

Review Article

Inflammatory Markers in Cerebrospinal Fluid from Patients with Hydrocephalus: A Systematic Literature Review

Sara Diana Lolansen ¹, Nina Rostgaard ², Eva Kjer Oernbo ¹, Marianne Juhler ²,
Anja Hviid Simonsen ³ and Nanna MacAulay ¹

¹Department of Neuroscience, University of Copenhagen, Copenhagen, Denmark

²Department of Neurosurgery, Rigshospitalet, Copenhagen, Denmark

³Danish Dementia Research Centre, Department of Neurology, Rigshospitalet, Copenhagen, Denmark

Correspondence should be addressed to Nanna MacAulay; macaulay@sund.ku.dk

Received 11 September 2020; Accepted 6 January 2021; Published 3 February 2021

Academic Editor: Roberta Rizzo

Copyright © 2021 Sara Diana Lolansen et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. The aim of this systematic review was to evaluate existing literature on inflammatory markers in CSF from patients with hydrocephalus and identify potential markers capable of promoting hydrocephalus development and progression. **Methods.** Relevant studies published before December 3rd 2020 were identified from PubMed, Embase, and reference lists. Studies were screened for eligibility using the predefined inclusion and exclusion criteria. Data from eligible studies were extracted, and sources of bias were evaluated. We included articles written in English investigating inflammatory markers in CSF from patients with hydrocephalus and control subjects. The review was conducted according to the PRISMA guidelines by three independent reviewers. **Results.** Twenty-two studies analyzed CSF from 311 patients with idiopathic normal pressure hydrocephalus (iNPH), 178 with posthemorrhagic hydrocephalus (PHH), 151 with other hydrocephalus diagnoses, and 394 control subjects. Fifty-eight inflammatory markers were investigated. The CSF of iNPH patients had increased CSF levels of IL-6, IL-1 β , and LRG compared with control subjects, whereas the CSF of PHH patients had increased levels of IL-6, IL-18, and VEGF. CSF from patients with “other hydrocephalus diagnoses” had elevated IFN- γ compared to control subjects, and VEGF was increased in congenital hydrocephalus, spina bifida, and hydrocephalus associated with tuberculous meningitis compared with controls. **Conclusion.** IL-6, IL-1 β , 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. They may serve as novel disease biomarkers, and their signaling pathways may represent targets for pharmacological management of hydrocephalus.

1. Introduction

Hydrocephalus is characterized by excessive amounts of cerebrospinal fluid (CSF) accumulating within the brain ventricles resulting in an increased intracranial pressure [1]. The CSF accumulation can have detrimental consequences and results in severe cognitive and neurological impairments and ultimately death if untreated [2]. Hydrocephalus occurs in all age groups, but onset is the most frequent in infants and elderly, i.e., in age groups where the brain is the most vulnerable [3]. Although the term “hydrocephalus” applies to all, the clinical presentations, radiological features, and etiologies greatly vary [2]. In infants, hydrocephalus is most often

caused by undiagnosed intrauterine conditions, congenital malformations, perinatal/infantile infections, or secondary intraventricular hemorrhage (IVH) due to germinal matrix bleeding [4–6]. Posthemorrhagic hydrocephalus (PHH) occurs in infants as well as older children and adults often as results of head trauma, IVH, or ruptured aneurysms (subarachnoid hemorrhage, SAH) [6]. In elderly, hydrocephalus often presents as idiopathic normal pressure hydrocephalus (iNPH), a condition without known etiology characterized by no or only slight increase in intracranial pressure, gait disturbances, urinary incontinence, and dementia. Although the etiologies vary tremendously in different types of hydrocephalus, emerging evidence indicates that neuroinflammation

contributes to the pathogenesis [7]. Elevated levels of inflammatory cytokines have been found in serum and CSF from patients with hydrocephalus [8–10], and the brain tissue from patients with hydrocephalus shows signs of inflammation such as macrophage and microglial activation [11, 12]. In addition, anti-inflammatory drugs can reduce the incidence of hydrocephalus in humans and attenuate ventriculomegaly in animals [13, 14]. Activation of inflammatory pathways in the CSF-producing tissue, the choroid plexus, can promote CSF hypersecretion sufficient to cause ventriculomegaly in animals [15]. Moreover, prolonged inflammation can contribute to scarring and fibrosis [16, 17], which is proposed to disrupt CSF flow and impair CSF reabsorption [7, 18]. Inflammation may thus promote the development and progression of hydrocephalus via CSF hypersecretion and/or impaired CSF drainage. To identify potential inflammatory markers involved in the pathogenesis of hydrocephalus, we systematically reviewed the existing literature on inflammatory markers in CSF from patients with hydrocephalus. Hereby, we identified promising inflammatory markers for future experimental research that aims to elucidate whether inflammatory markers can alter CSF dynamics and contribute to hydrocephalus development and progression. If inflammatory markers are involved in the pathogenesis of hydrocephalus, they may serve as novel disease biomarkers, and their signaling pathways may represent potential targets for pharmacological management of hydrocephalus. There is a great need for new potential biomarkers in diagnostic and prognostic settings within the field of hydrocephalic conditions.

2. Methods

The systematic literature review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [19], but is not registered in PROSPERO (as it does not include clinical trials).

2.1. Eligibility Criteria. Studies were included in the review if they were (1) original research, (2) written in English, and (3) available in full text. Studies were excluded from the review if they (1) were reviews, meta-analyses, conference abstracts, comments, notes (i.e. gray literature), case reports, duplicates, or not published in English; (2) were not available in full text; (3) were animal or postmortem studies; (4) did not focus on patients with hydrocephalus; (5) did not analyze CSF for presence of inflammatory markers; (6) did not include a control group; (7) did not separate patients with hydrocephalus of different etiologies into separate groups; or (8) did not make a statistical comparison between patients with hydrocephalus and control subjects (Figure 1).

2.2. Search Strategy. PubMed and Embase were searched for relevant studies published before December 3rd 2020. The following search terms were applied in all four searches: “hydrocephalus” AND “cerebrospinal fluid” OR “CSF” OR “cerebrospinal fluid” AND “inflammation” OR “inflammatory” OR “inflammatory marker” OR “biomarker” OR

“marker” OR “cytokine.” A unique search term was, in addition, applied to each of the four searches.

Search 1 “normal pressure hydrocephalus” OR “NPH” OR “idiopathic normal pressure hydrocephalus” OR “iNPH.” The search was limited to studies published after January 1st 2005 where the international iNPH guidelines were first presented and later published [20].

Search 2 “hemorrhage” OR “bleeding” OR “posthemorrhagic” OR “post-hemorrhagic” OR “post hemorrhagic.”

Search 3 “trauma” OR “traumatic” OR “post-traumatic” OR “posttraumatic” OR “post traumatic” OR “post head injury.”

Search 4 “meningitis” OR “infection” OR “infectious” OR “bacterial” OR “viral” OR “encephalitis.” Additional relevant studies were identified and manually curated from reference lists.

2.3. Data Items. The following data were extracted from each study: first author’s name, publication year, study design, patient groups, number of patients in each group, diagnostic criteria including inclusion and exclusion criteria, age, sex, type of medication given, type of surgery performed, CSF source, preanalytical CSF handling, analytical method, CSF marker(s) analyzed, and result of CSF analysis.

2.4. Data Extraction and Quality Assessment. Authors SDL, NR, MJ, AS, and NM designed the protocol and search strategy.

Initially, authors SDL and NR independently screened the studies by title and abstract. Studies that clearly violated the eligibility criteria were excluded. Afterwards, SDL and NR independently assessed the studies for eligibility by full-text review. After the independent assessment, SDL and NR collectively selected the studies for the final analysis. Authors SDL and EKO extracted data from relevant studies independently. After independent data collection, SDL and EKO collectively screened the extracted data, discussed any disagreements, and corrected any inconsistencies.

2.5. Synthesis of Results. Inflammatory markers were separated into two categories based on the degree of consistency in results among studies. Inflammatory markers were categorized as having *most evidence* when two or more studies reported consistent results, and a maximum of one study reported a contradictory result. Inflammatory markers were categorized as having *limited evidence* when results were reported from a single study.

2.6. Risk of Bias. The potential risk of bias was assessed by the evaluation of the following data from each study: study design, sample size, age and sex, diagnostic criteria, inclusion and exclusion criteria, medication, circumstances of CSF sampling, pre-analytical CSF handling, selection of inflammatory markers for CSF analysis, and analytical methods.

