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Research Article

Keywords: hypoxic-ischemic encephalopathy, neonate, magnetic resonance imaging, brain volumetry, therapeutic hypothermia, blood gas analysis, Rutherford MRI score

Posted Date: October 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11322188/v1>

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Additional Declarations: No competing interests reported.

Associations of initial blood gas parameters with post-cooling MRI-derived brain volumes and Rutherford injury scores in neonatal hypoxic-ischemic encephalopathy: a retrospective exploratory study

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Abstract

Background: Quantitative magnetic resonance imaging (MRI) may reveal structural alterations not captured by conventional visual assessment in neonatal hypoxic-ischemic encephalopathy (HIE). This study investigated associations between initial blood gas parameters and post-cooling MRI-derived brain volumes and Rutherford MRI injury scores in neonates treated with therapeutic hypothermia.

Methods: This retrospective exploratory study included 79 neonates with HIE. Initial pH, partial pressure of carbon dioxide, bicarbonate, base excess, and lactate were evaluated in relation to 50 absolute MRI-derived brain-volume measures and the consensus Rutherford score. Associations were assessed using Spearman correlations and separate multivariable linear regression models adjusted for gestational age at birth, postnatal age at MRI, and sex, with HC3 heteroskedasticity-robust standard errors. Two blinded radiologists independently assigned Rutherford scores and resolved disagreements by consensus. Secondary analyses compared neonates with pH ≤ 7.00 and pH > 7.00 . The Benjamini-Hochberg procedure

controlled the false discovery rate within prespecified analysis families.

Results: Consensus Rutherford scores were available for all 79 neonates (mean, 6.91 ± 1.34 ; median, 7; interquartile range, 6–8; range, 4–9). Interobserver agreement was good (absolute-agreement intraclass correlation coefficient, 0.789; quadratic-weighted κ , 0.787); exact agreement occurred in 50.6% of examinations, and scores differed by no more than 1 point in 88.6%. After false-discovery-rate correction, no initial blood gas parameter was significantly associated with the consensus Rutherford score in either unadjusted or adjusted analyses. The adjusted mean score difference between the pH groups was -0.20 (95% confidence interval, -0.93 to 0.54 ; $p = 0.597$). No blood gas–volume association remained significant after correction, and no absolute or intracranial-volume-normalized measure differed significantly between the pH groups.

Conclusions: This exploratory cohort provided no evidence of associations between initial blood gas parameters and post-cooling Rutherford MRI injury scores or global and regional brain volumes after correction for multiple comparisons. These findings do not exclude clinically relevant associations and require confirmation in larger studies using neonatal-validated segmentation methods.

Keywords: hypoxic-ischemic encephalopathy; neonate; magnetic resonance imaging; brain volumetry; therapeutic hypothermia; blood gas analysis; Rutherford MRI score

Background

Hypoxic-ischemic encephalopathy (HIE) remains a major cause of neonatal mortality and long-term neurodevelopmental impairment. Therapeutic hypothermia improves survival without disability in eligible term and near-term neonates. Nevertheless, outcomes remain heterogeneous, underscoring the need for reliable methods of early risk stratification [1].

Conventional MRI assessment of neonatal HIE focuses on characteristic injury patterns involving the basal ganglia, thalami, posterior limb of the internal capsule, cerebral cortex, and watershed white matter [2,3]. Although these qualitative patterns provide clinically valuable information, quantitative volumetric MRI may complement visual assessment by providing objective measurements of global and regional brain structures and identifying subtle structural alterations [4–7].

Initial blood gas parameters reflect systemic biochemical disturbances associated with perinatal hypoxia-ischemia. A previous study reported associations between these parameters and the clinical severity of encephalopathy, but not with a validated qualitative MRI injury score [8]. Their relationship with quantitative brain volumes measured after therapeutic hypothermia remains unclear.

Interpretation of neonatal brain volumes requires consideration of several developmental and technical factors, including gestational age at birth, postmenstrual and postnatal age at MRI, sex,

intracranial size, acquisition timing and protocol, and segmentation method [9–13]. These factors may influence associations between biochemical markers and quantitative MRI findings.

Recent evidence suggests that neonates with HIE treated with therapeutic hypothermia may exhibit volumetric alterations in the hippocampus, thalamus, basal ganglia, and cerebellum. However, findings vary with developmental age, volume normalization, MRI acquisition, and segmentation method [14]. It is therefore important to distinguish case–control differences associated with HIE from within-cohort associations between brain volumes and the initial biochemical severity of the hypoxic-ischemic insult.

This study investigated associations of initial pH, partial pressure of carbon dioxide ($p\text{CO}_2$), bicarbonate (HCO_3^-), base excess, and lactate with global and regional brain volumes measured after therapeutic hypothermia in neonates with HIE. Associations with consensus Rutherford MRI injury scores were also evaluated. To our knowledge, these blood gas parameters have not been systematically examined in relation to automated whole-brain volumetric measurements in this population.

The biological hypothesis was that greater initial biochemical derangement would be associated with lower total brain, gray matter, white matter, basal ganglia, thalamic, hippocampal, and cerebellar volumes.

Methods

Study population and patient selection

This retrospective, single-center study screened 198 neonates diagnosed with HIE at Etlik City Hospital, Ankara, Turkey, between December 2022 and October 2025. Recorded reasons for exclusion were no therapeutic hypothermia ($n = 14$), death before completion of the required clinical and imaging assessments ($n = 22$), transfer to another center ($n = 11$), a metabolic or genetic disorder ($n = 13$), no brain MRI within the first 20 days of life ($n = 18$), preterm birth ($n = 17$), and grade-C automated image quality ($n = 24$). The final cohort comprised 79 neonates: 51 boys (64.6%) and 28 girls (35.4%).

Eligibility required clinical evidence of neonatal encephalopathy following a suspected perinatal hypoxic-ischemic event and fulfillment of the institutional criteria for therapeutic hypothermia. Isolated perinatal acidemia without clinical signs of encephalopathy was insufficient for inclusion.

