Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods: Study Enrollment, Placental, and White Matter Injury Analyses, and Clinical Measures of Chorioamnionitis

As our total cohort consisted of a combination of three individual cohorts, enrollment criteria differed somewhat between the three centres. The UBC cohort consisted of a prospective cohort of preterm newborns born between 24 and 32 weeks gestation at Children's & Women's Health Centre of British Columbia, from April 2006 to September 2013, who were examined with placental pathology, early MRI scans, and neurodevelopmental follow-up. The UCSF cohort consisted of all inborn preterm infants born between 24 and 32 weeks gestation at the University of California San Francisco Benioff Children's Hospital from January 2004 to August 2011 who provided consent. Enrolment at UCSF was limited to inborn infants to ensure that placental pathology was reviewed by a specialized placental pathologist. At UMCU, MRI scans are clinically performed on neonates born at less than 28 weeks' gestation who are admitted to the NICU. For this study we thus retrospectively selected all infants born at UMCU between 24 and 28 weeks' gestation from March 2007 to March 2013.

The degree of placental inflammation was scored by experienced pathologists for stage (extent) and grade (severity) of both the maternal and fetal inflammatory responses, using the scale proposed by Redline et al.[1] For the maternal and fetal inflammatory responses, the stage (Score 1-3) ranges from early (subchorionitis, umbilical/chorionic vasculitis) to advanced (necrotizing chorioamnionitis, necrotizing funisitis), and the grade from mild/moderate (1) to severe (2), according to specific histopathological features.[1] For the purpose of this analysis, a single measure was constructed to quantify the extent of the maternal and fetal inflammatory responses, respectively, incorporating Redline's measures of stage and grade and was defined as none (stage and grade of 0), mild (stage or grade of 1), and severe (stage of 2-3 or grade of 2).

Labelling of WMI was carried out with simultaneous coronal, sagittal, and axial views of the brain using Display software (http://www.bic.mni.mcgill.ca/ServicesSoftwareVisualization). Tricubic interpolation was used for visualization as it permitted accurate and consistent segregation of the WMI from the surrounding structures. Images were inspected to ensure that voxels with ambiguous signal intensity were not erroneously considered within the WMI segmentation. An experienced neonatal neurologist reviewed the segmented WMI from all centres for quality control.

Due to inconsistencies in the definition of clinical chorioamnionitis, a descriptive term, "intrauterine inflammation or infection or both", or "Triple I" has been proposed.[2] Although we lack the clinical data to evaluate either "Triple I" or "suspected Triple I" as they have been defined, for the UBC and UMCU cohorts we were able to calculate a modified "Suspected Triple I" score as maternal fever plus baseline fetal tachycardia or maternal WBC according to clinical definitions used in our cohort: fever defined as temperature greater than 37.8 degrees Celsius, fetal tachycardia defined as HR > 160, and maternal leukocytosis defined as a WBC greater than 14,000/mL. We calculated the incidence of this measure in the combined UBC and UMCU cohort and, in a univariable analysis, its association with motor and cognitive outcomes.

eTable 1: *Motor* Outcomes at 18 to 24 months' Corrected Age From Multivariable Analysis of Newborns With and Without Chorioamnionitis

	Model with preeclampsia		**Full Model with preeclampsia, sepsis, BPD, and brain injury**	
	Points, Bayley-III motor scale	**95% conf. int.**	**Points, Bayley-III motor scale**	**95% conf. int.**
Chorioamnionitis	-3.6	-7.1 – -0.1	-2.2	-5.6 – 1.3
Centre UBC	(baseline)		(baseline)	
UMCU	13.8	10.2 – 17.4	12.2	8.5 – 15.8
UCSF	-0.4	-5.4 – 4.5	-0.1	-5.0 – 4.8
University educated	3.9	0.76 – 7.0	2.5	-0.5 – 5.6
Preeclampsia	0.05	-3.9 – 4.0	-0.2	-4.0 – 3.6
GA at birth	0.7	-0.9 – 1.6	0.4	-0.5 – 1.3
Postnatal Sepsis	--	--	-3.6	-6.9 – -0.2
BPD	--	--	-2.8	-6.7 – 1.1
WMI (% TBV)	--	--	-18.3	-25.0 – -11.6
IVH, Grade 1-2	--	--	1.6	-1.7 – 4.8
IVH, Grade 3-4	--	--	-3.4	-9.4 – 2.6

eTable 2: *Cognitive* Outcomes at 18 to 24 Months' Corrected Age From Multivariable Analysis of Newborns With and Without Chorioamnionitis

