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Comparative analysis of postmortem cerebral ammonia level and Alzheimer type 2 astrocytes with intravital blood laboratory parameters of deceased patients with liver cirrhosis of varying degree

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Abstract

Purpose - correlation analysis of the postmortem ammonia level and numbers of Alzheimer type 2 astrocytes in different parts of the brain with intravital laboratory blood parameters of deceased patients with liver cirrhosis of varying severity.

Material and methods. Autopsied brains of 90 patients with non-alcoholic liver cirrhosis (LC) of class A, B and C according to Child-Pugh formed 3 groups: «A» (n = 30), «B» (n = 30) and «C» (n = 30). Control group - 30 patients deceased from cardiovascular failure.

Cortex, white matter, hippocampus, thalamus, striatum and cerebellum were histochemically (HC) studied with Nessler's reagent for ammonia level. Correlation analysis of ammonia expression, Alzheimer type 2 astrocytes (AA2) numbers and 14 intravital laboratory blood parameters was performed.

Results. HC method reveals region-dependent ammonia expression in the brain neuropil. The most significant correlations are observed in thalamus, striatum, cerebellum, and cortex between ammonia expression (A_{CUOD}), AA2-astrocytosis, and lifetime values of total bilirubin (for Bil/A_{CUOD} & /AA2 r = 0.31-0.97), AST (for AST/A_{CUOD} & /AA2 r = 0.30-0.81), ALT (for ALT/A_{CUOD} & /AA2 r = 0.30-0.78), leukocyte index of intoxication (for LII/A_{CUOD} & /AA2 r = 0.34-0.76), albumin (for Alb/A_{CUOD} & /AA2 r = -0.34 to -0.78), leukocytes (for Wbc/A_{CUOD} & /AA2 r = 0.31-0.68), prothrombin index (for PI/A_{CUOD} & /AA2 r = -0.31 to -0.45) and potassium (for K^+/A_{CUOD} & /AA2 r = -0.32 to -0.45), $p < 0.05$.

Conclusion. During LC ammonia expression and AA2-astrocytosis in cerebellum, thalamus, striatum and cortex significantly correlate with each other, as well as with indicators of total bilirubin, AST, ALT, albumin, leukocytes, leukocyte index of intoxication, prothrombin index and blood potassium.

Keywords: liver cirrhosis; hepatic encephalopathy; ammonia; histochemistry; Alzheimer type 2 astrocytes; blood laboratory parameters

Introduction

Cirrhosis and chronic liver disease are the 10th leading cause of death in the world [1]. One of the life-threatening complications of decompensated liver cirrhosis (LC) is the development in 30-45% of patients of hepatic encephalopathy (HE) and hepatic coma [2]. The key role in the mechanisms of hepatogenic brain injury plays neurotoxicity of ammonia [3], which during hyperammonemia penetrates the blood-brain barrier (BBB) in large amounts [4]. During the glutamate-glutamine mechanism of ammonia detoxification, brain astroglia is oversaturated with glutamine, which leads to osmotic swelling of astrocytes, the appearance of Alzheimer type 2 astrocytes, dysfunction of astrocytic aquaporin channels and mitochondrial energetic failure [5, 6, 7]. Severe homeostatic insufficiency of astroglia determines the development of generalized edema-swelling of the brain [8], disruption of the energy and metabolic neuronal supply, as well as neurotransmission imbalance.

Histochemical determination of the ammonia level in sectional material of the nervous tissue is described in a single study by Gutiérrez-de-Juan, V. et al. [9]. While the relationship between plasma ammonia levels and the clinical symptoms of HE has been studied

extensively, there are no postmortem studies on the relationship between the brain ammonia level, numbers of Alzheimer type 2 astrocytes and intravital laboratory blood parameters of deceased cirrhotic patients.

Purpose of the study

Correlation analysis of the postmortem ammonia level and numbers of Alzheimer type 2 astrocytes in different parts of the brain with intravital laboratory blood parameters of deceased patients with liver cirrhosis of varying severity.

Materials and methods

Postmortem material of the brain and liver was studied in 90 patients (aged 65±3 years) who suffered from non-alcoholic cirrhosis of classes A, B, and C according to the Child-Pugh classification [10], which comprised 3 groups: «A» (n = 30, compensated LC), «B» (n = 30, subcompensated LC) and «C» (n = 30, decompensated LC). 59 (65.55%) patients had clinical symptoms of grade I-IV HE. There were excluded cases of combined pathology with systemic intoxication, endocrine disorders, as well as cases of cancerous and alcoholic liver pathology. The control group consisted of 30 patients (aged 59±2.5 years) died from acute cardiovascular failure and did not suffer from liver diseases or intoxication.