MRI exclusion criteria

MRI examinations were excluded from volumetric analysis if the three-dimensional T1-weighted sequence was absent or incomplete, did not provide full brain coverage, or contained moderate-to-severe motion, ghosting, blurring, susceptibility, or other artifacts that compromised anatomical delineation or tissue contrast.

Examinations were also considered unsuitable for automated volumetry when major congenital central nervous system malformations, severe hydrocephalus, extensive parenchymal destruction, large intracranial hemorrhage, or substantial mass effect distorted normal anatomy and prevented reliable segmentation.

After processing with AssemblyNet through the volBrain platform, native images and segmentation overlays were visually reviewed in the axial, coronal, and sagittal planes. Examinations were excluded if processing failed or quality control identified substantial registration errors, inaccurate intracranial cavity extraction, inclusion of extracranial tissue, tissue-classification failure, or anatomically implausible regional boundaries likely to affect volumetric measurements. Typical HIE-related signal abnormalities, including diffusion restriction and T1- or T2-weighted signal changes in the basal ganglia, thalami, perirolandic cortex, watershed cortex, or white matter, were not exclusion criteria if anatomical structures remained amenable to segmentation.

Therapeutic hypothermia and clinical data

Initial blood gas samples were obtained from the umbilical cord immediately after birth or from a peripheral artery within the first hour of life, before therapeutic hypothermia was initiated.

Clinical variables extracted from medical records included gestational age at birth, postnatal age at MRI, maternal age, and sex. Gestational age, postnatal age at MRI, and sex were prespecified covariates because of their potential associations with brain volume and injury severity. Maternal age was summarized descriptively but excluded from the primary models to limit model complexity.

Whole-body cooling was initiated within the first 6 hours of life. Core body temperature was maintained at 33.0–34.0°C for 72 hours, followed by controlled rewarming at approximately 0.25–0.5°C per hour until normothermia was achieved. During treatment, amplitude-integrated electroencephalography or conventional electroencephalography, vital signs, laboratory parameters, and organ function were monitored according to routine clinical practice. No

clinically relevant deviations from the institutional therapeutic hypothermia protocol were identified in the included cohort.

MRI acquisition

Brain MRI was performed using a 1.5-T GE SIGNA Champion scanner with a 32-channel head coil. Whenever possible, neonates were imaged during natural sleep using a feed-and-wrap technique. Sedation was administered only when clinically required, in accordance with institutional neonatal sedation and monitoring protocols.

MRI was performed at a mean postnatal age of 8.54 ± 2.80 days (median, 8 days; IQR, 7–9.5 days; range, 4–16 days). Gestational age at birth and postnatal age at MRI were entered separately in the primary models. Postmenstrual age was not included simultaneously because it is derived from these two variables and would introduce exact collinearity.

The neonatal brain MRI protocol included sagittal three-dimensional T1-weighted spoiled gradient-recalled echo imaging (TR/TE, approximately 20–30/4–5 ms; flip angle, 15–30°; field of view, 180–220 mm; acquisition matrix, 256×256 ; section thickness, 1–1.6 mm; no interslice gap); axial and multiplanar T2-weighted fast spin-echo imaging (TR/TE, approximately 4,500–6,000/120–210 ms; field of view, 180–220 mm; acquisition matrix, 256×256 ; section thickness, 3–4 mm; interslice gap, 0–0.5 mm); and axial diffusion-weighted single-shot echo-planar imaging (TR/TE, approximately 5,000–6,000/80–100 ms; b values, 0 and 1,000 s/mm²; section thickness, 3–5 mm). Apparent diffusion coefficient maps were generated automatically.

Only the three-dimensional T1-weighted images were used for automated volumetric processing. The remaining sequences were used for routine clinical assessment of HIE-related brain injury and were not included in the quantitative volumetric analysis.

Image processing, volumetric analysis, and quality control

Anonymized three-dimensional T1-weighted images were converted to Neuroimaging Informatics Technology Initiative format and processed using AssemblyNet version 1.0 through the volBrain platform [15,16]. The images used for processing had a voxel size of $1.2 \times 1.2 \times 1.0$ mm³.

Automated processing included denoising, correction of intensity inhomogeneity, intracranial cavity extraction, tissue classification, and regional segmentation [15,16]. Reports provided absolute volumes in cubic centimeters and relative volumes normalized to intracranial cavity volume (ICV).

The extracted measurements included global and regional brain volumes, ventricular and external cerebrospinal fluid compartments, cerebellar vermician lobules I–V, VI–VII, and VIII–X, and asymmetry indices for paired structures. Representative native images and detailed-structure and macrostructure segmentation outputs are shown in Figures 1 and 2.

Image quality was classified by AssemblyNet as grade A, B, or C. Grade-A and grade-B examinations were included after visual quality control; all grade-C examinations were excluded. Two radiologists, blinded to the blood gas results, independently evaluated native T1-weighted images and segmentation overlays in the axial, coronal, and sagittal planes. Using predefined criteria, they assessed image coverage, artifacts, registration accuracy, intracranial cavity extraction, tissue classification, and the anatomical plausibility of regional boundaries. Disagreements were resolved by consensus. Automated segmentations were not manually corrected. For sensitivity analyses, regional volumes were normalized to ICV as (raw volume/ICV) $\times$ 1000, and between-group analyses were repeated using these values.

The platform also generated asymmetry indices, but these were excluded from the primary analysis because the study focused on absolute volumes and signed ratio measures have different distributional properties. No confirmatory inference was made regarding asymmetry.

Qualitative MRI injury scoring

Two radiologists, blinded to the initial blood gas and volumetric results, independently evaluated conventional MRI examinations using the Rutherford MRI scoring system [2]. They assessed abnormalities in the basal ganglia and thalami, posterior limb of the internal capsule, cerebral white matter, and cerebral cortex according to predefined criteria and independently recorded total scores. Disagreements were subsequently reviewed jointly to determine a consensus score, which was used in all association and group-comparison analyses. Independent reader scores were used only to assess interobserver agreement, quantified using a two-way, absolute-agreement intraclass correlation coefficient for single measurements and quadratic-weighted Cohen κ . Exact agreement and agreement within 1 point were also reported.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as the number of available observations, mean, standard deviation, median, interquartile range, and range; categorical variables were summarized as frequencies and percentages. Missing values were excluded from the relevant analyses. Complete-case analysis was performed separately for each model, and the sample size was reported for each analysis. HC3 heteroskedasticity-consistent standard errors were used for robust statistical inference.

