Original Article

Early Post-cooling Brain Magnetic Resonance for the Prediction of Neurodevelopmental Outcome in Newborns with Hypoxic–Ischemic Encephalopathy

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ABSTRACT

Aim and Objectives: This study aimed to evaluate the predictive role of early post-cooling brain magnetic resonance for developmental outcome in newborns with hypoxic–ischemic encephalopathy. **Materials and Methods:** A retrospective cohort study was performed on 29 consecutive patients through magnetic resonance evaluation (visual analysis of the images and scoring of the detected lesions; mean diffusivity of semioval centre and lenticular nuclei; and area under the curve of basal ganglia *N*-acetylaspartate at proton magnetic resonance spectroscopic imaging) and Griffiths Mental Development Scales–third edition at 12 and 24 months. **Results:** Brain magnetic resonance was performed at a mean age of 5.7 ± 3.7 days. Newborns with no/minor magnetic resonance abnormalities had a better developmental outcome than patients with moderate or severe lesions. Structural and spectroscopic abnormalities in basal ganglia resulted in the most significant predictors for an unfavorable outcome. **Conclusion:** Normal magnetic resonance in early post-cooling phases is strongly associated with a favorable developmental outcome.

Keywords: *Development, hypoxic–ischemic encephalopathy, magnetic resonance, newborns, therapeutic hypothermia*

Background

The use of brain magnetic resonance (MRI) as a diagnostic tool for the prediction of developmental outcome in newborns with hypoxic–ischemic encephalopathy treated with therapeutic hypothermia has obtained a growing importance for the planning of posttreatment intensive care decisions and rehabilitation strategies.[1-7]

The literature is controversial about the optimal timing of neuroimaging for a reliable prediction power.[8-12] In the pre-hypothermia era, some authors suggested that an early-performed brain MRI (first week after birth) could underestimate the presence and the extension of brain lesions and that the outcome could be better evaluated through scans obtained in later stages (between the second week and the first month after birth).[2] A different orientation emerges from the few available studies in newborns treated with hypothermia who had received a complete developmental evaluation within the age of 24 months.[6,13-15] Charon *et al.*,[7] in particular, observed in 29 patients that early-performed brain MRI (between 1 and 6 days after birth) had the same sensitivity for adverse outcomes (100%) and a higher specificity (96.3% vs. 89.3%) than late-performed MRI (between 9 and 21 days after birth). Other authors reported similar data, despite relevant methodological differences in the MRI parameters that were used to

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define the severity of brain lesions and in the cognitive and motor scales that were used for the evaluation of developmental outcome.[13-15] All these papers were focused on isolated neuroradiological biomarkers (structural, diffusivity, or spectroscopic data were analyzed separately in different samples).[6,7,13-15]

This study aimed to evaluate, in a common sample, the prognostic role of main structural, diffusivity, and spectroscopic indices at brain MRI in the early stages after therapeutic hypothermia for the prediction of developmental outcome.

Materials and Methods

All patients with hypoxic–ischemic encephalopathy, who had received therapeutic hypothermia (whole body cooling) in the neonatal age in the Pediatric Intensive Care Unit of the Department of Pediatrics, Child Neurology and Psychiatry at "Sapienza"—University of Rome between January 1, 2011 and December 31, 2015, were enrolled for a retrospective cohort study when both a post-cooling MRI and a complete neurological and neuropsychological follow-up lasting more than two years were available.

In our institutional protocol, the realization of brain MRI was forecasted at the fourth day after birth (after the end of therapeutic hypothermia). MRI was anticipated in newborns with specific indications (i.e., worsening seizures or suspect of cerebral hemorrhage before the fourth day after birth) and postponed in patients whose clinical conditions were not stable enough for a safe transport to our Radiology Section (i.e., patients under nitric oxide).

Brain MRI examinations were performed with 1.5T Siemens Avanto, Erlangen, Germany, including superconductive magnets (six-channel coil) performing single, multiple, and volumetric acquisitions (sequences GRE, IR, STIR, FLAIR, SSFSE, MTC, and EPI; layer thickness: 0.1–2 mm; matrix: 256 × 512). Diffusion-weighted images (DWI) was obtained using a single-shot, echoplanar imaging sequence with diffusion

Table 1: Demographic, clinical, arterial blood gas values, and aEEG severity of our cohort

