



# Blood-Based Biomarkers Predict Cerebral Palsy and Cognitive Delay in Hypoxic-Ischemic Encephalopathy: A Secondary Analysis of the High-Dose Erythropoietin for Asphyxia and Encephalopathy Randomized Controlled Trial

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**Objective** To determine whether blood-based biomarkers in the first days of life could specifically predict cerebral palsy (CP) and/or cognitive/language delay in infants with moderate-severe hypoxic-ischemic encephalopathy (HIE).

**Study design** A secondary analysis of the High-dose Erythropoietin for Asphyxia and Encephalopathy trial, which examined erythropoietin as an adjunct to therapeutic hypothermia for moderate-severe HIE. Recruitment took place in the United States from 2017-2019. Analysis focused on 180 infants with biomarker measurements performed at baseline, day 2, and day 4 of life. Outcomes assessed were: a diagnosis of CP at 2 years and Bayley III cognitive/language scores <85 and, separately, <70 at 2 years. Magnetic resonance imaging (MRI) of the brain was performed on day 4-6 of life.

**Results** Mean gestational age was 39.0 weeks and 68 (44%) were female. After adjusting for multiple comparisons, 5 biomarkers: total tubulin-associated protein (Tau), glial fibrillary acidic protein, vascular endothelial growth factor, macrophage inflammatory protein 1b, and interleukin-13 were significantly altered in the CP group. On day 2 of life, Tau resulted in a receiver operating characteristic area under the curve (AUC) of 0.902 (95% CI: 0.812-0.992), specificity 91%, and sensitivity 88%, performing as well as detailed MRI scoring (AUC: 0.90 [95% CI: 0.80-1.0]). A different set of biomarkers showed altered expression in those with cognitive/language delay; Tau, ubiquitin carboxy-terminal hydrolase L1, S100 calcium-binding protein B, and neuron-specific enolase, with the best prediction seen with Tau on day 2 of life (AUC: 0.669 [95% CI: 0.564-0.774], sensitivity 40%, specificity 92%).

**Conclusions** Tau on day 2 of life demonstrated excellent prognostic value for CP in this cohort with moderate-severe HIE and gave similar prediction to detailed MRI score on day of life 4-6. Patterns of neurospecific biomarker perturbations were different for the prediction of cognitive/language delay, but Tau on day 2 remained the best performing biomarker. (*J Pediatr* 2026;297:115216).

**Trial registration** ClinicalTrials.gov NCT02811263.

|         |                                                          |          |                                         |
|---------|----------------------------------------------------------|----------|-----------------------------------------|
| aEEG    | Amplitude-integrated electroencephalography              | IL-12p70 | Interleukin-12p70                       |
| AUC     | Area under the curve                                     | IL-13    | Interleukin-13                          |
| cEEG    | Multichannel electroencephalography                      | IL-17    | Interleukin-17                          |
| CI      | Confidence interval                                      | IL-33    | Interleukin-33                          |
| CONSORT | Consolidated Standards of Reporting Trials               | IL-6     | Interleukin-6                           |
| CP      | Cerebral palsy                                           | IL-8     | Interleukin-8                           |
| EDTA    | Ethylendiaminetetraacetic acid                           | IQR      | Interquartile range                     |
| Epo     | Erythropoietin                                           | LMIC     | Low- and middle-income countries        |
| GFAP    | glial fibrillary acidic protein                          | MIP      | macrophage inflammatory protein         |
| GMA     | General Movements Assessment                             | MIP-1b   | macrophage inflammatory protein 1b      |
| GMFCS   | Gross motor function classification system               | MRI      | Magnetic resonance imaging              |
| HEAL    | High-dose erythropoietin for asphyxia and encephalopathy | NSE      | neuron-specific enolase                 |
| HIE     | Hypoxic ischemic encephalopathy                          | ROC      | Receiver operating characteristic       |
| IL      | Interleukin                                              | S100b    | S100 calcium-binding protein B          |
| IL-1    | Interleukin-1                                            | Tau      | tubulin-associated protein              |
| IL-10   | Interleukin-10                                           | TH       | Therapeutic hypothermia                 |
|         |                                                          | UCH-L1   | ubiquitin carboxy-terminal hydrolase-L1 |
|         |                                                          | VEGF     | vascular endothelial growth factor      |

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Cerebral palsy (CP) is the most common physical disability of childhood, with a prevalence of 1.6 per 1000 live births in high-income countries and higher in low- and middle-income countries (LMICs).<sup>1</sup> Hypoxic-ischemic encephalopathy (HIE) is an important cause of CP. Early prediction of outcome in HIE is complex and cannot be based on the severity of encephalopathy alone.<sup>2,3</sup> Prognosis is necessary both for counseling of families and also to identify candidates for emerging therapies which may have efficacy beyond the current narrow therapeutic window.<sup>4,5</sup> Current methods of prognostication include magnetic resonance imaging (MRI), lactate/N-acetyl aspartate peak area ratio on magnetic resonance spectroscopy, multichannel electroencephalography (cEEG), and amplitude-integrated electroencephalography (aEEG).<sup>6-11</sup> A combination of these, with expert interpretation combined with clinical information, can be highly predictive of outcome.<sup>12</sup> However, given the resources and expertise required, many centers do not have access to cEEG or aEEG. In LMICs, neither MRI nor cEEG/aEEG are widely available.<sup>1</sup> Specific patterns of brain injury on MRI are beneficial in providing individualized prognostication.<sup>12,13</sup> Even for those in high-income countries with access to MRI, the detailed published reporting systems are cumbersome in terms of the scanning protocols required and the level of reporting detail.<sup>14</sup> In addition, up to 30% of those with CP will have normal neuroimaging.<sup>13,15,16</sup>

Cognitive outcome following HIE is variable, even in the era of therapeutic hypothermia (TH), with or without a diagnosis of CP.<sup>17</sup> Despite detailed neuroimaging with expert interpretation, cognition can be even more difficult to predict than motor outcome.<sup>12</sup> This may be related to the fact that social and environmental factors have a significant impact on cognitive outcome, particularly maternal education level and an enriched literacy environment.<sup>18</sup>

Guidelines on early diagnosis of CP highlight the need for laboratory-based biomarkers. A combination of clinical information with biochemical measurements has been proposed as the path forward for the early prediction of outcomes in neonatal HIE.<sup>13,19</sup> Predictive biomarkers can identify which individuals or groups are most likely to experience a known outcome based on the presence or change in biomarker concentrations.<sup>20</sup> Alterations in the expression of inflammatory and neurospecific proteins have been previously reported in HIE and when assessing neurodevelopmental impairment and death as a composite outcome.<sup>19</sup> Blood-based biomarkers could offer a quantifiable measurement for the early prediction of CP as a specific outcome measure. The ideal predictor of outcome would be early, objective, accurate with a high sensitivity and specificity, inexpensive, and easily performed and interpreted by nonexpert users.

One of the challenges in developing a predictor of outcome relates to the heterogeneity in defining “outcome.” While the literature has historically reported a composite of death and disability, there is a shift toward predicting more specific measures of outcome. What may be referred to as “disability” is a broad spectrum, ranging from a simple hearing aid to

quadriplegic CP with a gross motor functional classification system (GMFCS) level 5. Increasing patient and public involvement in research has added momentum to this concept of precision prognostication and defining outcomes that are meaningful to families, with parents becoming advocates for change in health research.<sup>21,22</sup>

This study is an exploratory, secondary analysis of the previously published High-dose Erythropoietin for Asphyxia and Encephalopathy (HEAL) study.<sup>23</sup> We sought to determine if early blood-based biomarkers can specifically predict CP and separately to determine if they can predict cognitive/language delay.

## Methods

### Study Sample

This is an exploratory secondary analysis of data from the HEAL study, a multicenter, randomized controlled trial of erythropoietin (Epo) as an adjuvant neuroprotective agent for infants with neonatal encephalopathy undergoing TH, with a primary outcome of composite death and mild/moderate/severe neurodevelopmental impairment at 2 years of age ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT02811263) Identifier: NCT02811263).<sup>23-25</sup> Inclusion and exclusion criteria for the HEAL study have been previously documented.<sup>23</sup> In brief, infants were those born >36 weeks, with a diagnosis of moderate or severe encephalopathy. All infants received TH as standard of care. Demographic details, including maternal race and ethnicity as reported by the mother/father, are included in [Table I](#) and are presented here due to the known impact of social factors on infants' outcomes. Recruitment took place from January 25, 2017 to October 9, 2019 at 17 sites involving 23 hospitals in the United States, with follow-up finishing in October 2022. Written, informed consent was obtained prior to any study procedures taking place. The study was approved by the Food and Drug Administration and the institutional review boards at each site. This study follows the Consolidated Standards of Reporting Trials (CONSORT) guidelines (<https://www.equator-network.org/reporting-guidelines/consort/>).

