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Mild brain lesions do not affect brain volumes in moderate-late preterm infants

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ABSTRACT

Purpose: It is unknown whether frequently occurring mild brain lesions affect brain volumes in moderate (MP[2]; 32^{+0}-33^{+6} weeks' gestation) and late (LP[3]; 34^{+0}-35^{+6} weeks' gestation) preterm infants. Therefore, we aimed to investigate the effect of mild brain lesions on brain volumes in moderate-late preterm (MLPT[4]) infants and to compare brain volumes between MP and LP infants.
Methods: From August 2017 to November 2019, eligible MLPT infants born at Isala Women and Children's Hospital were enrolled in a prospective cohort study (Brain Imaging in Moderate-late Preterm infants 'BIMP-study'). MRI was performed around term equivalent age (TEA[5]). MRI scans were assessed for (mild) brain lesions. T2-weighted images were used for automatic segmentation of eight brain structures. Linear regression analysis was performed to compare absolute and relative brain volumes between infants with and without mild brain lesions and between MP and LP infants.
Results: 36 MP and 68 LP infants were included. In infants with mild brain lesions, intracranial volume (B = 27.4 cm^3, p = 0.02), cerebrospinal fluid (B = 8.78 cm^3, p = 0.01) and cerebellar volumes (B = 1.70 cm^3, p = 0.03) were significantly larger compared to infants without mild brain lesions. After correction for weight and postmenstrual age at MRI, these volumes were no longer significantly different. LP infants had larger brain volumes than MP infants, but differences were not significant. Relative brain volumes showed no significant differences in both analyses.
Conclusion: Neither having mild brain lesions, nor being born moderate prematurely affected brain volumes at TEA in MLPT infants.

1. Introduction

Preterm birth, defined as birth before 37 weeks' of gestation, is a growing public health concern. Worldwide annually 14.9 million infants are born prematurely [1]. Eighty-five percent of them are born between 32 and 36 weeks of gestation (i.e. moderate-late preterm; MLPT) [1]. Until recently, MLPT infants were thought to have a low risk of mortality, short- and long-term morbidities, such as neurodevelopmental problems [2,3]. However, recent studies have shown that they have a higher risk of developmental delay, cognitive impairment, behavioral and psychiatric problems as compared to term-born infants [4–9]. These problems may at least partly be related to altered brain development and/or (mild) brain

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injury, acquired during the perinatal and/or neonatal period. To identify MLPT infants with an increased risk of abnormal or suboptimal neurodevelopmental outcome and to optimize functional outcome, more knowledge about their brain development and possible brain injury is needed [10].

Several studies have demonstrated a negative effect of brain injury on brain volumes in very preterm infants (<32 weeks 'gestation) [11]. Especially moderate-severe brain lesions, such as intraventricular hemorrhage grade III, periventricular hemorrhagic infarction, post-hemorrhagic ventricular dilatation (>15 mm), arterial infarction, and/or cystic white matter lesions are associated with reduced brain volumes [12–14]. Moderate-severe brain lesions are only rarely encountered in MLPT infants, while mild brain lesions, such as intraventricular hemorrhage grade I, few punctate white matter lesions, irregularly shaped or mildly enlarged lateral ventricles, and punctate cerebellar hemorrhages are frequently seen [15]. To the best of our knowledge, the effect of these mild brain lesions on brain volumes in MLPT infants is yet unknown.

Previously, several groups reported smaller brain volumes at term equivalent age (TEA) in MLPT infants compared to term-born infants [16–19]. Smaller brain volumes in MLPT infants were associated with lower neurodevelopmental outcome scores at 2 years' corrected age [20]. This suggests that brain volumes can be used as a biomarker to identify MLPT infants with increased risk of suboptimal neurodevelopmental outcome. Hitherto, moderate (MP; 32^{+0}–33^{+6} weeks' gestation) and late (LP; 34^{+0} - 35^{+6} weeks' gestation) preterm infants have mainly been approached as one group, but their risks for developmental problems are not the same [21,22]. The brains of MP infants may be more vulnerable to injury and altered development than the brains of LP infants. However, Niwa et al. and Thompson et al. did not find a significant difference between brain volumes of MP and LP infants [18,23].

The aim of this study was to explore the effect of mild brain lesions on brain volumes in MLPT infants and to compare brain volumes at TEA between MP and LP infants. We hypothesized that around TEA brain volumes are smaller in infants with mild brain lesions than in infants without mild brain lesions and are smaller in MP infants than in LP infants.

