Cord Blood IL-16 Is Associated with 3-Year Neurodevelopmental Outcomes in Perinatal Asphyxia and Hypoxic-Ischaemic Encephalopathy

Caroline E. Ahearne^{a, b} Ruby Y. Chang^b Brian H. Walsh^{c, d}
Geraldine B. Boylan^{a, b} Deirdre M. Murray^{a, b}

^aThe Irish Centre for Foetal and Neonatal Translational Research (INFANT), and ^bClinical Investigations Unit, Department of Paediatrics and Child Health, University College Cork, Cork, Ireland; ^cDivision of Newborn Medicine, Boston Children's Hospital, and ^dDepartment of Pediatric Newborn Medicine Brigham and Women's Hospital, Boston, MA, USA

Keywords

Hypoxic-ischaemic encephalopathy · Perinatal asphyxia · Biomarkers · Developing brain · Umbilical cord blood · Neurodevelopment outcome

Abstract

Activation of the inflammatory pathway is increasingly recognized as an important mechanism of injury following neonatal asphyxia and encephalopathy. This process may contribute to the poor prognosis seen in some cases, despite therapeutic hypothermia. Our group has previously identified raised interleukin (IL)-6 and IL-16, measured in umbilical cord blood at birth, to be predictive of grade of hypoxic-ischaemic encephalopathy (HIE). Our aim in this study was to examine the ability of these cytokines to predict the 3-year neurodevelopmental outcome in the same cohort. As part of a prospective, longitudinal cohort study set in a single tertiary maternity unit, term infants with biochemical and clinical evidence of perinatal asphyxia were recruited at birth. Umbilical cord blood was collected and analyzed for IL-6 and IL-16 using a Luminex assay. The neurodevelopmental outcome of these infants was assessed at 3 years using the Bay-

ley Scales of Infant and Toddler Development (Edition 3). Early cord blood measurement of IL-6 and IL-16 and long-term outcome were available in 33/69 infants. Median (IQR) IL-16 differentiated infants with a severely abnormal outcome ($n = 6$) compared to all others ($n = 27$), (646 [466–1,085] vs. 383.5 [284–494] pg/mL; $p = 0.012$). IL-16 levels were able to predict a severe outcome with an area under the receiver-operating characteristic (ROC) curve of 0.827 (95% CI 0.628–1.000; $p = 0.014$). Levels ≥ 514 pg/mL predicted a severe outcome with a sensitivity of 83% and a specificity of 81%. IL-16 also outperformed other routine biochemical markers available at birth for the prediction of severe outcome. APGAR scores at 1 and 10 min were also predictive of a severe outcome ($p = 0.022$ and $p = 0.036$, respectively). A combination of IL-16 with these clinical markers did not improve predictive value, but IL-16 combined with electroencephalogram grading increased the area under the ROC curve. IL-6 did not show any association with 3-year outcome. This is the first report studying the association of IL-16 measured at birth with long-term outcome in a cohort of neonates with perinatal asphyxia. IL-16 may be an early biomarker of severe injury and aid in the long-term prognostication in infants with HIE.

© 2017 S. Karger AG, Basel

Introduction

Perinatal asphyxia is one of the most common causes of neonatal mortality and morbidity, with 20/1,000 deliveries requiring significant resuscitation [1]. In countries with low neonatal mortality rates, 1.6/1,000 of these will go on to develop hypoxic-ischaemic encephalopathy (HIE) [2]. On a global level, HIE causes nearly 1 million neonatal deaths each year [3]. Where resources are available, the use of therapeutic hypothermia (TH) has significantly improved mortality and neurodevelopmental outcomes in infants with moderate and severe HIE [4, 5]. Currently, the standard management for HIE is to commence TH within the first 6 h of birth. An early biomarker to guide diagnosis and prognostication in these infants could support clinical decision-making in this crucial timeframe.