Patient	Sex	Gestational age (weeks)	Birth weight (g)	Apgar score 1′	Apgar score 5′	Apgar score 10′	Sarnat stage	pH	BE	aEEG (according to Al Naqueeb *et al.*'s classification)
1	F	36.5	2600	4	7	/	2	7.09	–18.8	Moderately abnormal
2	M	40	3110	3	7	/	2	6.98	–21.9	Moderately abnormal
3	F	39.7	3450	3	4	8	2	7.24	–16.7	Moderately abnormal
4	M	41.1	3440	2	7	/	3	7.12	–20.7	Moderately abnormal
5	M	39	3300	3	Intubation	Intubation	3	6.93	–25	Moderately abnormal
6	F	38	3000	3	6	7	2	6.80	–27	Moderately abnormal
7	M	37.5	3150	0	3	Intubation	3	6.90	–20	Moderately abnormal
8	F	41	2950	0	4	7	2	6.9	–19	Moderately abnormal
9	M	39	4020	5	7	/	2	7.22	–8.6	Moderately abnormal
10	F	38	2700	4	6	Intubation	3	7.06	–15	Severely abnormal
11	F	37	3100	5	8	/	2	7.17	–10.7	Moderately abnormal
12	M	40	2960	1	5	8	2	7.13	–19.9	Moderately abnormal
13	F	39.8	2860	2	5	/	2	6.90	–23	Moderately abnormal
14	M	37.7	3770	1	5	8	2	6.96	–24	Moderately abnormal
15	M	42.4	3985	2	5	Intubation	3	6.80	–14.8	Moderately abnormal
16	F	35.8	2290	5	7	8	2	7.10	–13	Moderately abnormal
17	F	40.3	3510	3	7	/	2	7.30	–8.5	Moderately abnormal
18	F	40.7	3200	1	4	Intubation	2	7.06	–29	Moderately abnormal
19	M	34	2570	0	3	Intubation	3	6.80	–31	Severely abnormal
20	F	38	2700	1	4	7	2	6.86	–22.7	Severely abnormal
21	F	40	2900	1	4	Intubation	3	7.04	–17	Severely abnormal
22	M	40	2800	4	8	8	2	7.30	–6.7	Moderately abnormal
23	M	40	2900	2	5	Intubation	2	7.29	–13	Severely abnormal
24	F	38.2	2480	3	Intubation	Intubation	3	7.23	–17.5	Severely abnormal
25	M	40	3820	1	4	6	2	7.20	–14	Moderately abnormal
26	M	40.5	2670	4	8	8	2	7.28	–12	Moderately abnormal
27	F	40	3200	1	5	7	2	7.24	–9	Moderately abnormal
28	M	39	2950	5	8	8	2	7.10	–22.1	Moderately abnormal
29	M	39	3950	6	8	10	2	7.14	–16.7	Moderately abnormal

aEEG = amplitude-integrated electroencephalography, BE = base excess

sensitization obtained in 3–12 different directions and *b*-values of 0 and 1.000 s/mm^2. HM spatial resolution was 1.5 × 1.5 × 3 mm^3. Proton magnetic resonance spectroscopic imaging (^{1}HMRS) spectra were obtained through the Spectroscopy SV software version 1.0 on Leonardo Workstation (Siemens, Erlangen, Germany).

^{1}HMRS and diffusion coefficients were assessed according to postmenstrual ages of the patients.

Two expert pediatric neuroradiologists (CA and CC), who were blinded to the clinical history and to the developmental outcome of the patients (although they were informed about gestational age, it was essential for the evaluation of developmental stages at the scan), separately evaluated all the MRI examinations including visual analysis of severity and distribution of eventual MRI lesions, mean diffusivity of semioval centre and lenticular nuclei, and area under the curve of basal ganglia *N*-acetylaspartate (NAA) at ^{1}HMRS. The differences in the evaluations of nonquantitative parameters were then resolved by consensus between the two neuroradiologists.

The severity of MRI lesions was also systematically scored through Bednarek severity scores (a previously published semiquantitative system that considered regional subscores for basal ganglia, white matter, cortex, brainstem, and cerebellum and a global score resulting from the sum of the subscores; see Table 2).[3]

The mean diffusivity of semioval centre and lenticular nuclei through DWI was available for 27/29 patients. ^{1}HMRS was performed in 24/29 patients.

All patients underwent periodical neurodevelopmental evaluations and Griffiths Mental Development Scales–third edition (GMDS-III) were used to assess neurocognitive outcome at the age of 24 months. Of the 29 patients, 28 underwent GMDS-III also at the age of 12 months. GMDS-III was administered by an expert infantile neuropsychologist (AR) who was blinded to MRI features of the patients. The developmental outcome was considered unfavorable if GMDS-III global quotient was <85.

The statistic analysis was performed using Stata Statistical software version 8.1, College Station, USA.

The negative and positive predictive values of early brain MRI were calculated. The Mann–Whitney *U* test was used to evaluate differences in neuromotor and cognitive outcome between patients with no/minimally abnormal brain MRI (Group A—Bednarek severity global score between 48 and 55) and patients with moderate or severe neuroradiological abnormalities (Group B—Bednarek severity global score between 56 and 186).

The correlations between different neuroradiological predictors (Bednarek severity global scores and subscores, mean diffusivity of semioval centre and lenticular nuclei, and area under the curve of basal ganglia NAA at ^{1}HMRS) and neuromotor and cognitive outcome (GMDS-III scores at 12 and 24 months) in the whole sample were assessed through Spearman's coefficient. Written informed consent was obtained from the parents of all selected patients and the study was approved by the ethics committee of Sapienza Università di Roma and Umberto I-Policlinico di Roma.

Results

In the selected temporal range (January 1, 2011–December 31, 2015), 61 newborns with hypoxic–ischemic encephalopathy underwent therapeutic hypothermia in our institution. Twenty-nine consecutive patients (15 men and 14 women) with all the required criteria were recruited for this study. Two newborns who did not complete therapeutic hypothermia because of complications, three newborns who died before MRI, sixteen newborns who did not reach 24 months of follow-up at the moment of the retrospective analysis, and eleven newborns who were lost in follow-up were excluded from this study.

Table 1 summarizes auxologic features, Apgar scores, Sarnat stages, arterial blood test values, and amplitude-integrated electroencephalography (aEEG) severity in the neonatal age of all selected patients, whereas neuroradiological and neurocognitive scores are reported, respectively, in Tables 2 and 3.

The mean age at MRI was 5.7 ± 3.7 days after birth (range 1–20 days). At visual evaluation of MRI, brain lesions were prominently ischemic [Table 2] with the involvement of different sites mainly including basal ganglia (nine patients) and periventricular area (nine patients). Intraventricular hemorrhages were seen in two patients (Patients 7 and 19) [Table 2]. No brain lesions were seen in 13 patients (Patients 4, 5, 8, 9, 10, 12, 13, 14, 18, 23, 24, 27, and 29) [Table 2]. Group A included 17/29 subjects (58.6%: 10 men and 7 women) whereas Group B included 12/29 subjects (41.4%: 5 men and 7 women).