3. Results

3.1. Study Selection. The PubMed and Embase search identified 1198 potential studies for the review, and 3 additional studies were identified from relevant reference lists. The

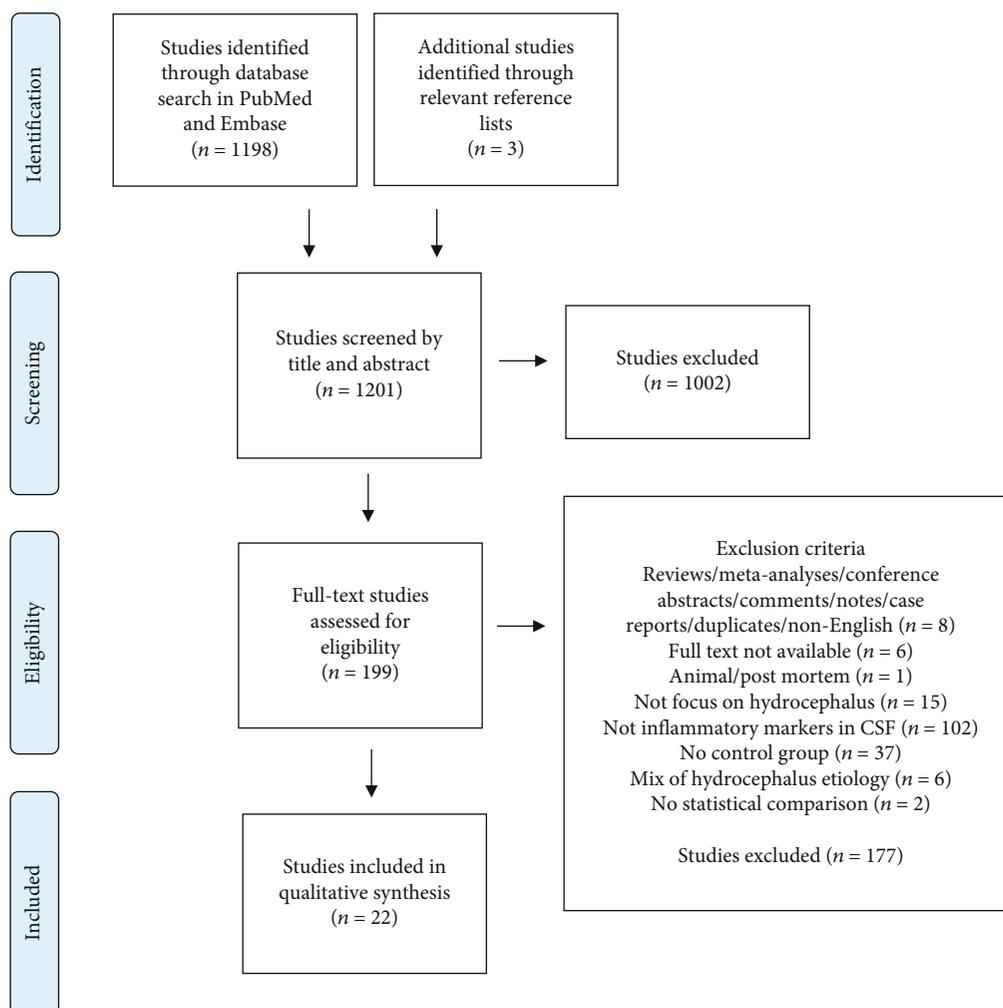


FIGURE 1: PRISMA flow diagram of the literature search strategy. The database search identified 1198 studies, and 3 additional studies were identified from reference lists. Screening by title and abstract led to the exclusion of 1002 studies. Full-text review for eligibility of the remaining 199 studies resulted in a final inclusion of 22 studies for the review.

initial search thus revealed 1201 potential studies for the review. Screening by title and abstract resulted in an exclusion of 1002 studies. Full-text review for eligibility of the remaining 199 studies led to the exclusion of 177 further studies. Hence, 22 studies were included in the final analysis (Figure 1).

3.2. Study Characteristics. The 22 studies included in the review were cross-sectional, case-control studies. Five studies were prospective [21–25], while two were retrospective [26, 27]. The remaining 15 studies did not report whether a prospective or retrospective strategy was performed. The 22 studies analyzed CSF from patients with hydrocephalus, which were sorted into three categories reflecting different hydrocephalic disorders: iNPH patients ($N = 311$), PHH patients ($N = 178$), and patients with other hydrocephalus diagnoses ($N = 151$). The latter patient group included hydrocephalus associated with tuberculous meningitis ($N = 44$), spina bifida ($N = 28$), aqueduct stenosis ($N = 25$), acute hydrocephalus of unknown origin ($N = 12$), congenital hydrocephalus ($N = 9$), hydrocephalus following emboliza-

tion of unruptured intracranial aneurysms ($N = 8$), fetal onset hydrocephalus ($N = 7$), late onset hydrocephalus ($N = 7$), arrested hydrocephalus ($N = 6$), and hydrocephalus with brain atrophy ($N = 5$). The CSF from patients with hydrocephalus was compared to CSF from control subjects ($N = 394$). The 22 studies investigated the presence of 58 inflammatory markers in total.

3.3. Analytical Methods. The 22 studies applied different analytical methods to detect presence of inflammatory markers in the CSF samples. The analytical methods included enzyme-linked immunosorbent assays (ELISAs) [21, 23–35], multiplex immunoassays [35–38], radioimmunoassays [32], electrochemiluminescence assays [39], a milliplex immunoassay [22], a cytokine antibody microarray kit [40], a quantitative sandwich enzyme immunoassay [41], and a bead-based human inflammatory cytokine kit [42]. Only three studies reported blinding of laboratory staff [21, 28, 36].

3.4. Inflammatory Markers in iNPH. CSF from iNPH patients was screened for presence of 36 inflammatory

TABLE 1: Inflammatory markers in iNPH patients.

Result	Marker	References
↑↑	IL-6	[37, 38, 42]
	IL-1 β	[37, 38]
	LRG	[33, 25]
↑	SP-G	[34]
	T β R-II	[33]
↓	TIMP-4	[26]
→ →	IL-8	[26, 36, 39]
	TNF- α	[36, 37, 42]
	CCL-2/MCP-1	[26, 36]
	IL-4	[37, 38]
	IL-33	[37, 38]
	sCD40L	[37, 38]
→	IL-12	[42]
	IL-12p40	[32]
	L-selectin	[26]
	MMP-9	[26]
	TGF- β 2	[33]
	TIMP-1	[26]
	TIMP-2	[26]
	YKL-40	[27]
Contr	IL-21	[37, 38]
	TGF- β 1	[26, 32, 33]
	IL-12p70	[32, 36]
	IL-17A	[37, 38]
	IL-22	[37, 38]
	IL-31	[37, 38]
	IL-10	[32, 36–39, 42]

↑↑ = most evidence: significantly increased in iNPH patients in two or more studies, and maximum one study reports a contradictory result. ↑ = limited evidence: significantly increased in iNPH patients in a single study. ↓ = limited evidence: significantly decreased in iNPH patients in a single study. → → = most evidence: no significant difference in two or more studies, and maximum one study reports a contradictory result. → = no significant difference in a single study. Contr = contradicting results. Abbreviations: IL: interleukin; LRG: leucine-rich α 2-glycoprotein; SP-G: surfactant protein-G; T β R-II: transforming growth factor beta type II receptor; TIMP: tissue inhibitor of metalloproteinases; TNF: tumor necrosis factor; CCL: C-C motif chemokine ligand; MCP: monocyte chemoattractant protein; sCD40: soluble CD40 ligand; MMP: matrix metalloproteinase; TGF: transforming growth factor; YKL40: chitinase-3-like protein.

markers spread across 11 studies [25–27, 32–34, 36–39, 42]. Detailed information from each study is provided in supplementary Table 1. The inflammatory markers were sorted according to the evidence criteria, and the results are presented in Table 1.

IL-6, IL-1 β , and LRG showed most evidence of increased levels in CSF from iNPH patients compared to control subjects (Table 1) [25, 33, 37, 38, 42], while SP-G and T β R-II showed limited evidence of increased levels. TIMP-4 showed limited evidence of decreased levels in CSF from iNPH patients compared to control subjects. The remaining

inflammatory markers showed no significant differences in CSF levels between iNPH patients and control subjects, were reported with contradictory results, or were below the detection limit (Table 1).