Intravital clinical and laboratory blood parameters were analyzed from the case histories (the level of erythrocytes, hemoglobin, leukocytes, platelets, prothrombin index, albumin, total bilirubin, ALT, AST, creatinine, urea, sodium, potassium, leukocyte index of intoxication), as well as symptoms HE and dynamics of ascites. Cirrhosis was verified as viral in 64 (71.12%), secondary biliary - in 13 (14.44%), congestive - in 9 (10.00%), drug-induced - in 2 (2.22%) %) and cryptogenic - in 2 (2.22%) cases. In group «A», cirrhosis in 8 (26.66%) patients was accompanied by symptoms of grade I HE. In group «B» 21 (70.00%) patients had HE I and II degree. In group «C», HE of II-III degree occurred in 23 (76.66%) patients, and IV degree HE (hepatic coma according to the Glasgow coma scale [11]) developed in 7 (23.33%) patients.

During autopsy, specimens of the liver, brain cortex and subcortical white matter, were taken from the frontal, parietal, temporal and occipital brain lobes, as well as hippocampus, thalamus, striatum (putamen, globus pallidus, caudate nucleus) and cerebellum. Specimens were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections of 4 μm thick were serially prepared using precision rotary microtome HM 3600 («MICROM laborerate GmbH» - Germany); after deparaffination they were treated with hematoxylin and eosin for a general assessment of histopsthology.

Histochemical (HC) expression of ammonia in paraffin sections was evaluated using Nessler's reagent by V. Gutierrez-de-Juan et al. method [9]. The expression level of HC-positive ammonia granules was determined in each brain region in conditional units of optical density (CUOD) in five standardized fields of view (SFV) of the A1 microscope «Carl Zeiss» (Germany) with a Jenoptik Progres Gryphax 60N-C1"1.0x426114 (Germany) camera at magnification x400 using automatic analysis system and standard color deconvolution plugin «H DAB» of ImageJ soft. When CUOD values are from 0 to 20, the degree of HC ammonia expression is regarded as negative («-»); from 21 to 50 – as weak («+»); from 51 to 100 – as moderate («++»); from 101 and above – as strong («+++»).

In twenty SFVs of each studied brain region (at x400), the numbers of astrocytes of Alzheimer type 2 astrocytes (AA2) was counted. The degree of AA2-astrocytosis is graded into 4 degrees; 0 degree (AA2-astrocytosis is absent) – 1-5 AA2/20 SFV; I (weak) degree of AA2-astrocytosis – 6-10 astrocytes AA2/20 SFV; II (moderate) degree of AA2-astrocytosis – 11-20 astrocytes AA2/20 SFV; III (pronounced) degree of AA2-astrocytosis – from 21 and more astrocytes AA2/20 SFV.

The results were analyzed using the Statistica ® package for Windows 13.0 (StatSoft Inc., License No. JPZ 804 I 382130 ARCN 10-J). Results were expressed as median (Me) with range (Q1; Q3). The Mann-Whitney U test was used to compare two groups, and the Kruskal-Wallis test was used to compare more than two groups. Correlation analysis was calculated using Spearman's rank correlation coefficient (r). The results were considered statistically significant at the level of 95% ($p < 0.05$).

Results

HC determination of ammonia with Nessler’s reagent according to V. Gutiérrez-de-Juan et al. reveals region-dependent fine-grained and extremely low optical density expression of ammonia in the neuropil of all six studied brain regions in control group, which corresponds to a «negative» expression in accordance with the ammonia scale we used. Herewith, relatively higher numerical values of optical density are observed in the cerebellum and thalamus, and the lowest values are observed in the white matter (Table 1).

In all studied brain regions of deceased cirrhotic patients, a more intense HC ammonia expression is observed accompanied by proportional increase with cirrhosis progression. In deceased patients of «A» group with compensated cirrhosis, weak HC ammonia expression is observed in the cortex, thalamus, striatum, and cerebellum (Table 1). In the brains of patients with subcompensated cirrhosis (group «B»), moderate HC ammonia expression is detected in the thalamus, striatum, and cerebellum which is higher than group «A» (Table 1).

