Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

Microbial invasion of the amniotic cavity is associated with impaired cognitive and motor function at school age in preterm children

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BACKGROUND: Chorioamnionitis is an important cause of preterm delivery. Data on neurodevelopmental outcome in exposed infants are inconsistent due to difficulties in diagnosing intrauterine infection/inflammation and lack of detailed long-term follow-up. We investigate cognitive and motor function in preterm infants at early school age and relate the findings to bacteria in amniotic fluid obtained by amniocentesis (microbial invasion of the amniotic cavity (MIAC)) or placenta findings of histological chorioamnionitis (HCA) or fetal inflammatory response syndrome (FIRS).

METHOD: Sixty-six infants with gestational age <34 weeks at birth and without major disabilities were assessed using WISC-III and the Bruininks–Oseretsky Test of Motor Proficiency. Results were corrected for gestational age and sex.

RESULTS: Children exposed to MIAC had significantly lower scores for full-scale IQ and verbal IQ compared to the non-MIAC group and the difference in full-scale IQ remained after correction for confounding factors. The MIAC group had also significantly lower motor scores after correction. In contrast, motor function was not affected in infants exposed to HCA or FIRS and differences between groups for cognitive scores were lost after corrections.

CONCLUSION: Exposure to bacteria in amniotic fluid is associated with lower motor and cognitive scores in school age preterm infants without major disabilities.

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INTRODUCTION

Preterm infants have a significantly increased risk of neurodevelopmental impairment. Studies show that 5–15% of infants born extremely preterm will have severe neurodevelopmental sequels such as cerebral palsy (CP) and intellectual disability.[1,2] A significantly larger proportion of children born preterm have learning difficulties[3,4] or minor motor dysfunctions[5,6] with a substantial need for assistance and support at school age.[3]

Intrauterine infection and/or inflammation, commonly referred to as chorioamnionitis, is an important cause of preterm labor (PTL), preterm pre-labor rupture of membranes (PPROM), and subsequent preterm delivery.[7–9] Several studies, including meta-analyses, also suggest that chorioamnionitis is an independent risk factor for preterm brain injury,[10] CP,[10,11] and cognitive impairment.[12] Data are, however, not consistent since other studies fail to show such an association and there are recent studies suggesting that intrauterine exposure to infection/inflammation may be of less importance or even protect the developing brain from injury.[13–16] The difficulties in assessing the results are illustrated by current meta-analyses reaching completely opposite conclusions.[17,18] Possible explanations for these inconsistencies are different definitions of chorioamniotis, limited sample sizes with varying inclusion criteria, confounding factors such as severe neonatal morbidities, short follow-up, and a lack of detailed follow-up data.

There are several ways of diagnosing or detecting intrauterine infection and/or inflammation apart from clinical symptoms in the mother.[19,20] Histological chorioamnionitis (HCA) with a maternal inflammatory response in the placenta at the time of birth is the most commonly used.[19] HCA may be associated with a fetal inflammatory response syndrome (FIRS) characterized by inflammation in fetal blood vessels and/or umbilical cord (funisitis).[19] FIRS is strongly linked to severe neonatal morbidity and several studies report a relation also to neurodevelopmental impairment.[21–23] Access to amniotic fluid could also detect bacteria present in the amniotic cavity (microbial invasion of the amniotic cavity (MIAC)) or intra-amniotic inflammation (IAI) with elevated cytokine levels in the amniotic fluid.[8,9,19,20] These processes may precede and affect the fetus before the development of HCA that

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represents an end stage of the inflammatory process diagnosed only after birth.[19]

While there are several studies reporting various and partly contradictory outcomes after exposure to HCA and/or FIRS, very few studies have addressed the consequences of MIAC or IAI, mainly due to the difficulties in obtaining amniotic fluid. Single studies report impaired motor outcome following IAI[11] and MIAC[24] and retrieval of microorganisms from the placenta is associated with increased risk of CP.[10]

There is also limited knowledge of the effects of intrauterine exposure to infection/inflammation on long term and more subtle outcomes in preterm infants. A small number of studies have assessed cognitive function in children up to 2 years of age. While single studies report lower cognitive scores on Bayley Scales of Infant Development at 18–24 months of corrected age following HCA and clinical chorioamnionitis,[12] several other studies do not find any significant associations between HCA and cognition.[14–16,25,26] Single studies also report lower cognitive scores at 2 years of age following MIAC.[24]

There are very few studies focusing on long-term neurodevelopmental sequels. Three studies of children born to mothers with HCA, FIRS, and/or clinical symptoms of chorioamnionitis present follow-up data from early school age regarding CP and cognitive deficits[22,27] or cognitive and behavioral outcomes at 8 and 18 years.[28] Only one of these studies found an association between FIRS and reduced cognitive and executive function.[22]

In this study, we investigated the long-term consequences of intrauterine exposure to infection/inflammation with a rare access to both amniotic fluid and placenta histology using strict definitions of MIAC, HCA, and FIRS. Preterm children up to 34 weeks' gestational age at birth with few severe neonatal morbidities and without major neurological or cognitive disabilities were examined at early school age with a focus on more subtle motor or cognitive deficits.

