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COMMENT

Biomarkers in neonatal encephalopathy: new approaches and ongoing questions

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Early therapeutic hypothermia is the cornerstone of neuroprotection for neonates with encephalopathy; the search is ongoing for better and adjunctive therapies. An obstacle to research efforts and clinical treatment alike is the need for rapid, accurate diagnosis of neonatal encephalopathy. Not all neonatal encephalopathy is due to hypoxia–ischemia; at the same time, identifying suspected hypoxic–ischemic encephalopathy (HIE) has been a particular focus because therapeutic hypothermia is most effective when initiated for neonates with suspected HIE within 6 h of birth. Conventional diagnosis relies on recognition of early indicators such as cord gas values suggestive of hypoxia–ischemia, in combination with examination findings of encephalopathy. This identifies those neonates with a sufficiently high likelihood of HIE to warrant intervention with therapeutic hypothermia. However, cord gas data may not be obtained in all cases, even a structured neurologic exam such as in the Sarnat scale or Thompson Score can be challenging and subjective, and thus a significant number of neonates with encephalopathy are not identified early enough for intervention. Conversely, not every neonate suspected of having neonatal encephalopathy at birth ultimately goes on to have an abnormal outcome; for these newborns escalation of therapy may be unnecessary. Biomarkers to reliably and rapidly identify neonates with the highest risk for brain injury are urgently needed in clinical practice. Furthermore, biomarkers are crucial for research, to help identify those at highest risk for inclusion in clinical trials, and to elucidate underlying mechanisms of injury.

In this issue of *Pediatric Research*, Piñeiro-Ramos and colleagues report findings from a multicenter study of urinary biomarkers in 55 newborns with neonatal encephalopathy enrolled in the HYPOTOP trial.[1] HYPOTOP is a randomized, controlled trial of topiramate in neonates with encephalopathy undergoing therapeutic hypothermia;[2] the authors capitalized on that infrastructure to investigate the urinary metabolome in enrolled neonates. Urine samples were collected before initiation of hypothermia, and then at 12, 24, 48, 72, and 96 h after initiation of hypothermia. Urine was analyzed using three mass spectrometry assays, identifying over 11,000 features used for further analysis. All neonates had magnetic resonance imaging (MRI) at days 4–8 after birth, interpreted by a blinded neuroradiologist using the NICHD NRN scoring system to characterize the primary outcome of injury on brain MRI.

The authors report that the urinary metabolome profile evolved longitudinally from birth through cooling, with characteristic "metabolomic fingerprints" associated with the severity of MRI injury. No single marker was highly predictive of outcome. Rather, there was an overall trajectory of abnormalities first identified before cooling, reduced at 12 h after the start of hypothermia, and then increasing again with the number of altered features ranging from 4 to 8% at subsequent time points. Models based on 398 of these features allowed prediction of MRI outcome specific to each of 4 degrees of severity, with areas under the curve ranging from 0.86 to 0.98.

Prior work has explored a variety of biomarkers with the potential to reliably identify neonates in the first hours after birth who are at high risk for brain injury or abnormal outcomes. The majority has analyzed molecules in the blood, although some studies have focused on cerebrospinal fluid (CSF) or urinary compounds. While these have provided some insight into pathogenesis, none is yet ready for clinical use. The BiHiVE (Biomarkers in Hypoxic-ischemic Encephalopathy) and BiHiVE2 studies identified umbilical cord metabolite and microRNA markers that were highly associated with HIE as identified by Sarnat exam and confirmed by electroencephalogram (EEG), improving the ability to predict HIE[3] and correlating with long-term developmental outcome.[4] Similarly, analysis of blood spots from a subcohort of neonates from the NICHD Neonatal Research Network trial of hypothermia studied cytokines/chemokines and identified two which predicted neurodevelopmental outcome at age 6–7 years.[5] Numerous further studies have identified individual or few blood or CSF biomarkers with the potential to predict brain injury on MRI or neurodevelopmental outcome. The majority of these have not been independently validated or studied for clinical utility.

The current work by Piñeiro-Ramos and colleagues is unique both in studying noninvasive biomarkers of neonatal encephalopathy and in using a much larger scale of analysis to present a dynamic picture of the metabolome from birth through completion of hypothermia. Urine sampling is noninvasive, preferable to serial blood sampling. Similarly, it has advantages over cord blood samples, which are not always available when concern for neonatal encephalopathy first arises, and over CSF, which is impractical for rapid decision making in some critically ill neonates. A potential limitation of urine sampling is that some severely affected neonates may have no or little urine output in the hours after birth; however, in this study neonates had urine adequate for testing before the initiation of hypothermia 6 h after birth. This study was also notable for an approach employing three complementary assays to assess several thousand candidate factors. This allows analysis to rely not on sole markers, but rather to incorporate a vastly larger data set. Longitudinal studies of biomarkers have the advantage of learning from the trajectory of results over time, rather than solely at birth or any other single

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time point. This has the potential to distinguish those neonates who, after starting from one presentation at birth with an associated risk of poor outcome, follow a course that portends worsening or increased risk, and therefore are most in need of additional interventions. In this way, monitoring of the dynamic evolution of brain injury through the metabolome might facilitate personalized medicine by indicating when and which babies would benefit from the escalation of treatment.

Among a large number of factors studied, the authors also note that pathway analysis demonstrated ten biochemical pathways impacted in this cohort. These findings likely reflect the disruption of multiple independent pathways underway in neonatal encephalopathy, each of which might be a target for intervention. Characterization of metabolic pathway disruptions among neonates with encephalopathy, particularly when paired with detailed phenotyping, may offer an avenue for future research to better understand the similarities and differences in underlying causes and processes of brain injury. Recent biomarker analysis found that despite similar presentations of neonatal encephalopathy with similar initial lactic acidosis, there are differing markers (vascular endothelial growth factor and interleukin-10) between those neonates who also had sentinel events vs those who did not, suggesting that the biomarkers might be useful to distinguish the causes of neonatal encephalopathy, leading to the common end clinical pathway.[6]

The findings reported here are promising, highlighting numerous opportunities for future work. A strength of this study is the inclusion of a cohort enrolled across multiple sites, which suggests generalizability. At the same time, as in all studies of biomarkers, the results must be independently validated in an external cohort prior. Similarly, the large quantity of data collection and analysis employed here has the potential to identify patterns with predictive power beyond what is possible from one or two biomarkers. At the same time, this larger-scale analysis would need to be proven feasible for a rapid turnaround to be clinically useful, and possible using equipment and analytic tools available to most neonatal intensive care units. Additional study would also be helpful to examine how tracking the urinary metabolome might best be used in combination with traditional tools for longitudinal assessment, such as clinical examination and EEG monitoring.

This research presents a new approach using numerous urinary analytes to identify biomarker patterns predictive of brain injury on MRI, with a very good ability to predict injury severity. Ultimately, converging lines of investigation may identify and validate candidate biomarkers for a "Neonatal Encephalopathy Panel" to provide a quantitative assessment of risk for brain injury at the bedside. While much work remains before that goal will be achieved, the addition of the large-scale urinary metabolome to the potential diagnostic arsenal is an encouraging step forward.

ADDITIONAL INFORMATION

Competing interests: The author declares no competing interests.

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