The correlation between Bednarek severity global score and GMDS-III scores showed that early post-cooling brain MRI had a negative predictive value of 93.75% at 12 months and 100% at 24 months and a positive predictive value of 36.36% at 12 months and 50% at 24 months for an unfavorable developmental outcome.

Table 2: Neuroradiological data of our cohort including age at MRI, visual evaluation, Bednarek severity scores, area under NAA curve in basal ganglia, and mean diffusivity in semioval centre and lenticular nuclei

Patient	Age at the MRI (days)	Type of brain lesions at visual evaluation	Bednarek severity scores[3]						[1]HMRS		DWI		Group
			Global score (r.- 48–186)	Basal ganglia score (r.24–96)	White matter score (r. 6–24)	Cortex score (r. 6–24)	Cerebellum score (r. 6–24)	Brainstem score (r. 6–18)	NAA–AUC right basal ganglia	NAA–AUC left basal ganglia	Semioval centre mean diffusivity	Lenticular nuclei mean diffusivity	
1	7	Left peritrigonal hemorrhage and bilateral T-2 hyperintensity in both thalami	80	42	20	6	6	6	NA	NA	1.9	1.06	B
2	4	Pale bilateral periventricular T2-hyperintensity	50	24	8	6	6	6	NA	82.8	1.7	1.1	A
3	1	Pale left lenticular T2-hyperintensity	50	26	6	6	6	6	85.4	93.3	1.6	1.1	A
4	5	None	48	24	6	6	6	6	NA	82	1.4	1.1	A
5	3	None	48	24	6	6	6	6	NA	88.8	1.6	1.1	A
6	4	Bilateral subcortical ischemic lesion involving parietal regions and pons	58	24	12	6	6	10	140	126	1.1	0.8	B
7	4	IVH grade I, pale bilateral periventricular and cerebellar T2-hyperintensity	58	24	10	6	12	6	NA	94.3	1.7	1.2	B
8	5	None	48	24	6	6	6	6	101	111	1.7	1	A
9	4	None	48	24	6	6	6	6	NA	NA	NA	NA	A
10	4	None	48	24	6	6	6	6	111	97.1	1.8	1	A
11	5	Bilateral hemispheric white matter and basal ganglia T2-hyperintensity	72	34	20	6	6	6	66.6	84.9	1.6	1.1	B
12	5	None	48	24	6	6	6	6	NA	30.4	1.6	1.13	A
13	4	None	48	24	6	6	6	6	NA	84.4	1.7	1	A
14	4	None	48	24	6	6	6	6	124	136	1.6	1	A
15	5	Pale periventricular T2-hyperintensity	49	24	7	6	6	6	148	89.8	1.6	1	A
16	5	Ischemic lesions in the right semioval centre and in periventricular zones	63	24	19	6	8	6	79.6	66	2	1.4	B
17	4	Pale T-2 hyperintensity involving both thalami and periventricular zones	62	26	18	6	6	6	80	65	1.8	1.08	B

Table 2: Continued

Patient	Age at the MRI (days)	Type of brain lesions at visual evaluation	Bednarek severity scores[3]						[1]HMRS		DWI		Group
			Global score (r.- 48–186)	Basal ganglia score (r.24–96)	White matter score (r. 6–24)	Cortex score (r. 6–24)	Cerebellum score (r. 6–24)	Brainstem score (r. 6–18)	NAA–AUC right basal ganglia	NAA–AUC left basal ganglia	Semioval centre mean diffusivity	Lenticular nuclei mean diffusivity	
18	4	None	48	24	6	6	6	6	8.391 pt	70	1.4	1.08	A
19	5	Diffuse T2-hyperintensity involving bilateral hemispheric white matter, cerebellum, and basal ganglia. IVH grade II	128	72	24	14	12	6	NA	24.9	1.7	1.2	B
20	3	T1-hypointensity involving thalami, posterior part of internal capsula, cerebral peduncles, temporal lobes, diencephalic region, optical radiations and periventricular areas	88	62	8	6	6	6	NA	72.4	1.66	0.9	B
21	4	Bilateral T2 hyperintensity in lenticular nuclei and periventricular areas	62	36	8	6	6	6	NA	77.7	1.8	1.2	B
22	5	Pale T2-hyperintensity involving periventricular areas and lenticular nuclei	50	24	8	6	6	6	NA	NA	1.6	1.1	A
23	20	None	48	24	6	6	6	6	NA	NA	0.8	0.7	A
24	6	None	48	24	6	6	6	6	NA	50.9	1.6	1.1	A
25	5	Ischemic lesions involving lenticular nuclei and pons	59	32	6	6	6	9	NA	77.3	1.4	1.2	B
26	7	Bilateral diffusion restriction in both thalami and in periventricular areas	64	24	22	6	6	6	NA	NA	NA	NA	B
27	14	None	48	24	6	6	6	6	101	111	1.7	1	A
28	11	T2-hyperintensity of both thalami	64	40	6	6	6	6	80.4	92.1	1.8	1.03	B
29	10	None	48	24	6	6	6	6	NA	86.8	1.4	1.3	A

[1]HMRS = proton magnetic resonance spectroscopy, NAA = *N*-acetylaspartate, AUC = area under the curve, R = range, IVH = intraventricular hemorrhage, DWI = diffusion-weighted images, NA = NA not available

Group A included patients with no/minimally abnormal brain MRI (Bednarek severity global score between 48 and 55), whereas Group B included newborns with moderate or severe neuroradiological abnormalities (Bednarek severity global score between 56 and 186)

