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ORIGINAL ARTICLE

# Selected pro- and anti-inflammatory cytokines in cerebrospinal fluid in normal pressure hydrocephalus

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## Abstract

**OBJECTIVES:** Normal pressure hydrocephalus (NPH) is a treatable neurological syndrome developing in the elderly. It is characterized by balance impairment, urinary incontinence and dementia development caused by disorders in the cerebrospinal fluid (CSF) circulation. The diagnosis can be easily mistaken for other neurodegenerative diseases, which are often accompanied by inflammation and the production of cytokines. The aim of our study was to determine and compare selected CSF and plasma cytokines with respect to their informative value for laboratory diagnostics of NPH.

**METHODS:** The levels of IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, INF-γ, sCD40L and TNF-α were measured in the CSF and plasma in age-matched subjects with NPH (n=20) and controls (n=20) by multiplex assay.

**RESULTS:** CSF IL-1β, IL-6 and IL-10 were significantly increased on the 1st day of lumbar drainage in NPH ($p<0.01$). No significant changes were observed in the plasma. The CSF cytokines were one to three orders of magnitude higher compared to the plasma.

**CONCLUSION:** CSF can better show the neurodegenerative changes in the brain. The cytokines IL-1β, IL-6 and IL-10 may be helpful in NPH diagnostics.

**Abbreviations:**

| | |
|---|---|
| AD | - Alzheimer disease |
| CNS | - central nervous system |
| CSF | - cerebrospinal fluid |
| HC | - hydrocephalus |
| IL | - interleukin |
| LD | - lumbar drainage |
| LLOQ | - lowest limit of quantification |
| MS | - Multiple sclerosis |
| NPH | - Normal pressure hydrocephalus |
| PD | - Parkinson disease |

## INTRODUCTION

Systemic and brain inflammatory processes have been associated with various neurodegenerative and neuropsychiatric diseases such as Alzheimer disease (AD), Parkinson disease (PD), multiple sclerosis (MS), schizophrenia and normal pressure hydrocephalus (NPH) (Blum-Degena *et al.* 1995; Seppi *et al.* 2014; Sasayama *et al.* 2013; Mogi *et al.* 1996; Tarkowski *et al.* 2003b; Li *et al.* 2007). NPH is a treatable neurological disorder affecting elderly people with the prevalence ranging from 0.2% to 5.9% increasing with age (Jaraj *et al.* 2014). It is caused by abnormal CSF reabsorption with ventricle enlargement, resulting in damaged brain tissues and several brain malfunctions. The degenerative changes may be reversible if recognized early and treated properly. Diagnosis is difficult and can be easily mistaken for other neurodegenerative disorders, which makes NPH one of the most significant misdiagnosed diseases (Jaraj *et al.* 2014; Brean *et al.* 2009).

Neurodegenerative diseases are often accompanied by inflammation, which usually contributes to disease progression by producing inflammatory mediators (Cunningham *et al.* 2009; Wyss-Coray & Mucke 2002). The most known mediators of inflammation are cytokines. They play key roles in inflammatory processes within and outside the brain and have both beneficial and detrimental actions on CNS (Rothwell 2003). The brain's response to varied stimuli differs from peripheral inflammation. Peripheral immune cells are not normally allowed to penetrate into the CNS across the blood brain barrier, while in an inflamed brain this barrier is breached, allowing immunocompetent cells to infiltrate into the injury site (Lossinsky & Shivers 2004).

The most discussed pro-inflammatory cytokine in the relation to neurodegenerative disorders is IL (interleukine)-1β (Simi *et al.* 2007). Other important cytokines are IL-6 and TNF-α which interplay with IL-1β (Kishimoto *et al.* 1992; Tobinick *et al.* 2006). The IL-17 cytokine family plays a role in inducing inflammation, except for one – IL 17E (IL-25), with anti-inflammatory properties (Jin & Dong 2013). Pro-inflammatory effects have also been reported for IL-21, IL-22, IL-23, IL-31 and IL-33, as well as for the soluble CD40 ligand (sCD40L). Another pro-inflammatory cytokine is INF-γ. In contrast to other interferons, INF-γ is not directly induced by viral infection, but produced later and shows mainly immunomodulatory functions (Schroder *et al.* 2004). The longest known anti-inflammatory cytokines are IL-4 and IL-10 involved in the downregulation of some pro-inflammatory cytokines (Gadani *et al.* 2012; Hart *et al.* 1989; Park *et al.* 2007).

