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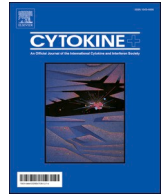
Lower levels of Th1 and Th2 cytokines in cerebrospinal fluid (CSF) at the time of initial CSF shunt placement in children are associated with subsequent shunt revision surgeries

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ABSTRACT

Objective: We compare cytokine profiles at the time of initial CSF shunt placement between children who required no subsequent shunt revision surgeries and children requiring repeated CSF shunt revision surgeries for CSF shunt failure. We also describe the cytokine profiles across surgical episodes for children who undergo multiple subsequent revision surgeries.
Methods: This pilot study was nested within an ongoing prospective multicenter study collecting CSF samples and clinical data at the time of CSF shunt surgeries since August 2014. We selected cases where CSF was available for children who underwent an initial CSF shunt placement and had no subsequent shunt revision surgeries during $>=24$ months of follow-up ($n = 7$); as well as children who underwent an initial CSF shunt placement and then required repeated CSF shunt revision surgeries ($n = 3$). Levels of 92 human cytokines were measured using the Olink immunoassay and 41 human cytokines were measured using Luminex based bead array on CSF obtained at the time of each child's initial CSF shunt placement and were displayed in heat maps.
Results: Qualitatively similar profiles for the majority of cytokines were observed among the patients in each group in both Olink and Luminex assays. Lower levels of MCP-3, CASP-8, CD5, CXCL9, CXCL11, eotaxin, IFN-γ, IL-13, IP-10, and OSM at the time of initial surgery were noted in the children who went on to require multiple surgeries. Pro- and anti-inflammatory cytokines were selected *a priori* and shown across subsequent revision surgeries for the 3 patients. Cytokine patterns differed between patients, but within a given patient pro-inflammatory and anti-inflammatory cytokines acted in a parallel fashion, with the exception of IL-4.
Conclusions: Heat maps of cytokine levels at the time of initial CSF shunt placement for each child undergoing only a single initial CSF shunt placement and for each child undergoing repeat CSF shunt revision surgeries demonstrated qualitatively similar profiles for the majority of cytokines. Lower levels of MCP-3, CASP-8, CD5, CXCL9, CXCL11, eotaxin, IFN-γ, IL-13, IP-10, and OSM at the time of initial surgery were noted in the children

who went on to require multiple surgeries. Better stratification by patient age, etiology, and mechanism of failure is needed to develop a deeper understanding of the mechanism of inflammation in the development of hydrocephalus and response to shunting in children.

1. Introduction

Hydrocephalus is characterized by the abnormal secretion, circulation, and/or absorption of cerebrospinal fluid (CSF) within the ventricles of the brain resulting in ventricular expansion and intracranial pressure [1]. Cerebrospinal fluid shunt placement is the standard of care for pediatric hydrocephalus [2]. While CSF shunts avoid further brain injury and allow children to survive, 30–40% of all pediatric shunts placed fail within the first year resulting in shunt revision and can cause the onset of new and often chronic surgical and medical problems [3–4].

Shunt system failures are generally classified as resulting from one of four causes: (1) the intricate and variable causes of the disorder [5–6] (2) shunt infection, (3) mechanical malfunction, or (4) shunt obstruction. Obstruction of the shunt catheters is multiplex and can be caused by cells originating from normal brain tissue (choroid plexus, ependyma, leptomeninges, and connective tissue) or by pathological cells and tissues (blood-borne or central nervous system [CNS] inflammatory cells, red blood cells, platelets, and cell debris) [7]. Interestingly, prior studies reveal that pathological inflammatory reactions occur in and around many obstructed CSF shunt systems, highlighting that inflammatory response biomarkers may be an important target for inhibiting CSF shunt failure [8–9]. Given our current understanding of the cellular environment of shunt obstruction, the role of inflammatory molecules (i.e., cytokines and chemokines) may be particularly relevant in shunted hydrocephalus.

Literature about the role of inflammation in the context of pediatric shunted hydrocephalus is limited. Sävman et al. investigated the levels of cytokines in preterm infants with post-hemorrhagic hydrocephalus (PHH) and revealed elevated levels of tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-8 (IL-8), and interferon-γ (IFN-γ) in CSF from infants with PHH [10]. Levels of IL-6, IL-4, TNF- α, tumor necrosis factor- α (TNF α), transforming growth factor-β1 (TGF β1), and other inflammatory markers in CSF and blood have been found to correlate with the likelihood of subsequent hydrocephalus development in infants and adults who have had an infection or brain hemorrhage [11–16]. Multiple studies have confirmed a degree of inflammatory response being involved in the pathophysiology mechanism of shunt failures [17–21] after surgical treatment for hydrocephalus is initiated. However, this evidence is limited and an improved understanding of the molecular physiology and the immune activation responses associated with CSF shunt failure may enhance our ability to prolong CSF shunt survival among children with hydrocephalus.

