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Angiogenic factors at diagnosis of late-onset small-for-gestational age and histological placental underperfusion

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ABSTRACT

Objective: This study was designed to explore the association between angiogenic factors levels at diagnosis of small-for-gestational age (SGA) and placental underperfusion (PUP).
Methods: In a cohort of SGA singleton pregnancies, each delivered at >34 weeks, uterine (UtA), umbilical (UA), and middle cerebral (MCA) arteries were evaluated by Doppler upon diagnosis of SGA status. In addition, maternal circulating concentrations of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were assayed by ELISA, and each placenta was evaluated for histologic signs of PUP using a hierarchical and standardized classification system. Logistic regression was applied to analyze independent relationships (at diagnosis) between angiogenic factors and Doppler parameters.
Results: A total of 122 suspected SGA pregnancies were studied, 70 (57.4%) of which ultimately met PUP criteria. In this group, 85 placental findings qualified as PUP. Both mean UtA pulsatility index z-values (1.26 vs. 0.84; $p = 0.038$) and PlGF multiples of normal median (0.21 vs. 0.55; $p = 0.002$) differed significantly in pregnancies with and without PUP, respectively. By logistic regression, PlGF alone was independently predictive of PUP (OR = 0.11 [95% CI 0.025–0.57]; $p = 0.008$).
Discussion: Histologic placental abnormalities in term SGA neonates reflect latent insufficiency in uteroplacental blood supply. The heightened risk of adverse perinatal outcomes in this context underscores a need for new Doppler or biochemical prenatal markers of placental disease. Angiogenic factors may be pivotal identifying SGA neonates.
Conclusions: Diminished circulating levels of placental growth factor, determined upon discovery of SGA status, are associated with histologic evidence of PUP.

1. Introduction

Small-for-gestational age (SGA) is defined by a birth weight (BW) below 10th centile, commonly used as a proxy to identify fetuses at risk of adverse outcomes [1–4]. SGA births typically are viewed as constitutionally small neonates displaying satisfactory perinatal outcomes [3,5]. However, this tenet is being challenged by recent studies attesting to poorer perinatal outcomes, suboptimal neurodevelopment, and higher postnatal cardiovascular risk in late-onset SGA babies, compared with adequate-for-gestational age (AGA) newborns [6–8]. Such findings support the concept that a subset of.

SGA fetuses in actuality reflect late-onset fetal growth restriction (FGR), where placental insufficiency goes undetected by umbilical artery (UA) Doppler. Therefore, it is impossible to distinguish those particular neonates whose perinatal courses are at higher risk [3,4,6,9,10]. Latent placental insufficiency thus is a key aspect in differentiating true FGR from constitutional smallness.

The placenta is generally regarded as a passive sieve, or conduit that does not provide clinically relevant information about the

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Abbreviations

SGA	small-for-gestational age
FGR	fetal growth restriction
GA	gestational age
UA	umbilical artery
CPR	cerebroplacental ratio
MCA	middle cerebral artery
UtA	uterine artery
PI	Doppler pulsatility index
BW	birth weight
EFW	estimated fetal weight
PUP	placental underperfusion
PlGF	placental growth factor
sFlt-1	soluble fms-like tyrosine kinase-1
PE	preeclampsia
MoM	multiples of normal median

condition of the fetus, although specific pattern of FGR have been associated with it [11]. Hypoxic/ischemic injury for maternal and fetal vascular obstructive lesions due to placental underperfusion (PUP) is clearly linked to adverse outcomes [12,13]. In early FGR, placental signs of maternal/fetal underperfusion have long been recognized as contributors [14,15]. Histologic manifestations of underperfusion have correlated with observed deficits in 2-year mental development [16]. A recent study similarly documents signs of PUP in roughly two-thirds of placentas from late SGA births with normal UA Doppler studies [17], implicating latent placental insufficiency. Even more recently, we have shown that placental signs of PUP in late SGA infants correspond with poorer perinatal outcomes [18] and compromised neurodevelopment in early infancy [19]. As such, it appears that PUP is a form of placental insufficiency that is worth targeting in clinical practice.

In the setting of uteroplacental insufficiency, angiogenic imbalance is common, with up-regulation of soluble fms-like tyrosine kinase 1 (sFlt-1) and down-regulation of placental growth factor (PlGF) [20,21]. The value of angiogenic biomarkers for predicting and characterizing early-onset preeclampsia (PE) and FGR is underscored in a large number of studies [20,22–24]. Likewise, several recent reports have provided evidence that angiogenic markers pertain to PE and FGR developing late in pregnancy as well [22–24]. One small series [25] found reduced maternal plasma levels of PlGF in late SGA births with placental abnormalities; although, instances of overt placental dysfunction (determinable by UA Doppler) may have been included. Otherwise, no prior investigations of late-onset SGA births have addressed PUP in terms of angiogenic factors, analyzing data on fetomaternal Doppler parameters. Herein, the relationship between angiogenic factors and PUP in late-onset SGA neonates is subsequently explored.

2. Materials and methods

2.1. Study population

At routine third-trimester (30- to 34-week) scan, a cohort of consecutive singleton pregnancies was generated, each requiring EFW <10th percentile (by local standards) [26]. All women were normotensive at recruitment. Fetal age was dated by crown-rump length at first trimester [27], and EFW was calculated via Hadlock formula [28]. Any qualifying pregnancies complicated thereafter by pregestational diabetes, premature rupture of membranes, chorioamnionitis, spontaneous preterm delivery, aneuploidies, or major structural abnormalities were excluded. The study protocol was approved by the local Ethics Committee, and written informed consent was granted by all enrollees (IRB 2012/7154).

