Evaluation of Feeding Tolerance in Intrauterine Growth Restricted Preterm Infants

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Evaluatie van Voedingstolerantie in te Vroeg Geboren Kinderen met Intrauteriene Groeiachterstand

(met samenvatting in het Nederlands)

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Una grande parte di quello che i medici sanno è insegnato loro dai malati.

Marcel Proust

Alle mie nonne

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Introduction

The term intrauterine growth restriction (IUGR) indicates the presence of a pathological process occurring in utero that inhibits fetal growth as documented by at least two fetal intra-uterine growth assessments. IUGR is an important and relatively common problem in obstetrics which may represent impaired placental function and associated placental nutrient transport function (1). In developed countries, 3-7% of newborns are classified as IUGR, the causes of which include, but are not limited to, maternal malnutrition, maternal hypertension and idiopathic placental insufficiency.

In IUGR fetuses placental insufficiency may be characterized by blood flow redistribution to the vital organs (brain, myocardium, and adrenal glands), while other organs, including the gastrointestinal tract, are deprived from sufficient blood flow. These fetuses are at increased risk of hypoxia, hypoglycemia and acidemia and also spontaneous preterm delivery (2,3).

The IUGR fetus: Detection and Monitoring

Fetal Monitoring

Serial ultrasound biometric recordings may be able to identify the fetus that does not reach its growth potential. Commonly used methods for estimating fetal size are clinical palpation, fundal height measurement and ultrasonic fetal biometry. Ultrasound must be considered the method of choice as it is highly reliable and reproducible (4). When IUGR is the consequence of a placental etiology (placental insufficiency), management is based on careful fetal assessment in order to detect the optimal time for delivery. The most commonly used methods of monitoring include Doppler assessment. Doppler velocity waveform in arteries is mainly influenced by the characteristics of the diastolic phase and reflects the peripheral resistance to blood flow. The pulsatility index (PI) (peak systolic velocity – end-diastolic velocity/mean

velocity) is commonly used. PI values increase as the peripheral resistance increases (1).

Increased placental resistance in the presence of placental failure leads to a reduction in end diastolic blood flow (EDF) through the umbilical arteries (UA), progressing to absent (AEDF) or reversed flow (AREDF) (5). Animal and human experiments have shown that there is an increase in blood flow to the brain in the IUGR fetus. This increase in blood flow can be evidenced by Doppler ultrasound of the middle cerebral artery (MCA). Longitudinal studies on deteriorating early-onset IUGR fetuses have reported that MCA PI progressively becomes abnormal. This phenomenon is defined as brain sparing (BS) effect. An UA PI to MCA PI ratio >1 is considered as index of BS and fetal hypoxia (6). Up to 20% of IUGR fetuses have severe Doppler abnormalities also associated with poorer perinatal outcome and suboptimal neurodevelopmental development at 2 years of age (7-9). As a consequence of the redistribution of the blood flow in IUGR infants, gut may be under-perfused and ischemia/hypoxia may occur; IUGR infants are thought to have impaired gut function after birth, which may result in intestinal disturbances, ranging from temporary intolerance to the enteral feeding to full-blown necrotizing enterocolitis (NEC) occurring up to 7% of very low birth weight infants (10-13). A meta-analysis of 14 observational studies demonstrated an increased incidence of NEC in preterm infants who had suffered fetal AREDF compared with controls, with an odds ratio of 2.13 (95% CI 1.49 to 3.03) (14).

Neonatal Monitoring

Doppler Findings

The superior mesenteric artery (SMA) is the major source of blood for the small intestine and for a portion of the large intestine. After birth, SMA blood flow velocity (BFV) increases to support the dramatic increase in intestinal growth and oxygen uptake that occurs during the first few postnatal weeks (15). There is increasing evidence that the rate of increase in SMA BFV may have clinical significance. Greater increases in postnatal SMA BFV during the first week of life in preterm infants are reported to be associated with less intestinal dysmotility, and with better tolerance to enteral feedings (16,17). Intestinal blood flow is regulated by numerous factors including cardiovascular status, neural control, humoral substances and local control. Extrinsic factors that can affect splanchnic blood flow in preterm infants include the type and volume of enteral nutrition given, and various pharmacologic agents, including indomethacin and caffeine. Although factors that can affect intestinal blood flow have been identified, little is known about factors that may affect the rate at which SMA BFV increases in newborn preterm infants (18-21).

Near Infrared Spectroscopy (NIRS)

The assessment of adequate perfusion in very low birth weight infants is commonly based on clinical parameters, as well as invasive measures requiring central venous and/or arterial catheter access with well-established associated risks. Since the pathogenesis of intestinal dysfunction in IUGR infants seems to be related to a redistribution of blood flow, monitoring the perfusion and the oxygenation of the splanchnic district in IUGR infants is mandatory. Additionally, most of these data are acquired intermittently, and

thus may only represent a delayed picture of oxygen delivery and consumption.

NIRS is a continuous, non-invasive, real-time and portable technique, which can be used to measure oxygenation in living tissue (22). NIRS has been reported to be useful in detecting changes in oxygen delivery as calculated by fractional tissue oxygen extraction (FTOE) ratio: (SaO2-rSO2)/SaO2. Thus, NIRS in the splanchnic district offers useful information on tissue oxygen delivery and may predict splanchnic ischemia in neonates by measuring, the cerebrosplanchnic oxygenation ratio (CSOR). Splanchnic oxygenation is compared with brain oxygenation as a reference, because under most of physiological conditions cerebral blood flow autoregulation minimizes changes in brain oxygenation during events affecting splanchnic perfusion (23). NIRS is able to detect changes in splanchnic oxygen delivery, which is curtailed during IUGR and may be used to predict feeding intolerance and NEC by measuring the splanchnic/cerebral oxygenation status ratio.

IUGR and Preterm Infants Feeding

Infants born prematurely and IUGR, especially those with extremely low birth weight (ELBW), are often considered to be too unstable to be fed enterally; early feeding is thought to increase the risk of NEC and feeding intolerance. Nevertheless, the use of a prolonged parenteral nutrition (PN) exposes infants to the metabolic and central line complications as well as to the deleterious effects of fasting on gastrointestinal system (24,25). Late introduction of feeding may be detrimental due to lack of stimulation of the gastrointestinal tract, resulting in villous atrophy and lack of hormone and enzyme production and may not reduce the incidence of NEC. Enteral fasting can also affect the timing of full enteral feeding (FEF) and may delay hospital discharge (26,27).

MEF is also known as *"trophic feeding or gut-priming or non nutritive feeding or hypocaloric feeding"* conventionally defined as giving small volumes of milk (typically 12 to 24 ml/kg/day) starting within the first few days after birth without advancing the feed volumes during the first week of life (28). MEF in combination with PN are employed to: i) promote the intestinal motility and the development of an appropriate microflora, ii) preserve intestinal barrier integrity, and iii) reduce infection's rate.

Early enteral feeding is advantageous because it improves the functional adaptation of the gastrointestinal tract by stimulating hormone secretion and gastrointestinal motility (29). MEF has also been shown to improve gastrointestinal disaccharidase activity, hormone release, blood flow, motility and microbial flora. Clinical benefits include improved milk tolerance, greater postnatal growth, reduced systemic sepsis and shorter hospital stay.

A meta-analysis of RCTs conducted on preterm infants did not detect a significantly different risk of NEC between infants randomized to delayed feeding (as later than day 5–7 after birth) and infants vs early feeding (less than 4 day after birth) (30-33).

Altogether there is evidence that feeding issue in preterm and IUGR infants still constitutes a hot topic in NICU daily practice opening-up to further studies in terms of: i) how and when start feeding in IUGR and preterm infants, and ii) usefulness of perinatal standard monitoring procedures for feeding start.

Therefore, the purpose of the present thesis was to investigate in preterm and IUGR infants:

1. Feeding issues in IUGR and preterm infants;
2. Clinical parameters and Doppler patterns as predictors of feeding tolerance in IUGR infants;

3. Splanchnic and cerebral NIRS pattern in IUGR and non IUGR infants in the first 72 hours from birth.
4. Splanchnic oxygenation and perfusion pattern in IUGR and non IUGR infants after feeding by bolus and by continuous enteral nutrition

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Feeding Issues in IUGR Preterm Infants

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Abstract

Intra-uterine growth restriction (IUGR) is a severe and quite common problem in obstetrics. A condition of placental dysfunction can lead to a cardiovascular adaptation in the fetus characterized by a redistribution of cardiac output to maintain oxygen supply to the heart, adrenal glands and brain – the so called brain sparing (BS) effect - at the expense of visceral organs (as the gastrointestinal system). This condition may predispose IUGR infants to impaired gut function after birth. A higher incidence of necrotizing enterocolitis (NEC) is documented in IUGR preterm infants; therefore a common practice in neonatal intensive care unit (NICU) is to delay feeds to reduce the risk of feeding tolerance. Recent trials, however, have shown that early enteral feeding in IUGR infants is safe and it would appear, on the basis of the few available data, that breast milk may offer protection against NEC.
The present mini-review offers an up-date on feeding in IUGR infants. Future perspectives on the usefulness of Doppler and regional splanchnic and cerebral saturation monitoring for the time-decision of starting feeding are also provided.

Abbreviations: Intra-uterine growth restriction (IUGR); brain sparing (BS); necrotizing enterocolitis (NEC); neonatal intensive care unit (NICU); small for gestational age (SGA); American College of Obstetricians and Gynecologists (ACOG); birth weight (BW); absent/reverse end-diastolic flow (ARED); Umbilical artery (UA); middle cerebral artery (MCA); cerebroplacental ratio (U/C); pulsatility index (PI); full enteral feeding (FEF); adequate for gestational (AGA); very low birth weight (VLBW); total parenteral nutrition (TPN); minimal enteral feeding (MEF); near infrared spectroscopy (NIRS).

Introduction

Intra-uterine growth restriction (IUGR) is a severe and quite common problem in the perinatal period; it is determined by placental insufficiency and impaired placental nutrient transport from mother to the fetus. In developed countries, 3-7% of newborns are classified as IUGR [1]. These fetuses are at major risk of hypoxic events, hypoglycemia and acidaemia and also spontaneous preterm delivery [2] hence IUGR represents an important clinical entity. It has been shown that 52% of stillbirths are associated with IUGR [3] and 10% of perinatal mortality is a consequence of IUGR [4]. Up to 72% of unexplained fetal deaths are associated with being IUGR and/or small for gestational age (SGA) [5].

A globally recognized definition of IUGR has been proposed by the American College of Obstetricians and Gynecologists (ACOG) [3] ‘‘a fetus that fails to reach his potential growth’’. Small for gestational age, on the other hand, is a different entity, but it is also associated with adverse perinatal outcomes. SGA is defined as a birth weight (BW) below the 10th percentile for gestational age. SGA and IUGR are not synonymous. Evaluation of fetal-placental hemodynamic patterns, by means of Doppler velocimetry recordings in the umbilical and cerebral arteries, associated with longitudinal monitoring of fetal growth and maternal clinical conditions, constitute the main issue for IUGR and SGA early detection [6-9].

In IUGR pregnancies with impaired placental perfusion, transfer of oxygen and nutrients from the mother to the fetus is reduced. In severe IUGR, absent/reverse end-diastolic flow (ARED) in the umbilical artery may be observed. Perinatal mortality and morbidity are markedly increased in the presence of ARED flow [10]. Abnormal umbilical artery Doppler is also associated with a higher risk for adverse perinatal and neurodevelopmental outcome [11-14].

This condition of placental dysfunction may lead to a cardiovascular adaptation of the fetus, characterized by a redistribution of cardiac output to maintain oxygen supply to the heart, adrenal glands and brain – the so called brain sparing (BS) effect - at the expense of visceral organs (as the gastrointestinal system). Doppler patterns suggestive of BS are characterized by and abnormal cerebroplacental ratio (U/C) as defined by umbilical (UA) and middle cerebral arteries (MCA) pulsatility index (PI: peak systolic velocity-end-diastolic velocity/mean velocity) ratio. An U/C >1 is considered as index of fetal hypoxia [15]. In animal and clinical models, U/C has been shown to be a sensitive marker of hypoxia and to correlate with adverse perinatal outcome [16,17]. As a consequence of BS, gut ischemia/hypoxia can occur and therefore infants with ARED flow are thought to have impaired gut function after birth. IUGR infants are at risk for intestinal disturbances, ranging from temporary enteral feeding intolerance to necrotizing enterocolitis (NEC).

Risk of NEC

Literature data provides evidence that feeding tolerance improves as gestational age increases and it worsens in IUGR infant. SGA infants spend more time to achieve full enteral feeding (FEF) than adequate for gestational (AGA) infants do. Feeding tolerance is influenced by gestational age; it constitutes a significant determinant of perinatal morbidity affecting an adequate intrauterine growth and maturation of the gastrointestinal tract. This especially holds for SGA infants in whom the suspected placental insufficiency and subsequent hemodynamic compensatory mechanisms reasonably have a somewhat negative impact on feeding tolerance [18].

Analysis of the effect of IUGR on outcome of 19759 singleton infants born at 25–30 weeks gestation and enrolled in the Vermont-Oxford Database revealed an increased risk of NEC when corrected for significant covariates

[19]. A meta-analysis of 14 observational studies confirmed an increased incidence of NEC in preterm infants who had exhibited fetal ARED flow compared with controls, with an odds ratio of 2.13.[20]

Early versus Delayed Feeding

To date, the timing of starting enteral feeding in IUGR very low birth weight (VLBW) infants to prevent NEC and feeding intolerance is still controversial and matter of debate. Several strategies have been promoted such as: i) late introduction of enteral nutrition, ii) slowly increasing feeds, iii) use of enteral fasting and total parenteral nutrition (TPN) and prophylactic antibiotics [21]. In this regard, since there are pros and cons issues for early and late introduction of enteral feeds, no conclusive consensus has been obtained. From one side early introduction may improve nutrition and growth, but from the other side may also increase the risk of NEC and feeding intolerance [22]. Conversely, late introduction of feeding may be detrimental due to lack of stimulation of the gastrointestinal tract, resulting in villous atrophy and lack of hormone and enzyme production and may not reduce the incidence of NEC [23].

Literature reported inconclusive data about the time of starting enteral feeding, because of: i) no available data provided evidence that delayed introduction of progressive enteral feeds may reduce the risk of NEC in VLBW infants, ii) delay in introducing progressive enteral feeds affects FEF timing but the clinical importance of this effect is still unclear, iii) IUGR infants are excluded from many trials of early enteral feeding practices. Altogether, data on timing of minimal enteral feeding (MEF) for IUGR preterm infants in the trials included in the Cochrane review [24] are lacking.

Recently, an open-label randomized control trial in preterm infants with IUGR and abnormal antenatal Doppler suggested that early introduction of MEF may not have a significant effect on the incidence of feeding

intolerance [25]. Nevertheless a larger sample size is needed to draw definitive conclusions regarding the effect of early MEF on measures of clinical outcome.

A larger sample size (including 404 preterm infants with IUGR and abnormal antenatal Doppler) was enrolled in a randomized trial by Leaf et al. [26] which compared the effect of “early” versus “late” commence of enteral feeding on NEC incidence. They concluded that there was no difference in the incidence of NEC [early group: 18% vs in late group: 15%; (relative risk: 1.2 95% confidence interval: 0.77–1.87)]. Early feeding resulted in shorter duration of TPN and high-dependency care, lower incidence of cholestatic jaundice, and improved SD score for weight at discharge. Early introduction of enteral feeds in growth-restricted preterm infants resulted in earlier achievement of full enteral feeding and did not appear to increase the risk of NEC.

A more recent randomized trial, on 133 IUGR infants, comparing the effects of an "early" enteral feeding regimen versus “late” enteral feeding on the incidence of NEC and feeding intolerance concluded that early MEF of preterm infants with IUGR and abnormal antenatal Doppler results may not have a significant effect on the incidence of NEC or feeding intolerance [27].

Finally, American Society for Parenteral and Enteral Nutrition (ASPEN) guidelines about the nutrition support of preterm infants at risk for NEC state that, although the majority of the studies in the literature have recommended larger, multicentre prospective trials to evaluate issues on enteral nutrition initiation and advancement, on the basis of the available data, early MEF within the first 2 days of life and advancement at 30 mL/kg/d in infants ≥1000 g can be suggested [28].

New perspectives

Postnatal physiological studies have shown persistent flow abnormalities in

the superior mesenteric artery blood flow velocity in IUGR infants during the first days of life [29,30]. Neonates with increased resistance patterns of blood flow velocity in the superior mesenteric artery on the first day of life are at higher risk of developing NEC [31]. Therefore, the gradual recovery of intestinal perfusion during the first days of life provides a sound rationale for a modest delay in enteral feeding. A randomized trial is actually ongoing in our NICUs to detect the physiological changes (and those induced by feeding) in splanchnic oxygenation and perfusion in IUGR infants trough near infrared spectroscopy (NIRS) monitoring. We expect to clarify the timing and the entity of the postnatal intestinal adaptation in infants who experienced intrauterine growth restriction [32]. Additional informations can be provided from splanchnic and cerebral saturation values comparison between IUGR VLBW infants and NON IUGR VLBW infants in the transitional period (first 72 hours of life). Preliminary results showed that in the first 72 hours from birth adaptation phase is significantly different in IUGR infants with changes in cerebral and splanchnic oxygenation patterns. Data suggest that the BS lasts 24 hours after birth and then it vanishes with 3-days from birth.

Bozzetti et al. (unpublished data) in a case-control study enrolled 70 IUGR infants of whom 35 were complicated by BS and hypoxia. The control group consisted of 35 IUGR preterm infants with no BS matched for gestational age. Clinical data and parameters of feeding tolerance (days to achieve FEF) were compared between IUGR infants with BS versus IUGR infants without BS. Results showed that the interval from birth to the achievement of FEF was significantly shorter in IUGR no BS than IUGR with BS. Multivariate analysis showed significant correlations between FEF and the occurrence of BS.

Which Milk

Due to the difficulty of recruiting infants to a randomized trial of human or formula milk (mothers usually have strong preferences) trials are to date lacking. Lucas and Cole [33], in a large prospective randomized trial that dates back in the 90s, identified a protective effect of breast milk on NEC.

McGuire et al. performed a review to determine whether enteral feeding with donor human milk compared with formula milk reduced the incidence of NEC in preterm or VLBW infants. Four small trials fulfilled the pre-specified inclusion criteria. None of them found any statistically significant difference in the incidence of NEC. However, meta-analysis found that feeding with donor human milk was associated with a significantly reduced relative risk of NEC. Infants who received donor human milk were three times less likely to develop NEC and four times less likely to have confirmed NEC than infants who received formula milk [34].