In subjects suffering from NPH, only few studies on various cytokines in the CSF were reported. A brief summary on CSF cytokines in adult hydrocephalus is shown in Table 1.

The aim of our study was to determine and compare selected CSF and plasma cytokines with respect to their informative value for laboratory diagnostics of NPH. The levels of the following cytokines were measured: IL-1β, Il-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, INF-γ, sCD40L and TNF-α in patients diagnosed with NPH comparing to controls.

## MATERIALS AND METHODS

*Subjects*

The patient group consisted of 20 subjects aged 65–80 years with non-obstructive idiopathic normal-pressure hydrocephalus diagnosed on the basis of a combination of NMR imaging and a lumbar drainage (LD) (Walchenbach *et al.* 2002). CSF was collected during a five-day LD. Plasma was collected before the LD. The shunt was introduced in all patients diagnosed with NPH after LD. The shunt implementation led to an improvement of the characteristic symptoms (bal-

**Tab. 1.** Brief survey of studies reporting cerebrospinal fluid (CSF) cytokine levels in various types of adult hydrocephalus (HC).

| Author and year | Number of subject, disease | Cytokines measured in CSF | Main Findings – comparison with controls |
|---|---|---|---|
| Kitazawa & Tada 1994 | 11, communicating HC after subarachnoid hemorrhage (SAH) | TGF-β1 | Higher levels compared to controls |
| Tarkowski *et al.* 2003b | 35, NPH | TNF-α | Increased levels before shunt operation, which were reversed after operation in parallel with the clinical improvement |
| Rota *et al.* 2006 | 14, NPH | IL-12, INF-γ, IL-10, TGF-β | No differences |
| Li *et al.* 2007 | 21, NPH | TGF-β | Elevated TGF-β CSF levels in NPH |
| Killer *et al.* 2010 | 34, various types of adult HC | IL-6 and IL-8 | Increased concentration of IL-6 and IL-8 |
| Leinonen *et al.* 2011 | 26, NPH | TNF-α, TGF-β | TNF-α concentration was lower in NPH |
| Lee *et al.* 2012 | 24, various types of adult HC | TNF-α, TGF-β | No differences |
| Pyykko *et al.* 2014 | 53, NPH | IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, INF-γ, MCP-1, TNF-α | No differences |

ance impairment, urinary incontinence and dementia development) in all patients.

The control group (20 subjects; 65–80 years) consisted of subjects tested for suspected NPH, where this diagnosis was excluded on the basis of NMR and LD. They underwent CSF collections as patients with diagnosed NPH. The surgeries were performed at the Department of Neurosurgery of Central Military Hospital in Prague.

Samples were collected in plastic tubes, subsequently frozen, stored at –79 °C and transported to the Institute of Endocrinology. The protocol was approved by the Ethical Committee of the Institute of Endocrinology. Informed and written consent with the use of biological materials for research reasons was obtained from all subjects participating to the project.

*Sample analysis*

Multiplex immunoanalytic xMAP (Luminex Corporation) technology employing Bio-Plex® 200 system (Bio-Rad Laboratories, Inc.) was used for the measurement of all analytes. The measurements were performed using multiplex assay (Bio-Plex ProTM Human Th17 Cytokine Panel, catalog #171AA001M (Bio-Rad, Hercules, CA, USA) designed for the determination of IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, INF-γ, sCD40L and TNF-α (Geng *et al.* 2012).

Immediately before analysis, samples were thawed and centrifuged at 10000 × *g* (4 °C) for 10 minutes to eliminate cells and other insoluble material. All preparatory steps with the samples were performed on ice. The samples were processed according to the manufacturer's protocol with minor modifications: the first incubation was extended to 2 hours and CSF samples were diluted with the sample diluent enriched with 0.5% of bovine serum albumin. Plasma samples were diluted in the ratio 1:4 (25 µL of plasma and 75 µL of sample diluent). CSF samples were diluted in the ratio 1:1 (50 µL of CSF and 50 µL of the above-mentioned diluent).

