Cerebrospinal Fluid Biomarkers of Neuroinflammation in Children with Hydrocephalus and Shunt Malfunction

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Abstract

Background: Cerebrospinal fluid (CSF) shunt systems fail approximately 30% of the time within the first year of shunt implantation, while 98% of all patients with hydrocephalus (HCP) will suffer shunt failure in their lifetime. Perhaps due to the heterogeneity in etiology or the unique environment housing CSF shunt systems, the underlying reasons for shunt failure continue to elude researchers and clinicians. Still, obstruction remains the most common reason for shunt failure and it is vital to understand what factors may be contributing or driving shunt failure for improving patient outcomes and ultimately quality of life.

Methods: Using multiplex ELISA, this study first examined the protein concentration profiles of select pro-inflammatory and anti-inflammatory cytokines, as well as select matrix metalloproteinases (MMPs) in 38 pediatric patients and their CSF obtained at shunt revision operation; all patients pediatric (<18). Shunt failure was further examined for interdependencies between the past number of previous revisions, length of time implanted, patient age, and the surgeon-described reason for revision noted as obstruction or non-obstruction. The pro-inflammatory cytokines were IL-1β, IL-2, IL-5, IL-6, IL-8, IL-12, IL-17, TNF-α, GM-CSF, IFN-γ. The anti-inflammatory cytokines were IL-4 and IL-10, and the MMPs are MMP-2, MMP-3, MMP-7, MMP-9. protein concentration is reported as pg/mL for each analyte.

Results: IL-10, IL-6, IL-8 and MMP-7 demonstrated significantly increased concentrations in patient CSF for the non-obstructed subgroup compared to the obstructed subgroup ($p=<0.05$). Under generalized etiology, these analytes present within communicating HCP cases (15 of which were diagnosed with PHH) compared to non-communicating HCP or traumatic brain injury (TBI) cases. Further examination revealed that CSF IL-6 was significantly increased in both obstructed and non-obstructed cases, predominately for PHH and congenital HCP patients while IL-8 was significantly higher only in PHH patients. In terms of number of past revisions, IL-10, IL-6, IL-8, MMP-7 and MMP-9 significantly and progressively increased from zero to two past revisions and then significantly declined and remained low for subsequent revisions ($p=<0.05$). In terms of implantation time, CSF from shunts implanted for three months or less show significantly increased concentration for IL-6, IL-8, and MMP-7 ($p=<0.05$). Lastly, Six months or less was the age identified to coincide with significantly increased concentration of IL-6, IL-8, and MMP-7 ($p=<0.05$).

Conclusion: Of all the cytokines and MMPs tested, IL-10, IL-6, IL-8, MMP-7 and MMP-9 were significantly elevated compared to other cytokines and MMPs in the various dependencies evaluated. IL-6 and IL-8 stand out in our study with significantly increased concentration in select etiologies, age, length of time implanted. The aforementioned cytokines and MMPs all showed an interesting relation with number of past revisions which warrants further examination. Non-obstruction cases were determined to be accompany significantly higher CSF cytokine MMP presence compared to obstructive cases. This suggests closer examination to the extent of the role neuroinflammation plays for causing obstruction in HCP patients. Additionally, IL-10 was associated with the length of time implanted as well as number of past revisions, and higher in non-obstructed cases compared to obstructed cases. A severity-dependent

interplay between IL-10, IL-6 and IL-8 is an area for expansion and may provide foundation for therapeutic control of neuroinflammation.

Trial registration: Not applicable.

Background

Hydrocephalus predominately presents as an abnormal expansion of cerebral ventricles due to cerebrospinal fluid (CSF) build up [1, 2, 3] . Left untreated, increased intracranial pressure and consequent compression of brain structures can lead to neurological defects, nausea, headaches, lethargy and ultimately be fatal [3,4,7] . Globally, hydrocephalus has a prevalence between 11–175/100,000 depending on the age [5,6] . In the United States, hydrocephalus is responsible for 69,000 annual discharges, incurring a burden greater than $1.4-2.0 billion to the healthcare system [6,8,9] . Upon deeper examination, clinicians and researchers have observed hydrocephalus to be a multifactorial and more complicated condition than its initial impression of being a “plumbing problem”, containing a heterogenous group of etiologies [8] . Hydrocephalus can be caused by genetic defects or congenital malformation, or it can be acquired through hemorrhage, trauma, tumors, infection, or other causes [4, 5, 8] .

To date, no 100% effective treatment of hydrocephalus exists. However, the most effective interventions for treating symptoms of hydrocephalus are surgical, typically Endoscopic Third Ventriculostomy (ETV) or CSF shunting; the gold standard and most common treatment for hydrocephalus[9,10]. In recent decades, ventriculoperitoneal (VP) shunts have been used in concert with valves to redirect excess CSF from the cerebral ventricles into the peritoneum[10]. However, regardless of design or technical issues, shunts are plagued by a number of complications that usually result in their failure, requiring their subsequent revision and eliciting costly financial and healthcare burdens[11, 12, 23].

CSF shunt systems fail approximately 30% of the time within the first year of shunt implantation, and 40%-50% within the first two years of shunt placement[12, 13]. 85% require revision surgery within the first ten years of shunt placement while 98% of all patients with hydrocephalus will suffer shunt failure in their lifetime[13, 14]. Still, shunt failure is most commonly seen among pediatric hydrocephalus patients[8, 15]. It has been difficult to discern a unifying feature underlying CSF shunt systems failures as they can fail anywhere between days to months to years, but also due to a heterogeneity in complications including infection, obstruction due to clot formation, scar tissue or choroid plexus, disconnection or migration of shunt, loculation, as well as over/under-drainage of CSF due to valve malfunction, [8, 12–18]. Obstruction has been reported to be the most common reason for shunt failure[19].

As many mechanisms underlying shunt failure elude researchers and clinicians, it is important to thoroughly investigate the CSF and environment surrounding the shunt catheters in addition examining to the efficacy of the catheters themselves[24, 25]. To that end, this study first looks at the protein concentration profiles of select pro-inflammatory and anti-inflammatory cytokines, as well as select

matrix metalloproteinases (MMPs) in CSF at the time of shunt failure from patients with various hydrocephalus etiologies. Subsequently, in order to gain a deeper understanding of the various interdependencies within this cohort, the role of suspect factors which may be influencing shunt failure is examined here, namely: etiology, shunt revision history, length of time implanted, and age. Lastly, the study compares the presentation of pro-inflammatory and anti-inflammatory cytokines as well as select MMPs differentially if the reason for revision is noted by the clinician was obstruction or non-obstruction. Identifying and understanding the relationship between the CSF and environment surrounding the shunt catheters at the molecular and cellular level is imperative towards gaining an understanding of shunt failure and facilitating better informed clinical decisions for the future.

