm infants. The presence and severity of FIR were shown to be significant contributors to adverse neonatal outcomes such as sepsis, IVH, and BPD. Moreover, the specific localization of IUI was found to carry important prognostic value, highlighting the importance of detailed placental examination in clinical practice.

In parallel, this work addressed one of the most critical challenges in neonatal care - ensuring successful extubation in preterm infants. Recognizing the vulnerability of these infants during the peri-extubational period, we proposed a targeted therapeutic approach using an additional pre-extubational loading dose of caffeine citrate to support respiratory transition and reduce the risk of reintubation.

Together, the findings presented in this thesis underscore the importance of integrating precise pathological assessment with evidence-based clinical interventions to improve neonatal outcomes.

11. IMPLEMENTATION FOR PRACTICE

The results presented in this thesis highlight practical pathways for improving neonatal care in the context of preterm birth. First, a more detailed assessment of the placenta and UC - especially the localization of inflammatory processes - can enhance early risk stratification of preterm infants. The integration of pathological findings into clinical decision-making can ultimately contribute to more tailored and timely neonatal care.

Additionally, careful timing and dosing of supportive therapies, such as respiratory stimulants around extubation, may help reduce the incidence of reintubation and its associated risks.

12. IMPLEMENTATION FOR RESEARCH

This work opens several new directions for future research. The findings encourage the validation and broader adoption of more detailed placental evaluation protocols, as well as the exploration of how inflammatory patterns relate to both short- and long-term outcomes.

However, the clinical utility of histological associations remains limited, as these are established only postnatally - after the fetus has already been exposed to the inflammatory environment. This limitation underscores the urgent need to develop prenatal diagnostic tools, such as ultrasound markers or biochemical indicators, that can reliably detect intrauterine inflammation before delivery.

Additionally, identifying evidence-based antenatal interventions could play a critical role in mitigating the effects of intrauterine inflammation and improving neonatal outcomes.

Further clinical investigations are needed to refine therapeutic strategies that support preterm infants during vulnerable transitions, such as extubation. In particular, optimizing caffeine therapy remains a key area of interest, given the significant variability in its dosing and timing practices both across countries and among neonatal intensive care units within the same healthcare systems. Standardizing and individualizing its use could offer substantial benefits for respiratory stability and neurodevelopment in this population.

13. IMPLEMENTATION FOR POLICYMAKERS

The insights from this thesis support actions aimed at improving neonatal outcomes through standardization and investment in perinatal care. Promoting more detailed and consistent placental examination protocols in centers managing preterm deliveries could significantly improve early identification of at-risk newborns. Additionally, the variability observed in extubation practices and supportive therapies - such as caffeine administration - underscores the need for the development and revision of standardized national protocols. Although pre-extubational caffeine therapy is currently under investigation, establishing consistent, evidence-informed care bundles across neonatal units would help ensure equitable and high-quality care.

14. FUTURE PERSPECTIVES

Future work will focus on expanding the NEOKOFF trial. The trial is currently being conducted at Semmelweis University, and we are in the process of involving additional Hungarian centres. We already initiated the process of obtaining ethical approval for several NICUs in Hungary. This expansion will not only enhance recruitment and external validity but also foster a broader research network for neonatal care.

In parallel, we plan to continue expanding our database with prospectively collected histological and clinical data. A standardized and forward-looking data collection approach will allow more accurate analysis of intrauterine inflammation and its neonatal consequences. Additionally, future efforts will explore the use of digital histopathology and machine learning–based image analysis to support earlier, more objective detection of inflammatory lesions.

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16.1. Publications related to this thesis

The histologic fetal inflammatory response and neonatal outcomes: systematic review and meta-analysis

Kovács K, Kovács ŐZ, Bajzát D, Imrei M, Nagy R, Németh D, Kói T, Szabó M, Fintha A, Hegyi P, Garami M, Gasparics Á.

Am J Obstet Gynecol. 2024 May;230(5):493-511.e3.

DOI: 10.1016/j.ajog.2023.11.1223.

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doi: 10.1016/j.ajog.2024.05.024.

IF: 8,7 (2023)

The effect of an additional pre-extubational loading dose of caffeine citrate on mechanically ventilated preterm infants (NEOKOFF trial): Study protocol for a multicenter randomized clinical trial

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PLoS One. 2025 Jan 13;20(1):e0315856.

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16.2. Publications not related to this thesis

Association of meconium-stained amniotic fluid and histological chorioamnionitis with fetal inflammatory response in preterm deliveries

Balogh DC, **Kovács K**, Kovács ŐZ, Regős E, Fintha A, Harmath Á, Szabó M, Gasparics Á, Varga P.

Children (Basel). 2025 Apr 7;12(4):477.

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Safety analysis of preoperative anti-TNF-α therapy in pediatric IBD after intestinal

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Inflamm Bowel Dis. 2023 Dec 5;29(12):1971-1980.

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