	Model with preeclampsia		**Full Model with preeclampsia, sepsis, BPD, and brain injury**	
	Points, Bayley-III cognitive scale	**95% conf. int.**	**Points, Bayley-III cognitive scale**	**95% conf. int.**
Chorioamnionitis	-4.2	-7.6 – -0.9	-3.0	-6.4 – 0.4
Centre UBC	(baseline)		(baseline)	
UMCU	1.2	-2.2 – 4.6	0.2	-3.4 – 3.8
UCSF	-4.1	-8.8 – 0.5	-4.2	-9.0 – 0.5
University educated	6.4	3.4 – 9.4	6.2	3.2 – 9.2
Preeclampsia	-2.8	-6.5 – 1.0	-2.8	-6.5 – 1.0
GA at birth	1.2	0.4 – 1.9	0.8	-0.1 – 1.7
Postnatal Sepsis	--	--	-4.3	-7.6 – -0.9
BPD	--	--	-1.3	-5.1 – 2.6
WMI (% TBV)	--	--	-10.3	-16.9 – -3.7
IVH, Grade 1-2	--	--	2.9	-0.4 – 6.1
IVH, Grade 3-4	--	--	-1.8	-7.7 – 4.2

eTable 3: *Cognitive* Outcomes at 18 to 24 Months' Corrected Age From Multivariable Analysis of Newborns With Mild and Severe Inflammation of *Maternal* Placental Tissues

	Model with preeclampsia		Full Model with preeclampsia, sepsis, BPD, and brain injury	
	Points, Bayley-III cognitive scale	95% conf. int.	Points, Bayley-III cognitive scale	95% conf. int.
Maternal inflammation				
Mild	-2.7	-6.6 – 1.2	-2.4	-6.4 – 1.5
Severe	-5.9	-10.0 – -1.8	-3.3	-7.5 – 0.8
Centre				
UBC	(baseline)		(baseline)	
UMCU	1.3	-2.0 – 4.7	0.5	-3.1 – 4.1
UCSF	-4.5	-9.1 – 0.1	-4.2	-9.0 – 0.5
University educated	6.6	3.7 – 9.6	6.3	3.4 – 9.3
Preeclampsia	-2.8	-6.5 – 0.9	-2.7	-6.4 – 0.9
GA at birth	1.1	0.3 – 1.9	0.9	-0.0 – 1.8
Postnatal Sepsis	--	--	-4.2	-7.5 – -0.9
BPD	--	--	-1.2	-5.0 – 2.6
WMI (% TBV)	--	--	-10.3	-16.9 – -3.7
IVH, Grade 1-2	--	--	3.3	0.04 – 6.6
IVH, Grade 3-4	--	--	-1.7	-7.5 – 4.2

eTable 4: *Cognitive* Outcomes at 18 to 24 Months' Corrected Age From Multivariable Analysis of Newborns With Mild and Severe Inflammation of *Fetal* Placental Tissues

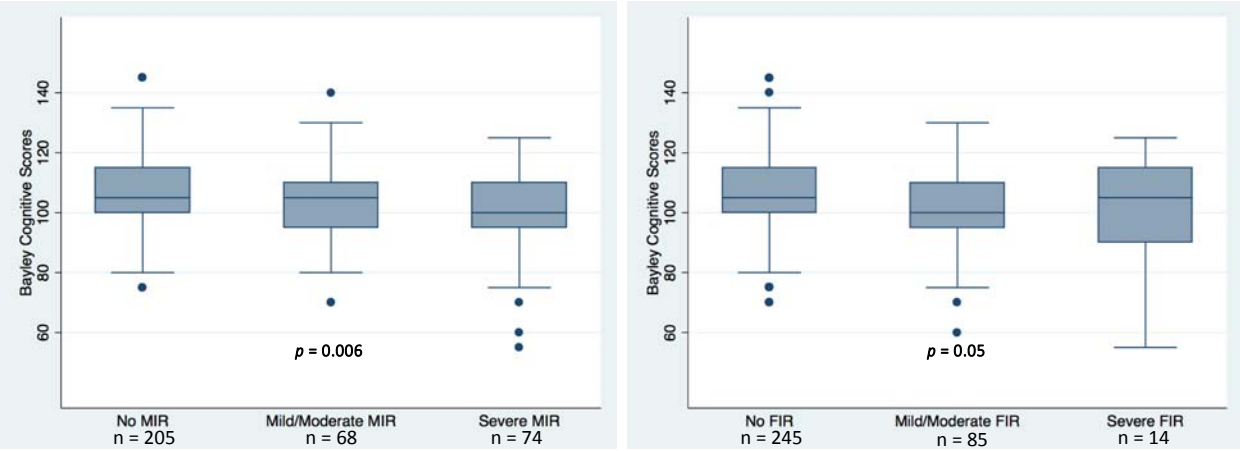
	Model with preeclampsia		**Full Model with preeclampsia, sepsis, BPD, and brain injury**	
	Points, Bayley-III cognitive scale	**95% conf. int.**	**Points, Bayley-III cognitive scale**	**95% conf. int.**
Fetal inflammation Mild	-3.5	-7.0 – 0.1	-2.2	-5.8 – 1.5
Severe	-4.2	-11.7 – 3.2	-4.3	-11.7 – 3.1
Centre UBC	(baseline)		(baseline)	
UMCU	0.8	-2.6 – 4.3	-0.3	-4.0 – 3.3
UCSF	-3.4	-8.3 – 1.4	-3.8	-8.8 – 1.1
University educated	5.9	2.9 – 8.9	5.8	2.8 – 8.8
Preeclampsia	-2.2	-5.8 – 1.5	-2.3	-6.0 – 1.3
GA at birth	1.3	0.5 – 2.0	0.9	-0.0 – 1.8
Postnatal Sepsis	--	--	-4.5	-7.8 – -1.1
BPD	--	--	-0.9	-4.8 – 3.1
WMI (% TBV)	--	--	-10.5	-17.2 – -3.9
IVH, Grade 1-2	--	--	2.8	-0.5 – 6.1
IVH, Grade 3-4	--	--	-0.5	-6.7 – 5.7