A more recent Cochrane review on this issue stated that there are no sufficient data from randomized trials comparing formula milk versus maternal breast milk for feeding preterm or VLBW infants [35]. This may be related to a perceived difficulty of allocating an alternative feed to an infant whose mother wishes to feed with her own breast milk. Maternal breast milk remains the default choice of enteral nutrition because observational studies, and meta-analyses of trials comparing feeding with formula milk versus donor breast milk, suggest that feeding with breast milk has major non-nutrient advantages for preterm or low birth weight infants.

Conclusions

IUGR is considered to be a serious yet compensated state of decreased placental function with fetal circulatory redistribution in favor of the most vital organs. Blood flow redistribution does have postnatal clinical impact, especially in very preterm infants, predisposing to NEC [36]. Abnormalities

of splanchnic blood flow persist post-natally, with some recovery during the first week of life, providing physiologic justification for a delayed and careful introduction of enteral feeding. Nevertheless, recent trials comparing early versus delayed introduction of enteral feeding suggest that prolonging enteral fasting after birth is not justified with regards of NEC incidence or feeding tolerance.

Breast milk has been shown to offer protection against NEC, thus promoting breastfeeding in the NICU setting is mandatory. Further investigation in this area, with well-defined end-points of clinical outcome is warranted.

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Declaration of interest

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Feeding Tolerance of Preterm Infants Appropriate for Gestational Age (AGA) as Compared to those Small For Gestational Age (SGA)

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Abstract

Preterm infants are often considered too unstable to be fed enterally so they are exposed to complications related to a prolonged enteral fasting.

Our study aims to compare feeding tolerance of AGA versus SGA infants and to evaluate which perinatal factors affect feeding tolerance (measured as time to achieve full enteral feeding, FEF).

Inborn infants with a gestational age (GA) less than 32 weeks, born from January 2006 to December 2010, were eligible to this study.

We enrolled 310 infants. The time to FEF was longer for SGA infants than for AGA, while a longer GA was associated to a reduced time to FEF.

A beneficial effect was observed for antenatal steroids, while Apgar score below 7, the administration of inotrops or caffeine, the occurrence of sepsis or NEC, and the presence of PDA were associated to a longer time to FEF. When evaluated jointly with a multivariate analysis, GA ($p<0.0001$), antenatal steroids prophylaxis ($p=0.002$), SGA ($p<0.0001$), and occurrence of NEC ($p=0.0002$) proved to have independent prognostic impact on the time to FEF.

Feeding tolerance is better as GA increases, and worsen in SGA infants. Antenatal betamethasone is effective in reducing the time to FEF in both AGA and SGA.

Keywords: Minimal Enteral Feeding, Full Enteral Feeding, antenatal steroids, prematurity, necrotizing enterocolitis.

Abbreviations: AGA, adequate for gestational age; GA, gestational age; BW, birth weight; SGA, small for gestational age; VLBW, Very Low Birth Weight; PN, Parenteral Nutrition; MEF, Minimal Enteral Feeding; NEC, Necrotizing EnteroColitis; FEF, Full Enteral Feeding

Introduction

Infants born prematurely, especially those with extremely low birth weight (ELBW), are often considered to be too unstable to be fed enterally (1). Furthermore, an early oral feeding may expose them to the occurrence of necrotizing enterocolitis (NEC) known to have a high impact on mortality and morbidity rates (2-3). Conversely, the use of a prolonged parenteral nutrition (PN) exposes infants to the metabolic and central line complications as well as to the deleterious effects of fasting on gastrointestinal system (4). Enteral fasting can predispose to impaired intestinal growth, mucosal atrophy, intestinal barrier dysfunction, decreased digestive and absorptive capacity, increased colonization with pathogenic bacteria, and systemic inflammation. Enteral fasting can also affect the timing of full enteral feeding (FEF) and may delay hospital discharge (5). In this regard, minimal enteral feeding (MEF) in combination with PN is employed to: i) promote the intestinal motility and the development of an appropriate microflora, ii) preserve intestinal barrier integrity and iii) reduce infection's rate (6). MEF has also been shown to improve gastrointestinal disaccharidase activity, hormone release, blood flow, motility and microbial flora. Clinical benefits include improved milk tolerance, greater postnatal growth, reduced systemic sepsis and shorter hospital stay (7-10).

Infants born SGA are known to be complicated by placental insufficiency. This impairment of the placenta's functions should cause a redistribution of blood flow to heart and brain with a consequent blood flow deprivation in the splanchnic district (11-17). Therefore, in SGA infants, an altered intestinal perfusion may occur leading to impaired gut function and intolerance of progression of the enteral feeding up to even full-blown NEC. However, data on potential association between the above mentioned perinatal factors and the feeding tolerance in SGA infants are, to date, lacking.

The aim of the present study was twofold: (i) to assess feeding tolerance of AGA versus SGA infants with gestational age (GA) below 32 weeks; (ii) to evaluate if any parameter of perinatal morbidity affects the progression of oral intake.

Patients & Methods

We selected all inborn babies with a GA less than 32 weeks admitted at the Neonatal Intensive Care Unit (NICU) of San Gerardo Hospital from January 2006 to December 2010. During the study period 423 infants with a GA below 32 weeks and no major congenital malformations or anomalies were admitted to our NICUs. Three-hundred ten infants were eligible for the study. One-hundred thirteen were excluded for the following reasons: outborn (n= 41), death (n= 41) or transfer to other Institution (n= 31) before the achievement of FEF due to surgical intervention. We excluded infants with major congenital malformations or anomalies that may interfere with enteral nutrition and those who didn't achieve full enteral feeding (FEF, defined as an enteral intake of 160 mL/Kg/day) before discharge (because of death or transfer to another hospital). Data were collected from NICU's medical records (Metavision IMD System).

We recorded prenatal data regarding maternal health status, demographic variables and clinical data such as percentiles for birth weight, length and head circumference, gestational age, Apgar score, umbilical cord pH, occurrence of sepsis episodes, presence of haemodynamically significant ductus arteriosus, use of inotropic drugs and/or caffeine, occurrence and severity of neonatal distress respiratory syndrome (RDS) with or without the need for mechanical ventilation longer than 96 hours, occurrence of necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), periventricular/intraventricular hemorrhage (PIVH) and periventricular

leukomalacia (PVL). Perinatal and clinical characteristics of the study cohort of 310 infants are described in Table 1.

Maternal steroid prophylaxis was defined as complete when betamethasone 12 mg was given twice at 12-h interval (18) with the last dose administered at least 24 hours before the delivery.

Infants were classified as SGA when, due to placental insufficiency, the birth weight (BW) was under the 10' percentile according to the Italian Neonatal Study charts (19).

Categories of GA were defined, when needed, on the basis of the CRIB score's gestational age subdivision (20).

To assess the feeding tolerance, we registered the time (in days) from birth to FEF achievement, the age at the start of MEF and the time necessary to achieve full enteral feeding from the start of MEF. All infants were fed according to our institutional guidelines. Infants without severe hemodynamic instability, suspected or confirmed NEC, evidence of intestinal obstruction/ perforation or paralytic ileus, were given minimal enteral feedings (< 20 ml/kg/day) that were advanced at 15 to 20 ml/kg/day if tolerated. Feedings were given as a bolus every 3 hours or continuously and included breast milk, when available, or preterm formula (80 Kcal/100ml). Human milk was fortified when an enteral intake of 100 ml/kg/day was tolerated.

Statistical Analysis

We applied the Fisher's exact test to assess the association between patients' clinical features and AGA versus SGA classification. Medians of the time from birth to FEF, from birth to MEF and between MEF and FEF in these two subgroups were compared by the Wilcoxon rank sum test, while we

applied the Kruskall-Wallis test to perform the analogous comparisons by GA.

The impact on feeding tolerance (i.e. on the three time spans mentioned above) of each perinatal factor, taken separately, was assessed by means of the Wilcoxon rank sum test. After taking the logarithms of the times expressing feeding tolerance, we applied separate multiple linear regression models and the t-test to jointly analyse the impact of the following perinatal factors: GA, SGA, Antenatal steroids, Apgar at five minutes, Inotropic drugs, Caffeine, Sepsis, PDA and NEC. All tests were two sided.

All analyses were performed with the software SAS 9.2. A $p<0.05$ was considered as significant.

Results

Perinatal and clinical characteristics of the study cohort of 310 infants are described in Table 1.

Overall, the 310 infants had a median GA of 29+5 weeks+days (range, 23+6 to 31+6 weeks+days), with a median BW of 1153 g (range, 495 to 2230 g). Male infants were 160 (51.6%); Apgar score at fifth minute was 8 (range, 1 to10). PDA and RDS occurred in 76 (24.5%) and 178 (57.4%) infants, respectively. Two hundred and twenty-six (72.9%) fetuses received a complete course of prenatal steroids. The cohort included 32 SGA infants (10.3%), who were not different from AGA infants, in terms of all considered perinatal and clinical features.

	Total (n = 310)	**SGA** (n = 32)	**AGA** (n = 278)	P
Perinatal Characteristics				
GA (wks), median (range)	29+5 (23+6-31+6)	30+1 (26+1-31+5)	29+5 (23+6-31+6)	0.28
BW (g), median (range)	1153 (495 - 2230)	815 (505 - 1065)	1220 (495-2230)	P<0.05
BW (centile), median (range)	54.5 (1-100)	5.5 (1-10)	59 (11-100)	P<0.05
Gender, male (%)	160 (51.6%)	15 (46.9%)	145 (52.1%)	0.58
Apgar 5'<7' (%)	43 (14.1%)	2 (6.3%)	41 (15.0%)	0.28
UA pH§, median (range)	7.30 (6.83-7.59)	7.29 (7.01-7.40)	7.30 (6.83-7.59)	0.48
Clinical Conditions				
RDS	178 (57.4%)	23 (71.9%)	155 (55.8%)	0.09
PDA	76 (24.5%)	6 (18.8%)	70 (25.1%)	0.52
Sepsis	75 (24.2%)	7 (21.9%)	68 (24.4%)	0.83
NEC	12 (3.9%)	1 (3.1%)	11 (4.0%)	0.99
IVH	77 (24.8%)	7 (21.9%)	70 (25.2%)	0.83
PVL	6 (1.9%)	0 (0%)	6 (2.2%)	0.99
BPD	29 (9.4%)	4 (12.5%)	25 (9.0%)	0.52
Treatments				
Antenatal steroids	226 (74.8%)	27 (90.0%)	199 (73.2%)	0.05
Inotropic therapy	39 (12.6%)	1 (3.1%)	38 (13.7%)	0.10
Caffeine	191 (61.6%)	20 (62.5%)	171 (61.5%)	0.99
MV > 96h	130 (41.9%)	7 (21.9%)	123 (44.2%)	0.32

Table 1: Perinatal and clinical features of the cohort, overall and in SGA and AGA subgroups. All data are number (%) unless otherwise stated. SGA: small for gestational age. AGA: adequate for gestational age. RDS: Respiratory Distress Syndrome; PDA: presence of haemodynamically significant ductus arteriosus; NEC: Necrotizing Enterocolitis, Bell stage ≥ 2; IVH: periventricular/intraventricular haemorrhage; PVL: periventricular leukomalacia; sepsis: clinical diagnosis or positivity of the blood colture; BPD: bronchopulmonary dysplasia; antenatal steroids: complete prophylaxis with betamethasone; MV: Mechanical Ventilation.

The time needed to achieve FEF in AGA and SGA infants is described according to clinically relevant classes of GA (Table 2). The increase in GA was associated with a reduction in the time to FEF ($p < 0.0001$) and SGA infants tended to achieve FEF later than AGA ($p=0.008$).

	Total	SGA	AGA
GA<28 wks			
N. Infants	77	3	74
BW (g)	850 (495-1490)	505, 530, 660	865 (495-1490)
time birth-FEF (days)	39 (14-91)	36, 38, 52	39 (14-91)
time birth-MEF (days)	5 (1-19)	6, 8, 15	5 (1-19)
time MEF-FEF (days)	32 (11-89)	23,30,44	32 (11-89)
GA from 28 to 29+6 wks			
N. Infants	87	12	75
BW (g)	1090 (615-1725)	755 (615-900)	1140 (820-1725)
time birth-FEF (days)	22 (10-50)	36 (20-50)	22 (10-50)
time birth-MEF (days)	4 (1-14)	7 (2-14)	3 (1-12)
time MEF-FEF (days)	18 (8-47)	28 (16-44)	18 (8-47)
GA from 30 to 31+6 wks			
N. Infants	146	17	129
BW (g)	1450 (700-2230)	930 (700-1065)	1500 (960-2230)
time birth-FEF (days)	16 (7-54)	20 (13-42)	15 (7-54)
time birth-MEF (days)	2 (0-18)	4 (1-10)	2 (0-18)
time MEF-FEF (days)	14 (6-42)	17 (11-32)	13 (6-42)
Total			
N. Infants	310	32	278
BW (g)	1153 (495-2230)	815 (505-1065)	1220 (495-2230)
time birth-FEF (days)	22 (7-91)	27 (13-52)	21 (7-91)
time birth-MEF (days)	3 (0-19)	5 (1-15)	3 (0-19)
time MEF-FEF (days)	18 (6-89)	21 (11-44)	18 (6-89)

Table 2 Time from birth to Full Enteral Feeding and birth weight in SGA and AGA infants by gestational age.
Numbers are median (range) unless otherwise stated. When the number of infants is less than 5, actual observations are reported instead of the median (range). SGA: small for gestational age. AGA: adequate for gestational age. GE=gestational age. BW=birth weight. FEF=Full Enteral Feeding. MEF=Minimum Enteral Feeding.

According to the univariate analysis (see Table 3), we analyzed other perinatal factors influencing feeding tolerance as measured by the time from birth to FEF.

Prognostic Factor	Birth-FEF	Birth–MEF	MEF-FEF
Antenatal steroids			
Yes	21 (7-91)	3 (0-19)	17 (6-89)
No	25 (7-79)	3 (0-18)	22 (7-73)
p-value	0.027	0.4532	0.0019
Apgar 5' < 7			
Yes	28 (8-79)	4 (1-19)	23 (7-73)
No	21 (7-91)	3 (0-19)	18 (6-89)
p-value	0.012	0.0028	0.0028
Inotropic drugs			
Yes	33 (11-90)	6 (0-19)	25 (11-76)
No	21 (7-91)	3 (0-19)	18 (6-89)
p-value	<0.0001	<0.0001	0.0008
Caffeine			
Yes	24 (7-91)	4 (0-19)	20 (7-89)
No	17 (7-79)	2 (0-15)	15 (6-72)
p-value	<0.0001	<0.0001	<0.0001
Sepsis			
Yes	31 (8-79)	5 (0-19)	27 (7-72)
No	20 (7-91)	3 (0-18)	17 (6-89)
p-value	<0.0001	<0.0001	<0.0001
PDA			
Yes	29 (12-90)	5 (1-19)	24 (9-76)
No	20 (7-91)	2 (0-19)	17 (6-89)
p-value	<0.0001	<0.0001	<0.0001
NEC			
Yes	51 (12-91)	3 (0-18)	45 (11-89)
No	21 (7-90)	3 (0-19)	18 (6-86)
p-value	0.0004	0.3270	0.0006

Table 3 Univariate analysis of the time from birth to FEF achievement, the age at the start of MEF and the time from the start of MEF to FEF achievement by relevant prognostic factors. Numbers are median days (range) unless otherwise stated. PDA: presence of haemodynamically significant ductus arteriosus; NEC: Necrotizing Entercolitis, Bell stage ≥ 2; FEF=Full Enteral Feeding; MEF=Minimum Enteral Feeding.

Protective effect was related to antenatal steroids prophylaxis (p=0.027), while Apgar score below 7 at five minutes (p=0.001), the administration of inotropic drugs (p<0.0001) and caffeine (p-value<0.0001), the occurrence of sepsis (p <0.0001), the presence of PDA (p <0.0001) and the occurrence of NEC (p=0.0004) were negative factors prolonging FEF achievement.

The multivariate analysis performed on 297 infants with complete data showed that GA, SGA, antenatal steroids prophylaxis and occurrence of NEC were the only independent prognostic factors for the time to FEF. A more advanced GA and the administration of antenatal steroids were both associated with a significant reduction of the time to FEF (p< 0.0001 and 0.002, respectively), while SGA infants needed a longer time to reach full enteral feeding (p < 0.0001) as well as infants who suffered from NEC (p=0.0002). For instance, the estimated time to FEF for an infant born at 24 weeks who does not carry any other risk factor, is 48 days, which increases to 69 if the baby is SGA or to 72 if NEC occurs. Should antenatal steroids be given, the projected time to FEF reduces to 41. At 28 weeks of GA, the estimated time to FEF almost halves to 26 days, and becomes 37 or 39 if SGA or NEC occurs, while it reduces to 22 days if antenatal steroids were administered.

Moreover, we evaluated age at the start of MEF and the between MEF and FEF. The results of the univariate comparisons by relevant perinatal features are described in Table 2 and 3, and are superimposable to the findings described above for the time from birth to FEF, except for antenatal steroids prophylaxis and NEC, which do not reach statistical significance for the time from birth to MEF.

The multivariate analysis of the interval from birth to MEF showed that the lower the gestational age, the later the MEF was started (p < 0.0001). SGA

infants started the MEF later than AGA infants ($p < 0.0001$). The presence of a hemodinamically significant ductus arteriosus, the use of caffeine therapy and of inotropic drugs negatively affected the age at start of MEF ($p = 0.002$, 0.006 and 0.012, respectively). Of note, antenatal steroids therapy did not show any correlation with the age at starting MEF.

For what concerns the interval of time between MEF and FEF, the multivariate analysis revealed that it is shorter with higher GA ($p < 0.0001$) and with administration of antenatal steroid prophylaxis ($p =0.003$) while it is longer in SGA infants ($p = 0.0001$) and in patients who suffered from sepsis ($p =0.036$) and NEC ($p=0.0001$).

Discussion

There is growing evidence that in preterm infants an accurate evaluation of feeding tolerance and of the timing of achievement of full enteral feeding is factor of utmost importance in a successful management of high risk newborns. To the best of our knowledge data on perinatal events improving or affecting minimal and/or FEF are controversial and still matter of debate.

The present study provides evidence that feeding tolerance improves as gestational age increases and impairs in small for gestational age infants. Furthermore, SGA infants spent more time to achieve FEF than AGA infants did.

