Ludovica (2008)
X, 4:121-140

Postnatal cerebellar development in preterms with postconceptional age at term equivalent A neuropathological study

Desarrollo post-natal del cerebelo en recién nacidos pretérmino con edad post-concepcional equivalente al término. Estudio neuropatológico

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Abstract

To evaluate postnatal development, we analyzed 65 cerebella from preterm neonates at term-gestational-age equivalent, separated into 2 groups: Group I (GI), cerebellar weight (cw) up to 14 g; Group II (GII), cw more than 14 g. The control group (CG) consisted of 20 term neonates up to 6 days old. Morphometry showed: diminished size, expressed as a coefficient (GI: 2.2; GII:2.9; CG:3.68) and cw [g] (GI:9.8; GII:17.9; CG:26.71); in the lobes, diminished foliar height [μm] (inferior folia; GI: 3.486; GII:4.764; CG:6.458) and foliation; diminished cortical width [μm] (GI:63.9; GII:74.5; CG:92.27), and a high number of Purkinje cells per segment (GI:33.6; GII:21.4; CG:13.95). These results correlated significantly with each other, with brain weight, and to a lesser degree with body weight. No high correlation with gestational age was found. Brain lesions and different serious and protracted illnesses were more frequent in GI. Histology: necrosis and apoptosis of the immature cerebellar cortex as well as reactive astrocytosis and gliosis of the white matter were observed. These findings related to hypoxia-ischemia, infections, and therapies.

In summary, the cases examined in this study evinced cerebellar patterns similar to those of 30-32 (GI) and 33-35 (GII) weeks of gestational age, although these preterm neonates had completed a postconceptional age of 37 to 42 weeks. These findings may be interpreted as having resulted from the action of noxa during the cerebellar lobes' vulnerability window. Direct injury of cerebellar cortex and white matter is a fundamental and poorly recognized cause of impaired cerebellar growth.

Keywords: cerebellum, foliation, white matter, hypoxia-ischemia, preterm neonates, neuropathology.

Resumen

Para evaluar el desarrollo postnatal se analizaron 65 cerebelos de recién nacidos pretérmino con edad postconcepcional equivalente al término en dos grupos (Grupo I (GI): peso cerebeloso (pc) hasta 14grs; Grupo II (GII): pc superior a 14grs), y 20 con-

troles (C) (recién nacidos de término hasta 6 días de vida). Se halló: reducción del tamaño (medido con un coeficiente) (GI:2,2; GII:2,9; C:3,68), y pc (en grs) (GI:9,8; GII:17,9; C:26,71); en hemisferios, reducción de altura de folias (en μm) (folias inferiores: GI:3486; GII:4764; C:6458) y su ramificación, del espesor cortical (mo-lecular + granos externa en μm) (GI:63,9; GII:74,5; C:92,27), y elevado número de células de Purkinje por segmento (GI:33,6; GII:21,4; C:13,95). Estos resultados se correlacionaron significativamente entre sí y con el peso corporal y cerebral. No se halló una alta correlación con la edad gestacional. Las lesiones cerebrales, y enfermedades ocurridas durante la hospitalización fueron más frecuentes en el GI. Además se halló necrosis y apoptosis en la corteza cerebelosa inmadura, y astrocitosis reactiva y gliosis en la sustancia blanca, vinculables a hipoxia-isquemia, infecciones y/o tratamientos.
En conclusión, los casos analizados presentaron una detención del desarrollo con un patrón similar al de cerebelos de 30-32 (GI) y 33-35 (GII) semanas de EG, a pesar de haber completado una edad postconcepcional equivalente al término. Los hallazgos pueden ser interpretados como resultado de la acción de noxas durante la *ventana de vulnerabilidad* de los hemisferios cerebelosos. Las lesiones primarias y directas de la corteza cerebelosa y de la sustancia blanca constituyen una causa fundamental y poco jerarquizada de alteración del desarrollo cerebeloso.

Palabras clave: cerebelo, foliación, sustancia blanca, encefalopatía hipóxico-isquémica, recién nacidos pretérmino, neuropatología.

Introduction

Extensive reports dealing with hypoxic-ischemic encephalopathy (HIE) describe basically the cerebral lesions, leaving but little space for the cerebellum [1]. Whereas the cerebrum is frequently the target for severe injuries, the cerebellum, particularly in the term neonate, remains apparently "protected" in the posterior cranial fossa [2, 3].
Although cerebellar compromise in the preterm neonate (PTN) is no new concept [4-11], in recent years reports have rediscovered that the acquired lesions especially HIE, and in particular those associated with periventricular leukomalacia (PVL) and periintraventricular hemorrhage (PIVH) are not the cerebrum's exclusive domain [12-18]. The later development of the cerebellum would be subsequently damaged as a consequence of preterm birth [12, 15, 19, 20-23], even in cases in which the lesion is slight and perhaps would not be recognized by magnetic-resonance imaging (MRI). This situation would be aggravated by the complications that frequently occur during postnatal life. Premature birth apparently opens windows of vulnerability that expose the immature cerebellum to multiple external risks.
Neurological and neuropsychological studies along with clinical and imaging follow-up in this group of patients have yielded surprising results not only in the immediate postnatal period but also throughout infancy and during puberty [8, 21-26]. This circumstance is important considering recent findings pointing to the role of the cerebellum in cognitive function as well as the relationship between cerebellar compromise and difficulties in academic development and the pursuit of a normal social life [25, 27-29].
Although in recent years MRI has contributed enormously to our understanding of the pathology of the CNS, the associated histologic and cellular changes can only be definitively ascertained through histopathological examination [30].

The present report describes the pathology of cerebella of PTNs who survived up to term equivalent postconceptional age with an aim at evaluating grossly and histologically whether there was an impairment of the cerebellar postnatal development. In such a case, and as a secondary hypothesis, this study attempted to find the real developmental stage reached by those cerebella. The results were compared to controls as well as to published data, in particular those pertaining to MRI.

Materials and methods

Sixty-five cerebella coming from PTNs —gestational age (GA) 28-36 weeks, postnatal age (PNA) 5-75 days— at term-GA equivalent (TGAE, where TGAE = GA + PNA) were studied. All necropsies were performed at "Superiora Sor María Ludovica" Children's Hospital (La Plata, Argentina) between March 1977 and June 2002 (most of our cases date back to the

period 1980-2000). The cases were selected depending only on the basis of GA and PNA. PTNs with genetic, malformative and/or disruptive CNS syndromes, multimalformative syndromes with evident CNS compromise, and CNS prenatal infectious diseases were not included.

The following data were also submitted to analysis: GA, PNA, postconceptional age (PCA), birth body weight (BBW), obstetric and perinatal data, and diseases suffered during the postnatal period. Low body weight (LBW) was defined as BBW of 1000 to 1500g, and extremely low body weight (ELBW) was considered as being a BBW of less than 1000 g, regardless of GA [31]. Additionally, BBW was analyzed in relation to GA.

Control cases (CG) were 20 cerebella coming from term newborns (GA: 37-42 weeks) up to 6 days PNA, with normal values for post-mortem body weight (BW), cerebral weight (CW), and cerebellar weight (cw). They presented congenital diaphragmatic hernia, bronchopneumonia, cardiovascular malformations of diverse complexities, adrenal hypoplasia, or bilateral renal hemorrhagic infarct as main diseases. Severe neuropathological changes were not found (see Results). These necropsies were performed between November 1978 and March 2006, and their results statistically matched with normal values for gross and histological measurements previously performed [32].

Preterm controls of 30-35 weeks of GA and up to 7 days of PNA were used for comparison with the developmental stage reached by all 65 cases. Those preterm controls had normal values for BW, CW and cw in relation to their corresponding GA. Main diseases were bronchopneumonia, hyaline membrane disease, renal infarct, necrotizing colitis and focal periventricular hemorrhage. Severe neuropathological changes were not found. Particularly, the cerebella were grossly and histologically unremarkable.

PATHOLOGY

1) GROSS PATHOLOGY

BW and CW were recorded in 64 cases, cw and other necropsy findings in 65, and the Jk coefficient (Jkc) [33] in 16. The calculation of the Jkc involves the product of three-dimensional diameters (transversal [a], craniocaudal [b], and dorsoventral [c] giving an estimate of cerebellar size, and results from [33]:

$$[(a).(b).(c)]^{1/3}$$

Extremely low cerebellar weight (ELcw) was defined as a cw of less than 10 g. Ventriculomegaly was diagnosed when ventricular enlargement was clearly evident.

2) HISTOLOGY

Morphometry and neuropathological changes were analyzed on 5-μm thick hematoxylin and eosin-stained sections of buffered-formalin-fixed, paraffin-embedded cerebellar tissue. Immunomarkers (GFAP and CD68) were used only in a few cases.

a) Morphometry

Measurements were made on slices of cerebellar lobes which included posterior (a folia) and inferior (b folia) areas and dentate nucleus [33], as follows (Figure 1):

(a) The degree of foliation was measured on b folia and was determined by means of a slight modification of standard histological criteria that classify the folia as primary, secondary, tertiary [33].