Table 1. HC ammonia expression (in CUOD), HC ammonia scale, AA2 numbers and AA2-score in the brain of deceased cirrhotic patients of «A», «B», «C» groups and in control

Parameter	Group «A»	Group «B»	Group «C»	Control group
Cerebral cortex				
Ammonia expression (CUOD)	27.11 (23.82; 28.85) *	29.5 4 (24.34; 35.12) *	64.23 (53.12; 76.07) *#	18.14 (15.26; 19.53)
Ammonia scale	+	+	++	-
AA2 numbers	3.50 (2.40; 5.20)	12.50 (6.30; 14.20) *	21.10 (15.50; 26.20) *#	3.10 (1.20; 4.50)
AA2-score	0	II	III	0
Subcortical white matter				
Ammonia expression (CUOD)	11.23 (10.75; 15.81)	12.47 (11.15; 16.25)	21.15 (20.22; 22.73) *#	11.10 (10.34; 2.26)
Ammonia scale	-	-	+	-
AA2 numbers	3.60 (2.70; 5.40)	3.70 (2.90; 5.90)	14.50 (7.20; 15.60) *#	3.40 (1.80; 4.30)
AA2-score	0	0	II	0
Hippocampus				
Ammonia expression (CUOD)	18.12 (15.57; 19.37)	22.48 (21.39; 33.79) *	55.43 (52.61; 61.48) *#	17. 25 (14.68; 18.72)
Ammonia scale	-	+	++	-
AA2 numbers;	2.60 (1.80; 3.40)	3.40 (2.70; 5.20)	6.10 (4.80; 9.50) *	2.10 (1.50; 3.20)
AA2-score	0	0	I	0
Thalamus				
Ammonia expression (CUOD)	26.68 (24.72; 29.35) *	65.47 (51.71; 78.89) *†	110.23 (99.22; 112.35) *#	19.25 (16.58; 19.72)
Ammonia scale	+	++	+++	-
AA2 numbers	9.30 (7.40; 10.60) *	15.20 (12.60; 19.90) *†	35.70 (21.50; 54.10) *#	4.10 (3.70; 6.20)
AA2-score	I	II	III	0
Striatum				
Ammonia expression (CUOD)	24.37 (22.68; 28.61) *	55.52 (52.48; 65.57) *†	101.56 (100.48; 103.27) *#	18.46 (15.69; 18.93)
Ammonia scale	+	++	+++	-
AA2 numbers	7.60 (6.30; 9.70) *	8.30 (6.70; 10.20) *	25.20 (21.30; 45.20) *#	3.80 (3.20; 5.90)
AA2-score	I	I	III	0
Cerebellum				
Ammonia expression (CUOD)	29.27 (26.48; 31.43) *	67.08 (54.29; 84.27) *†	122.16 (107.37; 131.27) *#	19.74 (18.32; 19.83)
Ammonia scale	+	++	+++	-
AA2 numbers	9.10 (7.50; 10.20) *	16.10 (14.60; 20.40) *†	32.40 (20.9 0; 52.20) *#	3.90 (3.80; 6.10)
AA2-score	I	II	III	0

Notes: (*) - reliable difference compared to control ($p < 0.05$); (†) - reliable difference compared to group «A» ($p < 0.05$); (#) - reliable difference compared to groups «A» and «B» ($p < 0.05$). Data are presented as median with lower and upper quartiles: Me (Q1; Q3).

In the group «C» (decompensated cirrhosis) all studied brain regions show the highest HC ammonia expression relative to control, groups «A» and «B», wherein, the highest values are observed in *the cerebellum* (6.18 times higher than control); *thalamus* (higher by 5.72 times relative to control); *striatum* (higher by 5.50 times relative to control). Moderate HC expression is observed in *the cortex* (3.54 times higher relative to control), as well as in *the hippocampus* (3.21 times higher relative to control); weak HC expression is observed in *the white matter* (higher by 1.90 times relative to control) (Table 1).

Alzheimer type 2 astrocytes (AA2) have an enlarged, watery nucleus with a prominent nucleolus and punctate accumulations of chromatin at the nuclear membrane, as well as an inconspicuous rim of the cytoplasm; they often form paired figures and triplets. AA2 nuclei can be 2 times the size of the nuclei of neighboring oligodendrocytes. In control group, only single AA2-s are observed per 20 SGVs of different brain regions, which corresponds to «0» degree of AA2-astrocytosis according to the AA2-score we used.

