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Extra-uterine growth in preterm: an independent factor for neurological outcome.

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Abstract:

Introduction: Extrauterine growth restriction (EUGR) is a common complication in preterm. Literature reports that EUGR is a risk factor for cerebral palsy and worse long-term neurological outcome. We report our experience of EUGR and neurological outcome in a large population of very low birth weight (VLBW) preterms without major lesions at MRI.

Methods: we selected all VLBW patients born between 2012 and 2018 who perform an MRI study at term age. We exclude all patients with congenital malformations or major brain lesions (patients with low-grade IVH, punctate lesions, and micro-cerebellar hemorrhage were included). Neurological outcomes were evaluated with Griffiths II scale (GMDS II) at 2 and 3 years of age. Perinatal and neonatal risk factors such as gestational age (GA), born weight, Apgar, sepsis, NEC, bronchopulmonary dysplasia, and surgery were collected. Weight growth was evaluated at term age, 6 months, and 12 months of correct age. EUGR was defined as "cross-sectional" if the weight z-score was < 1,282 (<10° percentile) at TEA, 6 months, and 12 months; "longitudinal" EUGR was defined if the z-score decreased by 1-point SDS from birth to TEA and from TEA to 6 months. Multivariate analysis was done.

Results: Of 498 VLBWs, 210 were excluded for severe brain lesions, congenital malformation, or incomplete data. Statistical analysis was performed in 288 patients (mean GA 28,9 + 2,1 weeks). "Cross-sectional" EUGR was found in 50% at term age, 46.2% at 6 months, 48,3% at 12 months while "longitudinal" EUGR was found in 43,8% at TEA and 16% at 6 months. Multivariate analysis showed that a higher weight z-score at 6 months is protective for global developmental quotient (DQ) at 2y of age (OR 0.74; CI 95% 0.59-0.93; p=0,01). In particular, "cross-sectional" EUGR at 6 months had a higher risk for worse locomotor (OR 1.96; CI 95% 1.10-3.47; p=0,02), language (OR 1,87; CI 95% 1,05-3,29 p=0,02) and adaptive behavior DQ (OR 1,94; CI 95% 1.12-3.37; p=0,02) at 2 years GMDS, and for worse language DQ (OR 1.63; 0.99-2.69 p 0,05) at 3 years of age. Furthermore, "longitudinal" EUGR from TEA to 6 months had a higher risk for worse performance DQ (OR 2,10; CI 95% 1,03-4,30; p=0,04) at 2 years and practical reasoning DQ (OR 2,07; CI 95% 1,02-4,17; p=0,04) at 3 years. NEC (OR 2,55; CI 95% 1.11-5.86; p= 0.03) and male sex (OR 1.94; CI 95% 1.16 - 3.24; p= 0,01) seems to be the major risk factors for lower global DQ at 3 years of age.

Conclusion: EUGR has a high incidence in the preterm population. Both "cross-sectional" and "longitudinal" EUGR at 6 months of age seems to be an independent risk factor for worse GMDS in many areas. These results draw attention to nutrition after discharge and complementary feeding that can affect neurological outcome in preterm infants.

Introduction:

Preterm birth and neurological outcome: dimension of the problem.

Preterm birth is defined as birth before 37 completed gestation weeks and is estimated that each day, over 41000 neonates, about 10% of all births, are preterm (1). Preterm is classified according to gestational age into extreme preterm (<28 weeks), very preterm (28-32 weeks), and moderate-late preterm (32 to 37 weeks) (1). We can also classify preterm babies according to their birth weight (BW) in extremely low birth weight (ELBW, with BW < 1000g) and in very low birth weight (VLBW, with BW <1500g). The survival rate in these smaller preterm babies has drastically increased over the years from 5% in the 1960s (2) to over 90% in high-income countries in the new millennium (1) (3). However, the incidence of neurological sequelae has remained high due to increased survival at the lower gestational age (4). Rates of neurological sequelae in preterms born after 1990were estimated at a quarter of survivors, with the highest rate of most immature survivals (4). The most common long-term neurodevelopmental disabilities associated with preterm births are cerebral palsy (14,6% in extreme preterm, 6,2% in very preterm (5)), cognitive impairment (about 30%; 7% severely impaired (4)), developmental co-ordination disorder including attention and activity disorder and behavioral sequelae (about 30% of born before 30 weeks gestation, six-fold increase risk when compared with term-born children (6)).

Prognostic factors for neurodevelopment in preterm.

Neurodevelopment can be affected by several neonatal complications in preterm, and brain lesions are one of the most important. White matter damage is the most frequent form of neurological impairment with long-lasting consequences in VLBW (7). Two main kinds of lesions have been described to affect white matter and largely recognized as acquired causes of motor impairment in preterms: cystic periventricular leukomalacia (PVL), and periventricular

hemorrhagic venous infraction (PVHI) complicating germinal matrix-intraventricular hemorrhage (GMH-IVH) (8) (9).

PVL is a white matter injury characterized by focal necrosis and diffuse gliosis. It has multifactorial etiology and typically affects preterm of 26-32 weeks of gestation (10) (11). PVL is a strong predictor of motor disability such as cerebral palsy (90% of children with cerebral palsy born preterm have PVL), but can also have an impact on later cognitive function (12) (13). During recent years, rates of PL have drastically decreased (from 4,5% in 1991 to 1,6% in 2006) (14) but on the other hand a milder form of white matter damage, punctate white matter lesion (PWML), is more frequently diagnosed and seems to affect about 20% of the preterm population. PWML can affect brain maturation with delayed myelination and reduced cortical infolding (15) but the role on long-term outcome are still debated. Number of lesions, their pattern, and their localization seem to be important (16).

