# Ultrasonographic evaluation of the early brain growth pattern in very low birth weight infants

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**Impact Statement:** In our study we have evaluated brain volume as an absolute value and brain growth rate during early life in preterm infants, which has not been previously studied. We describe the brain growth pattern in very low birth weight infants at-risk during their first postnatal weeks. We have found that in the presence of certain perinatal factors and comorbidities, brain growth may be affected, conditioning a deviation of the normal growth pattern. The serial ultrasound follow-up of these at-risk patients has allowed us to identify these brain growth patterns early, which offers a window of opportunity for implementing earlier interventions.

# Summary

**Background**: Preterm infants develop smaller brain volumes compared to term newborns. Our aim is to study early brain growth related to perinatal factors in very low birth weight infants (VLBWI).

**Methods:** Manual segmentation of total brain volume (TBV) was performed in weekly 3D-ultrasonographies in our cohort of VLBWI. We studied the brain growth pattern related to term magnetic resonance image (term-MRI).

**Results**: We found different brain growth trajectories, with smaller brain volumes and a decrease in brain growth rate in those VLBWI who would later have an abnormal term-MRI (mean TBV 190.68 vs. 213.9 cm3; P=0.0001 and mean TBV growth rate 14.35 (±1.27) vs. 16.94 (±2.29) cm3/week; P=0.0001).

TBV in those with normal term-MRI was related to gestational age (GA), being small for gestational age (SGA), sex, and duration of parenteral nutrition (TPN) while in those with abnormal term-MRI findings it was related to GA, SGA, TPN, and comorbidities. We found a deceleration in brain growth rate in those with ≥3 comorbidities.

**Conclusions**: An altered brain growth pattern in VLBWI who subsequently present worst scores on term-MRI is related to GA, being SGA and comorbidities. Early ultrasonographic monitoring of TBV could be useful to detect deviated patterns of brain growth.

# Introduction

Advances in perinatal care in recent years have led to a considerable increase in the survival rates of very low birth weight infants (VLBWI) [1-3]. However, the prevalence of long-term neurological disabilities in these preterm infants has remained stable over time, in part, due to increased survival of those born at extreme gestational ages (GA) [1]. VLBWI continue to be a vulnerable population with a high prevalence of moderate to severe disability, resulting from neurodevelopmental disorders including intellectual deficits, executive function disorders, autism spectrum disorders, cerebral palsy and epilepsy [2-6]. These sequelae are often associated with brain injury and/or impaired maturation and growth of different brain structures [7].

Brain ultrasound (US) and magnetic resonance imaging (MRI) allow for the early diagnosis of brain injury, including germinal matrix-intraventricular hemorrhage (GM-IVH), parenchymal hemorrhagic infarction (PHI), and white matter injury (WMI). Regarding the latter, diffuse WMI is the most frequently observed abnormality, found in up to 50 to 80% of extremely preterm infants [8] and it is associated with altered brain maturation and growth, [9] with a variable impact on neurodevelopment including cognitive, psychomotor, language, executive functioning and sensorineural impairment [10]. This pattern of brain injury can be identified in neuroimaging studies as a significant decrease of the white matter volume, ventriculomegaly, immature gyral development and increased subarachnoid space [11,12].

Neuroimaging can also identify alterations in brain growth and maturation patterns. Brain growth after preterm birth deviates from that of term infants, with those being preterm having smaller total and regional brain volumes as well as alterations in brain connectivity at the microstructural level [13,14]. It has been suggested that there is a specific pattern of brain

dysmaturation after preterm birth given the interruption of brain development at a critical period for axonal and synaptic development[15].

MRI allows detailed evaluation of brain morphometry, development and brain growth has been extensively studied with MRI over the past years. Despite MRI being considered the gold standard technique, brain US remains the first tool for diagnosis and follow-up during the neonatal period due to its advantages in terms of accessibility and cost. Total brain volume (TBV) can be accurately estimated by brain US which can enable a longitudinal study of early brain growth during Neonatal Intensive Care (NICU) admission [16]. Our aim in this study is to describe early brain growth trajectory imaged by brain US in VLBWI and evaluate its association with term equivalent-age MRI (term-MRI) scan findings. We will explore how different perinatal factors impact this trajectory of brain growth.

# Methods

## Study population

This study is part of a longitudinal cohort that includes VLBWI born at Hospital Puerta del Mar, Cádiz, Spain as of May 2018 with recruitment still ongoing. The aim is to investigate brain growth trajectory in the neonatal period in VLBWI. This study was reviewed and approved by Research and Ethics Committee and informed consent was obtained from all participants included in the study. We consecutively enrolled VLBWI who met inclusion criteria (weight at birth equal or less than 1500 grams, GA at birth equal or less than 32 weeks) and whose parents or legal guardians had signed informed consent. Exclusion criteria consisted of congenital and chromosomal anomalies, metabolic disorders, and central nervous system infections. We included those who were born from May 2018 to January 2021 and further excluded those with post hemorrhagic ventricular dilatation and those that died during the neonatal period.

Perinatal data and details of the infants' clinical course were prospectively collected. We used maternal level of education as a measure of socioeconomic status (SES) and grouped it in three categories: low (including primary and secondary education), medium (high school studies and professional training modules) and high (university and postgraduate studies) [17,18].

We recorded the following clinical variables:

- Sepsis: Clinical condition consisting of systemic signs of infection and isolation of a bacterial pathogen in blood culture, being considered early if occurring in the first 72 hours of life and late onset sepsis if occurred after the first 72 hours of life.

- Necrotizing enterocolitis (NEC): Confirmed necrotizing enterocolitis (Bell Stage II or higher) [19].
- Moderate to severe bronchopulmonary dysplasia (BPD): the need for supplemental oxygen and/or positive pressure at 36 weeks postmenstrual age or at discharge among VLBWI born < 32 weeks and at 56 days postnatal age or at discharge in VLBWI born >32 weeks [20]. We also considered total days of respiratory support: duration of invasive and non-invasive respiratory support in days.
- Significant patent ductus arteriosus (PDA): PDA requiring surgical or pharmacological closure.
- Severe retinopathy of prematurity (ROP): Retinal vasculopathy stage 3 or higher [21].
- Severe brain injury: grade 3 GM-IVH, PHI and/or moderate to severe WMI [22,23].
- Small for gestational age (SGA): Those with birth weight bellow the 10$^{th}$ percentile for gestational age.
- Time of parenteral nutrition (TPN): Total days of TPN received (in our center, TPN is started at admission and also trophic enteral nutrition (20ml/kg/day) is offered as soon as the patient is considered stable, maintained for 3-5 days, and then volumes are progressively increased until full enteral nutrition is achieved.

