Angiotensinogen and interleukin-18 as markers of chronic kidney damage in children with a history of hemolytic uremic syndrome

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Short title: Angiotensinogen and interleukin-18 in children with HUS

Summary

Hemolytic uremic syndrome (HUS) is a type of thrombotic microangiopathy, in the course of which some patients may develop chronic kidney disease (CKD). It is clinically important to investigate the markers of a poor prognosis.

The levels of angiotensinogen (AGT) and interleukin-18 (IL-18) in serum and urine were evaluated. Study was conducted in 29 children with a history of HUS.

Serum and urine AGT concentration was significantly higher in children after HUS as compared to the control group. No differences depending on the type of HUS and gender were noted. The serum concentration of IL-18 in children after HUS was significantly lower, whereas in urine did not differ significantly between the sick and healthy children. A negative correlation between the concentration of AGT in serum and albuminuria in patients after HUS was detected.

The results indicate that the concentration of AGT in serum and urine in children after HUS increases, which may indicate the activation of the intrarenal renin-angiotensin-aldosterone system. The statement, that AGT may be a good biomarker of CKD after acute kidney injury due to HUS requires prospective studies with follow-up from the acute phase of the disease on a larger group of patients. Reduced IL-18 serum concentration in children after HUS with no difference in its urine concentration may indicate a loss of the protective effects of this cytokine on renal function due to previously occurred HUS.

Keywords: hemolytic-uremic syndrome, chronic kidney disease, children, angiotensinogen, IL-18

Introduction:

Hemolytic uremic syndrome (HUS) is the most common cause of acute kidney injury in previously healthy infants and young children. Together with thrombotic thrombocytopenic purpura (TTP) HUS belongs to a group of thrombotic microangiopathies (TMA). It is characterized by a triad of symptoms - hemolytic anemia, thrombocytopenia and acute renal injury. Due to the different etiology and clinical course of HUS is divided into typical and atypical form (Picard et al. 2015). There are also many forms of HUS associated with coexisting disease or condition such as: drug induced, cancer-related, **occurring** after bone marrow and solid organs transplantation, in the course of autoimmunologic diseases, caused by HIV infection etc. (Adamczuk et al. 2009, Jander et al. 2013, Zurowska 2012).

Typical form of HUS, which constitutes 90% of cases of the disease, occurs most often in younger children (Adamczuk et al. 2009, Zurowska 2012, Franchini 2015). Verotoxin or Shiga-like toxin, produced by entero-hemorrhagic strains of *Escherichia coli* or *Shigella dysenteriae* is responsible for the damage of endothelial cells in this condition (Picard et al. 2015, Jander et al. 2013, Nester et al. 2015). Current mortality in typical form of HUS does not exceed 10%. In the vast majority of patients the recovery of renal function with normal glomerular filtration rate is observed. In some patients the disease leads to chronic kidney disease (CKD) **including** end-stage renal failure (Lumbreras Fernandez et al. 2010).

Atypical hemolytic-uremic syndrome (aHUS) in 2/3 of cases is not preceded by diarrhea, may be of family occurrence and is characterized by recurrent course (Adamczuk et al. 2009, Zurowska 2012). aHUS is an heterogeneous group of disorders related to defects in immune and coagulation systems (Jander et al. 2013). **We know that in majority of patients** aHUS relates to permanent abnormal activation of the complement system (the alternative pathway), caused by mutations of genes encoding proteins of the complement system (C3 protein, factor H, factor I, factor B, membrane cofactor protein (MCP), thrombomodulin) and the presence of antibodies against factor H. These mutations are both of inactivation (gene encoding factor H, MCP, thrombomodulin) or activation (C3 protein, factor B) (Wong et al. 2016) (Nester et al. 2015). There are also mutations in other genes, e.g. newly discovered mutations of the gene encoding diacylglycerol kinase (DGKE) (Loirat et al. 2016), and some patients may have the causative mutations in more than one gene (Wong et al. 2016). aHUS is characterized by a poor prognosis, is burdened with significant mortality and 50% of the patients require renal replacement therapy (Adamczuk et al. 2009).

