Perinatal and neurodevelopmental outcome of late-onset growth restricted fetuses

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PhD THESIS

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Perinatal and neurodevelopmental outcome of late-onset growth restricted fetuses

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A thesis submitted by Daniel Orós López for the degree of Doctor in Medicine (Faculty of Medicine, University of Barcelona) under the direction of Francesc Figueras Retuerta, Asociated Professor of Obstetrics and Gynecology at Barcelona University, and Eduard Gratacós Solsona, Professor of Obstetrics and Gynecology at Barcelona University.

Signed: Daniel Orós López

Barcelona, 22nd February 2010.

Francesc Figueras Retuerta, Asociated Professor of Obstetrics and Gynecology at Barcelona University, and Eduard Gratacós Solsona, Professor of Obstetrics and Gynecology in the Barcelona University.

DECLARE:

That Daniel Orós López has carried out the study entitled “Perinatal and neurodevelopmental outcome of late-onset growth restricted fetuses” under our direction for the degree of Doctor in Medicine, and that the mentioned study is henceforth ready to be presented.

Signed,

Francesc Figueras Retuerta

Eduard Gratacós Solsona

Barcelona, 22nd February 2010.

This thesis project has been structured following the normative for PhD thesis as a compendium of publications. The studies included in the thesis belong to the same research line leading to three papers already published or submitted for publication in international journals:

1. Oros D, Francesc Figueras, Rogelio Cruz-Martinez, Eva Meler, Meritxell Munmany, Eduard Gratacos. ***"Longitudinal changes in uterine, umbilical and cerebral Doppler in late-onset small-for-gestational age fetuses."*** Ultrasound Obstet Gynecol. (submitted)

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3. Daniel Oros; Francesc Figueras; Rogelio Cruz-Martinez; Nelly Padilla; Eva Meler; Edgar Hernandez-Andrade; Eduard Gratacos. ***"Middle versus anterior cerebral artery Doppler for the prediction of perinatal outcome and neonatal neurobehavior in term small-for-gestational-age fetuses with normal umbilical artery Doppler."*** Ultrasound Obstet Gynecol (in press)

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1) INTRODUCTION

1.1. Definition

Conceptually, intrauterine growth restriction (IUGR) is failure of a fetus to achieve its genetically endorsed growth potential. However, the difficulty in accurately determining the growth potential for a given fetus limits the applicability of this definition, although birthweight centiles (1) and estimated fetal weight charts, customized for individual maternal and fetal characteristics (2) represent attempts to use this concept in clinical practice.

Hence, in clinical pratice birthweight is classified using population-based sex-adjusted centiles, with a newborn being small for gestational age (SGA) if the birthweight is <10th centile. However, this definition includes a large proportion of cases that are constitutionally small and otherwise healthy newborns.

Currently, the most accepted clinical classification of SGA, to further tailor their monitoring and management, is (Figure 1):

- ***IUGR:*** this category represents those fetuses that fail to reach their growth potential, mostly because of chronic placental insufficiency.
- ***Abnormal SGA fetuses:*** this category comprises cases with congenital anomalies, including chromosomopaties, genetic syndromes and fetal infections.
- ***Normal SGA fetuses:*** this category includes constitutionally small fetuses with a genetically determined lower growth potential. This group is defined after negative screening for congenital anomalies, fetal infeccion and signs of placental insufficiency.

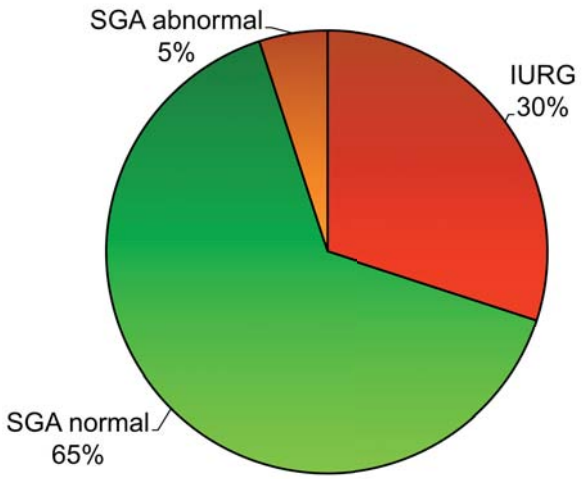

Figure 1. Proporcion of fetuses with estimated fetal weigth < 10th centile (3)

1.2. Clinical impact of SGA

Small for gestational age, defined as a birthweight below the 10th centile for the gestational age at delivery represents in Europe 600,000 cases amongst the 6,000,000 deliveries/year. Intrauterine hypoxia and hyponutrition promote a wide and different espectrum of adaptative and pathologycal changes that manifest at different periods during postnatal life (Table 1). (4)

1.2.1. Perinatal impact

SGA is considered a major contributor to perinatal morbidity and mortality, and has been described as responsible for 50% of perinatal deaths occurring preterm and 20% at term. (5)

SGA neonates have a higher risk of respiratory distress syndrome, intraventricular haemorrhage and necrotizing enterocolitis. (6) SGA are also associated with an increased need for prolonged respiratory support and retinopathy of prematurity, and a four to six times higher risk of cerebral palsy among near term and term neonates. (7)

Table 1. Systemic effects of intrauterine growth restriction. (4)

Cardiovascular	• Increased afterload • Cardiac hypertrophy • Initial elevation of left ventricular output • Elevated brain natriuretic peptide (BNP) • Higher shunting through Ductus venosus • Decreases cardiac compliance
Lungs	• Accelerated lung maturity • Decreased lung compliance
Kidneys	• Reduced number of nephrons • Alterations in rennin–angiotensin–aldosterone axis, hypertension • Accelerated nephrosclerosis • Shortened life span.
Skeletal muscle	• Increase in thyroxine secretion • Altered mitochondrial function • Reduction and then cessation of foetal motor activity
Brain	• Increase in cerebral blood flow • Reduction in metabolic rate • Dishminuish oxigenation of temporal and occipital cortex, hippocampus and cerebellum • Altering nitric oxide synthases (NOSs) genes and protein expression. • Affect myelination and growth • White-matter injury • Increase in the dopaminergic system
Gastrointestinal tract	• Alter blood flow in the mesenteric artery • Postnatal intestinal motility syndrome and poor nutrient absorption

1.2.2. Impact on childhood

In childhood, SGA is linked with an increased mortality due to infectious diseases, congenital anomalies, central nervous system anomalies and cardiovascular diseases. (8)

Furthermore, SGA neonates are at higher risk of developing cognitive deficits at school age with impaired learning capacities. Even in the absence of overt perinatal brain lesions, minor neurologic deficits have been reported. (7) The most frequent cognitive difficulties are in memory performance, learning abilities, visuomotor functions and attention span. (9) Both in the animal model and in human pregnancies complicated by IUGR, autopsy of the brain showed a significant reduction in myelination, whereas visual and frontal cortical synaptogenesis seemed to be decreased. (10) In addition, magnetic resonance imaging (MRI) studies have demonstrated specific differences in the brain composition of neonates with IUGR in comparison with those without this condition. Thus, significant volume reductions in cortical grey matter (11) and both hippocampi (12) have been demonstrated. Moreover an important delay in cortical development, a discordant pattern of gyrification and a pronounced reduced cortical expansion has been observed. (13) In contrast, white matter (WM) seems to be affected only in cases of evident ischemic injury. (11)

1.2.3. Impact on adulthood

Epidemiological evidence has long suggested a link between low birth weight and increased cardiovascular mortality in adulthood. (14) This association is essentially mediated through fetal growth restriction. (15) The mechanistic pathways underlying the relationship between IUGR and cardiovascular risk are poorly understood. A number of studies support that it might be partially explained by fetal metabolic programming leading to conditions associated with cardiovascular disease, such as obesity, diabetes and hypertension. (16-17)

1.3. SGA versus IUGR: the value of umbilical artery Doppler

Clinical management and prognosis differ in abnormal SGA fetuses depending of their specific aetiology. SGA and IUGR have been used interchangeably, but the two terms are not synonymous; not all small babies are growth-restricted and not all growth-restricted infants are small. (18) Intrauterine placental

function evaluation by umbilical artery Doppler (UA) is currently the clinical standard to distinguish between SGA and IUGR. (19)

1.3.1. Physiopatology of UA Doppler abnormalities

The vasoconstriction phenomena of the tertiary stem villi are considered responsible for the up river modifications in the normal wave flow velocity of the UA, with a decrease in the diastolic velocities and an increase in the resistance and impedance indices. (20) Umbilical artery Doppler resistance index only increase when approximately 30% foetal villous vasculature is abnormal. At the end of the spectrum, a pattern of absent or reversed end-diastolic flow velocities (EDFV) appears when 60–70% of the villous vasculature is damaged. (21) Accordingly, clinical studies on IUGR have established that UA impedance progressively increases and, in advanced stages of placental histological and functional damage, diastolic velocities become absent or even reversed (Figure 2).

Figure 2. Site of insonation of the umbilical artery (a). Progressive waveform patterns with advancing severity: normal UA waveform(b), increased impedance to flow(c), absent end-diastolic flow (d) and reversed end-diastolic flow (e). (20)

UA Doppler index correlate with foetal levels of glucose, aminoacids and blood gases and, therefore, it could be considered a surrogate measurement of placental functionality. (22).

1.3.2. Outcome of SGA with abnormal umbilical artery Doppler

There is an extensive body of evidence that those SGA fetuses with abnormal UA flow are at higher risk of adverse perinatal outcome than those with normal

flow. (23-25) This association is independent of gestational age at delivery. (23,26) At the end of the spectrum, absent and reversed end-diastolic velocities are independently correlated with perinatal morbidity and mortality, with a relative risk of 4.0 and 10.6, respectively. (27) In addition, this pattern is also associated with increased risk of long-term abnormal neurodevelopment. (28)

A meta-analysis, including nearly 7000 high-risk pregnancies monitored with UA Doppler, has demonstrated a significant improvement of a number of perinatal outcomes, with an overall reduction of perinatal mortality of 30%. (29) Thus, there is a sound basis for the use of umbilical artery Doppler as a risk-discriminator tool in the management of SGA fetuses.

1.3.3. Outcome of SGA with normal umbilical artery Doppler

While abnormal umbilical Doppler is associated with adverse perinatal and neurodevelopmental outcome, (29) small fetuses with normal umbilical artery Doppler have been traditionally considered to represent one end of the normal size spectrum and the importance of managing them completely differently from true IUGR babies has been stressed. (30)

However, recent evidence suggests that a substantial proportion of these fetuses have true growth restriction. (6) Studies over the last decade have provided evidence that perinatal outcome may be significantly poorer in SGA fetuses. (25,31-32) Trudinguer et al (23) showed that SGA fetuses with normal UA Doppler born beyond 34 weeks still had a mean neonatal intensive care unit stay of 5 days. It has also been reported a 3% mortality rate and a 6% rate of intraventricular hemorrhage in a series of SGA fetuses with normal Doppler. (24) In accordance with these results, other studies found that one-quarter of small-for- gestational-age babies with normal umbilical Doppler were hypoglycemic, and even after adjusting for gestational age the odds of SGA with normal Doppler requiring prolonged newborn care was two-fold greater than the general population. (25) Therefore, morbidity and mortality among these fetuses could be increased in relation with normally grown fetuses. (6)

Furthermore, there is an increased prevalence of abnormal neurobehavioral and neurodevelopmental tests in childhood, (33) with similar features to those described for preterm children who had intrauterine growth restriction. (34) (11) Ley et al. (35-36) reported that 46% and 11% of SGA fetuses with normal Doppler have minor neurological abnormalities and an intelligence quotient below 85 at 7 years. Scherjon et al. (10) reported that among preterm neonates without Doppler signs of centralization, 20% have an intellectual quotient below 85 at 5 years. Finally, in a cohort of 282 neonates born SGA, McCowan et al. (37) reported that about 15% of those with normal umbilical flow on Doppler examination had suboptimal neurodevelopmental outcome at 18 months, corrected for gestational age. Thus, it seems likely that neurological outcome may also be mildly impaired in small-for-gestational-age fetuses with normal umbilical artery Doppler.

From this evidence it could be inferred that umbilical artery Doppler might not accurately identify mild forms of placental insufficiency. In keeping with this concept, umbilical vein flow is already reduced by the time the UA Doppler is abnormal in SGA, suggesting that UA Doppler is not an early sign of placental insufficiency. (38) In addition, it has been demonstrated by animal (39) and mathematical (40) experimental models of placental vessel obliteration that umbilical Doppler resistance indices become abnormal only in advanced stages of placental dysfunction. Since the identification of late-onset SGA fetuses at risk for adverse perinatal outcome cannot be relied on umbilical artery Doppler, other vascular territories have been proposed.

1.4. Uterine artery Doppler

1.4.1 Physiopathology

The vascular bed of the placenta starts to develop early in pregnancy. Invasion of cytotrophoblastic cells into the decidual tissue transforms the subplacental vasculature into a low resistance circulation. In pregnancies complicated by preeclampsia and/or fetal growth restriction, the transformation of these vessels is incomplete. (41) Furthermore, morphological evaluation of the placental bed

has shown that this lack of vessel transformation is associated with increased vascular impedance of the uterine artery. (42)

Doppler velocimetry of the uterine arteries reflects vascular impedance on the maternal side of the placental circulation. Increasing impedance, due to anomalous invasion of cytotrophoblastic cells into the decidual tissue of the placental bed and defective remodelling of the spiral arteries, is reflected in decreasing diastolic blood flow velocities and/or persistent early diastolic notch in the uterine artery blood flow waveform. (43) (Figure 3).

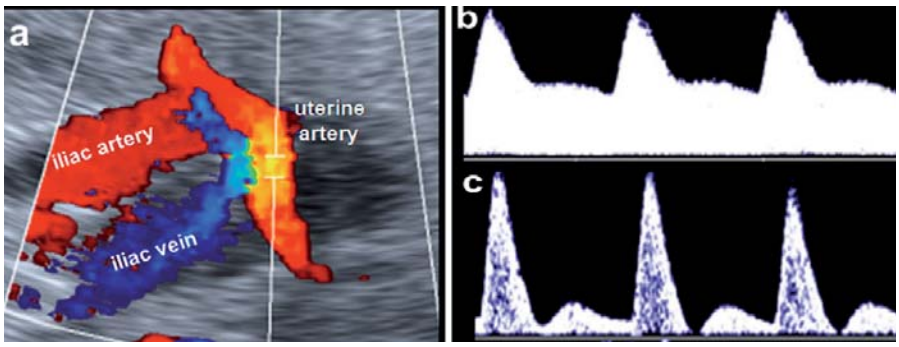

Figure 3. Site of insonation of the uterine artery with color Dopper at the crossover of the iliac artery (a). Normal (b) and abnormal (increased impedance flow with early diastolic notching) (c) waveforms. (20)

1.4.2. Uterine Doppler as a screening tool for placental-related diseases

Increased pulsatility index (PI) and/or persistent notches in the blood flow waveform can be detected early in the second trimester. Uterine artery (UtA) Doppler evaluation has been proposed as a screening tool for early-onset IUGR and has been associated with detection rates of approximately 25% and 75%, for a false-positive rate of 10%. (44-45) These sensitivities are higher for the prediction of early-IUGR associated with preeclampsia. Different strategies combining maternal risk factors, blood pressure and biochemical markers have been published, with detection rates greater than 90% for early-onset preeclampsia-associated IUGR. (46-47)

1.4.3. Uterine artery in the management of SGA

In pregnancies complicated by pre-eclampsia, increased uterine artery vascular impedance in the third trimester has been correlated with small-for-gestational-

age (SGA) newborns, delivery by caesarean section, premature delivery, admission to a neonatal intensive care unit (NICU) and maternal complications. (48-49) In late-onset IUGR, abnormal uterine artery Doppler has shown to be comparable to umbilical artery Doppler as a predictor of adverse outcome in late-onset IUGR. (32,43,50) Futhermore, it has been suggested that UtA Doppler could provide additional value to the umbilical and cerebral arteries in predicting the occurrence of adverse perinatal outcome (32,51)

Despite these encouraging reports, uterine artery Doppler is not routinely used in the evaluation of pregnancies suspected for fetal growth restriction. Futhermore, a meta-analysis (52) showed a limited capacity of this parameter to predict perinatal mortality.

1.5. Middle cerebral artery Doppler

1.5.1 Pathophysiology

Several studies in IUGR fetuses show redistribution of fetal blood flow in response to hypoxia. Selective vasoconstriction results in a primary reduced perfusion of the gastrointestinal organs, kidneys and skin. On the other hand, blood supply to the brain, spleen and heart is increased to ensure adequate oxygenation. (53) This vasodilatation leads to an increase in the diastolic velocities and a decrease in the resistance and impedance indices in the vessels supplying these organs. This centralisation of the fetal circulation with the described brain sparing effect represents an essential development in the hemodynamic adaptation of the fetus to an increasingly hypoxic environment. (28,54) Accordingly, longitudinal monitoring of IUGR demonstrated a progressive decrease in the impedance indices of the brain arteries. (55) In clinical practice the key sign to identify brain vasodilation is the demonstration of a reduction in the pulsatility index of the middle cerebral artery (MCA). Doppler evaluation of this artery has become a standard parameter for fetal assessment and management. (Figure 4)

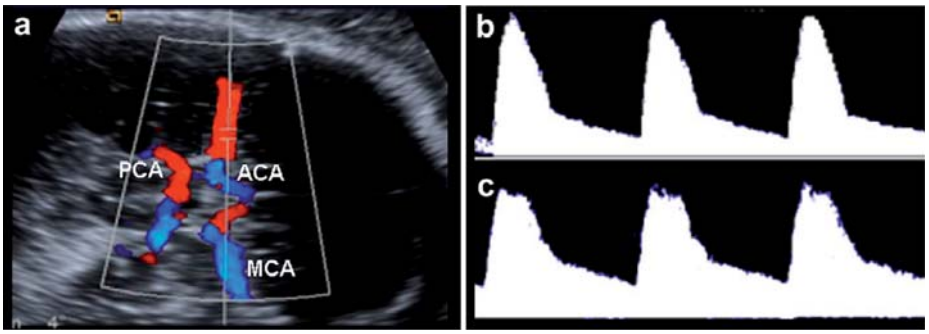

Figure 4. Color Doppler assessment of the middle cerebral artery at the level of the circle of Willis (a). Normal (b) and abnormal (high diastolic velocities and decreased pulsatility index) (c) waveforms. (20)

1.5.2. Clinical significance of brain sparing

It remains under debate whether brain sparing is an entirely protective mechanism or whether it indicates a higher risk for brain injury. Early studies on neurodevelopmental outcome suggested that fetal brain sparing in IUGR was a benign adaptive process preventing severe brain damage. (56) More recently, it has been reported that a reduced pulsatility index of the middle cerebral artery may be associated with poorer perinatal outcome (32,51,57) and with an increased risk of abnormal neurodevelopment. (10,33) In the subgroup of late-onset SGA fetuses, up to 20% have reduced PI in the MCA, and this sign is associated with poorer perinatal outcome (32,58) and with an increased risk of abnormal neurobehaviour at birth (59) and at two years of age, (33) independently of the umbilical artery Doppler findings. (60) In a longitudinal cohort of infants born preterm (26–33 weeks), accelerated visual maturation was found using visual evoked cortical potentials at 3 years. (56) At 5 years, these series demonstrated that both changes in cerebral Doppler and the acceleration of visual maturation were associated with a deficit in cognitive scores. (10) Recently, studies in the same cohort confirmed that brain sparing was associated with impaired visual function and visual motor capabilities at 11 years of age. (61)

1.6. Anterior cerebral artery Doppler

1.6.1. Pathophysiology

In animal models under hypoxia, blood supply differs substantially between different brain areas depending on the gestational age and the type and severity of the insult. (62) Intra-brain regional hemodynamic redistribution could be one of the mechanisms behind the existence of a regional hierarchy in brain deterioration, whereby certain areas are more susceptible than others to hypoxic damage. (63) Frontal areas are philogenetically recently acquired and, therefore, maturation and myelinization processes of these areas occur late in the fetal development, making these structures vulnerable during a long period. (64) Long-term outcome of growth-restricted fetuses revealed a specific profile of neurocognitive difficulties with poor executive functioning, inflexibility-creativity and language problems, (9) as signs of frontal lobe dysfunction. (65) Consistently, studies using magnetic resonance imaging (11) and 3-D ultrasound (66) demonstrated specific disruption of this area in growth-restricted fetuses. (Figure 5)

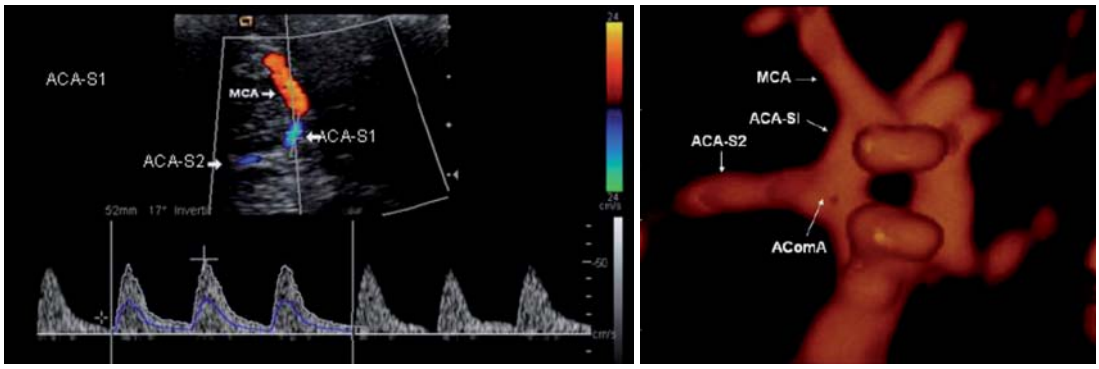

Figure 5. *Doppler assessment of the anterior cerebral artery at the level of the circle of Willis (a); power Doppler image of the circle of Willis (b)*

1.6.2. Clinical significance of Anterior Cerebral Artery Doppler

Cortical and subcortical areas of the frontal lobe are supplied by the anterior cerebral artery (ACA) and this vessel has been found to be vasodilated in a proportion of SGA fetuses with normal MCA Doppler. (67) Studies assessing

the temporal evolution of brain arteries in IUGR fetuses suggest that the ACA shows vasodilatory changes earlier than the MCA (67) (62) Established vasodilation of the MCA seemed to coincide with a decline in the relative perfusion to the frontal lobe in relation with other regions such as the basal ganglia. Furthermore, tissue perfusion studies in fetuses with IUGR suggest that increased frontal perfusion occurs weeks before the MCA PI both in early and late-onset IUGR. (59,68)

1.7. Cerebro-placental ratio

1.7.1 Pathophysiology

The unique arrangement of the fetal circulation allows afterload to affect ventricles individually. Accordingly, relative contributions of individual ventricles to the common cardiac output may change with individual alterations in afterload. (69) In this setting, improved oxygen delivery to the brain is thought to result from decreased left ventricular afterload. The cerebroplacental ratio (CPR) quantifies redistribution of cardiac output by dividing Doppler indices from representative cerebral and fetoplacental vessels: the MCA and the UA. (70)

1.7.2. Clinical significance

Studies on animal models suggest that the CPR most closely reflects acute changes in pO2 (71) than its individual components, that is the UA and MCA. Finally, as supported by an increasing number of studies, cerebroplacental ratio is an earlier and more sensitive predictor for adverse outcome than either the middle cerebral artery or umbilical artery alone, both in severe (70,72-74) as in mild forms of IUGR, (75) and it correlates better with adverse outcomes. (76)

1.8. Relevance and justification of the research project

The studies included in this project are part of a research line on fetal brain circulation in growth-restricted fetuses and its prediction capacity for neurological damage.

In early-onset IUGR, the sequence of changes in Doppler indexes has been described. (27,55,75,77) However, in late-onset cases, these longitudinal trends have not been investigated before. This information is essential to ascertain whether there is a sound basis to use these parameters in the monitoring of late-onset SGA. The first project was aimed at determining the longitudinal trends and rate of conversion of normal to abnormal Doppler pulsatility indexes of the uterine, umbilical and middle cerebral artery in late-onset SGA fetuses from diagnosis to delivery.

Many studies have found associations between premature growth restricted infants and later behavioral, (11,34,78) sensorial (10,79) and cognitive dysfunctions. (9,65,80,81) Long-term outcome of preterm growth-restricted infants has revealed a specific profile of neurocognitive difficulties with poor executive functioning, inflexibility-creativity and language problem. (9,80) Some studies have correlated these difficulties during childhood with behavioral disruptions already present in the neonatal period (11,12,34) a time when environmental influences are still minimal. Neurobehavior is a neurological function that in the neonate is mainly related to neurological maturation. (82) In low-risk preterm infants, individualized developmental interventions reportedly prevent short-term neurobehavioral dysfunction. (83) Some studies have also reported long-term cognitive disadvantages for full-term SGA, (84-85) but there is no information on neurobehavioral performance of full-term SGA babies with normal placental function. The purpose of the second project was to evaluate the neonatal neurobehavior performance of full-term SGA fetuses with normal placental function.

