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● *Original Contribution*

3-D ECHO BRAIN VOLUMES TO PREDICT NEURODEVELOPMENTAL OUTCOME IN INFANTS: A PROSPECTIVE OBSERVATIONAL FOLLOW-UP STUDY

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Abstract—Prematurity and intra-uterine growth restriction (IUGR) are risk factors for long-term poor neurodevelopmental outcomes and are associated with reductions in regional brain volumes. In this study, the aim was to determine the possible role of 3-D ultrasonography (3-DUS) volumes of whole brain, thalamus, frontal cortex and cerebellum, measured at postnatal days 30–40, as early predictors of long-term risk for neurobehavioral disorders. To this purpose, a heterogeneous population of full-term, preterm, IUGR and preterm IUGR (pre-IUGR) born individuals (n = 334), characterized by gestational age and birth weight in the ranges 24–41 wk and 860–4000 g, respectively, was followed from postnatal days 30–40 to the second year of life. At enrollment, brain volumes were measured using 3-DUS, whereas neurodevelopment was assessed at 2 y using the Griffiths III test. Cerebral volumes were strictly and significantly lower in infants characterized by a negative outcome and had excellent diagnostic accuracy. The 3-DUS volume of whole brain, thalamus, frontal cortex or cerebellum may be an early predictor of neonates at major risk for neurobehavioral disorders in later life. (E-mail: maria.aisa@unipg.it)

Key Words: **Neurodevelopment impairment, Prematurity, Intra-uterine growth restriction, 3-D ultrasonography, Cerebral volumes, Griffiths III test.**

INTRODUCTION

Compared with full-term neonates, preterm, very low birth weight (VLBW) and intra-uterine growth restriction (IUGR) born individuals are at higher long-term risk of neurodevelopmental disorders (Aisa et al. 2020), which tends to increase with decreases in gestational age (GA) and birth weight (BW) (Thompson et al. 2019) and impair quality of life (Theunissen et al. 2001). These deficits often emerge later in life (Hack et al. 2005; Serenius et al. 2016; Allotey et al. 2018), illustrating how prematurity, VLBW and IUGR may have negative consequences on children who do not present clear clinical manifestations that can be diagnosed early. Additionally, in the first 3 y after birth, the brain undergoes dramatic growth, and multitudes of synaptic connections are laid down (Dobbing and Sands 1973). These sensitive early years are critical for neuroplasticity (Johnston 2009). In the light of the above observations, the need for the early recognition of neonates at high long-term risk of neurodevelopmental impairments appears evident, along with the advance of early stimulation programs or neuroprotective intervention attempting to improve the outcome of delayed neurodevelopment (Nordhov et al. 2010; Spittle et al. 2015).

Early diagnosis of developmental impairment or disabilities using traditional means is, however, unlikely because neurologic function is still very immature at

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birth (Parikh 2016). The recent introduction of new instrumental techniques for the morphological, functional and metabolic study of the brain in the perinatal period has provided important knowledge and perspective for both diagnosis and prognosis of neurological damage (Parikh 2016), and in this context, the regional volumetric evaluations, by magnetic resonance imaging (MRI) or ultrasonography (US), seem to offer the best opportunities to identify and develop powerful prognostic indicators (Tao and Neil 2014; Parikh 2016). The strong correlation of prematurity and IUGR with both poor motor and cognitive development in later life (Levine et al. 2015; Murray et al. 2015; Wang et al. 2016; Taine et al. 2018; Sacchi et al. 2020) and reduction of regional brain volumes (García-Alix et al. 2004; Cheong et al. 2016; Keunen et al. 2016; Monson et al. 2016; Bruno et al. 2017; Matthews et al. 2018; Chau et al. 2019; Wu et al. 2019; Aisa et al. 2020; Hammerl et al. 2020) has indicated the strong relationships between cerebral volumes and neurodisorders, as well as the prognostic significance of the volumetric measurements in preterm and IUGR subjects (García-Alix et al. 2004; Keunen et al. 2016; Matthews et al. 2018; Wu et al. 2019; Aisa et al. 2020; Hammerl et al. 2020). At present, however, data on 3-DUS, which is now emerging as a sensitive, valuable, non-time-consuming or non-cost-consuming method capable of estimating brain structures and volumes in neonates without restrictions (Rizzo et al. 2011; Kleber-mass-Schrehof et al. 2013; Riccabona 2014; Businelli et al. 2015; Green et al. 2016; Ximenes et al. 2019), are very limited, particularly with respect to the early postnatal period.

Using a well-established experimental approach (Aisa et al. 2020), to investigate, further, the potential significance of 3-DUS whole brain volume (WBV), thalamus volume (TV), frontal cortex volume (FCV) and cerebellum volume (CV) as early and sensitive predictors of adverse neurodevelopment in later life (Aisa et al. 2020), and to look for a procedure capable of identifying subjects at long-term risk of neurological diseases, in general, we examined the aforementioned variables in a wide and heterogeneous population. As prematurity and IUGR are two diverse conditions that may induce similar or distinctive effects in many circumstances (Aisa et al. 2016, Aisa et al. 2019), including neurodevelopment (Aisa et al. 2020), to reveal possible comparable or differential consequences related to prematurity and IUGR, alone or in combination, we selected preterm, IUGR and pre-IUGR neonates. In addition, because the risk of morbidity varies according to the spectrum of GA and BW (Thompson et al. 2019), the enrolled subjects significantly differed in GA and BW. Infants were followed from postnatal days 30 to 40 to the second year of life. At these time points, 3-DUS volumetric and neurodevelopmental assessments were carried out, respectively. Neurodevelopment was assayed using the Griffiths III test (Green et al. 2016). Data on 3-DUS cerebral volumes were then related to neurodevelopment outcome. The variability of volumes with respect to different gestational ages and IUGR was also considered.

METHODS

Study design and neonate population

Characteristics of the present study are summarized and graphically represented in Figure 1.

Briefly, our observational population was made up of 334 neonates of healthy mothers recruited at the Centre of Perinatal and Reproductive Medicine, University of Perugia, Perugia, Italy. Neonates with GA and BW in the ranges 24−41 wk and 860−4000 g, respectively, were enrolled at postnatal days 30−40 or corrected age. The exclusion criteria were genetic disease, rescue hypothermia, severe perinatal asphyxia, cerebral hemorrhage, intraventricular hemorrhage greater than first grade, periventricular leukomalacia, sepsis, infectious diseases of the brain, maternal drug use and/or alcohol abuse in pregnancy and maternal congenital infections (*i.e.,* toxoplasmosis, cytomegalovirus).