In recent years **rapid** progress both in understanding the pathophysiology and the treatment of aHUS has been done. By the year 2011, the basic tool for the treatment of aHUS

was plasma therapy - prophylactic and therapeutic infusions of fresh frozen plasma or therapeutic plasma exchange (Picard et al. 2015,Nester 2015). Currently, the most effective treatment in aHUS is eculizumab therapy, which improved significantly the outcome (Picard et al. 2015, Franchini 2015, Loirat et al. 2016, Nester 2015).

Children with a history of HUS require continuous monitoring because they may reveal the features of reduced renal function even many years after the acute phase of the illness. An important concern is early identification of patients at risk of poor prognosis. Serum creatinine evaluation with the calculation of estimated glomerular filtration rate, albuminuria assessment (albumin/ creatinine ratio - **ACR**) or ultrasound of the kidneys are not sufficient to reach that aim. Hence there is a need to look for sensitive and specific markers that would identify the early stages of kidney damage. It seems that angiotensinogen (AGT) and interleukin -18 (IL-18) appear among such markers.

The aim of the study

We evaluated the concentration of AGT and IL-18 in serum and urine in children with a history of HUS as compared to the concentrations in healthy children. We **raised hypothesis if these markers may be used as indicators of renal function deterioration.** We have also tried to answer whether AGT and IL-18 could be early predictors of worsening of renal function.

Material and methods

The study group (HUS) consisted of 29 patients (9 girls and 20 boys) aged 1 to 15 years with **confirmed past** history of HUS diagnosed **based on standard criteria (hemolytic anemia, thrombocytopenia and acute renal injury),** treated at the Department and Clinic of Pediatrics in Zabrze, Medical University of Silesia in Katowice. In this group 86% children required renal replacement therapy in the acute phase of the disease, 62% of them - peritoneal dialysis, 14% - hemodialysis, 10% - both peritoneal dialysis and hemodialysis and 14% of children did not require dialysis. In 15 children atypical HUS (aHUS) was diagnosed **(negative stool culture, not coexisting defined disease, or condition, ADAMTS13 activity greater than 10%).** All the children from the study group at the time of study were treated pharmacologically (diuretics (4 children), calcium channel blockers (21 children), angiotensin converting enzyme inhibitors (ACE-I) (14 children), supplementation of bicarbonate (4 children), supplementation of iron and folic acid (9). Weight, height, blood pressure and routine biochemical tests were performed on admission. Body mass index (BMI) was

calculated using the formula BMI = body weight [kg] / height^2 [m^2]. **To compare the values of blood pressure between diverse groups of children SDSs' for systolic and diastolic blood pressure were calculated.**

The control group consisted of 21 healthy children (11 girls and 10 boys) aged 1 to 15 years hospitalized for nocturnal enuresis or presenting with surgical procedures of one-day surgery. All children participating in the study were in good clinical condition, without signs of acute infection. The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (Resolution No. CDF / 0022 / KB1 / 111/13 of 10.22.2013 year) and written consent from parents or legal guardians, and / or patients was obtained.

Laboratory tests

Blood samples (3-5 ml) for laboratory tests were drawn in Eppendorf tubes in the morning (8.00-9.00) during examination related to periodic control in out-patients clinic. After centrifugation 1000x for 15 min at 4 ° C, the serum was stored at -20 ° C until assayed. Urine samples (50-100 ml) were collected at the same time as the blood samples, and also kept at -20 ° C until evaluated. Determination of concentrations of IL-18 and AGT was performed in the Chair and Department of Medical and Molecular Biology, SMDZ in Zabrze, SUM in Katowice.

