L-FABP and IL-6 as markers of chronic kidney damage in children after hemolytic uremic syndrome

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Abstract

Background. Hemolytic-uremic syndrome (HUS) is a form of thrombotic microangiopathy, in the course of which some patients may develop chronic kidney disease (CKD). From a clinical point of view, it is important to search for markers that allow for early identification of patients at risk of a poor prognosis.

Objectives. The study evaluated the serum and urine levels of liver-type fatty acid binding protein (L-FABP) and interleukin 6 (IL-6).

Material and methods. The study was conducted in 29 children with a history of HUS. The relationship between L-FABP and IL-6 and anthropometric measurements, the value of estimated glomerular filtration rate (eGFR) and albuminuria were additionally evaluated.

Results. In children after HUS, L-FABP and IL-6 concentration in both serum and urine was significantly higher in comparison to the control group. No differences in L-FABP and IL-6 concentration in serum and urine depending on the type of HUS and gender were noted. Correlation between L-FABP and IL-6 in serum and urine with eGFR and urine albumin-creatinine ratio (ACR) in the total group of patients after HUS was not detected. In the group of children after 6 month observation after HUS, a negative correlation of L-FABP concentration with eGFR was found.

Conclusions. The results indicate that the higher concentration of L-FABP in serum and urine of children with a history of HUS can be the result of protracted injury initiated during the acute phase of the disease. Lack of correlation of L-FABP concentration with the ACR may be associated with a short (less than 6 months) observation after acute renal failure or merely temporary renal tubular damage in the acute phase of the disease. In contrast, higher levels of IL-6 in serum and urine in children after HUS compared to healthy children and the negative correlation of L-FABP concentration and eGFR in children after 6 month observation after HUS may confirm their participation in CKD. Thus, L-FABP and IL-6 seem to be good biomarkers of chronic kidney damage in survivors of the acute phase of HUS.

Key words: children, interleukin 6, chronic kidney disease, hemolytic-uremic syndrome, liver-type fatty acid binding protein

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Introduction

Hemolytic uremic syndrome (HUS) is the leading cause of acute kidney injury in previously healthy infants and younger children. It is characterized by a triad of symptoms – hemolytic anemia, thrombocytopenia, and acute kidney injury.¹ The most common form of HUS is a typical form, which traditionally is expected to represent up to 90% of cases of the disease.^{1–3} A factor responsible for its occurrence is a bacterial toxin called verotoxin (VTEC) or Shiga-like toxin, produced by enterohemorrhagic *Escherichia coli* strains (STEC).^{2,4–6}

Atypical hemolytic-uremic syndrome (aHUS) is a mixed group of disorders related to disturbances in the coagulation cascade and immune system.⁴ aHUS in most cases is characterized by abnormal activation of the complement system (the alternative pathway), caused by, among other factors, the mutation of genes encoding protein components of the complement system (C3 protein, factor H, I, and B, membrane cofactor protein – MCP, thrombomodulin) and the presence of antibodies against factor H. There are also mutations in other genes, e.g. mutations of the gene encoding diacylglycerol kinase (DGKE).⁷ Some patients may have more than one causative mutation in different genes.⁸ aHUS is characterized by a poor long-term prognosis, is burdened with significant mortality, reaching 25%, and 50% of the patients require chronic renal replacement therapy.^{1,9}

Prognosis in the typical form of HUS is much better since renal replacement therapy had become widely available and mortality does not exceed 10%. In the majority of patients, the recovery of renal function with normal glomerular filtration rate is observed. However, in some patients the disease has consequences which may lead to developing chronic kidney disease (CKD) and even end-stage renal failure (ESRF).¹⁰ Children with a history of HUS require continuous monitoring, since the features of reduced renal function may appear many years after the outbreak of the illness. Commonly used diagnostic tools such as serum creatinine measurement (with calculation of estimated glomerular filtration rate – eGFR), albuminuria assessment (including the urine albumin/creatinine ratio – ACR) or ultrasound examination of the kidneys do not give the opportunity for early detection of chronic kidney injury. There is a need to look for sensitive and specific markers that would be sufficient to identify initial stages of kidney damage. It seems that liver-type fatty acid binding protein (L-FABP) and interleukin 6 (IL-6) may be among those markers.