2. Methods

This study was part of a prospective cohort study, the 'BIMP-study' (the Netherlands trial register; NL6310). From August 2017 to November 2019, MLPT infants born between 32^{+0} and 35^{+6} weeks' gestation were recruited at the neonatal units (medium, high or intensive care) of Isala Women-Children's Hospital, Zwolle, The Netherlands [15]. MP infants were defined as infants born between 32^{+0} and 33^{+6} weeks', and LP infants as infants born between 34^{+0}-35^{+6} weeks' gestation. Infants with congenital anomalies of the nervous system, inborn errors of metabolism, congenital infections and chromosomal disorders, or whose parents did not speak sufficient Dutch or English were excluded. Signed informed consent was obtained from both parents. Ethical approval was given by the Central Committee in Research Involving Human Subjects, The Hague, The Netherlands (NL52323.075.15).

2.1. Data collection

All MRI scans were acquired around TEA with a 3 T MRI scanner (Ingenia, Philips Healthcare, Best, The Netherlands). The scan protocol included 3-dimensional T1-weighted (voxel size 0.47 × 0.47 × 2mm), coronal and axial T2-weighted (voxel size 0.35 × 0.35 × 2mm), diffusion weighted (DWI; voxel size 0.80 × 0.80 × 3mm), and susceptibility weighted imaging (SWI; voxel size 0.31 × 0.31 × 1mm). Infants were scanned without sedation; natural sleep was induced by feeding and swaddling. Infants were subsequently immobilized with a MedVac vacuum-bag immobilizer (CFI Medical Solutions/Contour Fabricators, Fenton, MI, USA). Ear protection was provided by earmuffs (Natus MiniMuffs; Natus Medical Inc., San Carlos, CA, USA), headphones (EMS for kids, Hornchurch UK) and a polystyrene noise-insulating mattress covering the coil. Axial T2-images were acquired with a turbo spin-echo sequence with sensitivity encoding, repetition time 5483 ms, echo time 110 ms, flip angle 90°, matrix size 512 × 512, pixel spacing 0.35 × 0.35 mm, 54 axial slices and slice thickness 2 mm. Infant characteristics including sex, gestational age (GA), weight and head circumference at birth and MRI, and post-menstrual age (PMA) at MRI were collected.

2.2. Brain lesions

All MRI sequences were assessed for mild brain lesions as previously described by Boswinkel et al. [15]. These included: <6 punctate white matter lesions (PWMLs) on T1-weighted images [24], inhomogeneous and/or increased white matter signal intensity on T2-weighted images [25], irregularly shaped lateral ventricles and/or ventricular index 13–15 mm [26], increased width of the interhemispheric fissure (>3 mm) [27], (remnants of) intraventricular hemorrhage [28], choroid plexus hemorrhage, punctate cerebellar hemorrhages [29], multiple smaller or single larger (≥6 mm) choroid plexus or germinolytic cysts [15]. The ventricular index and interhemispheric fissure were measured on a coronal T2-weighted image at the level of the foramen of Monro.

Infants with more severe brain lesions were excluded from this part of the BIMP-study (e.g. ≥6 PWMLs, cystic white matter lesions, periventricular hemorrhagic infarction, post-hemorrhagic ventricular dilatation and/or arterial infarction). Immediately after the MRI procedure, scans were assessed by MFB (pediatric radiologist with >10 years of experience) for moderate-severe lesions with a potential need for intervention. Afterwards, three investigators (VB: research physician, JN: radiologist with >3 years of experience and GvWM: neonatologist with >25 years of experience in neonatal neuro-imaging) assessed the MRI scans by consensus. If no agreement could be achieved, the opinion of GvWM was leading. Investigators were unaware of (JN) or blinded to (VB and GvWM) the clinical course and head ultrasound findings.

2.3. MRI processing

FMRIB Software Library's (FSL) brain extraction tool (BET) (version 6.0.2, FMRIB, Oxford, UK) was used to remove skulls from the MRI scans (threshold = 0.5) [30–32]. The brain extracted volume was used to calculate intracranial volume. Segmentation was performed on the axial T2-weighted images, using the morphologically adaptive neonatal tissue segmentation toolbox (MANTiS; Murdoch Children's Research Institute, Melbourne, Australia) [33]. MANTiS was adapted to optimize segmentation in this specific MLPT cohort with mainly mild or no brain lesions. This adapted version of MANTiS is available at: https://github.com/DevelopmentalImagingMCRI/mantis/tree/BIMP-NoLargeVentricles2. Eight brain volumes were segmented: cortical gray matter (cGM), white matter (WM), cerebrospinal fluid (CSF), deep gray matter (dGM), hippocampus, amygdala, brainstem and cerebellum. Segmented structures, without CSF, were used to compute total tissue volume. A visual quality check of the segmentation was performed by MFB (radiologist), VB (research-physician) and ASV (technical medicine researcher). If no agreement could be achieved, the opinion of MFB was leading. In 25/104 scans mislabeling occurred due to severe motion artifacts. The motion affected MRI slices were re-estimated by cubic

interpolation of adjacent slices [34]. Subsequently, corrected scans were re-segmented and a second visual quality check was done by the same investigators [34]. Scans with failed cubic interpolation attempts (n = 8) or severe segmentation errors (n = 5) were excluded from further analysis. Absolute and relative brain volumes were calculated with MATLAB (version 9.6, MathWorks, Natick, Massachusetts, USA). Relative volumes were defined as proportion of intracranial volume.