MATERIALS AND METHODS

Subjects

The study included singleton infants born before 34 weeks' gestational age whose mothers participated in a study of intrauterine infection/inflammation and spontaneous preterm delivery at Sahlgrenska University Hospital, Gothenburg, Sweden.[8,9] The mothers enrolled in the study presented with either PTL or PPROM. Amniotic fluid was retrieved by ultrasound-guided transabdominal amniocentesis under antiseptic conditions within 12 h of admittance. After delivery, placenta and fetal membranes were histologically examined. Gestational age was determined by ultrasound in the second trimester (between 16 and 20 weeks of gestation). Children with congenital malformations were excluded in the perinatal period.

The study was approved by the Regional Ethics Committee (EPN Gbg 349-95 and 538-05) at the Sahlgrenska University Hospital, Gothenburg, Sweden, and children were enrolled following informed parental consent.

Analyses of amniotic fluid and placenta

MIAC was defined as positive polymerase chain reaction for *Ureaplasma urealyticum* or *Mycoplasma hominis* and/or growth of any bacteria in amniotic fluid. However, growth of *Staphylococcus epidermidis* was considered as skin contamination unless the mother also had elevated cytokine levels.[9] The most commonly detected pathogen in MIAC in this study was Ureaplasma in one third (5/16) of the included cases while two thirds had positive anaerobic and/or aerobic amniotic fluid cultures for other pathogens, including Listeria, Hemophilus and Gram-negative rods.

The diagnosis of HCA was based on analyses by a perinatal pathologist. Tissue samples were obtained from umbilical cord (proximal and distal samples), roll of chorioamniotic membranes, umbilical cord insertion, and full-thickness samples of placenta. Histological examination of the placenta was performed following College of American Pathologists guidelines and findings were classified according to the ELGAN protocol as previously described.[29] HCA was defined as a maternal inflammatory response with neutrophil infiltration of the chorionic plate, chorion/decidua, and/or amnion while FIRS was defined as inflammation of the umbilical cord (funisitis) and/or eosinophilic and neutrophilic infiltration of fetal stem vessels. Amniotic fluid cytokine levels of interleukin (IL)-6 and IL-8 were determined by Luminex® (Invitrogen, Waltham, MA USA), according to the manufacturer's instructions.

Neonatal data

The children were identified in the newborn period and ultrasound of the brain was performed at 1 and 4 weeks of age. Severe infant morbidity was recorded, including intraventricular hemorrhage (IVH) grade 3–4, periventricular leukomalacia (PVL), necrotizing enterocolitis (NEC) needing surgical intervention, patent ductus arteriosus (PDA) requiring medical or surgical treatment, chronic lung disease (CLD) with need for extra oxygen at 36 weeks gestational age, and early-onset sepsis within the first week of life. Sepsis was diagnosed by positive bacterial cultures in blood, except for *Staphylococcus epidermidis* where sepsis diagnosis required an elevated C-reactive protein value of >20 mg/L.

Follow-up study

Eighty-three children eligible for inclusion and with data available from amniotic fluid and/or placenta were identified in the neonatal period and followed prospectively. Follow-up examinations of cognitive and motor function were scheduled at 7 years of age. All children were accompanied by at least one parent. The assessors were blinded to maternal and neonatal data. Since the focus of the study was subtle developmental deficits, children with severe motor or cognitive disabilities (previously diagnosed CP, intellectual disability, or blindness) were excluded.

Cognitive assessment

The children were examined by the same experienced and trained psychologist. The Wechsler Intelligence Scale for Children, WISC-III, was used. Ten subtests were administrated and full-scale intelligence quotient (FSIQ), verbal IQ (VIQ), and performance IQ (PIQ) scores were calculated.

Motor assessments

Motor function was examined by an experienced and trained physiotherapist using the Bruininks–Oseretsky Test of Motor Proficiency at School Age, Second Edition (BOT-2). This test offers a detailed and comprehensive assessment of motor function[30] by administering several subtests for each specified motor function and with the scores for several functions in turn resulting in four composite scores: (1) Fine manual control (motor skills involved in writing and drawing tasks requiring precise control); (2) Manual coordination (reaching, grasping, and object manipulation, with emphasis on speed, dexterity, and coordination of upper extremities); (3) Body coordination (sequential and simultaneous coordination of the upper limbs with the lower limbs, static balance, and balance while walking); and (4) Strength and agility (strength, running speed, and postural control during walking and running). The four composite scores were combined to yield a total motor composite score. Raw scores on subtests were converted to an age-adjusted scale and sex-specific norms were used as previously described.[30] Routine neurological examinations were performed by one of two trained and experienced pediatricians in order to exclude CP.

