Clinical Study

Cerebrospinal Fluid Biomarkers in Idiopathic Normal Pressure Hydrocephalus

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The diagnosis of idiopathic normal pressure hydrocephalus (iNPH) is still challenging. Alzheimer's disease (AD), along with vascular dementia, the most important differential diagnosis for iNPH, has several potential cerebrospinal fluid (CSF) biomarkers which might help in the selection of patients for shunt treatment. The aim of this study was to compare a battery of CSF biomarkers including well-known AD-related proteins with CSF from patients with suspected iNPH collected from the external lumbar drainage test (ELD). A total of 35 patients with suspected iNPH patients were evaluated with ELD. CSF was collected in the beginning of the test, and the concentrations of total tau, ptau$_{181}$, Aβ_{42}, NFL, TNF-α, TGFβ1, and VEGF were analysed by ELISA. Twenty-six patients had a positive ELD result—that is, their gait symptoms improved; 9 patients had negative ELD. The levels of all analyzed CSF biomarkers were similar between the groups and none of them predicted the ELD result in these patients. Contrary to expectations lumbar CSF TNF-α concentration was low in iNPH patients.

1. Introduction

Normal pressure hydrocephalus (NPH) is characterized by a clinical triad of symptoms including cognitive impairment, gait difficulty, and urinary incontinence along with ventricular enlargement in brain imaging [1]. NPH is considered as idiopathic (iNPH) when there are no known predisposing factors such as subarachnoid haemorrhage or brain trauma [1]. NPH can be treated by shunt [1] but the response rate is highly sensitive for selection of patients [2, 3]. Alzheimer's disease (AD) is along with vascular dementia (VaD) the most frequent differential diagnosis for iNPH [4].

Several supplementary diagnostic tests of cerebrospinal fluid (CSF) dynamics are used in the selection of patients to shunt surgery. The CSF tap test or external lumbar drainage test (ELD) can predict the shunt response with high specificity and are widely used [5]. Infusion tests where usually Ringer^R-solution is infused into CSF space with simultaneous CSF pressure monitoring to calculate outflow conductance or outflow resistance are also used [6]. In addition, intracranial pressure monitoring alone [7]

or together with cortical brain biopsy to detect AD-related pathological findings [4] has been suggested.

CSF biomarkers reflecting ongoing pathophysiological processes might further help in the evaluation of patients with suspected iNPH. Previous reviews have pointed out several potential CSF biomarkers associated with NPH [8] but all of them still requiring further verification [9]. There are numerous experimental studies in both acute and chronic hydrocephalus that provide translational evidence for the role of metabolic changes and markers in parallel with hydrocephalus and disturbed CSF dynamics, for example, as reported by Kondziella et al. [10].

Several CSF proteins are potentially important in iNPH or Alzheimer's disease.

Tumour necrosis factor-α (TNF-α), a proinflammatory cytokine, seems to be one of the most promising since up to 45-fold increased CSF concentrations compared with healthy controls have been observed in NPH prior to treatment along with normalization after shunt [11]. However, the patients with known aetiology that is secondary NPH were mixed

TABLE 1: Characteristics of the patients and CSF concentrations of the analyzed biomarkers.