The role of inflammation in neonatal hypoxic-ischaemic (HI) injury has long been recognized, though incompletely defined. Experimental models of perinatal injury have consistently shown evidence of microglial activation in response to hypoxia-ischaemia followed by the release of diverse inflammatory mediators including cytokines [6]. The effect of this inflammatory response is potentially either advantageous or deleterious and the debate continues on the effect of this balance in many forms of central nervous system (CNS) injury [7]. Raised inflammatory mediators, including interleukin-6 (IL-6), measured in postnatal blood and cerebrospinal fluid samples as well as on magnetic resonance spectroscopy have previously been shown to be associated with adverse outcomes [8–10]. Inflammation and infectious sensitization appears to increase injury, and may also reduce the response to TH [11].

Work previously published by our group, following the analysis of multiple candidate analytes, reported 2 cytokines measured in umbilical cord blood, IL-6 and IL-16, which were significantly increased in infants with subsequent moderate-to-severe clinical and electroencephalographic (EEG) grades of encephalopathy. The combination of 10-min APGAR and IL-16 was able to predict an abnormal EEG better than other markers utilized in current practice [12]. Raised IL-6 in postnatal samples has been previously described in HIE to be associated with more severe injury and a poor long-term outcome [9, 13]. Prior to this work, levels of IL-16 in neonatal hypoxia-ischaemia had not been described. However, IL-16 alterations have been identified in other pathologies of the nervous system, specifically multiple sclerosis, autoimmune encephalomyelitis, cerebral infarctions, spi-

nal cord injuries and astrocytic brain tumours [7, 14–16]. The role of IL-16 remains poorly understood. No study to date has examined the relationship between the cord blood levels of IL-16 or IL-6 and long-term neurodevelopmental outcome following HIE.

The primary aim of this study was to explore the association between the levels of the cord blood inflammatory proteins IL-16 and IL-6 collected at birth and the neurodevelopmental outcome at 3 years in the same cohort of infants with perinatal asphyxia and HIE.

Materials and Methods

Recruitment took place from September 2009 to June 2011 in a single maternity hospital and was designed to recruit all grades of HIE; mild, moderate, and severe. Infants of ≥ 36 weeks' gestation were recruited if they had any of the following risk factors for perinatal asphyxia: umbilical cord pH < 7.1 , 5-min APGAR score ≤ 6 , or the need for intubation or cardiopulmonary resuscitation at delivery. The presence and grade of HIE was determined by modified Sarnat score, assigned at 24 h after birth by a dedicated research fellow (B.H.W.). Written informed parental consent was obtained for inclusion. The Clinical Research Ethics Committee of the Cork Teaching Hospitals provided ethical approval.

In clinically identified cases of neonatal encephalopathy, infants underwent multi-channel video-electroencephalogram (EEG) monitoring as previously described [17]. The entire video-EEG was reviewed by an experienced neonatal neurophysiologist (G.B.B.). One-hour epochs of the background EEG were graded at 6 and 24 h after birth where available. An EEG grade was assigned at these time points, based on a previously described classification system [18, 19] and designated as: (0) normal, i.e. sleep cycles present on continuous background, (1) mildly abnormal, i.e. continuous, mild asymmetry but disrupted sleep cycles, (2) moderately abnormal, i.e. excessive discontinuity or the presence of seizures, and (3) severely abnormal, i.e. suppressed/isoelectric tracing and/or high seizure burden/status epilepticus.

Demographic and clinical details were collected prospectively. These included perinatal measures routinely used in the evaluation of infants with perinatal asphyxia such as APGAR scores at 1, 5, and 10 min, as well as pH, lactate, and base deficit measured on first postnatal sampling. TH was commenced at the discretion of the supervising clinician on duty based on compliance with the Total Body Hypothermia (TOBY) criteria [20] within the first 6 h of life.