For the current analysis, the intervention and placebo groups were pooled, as in the primary outcome analysis there was no significant difference in the primary outcome measure or circulating biomarker concentrations based on treatment group.<sup>24,25</sup>

### Biomarkers

Plasma biomarkers were obtained from a subset of infants at 3 time points: within 24 hours of birth, day 2, and day 4 of life.<sup>23,25</sup> As planned in the HEAL study protocol, the first 180 infants recruited who met the following inclusion criteria were selected for biomarker analysis: adequate volumes of plasma obtained at each time point, brain MRI data during the initial hospitalization, 2-year Bayley Scale of Infant and Toddler Development III with standardized neurologic examination, and GMFCS completed or who had died during

**Table I. Participant baseline characteristics**

| Variable                                   | Overall, n = 153 (100%)* |
|--------------------------------------------|--------------------------|
| Maternal race                              |                          |
| Asian                                      | 8 (5.2%)                 |
| Black                                      | 18 (12%)                 |
| Other                                      | 16 (10%)                 |
| White                                      | 111 (73%)                |
| Maternal ethnicity                         |                          |
| Hispanic ethnicity                         | 44 (29%)                 |
| Not Hispanic/not reported                  | 109 (71%)                |
| Maternal language                          |                          |
| English                                    | 121 (79%)                |
| Other                                      | 9 (5.9%)                 |
| Spanish                                    | 23 (15%)                 |
| Maternal age (y)                           | 30.1 (6.9)               |
| Maternal education                         |                          |
| High school or less                        | 62 (41%)                 |
| Some college or university                 | 33 (22%)                 |
| College graduate or postgraduate           | 54 (35%)                 |
| Not reported                               | 4 (2.6%)                 |
| Maternal parity                            |                          |
| >2                                         | 64 (42%)                 |
| 1                                          | 89 (58%)                 |
| Gravida                                    | 2.21 (1.77)              |
| Number of fetuses                          |                          |
| 1                                          | 149 (98%)                |
| 2                                          | 3 (2.0%)                 |
| Chorioamnionitis                           |                          |
| Yes                                        | 25 (16%)                 |
| No                                         | 128 (84%)                |
| Maternal fever                             |                          |
| Yes                                        | 21 (14%)                 |
| No                                         | 132 (86%)                |
| Maternal thyroid disease                   |                          |
| Yes                                        | 14 (9.2%)                |
| No                                         | 139 (91%)                |
| Preeclampsia                               |                          |
| Yes                                        | 12 (7.8%)                |
| No                                         | 141 (92%)                |
| Gestational diabetes                       |                          |
| Yes                                        | 21 (14%)                 |
| No                                         | 132 (86%)                |
| Maternal obesity                           |                          |
| Yes                                        | 28 (18%)                 |
| No                                         | 125 (82%)                |
| Maternal employment status                 |                          |
| Employed any                               | 102 (67%)                |
| Full-time stay at-home mother or caregiver | 18 (12%)                 |
| Student                                    | 6 (3.9%)                 |
| Unemployed any                             | 25 (16%)                 |
| Unknown                                    | 2 (1.3%)                 |
| Paternal employment status                 |                          |
| Employed any                               | 132 (90%)                |
| Full-time stay at-home father or caregiver | 2 (1.4%)                 |
| Student                                    | 2 (1.4%)                 |
| Unemployed any                             | 7 (4.8%)                 |
| Unknown                                    | 3 (2.1%)                 |
| Maternal drug use                          |                          |
| Yes                                        | 9 (5.9%)                 |
| No                                         | 137 (90%)                |
| Unknown                                    | 7 (4.6%)                 |
| Maternal alcohol use                       |                          |
| Yes                                        | 8 (5.2%)                 |
| No                                         | 138 (90%)                |
| Unknown                                    | 7 (4.6%)                 |
| Maternal tobacco use                       |                          |
| Yes                                        | 11 (7.2%)                |
| No                                         | 135 (88%)                |
| Unknown                                    | 7 (4.6%)                 |

(continued)

**Table I. Continued**

| Variable                               | Overall, n = 153 (100%)* |
|----------------------------------------|--------------------------|
| Prenatal care                          |                          |
| Yes                                    | 150 (98%)                |
| No                                     | 1 (0.7%)                 |
| Unknown                                | 2 (1.3%)                 |
| Delivery modality                      |                          |
| Spontaneous vaginal                    | 39 (25%)                 |
| Vacuum or forceps (vaginal assisted)   | 12 (7.8%)                |
| Elective cesarean                      | 3 (2.0%)                 |
| Emergency or urgent cesarean           | 99 (65%)                 |
| Sentinel event                         |                          |
| Yes                                    | 45 (29%)                 |
| No                                     | 108 (71%)                |
| Infant sex                             |                          |
| Female                                 | 68 (44%)                 |
| Male                                   | 85 (56%)                 |
| Infant birth weight                    | 3361.3 (604.3)           |
| Gestational age                        | 39.0 (1.4)               |
| Apgar score at 5 min, median (Q1, Q3)  | 3.0 (2.0, 5.0)           |
| Apgar score at 10 min, median (Q1, Q3) | 5.0 (4.0, 7.0)           |
| Infant resuscitation                   |                          |
| Yes                                    | 136 (89%)                |
| No                                     | 17 (11%)                 |
| Lowest pH                              | 7.0 (0.2)                |
| Worst base excess                      | −17.0 (5.4)              |
| Grade of encephalopathy                |                          |
| Moderate                               | 126 (82%)                |
| Severe                                 | 27 (18%)                 |
| Treatment assignment                   |                          |
| Placebo                                | 72 (47%)                 |
| Erythropoietin                         | 81 (53%)                 |
| Cerebral palsy status                  |                          |
| No                                     | 135 (88%)                |
| Yes                                    | 18 (12%)                 |
| Bayley motor <85                       |                          |
| No                                     | 118 (78%)                |
| Yes                                    | 33 (22%)                 |
| Bayley cognitive <85                   |                          |
| No                                     | 112 (74%)                |
| Yes                                    | 39 (26%)                 |
| Bayley language <85                    |                          |
| No                                     | 86 (57%)                 |
| Yes                                    | 65 (43%)                 |
| Bayley motor <70                       |                          |
| No                                     | 136 (90%)                |
| Yes                                    | 15 (9.9%)                |
| Bayley cognitive <70                   |                          |
| No                                     | 131 (87%)                |
| Yes                                    | 20 (13%)                 |
| Bayley language <70                    |                          |
| No                                     | 116 (77%)                |
| Yes                                    | 35 (23%)                 |

\*n (%); mean (SD).

the study period. Whole blood (1.5 mL) was collected in polypropylene K<sub>2</sub> ethylenediaminetetraacetic acid (EDTA) tubes (BD Microtainer), centrifuged at 2000 g for 8 minutes with plasma collected and stored at −80°C within 4 hours of collection.<sup>25</sup> The panel of biomarkers analyzed included neurospecific proteins (glial fibrillary acidic protein [GFAP], total tubulin-associated protein [Tau], ubiquitin carboxy-terminal hydrolase L1 [UCH-L1], S100 calcium-binding protein B [S100B], and neuron-specific enolase [NSE]), proinflammatory mediators (complement component 5a, fractaline, intercellular adhesion molecule 1, interferon-

gamma, interleukin [IL] 1 beta, IL-6, IL-8, IL-12p70, IL-17, monocyte chemoattractant protein-1, macrophage inflammatory protein [MIP] 1a, MIP-1b, vascular cell adhesion molecule 1, and tumor necrosis factor  $\alpha$ ), anti-inflammatory mediators (IL-1 receptor agonist, IL-10, IL-13, and IL-33), and other growth factors (brain-derived neurotrophic factor, neural cell adhesion molecule 1, neuroglobin, vascular endothelial growth factor [VEGF], and Epo). Analysis was performed using the MILLIPLEX MAP Human High Sensitivity T Cell Panel from Millipore-Sigma, the Luminex Multiplex assay from R&D Systems, and the R-PLEX Human GFAP Assay and the R-PLEX Human Tau Assay, both Meso Scale Diagnostics. All assays were performed centrally according to the manufacturer's instructions, in duplicate, and the results were averaged for analyses. Epo concentrations were excluded from the current analyses as this was the drug of investigation in the trial.

For the purposes of brevity in this article, we developed a shorthand, for example, GFAP 1 is used to refer to GFAP measurement at time point 1, that is, within 24 hours of birth; Tau 2 refers to total Tau measurement at time point 2, that is, day 2 of life; and UCH-L1 3 refers to UCH-L1 measurement at time point 3, that is, day 4 of life. Infants included in the biomarker cohort did not differ from the overall HEAL cohort.<sup>25</sup>

## Outcome

For the current analysis, we have chosen to separate outcome measures from the HEAL study and focused initially on the prediction of CP. The diagnosis of CP was determined by a validated standardized neurologic examination.<sup>23,24,26</sup> GMFCS was assessed using the modified algorithm used by the National Institute of Child Health and Human Development Neonatal Research Network.<sup>27</sup> Second, the outcome of Bayley III cognitive or language score  $<85$  ( $-1$  SD) and  $<70$  ( $-2$  SD) was chosen to assess the ability of biomarkers to predict cognitive/language outcome. A composite of cognitive or language scores was chosen due to the intrinsic links between cognition and early language development. This was derived from either a Bayley III cognitive score or a Bayley III language score (or both) below the stated cutoff ( $<85$  or  $<70$ ). Outcome assessments were performed between 22 and 36 months of age. MRI findings were used as a comparative predictor of outcome. The HEAL study used a harmonized MRI protocol performed on day 4-6 of life when possible and a previously validated MRI scoring system.<sup>28-30</sup> The severity of brain injury was determined by 3 independent reporters, yielding scores of 0-138, where a higher score indicates a more severe injury.

