Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com

Research article | SOJ Immunology | Open Access

Heterogeneous Nuclear Ribonucleoprotein K Autoantibodies in Patients who Suffered Severe Traumatic Brain Injury

María J. Acevedo-Calado[1,4], Marco A. Montes-Cano[1,4]*, Francisco Murillo-Cabezas[1,3], Ainara Madrazo-Atutxa[2,4], Salvador Toro- Llamas[1,4], Ángel Luis Castaño Núñez[1,4], José L. Caro Oleas[1,4], Alfonso Leal-Cerro[2,4] and Antonio Núñez-Roldán[1,4]

[1]Servicio de Inmunología, Hospital Universitario Virgen del Rocío, Sevilla, Spain
[2]Unidad de Gestión de Endocrinología, Hospital Universitario Virgen del Rocío, Sevilla, Spain
[3]División de Cuidados Críticos, Hospital Universitario Virgen del Rocío, Sevilla, Spain
[4]Instituto de Biomedicina de Sevilla (IBiS), Sevilla, Spain

Received: March 26, 2014; Accepted: August 28, 2014; Published: September 15, 2014

***Corresponding author:** Marco Antonio Montes-Cano, Servicio de Inmunología, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío, 41013-Seville, Spain, Tel: +34-95-501-3231; Fax: +34-95-501-3221; E-mail: marcoa.montes.sspa@juntadeandalucia.es*

Abstract

The immune-inflammatory response as well as cerebral endothelial activation has been described after Traumatic Brain Injury (TBI). Although inflammation can have both beneficial and detrimental effects in TBI, the mechanisms underlying this dichotomy are mostly unknown. Moreover, emerging data indicates that chronic alterations produced after TBI are probably the result of systemic and persistent inflammation that activates immune response, culminating in the production of different specific auto antibodies against central nervous system antigens. In the previous study we demonstrated the production of anti-hnRNPK antibodies, belonging to Anti-Endothelial Cell Antibodies (AECA), in heart transplanted patients who developed Cardiac Allograft Vasculopathy (CAV). These antibodies could be produced in response to the vascular endothelial damage that is also present in TBI patients. In the current study we analyse the presence of AECA by Indirect Immunofluorescence assay (IFI) and anti-hnRNPK IgG antibodies by Enzyme-Linked Immunosorbent Assay (ELISA) in a cohort of 19 patients who suffered TBI. We detected a significant increase in the number of patients with AECA and anti-hnRNPK antibodies after TBI. Moreover, anti-hnRNPK antibody levels were also higher during follow-up, being the values obtained after TBI significantly higher than those detected at the time of trauma ($p = 0.001$). We also found that AECA and anti-hnRNPK antibodies were mostly present in the sera of patients with a worse outcome ($p = 0.018$ and $p = 0.04$ respectively). Taking into account, measuring these auto antibodies could be useful for evaluating endothelial injury and/or the outcome after TBI.

Keywords: Traumatic brain injury; Anti-endothelial cell antibodies; Heterogeneous nuclear ribonucleoprotein K

Introduction

In recent year's the knowledge about the alterations occurred after Traumatic Brain Injury (TBI) has undergone significant developments [1]. These advances have contributed to clarify the relationship between post-traumatic edema and neuropathological sequelae that are largely responsible for adverse outcome. Several investigations have been conducted to clarify the role of immunological and inflammatory response produced after TBI. Thus, it has been described two types of brain damage: an immediate and irreversible primary lesion, followed by a secondary lesion, which begins at the time of the injury and continues in the ensuing days to weeks. Primary and secondary events lead to a variety of physiological, cellular and molecular responses aimed at restoring the homeostasis of the damaged tissue which, if not controlled, could increase the brain lesion [2]. Therefore, TBI initiates a series of related events including edema, cytotoxicity and an intense inflammatory response which affects the injured cerebral tissue as well as the healthy one. The immuno-inflammatory response that occurs after TBI persists and auto amplifies by complement activation, proinflammatory cytokine production, increased expression of endothelial cell adhesion molecules, as well as the production of processes of necrosis and apoptosis; these processes could be a potential source for presentation and generation of autoimmune responses [3]. In this way, Anti-Pituitary Antibodies (APA), Neuron-Specific Enolase (NSE), S100β, heat shock protein-70, among other possible biomarkers, has been studied in an effort to reach a more accurate of prognosis [4,5], and more recently it has been described different molecules as biomarkers that are thought to play a part in secondary injury following severe TBI [6-8].