Data collection and definition of terms Maternal characteristics, including age, ethnicity, body mass index (BMI), parity, smoking status, known chronic disease (i.e., hypertension, diabetes mellitus, renal disease, and autoimmune disorders), and obstetric history (including complications) were recorded in the hospital database upon enrollment.

EFW <10th percentile (by local standards) and Doppler study abnormalities (cerebroplacental ratio [CPR] <5th percentile or mean uterine artery [mUtA]-pulsatility index [PI] >95th percentile) constituted FGR criteria [26,29–31].

In accordance with guidelines of the International Society for the Study of Hypertension in Pregnancy [32], PE was stipulated by two elevated blood pressure readings (systolic ≥140 mmHg or diastolic ≥90 mmHg) ≥4 h apart in previously normotensive females at >20 weeks' gestation; plus proteinuria ≥300 mg in 24 h [32].

Non-reassuring fetal status was defined by an abnormalities in heart rate and scalp blood pH during intrapartum fetal monitoring [33].

2.2. Ultrasonography and Doppler assessment

Prenatal Doppler ultrasound examinations were performed using either a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA) or a General Electric Voluson E8 (GE Medical Systems, Zipf, Austria) ultrasound machine equipped with a 6-2 MHz linear curved-array transducer. Doppler recordings were obtained in the absence of fetal movements and with voluntarily suspended maternal breathing.

Spectral Doppler parameters were calculated automatically from three or more consecutive waveforms at an angle of insonation approximating 0° as feasible. Uterine artery (UtA) evaluation, involved lower abdominal quadrants, with medial angling of the probe, and color Doppler imaging was used to locate vessels at apparent junctions with external iliac artery. As an average of right and left values, UtA-PI determinations >95th percentile were considered abnormal [29]. UA-PI was obtained from a free floating cord loop (insonation angle <30°) and was interpreted as normal at <95th percentile for gestational age (GA) [34]. Middle cerebral artery (MCA)-PI was measured at point of origin from circle of Willis in a transverse view of fetal head [29]. CPR (the ratio of MCA-PI to UA-PI) was considered abnormal at <5th percentile [31]. For the purpose of this study, only the first Doppler examination at the time of SGA diagnosis was considered.

2.3. Management

Indications for labor induction were as follows: 1) persistent (12 h apart) MCA-PI <5th percentile or UA PI > 95th percentile at >37 weeks' gestation [34]; 2) UtA-IP >95th percentile at >39 weeks' gestation [29,30,34]; and 3) 40 weeks' gestation, even with normal Doppler parameters. Induction of labor was achieved by cervical ripening with a slow-release prostaglandin E2 vaginal pessary (10 mg). If onset of labor did not follow within 18 h, oxytocin induction was initiated. Each delivery was attended by a staff obstetrician blinded to angiogenic factor determinations. Continuous fetal heart monitoring was performed during labor, graded (normal, suspicious, or abnormal) in terms of presence, type, and length of decelerations; bradycardia; tachycardia; and assessment of variability (detailed elsewhere) [34]. In instances of two or more suspicious variables or with at least one abnormal parameter unresponsive to digital fetal scalp stimulation, fetal scalp blood pH was tested. Any pH value <7.15 or <7.20 on two attempts 30 min apart was regarded as abnormal [32]. If cervical conditions 185 prevented sampling of 186 fetal scalp blood. Cesarean section was done for non-reassuring fetal status, based on intrapartum fetal abnormalities in heart rate [33,35] and scalp blood pH. Cesarean delivery for non-reassuring fetal status was indicated for a persistently abnormal heart tracing after pessary withdrawal, oxytocin suspension, and a 10-minute intravenous infusion of ritrodine (200 μg/min).

2.4. Blood sampling and testing procedures

Venous blood samples were obtained upon establishing SGA status. Serum separated by centrifugation (3000× *g* for 10 min) and stored at −80 °C ultimately was subjected to PlGF and sFlt-1 quantification by enzyme-linked immunosorbent assay (ELISA) (R&D Systems Europe Ltd, Abingdon, UK). The assay kits used detect only free analytes, not the bound fractions. All samples were collected, handled, and stored under the same conditions and tested in duplicate. Plate-to-plate variability was controlled through independent plate-specific standard curves. Test sera and matched controls were simultaneously and blindly run on the same plates. Intra-assay (<5%) and inter-assay (10%) precision of all kits were consistent. Linear regression coefficients of standard curves were never <0.99. Maternal serum levels of PlGF and sFlt-1 were expressed as multiples of normal median (MoM) for GA. Normal median reference values were calculated from controls, utilizing reciprocal-linear regression in *R* [36].

2.5. Placental evaluation

Placental examinations adhered to standard laboratory protocol. Fresh and trimmed (after removal of the membranes, cord, and any blood clots, the placenta) placental weight was recorded. Trimmed placental weight centiles were assigned based on GA-specific placental weight charts [37]. The fetoplacental weight ratio (BW/fresh placental weight) was also expressed as a percentile, drawn from GA-specific ranges [38].