## Statistical Analysis

Descriptive statistics were used for demographic and baseline characteristics. Statistical analyses were performed using R software, version 4.2.2 (R Foundation for Statistical Computing).<sup>31-37</sup> A  $P$  value of  $<.05$  was deemed significant.

A Wilcoxon test was performed for all 27 biomarkers at each of the 3 time points to determine significant differ-

ences between groups for CP diagnosis. Bonferroni correction was performed to account for multiple (81) comparisons and those that remained statistically significant ( $P < .05$ ) were subject to further analysis. Complete case analysis was performed for each individual biomarker, excluding cases with missing data. Wilcoxon testing was performed for CP diagnosis, and logistic regression was used to produce receiver operating characteristic (ROC) curves. Bootstrapping was performed for 95% CIs, and diagnostic sensitivities and specificities were calculated. ROC curves were produced for MRI injury score alone for comparison with biomarkers, and subsequently, MRI injury scores were combined with biomarker data, producing further ROC curves. For the ROC curves, no more than 2 predictors were fit to the logistic regression analysis, to avoid overfitting.<sup>38</sup>

Spearman's correlation coefficient was calculated for Bayley cognitive and language scores for all 27 biomarkers at each of the 3 time points. After correcting for multiple comparisons using Bonferroni, those which remained significant were selected for further analyses for cognitive/language outcomes. Again, a complete case analysis was performed for each individual biomarker due to a small amount of missing data. Wilcoxon testing was performed for Bayley cognitive/language scores  $<85$ , and logistic regression was used to produce ROC curves, with bootstrapping for 95% CIs, and sensitivities and specificities were calculated. Analyses were repeated for Bayley cognitive/language score  $<70$ . ROC curves were produced for MRI injury score alone for comparison with biomarkers, and subsequently, the logistic regression model was trained to combine MRI injury scores with biomarker data, producing further ROC curves.

For theoretical cutoff determination, thresholds were established with the goal of achieving 90% sensitivity for lower cutoffs and 90% specificity for upper cutoffs. In practice, this meant that for determining a lower cutoff value, a high sensitivity was prioritized to minimize false negatives, whereas for the upper cutoff value, a high specificity was prioritized to reduce false positives. Measurements falling between these theoretically derived cutoffs have been termed 'indeterminate' due to the outcome being less predictable in this region.

## Results

### Demographics

Of the 180 infants enrolled in the HEAL trial who had biomarkers performed, 153 were included in this study (Figure 1). The baseline characteristics of this group are summarized in Table 1. All measured biomarkers were compared between outcome groups: CP vs non-CP and cognitive/language delay vs no cognitive/language delay (Supplemental Table 1; available at [www.jpeds.com](http://www.jpeds.com)). A diagnosis of CP was present in 18 (12%) of the 153 infants included in the analysis (Supplemental Table 2; available at [www.jpeds.com](http://www.jpeds.com)). Of the 151 who had Bayley assessments performed, 78 had Bayley cognitive/language scores  $<85$

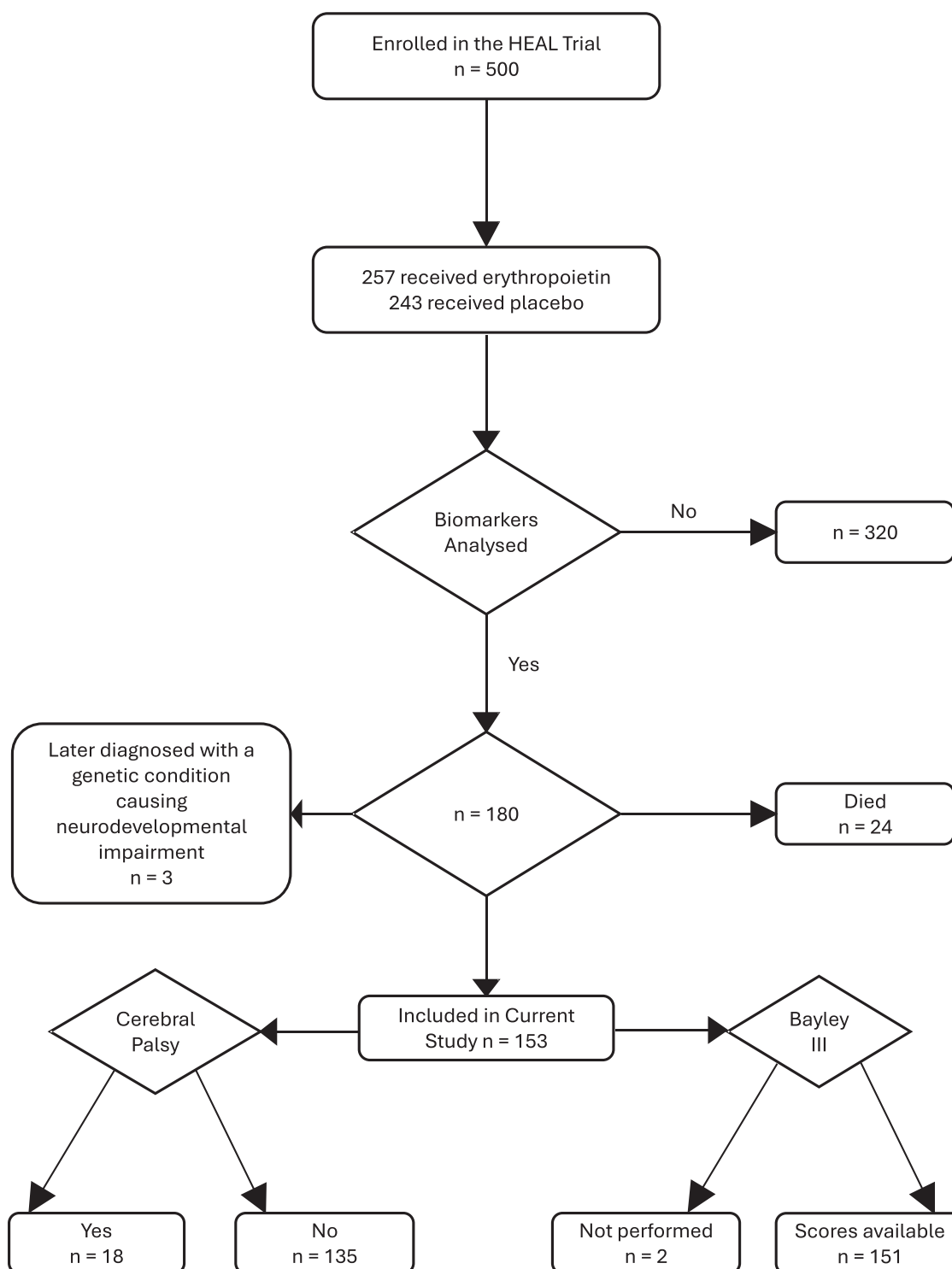


**Figure 1.** Study flowchart.

and 40 had scores <70. Between 147 and 153 infants had individual data available for each biomarker. Statistically significant differences were seen for outcomes in 18 measured biomarkers at enrollment, 13 at day 2, and 10 at day 4 ([Supplemental Table 1](#); available at [www.jpeds.com](http://www.jpeds.com)). Following Bonferroni correction, only GFAP, NSE, S100B,

UCH-L1, total Tau, VEGF, MIP-1b, and IL-13 remained significant.

### Prediction of Cerebral Palsy

The biomarkers which remained significantly altered in children who developed CP were Tau 2 and 3; GFAP 1, 2, and 3;

**Table II.** ROC analysis for biomarkers and cerebral palsy

| Predictor              | AUC              | Sensitivity (%) | Specificity (%) |
|------------------------|------------------|-----------------|-----------------|
| Tau 2                  | 0.90 [0.81-0.99] | 88              | 91              |
| Tau 3                  | 0.88 [0.76-0.99] | 88              | 80              |
| GFAP 3                 | 0.80 [0.66-0.94] | 71              | 86              |
| GFAP 2                 | 0.78 [0.64-0.92] | 78              | 75              |
| IL-13 2                | 0.75 [0.64-0.87] | 78              | 67              |
| MIP-1b 2               | 0.74 [0.63-0.84] | 94              | 51              |
| IL-13 1                | 0.74 [0.62-0.85] | 78              | 65              |
| VEGF 1                 | 0.72 [0.61-0.83] | 88              | 54              |
| GFAP 1                 | 0.72 [0.58-0.85] | 78              | 65              |
| MRI injury score       | 0.90 [0.80-1.0]  | 83              | 95              |
| MRI score and Tau 2    | 0.95 [0.90-0.99] | 88              | 94              |
| MRI score and MIP-1b 2 | 0.93 [0.88-0.98] | 78              | 96              |
| MRI score and IL-13 1  | 0.93 [0.86-0.99] | 83              | 94              |
| MRI score and IL-13 2  | 0.92 [0.84-0.99] | 83              | 93              |
| MRI score and VEGF 1   | 0.90 [0.82-0.99] | 77              | 95              |
| MRI score and Tau 3    | 0.88 [0.76-0.99] | 88              | 80              |
| MRI score and GFAP 3   | 0.80 [0.66-0.93] | 67              | 86              |
| MRI score and GFAP 2   | 0.78 [0.64-0.92] | 78              | 75              |
| MRI score and GFAP 1   | 0.72 [0.58-0.85] | 78              | 65              |

VEGF 1; MIP-1b 2; and IL-13 1 and 2 (**Supplemental Table 1**; available at [www.jpeds.com](http://www.jpeds.com)). Of these, the most significantly differentiating biomarkers were GFAP and total Tau. These neurospecific biomarkers were then examined for their ability to predict outcomes.