Some studies on IUGR fetuses have demonstrated a regional redistribution of blood supply within the brain, (62) which contributes to the regional hierarchy in brain deterioration, making certain areas more susceptible than others to hypoxic damage. The frontal brain lobe, mainly supplied by the ACA, is one of these highly susceptible structures in chronically hypoxic infants. The study of this vessel may be superior to the standard parameter used to detect brain redistribution, the MCA, detecting those fetuses at an early stage of brain hypoxia. This may allow earlier interventions such as closer monitoring and timely delivery. The third project was aimed to evaluate whether ACA Doppler

investigation is superior to middle cerebral artery Doppler investigation in the prediction of adverse perinatal outcome in term SGA fetuses with normal umbilical Doppler.

2) GENERAL HYPOTHESIS

2.1. Conceptual hypothesis

- A proportion of late-onset growth restricted fetuses with normal placental function have been exposed to mild in utero hypoxia.

2.2. Secondary hypothesis

- Longitudinal monitoring of late-onset growth restricted fetuses show that Doppler pulsatility indices of the anterior cerebral artery (ACA), middle cerebral artery (MCA) and cerebro-placental ratio (CPR) present earlier and more frequent modifications than umbilical artery (UA) and maternal uterine arteries (AUT).
- Late-onset growth restricted fetuses with normal umbilical artery Doppler, have worse perinatal outcomes as well as a suboptimal neonatal neurobehavior.
- Late-onset growth restricted fetuses with signs of cerebral hemodynamic redistribution present neurological disrruptions affecting neonatal neurobehavior.

3) GENERAL OBJECTIVES

3.1. Main objective

- To explore the temporal evolution of Doppler parameters in late-onset growth restricted fetuses and to assess its association with adverse perinatal and neurobehavioral outcome.

3.2. Specific objectives

- To describe during late pregnancy the trend of the Doppler longitudinal pulsatility indices of the middle cerebral artery, umbilical and maternal uterine arteries in late-onset growth restricted fetuses.
- To assess the neurobehavior and perinatal outcome of fetuses with an estimated fetal weight less than p10 and normal umbilical artery Doppler.
- To assess the neurobehavior and perinatal outcome of late-onset growth restricted fetuses with signs of intrauterine brain Doppler redistribution defined by the anterior and middle cerebral arteries.

4) METHODS

4.1. Study design

Between November 2007 and August 2009, a prospective longitudinal cohort study was performed in the Fetal Growth Unit of the Maternal-Fetal Medicine Department of Hospital Clinic of Barcelona.

4.2. Study population

Study population was divided in two cohorts.

a) Case cohort

a.1) Inclusion criteria:

- Consecutive suspected SGA singleton fetuses at routine third trimester ultrasound (30-36 weeks), with an estimated fetal weight <10 percentile according to local standards (1).
- Normal admission Doppler examination, with mean uterine artery pulsatility index (PI) < 95th percentile (86), umbilical artery pulsatility index < 95th percentile (87), middle cerebral artery pulsatility index >5th percentile and cerebroplacental ratio >5th percentile (76).

a.2) Exclusion criteria:

- Congenital defects, chromosomal abnormalities and infections, birthweight > 10th percentile according to local standards (1).

b) Control cohort

b.1) Inclusion criteria:

- Adequate for gestational age controls were defined as singleton neonates with birthweight between the 10th and 90th percentile according to local standards. (1)

Controls were selected from our general population, with previous adequate ultrasound estimated fetal weigth, individually matched with cases for

gestational age at inclusion (± 1 week), corrected by first trimester ultrasound. (88)

b.2) Exclusion criteria:

- Congenital defects, chromosomal abnormalities and infections.

4.3. Sample size

Based on results of previous studies, the number of subjects that should be included in the study to detect differences equal to or greater than 0.4 points in the result of the Neonatal Behaviour Assesment Scale (NBAS) test and differences equal to or greater than 15% for the onset of intrauterine cerebral redistribution, assuming an alpha error of 5% and a beta error of 20%, was 102 children per branch.

Assuming an acceptance rate of 90% we aimed to include in the initial sample 116 patients in each branch.

4.4. Predictive variables

1. Maternal age at delivery; Continuous (years)
2. Smoking consumption during pregnancy; Continuous (cigarettes / day)
3. Maternal weight at the beginning of pregnancy; Continuous (kg)
4. Maternal height; Continuous (cm)
5. Maternal ethnicity; Categorical (Europe, Africa, South America, North Africa, Asia, Others)
6. Parity (number of births> 22 weeks); Discrete
7. History of preeclampsia (89) Binary (Yes / No)
8. History of gestational hypertension (89)(Binary (Yes / No)
9. Previous history of intrauterine growth restriction in previous pregnancies (birth weight below the 10th percentile (1); Binary (Yes / No)

10. Gestational age at inclusion; Continuous (weeks)
11. Diastolic blood pressure at inclusion; Continuous (mmHg)
12. Sistolic blood pressure at inclusion; Continuous (mmHg)
13. Pulsatility index of uterine arteries at each examination (86); Continuous (standardized for gestational age)
14. Pulsatility index of umbilical artery at each examination (87); Continuous (standardized for gestational age)
15. Pulsatility index of middle cerebral artery at each examination (76); Continuous (standardized for gestational age)
16. Pulsatility index of anterior cerebral artery at each examination (66); Continuous (standardized for gestational age)
17. Cerebro-placental ratio at each examination (76); Continuous (standardized for gestational age)

4.5. Result variables

a) Main result variable:

Neonatal Behavior Assessment Scale (90); Continuous (standardized)

b) Secondary result variables

1. Preeclampsia: Diastolic blood pressure (DBP) ≥ 90mmHg and/or systolic (SBP) ≥ 140 in 2 separate determinations (≥ 4h), and proteinuria> 300 mg/24 h; Binary (Yes / No)
2. Severe preeclampsia: preeclampsia criteria DBP ≥110 mmHg, proteinuria> 5 g./24 h, oliguria (<400 ml/24h), neurological symptoms (cerebral or visual), acute pulmonary edema (radiological and blood gas test) persistent epigastric pain, abnormal liver function (AST or ALT> 70 IU) Laboratory evidence of hemolysis (LDH> 700 U / L) and / or thrombocytopenia (<100,000 / mL); Binary (Yes / No)
3. Gestational age at delivery; Continuous (weeks)
4. Intervention for fetal distress; Binary (Yes / No).

5. Cesarean for fetal distress; Binary (Yes / No)
6. Neonatal weight; Continuous (g)
7. Neonatal acidosis (arterial pH <7.10 EB> 12mEq / L), Binary (Yes / No)
8. Perinatal mortality (> 22 weeks gestation - <28 days postpartum), Binary (Yes / No)
9. Admission length in Neonatal Intensive Care Unit, Continuous (days)
10. Significant neonatal morbidity (seizures, intraventricular hemorrhage> grade III, periventricular leukomalacia, hypoxic ischemic encephalopathy, abnormal electroencephalogram, necrotizing enterocolitis, acute renal failure (serum creatinine> 1.5 mg / dL) or heart failure (requiring inotropic agents), Binary (Yes / No)

4.6. Ethical approvement

The research protocol was approved by the Local Ethics Committee. Informed consent complies with the provisions of the RD 561/1993. A copy of the approval of the CEIC, and patient or controls informed consent is given in the annexes. (Annex 1)

4.7. Study protocol

1. Patients were recruited at routine third trimester ultrasound (30-36 weeks).

2. In those patients meeting the inclusion criteria, a study information sheet was given to those agreeing to participate (Annex 2).

3. At a first visit within 7 days of inclusion, variables previously defined were collected. (Annex 3)

4. From diagnosis to delivery, a biweekly ultrasound examination was scheduled. Prenatal Doppler was performed using an Acuson Antares Premium Edition (Siemens, Mountain View, CA) ultrasound machine equipped with a 2.3–4 MHz transabdominal transducer. A high pass wall filter of 70 Hz was used to avoid artefacts.

Uterine arteries were examined transabdominally. The probe was placed on the lower quadrant of the abdomen, angled medially, using color Doppler to identify the uterine artery at the apparent crossover with the external iliac artery. Measurements were taken approximately at 1 cm distal to the crossover point. The pulsatility index of the left and right arteries were measured, and the mean index was calculated.

The umbilical artery Doppler flow spectrum was recorded from a free-floating portion of the umbilical cord.

The middle cerebral artery Doppler was recorded in a transverse view of the fetal brain, with the Doppler gate placed on the vessel 1 cm distal from the circle of Willis.

For the first segment of ACA, the Doppler gate was placed immediately after the origin of the ACA from internal carotid artery.

The cerebroplacental ratio was calculated as MCA PI / UA PI.

In these vessels, once it had been ensured that the angle was less than 30º, the pulsed Doppler gate was placed over the whole width of the vessel. Angle correction was then applied and the signal updated until three similar consecutive waveforms were obtained in the absence of fetal movements and voluntary maternal suspended breathing. All cases had a Doppler examination within 7 days of delivery.

Either the MCA PI, the ACA PI and CPR values below the 5th centile were considered indicative of cerebral blood flow redistribution and were reported as abnormal. Values over the 95th centile were considered reported as abnormal for both UA PI and mean uterine artery PI.

5. Labour induction was performed by cervical ripening with prostaglandins for cases when (i) at 37 weeks of gestation there was an estimated fetal weight below the 3rd centile; or (ii) at 40 weeks of gestation when estimated fetal weight was below the 10th centile; (iii) at 37 weeks with abnormal umbilical artery Doppler. Cases were managed at the discretion of an attending senior obstetrician, following standard management guidelines, who was blinded to the cerebral Doppler. A staff obstetrician assisted all the deliveries.

6. After delivery previously defined outcome variables were collected from the clinical history. (Annex 3)

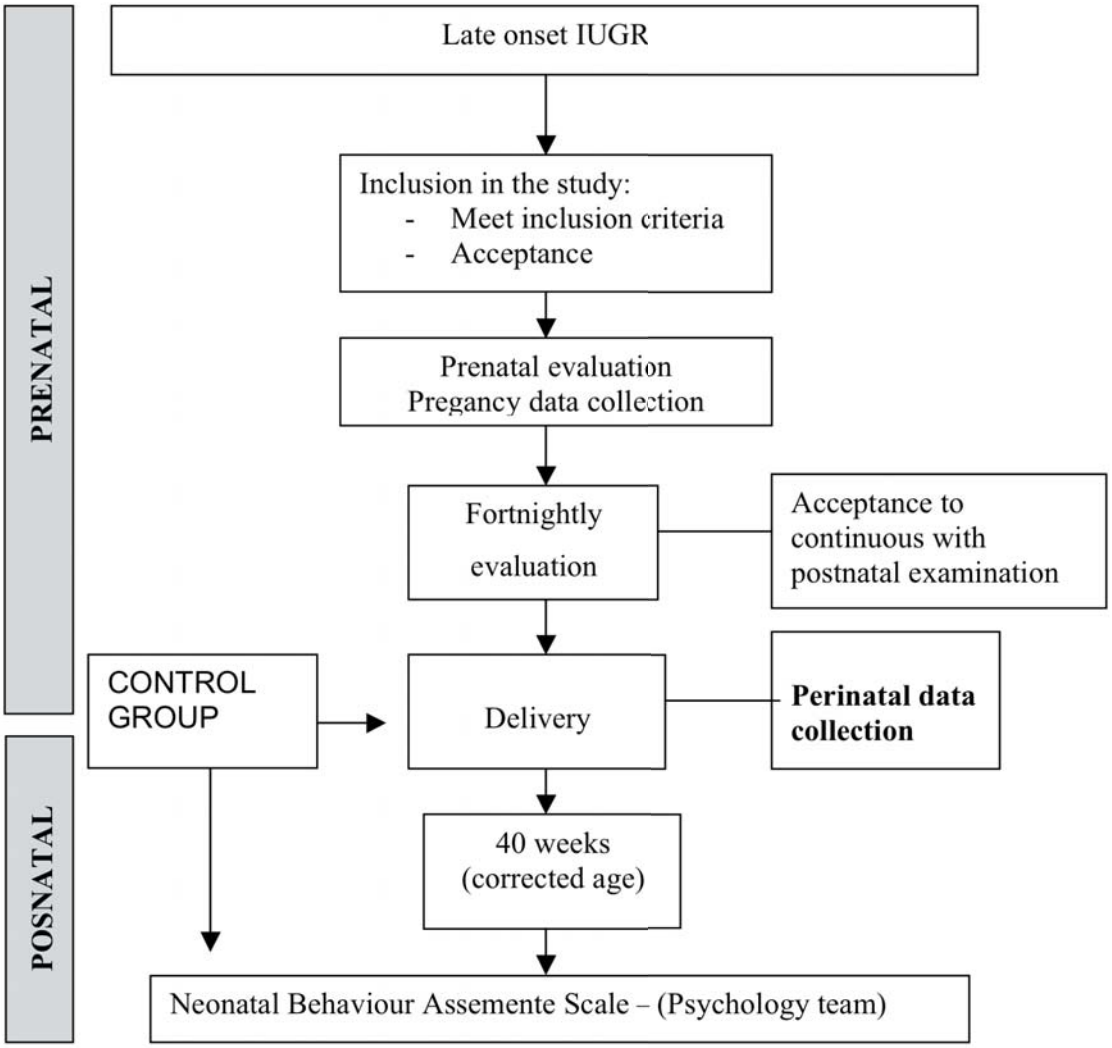

Figure 6. Protocol algorithm

7. The Neonatal Behavioral Assessment Scale (NBAS) was prospectively performed in all cases and controls at 40 weeks (±1) of corrected age by one of three observers accredited by The Brazelton Institute (Harvard Medical School, Boston, USA). The observers were blinded to the study group and to Doppler status. The examination consisted of 4 behavioral areas rated on a 1 to 9 scale where 9 is the best performance, except for some curvilinear scale items which, according to the manual, were re-scored as linear on a 5, 6, or 8-point scale. (91) With the newborn between two feedings, in a small, quiet, semi-dark room

at a temperature of between 22 to 27°C and in the presence of at least one parent, the following areas were analyzed: habituation (habituation to light, rattle, bell and tactile stimulation of the foot items), motor (which includes general tone, motor maturity, pull-to-sit, defensive movements and level of activity) social-interactive (which includes response to visual and acoustic stimuli), state organization (which includes peak of excitement, rapidity of build-up, irritability and lability of states). The behavioral items were converted into percentiles according to normal curve references for our population, (90) and each area was considered abnormal at a score below the 5th percentile.

8. Controls were recruited, matched for gestational age at delivery and gender, of babies born at the hospital during the study period. After acceptance of informed consent, Neonatal Behavior Assesment Scale was performed. The psychology team was blinded to the study group.

4.8. Specific projects

4.8.1. Project 1: Longitudinal evaluation of fetal hemodynamic status in late-onset IUGR fetuses.

a) Hypothesis: In late-onset growth restricted fetuses cerebral haemodinamic redistribution appears earlier and more frequently than umbilical artery (UA) and maternal uterine arteries (AUT) haemodynamic changes.

b) Objective: To describe during late pregnancy the trend of the Doppler longitudinal pulsatility indices of the middle cerebral artery, umbilical and maternal uterine arteries in late-onset growth restricted fetuses without hemodynamic changes at the time of diagnosis.

c) Study design: propective longitudinal cohort study.

d) Study population: a prospective cohort was created of consecutive suspected late-onset IUGR singleton babies at routine third trimester ultrasound (30-36 weeks) with confirmed birth weight < 10 percentile according to local standards. (1) Only cases presented upon admission with mean uterine artery pulsatility index (PI) < 95^{th} percentile (86), umbilical artery pulsatility index < 95^{th} percentile (87), middle cerebral artery pulsatility index >5^{th} percentile and cerebroplacental ratio >5^{th} percentile (76) were included. Exclusion criteria were congenital malformations (including chromosomopathies and infections) and the diagnosis of preeclampsia.

e) Statistical analysis: All Doppler parameters were transformed into z-values according to normative references. The longitudinal changes were analyzed by Kaplan-Meier survival analysis, in which the endpoint was defined as an abnormal Doppler value (middle cerebral artery pulsatility index and cerebroplacental ratio < 5th percentile; umbilical and uterine arteries pulsatility index > 95th percentile). The McNemar test was used to compare paired group proportions. Statistical and survival analyses were performed using the Statistical Package for Social Sciences (SPSS 15.0, SPSS Inc., Chicago, IL) statistical software.

Longitudinal changes in z-values during the last 10 weeks before delivery were modeled by means of multilevel analysis, fitting to second-degree polynomials:

$\alpha + \beta t + \gamma t^2$, where α, β and γ were the parameters characterizing the individual fetus and t the days before delivery. These parameters were calculated assuming its randomly normal distribution in the population (random effect model), which allows us to assume that both the individuals and the days to delivery at which the scan was performed are random samples of their respective populations. Standard errors of these parameters were used to construct confidence intervals (CI). The software MLwiN 2.1 (Centre for Multilevel Modelling, University of Bristol, UK) was used for the parameters´ estimation. Repeated measurements at different time points (gestational age) in the same fetus comprised Level 1 and those in different fetuses comprised Level 2. Individual regression lines for each variable were calculated for each fetus and from these the regression lines for the whole group were derived.

4.8.2 Project 2: Evaluation of perinatal outcome and neonatal neurobehaviour of late-onset IUGR fetuses with normal UA Doppler versus controls.

a) Hypothesis: Late-onset growth restricted fetuses with normal umbilical artery Doppler, have worse perinatal outcomes as well as a suboptimal neonatal neurobehavior.

b) Objective: To assess the neurobehavioral and perinatal outcome of fetuses with an estimated fetal weight less than p10 and normal umbilical artery Doppler.

c) Study design: propective cohort study.

d) Study population: patients were divided in two cohorts:

A cohort was created of consecutive, suspected SGA; singleton infants delivered at term with confirmed birth weights of <10th percentile according to local standards. (1) Exclusion criteria included congenital malformations (including chromosomopathies and infections) and umbilical artery pulsatility index values of >95th percentile. (87)

Control subjects were defined as singleton term infants with size appropriate for gestational age (AGA) (>10th percentile, according to local standards (1)) and were sampled from our general neonatal population during the same period; they were matched with case subjects according to the date of delivery (±7 days).

e) Statistical analysis: Student's t test for independent samples and Pearson's χ^2 test were used to compare quantitative and qualitative data, respectively. Multivariate analyses were conducted through multivariate analysis of covariance in which a model was run for each different set of skills (attention, habituation, motor, state organization, state regulation, and autonomic nervous system), with the study group included as a factor and the following variables as covariates: (1) smoking during pregnancy (no smoking, 1–9 cigarettes per day, or 10 cigarettes per day); (2) maternal BMI at booking; (3) low socioeconomic level (routine occupations, long-term unemployment, or never worked; United Kingdom National Statistics Socio-economic Classification); (4) onset of labor (spontaneous versus induction); (5) mode of delivery (vaginal delivery versus cesarean section); (6) number of doses of epidural anesthetic medication (bupivacaine, 1.2–1.8 mg) during labor (none, 1–3 doses, or 4 doses); (7) gestational age at delivery; (8) postnatal age (in days) at evaluation; and (9) gender.

For each model, assumptions for the multivariate analysis of covariance were checked and the multivariate significance of the F value was assessed with Wilks' P value. Also, the χ^2 value was provided, which could be interpreted as the proportion of the total variance of the dependent variables explained by each factor and covariate. To rule out an expectation bias, the association between birth weight and NBAS scores was evaluated through Pearson correlation within each study group.

The software package SPSS 15.0 (SPSS, Chicago, IL) was used for the statistical analyses.

4.8.3) Project 3: Evaluation of the anterior and middle cerebral arteries for the prediction of perinatal outcome and neonatal neurobehavior in late-onset IUGR fetuses with normal UA Doppler.

a) Hypothesis: Late-onset growth restricted fetuses with signs of cerebral hemodynamic redistribution present neurological alterations affecting neonatal neurobehavior as a result of secondary damage due to chronic hypoxia during fetal development.

b) Objective: To assess the neurobehavioral and perinatal outcome of late-onset growth restricted fetuses with signs of intrauterine brain Doppler redistribution defined by the anterior and middle cerebral arteries.

c) Study design: prospective cohort study.

d) Study population: A prospective cohort was created of all suspected SGA fetuses (estimated fetal weight below the 10th centile (1) at a routine third trimester ultrasound) meeting inclusion and exclusion criteria previously defined. Cases were classified according to their brain arteries vasodilation at last examination before delivery into:

Group 1. SGA with MCA vasodilation: defined as SGA fetuses presented MCA PI values below the 5th centile. (87)

Group 2. SGA with ACA vasodilation: defined as SGA fetuses presented ACA PI values below the 5th centile. (66)

Group 3. Adequate for gestational age controls: defined as singleton neonates with birthweight between the 10th and 90th percentile according to local standards. (1) Controls were selected from our general population, with previous adequate ultrasound estimated fetal weigth, individually matched with cases for gestational age at inclusion (± 1 week), corrected by first trimester ultrasound.

e) Statistical analysis: Student's t-test and Pearson Chi-squared test or exact Fisher test were used to compare quantitative and qualitative data, respectively. Receiver operating characteristic curves evaluated diagnostic performance for

adverse perinatal outcome of both arteries. Following standard methodology, neurobehavioral outcome was adjusted for smoking during pregnancy (no smoking; 1-9 cigarettes/day; 10+ cigarettes/day), labor induction, mode of delivery (cesarean section vs. vaginal delivery), gestational age at birth, gender and postnatal days at evaluation by multiple linear or logistic regression. Statistical analysis was performed using the SPSS 15.0 (Chicago, IL, USA) and MedCalc 8.0 (Broekstraat, Belgium) statistical software.

5) RESULTS

5.1. Project 1: Longitudinal evaluation of fetal hemodynamic status in late-onset IUGR fetuses.

The results of this project have been submitted for publication in an international Journal:

Daniel Oros, Francesc Figueras, Rogelio Cruz-Martinez, Eva Meler, Meritxell Munmany, Eduard Gratacos. "Longitudinal changes in uterine, umbilical and cerebral Doppler in late-onset small-for-gestational age fetuses." Ultrasound in Obstetrics & Gynaecoly. (submitted)

These results have also been presented at the 19th World Congress of Ultrasound in Obstetrics and Gynecology (October 2009, Hamburg, Germany):

M. Munmany, F. Figueras, E. Meler, R. Cruz-Martinez, D. Oros, E. Gratacos "Rate of conversion of normal to abnormal umbilical, middle cerebral and uterine arteries in late-onset small-for-gestational-age fetuses". Ultrasound in Obstetrics & Gynecology 2009; 34 (Suppl. 1): 62 – 176.

5.1.1. Study population

During the study period a total of 616 scans were performed on 171 SGA fetuses. The median number of Doppler examinations was 3 (range 2-9). In 124 (62.5%) women more than two examinations were performed.

5.1.2. Clinical characteristics of the population

The mean gestational ages at inclusion and delivery were 34.1 (SD 1.6; range 30.0-35.6) and 38.7 (SD 1.7; range 37-41.6) weeks, respectively. The median interval between the last examination and delivery was 3 (range 0-6) days. Table 2 shows the maternal and neonatal clinical characteristics of the population.

Table 2. Clinical characteristics of the population.

	SGA, n = 171
GA at inclusion (weeks); mean (SD); rank	34.1 (1.6); 30.0-35.6
GA at last scan (weeks); mean (SD)	37.7 (1.6)
Maternal age (years); mean (SD)	31 (5.2)
Low socio-economic class*;n (%)	40 (23.4)
Primiparity; n (%)	118 (69)
Non-Caucasian ethnicity; n (%)	38 (22.2)
Smoking; n (%)	33 (19.3)
1-10 cigarettes/day; n (%)	18 (10.5)
10-19 cigarettes/day; n (%)	11 (6.4)
≥20 cigarettes/day; n (%)	4 (2.3)
Labor induction; n (%)	114 (66.7)
Cesarean delivery; n (%)	60 (35.1)
Cesarean delivery for fetal distress; n (%)	32 (18.7)
GA at delivery (weeks); mean (SD); rank	38.7 (1.7);37.0-41.6
Birth weight (g) ; mean (SD)	2433 (268)
Birth weight percentile; mean (SD)	4 (3.5)
Umbilical artery pH<7.15 at delivery; n (%)	12 (7%)
5-minute Apgar score<7; n (%)	0
Admission in the Neonatal Unit; n (%)	8 (4.7)

5.1.3. Outcomes

5.1.3.a) Proportion of abnormal Doppler at 37 weeks and at the last examination before delivery.