Multivariable analysis with minimal and full enteral feeding achievement as dependent variables showed a series of perinatal events that positively/negatively correlated with MEF and FEF. In details, antenatal steroids prophylaxis was beneficial on FEF achievement, whilst an Apgar score <7 at 5’ minutes, the administration of inotropic drugs and caffeine, the

occurrence of sepsis, PDA and NEC negatively affected MEF and FEF achievement.

The finding of an influence of gestational age on MEF and FEF is noteworthy since preterm birth constitutes a significant determinant of perinatal morbidity affecting an adequate intrauterine growth and maturation of the gastrointestinal tract. This especially holds for SGA infants in whom placental insufficiency and subsequent hemodynamic compensatory mechanisms reasonably had a somewhat negative impact on feeding tolerance. The placental insufficiency may deprive the gastrointestinal tract from sufficient blood flow. As a consequence of gut ischemia/hypoxia, SGA infants are thought to have impaired gut function after birth, which may result in feeding intolerance (21). Therefore, the prolonged time necessary to achieve FEF in SGA infants may be ascribed to a chronic prenatal intestinal hypoxic injury.

In our study the presence of a haemodynamically significant ductus arteriosus and the use of such drugs as caffeine or dopamine and dobutamine adversely affect the start of MEF. This finding is not unexpected because the use of these drugs, according to our Department's guidelines, testifies a severe clinical instability of the neonates and induces clinicians to avoid the beginning of enteral nutrition. Our data showed that a full course of antenatal betamethasone reduced the time necessary to achieve full enteral feeding from birth and from the start of MEF.

Antenatal steroid treatment for women who are at risk of preterm delivery has emerged as the most effective intervention for the prevention of RDS, reducing early neonatal mortality and morbidity, and the risk of abnormal neurodevelopmental outcome as indicated in a Cochrane systematic review (22). In a large multicenter, blinded randomized trial on antenatal

corticosteroid treatment, a significant decrease in incidence of NEC ($p< 0.01$) was found in the infants treated with steroids (23).

In the literature several reports demonstrate the impact of steroid treatment on gastrointestinal maturation increasing production of intestinal enzymes, hormones and ameliorating intestinal motility (24-28). Data from animal studies suggest that developing intestinal cells are most responsive to steroids in the prenatal and immediate postnatal period and that the activities of several small intestinal enzymes correlated positively with plasma cortisol levels (29-30). Studies in both human fetuses and preterm infants suggest that similar maturational effects may be seen also in the human infant (31-33).

Our study reports the beneficial effects of antenatal steroid therapy on gut maturation from a clinical point of view describing the positive effects of antenatal steroid therapy on progression of enteral nutrition. Although we were unable to trace a study in literature that reported on the correlation between antenatal steroids therapy and feeding tolerance, we suppose that steroids may exert a direct effect on the gut promoting intestinal maturation.

In our study the beginning of MEF is not influenced by the antenatal steroid therapy. The decision to start MEF depends on the clinical condition of the infant; MEF is started only if the infant is clinically stable (as cardiocirculatory and respiratory parameters are stable); on the contrary, the progression of the enteral nutrition is strictly related to the gut's ability to tolerate the feeding (which, according to our NICUs guidelines, is observed by evaluations of gastric residuals, occurrence of vomiting and abdominal discomfort). As only the time to achieve FEF is influenced by the antenatal steroid therapy, this seems to indicate that steroids may affect directly the gut.

This study suggests that also in the SGA infants an earlier start of MEF and timely increase in oral feeding can be safe. An individualized approach to

enteral nutrition seems warranted, considering the gestational age of the infant, the clinical condition and prenatal factors as intrauterine growth restriction and the steroid prophylaxis. The perfusion of the gastrointestinal tract, that is thought to be compromised in SGA infants and improved by antenatal steroid therapy, is the fundamental variable to consider when enteral feeding is administered in preterm infants.

Although we reported on the consecutive series of infants followed in our Department until achievement of FEF, we are aware of the limitations related to the retrospective nature of this study. Therefore, a prospective trial that addresses various issues related to feeding tolerance in preterm infants is currently ongoing in our NICU (34).

In conclusion, the time necessary to achieve full oral feeding is strictly related to gestational age. SGA infants needed more time to achieve FEF as compared to AGAs with the same gestational age. Antenatal betamethasone is effective in reducing the time to FEF in both AGA and SGA.

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Monitoring Doppler Patterns and Clinical Parameters may Predict Feeding Tolerance in Intrauterine Growth-Restricted Infants.

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Abstract

Aim: to detect predictors of feeding tolerance in IUGR infants with or without brain sparing effect (BS).

Methods: We conducted a case-control study in 70 IUGR infants (35 IUGR with BS matched for gestational age with 35 IUGR infants no BS). BS was classified as pulsatility index (PI) ratio [umbilical artery (UAPI) to middle cerebral artery (MCAPI) (U/C Ratio)] > 1. Clinical parameters of feeding tolerance [days to achieve full enteral feeding (FEF)] were compared between IUGR with BS versus IUGR without BS infants. Age at beginning of minimal enteral feeding (MEF) was analyzed.

Results: Achievement of FEF was significantly shorter in IUGR without BS than in IUGR with BS. IUGR with BS started MEF later than IUGR without BS infants. Significant correlation of MEF and FEF with UA PI, U/C ratio and CRIB score was found. Multiple linear regression analysis showed significant correlations with CRIB score and caffeine administration (MEF only), sepsis (FEF only) and U/C Ratio (for both).

Conclusion: impaired gut function can be early detected by monitoring Doppler patterns and clinical parameters.

Keywords: IUGR, Feeding Tolerance, Doppler velocimetry, Minimal Enteral Feeding, Full Enteral Feeding.

Key Notes: IUGR may predispose infants to impaired gut function. Prenatal Doppler patterns and clinical data may suggest which infants are prone to develop feeding intolerance.

Introduction

Intra-uterine growth restriction (IUGR) occurs in 8-10% of pregnancies and still remains one of the major complications contributing to perinatal mortality and morbidity (1). Fifty-two percent of stillbirths are associated with IUGR with a rate of perinatal mortality of 10% (2).

IUGR is widely accepted as an expression of persistent suppression of genetic growth potential caused by decreased oxygen and substrate supply. It is characterized by a decrease in uteroplacental blood flow (up to 50%) because of impaired trophoblast invasion of spiral arteries, which are not transformed to low resistance vessels (3). Thus, transfer of oxygen and nutrients from the mother to the fetus is reduced, leading to a cardiovascular response characterized by a redistribution of cardiac output to maintain oxygen supply to the brain, heart, and adrenal glands – the so called brain sparing effect - at the expense of visceral organs including gastrointestinal tract. Therefore, IUGR infants are thought to have impaired gut function after birth. Whenever direct tissue injury does not occur, prolonged exposure to these conditions of impaired blood flow may modulate the development of motor, secretory and mucosal function, so that in the postnatal period the intestine is more susceptible to stasis, abnormal colonisation and bacterial invasion (4). In this regard, no data on the relationships among prenatal monitoring parameters such as Doppler' patterns and the tolerance of enteral nutrition have been provided.

In the present case-control study we aimed at investigating whether in IUGR infants the occurrence or not of prenatal hemodynamic patterns suggestive of brain sparing effect, alone or associated with perinatal parameters, interfere with the timing necessary to achieve full enteral feeding (FEF). We also evaluated the age at the starting the minimal enteral feeding (MEF).

Materials and Methods

Patients

We conducted a retrospective case-control study, from January 2007 to December 2011, at our third level referral Centre for Perinatal Medicine involving infant born at a gestational age less than 32 weeks who are at higher risk for feeding intolerance. From our database, we were able to retrieve a complete perinatal data set on 70 IUGR infants of whom 35 were complicated by brain sparing effect (BS). The control group consisted of 35 IUGR preterm infants with no BS.

We recorded data regarding Doppler sonography that was performed according to the recommendations of the American College Obstetrics and Gynecology ACOG. The Doppler sonography was performed within 7 days before delivery. The fetal abdominal circumference (AC) was measured and considered normal if over 10^{th} percentile, and pathological if below or equal the 10^{th} percentile. Infants were defined IUGR when the AC measurement deviate 10% or more from the expected from the individual projected curve of growth (5).

Flow velocity waveform patterns of the main branch of the uterine artery bilaterally, umbilical artery (UA), and fetal middle cerebral artery (MCA) were recorded by means of a duplex pulsed color Doppler ultrasound (Philips IU 22, Eindhoven, The Netherlands) with a convex 3.5-MHz transducer, and the RI (peak systolic velocity - end-diastolic velocity / peak systolic velocity) and PI (peak systolic velocity – end diastolic velocity / mean velocity) were calculated automatically by the built-in software. A spatial peak temporal average <100 mW/cm^2 was used for blood flow measurements in the middle cerebral artery. A 100-Hz high-pass filter was used, and Doppler waveforms were obtained in the absence of fetal body or breathing movements. In every record, three to five consecutive cardiac cycles were examined, and the mean

of at least three values from each vessel was used for subsequent analysis. An umbilical artery PI to middle cerebral artery PI ratio >1 was considered as index of BS and fetal hypoxia (6).

Prenatal glucocorticoids prophylaxis for lung immaturity were administrated in the two studied groups according to the ACOG guidelines (betamethasone 12 mg/24 h for 2 d i.m., Bentelan, Glaxo Wellcome, Verona, Italy).

At birth the perinatal outcomes [i.e. birthweight, length, head circumference, gestational age centiles, Apgar score, umbilical cord blood pH, CRIB score (7) the incidence of sepsis and of hemodynamically significant ductus arteriosus (PDA)] and main interventions (inotropic drugs and/or caffeine administration) were recorded in the two studied groups by MetaVision ICU X-Edition (i-MD soft Ltd., Tel Aviv, Israel).

Infants with major congenital malformations, congenital viral infections or anomalies that may interfere with enteral nutrition and those who didn't achieved full enteral feeding (FEF) were excluded from the study.

According to our internal guidelines, caffeine was administered if 3, or more, apneic episodes, requiring manual ventilation, occurred in 24 hours.

Full enteral feeding was defined as an enteral intake of 150 mL/kg/day sustained for 72 hours (8). Minimal enteral feeding refers to small amounts of enteral feedings of formula and/or breast milk intakes of 5–20 ml/kg/day. All patients were on intravenous nutritional support till achievement of full enteral feeding.

To assess the feeding tolerance we reviewed the time (in days) to achieve full enteral feeding from birth. We moreover evaluated the age at the starting of the minimal enteral feeding (MEF).

Infants with no adverse indication to enteral feeding, severe hemodynamic instability, suspected or confirmed necrotizing enterocolitis (NEC), evidence of intestinal obstruction/perforation or paralytic ileus, were given minimal

enteral feedings (< 20 ml/kg/day) that were advanced at 15 to 20 ml/kg/day as tolerated. Feedings were given as bolus every 3 hours or as continuous nutrition, and included breast milk, when available, or preterm formula (80 Kcal/100ml). Human milk was fortified when an enteral intake of 100 ml/kg/day was tolerated.

Statistical Analysis

Perinatal characteristics are expressed as mean (ranges) and with rates and percentages. We used Student's t-test for continuous variables and Mann-Whitney U two-sided test when parameters were not normally distributed. Categorical data were analyzed by means of Fisher's exact test or chi-square analysis as appropriate. Univariate regression analysis was used for correlation between MEF and FEF and various monitoring and clinical parameters (UA PI, MCA PI, U/C ratio, antenatal glucocorticoids prophylaxis, weight and gestational age at birth, gender, CRIB score, cord blood pH, lactate, inotropic drugs and caffeine administration, sepsis). Multiple regression analysis was performed with MEF and FEF as the dependent variables for the analysis of several variables predictors of MEF and FEF (U/C ratio, antenatal glucocorticoids prophylaxis, gender, CRIB score, caffeine administration, sepsis). Statistical significance was set at $P<0.05$.

Results

Perinatal characteristics of the two studied populations are reported in Table 1.

Parameter	IUGR BS Group (n=35)	IUGR no-BS Group (n=35)	P
Gestational age (wks)	30 (26.1–31.5)	29.6 (26.1–31.6)	0.60
Birth weight (g)	876 (530-1480)	1054 (680-1630)	<0.001
Gender (male/total)	17/35	13/35	0.47
Apgar 5' ≥ 7 n/total (%)	32/35 (91.4%)	31/35 (88.6%)	1.00
Prenatal steroids n/total (%)	29/35 (82.8%)	32/35 (91.4%)	0.31
UA pH	7.29 (6.93-7.36)	7.29 (6.83-7.40)	0.45
CRIB score	2.5 (1-12)	4 (1-12)	<0.01
Doppler Patterns			
UA PI	1.83 (1.23-5.22)	1.20 (0.51-1.91)	<0.01
MCA PI	1.31 (0.75-1.95)	1.68 (1.04-2.87)	<0.01
U/C ratio	1.33 (1.02-6.14)	0.70 (0.28-1.00)	<0.01
Primary Outcomes			
Birth – MEF interval (d)	5 (1-18)	3 (1-14)	<0.01
Birth – FEF interval (d)	27 (11-66)	22 (10-45)	0.028
Main Interventions			
Inotropic drugs n/total (%)	4/35 (11.4%)	0/35 (0.0%)	0.11
Caffeine n/total (%)	21/35 (60.0%)	20/35 (57.1%)	1.00
Sepsis n/total (%)	10/35 (28.6%)	7/35 (20.0%)	0.58
PDA n/total (%)	7/35 (20.0%)	7/35 (20.0%)	1.00

Table 1 Perinatal clinical and monitoring parameters in the two IUGR groups with or without brain sparing effect (BS). Data are expressed as median (ranges).
Abbreviations: UA, Umbilical Artery; UA PI, Umbilical Artery Pulsatility Index; MCA, Middle Cerebral Artery; U/C, umbilical/cerebral; PDA, Patent Ductus Arteriosus.

As expected, Doppler velocimetry waveform patterns significantly differed (P<0.01, for all) between groups as well as birth-weight was lower (P<0.05) in IUGR BS group. No differences (P>0.05, for all) in gender, Apgar score at 5th minute, antenatal steroid therapy, cord blood pH, the need of inotropic

drugs and caffeine, the occurrence of sepsis and of PDA, were observed in the two groups. Of note, the interval from birth to the achievement FEF was significantly shorter (P<0.05) in IUGR no BS than IUGR BS infants. Moreover IUGR infants with BS started MEF significantly later than IUGR no BS (5, r 1-18 versus 3, r 1-14).

Linear regression analysis performed in order to identify which variables correlated with FEF showed a significant correlation with UA PI (R= 0.43: P<0.01), with U/C ratio (FEF: R= 0.44: P<0.01) and with CRIB score (R= 0.48: P<0.01).

Linear regression analysis performed to identify correlation between clinical parameters and MEF starting time showed a significant correlation with UA PI (R=0.40; P<0.01), with U/C ratio (R=0.37; P<0.01), with CRIB score (R=0.47; P<0.01) and with cord blood pH (R=-0.27; P<0.05).

Data on multiple linear regression analysis performed with FEF and MEF as dependent variables in order to analyze the influence of various clinical and monitoring parameters on the timing of FEF and MEF are reported in Table 2.

Variable	Coefficient	Standard Error	T	P	VIF
CONSTANT	12.9	3.4	3.78	<0.01	0.000
GENDER	0.79	2.01	0.39	0.69	1.06
CRIB SCORE	0.7	0.39	1.76	0.08	1.36
SEPSIS	5.3	2.51	2.13	0.036	1.23
STEROIDS	-0.36	2.72	-0.13	0.89	1.24
CAFFEINE	3.99	2.16	1.84	0.07	1.21
U/C RATIO	2.99	1.1	2.52	0.014	1.2

Table 2. Multiple linear regression analysis with full enteral feeding (FEF) as dependent variable for the analysis of several variables (U/C ratio, antenatal glucocorticoids prophylaxis, gender, CRIB score, caffeine administration, sepsis) in order to analyse the influence of various clinical and monitoring parameters on the timing of FEF. FEF

achievement time was correlated with sepsis occurrence (P=0.036) and U/C Ratio (P=0.014). No redundant information in the other independent variables have found as shown by the Variance Inflation Factor (VIF).

FEF achievement time was correlated with sepsis occurrence (P=0.036) and U/C Ratio (P=0.014). MEF starting time was correlated with CRIB score (P=0.006), caffeine administration (P=0.04) and U/C Ratio (P=0.004).

Discussion

It is well known that adverse sequelae, especially those related to impaired gut function, are more frequent in IUGR infants due to their condition of chronic fetal hypoxia. Based on this statement the scope of our study was to investigate the differences in feeding tolerance among IUGR preterm infants with or without brain sparing.

In the present study we found that IUGR infants with brain sparing need more time to achieve FEF than IUGR infants with no fetal hypoxia. Furthermore, multiple linear regression analysis performed with FEF as dependent variable, in order to analyze the influence of various clinical parameters on the timing to FEF, showed significant correlations with U/C Ratio and sepsis.

We found that FEF achievement is influenced by abnormal Doppler patterns suggestive of fetal hypoxia characterized by the so-called brain sparing effect. BS is characterized by redistribution towards the brain and away from the viscera and placenta, culminating in umbilical artery or aortic absence of end-diastolic flow in the most severely affected. Thus our finding of BS occurrence suggests the possibility of: i) an increased mesenteric vascular resistance causing an hypoxic-ischemic injury of the intestine prior to the

delivery (9), ii) postnatal flow abnormalities in superior mesenteric artery blood flow velocity (10).

The finding of correlation between abnormal U/C ratio and feeding intolerance is consistent with previous observations reporting in IUGR infants a higher incidence of feeding intolerance and NEC (11-19). In detail: i) Vermont-Oxford Database revealed an increased risk of feeding intolerance and NEC in 759 singleton IUGR infants born at 25–30 weeks gestation with a OR of 1.27 (95% CI 1.05 to 1.53) (13); ii) the presence of absent or reversed end diastolic flow velocities (ARED) in umbilical arteries correlated with prenatal hypoxia, with postnatal gastrointestinal impairment and increased risk for NEC (OR 6.9, 95% CI 2.3 to 20) (3,20-21). All together it is reasonable to suggest that an accurate fetal monitoring can provide useful informations to neonatologists for a more accurate postnatal management including feeding.

Pathophysiologically, brain sparing is a serious yet compensated state of circulatory redistribution in favour of the most vital organs at the expense of visceral organs (22). Our study confirms that this redistribution does have postnatal clinical impact, in terms of feeding tolerance. This finding is in accordance with the results of the study by Robel-Tillig et al. (23,24) that studied postnatal gastrointestinal adaptation and haemodynamics in 124 infants born < 1500 g. Eighty-eight per cent of the prenatally disturbed infants developed signs of intestinal motility disturbances compared to 20% of the infants without prenatal Doppler pathology.