The sample analysis, calibration curve, blanks and controls were performed in duplicates. For each analysis, one more in-house control employing a sample-similar matrix was prepared. For the construction of calibration curves, the first two calibration points that were out of the sample range were excluded. Instead, one more standard was added using the serial dilution of the lowest calibrator.

Inter- and intra-assay coefficients of variation were within the ranges given by the manufacturer (Geng *et al.* 2012). By diluting the lowest calibrator and adding one more calibration point, we lowered the lowest limit of quantification (LLOQ). The LLOQ for individual cytokines were (pg/mL): IL-1β (0.059), IL-4 (1.576), IL-6 (0.623), IL-10 (3.124), IL-17A (0.530), IL-17F (7.572), IL-21 (4.495), IL-22 (1.356), IL-23 (5.802), IL-25 (0.302), IL-31 (2.785), IL-33 (1.977), INF-γ (2.468), sCD40L (0.949) and TNF-α (0.577).

*Data statistical analysis*

The relationships between dependent variables and effects of NPH and stage were evaluated using repeated measures of the ANOVA model consisting of the following factors: status (NPH vs. controls), stage (1st, 2nd, 3rd, 4th and 5th day of LD), subject (explaining inter-individual variability) and status × stage interaction. The ANOVA model was followed by least significant difference multiple comparisons. To eliminate skewed data distribution and heteroscedasticity, the original data were transformed by a power transformation to attain Gaussian distribution and constant variance before further processing. Statistical software Statgraphics Centurion, version XV from Statpoint Inc. (Herndon, Virginia, USA), was used for data processing.

## RESULTS

The CSF levels of IL-17F, IL-23, IL-25 and INF-γ were under the LLOQ. All other cytokines included in the used cytokine panel were within the measurable range. The levels of cytokines in the CSF and their changes during LD are shown in Table 2 and Figure 1.

The CSF levels of IL-1β and IL-6 were significantly increased on the 1st day of LD in NPH patients compared to the controls ($p<0.01$). After that, the levels decreased significantly and approached the levels of the controls.

The CSF cytokine levels of IL-10 were significantly higher ($p<0.01$) on the 1st day of LD in NPH patients compared to the controls, on the 2nd day, the levels of the controls increased to NPH; from the third day the levels in both groups decreased sequentially.

CSF IL-4, IL-17A, IL-21, IL-22, IL-31 and TNF-α were insignificantly lower in NPH patients during all days of LD.

The CSF cytokine levels of IL-33 and sCD40L were higher in NPH on the 1st day of LD; on the 2nd day, the levels of controls increased over NPH and remained higher during all subsequent LD days.

The plasma levels of IL-10, IL-17A, IL-17F, IL-21, IL-22 and IL-23 were under LLOQ. Other cytokines were within the measurable range. All cytokines measured in the plasma were not statistically different between patients and controls. The levels of cytokines in the plasma are reported in Table 3.

## DISCUSSION

The analysis of CSF has been used in diagnostics of various neurodegenerative disorders. CSF is the main component of the brain's extracellular space providing an insight into changes in the brain milieu associated directly with the patient's condition (Tarnaris *et al.* 2006). It participates in the free exchange of many biochemical products with the brain and therefore can better reflect the biological processes occurring in the CNS (Li *et al.* 2007). CSF is an easily accessible biologi-

**Tab. 2.** The levels (pg/mL) of individual cytokines in cerebrospinal fluid (CSF) from subjects with normal pressure hydrocephalus and controls. The levels are given during five-day lumbar drainage test (LD). The numbers express the LD day of CSF collection. Medians, quartiles and significancy are provided.

