Published in final edited form as:

Acta Paediatr. 2016 March ; 105(3): 274–280. doi:10.1111/apa.13286.

Antecedents of inflammation biomarkers in preterm newborns on days 21 and 28

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Abstract

Aim—Most studies of systemic inflammation in very preterm newborns focus on assessments made during the first two weeks. The purpose of this study was to identify some of the antecedents of systemic inflammation evident during postnatal weeks three and four.

Methods—We measured the protein concentrations in blood spots collected on postnatal days 21 (N = 176) and 28 (N = 157) from infants born before the 28th week of gestation and sought correlates of measurements in the top quartile. Odds ratios of elevated concentrations were calculated for the most obvious correlates.

Results—Infants born for maternal and fetal indications were more likely than their peers to have top quartile concentrations of IL-beta, IL-8, TNF-alpha, and ICAM-1 on both days 21 and 28. Similarly, infants whose birthweight Z-score was < −2 or between −1 and −2 were also more likely than their peers to have elevated concentrations of these proteins.

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Conclusion—Markers of systemic inflammation in the very preterm newborn during the third and fourth post-natal weeks are most strongly associated with maternal and fetal indications for (very preterm) delivery and their common correlate/consequence, fetal growth restriction.

Introduction

To document that our measurements of cytokines and other inflammation-related proteins in blood specimens collected during the first two postnatal weeks from very preterm newborns measured what we thought they should, we assessed the relationships relatively-elevated concentrations had with maternal pre-pregnancy obesity and overweight,(1) maternal disorders during pregnancy,(2) maternal pregnancy disorders,(3) fetal growth restriction, (4) placenta histology,(5, 6) placenta bacteriology,(7) early postnatal exposures,(8, 9) and early illnesses.(10–12) Subsequently, the availability of additional funds provided an opportunity to measure the concentrations of many of these same proteins in blood specimens collected on days 21 and 28. We conducted the following study to see if elevated concentrations on days 21 and 28 conveyed similar or different information.

Methods

Participants

The subjects of this report were enrolled in the ELGAN Study and had blood collected for clinical indications on either day 21 (N = 749), or day 28 (N = 697), or on both days 21 and 28 (N = 609). These drops of blood were blotted on paper, frozen for 10 years, then eluted and the concentrations of 16 proteins measured. Details about enrollment, definitions of variables, and protein measurements are presented in the supplement. Additional details about the methods are in the supplement.

Data analyses

We began data exploration by tabulating the proportion of infants with given characteristics who had a protein concentration in the top quartile on individual days (Tables S1–S4). Because approximately 25% of newborns with that characteristic could be expected to have such a high concentration, we identified the characteristics beyond screening frequency bounds of 20% or less, and 30% or higher. We then repeated this exercise for the occurrence of protein concentrations in the top quartile on both days 21 and 28 (Tables S5 and S6). Here we flagged percents of 6 or less, as well as 19 or above.

Based on what we saw in the tables, we then evaluated two null hypotheses. The first one is that infants delivered for maternal or fetal indications are no more likely than their peers delivered for spontaneous indications to have a protein concentration in the top quartile on day 21, day 28, and both days 21 and 28. The second hypothesis is that infants who had a birth weight Z-score < -2, or ≥ -2, < -1 were no more likely than their peers who had a higher birth weight Z-score to have a protein concentration in the top quartile on day 21, day 28, and both days 21 and 28.

Because elevated concentrations of the proteins studied are associated with major early morbidities,(8–12) we view the elevated concentrations as undesirable. Consequently, we

quantify the magnitude of associations between antecedents and elevated concentrations of proteins using odds (risk) ratios. To calculate these ratios and their 95% confidence intervals, we created multinomial regression models, first comparing infants delivered for maternal indications and those delivered for fetal indications to infants delivered for spontaneous indications, and then comparing infants whose birth weight Z-score was < -2, and those whose birth weight Z-score was ≥ -2, < -1 to infants whose birth weight Z-score was ≥ -1.