Infants meeting the above criteria had umbilical cord blood drawn at birth and processed according to strict, standardized operating procedures. The plasma was then frozen at -80°C within 3 h of birth. IL-6 and IL-16 were measured using Luminex technology (Rules-Based Medicine, Austin, TX, USA). Further information regarding sample processing and analysis is available from Walsh et al. [12].

Developmental outcome was assessed at 36–42 months of age using the Bayley Scales of Infant and Toddler Development, Edition 3 (BSID-III). A research fellow (C.E.A) blinded to the clinical history, EEG findings, and cord blood cytokine levels measured at the birth of the patients performed all BSID-III assessments.

Table 1. Demographic details of the cohort

	Non-severe outcome (<i>n</i> = 27)	Severe outcome (<i>n</i> = 6)	<i>p</i> value ¹
Gestational age, weeks	40.4 (39.2–41.1)	40.8 (39.7–41.4)	0.372
Male/female gender	17/10	5/1	0.637
Birth weight, kg	3.5 (3.2–4.1)	3.4 (3.1–3.7)	0.569

Values express median (IQR).

¹ Derived with the Mann-Whitney U test, the χ^2 test, or the Fisher exact test as appropriate.

Neurodevelopmental outcome severity was defined a priori. Three-year outcome was designated as normal if the child scored >85 on all 3 BSID-III subscales, i.e. cognitive, language, and motor. A mildly abnormal outcome was determined if 1 or 2 subscales scored ≤ 85 but >70, and a moderately abnormal outcome if all 3 scored ≤ 85 but >70. Infants were deemed to have a severely abnormal outcome if they scored ≤ 70 in all subscales, or suffered dyskinetic or spastic quadriplegic cerebral palsy or autism, or if they died [2, 21, 22].

Statistical analysis was performed using IBM SPSS v22.0. Patient demographic information is presented as median (interquartile range, IQR) and *n/n* (%) as appropriate. Differences between groups were examined using the Kruskal-Wallis and Mann-Whitney U tests as appropriate. Correlation of data was performed using the Pearson correlation coefficient or the Spearman rho (ρ) correlation coefficient as appropriate. The ability of the cord blood proteins to predict a severe outcome at 3 years was assessed using receiver-operating characteristic (ROC) curve analysis. Biomarker utility was determined using the area under the ROC curve (AUC), and the best cut-off was determined using the coordinates of the curve. Sensitivity and specificity for a given cut-off are reported. A *p* value <0.05 was considered significant. Binomial logistical regression was used to examine combinations of significant markers for associations with outcome, and the predicted probabilities resulting from the model were also assessed for their ability to predict a severe outcome upon analysis of the ROC curve.

Results

Umbilical cord blood analysis and neurodevelopmental outcome were available for 33 infants out of 69 recruited at birth. There were 22 males and 11 females, the median (IQR) gestational age at birth was 40.4 (39.6–41.2) weeks and the median (IQR) birth weight was 3.49 (3.12–4.09) kg. Of these, 20/33 (60%) were given a clinical diagnosis of HIE in the neonatal period (11 mild, 4 moderate, and 5 severe) while the remaining 13/33 (40%) were those with clinical or biochemical signs of perinatal asphyxia at birth who recovered quickly with no clinical signs of encephalopathy, i.e. the “non-encephalopathic” group. Two infants (6%), both from the non-encephalopathic group,

were born following prolonged rupture of membranes (>18 h). A total of 7/33 (21%) infants, 4 with moderate HIE and 3 with severe HIE, were treated with TH. Of the 2 severe HIE cases that were not treated, 1 died before the initiation of cooling and the other developed symptoms outside the therapeutic window for cooling. Two of the 7 cooled infants died in the neonatal period (Table 1).