The population comprised 158 full-term, 113 preterm, 30 IUGR and 33 pre-IUGR infants (Fig. 2). In the case of prematurity, the postnatal age was corrected to equivalent age. Once the newborns had been included in the study, WBV, TV, FCV and CV were estimated using 3-DUS and recorded. At 2 y of age (or corrected age in the case of prematurity), the infants underwent a neurobehavioral assessment using the Griffiths III test (Cirelli et al. 2015; Green et al. 2016) (Fig. 2). Two subgroups with different outcomes, namely, the normal neurodevelopment subgroup (301 subjects: 158 full-term, 100 preterm, 25 IUGR and 18 pre-IUGR) and abnormal neurodevelopment subgroup (33 subjects: 13 preterm, 5 IUGR and 15 pre-IUGR), were then recognized (Fig. 2).

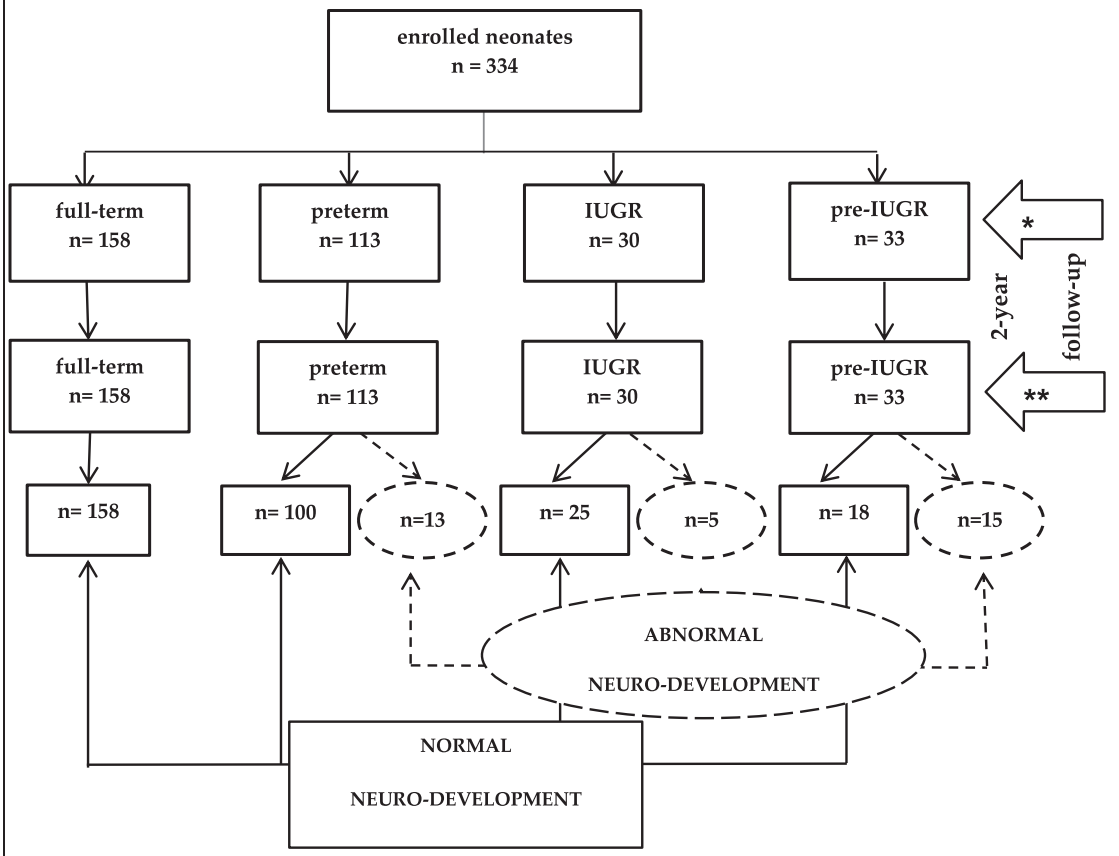
Institutional review board approval was obtained for data collection, and mothers were informed and gave specific consent to the study.

Diagnosis of IUGR and assessment of GA, postnatal corrected age and 3-DUS cerebral volumes

Diagnosis of IUGR was based on biometric US evaluations during the second and third trimesters when decreases in the assessed fetal weight below the 10th percentile from the reference curves were detected using Hadlock's formula and reference values (Hadlock et al.1985).

Type	- observational prospective study with two years of follow-up, from 30-40 days of postnatal period to 2 years of age, or corrected age in case of prematurity.
Aims	- evaluation of the prognostic significance as indicators of impaired neuro-development in later life by 3DUS WBV, TV, FCV and CV.
Population	- 334 neonates, 30-40 days old with GA and BW in the ranges of 24-41 wks and 630-4000 g.
Primary Endpoints	- evaluation of the prognostic accuracy of the 3DUS cerebral volumes as early predictors for impaired neuro-development at two years.
Secondary Endpoints	- preliminary cut-off values of the 3DUS cerebral volumes.

Case selection and experimental phase diagram.