3.5. Inflammatory Markers in PHH. CSF from PHH patients was screened for presence of 37 inflammatory markers spread across 12 studies [21, 23, 24, 26, 28–31, 34, 35, 40, 41]. Detailed information is provided in supplementary Table 2. The studies analyzed CSF from infants as well as adults and the pathological events preceding hydrocephalus thus differ between the included studies. However, due to the limited number of studies with adult PHH patients and the absence of any obvious differences in inflammatory CSF profiles, the results were not separated according to age. The inflammatory markers were sorted according to the evidence criteria and the results are presented in Table 2.

IL-6, IL-18, and VEGF showed most evidence of increased levels in CSF from PHH patients compared to control subjects [21, 26, 29, 30, 35, 40].

Sixteen inflammatory markers, CCL-3/MIP-1 α , CCL-19, CXCL-10/IP-10, HGF, HMGB1, IL-1 α , IL-4, IL-12, L1CAM, MMP-9, FasR, sFas, SP-G, sRAGE, TGF- β 2, and TIMP-1, showed limited evidence of significantly increased levels in CSF from PHH patients compared to control subjects. Two inflammatory markers, TIMP-4 and XCL-1, showed limited evidence of significantly decreased levels in CSF from PHH patients compared to control subjects. The remaining inflammatory markers showed no significant differences in CSF levels between PHH patients and control subjects, were reported with contradictory results, or were below the detection limit.

3.6. Inflammatory Markers in Other Hydrocephalus Diagnoses. CSF from the mixed patient group with other hydrocephalus diagnoses was screened for presence of 30 inflammatory markers spread across seven studies [21, 22, 26, 30, 34, 40, 42]. As the patient groups and the studies included in this category are extremely heterogeneous, it is not possible to summarize data in a simple table. Detailed information is provided in supplementary Table 3. However, an interesting marker is IFN- γ , which showed most evidence of increased levels in CSF from patients with hydrocephalus compared to control subjects [22, 30].

Further, VEGF was found increased over that of control subjects in congenital hydrocephalus, spina bifida, and hydrocephalus associated with tuberculous meningitis [21, 22, 40].

3.7. Risk of Bias. Sources of bias that could potentially influence the findings presented in the review are assessed in the following.

3.7.1. Sample Size. Ideally, the sample size should be large enough to detect differences that are clinically relevant [43]. However, practical issues such as costs and patient availability often limit the sample size [43]. The sample size of the patient groups included in the review ranged from 2 to 100 individuals. In some of the studies, the small sample size may have reduced the chance of detecting a significant

TABLE 2: Inflammatory markers in PHH patients.

Result	Marker	Reference
↑↑	IL-6	[26, 35]
	IL-18	[29, 30]
	VEGF	[21, 40]
↑	CCL-3/MIP-1 α	[35]
	CCL-19	[35]
	CXCL-10/IP-10	[35]
	HGF	[40]
	HMGB1	[28]
	IL-1 α	[35]
	IL-4	[35]
	IL-12	[35]
	L1CAM	[31]
	MMP-9	[26]
	FasR	[40]
	sFas	[23]
	SP-G	[34]
	sRAGE	[24]
	TGF- β 2	[41]
TIMP-1	[26]	
↓	TIMP-4	[26]
	XCL-1	[35]
→ →	TGF- β 1	[21, 35]
→	CXCL-11	[35]
	CXCL-12	[35]
	IL-10	[35]
	L-selectin	[26]
	SCF	[40]
→	FasL	[40]
	sFasL	[30]
	TIMP-2	[26]
Contr	CCL-2/MCP-1	[26, 35]
	IL-1 β	[29, 35]
	IL-8	[26, 35]
	IFN- γ	[30, 35]
	TNF- α	[26, 35, 40]

↑↑ = most evidence: significantly increased in PHH patients in two or more studies, and maximum one study reports a contradictory result. ↑ = limited evidence: significantly increased in PHH patients in a single study. ↓ = limited evidence: significantly decreased in PHH patients in a single study. → → = most evidence: no significant difference in two or more studies, and maximum one study reports a contradictory result. → = no significant difference in a single study. Contr = contradicting results. Abbreviations: IL: interleukin; VEGF: vascular endothelial growth factor; CCL: C-C motif chemokine ligand; MIP: macrophage inflammatory protein; CXCL: C-X-C motif chemokine ligand; IP: interferon gamma inducible protein; HGF: hepatocyte growth factor; HMGB1: high-mobility group box 1; L1CAM: L1 cell adhesion molecule; MMP: matrix metalloproteinase; FasR: Fas receptor; sFas: soluble Fas; SP-G: surfactant protein-G; sRAGE: soluble receptor for advanced glycation end products; TGF: transforming growth factor; TIMP: tissue inhibitor of metalloproteinases; XCL: X-C motif chemokine ligand; SCF: stem cell factor; FasL: Fas ligand; sFasL: soluble fas ligand; MCP: monocyte chemoattractant protein; IFN: interferon; TNF: tumor necrosis factor.

difference due to low statistical power. A small sample size may thus explain some of the instances where levels of inflammatory markers were reported similar in patients with hydrocephalus and control patients. However, the systematic

review was designed not to exclude studies based on the low sample size as such exclusion would itself bias the results and risk potential loss of information.

3.7.2. Age and Sex. Studies are generally recommended to match for age and sex to control for confounding elements and increase study efficiency [44]. Of the 22 included studies, nine studies reported that patients were age-matched or similar in age [22, 27–32, 37, 38]. The age distribution was given in 10 of the remaining studies [21, 23, 25, 33–36, 39–41 and was not reported in three studies [24, 26, 42]. Of the 22 studies, four studies examined the correlation between age and levels of inflammatory markers in the CSF [23, 25, 32, 34]. Of these, only one study reported a significant correlation with age [25]. Of the three categories of patients with hydrocephalus, PHH patients exhibited the largest variation in the age distribution ranging from preterm infants [21, 23, 29–31, 35, 40, 41 to adults [24, 28, 34]. In addition to variations related to age-matching, findings presented in the PHH category may have been particularly prone to age variation across studies. Eleven of the 22 studies reported a sex distribution among the included patient groups [22, 25, 27, 32–36, 38–40] while it was not reported in the remaining studies [21, 23, 24, 26, 28–31, 37, 41, 42]. Only three studies reported that groups were sex-matched or had a similar sex distribution [22, 27, 28]. None of the 22 studies included in the review reported any effect of sex on the levels of inflammatory markers.

3.7.3. Diagnostic Criteria

(1) iNPH Patients. In 2005, English international guidelines for the clinical diagnosis of iNPH were published [20]. Studies published prior to the publication of the international guidelines were excluded from the review to limit patient heterogeneity. Of the 11 studies investigating iNPH, two studies diagnosed patients according to the international guidelines from 2005 [27, 39]. Two studies [25, 33] referred to another set of iNPH guidelines, which were published in Japan in 2004 and translated to English in 2008 [45, 46]. Five studies did not refer to any guidelines but diagnosed patients based on imaging, presence of clinical symptoms, and physiological criteria [32, 36–38, 42]. The last two studies did not report how iNPH patients were diagnosed [26, 34].

(2) PHH Patients. Patients diagnosed with PHH are heterogeneous in relation to age of onset, underlying pathological event, and clinical presentation. The internationally accepted diagnostic criteria for PHH are yet to be established [6]. However, criteria and grading scales exist for the hemorrhagic events underlying PHH development (e.g., subarachnoidal hemorrhage; SAH, intraventricular hemorrhage; IVH). Of the 12 studies investigating PHH, six studies reported that patients developed PHH secondarily to IVH, and four studies reported the cause to be SAH, while one study reported PHH to develop from either SAH or IVH [21, 23, 24, 26, 28, 29, 31, 34, 35, 40, 41]. One study reported that patients developed PHH secondarily to intracranial hemorrhage but did not specify further [30]. Of the six

IVH-related studies, different grading systems and criteria were used in three studies [21, 29, 31], while two of the four SAH-related studies reported use of a specific grading scale [24, 28].

