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REGULAR ARTICLE

Perinatal systemic inflammatory responses of growth-restricted preterm newborns

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ABSTRACT

Aim: To compare the early post-natal pattern of systemic inflammation in growth-restricted infants born before the 28th week of gestation to that of appropriately grown peers.

Methods: We measured the concentrations of 25 inflammation-related proteins in blood spots collected from 939 newborns during the first 2 post-natal weeks. We calculated the odds ratios (99% confidence intervals) that concentrations would be in the highest quartile.

Results: Severely growth-restricted infants (birth weight *Z*-score <−2) were not at increased risk of systemic inflammation shortly after birth. On post-natal day 14, however, they were significantly more likely than their peers to have a CRP, IL-1β, IL-6, TNF-α, IL-8, MCP-4, ICAM-1, ICAM-3, E-SEL, MMP-9, VEGF-R2 and/or IGFBP-1 concentration in the *highest* quartile. These increased risks could not be attributed to delivery indication, bacteremia or duration of ventilation.

Conclusion: Growth-restricted preterm newborns appear to be at increased risk of elevated concentrations of inflammation-associated proteins by post-natal day 14.

INTRODUCTION

Growth-restricted preterm newborns are at increased risk of systemic inflammation (1). Some of this increased risk might be a consequence of their greater tendency to be exposed to inflammatory stimuli, including sepsis/bacteremia (2) and prolonged ventilation (3). But what if some of the systemic inflammation that characterizes the growth-restricted, preterm newborn is beyond what might be expected in light of obvious inflammatory stimuli? What if the systemic inflammation is an early indicator of the 'foetal programming' processes that lead to later-life increased risk of diseases such as obesity, type 2 diabetes and atherosclerotic cardiovascular disease? (4) While each of these diseases is believed to have a prominent systemic inflammatory component in the adult (5), we are not aware of evidence that the growth-restricted foetus or newborn is at increased risk of systemic inflammation. If the adult consequences of foetal growth restriction are associated with increased systemic inflammation, but the associated intrauterine environment is not characterized by inflammation, then when does the post-natal inflammation first become manifest?

Abbreviations

E-SEL, E-selectin (CD62E); ICAM-1, Intercellular Adhesion Molecule -1 (CD54); ICAM-3, Intercellular Adhesion Molecule -3 (CD50); IGFBP-1, Insulin-like Growth Factor Binding Protein-1; IL-1β, Interleukin-1β; IL-6-6R, Interleukin-6 & Receptor; IL-8, Interleukin-8 (CXCL8); I-TAC, Interferon-inducible T-cell Alpha-Chemoattractant (CXCL11); MCP-1 -4, Monocyte Chemotactic Protein-1 & -4 (CCL2, CCL13); MIP-1β, Macrophage Inflammatory Protein-1β (CCL4); MMP-1 – 9, Matrix Metalloproteinase-1 & -9; MPO, Myeloperoxidase; RANTES, Regulated upon Activation, Normal T-cell Expressed, and Secreted (CCL5); SAA, Serum Amyloid A; TNF-R1 –R2, Tumour Necrosis Factor Receptor-1 & -2; TNF-α, Tumour Necrosis Factor-α; VCAM-1, Vascular Cell Adhesion Molecule-1 (CD106); VEGF-R1 – R2, Vascular Endothelial Growth Factor Receptor-1 & -2; VEGF, Vascular Endothelial Growth Factor.

METHODS

Details about the ELGAN Study are presented elsewhere (6,7). Inflammation-related proteins were measured only in the blood of children who might have had the neurological

Key notes

- Among infants born before 28 weeks, those that were severely growth restricted (Z-score <-2) were not at increased risk of systemic inflammation at birth.
- By post-natal day 14, however, severely growth restricted infants demonstrated increased concentrations of inflammation associated proteins.
- This increase could not be attributed to delivery indication, bacteremia or duration of ventilation.

dysfunctions that were the focus of the ELGAN Study. Thus, the sample for the analyses presented here consists of the 939 children who had a developmental assessment at age 2 years. Delivery indications and foetal growth parameters were equivalent in both the protein measured and unmeasured groups. Drops of blood from these newborns were collected on filter paper on post-natal day 1 (range: 1–3 days; N = 861), 7 (range: 5–8 days; N = 867) and 14 (range: 12–15 days; N = 786). We evaluated two groups of babies whose birth weight was low for gestational age. We identify babies as growth-restricted infants if they had a birth weight *Z*-score <−2, and as growth-limited if they had a birth weight *Z*-score ≥−2 and <−1. We classified infants by whether or not they were mechanically ventilated during every one of the first 14 post-natal days. Infants were classified as having bacteremia if an organism was recovered from blood obtained during the first 14 days.