As cirrhosis progresses, increasing rates of AA2-astrocytosis is noted in the thalamus, cerebellum, striatum, cortex, white matter, and hippocampus. In group «A», mild grade (I degree) AA2-astrocytosis is found in the thalamus, striatum, and cerebellum. In group «B», moderate AA2-astrocytosis of the II degree is noted in the cortex, thalamus and cerebellum; weak AA2-astrocytosis of I degree is determined in the striatum. In the brains of patients of group «C», there is a pronounced grade III AA2-astrocytosis in the thalamus, cerebellum, striatum and cortex; moderate II degree - in the white matter; weak I degree AA2- astrocytosis is observed in the hippocampus. In the group «C», the average indicators of AA2-astrocytosis are higher compared to control: in *thalamus* - by 8.71 times, in *cerebellum* - by 8.30 times; in *cortex* – 6.81 times; in *striatum* - 6.63 times, in *white matter* - 4.26 times, in *hippocampus* - 2.90 times higher (Table 1).

Correlation analysis showed a moderate, strong, and very strong direct relationship between the AA2 numbers and values of HC ammonia expression in the thalamus, striatum, and cerebellum. Among aforementioned regions, correlation coefficient (r) between AA2 numbers and ammonia expression in group «A», ranges from 0.47 to 0.75; in group «B», r ranges from 0.61 to 0.86; in group «C», r ranges from 0.79 to 0.93. While a moderate and

weak direct correlation between indicators is present in the cortex, white matter, and hippocampus in all three observational groups.

A retrospective analysis of the latest intravital laboratory blood parameters, which are of particular diagnostic value in cirrhosis, revealed the following trends (Table 2). Starting from group «A», there is a tendency to anemia and thrombocytopenia development, reaching significant values in group «C» (decrease in the of erythrocytes level by 1.51 times, 50.85%; hemoglobin - by 1.46 times, 45.79%; platelets - by 2.74 times, 174.01%). The level of leukocytes shows a significant decrease in group «B» compared to control, however, in group «C» the average indicators reflect the development of leukocytosis (an increase by 1.78 times, 78.19%). The levels of total bilirubin, AST and ALT reflect a progressive increase in relation to control data. In group «A», the level of bilirubin increased by 1.92 times (by 92.07%), in group «B» - by 7.34 times (by 633.83%), in group «C» - by 15.33 times (by 1432.68%). The level of AST in group «A» increases by 2 times (by 100%), in group «B» - by 4 times (by 300%), in group «C» - by 9.78 times (by 878.26%). The level of ALT in group «B» increased by 2.21 times (by 120.67%), in group «C» - by 5.12 times (by 411.76%). The level of plasma albumin is characterized by a decrease relative to control values: in group «B» - by 1.30 times (by 29.51%), in group «C» - by 1.67 times (by 67.31%). Along with decrease in the prothrombin index (PTI) (in group «B» - by 1.46 times, 45.71%; in group «C» - by 2.04 times, by 104.30%), the above data indicate the decompensation of hepatocellular insufficiency. A significant increase in the levels of creatinine (by 3.06 times, 205.82%) and blood urea (by 4.26 times, 325.59%) in group «C», reflects the addition of renal failure as part of the hepatorenal syndrome in this group of patients. Following a slight increase in the level of sodium in the blood plasma of patients with subcompensated cirrhosis of group «B», patients of group «C» show hyponatremia (a decrease by 1.05 times, 4.64%). The blood plasma potassium level also reflects a significant decrease relative to control (by 1.26 times, 26.27%) in decompensated cirrhosis. The leukocyte index of intoxication (LII), calculated by Ya.Ya. Kalf -Khalif's formula, is increased in decompensated cirrhosis by an average of 3.69 times (by 269.23%) compared to control values (Table 2).

Correlation analysis of the indicated laboratory data and HC indices of ammonia expression (A_{CUOD}), as well as AA2-astrocytosis scores in the studied brain regions in compensated, subcompensated and decompensated cirrhosis (groups «A», «B» and «C») revealed the following relationships.

Table 2. *In vivo* blood parameters of cirrhotic patients of «A», «B», «C» and control groups