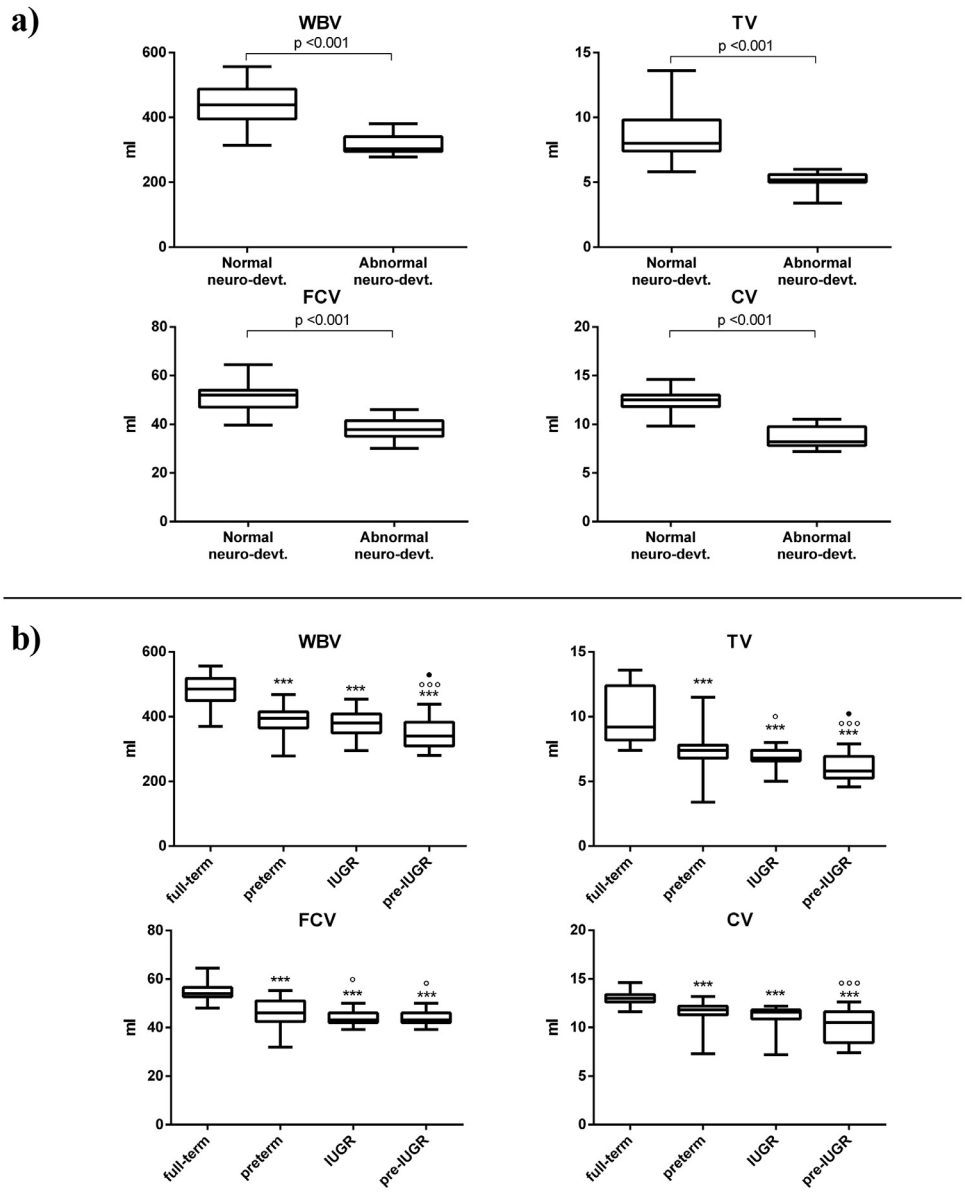
*** 30-40 days of postnatal age or corrected age: evaluation of 3DUS cerebral volumes.**
**** 2 years of age or corrected age: neuro-development assessment.**

Fig. 1. Characteristics of the study. 3-DUS = 3-D ultrasonography; WBV = whole brain volume; TV = thalamus volume; FCV = frontal cortex volume; CV = cerebellum volume; IUGR = intra-uterine growth restriction.

GA was correctly dated during the first trimester of pregnancy by US estimates.

Postnatal corrected age was calculated by subtracting from the chronological age the number of weeks before 40 wk at which the neonate was born.

Cerebral volumes were estimated using Virtual Organ Computer-Aided Analysis software (VOCAL; Vocal II, General Electric Ultrasound Systems, USA; probe RIC5, frequency 5 Hz) through transfontanellar 3-DUS. This technology is in accordance with the



WBV: whole brain volume, TV: thalamus volume; FCV: frontal cortex volume; CV: cerebellum volume.

*, significant different from control; ***, $p \leq 0.001$. °, significant different from preterm; °, $p \leq 0.05$, °°°, $p \leq 0.001$. •, significant different from IUGR; • $p \leq 0.05$, ••• $p \leq 0.001$.

Fig. 2. Three-dimensional ultrasonography cerebral volumes in the normal and abnormal neurodevelopment subgroups (a) and in neonates of different gestational age and intrauterine growth restriction (IUGR) status (b). WBV = whole brain volume, TV = thalamus volume; FCV = frontal cortex volume; CV = cerebellum volume.

International US Scans guidelines and seems to be highly reproducible and accurate for the assessment of organ volumes in fetal life and throughout childhood (Rizzo et al. 2011; Riccabona 2014; Businelli et al. 2015; Aisa et al. 2016, Aisa et al. 2019, Babucci et al. 2019; Ximenes et al. 2019; Dudink et al 2020). It consists of coronal, axial and sagittal scans with additional A, B and C image plans automatically produced by VOCAL II Software.

Measurements were obtained as an average of four repeated estimations by a blinded sonographer. Both intra- and inter-operator variability were <2% for WBV, TV and CV and <5% for FCV.

Neurodevelopmental assessment at 2 y

Neurodevelopment was assessed using the Griffiths III test (Cirelli et al. 2015; Green et al. 2016).

The total developmental quotient (DQ) was calculated according to the instructions in the manual. The children were evaluated by psychologists and pediatricians experienced in neurodevelopmental examination, who had completed an accredited training course on the Griffiths scales. All of them were blinded to the data on cerebral volumes. At the cutoff value of 85, which was obtained using DQ mean − 1SD, a DQ ≥85 was considered normal. Infants with DQs ≥85 or <85 were then included in the normal or abnormal neurodevelopment groups, respectively (Cirelli et al. 2015; Green et al. 2016).

Statistical analysis

Data were analyzed and graphs made using GraphPad Prism (Version 6.01, GraphPad, San Diego, CA, USA) and SPSS Version 23 (IBM, Armonk, NY, USA) statistical software.