Using data from an ongoing prospective, multicenter study collecting CSF samples and clinical data at the time of CSF shunt surgeries since August 2014, we recognized a unique opportunity to investigate the utility of cytokines as diagnostic markers for pediatric CSF shunt failure (not due to CSF shunt infection). We identified two objectives for this pilot study. First, we sought to compare the cytokine profiles at the time of initial CSF shunt placement between children who required no subsequent shunt revision surgeries and children who ultimately required repeated CSF shunt revision surgeries for CSF shunt failure. Second, we sought to describe the cytokine profiles across surgical episodes for children who ultimately undergo multiple subsequent revision surgeries for CSF shunt failure. We hypothesized that, unlike with CSF from children requiring no revision surgeries, Olink based assays would identify relatively higher abundances of pro-inflammatory cytokines in the CSF from children requiring subsequent revision surgeries for CSF shunt failure.

2. Methods

2.1. Study subjects

Enrollment in the CLIMB study occurred from August 2014 to present; CLIMB enrolled children with hydrocephalus across 4 centers and collected clinical data, CSF, and hardware [22–26] to investigate biomarkers of CSF shunt infection. Children with hydrocephalus who were ≤ 18 years old and undergoing initial CSF shunt placement surgery at Seattle Children's Hospital (SCH) were eligible for enrollment in this study. For this study, we selected cases where CSF was available for children who underwent an initial CSF shunt placement and had no subsequent shunt revision surgeries during >=24 months of follow-up (n = 7); as well as children who underwent an initial CSF shunt placement and then required repeated CSF shunt revision surgeries (n = 4). CSF shunt system(s) included ventriculoperitoneal, ventriculoatrial, ventriculopleural, arachnoid cyst shunts, subdural shunts, and lumboperitoneal shunts; temporary devices only such as external ventricular drain(s), Ommaya reservoir(s), ventricular access devices (reservoirs) and subgaleal shunts were excluded. The study was limited to those for which CSF samples were available from both initial CSF shunt placement and the majority of subsequent CSF shunt revisions.

The cytokine profiles of select cases were examined in two distinct lines of inquiry: 1) children who undergo placement of an initial CSF shunt and require no revision surgeries over the subsequent two years compared to those who undergo placement of an initial CSF shunt and require multiple revisions and 2) children who undergo placement of an initial CSF shunt and require multiple subsequent revision surgeries compared within child and across surgical episodes.

2.2. Ethics statement

The study received Institutional Review Board approval from the Seattle Children's Research Institute (13346, approved February 9, 2011) and the Children's Hospital Los Angeles (CHLA-20–00069, approved March 13, 2020). For all study subjects, written consent was obtained from parents or guardians, and assent when age- and developmentally-appropriate from study subjects, for leftover CSF to be collected on each occasion that regular CSF samples were obtained during treatment for hydrocephalus.

2.3. Clinical data

Clinical data were extracted from the electronic medical record for each patient enrolled into the study at the participating center by trained research staff. Data included eligibility, demographics, hydrocephalus etiology, prior central nervous system surgeries, surgical treatment details, shunt information, antibiotic treatment, post-operational complications, CSF microbiological and laboratory results.

Data regarding subsequent revision and infection interventions were also abstracted from the electronic medical record of eligible patients. A subsequent intervention was defined by any medical complication or surgical procedure that a patient had to undergo secondary to shunt malfunction or failure but not infection. Data included indication of subsequent intervention, type of surgical intervention, surgical treatment details, antibiotic treatment, post-operational complications, CSF microbiological and laboratory results.

2.4. CSF specimen collection

CSF was collected from patients during surgical intervention. Sterile conditions were standard practice throughout recovery and storage of CSF. After collection, CSF samples were stored at 4 °C for up to 5 days. CSF was then aliquoted into vials of ∼ 100 μl for the study and stored at −70 °C. After identification for this study, samples were shipped overnight to Palo Alto, California on dry ice for analysis.

2.5. Laboratory analyses

The levels of 92 human cytokine molecules were measured using the Olink immunoassay and 41 human cytokine molecules were measured using Luminex based bead array on CSF obtained at the time of each child's initial CSF shunt placement. For clarity, we focus here on the Olink immunoassay; [27–30] however, Luminex assay methods and results are provided in **supplemental materials**.

The Olink immunoassay was performed by the Human Immune Monitoring Center at Stanford University. The samples were subjected to Olink multiplex assay with Inflammatory panel (Olink Bioscience, Uppsala, Sweden), according to the manufacturer's instructions. Briefly, an incubation master mix containing pairs of oligonucleotide-labeled antibodies to each protein was added to the samples and incubated for 16 h at 4 °C. Each protein was targeted with two different epitope-specific antibodies increasing the specificity of the assay. Presence of the target protein in the sample brought the partner probes in close proximity, allowing the formation of a double strand oligonucleotide polymerase chain reaction (PCR) target. On the following day, the extension master mix in the sample initiated the specific target sequences to be detected and generated amplicons using PCR in 96 well plates. For the detection of the specific protein, Dynamic array integrated fluidic Circuit (IFC) 96x96 chip was primed, loaded with 92 protein specific primers and mixed with sample amplicons including three inter-plate controls and three negative controls. Real time microfluidic qPCR was performed in Biomark (Fluidigm, San Francisco, CA) for the target protein quantification. Data were analyzed using Real time PCR analysis software via ΔΔCt method and Normalized Protein Expression (NPX) manager. One NPX difference equals to the doubling of the protein concentration.