eResults: Associations of Maternal and Fetal Inflammatory Responses, and of Clinical Features of Chorioamnionitis With Outcome

To further examine the relationship of chorioamnionitis with cognitive outcomes, we explored the severity of the inflammation defined on histopathological examination of the placental tissues. The severity of the maternal inflammatory response was associated with worse cognitive scores in the univariable model ($p = 0.006$; eFigure 1) but not in the final multivariable model (-3.3 points, Bayley-III cognitive scale; 95% CI [-7.5, 0.8]; eTable 3). Likewise, the severity of the fetal inflammatory response was associated with worse cognitive scores in the univariable model ($p = 0.05$; Figure 1) but not in the final multivariable model (-2.2, Bayley-III cognitive scale; 95% CI [-5.8, 1.5]; eTable 4).

The incidence of the modified "suspected Triple I" measure we calculated was only 7.2% in the combined UBC and UMCU cohort, and, in a univariable analysis, this measure was not related to either motor ($p = 0.7$) or cognitive ($p = 0.2$) outcomes.

eFigure: Associations Between Cognitive Outcome and Severity of Maternal and Fetal Inflammatory Response



Legend
Horizontal line: median
Box edges: interquartile range
Whiskers: upper and lower adjacent lines

MIR: maternal inflammatory response
FIR: fetal inflammatory response

eDiscussion: Associations of Maternal and Fetal Inflammatory Responses, and of Clinical Features of Chorioamnionitis With Outcome

In the univariable analysis, the severity of the maternal inflammatory response was more strongly associated with cognitive outcome than that of the fetal inflammatory response. These findings seem to affirm those of Ylijoki et al. where, in multivariable models accounting for perinatal factors, worse cognitive performance at 5 years was associated with histological chorioamnionitis, but not with the fetal inflammatory response specifically.[3] Likewise, in the study by Hendson et al., there was no difference in rates of neuromotor, neurosensory, or neurocognitive disability at 18-months CA between children with chorioamnionitis with and without a fetal inflammatory response.[4] The more limited ability of the preterm fetus, relative to the term neonate, to mount an overt inflammatory response might contribute to these findings.[5] Results from the ELGAN study have suggested the humoral inflammatory response in the fetus to be an important mediator of perinatal brain injury,[6] particularly in settings of microbiological infection in the placenta without histological inflammation.[7,8] In addition, an important mediator of perinatal brain injury in the setting of chorioamnionitis may be placental vascular effects,[9,10] which could potentially be uncovered through better placental imaging. Thus, the humoral response or the placental vascular response to inflammation may be the more significant pathways to white matter injury and warrant further attention.

Our modified “suspected Triple I” measure was not associated with motor or cognitive outcomes in the UBC and UMCU combined cohort, in contrast to histological chorioamnionitis which was associated with worse cognitive outcome in the univariable analysis. As acknowledged in the report, these clinical findings are not specific to placental inflammation due to an infectious process, suggesting that placental pathology may remain the gold standard for definitive diagnosis of acute chorioamnionitis.

eReferences

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