The probability of occurrence of a neurobehavioral disorder associated with different gestational ages and IUGR was defined by the risk ratio. This was calculated as the ratio of the probability of disease occurrence in the category of infants born preterm, IUGR or pre-IUGR to that of the infants born at full term (used as reference control). As the occurrence of neurobehavioral disorders in the latter category was zero, its value was conventionally set to 0.5 to obtain a value mathematically. The normal distribution of variables under investigation was assessed using the D'Agostino−Pearson normality test. As these were not found to be distributed normally, comparison between two groups was performed using the non-parametric Mann−Whitney test, whereas multiple comparisons between more than two groups were performed using the non-parametric Kruskal−Wallis test with Dunn's *ad hoc* post-test. Correlations between variables under investigation were checked with Spearman's rho (r) rank correlation coefficient analysis. Bivariate and multivariate binary logistic regression was performed, and odds ratios with 95% confidence intervals (CIs) were calculated to assess the relationship between the parameters studied and the neurodevelopmental outcome, as well as to look for a predictive model. Prognostic accuracy of cerebral volumes, GA and BW as indicators of disease was quantified as the area under (AUC) the specific receiver operating characteristic (ROC) curves. These were constructed considering values of infants who had not had not or had experienced impaired neurodevelopment, respectively. The cutoff and corresponding sensitivity and specificity values were calculated. AUCs were compared using the Delong test.

Table 1. 3-D ultrasonography cerebral volumes in the whole population and in the normal and abnormal neurodevelopment subgroups

	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)
	Whole population			
Median	425	7.8	51	12.4
IQR	381.8−484.3	7−9	44−54	11.6−13
Mean	428.1	8.4	49.6	12.03
SD	67.8	2.2	6.5	1.45
	Normal neurodevelopment subgroup			
Median	438	8	52	12.5
IQR	395−487	7.41−9.8	47−54	11.8−13
Mean	440	8.76	50.9	12.41
SD	59.2	2.03	5.4	0.9
	Abnormal neurodevelopment subgroup			
Median	303	5.2	37.8	8.2
IQR	295−340	5−5.6	35−41.5	7.8−9.75
Mean	316.5	5.21	38.2	8.6
SD	24.5	0.55	4.1	0.98

IQR = interquartile range; SD = standard deviation; WBV = whole brain volume; TV = thalamus volume; FCV = frontal cortex volume; CV = cerebellum volume.

RESULTS

Occurrence of neurobehavioral disorders and risk ratios associated with different gestational ages and IUGR

At 2 y, the occurrence of neurobehavioral disorders, as well as the risk ratios associated with different gestational ages and IUGR, were considered.

The occurrence of abnormal neurodevelopment in our population was 9.9% (3.9% preterm, 1.5% IUGR and 4.5% pre-IUGR). Risk ratios were as follows; pre-IUGR, 143.6 (95% CI: 9.4−2196, $p < 0.001$); IUGR, 52.7 (95% CI: 3.75−740.6, $p < 0.001$), preterm, 36.3 (95% CI: 2.4−552.8, $p < 0.001$)].

Descriptive statistics of variables under investigation

Data concerning the variability of 3-DUS cerebral volumes in normal and abnormal neurodevelopment subgroups were reported (Table 1) also with respect to gestational ages and IUGR (Table 2).

3-DUS cerebral volumes in the subgroups of normal and abnormal neurodevelopment and with respect to GA and IUGR

Comparison analysis of WBV, TV, FCV and CV in the subgroups with different neuro-development outcomes revealed that all cerebral volumes examined were significantly lower in neonates characterized by abnormal neurodevelopment (Fig. 2a).

Multicomparison analysis of WBV, TV, FCV and CV in the whole population showed that all variables were significantly higher in full-term neonates (Fig. 2b). Pre-IUGR newborns had significantly greater reductions in WBV and TV or FCV and CV in comparison with preterm and IUGR or preterm newborns, respectively

Table 2. 3-D ultrasonography cerebral volumes for the whole population and the normal and abnormal neurodevelopment subgroups with respect to gestational age and intra-uterine growth restriction

	Whole population							
	Full term				Preterm			
	WBV (mL)	TV (ml)	FCV (mL)	CV (mL)	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)
Median	485	9.2	54	13	395	7.4	46	11.8
IQR	450−518	8.2−12.4	52.7−56.6	12.6−13.4	365−415	6.8−7.8	42.4−51	11.3−12.2
Mean	482.5	10	54.6	13	387.7	7.2	46.2	11.5
SD	43.1	2	3.2	0.59	42.6	1	5.4	1.3
	IUGR				Pre-IUGR			
	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)
Median	380.5	6.8	43.2	11.5	340	5.8	43	10.5
IQR	350−408	6.6−7.4	42−46	10.9−11.8	309−383	5.2−6.9	36.5−48.5	8.4−11.6
Mean	381.1	6.8	43.6	11.1	348.0	6,152	42.96	10.13
SD	39.5	07	2.7	1.2	43.83	1.008	6.3	1.5
	Normal neurodevelopment subgroup							
	Full-term				Preterm			
	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)
Median	485	9.2	54	13	396	7.4	48	11.8
IQR	450−518	8.2−12.4	52.7−56.6	12.6−13.4	378−419	7.1−7.8	43.8−51	11.6−12.3
Mean	482.5	10	54.6	13	398.1	7.5	47.4	11.9
SD	43.1	2	3.2	0.59	32.1	0.67	4.3	0.6
	IUGR				Pre-IUGR			
	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)
Median	388	7	43.8	11.6	378.5	6.85	48	11.4
IQR	369−414	6.8−7.45	42−46	11.3−11.9	343−401	6.8−7.3	42.7−50	10.9−11.8
Mean	389.5	7.1	44	11.5	374.6	6.9.	47	11.3
SD	36.7	0.44	2.8	0.5	36.8	0.6	4.5	0.7

	Abnormal neurodevelopment subgroup											
	Preterm				IUGR				Pre-IUGR			
	WBV (mL)	TV (mL)	WBV (mL)	TV (mL)	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)	WBV (mL)	TV (mL)	FCV (mL)	CV (mL)
Median	298	5.2	305	5.2	352	5.3	42	9.8	305	5.2	36	8.4
IQR	292−328	4.8−5.8	295− 337	4.8−5.6	318− 353	5.1−5.5	40.1− 43.5	7.5−10	295− 337	4.8−5.6	35.6− 36	7.4−8.4
Mean	308.3	5.2	316	5.2	339	5.3	41.8	8.9	316	5.2	38	8.7
SD	25.7	0.8	27.3	0.42	25	0.2	1.8	1.4	27.3	0.42	4.5	0.9

IQR = interquartile range; SD = standard deviation; WBV = whole brain volume; TV = thalamus volume; FCV = frontal cortex volume; CV = cerebellum volume.