The concentration of IL-18 in serum and urine was performed by ELISA using a set of e-Bioscence (USA) according to the manufacturer's protocol. Determination of concentrations of AGT was carried out using a kit from Diaclone (France) according to the manufacturer's protocol. In children with a history of HUS panel of routine laboratory tests were collected, and estimated glomerular filtration rate (eGFR) was calculated by the Schwartz formula [ml/min / 1.73 m ^{2}]. Albuminuria [mg/day] was evaluated using 24h urine collection. The relationship between the two markers and anthropometric measurements, the value of eGFR and albuminuria (expressed as albumin / creatinine ratio and daily urinary albumin excretion) have been evaluated.

Statistical analysis

The database was prepared in an Excel spreadsheet from Microsoft. For statistical calculations licensed version 10.0 software Statistica (StatSoft Inc., USA) was used. In the statistical analysis the level of significance p = 0.05 was set. The arithmetic mean, median, minimum and maximum value, lower and upper quartile and standard deviation were chosen as the parameters of descriptive statistics. For all parameters the compatibility of their distributions with a normal distribution was checked using the Shapiro-Wilk test.

Homogeneity of variations was tested by Levene test. For variables with normal distribution parametric tests were used with a separate estimate of the variance. Mann - Whitney nonparametric test was used for comparisons of variables with distribution diverged from the normal one. In the analysis of correlation Pearson's test or Spearman's rank correlation test was conducted - according to the distribution of the considered variables.

Results:

Anthropometric measurements and the age of the children are presented in Table I. The mean age did not differ significantly between the study group and the control group. The mean age of children in the study group at HUS onset was 3.4 ± 3.5 years, while the mean time from the HUS onset with acute renal failure until the current examination was 4.9 ± 3.9 years. The mean weight, height and BMI in the study group did not differ significantly from the values in comparison group. The results of laboratory tests in the group of children after HUS are reported in Table II. Increased ACR (122.0 ± 378,7mg/g) and 24h albumin excretion was noted. The mean value of eGFR in the study group was 96.5 ± 19.8 ml/min/1.73m^2 and in the majority of children remained within the normal range. Concentrations of the examined markers (AGT, IL-18) in serum and urine are shown in Table III. Children with HUS had significantly higher concentrations of AGT in serum and urine as compared to healthy children. The concentration of IL-18 was significantly lower in the serum of children in the study group compared to the control group, whereas there was no difference between IL-18 level in the urine between the study group and the control group. A negative correlation between serum AGT levels and ACR was documented. The concentration of IL-18 in serum and urine did not correlate with anthropometric and biochemical parameters in children after HUS. **To eliminate the influence of results obtained in children early after HUS acute phase, we have evaluated additionally the data from 24 children, who were sampled more than 6 months after HUS in which calculation was repeated (data not shown). We have obtained similar results as presented in tables with a negative correlation between serum AGT level in blood with ACR. Negative correlation between MAP (mean arterial pressure) and eGFR was additionally demonstrated (r=-0.4143, p=0.044).**

Discussion

Renin-angiotensin-aldosterone system (RAS) as a system of hormone-enzyme properties controls the circulating blood volume and regulates the concentration of sodium and potassium ions in body fluids. The first of studied markers – AGT is a prohormone

involved in RAS, produced in the liver and kidneys. In the renal tissue AGT is produced primarily by proximal tubular cells. Inside the lumen of the collecting ducts AGT can be converted to angiotensin I and II, by locally produced renin, prior to reaching the final urine (Sparks et al. 2014). To our knowledge, in the current study for the first time AGT levels in serum and urine in children with a history of HUS have been evaluated.