The proportions of abnormal uterine artery pulsatility index and abnormal umbilical artery pulsatility index were not significantly different between 37 weeks and before delivery; (2.3% vs. 4.1%; p=0.36) and (2.3% vs. 2.9%; p=0.65) respectively. On the contrary, the proportions of abnormal middle cerebral artery pulsatility index (4.1% vs. 13.5%; p=0.02) and abnormal cerebroplacental ratio (7% vs. 22.8%; p=0.01) were significantly different between these two examinations.

Before delivery, the proportion of abnormal umbilical artery index was significantly lower than the proportion of abnormal middle cerebral artery index (2.9% vs. 13.5%; p<0.01) and abnormal cerebroplacental ratio (2.9% vs. 22.8%; p<0.001). Also, the proportion of abnormal middle cerebral artery index and cerebroplacental ratio significantly differed (13.5% vs. 22.8%; p=0.002). No cases of absent or reversed end-diastolic umbilical artery Doppler occurred (Figure 7)

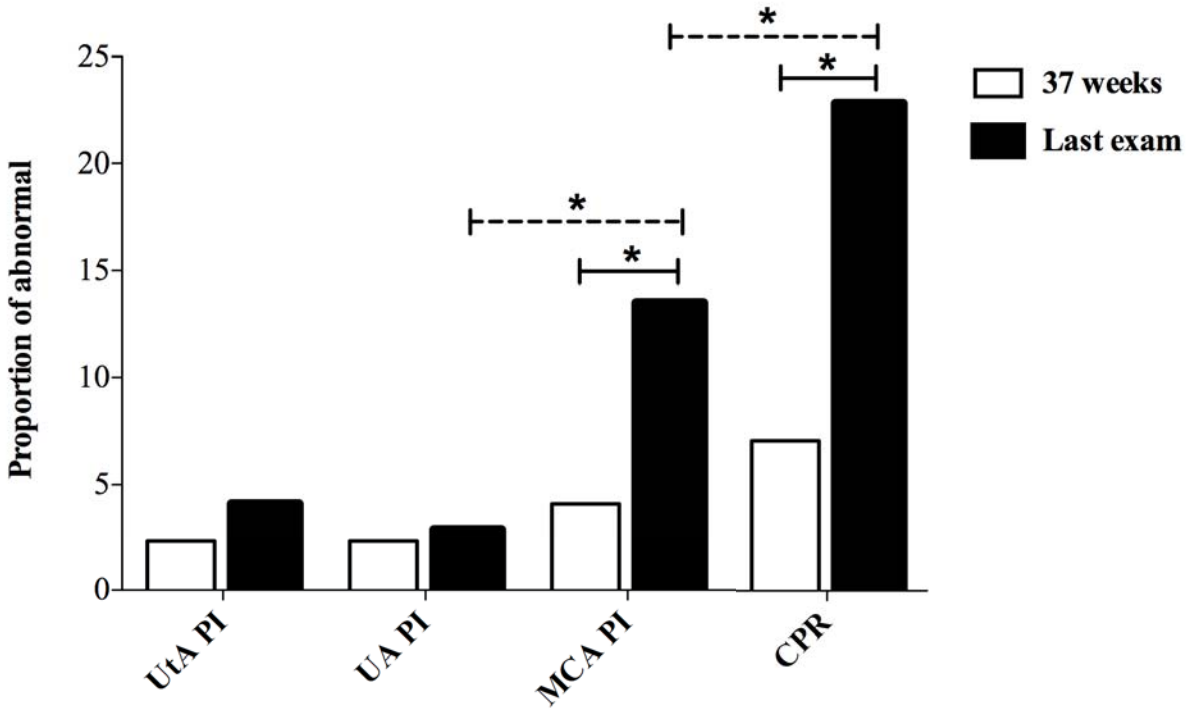

Figure 7. Abnormal Doppler at 37 weeks and at the last examination before delivery.

(* McNemar p<0.001). PI: Pulsatility Index; UtA: Uterine Artery; UA: Umbilical Artery; MCA: Middle Cerebral Artery; CPR: Cerebroplacental Ratio.

5.1.3.b) Description of abnormal Doppler parameters throughout the study period.

The remaining proportions (95% CI) of cases with normal Doppler at 40 weeks were:

- uterine artery: 98.6% (96-100)
- umbilical artery: 94.5% (85.3-100)

- middle cerebral artery: 85% (76.2-93.8)
- cerebroplacental ratio: 9.6% (35.1-64.1)

Figure 8 shows the survival graph of the Doppler parameters throughout the study period, plotted against gestational age, which could be interpreted as the remaining proportion of normal Doppler at each week of gestational age for each of the parameters.

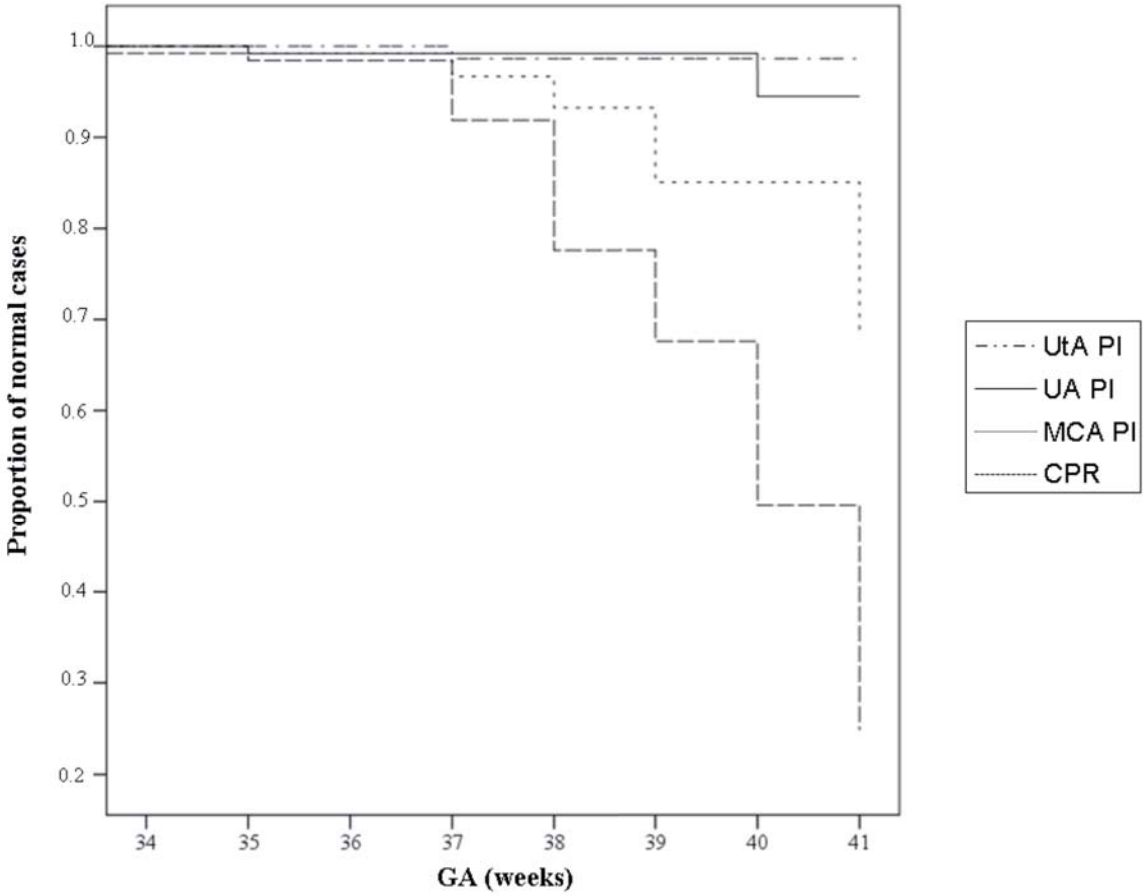

Figure 8. Survival graph of the Doppler parameters throughout the study period PI: Pulsatility Index; UtA: Uterine Artery; UA: Umbilical Artery; MCA: Middle Cerebral Artery; CPR: Cerebroplacental Ratio

5.1.3.c) Doppler longitudinal trends

Figure 9 shows the longitudinal trends of uterine, umbilical, middle cerebral artery, and CPR from enrolment to delivery. Umbilical artery pulsatility index (β=0.01; 95%CI 0.005-0.014) and uterine artery pulsatility index (β=0.002; 95%CI 0.0009-0.032) showed a slight, but almost negligible trend of increase, while middle cerebral artery pulsatility index (β=0.044, 95%CI 0.029-0.6) and

cerebroplacental ratio (β=0.124; 95% CI 0.099-0.0225) experimented a clear and progressive decrease in values from inclusion to delivery.

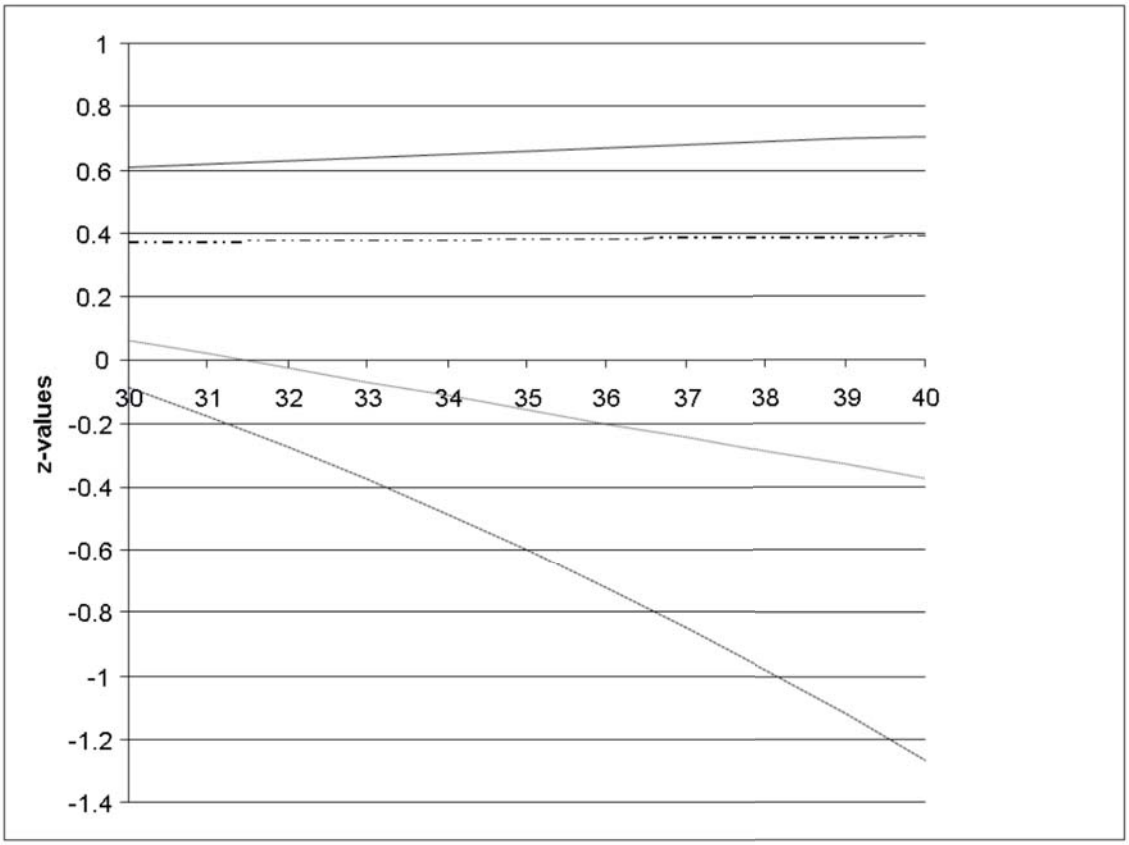

Figure 9. Longitudinal trends of Doppler Pulsatility Index

PI: Pulsatility Index; UtA: Uterine Artery; UA: Umbilical Artery; MCA: Middle Cerebral Artery; CPR: Cerebroplacental Ratio.

5.2. Project 2: Evaluation of perinatal outcome and neonatal neurobehaviour of late-onset IUGR fetuses with normal UA Doppler versus controls.

The results of this project have been published in an international Journal:

Francesc Figueras, Daniel Oros, Rogelio Cruz-Martinez, Nelly Padilla, Edgar Hernandez-Andrade, Francesc Botet, Carmen Costas, Eduard Gratacos. "Neurobehavioral performance of full-term small-for-gestational age infants with normal placental function". Pediatrics. 2009 Nov;124(5):e934-41. Epub 2009 Oct 26)

These results also have been presented at the 19th World Congress on Ultrasound on Obstetrics and Gynecology (October 2009, Hamburg, Germany)

F. Figueras, R. Martinez-Cruz, D. Oros, N. Padilla, F. Botet, E. Gratacos. "Neurobehavioral performance of term small-for-gestational age newborns with normal umbilical artery Doppler". Ultrasound in Obstetrics & Gynecology 2009; 34 (Suppl. 1): 1 – 61.).

This communication was selected as a top-10 for oral presentation in plenary session.

5.2.1 Study population

A final population of 202 infants (102 SGA and 100 AGA) was studied. A total of were of 216 infants were initialy included. Neurobehavioral assessment visit was scheduled at corrected age of 40 ±1 weeks. Parents of 6 case subjects and 8 control subjects later declined to participate. The habituation area could not be assessed for 50 newborns (21 SGA and 29 AGA) because of the absence of a sleeping period during the evaluation.

5.2.2. Clinical characteristics of the population

Mothers in the SGA group had a lower body weight and more frequently were from a low socioeconomic level. There were no cases of drug consumption other than tobacco or alcohol. Table 3 depicts the clinical characteristics of the

population.

Table 3. Clinical characteristics of the population

	AGA (n=100)	SGA (n=102)	p+
Primiparity (%)	61	67.6	0.32
Non-caucasian ethnicity (%)	21.2	16	0.34
Maternal age (years)	31.8(4.9)	33.6(4.9)	0.79
Body Mass Index (kg/m2) at booking	23.4	21.9	0.003
Low socioeconomic level* (%)	31	52	0.003
Smoking (%)			
Non-Smoking	85	83.3	0.75
1-10 cigarettes/day	12	5.9	
11-20 cigarettes/day	2	7.8	
> 20 cigarettes/day	1	2.9	

SD: Standard deviation; + Student's t-test for independent samples or Pearson-$\chi 2$ test; Routine occupations, long-term unemployment or never worked (UK National Statistics Socio-Economic Classification

5.2.3. Outcomes

5.2.3.a) Perinatal outcome

SGA newborns had a lower birth weight and a smaller head circumference. Delivery in the SGA group was at an earlier gestational age (SGA: 38.5 weeks; AGA: 39.7 weeks). SGA fetuses were more frequently induced. Operative delivery because of fetal distress was twice as frequent in the SGA group. Although no infants in the AGA group were admitted to the neonatal unit 3% in the SGA group were. (Table 4)

5.2.3.b) Neonatal neurobehavior

Neonatal neurobehavior was assessed at 7.8 (±7.2) in the AGA group and at 10.5 (±9.6) days of life in the SGA groups.

All of the neurobehavioral areas studied were poorer in the SGA group, (Figure 10) with univariate significance being achieved for attention, habituation, motor,

social-interactive and regulation of state areas.

Table 4. Perinatal outcome

	AGA (n=100)	SGA (n=102)	p*
GA at delivery (weeks)	39.7(1.1)	38.5(1.1)	<0.001
Neonatal weight (g)	3338(390)	2354(266)	<0.001
Neonatal weight centile	52.2(26.6)	3.3(2.2)	<0.001
Head circumference (mm)	346(12.5)	325(11.4)	<0.001
Boys (%)	50	56.9	0.33
Cesarean section (%)	25	35.5	0.11
Labor induction (%)	18	65.7	<0.001
Operative delivery for fetal distress (%)	8	16.7	0.06
5-min Apgar score <7 (%)	0	0	-
UA pH<7.15 at delivery (%)	3.3	10.2	0.07
Neonatal unit admission (%)	0	3	0.08
Neonatal unit (days)	0(0)	0.7(3)	0.025

UA: Umbilical artery; + Student's t-test for independent samples or Pearson-χ2 test

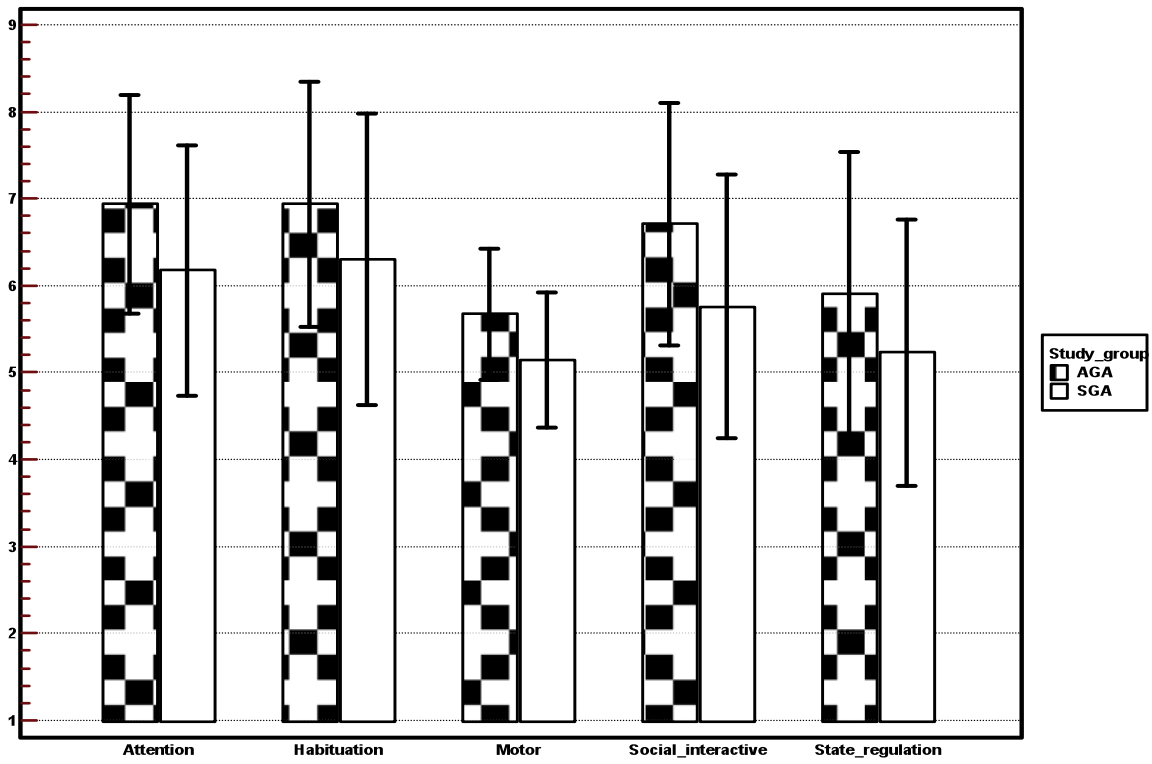

Figure 10. NBAS scores according to study group. (mean/sd)

Differences remained significant after adjustment for potential confounders (smoking during pregnancy, maternal BMI, low socioeconomic level, onset of labor, mode of delivery, use of epidural anesthetic medication, gestational age at delivery, postnatal age at evaluation, and gender) (Table 5).

Table 5. NBAS scores according to study group.

	AGA (n=100)	SGA (n=102)	p*	p**
Attention	6.94(1.3)	6.17(1.4)	<0.0001	<0.0001
Habituation†	6.94(1.4)	6.3(1.7)	0.014	0.016
Motor	5.67(0.8)	5.15(0.8)	<0.0001	<0.0001
Social-interactive	6.71(1.4)	5.76(1.5)	<0.0001	<0.0001
Visual	6.71(1.5)	5.35(1.8)	<0.0001	<0.0001
Auditory	7.03(1.4)	6.15(1.6)	<0.0001	<0.0001
Organization of state	4.09(0.8)	4.02(0.8)	0.48	0.92
Regulation of state	5.91(1.6)	5.23(1.5)	0.003	<0.0001
Autonomic nervous system	7.23(0.9)	7(0.99)	0.08	0.12

†: n=152 (81 SGA and 71 AGA); * Student´s t-test; ** Adjusted for maternal smoking, mode of delivery, onset of labor, gestational age at delivery, gender and postnatal days at test performance, by multiple linear regression.

Multivariate analysis results (MANCOVA), where the adjusted^{+} effect of the study group (SGA vs. AGA) on each NBAS area is shown. In addition, covariates which explained more than 3% of the total variance of dependent variables (squared eta) are also displayed. (Table 6)

Of these covariates, induction of labor and cesarean delivery significantly accounted for lower habituation scores; gestational age at delivery for higher habituation scores; low socioeconomic level for lower social-interactive scores; and age at NBAS evaluation for higher habituation and social scores.

Within each study group, no significant correlations were observed between birth weight and any of the neurobehavioral scores, except for organization of state (R=0.24; P= 0.03) in the SGA group.

Table 6. Multivariate analysis of covariance results (MANCOVA)

Attention	F	Wilk´s lambda p-value	Squared Eta (%)
SGA	5.96	0.001	9.2
Smoking	0.65	0.59	1.1
BMI	0.35	0.79	0.6
Low socioeconomic level	0.67	0.57	1.1
Induction	2.14	0.1	3.5
Cesarean section	0.24	0.87	0.4
Epidural anesthetic medication	0.46	0.71	0.8
Gestational age at delivery	0.25	0.86	0.4
Days at NBAS evaluation	1.9	0.12	3.2
Male gender	0.04	0.99	0.1
Habituation†			
SGA	4.59	0.002	17
Smoking	1.03	0.39	4.5
BMI	0.73	0.57	3.3
Low socioeconomic level	0.17	0.95	0.8
Induction	2.54	0.046	10.4
Cesarean section	2.44	0.05	10.1
Epidural anesthetic medication	0.32	0.86	1.5
Gestational age at delivery	2.59	0.04	10.6
Days at NBAS evaluation	3.57	0.01	14.1
Male gender	1.26	0.29	5.5
Motor			
SGA	5.18	<0.001	12.8
Smoking	1.16	0.33	3.2
BMI	1.13	0.34	3.1
Low socioeconomic level	0.85	0.52	2.4
Induction	1.1	0.36	3

Cesarean section	0.57	0.72	1.6
Epidural anesthetic medication	0.29	0.92	0.8
Gestational age at delivery	0.44	0.82	1.2
Days at NBAS evaluation	2.19	0.06	5.8
Male gender	0.75	0.58	2.1

Social-interactive

SGA	4.6	<0.001	18
Smoking	1.05	0.4	4.8
BMI	0.77	0.61	3.6
Low socioeconomic level	2.49	0.02	10.6
Induction	0.59	0.76	2.7
Cesarean section	0.44	0.88	2
Epidural anesthetic medication	0.81	0.59	3.7
Gestational age at delivery	0.49	0.84	2.3
Days at NBAS evaluation	2.86	0.008	12
Male gender	0.77	0.61	3.6

Organization of state

SGA	1.57	0.18	3.4
Smoking	0.22	0.93	0.5
BMI	0.27	0.9	0.6
Low socioeconomic level	2.16	0.08	4.6
Induction	0.95	0.44	2.1
Cesarean section	0.1	0.98	0.2
Epidural anesthetic medication	0.78	0.54	1.7
Gestational age at delivery	0.86	0.49	1.9
Days at NBAS evaluation	0.90	0.47	2
Male gender	0.13	0.97	0.3

Regulation of state

SGA	7.85	<0.001	15.8
Smoking	1.09	0.36	2.5

BMI	0.37	0.83	0.9
Low socioeconomic level	0.87	0.79	2
Induction	1.06	0.38	2.5
Cesarean section	0.91	0.46	2.1
Epidural anesthetic medication	0.19	0.94	0.5
Gestational age at delivery	1.73	0.15	4
Days at NBAS evaluation	1.01	0.4	2.4
Male gender	0.25	0.9	0.6

Autonomic nervous system

SGA	2.14	0.1	3.5
Smoking	2.5	0.06	4
BMI	1.14	0.33	1.9
Low socioeconomic level	0.12	0.95	0.2
Induction	0.81	0.49	1.3
Cesarean section	0.04	0.99	0.1
Epidural anesthetic medication	0.55	0.65	0.9
Gestational age at delivery	0.29	0.84	0.5
Days at NBAS evaluation	0.1	0.96	0.2
Male gender	0.15	0.93	0.3

SGA: Small-for-gestational age; + Adjustment performed by smoking during pregnancy, maternal body mass index, socioeconomic level, onset of labor, mode of delivery, use of epidural anesthetic medication, gestational age at delivery, postnatal days at evaluation and gender; * Positive associations; ++ Wilks´ lambda; †: n=152 (81 SGA and 71 AGA)

5.3. Project 3: Evaluation of the anterior and middle cerebral arteries for the prediction of perinatal outcome and neonatal neurobehavior in late-onset IUGR fetuses with normal UA Doppler.