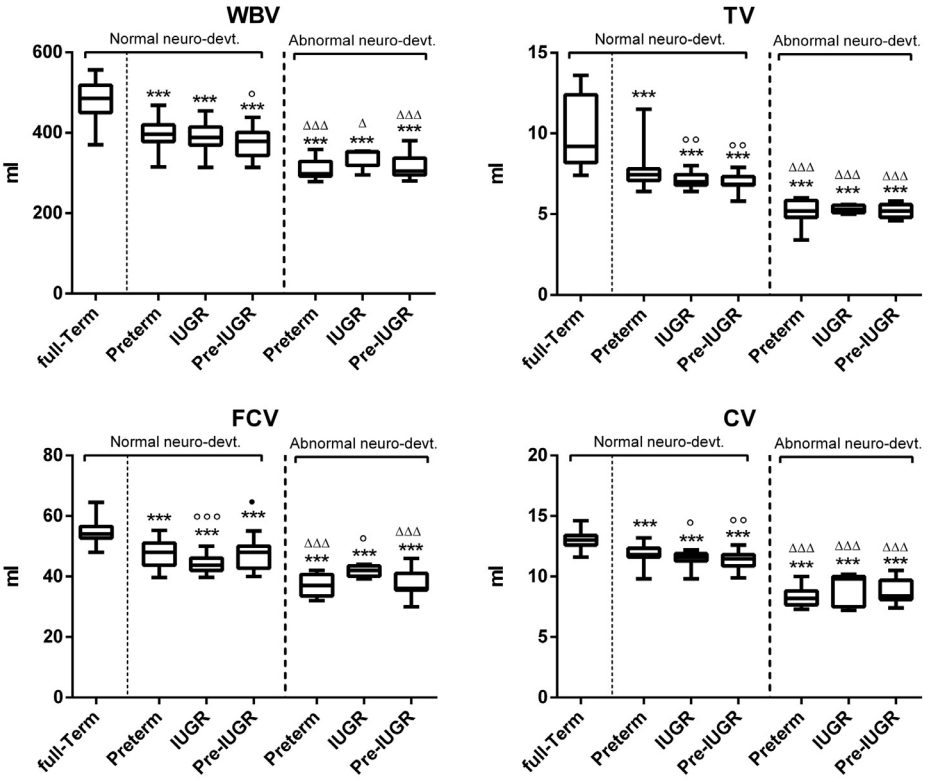
(Fig. 2b). Also, TV and FCV levels were significantly lower in IUGR versus preterm neonates (Fig. 2b).

3-DUS cerebral volumes in the normal and abnormal neurodevelopment subgroups with respect to GA and IUGR

When the cerebral volumes of full-term, preterm, IUGR and pre-IUGR neonates in the normal and impaired neurodevelopment outcome subgroups were compared, we found that full-term neonates had significantly higher levels of all variables in both subgroups (Fig. 3). On examination of WBV, TV, FCV and CV in the normal and abnormal neurodevelopment subgroups, newborns with impaired outcome manifested significant reductions, except in the case of FCV in IUGR neonates (Fig. 3). In the normal neurodevelopment subgroup, in addition, significant differences in WBV, TV, FCV and CV were noted between IUGR and/or pre-IUGR versus preterm neonates, these variations were not more seen in the abnormal neurodevelopment subgroup (Fig. 3).

GA and BW in normal and abnormal neurodevelopment subgroups and correlation between variables

Once the reduction in cerebral volumes in neonates with impaired neurodevelopment was determined, and because the risk of morbidity varies with the spectrum of GA and BW (Thompson et al. 2019), the variability of the



WBV: whole brain volume, TV: thalamus volume; FCV: frontal cortex volume; CV: cerebellum volume. *, significant different from full-Term; ***, $p \leq 0.001$. °, significant different from Preterm of the same subgroup; °, $p \leq 0.05$, °°°, $p \leq 0.001$. •, significant different from IUGR of the same subgroup; • $p \leq 0.05$, ••• $p \leq 0.001$. Δ, significant different from respective category in Normal neuro-development subgroup; Δ, $p \leq 0.05$, ΔΔΔ, $p \leq 0.001$.

Fig. 3. Three-dimensional ultrasonography cerebral volumes in the normal and abnormal neurodevelopment subgroups with respect to gestational age and intra-uterine growth restriction (IUGR). WBV = whole brain volume, TV = thalamus volume; FCV = frontal cortex volume; CV = cerebellum volume.

latter parameters in the two subgroups (Table 3) and their possible correlation with cerebral volumes were evaluated.

Statistically significant reductions in GA and BW were determined in neonates with impaired neurodevelopment (Table 3). Analysis of the correlation indicated that these variables strongly correlated with WBV, TV, FCV and CV, with Pearson's coefficients (r) of 0.739, 0.723, 0.687 and 0.707 or 0.846, 0.853, 0.810 and 0.827, respectively. A strong and mutual correlation was also found between all cerebral volumes; *r* was 0.868, 0.791, 0.841, 0.812, 0.855 and 0.826 for the associations of WBV with TV, WBV with FCV, WBV with CV, TV with FCV, TV with CV and CV with FCV, respectively.

Cerebral volumes, GA and BW as predictors of impaired neurodevelopment at 2 y

Bivariate and multivariate analyses were then conducted to further evaluate the relationship between brain volumes and neurodevelopmental outcome at 2 y, as well as to determine their ability as predictors of disease or to look for a predictive model. The two risk factors GA and BW were also included in the examination.

Results of univariate analysis revealed a significant association of all cerebral volumes, GA and BW with neurodevelopmental outcome (Table 4). No significant logistic regression multivariate models were achievable as a consequence of multicollinearity problems outstanding the strong correlation between the variables studied (data not shown).

