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Antecedents and early correlates of high and low concentrations of angiogenic proteins in extremely preterm newborns

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ABSTRACT

Background: To identify the antecedents and very early correlates of low concentrations of angiogenic proteins in the blood of extremely preterm newborns during the first postnatal month.
Methods: Using multiplex immunoassays we measured the concentrations of vascular endothelial growth factor A (VEGF), VEGF receptor-1 (VEGFR-1), VEGF receptor-2 (VEGFR-2), placenta growth factor (PIGF), and angiopoietins 1 and 2 (Ang-1, Ang-2), as well as 21 other proteins in blood spots collected on postnatal days 1 (N = 1062), 7 (N = 1087), 14 (N = 989), 21 (N = 940) and 28 (N = 880) from infants born before the 28th week of gestation. We then sought the protein-concentration correlates of concentrations in the top and bottom quartile for gestational age and day the specimen was collected.
Results: Children who were delivered for medical indications and those who were severely growth restricted were more likely than others to have low day-1 blood concentrations of VEGF, VEGF-R2, Ang-1, and PIGF. Systemic inflammation accompanied top quartile concentrations of every one of the 6 angiogenic proteins.
Conclusions: Low day-1 concentrations of most angiogenic proteins are associated with disorders linked to placenta insufficiency/dysfunction. High concentrations, on the other hand, are associated with systemic inflammation throughout the first postnatal month.

1. Introduction

Placental dysregulation of angiogenic growth factors has been implicated in the etiology of preeclampsia and fetal growth restriction [1]. In very preterm newborns, deficiency or excess of some of these proteins have been implicated in promoting damage or repair of damage to the bowel [2], brain [3], lung [4], and retina [5]. What remains unknown is to what extent low blood levels of angiogenic proteins in the very preterm newborn reflect preeclampsia and fetal growth restriction.

These angiogenic proteins, including vascular endothelial growth factor (VEGF) [6], placenta growth factor (PIGF) [7], VEGF-receptor 1(VEGFR-1)(sFlt-1) [8], VEGF-R2 [9], as well as angiopoietins 1 and 2 (Ang-1, Ang-2) [10], have inflammatory characteristics. In addition, the placenta is capable of synthesizing cytokines and other inflammatory proteins [11,12], growth factors [13], including insulin growth factor-1 (IGF-1) and its binding protein-1 (IGFBP-1) [14], as well as proteins with neurotrophic properties [15,16]. What remains unknown is to what extent elevated concentrations of angiogenic proteins accompany elevated concentrations of proteins with inflammation-related and neurotrophic properties.

The ELGAN Study of infants born before the 28th week of gestation obtained blood specimens on postnatal days 1, 7, 14, 21, and 28, and measured the concentrations in these specimens of VEGF, VEGFR-1, VEGFR-2, PIGF, Ang-1 and Ang-2, as well as the concentrations of 21 other proteins. This information allowed us to assess to what extent neonatal concentrations of angiogenic proteins are related to pregnancy disorders and postnatal inflammation.

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2. Methods

The ELGAN (extremely low gestational age newborn) study is a multi-center prospective, observational study of the risk of structural and functional neurologic disorders in infants born before the 28th week of gestation [17]. A total of 1506 infants born before the 28th week of gestation were enrolled during the years 2002–2004. Enrollment and consent procedures for this follow up study were approved by the institutional review boards of all participating institutions.

2.1. Pregnancy variables

The clinical circumstances that led to each maternal admission and ultimately to each preterm delivery were operationally defined using both data from an interview of the mother shortly after delivery and data abstracted from the medical record [18]. Each mother/infant pair was assigned to the category that described the primary reason for the preterm delivery. Maternal indication (invariably preeclampsia) was defined as new onset hypertension and proteinuria of sufficient severity to warrant delivery for the gravida's wellbeing. Presentations under the category of fetal indication included severe intrauterine growth restriction based on antepartum ultrasound examination, non-reassuring fetal testing, oligohydramnious, and Doppler abnormalities of umbilical cord blood flow.

2.2. Newborn variables

The gestational age estimates were based on a hierarchy of the quality of available information. Most desirable were estimates based on the dates of embryo retrieval or intrauterine insemination or fetal ultrasound before the 14th week (62%). When these were not available, reliance was placed on a ≥ 14 weeks fetal ultrasound (29%), date of last menstrual period (7%), and gestational age recorded in the log of the NICU (1%). The birthweight *Z*-score is the number of standard deviations the infant's birthweight is above or below the median weight of infants at the same gestational age in a standard data set [19].

