Cerebrospinal Fluid Neopterin: potential marker for central nervous system inflammation in Sudden Infant Death Syndrome

Molly Riehs[1]; Hani Harb, PhD[2]; Benjamin W. Okaty, PhD[4]; Elisabeth Haas, MPH[5]; Brankica Paunovic, MD[6]; Keith Hyland, PhD[7]; Mark Gorman, MD[3]; Gerard T. Berry, MD[8]; *Richard D. Goldstein, MD[9]; and *Robin L. Haynes, PhD[1]

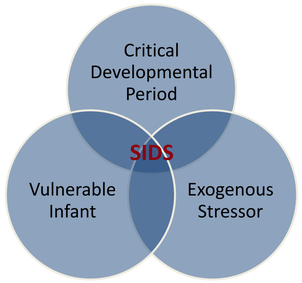
Departments of Pathology[1], Immunology[2], Neurology[3], and Pediatrics[9], and Division of Genetics and Genomics[8], Boston Children's Hospital, Boston, MA; Department of Genetics, Harvard Medical School[4], Boston, MA
Rady Children's Hospital[5] and San Diego County Office of the Medical Examiner[6], San Diego, CA; MNG Labs[7], Atlanta, GA [* Co-Senior Authors]

1. Introduction

Sudden Infant Death Syndrome (SIDS) is the leading cause of postneonatal infant death in the United States with an incidence rate of 0.39/1000 live births [1].

The *triple risk model* for SIDS posits an infant dies of SIDS when 3 factors converge; an infant with a biological abnormality passes through a critical developmental window (1st year of life), and faces an exogenous stressor (**Fig. 1**). Our laboratory is specifically interested in abnormalities within the serotonergic system of the brainstem regions critical to respiratory and autonomic regulation [2].

Figure 1. Triple Risk Model



Epidemiological and pathological evidence suggests a role for risk factors that potentially elicit an inflammatory response within the brain (i.e, illness prior to death and hypoxia). To begin to examine the relationship between neuroinflammation and brainstem abnormalities, we first tested the following hypothesis: **SIDS, or a subset of SIDS, involves central nervous system inflammation, as identified by increased levels of the cellular immune system marker, neopterin, in the cerebrospinal fluid (CSF).**

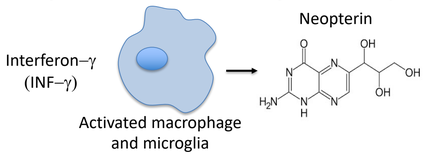


Figure 2. Neopterin is considered a specific marker for CNS inflammation.

References

1. Shapiro-Mendoza, C.K., et al., *SIDS Sudden Infant and Early Childhood Death: The Past, the Present and the Future* 2018: Adelaide (AU).
2. Kinney, H.C. and R.L. Haynes, J Neuropathol Exp Neurol, 2019. **78**(9): p. 765-779.
3. Habib, N., et al., Nat Methods, 2017. **14**(10): p. 955-958.

2. Materials and Methods

	SIDS Mean (SD) n=64	Controls Mean (SD) n=15	p-value
Gestational Age (wks)	38.0 (3.4)	38.8 (2.5)	ns
Postconceptional age (wks)	54.5 (11.3)	61.5 (17.4)	0.06
Postnatal age (wks)	16.5 (11.9)	22.3 (16.9)	ns
Postmortem interval (hrs)	23.5 (7.5)	22.5 (6.9)	ns

Neopterin Levels: Sequential electrochemical/fluorescence detection following reversed phase HPLC separation (MNG Laboratories, Atlanta, GA)
INF-γ/Cytokine Levels: Bio-Plex Pro Human Cytokine 27-plex assay
Single-nuclei RNA-seq: Habib et al. [3] nuclei extraction, 10x Genomics Chromium Single Cell 3' v3 protocol followed by Illumina NextSeq 500 sequencing

3. Results

SIDS outliers with elevated levels of CSF neopterin

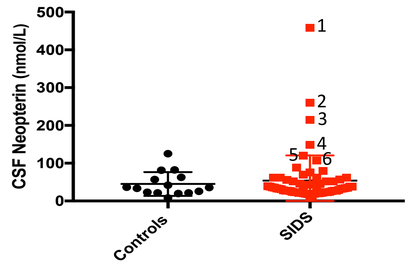


Figure 3. CSF neopterin in SIDS (n=64) vs Controls (n=15) with identified SIDS outliers. Outliers have neopterin levels > 2 standard deviations above the mean of controls (>107 nmol/L). There is no statistical difference in SIDS and controls.

