

Xu Hao<sup>1,2</sup>,  
Wang Junwen<sup>1</sup>,  
Li Jiaqing<sup>1</sup>,  
Li Ran<sup>1</sup>,  
Zhang Zhuo<sup>1</sup>,  
Huang Yimin<sup>1</sup>,  
Jiao Wei<sup>1</sup>,  
Sun Wei<sup>1</sup>,  
Lei Ting<sup>1\*</sup>

<sup>1</sup>Department of Neurosurgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P. R. China

<sup>2</sup>Department of Neurosurgery, Anhui Provincial Hospital, Hefei, P. R. China

Received 6 June 2016  
accepted 20 June 2016

## Introduction

Chronic hydrocephalus is a common complication after craniocerebral trauma. It is characterized by abnormalities in the circulation and absorption of cerebrospinal fluid (CSF) that result in ventricular dilatation. Most patients with malabsorption of CSF will become shunt-dependent. Previous research has implicated the malabsorption of CSF, caused by fibrosis along the route of CSF flow, in the pathogenesis of hydrocephalus [8, 9, 10, 18, 20, 34-36].

PICP, PIIINP, HA, and LN are widely recognized as biomarkers of fibrotic disease and elevated levels of these substances have been detected in the CSF of hydrocephalus animal models [11, 12, 39, 42]. However, the role that fibrosis of the CSF circulation pathway plays in patients with post-traumatic hydrocephalus is still unclear. Our goal in the present study

# HIGH FIBROSIS INDICES IN CEREBROSPINAL FLUID OF PATIENTS WITH SHUNT-DEPENDENT POST-TRAUMATIC CHRONIC HYDROCEPHALUS

## Abstract

**Objective:** A possible relationship between fibrosis along the route of cerebrospinal fluid (CSF) flow and the subsequent development of hydrocephalus has been indicated in previous studies. These changes in the fibrosis index may reflect the severity of hydrocephalus and could potentially become a diagnostic tool. The object of this study was to analyze the levels of procollagen type I C-terminal propeptide (PICP), procollagen type III N-terminal propeptide (PIIINP), hyaluronic acid (HA), and laminin (LN) in the CSF of patients with post-traumatic hydrocephalus and determine the significance of their presence. **Subjects and methods:** Forty-four patients were included in the study: 24 patients with shunt-dependent post-traumatic hydrocephalus (group A - hydrocephalus group); ten brain trauma patients without any sign of hydrocephalus (group B - trauma group); ten patients without brain trauma and hydrocephalus (group C - normal control group). CSF levels of PICP, PIIINP, HA, LN and transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) were detected using enzyme-linked immunosorbent assay (ELISA). **Results:** Levels of PICP, PIIINP, HA, and LN in the group of hydrocephalus patients were significantly higher than those in the post-trauma patients without hydrocephalus ( $p < 0.05$ ) and normal control patients ( $p < 0.05$ ). Moreover, the increased levels of PICP, PIIINP, HA, and LN were positively correlated with the level of TGF- $\beta$ 1 ( $p < 0.05$ ). **Conclusion:** We demonstrated an increase of fibrosis factors including PICP, PIIINP, HA, and LN, that was positively correlated with TGF- $\beta$ 1 levels. This indicates an important role for the process of fibrosis in the development of post-traumatic chronic hydrocephalus and shows the potential utility of PICP, PIIINP, HA, and LN as a diagnostic index in shunt-dependent post-traumatic chronic hydrocephalus.

## Keywords

• Fibrosis • Hyaluronic acid • Hydrocephalus • Laminin • Procollagen • TGF- $\beta$ 1

was to determine the fibrosis index in the CSF of patients with shunt-dependent post-traumatic hydrocephalus in order to further understanding of the pathophysiological mechanisms involved in hydrocephalus, and to promote the development of better therapies and diagnoses.