Both superior mesenteric artery (SMA) and coeliac axis blood flow velocity are dramatically reduced after birth. There is a slow recovery in baseline values during the first days of life, with SMA values at day 7 comparable to those found in unfed non IUGR infants (10,25).

We observed also that infants who experienced clinical sepsis achieved FEF later than non-septic IUGR infants. The finding is not surprising since during septic episodes infants are unstable and hence enteral nutrition has to be often withdrawn. Moreover, pregnancy induced hypertension with fetal growth restriction is associated with neutropenia in early postnatal life, which may affect susceptibility to infective factors (26). However, the mechanism through which sepsis primarily affected FEF and/or participated in a cascade of events triggered by IUGR and its hemodynamic patterns is still not completely elucidated. Further investigations in this regard are so requested.

With regard to MEF starting time, multiple linear regression analysis showed significant correlations of age at MEF with CRIB score, caffeine administration and U/C Ratio.

Timing for MEF in IUGR infants is a topic highly debated. From one hand delayed enteral feeding is protective against feeding intolerance and NEC (27), from the other hand prolonging enteral fasting could be detrimental, with an higher risk of parenteral nutrition related side effects. In a recent randomized non blinded pilot trial, it has been suggested that early introduction of MEF, in IUGR infants with abnormal antenatal Doppler, may not have a significant effect on the incidence of feeding intolerance (28). Furthermore Leaf et al. (8) showed that IUGR infants who started feeds earlier achieved sustained enteral feeding at a significantly earlier age, with an average difference of three days and without an increased incidence of NEC.

In our study IUGR infants started MEF later than IUGR infants no BS. The influence of CRIB score on MEF starting time warrants consideration since CRIB score is a valuable predictor for neonatal mortality and morbidity in very low birth weight infants (7). Thus, a high CRIB score testifies a severe clinical instability of the neonates inducing clinicians to avoid enteral

nutrition and to perform the due therapeutic strategies. This holds also for caffeine administration that correlates with a later beginning of MEF. Caffeine administration reflects infants' unstable clinical conditions (i.e. respiratory distress) with a later starting of the feeding and/or intestinal discomfort during therapy. Lane et al. reported a significant fall in blood flow velocity in intestinal arteries (superior mesenteric and celiac arteries) after intravenous infusion of caffeine (29). The finding of caffeine hemodynamic side effects can be of relevance enhancing blood flow impairment due to IUGR. This latter phenomenon is known to affect development of motor, secretory and mucosal intestinal function.

The decision to start MEF depends on the clinical condition of the infant; MEF is started only if the infant is clinically stable (as cardiocirculatory and respiratory parameters are stable); not as the progression of the enteral nutrition that is strictly related to the gut's ability to tolerate the feeding. The gradual recovery of intestinal perfusion during the first days of life provided a rationale for a delay in enteral feeding and it may justify the delayed starting of MEF in IUGR infants with BS in the present study. A randomized trial is actually ongoing in our NICU to detect the physiological changes (and those induced by feeding) in splanchnic oxygenation and perfusion in IUGR infants; data from this trial should clarify the timing and the entity of the post natal intestinal adaptation in infants who experienced growth restriction (30).

In conclusion, this study shows that diagnosing an intra-uterine growth restriction should alert the clinician to further investigate the fetus through cerebral and umbilical Doppler and to estimate the umbilical to cerebral ratio. These parameters identify population at high risk for gastrointestinal impairment even if adequate preventing measures are still lacking and studies on larger number of patients are warranted.

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List of abbreviations

IUGR: intrauterine growth restriction, FEF: full enteral feeding, MEF: minimal enteral feeding, BS: brain sparing, PI: pulsatility index, NEC: necrotizing enterocolitis, UA: umbilical artery, AC: abdominal circumference, MCA: fetal middle cerebral artery, U/C: umbilical to cerebral

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Cerebral and Somatic NIRS-determined Oxygenation in IUGR Preterm Infants during Transition.

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Abstract

Background. Fetal growth restriction (IUGR) has a considerable impact on perinatal morbidity and mortality. Preterm IUGR infants are prone to impaired intestine function. Near InfraRed Spectroscopy (NIRS) has been recently used to monitor oxygenation status of the brain and of splanchnic organs such as the intestine.

Patients & Methods We conducted a prospective case-control study at our NICU in 20 preterm infants of whom 10 infants complicated by IUGR were matched for gestational age at birth with 10 non-IUGR preterm infants (1 IUGR to 1 non-IUGR). Splanchnic and cerebral regional oximetry values were measured by means of near infrared spectroscopy. Three hours of consecutive recordings were performed in the first 24 hours of life, T0, and during the transitional period, T1, (at 48-72hrs from birth). Arterial Oxygen Saturation Monitoring (SaO2) was performed at the same time-points as the NIRS monitoring. The cerebral/splanchnic oxygenation ratio, CSOR, ($rScO_2/rSsO_2$) was also calculated.

Results Both in the IUGR and the non-IUGR infants, at T0 and T1 monitoring time-points, the rSO_2 values were higher in the cerebral district when compared to those of the splanchnic area. Comparison of the NIRS parameters between the IUGR and non-IUGR infants at T0 showed no difference in $rScO_2$,whilst $rSsO_2$ was significantly lower in the IUGR group. At T1 $rScO_2$ was significantly lower and $rSsO_2$ higher in the IUGR group.

Conclusions Cerebral/splanchnic vascular adaptation of IUGR infants to the extra-uterine environment is characterized by a postnatal persistence of the brain sparing effect with reperfusion in the transitional period.

Abbreviations: IUGR - Intra-uterine growth restriction, BS - Brain Sparing, NEC - Necrotizing Enterocolitis, NIRS- Near-infrared spectroscopy, CSOR -

cerebrosplanchnic oxygenation ratio, VLBWi - very low birth-weight infants, rSsO2 - splanchnic regional saturations, rScO2 - cerebral regional saturations, FTOE - Fractional Tissue Oxygen Extraction

Keywords: IUGR, NIRS, cerebral and splanchnic oxygenation, brain sparing.

Introduction

Intra-uterine growth restriction (IUGR) has a considerable impact on perinatal morbidity and mortality (1,2). Placental insufficiency curtails oxygen and nutrient transport from the mother to the fetus leading to chronic fetal hypoxia and under-nutrition. Hemodynamically, IUGR is characterized by a redistribution of the circulation assuring adequate perfusion to the vital organs (brain, myocardium, and adrenal glands) at the expense of other organ systems such as the gastrointestinal tract. This is mostly called “the brain sparing” effect (BS).

These phenomena can be monitored by Near InfraRed Spectroscopy (NIRS) to assess oxygenation status of the brain and of splanchnic organs such as kidneys and the intestine (3). Splanchnic oxygenation was compared with brain oxygenation as a reference, because, under most of physiological conditions, cerebral blood flow auto-regulation minimizes changes in brain oxygenation during events affecting splanchnic perfusion (4). Further investigations, both in human and animal models, showed that NIRS is able to detect changes in splanchnic oxygen delivery, which is curtailed during IUGR and may be used to predict NEC by measuring the splanchnic/cerebral oxygenation status ratio (CSOR) (5).

Although hemodynamic patterns related to BS have been widely described (6,7), data on perinatal adaptation in IUGR infants and the timing of the exhaustion of the BS effect are still matters of investigation. Of note, data for the BS effect on splanchnic perfusion and oxygenation status after birth and in the transitional period are still lacking in IUGR infants. Therefore, the present study aims to investigate cerebral and splanchnic oxygenation status and hypothesizes that during the transitional period (i.e. the first 72 hours after birth) oxygenation of the brain and the intestine differ between IUGR and non-IUGR very low birth-weight (VLBW) infants.

Materials and Methods

From January 2011 to October 2011 we conducted a prospective case-control study in our third referral center for NICU: 10 very low birth weight preterm infants with IUGR were matched for gestational age at birth with 10 non-IUGR very low birth weight preterm infants.

We recorded the prenatal Doppler sonography data in accordance with the recommendations of the American College of Obstetrics and Gynecology (ACOG 8,9). The Doppler sonography was carried out within 7 days before delivery. The fetal abdominal circumference (AC) was measured and considered normal if above the 10^{th} percentile, and abnormal if below or equal to the 10^{th} percentile. Infants were considered IUGR when the AC measurement deviated 10% or more from the expected individual projected growth curve (8,9).

Flow velocity waveform patterns of the main branch of the uterine artery bilaterally, umbilical artery (UA), and fetal middle cerebral artery (MCA) were recorded using duplex pulsed color Doppler ultrasound (Philips IU 22, Eindhoven, The Netherlands) with a convex 3.5-MHz transducer, and the RI (peak systolic velocity - end-diastolic velocity / peak systolic velocity) and PI

(peak systolic velocity – end diastolic velocity / mean velocity) were calculated automatically by the built-in software. A spatial peak temporal average <100 mW/cm^2 was used for blood flow measurements in the middle cerebral artery. A 100-Hz high-pass filter was used and Doppler waveforms were obtained in the absence of fetal body or breathing movements. In each recording, 3 to 5 consecutive cardiac cycles were examined, and the mean of at least 3 values from each vessel was used for subsequent analysis. An UA/MCA PI ratio >1 was considered to indicate BS due to fetal hypoxia (10).

At birth, prenatal data [i.e. preterm premature rupture of membranes (pPROM), antenatal steroid prophylaxis (11)], perinatal outcomes [i.e. birthweight, length, head circumference, gestational age centiles, Apgar score at 5th minute <7, umbilical cord blood pH, maximum blood lactate levels in the first 12hrs. from birth, CRIB score, Clinical Risk Index for Babies (12)], and important interventions [i.e. inotropic drugs and/or caffeine administration, antibiotic and surfactant treatment, the occurrence of patent ductus arteriosus (PDA), of respiratory distress syndrome (RDS) and of early onset sepsis (EOS)]were recorded (Table 1).

Exclusion criteria: major congenital abnormalities, perinatal asphyxia with multi-organ failure and pre-existing cutaneous disease impeding the placement of the probe.

Informed consent was given by all parents of the patients prior to inclusion in the study. Approval was granted by the Human Investigations Committees of the participating Institution.

NIRS-determined oxygenation

Splanchnic and cerebral regional oxygen saturations were monitored via NIRS (Somanetics 5100 INVOS System, Troy, MI, USA). NIRS sensors

were placed over the abdomen (splanchnic bed) and on the forehead (cerebral bed) to measure cerebral ($rScO_2$) and infra-umbilical abdomen splanchnic ($rSsO_2$) regional saturations. Due to the contour of the neonatal abdomen and the potentially complicating contribution of the liver, infra-umbilical probe placement was chosen in order to sample the mesenteric $rSsO_2$in the most optimal way. The CSOR ($rScO_2/rSsO_2$) was also calculated.

Three hours of consecutive recordings were performed in the first 24hrs (T0 mean/SD: 12.3 ± 4.4 hrs) and at 48-72hrs (T1 mean/SD: 62.7 ± 4.0 hrs) from birth. No differences ($P>0.05$, for both) at time of monitoring recordings were observed. The infants were not administered oral feeding during the study period. All infants were in stable clinical conditions at the T0-T1 time-points. Fractional tissue oxygenation extraction (FTOE) was calculated both in splanchnic (sFTOE) and cerebral (cFTOE) districts according to the following formula: $FTOE= (SaO_2-rSO_2)/SaO_2$.

Standard Monitoring Parameters

Heart and respiratory rates, and arterial oxygen saturation (SaO_2) monitoring were continuously recorded by a Masimo Datascope Radical (Masimo Corporation, Irvine, CA, USA) at a 1 minute interval in the two study groups and recorded by MetaVision ICU X-Edition software (i-MDsoft Ltd., Tel Aviv, Israel).

Statistical Analysis

Perinatal characteristics are expressed as median (ranges) and with rates and percentages. We used Student's t test for continuous variables and Mann-Whitney U two-sided test and Kruskal-Wallis test when parameters were not normally distributed. Categorical data were analyzed by means of Fisher's exact test or chi-square analysis as appropriate. Statistical significance was set at $P<0.05$.

Results

Perinatal and clinical characteristics of the study groups are reported in Table 1.

Parameters	IUGR (n=10)	NON-IUGR (n=10)	P
PRENATAL DATA			
Glucocorticoids n (%)	6 (60%)	8 (80%)	NS
Chorionamnionitis (%)	3 (30%)	6 (60%)	NS
pPROM	0 (0%)	2 (20%)	NS
DOPPLER PATTERNS			
UA PI	1.93 (1.33-4.82)	1.40 (0.6-1.78)	<0.01
MCA PI	1.31 (0.75-1.95)	1.52 (1.01-2.74)	<0.01
U/C RATIO	1.41 (1.01-6.21)	0.72 (0.31-1.00)	<0.01
PERINATAL DATA			
Gestational age	31+5 (29-33)	30 +4(29-32)	NS
Weight (g)	992.5	1322.5	P<0.05
Gender (n° of males)	5 (50%)	3 (30%)	NS
Caesarean delivery n° (%)	9 (90%)	8 (80%)	NS
Apgar 5'	9 (7-10)	9 (6-9)	NS
CRIB score	2 (0-5)	1 (0-3)	NS
Umbilical cord PH	7,3 (7.15-7.34)	7,3 (7.26-7.40)	NS
Lactate max <12h	4.5 (1.7-9.4)	2.6 (1.4-7.3)	NS
CLINICAL FEATURES			
RDS n° (%)	9 (90%)	6 (60%)	NS
PDA 24-h n° (%)	4 (40%)	8 (80%)	NS
PDA 48-h n° (%)	2 (20%)	4 (40%)	NS
PDA n° (%)	2 (20%)	3 (30%)	NS
EOS n° (%)	0 (0%)	1 (10%)	NS
IVH n° (%)	1 (10%)	1 (10%)	NS
Surfactant n° (%)	3 (30%)	5 (50%)	NS

Table 1. Baseline characteristics of the series. Abbreviations: preterm premature rupture of membranes; Glucocorticoids: complete prophylaxis with Betamethasone; PDA 24h: patent ductus arteriosus in the first 24h of life : PDA 48H: patent ductus arteriosus at 48h of life; PDA: patent ductus arteriosus>48h requiring pharmacological closure; surfactant: surfactant administration needed during the study; EOS (early-onset sepsis): clinical sepsis in the first 72 hours of life; RDS (respiratory distress syndrome): radiologic evidence of type 1 RDS

No differences in antenatal glucocorticoids treatment, occurrence of chorioamnionitis and pPROM were observed. As expected, Doppler patterns suggestive of BS were significantly different between groups ($P<0.01$, for all). No difference in gestational age at birth was found but weight at birth was significantly lower ($P<0.05$) in IUGR newborns. Gender, the incidence of caesarean section, Apgar score at 5 min <7, CRIB score, umbilical cord blood gas analysis and lactate did not differ between the groups. Furthermore, no differences between the groups were detected regarding the occurrence of RDS, PDA, EOS, IVH and surfactant administration.

SaO_2 recordings were performed at the same time-points of NIRS monitoring.

NIRS-determined regional oxygen saturation patterns

NIRS recordings in splanchnic and cerebral districts were performed in all infants admitted to the study and no differences between groups were observed in terms of timing and length of recordings.

In the **IUGR** infants, at T0 and T1, the rSO_2 values were higher in the brain as compared to splanchnic rSO2 as confirmed by the CSOR ratio ($P<0.001$, for all values). $rScO_2$ was higher ($P<0.001$) at T0 than T1, whilst no differences were found for $rSsO_2$. Moreover, cFTOE was lower ($P<0.001$) at T0 than T1, whilst no differences were found for sFTOE.

In **non-IUGR** infants at the T0 and T1 monitoring time-points, rSO_2 values were higher in the cerebral district when compared to the splanchnic district as confirmed by the CSOR ratio ($P<0.001$, for all values). $rScO_2$ did not differ between T0 and T1, whilst $rSsO_2$ was ($P<0.001$) higher at T0. Indeed, sFTOE was lower ($P<0.001$) at T0 than T1, whilst no differences were found for cFTOE.

The NIRS parameter comparison between the IUGR and the non-IUGR infants at T0 showed no difference in $rScO_2$ whilst $rSsO_2$ was significantly ($P<0.001$) lower in the IUGR group. In fact, the CSOR differed ($P<0.001$) between the groups. Furthermore, at T0 the cFTOE was ($P<0.01$) lower and the sFTOE ($P<0.001$) higher in the IUGR infants, respectively. At T1 the $rScO_2$ was lower and the $rSsO_2$ ($P<0.001$, for both) higher in the IUGR group. The CSOR ($P<0.001$) differed between the groups. Moreover, at T1 the cFTOE was ($P<0.01$) higher and the sFTOE lower in the IUGR infants ($P<0.001$, for both), respectively.

Data regarding saturation and NIRS values of the population are reported in Table 2.

Parameter	IUGR (n=10)			Non-IUGR (n=10)		
	Median	**25°**	**75°**	**Median**	**25°**	**75°**
arterial oxygen saturation						
SaO_2 T0	95	92	97	96	94	98
SaO_2 T1	97	96	98	97	94	99
regional cerebral oxygen saturation						
$rScO_2$ T0	86	75	91	85	75	90
$rScO_2$ T1	79	74	84	85	81	89
cFTOE T0	0.10	0.03	0.18	0.12	0.06	0.21
cFTOE T1	0.18	0.13	0.23	0.12	0.08	0.16
regional splanchnic oxygen saturation						
$rSsO_2$ T0	48	33	71	59	50	71
$rSsO_2$ T1	56	34	68	49	36	58
sFTOE T0	0.49	0.26	0.65	0.38	0.25	0.47
sFTOE T1	0.41	0.30	0.65	0.49	0.40	0.61
Cerebral/Splanchnic Ratio						
CSOR T0	1.66	1.20	2.53	1.31	1.11	1.69
CSOR T1	1.33	1.16	1.67	1.70	1.43	2.29

Table 2. Saturation and NIRS values. Abbreviations: SaO2 - arterial oxygen saturation, rScO2 - cerebral regional saturations, rSsO2 - splanchnic regional saturations, FTOE - Fractional Tissue Oxygen Extraction, CSOR - cerebrosplanchnic oxygenation ratio.

Discussion

Fetal ultrasound and Doppler velocimetry recordings in the maternal and fetal vessels provided useful information of changes occurring in the third trimester of the IUGR pregnancies. Conversely, hemodynamic changes occurring in the so-called transition phase are still controversial and virtually unknown. Higher cerebral than splanchnic oxygenation values were observed

in the study groups at birth and at 72 hours of age. These findings are consistent with previous observations (13).