(b) Folia a height and folia b height was measured in μm from folium base to tip using a Zeiss measurement reticule with a scale of 1/100 mounted on a Zeiss Standard 18 light microscope with a 2.5X Planapochromatic objective and 10X oculars.

(c) The thickness of the external granular layer and of the molecular layer was measured in μm at b folia (top and lateral aspects) and expressed as the mean values of 5 determinations for each one of the layers through the use of a 25X Planapochromatic objective and 10X oculars.

(d) Cell density in the internal granular layer of b folia was scored qualitatively on a scale of one to three (+/+++) according to a published method [33] (Figure 2).

(e) The number of Purkinje cells corresponded to the cells counted in a linear segment of 980 μm in the

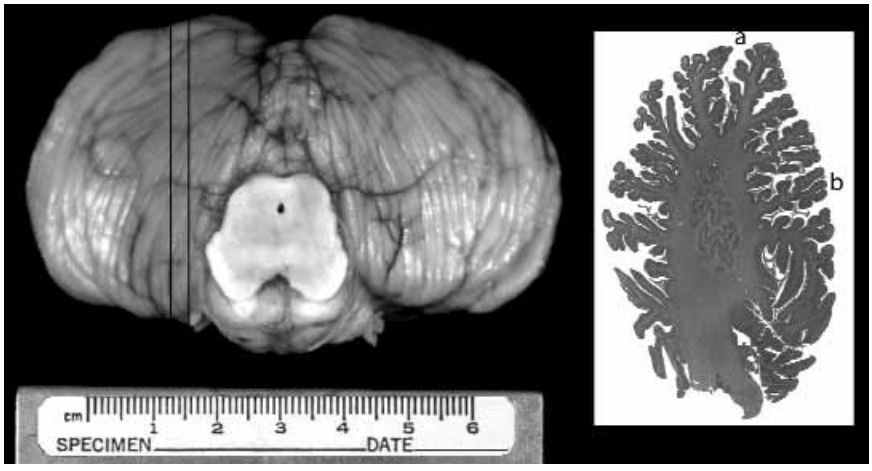

Figure 1. Left: dorsal aspect of the cerebellum. The parallel lines represent the slice of tissue shown at the right side of the figure. At this level the slice includes *lobulus semilunaris superior*, *lobulus semilunaris inferior*, *lobulus biventer* and *lobulus gracilis*, all of them belonging to the posterior lobule of the cerebellum (with permission) [33]. Right: photograph of the entire cerebellar hemispheric slice showing a folia, b folia and dentate nucleus as is usually used in our laboratory (with permission) [33].

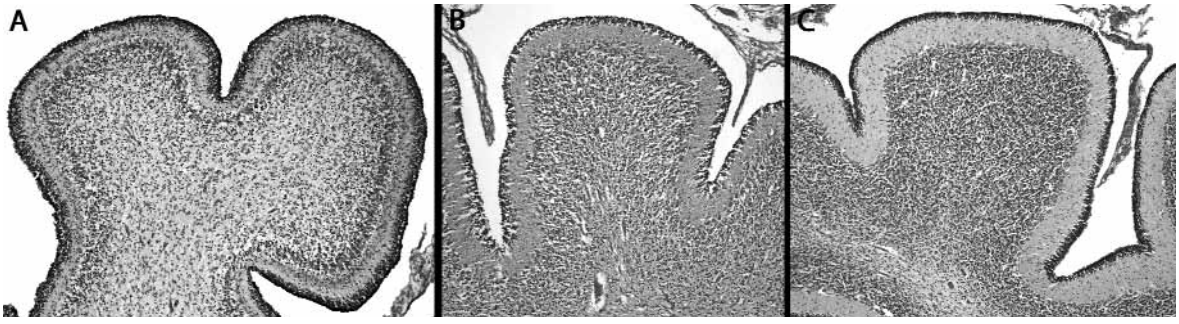

Figure 2. Nonparametric ranking of cell density in the internal granular layer; **A:** +/+++; **B:** ++/+++; **C:** +++/+++. Note that cell density has a parallel with internal granule layers' thickness (with permission) [33].

b folia. Only those cells whose nuclei were clearly visible were scored. In each case, the data were the average of 5 determinations in different areas of the b folia (top and lateral aspects).

(f) The number of neurons in the dentate nucleus corresponded to those counted within a segment of the dentate nucleus of 980 μm long. Only those cells whose nuclei were clearly visible were scored. In each instance, the data were expressed as the mean value of 5 determinations from different areas of the dentate nucleus.

b) Neuropathological findings

The cortex, white matter, and dentate nucleus of each specimen were examined although not quantified for the presence of neuronal necrosis, apoptosis, astroglial reactions, macrophages, inflammatory infiltrates, focal necrosis, edema and hemorrhage. The above measurements and neuropathological findings in PTNs and CG were analyzed without knowing the clinical and gross-pathological data.

3) **Groups**

The 65 cases were arranged according to increasing values of cw: Group I (GI), cw ≤ 14 g (35 cases, 54%); Group II (GII), cw ≥ 15 g (30 cases, 46%). To our knowledge, in the existent literature there is no classification of "normal" cw in preterms at TGAE that could serve as a guide to define the aforementioned groups; therefore, the limit between the 2 groups was defined by taking into account some statistical characteristics of the present cohort.

4) **Subgroups**

Two additional subgroups were designed: ELcw

(GI: 14 cases), and LBW/ELBW (GI: 22 cases; GII: 9 cases).

5) CEREBRUM

Gross and histological findings of the 65 cases were compiled for comparison with data coming from cerebellar tissue.

6) STATISTICS

All values were expressed as mean +/- standard deviation. Statistical analyses were undertaken with SPSS for Windows. Calculations included comparisons of quantitative measurements across GI and GII and between them and CG through the use of the Mann-Whitney test. For comparisons of qualitative data the Chi-Square test was used. The correlation between quantitative data was evaluated through the Spearman coefficient. Scatter plots and Box plots were performed.

CG data were compared with those already published; the latter were taken as normal [32, 34-47]. Differences were considered statistically significant at $p < 0.05$.

Results

COMPARISON BETWEEN CONTROL GROUP AND NORMAL DATA

No statistically significant differences were found between the gross and histological values of CG and already published normal data [32].

CLINICAL DATA AND GROSS OBSERVATIONS

Compared to GII, GI showed a greater incidence of idiopathic respiratory distress syndrome (IRDS) (GI: 19/35, 54.3%; GII: 9/30, 30%; p=0.0487), and lesions of the CNS; the most frequent of the latter being PVL (GI: 20/35, 57.1%; GII: 11/30, 36.7%)

Table 1a. Number (n) and proportion (%) of cases with perinatal complications and lesions in CNS, and in other sites in two groups of preterm infants (GI, GII)

		Clinical data			Necropsy findings	
Group (cases)		**Obstetrical** (*)	**IRDS**	**Others** (**)	**CNS (+)**	**Others (++)**
G I	%	51.4	54.3	68.6	82.8	94.3
(35)	**(n)**	(18)	(19)	(24)	(29)	(33)
G II	%	46.7	30	53.3	63.3	83.3
(30)	**(n)**	(14)	(9)	(16)	(19)	(25)

IRDS, idiopathic respiratory distress syndrome. (*) Premature rupture, chorioamnionitis, placenta previa, abruptio placentae, nuchal umbilical chord entanglement, gestosis, cesarea, breech presentation. (**) Developed while in hospital: necrotizing enterocolitis, meconium ileus, gastric perforation, gastrostomy, ileostomy, peri-intraventricular hemorrhage (PIVH), jaundice, hyperbilirubinemia, hyper/hypoglicemia, poliglobulia, anemia, seizures. (+) Hypoxic-ischaemic encephalopathy (HIE), sequelar HIE, periventricular leukomalacia, PIVH, sequelae of PIVH, cerebral cortical necrosis, massive cerebral necrosis, pontosubicular necrosis, leptomeningitis, meningoencephalitis, septic emboli, hidrocephalus. (++) Necrotizing enterocolitis, bronchoneumonia, bronchopulmonary dysplasia, amniotic fluid aspiration, necrotizing tracheobronchitis, necrotizing gastroenteritis, disseminated intravascular coagulation, sepsis, patent ductus arteriosus. Some patients had more than one disease.