In the previous study, we identify a new antigen that seems to be associated with endothelial damage, related auto-antigens with a significative increase in the production of antibodies against heterogeneous nuclear Ribonucleoprotein K (hnRNPK), and a type of AECA, in heart transplanted patients who developed Cardiac Allograft Vasculopathy (CAV) [9]. This protein, is a member of the hnRNP family which has several different cellular roles including transcription, mRNA shuttling, RNA editing and translation. These cellular functions might be related to the

involvement of this protein in apoptosis, tumors development, angiogenesis, cell invasion [10-12], and also altered gene expression patterns of hnRNPK have been found in many human cancers [13,14]. Additionally it has been described, as a high expression of this protein during smooth muscle cell proliferation in both, aortas from animal models of atherosclerosis and in human occluded veins [15]. We cannot rule out that hnRNPK auto antibodies could be also over expressed after endothelial damage and inflammation produced after TBI. However, at present there is no information on the involvement of anti-hnRNPK antibodies generation and the outcome of TBI. Therefore, the aim of this work was to analyze the presence of hnRNPK auto antibodies in patients who suffered different severity of TBI and its possible role in the outcome.

Materials and Methods

This study included patients with severe TBI admitted to neurosurgical ICU at the Virgen Del Rocio University Hospital, Seville, Spain, between July 2004 and Jan 2006. The study was approved by the hospital ethics committee and informed consent was obtained from a next of kin, given that all eligible patients were in coma. Inclusion criteria were: (1) male or female over 14 years of age with severe TBI (GCS score ≤ 8 after resuscitation) diagnosed by history and clinical examination with at least one reactive pupil; (2) to obtain at least two serum samples from each patient, one of them done within 24 hours after accident and a second serum sample at least six months post-TBI and (3) haemodynamically stable (mean arterial pressure > 75 mmHg with no or low-dose vasoactive drugs). Patients were excluded based on the following criteria: (1) presence of two reactive pupils; (2) suffered cardiac arrest after TBI; (3) any spinal cord injury, pregnancy, or coma suspected to be primarily due to other causes (E.g: alcohol); (4) suffered multiple injury as measured through the Abbreviated Injury Score (AIS) > 2; (5) no possibility of follow-up during one year and (6) presence of chronic or auto-immune diseases.

Data collection included demographic and clinical variables (age, sex, cause of injury, GCS and pupil reaction after resuscitation, occurrence of prehospital hypotension (systolic blood pressure < 90 mmHg), hypoxia (peripheral oxygen saturation (SpO_2)) < 90% and occurrence of sepsis (< 4 days). Sepsis was defined according to the Sepsis Consensus Conference criteria. Assessment of overall injury severity was based on the Injury Severity Score (ISS). Patients underwent an initial CT scan after resuscitation. Neuroradiological findings were classified according to the Traumatic Coma Data Bank (TCDB). This classification is divided into six groups: the first four indicate the presence and severity of the diffuse injury and the rest of the categories indicate the presence of an evacuated or non-evacuated mass lesion. A neuroradiologist, blind to the study goals and data reviewed and completed this classification.

Intra-Parenchymal Intra-Cranial Pressure (VENTRIX, INTEGRA Neuroscience, and Plainsboro, NJ), mean arterial blood pressure (obtained with a radial artery fluid-coupled system), Cerebral Perfusion Pressure (CPP), brain tissue oxygen pressure (LICOX, IMC System, GMS Kiel-Mielkendorf, and Germany), end-tidal carbon dioxide and SaO_2 were continuously monitored in the ICU. All patients were managed according to Brain Trauma Foundation guidelines and local protocols. Treatment was targeted at maintaining intracranial pressure at < 25 mmHg, CPP at > 60 mmHg, and brain tissue oxygen pressure at > 15 mmHg. The outcome assessment was carried out 12 months after patient discharge using the Glasgow Outcome Score (GOS). To relate our findings with the auto antibodies we dichotomized our results according to the GOS into two groups, severe (2-3) and mild (4-5) sequelae.