Regarding hemorrhagic lesions, GMH-IVH occurs in 15-20% of VLBW preterms, is secondary to bleeding of the germinal matrix with a multifactorial etiology, is typical of the first 48 hours of life and has a higher incidence as gestational age decrease (10). GMH-IVH is classified according to the percentage of ventricular involvement according to Volpe's classification (grade 1: <10%; grade 2: between 10% and 50%; grade 3: >50%), any grade of IVH can be complicated by periventricular hemorrhagic infarction (PVHI; grade 4 according to the old classification) (17; 18) with a rate of 15% of all GMH-IVH and a high incidence of cerebral palsy and severe cognitive impairment (19). In addition to PVHI, another complication of GMH-IVH is posthemorrhagic hydrocephalus (PHVD) which occurs in about 25% of infants with IVH, and typically appears in grade 3-4 hemorrhages, but can potentially complicate all grades of IVH. It has a strong association with neurodevelopmental impairment, in particular in infants with persistent PHVD who require neurosurgery, although it depends from the timing of management (19) (20) (21). The role of a lower grade of GMH-IVH in neurodevelopment is still

debated but a recent work of our group determines that low grade IVH is associated with worse Griffiths Mental Development Scale (GMDS, OR of 2,67, 95% CI 1,16-6,13; p=0,02) (22).

Another type of hemorrhagic lesion is cerebellar hemorrhage (CBH) which is typical of extremely preterm (10) and can negatively impact neurological outcome. Massive CBH involves more than 1/3 of the cerebellar hemisphere and is associated with cerebral palsy and motor, language, and cognitive delays. Limited (or "medium-sized") CBH involve an area of <1/3 of the cerebellar hemisphere with a still debated prognostic meaning. Punctate or micro-CBH that had diameter < than 4 mm and good prognosis (23), even if in our recent work seem to be associated with worse GMDS (22).

Lastly, other perinatal and postnatal complications can play a role in developing neurological sequelae in preterm babies:

- hypoxic-ischemic encephalopathy (HIE) with an estimated incidence of 1,5 per 1000 live births, has an incidence of 25% of long-term neurodevelopmental problems (24);
- neonatal sepsis, in particular, late-onset sepsis (LOS), which has an incidence of 24% in NICU, showed an increased risk for cerebral palsy (15% vs 10%) and pathologic development quotient (30% vs 25%) in preterm (25)
- necrotizing enterocolitis (NEC) that has an incidence between 4 and 10% in preterm and that increases the incidence of major neurodevelopmental disability from 10% to 24% in the population with a significantly higher risk of cognitive delay (26) (27);
- bronchopulmonary dysplasia that had an incidence of 22% in extremely preterm and was associated with a higher risk of cerebral palsy (ORs 2.10; 95% CI 1.57 - 2.82 in a recent meta-analysis) and major neurological sequelae (28) (29) (30);
- surgical procedure, in general, due to surgical NEC or patent ductus arteriosus correction, was associated with a higher rate of neurosensory disability (33% vs 10%;

OR 4.28, 95% CI 2.61 to 7.03) (31)and abnormal functional connectivity at MRI study (32).

Extrauterine growth restriction (EUGR) and neurodevelopment.

Extrauterine growth restriction (EUGR) is a common complication in preterm infants (33) and is commonly considered a risk factor for poor development. It is defined as "cross-sectional" when the patient weights below a specific cut-off at a specific time-point (34) or “longitudinal” when there’s a growth difference from birth concerning another defined time-point (35) (36). Its incidence varies from 13% to 97% in different populations and according to relative definitions (37). About its influence on neurodevelopment, Ehrenkranz RA et al. observed an increase in the incidence of cerebral palsy, pathologic mental development index and psychomotor development index, and neurodevelopment impairment in patients with EUGR during NICU hospitalization in a large population of ELBW infants (38). Furthermore, Guellec I et al. demonstrates an increased risk of cerebral palsy in "longitudinal" EUGR between birth and 6 months in a large population of preterms from the EPIPAGE study cohort (39). Even if the influence of EUGR in neurodevelopment is widely shared, there is no univocal agreement on which definition (“cross-sectional” or “longitudinal”) and time-point (36 weeks, TEA, 6 months) better predict neurological outcome (36). Furthermore, the most important studies donot consider MRI which is the gold standard to define the diagnosis and severity of brain lesions.

Aims of the study.

The principal aim of our study is to define if EUGR can be an independent prognostic factor for neurological outcome in VLBW preterm without major lesions at MRI.

The secondary aim is to identify if there is a better definition and time-point of EUGR that can better predict neurological outcomes in this population.

Material and Methods:

Study population and MRI

All VLBW infants consecutively admitted to Neonatal Intensive Care Unit of IRCSS Istituto Giannina Gaslini who underwent routine brain MRI at term-equivalent age from January 2012 to December 2018were selected for the study.

As per standard internal protocol, MRI scans were performed at term-equivalent age (TEA, between 38 and 42 weeks postmenstrual age) as a part of the screening program for identification of prematurity-related lesions.“Feed and wrap” technique was used to perform the MRI (40). The need for sedation (oral midazolam, 0,1 mg/kg) to prevent head motion was agreed with the neuroradiologist case by case. Scans were performed with a 1.5 Tesla MR system (Intera Achieva 2.6; Philips, Best, The Netherlands) using a dedicated pediatric head/spine coil. Our institutional standard MRI protocol included 3 mm thick axial T2-weighted and T1-weighted images, coronal T2-weighted images, sagittal T1- weighted images, axial diffusion-weighted images (b value:1000 s/mm2) and axial SWI(susceptibility weighted imaging) that is the gold standard to identify hemosiderin and low-grade hemorrhage (41).

Patients with major brain lesions such as periventricular leukomalacia (PVL), periventricular hemorrhagic infarction (PVHI), post-hemorrhagic hydrocephalus with ventricular shunt, and massive or limited cerebellar hemorrhage (CBH) (42) or with congenital brain malformations were excluded from the present study.

Patients with minor brain lesions such as low-grade intraventricular hemorrhage (I-II grade for Volpe's Classification (17)), punctate white matter lesions (PWML), or punctate cerebellar hemorrhage (micro-CBH) (42)were included in the study.

Data collection and analysis

Demographic and clinical data of the enrolled patients were extracted from clinical charts. Collected data included demographic characteristics: birth weight, gestational age, and sex; perinatal data: type of delivery, Apgar at 5th minute; postnatal data: diagnosis of sepsis (defined by the need for antibiotic therapy for clinical and laboratory findings suggesting blood infection), necrotizing enterocolitis (NEC, defined by clinical and imaging signs suggesting enterocolitis), pulmonary bronchodysplasia (defined by the need of any ventilatory support or O2 supplementation at 36 weeks), major surgery for NEC or patent ductus arteriosus (PDA), type of alimentation at TEA (formula milk or mother milk).