| Cytokines (pg/mL) | NORMAL PRESSURE HYDROCEPHALUS | | | | |
|---|---|---|---|---|---|
| | LD1 | LD2 | LD3 | LD4 | LD5 |
| IL-1β | 21.6 (11.36; 107.43)** | 2.91 (1.27; 10.51) | 1.51 (1.16; 5.86) | 1.79 (0.71; 3.19) | 0.36 (0.13; 0.59) |
| IL-4 | 19.17 (6.21; 38.42) | 28.03 (8.02; 47.99) | 38.21 (24.86; 66.8) | 27.62 (17.86; 36.51) | 37.42 (27.2; 80.51) |
| IL-6 | 11247.3 (937.3; 32044)** | 2888.7 (879.4; 11248.7) | 589.69 (273.9; 1121.9) | 234.57 (162.81; 353.87) | 88.72 (58.86; 195.78) |
| IL-10 | 45.08 (31.42; 72.09)** | 36.79 (29.47; 59.97) | 14.26 (4.61; 31.62) | 3.13 (3.13; 7.77) | under LLOQ |
| IL-17A | 24.56 (12.5; 43.58) | 52.16 (40.5; 66.85) | 40.01 (25.87; 47.9) | 18.79 (15.82; 43.66) | 23.685 (9.67; 71.92) |
| IL-21 | 113.7 (79.62; 219.59) | 218.11 (130.34; 337.45) | 292.22 (122.78; 481.94) | 127.58 (97.32; 168.23) | 282.1 (96.96; 770.78) |
| IL-22 | 23.48 (15.31; 35.04) | 35.8 (27.32; 49.65) | 33.97 (30.03; 65.88) | 19.21 (13.52; 36.69) | 35.83 (13.11; 122.21) |
| IL-31 | 41.12 (24.23; 63.86) | 48.64 (10.14; 89.38) | 75.42 (39.84; 166.2) | 27.54 (20.85; 96.25) | 31.93 (16.24; 49.22) |
| IL-33 | 21.12 (11.39; 27.43) | 11.41 (7.66; 28.38) | 9.32 (2.35; 19.83) | 4.1 (2.0; 8.91) | 2.215 (2.0; 4.58) |
| sCD40L | 38.16 (30.7; 46.64) | 29.4 (21.87; 41.61) | 23.51 (19.99; 41.75) | 17.4 (12.17; 29.87) | 17.54 (10.51; 43.13) |
| TNF-α | 45.13 (20.9; 70.47) | 37.77 (28.95; 49.24) | 33.51 (22.53; 43.97) | 21.14 (17.56; 36.44) | 27.53 (16.99; 68.69) |
| | CONTROL | | | | |
| IL-1β | 0.36 (0.22; 10.9) | 8.6 (3.67; 29.08) | 0.97 (0.48; 1.42) | 0.59 (0.34; 2.29) | 0.83 (0.38; 2.46) |
| IL-4 | 32.51 (11.99; 55.45) | 45.79 (33.09; 72.29) | 54.47 (36.6; 72.6) | 44.32 (24.04; 86.44) | 36.52 (20.35; 92.12) |
| IL-6 | 14.29 (12.75; 102.21) | 1637.8 (915.95; 4903.0) | 747.38 (373.45; 2671.5) | 174.96 (77.14; 563.08) | 78.56 (52.22; 201.28) |
| IL-10 | 3.22 (3.2; 30.79) | 38.52 (29.1; 64.86) | 16.85 (6.75; 50.13) | under LLOQ | under LLOQ |
| IL-17A | 22.35 (12.69; 29.04) | 74.34 (42.55; 122.06) | 52.2 (26.47; 72.78) | 34.55 (12.59; 71.2) | 27.0 (17.58; 60.52) |
| IL-21 | 151.94 (71.23; 392.63) | 335.72 (192.07; 493.19) | 315.97 (206.89; 531.36) | 186.57 (134.97; 708.42) | 205.5 (72.74; 654.84) |
| IL-22 | 25.46 (17.98; 58.34) | 83.86 (47.65; 143.65) | 38.75 (30.96; 50.79) | 31.05 (13.4; 88.74) | 40.07 (13.41; 81.76) |
| IL-31 | 38.77 (22.22; 127.3) | 114.11 (72.49; 227.14) | 67.28 (49.64; 106.41) | 31.8 (23.64; 43.17) | 32.15 (25.81; 148.68) |
| IL-33 | 15.17 (2.1; 24.32) | 23.44 (11.03; 35.01) | 6.75 (2.14; 12.22) | 3.62 (2.0; 10.51) | 4.99 (2.0; 6.13) |
| sCD40L | 33.68 (17.83; 39.54) | 44.86 (35.89; 68.18) | 29.96 (22.44; 45.8) | 19.6 (11.368; 43.99) | 16.24 (11.23; 46.33) |
| TNF-α | 37.19 (28.15; 64.05) | 51.7 (37.79; 62.13) | 40.72 (27.87; 45.46) | 37.99 (21.82; 60.33) | 40.13 (17.56; 65.78) |