Statistical analyses

We evaluated the null hypothesis that growth-restricted and growth-limited infants were no more likely than their appropriately grown peers (i.e. those who had a birth weight *Z*-score ≥−1) to have early post-natal concentrations of 25 inflammation-associated proteins in the highest quartile for gestational age and day of blood specimen collection. Consistent with prior analysis, we present the data with a minimum of reduction (8).

To assess the potential confounding that might be due to delivery indication, or inflammatory stimuli such as bacteremia and 'prolonged' ventilation, we conducted additional analyses limited to four proteins in strata defined by potential confounders. Because bacteremia and prolonged ventilation did not appear to be confounders, we did not adjust for them in the multinomial models, but did adjust for gestational age (23–24, 25–26, 27 weeks). Not presented here are tables of results when we also adjusted for bacteremia and prolonged ventilation. The odds ratios in those analyses are minimally different from those presented (9). We created multinomial logistic regression models comparing infants in each of two groups to infants whose birth weight *Z*-score as >−1. This enabled us to calculate odds ratios (and 99% confidence intervals) using the top quartile concentration of each protein as our indicator of elevated concentration.

RESULTS

Sample description (Table S1)

In this sample of 939 newborns, 50 had a birth weight for gestational age that placed them more than two standard deviations below the median of a referent sample (left data column), hereafter identified as growth restricted. An additional 122 had a birth weight *Z*-score that placed them between two and just less than one standard deviations below the median of the referent standard (*i.e.* ≥−2, <−1; middle data column), hereafter identified as growth limited.

The majority of the growth-restricted infants were born during the 25th and 26th weeks of gestation. While only 6% of infants who were not growth-restricted were born to women who had severe preeclampsia, 39% of the growth limited and 58% of the growth-restricted had a preeclamptic mother. Both groups of small babies were more likely than their peers to have been ventilated during the first 2 weeks.

Odds ratios of elevated protein concentrations associated with foetal growth restriction in strata defined by ventilation status, bacteremia and pregnancy disorder (Table S2)

Because infants who received mechanical ventilation for 14 days were at increased risk of systemic inflammation in this sample (10), we explored the relationship between low birth weight *Z*-scores and inflammation in strata defined by whether the infant was mechanically ventilated every day through day 14. For ease of presentation, we restricted these analyses to four proteins (*i.e.* IL-1β, IL-6, TNF-α and IL-8). Regardless of ventilation, gestational age and bacteremia strata, growth-restricted and growth-limited infants were more likely than their peers to have elevated concentrations of the IL-1β, IL-6, TNF-α and IL-8 on post-natal day 14.

The inflammatory response among growth-restricted newborns is more prominent among those born after a spontaneous delivery than after delivery for severe preeclampsia. The inflammatory response is also seen among children whose birth weight for gestational age is not as low, but only if birth followed a spontaneous indication.

Odds ratios of elevated protein concentrations on days 1, 7 and 14 associated with foetal growth restriction (Table S3)

Both growth-restricted and growth-limited infants were at increased risk of elevated concentrations of three proteins in day-7 blood. On day 14, the growth-restricted infants were at increased risk of elevated concentrations of 11 proteins, while the growth-limited infants were at increased risk of elevated concentrations of seven proteins.

Odds ratios of elevated protein concentrations on at least 2 days 1 week apart associated with restricted growth (Table 1)

Growth-restricted newborns were more likely than others to have prominently elevated odds ratios of highest-quartile concentrations of six proteins on two separate days. Elevated concentrations of three of these six proteins on only 1 day also characterized these growth-restricted newborns. Growth-limited newborns had elevated concentrations of three proteins on 2 days.