2.3. Blood spot collection and storage

Drops of blood collected for clinical indications. The specimens about to be discarded were blotted on filter paper on the first postnatal day (N = 1121, range: 1–3 days), the 7th postnatal day (N = 1142; range: 5–8 days), the 14th postnatal day (N = 1033; range: 12–15 days), the 21st postnatal day (N = 940; range: 19–23 days), and the 28th postnatal day (N = 880; range: 26–29). All blood was from the remainder of specimens obtained for clinical indications. Dried blood spots were stored at − 70 °C in sealed bags with a desiccant until processed.

2.4. Protein measurements

Details about the elution of proteins from the blood spots are provided elsewhere [20]. The Genital Tract Biology Laboratory at the Brigham and Women's Hospital in Boston Massachusetts eluted all blood spots as previously described and measured all proteins reported here. The concentrations of the following proteins were measured with the Meso Scale Discovery (MSD) electrochemiluminescence multiplex platform and Sector Imager 2400: C-Reactive Protein (CRP), serum amyloid A (SAA), Myeloperoxidase (MPO), Interleukin-1 β (IL-1β), IL-6, IL-6 Receptor (IL-6R), Tumor Necrosis Factor-α (TNF-α), TNF Receptor-1 (TNFR-1), TNFR-2, IL-8 (CXCL8), Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES; CCL5), Intercellular Adhesion Molecule − 1 (ICAM-1; CD54), Vascular Cell Adhesion Molecule-1 (VCAM-1; CD106), VEGF, VEGFR-1, VEGFR-2, IGF-1, IGFBP-1, thyroid stimulating hormone (TSH), Metalloproteinase (MMP)-9, and erythropoietin (EPO). The MSD platform has been validated by comparisons with traditional ELISA [21], and produces measurements that have high content validity [22–26].

A multiplex immunobead assay manufactured by R & D Systems (Minneapolis, MN) and a MAGPIX Luminex reader (R & D Systems) were used to measure Ang-1, Ang-2, PIGF, Neurotrophin-4 (NT-4), Brain Derived Neurotrophic Factor (BDNF), and basic Fibroblastic Growth Factor (bFGF). ELISA (R & D Systems) was used to measure IGF-1.

The total protein concentration in each eluted sample was determined by BCA assay (Thermo Scientific) using a multi-label Victor 2 counter (Perkin Elmer) and the measurements of each specific analyte were normalized to mg total protein.

The concentrations of inflammation-related and angiogenic-related proteins in the ELGAN Study varied with gestational age, and with the postnatal day of collection. Consequently, we divided our sample into 15 groups defined by gestational age category (23–24, 25–26, 27 weeks), and postnatal day of blood collection (1, 7, 14, 21 and 28). Because we were interested in the contribution of both high and low concentrations, and the concentrations of most proteins did not follow a normal distribution, the distribution of each protein's concentration was divided into quartiles among children in each of the 15 groups (3 gestational age groups, 5 collection days).

2.5. Data analyses

In light of the literature that supports the view that an abundance of individual angiogenic proteins might promote brain damage and/or repair [27,28], we wanted to identify the antecedents and correlates of top quartile concentrations. We specifically tested the hypothesis that infants delivered for maternal or fetal indications are no more likely than their peers to have a protein concentration in the bottom quartile on each day, and the hypothesis that infants with severe fetal growth restriction (birth weight *Z*-score < -2) are no more likely than their normal birth weight peers (birth weight Z-score ≥ -2) to have a protein concentration in the bottom quartile on each day.

Both hypotheses were tested with logistic regression models that included variables for top quartile concentrations so that we could also see if elevated concentrations were associated with decreased risks of indicated delivery and severe fetal growth restriction. These models, which had children in the middle two quartiles as the referent group allowed us to calculate odds ratios and 95% confidence intervals (Tables 1 and 2).

Because angiogenic proteins appear to be involved in inflammation, we wanted to explore the relationships between elevated concentrations of angiogenic factors and high concentrations of inflammation-related proteins. Consequently, our second null hypothesis postulates that postnatal systemic inflammation is not associated with top quartile concentrations of angiogenic proteins. Our logistic regression models adjusted for 3 variables: indicated delivery only, birth weight *Z*-score < -2 only, and both indicated delivery and birth weight Z-score < -2 (Tables 3–8).

3. Results

The subjects of this report had blood collected for clinical indications on postnatal days 1(N = 1121), 7 (N = 1142), 14 (N = 1033), 21 (N = 940) and 28 (N = 880).

3.1. Maternal and fetal indications for delivery (Table 1)

Children delivered for a medical (maternal or fetal) indication were at increased risk of a bottom quartile concentration of VEGF on days 1 and 7, and at reduced risk of a top quartile concentration of VEGF on days 1 and 7. A similar pattern of increased risk of a bottom quartile concentration on one day and a reduced risk of a top quartile concentration on the same day was seen for Ang-1 on day 1.