## Methods

### Patient selection


The patients were divided into 3 groups: group A (hydrocephalus group) consisted of 24 patients with shunt-dependent post-traumatic chronic hydrocephalus; group B (trauma group) consisted of 10 brain trauma patients without any sign of hydrocephalus determined by clinical exam and imaging; group C (normal control group) consisted of 10 patients without any trauma or hydrocephalus. Exclusion criteria included

the following: congenital hydrocephalus, post intracranial infection hydrocephalus, tumor obstructive hydrocephalus and other causes of hydrocephalus. Ethics approval and informed consent were obtained for this study from Anhui Medical University and from either patients or their relatives. All procedures performed were in accordance with the ethical standards of national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The diagnosis of hydrocephalus was made by neurosurgeons and neuroradiologists.

### Sample collection and detection

CSF samples from the hydrocephalus group were collected during shunt surgery; trauma group samples were collected during brain trauma surgery; normal control groups samples were collected in epilepsy and trigeminal neuralgia surgeries. All samples were stored

\* E-mail: tony\_xuhao@163.com

 © 2016 Xu Hao et al., published by De Gruyter Open.

This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 License.

at -70 for the ELISA test. For the quantitative detection of PICP, PIIINP, HA, LN and TGF- $\beta$ 1 in serum and CSF, a commercial ELISA was used (Wuhan Boster Biological Technology Ltd., Wuhan, P.R. China) according to the manufacturer's manual. In short, microtiter plates pre-coated with PICP, PIIINP, HA, LN, or TGF- $\beta$ 1 antibodies were filled with standard and undiluted samples and incubated for 2 hours at 37°C. After removal of both the standard and undiluted samples, we added a biotin-conjugated polyclonal antibody preparation specific to PICP, PIIINP, HA, LN and TGF- $\beta$ 1, and incubated the plates for 1 hour. Following the washing cycles, the plate was incubated with avidin conjugated to horseradish peroxidase for another hour at 37°C. After additional washing cycles, 3',3',5',5'-tetramethylbenzidine was added and the plates were incubated at 37°C for 30 minutes. The enzyme-substrate reaction was stopped by H<sub>2</sub>SO<sub>4</sub>. The optical density was measured immediately at 450 nm. According to the manufacturer's specifications, no significant cross-reactivity or interference was observed.

### Statistical analysis

Data were generally expressed as mean  $\pm$  standard deviation (SD) values. Groups of data were compared by standard t-test. Comparisons were made between the hydrocephalus, trauma and normal control groups. The relationships between PICP, PIIINP, HA, LN and TGF- $\beta$ 1 levels were determined by Pearson correlation analysis. A difference between means was considered significant at  $p < 0.05$ .

## Results

### Clinical states

General information about the patients in all 3 groups is shown in Table 1. The clinical profiles of patients in the hydrocephalus group is shown in Table 2. The etiologies of primary brain trauma included SAH, intracerebral hematoma and slight ventricular hemorrhage. All patients were followed up for a period of at least 3 months. The Glasgow Coma Scale (GCS) of patients in the presence of brain trauma was  $7.9 \pm 2.8$ . Hydrocephalus occurred between

the first and the tenth month post-trauma with the majority of cases developing in the second or third month. Once the symptom of hydrocephalus occurred, it progressed rapidly and become surgery dependent shortly thereafter. All patients' symptoms were ameliorated following shunt surgery.

### Fibrosis in CSF of post-traumatic chronic hydrocephalus

The level of the fibrosis index was calculated by ELISA. Group A expressed increased levels ( $*p < 0.05$ ) of PICP, PIIINP, HA, and LN protein

compared with Groups B and C (Fig. 1). The relationship between TGF- $\beta$ 1 and PICP, PIIINP, HA, and LN levels was analyzed by Pearson correlation. The increase in the concentration of PICP, PIIINP, HA, and LN was positively correlated with the level of TGF- $\beta$ 1 (Fig. 2).