(3) *Patients with Other Hydrocephalus Diagnoses.* Seven studies investigated patients with other hydrocephalus diagnoses [21, 22, 26, 30, 34, 40, 42]. The diagnoses were hydrocephalus associated with tuberculous meningitis, spina bifida or aqueduct stenosis, acute hydrocephalus of unknown origin, congenital hydrocephalus, hydrocephalus following embolization of unruptured intracranial aneurysms, fetal onset hydrocephalus, late onset hydrocephalus, arrested hydrocephalus, and hydrocephalus with brain atrophy. None of the studies reported use of specific guidelines.

3.7.4. Inclusion and Exclusion Criteria

(1) *All Patients with Hydrocephalus.* The majority of the studies enrolled patients with hydrocephalus according to a certain diagnostic criteria. Only two studies specified the inclusion criteria that extended beyond the diagnostic criteria [32, 42]. One study included patients if they were free from other disorders of the central nervous system (CNS) and their mental status allowed neurophysiological tests to be performed [42]. The other study included patients if they were free from comorbidities with inflammatory or immunological diseases and had not received treatment with steroids or immunosuppressive drugs three months prior to the CSF sampling [32]. Four studies applied the exclusion criteria [23, 24, 27, 28]. The exclusion criteria were complications related to shunt surgery [27], history of CNS disease or active systemic disease [24, 28], presence of infections [23, 24], development of inflammatory reactions not associated with surgery [28], and exclusion of SAH patients below the age of 18 due to differences in CSF content, distinct SAH presentations, morphology of aneurysms, and outcome [24]. The absence of the specific inclusion and exclusion criteria in the majority of the studies suggests that the patient groups are heterogeneous. Hence, the results presented in this review may be compromised by patient heterogeneity. Only a few studies addressed presence of inflammation or other CNS disorders in the patient population [23, 24, 28, 32, 42].

(2) *Control Subjects.* The ideal control group should be the representative of the population from which patients arise [47]. Of the 22 studies, ten studies enrolled control patients who underwent workup for exclusion of sepsis or meningitis, or pathologies such as SAH, demyelinating disorders, and rhinorrhea [21, 23, 29–31, 34, 35, 40–42]. Five studies included control patients that underwent surgeries unrelated to hydrocephalus [22, 24, 26, 27, 38]. In addition, studies included control patients with noninflammatory conditions (headache, spinal disc herniation, dementia, and occipital neuralgia) [28, 32, 33, 37]. Two studies did not describe the control patients [25, 36]. One study reported that controls patients were healthy volunteers recruited from a population registry [39]. Of the 22 studies, 17 studies reported that control patients were free from neurological injuries, deficits, or

disorders [21–25, 27–29, 31–33, 35, 38–42]. In addition to the studies where control subjects underwent workup for sepsis and meningitis, two studies reported absence of inflammation in control patients [22, 42]. Additionally, the specific exclusion criteria were reported in three studies [23, 28, 39]. In general, the 22 studies enrolled control patients with conditions that required medical examination or surgery.

3.7.5. *Medication.* Ideally, patients should not receive medication that is expected to influence the levels of the investigated inflammatory markers [48]. However, only six of the 22 studies provided information regarding medication of patients [22, 24, 32, 38, 39, 41]. Four studies reported that patients received medication [22, 24, 38, 41], while two studies reported absence of medication [32, 39]. One study reported that patients with PHH secondarily to IVH received thrombolytic agents [41]. Another study reported that patients with PHH secondarily to SAH received a combination of nimopidine, a calcium channel blocker, and vasopressors [24].

3.7.6. Preanalytical and Analytical Methods

(1) *CSF Sampling.* It remains controversial whether a protein concentration gradient exists from the ventricular to the lumbar space [49–53]. To rule out variations related to sample origin, CSF samples should originate from the same space [54]. Ten studies compared lumbar CSF from patients with hydrocephalus and control patients [25, 27, 31–33, 35, 37–39, 42], while eight studies compared ventricular CSF from patients with hydrocephalus to lumbar CSF from control patients [21, 23, 26, 28–30, 40, 41]. One study separately compared ventricular and lumbar CSF from patients with hydrocephalus to lumbar CSF from control patients [22], whereas another study mixed ventricular and lumbar CSF from a subset of patients with hydrocephalus [26]. A third study acquired ventricular and lumbar CSF from both patient groups and compared the ventricular and lumbar CSF samples separately [36]. Two studies did not adequately describe the origin of the CSF from one of the included patient groups [24, 34]. Two studies reported higher levels of inflammatory markers in lumbar CSF compared to ventricular CSF within patient groups [22, 36].

(2) *Preanalytical CSF Handling.* Preanalytical factors such as centrifugation, time from sampling to storage, storage material, and storage temperature are known to influence CSF analyses [55–58]. CSF samples are recommended to be centrifuged at $2000 \times g$ for 10 min at 4°C or room temperature prior to storage at -80°C [55]. Fourteen studies centrifuged CSF samples, either prior to storage [21, 23–25, 28–30, 32, 33, 39] or after storage [31, 35, 37, 38]. Seven studies reported centrifugation speed and duration ($2000\text{--}10,000 \times g/2500\text{--}3000$ rpm for 6–10 min) [28, 31–33, 35, 37, 38]. Four studies reported centrifugation temperature ($4\text{--}7^{\circ}\text{C}$) [32, 33, 37, 38]. CSF samples are recommended to be stored within 1–2 hours of withdrawal to prevent variable results [55]. Eight studies reported that CSF samples were stored immediately

[21–23, 26, 28–30, 32], while one study reported storage within 30 minutes [40]. The remaining studies did not specify the time from CSF sampling to storage. Polypropylene tubes are generally recommended for CSF storage as their low protein binding capacity minimizes the degree of adherence to tube walls [55–58]. However, only five studies provided information regarding the type of storage tubes used [25, 26, 37–39]. Two studies stored CSF in polypropylene tubes as recommended [25, 39]. Two studies used regular plastic tubes [37, 38]. The last study only reported use of cryogenic vials [26].

(3) *Selection of Inflammatory Markers for CSF Analysis.* The majority of the studies provided information in the introduction of their publication explaining why the inflammatory markers, or at least why some of them, were selected for analysis. Eight studies stated that some of the selected inflammatory markers had been detected previously in CSF from patients with hydrocephalus [23, 31, 33, 35–38, 41]. None of the studies stated that CSF was screened for presence of inflammatory markers in an unbiased manner, although one study stated that they “broadly investigated neuro-inflammatory processes” [35]. The study investigated 17 inflammatory markers, the highest number across all of the included studies [35]. Biased selection of inflammatory markers may hinder discovery of new markers that are worth investigating in relation to hydrocephalus development. Hence, as one study proposed, an unbiased exploration may be preferable in the future [22].

4. Discussion

In the current review, we systematically evaluated the existing literature on inflammatory markers in CSF from patients with hydrocephalus. We sought to identify promising inflammatory markers for future experimental research that aims to elucidate whether inflammatory markers can alter CSF dynamics and contribute to hydrocephalus development and progression. If neuroinflammation is involved in the pathogenesis of hydrocephalus, inflammatory markers may serve as novel disease biomarkers. Modulation of inflammatory pathways affects a range of systemic illnesses [59, 60] and may well serve as potential targets for pharmacological management of hydrocephalus. We reviewed the results presented in 22 studies, which investigated the presence of 58 inflammatory markers. Thirty-six inflammatory markers were investigated in iNPH patients, 37 were investigated in PHH patients, and 30 were investigated in patients with other hydrocephalus diagnoses. The inflammatory markers with similar CSF levels in hydrocephalus patients compared to control subjects were considered less promising, as they may not be relevant in the pathogenesis of hydrocephalus. For iNPH patients, IL-6, IL-1 β , and LRG were associated with the most evidence of significantly increased CSF levels. For PHH patients, IL-6, IL-18, and VEGF were associated with the most evidence of significantly increased CSF levels. For patients with other hydrocephalus diagnoses, IFN- γ and VEGF were significantly increased in several types of hydrocephalus compared to controls.

4.1. Inflammatory Markers and Signaling Pathways in Hydrocephalus. Activation of the NF- κ B pathway was recently demonstrated to cause CSF hypersecretion and subsequent hydrocephalus in rats [15]. IL-6 dramatically rises in inflammatory conditions [61, 62], where IL-6 exerts chemotactic effects, stimulates production of acute-phase proteins, and regulates immune responses [63]. IL-6 has also been reported to activate the NF- κ B pathway [64]. IL-6 may therefore potentially promote choroidal CSF hypersecretion and hydrocephalus development by activating the NF- κ B pathway. IL-1 β is secreted primarily by immune cells in situations of infection, cell injury, and inflammation [65]. Similarly to IL-6, the NF- κ B pathway can be initiated by IL-1 β [66]. Hence, IL-1 β also appears to have the prerequisites to promote choroidal CSF hypersecretion and cause hydrocephalus development.