The present study provides evidence that in IUGR infants the pattern of regional cerebral and splanchnic oxygen status, suggestive of a brain sparing effect, is still present in the transition phase and differs from controls as suggested by the cerebral/splanchnic ratio.

Fractional tissue oxygen extraction values, expression of tissue stress/damage, are higher in the cerebral district up to 72 hours from birth. Furthermore, NIRS patterns in non-IUGR infants show stable cerebral oxygenation and significant changes in the splanchnic area during the transition phase.

van Bel et al (14), by means of Doppler measurements, first observed the persistence of the brain sparing effect in IUGR infants in early postnatal life. They found significantly lower cerebrovascular resistance and higher cerebral blood flow velocity, indicating vasodilatation and increase of cerebral blood flow, in small for gestational age infants compared with appropriate for gestational age infants during the first days of life. By measuring regional oxygenation with NIRS, our data likewise reveal higher cerebral than splanchnic saturation values in IUGR infants; this is suggestive of hemodynamic redistribution as a compensatory mechanism to fetal hypoxia; the impaired cerebral/splanchnic ratio (CSOR) also supports this condition.

In the IUGR infants, a lower oxygenation of the splanchnic district in association with a significant difference between the fractional tissue oxygenation extraction values in the cerebral and splanchnic regions should be responsible for the increased risk of intestinal damage. The cFTOE rises in IUGR infants from T0 to T1 suggests an increase of oxygen extraction by the brain due to higher oxygen consumption in relation to oxygen delivery. This was confirmed by a decrease in brain oxygenation patterns whilst no

changes have been shown in the splanchnic district. Conversely, cerebral and splanchnic patterns still remained significantly different than controls. We found a late "compensatory" mechanism which deserves further consideration since the NIRS parameters in the IUGR infants shows: i) a CSOR pattern characterized by a significant decrease in cerebral oxygenation and stable lower splanchnic oxygenation in agreement with previous observations (4), ii) increased cFTOE and decreased sFTOE suggestive of a loss in the maintenance of cerebral oxygen stability and of an attempt to preserve the splanchnic district. Therefore, it is reasonable to argue that at the 72 hour time-point the NIRS patterns are suggestive of an expired brain sparing effect but, more interestingly, of a new/occurring reperfusion phase during which the brain seems to be less perfused at the expense of the splanchnic district. The explanation resides in an impaired oxygen delivery due to a decreased cerebral blood flow. Conversely, low sFTOE can be the expression of low oxygen consumption in relation to oxygen delivery.

We also found that in the non-IUGR infants the splanchnic saturation values fell over time. This could reflect a progressive reduction of mesenteric perfusion after birth due to a low metab olic demand induced by fasting (all infants were at nulla per os during the study period). As a consequence, an increase of the sFTOE occurred in non-IUGR infants and it reflects the ability of the splanchnic tissue to extract oxygen in the condition of reduced perfusion (eg. during fasting).

Limitations of the present study reside in the small number of infants studied, in the high variability of the regional splanchnic oxygenation and in the lack of a reference curve for VLBW infants. In this regard, the interpretation of the rSO2 of the mesenteric bed is challenging because of the hollow structure of the intestine, the presence of peristalsis and the large surface area. However, further investigations in a wider population should be able to

elucidate the usefulness of NIRS alone or combined with mesenteric artery Doppler velocimetry in the monitoring of splanchnic perfusion, in IUGR infants (15).

In conclusion, our results highlight that the cerebral/splanchnic vascular adaptation of IUGR infants to the extra-uterine environment is characterized by a persistence of the brain sparing effect and reperfusion. The present data provide additional confirmation of the need for a specific approach and feeding management of IUGR infants.

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Evaluation of Splanchnic Oximetry, Doppler Flow Velocimetry in the Superior Mesenteric Artery and Feeding Tolerance in Very Low Birth Weight IUGR and non-IUGR Infants Receiving Bolus *versus* Continuous Enteral Nutrition

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Abstract

Background. IUGR infants are thought to have impaired gut function after birth, which may result in intestinal disturbances, ranging from temporary intolerance to the enteral feeding to full-blown NEC.

In literature there is no consensus regarding the impact of enteral feeding on intestinal blood flow and hence regarding the best regimen and the best rate of delivering the enteral nutrition.

Methods/Design. This is a randomized, non-pharmacological, single-center, cross-over study including 20 VLBW infants. Inclusion criteria:

- Weight at birth ranging: 700–1501 grams
- Gestational age above 25 weeks and 6 days
- Written informed consent from parents or guardians

Exclusion criteria:

- Major congenital abnormality
- Patients enrolled in other trials
- Significant multi-organ failure prior to trial entry
- Pre-existing cutaneous disease not allowing the placement of the NIRS' probe

In the first 24 hours of life, between the 48th and 72nd hours of life, and during Minimal Enteral Feeding, all infants' intestinal perfusion will be evaluated with NIRS and a Doppler of the superior mesenteric artery will be executed.

At the achievement of an enteral intake of 100 mL/Kg/day the patients (IUGR and NON IUGR separately) will be randomized in 2 groups: Group A (n=10) will receive a feed by bolus (in 10 minutes); then, after at least 3 hours, they will receive the same amount of formula administered in 3 hours. Group B (n=10) will receive a feed administered in 3 hours followed by a

bolus administration of the same amount of formula (in 10 minutes) after at least 3 hours.

On the randomization day intestinal and cerebral regional oximetry will be measured via NIRS. Intestinal oximetry will be measured before the feed and 30 minutes after the feed by bolus; during the 3 hours nutrition the measurements will be performed before the feed, 30 minutes from the start of the nutrition and 30 minutes after the end of the gavage. An evaluation of blood flow velocity of the superior mesenteric artery will be performed meanwhile. The infants of the Group A will be fed with continuous nutrition until the achievement of full enteral feeding. The infants of the Group B will be fed by bolus until the achievement of full enteral feeding.

Discussion. Evaluations of intestinal oximetry and superior mesenteric artery blood flow after the feed may help in differentiating how the feeding regimen alters the splanchnic blood flow and oxygenation and if the changes induced by feeding are different in IUGR versus NON IUGR infants.

Trial registration number**:** NCT01341236

Keywords: Feeding tolerance, Near infrared spectroscopy, Minimal enteral feeding, Enteral nutrition, Parenteral nutrition, Intra-uterine growth restriction, Near infrared spectroscopy, Mesenteric artery Doppler, Bolus nutrition, Intermittent nutrition

Abbreviations: NEC, Necrotizing enterocolitis; NIRS, Near infrared spectroscopy; MEF, Minimal enteral feeding; EN, Enteral nutrition; NPT, Total parenteral nutrition; VLBW, Very low birth weight; IUGR, Intra-uterine growth restriction; TOI, Tissue oxygenation index; CSOR, Cerebrosplanchnic oxygenation ratio; CN, Continuous nutrition; BN, Bolus nutrition; NPO, Nothing per os; PSV, Peak sistolic velocity; EDV, End-diastolic velocity; Vmean, Mean velocity; RI, Resistance index; SMA, Superior mesenteric artery.

Background

Intra-uterine growth restriction (IUGR) caused by placental insufficiency is characterized by blood flow redistribution to the vital organs (brain, myocardium, and adrenal glands), while other organs, including the gastrointestinal tract, are deprived from sufficient blood flow. As a consequence of gut ischemia/hypoxia, IUGR infants are thought to have impaired gut function after birth, which may result in intestinal disturbances, ranging from temporary intolerance to the enteral feeding to full-blown NEC.
In literature, however, there is no consensus regarding the impact of enteral feeding on intestinal blood flow and hence regarding the best regimen and the best rate of delivering the enteral nutrition.
Doppler ultrasonography is the method currently used for the clinical assessment of velocity of superior mesenteric artery blood flow [1]. Blood flows parameters in the superior mesenteric artery (SMA) change with vasoconstriction or vasodilatation of the intestinal vascular bed. Prenatal

utero-placental insufficiency with chronic fetal hypoxia can lead to foetal growth retardation with a redistribution of blood flow favouring the cerebral circulation and reducing mesenteric perfusion [2]. This underlines the importance of chronic or acute hypoxia as the most intensively studied condition associated with disturbances of intestinal motility.

The possible association between the increase in blood flow velocity and change in tissue oxygenation is expected. Greater understanding of the rate of oxygen delivery and uptake in sick preterm infants undergoing intensive care is an important aim of neonatal medicine.

The assessment of adequate perfusion in very low birth weight infants is commonly based on clinical parameters, as well as invasive measures requiring central venous and/or arterial catheter access with well established associated risks. Additionally, most of these data are acquired intermittently, and thus may only represent a delayed picture of oxygen delivery and consumption.

Near-infrared spectroscopy (NIRS) is a continuous, non-invasive, real-time and portable techinique, which can be used to measure oxygenation in living tissue [3].

In 1985, Brazy and Lewis [4] reported the first pediatric application of NIRS monitoring of cerebral oxygenation in sick preterm infants. Since then the list of publications on NIRS for hemodynamic and oxygenation assessment in children and adults has rapidly expanded [5,6].

The technological background of NIRS technology has been reviewed in detail [7]. The main principle upon which NIRS device relies is the fact that most biological tissues, other than haemoglobin and cytochrome oxidase, are relatively transparent to infrared light in the range closest to the visual spectrum (700–1000 nanometers), and that the absorbance spectrum of the haemoglobin depends on its oxygenation status (deoxygenated haemoglobin

absorbs more red light and less infrared light than oxygenated haemoglobin). All devices emit lights at wavelengths within the above mentioned spectrum and analyze photons returning to the transducer. Because the change in the intensity of the reflected light is dependent upon the oxyhemoglobin to deoxyhemoglobin ratio, oxyhemoglobin saturation can be derived [8]. There are many different NIRS devices available. We use the INVOS cerebral oximeter (Somanetics Corporation, Troy, Michigan USA) that is FDA approved for adult and pediatric use including infants [9].

NIRS has been used to monitor oxygenation of the brain in neonates by measuring the ratio of oxygenated to deoxygenated hemoglobin (termed "tissue oxygenation index", TOI) [10]. NIRS has been reported to be useful in detecting changes in splanchnic oxygen delivery and predicting splanchnic ischemia in neonates by measuring the ratio of splanchnic to cerebral TOI, the cerebrosplanchnic oxygenation ratio (CSOR). Splanchnic oxygenation is compared with brain oxygenation as a reference, because under most of physiological conditions cerebral blood flow autoregulation minimizes changes in brain oxygenation during events affecting splanchnic perfusion [11].

A significant concern with the application of NIRS to the abdomen is the possibility of movement of the gut within the abdomen and also movement produced by peristalsis of the gut wall. These two movements can alter the scattering path of the near infrared light, resulting in absorption changes, which would swamp the signal of interest [12,13]. However TOI now offers a method of comparing the haemoglobin redox status within the splanchnic circulation, which is not path-length-dependent because it provides a simultaneous ratio of oxyhaemoglobin to deoxyhaemoglobin. Finally, by measuring the TOI of the brain, which is preferentially autoregulated with the splanchnic region under most physiological conditions, the resultant CSOR

ratio gives absolute values, which allow comparison between individual patients. CSOR had a 90% (56-100%) sensitivity to detect splanchnic ischaemia in neonates [14].

Regional tissue oxygenation of some other vascular beds and its clinical relevance is under review in extremely low birth weight infants [15].

In an effort to decrease the risk for development of NEC in preterm infants, enteral nutrition is often delayed when the neonate shows signs of feeding intolerance. However, enteral fasting can predispose a neonate to impaired intestinal growth, mucosal atrophy, intestinal barrier dysfunction, decreased digestive and absorptive capacity, increased colonization with pathogenic bacteria, and systemic inflammation. In addition, enteral fasting can prolong the time to establish full enteral feeding and the length of hospital stay [16]. Consequently, minimal enteral feeding (MEF) in combination with parenteral nutrition (PN) is often employed to alleviate the side effects of enteral fasting in premature infants. MEF is thought to promote intestinal motility, to maintain the intestinal barrier, to stimulate the development of “good” microflora, and to reduce infections.

Tube feeding is necessary for most premature infants less than 1500 grams because of their inability to coordinate sucking, swallowing, and breathing and the risk of aspiration. The conventional tube feeding method is intermittent bolus gavage feeding, where a prescribed volume of milk is given over a short period of time, usually over 10 to 20 minutes by gravity. Some clinicians prefer the continuous nasogastric route to feed premature infants less than 1500 grams birth weight, although, in practice, intermittent bolus gavage feeding is the method more commonly used [17,18]. In our Unit VLBW infants are fed by boluses, although they are often empirically switched to the continuous infusion method without an established rationale. Theoretical risks and benefits of both continuous nasogastric milk feeding

and intermittent bolus milk feeding have been proposed. Continuous nasogastric feedings may improve energy efficiency (by increasing energy absorbed and decreasing energy expenditure), reduce feeding intolerance, improve nutrient absorption, and improve growth. However, continuous infusion of milk into the gastrointestinal tract could alter the cyclical pattern of release of gastrointestinal tract hormones, which might affect metabolic homeostasis, and growth. Milk feedings given by bolus gavage method are thought to be more physiologic because they promote the cyclical surges of gastrointestinal tract hormones normally seen in healthy term infants. On the other hand, functional limitations of the premature infant's gastrointestinal system such as delayed gastric emptying or intestinal transit could hinder the premature infant's ability to handle bolus milk feeds, resulting in feeding intolerance.

Additionally, this feeding regimen that alternates between periods of feeding and fasting may challenge the premature infant's ability to maintain metabolic homeostasis and, therefore, decrease growth. There is still a debate about which is the best feeding regimen in order to prevent episodes of feeding intolerance and to promote a better growth.

Aims of the study

Primary aim of this study:

1. To evaluate the changes in the intestinal perfusion and oximetry determined by feeding in VLBW infants fed by 3 hours nasogastric nutrition (CN) versus infants fed by bolus nutrition (BN).

Secondary aims of the study:

2. To compare if changes in the intestinal perfusion and oximetry induced by feeding are different between IUGR and NON-IUGR infants.
3. To compare growth and nutritional status of the 2 groups by randomized

arm.

4. To test if changes in intestinal oximetry and perfusion can be reliable predictors of feeding intolerance (days necessary to achieve full enteral feeding).

Endpoints

The endpoint for the primary aim will be the cross-over difference of CSOR values, measured with NIRS before and at the end of the randomized feeds.

Aims 1 to 4 will be pursued analysing the following endpoints:

- The cross-over difference of CSOR values and of rSO2s values (i.e. the splanchnic saturation slope) in IUGR and non-IUGR infants.
- Growth and nutritional status will be measured by weight, length and head circumference. Main comparison will be between measures at randomization and at achievement of full enteral feeding. The growth at 28 days of life and at 36 weeks of gestational age will also be compared with the appropriate standard growth curves.
- The difference in the CSOR values pre- and post- feeding will be compared with the baseline CSOR value and the baseline Doppler flow velocimetry (both measured within the first 72 hours of life).
- Comparison of the time needed to reach full enteral feeding (i.e. days from randomization till enteral intake of 160 ml/kg/day of formula or fortified human milk) by randomized arm;

Methods and design

This is a randomized, non-pharmacological, single-center, cross-over study. Duration of the study: 24 months. The study takes place in the Neonatal Intensive Care Unit.

This study aims at recruiting about twenty very low birth weight infants, either IUGR or NON-IUGR.

Inclusion criteria:

- Weight at birth ranging: 700–1501 grams;
- Gestational age above 25 weeks and 6 days;
- Written informed consent from parents or guardians.

Exclusion criteria:

- Major congenital abnormalities (severe heart or cerebral disease, chromosomopathies, severe renal malformations, any malformation or disease of the gastroenteric tract)
- Patients enrolled in other trials
- Significant multi-organ failure prior to trial entry (perinatal asphyxia with renal, cardiac or cerebral impairment, DIC)
- Pre-existing cutaneous disease not allowing the placement of the probe

Eligibility to randomization

Infants who fulfill the following requirements are eligible to randomization:

- achievement of at least 100 mL/Kg/day of enteral nutrition
- adequate ventilation i.e. infants who are not intubated and not in-cPAP with a FiO2≥50% at the achievement of 100 mL/Kg/day of enteral nutrition;
- no NEC;
- written informed consent from parents or guardians.

Stratification

Randomization will be stratified in two groups: IUGR infants (approximately 10 children) and NON-IUGR infants (approximately 10 children).

At the achievement of an enteral intake of 100 mL/Kg/day the patients (IUGR and NON IUGR separately) will be randomized in 2 groups: Group A (n = 10) will start with bolus administration of nutrition (in 10 minutes); then, after at least 3 hours, they will be fed by the same amount of feed administered as continuous nutrition for 3 hours; Group B (n = 10) will start with continuous administration of nutrition for 3 hours followed by a bolus administration of the same amount of feed (in 10 minutes) after at least 3 hours.

The infants of the Group A will be fed with continuous nutrition until the achievement of full enteral feeding (enteral intake of 160 mL/Kg). The infants of the Group B will be fed with 7 or 8 boli/d until the achievement of full enteral feeding.

If, due to clinical instability (i.e. desaturation episodes or bradicardia) during the feeding, a change occurs in the modality of feeding, the results will be analyzed according to the "intention to treat" or the "by treatment" analysis.

All the patients will undergo a baseline evaluation in the first 72 hours of life including: cerebral ultrasound, cardiac ultrasound and abdominal ultrasound.

After birth, in the first 24 hours of life, and in the transitional period, between the 48^{th} and 72^{nd} hours of life, all infants' intestinal perfusion will be evaluated with NIRS and the echocolordoppler (evaluation of the superior mesenteric artery blood flow) will be performed. The evaluations with NIRS and with the echocolordoppler will be performed under condition of clinical stability (absence of arterial desaturation or instability of cardiocirculatory parameters). Data from NIRS will be collected for 3 hours. Other anamnestic and clinical data will be collected (gestational age, umbilical arterial pH, race, obstetrical anamnesis, mode of delivery, umbilical arterial and venous catheters, patent ductus arteriosus, respiratory distress syndrome, mechanical

ventilation, episodes of clinical sepsis). All the infants will be at nothing per os (npo) at the moment of the first two evaluations.

According to our protocol enteral nutrition will start after the 72nd hour of life as minimal enteral feeding (MEF), intake less than 20 mL/Kg/day of enteral feeding will be administered. The increase will be by 20 mL/Kg/day if enteral nutrition is well tolerated. All the infants, according to our standardized protocol, will start parenteral nutrition on the first day of life. The infants will be fed with human milk, if available, (human milk will be fortified at an achievement of an enteral intake of 100 ml/Kg/d), or with a preterm formula (75–80 Kcal/100 mL). The nutrition will be administered via the nasogastric route.