Statistical analyses

Statistical analyses were performed using Graph-Pad Prism© (version 5) and SPSS (version 24). For comparisons between groups, Fisher´s Exact test was used for dichotomous variables and Mann–Whitney *U* test was used for continuous variables. A p value < 0.05 was considered statistically significant.

Follow-up data were examined regarding known confounding factors, including gestational age at birth, fetal growth restriction (SDS; standard deviation score for weight at birth), severe neonatal morbidities, sex, and social background, as summarized in Tables 2 and 3. The size of the study groups limited the number of possible corrections. Two main independent confounders, gestational age and sex, significantly differed between groups and all comparisons were corrected for these confounders using logistic regression. A statistician was consulted throughout the analyses of data.

RESULTS

Eighty-three children fulfilled the neonatal inclusion criteria and 70/83 (84%) were examined at a median age of 7.6 years (range 7.0–10.7). Two children had moved abroad, four families declined, and seven families could not be reached by letter or phone. In addition, four children were excluded owing to severe disabilities. Sixty-six children were thus included in the study group. Data from amniotic fluid were available in 50 children and histological data from placenta were available in 50 children. Accordingly, data from amniotic fluid as well as placenta were available in 34 children. Inclusion and sample availability are summarized in Fig. 1. Antenatal data on the study group as a whole ($n = 66$), children lost to follow-up ($n = 13$), and excluded children ($n = 4$) are summarized in Table 1. No significant differences were seen for any of the parameters when comparing the study group with children lost to follow-up. Of the four excluded children, one child had bilateral spastic CP, intellectual disability, and autism without previous signs of HCA or FIRS; one child exposed to HCA and FIRS had severe IVH, unilateral spastic CP, and intellectual disability while the second child exposed to FIRS and HCA had severe IVH and bilateral CP. Finally, the only child for whom amniotic fluid was available was not exposed to MIAC but suffered from severe visual impairment and intellectual disability.

Background data

Descriptive data divided by exposure to MIAC, HCA, or FIRS are shown in Table 2. In summary, infants born to mothers with MIAC, HCA, or FIRS were significantly smaller and born at an earlier gestational age than infants to mothers without MIAC, HCA, or FIRS. The differences were most pronounced for FIRS. The SDS score did, however, not differ between groups. Children exposed to FIRS had also significantly higher neonatal morbidity, while only slight differences for days of respiratory support seen for MIAC and HCA. Need for ventilator was strongly associated with gestational age with mean (SD) 25.9 (2.1) weeks in the ventilated group and 31.2 (2.5) in the non-ventilated group. The overall infant morbidity was low, and no children had PVL, severe IVH, or NEC and only one child had early-onset sepsis. Infants with CLD were relatively few and mainly found in groups exposed to HCA and FIRS.

Background data relevant to long-term follow-up including growth and socioeconomic factors are shown in Table 3. In summary, all children regardless of intrauterine exposure had normal growth at school age and no differences were seen between the groups. Socioeconomic factors including maternal education, single parent household, and language spoken at home did not differ between the groups except for one measure of maternal education that was lower in the FIRS group. Very little long-term morbidity was seen except for 8/66 children diagnosed with asthma without relation to intrauterine exposure (data not shown) and one case of nephrosis unrelated to prematurity.

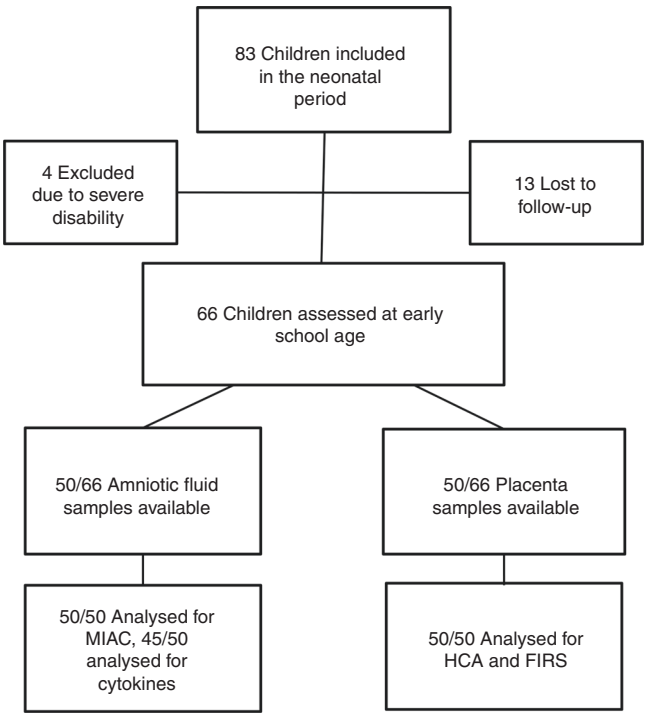

Fig. 1 Patient group and availability of samples.