2.4. Statistics

Statistical analyses were executed with IBM SPSS statistics (version 25.0, IBM SPSS Statistics for Windows, IBM Corp. Released 2017). Descriptive characteristics were calculated for infants with and without mild brain lesions, for MP and LP infants, and for excluded infants. Mean (SD) values were calculated for continuous variables and frequencies (%) for categorical variables. Differences in baseline characteristics between infants with and without brain lesions, between MP and LP infants, and between excluded and included infants were studied with the Fisher's exact (dichotomous variables) and independent-samples *t*-test (continuous variables). Linear regression analyses were used to compare absolute and relative brain volumes of infants with and without mild brain lesions, and MP and LP infants. Confounders 'weight at MRI' and 'PMA' were included in the analyses. Results were expressed in difference in volume with 95% confidence interval. Significance levels were set at $p < 0.05$.

3. Results

Participants A cohort of 167 MLPT infants was enrolled in the 'BIMP-study', of whom 127 infants (mean GA = 34^{+2} (±1) weeks, 69 boys) underwent MRI. Of these 127 infants, twenty-three (18.1%) were excluded from this part of the BIMP-study (Fig. 1). In total, MRI scans of 104 infants (mean PMA 41.1 (±1.6) weeks) without or with only mild brain lesions were included for analysis. Neonatal descriptive characteristics were not significantly different for excluded infants compared to included infants. Due to the high PMA (>47 weeks) at TEA MRI of two excluded infants, PMA ($p < 0.001$), weight ($p = 0.01$) and head circumference ($p = 0.006$) at TEA were significantly higher for the excluded infants (Table 1). In the included group of infants, head circumference at MRI (unadjusted for PMA) was significantly larger in infants with mild brain lesions than in infants without mild brain lesions ($p = 0.02$). Mean PMA ($p = 0.003$), weight ($p = 0.03$) and head circumference ($p = 0.049$) at MRI were significantly higher for LP infants than MP infants (Table 1).

3.1. Brain lesions

Of the twenty-tree excluded infants, four (17.4%) infants had no brain lesions (one infant with missing T2-weighted images, one infant with an MRI performed at PMA >47 weeks and two infants with irreparable motion artifacts) and thirteen (56.5%) had mild brain lesions (one infant with missing T2-weighted images,one infant with an MRI performed at PMA >47 weeks, five infants with severe segmentation errors and six infants with irreparable motion artifacts) (Table 2). As moderate-severe brain lesions were an exclusion criteria for this part of the BIMP-study, all six (5%) infants who had moderate-severe brain lesions on MRI were also excluded: one infant with a periventricular hemorrhagic infarction, one with a posterior cerebral artery infarction and moderate-severe ex-vacuo ventricular dilatation, one with isolated moderate-severe ex-vacuo ventricular dilatation, and three infants with ≥ 6 PWMLs.

Of the included infants, sixty-eight (65%) infants had mild brain lesions (Table 2). Mild brain lesions were more frequently seen in