Table 3: GMDS-III scores and equivalent ages at 12 and 24 months in our cohort

Patient	GMDS-III scores at 12 months						GMDS-III scores at 24 months					
	Locomotor development score (equivalent age)	Hand and eye coordination score (equivalent age)	Hearing and speech score (equivalent age)	Personal–social development score (equivalent age)	Performance tests score (equivalent age)	Global quotient score (equivalent age)	Locomotor development score (equivalent age)	Hand and eye coordination score (equivalent age)	Hearing and speech score (equivalent age)	Personal–social development score (equivalent age)	Performance test score (equivalent age)	Global quotient score (equivalent age)
1	100 (12 m)	92 (11 m)	92 (11 m)	92 (11 m)	92 (11 m)	**94 (11 m)**	100 (24 m)	92 (22 m)	100 (24 m)	96 (23 m)	92 (22 m)	**96 (23 m)**
2	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
3	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
4	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	96 (23 m)	87 (21 m)	100 (24 m)	98 (23 m)	**96 (23 m)**
5	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	96 (23 m)	87 (21 m)	100 (24 m)	98 (23 m)	**96 (23 m)**
6	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
7	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
8	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**102 (12 m)**	100 (24 m)	92 (22 m)	92 (22 m)	100 (24 m)	97 (23 m)	**96 (23 m)**
9	100 (12 m)	100 (12 m)	100 (12 m)	92 (11 m)	96 (11 m)	**98 (11 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
10	100 (12 m)	92 (11 m)	92 (11 m)	100 (12 m)	94 (11 m)	**95 (11 m)**	100 (24 m)	92 (22 m)	92 (22 m)	100 (24 m)	92 (22 m)	**95 (23 m)**
11	75 (9 m)	83 (10 m)	75 (9 m)	83 (10 m)	80 (10 m)	**80 (10 m)**	46 (11 m)	56(13m)	37 (9 m)	56 (13m)	56 (13m)	**49 (11 m)**
12	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
13	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
14	NP	NP	NP	NP	NP	**NP**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
15	100 (12 m)	92 (11 m)	92 (11 m)	100 (12 m)	100 (12 m)	**97 (11 m)**	100 (24 m)	96 (23 m)	83 (20 m)	100 (24 m)	97 (23 m)	**95 (23 m)**
16	100 (12 m)	83 (10 m)	92 (11 m)	92 (11 m)	92 (11 m)	**92 (11 m)**	100 (24 m)	92 (22 m)	100 (24 m)	100 (24 m)	97 (23 m)	**100 (24 m)**
17	100 (12 m)	75 (9 m)	83 (10 m)	83 (10 m)	83 (10 m)	**85 (10 m)**	87 (21 m)	75 (18m)	62 (15 m)	71 (17m)	75 (18m)	**74 (18 m)**
18	92 (11 m)	92 (11 m)	92 (11 m)	92 (11 m)	92 (11 m)	**92 (11 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	91 (22 m)	**100 (24 m)**
19	100 (12 m)	92 (11 m)	83 (10 m)	100 (12 m)	100 (12 m)	**95 (11 m)**	100 (24 m)	87 (21 m)	79 (19 m)	100 (24 m)	96 (23 m)	**92 (22 m)**
20	16 (2 m)	16 (2 m)	16 (2 m)	16 (2 m)	16 (2 m)	**16 (2 m)**	12 (3 m)	8 (2 m)	8 (2 m)	8 (2 m)	8 (2 m)	**9 (2 m)**
21	75 (9 m)	75 (9 m)	67 (8 m)	75 (9 m)	75 (9 m)	**73 (9 m)**	54 (13m)	75 (18m)	50 (12 m)	75 (18m)	79 (19 m)	**67 (16 m)**
22	83 (10 m)	83 (10 m)	83 (10 m)	83 (10 m)	83 (10 m)	**100 (12 m)**	90 (21 m)	87 (21 m)	90 (21 m)	90 (21 m)	91 (22 m)	**91 (22 m)**
23	100 (12 m)	92 (11 m)	83 (10 m)	92 (11 m)	94 (11 m)	**92 (11 m)**	100 (24 m)	96 (23 m)	92 (22 m)	94 (23 m)	94 (23 m)	**95 (23 m)**
24	92 (11 m)	92 (11 m)	83 (10 m)	94 (11 m)	94 (11 m)	**91 (11 m)**	96 (23 m)	96 (23 m)	92 (22 m)	96 (23 m)	96 (23 m)	**95 (23 m)**
25	42 (5m)	58 (7m)	58 (7m)	50 (6 m)	50 (6 m)	**52 (6 m)**	41 (10 m)	66 (16 m)	75 (18 m)	79 (19 m)	75 (18 m)	**68 (16 m)**
26	100 (12 m)	100 (12 m)	83 (10 m)	100 (12 m)	100 (12 m)	**99 (12 m)**	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	100 (24 m)	**100 (24 m)**
27	100 (12 m)	100 (12 m)	83 (10 m)	100 (12 m)	100 (12 m)	**99 (11 m)**	100(24 m)	96 (23 m)	80 (19 m)	100 (24 m)	100 (24 m)	**95 (23 m)**
28	100 (12 m)	100 (12 m)	83 (10 m)	100 (12 m)	100 (12 m)	**97 (11 m)**	100 (24 m)	79 (19 m)	62 (12 m)	79 (19 m)	79 (19 m)	**80 (19 m)**
29	100 (12 m)	100 (12 m)	92 (11 m)	100 (12 m)	100 (12 m)	**100 (12 m)**	100 (24 m)	96 (23 m)	87 (21 m)	96 (23 m)	92 (22 m)	**94 (23 m)**

NP = not performed, m = months

Table 4: Comparison between patients with normal/minimally abnormal brain MRI (Group A) and patients with moderate-to-severe neuroradiological abnormalities (Group B) through Mann–Whitney *U* test