Materials And Methods

The permission to collect CSF samples from failed shunt surgeries was approved by local ethics committees at each participating center; records of approval were sent to the coordinating center, Wayne State University (WSU), and submitted as amendments to our protocol under the Institutional Review Board (IRB). Washington University Human Research Protection Office (WU-HRPO) approval was obtained prior to beginning this study. Written informed consent was obtained from all patients or their legally authorized representative. Collection was performed in a manner consistent with the Declaration of Helsinki and represents no modification to the standard of treatment. The patient population includes a vulnerable group (children), but the study is aimed at addressing the health needs of this group and cannot be conducted in a non-vulnerable group.

Study population

38 samples were collected from neonates to those 17 years and younger. Samples were collected from individuals with any hydrocephalus etiology except normal pressure hydrocephalus. Patients were evaluated by local centers according to their individual guidelines, and samples were only collected if the shunt malfunction necessitated revision. For revision history, implantation time and age, binning was done with a focus on acute timeframes and early age groupings. This is in accordance with previous reporting that the highest risk of shunt failure is acute, which decreases over time and over number of revised shunts[43], and the fact that hydrocephalus is more common in infants[6,9]. Data for healthy controls was retrieved from previous publications where similar multiplex ELISAs were performed using age-matched patient CSF whenever possible [20–22, 47] and added to the figures as a dotted line. All pediatric subjects underwent routine MRIs and/or CT to assist in the diagnosis of a shunt malfunction. Those found to have a possible shunt malfunction were then at the discretion of the treating neurosurgeon whether surgery was required or not. At the time of shunt surgery, CSF was collected and transported to the Washington University Neonatal CSF Repository on ice. Samples were then centrifuged at 2500 rpm for 6 min and the supernatant was then stored in microcentrifuge tubes at − 80 °C until experimental analysis.

Data collection and Multiplex Assay

Multiplex assays were run by the Bursky Center for Human Immunology & Immunotherapy Programs (CHiiPs) at Washington University School of Medicine according to the manufacturer's instruction. Frozen CSF was slowly thawed and then analyzed in duplicate fashion with multiplex kits (Thermofisher Scientific, Waltham, MA) for the following pro-inflammatory cytokines: IL-1β, IL-2, IL-5, IL-6, IL-8, IL-12, IL-17, TNF-α, GM-CSF, IFN-γ; and anti-inflammatory cytokines: IL-4 and IL-10; as well as select MMPs: MMP-2, MMP-3, MMP-9, MMP-7 (catalog number EPXP130-10100-901, EPX03A-10829-901, PPX-01-MMP7). protein concentration is reported as pg/mL for each analyte.

Data presentation and statistical analysis:

All data presentation was performed using Graphpad Prism version 8.4.0 in MacOS. Mean difference between groups with a 95% confidence interval is shown. Two-way ANOVA comparisons were conducted to determine dependency of past revision number, length of time implantation, etiology and age on cytokine and MMP protein concentration in CSF collected, assuming unequal variances. A predetermined significance level of 0.05 was used in all statistical tests.

Results

Comparisons were made for protein concentration in CSF for the following pro-inflammatory cytokines: IL-1β, IL-2, IL-5, IL-6, IL-8, IL-12, IL-17, TNF-α, GM-CSF, IFN-γ; and anti-inflammatory cytokines: IL-4 and IL-10; as well as select MMPs: MMP-2, MMP-3, MMP-7, MMP-9. Figure 1 includes a distribution of patient etiology. Figure 2 shows the distribution of samples within the dataset in relation to length of time implanted, age and number of past revisions.

Obstruction vs. Non-Obstructed

All samples with protein concentration values for the aforementioned analytes within the dataset were separated according to clinical determination of whether the shunt catheter was being revised due to obstruction (n = 19) or non-obstruction reasons (n = 19). As Figs. 3A and 3B show, none but IL-10 ($p = 0.0051$), IL-6 ($p = < 0.0001$), IL-8 ($p = < 0.0010$) and MMP-7 ($p = 0.0494$) demonstrated significantly increased concentration in patient CSF in the non-obstructed subgroup compared to the obstructed subgroup. This is primarily observed data from an increase in expression from female individuals with PHH whose shunt was revised due to loculation. This demonstrated that the variance in the cytokine and MMP protein concentration in hydrocephalus patients needs further analysis. Therefore, the data was examined for the influence of the following factors on protein concentration: past number of previous revisions, length of time implanted, age and etiology in each category subdivided shunt catheter revised due to obstruction or other reasons.

Etiology

As Fig. 4A shows, IL-10, IL-8 and MMP-7 demonstrated significantly increased concentrations in patient CSF for communicating cases compared to non-communicating cases, while only IL-6 is significantly higher than both non-communicating and TBI cases. Further examination of the etiologies within the

cohort, as included in supplementary Fig. 4A: aqueductal stenosis (n = 3), congenital HCP (n = 3), infection (n = 2), myelomeningocele (n = 6), traumatic brain injury (TBI) (n = 3) and individuals with PHH being the most prevalent (n = 15). IL-6 was significantly increased in patients with PHH compared to those with aqueductal stenosis (p = 0.0295), myelomeningocele (p = 0.0002), and TBI (p = 0.0282). IL-8 was significantly higher only in PHH patients when compared to patients with myelomeningocele (p = 0.0144).

Subsequently, data was parsed according to the reason for revision: obstruction or non-obstruction (n = 19 for both) for Figs. 4B and 4C. In obstructed cases, no cytokines or MMPs are significantly changed within the general etiologies. Interestingly, only IL-6 was determined to be significantly higher and showed a similar profile as seen in Fig. 4A. In contrast, supplementary Fig. 4B shows, in individuals denoted with obstruction for the reason for revision, IL-6 is the sole cytokine with significantly higher expression in individuals with congenital HCP (p = 0.0126, p = 0.0003) and PHH (p = 0.0018) compared to other etiologies. For non-obstructed cases as Fig. 4C shows, it is to be noted IL-6 was also the only significantly higher expression in individuals with PHH compared to TBI in the non-obstruction subgroup (p = 0.0169).