LRG (leucine-rich α 2-glycoprotein) is produced by various cell types including hepatocytes, astrocytes, neutrophils, and epithelial cells in the inflamed tissue. LRG levels rise in inflammatory conditions such as meningitis, tuberculosis, and rheumatoid arthritis and are therefore suggested to be a marker of inflammation similar to acute-phase proteins [25, 67–72]. LRG levels can be induced by inflammatory cytokines including IL-6 and IL-1 β [67, 73]. Hence, the increased LRG levels in iNPH patients might be a secondary response to the increased IL-6 and IL-1 β levels. LRG signals by binding to endoglin (ENG), a component of the receptor complex for TGF- β , and LRG is therefore proposed to modulate TGF- β signaling [74]. Although the TGF- β signaling pathway has been reported to crosstalk with the NF- κ B pathway [75], it is uncertain whether LRG, rather than TGF- β , would initiate such crosstalk and hence potentially promote choroidal CSF hypersecretion and hydrocephalus development. IL-18 is secreted by immune cells such as macrophages and nonimmune cells such as epithelial cells, keratinocytes, and osteoblasts [76]. IL-18 belongs to the same family of cytokines as IL-1 β and therefore shares properties related to the receptor structure and signaling pathways, including activation of the NF- κ B pathway [77]. The increased IL-18 levels in PHH patients may therefore reflect presence of inflammation. VEGF serves a variety of roles during inflammation [78]. Amongst others, VEGF stimulates production of inflammatory cytokines [78]. Similarly to IL-6 and IL-1 β , VEGF is capable of activating the NF- κ B pathway [79, 80], and its main receptor VEGFR2 is found on ependymal cells lining the ventricles [81]. In rats, intraventricular VEGF infusion has been reported to cause ventriculomegaly associated with disruption of the ventricular ependymal cell lining and loss of cilia, which have previously been associated with hydrocephalus development [82–84]. Hence, VEGF may promote hydrocephalus development and possibly contribute to disease progression by disrupting the normal CSF flow. IFN- γ is secreted primarily by immune cells [85, 86]. Once inflammation is initiated, IFN- γ intensifies the inflammatory response. The elevated IFN- γ levels in patients with other hydrocephalus diagnoses suggest presence of an ongoing inflammatory condition. Although IFN- γ primarily activates the JAK/STAT pathway [85], IFN- γ is also capable of activating the NF- κ B pathway [87]. Hence, IFN- γ has the

prerequisites to promote choroidal CSF hypersecretion similarly to IL-6, IL-1 β , IL-18, and VEGF.

The molecular mechanisms underlying choroidal CSF secretion are still investigated [88]. Except for malignancy-related CSF hypersecretion, activation of the NF- κ B pathway is the only mechanism currently demonstrated to promote choroidal CSF hypersecretion and cause development of hydrocephalus [15, 89, 90]. The ability of the inflammatory markers, IL-6, IL-1 β , LRG, IL-18, VEGF, and IFN- γ , to promote choroidal CSF hypersecretion was therefore mainly discussed in relation to activation of the NF- κ B pathway. However, this does not exclude the possibility that activation of alternative, yet unidentified, pathways may promote choroidal CSF hypersecretion. Hence, future research may reveal additional molecular mechanisms involved in choroidal CSF secretion, which may be regulated by inflammatory markers. Inflammatory markers may also promote hydrocephalus development and contribute to disease progression in ways that do not involve choroidal CSF hypersecretion. Prolonged inflammation can lead to scarring and fibrosis [16, 17], which may disrupt the CSF flow and impair CSF reabsorption [7, 18].

4.2. Limitations. The systematic review included patients with hydrocephalus of various etiologies. Although the term “hydrocephalus” applies to all, the clinical presentations, radiological features, and etiologies vary between age groups. The classification of hydrocephalus is complex and ambiguous and thus an obvious challenge for a review of the present type, as the same clinical condition may be classified in more than one way—and the same classification can apply to different clinical conditions. In this review, we sought to overcome this by choosing etiologies as our defining parameter. Even though the results were separated into three main categories, patient heterogeneity within each category may have influenced the results. The category that comprised patients with other hydrocephalus diagnoses may especially have suffered from variations related to patient heterogeneity. Discrepancies in the diagnostic criteria combined with general lack of the stringent inclusion and exclusion criteria may also have contributed to patient heterogeneity. The control subjects were likewise heterogeneous in health status. The included studies had variations in sample sizes, and the majority of studies did not match patient groups on age and sex, which may also have influenced the results of the review. In addition, recruitment of patients from specialized clinics such as hydrocephalus units may have resulted in selection bias of patients with more pronounced cognitive and physiological impairments than encountered on average. The included studies analyzed CSF from children and adults, but the results were not separated accordingly. The inflammatory CSF profile of children and adults could possibly differ, and such information may thus have been lost. The category comprising PHH patients may have been particularly prone to variations related to age differences (preterm infants to adults). However, as some studies failed to report an age distribution or compared CSF from a mix of children and adults, results separated according to age would likewise have been associated with certain limitations.

Another limitation of this review is the heterogeneity in sampling and handling of CSF across the included studies. Several studies directly compare lumbar CSF with ventricular CSF which may have influenced the results due to differences in the protein content from the ventricles to the lumbar space. The influence of sample time on CSF protein concentration remains debatable [57]. However, only two studies reported that samples were collected at a specific time of day [38, 39]. The included studies also exhibited variation regarding the circumstances under which CSF samples were collected from patients with hydrocephalus. The variable circumstances of CSF sampling may raise concerns as to whether the CSF samples are compatible across the included studies. Hence, it cannot be excluded that the results presented in the review are affected by the heterogeneous circumstances of CSF sampling.

The review included inflammatory markers both directly and indirectly linked to inflammation. The definition of an indirect inflammatory marker falls within a grey area. Hence, some of the indirect inflammatory markers might be more relevant than others. The current review included indirect inflammatory markers that appeared relevant based on the identified studies in the original search. Regarding medication, it is surprising that only one study comment on the use of medication in relation to the investigated outcome [38]. It can be speculated whether some of the prescribed drugs such as prednisone can have influenced the results of the study [22]. However, since the majority of the included studies failed to report whether patients received medication, the bias introduced by medication cannot be properly assessed.

5. Conclusion

Despite the limitations, this systematic review presents a currently needed overview of the relevant literature and enables identification of promising inflammatory markers for future experimental research. In the current systematic review, we evaluated the existing literature on inflammatory markers in CSF from patients with hydrocephalus. We identified six inflammatory markers, IL-6, IL-1 β , LRG, IL-18, VEGF, and IFN- γ , which were associated with the most evidence of increased levels in CSF from patients with hydrocephalus compared to control subjects. Future research should investigate whether these markers directly contribute to hydrocephalus development and progression. Such research will elucidate whether inflammatory markers may serve as novel disease biomarkers and whether their signaling pathways represent potential targets for pharmacological management of hydrocephalus. Future studies investigating the presence of inflammatory markers in CSF from patients with hydrocephalus should strive to eliminate the potential sources of bias presented in the current review. Efforts should be made to minimize patient heterogeneity. Patient diagnosis should occur in accordance with published international guidelines, and the inclusion and exclusion criteria should be clearly stated. Control subjects should, if possible, resemble healthy individuals. Studies should avoid small sample sizes, and patients should be matched on age and sex. CSF samples

should preferably originate from the same space, be handled in the same manner, and if possible, be analyzed with the same analytical method. Furthermore, inflammatory markers should preferably be selected for analysis in an unbiased manner. The efforts done to eliminate the sources of bias may result in the identification of additional inflammatory markers, which are potentially involved in hydrocephalus development and progression.

Data Availability

All data is available on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Sara Diana Lolansen and Nina Rostgaard contributed equally to this work.

Acknowledgments

This study is supported by a Tandem grant from the Novo Nordisk Foundation [grant number NNF17OC0024718 to N.M. and M.J.], IMK Almene Fond (to N.M.) and the Absalon Foundation (to A.H.S.).