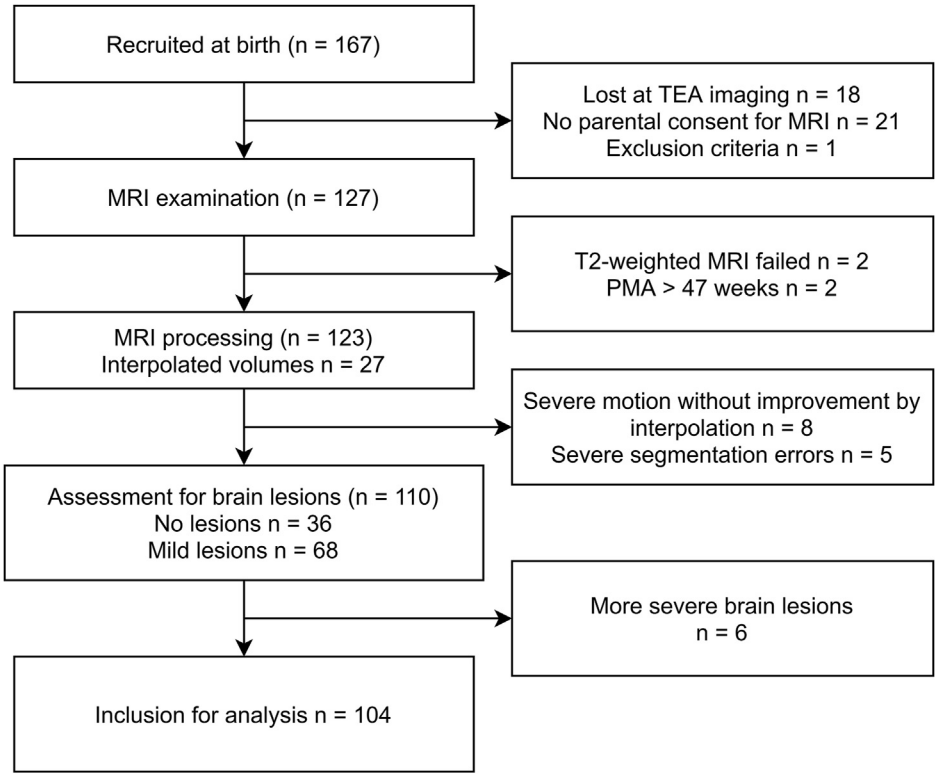

Fig. 1. In- and exclusion flowchart. Processing steps with number of participants and number of excluded infants are shown. PMA: Postmenstrual age.

Table 1
Participant characteristics. Values in bold are significant.

	Characteristics of infants without and with mild brain lesions			Characteristics of MP and LP infants			Excluded infants	
Characteristics	No lesions, n = 36	Mild lesions, n = 68	p-value	MP[b] n = 36	LP[c] n = 68	p-value	n = 23	p-value[d]
Neonatal period								
Gestational age in weeks, mean (SD)	34.3 (1.1)	34.3 (1.2)	0.87	33.0 (0.6)	35.0 (0.5)	< **0.001**	34.3 (1.1)	0.87
Birth weight in grams, mean (SD)	2229 (486)	2332 (446)	0.24	2012 (397)	2442 (424)	< **0.001**	2188 (388)	0.32
Head circumference in cm, mean (SD)	31.4 (1.7)	31.7 (1.8)	0.33	30.4 (1.9)	32.2 (1.4)	< **0.001**	31.7 (1.3)	0.79
Boys, n (%)	17 (47.2)	37 (54.4)	0.54*	19 (52.8)	35 (51.5)	>0.99*	16 (69.6)	0.17*
Girls, n (%)	19 (52.8)	31 (45.6)		17 (47.2)	33 (48.5)		7 (30.4)	
Singleton, n (%)	27 (75.0)	47 (69.1)	0.84**	26 (72.2)	48 (70.6)	0.71**	16 (69.6)	0.89**
Twin, n (%)	8 (22.2)	19 (27.9)		10 (27.8)	17 (25.0)		7 (30.4)	
Triplet, n (%)	1 (2.8)	2 (2.9)		0 (0)	3 (4.4)		0	
Admission to neonatal intensive care, n (%)	7 (19.4)	21 (30.9)	0.83	15 (41.7)	13 (19.1)	**0.02**	2 (8.7)	0.25
Characteristics at TEA MRI								
PMA[a] in weeks, mean (SD)	41.0 (1.5)	41.2 (1.3)	0.53	40.6 (1.3)	41.5 (1.6)	**0.003**	42.9 (3.1)	< **0.001**
Weight in grams, mean (SD)	3364 (592)	3588 (553)	0.06	3361 (411)	3589 (633)	**0.03**	3837 (784)	**0.023**
Head circumference in cm, mean (SD)	35.3 (1.5)	36.0 (1.4)	**0.02**	35.4 (1.3)	36.0 (1.5)	**0.049**	36.7 (1.8)	**0.006**

LP: late preterm; *p-value was calculated for sex; **p-value was calculated for plurality.
[a] PMA: postmenstrual age;
[b] MP: moderate preterm.
[c] LP: late preterm.
[d] Excluded compared to included infants.

Table 2
Overview of brain lesions in included and excluded MLPT infants.