MRI data were collected and patients were divided into two groups: patients with normal MRI and patients with low-grade brain lesions.

Anthropometric data were collected by clinical charts of patients enrolled in our preterm follow-up service. During the hospital stay, weight was measured daily and after discharging it was collected at term-equivalent age (TEA), and 1, 3, 6, 9, and 12 months of correct age. We collected only weight at birth, at TEA, and 6 and 12 months of corrected age. Z-scores of weight for age and sex were calculated for birth and TEA using INTERGROWTH-21strelativescharts for very preterm size at birth (43) and postnatal growth standard in preterm infants (44), while for 6 and 12 months of corrected age, we used CDC Growth Charts, 2000 (45). Patients who were lost in the follow-up phase or with incomplete anthropometric data were excluded from the present study.

The patients with birth weight z-score < 1,282 (<10° percentile) were considered small for gestational age (SGA). Extra-uterine growth restriction (EUGR) at TEA and 6 months was diagnosed by two different definitions: “cross-sectional” EUGR as a weight z-score < 1,282 (<10° percentile)and “longitudinal” EUGR as z-score decreased by 1-point SDS from birth to TEA and from TEA to 6 months (46). A pathologic weight with z-score <1,282 (10° percentile) was also defined as EUGR at 12 months of correct age.

The neurological development evaluation of patients was performed with Griffiths Mental Developmental Scales (GMDS) (47) at two years of corrected age and three years of chronological age. Patients lost in the follow-up phase were excluded from the study. Patients who did not perform only the 3-years assessment were included. These evaluations are a part of routine follow-up service offered to all VLBW patients after discharge from the hospital. GMDS was administered by a 10-year experienced single operator blinded to MRI results. Raw numbers were converted into standardized development quotients (DQ). Total development quotients (DQ), relating to global development, were derived from the mean of the results of different areas of assessment:

- scale A (locomotor): gross motor skills
- scale B (personal-social): adaptive behavior and social development
- scale C (language): receptive/expressive language
- scale D (hand-eye coordination): fine motor function and hand-eye coordination
- scale E (performance): precursors of reasoning and planning.
- scale F (practical reasoning): solve practical problems, understanding basic math concepts and moral issues(this scale is performed only at 3 years of age)

Resulting values were used to evaluate the level of neurodevelopment: values below 70 define a developmental delay, values from 70 to 84 evidence border-line condition, and values above 84 are considered normal (47).

Descriptive statistics were generated for the whole cohort; data were expressed as the mean and standard deviation for continuous variables and absolute and relative frequencies for categorical variables. Demographic and clinical characteristics were compared using the Chi-square or Fisher exact test and the Mann-Whitney U test for categorical and continuous variables. Univariate analysis determined the potential risk factors which were significantly associated with unsatisfactory scores in the GMDS (<85) at 2, and 3 years of CA. Logistic regression analysis was used for each variable, and odds ratios (ORs) were calculated with 95% confidence intervals (CIs). The absence of exposure to the factor or the variable that was less likely to be associated with the risk was used as a reference for each analysis. Multivariate analysis corrected for gestational age (GA) was then performed. The only variables that proved to be statistically or borderline significant in the univariate analysis (<0.08) were included in the model. The model which best fitted was based on the backward stepwise selection procedures, and each variable was removed if it did not contribute significantly. In the final model, a p-value of <0.05 was considered statistically significant, and all p-values were based on two-tailed tests. Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows (SPSS Inc. Chicago, IL).

Results:

498 very low birth weights underwent brain MRIs from January 2012 to December 2018. Of these patients, 65 were excluded for severe brain lesions, 18 for incidental findings of brain malformations and congenital diseases, and 127 for incomplete follow-up or missing data.

The final population included 288 patients. GA mean was 28.9 ± 2.1 weeks (range 23-34.6), with a mean birth weight of 1097±255g (z-score -0,449 ± 1,09; range 435-1490g). 139 patients (48.3%) were male. The incidence of small with respect to gestational age (SGA) was 21.5% (62 patients). 232 neonates were born by cesarean delivery (80.6%), mean Apgar score at 5 minutes was 8(range 2-10).

About the major neonatal complications of preterm: incidence of sepsis was 37.5% (n 108), NEC was present in 30 patients (10.4%) of these 15 who underwent surgical treatment. A total of 36 patients (12.5%) underwent surgery before discharge (15 for NEC, 17 for PDA). The MRI study at term age (TEA) showed that101 patients had low-grade lesions (35,1%). Of these 44 patients with low-grade IVH (15,3%), 47 had punctuate lesions of white matter (16.3%), 31 had cerebellar micro-hemorrhages (10,8%).

At term age, only 17% (n 49) were fed by mother milk exclusively and 41% were fed only by formula milk. The mean weight at TEA was 2600 ± 598g (range 1140-4180g), the mean z-score for weight was -1.407±1.415,and 50% (n=144) had “cross-sectional” EUGR(z score <-1.282; <10°centile). The incidence of “longitudinal” EUGR (SD minor than 1 point from birth) was 43.8% (n=126). At 6 months of age, the mean weight was 6.81± 1.03 kg, z-score -1.24±1.29, the incidence of patients with “cross-sectional” EUGR (z-score<-1.282; <10° percentile) was 46.2% (n=133), while the rate of "longitudinal" EUGR (SD z-score minor than 1 point from TEA) was 16,0% (n=46). At 12 months of age, the mean weight was 8.78± 1.16 kg, z-score -1.36±1.25, the incidence of patients with “cross-sectional” EUGR was 48.3% (n=139).*(Tab1)*