At the 3-year follow-up, 23/33 (70%) had a normal outcome, 1/33 (3%) had a mildly abnormal outcome, 3/33 (9%) had a moderately abnormal outcome, and 6/33 (18%) had a severely abnormal outcome (Table 2). Those with a normal outcome included 12 non-encephalopathic infants, 6 with mild encephalopathy, 4 with moderate encephalopathy, and 1 with severe encephalopathy according to the modified Sarnat score at 24 h. The child with a mildly abnormal outcome at 3 years had suffered a mild clinical encephalopathy. All 3 children with a moderately abnormal outcome had a mild clinical encephalopathy postnatally. The 6 children with a severely abnormal outcome included 1 from the non-encephalopathic group, 1 with mild encephalopathy, and 4 with severe encephalopathy. Those with a severely abnormal outcome included 3 neonatal deaths, 1 child who developed dyskinetic cerebral palsy, and 2 with an autism diagnosis. All of the surviving cooled infants had a normal neurodevelopmental outcome at follow-up.

Interleukin-16

While median (IQR) IL-16, measured in cord blood at birth, failed to differentiate across all 4 outcome groups as outlined above (*p* = 0.063; Table 2), it was able to differentiate infants with a severely abnormal outcome compared to all others; 646 (466–1,085) versus 383.5 (284–494) pg/mL (*p* = 0.012; Fig. 1). IL-16 levels were able to predict a severe outcome with an AUC of 0.827 (95% CI 0.628–1.000; *p* = 0.014). Levels ≥ 514 pg/mL predicted a severe outcome with a sensitivity of 83% and a specificity of 81%.

Table 2. Clinical data of the cohort according to details of outcome severity

Outcome	Normal (<i>n</i> = 23)	Mild (<i>n</i> = 1)	Moderate (<i>n</i> = 3)	Severe (<i>n</i> = 6)
Sarnat score				
Perinatal asphyxia	12	0	0	1
Mild	6	1	3	1
Moderate	4	0	0	0
Severe	1	0	0	4
EEG at 24 h (<i>n</i> = 18)				
Normal	4	0	0	0
Mild	3	1	2	0
Moderate	2	0	1	0
Severe	2	0	0	3
Median IL-6 (IQR), pg/mL	11 (5–24)	15	8 (7–8)	31 (5–255)
Median IL-16 (IQR), pg/mL	403 (284–517)	381	304 (139–304)	646 (466–1,086)

Fig. 1. Boxplot representing IL-16 levels of infants with a non-severe neurodevelopmental outcome compared to those with a severely abnormal outcome.

Other perinatal and neonatal markers were tested for the prediction of severe outcome in this cohort (Table 3). Biochemical markers of pH, lactate, and base deficit, measured at the first postnatal blood sampling did not differentiate between outcome groups. APGAR scores at 1 and 10 min of life were significant for the prediction of a severe outcome ($p = 0.022$ and $p = 0.036$, respectively). Among the early neonatal tools used to assess and prognosticate in perinatal asphyxia, the modified Sarnat score assigned at 24 h of life and EEG graded at 6 and 24 h of life performed well for the prediction of severe outcome

at 3 years, with EEG at both time points having an AUC ≥ 0.9 ($p = 0.033$ and 0.008 , respectively). Each significant variable was combined with IL-16 pairwise to attempt to identify an improved predictive model. Combining IL-16 and APGAR score at 10 min of life, the combination shown to be most promising in our previous work, did not improve the AUC for predicting severe 3-year outcome (i.e., 0.819 , 95% CI 0.593 – 1.000 ; $p = 0.017$). This is likely due to the high degree of correlation between them ($R^2 = 0.38$; $p < 0.01$). Other combinations are shown in Table 3. Combining IL-16 with EEG grade at 6 or 24 h of life improved the AUC to 0.978 (95% CI 0.913 – 1.000 ; $p = 0.011$) and 1.000 (95% CI 1.0 – 1.0 ; $p = 0.008$), respectively.