Table 1. Antenatal data in the study population and in children lost to follow-up or excluded.

	Study group $n = 66$	Lost to follow-up $n = 13$	Excluded $n = 4$
Gestational age (days), mean (SD)	211.2 (22.4)	216.5 (22.4)	180.3 (19.6)
Gestational age (weeks), median (range)	32.0 (24.0–33.9)	31.7 (23.1–33.6)	24.9 (23.4–29.7)
Birth weight (g), mean (SD)	1640 (582)	1584 (483)	873 (357)
Male sex, *n* (%)	37/66 (56)	7/13 (54)	2/4 (50)
MIAC, *n* (%)	16/50 (32)	3/12 (25)	0/1 (0)
HCA, *n* (%)	34/50 (68)	4/5 (80)	2/3 (67)
FIRS, *n* (%)	24/50 (48)	3/5 (60)	2/3 (67)

MIAC microbial invasion of the amniotic cavity, *HCA* histological chorioamnionitis with maternal inflammatory response, *FIRS* fetal inflammatory response syndrome with funisitis and/or fetal vasculitis

Table 2. Perinatal data in the study population.

	Non-MIAC $n = 34$	MIAC $n = 16$	*p* value	Non-HCA $n = 16$	HCA $n = 34$	*p* value	Non-FIRS $n = 26$	FIRS $n = 24$	*p* value
Gestational age (days), mean (SD)	220.4 (18.0)	203.4 (24.5)	<0.05	227.1 (10.3)	203.6 (21.8)	<0.0001	223.1 (14.7)	193.7 (19.1)	<0.0001
Gestational age (weeks), median (range)	32.0 (24.6–33.9)	30.2 (24.0–33.6)		32.4 (24.0–33.9)	28.6 (24.0–33.9)		32.6 (24.6–33.9)	26.5 (24.0–33.0)	
Birth weight (g), mean (SD)	1857 (507)	1479 (572)	<0.05	2016 (373)	1359 (537)	0.0001	1913 (422)	1197 (486)	<0.0001
Standard deviation score (SDS) for weight, mean (SD)	−0.26 (1.06)	0.17 (1.27)	ns	−0.28 (0.95)	−0.28 (1.06)	ns	−0.29 (0.93)	−0.27 (1.12)	ns
Male sex, *n* (%)	24 (71)	7 (44)	ns	12 (75)	14 (41)	<0.05	19 (73)	7 (29)	<0.01
Antenatal steroids, *n* (%)	33 (97)	14[a] (93)	ns	13[a] (87)	33 (97)	ns	25 (96)	22[a] (92)	ns
Apgar score <7 (5 min), *n* (%)	2 (6)	3 (19)	ns	0	4 (12)	ns	1 (4)	5 (21)	<0.05
Periventricular leukomalacia	0	0	ns	0	0	ns	0	0	ns
Intraventricular hemorrhage grade 3–4, *n* (%)	0	0	ns	0	0	ns	0	0	ns
PDA, requiring treatment, *n* (%)	1 (3)	2 (13)	ns	0	5 (15)	ns	0	5 (21)	<0.05
Mechanical ventilation, *n* (%)	2 (6)	7 (44)	<0.05	0	9 (26)	<0.05	1 (4)	9 (38)	<0.05
Early-onset sepsis, *n* (%)	0	0	ns	0	1 (3)	ns	0	1 (4)	ns
CLD, *n* (%)	3 (9)	4 (25)	ns	1 (6)	9 (26)	ns	2 (8)	7 (29)	<0.05
NEC, *n* (%)	0	0	ns	0	0	ns	0	0	ns

Comparisons were performed using Mann–Whitney *U* test or Fisher's exact test
MIAC microbial invasion of the amniotic cavity, *HCA* histological chorioamnionitis with maternal inflammatory response, *FIRS* fetal inflammatory response syndrome with funisitis and/or fetal vasculitis; *PDA* patent ductus arteriosus, *CLD* chronic lung disease defined as the need for extra oxygen at 36 weeks' gestational age, *NEC* necrotizing enterocolitis
[a]Data on antenatal steroids missing in one patient

Children exposed to MIAC, but not to HCA or FIRS, have lower cognitive scores when corrected for gestational age at birth and sex

Cognitive testing with WISC III was performed in 64/66 children. The MIAC group had significantly lower mean (SD) scores than the non-MIAC group with FSIQ 91 (18) vs 107 (13) ($p < 0.001$), VIQ 92 (18) vs 107 (14) ($p = 0.004$), and PIQ 92 (15) vs 105 (16) ($p = 0.014$). The differences between the two groups for FSIQ remained significant when controlled for gestational age at birth and sex ($p = 0.04$), while VIQ was borderline significant at $p = 0.06$, Fig. 2. No differences regarding cognitive function was seen for children exposed to Ureaplasma ($n = 5$) compared with other cultured bacteria ($n = 11$), data not shown.