Table 1b. Number (n) and proportion (%) of cases with perinatal complications and lesions in CNS, and in other sites in subgroups of preterm infants

		Clinical data			Necropsy findings	
Subgroup (cases)		**Obstetrical** (*)	**IRDS**	**Others** (**)	**CNS (+)**	**Others (++)**
LBW/ELBW	%	54.8	51.6	67.7	77.4	87
(31)	**(n)**	(17)	(16)	(21)	(24)	(27)
ELcw	%	35.7	57.1	85.7	85.7	92.8
(14)	**(n)**	(5)	(8)	(12)	(12)	(13)

LBW, low body weight; ELBW, extreme low body weight; ELcw, extremely low cerebellar weight. IRDS, idiopathic respiratory distress syndrome. (*) Premature rupture, chorioamnionitis, placenta previa, abruptio placentae, gestosis, cesarea, breech presentation. (**) Developed while in hospital: necrotizing enterocolitis, gastrostomy, ileostomy, jaundice, hyper/hypoglicemia, poliglobulia, anemia. (+) Hypoxic-ischaemic encephalopathy (HIE), sequelar HIE, periventricular leukomalacia, peri-intraventricular hemorrhage (PIVH), sequelae of PIVH, cerebral cortical necrosis, pontosubicular necrosis, leptomeningitis, meningoencephalitis, septic emboli, hydrocephalus with ventriculomegaly. (++) Necrotizing enterocolitis, bronchoneumonia, bronchopulmonary dysplasia, sepsis, patent ductus arteriosus.

and PIVH (GI: 12/35, 34.3% -6 with ventriculomegaly-; GII: 4/30, 13.3% -2 with ventriculomegaly-; p=0.05) (Table 1a; Table 1b; Figure 3).
The GA was similar in both groups, and BBW was the lowest in GI (Table 2a); 35 out of 64 cases (55%) presented with low body weight for their GA (GI: 25/34, 74%; GII: 10/30, 33%); 31 out of 64 cases (48%) had LBW/ELBW (Table 2b) (GI: 22/34, 65%; GII: 9/30, 30%), and 28 out of 64 cases (44%) showed low body weight for their GA and LBW/ELBW (GI: 22/34, 65%; GII: 6/30, 20%). The values for BW (similar to those for BBW) as well as the data for CW, and cw were also much lower in GI; 14 out of 65 cases (21.53%) with ELcw also belonged to this group (Table 2b). The cases in which the Jkc was calculated had values that were lower than the ones found for the CG (Table 2a; Table 2b). The percentage of the cases with normal values for BW, CW, and cw within the total sampling (GI + GII) was 3.12, 4.68, and 1.53, respectively.

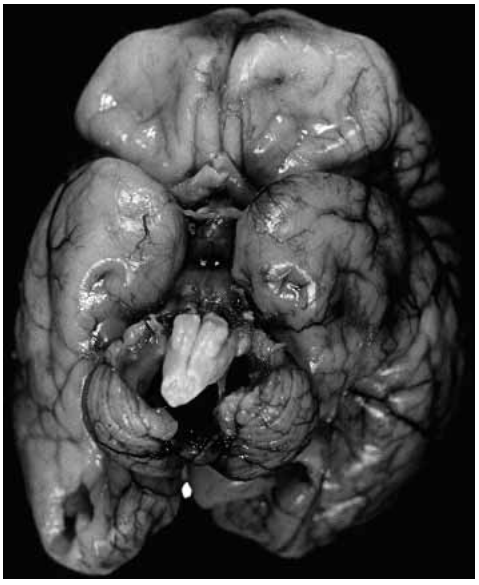

Figure 3. Case 21; GA: 32 weeks; PNA: 45 days; BW: 1120 g; CW: 210 g; cw: 10 g. Enlargement of the 4th ventricle as a result of post-hemorrhagic ventriculomegaly. The cerebellar parenchyma, particularly at the lobes, is very thin.

Table 2a. Mean and standard deviation of age and gross measurements for two groups of preterm infants (GI, GII) and control group (CG), and p-value for comparisons GI vs GII vs CG (Mann-Whitney test)

Group		PNA	GA	PCA	BBW	BW	CW	cw	Jkc
G I (35)	Mean	33	33.7	38.5	1402	1367	204	9.8	2.2
	± sd	± 13.6	± 1.8	± 1.5	± 478	± 423	± 40	± 2.5	± 0.3
	(n)	(35)	(35)	(35)	(34)	(35)	(35)	(35)	(11)
	p-value		0.085	0.035	0.002	0.000	0.000	0.000	0.004
G II (30)	Mean	34	34.3	39.3	1793	1758	293	17.9	2.9
	± sd	± 17.6	± 1.9	± 1.5	± 534	± 476	± 53	± 2.5	± 0.2
	(n)	(30)	(30)	(30)	(30)	(29)	(29)	(30)	(5)
	p-value		0.000	0.866	0.000	0.000	0.000	0.000	0.001
C G (20)	Mean	3,1	38.9	39.2	3197	3197	395	27	3.7
	± sd	± 2	± 1.2	± 1	± 514	± 514	± 43	± 5	± 0.3

PNA, postnatal age; GA, gestational age; PCA, post-conceptional age; BBW, birth body weight; BW, body weight at necropsy; CW, cerebral weight; cw, cerebellar weight; Jkc, Jk coefficient.

Table 2b. Mean and standard deviation of age and gross measurements in subgroups of preterm infants

Subgroup		PNA	GA	PCA	BBW	BW	CW	cw	Jkc
LBW/ELBW (31)	Mean	38	33.1	38.7	1140	1184	221	11.4	2.24
	± sd	± 15	± 1.9	± 1.4	± 181	± 221	± 52	± 4.4	± 0.44
	(n)	(31)	(31)	(31)	(31)	(30)	(31)	(31)	(8)
ELcw (14)	Mean	38	32.9	38.4	1204	1152	181	7.26	2.11
	± sd	± 16	± 2.1	± 1.3	± 323	± 307	± 36	± 1.11	± 0.32
	(n)	(14)	(14)	(14)	(13)	(14)	(14)	(14)	(7)

LBW, low body weight; ELBW, extreme low body weight; ELcw, extremely low cerebellar weight. PNA, postnatal age; GA, gestational age; PCA, post-conceptional age; BBW, birth body weight; BW, body weight at necropsy; CW, cerebral weight; cw, cerebellar weight; Jkc, Jk coefficient.

HISTOLOGY

Morphometry

The GI values for foliation and the height of a and b folia were below CG's values (Table 3; Table 4a; Table 4b; Figure 4). When 4,000 μm were taken as a height limit for b folia among the 58 cases where

Table 3. Number (n) and proportion (%) of cases for different grades of foliation (F) in two groups of preterm infants (GI, GII) and control group (CG), and p-value for Chi-square test (p = 0.000)

Group		F		
		1	2	3
G I (33)	% (n)	39.4 (13)	60.6 (20)	0.0 (0)
G II (27)	% (n)	3.7 (1)	70.4 (19)	25.9 (7)
C G (20)	% (n)	0.0 (0)	40 (8)	60 (12)

Chi-square p = 0.000

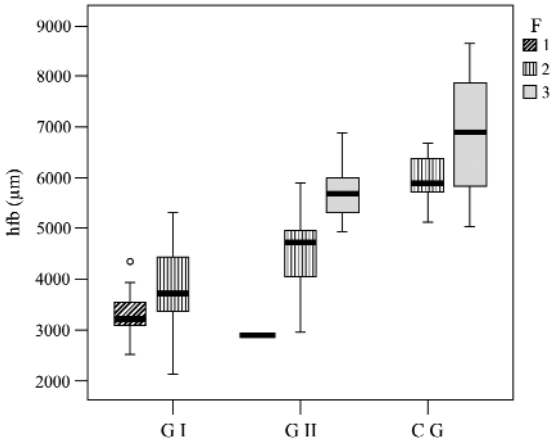

Figure 4. Box plot of folia b height for three grades of foliation within Group I, Group II and control group. Diagonal hashing: distribution of folia b height for cases with grade 1 foliation, corresponding to Group I (GI) and Group II (GII). There are no cases with grade 1 foliation in control group (CG). Vertical hashing: distribution of folia b height for cases with grade 2 foliation in GI, GII and CG. Gray shading: distribution of folia b height for cases with grade 3 foliation in GII and CG. There are no cases with grade 3 foliation in GI. F, foliation; hfb, folia b height.

Table 4a. Mean and standard deviation of histological measurements for two groups of preterm infants (GI, GII) and control group (CG), and p-value for comparisons GI vs. GII and GII vs CG (Mann-Whitney test)

Group		hfa	hfb	ext gr	mol	ext gr+mol	P	D
G I (35)	Mean ± sd (n)	4486 ± 1263 (27)	3487 ± 946 (32)	28.0 ± 7.9 (35)	35.8 ± 10.2 (35)	63.8 ± 14.0 (35)	33.5 ± 9.0 (32)	28.0 ± 7.1 (33)
	p-value	0.000	0.000	0.357	0.000	0.029	0.000	0.302
G II (30)	Mean ± sd (n)	6338 ± 1268 (22)	4764 ± 957 (26)	26.2 ± 6.9 (30)	48.4 ± 16.0 (30)	74.6 ± 17.9 (30)	21.5 ± 4.4 (30)	25.9 ± 5.7 (27)
	p-value	0.000	0.000	0.469	0.000	0.002	0.000	0.261
C G (20)	Mean ± sd	8704 ± 1704	6468 ± 1042	25.4 ± 8.3	68.9 ± 14.8	94.4 ± 19.6	13.7 ± 4.2	27.6 ± 4.0

hfa, folia a height; hfb, folia b height; ext gr, external granular layer thickness; mol, molecular layer thickness; P, Purkinje cells per segment; D, dentate nucleus neurons per segment.