	Positive ELD	Negative ELD	P
N. patients	26	9	
Median age at ELD (range)	74 (65–88)	77 (69–88)	.29
Median age at onset (range)	71.5 (62–86)	75 (68–88)	.14
Male/female	13/13	5/4	.77 (X^2)
Gait (n)	25	9	.55 (X^2)
Cognition (n)	21	9	.16 (X^2)
Incontinence (n)	14	6	.50 (X^2)
Major symptom gait/cogn/inc	21/2/1	7/1/1	.72 (X^2)
	2 cases n.d.*		
Shunt	25	1	
Shunt response fair/good/exc	1/3/21	0/0/1	
NFL pg/mL	1940** ± 2662 (280–>10000)	2046 ± 2886 (483–9306)	.92
Total tau pg/mL	274 ± 358 (44–1860)	291 ± 283 (89–983)	.90
$A\beta_{1-42}$ pg/mL	250 ± 179 (31–765)	209 ± 120 (102–486)	.52
Total tau/$A\beta_{1-42}$ > 1.15	7 (27%)	4 (44%)	.33 (X^2)
p-Tau$_{181}$ pg/mL	55 ± 45 (16–234)	53 ± 25 (24–95)	.88
TNF-α pg/mL	0.7 ± 1.1 (0–4.9)	1.3 ± 1.4 (0–3.4)	.16
TGFβ-1 pg/mL	61 ± 24 (29–146)	71 ± 62 (20–228)	.52
VEGF pg/mL	n.d.***	n.d.***	

The values are given as mean±SD (range) unless otherwise stated.

*not able to determine one major symptom.

** two cases with concentration >10 000 pg/mL (10 000 used as a value and therefore the real mean value is expected to be higher.)

***not determined due to low concentration.

ELD: external lumbar drainage test, NFL: neurofilament light chain, t-tau: total tau, p-tau$_{181}$: phosphorylated tau$_{181}$, $A\beta_{42}$: amyloid-β_{1-42}, TNF-α: tumour necrosis factor-α, TGFβ1: transforming growth factor β1, and VEGF: vascular endothelial growth factor.

with idiopathic cases and no further studies on this marker have been published in NPH.

Transforming growth factor-β (TGF-β) is associated with brain response to injury and inflammation, and increased CSF TGF-β concentrations are reported in AD patients [12]. Subarachnoid haemorrhage (SAH) increased TGF-β concentrations and correlated with the risk of shunt-dependent hydrocephalus [13] but in iNPH the role of TGF-β is somewhat controversial [14, 15].

Neurofilament protein is a marker of neurodegeneration especially axonal injury, and clearly increased NF light (NFL) concentrations have been detected both in iNPH and sNPH patients [16, 17].

Vascular endothelial growth factor (VEGF) is associated with cerebral ischemia and has been correlated negatively with neurofilament heavy chain (NFh) protein in iNPH patients and increased during ELD [18].

Increased total tau (t-tau) and phosphorylated-tau$_{181}$ (p-tau$_{181}$) in CSF are associated with neurodegeneration and AD [19]. In iNPH both normal [17] and increased [20] t-tau concentrations have been observed. Decreased amyloid-β_{42} ($A\beta_{42}$) in CSF is associated with AD and indicates risk of AD in mild cognitive impairment [21] but may be normal in iNPH [22].

In the present study, we correlated the concentrations of seven biomarkers, NFL, t-tau, p-tau$_{181}$ $A\beta_{42}$, TNF-α, TGFβ1, and VEGF in the CSF of 35 patients with suspected iNPH with the result of the lumbar drainage test.

TABLE 2: Black Scale for assessment of shunt outcome [3].

Excellent	Resumed preillness activity without deficit
Good	Resumed preillness activity with deficit, improved in two or more categories
Fair	Improved but did not return to previous work, improved in one category
Transient	Temporary major improvement
Poor	No change or worsening
Dead	Died within 6 wk of surgery or as a result of surgery

2. Material and Methods

2.1. Study Series. This study includes 35 patients referred to Brigham and Woman's Hospital (BWH) Neurosurgery with suspected idiopathic normal pressure hydrocephalus (iNPH) according to clinical and radiological examination. Patient characteristics are presented in Table 1. Patients with known cause of NPH (sNPH) were excluded.

2.2. External Lumbar Drainage Test (ELD). All patients were evaluated by standard previously described ELD [23] between 2007 and 2010. Continuous drainage was applied with targeted CSF drainage rate of 5 to 10 mL/h. Neurological (including Folstein Mini-Mental Status Examination in patients with cognitive symptoms) and physical therapy

TABLE 3: CSF biomarkers.