General features	Group A	Group B	*P* value
Number of patients	17	12	NA
Sex Males	10	5	0.362*
Females	7	7	
Mean gestational age	39.74 ± 1.19 w	38.05 ± 2.01 w	0.0339
(median gestational age)	(*M* = 40; Min = 37,7; Max = 42,4)	(*M* = 38; Min = 34; Max = 40,5)	
Mean birthweight	3239.70 ± 470.47 g	2938.33 ± 424.23 g	0.0881
(median birthweight)	(*M* = 3200; Min = 2480; Max = 4020)	(*M* = 2925; Min = 2290; Max = 3820)	
Mean Apgar score	2.52 ± 1.58	2.66 ± 1.96	0.8396
(median Apgar score)	(*M* = 2; Min = 0; Max = 6)	(*M* = 3; Min = 0; Max = 5)	
1′			
Mean Apgar score	5.66 ± 1.39	5.75 ± 2.00	0.9801
(median Apgar score)	(*M* = 5; Min = 4; Max = 8)	(*M* = 6,5; Min = 3; Max = 8)	
5′			
GMDS-III at 12 months	**Group A mean scores at 12 months (median sores at 12 months)**	**Group B mean scores at 12 months (median scores at 12 months)**	***P* value**
Locomotor development score	97.62 ± 4.45	82.54 ± 28.84	0.2708
	(*M* = 100; Min = 86; Max = 100)	(*M* = 100; Min = 16; Max = 100)	
Hand and eye coordination	95.93 ± 5.22	79.45 ± 24.76	0.0196
score	(*M* = 100; Min = 83; Max = 100)	(*M* = 83; Min = 16; Max = 100)	
Hearing and speech score	93.25 ± 7.05	75.63 ± 22.99	0.0053
	(*M* = 92; Min = 83; Max = 100)	(*M* = 83; Min = 16; Max = 100)	
Personal–social development	97.06 ± 5.02	81 ± 26.29	0.0294
score	(*M* = 100; Min = 83; Max = 100)	(*M* = 92; Min = 16; Max = 100)	
Performance tests score	96.75 ± 5.03	81 ± 26.33	0.0435
	(*M* = 100; Min = 83; Max = 100)	(*M* = 92; Min = 16; Max = 100)	
Global quotient	96.31 ± 4.86	80.27 ± 25.62	0.0199
	(*M* = 98.5; Min = 83; Max = 100)	(*M* = 92; Min = 16; Max = 100)	
GMDS-III at 24 months	**Group A mean scores at 24 months (median sores at 24 months)**	**Group B mean scores at 24 months (median scores at 24 months)**	***P* value**
Locomotor development score	99.76 ± 0.97	78.33 ± 31.32	0.0147
	(*M* = 100; Min = 96; Max = 100)	(*M* = 100; Min = 12; Max = 100)	
Hand and eye coordination	97.05 ± 3.32	77.5 ± 26.05	0.0069
score	(*M* = 96; Min = 90; Max = 100)	(*M* = 83; Min = 8; Max = 100)	
Hearing and speech score	93.64 ± 6.91	72.75 ± 30.11	0.0853
	(*M* = 92; Min = 80; Max = 100)	(*M* = 77; Min = 8; Max = 100)	
Personal–social development	98.58 ± 2.89	80.33 ± 27.173	0.0182
score	(*M* = 100; Min = 90; Max = 100)	(*M* = 87.5; Min = 8; Max = 100)	
Performance tests score	96.88 ± 3.68	79.75 ± 26.45	0.0303
	(*M* = 98; Min = 90; Max = 100)	(*M* = 85.5; Min = 8; Max = 100)	
Global quotient	97.17 ± 2.94	77.91 ± 27.46	0.0589
	(*M* = 96; Min = 91; Max = 100)	(*M* = 86; Min = 9; Max = 100)	

NA = not applicable, w = weeks, g = grams, M = median, Min = minimum, Max = maximum, GMDS-III = Griffiths Mental Development Scales-III rd edition

*Chi-squared test

The Mann–Whitney *U* test showed a significantly higher mean gestational age in Group A than in Group B and a higher birthweight (even if not statistically significant), whereas no significant differences were found in terms of Apgar score [Table 4]. Children belonging to Group A had higher mean values of global quotient, subitem scores, and equivalent ages at GMDS-III than children belonging to Group B [Table 4]. Patients of Group A had also higher mean area under NAA curve in basal ganglia than members of Group B (106.22 ± 24.70 vs.

89.32 ± 28.92 in the right side and 86.60 ± 25.35 in the left side) with no relevant differences in mean diffusivity in the analyzed structures.

The analysis of the whole sample through Spearman's coefficient showed a weak correlation between all Bednarek severity scores and GMDS-III scores both at the age of 12 and 24 months [Table 5]. Among severity regional subscores, basal ganglia score had the strongest correlation with both GMDS-III global quotient and subitem scores [Table 5]. A strong correlation was also recorded between area under NAA curve in basal ganglia and both GMDS-III global quotient and subitem scores with higher mean Spearman's coefficients at the age of 12 months than at the age of 24 months [Table 5]. None of the patients had detectable lactate peaks at ^{1}HMRS. No significant correlations were found between mean diffusivity data and neuropsychological indices [Table 5].

Discussion

The results of our study highlight the following [Tables 4 and 5]: (a) a normal early post-cooling brain MRI is strongly associated with a favorable cognitive and motor outcome (high negative predictive value for psychomotor delay both at the age of 12 and 24 months); (b) the presence of abnormalities at early post-cooling brain MRI is not necessarily related with an unfavorable outcome; and (c) structural and spectroscopic abnormalities in basal ganglia at early post-cooling brain MRI can be considered as a reliable predictor for an adverse motor and cognitive outcome, whereas structural lesions and diffusion abnormalities involving other cerebral sites (including cortex, cerebellum, brainstem, or white matter) often fail to predict developmental delay.