Finally, the logistic regression analysis was complemented with a predictive accuracy test that was quantified as AUCs. As full-term neonates are not at risk of impaired neurodevelopment (Levine et al. 2015; Murray et al. 2015; Wang et al. 2016; Taine et al. 2018), the ROC curves were calculated with data including (Figs. 4a and 5a) or excluding (Fig. 4b and 5b) the full-term category. Results indicated that the 3-DUS volumes had excellent prognostic accuracy in general (Fig. 4), which was higher with respect to GA and BW (Fig. 5). Comparison of the respective AUCs indicated that TV

Table 3. Gestational age and birth weight for the whole population and the normal and abnormal neurodevelopment subgroups

	Gestational age (wk)	Birth weight (g)
	Whole population	
Median	37	2400
IQR	34–38	1930–2970
Min/max	24–41	630–4000
Mean	35.9	2450
SD	3.2	778.4
	Normal neurodevelopment subgroup	
Median	37	2510
IQR	35–38	2065–2980
Min/max	24–41	670–4000
Mean	36.3	2567
SD	2.8	720
	Abnormal neurodevelopment subgroup	
Median	32*	1350*
IQR	28.5–35	1215–1660
min/max	24–37	630–2060
mean	31.9	1382
SD	3.8	376.5

IQR = interquartile range; SD = standard deviation.
* Statistically significant different compared with the normal neurodevelopment subgroup.

Table 4. Relationship of variables under investigation with neurodevelopment outcome at 2 y of age

Variable	Bivariate logistic regression analysis		
	Odds ratio	95% CI	*p* Value
WBV	0.923	0.91–0.952	<0.001
TV	0.00037	0.00003–0.05	0.002
FCV	0.55	0.446–0.682	<0.001
CV	0.017	0.002–0.166	>0.001
GA	0.686	0.609–0.773	<0.001
BW	0.997	0.995–0.998	<0.001

WBV = whole brain volume; TV = thalamus volume; FCV = frontal cortex volume; CV = cerebellum volume; GA = gestational age; BW = birth weight; CI = confidence interval.

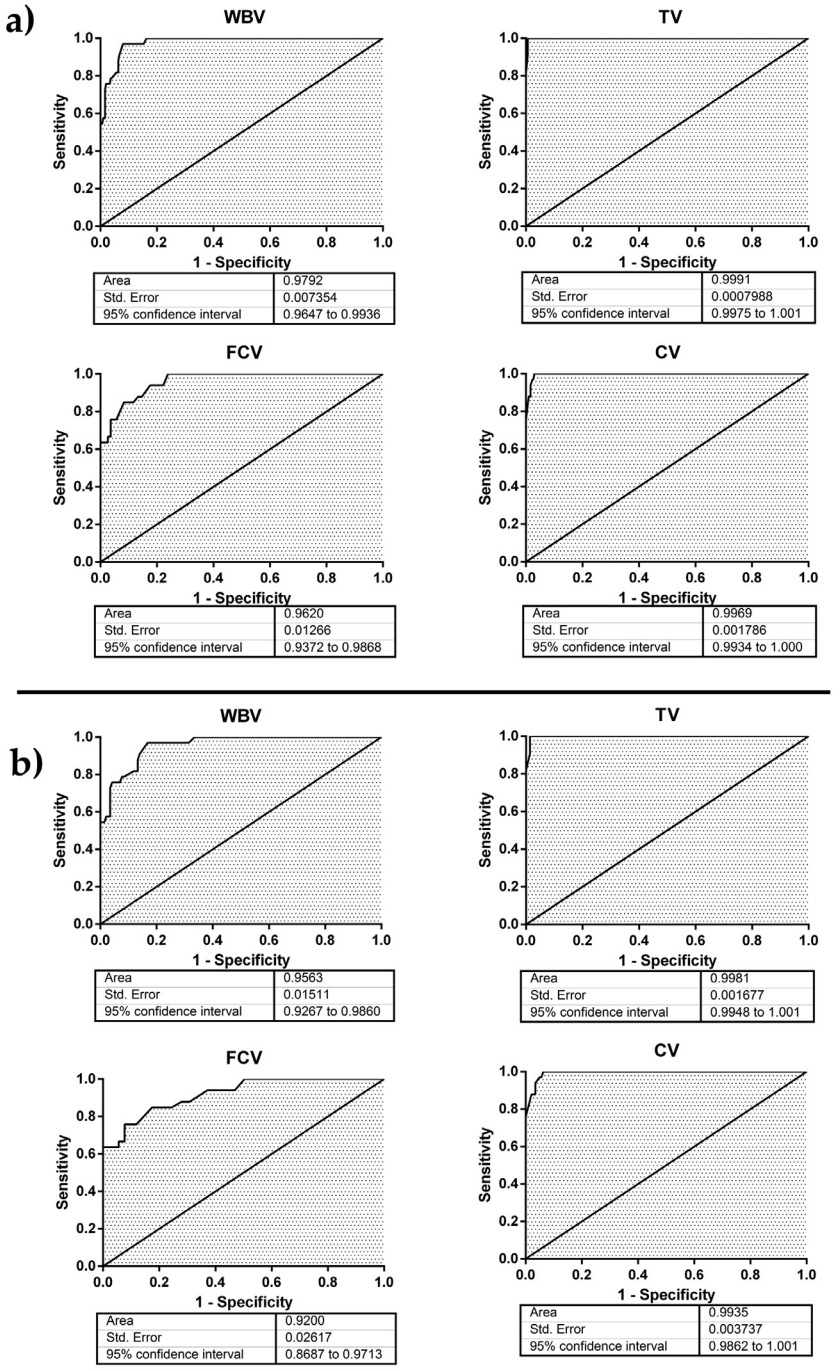
and CV were the best predictors in both evaluations, that is, including or excluding the values for the full-term category. In the first case, the prognostic accuracy was in the order TV∼CV > WBV∼FCV > BW > GA (with differences that were statistically significant different, $p \leq 0.01$). When values for the full-term category were omitted, the tendency was TV∼CV > WBV∼FCV, while WBV > BW > GA and FCV∼BW > GA (showing differences that were statistically significant different, $p \leq 0.01$).

Cutoff and corresponding sensibility and specificity values of variables were then reported (Table 5).