The results of this project have been published in two international journals:

Daniel Oros, Francesc Figueras, Rogelio Cruz-Martinez, Nelly Padilla, Eva Meler, Edgar Hernandez-Andrade, Eduard Gratacos. "Middle versus anterior cerebral artery Doppler for the prediction of perinatal outcome and neonatal neurobehavior in term small-for-gestational-age fetuses with normal umbilical artery Doppler." Ultrasound Obstet Gynecol (in press)

Benavides-Serralde J.A, Hernández-Andrade E, Figueroa-Diesel H, Oros D, Feria L.A, Scheier M, Figueras F, Gratacos E. Reference Values for Doppler Parameters of the Fetal Anterior Cerebral Artery throughout Gestation. Gynecol Obstet Invest 2010;69:33-39.

These results also have been presented at the 17th World Congress on Ultrasound on Obstetrics and Gynecology (October 2009, Florence, Italy):

Oros D, Figueras F, Padilla N, Hernandez-Andrade E, Gratacos E. Anterior cerebral artery improves the prediction of adverse perinatal outcome in small-for-gestational age fetuses with normal umbilical artery. Ultrasound Obstet Gynecol. 2007;30: 456-546.)

These results also have been presented at the 30th Congress of the Spanish Society of Obstetrics and Gynaecology. (Barcelona, June 2009):

Oros D; Figueras F, Cruz-Martínez R, Meler E, Padilla N, Gratacós E. "Utilidad de las arterias cerebral media y anterior para la predicción del resultado perinatal y neurocomportamiento neonatal en fetos pequeños para edad gestacional a término".

5.3.1. Study population

A final population of 199 babies (98 SGA and 101 AGA) was analyzed. Inclusion criteria were fulfilled for 118 SGA fetuses. Seven were excluded because of birthweight above the 10th centile, none of them had adverse perinatal outcome. The remaining 111 cases were matched at delivery with 111 adequate-for-gestational-age (AGA) babies. Of these, the parents of 6 cases and 7 controls later declined to participate in the neurobehavioral evaluation. Finally, in 7 cases and 3 controls the evaluation was not considered satisfactory by the examiner due to the absence of a sleeping state during the test.

5.3.2. Clinical characteristics of the population

Women in the SGA group had a lower body mass index and showed a non-significant trend corresponding to a lower socioeconomic level. (Table 7)

5.3.3. Outcomes

5.3.3.a) Perinatal outcome according to the MCA or ACA redistribution.

A total of 28 (29%) and 17 (17%) SGA fetuses had middle and anterior artery Doppler redistribution, respectively. It is of note that in 14 cases MCA and ACA simultaneously showed redistribution. Whereas ACA-redistributed fetuses had a lower birthweight than non ACA-redistributed, no differences were observed between MCA-redistributed and non ACA-redistributed fetuses. Both MCA and ACA redistribution accounted for significant differences in cesarean section, and only the MCA significantly differentiated cases at-risk for fetal distress. (Table 8)

Table 7 Demographic characteristics by study group.

	Control (n=101)	SGA (n=98)	p*
Maternal age at delivery (years)(mean,SD)	31.8 (4.9)	31.2 (5.1)	0.44
Primipara (%)	61.0	67.3	0.35
Caucasian (%)	78.2	78.6	0.95
Male (%)	49.5	54.1	0.52
Body mass index at booking (kg/m2) (mean,sd)	23.4 (3.7)	21.99 (3.5)	0.01
Heigth (cm) (mean,sd)	162.6 (6.3)	160.5 (7.3)	0.06
Low socioeconomic level** (%)	9.3	18.5	0.06
Smoking (%)	15	19.4	0.42
GA at birth (days) (mean,SD)	279 (7.9)	265 (9.5)	<0.001
Birthweight (gr) (mean,SD)	3339 (390.7)	2382 (263.7)	<0.001
Preeclampsia (%)	0	8.2	0.003
Cesarean Section (%)	25	37.1	0.06
Labor induction (%)	18	71.1	<0.001
Composite adverse outcome***	19.8	34.7	0.02

SD: Standard deviation; * Student's t-test for independent samples, Pearson $\chi 2$ or Fisher exact test, as appropriate. ** Routine occupations, long-term unemployment or never worked (UK National Statistics Socio-Economic Classification). *** Composite adverse outcome: intervention for fetal distress, umbilical artery < 7.10, need for neonatal resuscitation or admission to NICU.

5.3.3.b) Prediction performance of ACA and MCA

Figure 11 shows the ROC curves of MCA and ACA for the prediction of adverse outcome. Although both parameters showed significant areas under the curve (0.71 (CI 0.6-0.81) for the ACA PI and 0.72 (0.61-0.82) for MCA PI), pairwise

comparison of both areas showed no significant differences between the two parameters (p=0.82).

Table 8. Perinatal outcome according to the MCA or ACA redistribution.

	MCA			ACA		
	SGA not redistributed	**SGA redistributed**	**p+**	**SGA not redistributed**	**SGA redistributed**	**p++**
	(n=70)	(n=28)		(n=81)	(n=17)	
GA at birth (days)	266 (9.9)	265 (8.7)	1	266 (9.5)	262 (8.7)	0.1
Birthweight (gr)	2411 (246.6)	2310 (294.4)	0.54	2426 (235.2)	2173 (296.5)	0.01
Birthweight <3rd centile (%)	31 (44.1)	18 (64.3)	0.07	39 (48.1)	10 (58.8)	0.42
Head circumference (cm)	32.7 (1)	32.3 (1)	0.41	32.6 (1)	32.3 (1)	0.99
Preeclampsia (%)	5 (7.1)	3 (10.7)	0.68	5 (6.2)	3 (17.6)	0.14
Labor induction (%)	52 (75.2)	17 (60.5)	0.14	58 (73.4)	10 (58.8)	0.25
Cesarean Section (%)	18 (26.1)	18 (64.3)	0.001	24 (30)	12 (70.6)	0.002
Intervention for Fetal Distress (%)	10 (14.9)	12 (42.9)	0.006	15 (19.5)	7 (41.2)	0.06
5´Apgar <7 (%)	0	0	1	0	0	1
Umbilical artery pH <7.10 (%)	4 (6.1)	2 (8.3)	0.65	4 (5.4)	2 (13.3)	0.26
Neonatal resuscitation (%)	2 (3.7)	4 (14.8)	0.09	3 (4.5)	3 (20)	0.07
NICU admission (%)	1 (1.5)	2 (7.1)	0.2	1 (1.3)	2 (11.8)	0.08
Composite adverse outcome* (%)	14 (20)	13 (48.1)	0.01	20 (25)	8 (50)	0.05

GA: Gestational Age; NICU: Neonatal Intensive Care Unit; * Composite adverse outcome: intervention for fetal distress, umbilical artery < 7.10, need for neonatal resuscitation or admission to NICU. Student's t-test for independent samples, Pearson $\chi 2$ or Fisher exact test, as appropriate: +between the MCA groups; ++between the ACA groups;

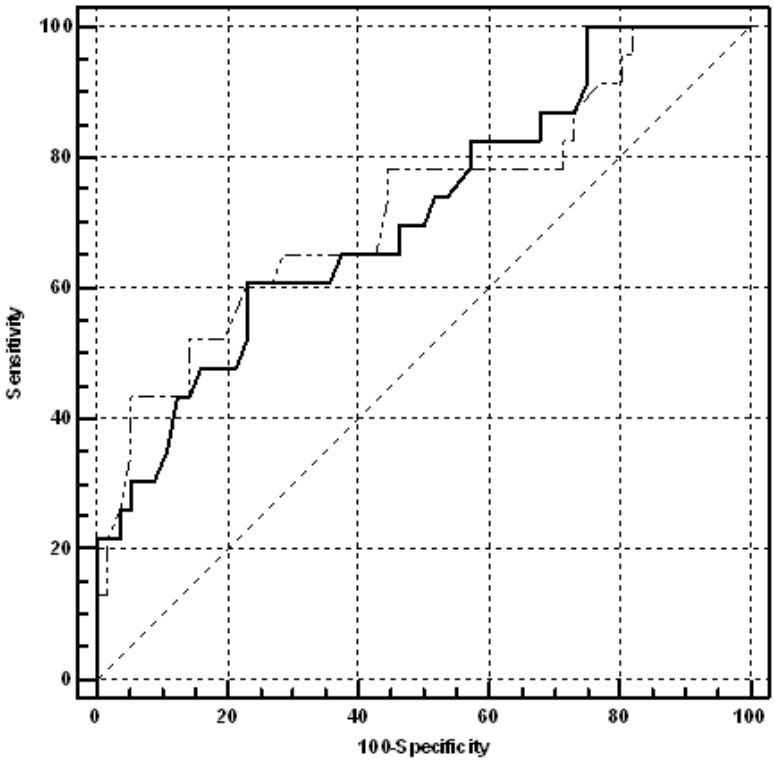

Figure 11. ROC curves of MCA and ACA for the prediction of adverse outcome.

5.3.3.c) Neurobehavioral outcome

Neurobehavior was assessed at 6.2 (SD 4.9) and 14.4 (SD 9.04) days of life in the AGA and SGA groups, respectively. Significant differences were found between AGA and SGA for:

- Motor organization
- Social organization
- State organization

These differences remained significant after adjustment for potential confounders for motor and state organization. (Table 9)

Among SGA fetuses, cases with MCA redistribution showed significantly lower NBAS in motor (adjusted p value 0.03) and state organization areas (adjusted p value 0.025) than the SGA without redistribution. On the other hand, a non-significant trend towards lower scores was observed in ACA-redistributed fetuses only in the state organization area. (Figure 12)

Table 9. Neurobehavioral scores by area and study group (mean and standard deviation)

	Control (n=101)	SGA (n=98)	p*	p**
Habituation	6.85 (1.6)	6.49 (1.67)	0.18	0.93
Social	6.72 (1.4)	5.59 (1.41)	<0.001	0.16
Motor	5.73 (0.75)	5.29 (0.84)	<0.001	0.04
State organization	4.39 (1.08)	3.86 (1.28)	0.002	0.012

SGA: small-for-gestational-age.

** Student´s t-test; ** Adjusted for smoking during pregnancy, mode of delivery, gestational age at birth, gender and postnatal days at evaluation by linear regression.*

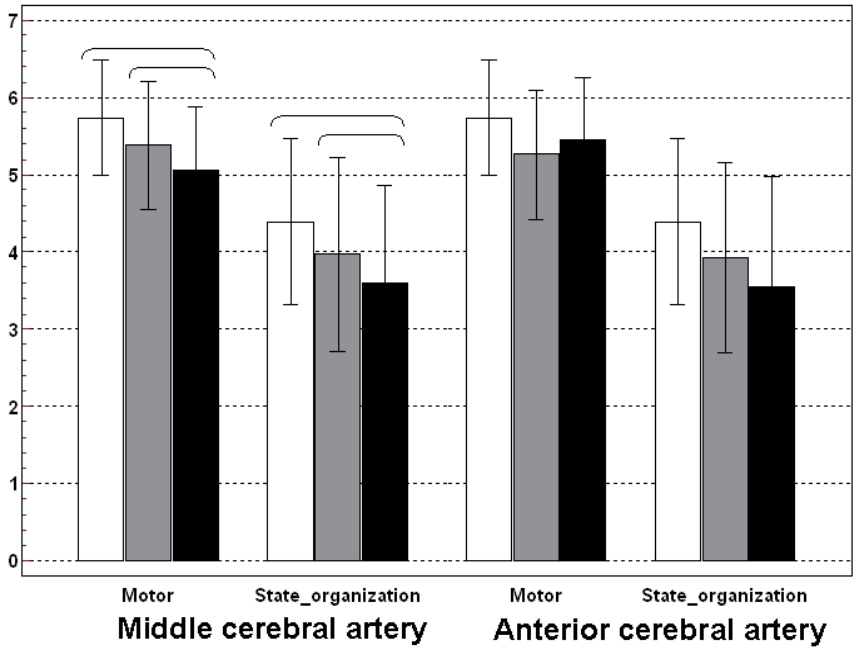

Figure 12. NBAS scores for AGA and SGA fetuses with and without redistribution according to MCA and ACA Doppler.

Among SGA fetuses, cases with MCA redistribution showed an increased risk for abnormal motor (36% vs. 20%; adjusted p value 0.023) (adjusted OR 3.94; 95% CI 1.21-12.8) and state organization (25% vs. 17.5%; adjusted p value

0.025) (adjusted OR 4; 95% CI 1.19-13.3) areas than the SGA without redistribution. (Figure 13)

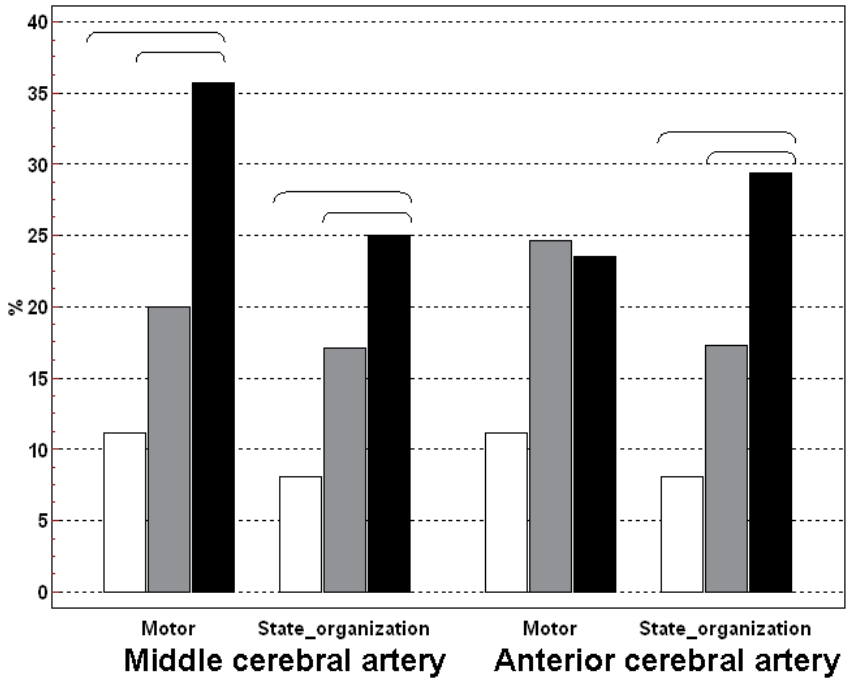

Figure 13. Frequency of abnormal NBAS.

The frequency of abnormal NBAS significantly differed between cases with and without ACA redistribution only for the organization of the state area (30% vs. 17.5%; adjusted p value 0.021) (adjusted OR 5; 95% CI 1.28–20).

6) DISCUSSION

6.1. General overview

This research project provides evidence to the concept that a proportion of late-onset small-for-gestational-age fetuses with no signs of placental insufficency are true forms of growth restriction. This is supported by our findings that brain redistribution occurs in a substantial proportion of late-onset SGA with normal umbilical artery Doppler and by the fact that those fetuses have delayed brain maturation.

Intrauterine growth restriction (IUGR) is predominantly a vascular disorder secondary to placental insufficency. The effects can be documented with Doppler ultrasound examination of a number of vessels: maternal uterine arteries and the fetal umbilical arteries for the placenta; middle cerebral artery (MCA) for preferential brain perfusion. As early-onset IUGR worsens, Doppler abnormalities in these vascular territories also deteriorate, (92) suggesting a sequential pattern of disease progression. This presumed sequence and the anticipation of fetal deterioration form the basis for Doppler surveillance in IUGR. Contrary to early-onset growth restriction, in late-onset cases, which are far more prevalent, most events of adverse outcome attributable to IUGR occur in fetuses with normal umbilical artery. (93) Thus, a substantial proportion of late-onset small-for-gestational age (SGA) fetuses with normal umbilical artery Doppler, considered to represent one end of the normal size spectrum, (30) may have true growth restriction and are at-risk of adverse perinatal outcome. (31,32,37) It seems that early and late-onset growth restricted fetuses followed different pathophysiologycal pathways. (58)

The importance of managing small fetuses with normal umbilical artery Doppler completely differently from true IUGR babies has been stressed. (30) The identification of fetal growth restriction depends on adequate risk assessment and surveillance. Failure to identify small-for-gestational age (SGA) fetuses has been described as a major cause of perinatal morbidity with a 4-fold risk for adverse perinatal outcome. (94) However, recent evidence suggests that umbilical artery Doppler might not accurately identify mild forms of placental insufficiency. (6) A substantial proportion of these so-called “normal” small

fetuses are actually growing under a pathological environment, but the consequences of this on fetal brain maturation had not been investigated before. Our results suggest the existence of subtle degrees of neurological injury from earlier stages of fetal hemodynamic adaptation to hypoxia.

6.2. Project 1: Longitudinal evaluation of fetal hemodynamic status in late-onset IUGR fetuses.

This is the first study describing the longitudinal changes of utero-placental and fetal brain hemodynamics in late-onset SGA fetuses. Our study shows that on average feto-placental Doppler indexes remain virtually unchanged from diagnosis to delivery in these fetuses, and consequently the proportion of cases progressing to abnormal Doppler in the umbilical and uterine arteries is negligible. On the contrary, average middle cerebral artery and, more markedly, cerebroplacental ratio values worsen significantly from 37 weeks until delivery, with 14% and 23% of abnormal values on the last scan before delivery.

The study provides evidence to support the notion that uteroplacental Doppler is of little clinical value in SGA fetuses and that cerebral Doppler may constitute the most sensitive exam to identify cases at high risk of adverse perinatal outcome.

6.2.1. Differencies between early-onset IUGR and late-onset IUGR fetal hemodinamics.

The findings of this study are in line with previous Doppler studies in IUGR and SGA fetuses.

Concerning umbilical artery Doppler, comparison of our findings in SGA fetuses with published longitudinal series on early-onset IUGR demonstrates that both conditions have a different pattern of Doppler deterioration. In early-onset IUGR, abnormal umbilical artery constitutes the mainstay for diagnosis and deteriorates progressively, followed by the development of brain sparing. (60) (75) On the contrary, as illustrated in this study, in most SGA fetuses the umbilical atery remains normal despite the development of brain sparing in up to 20% of cases. In a recent study on late-onset IUGR (beyond 30 weeks) with abnormal umbilical artery, Turan et al (75) reported that fetal rapid hemodynamic deterioration as observed in early-onset forms was very unlikely, and the median interval between admission and the appearance of any other Doppler abnormality was 33 days. The findings of Turan et al. together with

those reported in this study support that as gestational age at onset increases, abnormalities in the umbilical artery Doppler in small fetuses are more unlikely.

Regarding uterine artery Doppler, we and others (95-97) have demonstrated that progression of uterine Doppler during the second trimester correlates with perinatal outcome, but similarly to umbilical artery Doppler, this parameter remained almost constant throughout the third trimester in SGA fetuses.

Finally, concerning cerebral Doppler measurements our findings are in line with previous studies in early-onset IUGR reporting that cerebroplacental ratio becomes abnormal earlier than the middle cerebral artery, (60,75) and with animal models demonstrating that cerebroplacental ratio turns out to be the hemodynamic parameter that reflects most closely PO_2 changes. (71)

6.2.2. Clinical impact

Our study has several clinical implications.

First, since uterine and umbilical Doppler does not deteriorate from diagnosis to delivery, little benefit can be expected by serial monitoring. McCowan et al (98) demonstrated that fortnightly fetal surveillance by umbilical artery Doppler compared to twice-weekly surveillance resulted in fewer inductions without any impact on the perinatal outcome. They concluded that more studies are needed to determine the safety of less frequent surveillance of SGA with normal umbilical artery. Our findings suggest that in this population, isolated serial umbilical or uterine artery Doppler would be unnecessary. On the other hand, restricting the evaluation of near-term SGA to the umbilical artery Doppler could be potentially harmful since a normal result could give false reassurance of fetal wellbeing.

Secondly, cerebral Doppler seems to constitute the most sensitive parameter to detect late-onset SGA fetuses at risk for adverse perinatal outcome. Hershkovitz et al (58) first provided evidence that near-term SGA with isolated middle cerebral artery vasodilatation are at-risk of adverse outcome. Others have confirmed their findings. (32) In addition, cerebral Doppler studies seem to detect the group of SGA fetuses with the highest risk of suboptimal

neurodevelopmental outcome in these fetuses. (33) Notwithstanding its better predictiveness as compared with umbilical artery Doppler, the middle cerebral artery still fails to detect a substantial number of fetuses with abnormal neonatal outcome. (59) As supported by an increasing number of studies, cerebroplacental ratio is an earlier and more sensitive predictor for adverse outcome than either the middle cerebral artery or umbilical artery alone, both in severe (70-74) as in mild forms of IUGR. (75) The findings of this study are in line with these publications and further support the use of cerebroplacental ratio as a primary Doppler monitoring parameter in late-onset SGA.

6.2.3. Study limitations

This study has some limitations. Since abnormal umbilical artery Doppler was an indication for delivery for ethical reasons, the temporal relationship between the parameters studied may be biased. However, this occurred in a small proportion of cases and, therefore, the effect of this bias should be negligible. Any clinical study on the surveillance of fetal growth needs to be interpreted with caution because it can never be entirely blinded and the clinical management is influenced by the antenatal findings. However, clinicians attending the deliveries were unaware of the middle cerebral artery, uterine and cerebroplacental ratio Doppler status.

6.2.4. Conclusion

In conclusion, late-onset SGA with normal Doppler upon diagnosis show no changes in umbilical and uterine artery Doppler. In contrast, a progression from 37 weeks onward with worsening cerebroplacental ratio in up to 24 % and of middle cerebral artery vasodilation in 14% of cases is observed. These findings discourage the use of umbilical artery as a single parameter for surveillance in late-onset SGA and support the use of the cerebroplacental ratio as the most sensitive Doppler parameter for the clinical monitoring of these fetuses.

6.3. Project 2: Evaluation of perinatal outcome and neonatal neurobehaviour of late-onset IUGR fetuses with normal UA Doppler versus controls.

It is accepted that SGA with abnormal umbilical artery Doppler, as a surrogate sign of placental insufficiency, are at high risk of neurobehavioral and neurocognitive outcome. (87,99,100) Our study extends the consequences of being SGA on neurobehavior to full-term neonates with no signs of placental insufficiency, a subgroup of neonates traditionally considered to be one end of the size spectrum of normal babies. Contrary to the prevailing opinion, our findings challenge the concept that umbilical artery Doppler is a reliable tool to prenatally identify those constitutional term SGA babies at low risk. Subtle degrees of neurological injury seem to have already occurred before umbilical Doppler waveform becomes abnormal. In fact, animal (101) and mathematical (102) experimental models of placental vessel obliteration have suggested that umbilical artery Doppler become abnormal only in advanced stages of placental dysfunction.

6.3.1. Late-onset growth restricted fetuses neurobehavior

We found a poorer neonatal response to both visual and auditory stimuli in SGA babies. Long-term follow-up studies of SGA babies have found impaired visual-spatial stimuli processing. (80) Chronic fetal growth restriction animal models have found optic nerve reduced myelogenesis (103) along with reduced synaptogenesis in the visual cortex. (39) These factors could restrict the integration of cerebral cortex inputs. Additionally, mistimed cell migration could also have an effect on synaptic plasticity with functional and behavioral consequences. (40) Visual-spatial abilities also play a role in the neonatal capacity to select environmental stimuli to process and act on, which provide important scaffolding on which attention skills are constructed through early childhood. (104) Indeed, fetal growth restriction is also associated with the development of attention deficit symptoms in childhood and early adolescence.

(37) Our finding of lower attention capacities in SGA babies is in line with this hypothesis. Two previous studies (11,12) have found impaired attention skills among preterm SGA babies. Structural correlations have been made between attention scores and cortical grey matter (11) and hippocampal (12) volumes.

Whether NBAS predicts attention later in childhood is uncertain. However, some studies have found that infant attention is predictive of intelligence in childhood and adolescence. (105-106) Another study of temperament in early infancy (5-6 months) reported a significant prediction of attention deficits among 8-year-old children. (83) There is a substantial overlap between childhood attention disorders and the diagnosis of motor impairment, with poor movement abilities. (107) Our finding of lower motor performance among SGA babies is consistent with this association. In a series of 40 SGA preterm neonates Feldman et al (34) studied the correlation of neurobehavioral and cognitive function with the Bayley Scale of Infant Development. They found that neonatal motor maturity correlated with the Psychomotor Development Outcome at 24-months. Another interesting finding of our study is the lower level of self-regulation among SGA babies, as reflected by the regulation of state NBAS cluster. In a series of 38 full-term healthy babies, Lundqvist (108) reported that levels of self-regulation were also correlated with the infants' levels of cognitive development (personal-social development, speech development and eye and hand coordination, subvariables in Griffiths' Mental Development Test) and with sleeping disorders at 2 years of age.