## 2D and 3D US

All patients were followed prospectively and underwent weekly brain US until either discharge or term-equivalent age.

Weekly brain 2D US and 3D US were performed with the infant lying supine with his or her head turned right. Volume acquisition was carried out through the 3D option in the 3D/4D Voluson S8 BT18 (General Electric Healthcare, Buckinghamshire, United Kingdom) as

previously described by our group [16,24,25]. Through this option, with the transducer positioned in the third coronal plane, the beam moves from anterior to posterior planes using a center frequency of 6.5 MHz with a scan angle set at 90°. Scans were saved and analysis was performed off-line by using 4D View software (version 17.0; GE Healthcare).

TBV was measured by manual tracing the brain contour in 6 slices of 30 degrees rotation on the vertical axis using VOCAL (Virtual Organ Computer-Aided Analysis). This technique provides a reliable measure of TBV as previously published by our group [16].

## Brain MRI

All patients had a term-MRI scan. MRI scans were performed using 1.5 T scanner Magneton Symphony (Siemmens Health Care, Erlangen, Germany) located in the radiology unit. T1-weighted images were obtained using a three-dimensional (3D) spoiled gradient [repetition time 1,660 (TR)/echo time 5.16(TE)] and transverse T2-weighted turbo spin-echo imaging (4,180.00/98.00).

Term-MRI scans were evaluated using the scale published by Kidokoro et al. [12], which assesses development and injury of cortical and deep gray matter, white matter, and cerebellum. The overall score obtained through this scale classifies MRI findings as: normal (0-3 points) or abnormal: mild (4-7 points), moderate (8-11 points) and severe (≥12 points). For our study, these categories were grouped into two groups: those with a score of less than 8 points were classified as normal/mild abnormalities at term-MRI, and those with a score equal or greater than 8 points were classified as moderate/severe abnormalities at term-MRI.

## Statistical analysis

Clinical characteristics and demographic variables were compared using Pearson's chi-squared test or Fisher's exact test for categorical data and the Student's t test or Mann–Whitney U test for continuous variables after testing for normality. Multilevel linear regression models were used to study the relationship between TBV and clinical variables accounting for repeated measurements and time. The included variables were selected based on the theoretical background and a backward stepwise approach was performed to exclude the variables not significant if they were not considered variables needed to adjust for. TBV growth rate was calculated as the difference between volumes on serial US divided by time between US (in weeks).

Statistical analysis was conducted using Stata 16.0 (Stata Statistical Software: Release 16. College Station, TX: StataCorp LP). A result was considered statistically significant at $p < 0.05$.

# Results

## Study population

During the recruitment period we included 163 VLBWI admitted to the NICU at Hospital Puerta del Mar. Of these, 19 (11.7%) died during the neonatal period and 6 (3.7%) were excluded due to: post-hemorrhagic ventricular dilatation (PHVD), Down syndrome, and congenital cytomegalovirus infection (CMV). Our final study population included 138 patients (Fig. 1).

## Descriptive analysis of perinatal factors, comorbidities, and maternal level of education according to the severity of findings on term-MRI

Of the 138 patients included, 120 (86.9%) had a normal or mildly abnormal term-MRI with a Kidokoro score of less than 8 points, while 18 patients (13.1%) had a score equal to or higher than 8 points and were considered to have moderate to severe abnormal term-MRI findings. The distribution of our patients according to the findings in the term-MRI are described in Tables s1a and s1b in supplementary material. The clinical characteristics of both groups of the population studied are described below.

Those with moderate-severe MRI findings were of lower GA at birth (27.3 (± 2.8) vs. 29.5 (± 2.1) weeks; P=0.001), lower birth weight (967 (± 359) vs. 1176 (± 340) grams; P=0.013) and had a smaller head circumference at birth (25.3 (± 3.8) vs. 27.6 (± 3.1) cm; P=0.028). In this group we observed a higher proportion of clinical chorioamnionitis (6 (33.33%) vs. 16 (13.33%) P=0.042) and higher need for resuscitation (13 (72.22%) vs. 46 (38.33%); P=0.010)

as well as higher clinical severity during the first 12 hours of life, with a higher CRIB index (6.5 [1 - 7] vs. 1 [0 - 3]; P=0.014) (Table s2 in supplementary material).

Regarding comorbidities suffered during admission, those with more severe MRI abnormalities at term equivalent age had a higher proportion of moderate-severe BPD (11/18 (61.11%) vs. 19/117 (16.24%); P=0.0001), significant PDA (9 (50%) vs. 9 (7.5%); P=0.0001), severe ROP (5/18 (27.78%) vs. 4/119 (3.36%); P=0.002) and severe brain injury including GM-IVH grade III (6 (33.33%) vs. 2 (1.67%); P=0.0001), PHI (4 (22.22%) vs. 1 (0.83%); P=0.001), and moderate/severe WMI (2 (11.11%) vs. 0 (0%); P=0.016), as well as longer duration of respiratory support (median [IQR] 77 [27 - 111] vs. 15 [4 - 47] days; P=0.0001) and TPN (median [IQR] 29 [15 - 58] vs. 13 [8 - 20]; P=0.001). We found no differences between the two groups regarding maternal level of education (Table s3 in supplementary material).

## Evaluation of volumes and trajectories of brain growth related to perinatal factors and comorbidities.

To study the volumes and trajectories of brain growth in both groups, 889 brain US of these 138 patients were analyzed. Each patient had a median of 8 brain US (IQR 6 - 12). The median time interval between US for the same patient was 7 [IQR 4 – 9] days.

We chose the most parsimonious model, which showed a significant association between TBV and GA, with an increase of 16,69 cm3 per week of gestation (β coef =16.69; P= 0.0001), and a negative association with female sex (β coef = -8.92; P= 0.077), being SGA (β coef = -17.8; P= 0.011), TPN duration (β coef =-0.36; P= 0.020) and the presence of confirmed NEC (β coef =-111.32; P= 0.0001) (Table 1, Table s4 in supplementary material, and Figure 2).

## Evaluation of volumes and trajectories of brain growth related to term equivalent MRI.

In those patients with normal/mild term-MRI, TBV increases 17.34 cm3/week: TBV (cm3) = 219.14 + 17.34 x (PMA-33). We centered PMA at 33 weeks to enable the interpretation of the constant parameter. Thus, the mean TBV at 33 weeks PMA is 219.14 cm3.