Intrarenal expression of AGT increases in experimental models of hypertension (Urushihara et al. 2009,Kuroczycka-Saniutycz et al. 2013, Kobori et al. 2010). This increase was expressed by the activation of the AT1 receptor in the renal tubular cells via angiotensin II (Kobori et al. 2002, 2003). AGT elevation detected exclusively in serum does not provide the direct evidence for its increased renal production. Numerous studies have suggested that AGT excretion in the urine may be a reliable marker of the activity of the intrarenal RAS (Kobori et al. 2003, 2008, 2009). There are many reports that proved the relationship between AGT excretion in the urine and severity of CKD (Kobori et al. 2008,Yamamoto et al. 2007, Mills et al. 2012). Higher concentration of AGT in urine has also been demonstrated in patients with diabetic nephropathy (Fishea 2015,Satirapoj et al. 2014,Kamiyama et al. 2012), in the course of the membranous glomerulonephritis (Urushihara et al. 2011), after kidney transplantation (Mas et al. 2011), and in IgA nephropathy (Urushihara et al. 2015). Kobori et al. in another study showed that AGT levels in urine positively correlated with systolic and diastolic blood pressure in patients with hypertension not treated with ACE inhibitors (Urushihara et al. 2009). The results of our study in children with a history of HUS clearly indicate that AGT levels in serum and urine obtained after acute phase of the disease are significantly higher. Therefore, it seems that AGT determination as a predictor of progression of CKD, is potentially useful in children after acute kidney injury in the course of HUS, and can also help to choose the optimal antihypertensive therapy. In our study, a significantly higher concentration of AGT in serum and urine was also found in children treated with ACE-I. Kobori et al reported in another study that AGT excretion in the urine increases in adult patients with chronic kidney disease and additionally positively correlates with albuminuria and negatively with the value of eGFR (Kobori et al. 2008). In our study there was a **no** correlation between above mentioned parameters. Children, however, presented only a slight degree of kidney function deterioration and most of them were treated with ACE-I. Yamamoto et al. have shown that the level of AGT in urine was significantly higher in patients with a low eGFR and proteinuria (Yamamoto et al. 2007). Mills et al. demonstrated in adult patients with CKD the negative correlation between AGT levels in the urine and the value of the eGFR. In addition, they demonstrated that the level of AGT in the urine is

independent of the value of albuminuria. AGT presence in urine may also be conditioned by leakage of protein through the slit membrane, when glomerular proteinuria is present (Mills et al. 2012). Thus, the study mentioned above indicates the role of AGT excreted in the urine as a possible marker for the detection of early stages of renal impairment. Our study in children with a history of HUS also confirms this hypothesis, but certainly there is a need for more clinical trials, especially with longitudinal design, provided in larger groups of patients, including pediatric population. Small number of examined children is the limitation of the study.

IL-18 was first described in 1989 as a "factor inducing IFN gamma". In 1995 it was named interleukin-18. This cytokine belongs to the family of IL-1 (Okamura et al. 1995) and is synthesized as an inactive precursor, then converted to the active form by the caspase-1 (Dinarello et al. 2013). In addition, the active form of IL-18 is also released during cell apoptosis (Bossaller et al. 2012). The receptor for IL-18 is situated on T cells, dendritic cells, mast cells, basophils, macrophages, neutrophils, cells, "natural killer" (NK) cells, endothelial cells and smooth muscle cells (Boraschi and Dinarello 2006, Dinarello 2007). It has been shown that IL-18 is involved in the pathogenesis of many diseases - including its confirmed activity in inflammatory bowel diseases (Pizarro et al. 1999, Siegmund 2010, Monteleone et al. 1999, Banerjee and Bond 2008), obesity (Netea et al. 2006, Zorrilla et al. 2007), and heart diseases (Platis et al. 2008, Woldbaek et al. 2005, Pomerantz et al. 2001, Mallat et al. 2004, Raeburn et al. 2002). There are also reports about the protective role of elevated levels of IL-18 in certain disease conditions (Dinarello et al. 2013, Doyle et al. 2012, Campbell et al. 2014).

The usefulness of IL-18 evaluation in serum and urine as an additional marker of the early stages of renal damage was explored in our study as well. It was shown that the serum IL-18 in children who have previously had HUS and acute renal failure is significantly lower than in controls. However, we found no difference in the concentrations of IL-18 in the urine in patients with a history of HUS as compared to the control group of healthy children.