	Group «A»	Group «B»	Group «C»	control group
Red blood cells (Rbc; $\times 10^{12}$/L)	3.64 (3.30; 4.45)	3.55 (3.10; 3.82) *	2.93 (2.80; 3.12) *	4.42 (4.20; 4.90)
Hemoglobin (Hb, g/L)	115.50 (110.40; 127.20)	107.30 (86.00; 120.20) *	97.60 (62.00; 110.50) *	142.30 (125.40; 152.00)
Leukocytes (Wbc; $\times 10^9$/L)	5.80 (5.50; 6.70)	4.30 (3.80; 5.95) *	11.60 (10.20; 12.53) *	6.51 (6.10; 6.85)
Platelets (Plt, $\times 10^9$/L)	195.20 (165.00; 205.20)	136.00 (127.00; 153.40) *	93.50 (54.20; 1 3 6.40) *	256.20 (235.00; 287.00)
PI (Prothrombin index; %)	78.00 (75.30; 9 4.00)	6 5.20 (51.40; 67.30) *	46.50 (39.30; 65.30) *	95.00 (86.20; 103.00)
Albumin (Alb, g/L)	37.00 (36.30; 45.20)	33.20 (30.50; 35.30) *	25.70 (22.40; 32.20) *	43.00 (36.50; 46.30)
Bilirubin total (Bil, µmol/L)	21.57 (20.65; 32.84) *	82.41 (46.83; 97.72) *	172.12 (122.52; 315.23) *	11.23 (9.35; 16.43)
AST (aspartate aminotransferase; µmol/(sec*L)	0.46 (0.38; 0.55) *	0.92 (0.82; 1.34) *	2.25 (1.72; 2.93) *	0.23 (0.18; 0.37)
ALT (alanine aminotransferase; µmol/(sec*L)	0.35 (0.29; 0.71)	0.75 (0.57; 1.12) *	1.74 (1.33; 2.25) *	0.34 (0.20; 0.55)
Creatinine (Cre, µmol/L)	85.34 (73.26; 115.27)	118.62 (88.41; 146.74) *	227.16 (185.27; 329.63) *	74.28 (67.12; 95.12)
Urea (Ur, mmol/L)	6.82 (5.39; 9.79)	8.18 (6.28; 12.83)	23.11 (20.45; 28.18) *	5.43 (4.47; 7.28)
Sodium (Na+; mmol/L)	137.30 (135.00; 142.50)	145.00 (138.00; 146.40)	129.20 (126.10; 130.10) *	135.20 (134.30; 142.34)
Potassium (K+; mmol/L)	4.53 (3.52; 4.95)	4.67 (3.81; 5.15)	3.35 (3.21; 3.48) *	4.23 (3.74; 4.72)
LII (leukocytic intoxication index by Kalf-Kalif formula; units)	0.65 (0.57; 1.47)	0.93 (0.74; 1.78)	1.92 (1.57; 2.97) *	0.52 (0.34; 1.12)

Notes: (*) - significant differences relative to control. Data are presented as a median with the lower and upper quartiles: Me (Q1; Q3).

In the group «A» HC ammonia expression and AA2-astrocytosis *directly and weakly* correlate with the level of total plasma bilirubin in the *cortex* (r=0.43, r=0.34, respectively), *thalamus* (r=0.45, r=0.48), *striatum* (r=0.32 , r=0.30) and *cerebellum* (r=0.37, r=0.42); AA2-

astrocytosis also *directly and weakly* correlates with the level of AST (r=0.30) and LII (r=0.34) in *the thalamus*, $p<0.05$.

***In the group «B»**, a direct moderate* correlation is observed between the parameters Bil/A_{CUOD} and Bil/AA2 *in the cortex* (r=0.52, r=0.57), *thalamus* (r=0.56, r=0.62) *and cerebellum* (r=0.54, r=0.58); between AST/AA2 parameters – in *thalamus* (r=0.52), $p < 0.05$.

Direct weak correlation is noted *in the cortex* – for AST/A_{CUOD} (r=0.35); AST/AA2 (r=0.37); ALT/AA2 (r=0.32); LII/A_{CUOD} (r=0.35); LII/AA2 (r=0.32); *white matter* – AST/A_{CUOD} (r=0.33); *hippocampus* – Bil/AA2 (r=0.31); *thalamus* – AST/A_{CUOD} (r=0.48); ALT/A_{CUOD} (r=0.45); ALT/AA2 (r=0.48); LII/A_{CUOD} (r=0.36); LII/AA2 (r=0.38); *striatum* – Bil/A_{CUOD} (r=0.46); Bil/AA2 (r=0.48); AST/A_{CUOD} (r=0.35); AST/AA2 (r=0.41); ALT/AA2 (r=0.34); LII/A_{CUOD} (r=0.37); LII/AA2 (r=0.41); *cerebellum* – AST/A_{CUOD} (r=0.42); AST/AA2 (r=0.45); ALT/A_{CUOD} (r=0.37); ALT/AA2 (r=0.39); LII/A_{CUOD} (r=0.36); LII/AA2 (r=0.43); $p<0.05$.