6.3.2. Clinical implications

One strength of our study is that only included full-term SGA fetuses without signs of placental dysfunction, as are low-risk SGA defined in everyday clinical practice. No previous studies aimed to analyze the effect of growth restriction on neurobehavior have reported which proportion of cases had abnormal Doppler. This is a potential source of misinterpretation of the results, since an unknown part of the babies could have been exposed to a very prolonged period of intrauterine hypoxia. Another strong point of our study is that

confounding was accounted by adjustment by potential factors independently associated with SGA and the neonatal neurobehavioral performance.

In low-risk preterm infants, it has been reported that individualized developmental interventions prevent short-term neurobehavioral dysfunction. (83) It is unknown whether interventions could also be effective in term infants and whether they would influence long-term outcome. Nevertheless, identifying the at-risk infants is essential to understand the association between fetal wellbeing and later neurodevelopmental problems and lays the basis for possible preventive interventions.

6.3.3. Study limitations

This study also has some limitations. Although NBAS is a gold standard to evaluate the neonate's capacity to respond to the environment, reflecting brain maturation, it only assesses neurobehavioral and not cognitive function. However, several studies have demonstrated the correlation between neonatal neurobehavioral performance and later neurocognitive development. (11,12,34,108,109) It could also be argued that the study is prone to an expectation bias, since the examiners could have been influenced by baby size when assessing the neurobehavioral performance. However, within each study group, no significant correlations were found between birthweight and the NBAS scores, except for organization of state in the SGA group. This makes it unlikely that this potential bias could explain the differences observed between SGA and AGA babies in the other areas.

6.3.4. Conclusion

In summary, we found in a well-defined cohort of term SGA neonates with no signs of placental insufficiency poorer neurobehavioral performance, suggesting delayed neurological maturation. Whether earlier markers of placental insufficiency or hypoxia could predict this outcome requires further investigation.

6.4. Project 3: Evaluation of the anterior and middle cerebral arteries for the prediction of perinatal outcome and neonatal neurobehavior in late-onset IUGR fetuses with normal UA Doppler.

This is the first study to explore the ability of the ACA to predict perinatal and neurobehavioural outcome in term SGA fetuses. Contrary to the hypothesis of the study, we found no differences between MCA and ACA redistribution in terms of association with perinatal outcome or neonatal neurobehavioral performance. Therefore, MCA seems to remain unchallenged as a primary clinical tool to evaluate term SGA fetuses without signs of placental insufficiency.

6.4.1. Role of anterior cerebral artery Doppler for the prediction of neurological damage as a consecuence of intrauterine growth restriction

In preterm IUGR fetuses we have previously demonstrated that ACA vasodilation takes place earlier than MCA vasodilation. (62) Dubiel et al. reported similar results in pregnancies with pregnancy-induced hypertension. (67) These findings are not in line with the results of the present study. However, different population characteristics are likely to explain this inconsistency. The above studies included a substantial proportion of early onset growth restricted fetuses with abnormal umbilical artery Doppler. Since gestational age was considerably different we cannot exclude differences in the temporal patterns in the adaptation to chronic hypoxia due to maturity changes in brain hemodynamic regulation. Indeed, Dubiel et al (67) reported a better correlation of ACA PI with adverse perinatal outcome only for cases before 32 weeks' gestation. For those delivering beyond that gestational age MCA and ACA PI showed a similar association with perinatal outcome. Also in line with this reasoning, other studies (110) have found no differences between ACA and MCA in response to acute hypoxia in term SGA fetuses.

As a secondary explanation, the considerable degree of systemic fetal hemodynamic adaptation to hypoxia in early onset IUGR, which is not present

in term SGA, could also have influences in brain hemodynamics that we cannot interpret.

The findings of this study may appear inconsistent with evidences pointing to a higher vulnerability of frontal areas in fetuses with IUGR. (9,11,66,80,111) However, the evaluated arteries provide blood supply to ill-defined anatomical areas with a marked component of vascular shunting. Tissue perfusion depends on local arteriolar phenomena and therefore the data cannot be used to infer which territories are specifically involved by vascular changes. Using the fractional moving blood volume estimate to assess tissue perfusion we have previously suggested that increased perfusion in the frontal lobe seems to be the earliest response to hypoxia, rather than changes in basal ganglia perfusion. (68) It is unknown whether evaluation of tissue perfusion can detect subtle differences in blood perfusion between cerebral areas in this population of late-onset SGA. Studies evaluating the potential role of direct measurements of perfusion towards different brain regions in this population are under way.

6.4.2. Clinical implications

This study confirms previous reports suggesting that late-onset SGA is associated with an increased risk of abnormal neonatal neurobehavior (112-113) and neurodevelopment in childhood. (33,108,114) Furthermore, we have previously described that in late-onset SGA fetuses, MCA redistribution differentiates those at risk of long-term suboptimal neurodevelopment. (33) In a recent study, Roza et al. (114) showed that fetal ACA redistribution could be superior to MCA in the prediction of neurobehavioral problems in childhood. In the present study MCA and ACA Doppler were similarly associated with poorer neonatal neurobehavior. These findings are difficult to compare with those of Roza et al for two reasons. Our results refer exclusively to neonatal neurobehavior and therefore they do not exclude that long term assessment could demonstrate a higher sensitivity of ACA to predict poor neurodevelopment. A second important difference is that the study of Roza et al. (114) was based on a large cohort, which included all pregnancies, and not

only SGA fetuses. Therefore, the value of ACA to predict long-term outcome in small fetuses remains to be evaluated.

6.4.3 Study limitations

This study has some limitations. First, since no Doppler was performed in the group of AGA babies, we cannot rule out that some fetuses in this group might have shown brain redistribution. However, this potential bias would be conservative, attenuating the differences between AGA and SGA fetuses. Secondly, although NBAS is a gold standard to evaluate the neonate's capacity to respond to the environment, reflecting brain maturation, it only assesses neurobehavioral and not cognitive function. However, several studies have demonstrated the correlation between neonatal neurobehavioral performance and later neurocognitive development. (11,12,34,108,109) We admit that socioeconomic status may have confounded the association between SGA and abnormal neurobehaviour. However, although socioeconomic status is a major determinant of postnatal neurobehavior during childhood, its influence in the neonatal period is still minimal if smoking is accounted for in our population. Thirdly, any clinical study on the surveillance of fetal growth needs to be interpreted with caution because it can never be entirely blinded and the clinical management is influenced by the antenatal findings. This work-up bias could account for differences between AGA and SGA. However, the clinicians attending the deliveries were unaware of the MCA or ACA Doppler status. Thus this potential bias could not explain differences between the two groups of SGA fetuses. Besides, the neurobehavioral assessment could also have been biased by the examiners´ knowledge of perinatal factors. However, since these examiners were also blinded to the antenatal Doppler and due to the fact that birthweight was similar between the two groups, this expectation bias is unlikely to explain differences between Doppler groups.

6.4.4. Conclusion

In conclusion, the findings of this study do not support the inclusion of ACA Doppler investigation in the management of term SGA fetuses.

7) CONCLUSIONS

1. Umbilical artery Doppler failed as a single parameter for surveillance in late-onset growth restricted fetuses.

2. Term late-onset growth restricted fetuses neonates with no signs of placental insufficiency present poorer neurobehavioral performance, suggesting delayed neurological maturation.

3. Term late-onset growth restricted fetuses neonates with no signs of placental insufficiency present poorer perinatal outcome.

4. Cerebroplacental ratio is the most sensitive Doppler parameter for the clinical monitoring of late-onset growth restricted fetuses

5. The inclusion of ACA Doppler investigation in the management of term late-onset growth restricted fetuses does not improve neither perinatal nor neurobehavioral outcomes.

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10) ANNEXES

ANNEX 1. ETHIC COMMITTEE APPROVAL

CLÍNIC
Corporació Sanitària

CLÍNIC
BARCELONA
Hospital Universitari

Dña. Begoña Gómez Pérez , del Servicio de Farmacia del Hospital Clínic de Barcelona y Secretaria del Comité Ético de Investigación Clínica (CEIC)

CERTIFICA:

Que el Comité Ético de Investigación Clínica, según consta en el acta de la reunión celebrada en el día de hoy, ha analizado el proyecto de investigación titulado:

Clinical use of assessing regional brain circulation perfusion to identify long term neurodevelopment anomalies in small for gestational age fetuses (SGA).

cuyo investigador principal es el Dr. **Gratacós, Eduard**
del Servicio de ***Ginecología y Obstetricia***
entendiendo que dicho estudio se ajusta a las normas éticas esenciales y criterios deontológicos que rigen en este Centro, y, por tanto, ha decidido su aprobación.

Lo que firmo en Barcelona, a 12/06/2008

CLÍNIC
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Registro: 2008 / 4422

HOSPITAL CLÍNIC I PROVINCIAL DE BARCELONA
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ANNEX 2. INFORM CONSENT- PATIENT INFORMATION

CLÍNIC Corporació Sanitària

HOJA DE CONSENTIMIENTO INFORMADO

CLÍNIC BARCELONA Hospital Universitari

EVALUACION FETAL DE LA CIRCULACION CEREBRAL Y FUNCION CARDIACA Y SU ASOCIACION CON EVOLUCION NEUROLÓGICA POSNATAL

Como usted sabe, en la mayoría de hospitales y en particular en el Hospital Clínic, además del trabajo asistencial se lleva a cabo investigación biomédica. Esto requiere, entre otras cosas, recoger datos de pacientes para analizarlos y obtener conclusiones que puedan ser útiles para futuros pacientes. Le invitamos a participar en un estudio cuyo objetivo es estudiar diversas complicaciones de la gestación, como la restricción del crecimiento fetal en diferentes momentos del embarazo y la hipertensión o preeclampsia.

En la actualidad se acepta que la mayoría de estas complicaciones se deben a alteraciones en el funcionamiento de la placenta. Por el momento no existe un tratamiento eficaz para estas patologías. El manejo de estas gestaciones se basa en un control prenatal estricto y en la finalización del embarazo una vez asumida una madurez fetal aceptable y siempre antes de que se produzcan complicaciones fetales. Dentro del control fetal prenatal es especialmente importante el estudio de la circulación cerebral y del funcionamiento del corazón, ya que son los órganos que permiten la adaptación fetal a estas patologías y que advierten de la posible aparición de complicaciones futuras. Actualmente estamos intentando evaluar diferentes técnicas para estudiar el funcionamiento de estos órganos fetales y para mejorar el manejo del embarazo e incluso desarrollar posibles tratamientos futuros.

De acuerdo con las normas bioéticas y la legislación vigente, necesitamos su autorización para utilizar la información clínica (datos, imágenes, otros) y el material biológico que haya sobrado de las pruebas que formando parte del proceso asistencial normal, le hayamos hecho o le hagamos a partir de ahora. En ningún caso se le harán pruebas de carácter experimental.

¿En qué consiste el estudio? Se realizará un estudio ecográfico completo del bebé, que incluirá el flujo sanguíneo en el cerebro y el funcionamiento del corazón. El estudio se realiza utilizando un aparato de ecografía convencional, es totalmente inofensivo para el bebé y suele durar alrededor de 20-30 minutos de tiempo (dependiendo de la posición del bebé y de los movimientos que realice). En función de cómo se encuentre el bebé realizaremos un seguimiento ecográfico, que puede ser semanal o en algunos casos incluso diario. También le propondremos obtener muestras de sangre, orina y/o líquido amniótico, intentando aprovechar analíticas y/o exploraciones (amniocentesis) que usted se realice de forma programada durante el embarazo. En el momento del parto y de manera opcional se recogerán muestras de sangre del cordón umbilical (no directamente del bebé) y muestras de placenta. Inicialmente todas estas muestras se congelarán y serán estudiadas en un futuro, siempre en relación a la patología que usted ha presentado durante la gestación. Existen datos preliminares sobre la utilidad que podrían tener diferentes sustancias a la hora de identificar a aquellos fetos con mayor riesgo de presentar de problemas neurológicos tras el nacimiento.

Para conocer como acaba su embarazo, revisaremos su historia clínica y/o nos pondremos en contacto con usted telefónicamente. A su vez, nos interesa conocer como es el desarrollo neurológico de su bebé, para lo que le realizaremos una detallada evaluación en momentos claves del crecimiento (al nacimiento y durante el primer y segundo año de vida). Para ello utilizaremos diversas pruebas o tests de neuropsicología y técnicas de imagen cerebral como la ecografía o la resonancia magnética.

¿Cuales son los beneficios de participar en este estudio? Si identificamos una alteración de crecimiento del bebé y/o la aparición de hipertensión inducida por la gestación le realizaremos un mayor número de ecografías para estudiar el funcionamiento de la placenta, el crecimiento del bebé y la circulación en sus diferentes órganos. Esto será beneficioso para usted ya que podremos mejorar el control de su embarazo. Por otro lado, su bebé podrá optar a un seguimiento tras el nacimiento para identificar muy precozmente posibles alteraciones en su crecimiento y desarrollo e iniciar posibles tratamientos. Además, creemos que este estudio puede ayudarnos a comprender mejor porque se producen estas complicaciones para en un futuro ofrecer un mejor control del embarazo y posibles tratamientos.

¿Existe algún riesgo por participar en este estudio? No existen desventajas ni riesgos añadidos para usted ni para su bebé. Tanto la ecografía como la resonancia magnética son técnicas totalmente inofensivas para el bebé y no

implican ningún riesgo para la salud del niño. Intentaremos aprovechar analíticas o pruebas ya programadas para guardar muestras de suero, orina y/o o líquido amniótico. En cualquier momento puede usted cambiar de opinión y salir del estudio.

¿Qué ocurre si se niega a participar en el estudio? Nada. El seguimiento de su embarazo se realizará igualmente.

Le agradecemos su colaboración y estamos a su disposición para contestar cualquier pregunta que quiera realizar.

Sus datos serán utilizados siempre de forma anónima y absolutamente confidencial, de forma que únicamente miembros autorizados dispondrán de acceso a la información obtenida (Ley de protección de datos). Usted dispondrá en todo momento de toda la información obtenida y ésta se limitará a la mencionada en este texto. Por otro lado, ha de saber que cualquier estudio de investigación que se realiza en el Hospital Clínic dispone de la aprobación del Comité Etico de Investigación Clínica del hospital.

Yo, _____

He leído la hoja de información que se me ha entregado. He podido hacer preguntas sobre los posibles beneficios e inconvenientes de participar en el estudio, y he recibido suficiente información sobre el mismo.

He hablado con: _____
_____ comprendo que mi participación es voluntaria y que puedo retirarme del estudio: En cualquier momento, sin tener que dar explicaciones, y sin que ello repercuta en mis cuidados médicos.

Fecha: _____/_____/_____

Firma: _____Paciente

Firma: _____Médico

2

ANNEX 3. DATA FORM

CLÍNIC BARCELONA Hospital Universitari

PLACENTAL MEDIATED DISEASE: CLINICAL RECORD FORM

CLÍNIC Corporació Sanitària

IDENTIFICATION	MATERNAL DATA
Name & Surname:	**Age** (y) |__|__| **Smoking** (# cig./day) |__|__| (0 for non-smoking)
Hospital's ID |__|__|__|__|__|__|__|__|__|__|	**Height** (cm) |__|__||__| **Pregestational Weight** (kg) |__|__|__|
Date of birth (dd/mm/yy) |__|__| |__|__| |__|__|	**TPAL** : |__|__|__|__|
Phone numbers |__|__|__|__|__|__|__|__|__|__| |__|__|__|__|__|__|__|__|__|	**Ethnicity**. |__| 1.Caucasian 2.Black 3.Latin-American 4.Asian 5.Others
	Maternal History (0-No, 1-Yes):
	|__| Chronic hypertension |__| Previous PE / IUGR
	|__| Diabetes mellitus |__| Coagulation disorder
	|__| Autoimmune disease |__| Others
Address _____	Specify _____
_____	
Newborn´s name:	

CURRENT PREGNANCY	
LMP (dd/mm/yy) |__|__| |__|__| |__|__| (US corrected)	**Diagnosis (0-No, 1-Yes)**: GA ONSET |__|__| . |__| |__| IUGR-I |__| IUGR-II |__| IUGR-III |__| IUGR-IV |__| IUGR-V
Pregnancy:|__| 1.Singleton 2.Dichorionic twins	|__| PE – MILD |__| PE – SEVERE |__| HBP |__| SGA

PERINATAL OUTCOME

Death: \|__\| 0.NO 1.Intrauterine 2.Intrapartum 3.Neonatal Date of death (dd/mm/yy) \|__\|__\| \|__\|__\| \|__\|__\|

Date (dd/mm/yy) \|__\|__\| \|__\|__\| \|__\|__\| **Mode**:\|__\| 1.Spontaneous vaginal 2.Operative vaginal 3.Cesarean section

Indication for delivery : MATERNAL \|__\| FETAL \|__\|

Newborn gender: \|__\| 1.Male 2.Female **Birth weight** (g) \|__\|__\|__\|__\| **1-min/5-min Apgar score** \|__\|__\|/ \|__\|__\|

Cord arterial pH \|__\|.\|__\|__\| **venous pH** \|__\|.\|__\|__\| **Base Excess** (mmol/l) \|__\|__\|__\| **pO2**((mmol/l))\|__\|__\|__\|

Neonatal brain scan 3d: \|__\| 0.Not done, 1.Normal 2.IVH-I 3.IVH-II 4.IVH-III 5.IVH-IV 6.PV echodensity 7. Other

40w: \|__\| 0.Not done, 1.Normal 2.IVH-I 3.IVH-II 4.IVH-III 5.IVH-IV 6.PV echodensity 7. Other

Brazelton \|__\| 0.Not done, 1.Done

Specify if needed _____

PLACENTAL MEDIATED DISEASE: FOLLOW UP

IDENTIFICATION

Hospital's ID |__|__|__|__|__|__|__|__|__|__|	**Name & Surname:**

PRENATAL EVALUATION 1

Date (dd/mm/yy) |__|__| |__|__| |__|__| **Gestational age** (weeks.days) |__|__|. |__| **Proteinuria** |__|__|__|

BP |__|__|__| / |__|__|__| Drugs Yes |__| / No |__| Specify: **Date**(dd/mm/yy) |__|__| |__|__| |__|__|

Brain structure evaluation (3D volume): |__| 0.No 1.Yes **Fractional Blood Moving Volume evaluation**: |__| 0.No 1.Yes

Brain Doppler evaluation |__| 0.No 1.Yes **Heart evaluation**: |__| 0.No 1.Yes **Blood samples** |__| 0.No 1.Yes

PRENATAL EVALUATION 2

Date (dd/mm/yy) |__|__| |__|__| |__|__| **Gestational age** (weeks.days) |__|__|. |__| **Proteinuria** |__|__|__|

BP |__|__|__| / |__|__|__| Drugs Yes |__| / No |__| Specify: **Date**(dd/mm/yy) |__|__| |__|__| |__|__|

Brain structure evaluation (3D volume): |__| 0.No 1.Yes **Fractional Blood Moving Volume evaluation**: |__| 0.No 1.Yes

Brain Doppler evaluation |__| 0.No 1.Yes **Heart evaluation**: |__| 0.No 1.Yes **Blood samples** |__| 0.No 1.Yes

PRENATAL EVALUATION 3

Date (dd/mm/yy) |__|__| |__|__| |__|__| **Gestational age** (weeks.days) |__|__|. |__| **Proteinuria** |__|__|__|

BP |__|__|__| / |__|__|__| Drugs Yes |__| / No |__| Specify: **Date**(dd/mm/yy) |__|__| |__|__| |__|__|

Brain structure evaluation (3D volume): |__| 0.No 1.Yes **Fractional Blood Moving Volume evaluation**: |__| 0.No 1.Yes

Brain Doppler evaluation |__| 0.No 1.Yes **Heart evaluation**: |__| 0.No 1.Yes **Blood samples** |__| 0.No 1.Yes

PRENATAL EVALUATION 4

Date (dd/mm/yy) |__|__| |__|__| |__|__| **Gestational age** (weeks.days) |__|__|. |__| **Proteinuria** |__|__|__|

BP |__|__|__| / |__|__|__| Drugs Yes |__| / No |__| Specify: **Date**(dd/mm/yy) |__|__| |__|__| |__|__|

Brain structure evaluation (3D volume): |__| 0.No 1.Yes **Fractional Blood Moving Volume evaluation**: |__| 0.No 1.Yes

Brain Doppler evaluation |__| 0.No 1.Yes **Heart evaluation**: |__| 0.No 1.Yes **Blood samples** |__| 0.No 1.Yes

PRENATAL EVALUATION 5

Date (dd/mm/yy) |__|__| |__|__| |__|__| **Gestational age** (weeks.days) |__|__|. |__| **Proteinuria** |__|__|__|

BP |__|__|__| / |__|__|__| Drugs Yes |__| / No |__| Specify: **Date**(dd/mm/yy) |__|__| |__|__| |__|__|

Brain structure evaluation (3D volume): |__| 0.No 1.Yes **Fractional Blood Moving Volume evaluation**: |__| 0.No 1.Yes

Brain Doppler evaluation |__| 0.No 1.Yes **Heart evaluation**: |__| 0.No 1.Yes **Blood samples** |__| 0.No 1.Yes

ANNEX 4. ADMISSION LETTER PROYECT 3

Decision Letter (UOG-2009-0035.R2)

From: uog@isuog.org

To: FFIGUERA@clinic.ub.es

CC: shatcher@isuog.org

Subject: Accept - Manuscript UOG-2009-0035.R2

Body: Date:03-Jul-2009
Ref.: UOG-2009-0035.R2

Dear Dr Figueras

Thank you for submitting a further amended version of your manuscript, "Middle versus anterior cerebral artery Doppler for the prediction of perinatal outcome and neonatal neurobehavior in term small-for-gestational-age fetuses with normal umbilical artery Doppler". I am pleased to inform you that it has now been accepted for publication in Ultrasound in Obstetrics and Gynecology.

Please mail or fax a completed copyright transfer agreement (which can be downloaded from the journal website http://www3.interscience.wiley.com/cgi-bin/jabout/99020267/ForAuthors.html) to the address below.

Ultrasound in Obstetrics and Gynecology
4, Blythe Mews
Blythe Road
London W14 0HW
United Kingdom
Fax: 44 (0) 20 7471 9956

When the manuscript has been assigned an issue, it will be forwarded for typesetting and you will receive the proof via e-mail containing a link to a pdf file. Further instructions will be sent with the proof.

Thank you for choosing Ultrasound in Obstetrics and Gynecology for publication of your work.

Yours sincerely

Kurt Hecher
Editor, Ultrasound in Obstetrics and Gynecology

Date Sent: 03-Jul-2009

ANNEX 5. SUBMISSION LETTER PROYECT 1

Preview

From: uog@isuog.org

To: FFIGUERA@clinic.ub.es

CC:

Subject: Manuscript submitted to Ultrasound in Obstetrics and Gynecology - UOG-2010-0046, Authors Copy

Body: 29-Jan-2010

Manuscript number: UOG-2010-0046

Dear Dr Figueras

We are pleased to receive your manuscript entitled Longitudinal changes in uterine, umbilical and cerebral Doppler in late-onset small-for-gestational age fetuses by Oros, Daniel; Figueras, Francesc; Cruz-Martinez, Rogelio; Meler, Eva; Munmany, Meritxell; Gratacos, Eduard. We will shortly be assigning it to one of the Journal's Editors who will handle the peer review of the paper.

To track the progress of your manuscript through the editorial process using our web-based system, simply point your browser to:

http://mc.manuscriptcentral.com/uog

and log in using the following user ID and password:

(User ID): frafig
(Password): (Person not available)

Please remember in any future correspondence regarding this article to always include its manuscript ID number UOG-2010-0046.

If you experience problems associated with the submission web site, please contact the Wiley support staff directly at: uog@isuog.org

Many thanks for submitting your manuscript

Yours sincerely

Sarah Hatcher
Managing Editor

Date Sent: 29-Jan-2010

11) PAPERS

ULTRASOUND in Obstetrics & Gynecology
isuog
WILEY

Longitudinal changes in uterine, umbilical and cerebral Doppler in late-onset small-for-gestational age fetuses

Journal:	*Ultrasound in Obstetrics and Gynecology*
Manuscript ID:	Draft
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	
Complete List of Authors:	Oros, Daniel; Hospital Clinic - IDIBAPS, Department of Maternal-Fetal Medicine Figueras, Francesc; Hospital Clinic - IDIBAPS, Department of Maternal-Fetal Medicine Cruz-Martinez, Rogelio; Hospital Clinic - IDIBAPS, Department of Maternal-Fetal Medicine Meler, Eva; Hospital Clinic - IDIBAPS, Department of Maternal-Fetal Medicine Munmany, Meritxell; Hospital Clinic - IDIBAPS, Department of Maternal-Fetal Medicine Gratacos, Eduard; Hospital Clinic - IDIBAPS, Department of Maternal-Fetal Medicine
Manuscript Categories:	Obstetrics
Keywords:	Small-for-gestational-age, intrauterine growth restriction, longitudinal monitoring, uterine artery, Doppler, umbilical artery, Doppler, middle cerebral artery, Doppler, cerebroplacental ratio

Longitudinal changes in uterine, umbilical and cerebral Doppler in late-onset small-for-gestational age fetuses

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Short title: *Doppler trends in late SGA*

Key words: Small-for-gestational-age, Doppler, longitudinal monitoring, uterine artery, umbilical artery, middle cerebral artery, cerebroplacental ratio.