On the randomization day intestinal and cerebral regional oximetry will be measured via near infrared spectroscopy (NIRS) (INVOS- 5100 C) sensors placed over the abdomen (splanchnic bed) and on the forehead (cerebral bed). Recording of the tissues oximetry will start 30 minutes before the feed and will stop 30 minutes after the end of the nutrition. Intestinal oximetry will be measured before the feed (B_0) and 30 minutes after the feed (B_1) by bolus; during the continuous nutrition the measurements will be performed before (C_0), 30 minutes from the start of the nutrition (C_1) and 30 minutes after the end of the gavage (C_2).

The TOI will be obtained simultaneously at the two locations. The TOI measurement recordings will be obtained for 3 minutes from both the head and the abdomen. Those measurements will be combined as a ratio of abdominal TOI over brain TOI to produce a CSOR ($TOI_{abdomen}/TOI_{brain}$). Arterial haemoglobin oxygen saturation, measured by pulse oximetry, will be recorded during the NIRS tracing. NIRS tracing will be used only in the absence of desaturation (< 85% arterial saturation).

Capillar haemoglobin concentration will be measured on the day of the evaluation.

An evaluation of blood flow velocity of the superior mesenteric artery (peak sistolic and end-diastolic velocity, mean velocity, and pulsatility index) will be performed meanwhile. To achieve imaging of the SMA the transducer will be placed on the mid-abdomen above the umbilicus. The SMA will be identified at its emergency from the aorta and sample volume will be placed a few millimetres from its origin. Neonatologists with the same expertise and manual ability will perform the ecocholordoppler evaluations. The evaluations and the measurements will be performed on 5 contiguous homogeneous waves.

Measurements of body weight, length and head circumference will be performed at predefined times: at birth, at the beginning of MEF, on the randomization day, at the achievement of full enteral feeding, at 28 days of life and at 36 weeks of gestational age.

Parameters of feeding tolerance are those routinary used in the NICU (maximum gastric residual and residual appearance). The enteral nutrition will be discontinued or carried on according to our feeding protocol.

The follow-up will end at the achievement of a full enteral feeding, enteral intake of 160 mL/Kg/die or at 28 days of life, whichever occurs later. Patients may drop-out before this intended end because of the following withdrawal causes:

– Withdrawal of the consent by the relatives
– Severe skin reaction due to the skin probe
– Transferral to another hospital
– Death

After study end, patients will be fed according to the standard protocol of our Unit.

During the study, recommended diagnostic and therapeutic procedures are those usually performed as standard care in our Unit.

No new special measure for safety is planned since all the diagnostic and therapeutic procedures are part of the standard approach to the VLBW infant in our Unit.

Statistical considerations

Randomization

Randomized interventions are described in Section 'Methods and Design'. This section summarizes procedures and methodology, which will be adopted.

Patients eligible to randomization: all VLBW infants enrolled and eligible to the study, either IUGR or non-IUGR, who fulfill the following requirements:

1. achievement of 100 mL/kg/day of enteral nutrition;
2. adequate ventilation i.e. infants who are not intubated and not in-cPAP with a $FiO2 \geq 50\%$ at the achievement of 100 mL/Kg/day of enteral nutrition;
3. no NEC;
4. informed consent from parents or guardians.

When to randomize:

a. After achievement of 100 mL/kg/day of enteral nutrition in presence of adequate ventilation (see point 2 above)

Modalities of randomization:

b. Logistics: investigators will ask for randomization by access to a specific

software which will provide the assigned arm, after check on the eligibility criteria.

c. Methodology: randomization will be stratified (IUGR vs non-IUGR) and by blocks: the random assignment will be produced by an automatic procedure Ranlist [19] and will be based on random permuted blocks of small size, given the limited number of infants expected in each stratum.

Randomization refusals: if parents or guardians do not agree with randomization, patients will be excluded from the study and should be fed according to nutrition procedure regarded as the most appropriate for the child based on the Unit standard policy.

Statistical analysis

The primary aim of the study is to compare the impact on intestinal perfusion of 2 different nutrition modalities, bolus (BN, Group A) versus a 3-hour nasogastric nutrition (CN, Group B). The impact on intestinal perfusion is defined as the difference between pre- and post-prandial CSOR values measured with NIRS 30 minutes before feeding and at the expected peak after feeding (3 hours in CN and 30 minutes in BN). Pre- and post-prandial CSOR values will be calculated as the mean of 5-minute NIRS evaluations about the intended time-point (e.g. for baseline CSOR measurement, 2.5 minutes before and 2.5 minutes after the 30th minute before start of the randomized feed). Given that the study is a cross-over study, each infant will be evaluated both for CN and BN and the cross-over CSOR difference will be the primary endpoint.

As a secondary analysis, the cross-over difference of CSOR values as well as that of SO2s values (i.e. the splanchnic saturation slope) will be analysed comparing intra-uterine growth (IUGR vs. non-IUGR).

The impact of the 2 nutrition modalities on CSOR will also be studied in a multivariable context, adjusting for factors including the baseline CSOR value, the baseline Doppler flow velocimetry (both measured within the first 72 hours of life) and the intra-uterine growth (IUGR vs. non-IUGR).

Other secondary analyses include:

- Comparison of the time needed to reach full enteral feeding (i.e. days from randomization till enteral intake of 160 mL/Kg/day of formula or human milk) by randomized arm;
- Comparison of growth as measured by weight, length and head circumference by randomized arm. Main comparison will be between measures at randomization and at achievement of full enteral feeding. The growth at 28 days of life will also be compared with the appropriate standard growth curves.

The primary end-point analysis as well as all secondary analyses regarding randomized patients will be based on the ITT (intention-to-treat) principle. Comparisons of the two arms accounting for deviations from the assigned arm (analyses by “performed treatment”) will also be added to this main analysis. The primary endpoint will be analyzed with a two-tailed matched pairs t-test, after checking of appropriateness of assumptions. In particular, checking will concern the normality assumption, since period effect and cross-over effect appear both very unlikely in this study. Should normality be not adequate, non-parametric test such as the Wilcoxon signed ranks test will be applied [20]. An appropriate generalized linear model will be used to investigate the relationship between the CSOR cross-over difference and the candidate covariates. Should any raw CSOR observations be missing (e.g. NIRS instrument failed to measure or to save measurements), their estimation via imputation or other appropriate methods will be taken into account. The comparison of growth variables by randomized arm will be performed with

either the two-tailed t-test or a non-parametric alternative (e.g. Mann–Whitney test), should the normality assumption be not reasonable. The time from randomization till achievement of the full enteral feeding will be analysed with the Kaplan-Meier estimator, in which full enteral feeding as defined in the protocol will be considered as the sole 'event', while censoring will occur for withdrawal for any cause. Standard errors will be computed according to Greenwood formula and the log-rank test will be used for univariate comparisons.

Sample size

This single-center study will recruit patients for 2 years (24 months) since official opening. The expected accrual is about 20 VLBW infants, either IUGR or non-IUGR. The power calculations below are based on a two-tailed t-test with I-type error $\alpha = 0.05$ and show the power that the 20-patient study will achieve under various differences in CSOR from the 'baseline' impact observed under BN. On the basis of previous studies [21] this is expected to be 0.10 (mean of CSOR pre-post prandial difference), while various hypothesis are made about its standard deviation, σ_{CSOR}

Sample size: 20 infants

Impact on CSOR under BN = 0.10

σ_{CSOR}	CSOR difference	cross-over	Power
0.10	0.07		84%
0.15	0.10		80%
0.20	0.13		78%

The power calculations in different scenarios show that with 20 patients the study will achieve a good power in presence of a cross-over difference of at least 0.07.

Data collection

The case report form (CRF) attached to the protocol describes the data needed for each patient (see additional file 1). A specific database will be set up which will capture the data produced by the NIRS instrument and automatically saved in an exportable file and the demographic and clinical data routinely available in the electronic clinical chart used by the center. Other variables, collected ad hoc for this study, will be entered in the same database by the Trial Data Center.

Discussion

This is an explorative study. Evaluations of intestinal oximetry and superior mesenteric artery blood flow after the feed may help in differentiating how the feeding regimen alters the splanchnic blood flow and oxygenation and if the changes induced by feeding are different in IUGR versus NON IUGR infants.

We postulate that TOI will be altered in the vascular bed when blood flow is decreased; the resultant acidosis in poorly perfused tissues may also further increase the dissociation of oxygen from haemoglobin and increase the portion of reduced haemoglobin detected with NIRS. This condition may be present in IUGR infants and this condition may alter feeding tolerance thus requiring a longer time to achieve full enteral feeding and increasing the episodes of feeding intolerance.

Gut perfusion may depend on the way of administering feeding, bolus or continuous nutrition, so this study may suggest which is the best way to feed VLBW infants.

Legal and ethical requirements

Direct access to data/original documents

I/We hereby declare that the experimenter will grant examination, revision of the IRB/IEC, and the inspection by the competent authority through direct access to data/original documents.

'Liability

Legality of the study

The study will be conducted in conformity with the laws in force.

Protection of the patient's personal data.

Participants' personal data and the study results will be treated confidentially according to DL 30/06/2003 n.196.

Informed consent

Parents (or people with parental authority) of eligible patients will be informed and provided with details by any of the assigned doctors.

Ethics committee approval

The study received approval of the Ethics Committee.

Helsinki declaration

The study will be conducted in conformity with principles and regulations of the Helsinki Declaration and its amendments.

Quality assurance and quality control

Reference to the guidelines for Clinic Good Practice (CPMP/ICH/135/95)

The Center of Biostatistics for Clinical Epidemiology, University of Milano-Bicocca will analyze the outcomes of the study.

Regulation of the data promulgation

The final results of the study will be published even in case of non attainment of the goals. The publication will refer to the "CONSORT Statement" [22,23]

and will include complete analyses on security. Data of the study shall be published or reproduced upon Trial Steering Committee notice.

This study has received, on September 22th 2011, the ethical approval by the scientific and ethical committee of San Gerardo Scientific Institute of Monza, Italy.

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Impact on Splanchnic Perfusion of Two Feeding Regimens in Preterm Infants: a Randomized Trial.

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Abstract

Background. The different impact of bolus *versus* continuous nutrition on splanchnic blood flow is unknown. Preterm infants complicated by IUGR are considered to have impaired splanchnic perfusion.

Methods/Design. This is a randomized trial including 40 very low birth weight infants. At the achievement of an enteral intake of 100 mL/Kg/day the patients (IUGR and non-IUGR) were randomized in 2 groups: Group A (n = 20) received a feed by bolus (in 10 minutes); then, after at least 3 hours, they received the same amount of formula administered in 3 hours. Group B (n = 20) received a feed administered in 3 hours followed by a bolus administration of the same amount of formula after at least 3 hours.

On randomization day, intestinal and cerebral regional oximetry was measured via Near-infrared spectroscopy (NIRS) and superior mesenteric artery (SMA) Doppler was performed. Examinations were performed before the feed and at 30 minutes after the feed by bolus; for the 3-hour continuous feed, measurements were performed before the feed, at 30 minutes from the start of the nutrition and at 30 minutes after the end of the gavage.

Results SMA Doppler measurements showed higher perfusion values after bolus feed than after continuous feedings. NIRS values remained stable pre and post feedings. IUGR and NON IUGR infants showed the same perfusion and oxygenation patterns.

Conclusion According to Doppler results, bolus feeding is more effective in increasing splanchnic perfusion than continuous feeding.

Registered at **ClinicalTrials.gov** Identifier number: NCT01341236

Background

In literature there is no consensus regarding the impact of enteral feeding on intestinal blood flow and hence regarding the best rate of feeding delivery, by bolus or continuous nutrition.

In many Neonatal Intensive Care Units (NICUs), feeds are administrated by boluses or by continuous infusion without any standardized protocol.[1] There are theoretical benefits and risks with both kinds of feedings. Continuous enteral feeding might reduce feeding intolerance, improve nutrient absorption, and finally improve growth. However, it could also alter the cyclical pattern of release of gastrointestinal and pancreatic hormones and finally interfere with growth. Feeding by bolus on the contrary, promotes the cyclical surges of hormones, as in healthy term infants but, functional limitations of the premature infant's gastrointestinal system, such as delayed gastric emptying or intestinal transit, could hinder the premature infant's ability to handle bolus milk feeds, resulting in feeding intolerance. Additionally, this feeding regimen may challenge the premature infant's ability to maintain metabolic homeostasis and, therefore, decrease growth.[2-9]

Intra-uterine growth restriction (IUGR) is a severe and quite common problem in Obstetrics and consequently in NICU. A condition of placental dysfunction can lead to a cardiovascular adaptation in the fetus, characterized by a redistribution of cardiac output to maintain oxygen supply to the heart, adrenal glands and brain at the expense of visceral organs (as the gastrointestinal tract), the so called brain sparing (BS) effect. This condition, when associated with abnormal antenatal Doppler flow velocities in their descending aorta or umbilical arteries, may predispose IUGR infants to impaired gut function after birth.[10]

The superior mesenteric artery (SMA) is the major source of blood for the small intestine and large part of the colon. There is evidence that the rate of

increase of SMA blood flow velocity (BFV) as measured through Doppler ultrasound (US) correlates with tolerance to enteral feedings.[11] Although many factors[12-21] are known to affect intestinal blood flow, little is known about the response of SMA BFV of preterm infants according to different modalities of feeding administration.

Near-infrared spectroscopy (NIRS) is a continuous, non-invasive and real-time technique, which measures living tissues oxygenation.[22] NIRS is useful in detecting changes in splanchnic oxygen delivery and predicting splanchnic ischemia by measuring the ratio of splanchnic to cerebral saturation, the cerebrosplanchnic oxygenation ratio (CSOR).[23]

The purpose of this randomized, clinical trial is to detect the changes induced by two different feeding regimens (bolus *versus* continuous) in splanchnic oxygenation and perfusion through SMA Doppler and through NIRS in IUGR and non-IUGR preterm very low birth weight (VLBW) infants.

Patients and Methods

This is a single-center, randomized, cross-over study, performed at the NICU of the San Gerardo Hospital. The institutional Ethics Committee approved the study.

Inclusion criteria were: birthweight between 700 and 1500 grams, gestational age up to 25 weeks and 6 days and written informed consent from parents.

Exclusion criteria were: major congenital abnormalities (severe heart or cerebral disease, chromosomopathies, severe renal malformations, any gastrointestinal diseases), participation in other trials, significant multi-organ failure prior to trial entry (perinatal asphyxia with renal, cardiac or cerebral impairment, disseminated intravascular coagulation, DIC) or pre-existing cutaneous disease not allowing the placement of the probe.

Randomization

Infants who fulfil the following requirements were eligible to randomization: achievement of at least 100 mL/Kg/day of enteral nutrition, adequate ventilation (i.e. infants who are not intubated and not on nasal CPAP with a $FiO2 \geq 50\%$ at the achievement of 100 mL/Kg/day of enteral nutrition) and no evidence or suspect of NEC.

This study applied a randomized AB/BA crossover design. At the achievement of an enteral nutrition of 100 mL/Kg/day patients, IUGR and non-IUGR infants separately, have been randomly assigned to receive nutrition with bolus administration in 10 minutes and then, after at least 3 hours, the same amount of feed with continuous administration for 3 hours ('Bolus+Continuous' arm); or to receive nutrition in the reverse order ('Continuous +Bolus' arm).

Measurements

All patients underwent a baseline evaluation in the first 72 hours of life including cerebral, cardiac and abdominal ultrasound.

According to our protocol, enteral nutrition started after the 72nd hour of life as minimal enteral feeding (i.e. enteral feeding less than 20 mL/Kg/day). The increase was by 20 mL/Kg/day, if tolerated. All infants started parenteral nutrition on the first day of life. They were fed with human milk, if available, or with a preterm formula (75-80 Kcal/100 mL). Human milk was fortified at an achievement of an enteral intake of 100 ml/Kg/day. Nutrition was administered via the nasogastric route.

On randomization day, intestinal and cerebral regional oximetry were measured via NIRS (INVOS- 5100 C). NIRS sensors were placed over the abdomen (splanchnic bed) and on the forehead (cerebral bed) to measure cerebral (rScO2) and infra-umbilical abdomen splanchnic (rSaO2) regional saturations, respectively. The CSOR (rSaO2/rScO2) was also calculated. To investigate the balance between

oxygen delivery and oxygen consumption, splanchnic fractional tissue oxygen extraction (FTOE) was computed as (SaO2 – rsSO2)x100/SaO2. Tissues oximetry was measured before the feed and 30 minutes after the start of the feed by bolus; during the continuous nutrition the measurements were performed before, 30 minutes from the start and 30 minutes after the end of the feeding. Arterial haemoglobin oxygen saturation, measured by pulse oximetry, was recorded during the NIRS tracing. NIRS tracings were used only in the absence of desaturation (< 85% arterial saturation). Capillary haemoglobin concentration was measured on the day of the evaluation.

An evaluation of BFV of the SMA (peak sistolic and end-diastolic velocity, PSV and EDV, mean velocity, MV, and resistive index, RI) was performed meanwhile. To achieve imaging of the SMA, the transducer was placed on the mid-abdomen above the umbilicus. The SMA was identified at its origin from the aorta and measurements were performed a few millimetres from its origin. Echodoppler measurements were performed by two experienced operators. The measurements were performed on 5 contiguous homogeneous waves.