Reverse weak correlation is observed in *the thalamus* – Alb/AA2 (r= -0.34); *striatum* – PI/AA2 (r= -0.31); $p<0.05$.

***In the group «C»**,* a significant correlation is observed between the maximum numbers of compared blood parameters.

Direct very strong correlation is determined in Bil/A_{CUOD} and Bil/AA2 pairs *in the cortex* (r=0.93; r=0.95); *thalamus* (r=0.91; r=0.93); *cerebellum* (r=0.93; r=0.97); $p<0.05$.

Direct strong correlation takes place in *the cortex* – AST/A_{CUOD} (r=0.73); AST/AA2 (r=0.77); LII/A_{CUOD} (r=0.73); LII/AA2 (r=0.72); *thalamus* – AST/A_{CUOD} (r=0.75); AST/AA2 (r=0.79); ALT/AA2 (r= 0.72); LII/A_{CUOD} (r=0.74); LII/AA2 (r=0.76); *striatum* – Bil/A_{CUOD} (r=0.81); Bil/AA2 (r=0.83); AST/AA2 (r=0.77); *cerebellum* – AST/A_{CUOD} (r=0.72); AST/AA2 (r=0.81); ALT/AA2 (r= 0.78); $p<0.05$.

Direct moderate correlation is observed in the *cortex* – ALT/A_{CUOD} (r=0.53); *hippocampus* – Bil/A_{CUOD} (r=0.51); Bil/AA2 (r=0.54); *thalamus* – Wbc/A_{CUOD} (r=0.56); Wbc/AA2 (r=0.68); *striatum* – AST/A_{CUOD} (r=0.63); ALT/AA2 (r=0.52); *cerebellum* – Wbc/A_{CUOD} (r=0.53); Wbc/AA2 (r=0.65); ALT/A_{CUOD} (r=0.63); LII/A_{CUOD} (r=0.52); LII/AA2 (r= 0.63); $p<0.05$.

Direct weak correlation observed in the *cortex* – Wbc/A_{CUOD} (r=0.32); Wbc/AA2 (r=0.31); ALT/AA2 (r=0.38); *white matter* – Bil/A_{CUOD} (r=0.41); Bil/AA2 (r=0.46); AST/A_{CUOD} (r=0.32); AST/AA2 (r=0.33); ALT/AA2 (r=0.30); LII/A_{CUOD} (r=0.40); LII/AA2 (r=0.45); *hippocampus* – AST/A_{CUOD} (r=0.32); AST/AA2 (r=0.37); LII/A_{CUOD} (r=0.35); LII/AA2 (r=0.38); *thalamus* – Cre/A_{CUOD} (r=0.31); Cre/AA2 (r=0.32); Ur/A_{CUOD} (r=0.30);

Ur/AA2 (r=0.34); *striatum* – Wbc/A_{CUOD} (r=0.37); Wbc/AA2 (r=0.41); ALT/A_{CUOD} (r=0.49); Cre/AA2 (r=0.30); Ur/AA2 (r=0.34); LII/A_{CUOD} (r=0.45); LII/AA2 (r=0.47); $p<0.05$.

Inverse strong correlation observed in the *cortex and thalamus* – Alb/AA2 (r = -0.75, r = -0.78); *cerebellum* – Alb/A_{CUOD} (r = -0.74); Alb/AA2 (r = -0.75), $p < 0.05$.

Inverse moderate correlation takes place in the *cortex and thalamus* – Alb/A_{CUOD} (r= -0.63, r= -0.67); *striatum* – Alb/AA2 (r= -0.55); $p<0.05$.

Reverse weak correlation is observed in the *cortex* – PI/A_{CUOD} (r= -0.37); PI/AA2 (r= -0.45); K^+/A_{CUOD} (r= -0.33); K^+/AA2 (r= -0.35); *thalamus* – PI/AA2 (r= -0.35); Na^+/AA2 (r= -0.32); K^+/A_{CUOD} (r= -0.38); K^+/AA2 (r= -0.35); *striatum* – Plt/A_{CUOD} (r= -0.36); Plt/AA2 (r= -0.34); PI/A_{CUOD} (r= -0.34); PI/AA2 (r= -0.38); Alb/A_{CUOD} (r= -0.43); K^+/A_{CUOD} (r= -0.32); K^+/AA2 (r= -0.41); *cerebellum* – PI/AA2 (r= -0.32); K^+/A_{CUOD} (r= -0.35); K^+/AA2 (r= -0.45); $p<0.05$.