**Total Tau:** The *P* value corrected for multiple comparisons for both Tau 2 and Tau 3 was  $< .01$  (**Supplemental Table 1**; available at [www.jpeds.com](http://www.jpeds.com)). For the prediction of CP, total Tau displayed excellent and good area under the curve

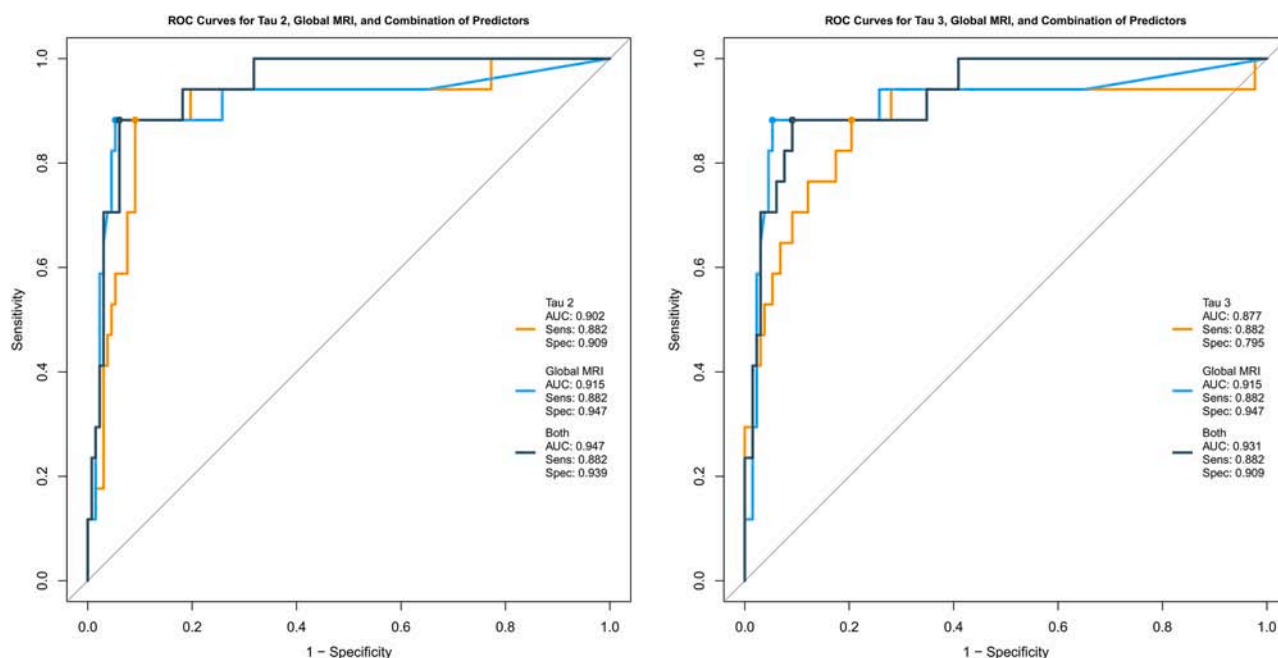
(AUC) values for Tau 2 and 3, respectively. Tau 2 displayed a sensitivity of 88% and a specificity of 91% for the prediction of CP (**Table II** and **Figure 2**).

For Tau 2, the lower theoretical cutoff for the prediction of CP was 245.04 pg/mL and the upper cutoff was 304.03 pg/mL (**Figure 3A**). Levels between 245.04 and 304.03 pg/mL were indeterminate (**Figure 3A**). For Tau 3, the calculated lower cutoff was 197.66 pg/mL and the upper was 447.19 pg/mL, with levels between these being indeterminate (**Figure 3A**).

The MRI injury score yielded an excellent AUC for CP with a sensitivity and specificity of 83% and 95%, respectively. In combination with MRI injury score, Tau 2 AUC further improved the prediction of CP (**Table II** and **Figure 2**). A scatterplot demonstrating each participant's change in total Tau concentration over time is displayed in **Figure 4A**. Results for Tau remained similar after outlier removal; therefore, we have chosen not to remove them from the analysis.

**GFAP:** GFAP 1, 2, and 3 were significantly increased in the CP group compared with the non-CP group, with corrected *P* values of .04,  $< .01$ , and .01, respectively (**Supplemental Table 1**; available at [www.jpeds.com](http://www.jpeds.com)). GFAP alone was investigated for its ability to predict CP, with GFAP 3 demonstrating the best prediction of outcome, producing a good AUC (**Table II**).

The theoretical lower cutoff level for GFAP 1 was 590.53 pg/mL and the upper cutoff was 2158.75 pg/mL, with levels between 590.53 and 2158.75 pg/mL being indeterminate for outcome prediction (**Figure 3B**). For GFAP 2, the lower

**Figure 2.** MRI injury score and outcome comparative ROC curves for CP diagnosis, Tau 2 and 3.

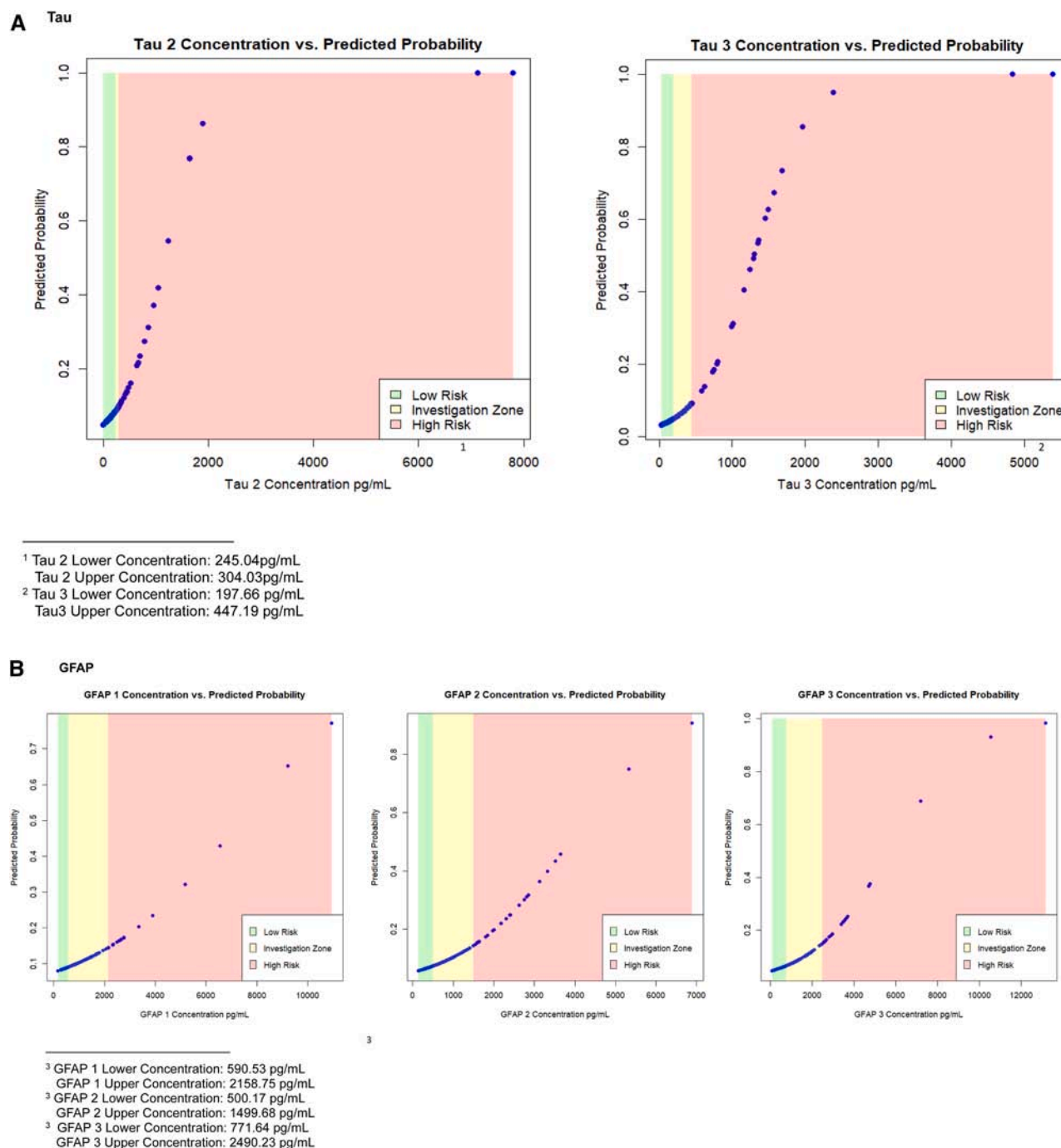


**Figure 3.** Theoretical cutoff values.

cutoff was 500.17 pg/mL and the upper was 1499.68 pg/mL, with values in between being indeterminate (**Figure 3B**). For GFAP 3, the lower cutoff level was 771.64 pg/mL. The theoretical upper cutoff level was 2490.23 pg/mL, with values between 771.64 and 2490.23 pg/mL being indeterminate for prediction (**Figure 3B**).