## Discussion

According to the classical hypothesis of CSF hydrodynamics, CSF is produced within the brain ventricles, circulates toward the cortical subarachnoid space, and is finally absorbed

Table 1. Information from patients included in this study.

Group	N	Age (years)	Gender (M/F)
Group A	24	48.8 $\pm$ 11.4	75%
Group B	10	43.5 $\pm$ 17.1	70%
Group C	10	52.6 $\pm$ 9.9	60%

Table 2. Clinical information from patients with traumatic brain injury requiring CSF shunt.

Patient number	Gender (M/F)	Age (years)	Duration of shunting (weeks)	GCS
1	F	23	8	7
2	M	52	12	14
3	M	47	12	6
4	M	59	12	5
5	M	39	12	6
6	M	47	8	8
7	F	43	16	4
8	F	57	4	10
9	M	56	8	6
10	M	40	4	6
11	M	66	16	15
12	F	60	32	8
13	M	44	32	8
14	M	47	8	8
15	F	60	8	10
16	M	43	12	8
17	F	25	8	4
18	M	54	16	8
19	M	67	4	8
20	F	61	28	10
21	M	44	20	8
22	F	55	4	4
23	M	45	4	9
24	M	39	8	11

CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale.

into the venous sinuses. But a more recent, well-accepted hypothesis suggests that CSF is permanently produced and absorbed in the whole CSF system as a consequence of filtration and reabsorption of water volume through capillary walls into the surrounding brain tissue [6, 15, 24, 25]. Therefore, we believe that the fibrosis may affect the whole CSF pathway and lead to malabsorption of CSF.

One of the main features of fibrotic disease is excessive extracellular matrix repair. It is generally accepted that when primary damage surpasses the repair capabilities of parenchymal cells, interstitial connective tissue (extracellular matrix) hyperplasia will repair the defect. Studies have shown that the pathological changes of the CSF circulation pathways found in hydrocephalus involve an increase in the quantity of extracellular matrix

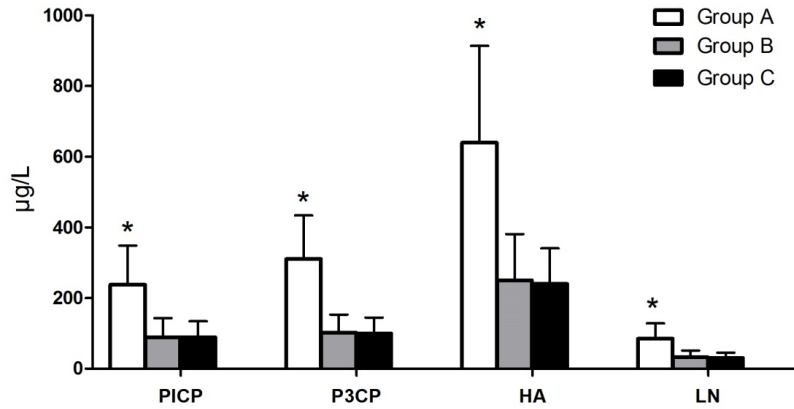


Figure 1. Mean ranking score for immunoreactivity of PICP, PIIINP, HA, and LN in CSF of Group A (hydrocephalus group), Group B (trauma group) and Group C (normal control group) (\*p < 0.05). Group A expressed increased levels (\*p < 0.05) of PICP, PIIINP, HA, and LN protein compared with Group B and C. PICP, procollagen type I C-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; HA, hyaluronic acid; LN, laminin; CSF, cerebrospinal fluid.