Two serum samples were obtained and analyzed from each patient; one at the time of TBI (basal sample, obtained within 24 hours after TBI), and the second within six months after trauma. As healthy controls, we included a total of 124 non related individuals with a range of age 26-53 (39.9 ± 13.9 years). Samples were immediately centrifuged, frozen at - 80°C and were stored for later analysis. Anti-hnRNPK IgG antibodies were detected by ELISA using hnRNPK recombinant protein previously purified in our laboratory [9]. Recombinant hnRNP-K protein, once affinity-purified by polyhistidine-tag, was plated at 2 µg/ml (in 0.1 mol/liter carbonate-bicarbonate buffer, p^H 9.5) onto a polystyrene flat-bottom ELISA plate (Nunc, Roskilde, Denmark) and incubated for 16 hours at 4°C. The non-specific binding of Igs was prevented by adding Tris-buffered saline with TBS-TM for 2 hours. The same serum-positive sample used in the library screening was used to construct a standard curve (dilutions of 1:100 to 1:1,600) to rule out non-specific antibody activities. For each ELISA, standards and samples (diluted 1:100 in TBS-TM) were added to duplicate wells and incubated for 1 hour at room temperature. Plates were washed, horseradish peroxidase conjugated rabbit anti-human IgG (Phadia AB, Uppsala, Sweden) was added, and the plates were for 1 additional hour at room temperature. The enzyme reaction was started by adding 100 µl of 3,3', 5,5'- tetramethylbenzidine and stopped 45 minutes later with 50 µl of 0.5 mol/liter H_2SO_4. Finally, the optical density was read at 450 nm in a micro titer plate reader (Bio-Tek Instruments, Winooski, VT). To calculate results, background reactivity of the reference mixture was subtracted to establish the frequency of newly identified antibodies in healthy individuals, a healthy control group of 124 individuals (72 men and 52 women; mean age, 40 ± 1.24 years) was also included. Patient serum samples with optical density values higher than the 95th percentile for control subjects (0.5966) were considered positive.

The study of Anti-Endothelial Cell Antibodies (AECA) was performed using the Indirect Immunofluorescence (IIF) method on commercially available slides of Human Umbilical Vein Endothelial Cells (HUVEC; Euroimmun, Lübeck, Germany). Antibodies present in patients' sera were detected with a fluorescein-conjugated secondary antibody against human IgG (Euroimmun, Lübeck, Germany) and hnRNP-K was localized using a rabbit anti-human hnRNP-K antibody (AbCam, Cambridge, UK) with a fluorescein conjugated secondary antibody against rabbit IgG (Jackson Immuno Research Laboratories Inc, West Grove, PA). After washing with phosphate-buffered saline-Tween,

slides were read on an epifluorescence microscope. Cutoff was set at 1:80 dilutions, at this dilution; all sera from 124 healthy individuals gave negative results. Positive samples were classified according to their IIF pattern.

Statistical analysis was performed with SPSS software version 18.0 (SPSS©, Chicago, IL). Qualitative variables were compared for statistically significant using the χ^2 test and Fisher's exact test. The Wilcoxon signed-rank test was used to evaluate the statistical significance between the levels of hnRNPK antibodies at basal time and after TBI. The p values below 0.05 were considered statistically significant.