A scatterplot demonstrating change in GFAP concentration levels over time for those with CP is displayed in **Figure 4B**.

### Biomarker Combinations

Finally, we combined pairs of biomarker measurements for the prediction of CP (**Table III**). These combinations demonstrated AUCs ranging from moderate for IL-13 1 combined with IL-13 2 to excellent for GFAP 1 with Tau 3.

### Prediction of Cognitive/Language Delay

Bayley cognitive and language scores were highly correlated (Spearman  $\rho = 0.8$ ,  $P < .01$ ). After correcting for multiple

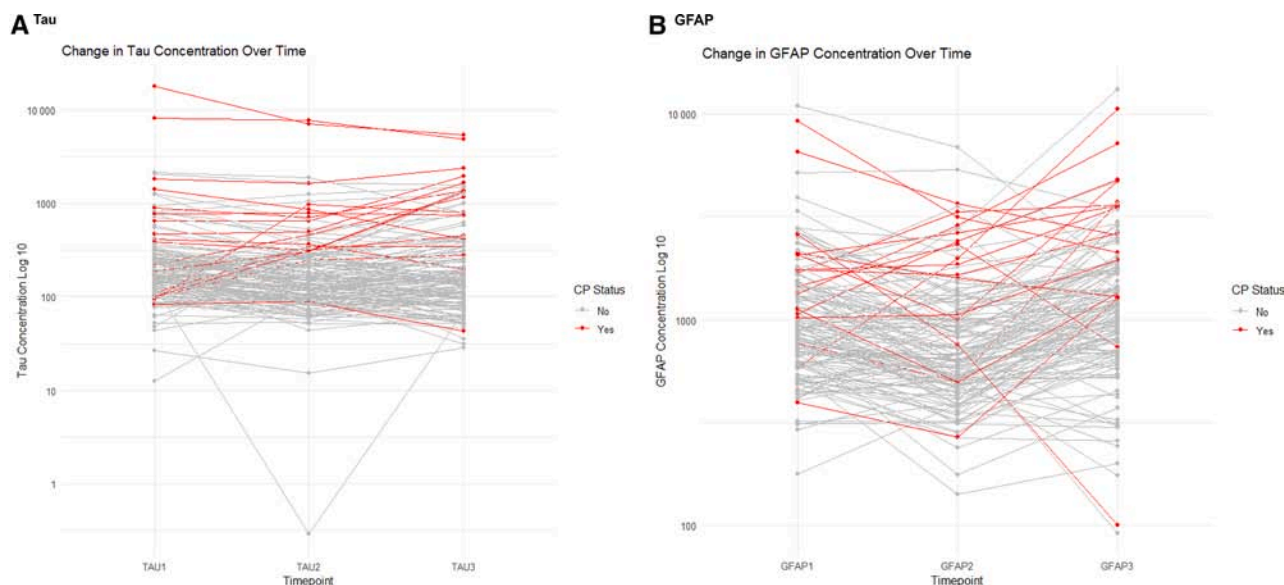


**Figure 4.** Biomarker scatterplot per participant.

comparisons, 5 biomarkers remained significant: Tau 2, Tau 3, UCH-L1 3, S100B 2, and NSE 3. Univariate analysis for individual biomarkers and Bayley's domains is displayed in [Supplemental Table 1](#); available at [www.jpeds.com](http://www.jpeds.com).

Infants who progressed to have a Bayley cognitive and/or language composite score <85 at 2 years had significantly higher values of Tau 2 and Tau 3 (median 160 [IQR: 201] vs 127 [132] pg/mL,  $P = .05$  and 158 [338] vs 130 [128] pg/mL,  $P = .03$ , respectively). They also showed increased levels of S100B 2 (1329 [2078] vs 791 [1275] pg/mL,  $P = .02$ ), UCH-L1 3 (1778 [1022] vs 1497 [822] pg/mL,  $P = .03$ ), and NSE 3 (36 389 [22 813] vs 29 251 [19 143] pg/mL,  $P = .02$ ). Ability to predict Bayley cognitive/language score <85 was assessed using AUC, but produced weak results ([Table IV](#)). When a more severe outcome was examined (Bayley cognitive/language <70), total Tau, UCH-L1, and S100B performed better (Tau 2 median 213 [339] vs 128 [124] pg/mL,  $P < .01$ ; Tau 3204 [915] vs 137 [150] pg/mL,  $P < .01$ , UCH-L1 31 953 [1070] vs 1577 [886] pg/mL,  $P = .04$ , S100B 21 554 [3006] vs 813 [1345] pg/mL,  $P < .01$ ), but AUC values remained weak ([Table V](#)).

MRI injury score correlated inversely with Bayley cognitive ( $\rho = -0.36$ ,  $P < .01$ ) and language scores ( $\rho = -0.30$ ,  $P < .01$ ). MRI injury score alone predicted a Bayley cognitive/language <85 with a weak AUC value and Bayley cognitive/language <70 with a moderate AUC ([Tables IV and V](#)).

We next examined the combination of biomarkers with MRI score to predict cognitive/language delay. For Bayley <85, the highest AUC value obtained was 0.67 (0.58-0.75), which was for Tau 3 with MRI score, with a specificity of 57% and sensitivity of 70% ([Table IV](#)). For Bayley cognitive/language <70, the combination of Tau 2 and MRI displayed the highest AUC of 0.74 (0.64-0.84), sensitivity 62%, and specificity 81% ([Table V](#)).

## Discussion

We have shown that blood-based biomarkers may provide valuable information for predicting CP and cognitive/language delay in a large cohort of term infants with moderate-severe HIE. Combining biomarkers with MRI findings further improved the prediction of both CP and cognitive/language delay in this group of high-risk infants. This builds on previous research in the same cohort, where machine-learning techniques have shown that a combination of several biomarkers has predictive ability for a composite outcome of death or neurodevelopmental disability.<sup>25</sup> The present study examines different neurospecific biomarkers for different outcomes, just as certain MRI patterns may be more predictive of particular outcomes.<sup>12</sup> These findings suggest that distinct biomarkers or combinations thereof are most predictive for specific neurodevelopmental outcomes, perhaps reflecting underlying differences in the pathophysiological mechanisms leading to motor vs cognitive and language impairment in neonates with HIE and potential earlier means of predicting outcomes. Furthermore, we show potential earlier means of predicting outcomes, which may be beneficial for precision medicine and additional therapies.<sup>4,5</sup>

Early prediction of CP following HIE remains challenging, and MRI is often used for counseling parents in this context and forming part of the diagnostic pathway.<sup>13</sup> Clear diagnostic pathways are now available and in place in many centers with the aim of diagnosing CP within the first year of life. This has allowed clinical trials of early CP-specific interventions with promising results.<sup>39,40</sup> These pathways use imaging and standardized early neurologic examination and General Movements Assessment (GMA) to allow diagnosis