It has been known for a long time that CKD regardless of its cause, is accompanied by inflammation, and the intensity of inflammation correlates negatively with renal function. The role of inflammation mediators, including pro-inflammatory cytokines (with IL-18 among them) in the progression of CKD is constantly discussed. In the literature there are only scarce reports on the role of IL-18 in the development of CKD (45). The most important and the earliest discovered function of IL-18 is its action as a part of a Th1-type immune response or the ability to induce IFN-gamma by Th1 and NK cells. IL-18 is also involved in the immune

response of Th2 type inducing the production of IL-4 and IL-13 in Th2 cells, NK cells, as well as in mast cells and basophils. (Nakanishi et al. 2991, Gołąb et al. 2001). IL-18 may also stimulate the population of Th17 cells to produce IL-17, thus promoting the autoimmune response. (Coccia et al. 2012,Lalor et al. 2011). Liang et al did not exclude that IL-18 might play a role in the process of interstitial fibrosis and tubular atrophy (renal tubulointerstitial fibrosis - TIF) through the activation of tubular epithelial cells as the result of their injury (Liang et al 2007). The role of IL-18 in the pathogenesis of renal disease is not fully understood, but it appears that it is more the marker of acute injury than the marker of CKD. Liu et al argue that increased concentrations of IL-18 in the urine could be a useful biomarker of acute kidney injury (Liu et al. 2013). On the other hand, in another report the authors are proving that IL-18 has a poor ability to predict acute renal failure and should not be used as a marker for predicting acute renal injury (Nisula et al. 2015).

In some pathologies IL-18 may act as a protective factor (Dinarello et.al 2013). In our study, IL-18 serum levels in children after HUS were significantly lower as compared to healthy children. This may also reflect the loss of the protective role of IL-18 after acute kidney injury in the course of HUS. **However the limitation of our study is that based on presented data no HUS specific trend in IL-18 and AGT development can be suggested.**

Conclusions

The results indicate that the concentration of angiotensinogen in serum and urine in children with a history of hemolytic-uremic syndrome increases, which may indicate the activation of the intrarenal RAS. **The statement**, that angiotensinogen may be a good biomarker of chronic kidney damage after acute kidney injury due to HUS **requires further studies with prospective follow-up from the acute phase of the disease on a larger group of patients.** Reduced IL-18 serum concentration in children after HUS with no difference in its urine concentration may indicate a loss of the protective effects of this cytokine on renal function due to previously occurred illness.

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Table I. Clinical characteristics of children evaluated after HUS and from control group

Parameter	HUS group			Control group
	Total group (n=29)	Girls (n=9)	Boys (n=20)	Total group (n=21)
Age (years)	8.4 ± 4.3 (0.9–16.7)	10.0 ± 4.3 (3.7–15.2)	7.6 ± 4.2 (0.9 – 16.7)	8.4 ± 4.1 (0.9 – 17.2)
Height (cm)	128.7 ± 25.5 (80 – 176)	142.5 ± 23.7 (106 – 168)	122.5 ± 24.3* (80 – 176)	128.2± 25.2 (76 – 172)
Height SDS	0.45 ± 0.93 (-1.86–1.92)	0.92 ± 0.73 (-0.16 – 1.92)	0.35 ± 1.30 (-1.86 – 1.6)	-0.06 ± 1.10 (-1.53 – 2.35)
Body weight (kg)	30.3 ± 17.1 (7.4 – 66)	42.5 ± 19.8 (17.3 – 66)	24.7 ± 12.7* (7.4 – 62)	31.9 ± 16.5 (9.7 – 63)
Weight SDS	0.15 ± 0.82 (-1.63 – 2.04)	0.85 ± 0.68 (-0.36 – 2.04)	-0.17 ± 0.68* (-1.63 – 1.3)	0.11 ± 1.3 (-1.87– 2.12)
BMI (kg/m^2)	16.7 ± 3.6 (10.0-28.6)	19.6 ± 4.7 (14.7– 28.5)	15.4 ± 2.0* (10 – 20)	15.4 ± 2.08 (10 - 20)
BMI SDS	-0.20 ± 1.00 (-2.36 – 2.08)	0.45 ± 0.86 (-0.68– 2.08)	-0.52 ± 0.92* (-2.36 – 1.01)	0.32 ± 1.02 (-1.22– 2.29)
Age at HUS onset (years)	3.4 ± 3.5 (0.2– 13.4)	5.0 ± 4.7 (0.9 -13.4)	2.7 ± 2.6 (0.2 – 11.1)	
Time after HUS (years)	4.9 ± 4.0 (0.04–13.9)	5.0 ± 5.0 (0.05 - 13.8)	4.9 ± 3.5 (0.04 – 11)	