In those with moderate/severe findings on term-MRI TBV increases 14.98 cm·/week, following the equation: TBV = 211.22 + 14.98 x (PMA-33), with a mean TBV at 33 weeks PMA of 211.22 cm3.

Visual representation of both trajectories can be seen in Fig. 3, showing how differences in TBV become greater with increasing PMA. The mean difference in predicted TBV among both groups is 23.21 cm3, with a mean TBV of 213.89 cm3 in those with normal/mild term-MRI scan vs. 190.68 cm3 in those with moderate/severe term-MRI involvement; P=0.0001 (for more details see Table s5 in supplementary material).

We found a significant increase in brain growth rate from 12 cm3/week at 25 weeks PMA to 24 cm3/week at term PMA in those with normal/mild term-MRI. In the group with moderate/severe abnormalities in term-MRI we found that after increasing during the first postnatal weeks, brain growth rate decreases after 34 weeks PMA (Fig. 4a).

The overall TBV growth rate is different between those that subsequently have normal/mild abnormalities in term-MRI and those that subsequently have moderate to severe brain abnormalities (mean TBV growth rate 16.94 (±2.29) vs. 14.35 (±1.27) cm3/week; mean difference 2.59; P=0.0001, Fig. 4a). Given the difference in the distribution we transformed TBV growth rate into a log scale for better comparison (mean TBV log growth rate 2.73 (± 0.85) vs. 2.63 (±0.04) cm3/week; mean difference 0,09; P = 0.0001, Fig. 4b).

We then compared the predicted TBV growth rate in both groups according to different PMA intervals. For those preterm infants with normal/mildly abnormal term-MRI we found that the rate was 15.08 before 32 weeks PMA, 18.77 from 32 to 37 weeks and 21.34 cm3/week after 37 weeks PMA. For those with moderate/severe term-MRI the growth rate was lower with 13.07 before 32 weeks, 16.27 from 32 to 37 weeks and 10.77 after 37 weeks PMA. We observed that the difference in growth rate was significantly higher in the last period of PMA with a mean difference of 10.57 cm3/week; P=0.0170 (Fig. 5 and Tab. 2).

## Association of perinatal factors, comorbidities, and maternal level of education on brain growth according to the severity of findings on term-MRI

In patients with normal or mildly abnormal term-MRI we found a positive association between TBV and GA, with an increase of 17.07 cm3 per week of gestation (β coef =17.07; P= 0.001), and negative association with female sex (β coef = -11.89; P=0.057), being SGA (β coef = -20.36; P= 0.035), and duration of TPN (β coef = -1.14; P= 0.001), while the presence of comorbidities in this group was not associated with TBV trajectory. In the group with moderate/severe term-MRI, TBV is also associated with GA (coef β = 14.59; P=0.0001), being SGA (coef β = -43.09; P=0.0001), and the presence of comorbidities such as sepsis (coef β = -32.17; P=0.0001) and NEC (coef β = -130.03; P=0.0001), the three last factors showing a negative impact on TBV. Interestingly, in this group, longer duration of TPN is associated with greater TBV (coef β = 0.61; P=0.002). In our cohort, maternal education level did not seem to be associated with brain volumes (Table 3).

Given the frequent association of multiple comorbidities in the same patient, we studied the relationship between the number of comorbidities in each patient and TBV. We again found that in the group with normal/mild term-MRI, the presence of comorbidities was not

associated with TBV: in this group TBV was influenced by GA, being SGA, sex, and days of TPN (Table s6 in supplementary material and Fig. 6a). However, in the group with moderate/severe term-MRI the presence of three or more comorbidities was related to a lower TBV (coef β = -45.85; P=0.006; -79.34; P=0.012 and -118.70; P=0.0001 respectively for having 3, 4 or 5 comorbidities). The remaining variables behaved similarly to the model previously described (Table s6 in supplementary material and Fig. 6b).

In addition, when we studied the impact of comorbidities on the rate of brain growth in the group with moderate/severe term-MRI, we found that preterm infants with three or more comorbidities display a progressive decrease in brain growth rate as PMA increases (Fig. 7 and Table 4).

Regarding moderate/severe brain injury detected by brain US, we see in the previous multivariate models that the presence of IPH was not statistically significant. We performed further analysis in those patients with an abnormal term-MRI to understand better the impact of moderate/severe brain injury on TBV. We found a statistically significant mean difference in TBV of 6.11 cm3 (P= 0.028), with those without moderate/severe brain injury having a mean TBV of 220.7013 versus 214.5932 cm3 in those that have moderate/severe brain injury, accounting for repeated measures and time and taking into account the same perinatal factors and comorbidities described (for more details see Table s7 in supplementary material).

# Discussion

Our study presents the volumetric trajectory of brain growth during early postnatal life in a cohort of VLBWI where TBV was measured through serial brain 3D US. We found a significant association between TBV and GA, sex, being SGA, confirmed of NEC and the duration of TPN.

We hypothesized that a deviated pattern of brain growth would be associated with abnormal findings in term-MRI. Despite finding a general increase in TBV, we found greater TBV among individuals with a subsequently normal or mildly abnormal term-MRI compared to those with moderate to severe abnormalities.

The trajectory of early brain growth was associated with different factors: GA, sex, and being SGA was related with TBV in the infants who would later have a normal/mildly abnormal term-MRI, while in preterm infants with moderate to severe abnormalities in their term-MRI, the presence of comorbidities (significant PDA, sepsis, confirmed NEC, severe ROP and PHI) played a major role. We found a greater negative impact in TBV when 3 or more comorbidities were concomitant in the same patient. Of note, we found that the duration of TPN had a different impact on brain trajectory in both groups. A greater duration in days of TPN was related to lower TBV in the group with normal/mild MRI, while in those with moderate/severe MRI TPN duration was associated to higher TBV.

Our study supports the hypothesis of a negative impact of early extrauterine life on early brain growth with comorbidities being an important determinant of a deviated brain growth trajectory. While brain growth related to a normal term equivalent MRI is related to perinatal factors such as GA, being born SGA and sex, the impact of these is outweighed by the

presence of comorbidities, which have a major impact on TBV in those that have an abnormal term MRI.

Previous studies have shown brain growth in preterm infants is slower than that of healthy fetuses assessed by fetal MRI, with smaller brain volume in preterm infants compared to term infants, even in the absence of structural brain damage [26]. There is evidence that preterm infants present, at term equivalent age, not only smaller brain volumes but also reduced cortical surface area, compared to term infants, with a greater impact at lower GA at birth [13,27]. Our study shows accordingly how GA at birth displays a great impact on brain volumes, even in the group of children without significant brain injury.