Revision History

Data was parsed according to revision history in order to determine if a correlation existed between the patient's revision history (quantified as the number of past revisions) and the protein concentration of neuroinflammatory cytokines and MMPs. Figure 5A includes all samples within the cohort without regard to reason for revision, while Fig. 5B includes protein concentration within the obstruction subgroup and Fig. 5C includes protein concentration within the non-obstruction subgroup. As Fig. 5A shows, in the case of all-inclusive data, only IL-6 (p = < 0.0001) and IL-8 (p = < 0.0051) are observed to hold significantly higher expression at the point of two previous revisions compared to the preceding and subsequent number of past revisions, but also a sharp drop in protein concentration is observed for individuals with three previous revisions and onward. Similarly, MMP-9 (p = < 0.0224) is observed to hold significantly higher expression from zero to two previous revisions but not for the next number of past revisions.

Interestingly, none of the cytokines or MMPs demonstrate significantly variant expression in terms of dependency on the number of past revisions in the obstructed subgroup. In contrast within the non-obstructed subgroup (Fig. 5C), IL-10, IL-6 IL-8, MMP-7 and MMP-9 show a similar protein concentration profile between the different numbers of past revisions. IL-10 (p = 0.0409) was observed to be significantly increased at two past revisions compared to zero past revisions, but within the error rate for one past revisions. IL-6 (p = < 0.03) and IL-8 (p = < 0.01) showed to have a similar protein concentration profile, where protein concentration progressively and significantly increases from zero to two previous revisions and then a sharp drop is observed for individuals with three previous revisions. MMP-7 (p = < 0.0333) was shown to be significantly increased at two past revisions compared to zero past revisions, but within the error rate for one past revisions, with a sharp decline following the point of two past revisions. MMP-9 (p = < 0.0364) was observed to have a similar protein concentration profile as IL-6 and IL-8, where protein concentration progressively and significantly increases from zero to two previous revisions and then a sharp drop is observed past that point.

Implantation Time

Next, protein concentration of neuroinflammatory cytokines and MMPs was examined to determine whether a dependency on the implantation time persisted. In Fig. 6A, within the unparsed data IL-10, IL-6 IL-8, and MMP-7 are observed to have significant fluctuations in protein concentration when implantation times are compared. IL-10 ($p = 0.0236$) was significantly more protein concentration within the 3 months or less period compared to 36 months+. IL-6 ($p = < 0.002$) and IL-8 ($p = < 0.014$) were observed to have significantly higher protein concentration in the 3 months or less period compared to all other time points. Lastly, MMP-7 ($p = < 0.0462$) was significantly higher in the 3 months or less period compared to 12 months and after period, but within error range for the 3–12 months period.

Next, the data was parsed with regard to reason for revision, i.e. obstruction or non-obstruction. As Fig. 6B shows, within the obstructed subgroup IL-6 ($p = < 0.0001$) and IL-8 ($p = < 0.0001$) were observed to have significantly higher protein concentration in the 3 months or less period compared to all other time points. As Fig. 6B shows, in the obstructed subgroup, MMP-7 ($p = 0.0369$) was observed to be relatively the same for the first year, and then significantly decreased by 36 months + time. In the non-obstructed subgroup, Fig. 6C, only IL-6 ($p = 0.0366$) was observed to be significantly higher in the 3 months or less period and then remained low for the following periods.

Age

Lastly, determining the dependency of age on CSF cytokines and MMP protein concentration, the ages were divided up into 6-month periods for the 1st year, then yearlong periods leading up to 3 years, while ages 3 years and more were grouped together. In the unparsed data, Fig. 7A, IL-8 and MMP-7 stand out. IL-8 ($p = < 0.0422$) is significantly higher in the first 6 months in our dataset compared to the rest of the ages, with the exception of 2–3 years of age. MMP-7 ($p = < 0.00261$) was observed to be significantly decreased in all ages in our data set, except for less than 6 months and 1–2 years of age.

Discussion

One of the reasons why we still have not yet identified a unifying, underlying reason for shunt failure is because of the unique positioning of shunt systems. They are inserted through cerebral cortex into the cerebral ventricles then bathed in CSF, where the shunt catheter is subject to protein adsorption, cells, tissues, and cellular debris with unknown variances in degree. Excluding shunt systems which fail due to distal effects such as displacement, disconnection, or valve disfunction, it is easy to imagine CSF shunt systems which fail must have failed in relationship to the factors present in the patient CSF. Previous evidence suggests elevated pro-inflammatory cytokine levels in CSF are conducive to worsening clinical outcomes in neuroinflammatory diseases[26–28]. However, to date there is no comprehensive hydrocephalus-focused CSF collection project focused on shunting and shunt failure and their relationship with neuroinflammation. Many cases of shunt failure are captured emergently or during off-peak hours, meaning routine CSF capture by research personnel may be expensive or have a latency in capture speed. The work presented here yields preliminary data to guide the direction of future

experiments by shedding light on the expression profile of cytokines and MMPs in patient CSF immediately prior to revision surgeries.

By looking at protein concentration levels, our results for the analytes tested suggest cytokine expression and MMP activation is generally decreased or within error in individuals with revised shunts due to symptoms of obstruction compared with those that failed for non-obstructed reasons (Fig. 3-Figure 8, supplementary Figs. 1–4). Meaning, that despite most of the pro-inflammatory cytokines and MMPs protein concentrations being close to healthy control levels or within deviation between obstructed and non-obstructed cases, other major cytokines and select MMPs stand out and consistently. These analytes with significant increase in non-obstructed cases at total protein concentration levels are IL-10, IL-6, IL-8, and MMP-7. Low sample size may preclude this as a predictor, however. Inclusion of referenced healthy controls as baseline shows the protein concentration levels for almost all of the cytokines and MMPs tested are either elevated or at baseline in all of the dependencies examined (Figs. 4–7).

Sorting by etiology reveals IL-6 as significantly increased in both obstructed and non-obstructed individuals and primarily for PHH and congenital HCP patients, while IL-8 is significantly higher in PHH patients when all of the data is considered. The lack of significant change in other cytokines and MMPs in other etiologies, considering protein concentrations or even after subdividing data according to obstructed or non-obstructed cases, suggests a high degree of variance in the patient population exists in terms of how their central nervous system and immune system responds towards recovering, compensating or aggravating hydrocephalus pathophysiology. This may be addressed by increasing the sample size to identify subsets, by standardizing and establishing a physiological grading system showcasing the stage/severity of hydrocephalus beyond clinical presentation[8, 10]. Additionally, in future work, it would be worthwhile to consider new classification or gradation schemes that correlate signs of physical obstruction of the shunt with the CSF quality or characteristics to eliminate out underlying subjectivity or variances.