Protein	Abbreviation	Mean detection limit	Manufacturer
Neurofilament light chain	NFL	31 pg/mL	Uman Diagnostics, Umeå, Sweden
Total tau	t-tau	12 pg/mL	Invitrogen, Camarillo, Calif, USA
Phosphorylated tau$_{181}$	p-tau$_{181}$	10 pg/mL	Invitrogen, Camarillo, Calif, USA
Amyloid-β_{1-42}	Aβ_{42}	10 pg/mL	Invitrogen, Camarillo, Calif, USA
Tumour necrosis factor α	TNF-α	0.106 pg/mL	Quantikine HS, R&D Systems, Minneapolis, Minn, USA
Transforming growth factor β1	TGFβ1	4.61 pg/mL	Quantikine HS, R&D Systems, Minneapolis, Minn, USA
Vascular endothelial growth factor-165	VEGF	5 pg/mL	Invitrogen, Camarillo, Calif, USA

NFL: neurofilament light chain, t-tau: total tau, p-tau$_{181}$: phosphorylated tau$_{181}$, Aβ_{42}: amyloid-β_{1-42}, TNF-α: tumour necrosis factor-α, TGFβ1: transforming growth factor β1, and VEGF: vascular endothelial growth factor.

(including Timed Up and Go test—the patient was timed while rising from a chair, walking 3 m, turning around, walking back to the chair, and sitting down) evaluations were completed prior to drainage and daily until the end of the test, which continued for a maximum of 5 days. If the patient demonstrated documented improvement before 5 days or if the patient experienced side effects of drainage refractory to conservative measures, the trial was considered complete and the drain was removed. Criteria for positive ELD were 20% improvement in objectively measured gait or cognition [23]. ELD test was negative in nine and positive in 26 patients.

2.3. Response to Shunt. Patients were shunted or not according to ELD result. One patient was shunted despite of primary negative response but later noted subjective improvement.

Clinical symptoms, history of any possible known cause of NPH, and other neurological disorders were recorded as well as clinically evaluated response for treatment in shunted patients. Shunt response was graded according to Black Scale (Table 2) as no response, fair, good, or excellent response after 3-month followup. All shunted patients responded to the treatment (Table 1).

2.4. CSF Analysis for Biomarkers. The cerebrospinal fluid (CSF) samples (4 mL) were collected in the beginning of the ELD immediately after the puncture, centrifuged, and stored in polypropylene tube at $-80°$C until analysis to ensure the stability of the CSF biomarker levels during the storage.

Measurements of CSF were performed in BWH Neurosurgery laboratory using commercially available solid phase sandwich enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's protocol and blinded to the ELD results (Table 3). All samples were analyzed as duplicates.

2.5. Ethical Aspects. The study was approved by the Partners Human Research Committee. Written informed consent was obtained from all patients.

2.6. Statistical Analysis. The CSF concentrations of the analyzed markers were compared between the positive and negative ELD groups by independent samples t-test. Dichotomized variables were compared by X^2-test. Pearson's correlation coefficients were calculated between the markers. Statistical analyzes were performed using SPSS 17.0.

3. Results

The biomarker concentrations in the CSF of 26 patients with a positive ELD result and nine patients with negative ELD result are presented in Table 1. Nine patients had initially negative ELD, and one of them was shunted with excellent response. The levels of all analyzed CSF biomarkers were similar between the groups, and none of them could predict the ELD result in these patients.

NFL concentrations were increased similarly both in patients with positive (range 280–>10000) and negative (range 438–9306) ELD (Table 1).

TNF-α concentration was equally low around 1 pg/mL in both groups (Table 1). In 10 cases the concentration was even under the mean detection limit (0.106 pg/mL).

TGFβ1 concentrations were similar (mean 61 versus 71 pg/mL, range 20–228, $P = .52$) in both groups (Table 1). TGFβ1 concentration had positive correlation with t-tau ($r = 0.413$, $P = .014$, Table 4).