The first marker chosen by us for testing in the HUS group, whose predictive value in renal diseases has recently attracted considerable attention, was L-FABP. It is a cytoplasmic protein belonging to the family of fatty acid binding proteins (FABPs).^{11,12} Fatty acid binding proteins are responsible for binding to an excess of accumulated free long-chain fatty acids and directing them to appropriate intracellular sites of utilization.¹³

L-FABP facilitates fatty-acid metabolism via β -oxidation and causes the excretion of lipid peroxidation products, which result in attenuating the release of inflammatory factors and inhibiting damage of tubulointerstitial tissue. L-FABP belongs to effective endogenous antioxidants. This protein, due to its low molecular weight (14 kDa), high tissue specificity, abundance in the tissue and rate of release into the blood stream, can serve as a specific marker of kidney damage.¹⁴ The L-FABP gene is located on chromosome 2 and encodes 127 amino acids.¹⁵ L-FABP is present in proximal tubule cells of the kidney.¹⁶ The circulating fraction of L-FABP is filtered by the glomeruli and afterwards reabsorbed in the proximal renal tubules, which explains the increase of its concentration in the urine when proximal tubule cell injury occurs.^{17,18} The increase in the concentration of L-FABP excreted in the urine in the course of kidney disease was confirmed in numerous clinical trials in adult patients.^{19–21}

The second of the markers analyzed by us was IL-6, one of the main factors regulating the body's defense mechanisms via multidirectional action.¹⁷ The most important role of IL-6 function covers its involvement in the immune response, hematopoiesis, and inflammatory processes.^{17,22} Many clinical trials carried out in adults and children have confirmed the association of elevated levels of IL-6 with kidney disease severity, especially chronic kidney disease progression.^{23–25} The cause of this phenomenon is believed to be ongoing chronic inflammation within the kidney tissue, which is a characteristic feature of kidney damage due to different reasons.²⁶ Interleukin 6, one of the major pro-inflammatory human cytokines, is also involved in such processes, which may explain the increase in serum level of this cytokine in the course of chronic kidney damage.

Objectives

The aim of the study was to evaluate the concentration of L-FABP and IL-6 in serum and urine in children with a past history of HUS and to compare the obtained results to a control group of healthy age-matched children. The relationship between the 2 selected markers and anthropometric measurements, the value of estimated glomerular filtration rate (eGFR) and albuminuria (expressed as ACR and daily urinary albumin excretion) were also evaluated.

Material and methods

The study group (HUS) consisted of 29 patients (9 girls and 20 boys) aged 1 to 15 years with a past history of HUS treated at the Department and Clinic of Pediatrics in Zabrze, Medical University of Silesia in Katowice. The children were also divided into 2 groups regarding the cause of HUS: typical HUS – 14, and atypical HUS – 15 children. In the group of children with a history of HUS,

86% required renal replacement therapy in the acute phase of the disease; 62% – were treated with peritoneal dialysis, 14% – hemodialysis, 10% – both peritoneal dialysis and hemodialysis and 14% of the children did not require dialysis. The mean anuria period observed in 12 children lasted for 4.6 ± 4.0 days. Mean acute kidney injury (AKI) duration was 28.6 ± 15.0 days (range 2–60 days) with final mean eGFR 71.9 ± 23.6 mL/min/1.73 m². Mean duration of renal recovery was 37.2 ± 21.4 days with mean eGFR at renal recovery of 95.0 ± 34.3 mL/min/1.73 m². Two children progressed to CKD. All the children from the study group were treated pharmacologically: diuretics (4 children), calcium channel blocking agents (21 children), angiotensin converting enzyme inhibitors (ACEI) (14 children), supplementation of alkali (4 children), iron formulas (1 child), and folic acid (8 children). On admission, weight, height and blood pressure were taken and routine biochemical tests were performed. Body mass index (BMI) was calculated using the formula BMI = body weight (kg)/height (m²). Estimated glomerular filtration rate (eGFR) was calculated according to the Schwartz formula (mL/min/1.73 m²).²⁷ Additionally, albuminuria (mg/day) using 24 h urine collection was evaluated.