The Luminex -EMD Millipore Magnetic kit assay was also performed by the Human Immune Monitoring Center at Stanford University. Kit Cat# HCYTMAG-60 K-PX41 was purchased from EMD Millipore Corporation, Burlington, MA., and used according to the manufacturer's recommendations with modifications described as follows. Briefly, samples were mixed with antibody-linked magnetic beads on a 96-well plate and incubated overnight at 4 °C with shaking. Cold and room temperature incubation steps were performed on an orbital shaker at 500–600 rpm. Plates were washed twice with wash buffer in a Bio-Tek ELx405 washer. Following one hour incubation at room temperature with biotinylated detection antibody, streptavidin-PE was added for 30 min with shaking. Plates were washed as described and PBS added to wells for reading in the Luminex FlexMap3D Instrument with a lower bound of 50 beads per sample per cytokine. Samples were measured in singlet. Custom Assay Chex control beads were purchased from Radix BioSolutions, Georgetown, Texas, and added to all wells.

2.6. Data analysis

Patient sociodemographic and clinical characteristics were summarized overall and separately for children who undergo placement of an initial CSF shunt and required no revision surgeries over the subsequent two years compared to those who undergo placement of an initial CSF shunt and required multiple revision surgeries.

Cytokine profiles at the time of initial shunt placement were examined qualitatively in a heatmap comparing children who required no revision surgeries over the subsequent two years (single) to those who required multiple revision surgeries (repeat). Cytokine profiles were further examined across multiple subsequent revision surgeries for those children who underwent placement of an initial CSF shunt and required multiple subsequent revision surgeries. One patient from the original group was removed as the available CSF samples from subsequent surgeries were not from distinct surgical events (i.e. were from staged revisions). A subset of pro- and anti-inflammatory cytokines were selected a priori for examination in spaghetti plots depicting temporal patterns across shunt surgeries. An approximate permutation test [31] was applied to inform the selection of cytokines with different median labels between the two groups. A test statistic, $S(X,G)$, for the permutation test was defined as the median difference divided by a common standard deviation where X represents a cytokine of interest and G takes the value one if children did not require revision surgeries and zero otherwise. The observed statistic, S_0, was first computed using non-permuted data. One thousand simulated samples were selected for each of the cytokines, and a cytokine-specific p-value was calculated as the proportion of values of the test statistic $S(X,G)$ exceeding the observed statistic S_0. Although p-values were not used for testing, the cytokines with the largest median difference ($p \leq 0.10$) were further examined in dot scatterplots. Dot scatterplots are useful for visualizing differences in the distribution of individual patient cytokine measures between groups (e.g., single versus repeat).

3. Results

A total of 11 individual patients who underwent initial CSF shunt surgeries were included in this study. The baseline characteristics of the 11 patients are shown in Table 1. Ages ranged from 4 days to 11 years with a median age of 8 months. The common causes of hydrocephalus in this cohort were post-intraventricular hemorrhage secondary to prematurity (3/11 [27%]) and myelomeningocele (3/11 [27%]). The details of surgical approach to initial shunt placement among the 11 patients are shown in Table 2. No statistically significant differences (p < 0.05) were observed between patients who did and did not require multiple revision surgeries in either baseline characteristics or details of surgical approaches.

Heatmaps of cytokine levels at the time of initial CSF shunt placement for each child undergoing only a single initial CSF shunt placement and for each child undergoing repeat CSF shunt revision surgeries are provided in Fig. 1 for Olink assays and Supplemental Figure 1 for Luminex assays. Qualitatively similar profiles for the majority of cytokines were observed among the patients in each group in both Olink and Luminex assays.

Of note, within each group at least one child was noted to demonstrate an outlier cytokine profile. The cytokine profile of SEA0158 appears qualitatively different from other 6 patients who underwent only a single initial CSF shunt placement. (Fig. 1 and Supplemental Figure 1) This child underwent initial CSF shunt close to birth (as did all others except SEA0181 who was treated at 7 months of age) and s/he experienced IVH (as did SEA0248). The cytokine profile of SEA0159 appears qualitatively different at initial CSF shunt placement from other 3 patients who underwent multiple CSF shunt revisions. (Fig. 1 and Supplemental Fig. 1) This child underwent initial CSF shunt close to birth (as did all others except SEA0142 who was treated at 11 months of age) and s/he experienced aqueductal stenosis. Neither age at time of CSF shunt placement nor etiology appears to correlate with outlier cytokine profiles in this limited dataset.