Moreover, it appears that these differences in brain growth are not only found at term-equivalent age but are maintained throughout childhood [28] and have been associated with impairments in the areas of language, memory, motor skills and executive functioning [29,30].

In addition to the impact of prematurity itself, multiple studies have shown how different perinatal risk factors such as sex, low birth weight, prolonged mechanical ventilation, BPD, PDA or sepsis impact brain volumes in the preterm population [30-32]; lower brain volumes are also associated with neurodevelopmental deficits throughout childhood [33-36]. Brouwer et al. [37] studied the association between perinatal factors, neuroimaging, and early neurodevelopmental outcomes, finding that longer duration of mechanical ventilation and TPN were associated with worse MRI imaging scores and these, in turn, with worse cognitive, fine motor and gross motor outcomes. Asztalos et al. [38] in a similar study also found that the absence of a major neonatal morbidity (particularly BPD, NEC and severe neurological injury) was predictive of better cognitive, language and motor neurodevelopmental outcomes. In consistency with what has been previously described, our

study shows how comorbidities can impact brain growth suggesting that brain growth trajectory is modified in those preterm infants critically ill during admission in NICU [30,32].

We have found opposite effects of the duration of TPN in both groups. Most studies to date have identified the duration of TPN as having a deleterious effect on central nervous system development [37,39]. However, it has also been studied that an adequate supply of nutrients (mainly proteins and lipids) is essential for adequate neurodevelopment [40-42]. It is possible that the need for TPN in the group with the highest incidence of comorbidities may have exerted a protective effect by providing an adequate nutrient supply. On the contrary, the additional days of TPN in the group of preterm infants without comorbidities seemed to have a deleterious effect.

In addition to comorbidities, it has also been widely studied that intrauterine growth restriction is associated with a decrease in TBV and especially in cortical gray matter volume, demonstrating a specific vulnerability of gray matter to chronic hypoxia, suggesting that the redistributive adaptive response in the context of placental insufficiency may be insufficient for the preservation of brain development [43]. We found that being SGA negatively impacts brain growth from the first postnatal weeks and those preterm SGA showed significantly lower brain volumes compared to those born with adequate birth weight. Tolsa et al. [44] reported a significant reduction in TBV and cortical gray matter measured by MRI in SGA preterm infants from the first two weeks of life to term equivalent age. These findings persist during childhood and adulthood and are associated with cognitive, motor and behavioral deficits [45-48].

In our cohort, we found early sexual dimorphism with females having smaller brain volumes than males and slower brain growth rates. Sexual dimorphism in infant brain development has also been previously investigated. Gilmore et al. [49] studied brain volumes in term infants

by MRI, finding that some of the sex differences in brain development were already present at birth, with greater absolute volumes of TBV, gray matter and white matter in males. In a similar study, Lehtola et al. [50] also found a significant difference with larger absolute brain volumes in males, however, after adjusting for TBV, they found that females, presenting smaller brain volumes, showed larger relative gray matter volumes. Similarly, Benavides et al. [51] found a greater brain volume in males, although there was a predominance of cortical gray matter volume in females.

We did not find a consistent impact of SES, measured by maternal level of education, on brain growth trajectory. The influence of SES on long-term neurodevelopmental outcomes in our population warrants further research as SES appears to be strongly related to later neurodevelopmental outcomes and could modify the effect of perinatal factors on long-term neurological prognosis [52,53]. Moreover, a higher SES has been shown to mitigate the effect of preterm brain injury on long-term neurodevelopment [54].

Regarding perinatal factors, our findings suggest the presence of comorbidities may be a key factor for impaired brain growth rate in the group with subsequent moderate/severe MRI abnormalities at term equivalent age. This could be due to multifactorial exposure to a proinflammatory state and hypoxia-ischemia, as persistent inflammation has been associated with poor brain growth [55-57].

The presence of moderate/severe brain injury determined a smaller TBV in those patients with abnormal term-MRI, with 6 cm3 difference to those that didn't have moderate/severe brain injury. While this finding supports the hypothesis of brain injury impacting brain growth our results should be taken cautiously. On the one hand only two patients had moderate/severe WMI detected by brain US, which could be expected to impact brain growth. On the other hand, brain US could underestimate the presence of diffuse WMI[58], the

most common finding in VLBWI[8,9,59]. The deviated pattern of brain growth detected in those infants that will later have an abnormal term-MRI could revealed the impact of the latter on TBV and the association of a more complicated NICU course with a suboptimal brain growth.

Our study assessed both brain volume as an absolute value, as well as brain growth rate, which has not been previously studied. We found a deviation in the expected pattern of brain growth in those preterm infants who subsequently have more severe brain injury scores on term MRI, with a significant decrease in the brain growth rate.

In summary, we suggest that incorporating the assessment of TBV and brain growth velocity by brain US during the first weeks of life into routine care, could facilitate early identification of those patients in whom brain growth is suboptimal and its associated factors, potentially provide a window of opportunity for neuroprotection and reduction of risk factors.

This study has some limitations. The low number of patients in the group of preterm infants with moderate to severe abnormalities in term-MRI (n=18) may have impacted our results by narrowing the extent of the studied associations. Future research is warranted to better elucidate the potential role and impact on brain growth of the studied variables, as some variables my act as effect modifiers or confounding factors (i.e TPN). Using US instead of MRI to estimate TBV in our study could potentially be seen as a limitation, as MRI is considered the gold standard. However, before carrying out this study, our group conducted a study to check the validity of this TBV segmentation method and to verify its accuracy and reproducibility. In addition, we compared the estimation of TBV through US and MRI, confirming that the estimation of TBV through US is reliable, accurate and reproducible [16]. Finally, we have relied on the MRI performed at term equivalent age to study different brain growth trajectories which can have a variable impact on long term outcome. As this study is

ongoing, we plan to further study early brain growth trajectory related to neurodevelopmental outcomes.

# Conclusions

Preterm infants are at risk of early brain growth impairment. We observed smaller brain volumes and a decrease in the brain growth rate in preterm infants related to more severe brain injury scores on term-MRI. Brain growth is related to GA, being SGA and the presence of comorbidities.

This deviated brain growth pattern can be distinguished from very early stages by serial US monitoring of preterm infants during their NICU admission.

# Data Availability Statement

The datasets generated and analyzed during the current study are available in the OSF repository: https://osf.io/hm9y7/?view_only=accdb3061be04b7b867512f29850cc39.

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# Figures

Fig. 1. Flow diagram of the included patients.