Among the 27 surviving children who attended and could perform the BSID-III, levels of IL-16 were not found to correlate with scores on the individual subscales (cognitive $\rho = 0.241$, $p = 0.236$; language $\rho = -0.016$, $p = 0.944$; motor $\rho = 0.108$, $p = 0.625$).

Interleukin-6

IL-6 was unable to differentiate between infants with differing severity of neurodevelopmental outcome ($p = 0.520$), and did not correlate with individual subscale composite scores (cognitive $\rho = 0.270$, $p = 0.173$; language $\rho = 0.221$, $p = 0.299$; motor $\rho = 0.018$, $p = 0.935$).

Discussion

Our analysis has shown an association between elevated cord blood IL-16 and severely abnormal neurodevelopmental outcome at 3 years in a cohort of infants with perinatal asphyxia and HIE. These results must be care-

Table 3. Values for clinical and biochemical markers available at delivery across the outcome groups and AUC

	Non-severe (<i>n</i> = 27)	Severe (<i>n</i> = 6)	<i>p</i> value ¹	Severe outcome	
				AUC (95% CI)	<i>p</i> value ²
IL-6, pg/mL	9.3 (5.5–19.0)	31 (5.2–255.8)	0.222	0.667 (0.388–0.945)	0.208
IL-16, pg/mL	384 (284–494)	646 (466–1,086)	0.012*	0.827 (0.628–1.000)	0.014*
Biochemical markers					
pH	7.04 (7.00–7.24)	6.97 (6.61–7.19)	0.429	0.636 (0.265–1.000)	0.394
Lactate	8.2 (5.4–11.3)	14.5 (10.6–21.8)	0.067	0.803 (0.597–1.000)	0.062
Base deficit	11.2 (8.9–13.6)	12.7 (10.4–19.0)	0.308	0.641 (0.391–1.000)	0.288
Clinical markers					
APGAR at 1 min	4 (2–6)	1 (0–3)	0.021*	0.802 (0.572–1.000)	0.022*
APGAR at 5 min	6 (4–8)	4 (0–7)	0.109	0.713 (0.480–0.946)	0.107
APGAR at 10 min	9 (7–10)	4 (0–9)	0.035*	0.780 (0.536–1.000)	0.036*
Sarnat score	–	–	–	0.796 (0.551–1.000)	0.025*
EEG at 6 h	–	–	–	0.900 (0.754–1.000)	0.033*
EEG at 24 h	–	–	–	0.933 (0.815–1.000)	0.008*
IL-16 + APGAR at 1 min				0.814 (0.554–1.000)	0.018*
IL-16 + APGAR at 10 min				0.819 (0.593–1.000)	0.017*
IL-16 + Sarnat				0.827 (0.596–1.000)	0.014*
IL-16 + EEG at 6 h				0.978 (0.913–1.000)	0.011*
IL-16 + EEG at 24 h				1.000 (1.000–1.000)	0.008*

Values express median (IQR), unless otherwise indicated; pH, lactate, and base deficit were measured at the first postnatal blood sampling. AUC, area under the ROC curve.

¹ Obtained with the Mann-Whitney U test. ² Shows the ability of the marker to predict a severe outcome. * *p* < 0.05.

fully interpreted in the light of only 50% follow-up data being available and the considerable overlap in IL-16 levels between infants with normal, mild, and moderate outcomes. Due to the low numbers in the mild- and moderate-outcome groups, we are limited in our ability to speculate upon reasons for this overlap.

The combination of IL-16 with other predictive clinical markers, APGAR score at 1 and 10 min, and EEG at 6 and 24 h, showed equivalent or improved predictive value. These additional measures may, in the future, form part of a clinical-decision algorithm for the management of infants with perinatal asphyxia, but the subjective nature of APGAR scoring and the demands on resources of providing and interpreting continuous multi-channel EEG may influence this.