Data are presented as mean ± standard deviation (minimum – maximum), HUS – hemolytic-uremic syndrome, BMI – body mass index.

*$p<0.05$ girls after HUS vs. boys after HUS,

Table II. Biochemical parameters eGFR and blood pressure values in children after HUS

PARAMETER	HUS total group (n=29)	HUS girls (n=9)	HUS boys (n=20)	aHUS	typical HUS
SERUM					
GPT (U/l)	14.31 ± 7.34 (8.3 – 47.3)	13.13 ± 3.48 (8.3 – 19.4)	14.84 ± 8.56 (8.5 – 47.3)	13.8 ± 4.4 (8.3 – 25.5)	14.78 ± 9.46 (8.5 – 47.3)
Albumin (g/l)	45.1 ± 4.34 (34.89 –54.71)	44.08 ± 4.52 (36.51 –50.58)	45.56 ± 4.3 (34.89–54.71)	44.1 ± 3.87 (36.51–50.58)	46.32 ± 4.7 (34.89 – 54.71)
Total proteins (g/l)	68.87 ± 5.45 (56.2 – 76.9)	70.54 ± 3.76 (63.1 – 76.9)	68.88 ± 5.39 (56.2 – 76.9)	69.35 ± 6.05 (56.2 – 76.9)	69.44 ± 3.86 (61.9 – 76.1)
Total cholesterol (mmol/l)	4.18 ± 0.88 (2.84 – 5.95)	4.02 ± 0.8 (2.99 – 5.58)	4.25 ± 0.92 (2.84 – 5.95)	3.98 ± 0.87 (2.84 – 5.58)	4.37 ± 0.88 (3.19 – 5.95)
HDL -cholesterol (mmol/l)	1.74 ± 0.33 (1.13 – 2.24)	1.49 ± 0.25 (1.15 – 1.73)	1.84 ± 0.32 (1.13 – 2.24)	1.6 ± 0.25 (1.13 – 1.88)	1.88 ± 0.36 (1.15 – 2.24)
LDL -Cholesterol (mmol/l)	2.1 ± 0.71 (1.31 – 3.59)	2.96 ± 0.5 (2.35 – 3.59)	1.76 ± 0.42 (1.31 – 2.46)	2.19 ± 0.83 (1.32 – 3.59)	2.01 ± 0.62 (1.31 – 2.95)
Triglycerides (mmol/l)	1.12 ± 0.59 (0.5 – 2.91)	1 ± 0.32 (0.6 – 1.58)	1.17 ± 0.68 (0.5 – 2.95)	1.18 ± 0.78 (0.5 – 2.91)	1.05 ± 0.33 (0.59 – 1.7)
Creatinine (umol/l)	50.27 ± 11.95 (34 – 82)	53.44 ± 12.02 (34 – 73)	48.85 ± 11.95 (34 – 82)	53.1 ± 14.2 (34-82)	47.67 ± 9.14 (34 – 64)
Uric acid (umol/l)	251 ± 61.7 (123-386)	260.9 ±74.0 (195 – 386)	247.9 ± 56.9 (123 – 369)	259.4 ± 74.9 (123 – 286)	244.9 ± 48.4 (174 – 328)
Urea (mmol/l)	4.3 ± 1.97 (2.1 – 11.2)	3.63 ± 1.12 (2.5 – 5.7)	4.61 ± 2.21 (2.1 – 11.2)	5.12 ± 2.41 (2.5 – 11.2)	3.54 ± 1.03 **#** (2.1 – 5.8)
URINE					
Daily albumin excretion (mg/24h)	52.69 ±108.37 (1.65 –477.75)	26.99 ± 25.99 (6.62 – 76.99)	62.97 ± 127.04 (1.65 – 477.7)	81.45 ± 161.6 (10.16– 477.7)	35 ± 58.72 (1.65 – 195.12)
Albuminuria (mg/l)	56.46± 157.26 (4.18 – 735)	17.4 ± 19.35 (4.41 – 54.99)	71.11 ± 183.46 (4.18 – 735)	106.47±254.46 (5.4 – 735)	27.89 ± 51.63 (4.18 – 186.55)
ACR (mg/g)	122.0 ± 378.7 (7.1 –1800.3)	40.4 ± 33.8 (10.0– 100.9)	152.6± 443.5 (7.1–1800.3)	257.6± 624.1 (13.5–1800.3)	44.6 ± 64.8 (7.1 – 208.6)
eGFR (ml/min/1.73 m^2)	96.53 ± 19.84 (38.25 – 127.78)	99.29 ± 14.38 (80.27 – 119.19)	95.28 ± 22.08 (38.29 – 127.78)	90.43 ± 22.44 (38.29 – 119.19)	102.22 ± 15.75 (73.02–127.78)
BLOOD PRESSURE					
SBP SDS	0.57 ± 1.22 (-1.29 – 3.49)	1.07 ± 1.09 (-0.52–2.97)	0.36 ± 1.24 (-1.29–3.49)	0.83 ± 1.36 (-1.29–3.49)	0.34 ± 1.06 (-0.98–2.97)
DBP SDS	0.41 ± 1.07 (-1.18–3.05)	0.44 ± 1.01 (-1.13–2.15)	0.40 ± 1.13 (-1.18–3.05)	0.66 ± 1.19 (-0.92–3.05)	0.18 ± 0.92 (-1.18–1.8)
MAP (mmHg)	76.9 ± 8.9 (61.7 – 99.3)	82.4 ± 10.2 (69.7 – 99.3)	74.5 ± 7.4 ***** (61.7 – 87.7)	78.83 ± 8.33 (64.33 – 91.67)	75.16 ± 9.41 (61.67 – 99.33)