Asterisks show differences between normal pressure hydrocephalus patients and controls. $^{**}p<0.01$

**Tab. 3.** The plasma levels (pg/mL) of selected cytokines in subjects with normal pressure hydrocephalus and controls on the first day of lumbar drainage test. Medians with quartiles are provided.

| Cytokines (pg/mL) | Normal pressure hydrocephalus | Control |
|---|---|---|
| IL-1β | 0.165 (0.02; 0.45) | 0.17 (0.01; 0.55] |
| IL-4 | 1.6 (1.6; 9.55) | 3.7 (1.6; 15.58) |
| IL-6 | 9.56 (6.1; 12.85) | 10.37 (7.83; 16.84) |
| IL-25 | 0.31 (0.3; 1.26) | 0.34 (0.3; 1.51) |
| IL-31 | 10.145 (7.64; 19.96) | 17.56 (7.6; 50.5) |
| IL-33 | 21.61 (5.76; 27.89) | 8.73 (2.2; 38.5) |
| IFN-Y | 3.02 (2.5; 14.36) | 3.54 (2.5; 18.3) |
| sCD40L | 39.12 (28.83; 57.58) | 69.93 (28.45; 134.96) |
| TNF-α | 6.27 (4.8; 7.12) | 4.93 (4.06; 7.83) |

cal material in NPH. Only a limited number of studies have been reported on the serum/plasma cytokines in NPH (Rota *et al.* 2006).

IL-1β is known to be a key driving force of CNS inflammation playing a major role in brain neurodegeneration (Simi *et al.* 2007). Higher levels have been reported in the CSF of patients suffering from various neurodegenerative diseases as AD and PD (Blum-Degena *et al.* 1995; Mogi *et al.* 1996), MS (Seppi *et al.* 2014; Hauser *et al.* 1990). IL-1β is not generally expressed in normal healthy tissues, its actions are limited to disease states (Dinarello & Thompson 1991). It initiates or augments multiple responses both within and outside CNS, resulting in inflammation. Subsequently, it activates resident immune cells and increases the formation of IL-6 (Chakraborty *et al.* 2010; Rothwell & Luheshi 2000). Consequently, together with ele-