Ten cytokines were observed in the Olink assays to differ at the time of initial shunt placement for children who undergo single versus repeated surgeries ($p < 0.10$). (Fig. 2) Lower levels of MCP-3, CASP-8, CD5, CXCL9, CXCL11, eotaxin, IFN-γ, IL-13, IP-10, and OSM at the time of initial surgery were noted in the children who went on to require multiple surgeries. In Luminex assays, lower levels of IP-10, IL-9, IL-5, and EGF at the time of initial surgery were noted in the children who went on to require multiple surgeries. (Supplemental **Figure 1**)

Table 1
Baseline Characteristics*.

	Entire Cohort (n = 11)	Patients Not Requiring Multiple Revision Surgeries (n = 7)	Patients Requiring Multiple Revision Surgeries (n = 4)
Median Age in years (interquartile range)	0.7 (0.1, 2.2)	0.7 (0.2, 2.2)	0.4 (0.1, 6.1)
Mean Gestational Age in weeks (standard deviation, (SD))	35.4 (4.6)	34.8 (5.3)	36.3 (3.8)
Mean Birth weight in kg (SD)	3.3 (1.5)	2.7 (1.3)	4.2 (1.5)
Sex, n (%)			
Male	5 (45)	4 (57)	1 (25)
Female	6 (55)	3 (43)	3 (75)
Race, n (%)			
White	4 (36)	2 (29)	2 (50)
More than one race	3 (27)	2 (29)	1 (25)
Unknown or not reported	4 (36)	3 (42)	1 (25)
Ethnicity, n (%)			
Not Hispanic or Latino	7 (64)	4 (57)	3 (75)
Hispanic or Latino	2 (18)	2 (29)	0
Unknown or not required	2 (18)	1 (14)	1 (25)
Hydrocephalus etiology, n (%) CNS tumor	1 (10)	2 (29)	1 (25)
Myelomeningocele	3 (27)	2 (29)	1 (25)
Communicating congenital hydrocephalus	2 (18)	2 (29)	0
Post-intraventricular hemorrhage secondary to prematurity	3 (27)	1 (13)	1 (25)
Aqueductal stenosis	2 (18)	0	1 (25)
Complex chronic condition in addition to hydrocephalus, n (%)	4 (36)	2 (29)	2 (50)

*No statistically significant differences (p < 0.05) were observed between patients who did and did not require multiple revision surgeries.

Table 2
Surgical Details*.

|	Entire Cohort (n = 11)	Patients Not Requiring Multiple Revision Surgeries (n = 7)	Patients Requiring Multiple Revision Surgeries (n = 4)
Mean surgery duration in minutes (standard deviation)	44.2 (8.4)	43.7 (9.7)	45.0 (6.9)
Ancef use as prophylactic perioperative antibiotics, n (%)	10 (91)	6 (86)	4 (100)
Valve type, n (%)			
Strata	9 (82)	5 (72)	4 (100)
Delta	1 (9)	1 (14)	0
Other	1 (9)	1 (14)	0
Simple shunt, n (%)	11 (100)	7 (100)	4 (100)
Proximal catheter placement in ventricle, n (%)	11 (100)	7 (100)	4 (100)
Distal catheter placement in peritoneum, n (%)	11 (100)	7 (100)	4 (100)
Ventricular catheter placement assistance, n (%)	0 (0)	0 (0)	0 (0)
Endoscopy			
Ultrasound	0 (0)	0 (0)	0 (0)
Stereotactic navigation	11 (100)	7 (100)	4 (100)
Post-operative complication, n (%) Sepsis	0 (0)	0 (0)	0 (0)
CSF leak	1 (9)	0 (0)	1 (25)
Pseudomeningocele	0 (0)	0 (0)	0 (0)
Wound infection	0 (0)	0 (0)	0 (0)
Meningitis	0 (0)	0 (0)	0 (0)
Bowel perforation	0 (0)	0 (0)	0 (0)
Other	1 (9)	0 (0)	1 (25)

*No statistically significant differences (p < 0.05) were observed between patients who did and did not require multiple revision surgeries.

Differences were not observed in MCP-3, eotaxin, IFN-γ, and IL-13 in the Luminex assay.

From this cohort of 11 patients, 4 (33%) underwent subsequent revision surgeries over the 2 years following initial CSF shunt placement. The number of revision surgeries ranged from 3 surgeries to 6 surgeries with a median of 4 revision surgeries. Shunt obstruction was the most common cause of required CSF shunt revision (55%). Median time between surgeries was 59 days with an interquartile range of 52–70 days (min 6, max 92 days). Two of the patients have experienced no additional revisions surgeries up to the time of publication; SEA0248 underwent their next revision surgery 22 months later.

A total of 7 CSF samples from subsequent revision surgeries for 3 patients were collected and analyzed using Olink (Fig. 3) and Luminex (Supplemental **Figure 2**) assays. Qualitatively similar profiles for the majority of cytokines were observed between surgeries for all 3 patients in both Olink and Luminex assays. Of note, the Luminex assay demonstrated similar findings for IP-10 and IL-6.