Heightened or aberrant inflammatory response in SIDS

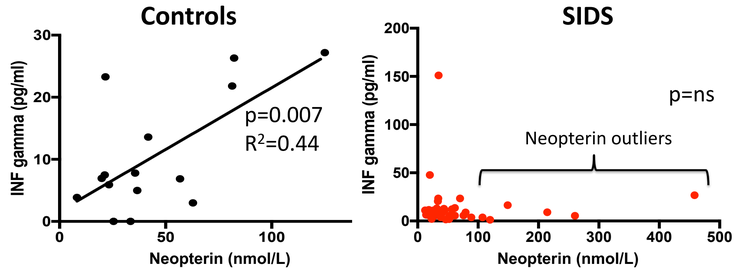


Figure 4. There is a linear relationship between neopterin and INF-γ in controls. This relationship is absent in SIDS cases suggesting that a subset of SIDS infants has a heightened response to an inflammatory trigger.

There is an increased prevalence of illness preceding death in SIDS outliers compared to SIDS with normal neopterin levels

Characteristic	% of Neopterin outliers	% of other SIDS	% of controls	p value (SIDS outliers vs other SIDS)
Premature birth	0/6 (0%)	14/58 (24%)	2/14 (14%)	ns
Illness 24- 48 hour prior to death	4/6 (66.7%)	12/57 (21%)	2/12 (17%)	**0.03**
Fever 24-48 hour prior to death	2/5 (40%)	1/57 (2%)	1/11 (9%)	**0.01**
Prone Sleep	1/6 (17%)	24/55 (44%)	3/11 (27%)	ns
Bedsharing	5/6 (83%)	26/58 (45%)	5/10 (50%)	ns

3. Results cont.

Microglial-mediated inflammatory response in the medulla of a SIDS neopterin outlier – Pilot analysis

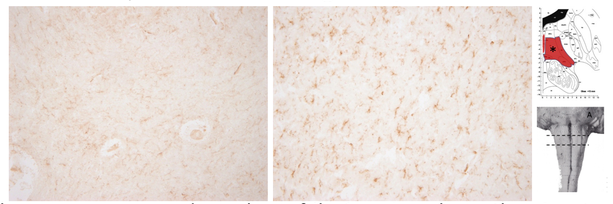


Figure 5. There is an increased number of Iba1+ microglia in the gigantocellularis (*) of the rostral medulla in the SIDS case with elevated CSF neopterin compared to a SIDS case with neopterin in the control range.

Cell-specific inflammatory response in the medulla of a SIDS case with high CSF levels of neopterin

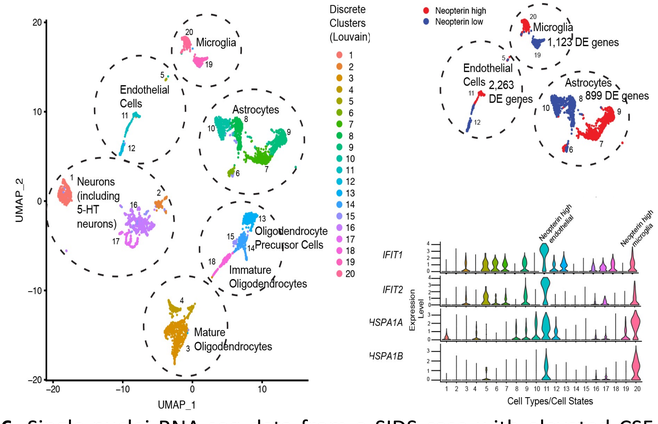


Figure 6. Single-nuclei RNA-seq data from a SIDS case with elevated CSF neopterin compared to a SIDS case with neopterin in the control range.

4. Summary

A subset of SIDS infants have an aberrant or heightened neuroinflammatory response, potentially to a subclinical infectious trigger.

High CSF levels of neopterin are associated with changes in expression of immune and stress response genes in specific medullary cell types.

Pilot analyses suggest a role for inflammatory responses within serotonergic regions of the medulla in the pathogenesis of some SIDS infants.