Discussion

Despite the key role of ammonia in the development of HE, *in vivo* determination of ammonia level in plasma of cirrhotic patients has shown heterogeneous and conflicting results in numerous clinical studies, due to the lack of generally accepted numerical criteria of its increase and upper normal limits [12]. The intravital laboratory determination of blood ammonia has significant limitations due to its logistical complexity, which strongly affects the objectivity of the analysis and makes the study more expensive [13, 14]. Furthermore, arterial blood is recommended for research, since the ammonia content in venous blood is significantly lower due to peripheral metabolism in muscle tissue and the brain. All this causes a substantial discrepancy in the interpretation of the relationship between plasma ammonia and HE development. The number of studies did not confirm such a relationship, showing that blood ammonia can be in the «normal» range in the presence of HE, and vice versa, it can have «high» values during mild, minimal HE and in its absence [15]. Therefore, AASLD and Choosing Wisely Campaign protocols are missing recommendations to evaluate blood on ammonia *in vivo* for confirmation or exception of HE diagnosis [16], although during severe HE ammonia blood level extremely rarely remains within conditionally normal values [17] and the immediate prognosis of patients with acute liver failure directly depends on their plasma ammonia levels [18, 19, 20].

HC method for ammonia determination, proposed by V. Gutierrez-de-Juan et al. [9], shows that the cerebellum, thalamus, and striatum are the richest in ammonia among the brain regions studied in all observational groups. Increased compared to the control ammonia level is already observed in the compensated cirrhosis in the cortex, thalamus, striatum, and

cerebellum, while in subcompensated cirrhosis, ammonia expression is increased in all studied brain regions. In decompensated cirrhosis, ammonia level reaches the maximum in the cerebellum, thalamus, and striatum. The latter may be due to the presence in this observation group the largest number of patients with severe HE and hepatic coma, as well as increased BBB permeability for ammonia due to neurotoxicosis caused by multiple organ failure in this category of patients. The revealed regional heterogeneity of ammonia level in the brain is most likely due to initially different levels of enzymes involved in ammonia metabolism, as well as different levels of glutamate, dopamine, GABA, and other neurotransmitters associated with ammonia metabolism.

The main histopathological hallmark of hepatogenic brain damage is the presence of so-called Alzheimer type 2 astrocytes, which manifest the primary dysmetabolic dystrophic astrocytopathy [5]. In our study, we confirmed this statement by showing that already at the stage of compensated cirrhosis, an increase in AA2 numbers in thalamus, striatum, and cerebellum is observed. With cirrhosis progression, the amount of AA2 increases, and at the stage of decompensation, pronounced AA2-astrocytosis is found in the cortex, thalamus, striatum, and cerebellum, while moderate one is observed in the white matter, and weak – in the hippocampus. The relationship between AA2 rates and HC ammonia expression in the same brain regions is confirmed by correlation analysis, reflecting the presence of a direct medium, strong, and very strong relationship between these indicators in the thalamus, striatum, and cerebellum.

The clinical diagnosis of HE is a diagnosis of exclusion, based on the clinical symptoms of HE and laboratory-instrumental confirmation of liver failure. In addition to the presence/absence of varying degrees of HE, the list of Child-Pugh assessment criteria of LC severity includes the presence/absence of ascites, plasma levels of total bilirubin, albumin, prothrombin index (%) (or prothrombin time/or International Normalized Ratio) [10].

Our study confirmed that cirrhosis progression is accompanied by trends to anemia, thrombocytopenia, hypoalbuminemia, and hypocoagulation with simultaneous increase in the level of total bilirubin, AST, and ALT. The leukocytes with initially normal levels at compensated cirrhosis, show a downward trend in subcompensated stage, and in decompensated stage their elevated levels reflect leukocytosis. An increase in LII and leukocytosis confirm high toxemia and systemic inflammation in patients of this observation group. Moreover, the indicators of leukocytosis and LII directly correlate with the tissue ammonia and AA2-astrocytosis in the same brain regions. As soon as tanatogenetically significant forms of HE are characteristic for decompensated cirrhosis, and infection is one of

the main precipitating factors for acute HE [21], systemic inflammation definitely plays a crucial role in acute neurotoxicosis and reinforces the inflammatory concept of HE [22, 23].