	Included infants n = 104	Excluded infants n = 23
No brain lesions, n (%)	36 (34.6)	4 (17.4)
Mild brain lesions present, n (%)	68 (65.4)	13 (56.5)
Moderate-severe brain lesions present, n (%)	0 (0)	6 (26.1)
Type of brain lesion and frequency		
Hemorrhages		
Remnants of IVH (grade 1–2), n (%)	5 (4.8)	3 (13.0)
Periventricular hemorrhagic infarction, n (%)	0 (0)	1 (4.3)
Choroid plexus hemorrhage, n (%)	4 (3.8)	1 (4.3)
Punctate CBH, n (%)	12 (11.5)	4 (17.4)
White matter		
Cystic white matter lesions, n (%)	0 (0)	0 (0)
Inhomogeneous and/or increased diffuse white matter signal changes °, n (%)	*(n = 99)*	*(n = 16)*
	19 (19.2)	8 (50.0)
PWML	(n = 101)	(n = 21)
< 6, n (%)	15 (14.9)	2 (9.5)
≥ 6, n (%)	0 (0)	3 (14.3)
Infarction	0 (0)	1 (4.3)
Arterial infarction, n (%)		
Deep gray matter lesions	0 (0)	1 (4.3)
Small focal lesion, n (%)	0 (0)	0 (0)
Moderate-severe lesion, n (%)		
Miscellaneous		
Choroid plexus cyst cyst ≥ 6 mm, n (%)		
Germinolytic or subependymal cyst ≥ 6 mm, n (%)	7 (6.7)	2 (8.7)
Signs suggestive of brain atrophy due to injury		
Ex-vacuo ventricular dilatation	*(n = 97)*	*(n = 21)*
Mild(13–15 mm), n (%)	26 (26.8)	8 (38.1)
Moderate-severe (>15 mm), n (%)	0 (0)	2 (9.5)
Irregular shape of the lateral ventricles¤, n (%)	(n = 103)	(n = 23)
	13 (12.6)	7 (30.4)
Widened interhemispheric fissure*, n (%)	(n = 97)	(n = 21)
	20 (20.6)	9 (42.9)

MRI assessment of diffuse white matter signal changes was missing in 12 infants due to poor imaging quality.
Assessment of PWML was missing in 5 infants on MRI due to poor imaging quality. ¤ MRI assessment of deep gray matter and irregular shape of the lateral ventricles was missing in 1 infant due to poor imaging quality.
MRI measurement was missing in 9 infants due to poor imaging quality.
IVH: intraventricular hemorrhage.
CBH: cerebellar hemorrhage.
PWML: punctate white matter lesions.

MP than in LP infants (respectively 61% and 51%), but this difference was not significant (p = 0.41). There was no significant difference between boys and girls for presence of mild brain lesions (p = 0.54). The other general characteristics of infants with and without mild brain lesions were also not significantly different (Table 1).

3.2. MRI processing

The adapted version of MANTiS visually restored over-segmentation of CSF in the cerebellum of all infants (n = 104; example in Fig. 2).