DISCUSSION

Data obtained in the study described here validate our previous findings and suggestions concerning the possible role of 3-DUS WBV, TV, FCV and CV in preterm and IUGR infants as early predictors of impaired neurodevelopment in later life (Aisa et al. 2020). In examining a heterogeneous population of full-term, preterm, IUGR and pre-IUGR neonates who significantly differed with respect GA and BW, in accordance with earlier reports, we found that at 2 y of age (or corrected age in the case of prematurity), the occurrence of impaired neurodevelopment was 9.9%, and this was strongly and significantly related to prematurity and IUGR in general (Gnanendran et al. 2015; Levine et al. 2015; Murray et al. 2015; Wang et al. 2016; Taine et al. 2018; Bolisetty et al. 2019; Aisa et al. 2020). The association was in the order pre-IUGR > IUGR > preterm and, conforming to GA and BW (Thompson et al. 2019), it was different from that recently obtained in a population of 37-wk GA full-term, moderate preterm and moderate IUGR neonates (Aisa et al. 2020). In the case of prematurity and IUGR, indeed, the correlation was not significant and less pronounced, respectively (Aisa et al. 2020). As observed before (Aisa et al. 2020), 3-DUS volumes of whole brain, thalamus, frontal cortex and cerebellum were significantly reduced in preterm, IUGR and pre-IUGR newborns in comparison to full-term newborns. In addition, with reference to prematurity or IUGR alone, the association of these two different conditions induced additional decreases in all cerebral volumes or in WBV and TV, respectively. As expected, the volumetric diminutions strongly correlated with GA and BW (Thompson et al. 2019). Evaluation of the 3-DUS volumes in the normal and abnormal neurodevelopment subgroups revealed significantly lower levels in neonates characterized by impaired outcome. This was also observed when comparing the respective categories of preterm, IUGR and pre-IUGR newborns (Aisa et al. 2020). Contrary to the normal neurodevelopment subgroup, the differences between categories were not evident in the abnormal neurodevelopment subgroup.

In agreement with the aforementioned findings, many cohort studies have highlighted similar evidence. Measurements of head circumference (a variable that correlates with WBV) at birth or of CV were found to be lower in preterm and VLBW or IUGR neonates and to associate with motor, cognitive and school performance in childhood (García-Alix et al. 2004; Cheong et al. 2008, 2016; Keunen et al. 2016; Matthews et al. 2018; Egashira et al. 2019; Wu et al. 2019; Bach et al. 2020; Hammerl et al. 2020). However, in contrast to our data, the association of IUGR with prematurity did not further impair CV in pre-IUGR newborns compared with preterm newborns, while TV was also affected (Bruno et al. 2017). To date, such a discrepancy in CV

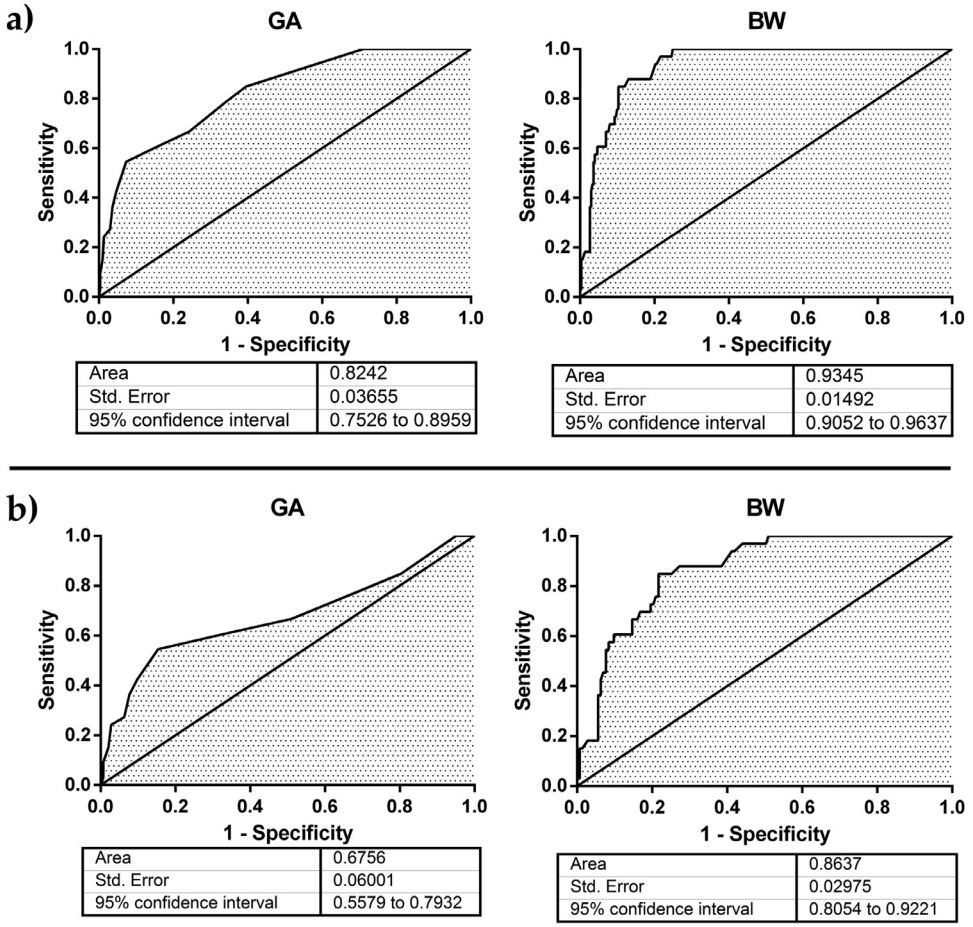


WBV: whole brain volume, TV: thalamus volume; FCV: frontal cortex volume; CV: cerebellum volume. ROC curves were assessed including (a) or excluding (b) values of full-term category.

Fig. 4. Receiver operating characteristic (ROC) curves of whole brain volume (WBV), thalamus volume (TV), frontal cortex volume (FCV) and cerebellum volume (CV).

is not clear; however, it may be owing to the different accuracy of MRI and US in volume detection.

Interestingly, thalamus growth in IUGR versus adequate-for-gestational-age participants followed the opposite trend depending on whether it occurred in fetal or perinatal life. In contrast to the reduction observed at 30−40 d of age, indeed, TV had a tendency to increase in IUGR fetuses (Green et al. 2016). The significance of this change is as yet unclear. However, it may be speculated that the reactive



GA: gestational age; BW: birth weight. ROC curves were assessed including (a) or excluding (b) values of full-term category.