Statistics

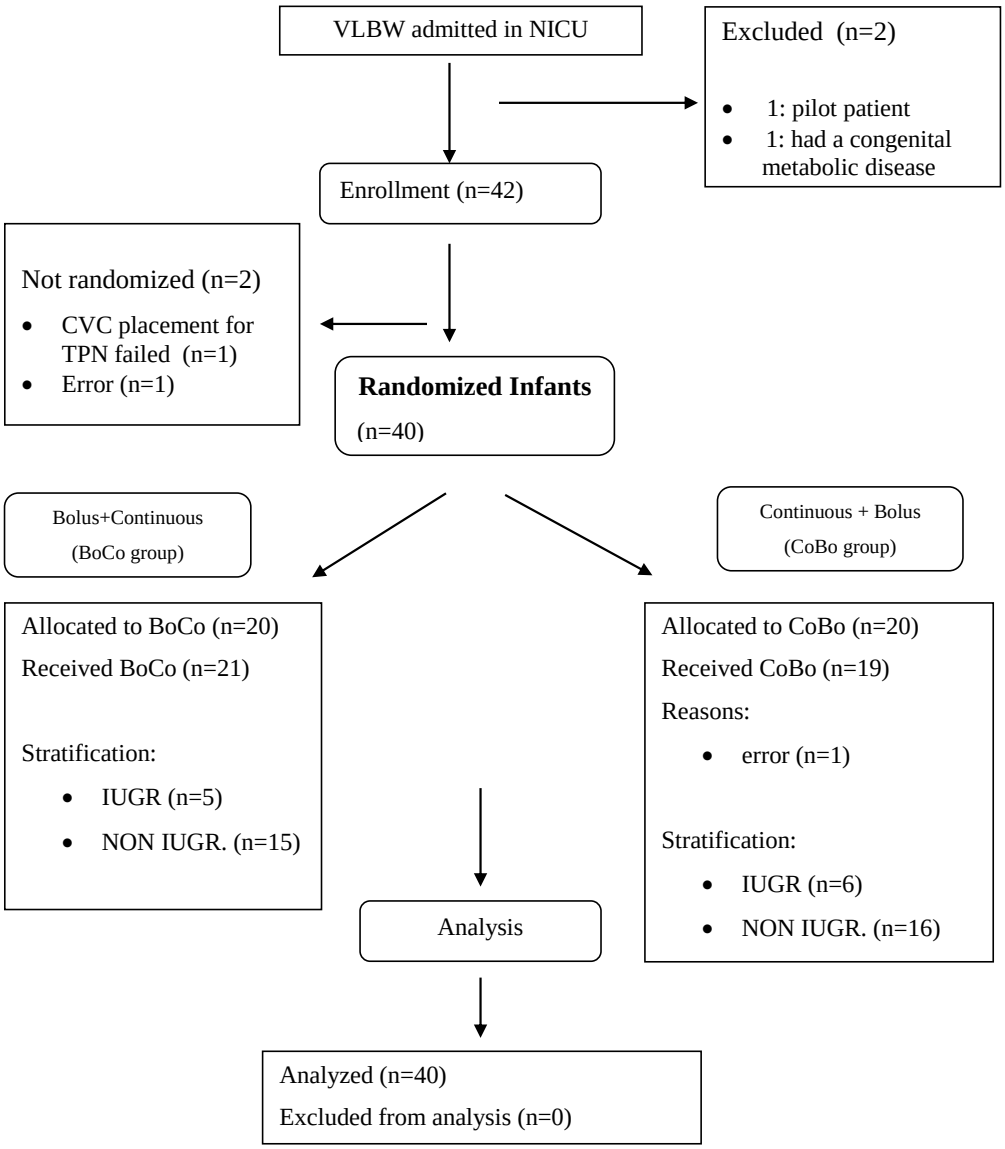
Randomization was based on random permuted blocks and was performed with the Ranlist software[24] at the trial data centre, at receipt of the randomized form, after checking of eligibility criteria. Recruitment was faster than expected, and 40 patients were randomized in one year, as compared to a minimum of 20 patients initially planned in 2 years (78% power to show a mean CSOR cross-over difference of at least 0.13, with standard deviation of 0.20, 2-sided test with alpha=0.05). The impact of the two nutrition modalities on splanchnic perfusion and oxygenation was defined as the difference between pre- and post-prandial CSOR and Doppler measurements, respectively. The primary endpoint was the cross-over CSOR difference,

defined as the difference of differences pre- and post-prandial CSOR under bolus and under continuous feeding. As secondary analyses, we evaluated the cross-over difference in terms of regional saturations rScO2 and rSaO2 and of FTOE, and Doppler parameters. We applied a generalized linear model to analyse the primary and secondary endpoints based on cross-over differences, after checking of Normality assumption.[25] Models including a term to account for period effect and to test for carry-over effect were also fitted to the data. We used the Fisher exact test to assess the association between patients' characteristics and intrauterine growth status. All tests were two sided. Analyses were performed using SAS 9.2 (SAS institute, Cary, NC, USA) at the trial data centre in the University of Milano-Bicocca.

Results

Forty-four VLBW infants were admitted to our Unit between November 2011 and November 2012. Forty-two were eligible and enrolled. Of these, 11 were IUGR and 31 NON-IUGR. Out of 42 patients, 40 were randomized and all but one received the assigned arm (Figure 1).

Figure 1. CONSORT DIAGRAM



ABBREVIATIONS

VLBW: Very Low Birth Weight

IUGR: Intra Uterine Growth Restriction

NICU: Neonatal Intensive Care Unit

CVC: Central Vascular Catether

TPN: Total Parenteral Nutrition

Characteristics of patients are described in detail in Table 1. Enrolled infants had mean gestational age of 29+4 weeks and mean birthweight of 1225 gr. Results of Doppler and NIRS examinations are summarized in Figure 2 and Figure 3.

Table 1: **Perinatal and clinical features of the randomized cohort, overall and by IUGR**

	Total (n = 40)	**IUGR** (n = 11)	**NON IUGR** (n = 29)	**p-value***
Prenatal Characteristics				
Preeclampsia	8 (20)	3 (27)	5 (17)	0.66
PROM	10 (25)	0	10 (34)	0.04
Chorionamniositis	3 (8)	0	3 (10)	0.55
Antenatal steroids°	32 (80)	6 (55)	26 (90)	0.02
Perinatal Characteristics				
C-section	26 (65)	10 (91)	16 (55)	0.06
Umbilical arterial pH, median (range)	7.30 (7.03-7.45)	7.29 (7.17-7.37)	7.30 (7.03-7.45)	0.29
Median Apgar Index 5 min (range)	9 (5-10)	9 (7-10)	8 (5-10)	0.08
Postnatal Characteristics				
Gestational age (weeks+days), median (range)	29+4 (26+2 – 36+0)	29+5 (28+2 – 36+0)	29+3 (26+2 – 32+1)	0.02
Birth weight (g), median (range)	1225 (780 – 1495)	1085 (780-1495)	1240 (866-1495)	0.51
Gender, male	16 (40)	4 (36)	12 (41)	0.99
Normal Cerebral US^	37 (95)	9 (90)	28 (97)	0.45
Normal Abdominal US§	38 (95)	10 (91)	28 (100)	0.28
Clinical Conditions				
RDS	27 (64)	8 (73)	19 (66)	0.99
PDA	13 (33)	1 (9)	12 (41)	0.07
Sepsis	13 (33)	4 (36)	9 (31)	0.99
NEC	2 (5)	1 (10)	1 (3)	0.45
IVH	5 (13)	3 (27)	2 (17)	0.11
BPD	1 (3)	0	1 (3)	0.99
Median age at randomized feeds (range) days	14 (8 – 40)	16 (10 – 40)	13 (8 – 26)	0.17
Human milk at randomized feeds	27 (68)	10 (91)	17 (59)	0.07

All data are number (%) unless otherwise stated. RDS: Respiratory Distress Syndrome; PDA: presence of haemodynamically significant ductus arteriosus; NEC: Necrotizing Enterocolitis, Bell stage ≥ 2; IVH: periventricular/intraventricular haemorrhage; Sepsis: clinical diagnosis or positivity of the blood culture; BPD: bronchopulmonary dysplasia; antenatal steroids: complete prophylaxis with betamethasone.; MV: Mechanical Ventilation; US:ultrasound .

* p-value of comparison of IUGR vs. non-IUGR; ^ 1 IUGR infant was excluded because exam was not performed; § 1 non-IUGR infant was excluded because exam was not performed.

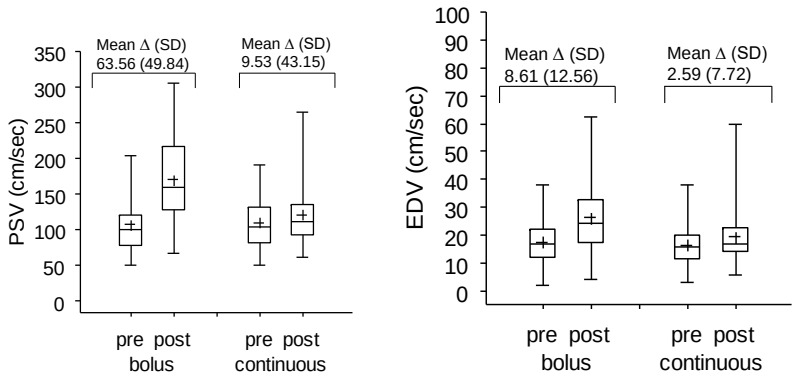


Figure 2 Doppler measurements taken pre and post bolus and pre and post continuous feeding in 40 randomized patients. Panel a) shows Peak Systolic Velocity (PSV), while panel b) End Dyastolic Velocity (EDV). Numbers above each plot show the mean[SD] of Δ= the difference between measurements taken pre and post each feeding).

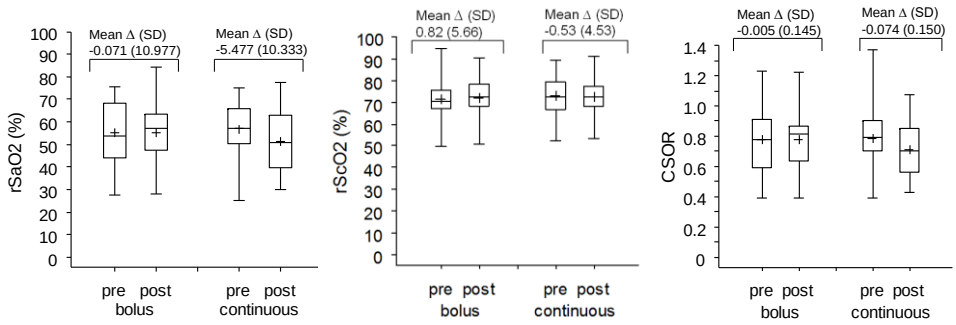


Figure 3 NIRS measurements taken pre and post bolus and pre and post continuous feeding in 40 randomized patients. Panel a) Abdominal oxygen saturation (rSaO2) Panel b) Cerebral oxygen saturation (rScO2), Panel c) cerebral to splanchnic ratio (CSOR) (numbers above each plot show the mean[SD] of Δ= the difference between measurements taken pre and post each feeding).

Doppler evaluation revealed that both PSV and EDV increased after bolus as compared to pre-bolus measurements, on average, by 60% and 50%, respectively (Figure 2, panel a and b, respectively). As a result, RI remains stable after bolus (mean absolute increase [SD] was 0.01 [0.06]). PSV and EDV increased also after continuous gavage, by 9% and 16% on average, respectively. RI after continuous thus showed a slight decrease (mean absolute decrease [SD] was -0.01[0.07]). Similarly to EDV, the MV showed

a more marked mean increase after bolus (44%) than after continuous nutrition (11%).

The mean PSV crossover difference (95% C.I.) was 54.03 (34.12; 73.94), $p<0.0001$, thus suggesting a significant increase in the PSV under bolus as compared to that under continuous nutrition. We also studied the impact of feeding on PSV by intrauterine growth: the mean PSV crossover difference (95% C.I.) was 35.56 (-3.86; 74.96) and 61.04 (37.02; 85.05) for IUGR and non-IUGR, respectively ($p=0.25$). The mean EDV crossover difference (95% C.I.) was 6.02 (0.89; 11.14), $p=0.02$, thus suggesting a significant increase in the EDV under bolus as compared to that under continuous nutrition. A non significant mean RI crossover difference (95% C.I.) was 0.02 (-0.01; 0.05) was observed ($p=0.16$). Results for the mean velocity were similar to those for EDV, with a mean velocity crossover difference (95% C.I.) of 6.04 (-0.09; 12.16), $p=0.05$. For all variables analyzed, no influence of period or carry-over effect was detected.

Response in terms of CSOR measured by NIRS remained stable after feeding as compared to pre-feeding values. Minor reductions were registered both after continuous and after bolus: the mean CSOR difference (SD) was -0.074 (0.150) after continuous and -0.005 (0.145) after bolus (Figure 3c). The resulting mean CSOR crossover difference (SD) was 0.069 (0.228) with 95% C.I. (-0.004; 0.142), thus suggesting no significant difference in the CSOR response under bolus as compared to that under continuous nutrition ($p=0.06$). Results did not change if adjustment by period effect ($p=0.06$) or carry-over effect ($p=0.06$) was considered in a regression model (data not shown).

We also studied the impact on CSOR by intrauterine growth: the mean CSOR crossover difference (95% C.I.) was -0.014 (-0.128; 0.010) and 0.100 (0.008; 0.192) for IUGR and non-IUGR, respectively ($p=0.16$).

Splanchnic oximetry evaluated in terms of FTOE confirmed previous findings and revealed no significant difference between continuous and bolus feeding. The mean crossover difference (SD) was -0.059 (0.177), 95% CI (-0.116; 0.002, p=0.05) and -0.017 (0.069), 95% CI (-0.039; 0.006, p=0.14) for abdominal and cerebral FTOE, respectively. IUGR had no impact on both abdominal and cerebral crossover difference of FTOE (p=0.20 and 0.72, respectively).

Discussion

It is well known that enteral feeding is one of the main factors involved in the onset of NEC[26,27] due to the imbalance between oxygen demand and supply. Oxygen supply is dependent on the route of feeding administration as this impacts on splanchnic perfusion. Thus, there is much interest to understand whether bolus has a different effect on splanchnic oxygenation and perfusion as compared to continuous feeding. A Cochrane Revision,[1] comparing clinical effects of continuous versus intermittent bolus nasogastric milk feeding in VLBW infants was inconclusive.

In this study, we aimed at comparing the impact of bolus versus continuous feeding on post prandial perfusion and oxygenation using, unlike the previous literature, a randomized cross-over design (stratified by IUGR).

Doppler examinations showed that BFV average measurements significantly increased both after bolus and after continuous gavage, but increase in all BFV parameters were significantly higher after bolus than after continuous feeding, consistently with previous studies.[28] The study of abdominal BFV in adults provides useful information about the mesenteric circulation in physiological and pathological situations.[29,30] Doppler US examination of the abdominal circulation in neonates has become a recognized method in the detection of impaired intestinal function. [31] Since studies have demonstrated

that drops of SMA BFV are associated with intestinal dysmotility, [32] feeding intolerance[11,33] and risk of NEC, [31,34-35] our findings suggest that in our cohort the hemodynamic response to the nutrients load was in a physiologic range. Blood flow in SMA increases after meal, to enable digestion and to stimulate the production of vascular endothelial growth factors responsible for anatomic growth of the intestinal vascular bed. [36] The increase in BFV is also associated with a further rise of enzymatic activities with a consequent increase of nitric oxide activity that is involved in intestinal functionality. [37,38] Moreover, it has been reported that SMA PSV change after feeding is lower in patients who had feeding intolerance compared with those who did not. [39] In our study, bolus feeding was more effective in increasing the flow in the mesenteric artery than continuous feeding. This suggests that feeding by bolus could be more protective on the gastrointestinal tract, since a low or absent increase in mesenteric blood flow would not support the additional metabolic demand that feeding imposes on the gut.[40] We speculate that this may occur because of the lower gastrointestinal workload occurring during continuous feeding.

It is noteworthy that IUGR and NON IUGR infants showed a similar increase in BFV after bolus and after continuous feedings. Also Fang et al. did not find any difference in the response to enteral feeding between SGA and AGA preterm infants. [11] These findings suggest that IUGR infants, who are unable to develop the physiological postprandial increase of BFV in the SMA after the first feeding, [28] may acquire this ability later on, when they tolerate full orogastric feeds. In fact, in our trial, the randomized evaluations were performed when infants were able to tolerate 100 ml/kg of enteral feeding, thus a time-point at which it is reasonable to think that the brain sparing effect was almost ceased. [41]

During feedings administered by bolus, NIRS examination revealed CSOR values and abdominal saturation values which were stable pre and post feeding. When feeding was administered as continuous nutrition, only minor reductions of CSOR and of abdominal saturation values were registered. We used the CSOR for comparison because this parameter is more reliable than the value of splanchnic oxygenation alone and it has been proposed as marker for abnormal perfusion processes affecting the gastrointestinal tract.

Similarly to previous studies, [42-44] brain tissue oxygenation in our study remained stable following feedings. This is probably due to the physiological mechanism of cerebral autoregulation, which keeps the levels of cerebral blood flow, and of consequent oxygen delivery, almost constant. Also FTOE, that gives an estimate of the amount of oxygen extracted and describes the balance between local oxygen delivery and consumption, remains stable pre and post feeding. Stable levels of splanchnic oxygenation, in presence of increased SMA BFV, would also support the view that the extra-demand of energy required by the gut for its digestive and endocrine[45] activity was adequately met by the splanchnic blood flow and that an additional increase in oxygen extraction by the intestine was not necessary.

However, the interpretation of NIRS data is difficult because of some limitations of the technique. [46] As already reported, [47] we observed NIRS series of measurements at the lower NIRS sensitivity threshold (rSaO2 at 15%), despite good sensor placement and absence of any pathologic conditions, as well as periods characterized by extreme variability.

Previous studies reported in the literature in this field are few, of small size and not randomized. Moreover, they report conflicting results. Dani et al. [44] demonstrated that bolus milk feeding induces an increase in splanchnic oxygenation without increasing oxygen blood extraction in both healthy AGA and SGA infants, whereas continuous feeding does not affect

gastrointestinal oxygenation. Faldella et al. [45] reported a significant decrease of splanchnic oxygenation occurring in the second half of continuous feeding and a slight trend toward increase in splanchnic Tissue Oxygenation Index during the final 10 minutes of continuous feeding. The study by Dave[46] shows that CSOR increases 1 hour after orogastric bolus feeding in stable preterm infants, without comparing the effect of bolus versus continuous feeding.

In our trial, IUGR and NON IUGR infants showed similar oxygenation patterns at NIRS measurements.

Since postnatal shunts through the PDA may significantly affect the intestinal circulatory adjustment causing a decreased superior mesenteric blood flow during the first day of life, all infants enrolled in our study underwent ecochardiography and the ductus was closed in all patients at randomization.

It is noteworthy from the practical point of view, that bolus feeding was more effective in increasing the splanchnic flow when compared to continuous feeding. The first procedure seems to be more prone to stimulate digestive and enzymatic activity of the gut and therefore to promote feeding tolerance, but the latter procedure appears to be a more prudent approach in those haemodinamically instable patients unable to balance the vascular response to the feeding by increasing the flow in the SMA.

The limitations of the study are the lack of clinical correlation between Doppler and NIRS findings. Doppler examination is usually considered suboptimal due to the intra-observer variability, but in our study this problem is limited because measurements were performed by only two experienced clinicians. Instead, our experience with NIRS would question its reliability to monitor gut oxygenation.