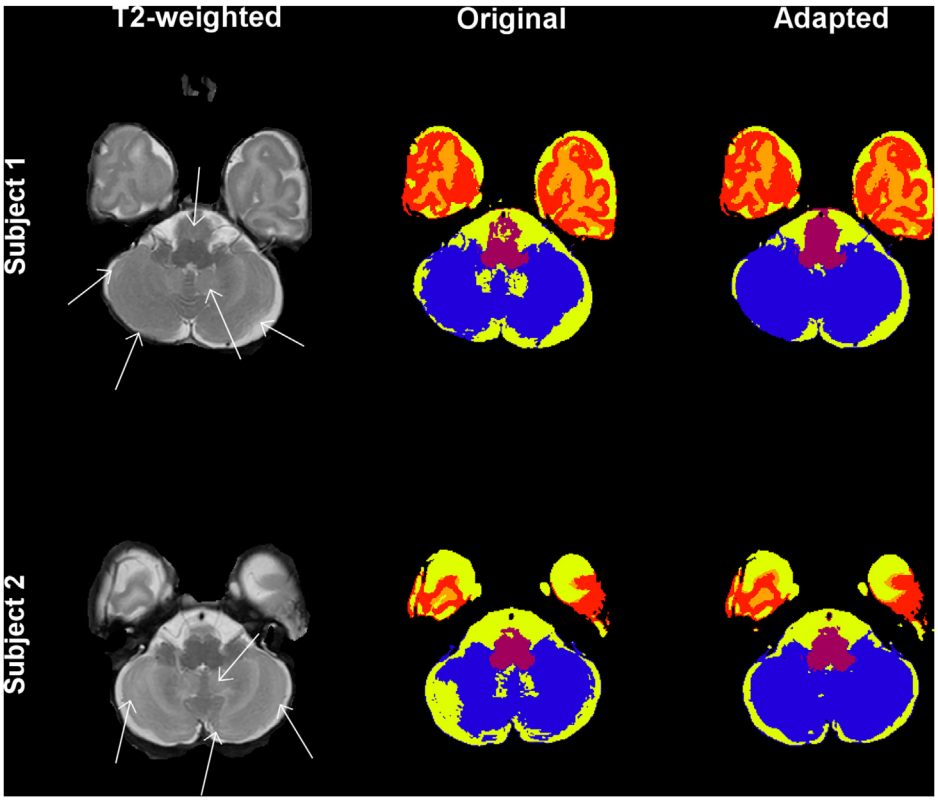

Fig. 2. Results after adapting MANTiS segmentation toolbox for data collected in the 'BIMP-study'. The left column shows T2-weighted MRI slices of two infants (subject 1: GA 35^{+6} weeks, MRI performed at PMA 40^{+6} weeks and subject 2: GA 35^{+4} weeks, MRI performed at PMA 38^{+4} weeks), the middle column shows segmentation results with the original MANTiS toolbox and the right column shows segmentation with the adapted MANTiS toolbox. Over-segmentation of CSF in the cerebellum is observed with the original MANTiS toolbox. Large errors are removed by adjusting the pipeline. Changes are indicated by arrows in the T2-weighted scans. In the online version, volumes are color-coded as follows: red: cortical gray matter, orange: white matter, yellow: cerebrospinal fluid, blue: cerebellum, purple: brainstem. GA: gestational age, PMA: postmenstrual age. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Comparison of brain volumes between infants with and without mild brain lesions

Without correction for weight and PMA at MRI, intracranial volume (difference in volume = 27.44 cm^3, p = 0.02), CSF (difference in volume = 8.78 cm^3, p = 0.01), and cerebellar volume (difference in volume = 1.70 cm^3, p = 0.03) were significantly larger in infants with mild brain lesions. Relative volumes were not different between infants with and without mild brain lesions. After correction for weight and PMA at MRI, none of the brain volumes were significantly different between infants with and without mild brain lesions (Table 3).

3.4. Comparison of brain volumes between MP and LP infants

Almost all absolute brain volumes were larger in LP infants than in MP infants. Only hippocampal and amygdala volumes were slightly smaller in LP infants. With regard to relative volumes, in LP infants only larger relative volumes of cGM and cerebellum were found. These differences between MP and LP infants were not significant, neither with nor without correction for weight at MRI and PMA (Table 4).

4. Discussion

Using an adapted MANTiS toolbox, we calculated brain volumes around TEA in MLPT infants and analyzed differences in brain volumes between MLPT infants with and without mild brain lesions, and between MP and LP infants. No differences were found between MLPT infants with and without mild brain lesions, or between MP and LP infants. These findings indicate that neither having mild brain lesions, nor being born MP had a measurable effect on brain volumes in MLPT infants.

In very preterm infants, moderate-severe brain injury is associated with a decrease in cGM, WM and cerebellar volumes, and an increase in CSF volumes [11,35]. Moderate-severe brain lesions are common in very preterm infants but are less frequently found in MLPT infants [15]. Although mild brain lesions were frequently seen in MLPT infants, we did not find significant differences in brain volumes between MLPT infants with and without mild brain lesions. Kelly et al. reported that regional cortical gray matter and white matter volumes and, in addition, white matter microstructural alterations were associated with poorer cognitive and language scores in MLPT infants at two years of age [36]. Future studies should also investigate the effect of mild brain lesions on microstructural alterations in the MLPT preterm brain.

Our study supports the findings by Niwa et al. and Thompson et al. who did not find a significant difference between brain volumes of MP and LP infants [18,23]. Nevertheless, we saw some differences in absolute brain volumes between our and their studies. Niwa et al. reported smaller average brain volumes, which is probably related to lower PMA at MRI (mean PMA = 38.6 versus 41.1 weeks in our study) [18]. Furthermore, CSF volumes were respectively 23% and 26% lower in MP and LP infants in our study than as reported by Thompson et al. [23]. These differences can probably be explained by MANTiS optimization, interpolation of motion artifacts and/or brain extraction threshold.

Table 3
Mean absolute and relative volumes in infants with and without mild brain lesions and linear regression analysis results before and after correction of confounding factors (i.e. weight and postmenstrual age at MRI). Values in bold are significant.