Results

During the present study 32 TBI patients were enrolled. Only 19 patients aged 19-64 (28.48 ± 10.3) met the inclusion criteria. None of the patients were excluded due to cardiac arrest. Twelve patients were excluded due to extra cerebral injury (AIS > 2), and 1- year follow-up could not be done on one patient due to administrative issues. Data concerning age, sex, initial GCS, GOS, mechanism of injury and other clinical information were displayed in Table 1. All patients showed pathological CT findings (none within TCDB category I). Six patients underwent neurosurgery, 4 of them at the admission and two require neurosurgery while in the ICU. No decompression craniectomy (duraplasty plus bone removal) was found in the sample. After discharge from the ICU, patients were admitted to the hospital´s Neuro-Rehabilitation Unit. In this series we did not have any death and the final outcome was evaluated one year after TBI by GOS and was performed by a Neuro radiologist not involved in the study.

AECA auto antibodies were present in 2 patients at basal time and also during post - TBI evolution, while 5 more patients developed de novo auto antibodies after trauma (Table 2). Additionally, after the screening of hnRNPK by ELISA assay, we observed that only 3 (15.8%) out of 19 patients had antibodies at the time of TBI, nevertheless 10/19 (52.6%) developed the antibodies during follow-up, showing a significant increase of anti-hnRNPK antibodies after TBI (Table 3). A statistically significant difference for the AECA factor ($p = 0.1$) was not reached, although in the case of hnRNPK the differences were statistically significant despite the small number of patients included ($p = 0.01$). Furthermore, when we analyze the concentration of anti-hnRNPK at the time and after TBI, we detected a significant increase of IgG hnRNPK, that were higher after TBI than the antibody levels found at the time of trauma $p = 0.001$, (Figure 1).

From our study cohort, eleven patients developed permanent injuries (ranked by GOS) after TBI and also, in all the cases it shows the presence of AECA auto antibodies in the serum samples obtained after trauma, and only one patient with sequelae was negative for hnRNPK antibodies. We found a significant correlation between the appearance of AECA and hnRNPK auto antibodies and the development of permanent injuries ($p = 0.018$ and $p = 0.040$ respectively).

Discussion

To our knowledge, this study first demonstrates the

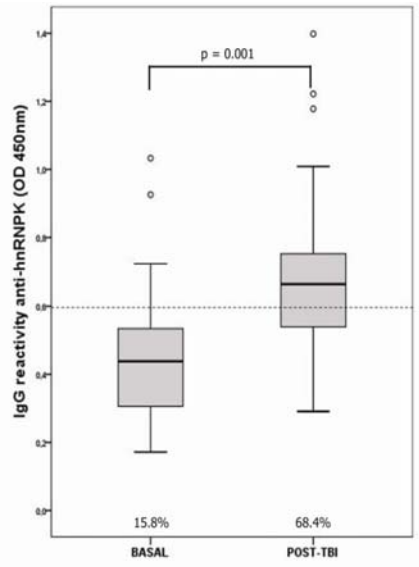

Figure 1: Immunoglobulin (IgG) titers against human recombinant hnRNP-K in TBI patients at the time of trauma (basal) and post-TBI. The box plots show the median and 25th .75th percentile range as well as the minimum and maximum values. Lines inside the boxes represent the mean. Circles indicate outliers. Values under the boxes are the percentage of patients with IgG titers above the cut-off value (0.5966), calculated as the 95th percentile from the control population.

Table 1: Demographic and clinical data for 19 patients with severe TBI.

Variable	n (%)
Age, years, median (range)	28 (19.0-64.0)
Sex n (%)	
Men	16 (84.2)
Women	3 (15.8)
Type of accident	
Traffic accident	12 (63.2)
Fall	12 (63.2)
Other	5 (26.3)
Hypoxia	
Absence	15 (78.9)
Presence	4 (21.1)
Hypotension	
Absence	15 (79.9)
Presence	4 (21.1)
GCS at admission	
3-4	2 (10.5)
5-6	3 (15.8)
7-8	14 (73.7)
Pupillary reaction	
Unilateral absence	4 (21.1)
Bilateral absence	1 (5.3)
Presence	14 (73.6)

CT-TCDB classification at admission	
II	5 (26.3)
III	9 (47.3)
IV	0 (0.0)
V	5 (26.3)
VI	0 (0.0)
Intracranial hypertension	
Yes	9 (47.4)
No	10 (52.6)
Neurosurgery	
Yes	6 (31.6)
No	13 (68.4)
Early sepsis	
Yes	12 (63.1)
No	7 (36.9)
GOS 1 year	
1	0 (0.0)
2	0 (0.0)
3	5 (26.3)
4	6 (31.5)
5	8 (42.1)
Sequelae	
Yes	11 (57.8)
No	8 (42.2)

Table 2: Evolution of AECA autoantibodies analyzed in basal and post-TBI sera.