Parsing the data according to past number of revisions reveals that none of the analytes are significantly increased in obstruction cases. However, in the non-obstructed cases an interesting trend persists. protein concentration levels for IL-10, IL-6, IL-8, MMP-7 and MMP-9 significantly and progressively increase from zero to two past revisions but then sharply drop for patients coming in with three past revisions. It should be noted those patients who came in with three past revisions only had their shunts implanted for less than four days and required revision. Whereas, the seven patients who came in with four or more past revisions had theirs left implanted for more than five years and needed revision after said extended length of time due to obstruction in four of seven cases. This presentation may very well be due to the fact that patient CSF was collected at the time of shunt failure/revision surgery. Meaning, that the elusive event(s) which may be responsible for CSF shunt system failure may already have come to pass prior to or as the patient's symptoms developed, and even decayed by the time CSF was collected. Additionally, small sample sizes per bin limit our ability to detect significant relationships in the obstruction cases as none of the cytokines significantly changed between the different revision attempts. However, as obstruction

remains the most common reported reason for shunt failure, a more comprehensive, larger sampled review of CSF cytokines would aid towards understanding the mechanisms driving shunt obstruction[19].

The lack of a clear neuroinflammatory response brings into question the role of neuroinflammation and CSF cytokines and MMPs as a driving force behind obstruction and consequent shunt failure. A longitudinal study with a large patient cohort and comprehensive in nature would be able to discern why protein concentration levels for major cytokines such as IL-10, IL-6 and IL-8, as well as MMP-7 and MMP-9 drop to healthy control/baseline levels by the third revision. Such a study would also answer whether revisions are still required despite the low presence of major neuroinflammatory cytokines in the patient CSF and thus, tell us of the extent of a role neuroinflammation plays in causing obstruction and/or shunt failure in HCP patients. Overall, IL-10, IL-6 and IL-8 consistently standout and may provide inspiration for therapeutic control of neuroinflammation and mitigating poor clinical outcomes such as shunt failure.

Investigations into the dependency of the length of time implanted suggests three months or less as the marked time with significantly increased concentration of IL-6, IL-8, and MMP-7. While the six months or less is identified as the most vulnerable age with significantly increased concentration of IL-6, IL-8, and MMP-7. It is intuitive that the shortest length of time has the highest protein concentration of neuroinflammatory cytokines as the risk for shunt failure is highest in the early stages of the disease and as it decreases over time and over number of revised shunts[43]. This indicates that the CSF cytokines evaluated in this paper, and their subsequent effects on activating immune responses *in vivo*, are measurable and could be useful in quantifying the degree of severity in the clinic as a useful biomarker. Thus, further arming physicians to make informed clinical decisions about their patients. It is most curious that IL-6, IL-8, and MMP-7 are significantly increased in both early implantation time and infant age groupings. As infants are arguably the most vulnerable group suffering from hydrocephalus[6,9], the presence of neuroinflammation and dysregulated MMPs could be contributing to loss of tissue integrity, worsening of clinical symptoms and signs, and consequently impeding neurological development[3,4]. Therefore, the interplay of IL-6, IL-8, and MMP-7 and the role they play in hydrocephalus pathophysiology is worth future investigations to improve our understanding of the molecular and cellular events driving hydrocephalus.

The pro-inflammatory cytokines that stand out as elevated are IL-6 and IL-8, which is in line with previously published literature on neurologic damage in hydrocephalus[29]. IL-6 is a major cytokine involved in development, neurogenesis, brain injury and neurodegeneration, in addition to mounting an immune response and inducing astrogliosis, astrocyte proliferation, and angiogenesis for recovery in the central nervous system[30,31]. While IL-8 behaves in many similar ways as IL-6, it retains a longer half-life[32–34]. IL-10 is recognized to promote neuronal and glial cell survival and by inhibiting pro-inflammatory cytokine production to decrease neuroinflammation[35, 36].

We show that in addition to pro- and anti-neuroinflammatory cytokines, as well as MMPs have heterogenous presentation in hydrocephalic CSF. Collectively, MMPs have been known to digest extracellular matrix proteins, but little is known about their neuroprotective or neuroinflammatory role in

hydrocephalus. MMPs in the brain play a role in neurogenesis, central nervous system survival and development, neuronal myelination, integrity of the blood-brain barrier, and neuroinflammation inhibition[37–40]. Some studies have shown increased MMP-9 disrupts the blood-brain barrier and could even be responsible for spontaneous hydrocephalus[44,45], while others have shown, in infants with PHH, MMP-9 can help them overcome symptoms of hydrocephalus[46]. Therefore, before claims can be made toward therapeutic directions, further investigations and characterization of MMP-7 and MMP-9 are required to understand the extent in which they influence hydrocephalus pathophysiology.

It has been purported that IL-8 and IL-10 have a dichotomy towards brain volume, as well as grey and white matter formation and that their interaction can significantly modulate neuroinflammatory responses[41]. In our study, with the exception of age CSF IL-8 and IL-10 protein concentrations are significantly increased in almost all of the interdependencies examined. It may be interesting to investigate if supplementing endogenous MMP-9 and IL-10 production in a time-sensitive manner can mitigate the onslaught and severity of hydrocephalus, in cases where an aggravated immune response is not warranted such as displacement, disconnection, loculation or valve disfunction.

Many questions remain about the nature and pathophysiology of hydrocephalus. Development of a suitable *in vivo* model of HCP to determine the degree to which glial cells are present and activated in a neuroinflammatory or neuroprotective phenotype would allow us to see if CSF IL-6 and IL-8 can serve as therapeutic targets in the hydrocephalus brain, serve as diagnostic markers for the degree of severity of hydrocephalus, or if they should be left unmitigated due to the myriad of events IL-6 and IL-8 are involved in the brain and their systemic integration in innate and adaptive immune responses[26, 28]. Suppression of pro-inflammatory CSF cytokines such as IL-6 and IL-8, perhaps through treatment with biologics with demonstrably efficacy to blunt subsequent immune responses, may bring reprieve to individuals suffering from hydrocephalus and lessen the blunt of their symptoms[42]. Additionally, future work can look towards relationships between CSF protein concentrations and patient physiology. For instance, comparisons with factors such as BMI, and lifestyle, as well as the relationship to ictus and ventricular size.

Limitations:

A likely potential side effect of CSF being collected at one center is the high occurrence of PHH in our dataset. This study also suffers from small sample size, which becomes apparent when data is parsed and grouped by obstruction or non-obstruction/other reasons for revision surgery. This study also heavily relies on the surgeon's observations at surgery –obstruction or non-obstruction, it does not evaluate the nature of the obstruction, the degree of the obstruction nor its localization.