Initial pH measurements were available for 75 of the 79 neonates (94.9%); 29 had a pH ≤ 7.00 , and 46 had a pH > 7.00 . The four neonates with missing pH values were excluded only from pH-specific analyses. Availability of the remaining blood gas parameters was as follows: partial pressure of carbon dioxide ($p\text{CO}_2$), 73/79 (92.4%); bicarbonate (HCO_3^-), 72/79 (91.1%); base excess, 73/79 (92.4%); and lactate, 74/79 (93.7%). Given the small amount of missing data, multiple imputation was not performed. Nevertheless, the possibility of a missing-not-at-random mechanism could not be excluded.

Because this was a retrospective study of all eligible cases, no a priori sample-size calculation was performed. A sensitivity analysis based on the smallest predictor-specific sample size ($n = 72$) indicated that, for a multiple linear regression model containing the blood gas parameter and three covariates, a two-sided α of 0.05 and 80% power would detect an incremental Cohen f^2 of 0.112 (equivalent partial $r = 0.318$), based on the noncentral F distribution. This calculation was not adjusted for multiplicity; power to detect associations surviving false-discovery-rate correction was lower.

Unadjusted associations of each initial blood gas parameter (pH, $p\text{CO}_2$, HCO_3^- , base excess, and lactate) with each of the 50 absolute MRI-derived brain-volume measures were assessed using Spearman rank correlations. This approach was selected because several biochemical variables and small regional-volume measures had non-normal distributions or were potentially affected by outliers. It also allowed assessment of monotonic associations without assuming linearity.

Correlations between postnatal age at MRI and the 50 absolute brain-volume measures were evaluated as a separate exploratory analysis family.

For the primary adjusted analysis, a separate ordinary least-squares regression model was fitted for each blood gas parameter–volume pair. Each model included the blood gas parameter of interest, gestational age at birth, postnatal age at MRI, and sex. Unstandardized regression coefficients, standardized β coefficients for the focal blood gas parameter, HC3-robust 95% confidence intervals, p values, and adjusted R^2 values were reported.

All 50 absolute brain-volume measures were treated as exploratory outcomes because no smaller confirmatory outcome set had been prespecified before analysis. False-discovery-rate correction was applied across the full primary test family. Effect estimates and confidence intervals were reported to support interpretation and inform future study design.

Collinearity was assessed in a representative model in which pH was the focal predictor.

Variance inflation factors for the focal predictor and covariates ranged from 1.03 to 1.33, and the correlation between gestational age at birth and postnatal age at MRI was 0.034. All variance inflation factors were substantially below the conventional threshold of 5, indicating negligible collinearity in the component-based age model.

For the secondary analysis, neonates were classified into pH ≤ 7.00 and pH > 7.00 groups. The threshold of 7.00 was selected a priori because profound acidemia at approximately this level is widely used as a biochemical criterion in therapeutic–hypothermia eligibility frameworks [1]. Modeling pH as a continuous variable remained the

primary analytical approach; dichotomization was used only as a secondary analysis to improve clinical interpretability.

Each absolute MRI-derived brain-volume measure was evaluated using multivariable linear regression adjusted for gestational age at birth, postnatal age at MRI, and sex, with HC3-robust inference. The group coefficient represented the adjusted mean difference for neonates with pH ≤ 7.00 relative to those with pH > 7.00 .

For sensitivity analyses, eligible regional volumes were normalized to ICV as (raw regional volume/ICV) $\times 1000$. These measures represent regional volume per 1000 units of ICV, not percentages. Adjusted comparisons between the pH groups were repeated using the normalized measures; ICV itself was excluded from these outcomes.

The Benjamini-Hochberg procedure was used to control the false discovery rate. Correction was applied separately to the following pre-specified analysis families: the 250 primary blood gas-volume regression tests, the 250 unadjusted blood gas-volume Spearman correlation tests, the 50 exploratory correlations between postnatal age at MRI and absolute brain volume, the 50 secondary pH-group models using absolute volumes, and the corresponding pH-group sensitivity models using eligible ICV-normalized volumes. All tests were two-sided. Nominal p values were reported for transparency; findings that did not remain significant after false-discovery-rate correction were interpreted as exploratory rather than confirmatory.

The consensus Rutherford total score was summarized descriptively and treated as the prespecified qualitative MRI injury outcome. Its unadjusted associations with pH, pCO₂, HCO₃⁻,

base excess, and lactate were evaluated using Spearman rank correlations. Separate HC3-robust linear regression models were subsequently fitted for each blood gas parameter, adjusting for gestational age at birth, postnatal age at MRI, and sex. The Benjamini-Hochberg procedure was applied across the five focal blood gas tests separately for the Spearman correlation and adjusted regression families. The comparison between the pH ≤ 7.00 and pH > 7.00 groups was performed using the same covariates.

Because the Rutherford total score is bounded, regression coefficients were interpreted as exploratory adjusted mean-score differences rather than as effects measured on a continuous biological scale.

Results

Study population and descriptive findings

The final analytic cohort comprised 79 neonates. The mean gestational age at birth was 39.0 ± 0.9 weeks (median, 39 weeks; IQR, 38–40 weeks; range, 37–41 weeks), and the mean maternal age was 31.3 ± 5.8 years (median, 31 years; IQR, 27–37 years; range, 21–42 years). The cohort included 51 boys (64.6%) and 28 girls (35.4%). The demographic and clinical characteristics of the study population are summarized in Table 1.

Mean total brain volume was 409.97 ± 107.38 cm³, with a coefficient of variation of 26.2%.

Mean white matter, gray matter, and total cerebellar volumes were 193.98 ± 61.07 cm³, $215.99 \pm$

63.37 cm³, and 40.55 ± 32.10 cm³, respectively. MRI-derived volumetric measures are presented in Table 2.