Children exposed to HCA had significantly lower mean (SD) scores for FSIQ and VIQ compared to children without exposure to HCA with FSIQ 94 (15) vs 105 (13) ($p = 0.008$) and VIQ 94 (14) vs 108 (17) ($p = 0.01$), while PIQ did not differ between groups with 95 (16) vs 100 (14) ($p = 0.22$). Similarly, children exposed to FIRS had lower mean (SD) scores than children not exposed to FIRS with FSIQ 91(14) vs 104 (14) ($p = 0.002$) and VIQ 91 (15) vs 106 (15) ($p = 0.003$). PIQ was lower in the FIRS group with 93 (15) vs 100 (15) but the difference was not significant ($p = 0.14$). None of the differences between infants exposed to HCA/FIRS or not remained significant after correction for gestational age at birth and sex, Figs. 3 and 4.

Exposure to MIAC, but not to HCA or FIRS, is associated with specific motor problems when corrected for gestational age at birth and sex

All children included performed the BOT-2 test. A similar pattern was seen for motor function as for cognition with reduced performance in children exposed to MIAC. Children in the MIAC group had lower total composite scores compared with children in the non-MIAC group ($p = 0.01$). They had also lower scores for fine manual control ($p = 0.03$) and for manual coordination ($p = 0.005$). The differences remained significant after adjustment for confounding factors (gestational age at birth and sex). Type of pathogen (Ureaplasma or other cultured bacteria) did not affect outcome, data not shown.

HCA and FIRS did not affect motor performances before or after correction except for one subscore reflecting Strength and agility in the FIRS group and the difference did not remain significant after correction for confounding factors. Data are summarized in Table 4.

The adverse effect of MIAC could not be explained by an increased inflammatory reaction when measured by IL-6 and IL-8

To determine whether the effect of MIAC on long-term outcome could be explained by degree of IAI, cytokines previously associated with MIAC and IAI[8,9] were analyzed in amniotic fluid. Absolute values of IL-6 and IL-8 were compared between the MIAC and the non-MIAC groups and no differences were found, Fig. 5.

DISCUSSION

In this study, we found that exposure to bacteria in amniotic fluid is associated with lower cognitive and motor scores at school age in a selected group of relatively mature preterm infants without major disabilities.

Our group consisted of infants up to 34 weeks' gestational age at birth, which differs from most studies of chorioamnionitis that focus on long-term follow-up of extremely or very preterm infants.[12,15,16] Contrary to these studies, our group had very little severe neonatal morbidities and we excluded infants with major disabilities. This is particularly important in a small study group since single postnatal events not related to

Table 3. Background data during follow-up.

	Non-MIAC $n = 34$	MIAC $n = 16$	p value	Non-HCA $n = 16$	HCA $n = 34$	p value	Non-FIRS $n = 26$	FIRS $n = 24$	p value
Growth									
Standard deviation for weight at school age, mean (SD)	0 (1.2)	0.2 (1.2)	ns	0 (1.2)	0.1 (1.3)	ns	0 (1.3)	0.2 (1.3)	ns
Standard deviation for height at school age, mean (SD)	0.2 (0.9)	0.1 (0.9)	ns	0.2 (1.2)	0.1 (1.1)	ns	0.2 (0.9)	0.1 (1.2)	ns
Socioeconomic factors									
Single parent household, *n* (%)	6 (18)	4 (25)	ns	2 (13)	9 (26)	ns	5 (19)	6 (25)	ns
Swedish as first language, *n* (%)	33 (97)	15 (94)	ns	15 (94)	30 (88)	ns	25 (96)	20 (83)	ns
Maternal education ≥12 years, *n* (%)	31 (91)	12 (75)	ns	15 (94)	26 (79)	ns	24 (92)	18 (75)	ns
Maternal education >12 years, *n* (%)	20 (59)	5 (31)	ns	10 (62)	12 (35)	ns	13 (58)	7 (29)	<0.05

Comparisons were performed using Mann–Whitney *U* test or Fisher's exact test
MIAC microbial invasion of the amniotic cavity, *HCA* histological chorioamnionitis with maternal inflammatory response, *FIRS* fetal inflammatory response syndrome with funisitis and/or fetal vasculitis