ABSTRACT

Objective: To determine the longitudinal trends and rate of conversion of normal to abnormal uterine, umbilical and cerebral Doppler throughout third trimester in late-onset small-for-gestational age (SGA) fetuses.

Methods: Uterine, umbilical and cerebral Doppler was serially performed in a cohort of singleton consecutive late-onset small-for gestational age fetuses with normal Doppler values at diagnosis. The rate of conversion of normal to abnormal Doppler values was evaluated by survival analysis. Longitudinal trends were modeled by means of multilevel analysis.

Results: A total of 616 scans were performed on 171 SGA fetuses. Mean gestational ages at inclusion and at delivery were 34.1 (standard deviation 1.6) and 38.7 (standard deviation 1.7) weeks. The proportions of abnormal uterine (2.3% vs. 4.1%) and umbilical (2.3% vs. 2.9%) artery indexes were not significantly different between 37 weeks and before delivery. On the contrary, the proportions of abnormal middle cerebral artery index (4.1% vs. 13.5%) and cerebroplacental ratio (7% vs. 22.8%) were significantly different between these two examinations. The remaining proportion of cases with normal uterine, umbilical and middle cerebral artery indexes, and cerebroplacental ratio at 40 weeks were 98.6%, 94.5%, 85% and 49.6%, respectively. Whereas a slight increasing trend was observed for the uterine (β=0.002) and umbilical (β=0.01) artery pulsatility indexes, middle cerebral artery pulsatility index (β=0.044) and cerebroplacental ratio (β=0.124) showed a progressive decrease until delivery.

Conclusion: Late-onset SGA with normal Doppler upon diagnosis show progression from 37 weeks with worsening cerebroplacental ratio followed by middle cerebral artery vasodilation.

INTRODUCTION

Placental function evaluation by umbilical artery Doppler is the clinical standard to identify early-onset intrauterine growth restriction (IUGR) [1-3] and there is evidence that its use in these pregnancies improves a number of obstetric care outcomes and reduces perinatal deaths [4]. On the contrary, in late-onset cases, which are far more prevalent, most events of adverse outcome attributable to IUGR occur in fetuses with normal umbilical artery [5]. Thus, a substantial proportion of late-onset small-for-gestational age (SGA) fetuses with normal umbilical artery Doppler may have true growth restriction and are at-risk of adverse perinatal outcome [6-8]. In line with this notion, we have reported an increased prevalence of suboptimal neurobehavioral [9,10] and neurodevelopmental outcome in children born SGA [10-11], with similar features to those described for children who had intrauterine growth restriction [11-12].

Since the identification of late-onset SGA fetuses at risk for adverse perinatal outcome cannot be relied on umbilical artery Doppler (UA), other vascular territories have been proposed, including the uterine and middle cerebral arteries. Abnormal uterine artery Doppler (UtA) has shown to be comparable with umbilical artery Doppler as a predictor of adverse outcome in late-onset IUGR [8,13,14]. On the other hand, a reduced pulsatility in the middle cerebral artery (MCA) is also associated with poorer perinatal outcome [8,15,16] and with an increased risk of abnormal neurodevelopment [17,18]. Finally, the cerebroplacental ratio CPR), (which combines the pulsatility index of the middle cerebral and umbilical arteries, has demonstrated to be more sensitive to hypoxia than its individual components in animal [19] and clinical [20] models, and it correlates better with adverse outcome [21].

In early-onset IUGR, the sequence of changes in Doppler indexes has been described [22-24]. However, in late-onset cases, these longitudinal trends have not been investigated. This information is essential to ascertain whether there is a sound basis to use these parameters in the monitoring of late-onset SGA. This study was aimed at determining the longitudinal trends and rate of conversion of normal to abnormal Doppler pulsatility indexes of the uterine, umbilical and middle cerebral artery in late-onset SGA fetuses from diagnosis to delivery.

METHODS

Between November 2007 and August 2009, a prospective cohort was created of consecutive suspected SGA singleton babies at routine third trimester ultrasound (30-36 weeks) with confirmed birth weight <10 percentile according to local standards [25]. Only cases presented upon admission with mean uterine artery pulsatility index (PI) <95th percentile [26], umbilical artery pulsatility index <95th percentile, middle cerebral artery pulsatility index >5th percentile and cerebroplacental ratio >5th percentile [21] were included. Exclusion criteria were congenital malformations (including chromosomopathies and infections) and the diagnosis of preeclampsia. The study protocol was approved by the Clinic Hospital Institutional Review Board and parents provided their written informed consent.

Prenatal Doppler was performed using an Acuson Antares Premium Edition (Siemens, Mountain View, CA) ultrasound machine equipped with a 2.3–4 MHz transabdominal transducer. Every Doppler measurement was obtained in all cases by one of three experienced observers (F.F, D.O and R.C.M). Uterine arteries were examined transabdominally. The probe was placed on the lower quadrant of the abdomen, angled medially, using color Doppler to identify the uterine artery at the apparent crossover with the external iliac artery. Measurements were taken approximately at 1 cm distal to the crossover point. The pulsatility index of the left and right arteries were measured, and the mean index was calculated. The umbilical artery Doppler flow spectrum was recorded from a free-floating portion of the umbilical cord. The middle cerebral artery Doppler was recorded in a transverse view of the fetal brain, with the Doppler gate placed on the vessel 1 cm distal from the circle of Willis. The cerebroplacental ratio was

calculated as MCA PI / UA PI. In these vessels, once it had been ensured that the angle was less than 30º, the pulsed Doppler gate was placed over the whole width of the vessel. Angle correction was then applied and the signal updated until three similar consecutive waveforms were obtained in the absence of fetal movements and voluntary maternal suspended breathing.

Pregnancies were dated by first-trimester crown-rump length measurement [27]. Doppler examination was performed fortnightly and biophysical profile was carried out weekly. All cases had a Doppler examination within 7 days of delivery. Indications for delivery included a persistent (12-hour apart) biophysical profile $\leq$6 and umbilical artery pulsatility index >95th percentile beyond 37 weeks. Otherwise, labor was induced at 40$\pm$1 weeks. A staff obstetrician assisted all the deliveries.

All Doppler parameters were transformed into z-values according to normative references [21-26].

The longitudinal changes were analyzed by Kaplan-Meier survival analysis, in which the endpoint was defined as an abnormal Doppler value (middle cerebral artery pulsatility index and cerebroplacental ratio <5th percentile; umbilical and uterine arteries pulsatility index >95th percentile). The McNemar test was used to compare paired group proportions. Statistical and survival analyses were performed using the Statistical Package for Social Sciences (SPSS 15.0, SPSS Inc., Chicago, IL) statistical software.

Longitudinal changes in z-values during the last 10 weeks before delivery were modeled by means of multilevel analysis, fitting to second-degree polynomials: $\alpha + \beta t + \gamma t^2$, where α, β and γ were the parameters characterizing the individual fetus and t the days before delivery. These parameters were calculated assuming its randomly normal distribution in the population (random effect model), which allows us to assume that both the individuals and the days to delivery at which the scan was performed are random samples of their respective populations. Standard errors of these parameters were used to construct confidence intervals (CI). The software MLwiN 2.1 (Centre for Multilevel Modelling, University of Bristol, UK) was used for the parameters´ estimation. Repeated measurements at different time points (gestational age) in the same fetus comprised Level 1 and those in different fetuses comprised Level 2. Individual regression lines for each variable were calculated for each fetus and from these the regression lines for the whole group were derived [28].

RESULTS

During the study period a total of 616 scans were performed on 171 SGA fetuses. The median number of Doppler examinations was 3 (range 2-9). In 124 (62.5%) women more than two examinations were performed.

The mean gestational ages at inclusion and delivery were 34.1 (SD 1.6; range 30.0-35.6) and 38.7 (SD 1.7; range 37-41.6) weeks, respectively. The median interval between the last examination and delivery was 3 (range 0-6) days. Table 1 shows the maternal and neonatal clinical characteristics of the population.

Figure 1 shows the proportion of abnormal Doppler at 37 weeks and at the last examination before delivery. Remarkably, the proportions of abnormal uterine artery pulsatility index (2.3% vs. 4.1%; p=0.36) and abnormal umbilical artery pulsatility index (2.3% vs. 2.9%; p=0.65) were not significantly different between 37 weeks and before delivery. On the contrary, the proportions of abnormal middle cerebral artery pulsatility index (4.1% vs. 13.5%; p=0.02) and abnormal cerebroplacental ratio (7% vs. 22.8%; p=0.01) were significantly different between these two examinations. Before delivery, the proportion of abnormal umbilical artery index was significantly lower than the proportion of abnormal middle cerebral artery index (2.9% vs. 13.5%; p<0.01) and abnormal cerebroplacental ratio (2.9% vs. 22.8%; p<0.001). Also, the proportion of abnormal middle cerebral artery index and cerebroplacental ratio significantly differed (13.5% vs. 22.8%; p=0.002). No cases of biophysical profile ≤6 or absent or reversed end-diastolic umbilical artery Doppler occurred.

Figure 2 shows the survival graph of the Doppler parameters throughout the study period, plotted against gestational age, which could be interpreted as the remaining proportion of normal Doppler at each week of gestational age for each of the parameters. The remaining proportions (95% CI) of cases with normal uterine, umbilical, middle cerebral artery and cerebroplacental ratio at 40 weeks were 98.6% (96-100), 94.5% (85.3-100), 85% (76.2-93.8) and 49.6% (35.1-64.1).

Figure 3 shows the longitudinal trends of uterine, umbilical, middle cerebral artery, and cerebroplancental ratio from enrolment to delivery. Umbilical artery pulsatility index (β=0.01; 95%CI 0.005-0.014) and uterine artery pulsatility index (β=0.002; 95%CI 0.0009-0.032) showed a slight, but almost negligible trend of increase, while middle cerebral artery pulsatility index (β=0.044, 95%CI 0.029-0.6) and cerebroplacental ratio (β=0.124; 95% CI 0.099-0.0225) demonstrated a clear and progressive decrease in values from inclusion to delivery.

DISCUSSION

This is the first study describing the longitudinal changes of utero-placental and fetal brain hemodynamics in late-onset SGA fetuses. Our study shows that on average feto-placental Doppler indexes remain virtually unchanged from diagnosis to delivery in these fetuses, and consequently the proportion of cases progressing to abnormal Doppler in the umbilical and uterine arteries is negligible. On the contrary, average middle cerebral artery and, more markedly, cerebroplacental ratio values worsen significantly from 37 weeks until delivery, with 14% and 23% of abnormal values on the last scan before delivery, respectively. The study provides evidence to support the notion that uteroplacental Doppler is of little clinical value in SGA fetuses and that cerebral Doppler may constitute the most sensitive exam to identify cases at high risk of adverse perinatal outcome.

The findings of this study are in line with previous Doppler studies in IUGR and SGA fetuses. Concerning umbilical artery Doppler, comparison of our findings in SGA fetuses with published longitudinal series on early-onset IUGR demonstrates that both conditions have a different pattern of Doppler deterioration. In early-onset IUGR, abnormal umbilical artery constitutes the mainstay for diagnosis and deteriorates progressively, followed by the development of brain sparing [29-30]. On the contrary, as illustrated in this study, umbilical atery remains normal in most SGA fetuses despite the development of brain sparing in up to 20% of cases. In a recent study on late-onset IUGR (beyond 30 weeks) with abnormal umbilical artery, Turan et al [30] reported that fetal rapid hemodynamic deterioration as observed in early-onset forms was very unlikely, and the median interval between admission and the appearance of any other

Doppler abnormality was 33 days. The findings of Turan et al. together with those reported in this study support that as gestational age at onset increases abnormalities in the umbilical artery Doppler in small fetuses are more unlikely. Regarding uterine artery Doppler, we and others [31-33] have demonstrated that progression of uterine Doppler during the second trimester correlates with perinatal outcome, but similarly to umbilical artery Doppler, this parameter remained almost constant throughout the third trimester in SGA fetuses. Finally, concerning cerebral Doppler measurements our findings are in line with previous studies in early-onset IUGR reporting that cerebroplacental ratio becomes abnormal earlier than the middle cerebral artery [29-30], and with animal models demonstrating that cerebroplacental ratio turns out to be the hemodynamic parameter that reflects most closely PO_2 changes [34].

Our study has several clinical implications. First, since uterine and umbilical Doppler does not deteriorate from diagnosis to delivery, little benefit can be expected by serial monitoring. McCowan et al [35] demonstrated that fortnightly fetal surveillance by umbilical artery Doppler compared to twice-weekly surveillance resulted in fewer inductions without any impact on the perinatal outcome. They concluded that more studies are needed to determine the safety of less frequent surveillance of SGA with normal umbilical artery. Our findings suggest that in this population, isolated serial umbilical or uterine artery Doppler would be unnecessary. On the other hand, restricting the evaluation of near-term SGA to the umbilical artery Doppler could be potentially harmful since a normal result could give false reassurance of fetal wellbeing. Secondly, cerebral Doppler seems to constitute the most sensitive parameter to detect late-onset SGA fetuses at risk for adverse perinatal outcome. Hershkovitz et al [15] first provided

evidence that near-term SGA with isolated middle cerebral artery vasodilatation are at-risk of adverse outcome. Their findings have been confirmed by others [8]. In addition, cerebral Doppler studies seem to detect the group of SGA fetuses with the highest risk of suboptimal neurodevelopmental outcome in these fetuses [17]. Notwithstanding its better predictiveness as compared with umbilical artery Doppler, the middle cerebral artery still fails to detect a substantial number of fetuses with abnormal neonatal outcome [10]. As supported by an increasing number of studies, cerebroplacental ratio is an earlier and more sensitive predictor for adverse outcome than either the middle cerebral artery or umbilical artery alone, both in severe [19,20,36,37] as in mild forms of IUGR [30]. The findings of this study are in line with these studies and further support the use of cerebroplacental ratio as a primary Doppler monitoring parameter in late-onset SGA.

This study has some limitations. Since abnormal umbilical artery Doppler was an indication for delivery for ethical reasons, the temporal relationship between the parameters studied may be biased. However, this occurred in a small proportion of cases and, therefore, the effect of this bias should be negligible. Any clinical study on the surveillance of fetal growth needs to be interpreted with caution because it can never be entirely blinded and the clinical management is influenced by the antenatal findings. However, clinicians attending the deliveries were unaware of the middle cerebral artery, uterine and cerebrolacental ratio Doppler status.

In conclusion, late-onset SGA with normal Doppler upon diagnosis show no changes in umbilical and uterine artery Doppler. In contrast, a progression from 37 weeks with worsening cerebroplacental ratio in up to 24 % and of middle cerebral artery

vasodilation in 14% of cases is observed. These findings discourage the use of umbilical artery as a single parameter for surveillance in late-onset SGA and support the use of the cerebroplacental ratio as the most sensitive Doppler parameter for the clinical monitoring of these fetuses.

ACKNOWLEDGMENTS

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Table 1. Clinical characteristics of the population

	SGA, n = 171
GA at inclusion (weeks); mean (SD); rank	34.1 (1.6); 30.0-35.6
GA at last scan (weeks); mean (SD)	37.7 (1.6)
Maternal age (years); mean (SD)	31 (5.2)
Low socio-economic class*;n (%)	40 (23.4)
Primiparity; n (%)	118 (69)
Non-Caucasian ethnicity; n (%)	38 (22.2)
Smoking; n (%)	33 (19.3)
1-10 cigarettes/day; n (%)	18 (10.5)
10-19 cigarettes/day; n (%)	11 (6.4)
≥20 cigarettes/day; n (%)	4 (2.3)
Labor induction; n (%)	114 (66.7)
Cesarean delivery; n (%)	60 (35.1)
Cesarean delivery for fetal distress; n (%)	32 (18.7)
GA at delivery (weeks); mean (SD); rank	38.7 (1.7);37.0-41.6
Birth weight (g) ; mean (SD)	2433 (268)
Birth weight percentile; mean (SD)	4 (3.5)
Umbilical artery pH<7.15 at delivery; n (%)	12 (7%)
5-minute Apgar score<7; n (%)	0
Admission in the Neonatal Unit; n (%)	8 (4.7)

SGA: small-for-gestational-age; GA: gestational age; *Routine occupations, long-term unemployment or never worked (UK National Statistics Socio-Economic Classification)

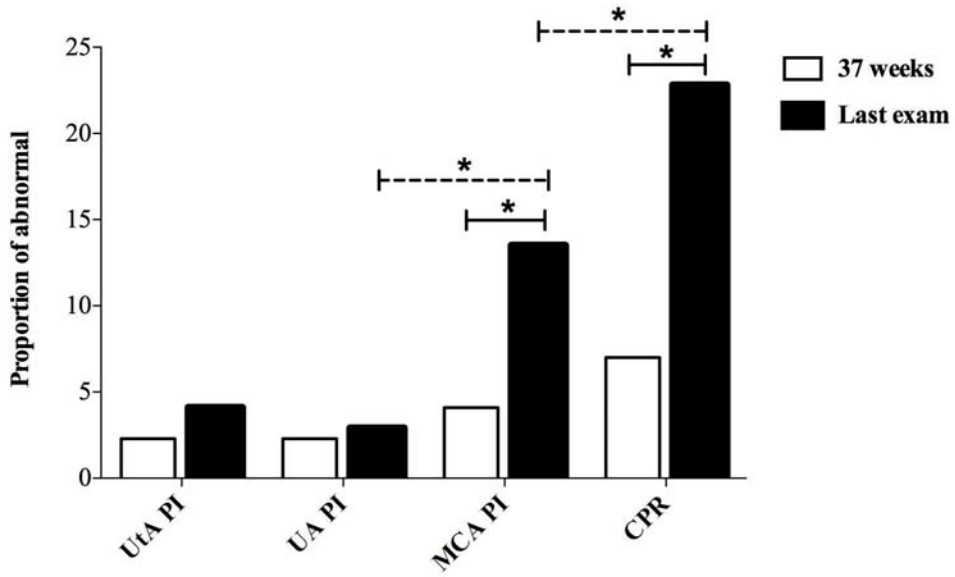

Figure 1. Proportion of abnormal Doppler at 37 weeks and last examination before delivery (*McNemar p<0.001). PI: Pulsatility Index; UtA: Uterine Artery; UA: Umbilical Artery; MCA: Middle Cerebral Artery; CPR: Cerebroplacental Ratio.
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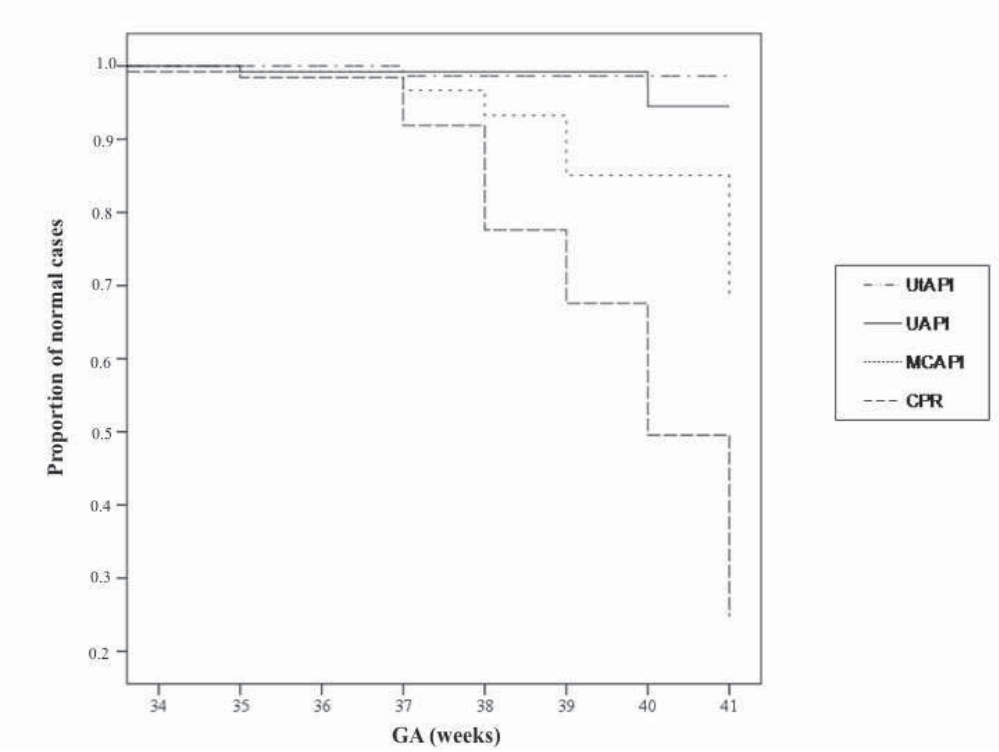

Figure 2. Kaplan–Meier plot showing the proportion of fetuses with normal Doppler parameters. PI: Pulsatility Index; UtA: Uterine Artery; UA: Umbilical Artery; MCA: Middle Cerebral Artery; CPR: Cerebroplacental Ratio
254x190mm (72 x 72 DPI)

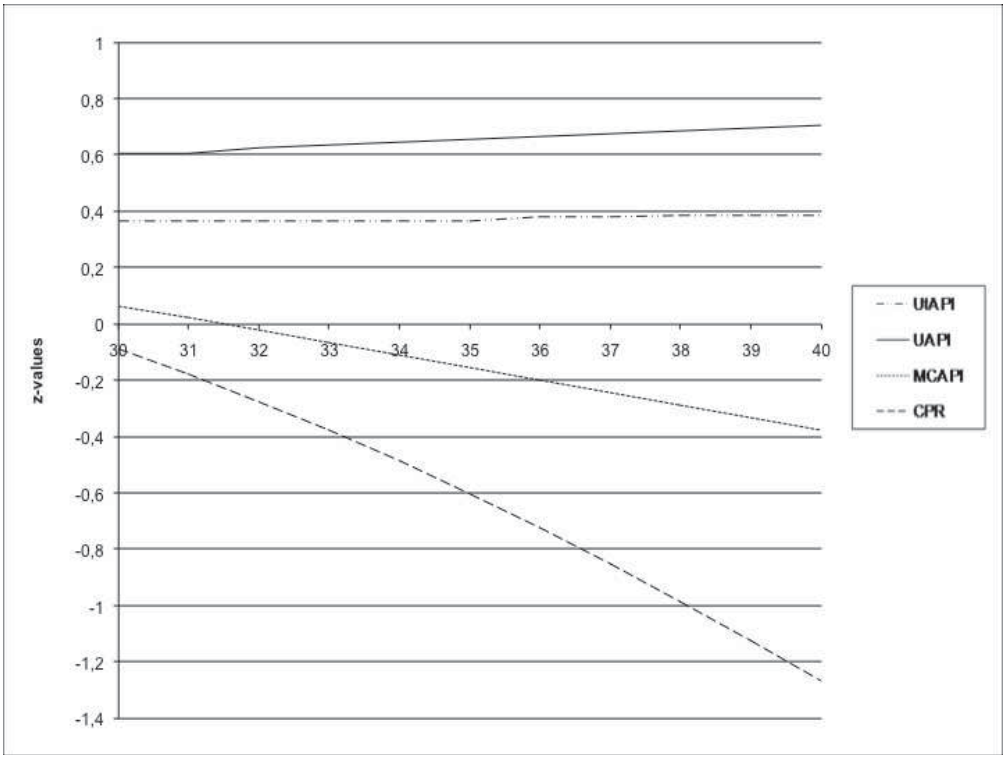

Figure 3. Longitudinal trends of the Doppler parameters (in z-values) during the study period. PI: Pulsatility Index; UtA: Uterine Artery; UA: Umbilical Artery; MCA: Middle Cerebral Artery; CPR: Cerebroplacental Ratio.
254x190mm (72 x 72 DPI)

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Neurobehavior in Term, Small-for-Gestational Age Infants With Normal Placental Function

Francesc Figueras, Daniel Oros, Rogelio Cruz-Martinez, Nelly Padilla, Edgar Hernandez-Andrade, Francesc Botet, Carme Costas-Moragas and Eduard Gratacos

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Neurobehavior in Term, Small-for-Gestational Age Infants With Normal Placental Function

WHAT'S KNOWN ON THIS SUBJECT: IUGR is associated with behavioral, sensorial, and cognitive dysfunctions in childhood and adolescence. Several studies on preterm infants correlated these difficulties with behavioral disruptions already present in the neonatal period.