Points of strength include the rigorous methodological and operative approach and the intensive investigation of multiple pathophysiologic parameters.

In conclusion, we found that bolus and continuous feeding achieve a qualitatively similar effect on the splanchnic blood flow, but the effect is more relevant after bolus feeding. Whether this translates in a clinical benefit for the patient is not known, even because more important than the absolute value of the splanchnic blood flow is the relationship between oxygenation and the required intestinal endocrine and digestive work of the gut.

Whereas future research should focus on the investigation of parallel hemodynamic and digestive/endocrine response to the nutrients' load and on the identification of factors which predict onset of the NEC, our results suggest to give bolus nutrition to infants in stable clinical conditions, and to switch to a continuous nutrition modality in case of any cardiocirculatory impairment.

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Summary and Conclusions

The feeding challenge

To date, the timing of starting enteral feeding to prevent NEC occurrence or feeding intolerance in IUGR and high-risk infants constitutes one of the major controversial concern. In the last decade several strategies have been proposed ranging from late or slow increase in introduction of enteral nutrition to the use of enteral fasting and TPN and prophylactic antibiotics. All these statements show *pros and cons* issues and therefore no conclusive consensus has been obtained.

As reported in the **Chapter II**, preterm IUGR infants present a significant feeding challenge. The incidence of NEC is increased in infants who exhibit fetal AREDF (1). Abnormalities of splanchnic blood flow persist postnatally, with some recovery during the first week of life, providing physiological justification for a delayed and careful introduction of enteral feeding. Such a policy exposes babies to the risks of parenteral nutrition, with no trials to date showing any benefit of delayed enteral nutrition (2). A meta-analysis of 14 observational studies confirmed an increased incidence of NEC in preterm infants who had exhibited fetal ARED flow compared with controls, with an odds ratio of 2.13 (3).

AGA versus SGA infants

In **Chapter III** a comparison of parameters of feeding tolerance between AGA and SGA infants is reported. Since preterm infants are often considered at high risk for gastrointestinal impairment (4) we retrospectively compared feeding tolerance of AGA versus SGA infants in order evaluate which perinatal factor may affect feeding tolerance (measured as time to achieve full enteral feeding, FEF). Gestational age resulted as an important determinant of feeding tolerance. It provides evidence that feeding tolerance improves as gestational age increases and impairs in small for gestational age

infants. The finding of an influence of gestational age on full enteral feeding achievement is noteworthy since preterm birth constitutes a risk factor for an inadequate intrauterine growth and maturation of the gastrointestinal tract. This is especially true in SGA preterm infants, in whom placental insufficiency and subsequent hemodynamic compensatory mechanisms reasonably had a somewhat negative impact on feeding tolerance. The placental insufficiency may deprive the gastrointestinal tract from sufficient blood flow. As a consequence of gut hypoperfusion, SGA infants have an impaired gut function after birth, which may result in feeding intolerance (5). Therefore, the prolonged time necessary to achieve FEF in SGA infants may be ascribed to a chronic prenatal intestinal hypoxic condition. Among the other factors involved in feeding tolerance, antenatal betamethasone plays a crucial role and it is effective in reducing the time to FEF in both AGA and SGA preterm infants. Antenatal steroid therapy seems to directly promote gut maturation.

Predictors of feeding tolerance in IUGR infants

In **Chapter IV** we conducted a case-control study in 70 IUGR infants complicated or not by redistribution o fetal-placental blood flow: the so called brain sparing effect. In the IUGR fetus, hypoxaemia produces circulatory redistribution towards the brain and away from the viscera and placenta, culminating in umbilical artery or aortic AREDF in the most severely affected. In this respect, there is evidence that in IUGR infants the gastrointestinal tract suffered, during the intrauterine life, of a condition of hypoperfusion. Whenever direct tissue injury does not occur, prolonged exposure to a condition of impaired blood flow may modulate the development of motor, secretory and mucosal function, so that in the postnatal period the intestine is more susceptible to stasis, abnormal

colonisation and bacterial invasion (4). In this regard, no data on the relationships among prenatal monitoring parameters such as Doppler' patterns and the tolerance of enteral nutrition have been provided.

Our study reveals that IUGR infants with brain sparing (defined as an umbilical artery PI to middle cerebral artery PI ratio >1) had worse feeding tolerance (longer time to achieve of full enteral feeding) than IUGR infants with no fetal hypoxia. The umbilical PI to cerebral PI ratio should be used as a tool to predict feeding tolerance. Impaired gut function can be early detected by monitoring Doppler patterns and clinical parameters.

Doppler sonographic examination of the fetal arterial circulatory system is established as a current standard for analysis of the degree of fetal compromise since it may diagnose a large spectrum of fetoplacental haemodynamic findings.

Cerebral and Somatic NIRS-determined Oxygenation pattern in IUGR Preterm Infants

The brain sparing effect in IUGR infant can be monitored by Near InfraRed Spectroscopy (NIRS) to assess oxygenation status of the brain and of splanchnic organs such as the intestine (9). Our study with NIRS technology, reported in **Chapter V**, demonstrates that cerebral/splanchnic vascular adaptation of IUGR infants to the extra-uterine environment is characterized by a postnatal persistence of the brain sparing effect with reperfusion in the transitional period. Other postnatal physiological studies have shown persistent flow abnormalities in the superior mesenteric artery blood flow velocity in IUGR infants during the first days of life (10). Neonates with increased resistance patterns of blood flow velocity in the superior mesenteric artery on the first day of life are at higher risk of developing NEC. Our experience revealed also that in the IUGR infants a lower oxygenation of

the splanchnic district occurs in association with a significant difference between the fractional tissue oxygenation extraction values in the cerebral and splanchnic regions; this should be responsible for the increased risk of intestinal damage in these infants.

Results from the RCT

Since there is no consensus regarding the impact of enteral feeding on intestinal blood flow and hence regarding the best regimen and the best rate of delivering the enteral nutrition we investigate the issue by the RCT conducted at our Department. The protocol is reported in **Chapter VI**.

Results from the RCT are reported in the **chapter VII**. The primary aim of the trial was to compare the effect induced by feeding by bolus and by continuos nutrition on splanchnic perfusion and oxygenation. Doppler of the Superior Mesenteric Artery showed that bolus feeding was more effective in increasing the splanchnic blood flow when compared to continuous feeding. The first procedure seems to be more prone to stimulate digestive and enzymatic activity of the gut and therefore to promote feeding tolerance, but the latter procedure appears to be a more prudent approach in those haemodinamically instable patients unable to balance the vascular response to the feeding by increasing the flow in the SMA. NIRS examination revealed CSOR values and abdominal saturation values which were stable pre and post feeding during feedings administered by bolus. When feeding was administered as continuous nutrition, only minor reductions of CSOR and of abdominal saturation values were registered. In our trial, IUGR and NON IUGR infants showed similar oxygenation and perfusion patterns at NIRS and SMA measurements.

Conclusions

There is limited evidence in scientific literature regarding the feeding policy in IUGR newborns. Currently available studies on this topic include ELBW/VLBW neonates, but are not focused specifically on IUGR infants. Furthermore there are not RCTs that made a clear distinction between SGA and IUGR neonates. A study, that focuses appropriately on preterm infants with impaired prenatal Doppler, and therefore at major risk for gastrointestinal impairment, is necessary. The inclusion criteria in a study involving IUGR infants may consider prenatal features of the subjects as well as Doppler patterns.

Non-invasive tools to assess gastrointestinal perfusion are available. Near-infrared spectroscopy (NIRS) is a non-invasive clinical tool with the potential to monitor splanchnic perfusion in very premature infants at increased risk for intestinal disorders. Moreover, blood flow velocities in the superior mesenteric artery (SMA) can be measured by doppler ultrasound and correlated with gestational and postnatal age, in-utero growth restriction, feeding, intestinal dysmotility and NEC. Nevertheless, there are currently very limited data on how abdominal NIRS monitoring affects patient outcomes.

Results from our randomized trial on feeding intervention, that is specifically targeted on IUGR infants, excluding constitutionally small newborns, provide evidence about which feeding method is to prefer according to the clinical condition of the patient.

Future studies need to focus on developing algorithms that calculate and describe patient-specific variability of intestinal perfusion (measured by NIRS and by SMA Doppler) over the first weeks of life in very premature infants. This may permit to modulate and to personalize enteral nutrition according to the perfusion patterns of the intestine.

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Nederlandse Samenvatting en Conclusions

Enterale voeding in de "high risk" te vroeg geboren (premature) pasgeborene: een uitdaging!

Intra-uteriene groeivertraging (IUGR) is een belangrijk en veel voorkomend probleem in de verloskunde. Het doel van dit proefschrift was om de volgende vragen te onderzoeken:

1. Voedingsproblemen bij te vroeg geboren pasgeborenen met IUGR;
2. Klinische en instrumentele parameters als voorspellers van voedingstolerantie bij te vroeg geboren pasgeborenen met IUGR;
3. Intestinale (darm) en cerebrale zuurstoftoevoerpatronen in IUGR en niet-IUGR baby's;
4. Zuurstofvoorziening en doorbloedings (perfusie) patronen van de darmen in IUGR en niet IUGR baby's na voeding met bolus of met continue enterale voeding.

De resultaten uit **Hoofdstuk II** lieten zien dat de langere tijd die nodig is in IUGR pasgeborenen om volledige enterale voeding te bereiken kan worden toegeschreven aan een chronische prenatale intestinale "hypoxische" toestand. Het voorkomen van darmontsteking (NEC) was toegenomen als er aanwijzingen waren van een afgenomen doorbloeding van de darm. Deze bevindingen geven aan dat een behoedzame introductie van enterale voeding terecht is als het gaat om IUGR pasgeborenen.

In **Hoofdstuk III** werden parameters van voedingstolerantie (gedefinieerd als de tijd nodig om tot volledige enterale voeding te komen) vergeleken tussen AGA (normaal gegroeide pasgeborenen) en SGA (pasgeborenen met groeiachterstand) pasgeborenen. De zwangerschapsduur bleek een belangrijke determinant te zijn van voedingstolerantie. Het bleek dat de voedingstolerantie beter werd met het toenemen van de zwangerschapsduur, maar slechter werd als het ging om SGA pasgeborenen. Dit laatste is waarschijnlijk te verklaren omdat placentainsufficientie (vaak [deels] de

oorzaak van IUGR) en een daarmee samenhangend hemodynamisch compensatiemechanisme een negatieve invloed kan hebben op de enterale voedingstolerantie. Antenatale behandeling met betamethasone verkort de tijd die nodig is om tot volledige orale voeding te komen, zowel in AGA als ook in SGA kinderen.

In **Hoofdstuk IV** werd gezocht naar voorspellers van enterale voedingstolerantie. Het bleek dat IUGR-baby's met een "brain sparing" effect (BS: gedefinieerd als een redistrubutie van de foetale en placentale perfusie) een grotere voedingsintolerantie hadden in vergelijking met IUGR pasgeborenen zonder een BS effect:. de tijd nodig om tot volledige enterale voeding te komen was significant korter in IUGR kinderen zonder het BS effect in vergelijking met kinderen die wel een BS fenomeen vertoonden. De doormiddel van het Doppler effect bepaalde pulsatility index (PI) van arteria umbilicalis en de CRIB score (een score die de mate van ziekzijn in de eerste dagen aangeeft) bleken de beste voorspellers te zijn om enterale voedings(in)tolerantie te kunnen inschatten.

In **Hoofdstuk V** werd met behulp van Near InfraRed Spectroscopy (NIRS) het zuurstofgehalte van de hersenen en het zuurstofgehalte van de darmen onderzocht in pasgeborenen met en zonder IUGR. In beide groepen kinderen was het zuurstofgehalte hoger in de hersenen in vergelijking met die van de darmen. Het zuurstof gehalte in de hersenen was niet verschillend in de hersenen tussen IUGR en niet-IUGR pasgeborenen, maar het zuurstofgehalte in de darmen was significant lager in de IUGR-groep indien vergeleken met de niet-IUGR-groep. Er werd geconcludeerd dat de vasculaire adaptatie van de IUGR kinderen aan het extra-uteriene bestaan wordt gekarakteriseerd door het persisteren van het BS-effekt met reperfusie in de transitieperiode.

Hoofdstuk VI beschrijft een voorgesteld onderzoek dat de "impact" wil onderzoeken van enterale voeding op darmdoorbloeding om op deze manier

tot het beste beleid van de opbouw van enterale voeding te komen. Evaluatie van de oxygenatie en de doorbloeding van de arteria mesenterica superior als indicator van de darm-oxygenatie (met behulp van NIRS en Doppler-onderzoek) na enterale voeding kan helpen om te achterhalen hoe een bepaald voedingsregime de doorbloeding en oxygenatie (zuurstofvoorziening) van de darmen kan beinvloeden en of de oxygenatie en doorbloedingsveranderingen, geinduceerd door deze regimes, verschillend zijn voor IUGR versus niet-IUGR kinderen.

Hoofdstuk VII onderzocht het verschil in effect dat enterale bolusvoeding versus enterale continue voeding heeft op de darmdoorbloeding. Dit mede en vooral in het licht van het gegeven dat tevroeg geboren kinderen met IUGR geacht worden een gestoorde darmdoorbloeding te hebben. Het bleek dat de darmdoorbloeding gemeten met Doppler onderzoek van de arteria mesenterica superior en de zuurstofvoorziening van de darmen gemeten met NIRS hoger waren na bolusvoeding in vergelijking met continue enterale voeding. IUGR en niet-IUGR pasgeborenen toonden dezelfde perfusie en oxygenatie patronen. Concluderend kan gezegd worden dat enterale voeding via het bolus principe effectiever is dan via continue enterale voeding.

Conclusies

De internationale literatuur biedt slechts beperkte richtlijnen hoe het enterale voedingsregime in IUGR pasgeborenen zou moeten zijn. Met name focust de literatuur niet zozeer op de IUGR pasgeborene maar op de extreme en ernstig premature zuigeling in zijn algemeenheid. Een studieopzet, zoals beschreven in dit onderzoek, dat zich vooral concentreert op het veel tevroeg geboren kind met IUGR (gedefinieerd door een abnormale prenatale met het Doppler effect bepaalde pulsatility index [PI] van arteria umbilicalis) was daarom

nodig. In dit onderzoek werd gebruik gemaakt van non-invasieve onderzoeksmethoden om de gastro-enterale perfusie te meten doormiddel van NIRS en de bloedstroomsnelheden in de arteria mesenterica superior (Doppler methode), als ook de hersenoxygenatie doormiddel van NIRS. Deze variabelen werden gecorreleerd met zwangerschapsduur en postnatale leeftijd, IUGR, enterale voeding motiliteitsproblemen van de darmen en het voorkomen van necrotiserende enterocolitis (darmontsteking).

De resultaten van onze gerandomiseerde studie ten aanzien van enterale voedingsinterventie, in dit geval gericht op IUGR pasgeborenen en constitutioneel te kleine pasgeborenen uitsluitend, geven meer duidelijkheid over welke enterale voedingsstrategie gevolgd zou moeten worden in IUGR kinderen, waarbij ook de klinische conditie van deze groep kinderen een rol speelt.

Toekomstige studies zouden zich moeten richten op het ontwikkelen van algoritmen die de patient-specifieke variabiliteit berekenen en beschrijven van de darmperfusie gedurende de eerste weken van het leven van de extreme en ernstig premature zuigeling (inclusief de IUGR pasgeborene). Op deze manier zou het optimale enterale voedingsregime kunnen worden bepaald voor de individuele prematuur-geboren zuigeling.

Short Summary and Conclusions

Intra Uterine Growth Restriction (IUGR) is an important and common problem in obstetrics.

The purpose of the present thesis was to investigate:

1. Feeding issues in IUGR preterm infants;
2. Clinical and instrumental parameters as predictors of feeding tolerance in IUGR preterm infants;
3. Splanchnic and cerebral oxygenation patterns in IUGR and non IUGR infants;
4. Splanchnic oxygenation and perfusion patterns in IUGR and non IUGR infants after feeding by bolus and by continuous enteral nutrition;

We outlined that the prolonged time necessary to achieve full enteral feeding may be ascribed to a chronic prenatal intestinal hypoxic condition of the IUGR infants. IUGR infants with brain sparing had worse feeding tolerance than IUGR infants without brain sparing, which may be due to poorer circulation towards the gastro-intestinal tract during prenatal life. We also demonstrated that cerebral and splanchnic vascular adaptation of IUGR infants with brain sparing to the extra-uterine environment was characterized by a postnatal persistence of the brain sparing effect with reperfusion occurring in the transitional period. Results from our randomized control trial revealed that bolus feeding was more effective in increasing the splanchnic blood flow when compared to continuous feeding. IUGR and NON IUGR infants showed similar oxygenation and perfusion patterns after feeding. Further results from the trial suggested that bolus feeding seems to be more prone to promote feeding tolerance by increasing intestinal perfusion, but continuous feeding appears to be a more prudent approach in haemodynamically unstable patients.

We conclude that these novel findings on feeding interventions in IUGR preterm infants, provide evidence regarding the preferred feeding method, in relation to the clinical condition of the young patient.

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Postgraduate training

Specialisation Fellowship in Pediatrics and Newborn Medicine, Universita' Vita-Salute, San Raffaele Scientific Institute, 2001-2006. Title of the postgraduation thesis: "Immunoprophylaxis anti-RSV in preterm newborn: our Department's experience" (grade: 70 cum laude)

Advanced Course of "Nutrition of the Developing Infant", Universita' degli Studi di Milano, academic year 2014-5

Residency Resident at the Division of Pediatrics and Neonatology Intensive Care Unit, San Raffaele Scientific Institute, 2001-2004

Professional Experience

2004-2007 San Raffaele Scientific Institute
member of the medical staff of Paediatrics and Neonatology

August 2009 Childrens Hospital, Boston, MA, U.S.A.
Observership at the Neonatal Intensive Care Unit

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Activity as clinical researcher at the Neonatal Intensive Care Unit

From 2007 — San Gerardo Hospital, Monza
Member of the medical staff of Neonatology, Outpatient Department and of the Neonatal Intensive Care Unit

Clinical practice

Responsibility and clinical management of the VLBW and ELBW, with a special familiarity with the following procedures:

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- incannulation and placement of central venous access
- neonatal resuscitation
- nutritional support of preterm babies
- follow-up of the high risk babies

Clinical research in the fields of neonatal nutrition, enteral and parenteral.

Clinical management, diagnosis and therapy of the more common paediatric endocrinopathies; wide experience in the paediatric diabetic area.

Knowledge and clinical experience in management of metabolic bone disease, both of children and of newborn.

Publications, Posters and Awards

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