Table 4b. Mean and standard deviation of histological measurements in subgroups of preterm infants

Subgroup		hfa	hfb	ext gr	mol	ext gr+mol	P	D
LBW/ELBW (31)	Mean ± sd (n)	4978 ± 1256 (23)	3894 ± 1034 (28)	28.2 ± 8.0 (31)	40.2 ± 14.8 (31)	68.5 ± 18.1 (31)	30.5 ± 10.9 (30)	27.7 ± 6.9 (29)
ELcw (14)	Mean ± sd (n)	3970 ± 1415 (13)	3015 ± 1074 (14)	27.4 ± 9.1 (14)	32.6 ± 8.8 (14)	60.0 ± 14.4 (14)	37.2 ± 11.6 (13)	27.0 ± 7.8 (13)

LBW, low body weight; ELBW, extremely low body weight; ELcw, extremely low cerebellar weight; hfa, folia a height; hfb, folia b height; ext gr, external granular layer thickness; mol, molecular layer thickness; P, Purkinje cells per segment; D, dentate nucleus neurons per segment.

this measurement could be made, 31 (53%) failed to attain that value (GI: 25/32, 78%; GII: 6/26, 23%) (Figure 5). In 29 (93.5%) of these latter cases (GI: 23/25, 92%; GII: 6/6, 100%), a serious cerebral lesion was present (massive cerebral necrosis, PIVH, PVL, or diffuse gliosis of the white matter); of those 29 cases, 15 (52%) presented foliar necrosis, hemorrhage, embolic microabscesses, reactive astrocytosis, gliosis and edema in the cortex and white matter, 11 (38%) revealed diffuse cellular lesions and edema, and the remaining 3 (10%) showed isolated neuronal necrosis in the Purkinje cells and dentate nucleus as the sole lesion. Of these last 3 cases, 2 exhibited bronchopulmonary dysplasia.

In both groups the height of the external granular layer was similar, and had little difference with the CG (Figure 6). In GI, the external granular and molecular layers combined had a height lower than 71.0 μm (CG: 92.3 ± 21.3 μm) in 25 out of 35 cases (71%) (Table 4a; Figure 7). The internal granular layer failed to show a value of 3 in any of the cases belonging to GI. In GII, 6 out of 30 cases (20%) reached a value of 3, whereas in CG 15 out of 20 cases (75%) got a value of 3, and another 5 cases (25%) got a value of 2 (Table 5). In GI and GII the number of Purkinje cells per segment was higher than the one of CG (p=0.000), being in GI higher than in GII (p=0.000) (Table 4a; Figure 8). The estimation of the number of neurons in the dentate nucleus yielded similar mean values in both groups and in controls. The proportion of cases with abnormal results was lower in this portion of

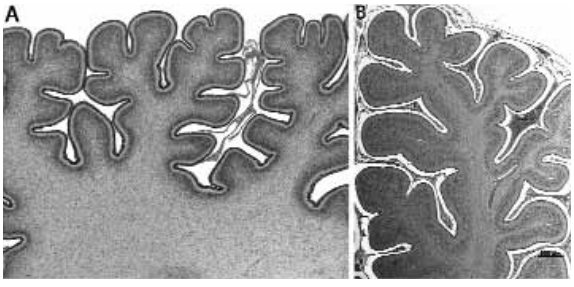

Figure 5. A: same case as Figure 3. Histological section of inferior portion of cerebellar lobe (b folia). Foliar height as well as foliation is diminished compared to control; **B:** control case. GA, 40 weeks; PNA, 2 days; BW, 2500 g; CW, 400 g. cw, 28 g. Scale bar: 500 μm (A, B).

Table 5. Number (n) and proportion (%) of cases with different grades of cell density in the internal granular layer (Int gr) in two groups of preterm infants (GI, GII) and control group (CG), and p-value for Chi-square test (p = 0.000)

Group		Int gr		
		1	2	3
G I (35)	% (n)	57.1 (20)	42.9 (15)	0.0 (0)
G II (30)	% (n)	23.3 (7)	56.7 (17)	20.0 (6)
C G (20)	% (n)	0.0 (0)	25 (5)	75 (15)

Chi-square p = 0.000

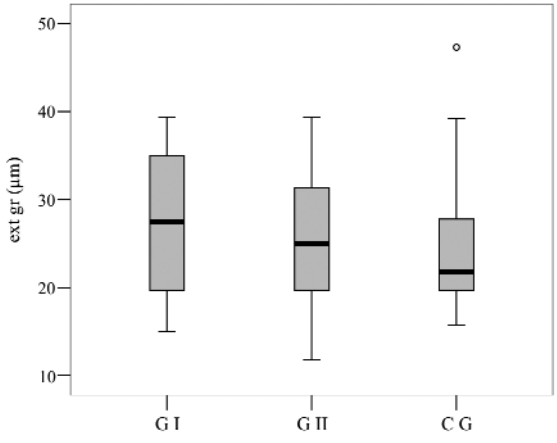

Figure 6. Box plot of external granular layer thickness, within Group I, Group II and control group. Ext gr, external granular layer thickness; GI, Group I; GII, Group II; CG, control group.

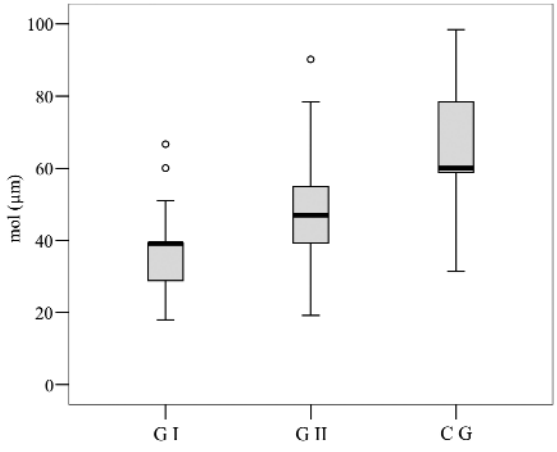

Figure 7. Box plot of molecular layer thickness, within Group I, Group II and control group. Mol: molecular layer thickness; GI, Group I; GII, Group II; CG, control group.

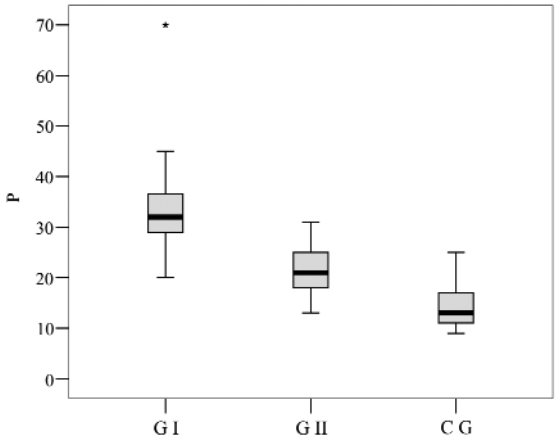

Figure 8. Box plot of Purkinje cells per segment, within Group I, Group II and control group. P, Purkinje cells per segment; GI, Group I; GII, Group II; CG, control group.

the cerebellum than that found for the rest of the histological parameters in both groups.

The proportion of the cases in GII that showed normal values was greater than that found in GI (Table 6).

Neuropathological findings

Neuropathological changes were found in cerebellar cortex as well as in white matter (Figure 9, A-I; Figure 10; Figure 11, A-I). Although not quantified, apoptosis was present in numerous cells of the external and internal granular layers. The cell density of the internal granular layer varied in direct proportion to its thickness, presenting both of them an

Table 6. Proportion (%) of cases that showed abnormal values in groups and subgroups of preterm neonates at term gestational age equivalent

Group	BBW	LBW GA	BW	CW	cw	Jkc	F	hfa	hfb	gr ext +mol	gr int	P
G I (35)	64.7	73.5	97.1	100	100	100	100	100	100	71.4	100	100
G II (30)	30.0	33.3	96.5	89.6	96.6	100	74	68.1	76.9	56.6	80	80
LBW/ELBW (31)	100.0	90.3	100	100	100	100	96.6	91.3	92.8	70.9	90	83.3
ELcw (14)	85.7	85.7	100	100	100	100	100	100	100	78.5	100	100

LBW, low body weight; ELBW, extreme low body weight; ELcw, extremely low cerebellar weight; BBW, birth body weight; LBWGA, low body weight in relation to gestational age; BW, body weight at necropsy; CW, cerebral weight; cw, cerebellar weight; Jkc, Jk coefficient; F, foliation; hfa, folia a height; hfb, folia b height; ext gr, external granular layer thickness; mol, molecular layer thickness; gr int, internal granular layer cell density; P, Purkinje cells per segment.