Rutherford MRI injury scores and interobserver agreement

Consensus Rutherford scores were available for all 79 neonates. The mean consensus score was 6.91 ± 1.34, the median was 7 (IQR, 6–8), and the range was 4–9. The score distribution was as follows: 4 in 2 neonates (2.5%), 5 in 11 (13.9%), 6 in 17 (21.5%), 7 in 23 (29.1%), 8 in 14 (17.7%), and 9 in 12 (15.2%).

The two readers assigned identical scores in 40 of 79 examinations (50.6%), and their scores differed by no more than 1 point in 70 examinations (88.6%). Interobserver agreement was good, with a single-measure, absolute-agreement intraclass correlation coefficient of 0.789 and a quadratic-weighted Cohen κ of 0.787 (Table 3).

Initial blood gas measurements

Initial pH measurements were available for 75 neonates (mean, 7.02 ± 0.15; median, 7.02; IQR, 6.95–7.10). pCO₂ measurements were available for 73 neonates (mean, 54.87 ± 24.89 mmHg; median, 52.00 mmHg; IQR, 36.40–73.00 mmHg). HCO₃⁻ measurements were available for 72 neonates (mean, 14.92 ± 5.42 mmol/L; median, 15.85 mmol/L; IQR, 10.70–19.45 mmol/L), base excess measurements for 73 (mean, -16.57 ± 6.31 mmol/L; median, -16.20 mmol/L; IQR, -19.80 to -12.80 mmol/L), and lactate measurements for 74 (mean, 10.20 ± 5.37 mmol/L; median, 9.00 mmol/L; IQR, 6.21–13.35 mmol/L).

Associations between blood gas parameters and brain volumes

Among the 250 unadjusted blood gas–volume Spearman correlation tests, three associations had nominal p values <0.05: pH with left cerebellar white matter volume, base excess with right cerebellar white matter volume, and pCO₂ with parietal operculum volume. One of the 50 exploratory correlations between postnatal age at MRI and absolute brain volume, involving vermian lobules VIII–X, also had a nominal p value <0.05. However, none of these associations remained statistically significant after family-specific Benjamini–Hochberg FDR correction.

Of the 250 adjusted blood gas–volume regression analyses, nine yielded nominal p values <0.05. The smallest nominal p value was observed for base excess and right cerebellar white matter volume.

After adjustment for gestational age at birth, postnatal age at MRI, and sex, each 1–mmol/L increase in base excess was associated with an estimated 0.062–cm³ decrease in right cerebellar white matter volume (unstandardized coefficient, –0.062 cm³ per mmol/L; standardized β = –0.356; 95% CI, –0.107 to –0.017; nominal p = 0.008).

None of the 250 associations remained significant after FDR correction (minimum FDR–adjusted p = 0.984). Results are summarized in Table 5; complete regression results are provided in Supplementary Table 1.

Associations with consensus Rutherford scores

None of the initial blood gas parameters was significantly associated with the consensus Rutherford score in the unadjusted analyses: pH (Spearman $\rho = -0.024$; $p = 0.837$), $p\text{CO}_2$ ($\rho = -0.033$; $p = 0.782$), HCO_3^- ($\rho = 0.154$; $p = 0.196$), base excess ($\rho = -0.002$; $p = 0.986$), or lactate ($\rho = -0.118$; $p = 0.318$). The results were also nonsignificant in the multivariable-adjusted HC3-robust regression models (all nominal $p \geq 0.452$; all FDR-adjusted $p = 0.973$).

Comparisons between pH groups

Among the 75 neonates with available pH measurements, 29 had $\text{pH} \leq 7.00$ and 46 had $\text{pH} > 7.00$. After adjustment for gestational age at birth, postnatal age at MRI, and sex, none of the 50 absolute MRI-derived brain-volume measures differed significantly between the groups after FDR correction.

The smallest nominal p value was observed for vermian lobules VI–VII. The adjusted mean difference for neonates with $\text{pH} \leq 7.00$ relative to those with $\text{pH} > 7.00$ was 0.133 cm^3 (standardized $\beta = 0.235$; 95% CI, -0.011 to 0.277 ; nominal $p = 0.070$; FDR-adjusted $p = 0.821$).

The consensus Rutherford score also did not differ significantly between the pH groups. The adjusted mean difference for neonates with $\text{pH} \leq 7.00$ relative to those with $\text{pH} > 7.00$ was -0.20 (standardized $\beta = -0.071$; 95% CI, -0.93 to 0.54 ; $p = 0.597$; adjusted $R^2 = -0.055$).

Sensitivity analysis using ICV-normalized brain volumes

As a sensitivity analysis, 49 regional and tissue-volume outcomes, excluding ICV itself, were normalized to ICV using the formula $\text{raw volume}/\text{ICV} \times 1000$. After adjustment for gestational age at birth, postnatal age at MRI, and sex, none of the ICV-normalized volume measures differed significantly between the pH groups.

The smallest nominal p value was again observed for vermian lobules VI–VII. The adjusted mean difference for neonates with $\text{pH} \leq 7.00$ relative to those with $\text{pH} > 7.00$ was 0.178 per 1000 ICV units (standardized $\beta = 0.204$; 95% CI, -0.046 to 0.402 ; nominal $p = 0.117$; FDR-adjusted $p = 0.859$; adjusted $R^2 = 0.000$). Thus, ICV normalization did not materially change the absolute-volume findings (Table 8). Complete ICV-normalized results are provided in Supplementary Table 3.

Overall, no association of initial blood gas parameters with post-cooling absolute brain volumes or consensus Rutherford scores remained significant after correction for multiple comparisons. The pH-group comparisons likewise showed no significant differences in these outcomes or in ICV-normalized regional volumes.

Discussion

In this exploratory cohort of 79 neonates with HIE treated with therapeutic hypothermia, no initial blood gas parameter was significantly associated with the consensus Rutherford MRI injury

score after adjustment for gestational age at birth, postnatal age at MRI, and sex. Nine adjusted blood gas–volume associations yielded nominal p values <0.05, but none remained significant after correction for multiple comparisons. Absolute and ICV–normalized brain volumes also did not differ significantly between neonates with pH $\leq$ 7.00 and those with pH >7.00. Thus, the analyses provided no multiplicity–adjusted evidence of an association between initial biochemical severity and post–cooling structural MRI findings. These results should not, however, be interpreted as evidence that clinically relevant associations are absent.