Whole population	**N= 288**
Gestationalage (weeks)	28.9 ± 2.1
Birth Weight (g)	1097 ± 255g (z-score -0,449 ± 1,09)
Small for gestational age (SGA)	62 (21,5%)
Male sex	139 (48,3%)
Cesarean delivery	232 (80,6%)
Apgarat 5 minutes	8± 1,2
Sepsis	108 (37,5%)
Necrotizingenterocolitis (NEC)	30 (10,4%)
Bronchodysplasia (BPD)	68 (23,6%)
Major Surgery	36 (12,5%)
NEC Surgery	15 (5,2%)
Patent ductus arteriosus surgery	17 (5,9%)
Exclusivemother-milkfeeding	49 (17%)
Exclusive formula feeding	118 (41%)
MRI low-grade lesions	101 (35,1%)
Low-grade intraventricularhemorrhage (GMH-IVH)	44 (15,3%)
Punctate white matter lesions (PWML)	47 (16,3%)
Cerebellar micro-hemorrage (micro-CBH)	31 (10,8%)
Weight at term age TEA (g)	2600 ± 598 (s-score -1.407 ±1.415)
"Cross-sectional" EUGR at TEA	144 (50%)
"Longitudinal" EUGR at TEA	126 (43,8%)
Weight at 6 months (kg)	6.81 ± 1.03 (z-score -1.240±1.29)
"Cross-sectional" EUGR at 6 months	133 (46,2%)
"Longitudinal" EUGR at 6 months	46 (16,0%)
Weight at 12 months (kg)	8.78± 1.16 (z-score -1.360±1.25)
"Cross-sectional" EUGR at 12 months	139 (48,3%)

Table 1: Population features. Data are reported in mean value ± SDS for continuous variables, absolute number, and percentage for categorical variables.

About the long-term neurological outcomes, Global DQ evaluated with Griffiths scale II was pathologic or borderline (<85) in 56 patients of 288 at 2 years of age (19,4%). Considering separately the different rating areas of the Griffiths II scale, incidence of pathologic or borderline score was 23,6% for locomotor, 23,2% for personal-social; 44,1% for hearing and language,13,2% for hand-eye coordination, 24,3% for performance. At 3 years of age, global DQ Griffiths scale was pathologic or borderline in 100 of 262 patients (26 patients were lost in

the follow-up phase) with an incidence of 38,2%. Considering separately the different rating areas incidence of pathologic or borderline score was 31,7 % for locomotor, 33,2% for personal-social; 53,0% for hearing and language, 31,6% for hand-eye coordination, 48,1% for performance, 38,5% for practical reasoning *(Table 2).*

GMDS at 2y (<85 vs ≥ 85);Total patients n=288		
Global DQ	56 *(19,4%)*	232 *(80,5%)*
Locomotor (scale A)	68 (*23,6%)*	220*(76,4%)*
Personal-social (scale B)	67*(23,2%)*	221*(76,8%)*
Language (scale C)	127*(44,1%)*	161*(55,9%)*
Hand-eyecoordination (scale D)	38*(13,2%)*	250*(86,8%)*
Performance (scale E)	70*(24,3%)*	218*(75,7%)*
GMDS at 3y (< 85 vs ≥ 85) total patients n= 262		
Global DQ	100 *(38,2%)*	162 *(61,8%)*
Locomotor (scale A)	83*(31,7%)*	179*(68,3%)*
Personal-social (scale B)	87*(33,2%)*	175*(66,8%)*
Language (scale C)	139*(53,0%)*	123*(47,0%)*
Hand-eyecoordination (scale D)	83*(31,6%)*	179*(68,4%)*
Performance (scale E)	126*(48,1%)*	136*(51,9%)*
Practicalreasoning(scale F)	101*(38,5%)*	161*(60,5%)*

Table 2: Griffith mental development scale II (GMDS) results at 2 and 3 years in the whole population for Global and sub-scales development quotient (DQ).

Considering the risk factors for worse neurological outcomes at 2 years, the univariate analysis showed that patients scoring below 85 on the Griffiths II scales had a lower weight z-score at 6 (-1,639 vs 1,140 p=0,03) and 12 months (-1,735 vs -1,264 p=0,03) than patients with normal results on the Griffiths assessment and the incidence of patients with “cross-sectional” EUGR at 6 months was higher even not statistically significant in patients with pathological or borderline global DQ at Griffiths (58,9 vs 43,5%; p=0,07). Furthermore, the incidence of surgical NEC appears to be higher in patients with pathological or borderline global DQ (10,7% vs 3,9%; p =0,08). Based on these data, multivariate analysis adjusted for gestational age showed that a higher z-score for weight at 6 months would be protective for global DQ deficit at 2 years of age (OR 0,74; CI 0,59-0,93; p=0,01)*(Table 3).*

2y GMDS GLOBAL DQ (< 85 vs ≥ 85)			
N	56	232	TOT 288
z-score 6month	-1,639 ± 1,582	-1,140 ±1,188	p=0,03
z-score 12 month	-1,735 ± 1,56	-1,264 ± 1,155	p=0,03
Surgical NEC	6 (10,7%)	9 (3,9%)	p=0,08
"Cross-sectional" EUGR at 6 months	33 (58,9%)	101 (43,5%)	p=0,07
Multivariate analysis (corrected for GA):			
z-score 6 month	OR 0,74 (CI95% 0,59-0,93)		p=0,01

Table 3: Univariate and Multivariate Analysis corrected for gestational age (GA); Global development quotient (DQ) at 2 years Griffith Mental Development Scale. Data are reported in mean value ± SDS for continuous variables, absolute number, and percentage for categorical variables. Odds ratios (OR) were calculated with a 95% confidence interval (CI). All p-values were based on two-tailed tests

The same type of analysis was performed separately for the different rating areas of the Griffiths II scale. Considering the locomotor area (Scale A) the multivariate analysis showed that "cross-sectional" EUGR at 6 months (OR 1.96; CI 95% 1.10-3.47; p=0.02), punctate white matter lesions (PWML)(OR 2,33; CI95% 1,15-4,71; p=0,02)and major surgery during NICU stay (OR 3.79; CI 95% 1.69-8.49; p=0.001) were a negative prognostic factor.*(Table4)*

For Personal-Social area (Scale B) "cross-sectional" EUGR at 6 months (OR 1,94; CI 95%; 1,12-3,37; p=0,02) and NEC (OR 2,6; CI 95% 1,14-5,92; p= 0,02) are the major risk factors. *(Table 4)*