Conclusion

In summary, this study is the first to examine the protein-level expression profile of MMPs in concert with select pro-inflammatory and anti-inflammatory cytokines implicated in neuroinflammation within a hydrocephalus patient population at the time of shunt revision. The study also examined dependencies

based on etiology, past number of previous revisions, length of time implanted, and age. We found PHH patients seemingly had the highest CSF protein concentration for all the analytes tested, and six months or less and 3 months or less being the most vulnerable age groups and length of time implanted groups, respectively. IL-10, IL-6, IL-8, MMP-7 and MMP-9 were observed to be significantly elevation compared to other cytokines and MMPs. Therefore, they are recommended for future investigations to understand their molecular and cellular role in hydrocephalus disease pathophysiology.

Understanding the disease pathophysiology of hydrocephalus as a lifelong condition must begin at the molecular and cellular level in order to see what factors present in the CSF environment and the presentation of those factors in relation to each other lead to shunt failure in cases of obstruction or otherwise. A more comprehensive review of CSF obtained from hydrocephalus patients is very much required to give researchers and clinicians a satisfactory looking glass into the inner workings of the diseased hydrocephalus brain because unveiling the mystery of hydrocephalus will undoubtedly give us a better understanding of means to mitigate the disease as well as elucidate us on the inner workings of the human brain.

List Of Abbreviations

CSF – Cerebrospinal fluid

MMP – Matrixmetalloproteinases

ETV – Endoscopic third ventriculostomy

PHH – Post-hemorrhagic hydrocephalus

IL – Interleukin

TNF-α – Tumor necrosis factor alpha

GM-CSF – Granulocyte-macrophage colony-stimulating factor

IFN-γ – Interferon gamma

VP – Ventriculoperitoneal

HCP – Hydrocephalus

Declarations

Authors’ Contributions:

CH: Conceptualization, Methodology, Investigation, Data curation, Project administration,

RA: Writing—original draft, data analysis, visualization, and writing—review & editing.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

Not applicable.

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Figures

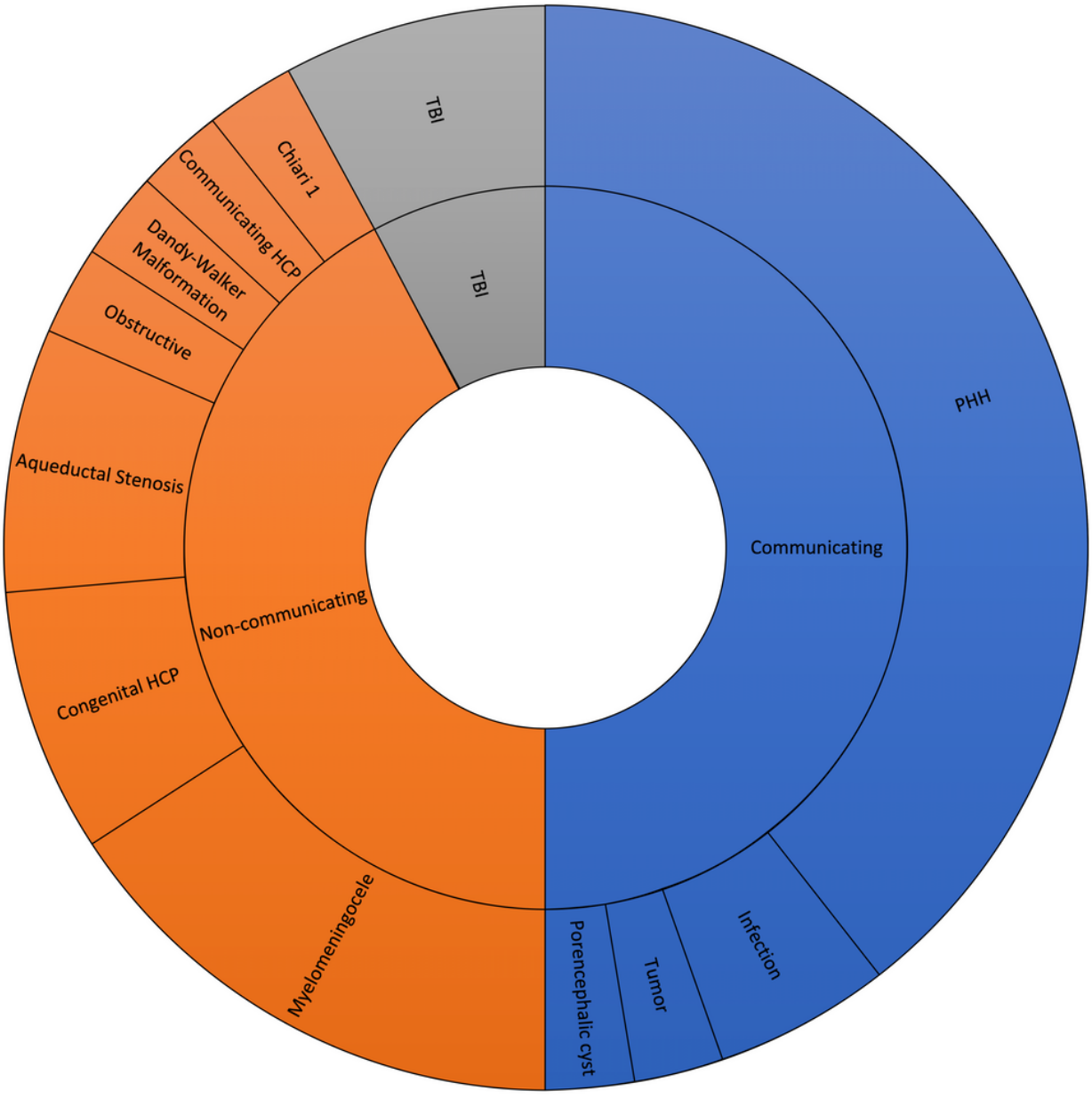


Figure 1

Distribution of etiologies and contained samples per etiology.