Table 7. Values of body weight, cerebral weight, and cerebellar weight in groups of preterm neonates at term gestational age equivalent (GI and GII), and in preterm neonates' controls (preterm neonates at 30-32/33-35 weeks gestational age, and up to 7 days postnatal age)

	BW	CW	cw	cw/CW	PCA	histol Eq
G I	1367 (850-3000)	204 (112-288)	9.8 (5-14)	0.05	37-42	30-32
G II	1758 (900-2780)	293 (185-421)	17.9 (15-25)	0.06	37-42	33-35
PTN (30-32 w)	1300 (1000-1600)	180 (115-306)	9.6 (6-15)	0.05	-	-
PTN (33-35 w)	1800 (1600-2000)	237 (150-342)	13.5 (9-21)	0.06	-	-

Group I and 30-32 weeks gestational age (GA) preterm neonates' mean values are similar. Mean values of Group II correlate well with those of 33-35 weeks GA preterm neonates. This equivalence was also seen histologically (see text and Figure 11). w, weeks GA; BW, body weight at necropsy; CW, cerebral weight; cw, cerebellar weight; PCA, post-conceptional age; histol Eq, GA histological equivalence.

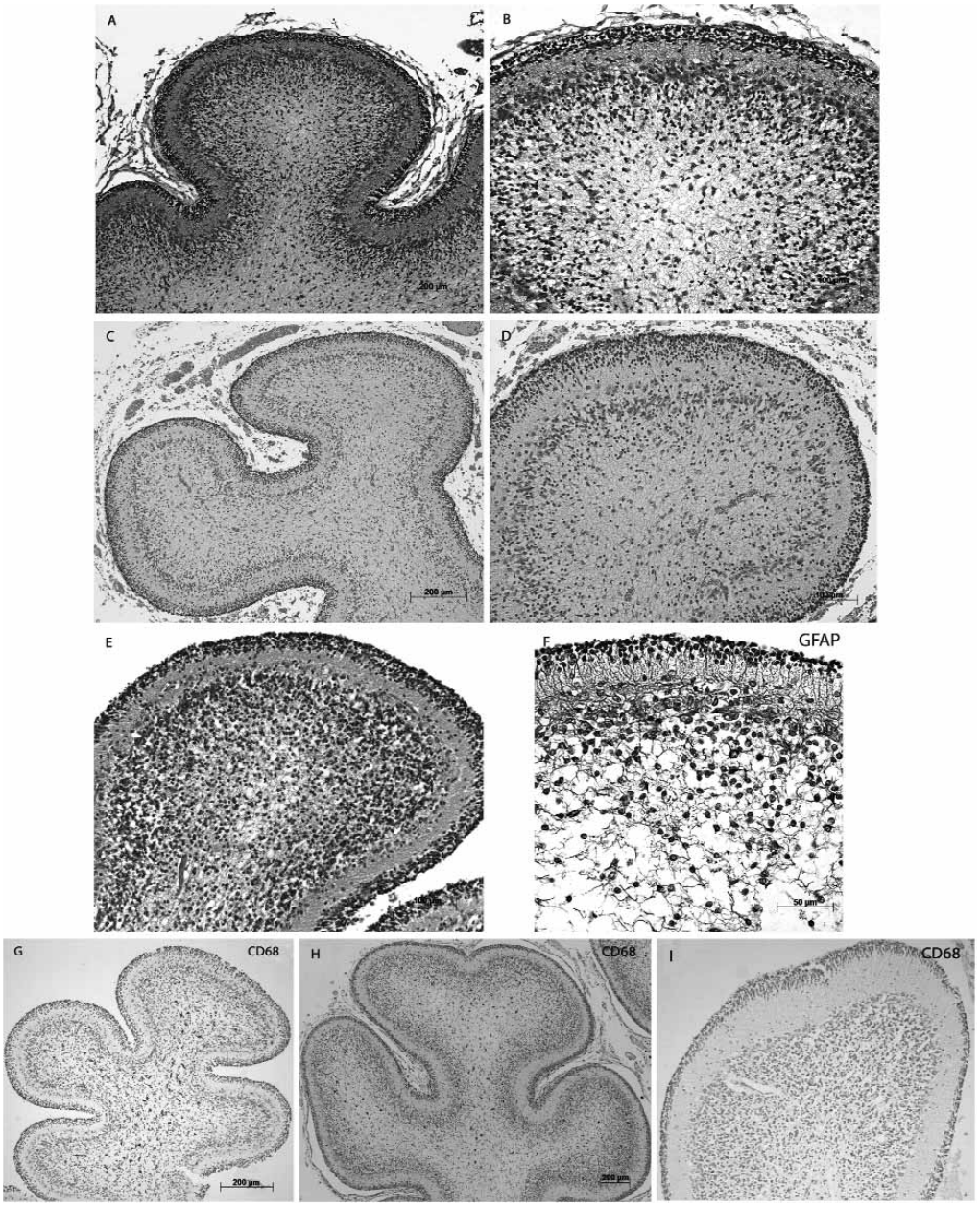

Figure 9. **A:** Case 41; GA: 36 weeks; PNA: 26 days; BW: 1200 g; CW: 310 g; cw: 15 g. Histological section of inferior folia (b folia). Immature cortical layers as well as edema and reactive astrocytosis of the foliar white matter are seen; **B:** Higher magnification shows preserved external granular layer, narrow molecular layer, high number of Purkinje cells per segment, and diminished internal granular layer with sparse neurons and interspersed reactive astrocytes. Some cells look apoptotic; **C:** Case 50; GA: 35 weeks; PNA: 27 days; BW: 2120 g; CW: 280 g; cw: 17 g. Histological section of b folia. Cortical layers look very immature and, notably, although external granule cells are greatly preserved, internal ones have nearly disappeared. *N.B.*, edema, microvacuoles and reactive astrocytosis in subcortical white matter; **D:** At a higher magnification, molecular layer shows external granule cells migrating inwards; Purkinje cells are small, numerous, and seem to be immature. Apoptosis is seen mostly in internal granular layer; **E:** Case 16. GA: 34 weeks; PNA: 26 days; BW: 1260 g; CW: 215 g; cw: 10 g. Histological section of b folia. White matter at the center of the folia shows edema, microvacuoles, and tiny foci of necrosis along with reactive astrocytosis. Molecular layer is thin but granule cells seem to be preserved; **F:** Case 15. GA: 33 weeks; PNA: 33 days; BW: 1640 g; CW: 246 g; cw: 10 g. This section of b folia shows a narrow cortex with Bergman glia traversing the molecular layer. Internal granule cells have nearly disappeared. Some interspersed reactive astrocytes are seen in the subcortical white matter as well as apoptotic cells, perhaps belonging to immature oligodendroglia. Note vacuolization and cystic change at that level; **G:** Same case as Figure 3. Histological section of b folia. White matter shows numerous cells with a positive reaction to CD68 antigen; scattered cells with similar characteristics are seen in the cortex; **H:** Same case as Figure 9 A. Histological section of b folia. Some CD68 positive cells are regularly distributed at the level of whitte matter and cortex; **I:** Control case. Histological section of b folia. Figure 9 G and 9 I have the same scale bar.

inverse proportion to the apparent (not quantified) number of apoptotic cells (Figure 9 A-D). The Purkinje cells and the neurons of the dentate nucleus frequently showed necrosis and only infrequently apoptosis.

The compromise of the white matter was evident in the majority of the cases as edema and reactive astrocytosis, which were more intense in the center of the folia than in the peripheral portion. Additionally diffuse gliosis and/or small foci of necrosis (with or without cystic transformation) were found (Figure 9 E,F). Microglial cells with rod-shaped nuclei and/or enlarged processes, as well as macrophages, were seen (Figure 9 G-I).

In general, the most severe compromise was observed in the specimens with lesser cerebellar development. Nineteen out of 65 cases (29%) (GI: 16/35, 46%; GII: 3/30, 10%) presented with necrosis and/or intraparenchymatous hemorrhage of diverse magnitude and chronicity, while 7/65 (11%) (GI: 5/35, 14%; GII: 2/30, 7%) showed embolic microabscesses or leptomeningitis. Otherwise, the cases with greater GA but with LBW/ELBW and a longer survival period were frequently accompanied by more serious cerebellar lesions, independently to which group they belonged (Figure 9 A,B; Figure 11, B).