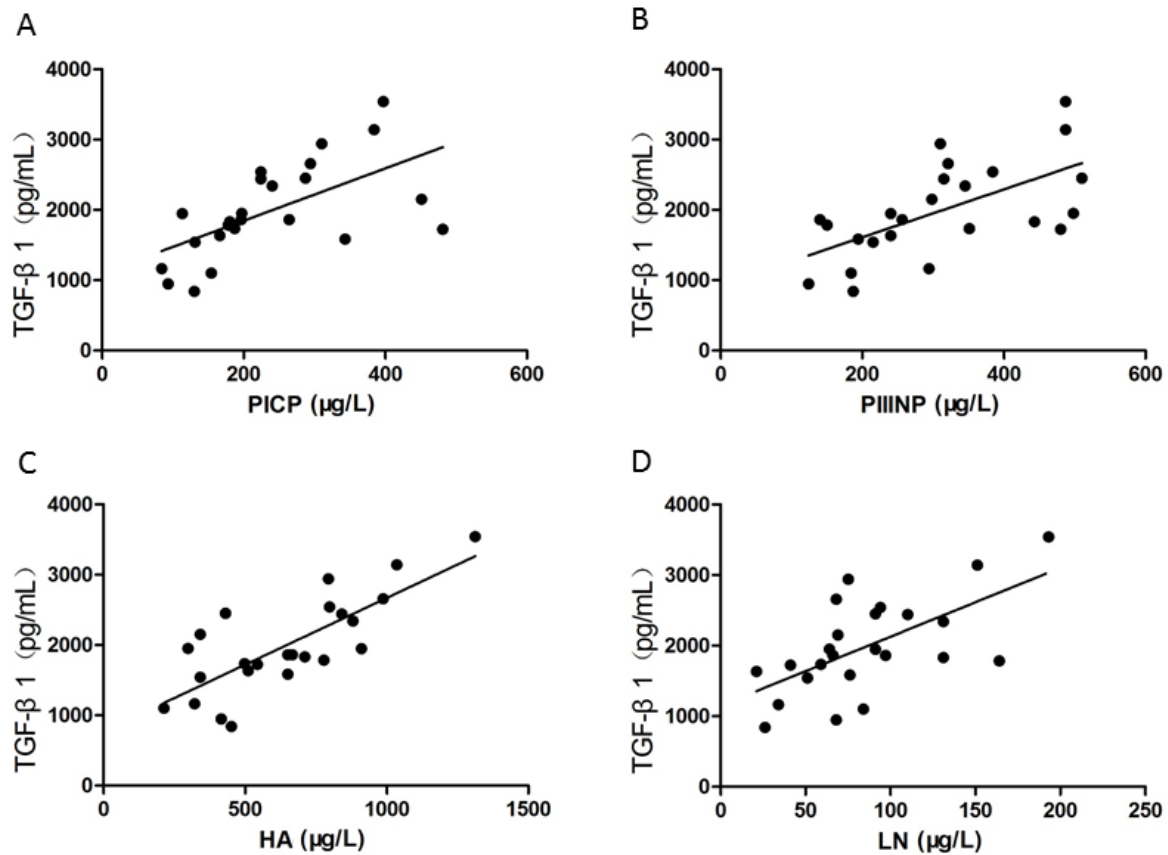


Figure 2. The Pearson correlation between TGF-β1 and A) PICP, B) PIIINP, C) HA, and D) LN. The PICP, PIIINP, HA, and LN concentration increases were positively correlated with the level of TGF-β1. PICP, procollagen type I C-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; HA, hyaluronic acid; LN, laminin; TGF, transforming growth factor.

and a decreased number of parenchymal cells [17, 28]. The hyperplasia of the extracellular matrix along the CSF circulation pathway may affect CSF absorption [7, 22, 26].

