Pediatric RESEARCH

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COMMENT

Comment on: Serial blood cytokine and chemokine mRNA and microRNA over 48 h are insult specific in a piglet model of inflammation-sensitized hypoxia–ischaemia

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Birth asphyxia resulting in neonatal hypoxic–ischemic encephalopathy (NE) remains a global burden for individuals and societies since it is the leading cause of death and neurodevelopmental morbidity in childhood. Therapeutic hypothermia (TH) initiated within the first 6 h of life has emerged as a promising standard of care for moderate-to-severe HIE following a large number of experimental and clinical trials. However, 40–50% of infants with HIE suffer from major neurological sequelae, despite cooling.

Infants with NE may also suffer from co-existing (multi-)organ dysfunction that modulates the severity of brain injury and may also have an impact on their response to TH.

There is strong evidence that intra- and/or extra-uterine infection/inflammation may modify key responses to injury and thereby significantly contribute to brain damage in a pathogen-dependent manner providing one explanation for poor response to cooling.[1,2] In addition, recent studies suggest adverse neurological outcome in children suffering from mild HIE, who are, according to current guidelines, not eligible for TH.[3]

Clinical assessments such as the modified Sarnat scoring system,[4] originally designed in 1976 for serial neurological examinations, and, when available, amplitude-integrated electroencephalography (aEEG) are helpful to stratify children into risk groups for mild, moderate, and severe NE within the first 6 h of life. However, in the clinical setting experienced scoring and also technical equipment is not always available in such a short time period considering the fact that a large proportion of asphyxiated babies gets referred from lower levels of care.

Management of infants suffering from HIE of all grades is currently hindered by the lack of quantifiable serum biomarkers representing the true classification of injury. Therefore, the development of specific biomarkers is highly warranted not only for classification and potential treatment with TH, but also for identification of non-responders to TH, which might benefit from additional personalized therapies. Furthermore, adequate biomarker profiles will significantly contribute to parental guidance for the prediction of outcome and will be of particular interest for conduction of future neuroprotective trials.

Since inflammation appears to play a key role in the pathogenesis of injury with potential for drugs with immunomodulatory properties as a feasible therapy for neonates, it is important to find biomarkers to identify infants at risk. The study presented by Lingam and co-workers[5] has embarked on this challenge by use of a piglet model of birth asphyxia (global hypoxia) complicated by inflammation induced by *Escherichia coli* lipopolysaccharide application to simulate Gram-negative sepsis. They investigated a large variety of biomarkers in the serum of experimental animals at clinically relevant time points in search to identify patterns that may facilitate decisions for treatment (baseline, 4 h following lipopolysaccharide (LPS) application at 0,1, 3, 6, 12, 24, and 48 h following global hypoxia). However, analysis of experimental time points was limited by a high mortality by 48 h in the hypoxia/LPS group. In parallel, apoptotic cell death in brains as quantified by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining and aEEG recordings was assessed. No single blood-based marker is currently robust enough to assess the severity of NE or to predict response to or outcome following TH. Therefore, the authors chose a selection of cytokines (*IL1A*, *IL6*, *CXCL8*, *IL10*, *TNFA*) and brain biomarkers (*ENO2*, *UCHL1*, *S100B*, *GFAP*, *CRP*, *BDNF*, *MAPT*). MicroRNAs were detected by microarray GeneChip (Affymetrix) and compared to the current literature. Combinations of biomarkers that reached peak levels at selected time points following the insult were *ENO2* and *CCL2* at 1–3 h; *IL1A* and *IL10* at 6 h; and *TNFA* and *MAPT* at 24 h. Of particular interest is the finding that interleukin-10 mRNA was differentially expressed in the three experimental groups, thereby serving as a marker to differentiate between inflammation exposure and hypoxia only.

Levels of cytokines, however, did neither correlate with TUNEL-positive cells nor with aEEG patterns, which is not surprising since cell death is a multifactorial mechanism unlikely to be dependent on single cytokines or brain markers found in the serum. However, two microRNAs (miRNAs) (mir-150-5p, mir-181c-3p) known as markers for acute ischemic stroke and hypoxia-inducible factor-1α response correlated positively with cell death. However, miRNAs expressed in specific species under certain experimental conditions might get differently regulated in humans where other types of miRNAs can play role as biomarkers.[6]

Predictive power varies very much depending on the timing of measurement of the biomarker and the sample type, which is very much standardized in an animal model. The combination of large panels of inflammatory and neuronal markers and specific patterns of microRNAs in addition to metabolomic profiling, all with the aim to develop bedside tools for better stratification of the degree of injury in NE, will continue to be under intensive investigation in neonatal research. In clinical biomarker studies,

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specific non-brain biomarkers, indicating endocrine, cardiac, renal, and liver function, need to be included in protocols in order to provide information on the extent of injury following HIE.

The present very nicely elaborated study by Lingam et al.[5] has significantly contributed to paving the way for the clinical utility of mRNA-based biomarkers, supporting the development of point-of-care devices to identify children at risk for inflammation shortly after birth asphyxia in order to guide personalized care.

ADDITIONAL INFORMATION

Competing interests: The author declares no competing interests.

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