2y GMDS LOCOMOTOR DQ (< 85 vs≥ 85)			
N	68	220	TOT 288
Sepsis	33 (48,5%)	75 (34,1%)	p=0,04
PDA surgery	11 (16,2%)	10 (4,5%)	p=0,003
Major surgery	17 (25,0%)	19 (8,6%)	p=0,001
Low-grade lesions at MRI	32 (47,0%)	69 (31,4)	p=0,02
Punctate white matter lesions (PWML)	19 (27,9%)	30 (13,6%)	p=0,04
"Cross-sectional" EUGR at 6 months	39 (57,4%)	93 (42,3%)	p=0,02
"Longitudinal" EUGR at 6 months	17 (25%)	29 (13,2%)	p=0,02
"Cross-sectional" EUGR at 12 months	40 (58,8%)	99 (45,0%)	p=0,05
Weight z-score at 6 months	-1,640 ±1,395	-1,113 ± 1,228	p=0,006
Weightz-score at 12 months	-1,757 ± 1,456	-1,231 ± 1,162	p=0,008
Multivariate analysis corrected for GA			
Major surgery	OR 3.79(CI 95% 1.69-8.49)		p=0,001
"Cross-sectional" EUGR at 6 months	OR 1.96(CI 95% 1.10-3.47)		p=0,02
Punctate white matter lesions (PWML)	OR 2,33 (CI 95% 1,15-4,71)		p=0,02

2y GMDS PERSONAL-SOCIAL DQ (< 85 vs > 85)			
N	67	221	TOT 288
Sex m	39 (58,2%)	100 (45,2%)	p=0,07
Surgical NEC	7 (10,4%)	8 (3,6%)	p=0,05
NEC	12 (17,9%)	18 (8,1%)	p=0,04
"Cross-sectional" EUGR at 6 months	39 (58,2%)	94 (42,5%)	p=0,03
Weight z-score at 6 months	-1,58 ±1,71	-1,13 ±1,11	p=0,06
Multivariate analysis corrected for GA			
"Cross-sectional" EUGR at 6 months	OR 1,94 (CI 95% 1,12-3,37)		p=0,02
NEC	OR 2,60 (CI 95% 1,14-5,92)		p=0,02

Table 4: Univariate and Multivariate Analysis corrected for gestational age (GA); Locomotor DQ (scale A) and Personal-Social DQ (Scale B) at 2 years Griffith Mental Development Scale. Data are reported in mean value ± SDS for continuous variables, absolute number, and percentage for categorical variables. Odds ratios (OR) were calculated with a 95% confidence interval (CI). All p-values were based on two-tailed tests.

Considering the hearing and language areas (Scale C), multivariate analysis identified higher gestational age (OR 0,5; CI 95% 0,27-0,92; p=0,02) and higher birth weight z-scores (OR 0,31; CI 95% 0,12-0,81; p=0,02) as protective factors, while the presence of NEC (OR 2,48; CI 95%1,07-5,71; p=0,03) and "cross-sectional" EUGR at 6 months (OR 1,87; CI 1,05-3,29; p= 0,02) were

also confirmed as negative prognostic factors.NEC was also a negative prognostic factor (OR 3,98; CI1,66-9,55; p=0,002)for the assessment of hand-eye coordination (Scale D)*(Table 5).*

2y GMDS LANGUAGE DQ (< 85 vs ≥ 85)			
n	127	161	TOT 288
Weight z-score at birth	-0,60 ± 1,21	-0,32 ± 0,97	p=0,04
Weight z-score at 6 months	-1,55 ± 1,47	-0,98± 1,05	p=0,001
Weight z-score at 12 months	-1,67 ± 1,39	-1,1 ±1,07	p=0,001
NEC	20 (15,7%)	10 (6,2%)	p=0,01
Major surgery	21 (16,5%)	15 (9,3%)	p=0,07
SGA	34 (26,8%)	28 (17,4%)	p=0,06
"Cross-sectional" EUGR at 6 months	73 (57,4%)	60 (37,3%)	p=0,001
"Cross-sectional" EUGR at 12 months	75 (59,0%)	65 (40,4%)	p=0,003
Multivariate analysis corrected for GA			
Gestational age (GA)	OR 0,50 (CI 95% 0,27, 0,92)		p=0,02
NEC	OR 2,48 (CI 95% 1,07-5,71)		p=0,03
"Cross-sectional" EUGR at 6 months	OR 1,87 (CI 95% 1,05-3,29)		p=0,02
Weight z-score at birth	OR 0,31 (CI 95% 0,12-0,81)		p=0,02

2y GMDS HAND-EYE COORDINATION DQ (< 85 vs≥ 85)			
n	38	250	TOT 288
NEC surgery	6 (15,8%)	9 (3,6%)	p=0,007
NEC	10 (26,3%)	20 (8%)	p=0,002
Major surgery	9 (23,7%)	27 (10,8%)	p=0,03
Multivariate analysis corrected for GA			
NEC	OR 3,98 (CI 95% 1,66-9,55)		p=0,002

Table 5: Univariate and Multivariate Analysis corrected for gestational age (GA); Language DQ (scale C) and Hand-eye coordination DQ (Scale D) at 2 years Griffith Mental Development Scale. Data are reported in mean value ± SDS for continuous variables, absolute number, and percentage for categorical variables. Odds ratios (OR) were calculated with a 95% confidence interval (CI). All p-values were based on two-tailed tests.