The high negative predictive value for a favorable developmental outcome of early post-cooling brain MRI, in our sample, agreed with similar previously published data by Charon *et al.*[6] and Kasdorf *et al.*[14] As additional points of strength, our study includes a more homogenous and adequate measurement of cognitive outcome for the considered age-range (Charon *et al.* used Brunet Lezine Revised Scale up to 30 months and, for children between 30 and 41 months, neurological examination only) and a large number of tested neuroradiological parameters (both the aforementioned studies did not include spectroscopic data).[6,14] A lower negative predictive value (74%) was reported in older newborns (mean age at MRI of 8 days) by Rollins *et al.*[10] and Chalak *et al.*[11] These authors observed a significant quote of cooled patients with normal or minimally altered MRI who developed moderate (20%–70% of patients) or severe developmental delay (6% of patients).[10,11] These conflicting results suggest caution in the parental counseling after a normal early post-cooling MRI and impose periodical developmental evaluations beyond the first two years after birth.[6,7,9-11,14,15] This approach could be useful for the choice of the best timing for performing subsequent MRI in patients with a normal early MRI and without progress in developmental milestones despite an optimal cooling and rehabilitative management.[11]

In our sample, the presence of moderate or severe MRI abnormalities after cooling (patients belonging to Group B) scarcely correlated with an unfavorable developmental outcome. The positive predictive values of MRI at 12 and 24 months were lower than in previous studies.[9-11,13] Single case inspection confirmed a remarkable interindividual variability in neuromotor and cognitive outcome independently from neuroradiological pattern. For example, Patient 19 [see Figure 1 in Supplementary Material for MRIs] had the highest Bednarek severity global score of our cohort (128/186), but he indicated only a mild delay at GMDS-III (at 24 months general quotient was 92 with an equivalent age of 22 months). These results can be interpreted through the frequent transient nature of several signal MRI abnormalities in the early post-cooling stages (especially the ones following less severe ischemic insults that are more responsive to therapeutic hypothermia). Another possible explanation is given by the assumption that prognostic meaning of MRI abnormalities varies according to the site and the extension of the lesions regardless of hypothermia as it was reported by Cheong *et al.*[9] and Rutherford *et al.*[13] in representative subgroups from two large controlled randomized trials.

Our study confirmed, according to the data of the literature the relevant role of basal ganglia involvement in ischemic perinatal damages as a reliable predictor of unfavorable outcome after cooling.[13-15] These data also showed that the lack of neuroradiological abnormalities in basal ganglia can be considered as a good marker of the neuroprotective action of therapeutic hypothermia against reperfusion damages.[13-16] Lesions involving basal ganglia in hypoxic–ischemic encephalopathy results in an impaired regulation of motor control, acquisition of language abilities, reasoning, and reward-based learning during the development.[17] Kasdorf *et al.*[14] reported that newborns with basal ganglia lesions after therapeutic hypothermia presented with a more frequent necessity of neonatal cardiopulmonary resuscitation, a more persistent severe acidosis, more

Table 5: Correlations between neuroradiological predictors (Bednarek severity scores, area under NAA curve in basal ganglia and mean diffusivity in semioval centre and lenticular nuclei) and GMDS-III scores in our cohort at 12 and 24 months through Spearman's coefficient