IL-16 is an unusual cytokine which appears to have a crucial role in the CNS inflammatory response to injury. It is a glycoprotein of 56-kDa relative molecular mass and is mapped to chromosome position 15q26.1, a position distinct from all other cytokines [23]. It was previously referred to as lymphocyte chemoattractant factor,

reflecting its important inflammatory actions. These actions are both proinflammatory and immunomodulatory. IL-16 is produced in a variety of immune cells, including CD4+ and CD8+ cells, monocytes, and microglial cells as the inactive pro-IL-16, which is then cleaved by caspase 3 to form the active C-terminal [15]. Recently, IL-16 has also been discovered to occur in a pre-formed state intracellularly in neutrophils. In normal circumstances, IL-16 becomes activated in a caspase-dependent manner during apoptosis. However, Roth et al. [24] have shown that during pathological processes such as infection and autoimmunity, IL-16 may be activated as part of the cascade of necrotic cell death.

Active IL-16 has previously been shown to have a role in CD4+ cell recruitment and activation, particularly in asthma and autoimmune conditions [25], but also following CNS injury, e.g. following cerebral infarction [15]. It is a potent chemoattractant and also has a regulatory function in the induction of cytokines including IL-6, TNF- α , and IL-1 β [26]. IL-16 is produced by microglia in response to CNS injury and, in turn, activates microglial

migration to the site of injury and repair [27]. Following ischaemic infarction, it reacts with CD4+ receptors and activates T cell migration, through the up-regulation of receptors such as T cell CD25 and MHC class 2 molecules. The neuronal variant, neuronal IL-16, is indistinguishable from uncleaved IL-16, and has been found to be expressed in cerebellar granular cells and hippocampal neurons. Neuronal IL-16 may be intrinsic to neuronal growth and survival mechanisms [14, 28, 29]. These areas of the brain are also among those most sensitive to hypoxia [30–32]. In studies on rat spinal cord injuries, IL-16 appears to accumulate significantly in cells within and adjacent to the lesion as well as in the local microvasculature for several days after injury, and it is therefore postulated to have a role in secondary damage [7]. In the relapsing form of multiple sclerosis, IL-16 has come under close scrutiny for its possible role in neuroinflammation and axonal damage [14]. Similar findings have been described when investigating the role of IL-16 in cerebral infarctions and astrocytic brain tumours [15, 16]. Along with its function in inflammatory cell activation and the induction of pro-inflammatory cytokine release, IL-16 has also been associated with induction of increased intracellular calcium or inositol-(1, 4, 5)-triphosphate and the translocation of protein kinase C, all of which can lead to neuronal ischaemic cell death [7]. There is, therefore, strong evidence of the involvement of IL-16 in the CNS response to injury. The results of this study suggest that there is a place for IL-16 in the pathophysiology of neonatal HI injury, although further investigation is required.

Based on our results, IL-16 appears to be a biomarker worthy of further study for the prediction of short- and long-term outcome following an HI insult. Raised levels are associated with more severe EEG findings in the neonatal period and a more severe neurodevelopmental outcome at 3 years.

The lack of an association between cord IL-6 and outcome in the study may be related to the time course of the IL-6 response to injury. Previous studies have shown an

association between postnatal IL-6 levels and injury severity, but not with cord blood levels [13]. Therefore, it may be that taking measurements at birth is too early, and a later rise may be more helpful and more predictive of long-term outcome. Further discussion of IL-6 is available in our previous work by Walsh et al. [12].

Unfortunately, low sample numbers limit the interpretation of these results. These findings require validation in another prospective cohort. The effect of treatment, where implemented, may modulate outcomes in these children, and it interferes with our full understanding of the meaning of a raised IL-16 level. However, this reflects the true clinical situation in a hospital with the resources to undertake such a study. Despite these results, there is still much left to be learned about the role of IL-16 in these infants, particularly about how the severity of injury impacts levels. Additional investigation in animal models may help to further elucidate this process.