Mean Aβ_{42} concentrations did not differ significantly between the groups (250 versus 209 pg/mL) (Table 1).

Mean t-tau concentration was 274 pg/mL (range 44–1860) in positive and 291 (range 89–983) in negative ELD groups without significant difference ($P = .90$, Table 1) and increased with age ($r = 0.38$, $P = .023$, Table 4). Also p-tau$_{181}$ concentrations were equal between positive and negative ELD groups (55 versus 53 pg/mL, $P = .88$, Table 1).

VEGF concentrations were under the detection limit (5 pg/mL) in all except two cases and therefore were not included in the correlation analysis.

4. Discussion

The most important finding of this study is the unexpectedly low CSF TNF-α concentrations observed in iNPH patients and the inability of CSF biomarkers to predict the ELD result.

Using a standard commercial ELISA, the CSF TNF-α levels between 0 and 5.0 pg/mL—close to the standard

TABLE 4: Correlations between CSF biomarkers.

	NFL	t-Tau	$A\beta_{1-42}$	p-Tau$_{181}$	TNF-α	TGFβ1
Age	−0.112 (0.52)	0.383* (0.023)	0.215 (0.22)	0.010 (0.95)	0.246 (0.15)	−0.009 (0.96)
NFL		0.030 (0.86)	−0.225 (0.19)	0.063 (0.71)	0.130 (0.46)	−0.013 (0.94)
t-Tau	0.030 (0.86)		−0.071 (0.69)	0.667* (<0.001)	0.143 (0.41)	0.413* (0.014)
$A\beta_{1-42}$	−0.225 (0.19)	−0.071 (0.69)		−0.169 (0.33)	−0.024 (0.89)	−0.103 (0.56)
p-Tau$_{181}$	0.063 (0.71)	0.667* (<0.001)	−0.169 (0.33)		−0.138 (0.43)	0.205 (0.24)
TNF-α	0.130 (0.46)	0.143 (0.41)	−0.024 (0.89)	−0.138 (0.43)		0.267 (0.12)
TGFβ1	−0.013 (0.94)	0.413* (0.014)	−0.103 (0.56)	0.205 (0.24)	0.267 (0.12)	

NFL: neurofilament light chain, t-tau: total tau, p-tau$_{181}$: phosphorylated tau$_{181}$, $A\beta_{42}$: amyloid-β_{1-42}, TNF-α: tumour necrosis factor-α, and TGFβ1: transforming growth factor β1.

levels previously observed in serum (0.5–2.8 pg/mL)—were detected. Concentrations up to 700 pg/mL would have been expected according to one previous study [11]. Differences in sampling and analyzing processes (different ELISA was used in the previous study [11]) might have effect on the results although likely not crucial. The most probable explanation could be that in the previous study [11] the idiopathic cases were not separated from the secondary cases. This indicates that the inflammatory reaction would be associated with secondary NPH rather than idiopathic form.

Our study did not include healthy controls, and our negative result on TNF-α should be reproduced in study were NPH patients with possible known cause of the disease are separated from idiopathic cases and compared with healthy controls. It would also be very interesting to study the possible role of inflammation in the formation of secondary hydrocephalus for example after SAH or trauma. Experimental studies clearly indicate the increased production of TNF-α as well as other proinflammatory cytokines due to accession of blood products to CSF [24].

Notably increased TGFβ1 levels after SAH indicated risk of persistent hydrocephalus [13]. We detected rather low TGFβ1 levels (varied from 20 to 228 pg/mL) in iNPH patients contrary to a previous observation of increased concentrations [14] but supporting other studies with low concentrations [15]. Interestingly TGFβ1 levels correlated with t-tau but not with the levels of other studied biomarkers. This is contrary to a previous study in AD patients and controls where TGFβ1 levels correlated with $A\beta_{42}$ but not with tau [12]. This can be explained by different patient population and by rather small number of cases in both studies. The clear correlation between p-tau$_{181}$ and total tau is expected since phosphorylated tau is an isoform included into total tau. Interestingly only total tau correlated with age and TGFβ1. The correlation analysis can be justified to indicate obvious associations between different biomarkers but has to be interpreted cautiously because of low statistical power due to rather small number of cases.