RESULTS

We present our results in two parts. The first part, which provides "a feel for the data," consists of 6 supplement tables that tabulate the percent of the population with and without a characteristic who had a concentration of each protein in the top quartile. Because the most consistent and impressive associations with systemic inflammation on days 21 and 28 were seen for low birth weight Z-scores, and to a lesser extent with maternal and fetal indications for preterm delivery, the second part consists of forest plots of the odds ratios and 95% confidence intervals of protein concentrations in the top quartile associated with low birth weight Z-scores and with maternal and fetal indications for delivery.

Maternal and pregnancy characteristics Day 21 (Table S1)

Elevated concentrations of the inflammation-associated proteins (also identified as "systemic inflammation") were most consistently seen for "antenatal magnesium for seizure prevention," maternal indication for delivery (preeclampsia), and fetal indication for delivery.

Newborn characteristics Day 21 (Table S2)

The newborn characteristics most consistently associated with systemic inflammation were birth weight Z-scores ≤ -2, and between -2 and -1. Hypercapnea (defined as pCO_2 measurements in the top quartile on two of the first three postnatal days), acidemia (defined as pH measurements in the lowest quartile on two of the first three postnatal days), and "late" bacteremia (defined as recovery of an organism from the blood during weeks 2–4) were associated with indicators of systemic inflammation, but less frequently than were categories of fetal growth restriction.

Maternal and pregnancy characteristics Day 28 (Table S3)

Elevated concentrations of the inflammation-associated proteins continued to be most consistently associated with "antenatal magnesium for seizure prevention" and maternal indication for delivery (preeclampsia). Fetal indication for delivery continued to be associated with systemic inflammation, but not as prominently as on day 21.

Newborn characteristics Day 28 (Table S4)

Birth weight Z-scores ≤ -2, and between -2 and -1 continued to be associated with elevated concentrations of inflammation-associated proteins on day 28, but hypercapnea, acidemia, and "late" bacteremia were no longer prominently associated with indicators of systemic inflammation.

Maternal and pregnancy characteristics Days 21 and 28 (Table S5)

Inflammation-associated protein concentrations in the top quartile on both days 21 and 28 were associated with “antenatal magnesium for seizure prevention,” maternal indication for delivery (preeclampsia), and fetal indication for delivery. Labor, but not spontaneous indications for delivery, was associated with systemic inflammation, but not as prominently.

Newborn characteristics Days 21 and 28 (Table S6)

The only two newborn characteristics/exposures associated with elevated concentrations of inflammation-associated proteins on both days 21 and 28 were a birth weight Z-score ≤ −2, and a birth weight Z-score between −2 and −1.

Forest plots for maternal (preeclampsia) and fetal indications for delivery (Figure 1)

On day 21, both maternal and fetal indications were associated with systemic inflammation (top panel). On day 28, maternal indication continued to be associated with systemic inflammation, while fetal indication was no longer associated with systemic inflammation (middle panel). Children were at increased risk of elevated concentrations of the many inflammation-associated proteins on both days 21 and 28 if they were delivered for maternal and fetal indications (bottom panel).

Children born preterm because their mother had very severe preeclampsia were at significantly increased risk of elevated concentrations on both days 21 and 28 of CRP, IL-1beta, IL-8, TNF-alpha, TNF–R2, and ICAM-1. Children born preterm because of fetal indications were at significantly increased risk of elevated concentrations on both days of MPO, IL-8, TNF-alpha, and ICAM-1 (bottom panel).

Forest plots for birth weight Z-scores < −2, and between −2 and −1 (Figure 2)

Newborns whose birth weight Z-score was < −2, or between −2 and −1 were at significantly increased risk of systemic inflammation on day 21 (top panel), on day 28 (middle panel), and on both days 21 and 28 (bottom panel).

Children whose birth weight Z-score was < −2 were at significantly increased risk of elevated concentrations on both days 21 and 28 of CRP, IL-1beta, IL-8, ICAM-1 and TSH. Children whose birth weight Z-score was between −2 and −1 were at significantly increased risk of elevated concentrations on both days of IL-8, TNF-alpha, TNF-R2, ICAM-1, and MMP-9.