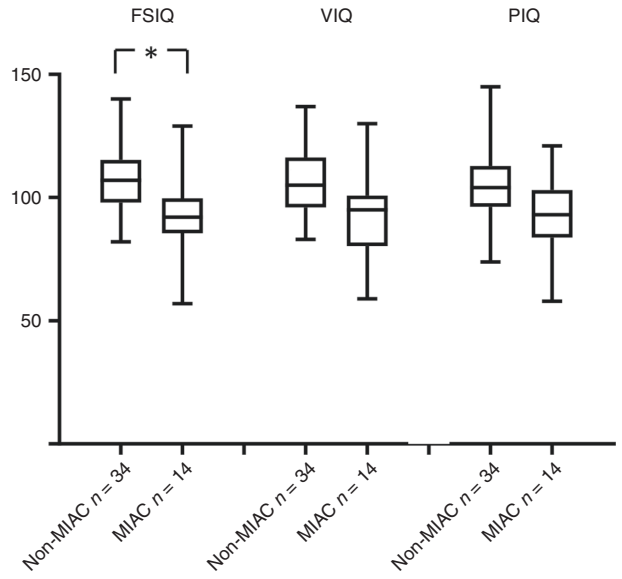

Fig. 2 Full-scale IQ was lower in school age preterm children exposed to MIAC also when corrected for confounding factors. Children examined by WISC III at 7 years of age. Data are given as median, quartiles, and range. Groups were compared using Mann–Whitney *U* test and adjusted for sex and gestational age at birth by logistic regression. FSIQ full-scale IQ, VIQ verbal IQ, PIQ performance IQ. *$p < 0.05$ after correction for confounding factors.

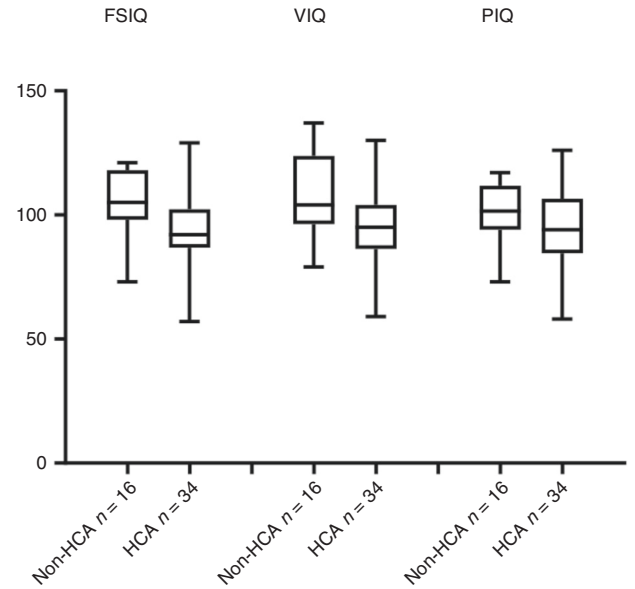

Fig. 3 IQ in school age preterm children was not affected by exposure to HCA when corrected for confounding factors. Children examined by WISC III at 7 years of age. Data are given as median, quartiles, and range. Groups were compared using Mann–Whitney *U* test and adjusted for sex and gestational age at birth by logistic regression. FSIQ full-scale IQ, VIQ verbal IQ, PIQ performance IQ. There were no differences between groups after adjusting for confounding factors.

intrauterine exposure may result in serious neurodevelopmental impairment and help to mask more subtle differences between groups. It should also be noted that almost all mothers received antenatal steroids. It has been suggested that antenatal steroids may protect from the adverse effect of inflammation on the developing brain, thereby explaining the increasing number of studies showing no correlation between chorioamnionitis and neurodevelopmental outcome.[31,32] Differences in antenatal steroid exposure could, however, not explain our results. Regarding confounding factors, we compensated for the relatively large gestational age span by correcting for gestational age at birth. Since we saw a significantly higher proportion of boys in groups exposed to infection and/or inflammation, we also corrected for sex as a confounding factor, in spite of our small study groups. All pregnancies were dated by ultrasound, and in the absence of differences in SD scores, the differences in birth weight between groups could largely be attributed to differences in gestational age. Similarly, we found that need for mechanical ventilation was strongly associated with lower gestational age. Regarding postnatal confounders, all children had normal growth, morbidities were rare, and socioeconomic factors did not significantly differ between groups. Our study is, however, limited by the sample size and our results need to be confirmed in larger studies were multiple confounding factors could be studied.