The extracellular matrix is predominantly composed of collagen, 85% of which is type I collagen, 11% type III, and 4% types IV and V. Therefore, PICP and PIIINP were selected as indicators of the formation of collagen fibrils. Hyaluronic acid is a glycosaminoglycan that is non-covalently bound by proteins in the extracellular space. It is the main component of proteoglycan. Laminin, one of the main glycoproteins of the basement membrane, is the index that reflects the level of glycoprotein. In liver fibrosis [19, 24, 29], idiopathic pulmonary arterial hypertension, cystic fibrosis [25], renal failure [38] and cardiac fibrosis [16, 30], serum concentrations of PICP, PIIINP, HA, and LN are believed to reflect the level of fibrosis. In experimental models of subarachnoid hemorrhage, increased concentrations of PICP and PIIINP in the CSF, as well as leptomeningeal deposition of collagen Types I and III, have been found [23, 30, 32, 41]. The fibril-forming collagens (types I–III) are synthesized by mesenchymal cells as procollagens. In searching for the origin of hyaluronan in CSF, studies have found that its concentration in the choroid plexus and leptomeninges is low and that it accumulates in the superficial layer of the cerebral cortex. Serial screening for hyaluronan in CSF may be valuable in cases of inflammatory diseases, tumors, and obstruction of CSF flow [14]. According to our results, increased levels of PICP, PIIINP, HA, and LN may indicate that hydrocephalus is a type of fibrotic disease.

Many factors have been demonstrated in the CSF of patients with hydrocephalus: enascin-C, GFAP, A $\beta$ , VEGF and TGF- $\beta$  [1, 19, 21, 33]. Concentrations of TGF- $\beta$ 1 have been found elevated in the CSF of adults with ventricular dilatation due to subarachnoid and intraventricular hemorrhage [39], and both TGF- $\beta$ 1 and - $\beta$ 2 have been shown to be elevated in infants with post hemorrhage ventricular dilation [37]. Ventricular dilatation

has been produced in mice by injecting TGF- $\beta$ 1 into the cerebral subarachnoid space [27].

The TGF- $\beta$ 1 superfamily of cytokines is comprised of several homologous polypeptides that transduce a range of signals involved in cell growth and differentiation, as well as in the response to inflammation and tissue damage. The family members TGF- $\beta$ 1, - $\beta$ 2 and - $\beta$ 3 are present in all mammalian tissues, including the central nervous system. They have potent desmoplastic activity, through increased synthesis of collagen and several other components of the extracellular matrix [40], and are involved in a number of serious diseases involving excessive deposition of collagen and extracellular matrix, including diabetic nephropathy [2] and cirrhosis [5, 8]. In patients with post-traumatic chronic hydrocephalus, TGF- $\beta$ 1 is released into the CSF, and evidence indicates that this cytokine stimulates the laying down of extracellular matrix proteins. This process may produce permanent obstruction of the CSF pathway [20]. According to our results, the increased concentrations of PICP, PIIINP, HA, and LN are positively correlated with TGF- $\beta$ 1 levels (a recognized fibrosis factor). These results further our understanding of the pathogenesis of hydrocephalus from the perspective of fibrosis.

The traditional theory of CSF absorption through arachnoid granulations may not be complete. There is evidence that CSF can cross the ependyma and endothelium to reach the surface of brain by accessory pathways including nasal lymphatics, nerve sheaths and the spinal subarachnoid space [4, 13, 27, 36, 39]. Previous research has shown that in HT-X hydrocephalus rat models, there is decreased CSF outflow to the nasal lymphatics compared with control animals [31]. Lymphatic CSF absorption is impaired in a kaolin-communicating hydrocephalus model and the degree of this impediment may contribute to the severity of the hydrocephalus [22]. Lymphatic circulation obstruction can also induce further fibrotic disease [42]. Therefore, continued investigation into the relationship between lymphatic circulation and CSF

pathway fibrosis will be an attractive research direction.

At the same time, we note that regulating fibrosis may delay the appearance of hydrocephalus. A recent finding by Botfield demonstrates that decorin can regulate factor- $\beta$ /Smad2/3 and its anti-fibrosis effect can delay the development of hydrocephalus [3]. It may also provide evidence for the role of CSF flow pathway fibrosis in the pathogenesis of hydrocephalus.