WHAT THIS STUDY ADDS: This study extends the neurobehavioral effects of being SGA to term neonates with no signs of placental insufficiency, a subgroup of neonates traditionally considered to be at one end of the size spectrum of normal infants.

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KEY WORDS
small for gestational age, intrauterine growth restriction, neurobehavioral outcome, Doppler ultrasonography

ABBREVIATIONS
SGA—small for gestational age
AGA—appropriate for gestational age
IUGR—intrauterine growth restriction
NBAS—Neonatal Behavioral Assessment Scale

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abstract

OBJECTIVE: The goal was to evaluate the neurobehavioral outcomes of term, small-for-gestational age (SGA) newborns with normal placental function.

METHODS: A cohort of consecutive term SGA newborns with normal prenatal umbilical artery Doppler ultrasound findings was created and compared with a group of term infants with size appropriate for gestational age, who were sampled from our general neonatal population. Neonatal behavior was evaluated at corrected age of 40 ± 1 weeks with the Neonatal Behavioral Assessment Scale. The effect of the study group on each Neonatal Behavioral Assessment Scale area was adjusted, through multivariate analysis of covariance, for smoking during pregnancy, maternal BMI, socioeconomic level, onset of labor, mode of delivery, use of epidural anesthetic medication, gestational age at delivery, postnatal age (in days) at evaluation, and gender.

RESULTS: A total of 202 newborns (102 SGA and 100 appropriate for gestational age) were included. All of the neurobehavioral areas studied were poorer in the SGA group, with significance for attention, habituation, motor, social-interactive, and regulation of state. The average mean differences in scores between the study groups were 0.77 (95% confidence interval: 0.38–1. 14) for attention, 0.64 (95% confidence interval: 0.13–1.14) for habituation, 0.52 (95% confidence interval: 0.31–0.74) for motor, 0.95 (95% confidence interval: 0.54–1.37) for social-interactive, and 0.68 (95% confidence interval: 0.23–1.13) for regulation of state. These differences remained significant after adjustment for potential confounders.

CONCLUSION: Term SGA newborns with no signs of placental insufficiency had poorer neurobehavioral competencies, which suggests delayed neurologic maturation. *Pediatrics* 2009;124:e934–e941

Small for gestational age (SGA) and intrauterine growth restriction (IUGR) have been used interchangeably. The 2 terms are not synonymous, however; not all small newborns have IUGR, and not all infants with IUGR are small.[1] Most instances of true IUGR correspond to cases of placental insufficiency.[2] Intrauterine placental function evaluation through umbilical artery Doppler ultrasonography is the clinical standard used to distinguish between SGA and IUGR.[3–5] In addition, there is evidence that umbilical artery Doppler ultrasound use in these pregnancies improves a number of obstetric care outcomes and reduces perinatal deaths.[6] Although abnormal umbilical artery Doppler ultrasound findings are associated with adverse perinatal and neurodevelopmental outcomes,[7–10] small fetuses with normal umbilical artery Doppler ultrasound findings are considered to represent one end of the normal size spectrum, and the importance of treating them in a completely different manner, compared with infants with true IUGR, has been stressed.[11,12]

Many studies have found associations between prematurity with IUGR and later behavioral,[13–15] sensorial,[16,17] and cognitive[18–21] dysfunctions. Long-term outcomes for such infants reveal a specific profile of neurocognitive difficulties, with poor executive functioning, cognitive inflexibility, poor creativity, and language problems.[18,19] Other studies also reported long-term cognitive disadvantages for term SGA infants.[22,23] Some studies on preterm infants with IUGR correlated these difficulties during childhood with behavioral disruptions already present in the neonatal period,[13,14,24] a time when environmental influences are still minimal. Behavioral competencies in newborns are mainly related to neurologic maturation, and increasing evidence supports a neurobiological basis for infant behavior.[25] Furthermore, several studies demonstrated correlations between neonatal neurobehavioral skills and later neurocognitive development for preterm[13,14,24] and term[26,27] infants. There is no information on the neonatal behavior of term SGA newborns with normal placental function.

Preliminary evidence suggested indirectly that a proportion of term SGA infants without Doppler ultrasound signs of placental insufficiency might have been exposed to mild hypoxia in utero,[8,28,29] but the effects of this on brain maturation have not been investigated. The hypothesis of this study is that term SGA infants with normal placental function might have neurobehavioral disruptions attributable to abnormal brain maturation. Accordingly, this study was aimed at evaluating the neurobehavior of term SGA newborns with normal placental function.

METHODS

Subjects

A cohort was created of consecutive, suspected SGA, singleton infants delivered at gestational ages of >37 weeks between November 2006 and August 2008, with confirmed birth weights of <10th percentile according to local standards.[30] Pregnancies were dated according to the first-trimester crown-rump length measurement.[31] Exclusion criteria included congenital malformations (including chromosomopathies and infections) and umbilical artery pulsatility index values of >95th percentile.[32] Control subjects were defined as singleton term infants with size appropriate for gestational age (AGA) (≥10th percentile, according to local standards[30]) and were sampled from our general neonatal population during the same period; they were matched with case subjects according to the date of delivery (±7 days). A total of 108 SGA newborns fulfilled the inclusion criteria and were matched with 108 AGA infants. The study protocol was approved by the ethics committee, and parents provided written informed consent.

Neurobehavioral Outcomes

Neurobehavioral performance was evaluated at corrected age of 40 ± 1 weeks with the Neonatal Behavioral Assessment Scale (NBAS),[33] which assesses both cortical and subcortical functions by evaluating 35 items; the items are rated on a scale of 1 to 9 (with 9 being the best performance) except for 8 curvilinear scale items, which, according to the manual, are rescored as linear on a 5-, 6-, or 8-point scale. Items are grouped into 6 clusters, including habituation (habituation to light, rattle, bell, and tactile stimulation of the foot), motor (general tone, elicited activity, spontaneous activity, and motor maturity), social-interactive (responses to visual, animate, and inanimate auditory stimuli and alertness), organization of state (irritability, state lability, maximal excitation, and reaction time), regulation of state (self-quieting and hand-to-mouth responses), and autonomic nervous system. The social-interactive cluster was subscored for visual and auditory stimuli. In addition, as reported recently by the authors of the NBAS,[34] an aggregation of individual items (alertness, quality of the alert responsiveness, and cost of attention) was used to evaluate the capacity of infant attention.

All evaluations were performed by 1 of 3 trained examiners accredited by the Brazelton Institute (Harvard Medical School, Boston, MA); they had been tested previously for reliability and achieved an interrater reliability level of >90%. The examiners were blinded to the study group and perinatal outcomes. Neonates were assessed in the afternoon, between feedings, in a small, semidark, quiet room with a

temperature between 22°C and 27°C, in the presence of ≥1 parent.

Placental Function Assessment

In all cases, prenatal Doppler ultrasound examinations were performed by 1 of 3 experienced observers (Drs Figueras, Oros, and Cruz-Martinez), with an Acuson Antares Premium Edition ultrasound system (Siemens, Mountain View, CA) equipped with a 2.3- to 4-MHz transabdominal transducer. The umbilical artery pulsatility index was calculated from ≥3 consecutive waveforms obtained from a free-floating portion of the umbilical cord during the absence of fetal movement, at insonation angles of <30°. All case subjects underwent a Doppler ultrasound examination within 7 days after delivery.

Statistical Analyses

Student's *t* test for independent samples and Pearson's χ^2 test were used to compare quantitative and qualitative data, respectively. Multivariate analyses were conducted through multivariate analysis of covariance in which a model was run for each different set of skills (attention, habituation, motor, state organization, state regulation, and autonomic nervous system), with the study group included as a factor and the following variables as covariates: (1) smoking during pregnancy (no smoking, 1–9 cigarettes per day, or ≥10 cigarettes per day); (2) maternal BMI at booking; (3) low socioeconomic level (routine occupations, long-term unemployment, or never worked; United Kingdom National Statistics Socio-economic Classification); (4) onset of labor (spontaneous versus induction); (5) mode of delivery (vaginal delivery versus cesarean section); (6) number of doses of epidural anesthetic medication (bupivacaine, 1.2–1.8 mg) during labor (none, 1–3 doses, or ≥4 doses); (7) gestational age at delivery; (8) postnatal age (in days) at evaluation; and (9) gender. For each model, assumptions for the multivariate analysis of covariance were checked and the multivariate significance of the F value was assessed with Wilks' λ *P* value. Also, the η^2 value was provided, which could be interpreted as the proportion of the total variance of the dependent variables explained by each factor and covariate. To rule out an expectation bias, the association between birth weight and NBAS scores was evaluated through Pearson correlation within each study group. The software package SPSS 14.0 (SPSS, Chicago, IL) was used for the statistical analyses.

TABLE 1 Clinical Characteristics of the Population

	AGA (*N* = 100)	SGA (*N* = 102)	*P*[a]
Primiparity, %	61	67.6	.32
Nonwhite ethnicity, %	21.2	16	.34
Maternal age, mean ± SD, y	31.8 ± 4.9	33.6 ± 4.9	.79
Maternal age of <21 y, %	5	2	.28
BMI at booking, mean, kg/m^2	23.4	21.9	.003
Low socioeconomic level, %[b]	31	52	.003
Smoking, %			
Nonsmoking	85	83.3	.75
1–9 cigarettes per d	12	5.9	
11–19 cigarettes per d	2	7.8	
≥20 cigarettes per day	1	2.9	
Alcohol consumption of >170 g/wk, %	5	3.9	.71

[a] Student's *t* test for independent samples or Pearson's χ^2 test.
[b] Routine occupations, long-term unemployment, or never worked (United Kingdom National Statistics Socio-economic Classification).

TABLE 2 Perinatal Outcomes for the Population

	AGA (*N* = 100)	SGA (*N* = 102)	*P*[a]
Gestational age at delivery, mean ± SD, wk	39.7 ± 1.1	38.5 ± 1.1	<.001
Epidural anesthesia, %	92	100	.004
Birth weight, mean ± SD, g	3338 ± 390	2354 ± 266	<.001
Birth weight percentile, mean ± SD	52.2 ± 26.6	3.3 ± 2.2	<.001
Head circumference, mean ± SD, mm	346 ± 12.5	325 ± 11.4	<.001
Male, %	50	56.9	.33
Cesarean section, %	25	35.5	.11
Labor induction, %	18	65.7	<.001
Operative delivery because of fetal distress, %	8	16.7	.06
5-min Apgar score of <7, %	0	0	
Umbilical artery pH of <7.15 at delivery, %	3.3	10.2	.07
Neonatal unit admission, %	0	3	.08
Neonatal unit stay length, mean ± SD, d	0 ± 0	0.7 ± 3	.025

[a] Student's *t* test for independent samples or Pearson's χ^2 test.

RESULTS

For all of the 216 included infants, a neurobehavioral assessment visit was scheduled at corrected age of 40 ± 1 weeks. Parents of 6 case subjects and 8 control subjects later declined to participate, leaving a final population of 202 infants (102 SGA and 100 AGA). The habituation area could not be assessed for 50 newborns (21 SGA and 29 AGA) because of the absence of a sleeping period during the evaluation.

Table 1 depicts the clinical characteristics of the population. It is noteworthy that the mothers in the SGA group had a lower body weight and more frequently were from a low socioeconomic level. There were no cases of drug consumption other than tobacco or alcohol. Table 2 shows the perinatal outcomes of the population. As expected, SGA newborns had a lower birth weight and a smaller head circumference. Delivery in the SGA group was at an earlier gestational age (SGA: 38.5 weeks; AGA: 39.7 weeks) and more

TABLE 3 NBAS Scores According to Study Group

	Score, Mean ± SD		*P*[a]
	AGA (*N* = 100)	SGA (*N* = 102)	
Attention	6.94 ± 1.3	6.17 ± 1.4	<.0001
Habituation[b]	6.94 ± 1.4	6.3 ± 1.7	.014
Motor	5.67 ± 0.8	5.15 ± 0.8	<.0001
Social-interactive	6.71 ± 1.4	5.76 ± 1.5	<.0001
Visual	6.71 ± 1.5	5.35 ± 1.8	<.0001
Auditory	7.03 ± 1.4	6.15 ± 1.6	<.0001
Organization of state	4.09 ± 0.8	4.02 ± 0.8	.48
Regulation of state	5.91 ± 1.6	5.23 ± 1.5	.003
Autonomic nervous system	7.23 ± 0.9	7 ± 0.99	.08

[a] Student's *t* test.
[b] *N* = 152 (81 SGA and 71 AGA).

frequently was induced. Operative delivery because of fetal distress was twice as frequent in the SGA group. Although no infants in the AGA group were admitted to the neonatal unit 3% in the SGA group were.

Neonatal neurobehavior was assessed at 7.8 ± 7.2 and 10.5 ± 9.6 days of life in the AGA and SGA groups, respectively. Table 3 and Fig 1 detail the neurobehavioral outcomes according to NBAS area. Interestingly, all of the neurobehavioral areas studied were poorer in the SGA group, with univariate significance being achieved for attention, habituation, motor, social-interactive, and regulation of state areas. As Table 4 shows, these differences remained significant after adjustment for potential confounders (smoking during pregnancy, maternal BMI, low socioeconomic level, onset of labor, mode of delivery, use of epidural anesthetic medication, gestational age at delivery, postnatal age at evaluation, and gender). Of these covariates, induction of labor and cesarean delivery significantly accounted for lower habituation scores; gestational age at delivery for higher habituation scores; low socioeconomic level for lower social-interactive scores; and age at NBAS evaluation for higher habituation and social scores. Within each study group, no significant correlations were observed between birth weight and any of the neurobehavioral scores,

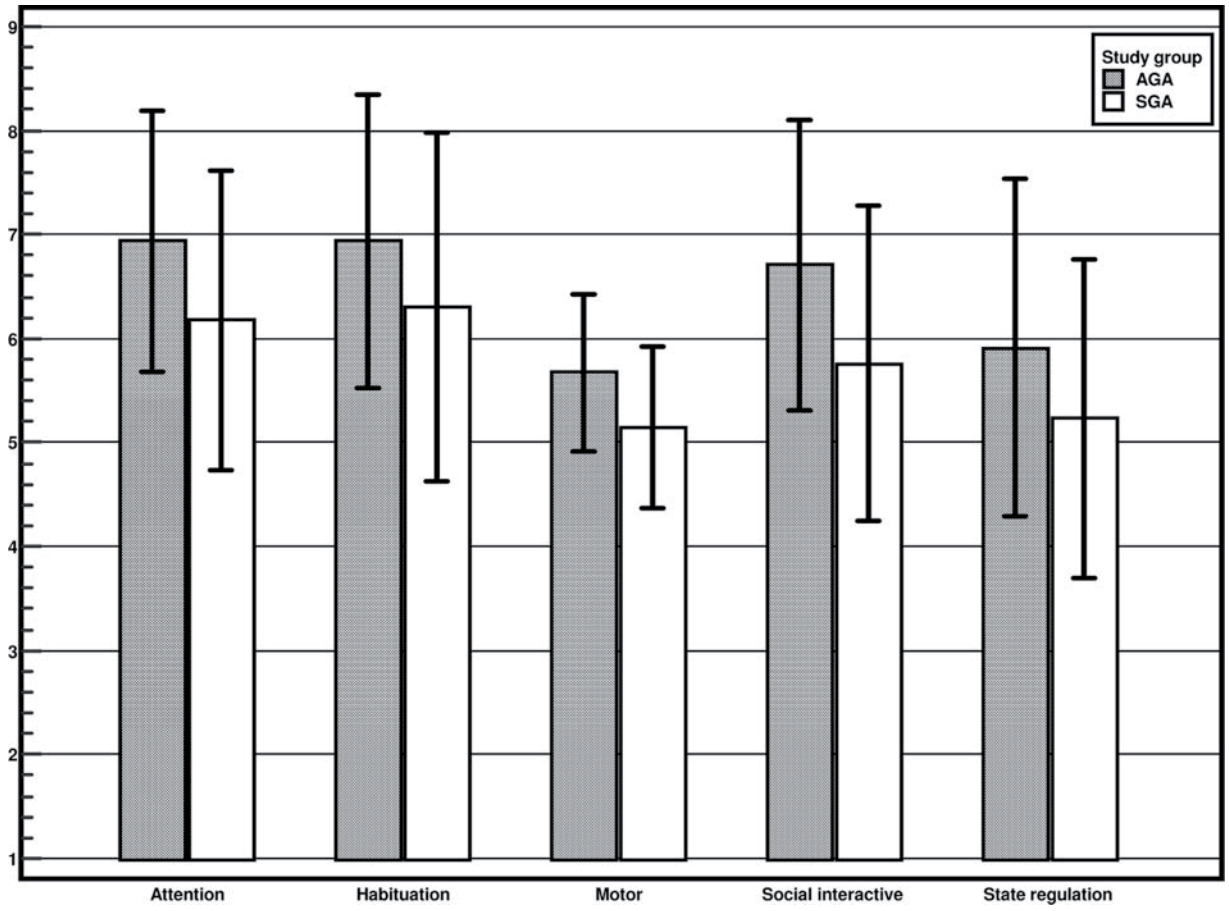

FIGURE 1
NBAS scores according to study group (mean and SD). Only significant associations are displayed (adjusted *P* value).

TABLE 4 Multivariate Analysis of Covariance Results

	F	P^a	η^2, %
Attention			
SGA	5.96	.001	9.2
Cesarean delivery	2.14	.1	3.5
Age at NBAS evaluation[b]	1.9	.12	3.2
Habituation[c]			
SGA	4.59	.002	17
Smoking	1.03	.39	4.5
BMI[b]	0.73	.57	3.3
Labor induction	2.54	.046	10.4
Cesarean delivery	2.44	.05	10.1
Gestational age at delivery[b]	2.59	.04	10.6
Age at NBAS evaluation[b]	3.57	.01	14.1
Male	1.26	.29	5.5
Motor			
SGA	5.18	<.001	12.8
Smoking	1.16	.33	3.2
BMI[b]	1.13	.34	3.1
Labor induction	1.1	.36	3
Age at NBAS evaluation[b]	2.19	.06	5.8
Social-interactive			
SGA	4.6	<.001	18
Smoking	1.05	.4	4.8
BMI[b]	0.77	.61	3.6
Low socioeconomic level	2.49	.02	10.6
Epidural anesthetic medication	0.81	.59	3.7
Age at NBAS evaluation[b]	2.86	.008	12
Male	0.77	.61	3.6
Organization of state			
SGA	1.57	.18	3.4
Low socioeconomic level	2.16	.08	4.6
Regulation of state			
SGA	7.85	<.001	15.8
Autonomic nervous system			
SGA	2.14	.1	3.5
Smoking	2.5	.06	4

The adjusted effect of the study group (SGA versus AGA) on each NBAS area is shown. Adjustment for smoking during pregnancy, maternal BMI, socioeconomic level, onset of labor, mode of delivery, use of epidural anesthetic medication, gestational age at delivery, postnatal age at evaluation, and gender was performed. Covariates that explained >3% of the total variance of dependent variables (η^2) also are presented.

[a] Wilks' λ.

[b] Positive associations.

[c] $N = 152$ (81 SGA and 71 AGA).

except for organization of state ($R = 0.24$; $P = .03$) in the SGA group.

DISCUSSION

It is well known that premature SGA infants with abnormal umbilical artery Doppler ultrasound findings, as a surrogate sign of placental insufficiency, are at high risk of neurobehavioral and neurocognitive outcomes.[35–37] Our study extends the neurobehavioral effects of being SGA to term newborns with no signs of placental insufficiency, a subgroup of newborns traditionally considered to be at one end of the size spectrum of normal infants. Our findings challenge the concept that umbilical artery Doppler ultrasonography is a reliable tool to identify prenatally the constitutional term SGA infants at low risk. Subtle degrees of neurologic injury seem to occur before umbilical artery Doppler ultrasound waveforms become abnormal. In fact, animal[38] and mathematical[39] experimental models of placental vessel obliteration suggested that umbilical artery Doppler ultrasound findings become abnormal only in advanced stages of placental dysfunction.

Identifying at-risk infants is essential for understanding the association between fetal well-being and later neurodevelopmental problems and lays the basis for possible preventive interventions. Infant early temperament and behavior have been demonstrated to have an impact on breastfeeding during the first 6 months of life.[40] The poorer regulation and organization of state observed in our series and others[27] may negatively influence adherence to breastfeeding, which has been proved to be a protective mechanism with respect to neurodevelopmental outcomes at 2 years for SGA infants, independent of the presence of signs of placental insufficiency.[41] Therefore, promoting and supporting breastfeeding may be of special importance for these infants. In addition, because these infants are at risk of poorer neurodevelopmental outcomes, they might benefit from early educational interventions. Early educational interventions have been documented to improve cognitive outcomes[42] and, in some cases, to reduce antisocial behavior early in the school experience.[43] For low-risk premature infants, it has been reported that individualized developmental interventions prevent short-term neurobehavioral dysfunction.[44] It is not known whether interventions also could be effective for term SGA infants and would influence long-term outcomes.

We found poorer neonatal responses to both visual and auditory stimuli in SGA newborns. Long-term follow-up studies with preterm and term SGA infants found impaired processing of visual-spatial stimuli.[19] Animal models of chronic IUGR demonstrated reduced myelogenesis in the optic nerve[45] and reduced synaptogenesis in the visual cortex.[46] These factors could restrict the integration of cerebral cortical inputs. In addition, mistimed cell migration in premature infants could have an effect on synaptic plasticity, with func-

tional and behavioral consequences.[47] Visual-spatial competencies also play a role in the neonatal capacity to select environmental stimuli to process and to act on, which provides important scaffolding on which attention skills are constructed through early childhood.[48]

IUGR also is associated with the development of attention-deficit/hyperactivity disorder symptoms in childhood and early adolescence.[49] Our finding of lower attention capacities in SGA newborn is in line with this finding. Two previous studies[13,24] found impaired attention skills among premature SGA infants. Structural correlations were made between attention scores and cortical gray matter[13] and hippocampal[24] volumes in the neonatal period. Whether NBAS findings predict attention later in childhood is not clear. However, some studies have found that, for term infants, attention is predictive of intelligence in childhood and adolescence.[50,51] A study of temperament in early infancy (5–6 months) performed with infants born at term reported significant prediction of attention deficits among 8-year-old children.[26]

There is substantial overlap between childhood attention disorders and the diagnosis of motor impairment, with poor movement skills.[52] Our finding of lower motor performance among SGA newborns is consistent with this association. With a series of 40 SGA premature neonates, Feldman and Eidelman[14] studied the correlation of neurobehavior with later cognitive function, as measured with the Bayley Scales of Infant Development. They found that neonatal motor maturity was correlated with Psychomotor Developmental Index values at 24 months.

Another interesting finding of our study was the lower level of self-regulation among SGA newborns, as reflected by the regulation of state NBAS cluster. For a series of 38 healthy term infants, Lundqvist-Persson[27] reported that levels of self-regulation were correlated with the infants' levels of cognitive development (personal-social development, speech development, and eye-hand coordination, subvariables in Griffiths' Mental Development Test) and with sleeping disorders at 2 years of age. We found no difference in autonomic nervous system functioning in SGA infants. This finding is consistent with previous studies that showed preserved sympathovagal balance in term SGA infants.[53] Autonomic nervous system problems are more likely to occur in preterm infants with acute and more-severe hypoxia.[54]

One strength of our study is that it included only term SGA infants without signs of placental dysfunction or congenital malformations, as low-risk SGA infants are defined in everyday clinical practice. No previous studies aimed at analyzing the effect of IUGR on neurobehavior reported the proportion of cases with placental insufficiency. This is a potential source of misinterpretation of results, because an unknown proportion of the newborns might have been exposed to a very prolonged period of intrauterine hypoxia. This study also has some limitations. Firstly, although NBAS assessment is a standard method for evaluating newborns' capacity to respond to the environment, reflecting brain maturation, it assesses only neurobehavior and not cognitive function.[55] However, several studies demonstrated correlations between neonatal neurobehavior and later neurocognitive development in preterm[13,14,24] and term[26,27] infants. Secondly, it could be argued the differences between SGA and AGA infants are likely to be clinically irrelevant, albeit statistically significant. However, because behavioral clusters are rated on a scale of 1 to 9, where 9 represents the best performance in some areas and 5 in others, a 1-point difference in absolute values represents a relative difference of 10% to 25%. Thirdly, it could be argued that the study design is prone to an expectation bias, because the examiners might have been influenced by the size of the infants during the neurobehavioral assessments. Within each study group, however, no significant correlations were found between birth weights and NBAS scores, except for organization of state in the SGA group. This makes it unlikely that this potential bias could explain the observed differences between SGA and AGA newborns in the other areas. In addition, our findings would be more valid if we had repeated the NBAS examinations to show that the behavior was consistent over time. Finally, mothers in the 2 study groups did not match with respect to sociodemographic conditions. It is likely that other factors associated with SGA also were different for these mothers. Although the effect of the study group on neurobehavior was adjusted with some of these potential confounders, we cannot rule out the possibility that some residual confounding is biasing our results.

