Acquired epilepsy after neonatal encephalopathy is associated with alterations in pathway-specific circulating inflammatory cytokines

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HEAL
HIGH-DOSE EPO FOR ASPHYXIA AND ENCEPHALOPATHY

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Objectives

To measure circulating biomarker levels and to evaluate their association with post-neonatal (acquired) epilepsy (PNE) among neonates undergoing therapeutic hypothermia (TH) for hypoxic-ischemic encephalopathy (HIE).

Methods

Participants: An ancillary to the High-Dose Erythropoietin for Asphyxia and Encephalopathy (HEAL) clinical trial for neonates with HIE and treated with TH and erythropoetin or placebo.

Inclusion Criteria:

✦ Born at ≥36 weeks' gestation with signs of perinatal depression & moderate or severe encephalopathy by modified Sarnat criteria.

✦ Passive or active therapeutic hypothermia started within 6 hours of birth.

Measurements:

✦ Whole blood specimens collected at three timepoints (12-24, 36-48, and 74-86 hours after birth) and then centrifuged at 6000rpm for 10-minutes. Plasma was stored at -80C within 4-hours of collection.

✦ Twenty-eight analytes were measured using the MILLIPLEX MAP Human High Sensitivity T-Cell Panel (catalog # HSTCMAG-28SK), R&D Systems Luminex Multiplex assays (catalog # LXSAHM-14/ DEPRUO), and MSD R-PLEX assay (catalog # K1511MR-2), all in duplicate per manufacturers' protocols.

Outcome: Longitudinal data was collected through parent survey. Presence of PNE at 2-years of age was determined by two board-certified child neurologists with outcome discrepancies adjudicated by consensus.

Results

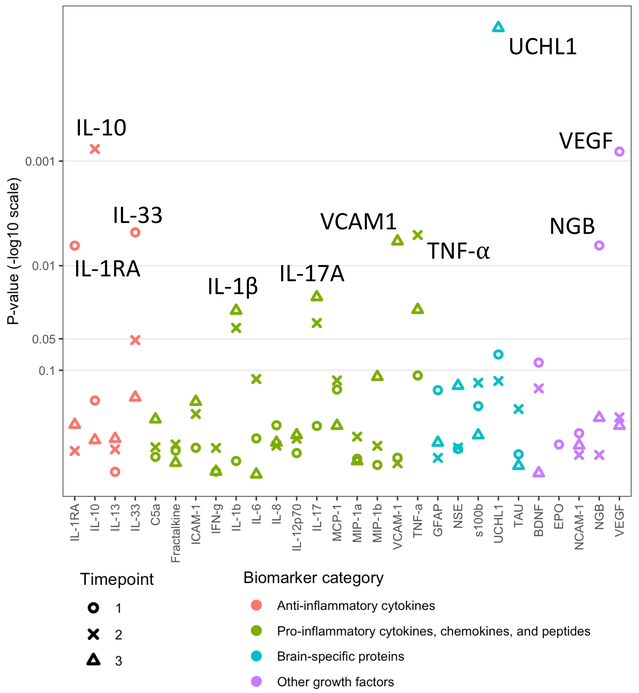
Table 1: No differences in baseline characteristics among neonates with and without post-neonatal epilepsy (PNE) at 2-years of age

	Overall (n=55)	No Epilepsy (n=47)	Epilepsy (n=8)	p-value
Maternal Characteristics				
Race, n (%)				
White	43 (78)	37 (79)	6 (75)	0.97
Black	6 (11)	5 (11)	1 (13)	
Other/Unknown	6 (11)	5 (11)	1 (13)	
Hispanic, n (%)	15 (27)	14 (30)	1 (13)	0.42
Maternal Age, yrs (SD)	29.9 (6)	30.4 (6)	26.9 (8)	0.13
Primiparous, n(%)	24 (44)	20 (43)	4 (50)	0.72
Pregnancy and delivery complications, n (%)				
Chorioamnionitis	7 (13)	7 (15)	0	0.58
(Pre)-Eclampsia	2 (4)	1 (2)	1 (13)	0.27
Sentinel Events	22 (40)	19 (40)	3 (38)	1.0
Cesarean section	41 (75)	35 (75)	6 (75)	1.0
Infant Characteristics				
Female sex, n (%)	25 (46)	23 (49)	2 (25)	0.27
Birth Weight, g (SD)	3346 (567)	3395 (553)	3058 (600)	0.12
Gest. Age, wks (SD)	38.8 (1.4)	38.8 (1.4)	38.8 (1.7)	0.97
5-min Apgar [IQR]	3 [1, 4]	3 [1, 4]	2 [2, 3]	0.42
10-min Apgar [IQR]	4 [3, 6]	4 [3, 6]	5 [3, 6]	0.96
Lowest pH (SD)	6.93 (0.21)	6.93 (0.21)	6.92 (0.24)	0.87
Worst base deficit (SD)	-19.6 (6.6)	-19.5 (6.7)	-20.5 (6.3)	0.72
Severe encephalopathy,	16 (29)	13 (28)	3 (38)	0.68
EPO Tx Group, n (%)	25 (46)	20 (43)	5 (63)	0.45
Acute Seizures, n(%)	40 (73)	34 (72)	6 (75)	1.0

✦ **Among 55 participants in the HEAL trial with biomarker measurements and follow-up for epilepsy, 8 (15%) were diagnosed with PNE by 2-years of age.**

✦ **There were no differences in maternal and infant characteristics in those with and without post-neonatal epilepsy (Table 1).**

Figure 1: Manhattan plot of biomarker associations between neonates with and without PNE. Kruskal-Wallis rank sum test p-values are shown in the y-axis.

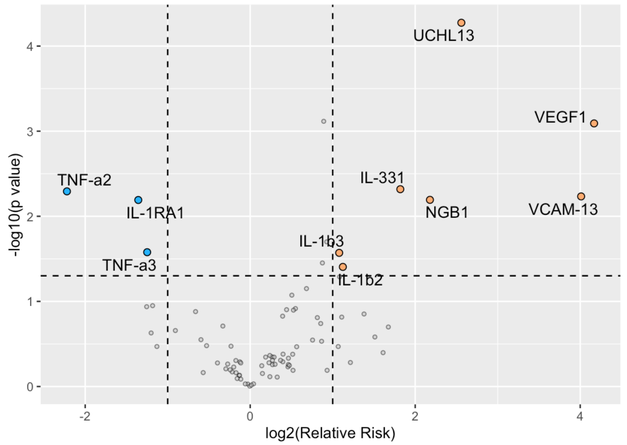


✦**13 of 82 biomarker concentrations (16%) differed between groups (p<0.05, Figure 1) with enrichment in the TNF-α, IL-17, and NF-κB signaling cascades on pathway analysis (p<0.05).**

✦**Eleven of 14 biomarkers were associated with at least a two-fold change in relative risk of PNE (Figure 2).**

✦**After multivariate regression, biomarker concentrations measured at timepoint 3 were more likely to be associated with PNE than biomarkers at timepoints 1 or 2.**

Figure 2: Volcano plot of biomarker expression differences between neonates with and without PNE. Numeric suffix after a biomarker name denotes collection timepoint. Dashed horizonal line represents p-value of 0.05. Dashed vertical lines represent two-fold increase or decrease in relative risk of PNE between groups.



Conclusion and Next Steps

✦ In neonates with HIE undergoing TH, subsequent PNE was associated with differences in concentrations of pro-inflammatory and brain injury proteins, which upon validation may serve as prognostic biomarkers with implications on novel therapeutics to prevent epilepsy.

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