This study has some limitations. To exclude the possibility of an increase in PICP, PIIINP, HA, and LN concentration caused solely by brain trauma, we used brain trauma patients without hydrocephalus in the trauma group. However, the timing of CSF sample collection could not be uniformly regulated between the hydrocephalus and trauma groups. Therefore, we cannot conclude that the absence of an increase in fibrosis indices indicates that brain trauma patients will not develop hydrocephalus in the future. At the least, our results indicate that the increase of PICP, PIIINP, HA, and LN is not caused by trauma but rather by hydrocephalus, and that before hydrocephalus occurs, the level of fibrotic factors remain normal in the CSF of traumatic brain injury patients.

## Conclusion

In summary, we confirmed that the levels of PICP, PIIINP, HA, and LN are increased in post-traumatic chronic hydrocephalus, but not increased in brain trauma patients without hydrocephalus. The fibrosis indices are positively related to TGF- $\beta$ 1 levels in hydrocephalus and have potential for use as biomarkers of post-traumatic hydrocephalus. Our results also demonstrated that fibrosis along the route of CSF flow may play a causal role in the development of chronic post-traumatic hydrocephalus and thus provide a new therapeutic orientation.

## Acknowledgments

*Conflict of interest statement:* All authors certify that they have no conflict of interest to disclose. No funding was received for this research.