Pro- and anti-inflammatory cytokines were selected *a priori* and shown across subsequent revision surgeries for the 3 patients. (Fig. 4) Cytokine patterns differed between patients, but within a given patient pro-inflammatory and anti-inflammatory cytokines appeared to be coordinately expressed, with the exception of IL-4, the levels of which varied independently.

4. Discussion

Heat maps of cytokine levels at the time of initial CSF shunt placement for each child undergoing only a single initial CSF shunt placement and for each child undergoing repeat CSF shunt revision surgeries demonstrate qualitatively similar profiles for the majority of cytokines. Lower levels of MCP-3, CASP-8, CD5, CXCL9, CXCL11, eotaxin, IFN-γ, IL-13, IP-10, and OSM at the time of initial surgery were noted in the children who went on to require multiple surgeries. While we focus our results on the Olink assay, the Luminex assay demonstrated similar findings for IP-10 and low detection for other cytokines in common between the two platforms. Cytokine patterns differed between patients, but within a given patient pro-inflammatory and anti-inflammatory cytokines act in a parallel fashion, with the exception of IL-4. While we focus our results on the Olink assay, the Luminex assay demonstrated similar findings for IP-10 and IL-6.

Type 1 T helper (Th1) cells stimulate cellular immune response, participate in the inhibition of macrophage activation, and stimulate B cells to produce antibodies. Migration of cells to the CNS is regulated by IFN-γ induced chemokines such as the IFN-γ induced protein (IP-10)/CXCL10 and the monokines induced by IFN-γ (MIG/CXCL9 and CXCL11), which are all 3 ligands of the CXC chemokine receptor 3 (CXCR3).[36] In physiological conditions, these chemokines are undetectable in most non-lymphoid tissues but they are strongly induced upon IFN-γ signaling, infection, or tissue injury. [32] The finding that lower levels of these chemokines are associated with subsequent shunt failure suggests upregulation of the Th1 pathway via all 3 ligands of
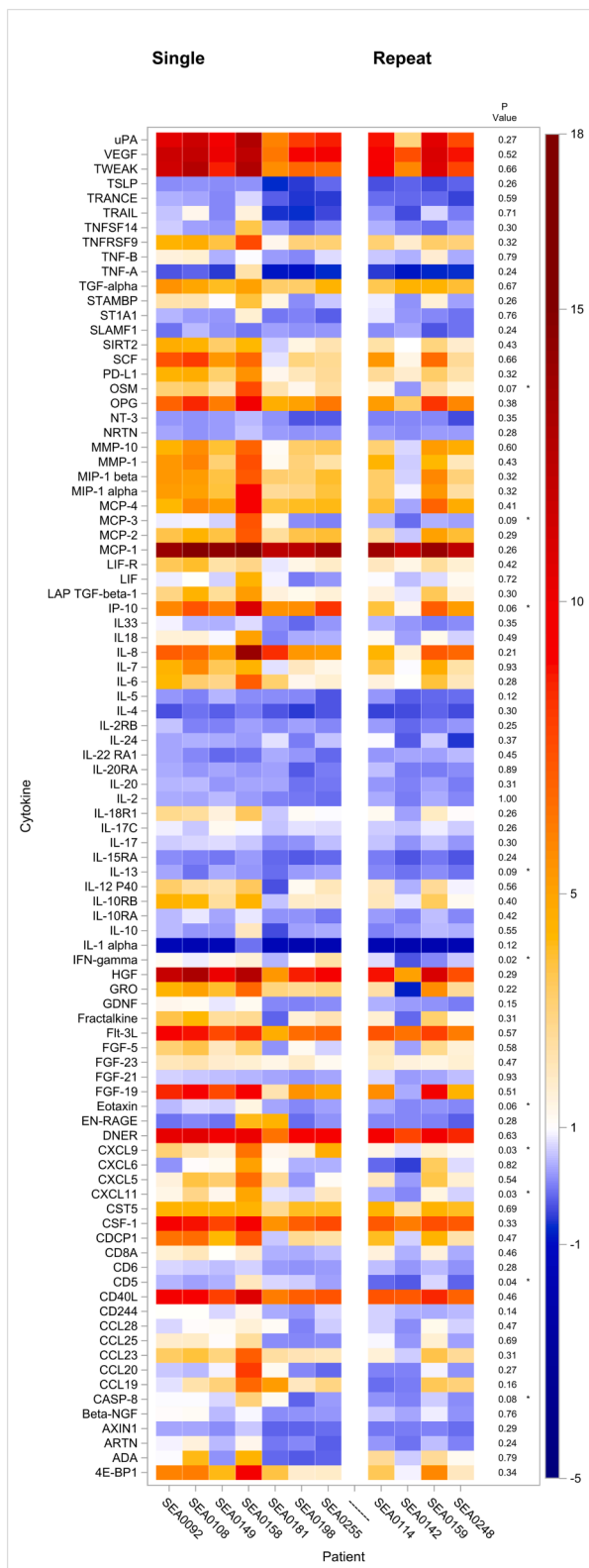
Fig. 1. Olink heatmap results showing the cytokine profiles at the time of initial surgery for children who undergo placement of an initial CSF shunt and require no revisions, vs. those requiring repeat revisions. Patients are listed along the x-axis, cytokines are listed on the y-axis, and p values generated from permutation tests are displayed next to the cytokines on the right side. Relative intensity of cytokine expression (NPX) is denoted by color gradient shown in legend to the right of the figure.