GMDS-III at 12 months	Basal ganglia score	White matter score	Cortex score	Cerebellum score	Brainstem score	Global score	NAA–AUC right basal	NAA–AUC left basal ganglia	Semioval centre mean diffusivity	Lenticular nuclei mean diffusivity
Locomotor development score	$R = -0.3207$ $P = 0.1029$	$R = -0.0487$ $P = 0.8093$	$R = 0.1249$ $P = 0.5349$	$R = 0.2248$ $P = 0.2597$	$R = -0.3746$ $P = 0.0542$	$R = -0.2048$ $P = 0.3054$	$R = 0.7028$ $P = 0.0234$	$R = 0.2936$ $P = 0.1847$	$R = 0.2896$ $P = 0.1602$	$R = -0.1251$ $P = 0.5513$
Hand and eye coordination score	$R = -0.4624$ $P = 0.0152$	$R = -0.3764$ $P = 0.0530$	$R = -0.0669$ $P = 0.7403$	$R = -0.0357$ $P = 0.8598$	$R = -0.3211$ $P = 0.1025$	$R = -0.4459$ $P = 0.0197$	$R = 0.4782$ $P = 0.1621$	$R = 0.4711$ $P = 0.0269$	$R = -0.0635$ $P = 0.7629$	$R = -0.0649$ $P = 0.7580$
Hearing and speech score	$R = -0.5511$ $P = 0.0029$	$R = -0.3153$ $P = 0.1091$	$R = -0.1435$ $P = 0.4753$	$R = 0.0939$ $P = 0.6413$	$R = -0.3130$ $P = 0.1119$	$R = -0.5144$ $P = 0.0060$	$R = 0.4112$ $P = 0.2377$	$R = 0.2853$ $P = 0.1981$	$R = 0.0106$ $P = 0.9600$	$R = 0.0453$ $P = 0.8297$
Personal–social development score	$R = -0.4246$ $P = 0.0273$	$R = -0.2724$ $P = 0.1693$	$R = 0.1667$ $P = 0.4061$	$R = 0.1302$ $P = 0.5173$	$R = -0.3333$ $P = 0.0893$	$R = -0.3751$ $P = 0.0539$	$R = 0.8282$ $P = 0.0031$	$R = 0.5003$ $P = 0.0177$	$R = 0.0289$ $P = 0.8910$	$R = -0.0430$ $P = 0.8383$
Performance tests score	$R = -0.3957$ $P = 0.0411$	$R = -0.2478$ $P = 0.2127$	$R = 0.1717$ $P = 0.3918$	$R = 0.1330$ $P = 0.5085$	$R = -0.3170$ $P = 0.1071$	$R = -0.3418$ $P = 0.0810$	$R = 0.7268$ $P = 0.0173$	$R = 0.4232$ $P = 0.0497$	$R = 0.0039$ $P = 0.9852$	$R = -0.0892$ $P = 0.6716$
General quotient	$R = -0.4700$ $P = 0.0134$	$R = -0.2951$ $P = 0.1351$	$R = -0.0128$ $P = 0.9496$	$R = 0.0721$ $P = 0.7209$	$R = -0.3065$ $P = 0.1200$	$R = -0.4230$ $P = 0.0279$	$R = 0.6718$ $P = 0.0334$	$R = 0.4295$ $P = 0.0461$	$R = 0.0002$ $P = 0.9992$	$R = 0.0044$ $P = 0.9834$
GMDS-III at 24 months	**Basal ganglia score**	**White matter score**	**Cortex score**	**Cerebellum score**	**Brainstem score**	**Global score**	**NAA–AUC right basal**	**NAA–AUC left basal ganglia**	**Semioval centre mean diffusivity**	**Lenticular nuclei mean diffusivity**
Locomotor development score	$R = -0.5553$ $P = 0.0018$	$R = -0.1886$ $P = 0.3271$	$R = 0.0957$ $P = 0.6215$	$R = 0.1718$ $P = 0.3729$	$R = -0.2169$ $P = 0.2585$	$R = -0.3981$ $P = 0.0325$	$R = 0.5331$ $P = 0.0743$	$R = 0.4047$ $P = 0.0498$	$R = -0.0201$ $P = 0.9206$	$R = -0.1252$ $P = 0.5336$
Hand and eye coordination score	$R = -0.6843$ $P = 0.0000$	$R = -0.2975$ $P = 0.1171$	$R = -0.1869$ $P = 0.3316$	$R = -0.0441$ $P = 0.8203$	$R = -0.0383$ $P = 0.8438$	$R = -0.5506$ $P = 0.0020$	$R = 0.4696$ $P = 0.1235$	$R = 0.2979$ $P = 0.1575$	$R = -0.3328$ $P = 0.0898$	$R = -0.0958$ $P = 0.6345$
Hearing and speech score	$R = -0.5763$ $P = 0.0011$	$R = -0.1123$ $P = 0.5619$	$R = -0.1898$ $P = 0.3241$	$R = 0.1057$ $P = 0.5852$	$R = -0.0071$ $P = 0.9710$	$R = -0.3615$ $P = 0.0540$	$R = 0.2461$ $P = 0.4407$	$R = 0.2156$ $P = 0.3116$	$R = -0.0847$ $P = 0.6746$	$R = -0.0101$ $P = 0.9600$
Personal–social development score	$R = -0.6191$ $P = 0.0003$	$R = -0.1867$ $P = 0.3322$	$R = 0.1425$ $P = 0.4609$	$R = 0.2558$ $P = 0.1805$	$R = -0.0649$ $P = 0.7379$	$R = -0.4546$ $P = 0.0132$	$R = 0.5609$ $P = 0.0578$	$R = 0.3199$ $P = 0.1275$	$R = -0.0410$ $P = 0.8389$	$R = -0.0352$ $P = 0.8616$
Performance tests score	$R = -0.6107$ $P = 0.0004$	$R = -0.1826$ $P = 0.3430$	$R = -0.0349$ $P = 0.8574$	$R = 0.1209$ $P = 0.5320$	$R = -0.0300$ $P = 0.8772$	$R = -0.4346$ $P = 0.0185$	$R = 0.6447$ $P = 0.0236$	$R = 0.4059$ $P = 0.0491$	$R = -0.0990$ $P = 0.6232$	$R = -0.0601$ $P = 0.7657$
General quotient	$R = -0.6258$ $P = 0.0003$	$R = -0.1556$ $P = 0.4203$	$R = -0.1645$ $P = 0.3938$	$R = 0.1331$ $P = 0.4912$	$R = -0.0309$ $P = 0.8735$	$R = -0.4056$ $P = 0.0290$	$R = 0.2342$ $P = 0.4638$	$R = 0.2498$ $P = 0.2391$	$R = -0.0948$ $P = 0.6381$	$R = -0.0319$ $P = 0.8743$