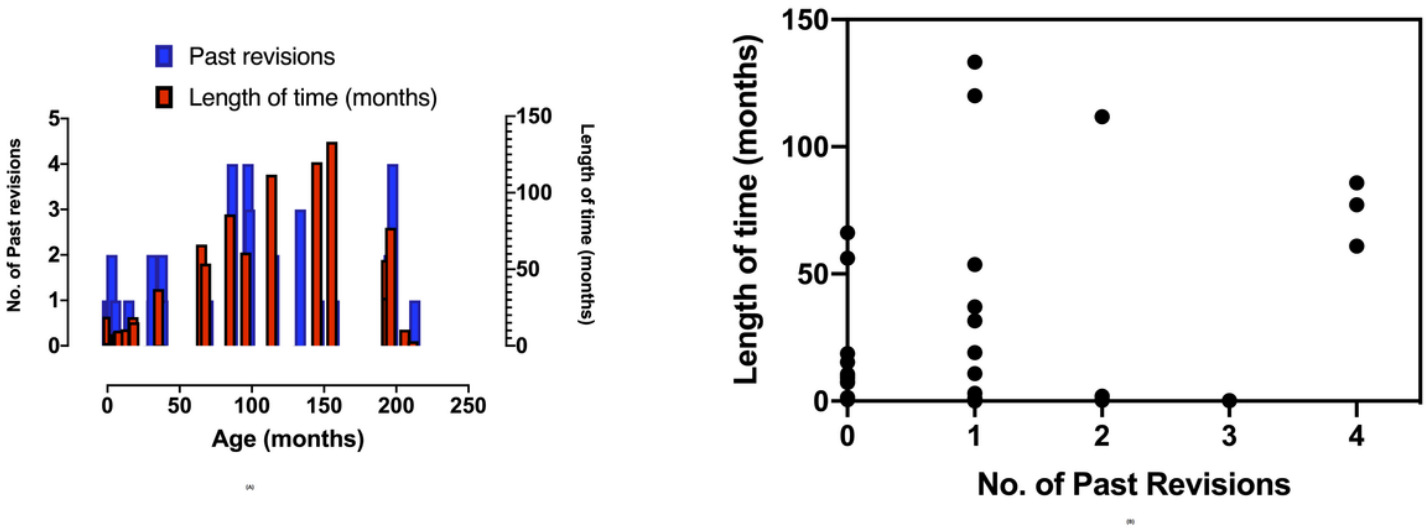


Figure 2

Distribution of samples per length of time implanted, age and number of past revisions.

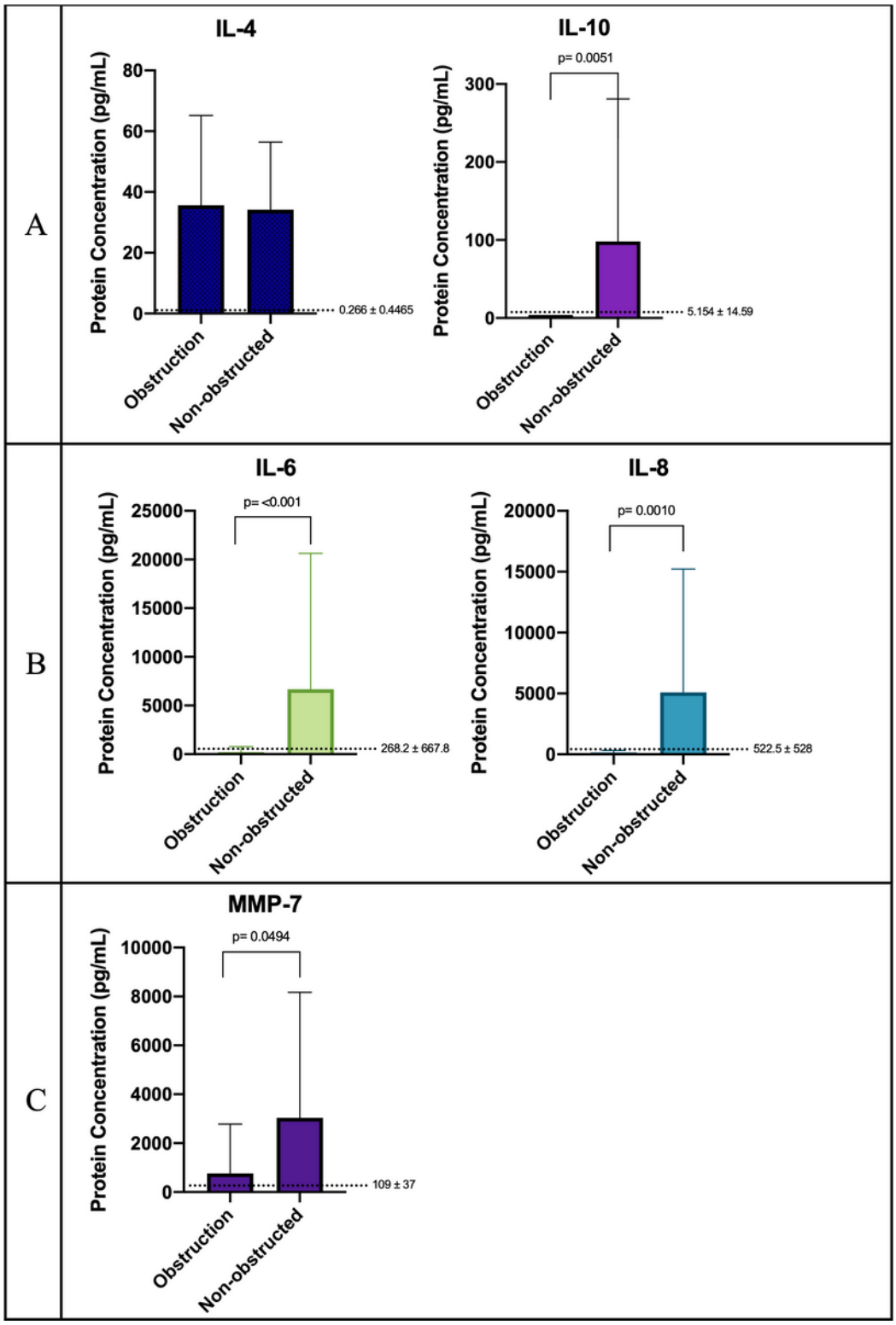
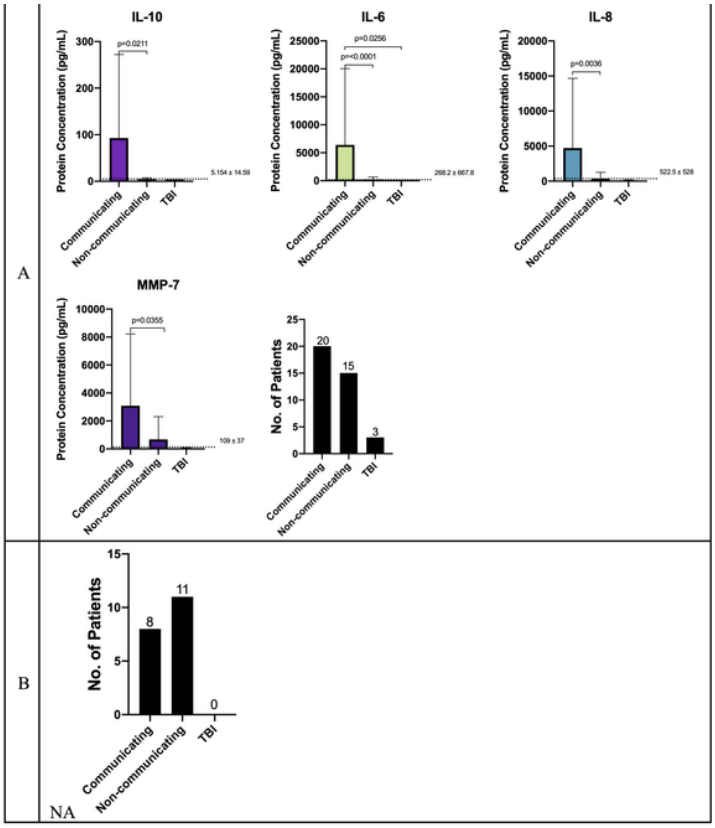