Patient	Sex/Age (yr)	AECA (basal)	AECA (post - TBI)
1	M/32	-	-
2	M/32	-	-
3	F/28	-	-
4	M/64	-	+
5	M/19	-	-
6	M/26	-	-
7	M/24	-	+
8	M/31	+	+
9	M/20	-	-
10	F/21	-	-
11	M/27	-	+
12	M/34	-	-
13	M/24	-	-
14	M/24	-	-
15	M/27	+	+
16	M/25	-	-
17	F/22	-	-
18	M/40	-	+
19	M/27	-	+

Table 3: Evolution of hnRNPK autoantibodies analyzed in basal and post-TBI sera.

Patient	Sex/Age (yr)	hnRNPK (basal)	hnRNPK (post-TBI)
1	M/32	-	+
2	M/32	-	+
3	F/28	+	+
4	M/64	-	+
5	M/19	-	+
6	M/26	-	-
7	M/24	-	-
8	M/31	-	+
9	M/20	-	+
10	F/21	-	-
11	M/27	-	+
12	M/34	-	-
13	M/24	-	-
14	M/24	+	+
15	M/27	-	-
16	M/25	+	+
17	F/22	-	+
18	M/40	-	+
19	M/27	-	+

development of AECA and anti-hnRNPK IgG antibodies after TBI and its likely relationship with the outcome. It is well established that the persistent inflammation produced after TBI activate the immune response and culminating in the production of different auto antibodies against endothelial and central nervous system antigens. In fact, the presence of AECA after TBI could be explained by an ulterior exposition of antigens produced by endothelial lesions. Similarly, in human and experimental models, the alterations produced in the blood-brain barrier after TBI have been related to neuronal damage caused by the activation of immune system cells that also impairs neurological recovery after TBI [16]. On the other hand, there are evidences of cell-mediated immune response within the brain and the systemic circulation after TBI, were antibodies and B cells are also pivotal players [1]. More specifically, the pathological sequelae that accompanies to CNS trauma has characteristics of a self-directed immunological disease, and the production of specific auto antibodies such as gangliosides, phospholipids, beta-tubulin III , nuclear antigens or anti pituitary antibodies has been related with neurological/neuroendocrinological diseases [17-22]. Although some of these antibodies have been found in healthy individuals, several findings suggest a pathogenic role of AECA auto antibodies in autoimmune rheumatic diseases such as systemic lupus erythematosus, scleroderma and vasculitis. These antibodies are able to induce proinflammatory and procoagulant effects on endothelium (increased expression of adhesion molecules and tissue factors, increased release of cytokines). In addition, in systemic vasculitis such as Takayasu arteritis and

antineutrophil cytoplasm antibody-positive vasculitis, AECA have been reported to correlate with disease activity [23]. These antibodies have been found after TBI in 15 out of 19 patients and only in five of them were preformed (Table 2). Thus, after trauma a specific immune response against endothelium cell tissue has been occurred. With respect to hnRNPK antibodies, the present study shows that 3 out of the 19 patients (15.8%) had antibodies against hnRNPK at the basal time sera, while after trauma these antibodies could be detected in serum sample from 13 of the patients (68.4%). Among the patients who were hnRNPK antibodies positive before the TBI, 2 suffered some type of trauma prior TBI (one suffered a serious stab wound and the other a motorcycle accident). These patients remained anti-hnRNPK positive antibodies during follow-up. Surprisingly, when patients were stratified by the presence/absence of any type of sequels, 100% and 91% of the patients with permanent injuries were AECA and hnRNPK antibodies positive respectively.