CXCR3 (IP-10, CXCL9, CXCL11) at the time of shunt placement may be associated with protection from shunt failure.

Type 1 T helper (Th2) cells stimulate the humoral immune response, promote B cell proliferation and induce antibody production. Some of the cytokines involved in the Th2 pathway include IL-13 and eotaxin. The finding that lower levels of these chemokines are associated with subsequent shunt failure suggests upregulation of the Th2 pathway may be associated with protection from shunt failure. Further study in larger cohorts of patients, ideally stratified by age, etiology, and/or mechanism of failure, is needed to better understand the roles of the Th1 and Th2 pathways in shunt failure.

Cytokine findings in hydrocephalus are variable, but most of the cytokines observed to be lower in children undergoing repeated future surgeries have been reported on in association with hydrocephalus. A systematic review of studies to date suggested that IL-6, IL-1β, LRG, IL-18, VEGF, and IFN-γ are elevated in CSF from patients with hydrocephalus and may be involved in promotion of hydrocephalus development and progression. [33] IFN-γ showed most promise in development of hydrocephalus due to diagnoses other than post-hemorrhagic hydrocephalus, whereas CSF of post-hemorrhagic hydrocephalus patients had increased levels of IL-6, IL-18, and VEGF. [33] Higher levels of IL-1β and other pro-inflammatory cytokines have been implicated in CSF cell levels observed in, [16] as well as development of, post-hemorrhagic hydrocephalus. [34] Cytokines in the Th1 pathway have been associated with increased CSF cell levels observed in post-hemorrhagic hydrocephalus. [16] A dysregulated host immune response with signaling via the IL-4, IL-13, and interferon pathways have been observed in inflammatory hydrocephalus. [35] Elevated levels of OSM were observed in idiopathic normal pressure hydrocephalus patients. [33] Several other cytokines such as MCP-3, CASP-8, and CD5 have not been reported to have an association with hydrocephalus previously.

However, this study differs from many of these studies as it includes only children with hydrocephalus and is more focused on shunt outcomes. Here the literature is more sparse. Eotaxin has been associated with CSF eosinophilia during CSF shunt infection [36]. Mixed findings were observed in pediatric patients with shunted hydrocephalus [37]. Our own findings in a very limited number of patients suggest patterns differed between patients, but within a given patient pro-inflammatory and anti-inflammatory cytokines act in a parallel fashion, with the exception of IL-4. For children with shunted hydrocephalus, better stratification by patient age, etiology, and mechanism of failure is needed to develop a deeper understanding of the mechanism of inflammation in shunted hydrocephalus [33]. There may also be benefit in comparing serum and CSF cytokine levels in future studies.

This study was subject to several limitations. Given the modest number of children and many cytokines tested, there is a risk of false discoveries with multiple comparisons within this pilot study. CSF sampling and preanalytical handling (centrifugation, time from sampling to storage, storage material, and storage temperature) all can influence CSF analyses. [33] These samples were all collected from the operating field and were kept at 4°for variable durations of time prior to storage at −80. Standardization of as many of the preanalytical handling of CSF prior to analyses will optimize our ability to draw meaningful conclusions about cytokines. In addition, we did not obtain information about use of anti-inflammatory medications in this patient population which may impact findings. Despite these limitations, we were able to explore a more diverse battery of cytokines in hydrocephalus, as well as change within patient over longer time, compared to earlier cohorts. [16,37].