DISCUSSION

This study has three major findings. First, compared with appropriate for gestational age infants, growth-restricted infants tend to have higher circulating concentrations of inflammation-related proteins 1–2 weeks after birth, but not earlier. Second, the systemic inflammation on post-natal day 14 does not appear to be due to the propensity for growth-restricted newborns to be exposed to such inflam-

Table 1 Odds ratios (99% confidence intervals) of a protein concentration sustained in the top quartile on one versus two or more days for newborns in each birth weight *Z*-score category compared with newborns whose birth weight *Z*-score was ≥−1*

	Birth weight *Z*-score			
	<−2		≥−2, <−1	
	1 day only	≥2 days	1 day only	≥2 days
CRP	1.9 (0.7, 5.1)	**3.8 (1.4, 9.9)**	1.5 (0.8, 2.7)	**2.0 (1.1, 3.9)**
SAA	2.1 (0.9, 5.0)	1.7 (0.5, 5.4)	1.2 (0.7, 2.2)	1.9 (0.97, 3.7)
MPO	1.4 (0.6, 3.1)	0.3 (0.1, 1.7)	0.9 (0.5, 1.6)	0.8 (0.4, 1.7)
IL-1β	2.1 (0.9, 4.9)	1.5 (0.5, 4.9)	1.1 (0.6, 2.0)	1.3 (0.6, 2.6)
IL-6	1.4 (0.6, 3.5)	**2.9 (1.1, 7.8)**	1.0 (0.5, 1.8)	1.8 (0.9, 3.6)
IL-6R	1.3 (0.5, 3.0)	0.7 (0.2, 2.3)	1.2 (0.6, 2.1)	1.1 (0.6, 2.2)
TNF-α	**3.1 (1.2, 8.5)**	**4.9 (1.7, 14)**	1.2 (0.6, 2.1)	1.3 (0.7, 2.7)
TNF-R1	1.8 (0.8, 4.4)	1.7 (0.6, 4.7)	1.8 (0.99, 3.1)	1.5 (0.7, 3.1)
TNF-R2	1.5 (0.6, 3.4)	1.0 (0.3, 3.2)	0.8 (0.4, 1.4)	1.2 (0.6, 2.3)
IL-8	**2.7 (1.03, 7.2)**	**4.7 (1.6, 13)**	1.7 (0.96, 3.1)	1.8 (0.9, 3.7)
MCP-1	1.4 (0.6, 3.6)	2.4 (0.9, 6.3)	1.1 (0.6, 2.0)	**2.1 (1.1, 4.0)**
MCP-4	1.5 (0.6, 3.8)	1.6 (0.6, 4.2)	1.4 (0.7, 2.5)	1.6 (0.9,, 3.1)
MIP-1β	0.9 (0.3, 2.3)	0.9 (0.3, 2.6)	1.3 (0.7, 2.3)	0.8 (0.4, 1.6)
RANTES	0.7 (0.3, 1.7)	0.3 (0.1, 1.3)	0.8 (0.4, 1.4)	0.3 (0.1, 0.8)
I-TAC	1.8 (0.8, 4.3)	1.5 (0.5, 4.5)	1.1 (0.6, 2.1)	1.3 (0.7, 2.6)
ICAM-1	1.2 (0.5, 3.2)	2.0 (0.8, 5.1)	1.0 (0.6, 2.0)	1.8 (0.96, 3.4)
ICAM-3	1.5 (0.6, 3.6)	0.7 (0.2, 2.3)	0.9 (0.5, 1.6)	0.5 (0.3, 1.1)
VCAM-1	09 (0.3, 2.2)	0.6 (0.2, 1.9)	1.2 (0.6, 2.1)	0.7 (0.3, 1.4)
E-SEL	1.7 (0.7, 4.4)	2.5 (0.9, 6.5)	1.5 (0.8, 2.7)	1.2 (0.6, 2.4)
MMP-1	0.7 (0.2, 2.0)	0.7 (0.2, 2.0)	1.1 (0.6, 2.1)	0.5 (0.2, 1.1)
MMP-9	1.0 (0.4, 2.3)	0.4 (0.1, 1.8)	0.8 (0.4, 1.4)	0.4 (0.1, 0.9)
VEGF	1.2 (0.5, 2.9)	0.4 (0.1, 1.5)	1.0 (0.5, 1.8)	0.5 (0.3, 1.1)
VEGF-R1	2.4 (0.9, 6.5)	**4.7 (1.7, 13)**	1.8 (0.98, 3.1)	1.8 (0.9, 3.5)
VEGF-R2	1.0 (0.4, 2.5)	1.3 (0.5, 3.3)	1.1 (0.6, 2.0)	1.1 (0.5, 2.1)
IGFBP-1	**4.2 (1.3, 13)**	**14 (4.2, 45)**	**2.0 (1.1, 3.6)**	**3.4 (1.7, 7.0)**

Bold items are significantly elevated at $p < 0.01$.
*Adjustment was made for gestational age (23–24, 25–26, 27 weeks).

matory stimuli as bacteremia and 'prolonged' ventilation. Third, the hyperinflammation among the growth-restricted infants was only minimally greater than among the growth limited.