DISCUSSION

Our main findings are that systemic inflammation in the very preterm newborn during the third and fourth post-natal weeks is most strongly associated with maternal and fetal indications for (very preterm) delivery and their common correlate/consequence of fetal growth restriction.

In this sample, day-1 blood specimens obtained from the newborn delivered for maternal or fetal indications tended NOT to have elevated concentrations of inflammation-associated

proteins.(3) Similarly, those who were severely growth restricted (birth weight Z-score <−2) were not at increased risk of systemic inflammation at birth.(4) By post-natal day 14, however, severely growth-restricted infants demonstrated increased concentrations of inflammation-associated proteins. Little of this increase could be attributed to delivery indication, bacteremia, or duration of ventilation. Less severely growth-restricted infants (birth weight Z-score between −2 and −1) had a less inflammatory blood protein profile.

Explanations for the prominent “late” inflammatory profiles

Prolonged inflammation—Prolonged inflammation can be attributed to persistent/recurrent exposure to inflammatory stimuli, impaired inflammation resolution, or diminished degradation of inflammation-related proteins. In the ELGAN Study, the prominent sources of systemic inflammation were “prolonged” assisted ventilation(9, 10) and bacteremia,(11) with or without necrotizing enterocolitis.(12) Yet, children with these characteristics were not more likely than others to display a strong inflammatory profile on day 28. In light of these exposures not adequately explaining what we see, we seek alternative explanations.

Epigenetics—Infants born to preeclamptic women and those who are severely growth restricted are at increased risk of cardiovascular disease, diabetes, and obesity.(13–15) These effects have been attributed to fetal programming/epigenetics, some of which regulate inflammation.(16–19) Some of these, in turn, can contribute to metabolic programming through epigenetic regulation of gene expression.(20, 21)

Evidence of presumed fetal programming in the ELGAN Study comes from the observation that severely growth-restricted newborns were at increased risk of bronchopulmonary dysplasia,(22) and lower scores on a cognition assessment.(23) In light of these observations, we raise the possibility that some of the early associations with systemic inflammation in our extremely preterm sample can be attributed to fetal programming.

Long half-life of the proteins—Compared to adults, premature (and full-term) newborns tend to have considerably longer half-lives of drugs.(24–26) We do not know the half-life of pro-inflammatory cytokines in the very preterm newborn. In light of what we know about the slow metabolism of drugs, however, we raise the possibility that delayed degradation, either of drugs that might influence inflammation, or of cytokines that indicate inflammation severity accounts for some of what we found.

Limitations and strengths

We measured a relatively small number of proteins, thereby providing a very sparse picture of the very broad and complex phenomenon we call inflammation.(27, 28) In addition, because the sickest infants were more likely than others to have blood drawn on days 21 and 28, selection bias might have resulted. Among the strengths of this study are the large number of uniformly-assessed infants born at such an early age and the measurement of protein concentrations by a very high quality laboratory.

Conclusion

In conclusion, very preterm newborns are at increased risk of systemic inflammation during the third and fourth post-natal weeks if they were delivered for maternal or fetal indications, or if they had moderate to severe fetal growth restriction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This study was supported by The National Institute of Neurological Disorders and Stroke (5U01NS040069-05; 2R01NS040069-06A2), The National Eye Institute (1-R01-EY021820-01), and the National Institute of Child Health and Human Development (5P30HD018655-28).