The average gross and microscopic data in the present study failed to reach normal values, remaining arrested at figures corresponding to cerebella of GA 30-32 weeks (GI) or 33-35 weeks (GII) [32, 41, 45] (Table 7; Figure 11, A-I).

When cerebellar neuropathological findings were compared with the ones compiled for the cerebrum, at least 3 instances seemed to appear.

a) Hemorrhage, necrosis, gliosis solely in the cerebellum; cerebrum with slight focal cellular lesions

This group included 8 cases (GI: 3; GII: 5) with a cw average of 15 g. These patients had had necrotizing enterocolitis, peritonitis, sepsis, bronchopneumonia, or pulmonary lesions due to oxygen toxicity.

b) Hemorrhage, necrosis, gliosis solely in the cerebrum; cerebellum with slight focal cellular lesions

Five cases presented this pattern (GI: 1; GII: 4; with a cw average of 13.2 g). These patients had had ventriculomegaly after PIVH, bronchopneumonia, pulmonary lesions due to oxygen toxicity, or patent ductus arteriosus with bilateral cardiac hypertrophy.

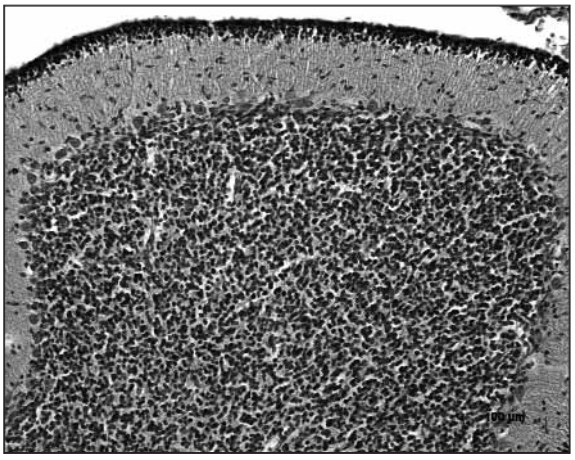

Figure 10. Case 65. GA: 30 weeks; PNA: 60 days; BW: 1800; CW: 320 g; cw: 25 g. Histological aspect of b folia appears greatly preserved in this case, although more Purkinje cells per segment were found compared to controls.

c) Focal cellular necrosis as the sole finding in cerebrum and cerebellum

This diagnosis comprised 4 cases (GII) with a cw average of 21.5 g (Figure 10). The associated illnesses were sepsis, bronchopneumonia or pseudomembraneous colitis.

The remaining cases (74%) presented variable degrees of supra- and infratentorial HIE as the main disease.

Subgroups

The 14 cases with ELcw showed extremely low values, both gross and histological; the 31 cases with LBW/ELBW presented values that, although very low, were similar to those found in GI (Table 1b, Table 2b, Table 4b and Table 6).

Control group

The control specimens consisted of 20 cases, of which 4 (20%) had suffered from congenital diaphragmatic hernia* and the remainder had had either bronchopneumonia, cardiovascular malformations of diverse complexities, adrenal hypoplasia, or bilateral renal

* Of the 43 cases with this diagnosis in our records, only 2 presented with low cw, one of these having trisomy 18, to which imbalance this alteration is usually attributed, and 5 were PTNs with cw in accordance to GA, PNA and BW.

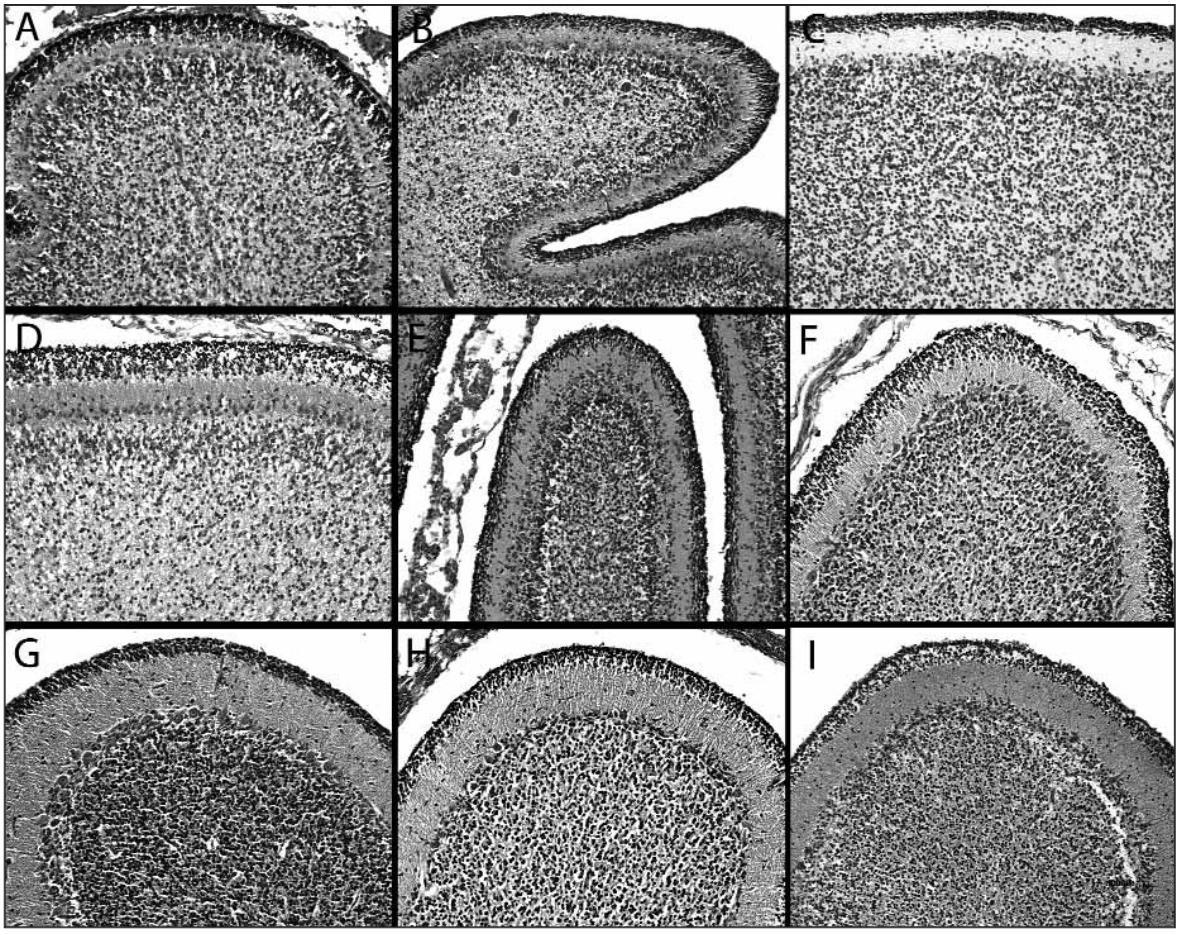

Figure 11. A, B, C correspond to histological sections of cases 8, 2, and 51, respectively. The images suggest an approximate maturation of 31, 32, and 35 weeks GA, although these patients have completed a TGAE. **D, E, F** correspond to histological controls of immature GA (GA: 31, 32, and 35 weeks, respectively; PNA up to 3 days). **G, H, I** are term neonates from CG (GA: 38 to 40 weeks; PNA: up to 2 days). **Data of the cases:** Case 8: GA, 28 weeks; PNA, 75 days; BW, 1530 g; CW, 242 g; cw, 8 g. Case 2: GA, 34 weeks; PNA, 33 days; BW, 930 g; CW, 130 g; cw, 6 g. Case 51: GA, 35 weeks; PNA, 40 days; BW, 1800 g; CW, 320 g; cw, 18 g. Scale bar: 100 μm (A-I).

hemorrhagic infarct as main diseases. The main and only neuropathological finding proved to be acute neuronal necrosis in 9 cases (45%). The results of the gross and microscopical measurements (Table 2a, Table 2b, Table 4a, Table 4b) were comparable to those previously published [32] (p for all values not significant).

Correlations BW correlated positively with CW, cw, Jkc, folia a height, and folia b height, and negatively with Purkinje cell layer. CW, cw, Jkc, folia a height, folia b height, and molecular layer correlated positively with each other. Molecular layer correlated negatively with Purkinje cell layer. GA correlated positively with CW and cw (the latter correlation being weak). GA did not correlate with histological measurements. The thickness of the external granular layer and the number of neurons in the dentate nucleus failed to correlate with any parameter (Table 8; Figure 12; Figure 13).