Several factors may explain these predominantly negative findings. A single initial blood gas measurement captures one point in a dynamic pathophysiological process and may not reflect the duration or timing of hypoxia-ischemia, reperfusion injury, seizure burden, or response to therapeutic hypothermia. Resuscitation and sampling conditions may also influence values obtained shortly after birth. A single measurement may therefore inadequately represent the cumulative cerebral injury subsequently detected on MRI.

MRI injury scores and volumetric measurements also describe different aspects of brain injury. The Rutherford score captures the presence and distribution of characteristic injury patterns, whereas volumetry measures tissue size and may be less sensitive to acute injury when substantial tissue loss has not yet developed. Diffusion-weighted imaging and proton MR spectroscopy may detect acute cytotoxic and metabolic abnormalities before measurable changes

in brain volume become apparent. Therefore, the absence of a volumetric association does not exclude acute structural or metabolic injury.

Previous quantitative MRI studies have demonstrated smaller subcortical structures in children with HIE and associations between selected regional volumes and later neurodevelopmental outcomes [4–7]. However, those studies generally compared patients with HIE with healthy controls or examined relationships between brain volumes and subsequent functional outcomes. In contrast, the present study investigated whether variation in initial blood gas parameters was associated with early post-cooling brain volumes within an exclusively treated HIE cohort. Because no healthy control group was included, the study could not determine whether the observed volumes differed from normal neonatal development. The absence of long-term neurodevelopmental follow-up also prevented assessment of the prognostic importance of the volumetric measurements.

A recent review described reduced hippocampal, thalamic, basal ganglia, and, less consistently, cerebellar volumes in children with HIE treated with therapeutic hypothermia [14]. Several regional differences were attenuated or became nonsignificant after adjustment for total brain volume or ICV. In the present study, ICV normalization did not materially alter the pH-group comparisons: none of the 49 normalized measures differed significantly between groups. This sensitivity analysis did not establish a between-group difference after accounting for intracranial size.

The nominal cerebellar findings warrant particular caution. The smallest nominal p value in the adjusted blood gas–volume analyses involved base excess and right cerebellar white matter volume; in the absolute and ICV-normalized pH-group analyses, it involved vermian lobules VI–VII. None of these findings remained significant after correction for multiple comparisons. Small

cerebellar substructures are particularly susceptible to partial-volume effects, registration inaccuracies, and segmentation error. These findings should not be interpreted as evidence of a specific cerebellar association without quantitative validation in neonates and independent replication.

All participants received therapeutic hypothermia before MRI. The study could not assess treatment effectiveness because neither pretreatment MRI nor an untreated contemporaneous HIE comparison group was available. The findings therefore do not establish that hypothermia normalized brain volumes, prevented structural injury, or eliminated associations between biochemical severity and cerebral injury.

Limitations

This study has several limitations. First, its retrospective, single-center design introduces the possibility of selection bias, limits generalizability, and leaves room for unmeasured confounding. Clinical characteristics of excluded neonates were not available for comparison with those included in the final cohort. Death, clinical instability, incomplete imaging, motion degradation, and severe anatomical distortion may occur more frequently in neonates with severe injury. Therefore, the exclusion process may have preferentially removed the most severely affected patients, potentially attenuating the observed associations and limiting the applicability of the findings to critically ill neonates.

Second, the AssemblyNet/volBrain segmentation pipeline was not developed or quantitatively validated specifically for neonatal HIE; neonatal-specific alternatives have been developed for infant MRI [12]. Neonatal brain segmentation is challenging because of incomplete myelination, high tissue water content, rapidly changing gray–white matter contrast, small anatomical

structures, and injury-related signal abnormalities. Although two blinded radiologists reviewed the native images and segmentation overlays using predefined quality criteria, manual reference segmentations and comparisons with a validated neonatal-specific pipeline were unavailable. Residual region-specific measurement error cannot therefore be excluded, and the volumetric findings should be regarded as exploratory.

Third, volumetric estimates may vary according to magnetic field strength, scanner platform, acquisition sequence, participant age, reconstruction method, and segmentation software. Measurements obtained using different processing pipelines may therefore not be directly interchangeable [14]. The absence of widely accepted neonatal normative values further limits the clinical interpretation of individual regional volumes.

Long-term neurodevelopmental outcomes were unavailable. The study therefore could not determine whether the MRI-derived volumes or initial blood gas measurements predicted later cognitive, motor, language, or behavioral outcomes. Future studies should combine component-level MRI injury scoring with serial blood gas measurements, electroencephalographic seizure burden, diffusion metrics, MR-spectroscopy biomarkers such as lactate/N-acetylaspartate, and standardized neurodevelopmental assessment.

Finally, missing blood gas measurements reduced the predictor-specific sample sizes to 72–75 neonates. With approximately 72 observations, the study had limited power to detect small associations, particularly after correction for multiple comparisons. The analysis of 50 volumetric outcomes also increased the likelihood of nominal false-positive findings. Although FDR correction reduced this risk, it also made the detection of modest true associations more difficult. The findings should therefore be interpreted as exploratory and hypothesis-generating.

Conclusions

In this exploratory cohort of neonates with HIE treated with therapeutic hypothermia, no initial blood gas parameter was significantly associated with the consensus Rutherford MRI injury score or post-cooling global and regional brain volumes after correction for multiple comparisons. Secondary analyses likewise identified no significant differences in Rutherford scores, absolute brain volumes, or ICV-normalized regional volumes between the pH groups. These findings do not exclude clinically relevant associations. Larger prospective studies should incorporate neonatal-validated segmentation methods, prespecified regional outcomes, serial biochemical measurements, multimodal acute-injury biomarkers, and standardized long-term neurodevelopmental follow-up.