Ultimately, male sex(OR 2,01; CI 95% 1,13-3,57; p=0,02); major surgery (OR 4,07: CI 95%1,78-9,33; p=0,001), white matter punctate lesions at MRI (OR 2,03; CI 95% 1,00-4,14; p=0,05), and "longitudinal" EUGR at 6 months (OR 2,10; CI 95% 1,03-4,30; p= 0,04) seemed to be negative prognostic factor relatively to the performance area (Scale E).*(Table 6).*

2y GMDS PERFORMANCE DQ (< 85 vs ≥ 85)			
n	70	218	TOT 288
Male gender	43(61,4%)	96 (44,0%)	p=0,01
Surgical NEC	9 (12,8%)	6 (2,7%)	p=0,03
NEC	12 (17,1%)	18 (8,2%)	p=0,04
Major surgery	16 (22,8%)	20 (9,2%)	p=0,006
Low-grade lesions at MRI	31 (44,3%)	70 (32,1%)	p=0,08
Punctate white matter lesions (PWML)	17 (24,3%)	30 (13,8%)	p=0,04
"Longitudinal" EUGRat 6 months	17 (24,3%)	29(13,3%)	p=0,04
Multivariate analysis corrected for GA			
Male sex	OR 2,01 (CI 95% 1,13-3,57)		p=0,02
Punctate white matter lesions (PWML)	OR 2,03 (CI 95% 1,00-4,14)		p=0,05
Major surgery	OR 4,07 (CI 95% 1,78-9,33)		p=0,001
"Longitudinal" EUGR at 6 months	OR 2,10 (CI 95% 1,03-4,30)		p=0,04

Table 6: Univariate and Multivariate Analysis corrected for gestational age (GA); Performance DQ (scale E) at 2 years Griffith Mental Development Scale. Data are reported in mean value ± SDS for continuous variables, absolute number, and percentage for categorical variables. Odds ratios (OR) were calculated with a 95% confidence interval (CI). All p-values were based on two-tailed tests.

Regarding the neurological outcome at 3 years of age, the Griffiths II scale was applied only in 262 patients of the 288 because 26 patients were lost in the follow-up phase. The univariate analysis showed a major incidence of Griffiths score <85 in the male babies (60% vs 43,2%; p= 0,01), and patients with NEC (17% vs 7,4%; p=0,02), while normal Griffiths had a higher incidence in patients born by cesarean delivery (76% vs 85,2%; p=0,05). Based on these data, the multivariate analysis adjusted for gestational age showed that only male sex (OR 1,94; CI 95% 1,16-3,24; p=0,01) and NEC (OR 2,55; CI 95% 1,11-5,86; p=0,03) was independent negative prognostic factors for worse global DQ. (Table 7)

3y GMDS GLOBAL DQ (< 85 vs ≥ 85)			
n	100	162	TOT 262
Male sex	60 (60%)	70 (43,2%)	p=0,01
Cesarean delivery	76 (76%)	138 (85,2%)	p=0,05
NEC	17 (17%)	12 (7,4%)	p=0,02
Multivariate analysis corrected for GA			
Male sex	OR 1,94 (CI 95% 1,16-3,24)		p=0,01
NEC	OR 2,55 (CI 95% 1,11-5,86)		p=0,03

Table 7: Univariate and Multivariate Analysis corrected for gestational age (GA); Global development quotient (DQ) at 3 years Griffith Mental Development Scale. Data are reported in mean value ± SDS for continuous variables, absolute number, and percentage for categorical variables. Odds ratios (OR) were calculated with a 95% confidence interval (CI). All p-values were based on two-tailed tests

Considering separately the areas of Griffith II at 3 years, multivariate analysis showed that the protective prognostic factors for locomotor area (Scale A) was higher gestational age at birth (OR 0,88; CI 95% 0,77-1; p=0,06) while male sex was a negative prognostic factor (OR 1,82; CI 95% 1,07-3,10; p=0,03). *(Table 8)*

Male sex was also a negative prognostic factor (OR 2,18; CI 95% 1,28-3,72; p=0,004) for personal-social area (Scale B) while cesarean delivery seemed to be a protective factor (OR 0,47; CI 95% 0,25-0,91; p=0,02).*(Table 8)*

About hearing and language area (Scale C)"cross-sectional" EUGR at 6 months (OR 1,63; CI 95% 0,99-2,68; p= 0,05) and male sex (OR1,88; CI 95% 1,14-3,10; p=0,01) seemed to be the only negative prognostic factors.*(Table 8)*

3y GMDS LOCOMOTOR DQ (< 85 vs≥ 85)			
n	83	179	TOT 262
Male sex	49 (59,0%)	81 (45,3%)	p=0,06
Surgical PDA	10 (12,0%)	9 (5,0%)	p=0,07
NEC	13 (15,7%)	14 (7,8%)	p=0,08
BPD	27 (32,5%)	34 (19,0%)	p=0,02
Major Surgery	16 (19,3%)	16 (8,9%)	p=0,02
"Cross-sectional" EUGR at 6 months	46 (55,4%)	76 (42,5%)	p=0,06
Weight z-score at 6 months	-1,49 ± 1,58	-1,10 ±1,07	p=0,02
Weight z-score at 12 months	-1,61 ± 1,51	-1,22 ± 1,06	p=0,03
Multivariate analysis corrected for GA			
Gestational Age (GA)	OR 0,88 (CI 95% 0,77-1)		p=0,06
Male sex	OR 1,82 (CI 95% 1.07-3,10)		p=0,03

3y GMDS PERSONAL-SOCIAL DQ (< 85 vs ≥ 85)			
n	87	175	TOT 262
sesso	54 (62,1%)	76 (43,4%)	p=0,06
TC	64 (73,6%)	149 (85,1%)	p=0,03
Multivariate analysis corrected for GA			
Male sex	OR 2,18 (CI 95% 1,28-3,72)		p=0,004
Cesarean delivery	OR 0,47 (CI 95% 0,25-0,91)		p=0,02

3y GMDS LANGUAGE DQ (< 85 vs ≥ 85)			
n	139	123	TOT 262
Male sex	79 (56,9%)	51 (41,5%)	p=0,01
"Cross-sectional" EUGR at 6 months	72 (52,1%)	49 (39,8%)	p=0,05
Multivariate analysis corrected for GA			
"Cross-sectional" EUGR at 6 months	OR 1,63 (CI 95% 0,99-2,68)		p=0,05
Male sex	OR 1,88 (CI 95% 1,14-3,10)		p=0,01

Table 8: Univariate and Multivariate Analysis corrected for gestational age (GA); Locomotor DQ (scale A), Personal-Social DQ (Scale B), and Language (Scale C) at 3 years Griffith Mental Development Scale. Data are reported in mean value ± SDS for continuous variables, absolute number, and percentage for categorical variables. Odds ratios (OR) were calculated with a 95% confidence interval (CI). All p-values were based on two-tailed tests.