In the previous study we describe that antibodies against hnRNPK were associated with a vascular endothelial damage in heart transplanted patients [9]. Similarly, in the current study, TBI patients who developed autoantibodies after trauma are probably due to vascular endothelium damage. Accordingly, a significant association between the presence and, in particular, higher levels of anti-hnRNPK antibodies were found. These differences could be explained by the endothelial damage/activation that occurs after of trauma triggering immune mechanisms. Our results can be added to other published studies that demonstrate the importance of the definition of a good marker and/or the combination of different markers in outcome prediction after TBI [5].

A major limitation of this study is the small number of patients included. However, the study included a very homogeneous cohort, with clinical follow-up of at least one year. Furthermore, this study addresses a pathway which is not sufficiently explored, and it is the role of the endothelium in the genesis of some accompanying phenomena to the pathology of TBI.

Summary

Measuring anti-hnRNPK antibodies in patients who suffered a severe TBI could be a helpful biomarker for evaluating endothelial injury as well as the pathophysiological events accompanying these traumas.

More studies including larger populations are needed to clarify whether these antibodies can predict the occurrence of secondary damage produced by the trauma.

Acknowledgement

This work was supported by grants: PI10-2332 from Fondo de Investigaciones Sanitarias, Ministerio de Ciencia e Innovación of Spain and P07-CTS-02886 from Incentivos a Proyectos de Investigación de Excelencia, Consejería de Economía, Innovación y Ciencia, Junta de Andalucía.

Declaration of interest

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the submitted manuscript or other conflict of interest to disclose.

References

1. Ankeny DP, Popovich PG. B cells and autoantibodies: complex roles in CNS injury. Trends Immunol. 2010; 31(9): 332-338. doi: 10.1016/j.it.2010.06.006.
2. Ziebell JM, Morganti-Kossmann MC. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. Neurotherapeutics. 2010; 7(1): 22-30. doi: 10.1016/j.nurt.2009.10.016.
3. Gopcevic A, Mazul-Sunko B, Marout J, Sekulic A, Antoljak N, Siranovic M, et al. Plasma interleukin-8 as a potential predictor of mortality in adult patients with severe traumatic brain injury. Tohoku J Exp Med. 2007; 211(4): 387-393.
4. De Oliveira CO, Ikuta N, Regner A. Outcome biomarkers following severe traumatic brain injury. Rev bras ter Intensiva. 2008; 20(4): 411-421.
5. Murillo-Cabezas F, Muñoz-Sánchez MA, Rincón-Ferrari MD, Martín-Rodríguez JF, Amaya-Villar R, García-Gómez S, et al. The prognostic value of the temporal course of S100beta protein in post-acute severe brain injury: A prospective and observational study. Brain Injury. 2010; 24(4): 609-619. doi: 10.3109/02699051003652823.
6. Stein DM, Lindell AL, Murdock KR, Kufera JA, Menaker J, Bochicchio GV, et al. Use of Serum Biomarkers to Predict Cerebral Hypoxia after Severe Traumatic Brain Injury. J Neurotrauma. 2012; 29(6): 1140-1149. doi: 10.1089/neu.2011.2149.
7. Lange RT, Iverson GL, Brubacher JR. Clinical Utility of the Protein S100B to Evaluate Traumatic Brain Injury in the Presence of Acute Alcohol Intoxication. J Head Trauma Rehabil. 2012; 27(9): 123-134. doi: 10.1097/HTR.0b013e31820e6840.
8. Czeiter E, Mondello S, Kovacs N, Sandor J, Gabrielli A, Schmid K, et al. Brain injury biomarkers may improve the predictive power of the IMPACT outcome calculador. J Neurotrauma. 2012; 29(9): 1770-8. doi: 10.1089/neu.2011.2127.
9. Acevedo MJ, Caro-Oleas JL, Álvarez-Márquez AJ, Sobrino JM, Lage-Gallé E, Aguilera I, et al. Antibodies against heterogeneous nuclear ribonucleoprotein K in patients with cardiac allograft vasculopathy. J Heart Lung Transplant. 2011; 30(9): 1051-1059. doi: 10.1016/j.healun.2011.02.014.
10. Lynch M, Chen L, Ravitz MJ, Mehtani S, Korenblat K, Pazin MJ, et al. hnRNPK binds a core polypyrimidine element in the eukaryotic translation initiation factor 4E (eIF4E) promoter, and its regulation of eIF4E contributes to neoplastic transformation. Mol Cell Biol. 2005; 25(15): 6436-6453.
11. Carpenter B, McKay M, Dundas SR, Lawrie LC, Telfer C, Murray GI, et al. Heterogeneous nuclear ribonucleoprotein K is over expressed, aberrantly localised and is associated with poor prognosis in colorectal cancer. Br J Cancer. 2006; 95(7): 921-927.
12. Klimek-Tomczak K, Mikula M, Dzwonek A, Paziewska A, Karczmarski J, Hennig E, et al. Editing of hnRNPK protein mRNA in colorectal adenocarcinoma and surrounding mucosa. Br J Cancer. 2006; 94(4): 586-592.
13. Zhou R, Shanas R, Nelson MA, Bhattacharyya A, Shi J. Increased expression of the heterogeneous nuclear ribonucleoprotein K in pancreatic cancer and its association with the mutant p53. Int J Cancer. 2010; 126: 395-404. doi: 10.1002/ijc.24744.