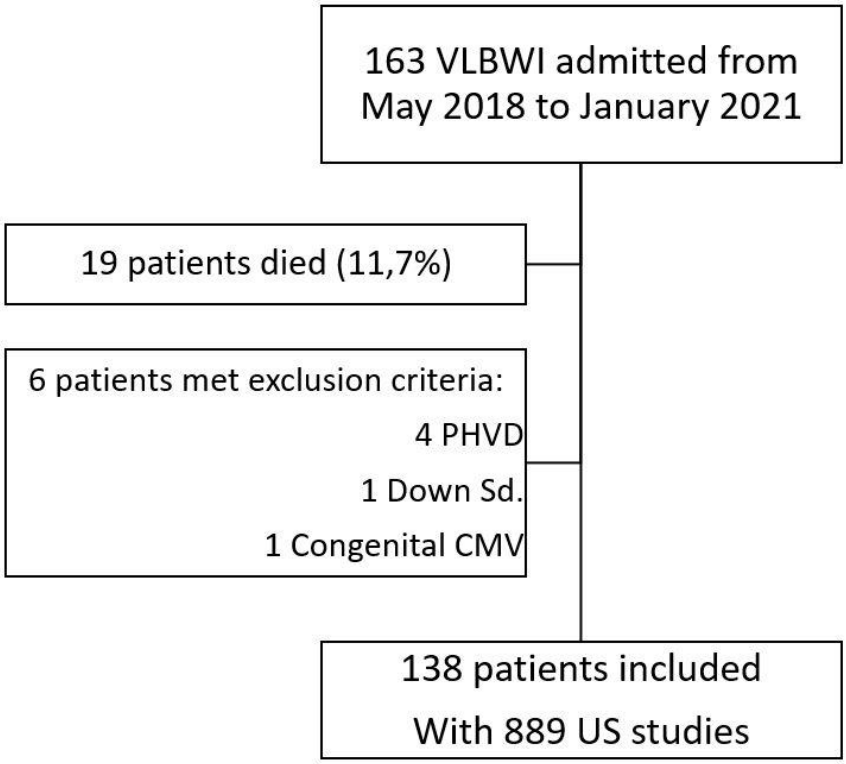


Fig. 2. Predicted total brain volume by postmenstrual age related to sex, being small for gestational age (SGA), confirmed necrotizing enterocolitis (NEC) and parenchymal hemorrhagic infarction (IPH). A full description of the model can be seen in table 1.

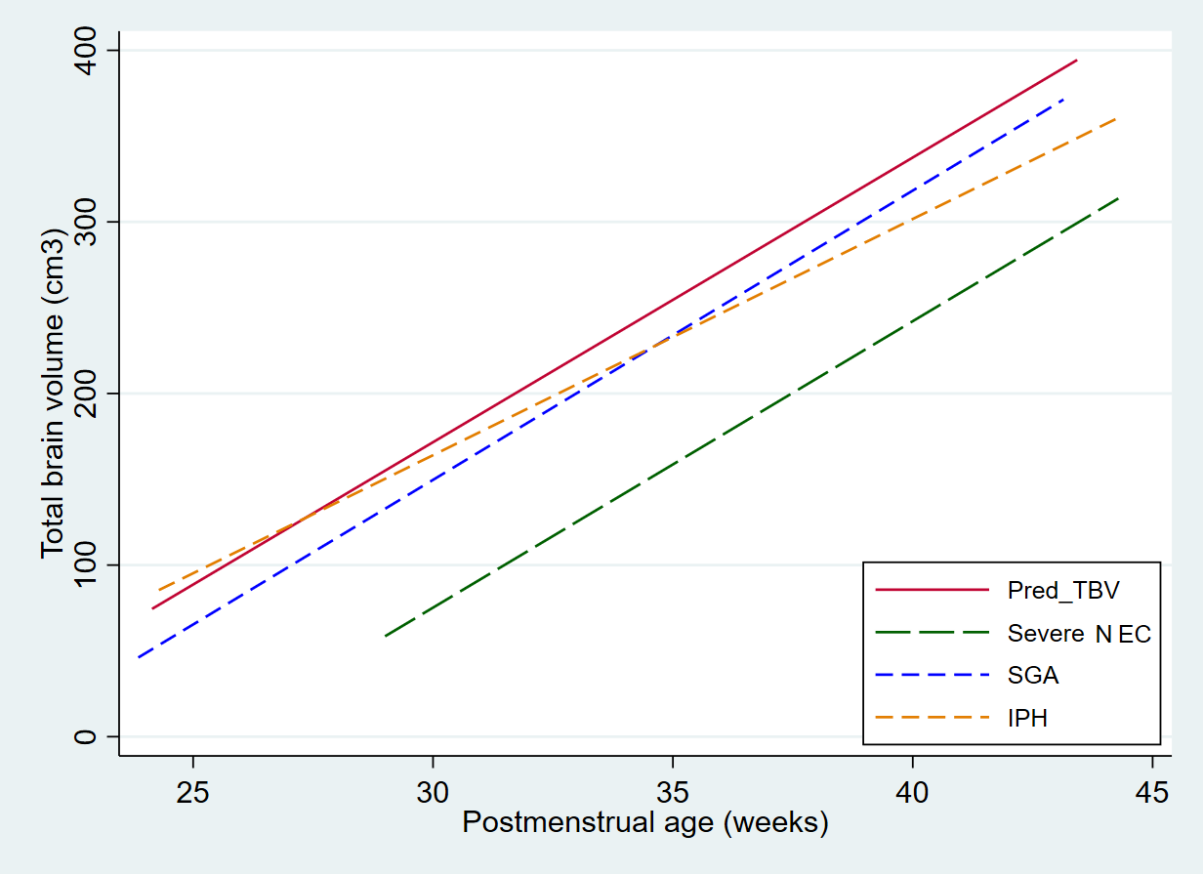


Fig. 3. Total brain volume trajectory (cm3) related to postmenstrual age in the group with normal/mild MRI (blue) and in the group with moderate/severe MRI (red).