Regarding hand-eye coordination (Scale D) and performance (Scale E) the major risk factors seemed to be male sex (OR 4,17; CI 95% 1,78-9,76; p=0,001 - OR 2,39; CI 95% 1,44-3,97; p=0,001 respectively)and NEC (OR 4,17; CI 95% 1,78-9,76; p=0,001 - OR 4,31; CI 95% 1,63-11,35; p=0,003 respectively). *(Table9).*

3y GMDS HAND-EYE COORDINATION DQ (< 85 vs≥ 85)			
n	83	179	TOT 262
Male sex	49 (59%)	81 (45,3%)	p= 0,05
Surgical NEC	8 (9,6%)	5 (2,8%)	p=0,03
NEC	17 (20,5%)	10 (5,6%)	p=0,001
Major surgery	17 (20,5%)	15 (8,4%)	p=0,008
Multivariate analysis corrected for GA			
NEC	OR 4,17 (CI 95% 1,78-9,76)		p=0,001
Male sex	OR 1,80 (CI95% 1,04-3,10)		p=0,03

3y GMDS PERFORMANCE DQ (< 85 vs ≥ 85)			
n	126	136	TOT 262
Male sex	76 (60,3%)	54 (39,7%)	p=0,001
Surgical NEC	10 (7,9%)	3 (2,2%)	p=0,004
NEC	21 (16,7%)	6 (4,4%)	p=0,002
Multivariate analysis corrected for GA			
NEC	OR 4,31 (CI 95% 1,63-11,35)		p=0,003
Male sex	OR 2,39 (CI 95% 1,44-3,97)		p=0,001

3y GMDS PRACTICAL REASONINGDQ (< 85 vs≥ 85)			
n	101	161	TOT 262
Male sex	58 (57,4%)	71 (44,4%)	p=0,06
NEC	19 (18,8%)	8 (5,0%)	p=0,001
"Longitudinal" EUGR 6 months	22 (21,8%)	19 (11,8%)	p=0,04
Multivariate analysis corrected for GA			
NEC	OR 4,47 (CI 95% 1,84-10,85)		p=0,001
"longitudinal" EUGR at 6 months	OR 2,07 (CI 95% 1,02-4,17)		p=0,04

Table 9: Univariate and Multivariate Analysis corrected for gestational age (GA); Hand-Eye Coordination DQ (scale D), Performance DQ (Scale E), and Practical Reasoning (Scale F) at 3 years Griffith Mental Development Scale. Data are reported in mean value ± SDS for continuous variables, absolute number, and percentage for categorical variables.Odds ratios (OR) were calculated with a 95% confidence interval (CI). All p-values were based on two-tailed tests.

For practical reasoning (Scale F) only “longitudinal” EUGR at 6 months (OR 2,07; CI95% 1,02-4,17; p= 0,04) and NEC (OR 4,47; CI 95% 1,84-10,85; p=0,001) seems to be significative *(Table9).*

Discussion:

EUGR is considered a risk factor for worse global mental development (33) (34) (35) (36) (37) (38). However, most studies tried to identify which definition and time-point of EUGR is better associated with poor neurological outcomes. Recently Zozaya et al. found that “longitudinal” EUGR at 36 weeks of CA is a worse negative prognostic factor than “cross-sectional” EUGR at the same time-point for mental development index (MDI) at 24 months (OR 5.6; 95% CI 1.7 - 9.4) (34). An Italian study guided by Domenico Umberto De Rose and Luca Maggio compared 48 definitions of EUGR, 24 "cross-sectional" and 24 "longitudinal" at different time points from births to TEA and with different growth charts (INeS charts and INTERGROWTH 21st) and found that “longitudinal” EUGR defined as loss of more than 1 point z-score from when physiological weight loss is over to discharge seemed to better predict neurodevelopmental outcomes (36). In our study, we didn't find an association between EUGR at TEA and worse neurodevelopment even if "longitudinal" and "cross-sectional" definitions were considered. On the other hand, we found that better weight z-score at 6 months is protective for pathologic-border line GMDS (cut off <85) at 2 years of age (OR 0,74; 95% CI 0,59-0,93; p=0,01). Furthermore, “Cross-sectional” EUGR at 6 months seemed to be associated with a worse neurodevelopment for some areas of GMDS at 2and 3 years in particular: 2y locomotor (scale A: OR 1.96; CI 95% 1.10-3.47; p=0.02), 2y personal-social (scale B: OR 1,94; 95% CI 1,12-3,37; p=0,02), 2y language (scale C: OR 1,87; CI 95% 1,05-3,29; p= 0,02), 3y language (scale C:OR 1,63; C.I. 95% 0,99-2,68; p= 0,05). “Longitudinal” EUGR from TEA to 6 months of CA was associated with a worse GMDS in 2y performance (scale E: OR 2,10; CI 95% 1,03-4,30; p= 0,04) and 3y practical reasoning

(scale F: OR 2,07; CI95% 1,02-4,17; p= 0,04). The 6-month time-point was not taken into consideration in the above-mentioned studies as well as MRI data.

A similar result was published by Guellec I at al., part of the EPIPAGE study, that found an association between "longitudinal" EUGR from birth to 6 months of age and cerebral palsy at 5 years in patients born AGA and cognitive deficiency-school difficulties in SGA at 5-8 years even if not significantly (39). This study too, however, didn't consider MRI.

Our results are interesting because it draws attention to post-discharge growth and feeding of VLBW preterms. Indeed, although there are evidence-based recommendations on VLBW nutrition in hospital (48), little is known about optimal nutrition after discharge and complementary feeding.

Regarding post-discharge nutrition in our center, we recommend mother milk feeding when it's available. Direct breastfeeding is rarely possible in our preterm babies so the initial indication is to feed with unfortified mother milk by bottle for a hydric quotient of 160 up to 200 ml/kg per day. In the absence of breastmilk, a post-discharge formula is prescribed for an energy quotient of between 120 and 140 kcal/kg/day until TEA and 3000 g of weight are reached, then it is replaced with common formula milk for an energy quotient of between 110 and 130 kcal/kg/day. These intakes are adjusted and individualized based on weight growth in the last hospital weeks and the first few post-discharge weeks and depending on the presence of co-morbidities.