## References

- [1] Beems T, Simons K.S., Van Geel W.J., De Reus H.P., Vos P.E., Verbeek M.M., Serum- and CSF-concentrations of brain specific proteins in hydrocephalus, *Acta Neurochir.*, 2003, 145, 37-43
- [2] Border W.A., Ruoslahti E., Transforming growth factor-beta 1 induces extracellular matrix formation in glomerulonephritis, *Cell Differ. Dev.*, 1990, 32, 425-431
- [3] Botfield H., Gonzalez A.M., Abdullah O., Skjolding A.D., Berry M., McAllister J.P.II, et al., Decorin prevents the development of juvenile communicating hydrocephalus, *Brain*, 2013, 136, 2842-2858
- [4] Bozanovic-Sosic R., Mollanji R., Johnston M.G., Spinal and cranial contributions to total cerebrospinal fluid transport, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2001, 281, R909-916
- [5] Brionne T.C., Tesseur I., Masliah E., Wyss-Coray T., Loss of TGF- $\beta$ 1 leads to increased neuronal cell death and microgliosis in mouse brain, *Neuron*, 2004, 40, 1133-1145
- [6] Bulat M., Klarica M., Recent insights into a new hydrodynamics of the cerebrospinal fluid, *Brain Res. Rev.*, 2011, 65, 99-112
- [7] Buonpensiero P., De Gregorio F., Sepe A., Di Pasqua A., Ferri P., Siano M., et al., Hyaluronic acid improves "pleasantness" and tolerability of nebulized hypertonic saline in a cohort of patients with cystic fibrosis, *Adv. Ther.*, 2010, 27, 870-878
- [8] Castilla A., Prieto J., Fausto N., Transforming growth factors  $\beta$ 1 and  $\alpha$  in chronic liver disease. Effects of interferon  $\alpha$  therapy, *N. Engl. J. Med.*, 1991, 324, 933-940
- [9] Chen X., Huang X., Li B., Zhao Z., Jiang L., Huang C., et al., Changes in neural dendrites and synapses in rat somatosensory cortex following neonatal post-hemorrhagic hydrocephalus, *Brain Res. Bull.*, 2010, 83, 44-48
- [10] Cherian S., Whitelaw A., Thoresen M., Love S., The pathogenesis of neonatal post-hemorrhagic hydrocephalus, *Brain Pathol.*, 2004, 14, 305-311
- [11] Del Bigio M.R., Enno T.L., Effect of hydrocephalus on rat brain extracellular compartment, *Cerebrospinal Fluid Res.*, 2008, 10, 12
- [12] Di Bello V., Santini F., Di Cori A., Pucci A., Palagi C., Delle Donne M.G., et al., Obesity cardiomyopathy: is it a reality? An ultrasonic tissue characterization study, *J. Am. Soc. Echocardiogr.*, 2006, 19, 1063-1071
- [13] Edsbacke M., Tisell M., Jacobsson L., Wikkelso C., Spinal CSF absorption in healthy individuals, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2004, 287, R1450-1455
- [14] Heep A., Bartmann P., Stoffel-Wagner B., Bos A., Hoving E., Brouwer O., et al., Cerebrospinal fluid obstruction and malabsorption in human neonatal hydrocephaly, *Childs Nerv. Syst.*, 2006, 22, 1249-1255
- [15] Klarica M., Orešković D., Božić B., Vukić M., Butković V., Bulat M., New experimental model of acute aqueductal blockade in cats: effects on cerebrospinal fluid pressure and the size of brain ventricles, *Neuroscience* 2009, 158, 1397-1405
- [16] Kosmala W., Przewlocka-Kosmala M., Szczepanik-Osadnik H., Mysiak A., Marwick T.H., Fibrosis and cardiac function in obesity: a randomised controlled trial of aldosterone blockade, *Heart*, 2013, 99, 320-326
- [17] Li F., Zhu C.L., Zhang H., Huang H., Wei Q., Zhu X., et al., Role of hyaluronic acid and laminin as serum markers for predicting significant fibrosis in patients with chronic hepatitis B, *Braz. J. Infect. Dis.*, 2012, 16, 9-14
- [18] Li X., Miyajima M., Arai H., Analysis of TGF- $\beta$ 2 and TGF- $\beta$ 3 expression in the hydrocephalic H-Tx rat brain, *Childs Nerv. Syst.*, 2005, 21, 32-38
- [19] Lipina R., Reguli S., Nováková L., Podesvová H., Brichtová E., Relation between TGF- $\beta$ 1 levels in cerebrospinal fluid and ETV outcome in premature newborns with posthemorrhagic hydrocephalus, *Childs Nerv. Syst.*, 2010, 26, 333-341
- [20] Massicotte E.M., Del Bigio M.R., Human arachnoid villi response to subarachnoid hemorrhage: possible relationship to chronic hydrocephalus, *J. Neurosurg.* 1999, 91, 80-84
- [21] Miller J.M., McAllister J.P. II, Reduction of astrogliosis and microgliosis by cerebrospinal fluid shunting in experimental hydrocephalus, *Cerebrospinal Fluid Res.*, 2007, 4, 5
- [22] Nagra G., Li J., McAllister J.P. II, Miller J., Wagshul M., Johnston M., Impaired lymphatic cerebrospinal fluid absorption in a rat model of kaolin-induced communicating hydrocephalus, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2008, 294, R1752-1759
- [23] Neuberger H.R., Cacciatore A., Reil J.C., Gräber S., Schäfers H.J., Ukena C., et al., Procollagen propeptides: serum markers for atrial fibrosis?, *Clin. Res. Cardiol.*, 2012, 101, 655-661
- [24] Orešković D., Klarica M., The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations, *Brain Res. Rev.*, 2010, 64, 241-262
- [25] Orešković D., Klarica M., Development of hydrocephalus and classical hypothesis of cerebrospinal fluid hydrodynamics: facts and illusions, *Prog. Neurobiol.*, 2011, 94, 238-258
- [26] Pang D., Sclabassi R.J., Horton J.A., Lysis of intraventricular blood clot with urokinase in a canine model: Part 3. Effects of intraventricular urokinase on clot lysis and posthemorrhagic hydrocephalus, *Neurosurgery*, 1986, 19, 553-572
- [27] Papaiconomou C., Bozanovic-Sosic R., Zakharov A., Johnston M., Does neonatal cerebrospinal fluid absorption occur via arachnoid projections or extracranial lymphatics?, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2002, 283, R869-876
- [28] Papakonstantinou E., Kouri F.M., Karakiulakis G., Klagas I., Eickelberg O., Increased hyaluronic acid content in idiopathic pulmonary arterial hypertension, *Eur. Respir. J.*, 2008, 32, 1504-1512
- [29] Parsian H., Rahimpour A., Nouri M., Somi M.H., Qujeq D., Assessment of liver fibrosis development in chronic hepatitis B patients by serum hyaluronic acid and laminin levels, *Acta Clin Croat.*, 2010, 49, 257-265
- [30] Qin S., Liu M., Niu W., Zhang C.L., Dysregulation of Kruppel-like factor 4 during brain development leads to hydrocephalus in mice, *Proc. Natl. Acad. Sci. USA*, 2011, 108, 21117-21121
- [31] Rammling M., Madan M., Paul L., Behnam B., Pattisapu J.V., Evidence for reduced lymphatic CSF absorption in the H-Tx rat hydrocephalus model, *Cerebrospinal Fluid Res.*, 2008, 5, 15