Supplementary Materials

Supplemental Table 1: overview of all inflammatory markers investigated in CSF from iNPH patients. Supplemental Table 2: overview of all inflammatory markers investigated in CSF from PHH patients. Supplemental Table 3: overview of all inflammatory markers investigated in CSF from patients with other hydrocephalus diagnoses. (*Supplementary Materials*)

References

- [1] S. Krishnamurthy and J. Li, "New concepts in the pathogenesis of hydrocephalus," *Translational Pediatrics*, vol. 3, no. 3, pp. 185–194, 2014.
- [2] V. Leinonen, R. Vanninen, and T. Rauramaa, "Cerebrospinal fluid circulation and hydrocephalus," *Handbook of Clinical Neurology*, vol. 145, pp. 39–50, 2018.
- [3] A. M. Isaacs, J. Riva-Cambrin, D. Yavin et al., "Age-specific global epidemiology of hydrocephalus: Systematic review, meta-analysis and global birth surveillance," *PLoS One*, vol. 13, no. 10, 2018.
- [4] H. M. Tully and W. B. Dobyns, "Infantile hydrocephalus: a review of epidemiology, classification and causes," *European Journal of Medical Genetics*, vol. 57, no. 8, pp. 359–368, 2014.
- [5] K. T. Kahle, A. V. Kulkarni, D. D. Limbrick, and B. C. Warf, "Hydrocephalus in children," *The Lancet*, vol. 387, no. 10020, pp. 788–799, 2016.
- [6] Q. Chen, Z. Feng, Q. Tan et al., "Post-hemorrhagic hydrocephalus: recent advances and new therapeutic insights," *Journal of the Neurological Sciences*, vol. 375, pp. 220–230, 2017.
- [7] J. K. Karimy, B. C. Reeves, E. Damisah et al., "Inflammation in acquired hydrocephalus: pathogenic mechanisms and therapeutic targets," *Nature Reviews. Neurology*, vol. 16, no. 5, pp. 285–296, 2020.
- [8] S. R. Chaudhry, B. Stoffel-Wagner, T. M. Kinfe et al., "Elevated systemic IL-6 levels in patients with aneurysmal subarachnoid hemorrhage is an unspecific marker for post-SAH complications," *International Journal of Molecular Sciences*, vol. 18, no. 12, article 2580, 2017.
- [9] K. Kitazawa and T. Tada, "Elevation of transforming growth factor-beta 1 level in cerebrospinal fluid of patients with communicating hydrocephalus after subarachnoid hemorrhage," *Stroke*, vol. 25, no. 7, pp. 1400–1404, 1994.
- [10] L. Sosvorova, M. Mohapl, J. Vcelak, M. Hill, J. Vitku, and R. Hampl, "The impact of selected cytokines in the follow-up of normal pressure hydrocephalus," *Physiological Research*, vol. 64, Supplement 2, pp. S283–S290, 2015.
- [11] N. Ulfig, J. Bohl, F. Neudörfer, and P. Rezaie, "Brain macrophages and microglia in human fetal hydrocephalus," *Brain & Development*, vol. 26, no. 5, pp. 307–315, 2004.
- [12] M. Fukumizu, S. Takashima, and L. E. Becker, "Glial reaction in periventricular areas of the brainstem in fetal and neonatal posthemorrhagic hydrocephalus and congenital hydrocephalus," *Brain & Development*, vol. 18, no. 1, pp. 40–45, 1996.
- [13] G. E. Thwaites, J. Macmullen-Price, T. T. H. Chau et al., "Serial MRI to determine the effect of dexamethasone on the cerebral pathology of tuberculous meningitis: an observational study," *The Lancet Neurology*, vol. 6, no. 3, pp. 230–236, 2007.
- [14] C. Gu, X. Hao, J. Li, Y. Hua, R. F. Keep, and G. Xi, "Effects of minocycline on epileptus macrophage activation, choroid plexus injury and hydrocephalus development in spontaneous hypertensive rats," *Journal of Cerebral Blood Flow and Metabolism*, vol. 39, no. 10, pp. 1936–1948, 2019.
- [15] J. K. Karimy, J. Zhang, D. B. Kurland et al., "Inflammation-dependent cerebrospinal fluid hypersecretion by the choroid plexus epithelium in posthemorrhagic hydrocephalus," *Nature Medicine*, vol. 23, no. 8, pp. 997–1003, 2017.
- [16] T. Wynn, "Cellular and molecular mechanisms of fibrosis," *The Journal of Pathology*, vol. 214, no. 2, pp. 199–210, 2008.
- [17] X. Hao, W. Junwen, L. Jiaqing et al., "High fibrosis indices in cerebrospinal fluid of patients with shunt-dependent post-traumatic chronic hydrocephalus," *Translational Neuroscience*, vol. 7, no. 1, pp. 92–97, 2016.
- [18] J. Strahle, H. J. L. Garton, C. O. Maher, K. M. Muraszko, R. F. Keep, and G. Xi, "Mechanisms of hydrocephalus after neonatal and adult intraventricular hemorrhage," *Translational Stroke Research*, vol. 3, Supplement 1, pp. 25–38, 2012.
- [19] D. Moher, A. Liberati, J. Tetzlaff, and D. G. Altman, "Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA statement," *PLoS Medicine*, vol. 6, no. 7, article e1000097, 2009.
- [20] N. Relkin, A. Marmarou, P. Klinge, M. Bergsneider, and P. M. Black, "Diagnosing idiopathic normal-pressure hydrocephalus," *Neurosurgery*, vol. 57, Supplement 3, pp. S2–16, 2005.
- [21] A. Heep, B. Stoffel-Wagner, P. Bartmann et al., "Vascular endothelial growth factor and transforming growth factor- β 1 are highly expressed in the cerebrospinal fluid of premature infants with posthemorrhagic hydrocephalus," *Pediatric Research*, vol. 56, no. 5, pp. 768–774, 2004.
- [22] U. K. Rohlwick, K. Mauff, K. A. Wilkinson et al., "Biomarkers of cerebral injury and inflammation in pediatric tuberculous meningitis," *Clinical Infectious Diseases*, vol. 65, no. 8, pp. 1298–1307, 2017.