List of abbreviations

CI, confidence interval; FDR, false discovery rate; HCO_3^- , bicarbonate; HIE, hypoxic-ischemic encephalopathy; ICV, intracranial cavity volume; IQR, interquartile range; MRI, magnetic resonance imaging; pCO_2 , partial pressure of carbon dioxide; TE, echo time; TR, repetition time.

Declarations

Ethics approval and consent to participate

This retrospective single-center study was approved by the Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval no. AEŞH-BADEK2-2026-136; February 10, 2026). The study was conducted in accordance with the Declaration of Helsinki and relevant institutional guidelines and regulations.

Consent for publication

Written informed consent for publication of the anonymized MRI images was obtained from the parents or legal guardians of the participants whose images are presented in this manuscript.

Availability of data and materials

The de-identified datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request, subject to institutional and ethics committee approval.

Competing interests

The authors declare that they have no competing interests.

Funding

The authors received no specific funding for this work.

Authors' contributions

Mehmet Burak Özkan drafted the manuscript and performed the quality assessment. Furkan Özdem and Enes performed the volBrain measurements. Elif Özkan collected the clinical data. All authors read and approved the final manuscript.

Acknowledgements

The authors thank Gülşen Avcioğlu for her assistance with data collection.

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Main Tables (Tables 1–8)
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Table 1. Demographic, clinical, and initial blood gas characteristics

Characteristic	Unit	n	Mean $\pm$ SD	Median (IQR)	Range / n (%)
Gestational age at birth	weeks	79	39.00 $\pm$ 0.89	39.00 (38.00–40.00)	37.00–41.00
Maternal age	years	79	31.34 $\pm$ 5.78	31.00 (27.00–37.00)	21.00–42.00
Postnatal age at MRI	days	79	8.54 $\pm$ 2.80	8.00 (7.00–9.50)	4.00–16.00
pH		75	7.02 $\pm$ 0.15	7.02 (6.95–7.10)	6.50–7.42
pCO ₂	mmHg	73	54.87 $\pm$ 24.89	52.00 (36.40–73.00)	10.00–139.00
HCO ₃ ⁻	mmol/L	72	14.92 $\pm$ 5.42	15.85 (10.70–19.45)	2.20–24.40
Base excess	mmol/L	73	-16.57 $\pm$ 6.31	-16.20 (-19.80–12.80)	-30.00–0.80
Lactate	mmol/L	74	10.20 $\pm$ 5.37	9.00 (6.21–13.35)	1.24–24.80
Female sex					28 (35.4%)
Male sex					51 (64.6%)
pH $\leq$ 7.00					29 (38.7%)
pH > 7.00					46 (61.3%)

Table 2. MRI-derived absolute volumes

MRI-derived volume (cm³)	n	Mean ± SD	Median (IQR)	Range
Total cerebrum	79	370.197 ± 96.416	362.480 (304.080–424.040)	171.020–786.680
Right cerebrum	79	186.621 ± 49.436	179.420 (154.180–217.755)	84.560–396.060
Left cerebrum	79	183.575 ± 47.236	179.300 (150.430–208.955)	86.370–390.620
Total cerebral white matter	79	186.807 ± 59.794	179.630 (146.300–226.390)	67.450–380.140
Right cerebral white matter	79	94.437 ± 30.350	90.610 (73.830–114.055)	32.670–192.060
Left cerebral white matter	79	92.848 ± 29.553	89.310 (72.485–112.195)	34.770–188.070
Total cerebral gray matter	79	183.043 ± 54.219	180.860 (160.260–196.460)	99.120–526.620
Right cerebral gray matter	79	102.334 ± 94.369	90.850 (80.480–99.310)	50.420–894.410
Left cerebral gray matter	79	90.714 ± 27.012	88.710 (78.755–96.635)	48.690–260.860
Total cerebellum	79	40.548 ± 32.098	35.370 (29.210–40.475)	20.110–302.300
Right cerebellum	79	20.644 ± 16.796	17.870 (15.140–20.550)	9.860–158.580
Left cerebellum	79	19.904 ± 16.025	17.100 (14.145–20.165)	10.250–149.830
Total cerebellar white matter	79	6.714 ± 2.461	6.060 (5.105–7.860)	2.650–13.940
Right cerebellar white matter	79	3.346 ± 1.208	3.050 (2.620–4.005)	0.980–6.960
Left cerebellar white matter	79	3.369 ± 1.318	3.050 (2.465–3.970)	1.320–7.800
Total cerebellar gray matter	79	30.290 ± 10.024	28.230 (24.160–33.245)	15.650–73.020
Right cerebellar gray matter	79	15.526 ± 4.980	14.490 (12.370–16.960)	8.210–36.500
Left cerebellar gray matter	79	14.896 ± 5.079	13.980 (11.760–16.060)	8.600–36.520
Total vermis	79	2.642 ± 1.047	2.520 (2.085–3.115)	0.490–7.490
Vermian lobules I–V	79	1.370 ± 0.540	1.260 (1.060–1.630)	0.250–3.870
Vermian lobules VI–VII	79	0.579 ± 0.297	0.530 (0.430–0.715)	0.000–1.620
Vermian lobules VIII–X	79	0.693 ± 0.353	0.650 (0.545–0.820)	0.070–2.200
Posterior insula, total	79	0.778 ± 0.510	0.750 (0.430–1.010)	0.090–3.730
Posterior insula, right	79	0.421 ± 0.271	0.370 (0.270–0.545)	0.020–1.870
Posterior insula, left	79	0.358 ± 0.301	0.310 (0.100–0.545)	0.010–1.870
Central operculum, total	79	1.981 ± 0.977	1.850 (1.375–2.280)	0.650–7.590
Central operculum, right	79	1.049 ± 0.512	0.990 (0.765–1.255)	0.120–3.730
Central operculum, left	79	0.923 ± 0.567	0.860 (0.545–1.085)	0.220–3.860
Frontal operculum, total	79	0.691 ± 0.366	0.610 (0.455–0.825)	0.140–2.540
Frontal operculum, right	79	0.382 ± 0.211	0.360 (0.240–0.465)	0.020–1.150
Frontal operculum, left	79	0.308 ± 0.214	0.270 (0.180–0.380)	0.020–1.380