The inverse correlation between plasma albumin and PTI on the one hand, and cerebral ammonia and AA2-astrocytosis on the other hand in thalamus, striatum and cerebellum in decompensated cirrhosis, indicates that hypoalbuminemia and hypocoagulation are indirectly involved in multicomponent mechanisms of cerebral damage in decompensated patients, however, the exact reasons for this relationship are not entirely clear. The effectiveness of albumin infusions in the complex treatment of hyponatremia in patients with decompensated cirrhosis indicates a pathogenetic relationship between these two parameters [24, 25, 26]. Decreased average levels of potassium, sodium, as well as increased levels of creatinine and urea at the last stage of cirrhosis indicates the development of hepatorenal insufficiency and osmotic dysregulation in these patients, as well as potential influence of additional factors causing breakdown of cerebral homeostasis and increased neurotoxicosis at this stage [27, 28, 29, 30]. Mikkelsen, A.C.D. et al. in their recent study have proved that experimental hypokalemia in rats is capable to raise concentration of blood ammonia by 8 times due to inhibition of genes regulating synthesis of urea cycle proteins and lowering its efficiency [31]. In our study, we confirmed this by indirect correlation between plasma potassium on the one hand, and ammonia expression, AA2-astrocytosis on the other, as well as the presence of a direct correlation between creatinine, urea, and cerebral ammonia, AA2-astrocytosis in decompensated cirrhosis.

Among the fourteen studied blood parameters, the most significant direct correlations, as well as their earliest establishment, are observed between the indicators of total bilirubin, AST, ALT, LII on the one hand, and tissue ammonia and AA2-astrocytosis on the other, which was confirmed in all 6 brain regions. The earliest and most significant correlation with ammonia level and AA2-astrocytosis is characteristic for plasma total bilirubin, while weaker correlation is referred for AST, ALT, and LII, wherein the strongest relationships are traced in cerebellum, thalamus, striatum, and cortex.

The strongest correlations with the most pronounced ammonia expression and AA2-astrocytosis in thalamus, cerebellum, striatum, and cortex, suggest that the level of intravital plasma bilirubin, as well as cell cytolysis indicators and leukocytic intoxication index, can be used as indirect laboratory markers of increased brain ammonia and brain hepatotoxic damage that develops under these conditions.

In the hippocampus and white matter, the lowest degree of ammonia expression and AA2-astrocytosis are characterized by the weakest correlations with *in vivo* altered laboratory parameters.

Depending on the severity of cirrhosis, the following pathomorphological criteria for hepatotoxic brain damage can be used:

For compensated cirrhosis: 1) weak HC ammonia expression in the cortex, thalamus, striatum, cerebellum; 2) weak AA2-astrocytosis in the thalamus, striatum and cerebellum; 3) *in vivo* increased total plasma bilirubin. *For subcompensated cirrhosis:* 1) moderate HC ammonia expression in the thalamus, striatum, and cerebellum; weak – in the cortex and hippocampus; 2) moderate AA2-astrocytosis – in the cortex, thalamus, cerebellum; weak – in the striatum; 3) *in vivo* elevated total bilirubin, AST, ALT, LII. *For decompensated cirrhosis:* 1) strong HC ammonia expression in the thalamus, striatum, cerebellum; moderate – in the cortex, hippocampus; weak – in the white matter; 2) pronounced AA2-astrocytosis in the cortex, thalamus, striatum, cerebellum; moderate – in the white matter; weak – in the hippocampus; 3) *in vivo* elevated total bilirubin, AST, ALT, LII, as well as leukocytosis, hypoalbuminemia, hypokalemia, and decrease in the prothrombin index.

Conclusions

1. In the deceased patients with liver cirrhosis of varying degree according to Child-Pugh, HC ammonia expression and AA2-astrocytosis in the cerebellum, thalamus, striatum and cortex directly correlate with each other and with *in vivo* plasma indicators of total bilirubin, AST, ALT, leukocytes, leukocyte index of intoxication, whereas negatively correlate with albumin, prothrombin index and potassium levels.
2. Different scores of tissue ammonia and Alzheimer-2-astrocytosis in the brain of deceased patients with cirrhosis of varying degree, combined with *in vivo* changes in plasma total bilirubin, AST, ALT, albumin, leukocyte index of intoxication, leukocytes, prothrombin index and potassium represent the novel pathomorphological criteria of hepatogenic toxic brain damage in the dynamics of decompensation of liver cirrhosis.

Prospects for further research

Further study on ammonia brain level and associated neuropathology in other somatogenic toxic encephalopathies, as well as its correlation with the key neuroglial markers are needed to improve our knowledge on the morphogenesis of acute and chronic toxic and metabolic disorders in the CNS.

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