Intrauterine infection or inflammation is more common in spontaneous onset of labor following PTL or PPROM. It is also inversely correlated with gestational age at birth and associated with severe neonatal morbidities.[19] In spite of our small sample size with comparatively healthy and mature preterm infants, our inclusion criteria of mothers with PTL or PPROM only, ensured a sufficient number of exposed infants. MIAC was present in close to 30% of the cases, HCA in 68%, and FIRS in 48% of the cases. This is a higher proportion than previously reported in cohorts with more

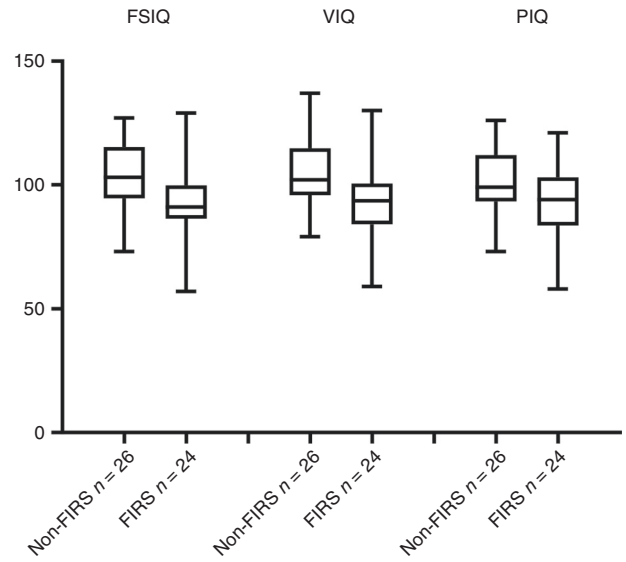

Fig. 4 IQ in school age preterm children was not affected by exposure to FIRS when corrected for confounding factors. Children examined by WISC III at 7 years of age. Data are given as median, quartiles, and range. Groups were compared using Mann–Whitney *U* test and adjusted for sex and gestational age at birth by logistic regression. FSIQ full-scale IQ, VIQ verbal IQ, PIQ performance IQ. There were no differences between groups after adjusting for confounding factors.

immature infants where mothers delivering after spontaneous as well as physician-initiated onset of labor were included.[15,16,26]

In this study, we provide data suggesting that exposure to bacteria in amniotic fluid already at or before onset of labor may be more important than histological findings of maternal or fetal inflammation at the time of birth. Since amniotic fluid is not easily accessible, there is very little data on long-term consequences of MIAC as opposed to the numerous articles on HCA. One study reports that positive cultures for Ureaplasma (but not other pathogens) obtained at cesarean section was associated with CP and adverse psychomotor development at 1 and 2 years of age.[24] In support of these findings, the ELGAN study reports that retrieval of low virulent microorganisms from the placenta predicts not only white matter injury and CP in preterm infants born before 28 weeks' gestational age[10] but also long-term behavioral difficulties.[33] In a recent study, where amniotic fluid was obtained by amniocentesis in women with PTL or PPROM like in our study, IAI with elevated IL-6 concentrations, but not MIAC, was associated with parent-reported delayed neurodevelopment at 2 years of age.[34] Similarly, Yoon et al. report that elevated IL-6 and funisitis, but not MIAC, are associated with white matter injury and CP.[11] The use of IAI as a predictor of outcome is, however, complicated by the need of relating cytokine cut-off values to a specific condition or outcome. IAI has been defined as elevated cytokine levels that predict MIAC,[35] delivery within 7 days,[8,9] or CP.[11] Therefore, in our study we did not use IAI but instead analyzed cytokine levels in relation to MIAC and found that MIAC was not associated with significantly higher levels of amniotic fluid cytokines previously associated with IAI.[8,9,11] These findings are surprising since previous studies show a strong correlation between MIAC and elevated levels of IL-6 and IL-8.[8,9,34] The discrepancy may be explained by a small sample size with large variability in cytokine concentrations, the timing of the sampling, or by the possibility that other cytokines, not measured in this study, may correlate better with MIAC. It is also possible that sterile inflammation may explain part of the cytokine elevations, thereby masking associations with MIAC.

Table 4. Motor scores at school age in preterm infants exposed to infection/inflammation in utero.

BOT-2	Non-MIAC $n = 34$	MIAC $n = 16$	*p* value	*p* value adjusted*	Non-HCA $n = 16$	HCA $n = 34$	*p* value	*p* value adjusted*	Non-FIRS $n = 26$	FIRS $n = 24$	*p* value	*p* value adjusted*
Total motor	52.2 (6.8)	46.0 (7.5)	**0.010**	0.069	51.3 (7.1)	48.4 (10.1)	0.304	0.672	51.3 (7.4)	47.2 (10.6)	0.127	0.903
Fine manual control	54.2 (7.3)	48.6 (9.2)	**0.026**	**0.048**	53.8 (9.5)	49.9 (9.4)	0.176	0.184	52.1 (9.3)	50.1 (9.8)	0.654	0.985
Manual coordination	50.0 (7.1)	43.1 (8.1)	**0.005**	**0.017**	49.6 (7.6)	47.7 (10.5)	0.588	0.999	50.0 (7.5)	46.4 (11.5)	0.294	0.520
Body coordination	52.9 (8.0)	50.5 (10.3)	0.383	0.936	51.9 (7.5)	50.6 (11.2)	0.473	0.829	52.3 (8.2)	49.6 (11.8)	0.203	0.921
Strength and agility	48.8 (8.2)	44.6 (8.4)	0.078	0.418	49.4 (9.3)	46.3 (8.5)	0.253	0.761	50.0 (8.7)	44.4 (8.1)	**0.021**	0.248