MRI-derived volume (cm³)	n	Mean ± SD	Median (IQR)	Range
Parietal operculum, total	79	0.598 ± 0.586	0.460 (0.290–0.790)	0.030–4.580
Parietal operculum, right	79	0.321 ± 0.293	0.300 (0.125–0.420)	0.000–2.190
Parietal operculum, left	79	0.277 ± 0.340	0.170 (0.060–0.435)	0.000–2.390
Inferior lateral ventricles, total	79	0.273 ± 0.318	0.200 (0.090–0.330)	0.030–1.920
Inferior lateral ventricle, right	79	0.136 ± 0.166	0.080 (0.050–0.165)	0.010–0.990
Inferior lateral ventricle, left	79	0.137 ± 0.167	0.090 (0.040–0.160)	0.010–0.980
Lateral ventricles, total	79	7.399 ± 5.418	6.210 (4.580–7.845)	2.000–38.810
Lateral ventricle, right	79	3.714 ± 2.798	3.050 (2.285–3.760)	0.350–19.240
Lateral ventricle, left	79	3.672 ± 2.750	2.930 (2.075–4.155)	0.830–19.570
Third ventricle	79	0.229 ± 0.238	0.190 (0.120–0.260)	0.000–1.740
Fourth ventricle	79	0.365 ± 0.217	0.310 (0.250–0.425)	0.100–1.650
External CSF	79	46.687 ± 28.444	36.830 (31.230–49.170)	0.900–164.970
White matter	79	193.977 ± 61.065	186.640 (152.130–236.725)	73.490–391.400
Gray matter	79	215.993 ± 63.367	217.290 (186.225–228.990)	116.680–607.140
Cerebellar gray matter	79	34.394 ± 16.605	31.020 (26.785–36.575)	15.300–144.290
Total brain	79	409.974 ± 107.377	397.850 (337.260–472.990)	191.890–880.910
CSF	79	55.548 ± 32.103	44.370 (37.220–57.550)	26.540–181.880
Intracranial volume	79	470.496 ± 123.505	447.410 (385.030–544.305)	252.370–984.700
Brainstem	79	4.974 ± 1.728	4.510 (4.080–5.145)	2.630–11.330

Table 3A. Consensus Rutherford score summary

Measure	n	Value
Consensus score, mean ± SD	79	6.91 ± 1.34
Consensus score, median (IQR)	79	7 (6–8)
Consensus score, range	79	4–9

Table 3B. Consensus Rutherford score distribution

Consensus score	n	%
4	2	2.5
5	11	13.9
6	17	21.5
7	23	29.1
8	14	17.7
9	12	15.2

Table 3C. Interobserver agreement

Interobserver agreement measure	n	Result
Exact agreement	79	40/79 (50.6%)
Agreement within 1 point	79	70/79 (88.6%)
Single-measure absolute-agreement ICC	79	0.789
Quadratic-weighted Cohen κ	79	0.787

Table 4. Nominally significant unadjusted Spearman correlations

Exposure	MRI outcome	n	ρ	Nominal p	BH-FDR p
MRI day	Vermian lobules VIII–X	79	0.256	0.023	0.333
pH	Left cerebellar white matter	75	-0.240	0.038	0.999
Base excess	Right cerebellar white matter	73	-0.237	0.043	0.999
pCO ₂	Parietal operculum, total	73	0.235	0.046	0.999
MRI day	Left cerebrum	79	0.224	0.047	0.333
MRI day	Right cerebrum	79	0.223	0.048	0.333

Table 5. Nominally significant adjusted blood gas–volume associations

Exposure	MRI outcome	n	B	Std β	95% CI	Nominal p	BH-FDR p	Adj. R ²
Base excess	Right cerebellar white matter	73	-0.062	-0.356	-0.107 to -0.017	0.008	0.984	0.104
pH	External CSF	75	-32.492	-0.189	-58.864 to -6.121	0.016	0.984	0.029
Base excess	Total cerebellar white matter	73	-0.111	-0.325	-0.204 to -0.018	0.020	0.984	0.084
pH	CSF	75	-38.108	-0.197	-70.492 to -5.724	0.022	0.984	0.067
pH	Lateral ventricle, right	75	-4.461	-0.260	-8.359 to -0.563	0.026	0.984	0.106
Lactate	Lateral ventricle, left	74	0.113	0.239	0.014 to 0.212	0.026	0.984	0.128
Lactate	Lateral ventricles, total	74	0.225	0.236	0.018 to 0.432	0.034	0.984	0.117
Base excess	External CSF	73	-0.823	-0.195	-1.635 to -0.011	0.047	0.984	0.045
Lactate	Right cerebellar white matter	74	0.065	0.320	0.001 to 0.130	0.047	0.984	0.070

Table 6. Associations between blood gas parameters and consensus Rutherford score

Exposure	n	Spearman ρ	Spearman p	Spearman FDR p	Adjusted B	Std β	95% CI	Adjusted p	Adjusted FDR p
pH	75	-0.024	0.837	0.986	0.124	0.014	-2.548 to 2.796	0.926	0.973
pCO ₂	73	-0.033	0.782	0.986	-0.001	-0.024	-0.015 to 0.013	0.857	0.973
HCO ₃ ⁻	72	0.154	0.196	0.795	0.020	0.081	-0.044 to 0.084	0.531	0.973
Base excess	73	-0.002	0.986	0.986	-0.001	-0.005	-0.057 to 0.055	0.973	0.973
Lactate	74	-0.118	0.318	0.795	-0.025	-0.101	-0.092 to 0.041	0.452	0.973

Table 7. Secondary pH ≤ 7.00 versus pH > 7.00 analyses: ten smallest nominal p values