Data are presented as mean ± standard deviation (minimum – maximum),

HUS – hemolytic-uremic syndrome, eGFR – estimated glomerular filtration rate, ACR - albumin/ creatinine ratio, SBP - systolic blood pressure, DBP - diastolic blood pressure, MAP - mean arterial pressure

*$p<0.05$ girls after HUS vs. boys after HUS,

#$p<0.05$ atypical HUS vs. typical HUS

Table III. Mean concentration of examined markers in children after HUS and in control group

Parameter	Children after HUS (n=29)	Girls after HUS (n=9)	Boys after HUS (n=20)	atypical HUS (n=15)	typical HUS (n=14)	Control group (n=21)
SERUM						
IL-18 (ng/ml)	50.55 ± 6.82 (36.88–60.12)	50.18 ± 6.29 (40.27–57.91)	50.71 ± 7.2 (36.88–60.12)	50.99 ± 6.28 (41.28–59.61)	50.13 ± 7.49 (36.88– 60.12)	75.25 ± 9.1* (60.57–98.72)
AGT (ng/ml)	3.84 ± 0.71 (2.18 – 5.23)	3.64 ± 0.84 (2.18 – 5.23	3.92 ± 0.64 (2.18 – 4.81)	3.7 ± 0.79 (2.18-4.81)	3.96 ± 0.62 (2.76 – 5.23)	2.42 ± 0.35* (1.87–3.11)
URINE						
IL-18 (ng/ml)	14.63 ± 3.35 (1.72–19.84)	15.48 ± 2.43 (10.46–17.9)	14.24 ± 3.67 (1.72 – 19.84)	15.4 ± 2.07 (12.41–19.8)	13.91 ± 4.15 (1.72–17.92)	13.41 ± 3.5 (9.87– 23.13)
AGT (ng/ml)	3.59 ± 0.63 (2.11 – 4.77)	3.73 ± 0.71 (2.67 – 4.77)	3.52 ± 0.60 (2.11 – 4.71)	3.67 ± 0.7 (2.11 – 4.77)	3.51 ± 0.58 (2.61 – 4.71)	2.31 ± 0.54* (1.78–3.71)