	Mean volumes (cm^3)		Univariate analysis		Multivariate analysis	
Brain region	No lesions (±SD)	Mild lesions (±SD)	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Intracranial volume	462 (63.5)	489 (50.9)	27.4 (4.74–50.1)	**0.02**	13.8 (−0.847–28.4)	0.07
Total brain tissue	398 (53.4)	417 (43.7)	18.7 (−0.658–38.0)	0.06	8.17 (−5.49–21.8)	0.24
cGM[a]	187 (34.0)	198 (31.6)	11.3 (−2.00–24.5)	0.10	4.45 (−4.74–13.6)	0.34
WM[b]	145 (16.1)	150 (12.1)	4.13 (−1.44–9.70)	0.14	2.05 (−3.17–7.26)	0.44
CSF[c]	64.3 (16.6)	73.0 (16.8)	8.78 (1.94–15.6)	**0.01**	5.62 (−0.290–11.5)	0.06
dGM[d]	26.3 (2.98)	27.3 (2.26)	1.03 (−0.007–2.06)	0.05	0.545 (−0.310–1.40)	0.21
Hippocampus	3.48 (0.80)	3.57 (0.82)	0.090 (−0.244–0.424)	0.59	0.027 (−0.309–0.364)	0.87
Amygdala	1.47 (0.25)	1.59 (0.35)	0.119 (−0.012–0.250)	0.08	0.089 (−0.041–0.281)	0.18
Cerebellum	27.5 (4.85)	29.2 (3.00)	1.70 (0.172–3.23)	**0.03**	0.856 (−0.119–1.83)	0.09
Brainstem	7.12 (0.77)	7.41 (0.75)	0.286 (−0.023–0.596)	0.07	0.124 (−0.143–0.39)	0.36
Ratio cGM[a]	0.403 (0.030)	0.403 (0.027)	−4.6E-5 (−0.012–0.011)	0.99	−0.003 (−0.013–0.008)	0.63
Ratio WM[b]	0.316 (0.026)	0.307 (0.027)	−0.009 (−0.020–0.001)	0.09	−0.005 (−0.014–0.004)	0.24
Ratio CSF[c]	0.138 (0.027)	0.149 (0.028)	0.011 (−0.001–0.022)	0.07	0.008 (−0.003–0.019)	0.15
Ratio dGM[d]	0.057 (0.004)	0.056 (0.004)	−0.001 (−0.003–0.0004)	0.16	−0.001 (−0.002–0.001)	0.44
Ratio Hippocampus	0.008 (0.002)	0.007 (0.002)	−0.0002 (−0.001–0.0005)	0.50	−0.0002 (−0.001–0.001)	0.67
Ratio Amygdala	0.003 (0.001)	0.003 (0.001)	0.00004 (−0.0002–0.0003)	0.78	0.0001 (−0.0002–0.0003)	0.62
Ratio Cerebellum	0.059 (0.004)	0.060 (0.004)	0.0005 (−0.001–0.002)	0.57	0.0004 (−0.001–0.002)	0.62
Ratio Brainstem	0.016 (0.002)	0.015 (0.001)	−0.0004 (−0.001–0.0002)	0.22	−0.0003 (−0.001–0.0002)	0.28

[a] cGM: cortical gray matter.
[b] WM: white matter.
[c] CSF: cerebrospinal fluid.
[d] dGM: deep gray matter.

Table 4
Mean absolute and relative volumes in MP and LP infants and linear regression analysis results before and after correction of confounding factors (i.e. postmenstrual age and weight at MRI).

	Mean volumes (cm^3)		Univariate analysis		Multivariate analysis	
Brain region	MP[a] (±SD)	LP[b] (±SD)	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Intracranial volume	469 (47.6)	486 (60.6)	−17.1 (−40.2–5.95)	0.14	9.10 (−6.10–24.3)	0.24
Total brain tissue	400 (42.1)	416 (50.1)	−15.6 (−35.0–3.80)	0.11	5.18 (−8.92–19.3)	0.47
cGM[c]	187 (26.8)	198 (35.1)	−10.7 (−24.0–2.56)	0.11	4.14 (−5.30–13.6)	0.39
WM[d]	146 (15.2)	149 (12.8)	−3.19 (−8.78–2.40)	0.26	−0.333 (−5.71–5.04)	0.90
CSF[e]	69.0 (14.0)	70.5 (18.7)	−1.52 (−8.57–5.53)	0.67	3.92 (−2.20–10.1)	0.21
dGM[f]	26.8 (2.22)	27.0 (2.74)	−0.260 (−1.31–0.791)	0.63	0.676 (−0.199–1.55)	0.12
Hippocampus	3.56 (0.71)	3.53 (0.87)	0.027 (−0.307–0.361)	0.87	0.105 (−0.241–0.450)	0.55
Amygdala	1.57 (0.30)	1.53 (0.34)	0.038 (−0.095–0.171)	0.57	0.100 (−0.033–0.233)	0.14
Cerebellum	27.7 (3.05)	29.13 (4.09)	−1.48 (−3.02–0.057)	0.06	0.326 (−0.688–1.34)	0.53
Brainstem	7.25 (0.68)	7.34 (0.81)	−0.086 (−0.400–0.228)	0.59	0.096 (−0.178–0.371)	0.49
Ratio cGM[c]	0.398 (0.026)	0.405 (0.029)	−0.007 (−0.019–0.004)	0.21	0.001 (−0.009–0.012)	0.83
Ratio WM[d]	0.312 (0.025)	0.309 (0.028)	0.003 (−0.008–0.014)	0.61	−0.008 (−0.017–0.001)	0.08
Ratio CSF[e]	0.147 (0.026)	0.144 (0.029)	0.003 (−0.008–0.014)	0.61	0.007 (−0.005–0.018)	0.25
Ratio dGM[f]	0.058 (0.003)	0.056 (0.004)	0.001 (−0.0002–0.003)	0.09	0.0003 (−0.001–0.002)	0.72
Ratio Hippocampus	0.008 (0.002)	0.007 (0.002)	0.0004 (−0.0003–0.001)	0.30	0.0001 (−0.001–0.001)	0.77
Ratio Amygdala	0.003 (0.001)	0.003 (0.001)	0.0002 (−0.0001–0.0004)	0.15	0.0001 (−0.0001–0.004)	0.31
Ratio Cerebellum	0.059 (0.004)	0.060 (0.004)	−0.001 (−0.003–0.001)	0.25	0.0004 (−0.0002–0.001)	0.60
Ratio Brainstem	0.016 (0.001)	0.015 (0.001)	0.0003 (−0.0002–0.001)	0.24	−0.0001 (−0.001–0.0004)	0.63