- [23] T. Schmitz, U. Felderhoff-Mueser, M. Sifringer, F. Groenendaal, S. Kampmann, and A. Heep, "Expression of soluble Fas in the cerebrospinal fluid of preterm infants with posthemorrhagic hydrocephalus and cystic white matter damage," *Journal of Perinatal Medicine*, vol. 39, no. 1, pp. 83–88, 2011.
- [24] B. Sokół, N. Waśnik, R. Jankowski et al., "Increase of soluble RAGE in cerebrospinal fluid following subarachnoid haemorrhage," *BioMed Research International*, vol. 2017, Article ID 7931534, 7 pages, 2017.
- [25] M. Miyajima, M. Nakajima, Y. Motoi et al., "Leucine-rich α 2-glycoprotein is a novel biomarker of neurodegenerative disease in human cerebrospinal fluid and causes neurodegeneration in mouse cerebral cortex," *PLoS One*, vol. 8, no. 9, article e74453, 2013.
- [26] M. Killer, A. Arthur, A. R. al-Schameri et al., "Cytokine and growth factor concentration in cerebrospinal fluid from patients with hydrocephalus following endovascular embolization of unruptured aneurysms in comparison with other types of hydrocephalus," *Neurochemical Research*, vol. 35, no. 10, pp. 1652–1658, 2010.
- [27] A. Jeppsson, M. Höllta, H. Zetterberg, K. Blennow, C. Wikkelso, and M. Tullberg, "Amyloid mis-metabolism in idiopathic normal pressure hydrocephalus," *Fluids and Barriers of the CNS*, vol. 13, no. 1, p. 13, 2016.
- [28] B. Sokół, A. Woźniak, R. Jankowski et al., "HMGB1 level in cerebrospinal fluid as a marker of treatment outcome in patients with acute hydrocephalus following aneurysmal subarachnoid hemorrhage," *Journal of Stroke and Cerebrovascular Diseases*, vol. 24, no. 8, pp. 1897–1904, 2015.
- [29] T. Schmitz, A. Heep, F. Groenendaal et al., "Interleukin-1 β , interleukin-18, and interferon- γ expression in the cerebrospinal fluid of premature infants with posthemorrhagic hydrocephalus—markers of white matter damage?," *Pediatric Research*, vol. 61, no. 6, pp. 722–726, 2007.
- [30] D. A. Sival, U. Felderhoff-Müser, T. Schmitz, E. W. Hoving, C. Schaller, and A. Heep, "Neonatal high pressure hydrocephalus is associated with elevation of pro-inflammatory cytokines IL-18 and IFN γ in cerebrospinal fluid," *Cerebrospinal Fluid Research*, vol. 5, no. 1, p. 21, 2008.
- [31] D. M. Morales, S. A. Silver, C. D. Morgan et al., "Lumbar cerebrospinal fluid biomarkers of posthemorrhagic hydrocephalus of prematurity," *Neurosurgery*, vol. 80, no. 1, p. 1, 2016.
- [32] E. Rota, G. Bellone, P. Rocca, B. Bergamasco, G. Emanuelli, and P. Ferrero, "Increased intrathecal TGF- β 1, but not IL-12, IFN- γ and IL-10 levels in Alzheimer's disease patients," *Neurological Sciences*, vol. 27, no. 1, pp. 33–39, 2006.
- [33] X. Li, M. Miyajima, C. Jiang, and H. Arai, "Expression of TGF- β s and TGF- β type II receptor in cerebrospinal fluid of patients with idiopathic normal pressure hydrocephalus," *Neuroscience Letters*, vol. 413, no. 2, pp. 141–144, 2007.
- [34] M. Krause, N. Peukert, W. Härtig et al., "Localization, occurrence, and CSF changes of SP-G, a new surface active protein with assumable immunoregulatory functions in the CNS," *Molecular Neurobiology*, vol. 56, no. 4, pp. 2433–2439, 2019.
- [35] G. Habiyaremye, D. M. Morales, C. D. Morgan et al., "Chemokine and cytokine levels in the lumbar cerebrospinal fluid of preterm infants with post-hemorrhagic hydrocephalus," *Fluids and Barriers of the CNS*, vol. 14, no. 1, p. 35, 2017.
- [36] O. T. Pyykkö, M. Lumela, J. Rummukainen et al., "Cerebrospinal fluid biomarker and brain biopsy findings in idiopathic normal pressure hydrocephalus," *PLoS One*, vol. 9, no. 3, article e91974, 2014.
- [37] L. Sosvorova, J. Vcelak, M. Mohapl, J. Vitku, M. Bicikova, and R. Hampl, "Selected pro- and anti-inflammatory cytokines in cerebrospinal fluid in normal pressure hydrocephalus," *Neuro Endocrinology Letters*, vol. 35, no. 7, pp. 586–593, 2014.
- [38] L. Sosvorova, R. Kanceva, J. Vcelak et al., "The comparison of selected cerebrospinal fluid and serum cytokine levels in patients with multiple sclerosis and normal pressure hydrocephalus," *Neuro Endocrinology Letters*, vol. 36, no. 6, pp. 564–571, 2015.
- [39] A. Jeppsson, H. Zetterberg, K. Blennow, and C. Wikkelso, "Idiopathic normal-pressure hydrocephalus: pathophysiology and diagnosis by CSF biomarkers," *Neurology*, vol. 80, no. 15, pp. 1385–1392, 2013.
- [40] I. Naureen, K. A. I. Waheed, A. W. Rathore et al., "Fingerprint changes in CSF composition associated with different aetiologies in human neonatal hydrocephalus: inflammatory cytokines," *Child's Nervous System*, vol. 30, no. 7, pp. 1155–1164, 2014.
- [41] A. Whitelaw, S. Christie, and I. Pople, "Transforming growth factor- β 1: a possible signal molecule for posthemorrhagic hydrocephalus?," *Pediatric Research*, vol. 46, no. 5, pp. 576–580, 1999.
- [42] K. Czubowicz, M. Głowacki, E. Fersten, E. Kozłowska, R. P. Strosznajder, and Z. Czernicki, "Levels of selected pro- and anti-inflammatory cytokines in cerebrospinal fluid in patients with hydrocephalus," *Folia Neuropathologica*, vol. 55, no. 4, pp. 301–307, 2017.
- [43] M. A. Pourhoseingholi, M. Vahedi, and M. Rahimzadeh, "Sample size calculation in medical studies," *Gastroenterology and Hepatology from Bed to Bench*, vol. 6, no. 1, pp. 14–17, 2013.
- [44] T. Faresjö and Å. Faresjö, "To match or not to match in epidemiological studies—same outcome but less power," *International Journal of Environmental Research and Public Health*, vol. 7, no. 1, pp. 325–332, 2010.
- [45] M. Ishikawa, "Clinical guidelines for idiopathic normal pressure hydrocephalus," *Neurologia Medico-Chirurgica (Tokyo)*, vol. 44, no. 4, pp. 222–223, 2004.
- [46] M. Ishikawa, M. Hashimoto, N. Kuwana et al., "Guidelines for management of idiopathic normal pressure hydrocephalus," *Neurologia Medico-Chirurgica*, vol. 48, pp. S1–S23, 2008.
- [47] S. Malay and K. C. Chung, "The choice of controls for providing validity and evidence in clinical research," *Plastic and Reconstructive Surgery*, vol. 130, no. 4, pp. 959–965, 2012.
- [48] F. A. Lisboa, M. J. Bradley, M. T. Hueman et al., "Nonsteroidal anti-inflammatory drugs may affect cytokine response and benefit healing of combat-related extremity wounds," *Surgery*, vol. 161, no. 4, pp. 1164–1173, 2017.
- [49] H. Reiber, "Dynamics of brain-derived proteins in cerebrospinal fluid," *Clinica Chimica Acta*, vol. 310, no. 2, pp. 173–186, 2001.
- [50] S. Brandner, C. Thaler, N. Lelental et al., "Ventricular and lumbar cerebrospinal fluid concentrations of Alzheimer's disease biomarkers in patients with normal pressure hydrocephalus and posttraumatic hydrocephalus," *Journal of Alzheimer's Disease*, vol. 41, no. 4, pp. 1057–1062, 2014.
- [51] S. Brandner, C. Thaler, P. Lewczuk, N. Lelental, M. Buchfelder, and A. Kleindienst, "Neuroprotein dynamics in the cerebrospinal fluid: intraindividual concomitant ventricular and