The control group consisted of 21 healthy children (11 girls and 10 boys) aged 1–15 years with monosymptomatic nocturnal enuresis or presented with surgical procedures of one-day surgery, without any signs of kidney diseases. 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) and written consent from parents or legal guardians, and/or the patients was obtained.

Anthropometric measurements and the age of the study subjects and controls are presented in Table 1. The average age, weight, height and BMI in the study group and the control group did not differ significantly. The average

age of children in the study group at HUS onset was 3.42 ± 3.49 years, while the average time from the HUS outbreak and acute kidney injury until the current examinations was 4.94 ± 3.94 years (range 1 month–13.9 years), including 24 from 29 patients more than 6 months after the onset of acute phase of the disease..

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 the outpatient clinic. After centrifugation $1000 \times$ 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 L-FABP and IL-6 was performed in the Chair and Department of Medical and Molecular Biology, SMDZ in Zabrze, SUM in Katowice.

The concentration of IL-6 in serum and urine was performed by ELISA using a Diaclone (Besancon Cedex, France) set according to the manufacturer's protocol. Determination of concentrations of L-FABP was carried out using a kit from BioVendor (Brno, Czech Republic) according to the manufacturer's protocol.

Statistical analysis

A database was prepared in a Microsoft Excel spreadsheet. For statistical calculations, licensed v. 10.0 STATISTICA software (StatSoft Inc., Tulsa, USA) was used. In the statistical analysis, the level of significance at $p \leq 0.05$ was assumed. As the parameters of descriptive statistics, the arithmetic mean, median, minimum and maximum value, lower and upper quartile and standard deviation were chosen. For all parameters, the compatibility of their distributions with a normal distribution were checked with a Shapiro-Wilk test. For variables with

Table 1. 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)	atypical HUS (n = 15)	typical HUS (n = 14)	total group (n = 21)
Age [years]	8.4 ± 4.3	10.0 ± 4.3	7.6 ± 4.2	7.7 ± 5.1	9.0 ± 3.4	8.4 ± 4.1
Height [cm]	128.7 ± 25.5	142.5 ± 23.7	$122.5 \pm 24.3^*$	125.8 ± 29.7	131.4 ± 21.6	128.2 ± 25.2
Height SDS	0.45 ± 0.93	0.92 ± 0.73	0.35 ± 1.30	0.47 ± 1.14	0.43 ± 0.75	-0.06 ± 1.10
Body weight [kg]	30.3 ± 17.1	42.5 ± 19.8	$24.7 \pm 12.7^*$	29.5 ± 19.0	30.9 ± 15.7	31.9 ± 16.5
Weight SDS	0.15 ± 0.82	0.85 ± 0.68	$-0.17 \pm 0.68^*$	0.25 ± 0.92	0.07 ± 0.74	0.11 ± 1.3
BMI [kg/m ²]	16.7 ± 3.6	19.6 ± 4.7	$15.4 \pm 2.0^*$	16.7 ± 3.6	16.8 ± 3.8	15.4 ± 2.08
BMI SDS	-0.20 ± 1.00	0.45 ± 0.86	$-0.52 \pm 0.92^*$	0.04 ± 0.86	-0.42 ± 0.09	0.32 ± 1.02
Age at HUS onset [years]	3.4 ± 3.5	5.0 ± 4.7	2.7 ± 2.6	3.4 ± 3.9	3.4 ± 3.1	
Time after HUS [years]	4.9 ± 4.0	5.0 ± 5.0	4.9 ± 3.5	4.3 ± 4.3	5.6 ± 3.7	

Data is presented as mean $\pm$ standard deviation; HUS – hemolytic-uremic syndrome; BMI – body mass index; SDS – standard deviation score; $p > 0.05$ HUS vs control group; * $p < 0.05$ girls after HUS vs boys after HUS.

normal distribution, parametric tests were used (t-test for independent variables in comparative analyses and Pearson's test for analyses of correlation). For other variables, nonparametric tests were applied (Mann-Whitney U-test for comparisons and Spearman's rank correlation test for analyses of correlation).

Results

The results of laboratory tests and mean arterial pressure values in the group of children after HUS are reported in Table 2. In children with a history of HUS, daily urine albumin excretion of 52.69 ± 108.4 mg/24 h and expressed as ACR 122.0 ± 378.7 mg/g were noted. The average value of eGFR in the study group was 96.5 ± 19.8 mL/min/1.73 m², and in the majority of children remained within the normal range. Girls after HUS had higher mean arterial pressure than boys after HUS. Children with atypical HUS showed higher values of serum urea as compared to children with typical HUS.