CONCLUSIONS

We found poorer neurobehavioral competencies, suggesting delayed neurologic maturation, in a well-defined cohort of term SGA infants with no signs of placental insufficiency. Whether earlier markers of placental insufficiency or hypoxia could be used to identify high-risk infants and whether timely interventions for these infants could be of benefit require further investigation.

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Neurobehavior in Term, Small-for-Gestational Age Infants With Normal Placental Function

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Middle versus anterior cerebral artery Doppler for the prediction of perinatal outcome and neonatal neurobehavior in term small-for-gestational-age fetuses with normal umbilical artery Doppler

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KEYWORDS: anterior cerebral artery; Doppler; intrauterine growth restriction; middle cerebral artery; perinatal outcome

ABSTRACT

Objective *To evaluate whether anterior cerebral artery (ACA) Doppler ultrasonography is superior to middle cerebral artery (MCA) Doppler in the prediction of perinatal outcome and neonatal neurobehavior in term small-for-gestational age (SGA) fetuses with normal umbilical artery (UA) Doppler.*

Methods *MCA and ACA Doppler ultrasonography was performed in a cohort of SGA term fetuses with normal UA Doppler. Perinatal outcome and neonatal neurobehavioral performance were compared with a group of term appropriate-for-gestational age (AGA) infants. Neurobehavior was evaluated at 40 (± 1) weeks of corrected age with the Neonatal Behavioral Assessment Scale. Differences between the study groups were adjusted for potential confounding variables by multiple linear or logistic regression analyis.*

Results *A total of 199 newborns (98 SGA and 101 AGA) were included. Among the SGA fetuses, 28.6 and 17% had MCA and ACA redistribution, respectively. Cases with either type of redistribution had an increased risk for adverse outcome, with no differences in predictive performance between the two parameters. SGA fetuses with MCA redistribution compared with controls had an increased risk for abnormal neurobehavioral performance in motor (36 vs. 20%; adjusted* $P = 0.02$*) and state organization (25 vs. 17.5%; adjusted* $P = 0.03$*) areas. SGA fetuses with ACA redistribution had only an increased risk for abnormal neurobehavioral performance area in state organization compared with controls (30 vs. 17.5%; adjusted* $P = 0.021$*).*

Conclusion *In term SGA newborns with no signs of brain-sparing, ACA Doppler investigation does not provide any benefit over MCA in terms of the prediction of adverse perinatal outcome.* Copyright © 2010 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Small fetuses with normal umbilical artery (UA) Doppler ultrasound findings are currently defined as normal small-for-gestational-age (SGA) fetuses[1,2]. Earlier reports suggested that this diagnostic category might essentially contain constitutionally small fetuses[3], but recent evidence suggests that a substantial proportion of fetuses have true growth restriction[4]. Studies over the last decade have provided evidence that perinatal outcome may be significantly poorer in SGA fetuses[2–5,6]. Furthermore, there is an increased prevalence of abnormal neurobehavioral and neurodevelopmental tests in childhood, with similar features to those described for preterm children who had intrauterine growth restriction[4,7–9]. Since the identification of SGA fetuses with milder forms of growth restriction cannot be based on UA Doppler, the use of middle cerebral artery (MCA) ultrasonography might help to identify these cases[6,10,11]. Up to 20% of SGA fetuses have a reduced pulsatility index (PI) in the MCA, a sign that is associated with poorer perinatal outcome[6,11] and with an

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increased risk of abnormal neurobehavior at birth and at 2 years of age[7,12].

Recent studies suggest that the anterior cerebral artery (ACA) could be a better predictor of adverse neurological outcome than the MCA[13]. The ACA supplies cortical and subcortical areas of the frontal lobe and has been found to be vasodilated in a proportion of SGA fetuses with normal MCA Doppler[14]. Studies assessing the temporal evolution of the brain arteries in intrauterine growth restricted (IUGR) fetuses suggest that the ACA shows vasodilatory changes earlier than does the MCA[14,15]. Furthermore, tissue perfusion studies in fetuses with IUGR suggest that increased frontal perfusion is the earliest response to brain hypoxia[16].

The aim of our study was to see whether ACA Doppler investigation is superior to MCA Doppler investigation in the prediction of adverse perinatal outcome in term SGA fetuses with normal UA Doppler.

METHODS

Subjects

A prospective cohort was created of all suspected SGA fetuses (estimated fetal weight below the 10th centile[17] at a routine third-trimester ultrasound scan) referred to our unit between January 2007 and October 2008. Inclusion criteria were: (1) singleton pregnancy; (2) absence of congenital malformations or chromosomopathies; and (3) normal UA-PI[18] at inclusion (PI $< 95^{th}$ centile for gestational age). Exclusion criteria were: (1) abnormal UA-PI[18] during the study period; and (2) birth weight above the 10^{th} centile according to local standards[19]. Appropriate-for-gestational age (AGA) controls were defined as singleton neonates with birth weight between the 10^{th} and 90^{th} percentiles according to local standards[19]. Controls were selected from our general population, with previous adequate ultrasound estimated fetal weight[17], individually matched with cases for gestational age at inclusion ($\pm$ 1 week), corrected by first-trimester ultrasound[20]. Written consent was obtained in all cases and the study design was approved by the local ethics committee.

Management

For Doppler studies Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA) or Voluson 730 Expert (GE Medical Systems, WI, USA) ultrasound devices with 6–2- and 7–4-MHz curved array probes were used. In all cases ultrasound examination was performed by one of the three experienced observers (F.F., R.C.M. and D.O.). Pulsed Doppler parameters were generated automatically from three or more consecutive waveforms, with the angle of insonation as close to 0° as possible. A high-pass wall filter of 70 Hz was used to avoid artifacts. Umbilical Doppler investigations were performed at a free-floating cord loop, by means of image-directed pulsed and color Doppler. The circle of Willis was located by color Doppler imaging in the axial view of the fetal head at the level of the cerebral peduncles. MCA flow velocity waveforms were recorded at 1–2 cm from the circle of Willis, during the absence of fetal movements, at insonation angles of less than 30°. For the ACA, the Doppler gate was placed immediately after the origin of the ACA from the internal carotid artery. Either the MCA-PI or the ACA-PI values below the 5^{th} centile were considered indicative of cerebral blood flow redistribution and were reported as abnormal[18,21].

Only the last examination within 1 week of delivery was included in the analysis. Cases were managed at the discretion of an attending senior obstetrician, following standard management guidelines, who was blinded to the cerebral Doppler results. Induction of labor was performed by cervical ripening with prostaglandins for cases when either estimated fetal weight was below the 3^{rd} centile at term or an ultrasound estimated fetal weight below the 10^{th} centile was suspected at 40 weeks' gestation.

Neurobehavioral outcome

The Neonatal Behavioral Assessment Scale (NBAS) was used to prospectively assess all cases and controls at 40 ± 1 weeks of corrected age by one of three observers accredited by The Brazelton Institute (Harvard Medical School, Boston, USA). The observers were blinded to the study group and to Doppler status. The examination consisted of four behavioral areas rated on a scale of 1–9, where 9 is the best performance, except for some curvilinear scale items which, according to the manual, were rescored as linear on a 5, 6 or 8-point scale[22]. With the newborn between two feeds, in a small, quiet, semi-dark room at a temperature of between 22 and 27°C and in the presence of at least one parent, the following areas were analyzed: habituation (habituation to light, rattle, bell and tactile stimulation of the foot items); motor (which includes general tone, motor maturity, pull-to-sit, defensive movements and level of activity); social–interactive (which includes response to visual and acoustic stimuli); and state organization (which includes peak of excitement, rapidity of build-up, irritability and lability of states). The behavioral items were converted into percentiles according to normal curve references for our population[23], and each area was considered abnormal at a score below the 5^{th} percentile.

Statistical analysis

Student's *t*-test and Pearson's chi-squared test or Fisher's exact test were used to compare quantitative and qualitative data, respectively. Receiver–operating characteristics (ROC) curves were used to evaluate the diagnostic performance for adverse perinatal outcome of both arteries. Following standard methodology, neurobehavioral outcome was adjusted for smoking during pregnancy (no smoking; 1–9 cigarettes/day; 10+ cigarettes/day); labor induction; mode of delivery

(Cesarean section vs. vaginal delivery); gestational age at birth; gender; and postnatal days at evaluation by multiple linear or logistic regression analysis. Statistical analysis was performed using the SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and MedCalc 8.0 (MedCalc Software, Broekstraat, Belgium) statistical software.

RESULTS

A total of 118 SGA fetuses fulfilled the inclusion criteria. Seven were excluded because of birth weight above the 10th centile; none had an adverse perinatal outcome. The remaining 111 cases were matched at delivery with 111 AGA babies. Of these, the parents of six cases and seven controls later declined to participate in the neurobehavioral evaluation. Finally, in seven cases and three controls the evaluation was not considered satisfactory by the examiner owing to the absence of a sleeping state during the test. Thus, a final total of 199 babies (98 SGA and 101 AGA) were tested.

Table 1 compares demographic characteristics by study group. Women in the SGA group had a lower body mass index and showed a non-significant trend to belong to a lower socioeconomic level.

Table 2 describes the clinical characteristics and perinatal outcome of the SGA group according to the MCA or ACA redistribution. A total of 28 (29%) and 17 (17%) SGA fetuses had MCA and ACA Doppler redistribution, respectively. It is of note that in 14 cases MCA and ACA simultaneously showed redistribution. Whereas ACA-redistributed fetuses had a lower birth weight than non ACA-redistributed fetuses, no differences were observed between MCA-redistributed and non MCA-redistributed fetuses. Both MCA and ACA redistribution accounted for significant differences in the incidence of Cesarean section, and only the MCA significantly differentiated cases at risk for fetal distress. Figure 1 shows the ROC curves of MCA and ACA for the prediction of adverse outcome. Both parameters showed significant areas under the curve (0.71 (CI, 0.6–0.81) for ACA-PI and 0.72 (CI, 0.61–0.82) for MCA-PI), and pairwise comparison of both areas showed no significant differences between the two parameters ($P = 0.82$).

Neurobehavior was assessed at 6.2 (±4.9) and 14.4 (±9.04) days of age in the AGA and SGA groups, respectively. Table 3 shows the NBAS score by area and study group, where motor, social and state organization differed significantly between the AGA and SGA groups. These differences remained significant after adjustment for potential confounders for motor and state organization. Figure 2 summarizes the NBAS scores for AGA and SGA fetuses with and without redistribution according to MCA and ACA Doppler. Among SGA fetuses, cases with MCA redistribution showed significantly lower NBAS in motor (adjusted $P = 0.03$) and state organization (adjusted $P = 0.025$) areas than the SGA fetuses without redistribution. On the other hand, a non-significant trend towards lower scores was observed in ACA-redistributed fetuses only in the state organization area.

Figure 3 shows the frequency of abnormal NBAS. Among SGA fetuses, cases with MCA redistribution showed an increased risk for abnormal motor (36 vs. 20%; adjusted $P = 0.023$) (adjusted OR 3.94

Table 1 Population demographic characteristics

Characteristic	*Control group (n = 101)*	*SGA group (n = 98)*	P*
MA at delivery (years)	31.8 ± 4.9	31.2 ± 5.1	0.44
Primiparous	61.0	67.3	0.35
Caucasian	78.2	78.6	0.95
Male: female ratio of fetuses	49.5 : 50.5	54.1 : 45.9	0.52
BMI at booking (kg/m^2)	23.4 ± 3.7	21.99 ± 3.5	0.01
Height (cm)	162.6 ± 6.3	160.5 ± 7.3	0.06
Low socioeconomic level†	9.3	18.5	0.06
Smoker	15	19.4	0.42
GA at birth (days)	279 ± 7.9	265 ± 9.5	< 0.001
Birth weight (g)	3339 ± 390.7	2382 ± 263.7	< 0.001
Pre-eclampsia	0	8.2	0.003
Cesarean section	25	37.1	0.06
Induction of labor	18	71.1	< 0.001
Composite adverse outcome‡	19.8	34.7	0.02

Values given as mean ± SD or% *Student's *t*-test for independent samples, Pearson's χ^2 test or Fisher's exact test, as appropriate. †Routine occupations, long-term unemployment or never worked (UK National Statistics Socio-Economic Classification[34]). ‡Composite adverse outcome based on the presence of at least one of the following: intervention for fetal distress, umbilical artery pH < 7.10, need for neonatal resuscitation or admission to neonatal intensive care unit. BMI, body mass index; GA, gestational age; MA, maternal age.

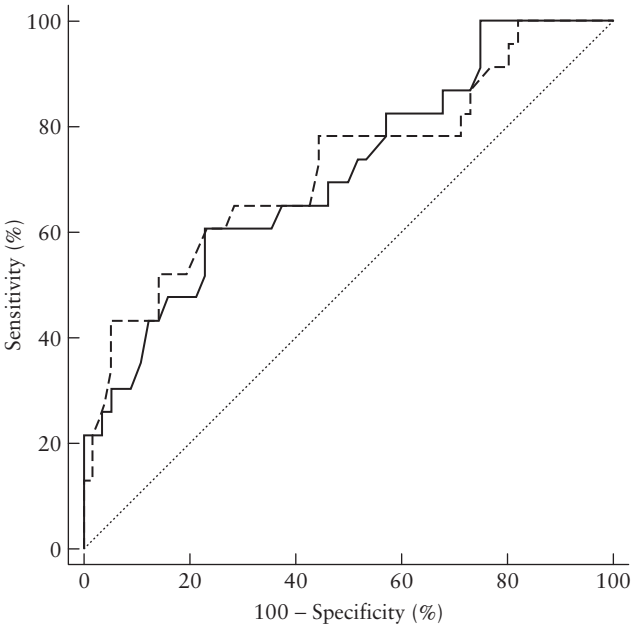

Figure 1 Receiver–operating characteristics curves of middle (– – –) and anterior (———) cerebral arteries for the prediction of adverse outcome.

Table 2 Clinical characteristics and perinatal outcome of small-for-gestational age neonates according to middle (MCA) or anterior (ACA) cerebral artery redistribution

	MCA			*ACA*		
Characteristic	*Not redistributed (n = 70)*	*Redistributed (n = 28)*	P*	*Not redistributed (n = 81)*	*Redistributed (n = 17)*	P†
GA at birth (days)	266 ± 9.9	265 ± 8.7	1	266 ± 9.5	262 ± 8.7	0.1
Birth weight (g)	2411 ± 246.6	2310 ± 294.4	0.54	2426 ± 235.2	2173 ± 296.5	0.01
Birth weight < 3rd centile	31 (44.3)	18 (64.3)	0.07	39 (48.1)	10 (58.8)	0.42
Head circumference (cm)	32.7 ± 1	32.3 ± 1	0.41	32.6 ± 1	32.3 ± 1	0.99
Pre-eclampsia	5 (7.1)	3 (10.7)	0.68	5 (6.2)	3 (17.6)	0.14
Induction of labor	52 (74.3)	17 (60.7)	0.14	58 (71.6)	10 (58.8)	0.25
Cesarean section	18 (25.7)	18 (64.3)	0.001	24 (29.6)	12 (70.6)	0.002
Intervention for fetal distress	10 (14.3)	12 (42.9)	0.006	15 (18.5)	7 (41.2)	0.06
5-min Apgar score < 7	0	0	1	0	0	1
Umbilical artery pH < 7.10	4 (5.7)	2 (7.1)	0.65	4 (4.9)	2 (11.8)	0.26
Neonatal resuscitation	2 (2.9)	4 (14.3)	0.09	3 (3.7)	3 (17.6)	0.07
NICU admission	1 (1.4)	2 (7.1)	0.2	1 (1.2)	2 (11.8)	0.08
Composite adverse outcome‡	14 (20.0)	13 (46.4)	0.01	20 (24.7)	8 (47.1)	0.05

Values given as mean ± SD or %. *P* determined by Student's *t*-test for independent samples, Pearson's χ^2 or Fisher's exact test, as appropriate: *between MCA groups; †between ACA groups. ‡Composite adverse outcome based on the presence of at least one of the following: intervention for fetal distress, umbilical artery pH < 7.10, need for neonatal resuscitation or admission to NICU. GA, gestational age; NICU, neonatal intensive care unit.

(95% CI, 1.21–12.8)) and state organization (25 vs. 17.5%; adjusted $P = 0.025$) (adjusted OR 4 (95% CI, 1.19–13.3)) areas than the SGA without redistribution. Finally, the frequency of abnormal NBAS significantly differed between cases with and without ACA redistribution only for the organization of the state area (30 vs. 17.5%; adjusted $P = 0.021$) (adjusted OR 5 (95% CI, 1.28–20)).

Table 3 Neurobehavioral scores by area and study group

Parameter	*Control group (n = 101)*	*SGA group (n = 98)*	P*	P†
Habituation	6.85 ± 1.6	6.49 ± 1.67	0.18	0.93
Social	6.72 ± 1.4	5.59 ± 1.41	< 0.001	0.16
Motor	5.73 ± 0.75	5.29 ± 0.84	< 0.001	0.04
State organization	4.39 ± 1.08	3.86 ± 1.28	0.002	0.012

Values given as mean ± SD. *Student's *t*-test. †Adjusted for smoking during pregnancy, mode of delivery, gestational age at birth, gender and postnatal days at evaluation by linear regression. SGA, small-for-gestational age.

DISCUSSION

This is the first study to explore the capacity of the ACA to predict perinatal and neurobehavioral outcome in term SGA fetuses. Contrary to the hypothesis of the study, we found no differences between MCA and ACA redistribution in terms of association with perinatal outcome or neonatal neurobehavioral performance. Therefore, MCA seems to remain unchallenged as a primary clinical tool for evaluating term SGA fetuses without signs of placental insufficiency.

We have previously demonstrated that in preterm IUGR fetuses ACA vasodilation takes place earlier than MCA vasodilation[15]. Dubiel *et al.*[14] reported similar results in pregnancies with pregnancy-induced hypertension. These findings are not in line with the results of the present study. However, different population characteristics are likely to explain this inconsistency. The above studies included a substantial proportion of early-onset growth restricted fetuses with abnormal UA Doppler. Since gestational age was considerably different we cannot exclude differences in the temporal patterns in the adaptation to chronic hypoxia due to maturity changes in brain hemodynamic regulation. Indeed, Dubiel *et al.*[14] reported a better correlation of ACA-PI with adverse perinatal outcome only for cases before 32 weeks' gestation. For those delivering beyond that gestational age MCA-PI and ACA-PI showed a similar association with perinatal outcome. Also in line with this reasoning, other studies have found no differences between ACA and MCA in response to acute hypoxia in term SGA fetuses[24]. As a secondary explanation, the considerable degree of systemic fetal hemodynamic adaptation to hypoxia in early-onset IUGR, which is not present in term SGA, could also influence brain hemodynamics in ways that we cannot interpret.

This study confirms previous reports suggesting that late-onset SGA is associated with an increased risk of abnormal neonatal neurobehavior and neurodevelopment in childhood[7,13,25–27]. Furthermore, we have previously described that in late-onset SGA fetuses, MCA redistribution differentiates those at risk of long-term suboptimal neurodevelopment[7]. In a recent study, Roza *et al.*[13] showed that fetal ACA redistribution could be superior to MCA in the prediction of neurobehavioral problems in childhood. In the present study MCA and ACA Doppler were similarly associated with poorer neonatal neurobehavior. These findings are difficult to compare with those

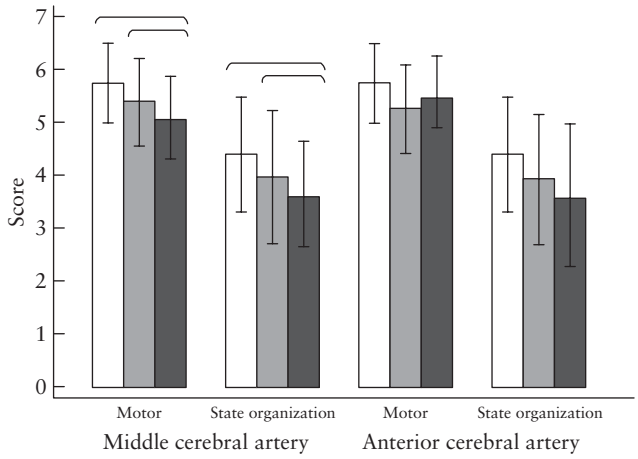

Figure 2 Neurobehavioral scores (mean and SD) according to area and study group: appropriate-for-gestational age fetuses (□), small-for-gestational age (SGA) fetuses without redistribution (▒) and SGA fetuses with redistribution (■). Long brackets indicate significant adjusted linear trend; short brackets indicate significant adjusted difference between SGA groups.

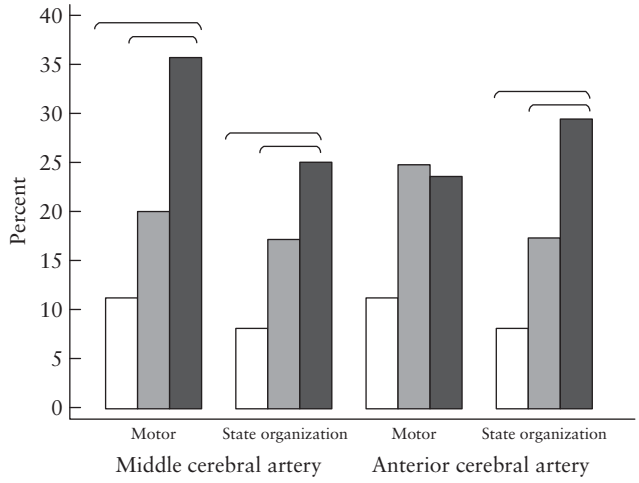

Figure 3 Frequency of abnormal neurobehavioral performance according to area and study group: appropriate-for-gestational age fetuses (□), small-for-gestational age (SGA) fetuses without redistribution (▒) and SGA fetuses with redistribution (■). Long brackets indicate significant adjusted linear trend; short brackets indicate significant adjusted difference between SGA groups.

of Roza *et al*. for two reasons. Our results refer exclusively to neonatal neurobehavior and therefore they do not exclude the possibility that long-term assessment could demonstrate a higher sensitivity of ACA in predicting poor neurodevelopment. A second important difference is that the study of Roza *et al*.[13] was based on a large cohort that included all pregnancies, not only SGA fetuses. Therefore, the value of ACA for the prediction of long-term outcome in small fetuses remains to be evaluated.

The findings of this study may appear inconsistent with evidence pointing to a higher vulnerability of frontal areas in fetuses with IUGR[9,28–31]. However, the arteries we evaluated provide the blood supply to ill-defined anatomical areas with a marked component of vascular shunting. Tissue perfusion depends on local arteriolar phenomena and therefore the data cannot be used to infer which territories are specifically affected by vascular changes. Using the fractional moving blood volume estimate to assess tissue perfusion we have previously suggested that increased perfusion in the frontal lobe seems to be the earliest response to hypoxia, rather than changes in basal ganglia perfusion[16]. It is not known whether evaluation of tissue perfusion can detect subtle differences in blood perfusion between cerebral areas in this population of late-onset SGA. Studies evaluating the potential role of direct measurements of perfusion towards different brain regions in this population are under way.

This study has some limitations. First, since Doppler ultrasonography was not performed in the AGA babies, we cannot rule out the possibility that some fetuses in this group might have shown brain redistribution. However, this potential bias would be conservative, attenuating the differences between AGA and SGA fetuses. Secondly, although NBAS is a gold standard for the evaluation of the neonate's capacity to respond to the environment, reflecting brain maturation, it only assesses neurobehavioral and not cognitive function. However, several studies have demonstrated the correlation between neonatal neurobehavioral performance and later neurocognitive development[8,9,27,32,33]. We admit that socioeconomic status may have confounded the association between SGA and abnormal neurobehavior. However, although socioeconomic status is a major determinant of postnatal neurobehavior during childhood its influence in the neonatal period is still minimal if smoking is accounted for in our population[23]. Thirdly, any clinical study on the surveillance of fetal growth needs to be interpreted with caution because it can never be entirely blinded, and the clinical management is influenced by the antenatal findings. This work-up bias could account for differences between AGA and SGA. However, the clinicians attending the deliveries were unaware of the MCA or ACA Doppler status. Thus this potential bias could not explain differences between the two groups of SGA fetuses. Besides, the neurobehavioral assessment could also have been biased by the examiners' knowledge of perinatal factors. However, since these examiners were also blinded to the antenatal Doppler findings and because birth weight was similar between the two groups, this expectation bias is unlikely to explain differences between the Doppler groups.

In conclusion, the findings of this study do not support the inclusion of ACA Doppler investigation in the management of term SGA fetuses.

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