In this work, we have shown, for the first time, the potential of an inflammatory protein measured in the cord blood at birth to predict the neurodevelopmental outcome at 3 years. With the development of a point-of-care testing platform, IL-16 levels could be measured quickly and contribute to clinical decision-making within the crucial 6-h time-frame.

Disclosure Statement

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

Funding Sources

C.E.A., D.M.M., and the BiHIVE2 study (Validation of Biomarkers in Hypoxic-Ischaemic Encephalopathy) are supported by the Health Research Board Clinical Scientist Award, CSA/2012/40 (www.medscinet.net/BIHIVE/, www.clinicaltrials.gov NCT02019147). This work was also supported by a Science Foundation Ireland Research Centre Award (INFANT – 12/RC/2272).

References

- 1 Vannucci RC: Hypoxic-ischaemic encephalopathy. *Am J Perinat* 2000;17:113–120.
- 2 Lee AC, Kozuki N, Blencowe H, Vos T, Bahalim A, Darmstadt GL, Niermeyer S, Ellis M, Robertson NJ, Cousens S, et al: Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. *Pediatr Res* 2013;74(suppl 1):50–72.
- 3 Lawn JE, Cousens S, Zupan J: Four million neonatal deaths: When? Where? Why? *Lancet* 2005;365:891–900.
- 4 Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG: Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2013;1:CD003311.