[a] MP: moderate preterm.
[b] LP: late preterm.
[c] cGM: cortical gray matter.
[d] WM: white matter.
[e] CSF: cerebrospinal fluid.
[f] dGM: deep gray matter.

Contrary to our findings and the findings by Niwa et al. and Thompson et al., other studies found a significant linear association between GA at birth and brain volumes at TEA [37–39]. An explanation for this might be that these studies used a wider GA spectrum (i.e. 24-42 weeks) and differences in brain volumes may have become more apparent between the two ends of the spectrum. However, as these studies used different statistical methods (i.e. student's independent *t*-test, linear regression and linear mixed-effects model) a reliable comparison between the results of these studies and our study is not possible.

Strengths of our study are the prospective design, careful evaluation of mild brain lesions in our cohort and the application of methodological improvements to determine brain volumes. Segmentation was enhanced by using an interpolation technique to handle motion artifacts [34]. Additionally, adjustments to MANTiS optimized segmentation of the data. Nevertheless, limitations should be mentioned. Ideally, data should have been compared to the data of a control group consisting of healthy full-term born infants, but inclusion of healthy full-term infants was not possible. Also, we were not yet able to investigate the association between brain volumes and neurodevelopmental outcome as follow-up is still ongoing. Secondly, infants born at GA 36^{+0} to 36^{+6} weeks were not routinely admitted and therefore not enrolled. Thirdly, motion artifacts were frequently encountered on MRI. Although infants were immobilized, fast imaging techniques were used and post-processing techniques were implemented, motion artifacts may

still have influenced estimated brain volumes in some cases. Fourthly, inter-rater reliability testing of the assessment of brain lesions was not performed as MRI scans were assessed by consensus. Fifthly, the relative small sample size and low incidence of specific brain lesions hampered sub-analysis at lesion level. Finally, volumes of small structures such as hippocampus and amygdala should be interpreted with care, since Beare et al. showed a Dice agreement between manual and MANTiS segmentation of respectively 0.66 and 0.51 for these structures [33]. For such tiny brain structures segmentation accuracy may not be sufficient to draw valid conclusions.

Development of the neonatal brain is influenced by a wide variety of peri- and/or postnatal factors, including brain lesions [11,40]. More insight into brain development and neurodevelopmental outcome in MLPT infants may be provided by 1) investigating the effect of perinatal factors on brain volumes as well as the effect of (mild) brain lesions at lesion level in a larger cohort of MLPT infants and 2) investigating the association between these factors and neurodevelopmental outcome. We aim to perform long-term neurodevelopmental follow-up in this MLPT cohort to investigate whether brain volumes and/or mild brain lesions are associated with neurodevelopmental outcome.

To conclude, neither having mild brain lesions, nor being born moderate prematurely have a measurable effect on brain volumes in moderate-late preterm infants. Further research is required to fully understand the effect of brain lesions and other perinatal factors on brain development in moderate-late preterm infants, and to investigate the association with neurodevelopmental outcome.

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Ethics approval

Ethical approval was given by the Central Committee in Research Involving Human Subjects, The Hague, The Netherlands (NL52323.075.15).

Consent to participate

Signed informed consent was obtained from all parents.

Conflicts of interest

There are no potential conflicts of interest.

Declarations of interest

None.

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