Discussion

During gestation the cerebellum undergoes a regulated and predictable development with a dynamic cortical growth and a changing morphology from week to week. This process is so consistent as to be used for a macroscopic [48] as well as histological [49]

Table 8. Spearman Correlation Coefficients

	BW	CW	cw	Jkc	hfa	hfb	ext gr	mol	P	D
GA	.536***	.338**	.282*	.339	.244	.177	-.016	-.081	-.102	-.123
BW	1.	.603***	.601***	.573*	.412**	.401**	-.048	.150	-.430**	-.017
CW		1.	.815***	.732**	.680***	.530***	-.117	.415**	-.632**	-.058
cw			1.	.811***	.733***	.661***	-.112	.542***	-.752***	-.023
Jkc				1.	.524*	.626**	.267	.483	-.506	-.052
hfa					1.	.783***	-.204	.633***	-.597***	-.003
hfb						1.	.005	.557***	-.539***	-.152
ext gr							1.	.004	.223	-.030
mol								1.	-.478***	-.011
P									1.	.308*
D										1.

Entire sample (Group I plus Group II) was used. Control group was not included. GA, gestational age; BW, body weight at necropsy; CW, cerebral weight; cw, cerebellar weight; Jkc, Jk coefficient; hfa, folia a height; hfb, folia b height; ext gr, external granular layer thickness; mol, molecular layer thickness; P, Purkinje cells per segment; D, dentate nucleus neurons per segment.
***: p<0.001; **: p<0.01; *: p<0.05

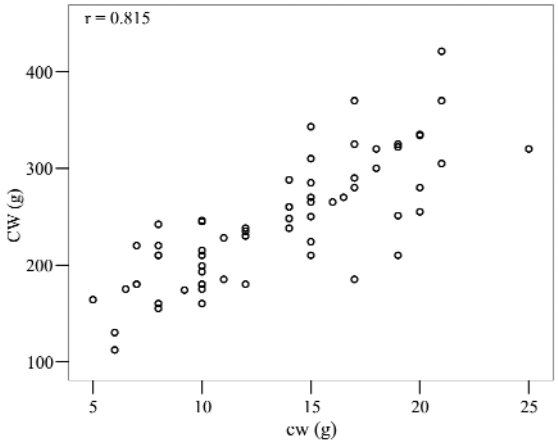

Figure 12. Scatter plot of cerebral weight against cerebellar weight. Entire sample (Group I plus Group II) was used. Control group was not included. CW, cerebral weight; cw, cerebellar weight.

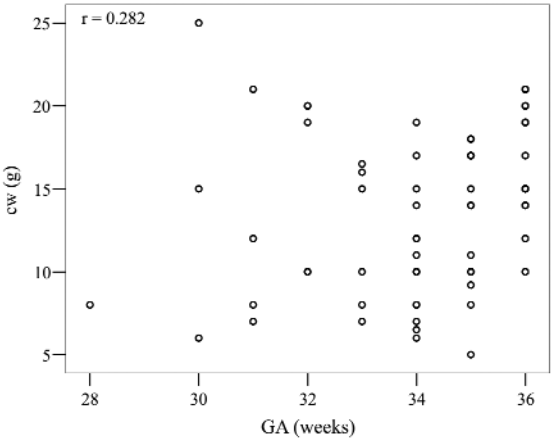

Figure 13. Scatter plot of cerebellar weight against gestational age. Entire sample (Group I plus Group II) was used. Control group was not included. cw, cerebellar weight; GA, gestational age.

index of GA. Nevertheless, the diverse parts of the cerebellum develop within individual time frames and possess different functions [22], and both of these characteristics can impinge on clinical pattern and histopathology [14, 16].

Preterm birth —of a steady incidence, and on the rise in certain parts of the world [31]— implies an increment in the risk of HIE, the principal cause of perinatal morbidity and mortality [1, 50]. The implications for neurodevelopment are still unclear [18, 51].

Even when the relationship between HIE and the immature cerebellum has been extensively documented [4-11, 52], the vulnerability windows are not so well understood as those in the cerebrum. The observable devastating lesions in the latter appear within the former in a less apparent and protracted fashion. Although the cerebellum seems to be partially protected from external insults which otherwise prove cataclysmic for the cerebral parenchyma, the condition of prematurity notably lowers the cerebellar threshold for lesion. The acceleration of

the growth speed in the late phase of cerebellar development (the last trimester of gestation with a peak at between 28 and 34 weeks) would imply an especial weakness in this regard [53, 54].

Clinical history, gestational age, weights, and Jk coefficient

Nearly half of the 65 cases in this study —the greatest number within GI— presented with LBW or ELBW at birth and low weight for GA. In these patients there converged severe obstetric and neonatal conditions plus a pronounced CNS participation (Table 1a and Table 1b). Specifically, patients who presented with LBW or ELBW frequently developed PVL or PIVH either with or without ventriculomegaly; in addition, the cases presenting ELcw were associated with LBW or ELBW [55]. In this way, these patients formed a group the characteristics of which were epidemiologically and pathogenically related. This specific group demonstrated a more pronounced delay in cerebellar development (Table 2b; Table 4b; Table 6).

Furthermore, analysis of the results from GI and GII also gave rise to the following conclusions: 1) Especially in GII, the percentage of cases with low BW was higher than that of the cases with low BBW (Table 6). 2) In GI as well as GII, all the parameters measured at the time of death showed a high proportion of cases with low values; the GA [16, 18] correlated significantly with the BBW and BW, weakly with the values of CW and cw, but not with Jkc. Quite possibly the developmental arrest here resulted from the pathology associated with premature birth itself (especially IRDS), and that occurring during the subsequent hospitalization as well as from diverse treatments. 3) In GI, the CW and the size and weight of the cerebellum proved to be particularly low. 4) The cases with ELcw presented with the lowest CW values, and those with the greatest cerebellar size and weight also had the most elevated CW [12]. It is not surprising that cw was strongly correlated with CW; the significant correlation between cw and CW demonstrates the clear anatomic and physiopathologic linkage between both organs. Just as the serious compromise in the cortical and subcortical gray-matter structures and in the white matter of the cerebrum must have influenced the shortfall of cerebellar development, so the pathology of the cerebellum very likely contributed to the stunting of cerebral growth [13, 24, 56]. Nevertheless, a serious supra- or infratentorial lesion along with milder changes at the supra- or infratentorial counterpart occurred in only 13 cases, with the most frequent abnormality being a lesion in overall encephalic structures from HIE, where the cerebellum constituted simply one additional part of the spectrum. Therefore, the significant correlation that existed in the present study between the CW and the cw is attributable, primarily, to the coexistence of direct supra- and infratentorial lesions and secondarily, to a probable lesion of an indirect nature through diaschisis [13, 16].

The value of the Jkc in the different cases varied in relation to cw, but showed a weaker correlation with a- and b-folia height. These latter two parameters express but a small sector of the anatomical parts that the Jkc measures (a portion of the craniocaudal and the dorsoventral diameters), so that the values for these measurements would not be expected to vary in parallel with this coefficient.

Histological results

The histological measurements showed values very far from normal in numerous cases with a great proportion of those belonging to GI, thus indicating an explanation identical to the one advanced for the gross observations. Under normal conditions, foliation is particularly active in the last trimester of gestation and occurs in parallel with the intense proliferative activity of the external granular layer and the widening of the internal granular and molecular layers [44]. Thus, the very low height of the b folia (corresponding to the lower portion of the cerebellum) that was observed in patients with severe supratentorial lesions was accompanied by a striking alteration in the thickness and cell density of the cortical strata in those same folia, and was associated, in the majority of cases, with very evident tissue and/or diffuse cellular lesions in the cortex and white matter of the cerebellum. Moreover, in these cases the CW and cw were low compared to the values for the

CG or for the rest of the cases in GI and GII. These findings would indicate that the concomitance of supra- and infratentorial lesions, both gross and microscopic, underlies the correlation between the values for CW and cw. The focal lesion of the lower portion of the cerebellar hemispheres in PTNs with ELBW has been detected by Johnsen *et al*. through MRI [18, 55].

Although the histological data failed to show the strong correlation with GA or BW, that is seen during normal cerebellum development [32], the highly significant correlation resulting between some of the histological parameters themselves, as well as between cw and the Jkc, reveals a certain degree of harmony in the developmental arrest of the cerebellum. The external granular layer and the neurons of the dentate nucleus, however, were exceptions to this observation. The thickness of the external granular layer did not constitute the reliable parameter as it has been described to be classically in normal cerebella [44]. Its preservation within folia, that otherwise showed necrosis of the inner neuronal layers and subcortical white matter is a remarkable observation [4]. On the contrary, the thickness of the molecular layer, or the value obtained upon summing its thickness to that of the external granular layer, gave a better measurement of attained development [47, 53, 54]. The thickness of the molecular layer, in turn, depends, in the last analysis, on the arborization of the Purkinje cells; therefore, the increase in this dimension occurred here in parallel with the reduction in the number of Purkinje cells per segment. In some cases, this reduction seemed to be exacerbated by the disappearance of cells through necrosis or apoptosis, which occurrence would explain the present correlation found between those two layers (r= -0.478), compared to the one found in normal cases (r= -0.660) [32]. The data for the neurons of the dentate nucleus showed a slight dispersion in the results, with figures reminiscent of those found in the normal cerebellum during the final months of gestation (from 30 weeks GA on) [32]. The intense lesion of this and other nuclei of the cerebellar roof are usually observed with very severe injuries in PTNs, and with greater frequency in term newborns [1].