- 5 Azzopardi D, Strohm B, Marlow N, Brocklehurst P, Deierl A, Eddama O, Goodwin J, Halliday HL, Juszczak E, Kapellou O, et al: Effects of hypothermia for perinatal asphyxia on childhood outcomes. *N Engl J Med* 2014;371:140–149.
- 6 Volpe JJ: *Neurology of the Newborn*, ed 5. Philadelphia, Saunders, 2008.
- 7 Mueller CA, Schluesener HJ, Conrad S, Pietsch T, Schwab JM: Spinal cord injury-induced expression of the immune-regulatory chemokine interleukin-16 caused by activated microglia/macrophages and CD8+ cells. *J Neurosurg Spine* 2006;4:233–240.
- 8 Bartha AI, Foster-Barber A, Miller SP, Vigneron DB, Glidden DV, Barkovich AJ, Ferriero DM: Neonatal encephalopathy: association of cytokines with MR spectroscopy and outcome. *Pediatr Res* 2004;56:960–966.
- 9 Foster-Barber A, Dickens B, Ferriero DM: Human perinatal asphyxia: correlation of neonatal cytokines with MRI and outcome. *Dev Neurosci* 2001;23:213–218.
- 10 Martin-Ancel A, Garcia-Alix A, Pascual-Salcedo D, Cabanas F, Valcarce M, Quero J: Interleukin-6 in the cerebrospinal fluid after perinatal asphyxia is related to early and late neurological manifestations. *Pediatrics* 1997;100:789–794.
- 11 Osredkar D, Thoresen M, Maes E, Flatebo T, Elstad M, Sabir H: Hypothermia is not neuroprotective after infection-sensitized neonatal hypoxic-ischemic brain injury. *Resuscitation* 2014;85:567–572.
- 12 Walsh BH, Boylan GB, Livingstone V, Kenny LC, Dempsey EM, Murray DM: Cord blood proteins and multichannel-electroencephalography in hypoxic-ischemic encephalopathy. *Pediatr Crit Care Med* 2013;14:621–630.
- 13 Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM: Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. *Pediatrics* 2002;110:673–680.
- 14 Skundric DS, Cruikshank WW, Drulovic J: Role of IL-16 in CD4+ T cell-mediated regulation of relapsing multiple sclerosis. *J Neuroinflamm* 2015;12:78.
- 15 Schwab JM: Human focal cerebral infarctions induce differential lesional interleukin-16 (IL-16) expression confined to infiltrating granulocytes, CD8+ T-lymphocytes and activated microglia/macrophages. *J Neuroimmunol* 2001;114:232–241.
- 16 Liebrich M, Guo LH, Schluesener HJ, Schwab JM, Dietz K, Will BE, Meyermann R: Expression of interleukin-16 by tumor-associated macrophages/activated microglia in high-grade astrocytic brain tumors. *Arch Immunol Ther Exp* 2007;55:41–47.
- 17 Reinke SN, Walsh BH, Boylan GB, Sykes BD, Kenny LC, Murray DM, Broadhurst DI: 1H NMR-derived metabolomic profile of neonatal asphyxia in umbilical cord serum: implications for hypoxic ischemic encephalopathy. *J Proteome Res* 2013;12:4230–4239.
- 18 Pressler RM, Boylan GB, Morton M, Binnie CD, Rennie JM: Early serial EEG in hypoxic ischaemic encephalopathy. *Clin Neurophysiol* 2001;112:31–37.
- 19 Murray DM, Boylan GB, Ryan CA, Connolly S: Early EEG findings in hypoxic-ischemic encephalopathy predict outcomes at 2 years. *Pediatrics* 2009;124:e459–e467.
- 20 Azzopardi D, Strohm B, Edwards AD: Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361:1349–1358.
- 21 Badawi N: Autism following a history of newborn encephalopathy: more than a coincidence? *Dev Med Child Neurol* 2006;48:85–89.
- 22 Badawi N, Felix JF, Kurinczuk JJ, Dixon G, Watson L, Keogh JM, Valentine J, Stanley FJ: Cerebral palsy following term newborn encephalopathy: a population-based study. *Dev Med Child Neurol* 2005;47:293–298.
- 23 Muhlhahn P, Zweckstetter M, Georgescu J, Ciosto C, Renner C, Lanzendorfer M, Lang K, Ambrosius D, Baier M, Kurth R, et al: Structure of interleukin 16 resembles a PDZ domain with an occluded peptide binding site. *Nat Struct Biol* 1998;5:682–686.
- 24 Roth S, Agthe M, Eickhoff S, Möller S, Karsten CM, Borregaard N, Solbach W, Laskay T: Secondary necrotic neutrophils release interleukin-16C and macrophage migration inhibitory factor from stores in the cytosol. *Cell Death Discov* 2015;1:15056.
- 25 Cruikshank WW: Interleukin 16. *J Leukoc Biol* 2000;67:757–766.
- 26 Mathy NL: Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. *Immunol* 2000;100:63–69.
- 27 Croq F, Vizioli J, Tuzova M, Tahtouh M, Sautiere PE, Van Camp C, Salzet M, Cruikshank WW, Pestel J, Lefebvre C: A homologous form of human interleukin 16 is implicated in microglia recruitment following nervous system injury in leech *Hirudo medicinalis*. *Glia* 2010;58:1649–1662.
- 28 Fenster CP, Chisnell HK, Fry CR, Fenster SD: The role of CD4-dependent signaling in interleukin-16 induced c-Fos expression and facilitation of neurite outgrowth in cerebellar granule neurons. *Neurosci Lett* 2010;485:212–216.
- 29 Kurschner C, Yuzaki M: Neuronal interleukin-16 (NIL-16): a dual function PDZ domain protein. *J Neurosci* 1999;19:7770–7780.
- 30 Le Strange E, Saeed N, Cowan FM, Edwards AD, Rutherford MA: MR imaging quantification of cerebellar growth following hypoxic-ischemic injury to the neonatal brain. *AJNR Am J Neuroradiol* 2004;25:463–468.
- 31 Gonzalez FF, Miller SP: Does perinatal asphyxia impair cognitive function without cerebral palsy? *Arch Dis Child Fetal Neonatal Ed* 2006;91:F454–F459.
- 32 Rennie JM, Hagmann CF, Robertson NJ: Outcome after intrapartum hypoxic ischaemia at term. *Semin Fetal Neonatal Med* 2007;12:398–407.