Fig. 5. Receiver operating characteristic (ROC) curves of gestational age (GA) and birth weight (BW).

vasodilation of cerebral circulation, with the relatively greater blood supply and possible edema formation in the cerebral areas (owing to oxygen deficiency in IUGR fetuses [Hernandez-Andrade et al. 2008]), may cause a false increase in brain volumes in fetal life. This, in the early postnatal period, may be quickly lost, allowing cerebral areas to exhibit the real conditions in which the hormonal changes in IUGR placenta have decreased the proliferation and differentiation of neuronal cells with consequent reduction of growth (Baud and Berkane 2019).

Table 5. Cutoff and corresponding sensitivity and specificity values of the variables under investigation

	Cutoff (mL)		Sensitivity [95% CI] (%)		Specificity [95% CI] (%)	
	With	Without	With	Without	With	Without
WBV	356	354.5	94 [80–99]	91 [76–98]	93 [89–95]	86 [79–91]
TV	6.2	6.2	100 [89–100]	100 [89–100]	99 [98–100]	99 [95–99.8]
FCV	44	42	94 [80–99]	85 [68–95]	82 [77–86]	83 [75–88]
CV	10.5	10.2	100 [89–100]	97 [84–99.9]	97 [94–99]	95 [91–98]
GA	34.5	33	67 [48–82]	61 [42–77]	76 [70–80]	77 [71–83]
BW	1790	1750	88 [72–97]	85 [68–95]	87 [83–91]	84 [79–89]

With = with data of full-term category; Without = without data of full-term category; AUC = area under the receiver operating characteristic curve; WBV = whole brain volume; TV = thalamus volume; FCV = frontal cortex volume; CV = cerebellum volume; GA = gestational age; BW = birth weight; CI: confidence interval.

Finally, to deeply evaluate the relationship of neurodevelopment at 2 y with the variables examined, as well as to assess their prognostic accuracy or to look for a possible predictive model, we performed logistic regression analyses and calculated the respective AUCs. Remarkably, all brain volumes had excellent prognostic accuracy in general, although TV and CV were the best predictors. A significant predictive multivariate model could not be achieved because of multicollinearity problems. This indicated that the 3-DUS cerebral volumes may be used alone to recognize neonates at risk of impaired neurodevelopment.

The cutoff values of the 3-DUS cerebral volumes were also reported, thus providing the preliminary tools of reference. As expected and in accordance with differences in GA and BW (Thompson et al. 2019), the cutoff values of 3-DUS WBV, TV, FCV and CV were lower than those found previously (Aisa et al. 2020), suggesting that identification of GA- or BW-specific ranges for 3-DUS regional brain volumes could be used to determine neonates at long-term risk of impaired neurodevelopment more accurately. Further studies in this direction with larger populations are needed to find optimized values.

CONCLUSIONS

Recent progress achieved in the preventive, diagnostic and therapeutic fields, concerning both assistance to pregnant women and care of newborns, has led to a significant increase in the survival of preterm and IUGR neonates with GA <32 wk and BW <1000 g. Despite this, concurrent reduction in the incidence of various developmental disorders in these children has not been described (Keunen et al. 2016; Cheong et al. 2017; Pierrat et al. 2017). In this context, the identification of early and accurate predictors of long-term risk of impaired psychophysical, behavioral and sociorelational development in infants is urgent and crucial because it would facilitate the implementation of targeted therapies to improve developmental issues when neuroplasticity is optimal (Cheong et al. 2016; Keunen et al. 2016; Monson et al. 2016; Aisa et al. 2020).

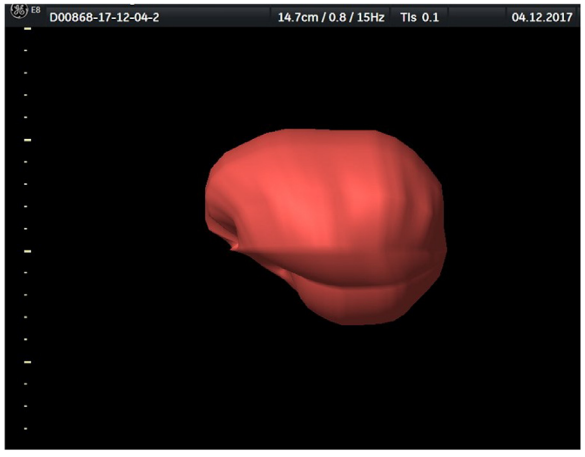


Fig. 6. Example of a 3-D ultrasonographic image of the whole brain volume.

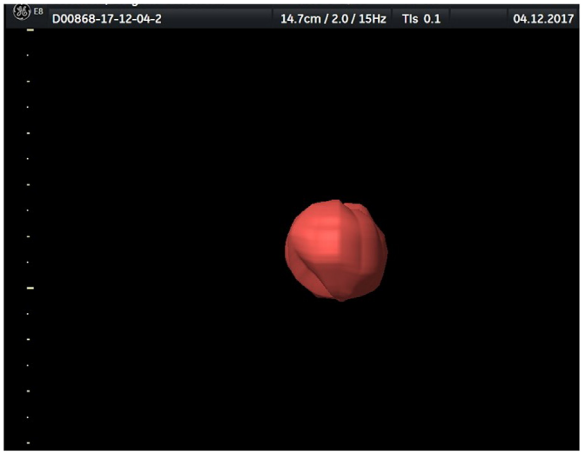


Fig. 7. Example of a 3-D ultrasonographic image of the thalmus.

Data from the present study may open wide perspectives in both pediatric neurology and pediatric neurobehavioral medicine, as well as highlight the fundamental role that the 3-DUS approach may play in neonatal cerebral imaging. The 3-DUS volumetric assessment at postnatal days 30–40 of the most important structures of the brain involved in neurodevelopment does indeed have all the features of a successful disease predictor, including excellent accuracy, high intra- and extra-operator reproducibility, little time and cost and ease of estimation. The possibility of easily and accurately measuring cerebral volumes (in Figs. 6 and 7 are two examples of 3-DUS images) at postnatal days 30–40 may provide specific long-term evaluations of the physical, behavioral or sociorelational areas, allowing a finer distinction between preterm and IUGR subjects at risk or not of delayed neurodevelopment. In addition, meeting the screening criteria, the 3-DUS assessment of these volumes may be used alone or in association with other approaches (Als et al. 2005; Provenzi et al. 2018) for the recognition of neonates eligible for early intervention therapies and neurodevelopmental follow-up during the first few years of life.

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