5. Conclusion

Given this and earlier study's mixed findings of the cytokine activity in children with hydrocephalus, [16,37] better stratification by patient age, etiology, and mechanism of failure is needed to develop a deeper
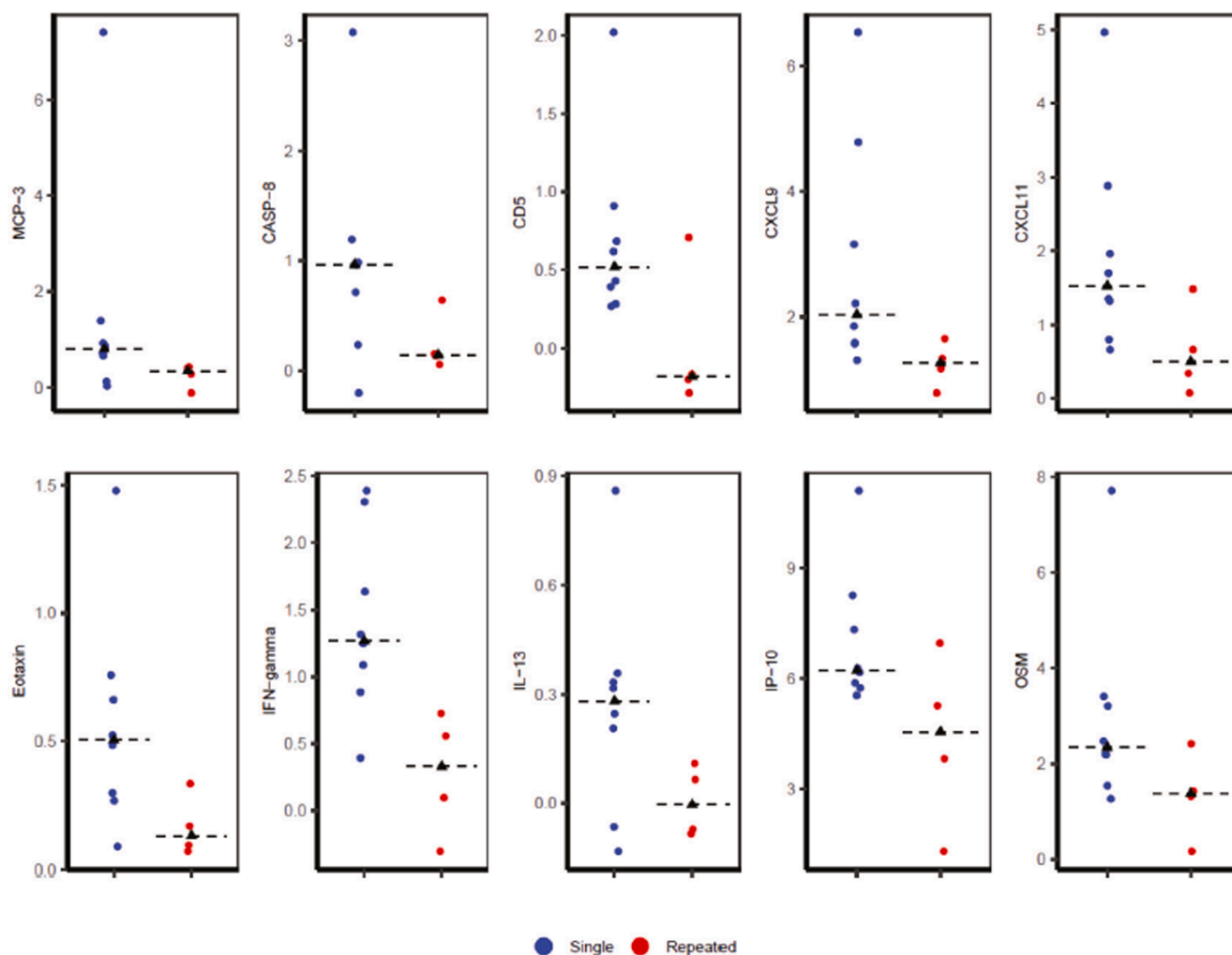
Fig. 2. Dot scatterplots showing the relative intensity of cytokine expression (NPX) (y-axis) for cytokines with differences (p < 0.10) noted between the time of initial surgery for patients who undergo single versus repeated surgeries (x-axis). Horizontal bars represent medians.

understanding of the mechanism of inflammation in the development of hydrocephalus, [33] the response to shunting of hydrocephalus, as well as the contribution of inflammation to the pathophysiology of CSF shunt infection.

Ethics approval and consent to participate

This study has received approval from Seattle Children's Hospital (FWA00002443) and the Childrens Hospital of Los Angeles (FWA00001914) institutional review boards.

Authors' contributions

The roles and responsibilities of the authors in this study are as follows:

TS: Conception and design of the study, analysis and interpretation of data, drafting the article, providing administrative, technical, and material support, and overseeing the study.

SS: Analysis and interpretation of data, drafting the article, providing administrative, technical, and material support.

YRH: Acquisition of data, analysis and interpretation of data, critically revising the article.

RDA: Analysis and interpretation of data, critically revising the article, and conducting statistical analysis.

KW: Analysis and interpretation of data, critically revising the article, and conducting statistical analysis.

PH: Analysis and interpretation of data and reviewing the submitted version of the manuscript.

JH, DL, PM, and JO: Reviewed the submitted version of the manuscript and provided study supervision.

HM: Acquisition of data, analysis and interpretation of data, and critically revising the article.