Data are presented as mean ± standard deviation (minimum – maximum), HUS – hemolytic-uremic syndrome,

IL-18 – interleukin-18, AGT - angiotensinogen

*p<0,05 children after HUS vs. control group

Table IV. Correlation between the studied markers of chronic kidney disease and the results of anthropometric measurements and biochemical parameters

Parameter	Examined children with HUS n=29 (HUS)			
	IL-18 serum	IL-18 urine	AGT serum	AGT urine
Body weight [kg]	r=-0,067 p=0,729	r=0,082 p=0,671	r=-0,01 p=0,957	r=-0,225 p=0,239
Height [cm]	r=-0,06 p=0,754	r=0,1 p=0,605	r=0,064 p=0,742	r=-0,236 p=0,216
Age [l]	r=0,009 p=0,962	r=0,076 p=0,692	r=0,145 p=0,453	r=-0,306 p=0,106
Age at HUS onset [l]	r=-0,026 p=0,172	r=0,023 p=0,905	r=-0,161 p=0,403	r=0,008 p=0,966
Time from HUS onset [l]	r=0,24 p=0,209	r=0,063 p=0,745	r=0,3 p=0,113	r=-0,34 p=0,071
MAP (mmHg)	r=-0,13 p=0,5	r=0,124 p=0,519	r=0,312 p=0,099	r=0,125 p=0,517
GPT (U/l)	r=-0,311 p=0,1	r=-0,106 p=0,583	r=-0,14 p=0,468	r=0,243 p=0,204
Serum albumin (g/l)	r=0,106 p=0,605	r=0,127 p=0,533	r=-0,084 p=0,68	r=-0,074 p=0,716
Total proteins (g/l)	r=0,124 p=0,519	r=-0,099 p=0,607	r=0,086 p=0,657	r=0,217 p=0,258
Total cholesterol (mmol/l)	r=-0,319 p=0,091	r=-0,019 p=0,919	r=-0,173 p=0,369	r=0,335 p=0,075
HDL cholesterol (mmol/l)	r=-0,012 p=0,967	r=-0,294 p=0,307	r=0,135 p=0,645	r=0,1 p=0,732
LDL cholesterol (mmol/l)	r=-0,081 p=0,783	r=0,072 p=0,804	r=-0,5 p=0,066	r=0,402 p=0,153
Triglycerides (mmol/l)	r=0,1 p=0,611	r=-0,059 p=0,764	r=0,238 p=0,221	r=0,288 p=0,137
Creatinine (umol/l)	r=-0,158 p=0,412	r=0,147 p=0,446	r=0,276 p=0,146	r=-0,08 p=0,669
Uric acid (umol/l)	r=-0,0003 p=0,999	r=0,092 p=0,632	r=0,27 p=0,157	r=0,206 p=0,283
Urea (mmol/l)	r=-0,039 p=0,838	r=0,07 p=0,716	r=0,15 p=0,435	r=0,046 p=0,812
ACR (mg/g)	r=-0,262 p=0,238	r=0,021 p=0,924	r=-0,456* **p=0,033**	r=0,162 p=0,471
eGFR (ml/ min/ 1,73m ^{2})	r=0,203 p=0,289	r=-0,059 p=0,76	r=-0,219 p=0,252	r= -0,109 p=0,57

* correlation coefficients are significant p <0.05

ACR - albumin/ creatinine ratio

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