**Table III.** Combination of biomarkers for predicting cerebral palsy

| Predictors           | AUC              | Sensitivity (%) | Specificity (%) |
|----------------------|------------------|-----------------|-----------------|
| GFAP 1 and Tau 3     | 0.93 [0.88-0.98] | 94              | 82              |
| GFAP 1 and Tau 2     | 0.91 [0.86-0.97] | 94              | 78              |
| Tau 3 and MIP-1b 2   | 0.91 [0.83-0.99] | 88              | 87              |
| GFAP 2 and Tau 3     | 0.91 [0.82-1.0]  | 94              | 86              |
| Tau 2 and IL-13 2    | 0.90 [0.82-0.97] | 88              | 83              |
| GFAP 2 and Tau 2     | 0.89 [0.82-0.97] | 88              | 84              |
| Tau 2 and MIP-1b 2   | 0.89 [0.83-0.96] | 94              | 74              |
| Tau 2 and IL-13 1    | 0.89 [0.80-0.98] | 94              | 83              |
| Tau 3 and VEGF 1     | 0.89 [0.79-0.99] | 94              | 83              |
| GFAP 3 and Tau 2     | 0.89 [0.77-1.0]  | 88              | 93              |
| Tau 2 and Tau 3      | 0.88 [0.77-0.99] | 94              | 74              |
| Tau 3 and IL-13 2    | 0.88 [0.78-0.98] | 77              | 89              |
| Tau 3 and IL-13 1    | 0.88 [0.77-0.99] | 94              | 74              |
| GFAP 3 and Tau 3     | 0.88 [0.76-1.0]  | 94              | 79              |
| Tau 2 and VEGF 1     | 0.87 [0.76-0.97] | 88              | 82              |
| GFAP 3 and MIP-1b 2  | 0.86 [0.78-0.94] | 94              | 61              |
| GFAP 2 and MIP-1b 2  | 0.84 [0.76-0.93] | 72              | 84              |
| GFAP 1 and GFAP 3    | 0.84 [0.73-0.94] | 65              | 92              |
| GFAP 3 and IL-13 1   | 0.84 [0.72-0.96] | 82              | 83              |
| GFAP 3 and IL-13 2   | 0.83 [0.71-0.95] | 82              | 79              |
| GFAP 3 and VEGF 1    | 0.83 [0.71-0.95] | 88              | 74              |
| GFAP 2 and GFAP 3    | 0.83 [0.70-0.96] | 82              | 82              |
| GFAP 2 and VEGF 1    | 0.81 [0.71-0.91] | 82              | 69              |
| GFAP 2 and IL-13 1   | 0.81 [0.69-0.93] | 83              | 73              |
| GFAP 2 and IL-13 2   | 0.81 [0.69-0.93] | 89              | 66              |
| VEGF 1 and MIP-1b 2  | 0.80 [0.71-0.88] | 100             | 60              |
| GFAP 1 and MIP-1b 2  | 0.80 [0.70-0.89] | 100             | 48              |
| MIP-1b 2 and IL-13 2 | 0.78 [0.68-0.88] | 67              | 81              |
| MIP-1b 2 and IL-13 1 | 0.78 [0.68-0.88] | 100             | 47              |
| GFAP 1 and GFAP 2    | 0.77 [0.63-0.91] | 61              | 91              |
| VEGF 1 and IL-13 2   | 0.76 [0.65-0.87] | 88              | 59              |
| GFAP 1 and IL-13 2   | 0.75 [0.62-0.88] | 72              | 80              |
| GFAP 1 and IL-13 1   | 0.75 [0.61-0.89] | 72              | 80              |
| VEGF 1 and IL-13 1   | 0.75 [0.64-0.86] | 88              | 64              |
| GFAP 1 and VEGF 1    | 0.75 [0.64-0.85] | 94              | 55              |
| IL-13 1 and IL-13 2  | 0.74 [0.62-0.86] | 89              | 54              |
| GFAP 1 and Tau 3     | 0.93 [0.88-0.98] | 94              | 82              |
| GFAP 1 and Tau 2     | 0.91 [0.86-0.97] | 94              | 78              |
| Tau 3 and MIP-1b 2   | 0.91 [0.83-0.99] | 88              | 87              |
| GFAP 2 and Tau 3     | 0.91 [0.82-1.0]  | 94              | 86              |
| Tau 2 and IL-13 2    | 0.90 [0.82-0.97] | 88              | 83              |
| GFAP 2 and Tau 2     | 0.89 [0.82-0.97] | 88              | 84              |
| Tau 2 and MIP-1b 2   | 0.89 [0.83-0.96] | 94              | 74              |
| Tau 2 and IL-13 1    | 0.89 [0.80-0.98] | 94              | 83              |
| Tau 3 and VEGF 1     | 0.90 [0.79-0.99] | 94              | 83              |
| GFAP 3 and Tau 2     | 0.89 [0.77-1.0]  | 88              | 93              |
| Tau 2 and Tau 3      | 0.88 [0.77-0.99] | 94              | 74              |
| Tau 3 and IL-13 2    | 0.88 [0.78-0.98] | 77              | 89              |
| Tau 3 and IL-13 1    | 0.88 [0.77-0.99] | 94              | 74              |
| GFAP 3 and Tau 3     | 0.88 [0.76-1.00] | 94              | 79              |
| Tau 2 and VEGF 1     | 0.87 [0.76-0.97] | 88              | 82              |

of CP as early as possible. However, this is not yet universally available as MRI requires intensive resources, both in terms of performing and acquiring the scan. The expertise required for detailed reporting remains a barrier for many centers. The GMA, a crucial part of the CP diagnostic guideline, similarly requires intensive training and expertise, with limited training available worldwide and resulting implications for scalability to widespread clinical use.<sup>13</sup> Constraints in access to both MRI and GMA are even more pronounced in resource-limited settings and LMICs. This study represents the analysis of blood-based biomarkers focusing on CP as a

**Table IV.** ROC analysis for biomarkers and Bayley <85

| Predictor              | AUC              | Sensitivity (%) | Specificity (%) |
|------------------------|------------------|-----------------|-----------------|
| S100B 2                | 0.62 [0.52-0.71] | 87              | 34              |
| NSE 3                  | 0.61 [0.52-0.70] | 72              | 46              |
| UCHL-1 3               | 0.61 [0.51-0.70] | 49              | 75              |
| Tau 3                  | 0.61 [0.52-0.70] | 60              | 60              |
| Tau 2                  | 0.60 [0.50-0.69] | 31              | 87              |
| MRI injury score       | 0.64 [0.55-0.73] | 29              | 97              |
| MRI score and Tau 3    | 0.67 [0.58-0.75] | 57              | 70              |
| MRI score and UCHL-1 3 | 0.66 [0.57-0.75] | 58              | 71              |
| MRI score and NSE 3    | 0.64 [0.55-0.73] | 29              | 97              |
| MRI score and Tau 2    | 0.64 [0.55-0.73] | 44              | 84              |
| MRI score and S100B 2  | 0.63 [0.54-0.72] | 55              | 71              |

specific outcome, in contrast to previous studies that have predominantly addressed composite outcomes, such as neurodevelopmental impairment or death.<sup>25,41</sup> The introduction to clinical practice of quantifiable blood-based biomarkers, some of which are available as point-of-care tests, such as GFAP and UCHL-1, would be of significant clinical value. This would enable the enrollment of infants into CP-specific interventions at the time of discharge.

In contrast, the prediction of cognitive disability is more complex, and therefore it is not surprising that the predictive ability of blood-based biomarkers was different in this context. There is clear evidence in preterm brain injury that environmental factors play a significant role.<sup>42-45</sup> Less has been reported on the effect of social factors on outcomes following HIE, but emerging evidence suggests a role.<sup>18</sup> It is likely that genetic factors, social factors, and early life environment are also important, and hence the perinatal injury is only part of the puzzle. We found weaker prediction of low average cognitive/language ability and better prediction of scores <70, especially in combination with MRI. MRI injury score in the HEAL study was reported by leading experts in the field of neonatal MRI, using a detailed published scoring system.<sup>30</sup> Again, this is not always available, and hence blood-based biomarkers may help guide clinicians on the prediction of outcomes and counseling of parents, but with less power than for the prediction of CP.

**Table V.** ROC analysis for biomarkers and Bayley <70

| Predictor              | AUC              | Sensitivity | Specificity |
|------------------------|------------------|-------------|-------------|
| Tau 2                  | 0.70 [0.56-0.77] | 40          | 92          |
| Tau 3                  | 0.65 [0.53-0.76] | 42          | 90          |
| S100B 2                | 0.64 [0.54-0.74] | 85          | 43          |
| UCHL-1 3               | 0.61 [0.50-0.72] | 54          | 70          |
| NSE 3                  | 0.59 [0.49-0.70] | 56          | 66          |
| MRI injury score       | 0.74 [0.64-0.84] | 58          | 81          |
| MRI score and Tau 2    | 0.74 [0.64-0.84] | 62          | 81          |
| MRI score and S100B 2  | 0.74 [0.63-0.84] | 60          | 80          |
| MRI score and Tau 3    | 0.73 [0.63-0.83] | 65          | 75          |
| MRI score and UCHL-1 3 | 0.73 [0.63-0.83] | 57          | 81          |
| MRI score and NSE 3    | 0.72 [0.61-0.83] | 59          | 80          |

Previous studies have highlighted key candidate biomarkers such as Tau, GFAP, and S100B for predicting neurodevelopmental impairment in neonatal encephalopathy.<sup>41,46,47</sup> Tau and GFAP are brain-specific proteins associated with neuronal axons and astrocytes, respectively. Elevated concentrations of these biomarkers in blood plasma may indicate neuronal and astrocytic damage, thus potentially allowing for the quantitative assessment of neurological injury in the neonate. The results of this secondary analysis illustrate the prognostic capability of total Tau as an early biological marker in both predicting CP and cognitive/language delay. Plasma measurements of total Tau on postnatal days 2 and 4 demonstrated equal sensitivity in the prediction of CP to that of detailed MRI scoring. Other studies investigating the utility of Tau as a biomarker for predicting poor neurodevelopmental outcomes have also identified measurements of Tau at 48 hours as a helpful prognosticator.<sup>25,46,48,49</sup> Among adults, there is growing evidence for the role of Tau in the detection and prediction of Alzheimer's disease, increasing the likelihood that a clinically validated assay may become available in the near future.<sup>50</sup> In addition, various phosphorylated forms of Tau, such as pTau-217 and pTau-181, are gaining prominence in Alzheimer's research, potentially opening new avenues for studying these biomarkers in neonatal populations.<sup>51,52</sup> The addition of Tau immuno-assays into pre-existing laboratory infrastructure, such as mainline clinical chemistry analyzers, would allow for ease of integration into both the developed and LMIC settings.