- lumbar measurements," *European Neurology*, vol. 70, no. 3–4, pp. 189–194, 2013.
- [52] E. Aasebø, J. A. Opsahl, Y. Bjørlykke, K. M. Myhr, A. C. Kroksveen, and F. S. Berven, "Effects of blood contamination and the rostro-caudal gradient on the human cerebrospinal fluid proteome," *PLoS One*, vol. 9, no. 3, article e90429, 2014.
- [53] A. H. Simonsen, S. Bech, I. Laursen et al., "Proteomic investigations of the ventriculo-lumbar gradient in human CSF," *Journal of Neuroscience Methods*, vol. 191, no. 2, pp. 244–248, 2010.
- [54] S. F. Khan, T. Macauley, S. Y. C. Tong et al., "When ventricular cerebrospinal fluid assessment misleads: basal meningitis and the importance of lumbar puncture sampling," *Open Forum Infectious Diseases*, vol. 6, no. 7, article ofz324, 2019.
- [55] C. E. Teunissen, A. Petzold, J. L. Bennett et al., "A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking," *Neurology*, vol. 73, no. 22, pp. 1914–1922, 2009.
- [56] O. Hansson, A. Mikulskis, A. M. Fagan et al., "The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: A review," *Alzheimer's & Dementia*, vol. 14, no. 10, pp. 1313–1333, 2018.
- [57] C. Cicognola, D. Chiasserini, and L. Parnetti, "Preanalytical confounding factors in the analysis of cerebrospinal fluid biomarkers for Alzheimer's disease: the issue of diurnal variation," *Frontiers in Neurology*, vol. 6, p. 143, 2015.
- [58] M. del Campo, B. Mollenhauer, A. Bertolotto et al., "Recommendations to standardize preanalytical confounding factors in Alzheimers and Parkinsons disease cerebrospinal fluid biomarkers: An update," *Biomarkers in Medicine*, vol. 6, no. 4, pp. 419–430, 2012.
- [59] M. Vahdat, S. A. Hosseini, G. Khalatbari Mohseni, J. Heshmati, and M. Rahimlou, "Effects of resistant starch interventions on circulating inflammatory biomarkers: a systematic review and meta-analysis of randomized controlled trials," *Nutrition Journal*, vol. 19, no. 1, p. 33, 2020.
- [60] M. Morvaridzadeh, S. Fazelian, S. Agah et al., "Effect of ginger (*Zingiber officinale*) on inflammatory markers: a systematic review and meta-analysis of randomized controlled trials," *Cytokine*, vol. 135, article 155224, 2020.
- [61] M. Rothaug, C. Becker-Pauly, and S. Rose-John, "The role of interleukin-6 signaling in nervous tissue," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1863, no. 6, pp. 1218–1227, 2016.
- [62] M. Erta, A. Quintana, and J. Hidalgo, "Interleukin-6, a major cytokine in the central nervous system," *International Journal of Biological Sciences*, vol. 8, no. 9, pp. 1254–1266, 2012.
- [63] M. Akdis, S. Burgler, R. Cramer et al., "Interleukins, from 1 to 37, and interferon- γ : Receptors, functions, and roles in diseases," *Journal of Allergy and Clinical Immunology*, vol. 127, no. 3, pp. 701–721.e70, 2011.
- [64] L. Wang, B. Walia, J. Evans, A. T. Gewirtz, D. Merlin, and S. V. Sitaraman, "IL-6 induces NF- κ B activation in the intestinal epithelia," *Journal of Immunology*, vol. 171, no. 6, pp. 3194–3201, 2003.
- [65] K. Ren and R. Torres, "Role of interleukin-1 β during pain and inflammation," *Brain Research Reviews*, vol. 60, no. 1, pp. 57–64, 2009.
- [66] S. E. Acuner Ozbabacan, A. Gursoy, R. Nussinov, and O. Keskin, "The structural pathway of interleukin 1 (IL-1) initiated signaling reveals mechanisms of oncogenic mutations and SNPs in inflammation and cancer," *PLoS Computational Biology*, vol. 10, no. 2, article e1003470, 2014.
- [67] R. Shirai, F. Hirano, N. Ohkura, K. Ikeda, and S. Inoue, "Up-regulation of the expression of leucine-rich α 2-glycoprotein in hepatocytes by the mediators of acute-phase response," *Biochemical and Biophysical Research Communications*, vol. 382, no. 4, pp. 776–779, 2009.
- [68] M. Nakajima, M. Miyajima, I. Ogino et al., "Brain localization of Leucine-Rich α 2-glycoprotein and its role," in *Acta Neurochirurgica, Supplementum*, vol. 113, no. 113pp. 97–101, Springer-Verlag, Wien, 2012.
- [69] L. C. O'Donnell, L. J. Druhan, and B. R. Avalos, "Molecular characterization and expression analysis of leucine-rich alpha2-glycoprotein, a novel marker of granulocytic differentiation," *Journal of Leukocyte Biology*, vol. 72, no. 3, pp. 478–485, 2002.
- [70] P. F. Chong, Y. Sakai, H. Torisu et al., "Leucine-rich alpha-2 glycoprotein in the cerebrospinal fluid is a potential inflammatory biomarker for meningitis," *Journal of the Neurological Sciences*, vol. 392, pp. 51–55, 2018.
- [71] S. Serada, M. Fujimoto, A. Ogata et al., "iTRAQ-based proteomic identification of leucine-rich α -2 glycoprotein as a novel inflammatory biomarker in autoimmune diseases," *Annals of the Rheumatic Diseases*, vol. 69, no. 4, pp. 770–774, 2010.
- [72] M. Fujimoto, T. Matsumoto, S. Serada et al., "Leucine-rich alpha 2 glycoprotein is a new marker for active disease of tuberculosis," *Scientific Reports*, vol. 10, no. 1, p. 3384, 2020.
- [73] S. Serada, M. Fujimoto, F. Terabe et al., "Serum leucine-rich alpha-2 glycoprotein is a disease activity biomarker in ulcerative colitis," *Inflammatory Bowel Diseases*, vol. 18, no. 11, pp. 2169–2179, 2012.
- [74] X. Wang, S. Abraham, J. A. G. McKenzie et al., "LRG1 promotes angiogenesis by modulating endothelial TGF- β signaling," *Nature*, vol. 499, no. 7458, pp. 306–311, 2013.
- [75] C. Freudlsperger, Y. Bian, S. Contag Wise et al., "TGF- β and NF- κ B signal pathway cross-talk is mediated through TAK1 and SMAD7 in a subset of head and neck cancers," *Oncogene*, vol. 32, no. 12, pp. 1549–1559, 2013.
- [76] K. Yasuda, K. Nakanishi, and H. Tsutsui, "Interleukin-18 in health and disease," *International Journal of Molecular Sciences*, vol. 20, no. 3, p. 649, 2019.
- [77] P. Bossu, A. Ciaramella, F. Salani et al., "Interleukin-18, from neuroinflammation to Alzheimers disease," *Current Pharmaceutical Design*, vol. 16, no. 38, pp. 4213–4224, 2010.
- [78] L. S. Angelo and R. Kurzrock, "Vascular endothelial growth factor and its relationship to inflammatory mediators: Fig. 1," *Clinical Cancer Research*, vol. 13, no. 10, pp. 2825–2830, 2007.
- [79] I. Kim, S.-O. Moon, S. Hoon Kim, H. Jin Kim, Y. Soon Koh, and G. Young Koh, "Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor- κ B activation in endothelial cells," *The Journal of Biological Chemistry*, vol. 276, no. 10, pp. 7614–7620, 2001.
- [80] T. Marumo, V. B. Schini-Kerth, and R. Busse, "Vascular endothelial growth factor activates nuclear factor-kappaB and induces monocyte chemoattractant protein-1 in bovine retinal endothelial cells," *Diabetes*, vol. 48, no. 5, pp. 1131–1137, 1999.
- [81] A. S. R. Maharaj, T. E. Walshe, M. Saint-Geniez et al., "VEGF and TGF- β are required for the maintenance of the choroid

- plexus and ependyma,” *The Journal of Experimental Medicine*, vol. 205, no. 2, pp. 491–501, 2008.
- [82] J. W. Shim, J. Sandlund, C. H. Han et al., “VEGF, which is elevated in the CSF of patients with hydrocephalus, causes ventriculomegaly and ependymal changes in rats,” *Experimental Neurology*, vol. 247, pp. 703–709, 2013.
- [83] M. D. Domínguez-Pinos, P. Páez, A. J. Jiménez et al., “Ependymal denudation and alterations of the subventricular zone occur in human fetuses with a moderate communicating hydrocephalus,” *Journal of Neuropathology and Experimental Neurology*, vol. 64, no. 7, pp. 595–604, 2005.
- [84] F. Tissir, Y. Qu, M. Montcouquiol et al., “Lack of cadherins Celsr2 and Celsr3 impairs ependymal ciliogenesis, leading to fatal hydrocephalus,” *Nature Neuroscience*, vol. 13, no. 6, pp. 700–707, 2010.
- [85] K. Schroder, P. J. Hertzog, T. Ravasi, and D. A. Hume, “Interferon- γ : an overview of signals, mechanisms and functions,” *Journal of Leukocyte Biology*, vol. 75, no. 2, pp. 163–189, 2004.
- [86] G. Tau and P. Rothman, “Biologic functions of the IFN-gamma receptors,” *Allergy*, vol. 54, no. 12, pp. 1233–1251, 1999.
- [87] Y. Lin, S. Jamison, and W. Lin, “Interferon- γ activates nuclear factor- κ B in oligodendrocytes through a process mediated by the unfolded protein response,” *PLoS One*, vol. 7, no. 5, article e36408, 2012.
- [88] A. B. Steffensen, E. K. Oernbo, A. Stoica et al., “Cotransporter-mediated water transport underlying cerebrospinal fluid formation,” *Nature Communications*, vol. 9, no. 1, p. 2167, 2018.
- [89] G. G. Hallaert, D. J. Vanhauwaert, K. Logghe et al., “Endoscopic coagulation of choroid plexus hyperplasia,” *Journal of Neurosurgery. Pediatrics*, vol. 9, no. 2, pp. 169–177, 2012.
- [90] H. M. Eisenberg, J. G. McComb, and A. V. Lorenzo, “Cerebrospinal fluid overproduction and hydrocephalus associated with choroid plexus papilloma,” *Journal of Neurosurgery*, vol. 40, no. 3, pp. 381–385, 1974.