Abbreviations

CRP	C-Reactive Protein
SAA	Serum Amyloid A
MPO	Myeloperoxidase
IL-1b	Interleukin-1 beta
IL-6	Interleukin-6
IL-6R	Interleukin-6 Receptor
TNF-alpha	Tumor Necrosis Factor-Alpha
TNF-R2	Tumor Necrosis Factor-Receptor 2
IL-8	Interleukin-8
RANTES	Regulated upon Activation Normal T cell Expressed and presumably Secreted
ICAM-1	InterCellular Adhesion Molecule-1
MMP-1	Matrix Metalloproteinase-1
MMP-9	Matrix Metalloproteinase-9
VEGF-R2	Vascular Endothelial Growth Factor-Receptor2
TSH	Thyroid Stimulating Hormone
EPO	Erythropoietin

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Key notes

- Systemic inflammation in very preterm infants during the first two postnatal weeks has been associated with fetal growth restriction.
- Here we show that elevated blood concentrations of IL-beta, IL-8, TNF-alpha, and ICAM-1 during the next two weeks are also associated with fetal growth restriction and its correlates, delivery for maternal and fetal indications.
- Some systemic inflammation in very preterm infants appears to be sustained rather than just intermittent.

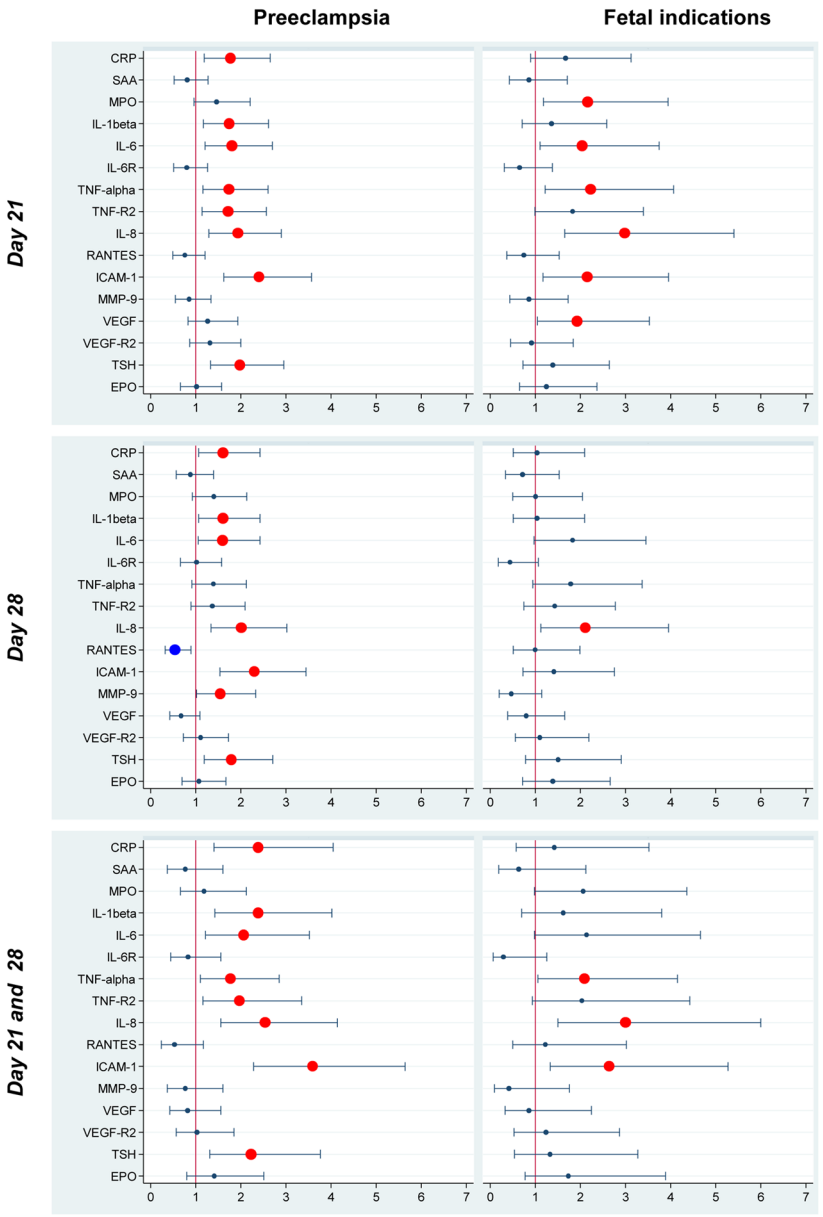

Figure 1.