- [32] Rekte H.L., A consensus on the classification of hydrocephalus: its utility in the assessment of abnormalities of cerebrospinal fluid dynamics, *Childs Nerv. Syst.*, 2011, 27, 1535-1541
- [33] Sajant J., Heikkinen E., Majamaa K., Rapid induction of meningeal collagen synthesis in the cerebral cisternal and ventricular compartments after subarachnoid hemorrhage, *Acta Neurochir.*, 2001, 143, 821-826
- [34] Silverberg G.D., Miller M.C., Machan J.T., Johanson C.E., Caralopoulos I.N., Pascale C.L., et al., Amyloid and tau accumulate in the brains of aged hydrocephalic rats, *Brain Res.*, 2010, 1317, 286-296
- [35] Sival D.A., Felderhoff-Müser U., Schmitz T., Hoving E.W., Schaller C., Heep A., Neonatal high pressure hydrocephalus is associated with elevation of pro-inflammatory cytokines IL-18 and IFN $\gamma$  in cerebrospinal fluid, *Cerebrospinal Fluid Res.*, 2008, 5, 21
- [36] Suzuki H., Kinoshita N., Imanaka-Yoshida K., Yoshida T., Taki W., Cerebrospinal fluid tenascin-C increases preceding the development of chronic shunt-dependent hydrocephalus after subarachnoid hemorrhage, *Stroke*, 2008, 39, 1610-1612
- [37] Tada T., Kanaji M., Kobayashi S., Induction of communicating hydrocephalus in mice by intrathecal injection of human recombinant transforming growth factor- $\beta$ 1, *J Neuroimmunol.*, 1994, 50, 153-158
- [38] Tan R.J., Zhou D., Zhou L., Liu Y., Wnt/ $\beta$ -catenin signaling and kidney fibrosis, *Kidney Int. Suppl.* 2011, 2014, 4, 84-90
- [39] Torvik A., Bhatia R., Murthy V.S., Transitory block of the arachnoid granulations following subarachnoid haemorrhage. A postmortem study, *Acta Neurochir.*, 1978, 41, 137-146
- [40] Upton M.L., Weller R.O., The morphology of cerebrospinal fluid drainage pathways in human arachnoid granulations, *J. Neurosurg.*, 1985, 63, 867-875
- [41] Xu H., Wang Z., Zhang S., Tan G., Zhu H., Procollagen type I C-terminal propeptide, procollagen type III N-terminal propeptide, hyaluronic acid, and laminin in the cerebrospinal fluid of rats with communicating hydrocephalus, *J. Neurosurg. Pediatr.*, 2013, 11, 692-696
- [42] Zhang T., Guan G., Liu G., Sun J., Chen B., Li X., et al., Disturbance of lymph circulation develops renal fibrosis in rats with or without contralateral nephrectomy, *Nephrology*, 2008, 13, 128-138