All authors reviewed the manuscript and approved it for submission.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tamara Simon reports financial support was provided by National Institutes of Health. Tamara Simon reports financial support was provided by National Center for Advancing Translational Sciences. David D. Limbrick reports a relationship with Mircobot Medical Inc. that includes: funding grants. No other conflict of interest to disclose.
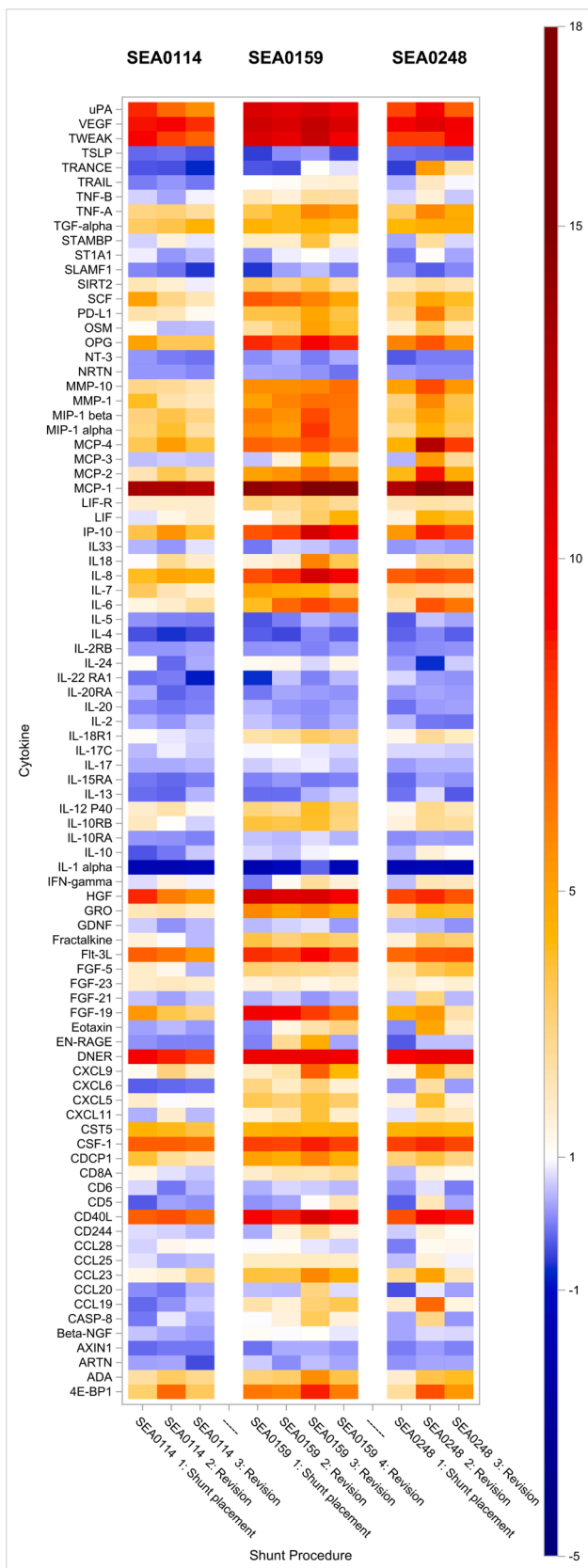
Fig. 3. Olink heatmap results showing the cytokine profiles of patients who undergo placement of an initial CSF shunt and require repeat revision surgeries. Procedures within each patient are listed along the x-axis, and cytokines are listed on the y-axis. Relative intensity of cytokine expression (NPX) is denoted by color gradient shown in legend to the right of the figure.
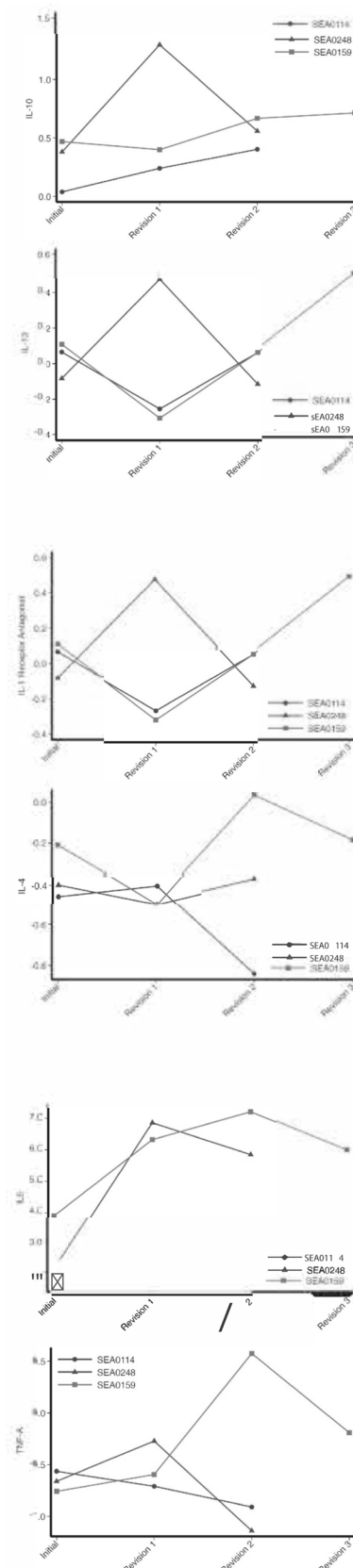
Fig. 4. Spaghetti plot of pro-inflammatory cytokines such as interleukin (IL) −6 and TNF-α; and anti-inflammatory cytokines such as IL-1 receptor antagonist, IL-4, IL-10, and IL-13 for patients who undergo placement of an initial CSF shunt and require repeat revisions surgeries.

Data availability

The data that has been used is confidential.

Acknowledgments

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2023.156310.

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