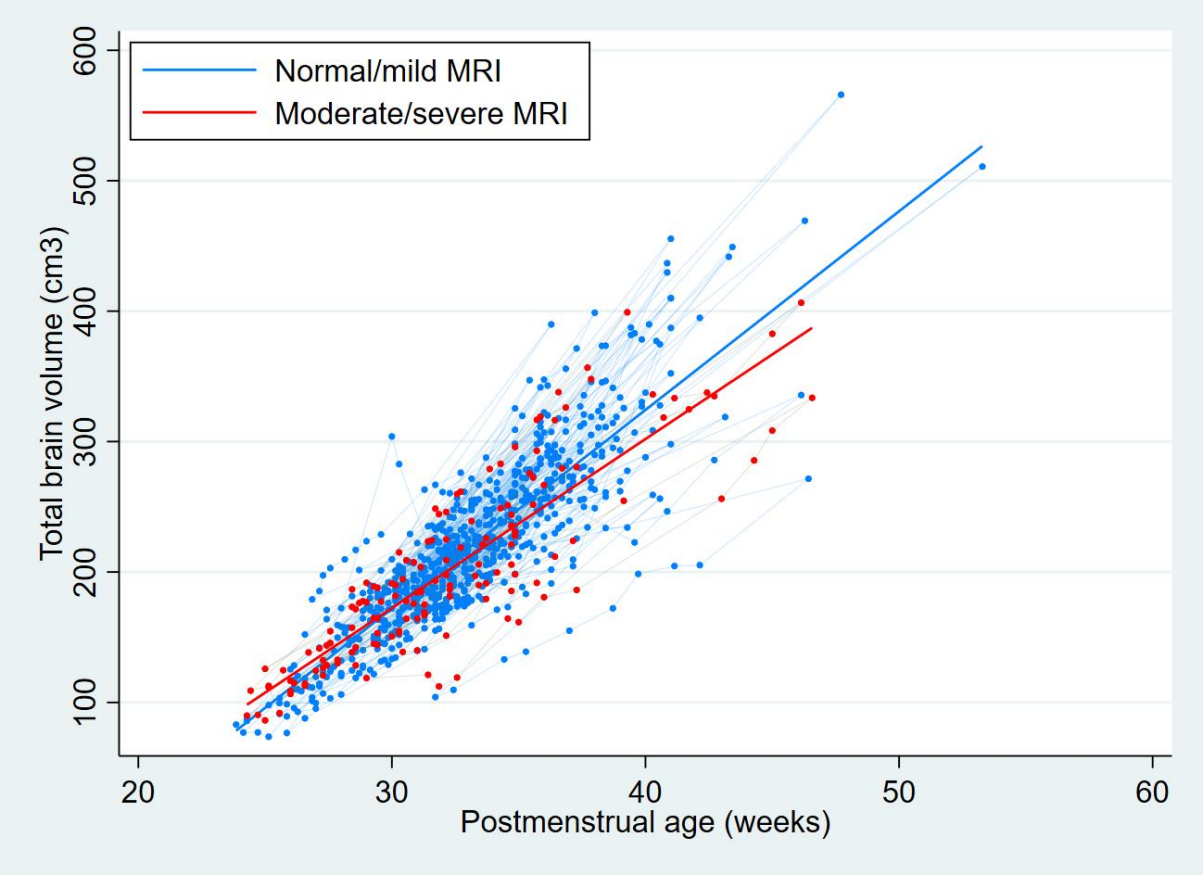


Fig. 4a. Total brain volume growth rate(cm3/week) in the normal/mild MRI group (blue) and in the moderate/severe MRI group (red).

Fig. 4b. Log scale for total brain volume growth rate (cm3/week) in the normal/mild MRI group (blue) and in the moderate/severe MRI group (red)

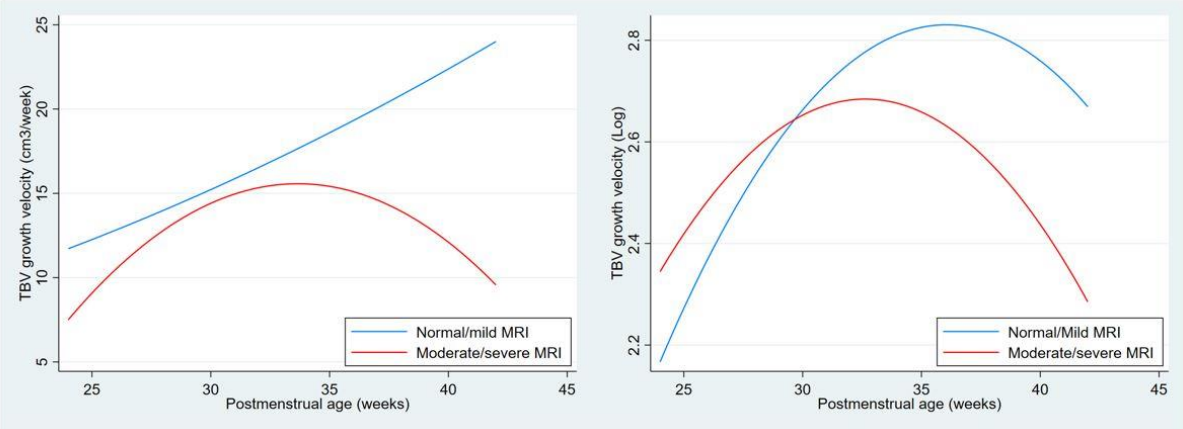


Fig. 5. Brain growth rate by postmenstrual age intervals in group with normal/mild MRI (blue) and moderate/severe MRI involvement (red).