Elevations in GFAP concentrations have been previously documented to correlate with abnormal MRI findings and poor neurological outcomes.<sup>47,53</sup> Our results highlight that GFAP, as an individual biomarker, displays good prognostication for CP. GFAP is expressed in response to reactive astrogliosis, a physiological response to brain injury. It is generally accepted that certain levels of postinjury gliosis are beneficial in the recovery from an insult.<sup>54</sup> However, excessive gliosis and its associated neuroinflammatory responses have a negative impact on brain structural and functional recovery.<sup>55</sup> This aligns with our findings, which demonstrate that increased measurements of GFAP on day 4 were most closely linked with adverse outcomes in neonates with HIE. When corrected for multiple comparisons, our results did not display statistical significance for the utility of GFAP in predicting cognitive/language delay, and so GFAP seems to be more intrinsically linked with later motor disability. In adults, GFAP is emerging as a prominent biomarker in traumatic brain injury, used for decision-making around computed tomography scans.<sup>56,57</sup> With the recent availability of a point-of-care device for GFAP measurements, this would also be a convenient candidate to introduce clinically in neonates.<sup>58</sup> To date, the use of GFAP as a biomarker has predominantly been in the adult population, with no validated pediatric normative reference intervals. Studies involving a healthy pediatric population have demonstrated that physiological levels of GFAP are much higher in early life and decrease toward adolescence.<sup>59,60</sup> A

better understanding of physiological levels in early life is required before the potential use of GFAP in a neonatal clinical setting can be fully exploited.

The HEAL study was a well-designed and carefully conducted randomized controlled trial with detailed standard operating procedures and robust data collection methods, allowing for the production of a high-quality data set on many infants with moderate-severe HIE. This large cohort of babies with detailed information on MRI findings and CP status allowed this exploratory secondary analysis to be conducted with minor limitations. A subset of infants were selected for biomarker analysis from the HEAL cohort, based on sample-size calculation using the primary outcome of death or neurodevelopmental impairment. Even in a large cohort of infants with moderate-severe HIE, only a small number of those with biomarkers available were diagnosed with CP. We were, therefore, unable to investigate the ability of biomarkers to predict the severity or topography of CP. The HEAL trial included infants with well-defined sentinel events. Our findings, therefore, may not apply to infants with partial, prolonged hypoxic insults. Our cognitive/language assessment of infants was limited to 2 years of age, and further longitudinal follow-up with school-age outcomes may provide further information. This cohort of infants was all diagnosed with moderate to severe HIE and treated with TH. We did not investigate which infants would benefit from TH, as biomarker measurements were performed in the first few days of life. However, our data may help to identify which infants would benefit most from novel or emerging adjunct therapies, such as mesenchymal stem cells, which could be administered several days after birth.<sup>61</sup> In addition, HIE is only one cause of CP, with preterm brain injury being a much more common cause. However, our data provide encouragement that robust neurospecific biomarkers may be found in other forms of perinatal brain injury.

This study shows that neurospecific, blood-based biomarkers, particularly total Tau protein on day 2, performed as well as detailed MRI scoring for the prediction of CP at 2 years of age. In addition, we have shown that while certain biomarkers may aid clinicians in prognostication and parental counseling, others may be more specific to either motor or cognitive/language outcomes. ■

## CRedit authorship contribution statement

**Danielle Clifford:** Writing – review & editing, Writing – original draft, Formal analysis. **Conor L. Vaughan:** Writing – review & editing, Writing – original draft, Formal analysis. **Seán J. Costelloe:** Writing – review & editing, Supervision. **Brian H. Walsh:** Writing – review & editing, Supervision. **Adam Numis:** Writing – review & editing, Methodology, Investigation, Data curation. **Sandra E. Juul:** Writing – review & editing, Resources, Investigation, Funding acquisition, Data curation. **Deirdre M. Murray:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

- McIntyre S, Goldsmith S, Webb A, Ehlinger V, Hollung SJ, McConnell K, et al. Global prevalence of cerebral palsy: a systematic analysis. *Developmental Med Child Neurol* 2022;64:1494-506.
- Conway JM, Walsh BH, Boylan GB, Murray DM. Mild hypoxic ischaemic encephalopathy and long term neurodevelopmental outcome - a systematic review. *Early Hum Dev* 2018;120:80-7.
- Chalak LF, Nguyen K-A, Prempunpong C, Heyne R, Thayyil S, Shankaran S, et al. Prospective research in infants with mild encephalopathy identified in the first six hours of life: neurodevelopmental outcomes at 18-22 months. *Pediatr Res* 2018;84:861-8.
- Baak LM, Wagenaar N, van der Aa NE, Groenendaal F, Dudink J, Tataranno ML, et al. Feasibility and safety of intranasally administered mesenchymal stromal cells after perinatal arterial ischaemic stroke in the Netherlands (PASSION): a first-in-human, open-label intervention study. *Lancet Neurol* 2022;21:528-36.
- Malhotra A, Thebaud B, Paton MCB, Fleiss B, Papagianis P, Baker E, et al. Advances in neonatal cell therapies: proceedings of the First Neonatal Cell Therapies Symposium (2022). *Pediatr Res* 2023;94:1631-8.
- Liu W, Yang Q, Wei H, Dong W, Fan Y, Hua Z. Prognostic value of clinical tests in neonates with hypoxic-ischemic encephalopathy treated with therapeutic hypothermia: a systematic review and meta-analysis. *Front Neurol* 2020;11:133.
- Ouwehand S, Smidt LCA, Dudink J, Benders MJNL, de Vries LS, Groenendaal F, et al. Predictors of outcomes in hypoxic-ischemic encephalopathy following hypothermia: a meta-analysis. *Neonatology* 2020;117:411-27.
- Van Laerhoven H, De Haan TR, Offringa M, Post B, Van Der Lee JH. Prognostic tests in term neonates with hypoxic-ischemic encephalopathy: a systematic review. *Pediatrics* 2013;131:88-98.
- Mitra S, Kendall GS, Bainbridge A, Sokolska M, Dinan M, Uriavallanal C, et al. Proton magnetic resonance spectroscopy lactate/N-acetylaspate within 2 weeks of birth accurately predicts 2-year motor, cognitive and language outcomes in neonatal encephalopathy after therapeutic hypothermia. *Arch Dis Child Fetal Neonatal Ed* 2018;104:F424-32.
- Lally PJ, Montaldo P, Oliveira V, Soe A, Swamy R, Bassett P, et al. Magnetic resonance spectroscopy assessment of brain injury after moderate hypothermia in neonatal encephalopathy: a prospective multicentre cohort study. *Lancet Neurol* 2019;18:35-45.
- Laptook A, Garvey AA, Adams C, Grant PE, Molloy EJ, Groenendaal F, et al. Magnetic resonance imaging and spectroscopy in neonatal encephalopathy: current consensus position and future opportunities. *Pediatr Res* 2025 <https://doi.org/10.1038/s41390-025-04448-5>
- Van Steenis A, Cizmeci MN, Groenendaal F, Thoresen M, Cowan FM, de Vries LS, et al. Individualized neuroprognostication in neonates with hypoxic-ischemic encephalopathy treated with hypothermia. *Neurol Clin Pract* 2025;15:e200370.
- Novak I, Morgan C, Adde L, Blackman J, Boyd RN, Brunstrom-Hernandez J, et al. Early, accurate diagnosis and early intervention in cerebral palsy: advances in diagnosis and treatment. *JAMA Pediatr* 2017;171:897-907.
- Weeke LC, Groenendaal F, Mudigonda K, Blennow M, Lequin MH, Meiners LC, et al. A novel magnetic resonance imaging Score predicts neurodevelopmental outcome after Perinatal asphyxia and therapeutic hypothermia. *J Pediatr* 2018;192:33-40.e2.
- Benini R, Dagenais L, Shevell MI. Normal imaging in patients with cerebral palsy: what does it tell us? *J Pediatr* 2013;162:369-74.e1.
- Ashwal S, Russman BS, Blasco PA, Miller G, Sandler A, Shevell M, et al. Practice parameter: diagnostic assessment of the child with cerebral palsy: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology* 2004;62:851-63.
- Schreglmann M, Ground A, Vollmer B, Johnson MJ. Systematic review: long-term cognitive and behavioural outcomes of neonatal hypoxic-ischaemic encephalopathy in children without cerebral palsy. *Acta Paediatr* 2020;109:20-30.
- Varga Z, Andorka C, Pataki M, Meder U, Szakmar E, Szabo AJ, et al. Higher parental education was associated with good cognitive outcomes in infants with hypoxic-ischaemic encephalopathy. *Acta Paediatr* 2024;113:417-25.
- Murray DM. Biomarkers in neonatal hypoxic-ischemic encephalopathy—Review of the literature to date and future directions for research. *Handb Clin Neurol* 2019;162:281-93.
- Califf RM. Biomarker definitions and their applications. *Exp Biol Med* 2018;243:213-21.
- Lemmon ME, Donohue PK, Parkinson C, Northington FJ, Boss RD. Communication challenges in neonatal encephalopathy. *Pediatrics* 2016;138:e20161234.
- Richter LL, Janvier A, Pearce R, Bourque CJ, Church PT, Luu TM, et al. Parental and medical classification of neurodevelopment in children born preterm. *Pediatrics* 2025;155:e2024066148.
- Juul SE, Comstock BA, Heagerty PJ, Mayock DE, Goodman AM, Hauge S, et al. High-Dose erythropoietin for asphyxia and encephalopathy (HEAL): a randomized controlled trial - background, aims, and Study protocol. *Neonatology* 2018;113:331-8.
- Wu YW, Comstock BA, Gonzalez FF, Mayock DE, Goodman AM, Maitre NL, et al. Trial of erythropoietin for hypoxic-ischemic encephalopathy in newborns. *New Engl J Med* 2022;387:148-59.
- Juul SE, Voldal E, Comstock BA, Massaro AN, Bammler TK, Mayock DE, et al. Association of high-dose erythropoietin with circulating biomarkers and neurodevelopmental outcomes among neonates with hypoxic ischemic encephalopathy. *JAMA Netw Open* 2023;6:e2322131.
- Kuban KC, Allred EN, O'Shea M, Paneth N, Pagano M, Leviton A. An algorithm for identifying and classifying cerebral palsy in young children. *J Pediatr* 2008;153:466-72.
- Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Developmental Med Child Neurol* 1997;39:214-23.