Reviewing the literature, there's a large consensus that mother milk represents the best choice due to its well-known positive effects on neurodevelopment (49) (48) (50) (51). Unfortunately, it's not always available and only 17% of patients in our population were fed by mother milk exclusively at 40 weeks of CA. The role of fortification with products that increase caloric and macro-micronutrient intake after discharge is still unclear. As a matter of fact, a quite recent Cochrane meta-analysis by Young et al. did not provide evidence that fortification of mother milk after hospital discharge affects growth rates and neurodevelopmental outcomes at 18 months CA (52). On the other hand, other studies (in particular a metanalysis and an RCT) have

shown some advantages in anthropometric parameters and no deleterious effect when fortified breast milk is used after discharge in VLBW preterm even if don't report evidence about neurodevelopment (53) (54).In our population, a great part of preterms was fed with formula milk at 40 weeks CA (41%) following the above-mentioned indications. Also, the choice of formula milk after discharge is controversial. Post-discharge formulas enriched in protein, LCPUFA, and micronutrients are commonly suggested for post-discharge nutrition in preterms (55) even if a recent Cochrane metanalysis didn't report advantages in the use of post-discharge formula, in particular for growth or neurodevelopment (56). In this context, it doesn't seem necessary to change our indications regarding fortification of breast milk or the use of special formula milk in the immediate post-discharge period. Instead, it would be necessary to improve the rate of patients fed exclusively by mother milk through careful education of mothers during hospitalization and after discharge.

Complementary feeding is also crucial for infantile nutrition and neurodevelopment (57) and is a real challenge in preterms because evidence-based recommendations are still lacking (58) and there's a wide variability in time of introduction, micronutrient supplementation and types of foods proposed center by center (59).

According to our internal protocol, we suggest a weaning time of 6 months of postnatal age (PA), quality of food proposed is the same as term babies in according to EPHGAN indications (60).

Different timing for weaning was proposed in the past such as 3-6 months of PA (61), 5-8 months of PA (62), or recently 3 months of CA (63) but these recommendations are mainly derived from observational studies. The few randomized controlled trials (RCT) do not report significant differences in weight growth when weaning is started at 4 vs 6 months of correct age (64) or 13 vs 17 weeks of age (65) but only an improvement in length at 12 months of age (65). Only another recent RCT guided by an Austrian group found that starting complementary feeding in VLBW at 10-12 weeks of CA (about 2 months and a half) instead of 16 -18 weeks of CA (about 4 months) had positive effects on weight z-score at 6 months (−0.49 mean; SD ± 1.2

vs −0.56 mean; SD ± 1.04, p = 0.03) (66).None of these RCTs considered neurodevelopment as outcome. The efficacy on the growth of this “early weaning policy” is still controversial with poor evidence as observed in different cohort studies (67) (68) but seems to be safe without increased risk of obesity (64) (69) and food allergy or atopic dermatitis (70).

At weaning time, it’s also important to consider the neurological skills reaching necessary for good swallowing. It can and should be stimulated already during hospitalization to not delay complementary feeding (71) (72) (73) (74).

A recent systematic review guided by the Italian Societies of Pediatrics (SIP), Neonatology (SIN), and Paediatric Gastroenterology, Hepatology and Nutrition (SIGENP) tried to draw up recommendations for complementary feeding in preterm infants and recommended to start weaning between 5 and 8 months of PA consider the limit of 3 months of CA to ensure the acquisition of developmental skills (75).Based on our data and what is reported in the literature, we speculate that an early weaning policy at about 3 months of correct age (i.e., between about 4 and 6 months of PA depending on GA), will be safe and could improve weight gain at 6 months and consequently the neurological outcome of VLBW patients. It’s also important to consider the child's neurological competence with a multidisciplinary approach (pediatric-logopedic-physiatrist-neuropsychiatric). This would modify our internal protocol, especially in preterm with major GA at birth.

About the type of food proposed in complementary feeding, specific recommendations for preterm doesn't exist and although the need to increase the intake of some nutrients is known, it’s not possible to define a weaning dietary scheme on a scientific basis for preterm infants. The guidelines used for term babies are actually accepted (60) (76).

An interesting result derives from the Cochrane systematic review that reports a decrease in risk of undernutrition and growth improvements when the family of term babies received adequate nutritional education even if the effects on neurodevelopment remain uncertain (77). A similar study was conducted in preterms families without evidence for lack of studies, but we can speculate that the same effects of term babies can occur in preterm (78). We

propose to offer families of preterm infants to be educated about complementary nutrition by a specialist.

Ultimately, other well-known risk factors like GA (4) (5) (6), NEC (26) (27), and major surgeries (31) (32)were identified by our study as risk factors for worse GMDS in different areas. In our population, males seemed to have worse GMDS in particular at 3 years. These data were already described in the literature and may be associated with the role of sexual hormones by regulating cortical growth and scaling and mediating the inflammatory responses (79) (80). About MRI findings, in our population, PWML was confirmed to be a risk factor for worse GMDS, in particular for locomotor development (scale A) and performance (scale E) at 2 years. This data is not new as it has already been reported in a recent systematic review our group was part of (81). By comparing our results with previous studies (22), we remain uncertain of the reason for the absence of negative effects due to minor IVH and CBH diagnosed with SWI. A possible explanation is the bias in sampling the population in these retrospective studies.

The strengths of our study are the large populationof VLBW and the presence of MRI data in all enrolled patients. This brings a new feature as the major studies on the evaluation of the relationship between EUGR and neurodevelopment present in the literature did not include MRI data.

The weakness of our study are the retrospective design and the long period of enrollment (about 7 years) with changes in the diagnostic and therapeutic approach over the years.

Conclusions

Our study confirms that extrauterine growth retardation affects neurological outcomes in preterm infants. The new finding is that the diagnosis of EUGR at 6 months seems to have a greater impact than EUGR at TEA on neurological outcome, particularly in a population of VLBW preterm infants with negative MRI or minor lesions. This finding draws attention to

post-discharge feeding and weaning. Although data regarding this matter in the literature are limited, it seems justified and safe to propose a post-discharge nutritional protocol for VLBW preterm based on exclusive mother milk feeding, early weaning, family education, and multidisciplinary approach also to corroborate data reported in literature through a prospective study.

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