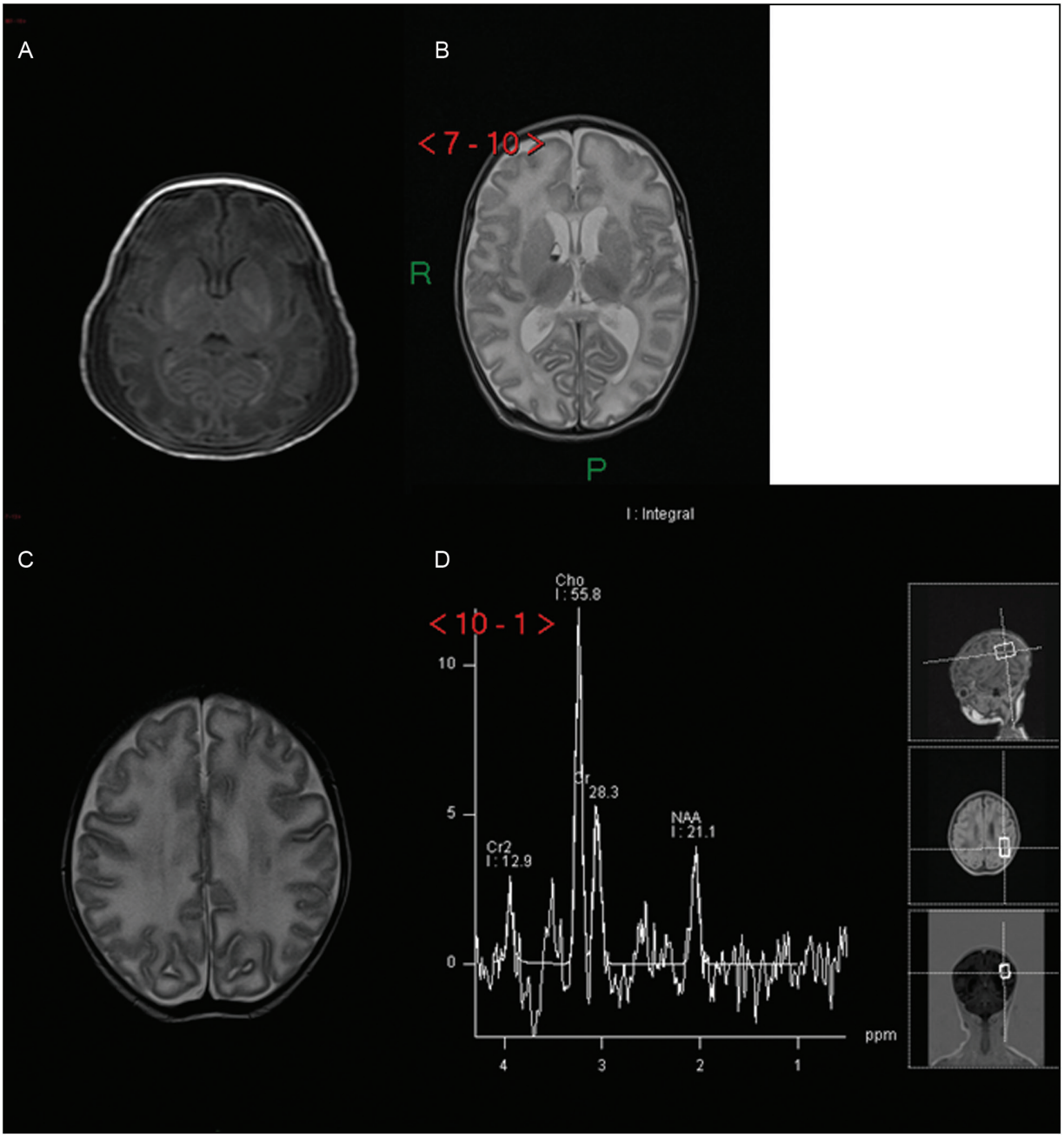

Figure 1: (A) T1-weighted axial image showing hyperintensity on basal ganglia and thalami. (B–C) T2-weighted axial images showing diffuse hyperintensity involving bilateral hemispheric white matter and basal ganglia. IVH grade II is seen on the right frontal horn of lateral ventricle. (D) Spectroscopy sequence obtained on the left basal ganglia

severe electroencephalographic abnormalities, a higher quote of encephalopathic features, and a more frequent abnormal developmental outcome than subjects with normal MRI and subjects with hippocampal injuries. Moreover, Alderliesten *et al.*[15] showed that low apparent coefficient diffusion values and high lactate/NAA ratios of basal ganglia at ^{1}HMRS were associated with a negative outcome at 24 months. No abnormalities of lactate were seen in our patients (none had advanced neuronal necrosis in the damaged areas because of the restricted temporal window between ischemic processes, cooling, and MRI), whereas area under the curve of NAA in basal ganglia represented a more useful prognostic index.

The lack of strong correlations between neurocognitive outcome and structural and diffusion abnormalities in cerebral structures other than basal ganglia partially agrees with previously reported data. Cheong *et al.*[13] reported a relatively low negative predictive value (between 54% and 63%) for lesions involving posterior limbs of internal capsula, white matter, and cortical grey matter. Charon *et al.*[6,7] reported that apparent coefficient diffusion measurements in posterior white matter, semioval centers, and posterior limbs of internal capsula added little to simple visual analysis of MRI for prognostic evaluations. Mean diffusivity and its variations are probably more reliable as a short-term index for the measurement of therapeutic response to hypothermia than for the prediction of long-term neurocognitive development.[3]

The limitations of our study include (a) its retrospective nature; (b) the limited number of patients in the analyzed sample; (c) the variable timing for performing MRI; (d) the semiquantitative tool that was used for the evaluation of severity of brain MRI lesions (Bednarek severity score allowed a systematic evaluation of all cerebral lesions but it has been previously used only in the original paper, that had a different design if compared with ours)[3]; (e) the technical limitations (better results for the detection of subtle lesions could be obtained with high-field 3T-MRI or functional MRI studies); (f) the selection bias that resulted from the exclusion of cases who did not reach 24 months of follow-up at the moment of the retrospective analysis (the minimum age that was considered to evaluate the progression of GMDS-III scores).This criterion could have facilitated the selection of less-severe cases because of the exclusion of newborns who died after hypothermia and of the patients who were lost in follow-up.

Conclusion

This study suggests that a normal MRI in early post-cooling phases is strongly associated with a favorable developmental outcome and confirms the negative prognostic value of basal ganglia lesions. Future trials should be focused on the definition of the optimal timing of MRI investigations in order to address the treatment protocols and the post-intensive care rehabilitation strategies.

Ethical policy and institutional review board statement

The study was approved by the ethics committee of Sapienza Università di Roma and Umberto I-Policlinico di Roma.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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