14. Barboro P, Repaci E, Rubagotti A, Salvi S, Boccardo S, Spina B, et al. Heterogeneous nuclear ribonucleoprotein K: altered pattern of expression associated with diagnosis and prognosis of prostate cancer. Br J Cancer. 2009; 100(10): 1608-1616. doi: 10.1038/sj.bjc.6605057.

15. Laury-Kleintop LD, Tresini M, Hammond O. Compartmentalization of hnRNP-K during cell cycle progression and its interaction with calponin in the cytoplasm. J Cell Biochem. 2005; 95(5): 1042-1056.

16. Lotocki G, de Rivero Vaccari JP, Pérez ER, Sánchez-Molano J, Furones-Alonso O, Bramlett HM, et al. Alterations in blood-brain barrier permeability to large and small molecules and leukocyte accumulation after traumatic brain injury: effects of post-traumatic hypothermia. J Neurotrauma. 2009; 26(7): 1123-1134. doi: 10.1089/neu.2008.0802.

17. Zhang Y, Popovich P. Roles of autoantibodies in central nervous system injury. Discov Med. 2011; 11(60): 395-402.

18. Davies AL, Hayes KC, Dekaban GA. Clinical correlates of elevated serum concentrations of cytokines and autoantibodies in patients with spinal cord injury. Arch Phys Med Rehabil. 2007; 88(11): 1384-1393.

19. Hayes KC, Hull TC, Delaney GA, Potter PJ, Sequeira KA, Campbell K, et al. Elevated serum titers of proinflammatory cytokines and CNS autoantibodies in patients with chronic spinal cord injury. J Neurotrauma. 2002; 19(6): 753-761.

20. Skoda D, Kranda K, Bojar M, Glosová L, Bäurle J, Kenney J, et al. Antibody formation against beta-tubulin class III in response to brain trauma. Brain Res Bull. 2006; 68(4): 213-216.

21. López-Escribano H, Miñambres E, Labrador M, Bartolomé MJ, López-Hoyos M. Induction of cell death by sera from patients with acute brain injury as a mechanism of production of autoantibodies. Arthritis Rheum. 2002; 46(2): 3290-3300.

22. Tanriverdi F, De Bellis A, Bizzarro A, Sinisi AA, Bellastella G, Pane E, et al. Antipituitary antibodies after traumatic brain injury: is head trauma-induced pituitary dysfunction associated with autoimmunity? Eur J Endocrinol. 2008; 159(1): 7-13. doi: 10.1530/EJE-08-0050.

23. Guilpain P, Mouthon L. Antiendothelial cells autoantibodies in vasculitis-associated systemic diseases. Clin Rev Allergy Immunol. 2008; 35(1,2): 59-65. doi: 10.1007/s12016-007-8069-3.