As previously referred (see Results and Plate), the average gross and microscopic data in the present study failed to reach normal values, remaining arrested at figures corresponding to cerebella of GA 30-32 weeks (GI) or 33-35 weeks (GII) [32]. Nevertheless, and particularly in GI, some cases showed a histological image of the cerebellum that corresponded to a lower GA than the actual one (Figure 11, B); a LBW or ELBW as well as low body weight in relation to GA was found in nearly all of these patients. Perhaps an early arrested maturation affecting the *in utero* development must be one possible explanation for the early stunted cerebellar development. Otherwise, the serious CNS injury found in some of these cases would have introduced an additional component such as atrophy, especially in those patients with a greater survival period. In both groups, a tendency was observed for the cases with higher GA but LBW/ ELBW and a longer survival period to be accompanied by more serious cerebellar lesions, as has already been observed in brains of very low birth weight PTs by Golden et al [57].

Neuropathological findings

Cell death in response to injury in the developing CNS is conceived nowadays as a continuous process that proceeds from apoptosis to necrosis [31]. Previous reports showed the extensive lesion of the internal granular cells as a component of disseminated cerebral necrosis during the perinatal period [58]. Likewise, Rorke [4] reported that in PTNs with low BW prolonged asphyxia produces more damage to external granular cells than to other neurons. The process of choice with the external and internal granular cells is apoptosis in PTNs, whereas with other cells of lesser maturity and of greater size (Purkinje cells, neurons of the dentate nucleus) the option becomes necrosis, and particularly so in term newborns [59-62]. These concepts concur with the findings from the present work. In addition, many of the cases presented here had developed sepsis, or lesions of the CNS from infection. Immature postmitotic and recently divided neurons are especially vulnerable to apoptosis in bacterial meningitis [63]. There-fore, it is possible that a mechanism similar to the cerebral one operates in the immature cerebellum as well in response to hypoxic, ischemic

and/or infectious lesions [51, 64-66]. The therapeutic measures to which these patients had been subjected (mechanical ventilation, administration of oxygen, corticoid medication) could also have participated in the induction of the above-mentioned process of apoptosis [16, 67, 68].

The apoptosis in the external and internal granular layers along with the narrowness of the molecular layer must have contributed to the reduction in the width of the cerebellar cortex, a remarkable phenomenon in the 14 cases of extreme hypoplasia presented here [4, 69, 70].
Although histological measurements on white matter were not performed, the relevant images of a clear *cerebellar leukoencephalopathy* show its compromise as well (Figure 9 E, F; Figure 11 G-I).

Oligodendroglial necrosis as well as apoptosis [71, 72] can diminish and even interrupt the development of white matter in the cerebrum. A mechanism similar to the cerebral one possibly operates in the cerebellum. In 8 of the cases that had suffered PIVH, atrophy of the parenchyma was found in addition to ventricular enlargement (*ex vacuo* ventriculomegaly) (Figure 3). Along with a cw considerably below normal values, small folia with scanty white matter, a reduced amount of granule cells, and/or severe degrees of histological immaturity were observed (Figure 5 A; Figure 9 A, B; Figure 11 A). To the effect of the cerebral white matter lesions usually observed in cases with post-hemorrhagic hydrocephalus [73, 74], it must be added then the compromise of the cerebellar tissue [18, 74]. Finally, intraparenchymatous cerebellar hemorrhage as part of the lesions in HIE [1], such as was found in 12 of the present cases, is not an infrequent phenomenon in the PTN [52]. This in turn, must have contributed to the observed under-development of the cerebellum.

From the combined analysis of the gross, histological, and neuropathological data, there seems to be a spectrum of lesions, one of its ends having a highly poor outcome. This was particularly represented by the cases presenting ELcw [52, 55] and some cases belonging to GII (Figure 9 A-D). Necrosis, hemorrhage, edema, macrophages, and/or diffuse reactive astrocytosis were the histological findings. Part of GI and a large portion of GII appear to be at the center of this continuum, these cases presenting mainly with cellular lesions, either focal or diffuse, along with infrequent necrosis and low-grade hemorrhage. The least impacted were 3 cases in GII without supra- or infratentorial lesions (see Results) (Figure 10), with relative preservation of the gross and microscopic parameters, and with only isolated cellular abnormalities. The aforementioned findings (i.e. necrosis, hemorrhage, macrophages, astrocytosis) suggest that the principal changes associated with the developmental arrest of the cerebellum are related to a primary and direct injury with a positive relationship between the severity of the damage and the degree of retention of cerebellar development. The gross and microscopic correlation allows the establishment of a second correspondence, this time between the pathological findings and those of MRI. It is evident that the most serious cerebellar lesions correlate with those visualized by MRI, while those situated at the other end of the spectrum, being visible only microscopically, very probably correspond to those cases in which MRI detected no lesion whatsoever. This situation was pointed to recently: The undersize cerebellum without cerebral (or cerebellar) lesion was observed by MRI in groups of PTNs [21, 23, 25]; Bodensteiner *et al.* [17] considered the probable existence of "milder forms of injury to the cerebellum", and Argyropoulou *et al.* [16] suggested that "functional disconnection from the cerebral lobes is thus probably not the only cause of cerebellar atrophy". Therefore, even though deafferentation and diaschisis cannot be discarded as operative mechanisms in the hypoplasia of the cerebellum [6, 13, 20, 68, 75], a primary and direct lesion to the cortex and white matter, though of a different intensity, constitutes a little appreciated but fundamental cause of the whole pathological process, especially in the group of PTNs with ELcw.

This study has a potential limitation. It seems likely that progress in therapy and other aspects of prenatal care during the period from which data were obtained could have effects upon the development

of the brain. The number of cases per year in our cohort, however, is too small to draw any conclusions as to the therapeutic effects on cerebellar development. Better therapies have undoubtedly increased the number of surviving PTNs. Presumably, those living PTNs could have a lesser compromise of their CNS and even a diminished incidence of their CNS lesions because of those improved therapies. Nevertheless, MRI studies in living patients frequently show a severe compromise of the cerebellum [8, 12, 13, 16-20]. Moreover, preterm birth continues to have a steady incidence all over the world. Therefore, if cerebellar lesions exist in the way they are presently shown to by MRI, it is not obvious that those changes would have a pathological background that is very different from the lesions described in this work. Upon comparing these cases with the results proceeding from MRI, it is appropriate to recall that the latter studies were performed on patients who survived a premature birth and then managed to attain a PCA greater than term. For this reason, MRI studies in living patients implies, to a certain extent, a filtering out of the severe pathology found on necropsy. Beyond these differences, both procedures show that in PTNs a deficient growth, with body weight being a reliable marker, can imply deterioration of the CNS in general and of the cerebellar developmental milestones in particular.

Conclusions

In this group of PTNs, the cerebellum maturation was arrested in late stages of development (30-35 weeks GA), perhaps that of the preterm birth itself, or at the most a period only shortly postnatal. The greater histological changes were found in the smaller cerebella. Nevertheless, the arrest of cerebellar growth occurred *pari passu* in both the folia and some parts of the cortex, mainly the molecular layer and Purkinje cells. GA was found to have little impact in this series. The main histological changes were related to direct injuries. The cerebellar lesions comprised just one additional part of the spectrum of primary injuries that were observed in the CNS in this group of PTNs.

Abbreviations	
BBW	birth body weight
BW	body weight at necropsy
CG	control group
CW	cerebral weight
cw	cerebellar weight
ELBW	extremely low body weight
ELcw	extremely low cerebellar weight
GA	gestational age
GI	Group I
GII	Group II
HIE	hypoxic-ischemic encephalopathy
IRDS	idiopathic respiratory distress syndrome
Jkc	Jk coefficient
LBW	low body weight
MRI	magnetic resonance imaging
PCA	post-conceptional age
PIVH	peri-intraventricular hemorrhage
PNA	postnatal age
PTN	preterm neonate
PVL	periventricular leukomalacia
TGAE	term gestational age equivalent

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Acknowledgements

The author wishes to thank Dr. Ricardo Drut for critically reviewing the manuscript, Dr. César Gómez Dumm for advice, Adriana Mijalovsky and Marina Valencia for their excellent technical assistance, and Dr. Joaquín E. Drut for assistance with images. Statistical analysis was performed by Dr. María Apezteguía, and Dr. Joaquín E. Drut and Dr. Donald F. Haggerty collaborated in the translation to the English version. ◈