Equally increased CSF VEGF levels are detected both in AD and VaD patients also correlating with TGFβ1 levels [25]. Decreased cerebral blood flow associated with chronic hydrocephalus potentially leads to hypoxia, which could induce increased VEGF formation also in iNPH. In an experimental study the increased CSF VEGF levels were

seen only in a short-term model of chronic hydrocephalus but not in the long-term model [26]. Therefore low VEGF concentrations detected here are not very surprising.

Based on studies comparing AD patients and healthy subjects it could be expected that iNPH patients with low $A\beta_{42}$ and increased t-tau and/or p-tau might have concomitant early AD or could be in the risk of developing AD. CSF tau and $A\beta_{42}$ protein levels might help in the detection of the patients who have AD instead of NPH or may have significant risk for concomitant AD. The low $A\beta_{42}$ together with increased tau concentrations (t-tau/$A\beta_{42}$ ratio >1.15) seems to indicate increased risk of AD [19]. Thus total tau/$A\beta_{42}$ ratio over 1.15 could be a potential cut-off limit for increased risk of AD [19] and according to that one-third of the current iNPH patients diagnosed by LDT would be in increased risk of future AD. However, increased CSF tau and decreased $A\beta_{42}$ could be detected also in other neurodegenerative disorders than AD [27]. In any case, shunted iNPH patient with still progressing amnestic cognitive impairment and the profile of increased tau and decreased $A\beta_{42}$ should be noted and managed interdisciplinary with a thorough dementia workup.

Specific CSF biomarkers of iNPH indicating shunt response and the possible "point of no return" in the course of the disease would be valuable. Also indicators of the long-term prognosis especially those predicting risk of dementia despite of shunting would be useful. However, this would need a battery of biomarkers and long-term follow-up studies since causes of dementia in these patients are variable from AD to dementia with vascular origin and perhaps dementia due to iNPH itself. Markers indicating possibility of AD are already available but their predictive value of concomitant AD in iNPH patients still needs to be shown in follow-up studies.

Currently there seems to be no CSF biomarker available which could clearly predict the result of lumbar CSF drainage test. It should also be kept in mind that the sensitivity of ELD is not 100% and as also in this series there can be patients who benefit from shunt despite negative test result. Therefore we can not exclude the possibility that some of the markers analyzed here could somehow predict response for shunt treatment in case of negative ELD. In tau and NFL the current results are in line with the previous study where they did not correlate with outcome of shunt [28].

Further research is needed to evaluate the molecular biological basis of idiopathic NPH and to obtain CSF biomarkers for the clinical diagnosis of iNPH. New methods may detect novel proteins to clarify the pathophysiology of iNPH [29]. The CSF, blood, and even brain tissue samples which are possible to obtain without significant additional risk for the patient during diagnosis and treatment of NPH may be useful in differential diagnosis and further research [4]. Also they can be useful for validation of less invasive methods to detect for example Aβ and other possible markers of neurodegeneration and help in the discovery of new surrogate markers. Neurosurgeons are encouraged to collect blood, CSF, and tissue samples for future biobanks with detailed clinical characterisation of patients with careful history taking and use of standardized outcome scales. It is also obligatory to present the results of the analyses separately in different entities of NPH since idiopathic and secondary forms of the disease have different molecular biological background. The secondary forms of NPH should also be separated depending on the specific aetiology.

In conclusion the CSF biomarkers analyzed here could not predict the ELD result. Contrary to the expectations lumbar CSF TNF-α concentrations were low in iNPH patients.

Acknowledgment

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