28. Trivedi SB, Vesoulis ZA, Rao R, Liao SM, Shimony JS, McKinstry RC, et al. A validated clinical MRI injury scoring system in neonatal hypoxic-ischemic encephalopathy. *Pediatr Radiol* 2017;47:1491-9.
29. Wisnowski JL, Bluml S, Panigrahy A, Mathur AM, Berman J, Chen P-SK, et al. Integrating neuroimaging biomarkers into the multicentre, high-dose erythropoietin for asphyxia and encephalopathy (HEAL) trial: rationale, protocol and harmonisation. *BMJ Open* 2021;11:e043852.
30. Wisnowski JL, Monsell SE, Bluml S, Goodman AM, Li Y, Comstock BA, et al. Brain injury outcomes after adjuvant erythropoietin neuroprotection for moderate or severe neonatal hypoxic-ischemic encephalopathy: a report from the HEAL trial. *Dev Neurosci* 2024;46:285-96.
31. Team RCR. A Language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2024.
32. Gohel D, Skintzos P. *flextable: Functions for Tabular Reporting* [Internet]. Accessed April 17, 2025. 2017. <https://CRAN.R-project.org/package=flextable>
33. Sjoberg DD, Whiting K, Curry M, Lavery JA, Larmarange J. Reproducible summary tables with the gsummary Package. *R J* 2021;13:570.
34. Wickham H, François R, Henry L, Müller K, Vaughan D. *dplyr: A Grammar of Data Manipulation* [Internet]. p. 1.2.1. Accessed April 17, 2025. 2014. <https://CRAN.R-project.org/package=dplyr>
35. Wickham H, Vaughan D, Girlich M. *tidyr: Tidy Messy Data* [Internet]. p. 1.3.2. Accessed April 17, 2025. 2014. <https://CRAN.R-project.org/package=tidyr>
36. HW ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag; 2016.
37. Xavier Robin NT, Hainard A, Tiberti N, Lisacek F, Sanchez JC, Müller M. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011;12:77.
38. Mohammad Ziaul Islam C, Tanvir CT. Variable selection strategies and its importance in clinical prediction modelling. *Fam Med Community Health* 2020;8:e000262.
39. Morgan C, Fetters L, Adde L, Badawi N, Bancale A, Boyd RN, et al. Early intervention for children aged 0 to 2 years with or at high risk of cerebral Palsy: International Clinical Practice Guideline based on systematic reviews. *JAMA Pediatr* 2021;175:846-58.
40. Novak I, McIntyre S, Morgan C, Campbell L, Dark L, Morton N, et al. A systematic review of interventions for children with cerebral palsy: state of the evidence. *Developmental Med Child Neurol* 2013;55:885-910.
41. Massaro AN, Wu YW, Bammler TK, Comstock B, Mathur A, McKinstry RC, et al. Plasma biomarkers of brain injury in neonatal hypoxic-ischemic encephalopathy. *J Pediatr* 2018;194:67-75.e1.
42. Mallinson DC, Grodsky E, Ehrenthal DB. Gestational age, kindergarten-level literacy, and effect modification by maternal socio-economic and demographic factors. *Paediatr Perinat Epidemiol* 2019;33:467-79.
43. Beauregard JL, Drews-Botsch C, Sales JM, Flanders WD, Kramer MR. Preterm birth, poverty, and cognitive development. *Pediatrics* 2018;141:e20170509.
44. Twilhaar ES, Pierrat V, Marchand-Martin L, Benhammou V, Kaminski M, Ancel PY. Profiles of functioning in 5.5-Year-Old very preterm born children in France: the EPIPAGE-2 Study. *J Am Acad Child Adolesc Psychiatry* 2022;61:881-91.
45. Vohr BR, McGowan EC, Bann C, Das A, Higgins R, Hintz S. Association of high screen-time use with school-age cognitive, executive function, and behavior outcomes in extremely preterm children. *JAMA Pediatr* 2021;175:1025-34.
46. McGowan MM, O'Kane AC, Vezina G, Chang T, Bendush N, Glass P, et al. Serial plasma biomarkers of brain injury in infants with neonatal encephalopathy treated with therapeutic hypothermia. *Pediatr Res* 2021;90:1228-34.
47. Ennen CS, Huisman TAGM, Savage WJ, Northington FJ, Jennings JM, Everett AD, et al. Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic encephalopathy treated with whole-body cooling. *Am J Obstet Gynecol* 2011;205:251.e1-e7.
48. Takahashi K, Hasegawa S, Maeba S, Fukunaga S, Motoyama M, Hamano H, et al. Serum tau protein level serves as a predictive factor for neurological prognosis in neonatal asphyxia. *Brain Development* 2014;36:670-5.
49. Li R, Lee JK, Govindan RB, Graham EM, Everett AD, Perin J, et al. Plasma biomarkers of evolving encephalopathy and brain injury in neonates with hypoxic-ischemic encephalopathy. *J Pediatr* 2023;252:146-53.e2.
50. Ankeny SE, Bacci JR, Decourt B, Sabbagh MN, Mielke MM. Navigating the landscape of plasma biomarkers in Alzheimer's Disease: focus on past, present, and future clinical applications. *Neurol Ther* 2024;13:1541-57.
51. Moloney CM, Labuzan SA, Crook JE, Siddiqui H, Castanedes-Casey M, Lachner C, et al. Phosphorylated tau sites that are elevated in Alzheimer's disease fluid biomarkers are visualized in early neurofibrillary tangle maturity levels in the post mortem brain. *Alzheimers Dement* 2023;19:1029-40.
52. Gonzalez-Ortiz F, Vávra J, Payne E, Kirsebom B-E, Sjöbom U, Santos C, et al. The potential dual role of tau phosphorylation: plasma phosphorylated-tau217 in newborns and Alzheimer's disease. *Brain Commun* 2025;7:fcaf221.
53. Stewart A, Tekes A, Huisman TAGM, Jennings JM, Allen MC, Northington FJ, et al. Glial fibrillary acidic protein as a biomarker for periventricular white matter injury. *Am J Obstet Gynecol* 2013;209:27.e1-e7.
54. Yang Z, Wang KKW. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends Neurosciences* 2015;38:364-74.
55. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosciences* 2009;32:638-47.
56. Legramante JM, Minieri M, Belli M, Giovannelli A, Agnoli A, Bajo D, et al. Evaluation of GFAP/UCH-L1 biomarkers for computed tomography exclusion in mild traumatic brain injury (mTBI). *Int J Emerg Med* 2024;17:164.
57. Lagares A, de la Cruz J, Terrisse H, Meján O, Pavlov V, Vermorel C, et al. An automated blood test for glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) to predict the absence of intracranial lesions on head CT in adult patients with mild traumatic brain injury: BRAINI, a multicentre observational study in Europe. *eBioMedicine* 2024;110:105477.
58. Okonkwo DO, Puffer RC, Puccio AM, Yuh EL, Yue JK, Diaz-Arrastia R, et al. Point-of-Care platform blood biomarker testing of glial fibrillary acidic protein versus S100 calcium-binding protein B for prediction of traumatic brain injuries: a transforming research and clinical knowledge in traumatic brain injury Study. *J Neurotrauma* 2020;37:2460-7.
59. Cooper JG, Stukas S, Ghodsi M, Ahmed N, Diaz-Arrastia R, Holmes DT, et al. Age specific reference intervals for plasma biomarkers of neurodegeneration and neurotrauma in a Canadian population. *Clin Biochem* 2023;121-122:110680.
60. Mannix R, Borglund E, Monashefsky A, Master C, Corwin D, Badawy M, et al. Age-Dependent differences in blood levels of glial fibrillary acidic protein but not ubiquitin carboxy-terminal hydrolase L1 in children. *Neurology* 2024;103:e209651.
61. Mintoft A, Vallatos A, Robertson NJ. Mesenchymal Stromal cell therapy for Hypoxic Ischemic Encephalopathy: future directions for combination therapy with hypothermia and/or melatonin. *Semin Perinatol* 2024;48:151929.