Forest plot of odds ratios and 95% confidence intervals of a protein concentration in the top quartile on postnatal day 21 (top row), day 28 (middle row), and on both of these days (bottom row) associated with maternal (preeclampsia)(left panels) and fetal (right) indications for preterm delivery. Infants delivered for spontaneous indications are the referent group. The dot in the center of each line is the point estimate of the odds ratio. The vertical anchors of each horizontal line indicate the location of the 95% confidence intervals.

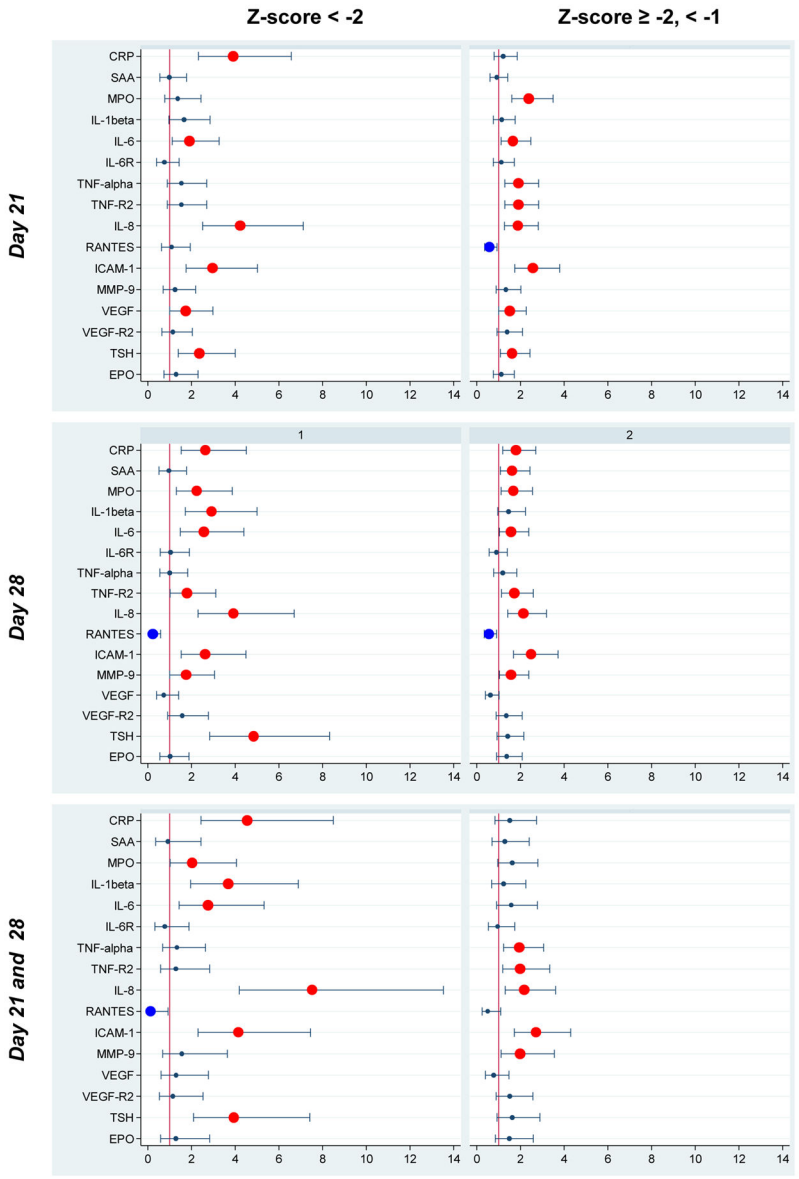

Figure 2.

Forest plot of odds ratios and 95% confidence intervals of a protein concentration in the top quartile on postnatal day 21 (top row), day 28 (middle row), and on both of these days (bottom row) associated with a birth weight Z-score < −2 (left panels) and a birth weight Z-score ≥ −2, < −1. Infants whose birth weight Z-score was ≥ 1 are the referent group.