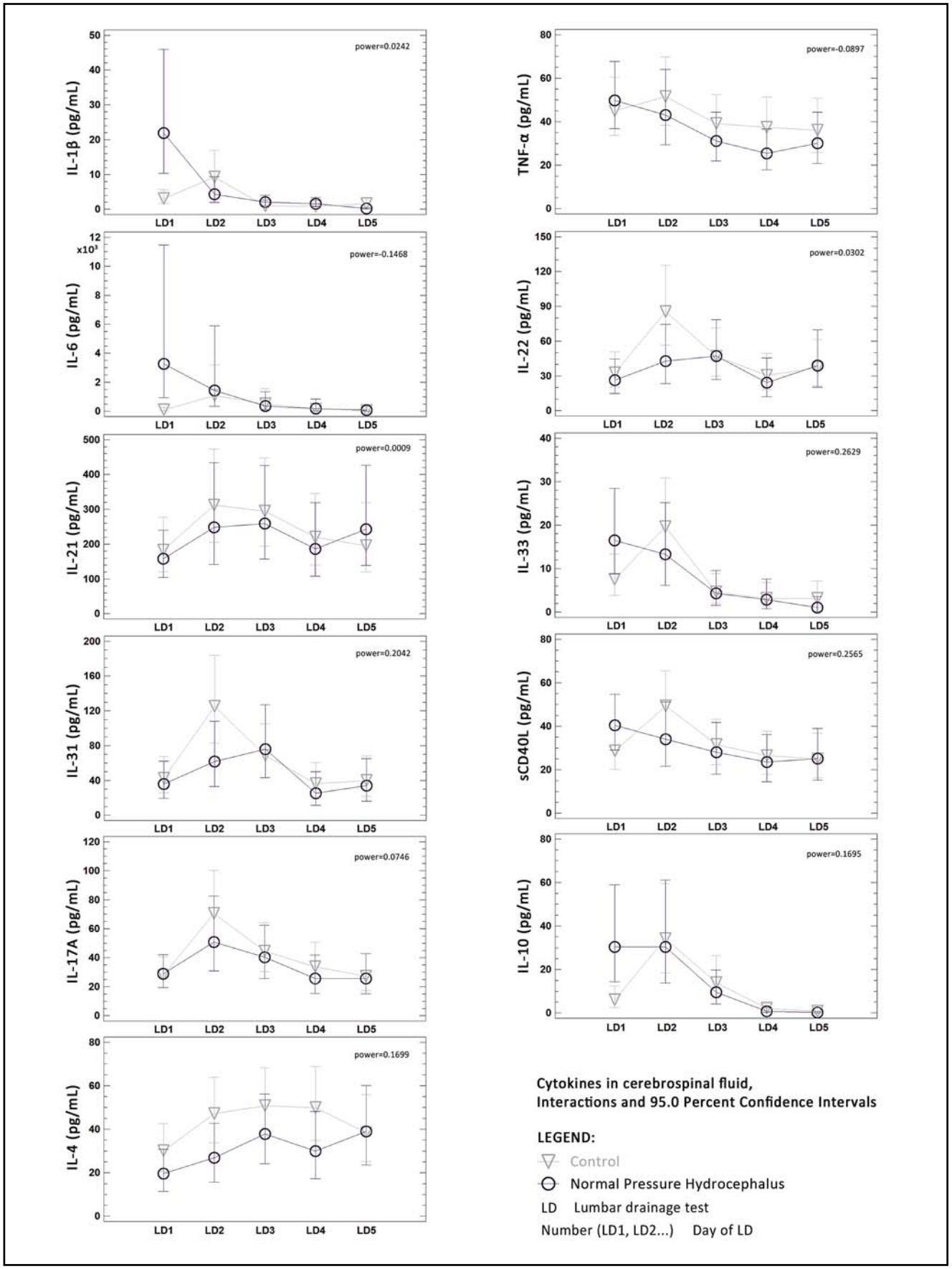


**Fig. 1.** The cerebrospinal fluid levels (pg/mL) of cytokines in patients with normal pressure hydrocephalus (black circle) and controls (grey triangle). The individual graphs show the levels of cytokines during 5 days of lumbar drainage test (LD1–LD5). The medians and 95% confidence intervals are presented after Box-Cox data transformation. The transformation power is showed with each cytokine graph.

vated IL-1β CSF levels in neurodegenerative diseases, elevated levels of pro-inflammatory cytokine IL-6 were reported (Blum-Degena *et al.* 1995; Mogi *et al.* 1996). In our group of subjects with diagnosed NPH, the CSF levels of IL-1β and IL-6 were significantly changed in accordance with conclusions of other authors on various degenerative diseases including NPH (Killer *et al.* 2010; Blum-Degena *et al.* 1995; Mogi *et al.* 1996). In general, we observed elevated levels of cytokines in the CSF compared to plasma. The highest levels were detected for IL-6 in the CSF.

The brain production of IL-1β and IL-6 are upregulated by TNF-α, the key initiator of immune-mediated inflammation in multiple organ systems including brain (Tobinick *et al.* 2006). TNF-α can be synthesized in the CNS and its expression increases among others in neurodegenerative disorders (Viviani *et al.* 2004). CSF levels of TNF-α were elevated in MS (Sharief & Hentges 1991), PD (Mogi *et al.* 1994), AD and VD (Tarkowski *et al.* 2003a). Controversial results have been reported concerning NPH (Table 1). In our study, the CSF levels of TNF-α were insignificantly lower in NPH patients during LD.