Figure 3

CSF cytokine and MMP protein concentrations between obstructive cases or non-obstructive cases (n=19 per group). Analytes showed include (A) anti-inflammatory cytokines, (B) pro-inflammatory cytokines and (C) MMPs.



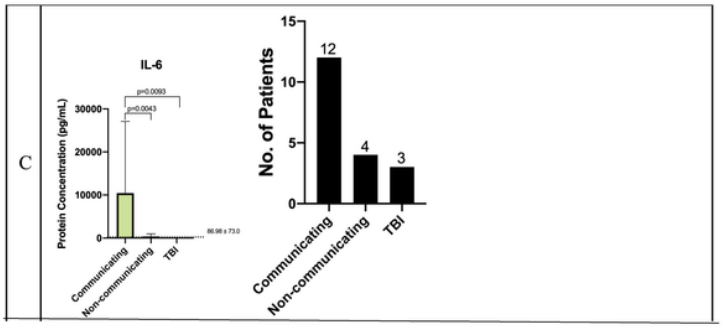
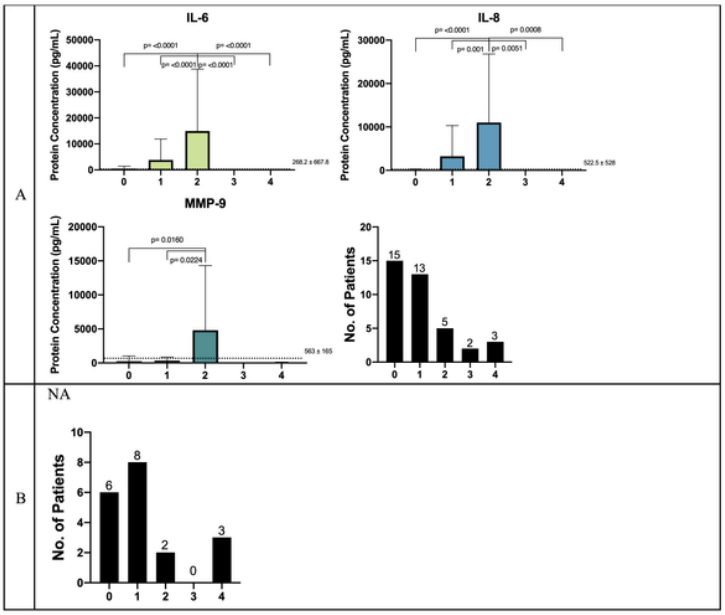


Figure 4

Following subdivision by general etiology, protein concentration values of select cytokines and MMPs and sample count per etiology of each of the following groups are reported: (A) unparsed data, (B) then for obstructed vs. (C) non-obstructed cases.



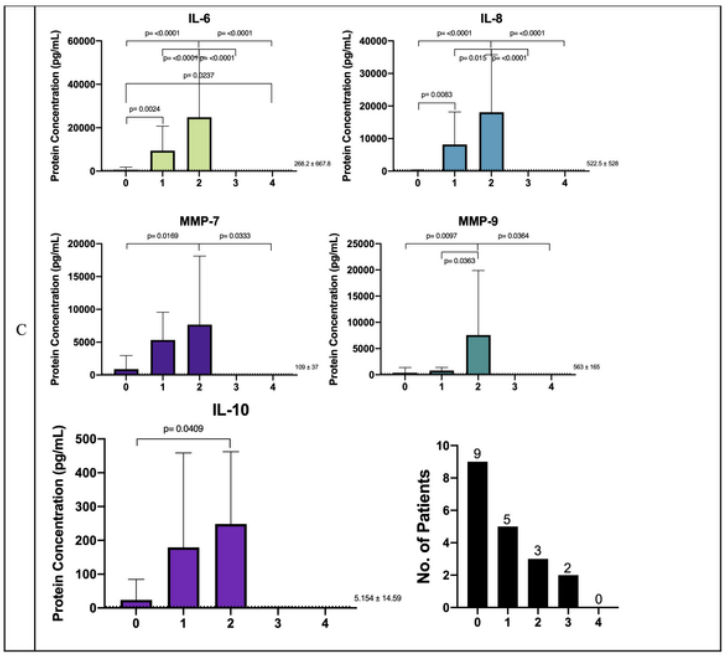


Figure 5

protein concentration values of CSF cytokines and MMPs after samples were subdivided by number of past revisions, as well as sample count per revisions are reported: (A) unparsed data, then (B) obstructed vs. (C) non-obstructed cases.