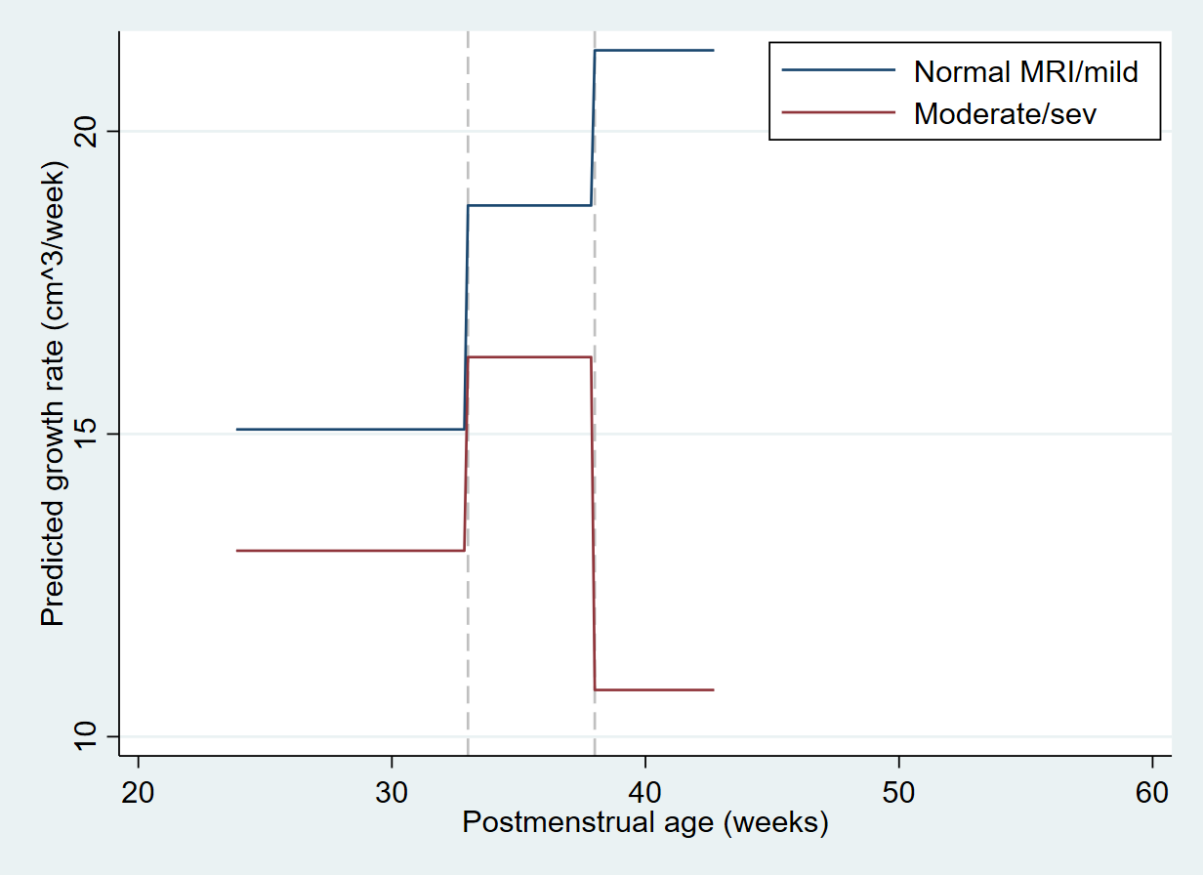


Fig. 6a. Total brain volume trajectory (cm3) in the normal/mild MRI group related to sex and being small for gestational (SGA) or having appropriate weight for gestational age (AGA): males AGA (blue, continuous), males SGA (blue, dashed), females AGA (red, continuous), and females SGA (red, dashed).

Fig. 6b. Total brain volume trajectory (cm3) in the group with moderate/severe MRI related to comorbidities: less than two comorbidities (blue), more than two comorbidities (red).

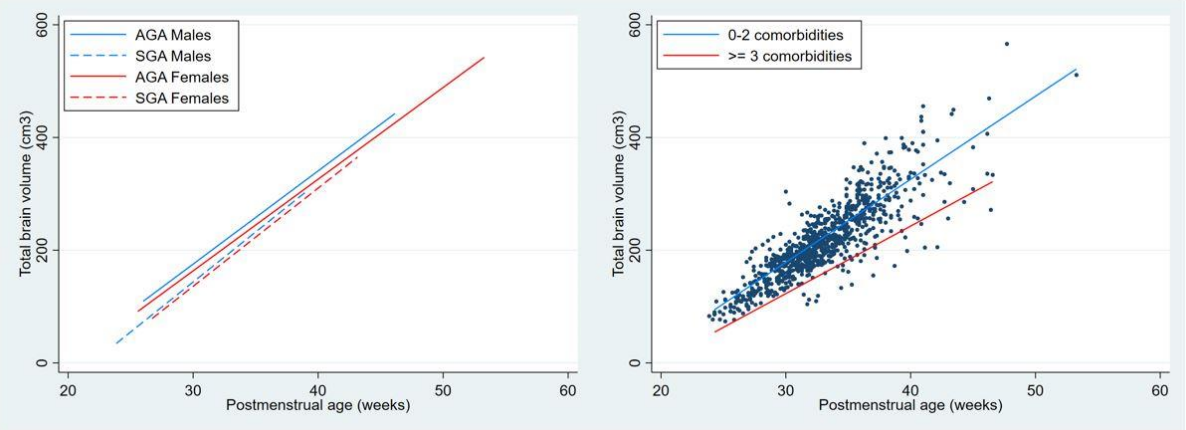
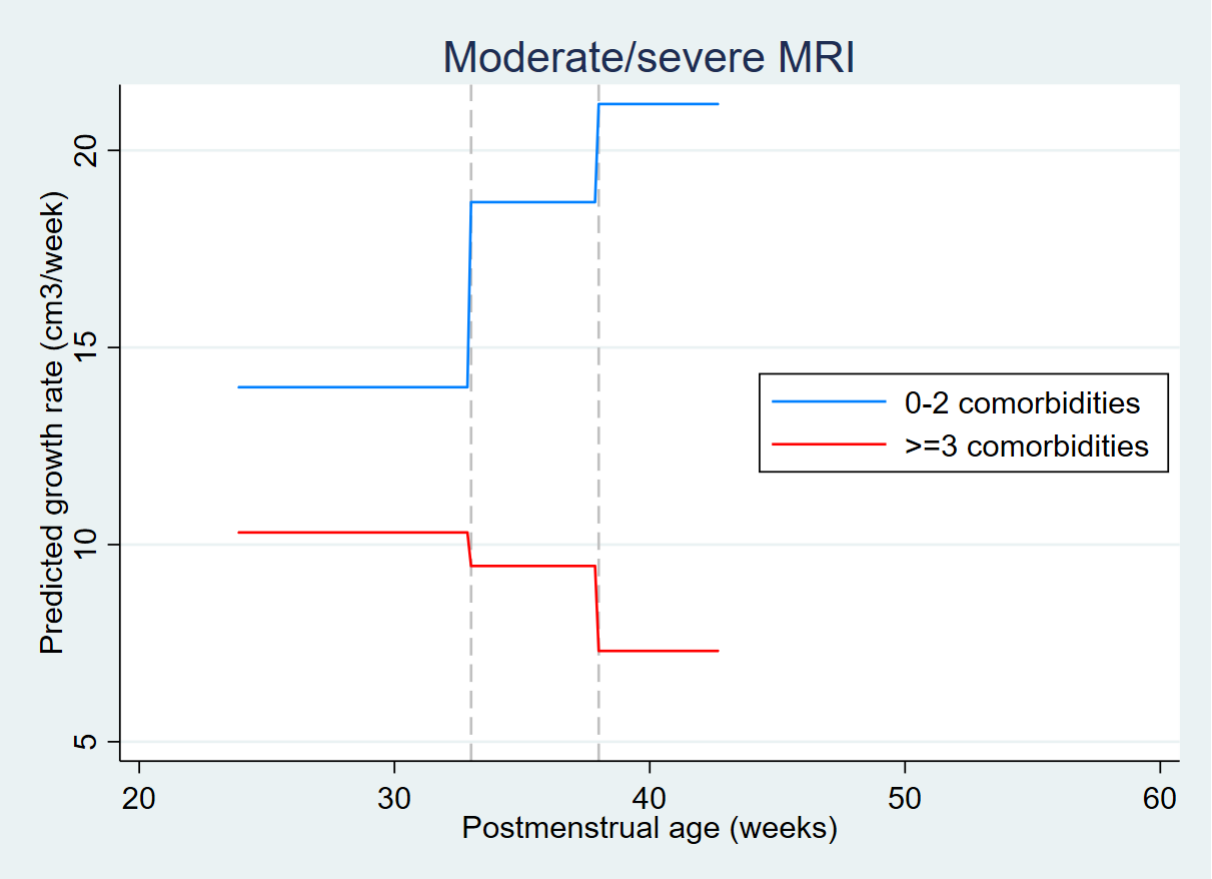