Comparisons were performed using Mann–Whitney *U* test and data are presented as mean (SD). Significant *p* values ($p \leq 0.05$) are shown in bold font
BOT-2 Bruininks–Oseretsky Test of Motor Proficiency, Second Edition, *MIAC* microbial invasion of the amniotic cavity, *HCA* histological chorioamnionitis with maternal inflammatory response, *FIRS* fetal inflammatory syndrome with funisitis and/or fetal vasculitis
*p value adjusted for gestational age and sex using logistic regression

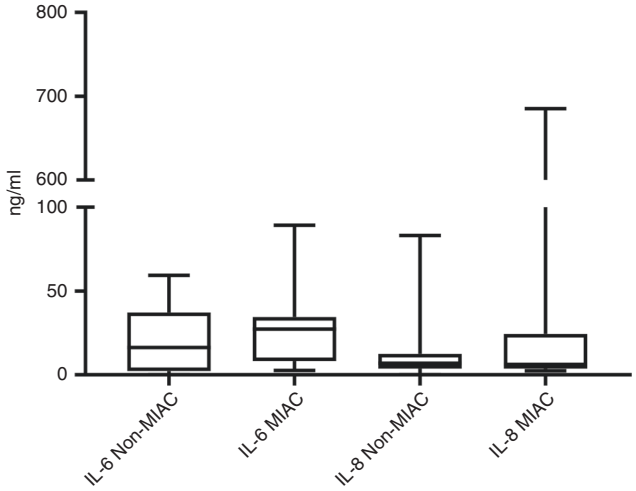

Fig. 5 The effect of MIAC on school age performance in preterm infants could not be explained by increased inflammation measured by IL-6 and IL-8 concentrations in amniotic fluid. Data are given as median, quartiles, and range. Groups were compared using Mann–Whitney *U* test. No significant differences were seen between groups.

We found that MIAC affected cognitive and motor performances in otherwise healthy preterm infants at school age. Interestingly, exposure to HCA and FIRS was not associated with motor problems, suggesting a specific role for bacteria as suggested also in the ELGAN study.[10] FIRS and HCA were associated with significantly lower cognitive scores, but the differences between groups were lost when corrected for gestational age at birth and sex. This is in accordance with previous findings of gestational age as a strong confounding factor, and the importance of gestational age may be emphasized in this small patient material including infants up to 34 weeks' gestational age at birth. Our findings are also in line with several recent studies and meta-analyses that find no association between HCA and neurodevelopmental outcome.[15,16,26,31] The combined effect of MIAC and FIRS/HCA also warrants further studies, since our limited number or children did not allow such analyses.

The effect of MIAC on cognition was seen as lower IQ, but the mean performance was within the normal range and >−1 SD. Similarly, motor problems did not constitute any major disability. It is, however, becoming increasingly clear that minor motor and cognitive impairments are present in a large proportion of preterm infants and may have profound effects on daily life.[3,4,6,36] When testing, minor motor problems are found in seemingly healthy preterm infants, attending normal school, and is commonly associated with behavioral problems and executive dysfunction.[36,37] Thus the association between MIAC, or any exposure to intrauterine infection or inflammation, and later behavior and executive function warrants further investigation.

In summary, we show that exposure to bacteria in amniotic fluid (MIAC) is associated with impaired motor and cognitive performance at school age in preterm infants without major disabilities. Access to amniotic fluid as well as placenta enabled strict diagnostic criteria for intrauterine infection and/or inflammation and the long-term follow-up combined with exclusion of children with major disabilities allowed for detection of more subtle differences between groups. The small sample size is the major limitation of the study and results need to be confirmed in larger populations.

An understanding of perinatal inflammation, and MIAC in particular, as a risk factor for adverse long-term neurodevelopmental outcome may help us discover underlying mechanisms and identify infants at risk.

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AUTHOR CONTRIBUTIONS

A.T., H.H., B.J. and K.S. were responsible for concept and design. A.T., M.H., H.H., I.-M.F., P.T., J.E.C., B.J. and K.S. contributed to data acquisition, analysis, and interpretation. A.T., I.O., C.M., B.J. and K.S. drafted and revised the manuscript. All authors approved the final manuscript.

ADDITIONAL INFORMATION

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