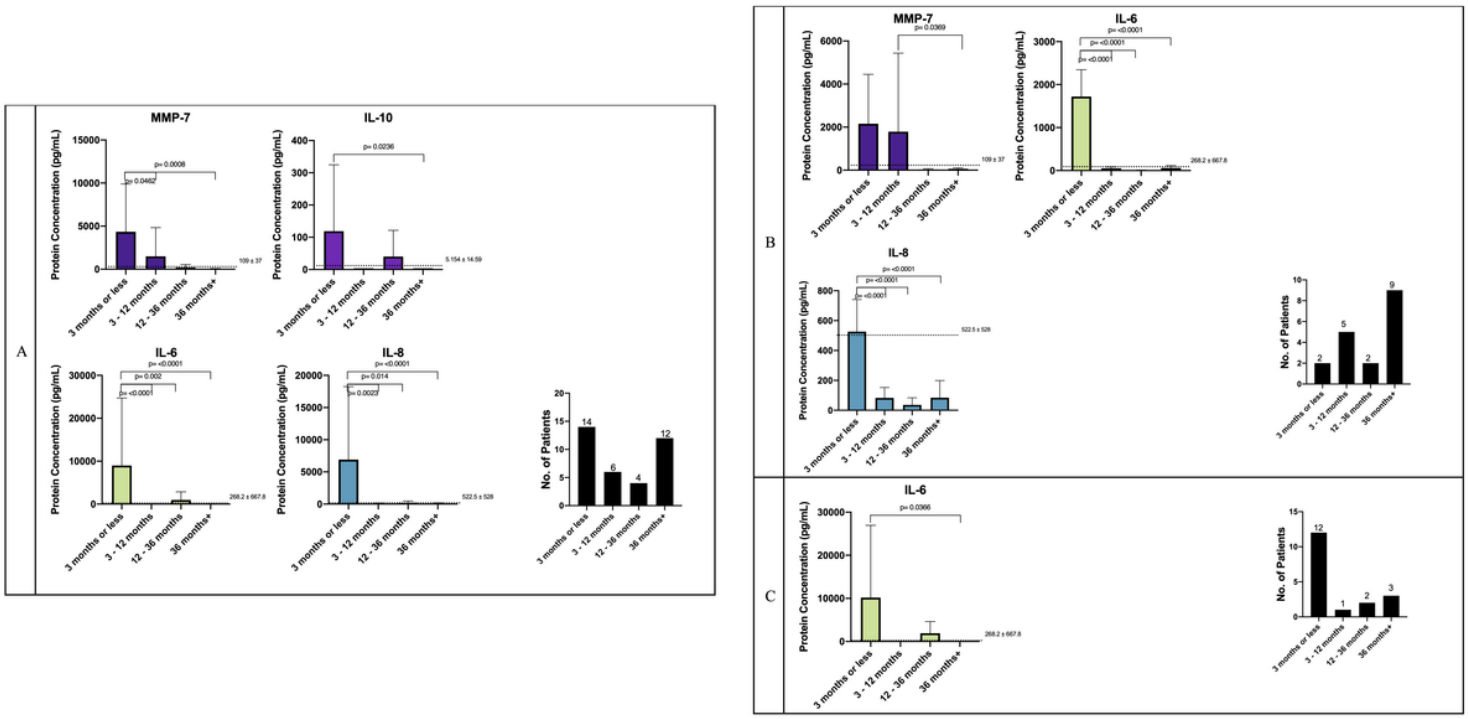


Figure 6

Frequency of patients in the biobank and CSF cytokine and MMP protein concentrations based on length of implantation are reported: (A) unparsed data, then (B) obstructed vs. (C) non-obstructed cases.

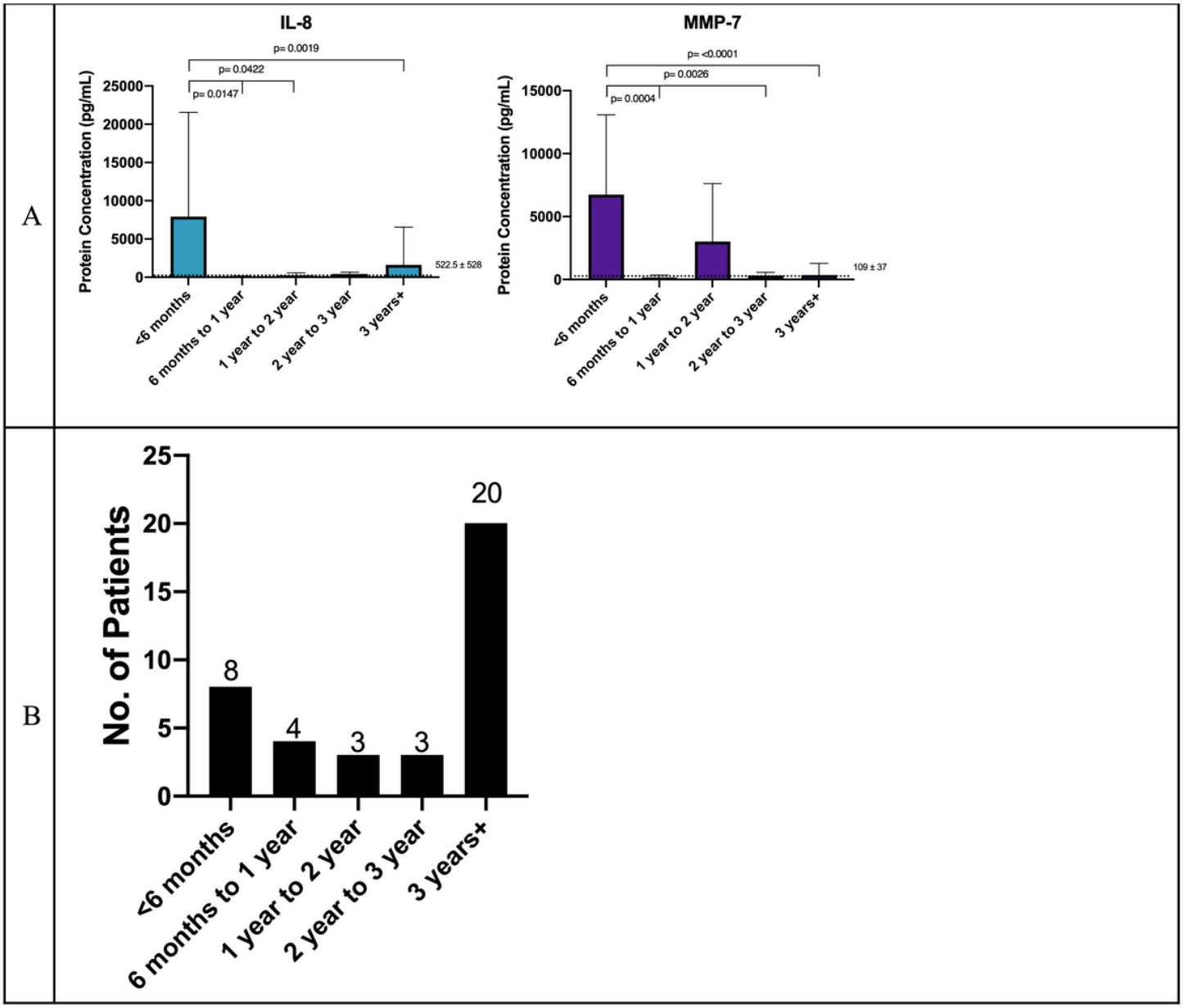


Figure 7

Frequency of (A) CSF cytokine and MMP protein concentrations in terms of age and (B) number of patients in the biobank are reported.

Supplementary Files

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- rawayneSupplementaryFigure4ABC.png
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- rawayneSupplementaryFigure3B.png
- rawayneSupplementaryFigure3A.png

- rawayneSupplementaryFigure2C.png
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- rawayneSupplementaryFigure2A.png
- rawayneSupplementaryFigure1ABC.png