MRI outcome	n	Adjusted difference	Std β	95% CI	Nominal p	BH-FDR p	Adj. R ²
Vermian lobules VI–VII	75	0.133	0.235	-0.011 to 0.277	0.070	0.821	0.027
Lateral ventricle, right	75	0.995	0.183	-0.501 to 2.491	0.189	0.821	0.079
Posterior insula, left	75	-0.077	-0.158	-0.197 to 0.042	0.200	0.821	0.003
Parietal operculum, left	75	-0.071	-0.159	-0.183 to 0.040	0.206	0.821	-0.033
Total vermis	75	0.253	0.154	-0.155 to 0.660	0.220	0.821	0.050
Vermian lobules I–V	75	0.129	0.148	-0.089 to 0.347	0.241	0.821	0.042
Lateral ventricles, total	75	1.684	0.163	-1.194 to 4.561	0.247	0.821	0.090
Left cerebellar white matter	75	0.339	0.147	-0.248 to 0.926	0.253	0.821	-0.003
Inferior lateral ventricle, right	75	0.047	0.166	-0.037 to 0.130	0.267	0.821	-0.021
Fourth ventricle	75	0.060	0.147	-0.058 to 0.178	0.316	0.821	-0.004

Table 8. ICV-normalized pH-group analyses: ten smallest nominal p values

MRI outcome	n	Adjusted difference	Std β	95% CI	Nominal p	BH-FDR p	Adj. R ²
Vermian lobules VI–VII	75	0.178	0.204	-0.046 to 0.402	0.117	0.859	-0.000
Lateral ventricle, right	75	1.606	0.181	-0.786 to 3.997	0.185	0.859	0.006
Parietal operculum, left	75	-0.170	-0.156	-0.433 to 0.093	0.202	0.859	-0.003
Frontal operculum, right	75	-0.125	-0.157	-0.324 to 0.073	0.211	0.859	-0.025
Right cerebellar gray matter	75	-1.400	-0.149	-3.668 to 0.868	0.222	0.859	0.013
Inferior lateral ventricle, right	75	0.082	0.167	-0.054 to 0.219	0.234	0.859	-0.001
Posterior insula, left	75	-0.184	-0.150	-0.490 to 0.122	0.234	0.859	0.034
Vermian lobules I–V	75	0.180	0.152	-0.132 to 0.491	0.253	0.859	-0.035
Lateral ventricles, total	75	2.527	0.152	-1.894 to 6.949	0.258	0.859	0.012
Right cerebellum	75	-7.992	-0.116	-22.068 to 6.083	0.261	0.859	-0.013

Abbreviations: B, unstandardized coefficient; BH, Benjamini–Hochberg; CI, confidence interval; CSF, cerebrospinal fluid; FDR, false discovery rate; HC3, heteroskedasticity-consistent standard errors type 3; ICV, intracranial volume; MRI, magnetic resonance imaging; SD, standard deviation.

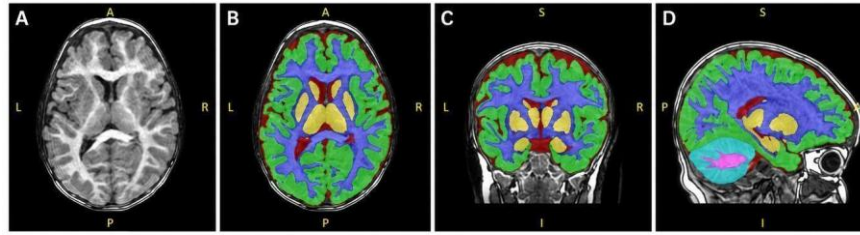


Figure 1. Representative volBrain AssemblyNet segmentation output in a neonate with hypoxic-ischemic encephalopathy. (A) Axial native three-dimensional T1-weighted image, (B) Axial segmentation overlay, (C) Coronal segmentation overlay, (D) Sagittal segmentation overlay. The automated output demonstrates global tissue and regional brain segmentation used for volumetric analysis.

Figure 1. Representative volBrain AssemblyNet segmentation in a neonate with hypoxic-ischemic encephalopathy. (A) Native axial three-dimensional T1-weighted image. (B–D) Segmentation overlays in the axial (B), coronal (C), and sagittal (D) planes.

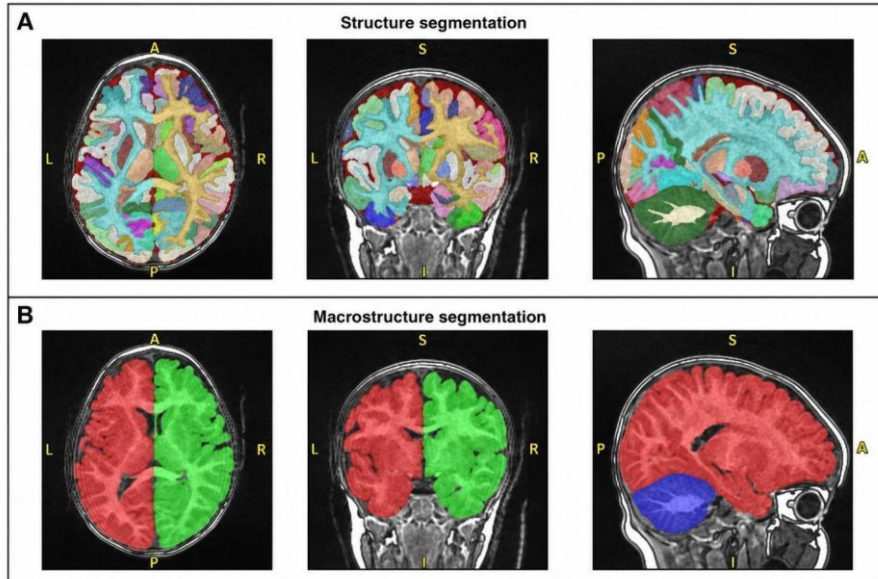


Figure 2. Representative *AssemblyNet* structure and *macrostructure segmentation* outputs in a neonate with hypoxic-ischemic encephalopathy. (A) Structure segmentation showing detailed regional parcellation in axial, coronal, and sagittal planes. (B) Macrostructure segmentation showing global parcellation in axial, coronal, and sagittal planes.

Figure 2. Representative AssemblyNet segmentation outputs in a neonate with hypoxic-ischemic encephalopathy. (A) Detailed regional parcellation in the axial, coronal, and sagittal planes. (B) Global cerebral and cerebellar parcellation in the same planes.

Supplementary Files

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