Inflammation not present at birth

The placentas of infants delivered for maternal or foetal indications are much less likely to harbour bacteria than the placentas of infants delivered for spontaneous indications and are also much less likely to have histologic inflammation (11). From these observations, we infer that the intra-uterine environment of infants delivered for spontaneous indications can be characterized as inflammatory (7). Indeed, growth-restricted infants, who are typically delivered for maternal or foetal indications, tend to have low circulating concentrations of inflammation-related proteins in blood collected during the first four post-natal days (12–14).

The first strong inflammatory stimulus for growth-restricted infants occurs post-natally

Since the intense inflammatory response appears to begin 1–2 weeks after delivery, we infer that the stimulus for this response probably also begins after delivery. One plausible stimulus is assisted ventilation (10,15). Stratification of our sample by ventilation every day through day 14 (Table S2) and by culture-proven bacteremia documented that ventilation status and bacteremia accounted for some, but not all of the increased propensity of growth-restricted and growth-limited infants to have systemic inflammation 2 weeks after birth. These observations raise the possibility of other inflammatory stimuli.

Endogenous propensity to post-natal hyper-inflammation

Support for endogenous contributions to post-natal hyper-inflammation comes from a study of rat pups, which found that those who were growth restricted at birth, tended to respond more vigorously than controls to an inflammatory stimulus (16). Additional support comes from a study of human infants born before the 32nd week of gestation who were exposed to mechanical ventilation, which showed that those delivered because of severe early-onset preeclampsia (and therefore at prominently elevated risk of severe growth restriction) had more intense systemic inflammatory responses than their peers delivered for other indications (*i.e.* preterm labour) (17). This latter report of a preeclampsia-post-natal inflammation relationship and awareness that preeclampsia appears to be characterized by a spectrum of immunologic characteristics, including hyper-responsiveness to inflammatory stimuli (18), prompted us to stratify our sample by whether delivery was for spontaneous indications or preeclampsia (Table S2). Growth-restricted and growth-limited infants of pre-eclamptic pregnancies did not show a greater propensity to inflammation than their peers born to women whose delivery was considered spontaneous. These findings support the view that the association between growth restriction and inflammation is not due solely to preeclampsia, or by inference, the aberrant placenta implantation associated with this disorder (19).

Might hyper-inflammation account for the late putative consequences of growth restriction?

Adult diseases whose risk appears to be influenced by foetal growth restriction (*e.g.* obesity, type 2 diabetes and atherosclerotic cardiovascular disease) tend to have a prominent inflammatory component (5,20). In light of our finding that the earliest manifestations of hyperinflammation in growth-restricted very preterm newborns are prominent by the end of the second post-natal week, we raise the possibility that this early hyperinflammation is the first indicator of the processes that might account for the diseases that occur preferentially in adults who were severely growth restricted at birth.

Limitations and strengths

The weaknesses of this study are those of all observational studies. We are unable to establish causation and report only associations. Our study has several strengths. First, we included a large number of infants, making it unlikely that we have missed important associations due to lack of statistical power, or claimed associations that might have reflected the instability of small numbers.

Second, we selected infants based on gestational age, and not birth weight, in order to minimize confounding due to factors related to foetal growth restriction (21).

CONCLUSION

Very preterm growth-restricted infants appear to be at increased risk of systemic inflammation by post-natal day 14.

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CONFLICT OF INTEREST

None of the authors has any financial issue or conflict of interest to disclose.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Percent of children classified by their birth weight *Z*-score who had the characteristics listed on the left (column percents).

Table S2 Odds ratios (and 99% confidence intervals) of a protein concentration in the top quartile on postnatal day 14 for newborns in each birth weight *Z*-score category compared to newborns whose birth weight *Z*-score was ≥ -1.

Table S3 Odds ratios (and 99% confidence intervals) of a protein concentration in the top quartile for newborns in each birth weight *Z*-score category compared to newborns whose birth weight *Z*-score was ≥ -1*.