Fig. 7. Brain growth velocity by corrected gestational age intervals in moderate/severe MRI group related to comorbidities.



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# Authors' contribution

Benavente Fernandez, Isabel has played a fundamental role in the conception and design of the work, in the analysis and interpretation of the study data, in the editing of the manuscript and in the approval of its final version.

Lubián Gutiérrez, Manuel has actively contributed to the data acquisition, interpretation/measurement of images, and has been involved in the approval of the final version of the manuscript.

Segado Arenas, Antonio has actively contributed to the data acquisition, medical assistance in the performance of MRI scans, and has been involved in the approval of the final version of the document.

Zafra Rodríguez, Pamela has actively contributed to the data acquisition, medical assistance in the performance of MRI scans, and has been involved in the approval of the final version of the document.

Mendez Abad, Paula has actively contributed to the data acquisition, medical assistance in the performance of MRI scans, and has been involved in the approval of the final version of the document.

Lubián López, Simón P has played a fundamental role in the conception and design of the work, in the interpretation of the study data, and in the correction of the manuscript and approval of its final version.

# Competing interests

The first author of the manuscript, on behalf of himself and all the authors, declares that there is no potential conflict of interest related to the article.

# Consent statement

Ethics approval and consent to participate. Informed consent was obtained from all participants included in the study.

# Tables

**Table 1. Association of total brain volume to gestational age, sex, being small for gestational age (SGA), total parenteral nutrition (TPN), confirmed necrotizing enterocolitis (NEC) and parenchymal hemorrhagic infarction (IPH).**

| VARIABLES | Coefficient | p |
|---|---|---|
| **Gestational age (weeks)** | **16,69** | **0,000** |
| **Sex (F)** | **-8,92** | **0,077** |
| **SGA** | **-17,81** | **0,011** |
| **TPN (days)** | **-0,36** | **0,020** |
| **Confirmed NEC** | **-111,32** | **0,000** |
| **PHI** | 24,98 | 0,109 |
| | Nº obs = 872 / Nº groups = 135<br>Wald chi2 = 9699,61 / P model = 0,00001 | |

*SGA: Small for gestational age. TPN: Parenteral nutrition. NEC: Necrotizing enterocolitis. PHI: parenchymal hemorrhagic infarction.*

**Table 2. Mean brain growth rate (cm3/week) in preterm infants with normal/mild findings versus moderate/severe findings in term-MRI.**

| PMA | Normal/mild MRI | Moderate/severe MRI | Difference | p |
|---|---|---|---|---|
| **< 32 weeks** | 15,08 | 13,07 | -2,01 | 0,2171 |
| **32-37 weeks** | 18,77 | 16,27 | -2,5 | 0,0908 |
| **> 37 weeks** | **21,34** | **10,77** | **-10,57** | **0,0170** |

**Table 3. Total brain volume related to perinatal factors, comorbidities and maternal level of education in those preterm infants with normal/mild term-MRI findings and those with moderate/severe MRI findings.**

| VARIABLES | | NORMAL/MILD MRI (n=120) | | MODERATE / SEVERE MRI (n=18) | |
|---|---|---|---|---|---|
| | | Coefficient | p | Coefficient | p |
| **Gestational age (weeks)** | | **17,07** | **0,001** | **14,59** | **0,0001** |
| **Small for gestational age** | | **-20,36** | **0,035** | **-43,09** | **0,0001** |
| **Sex (F)** | | **-11,89** | **0,057** | 1,18 | 0,911 |
| **Maternal level of education** | **Low** | Ref | Ref | Ref | Ref |
| | **Medium** | -6,75 | 0,315 | **-43,73** | **0,0001** |
| | **High** | -5,27 | 0,575 | **-28,35** | **0,011** |
| **Total respiratory support (days)** | | 0,068 | 0,630 | -0,20 | 0,080 |
| **TPN (days)** | | **-1,14** | **0,001** | **0,61** | **0,002** |
| **Severe ROP** | | 8,45 | 0,668 | -14,12 | 0,126 |
| **Late onset sepsis** | | -1,24 | 0,872 | **-32,17** | **0,0001** |
| **Significant PDA** | | 2,19 (p) | 0,856 | 0,05 | 0,992 |
| **Confirmed NEC** | | -------- | -------- | **-130,03** | **0,0001** |
| **PHI** | | -1,22 | 0,967 | 13,36 | 0,553 |
| | | Nº obs = 600 / Nº groups = 97<br>Wald chi2 = 6210,02 / P model = 0,00001 | | Nº obs = 116 / Nº groups = 15<br>Wald chi2 = 1882,99 / P model = 0,00001 | |

*MRI: Magnetic resonance imaging. TPN: Parenteral nutrition. ROP: Retinopathy of prematurity. PDA: Patent ductus arteriosus. NEC: Necrotizing enterocolitis. PHI: parenchymal hemorrhagic infarction.*

**Table 4. Mean brain growth rate (cm3/week) in preterm infants with moderate/severe findings in term-MRI related to the presence of 3 or more comorbidities.**

| PMA | 0 – 2 comorbidities | ≥ 3 comorbidities | Difference | p |
|---|---|---|---|---|
| **< 32 weeks** | 13,99 | 10,31 | -3,68 | 0,1756 |
| **32-37 weeks** | **18,69** | **9,46** | **-9,23** | **0,0138** |
| **> 37 weeks** | **21,17** | **7,30** | **-13,87** | **0,0295** |