A newly discovered member of IL-1 family – IL-33 – plays an important role in inflammation, infection and autoimmune diseases. The highest expression of IL-33 was observed in the brain and spinal cord (Schmitz *et al.* 2005). This cytokine can induce a proliferation of brain microglia and also improves the expression of pro-inflammatory cytokines IL-1β and TNF-α, while at the same time increasing the expression of anti-inflammatory IL-10. In our study, its levels in NPH decreased during LD (see Figure 1). The same trend was observed for sCD40L, another member of the TNF family (Schlom *et al.* 2013). It was observed that circulating levels of sCD40L are associated with a poor outcome in diseases with combined inflammatory and vascular pathology (Johansson *et al.* 2012), which agrees with our observations.

Another interesting group of pro-inflammatory cytokines is the IL-17 family. It is composed of six members – IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (known as IL-25) and IL-17F – out of which the first and last are best understood. IL-17A and IL-17F are known to mediate proinflammatory responses (Ishigame *et al.* 2009; Jin & Dong 2013). IL-17A, induces the production of IL-1β, IL-6 and TNF-α (Witowski *et al.* 2004). It was reported that the uncontrolled production of IL-17A can result in excessive pro-inflammatory cytokine expression and chronic inflammation leading to tissue damage. All IL-17 family cytokines are associated with autoimmune diseases, including MS (Jin & Dong 2013). In our study, we did not find differences between patients and controls. The CSF IL-17A levels were only slightly lower in NPH during all days of LD as well as for the less known IL-21, IL-22 and IL-31.

IL-21 is a cytokine produced by activated T-cells and has potent regulatory effects on all classes of lymphocytes (Parrish-Novak *et al.* 2000; Wang *et al.* 2003). Significantly increased levels of IL-21 were reported in MS, neuromyelitis optica and experimental inflammation (Wu *et al.* 2012; Nohra *et al.* 2010). IL-22 plays an important role in inflammation. It is produced by T cells and natural killer cells and acts synergistically with TNF-α, IL-1β, or IL-17 (Zenewicz & Flavell 2011). IL-31 is a recently characterized cytokine with potential pro-inflammatory actions, closely related to the IL-6 family (Zhang *et al.* 2008).

Out of the anti-inflammatory interleukins, the best understood are IL-4 and IL-10. IL-4 plays a crucial role in the regulation of brain immunity, with downstream effects on spatial learning/memory and neurogenesis, and with an implication for neurological disorders (Gadani *et al.* 2012). It is known to downregulate the production of some inflammatory cytokines as TNF-α (Hart *et al.* 1989). Of importance is its influence on neurological diseases as AD and MS (Gadani *et al.* 2012). In our study, IL-4 in the CSF was lower in NPH during LD, which concurs with our expectations taking into account its anti-inflammatory properties. The second anti-inflammatory cytokine of interest is IL-10, which is able to suppress the production of IL-1β and TNF-α and vice versa (Park *et al.* 2007). The neuroprotective effect of IL-10 was reported in PD (Qian *et al.* 2006), and lower levels of IL-10 were found in AD (Remarque *et al.* 2001). Owing to its occurrence in CNS, IL-10 is considered to be an important anti-inflammatory modulator of glial activation, by maintaining a balance between pro- and anti-inflammatory cytokines in the CNS (Qian *et al.* 2006; Park *et al.* 2007; Sawada *et al.* 1999). In our group, the elevated levels on the first day of LD differed from the lower levels reported in AD and might be an interesting analyte in the differential diagnosis of these two diseases.

Although serum/plasma is an easily accessible biological fluid, only one study demonstrating cytokines in the serum of NPH patients was reported (Rota *et al.* 2006). The overall human circulation does not reflect the brain neurodegenerative changes, as well as CSF. All cytokines measured in the plasma in our project were not statistically different between patients and controls.

## CONCLUSION

We measured selected cytokines in the CSF and plasma of patients with NPH and in controls. The elevated CSF levels of pro-inflammatory cytokines IL-1β and IL-6 on the first day of LD reflects the inflammatory changes in the brain. Its reversibility is shown by a rapid decrease of the above-mentioned cytokines during the LD. The CSF IL-10 levels on the first day of LD were higher in NPH patients, which can be explained by the human body's potential to activate defense mechanisms to suppress the detrimental process in the brain. These results show IL-10 as an interesting cytokine in the NPH disease progression having potential in the diagnosis of this disease.

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