Serum and urine concentrations of L-FABP and IL-6, as the CKD markers in the group of children after HUS, are shown in Table 3. A significantly higher concentration of L-FABP in serum and urine was found as compared to healthy children. IL-6 level was also significantly higher in serum and urine in the study group in contrast to the control group. In the whole HUS group, no correlation between the studied markers in serum and urine and eGFR or albuminuria expressed as ACR was found, except the value of eGFR at the end of AKI, which positively correlated with serum IL-6 concentration (Table 4). For additional analyses, the subgroup of children after HUS limited to subjects with a minimal time period since resolution of the disease onset until sample collection established at 6 months was extracted. In this subgroup (n = 24), a negative correlation between the concentration of L-FABP in the serum and eGFR, as well as between the concentration of L-FABP in urine and eGFR were documented (Fig. 1, 2). The concentration of L-FABP in serum and urine positively correlated with standard deviation score (SDS) for growth in children with a history of HUS,

Table 2. Biochemical parameters and mean arterial pressure in children after HUS

Parameter	HUS total group (n = 29)	HUS girls (n = 9)	HUS boys (n = 20)	aHUS (n = 15)	Typical HUS (n = 14)
Serum albumin [g/L]	45.1 $\pm$ 4.34	44.08 $\pm$ 4.52	45.56 $\pm$ 4.3	44.1 $\pm$ 3.87	46.32 $\pm$ 4.7
Total proteins [g/L]	68.87 $\pm$ 5.45	70.54 $\pm$ 3.76	68.88 $\pm$ 5.39	69.35 $\pm$ 6.05	69.44 $\pm$ 3.86
Total cholesterol [mmol/L]	4.18 $\pm$ 0.88	4.02 $\pm$ 0.8	4.25 $\pm$ 0.92	3.98 $\pm$ 0.87	4.37 $\pm$ 0.88
Triglycerides [mmol/L]	1.12 $\pm$ 0.59	1 $\pm$ 0.32	1.17 $\pm$ 0.68	1.18 $\pm$ 0.78	1.05 $\pm$ 0.33
Creatinine [mmol/L]	50.27 $\pm$ 11.95	53.44 $\pm$ 12.02	48.85 $\pm$ 11.95	53.1 $\pm$ 14.2	47.67 $\pm$ 9.14
Uric acid [mmol/L]	251 $\pm$ 61.7	260.89 $\pm$ 74.0	247.85 $\pm$ 56.9	259.36 $\pm$ 74.9	244.9 $\pm$ 48.4
Urea [mmol/L]	4.3 $\pm$ 1.97	3.63 $\pm$ 1.12	4.61 $\pm$ 2.21	5.12 $\pm$ 2.41	3.54 $\pm$ 1.03 [#]
Daily urine albumin excretion [mg/24 h]	52.69 $\pm$ 108.4	26.99 $\pm$ 25.99	62.97 $\pm$ 127.0	81.45 $\pm$ 161.6	35 $\pm$ 58.72
Albuminuria [mg/L]	56.46 $\pm$ 157.3	17.4 $\pm$ 19.35	71.11 $\pm$ 183.5	106.47 $\pm$ 254.46	27.89 $\pm$ 51.63
ACR [mg/g]	122.04 $\pm$ 378.69	40.43 $\pm$ 33.77	152.6 $\pm$ 443.5	257.6 $\pm$ 624.1	44.6 $\pm$ 64.83
eGFR [mL/min/1.73 m ²]	96.53 $\pm$ 9.84	99.29 $\pm$ 14.38	95.28 $\pm$ 2.08	90.43 $\pm$ 2.44	102.2 $\pm$ 15.75
Mean arterial pressure [mm Hg]	76.93 $\pm$ 8.94	82.4 $\pm$ 10.15	74.47 $\pm$ 7.35*	78.83 $\pm$ 8.33	75.16 $\pm$ 9.41

Data is presented as mean $\pm$ standard deviation (min–max); HUS – hemolytic-uremic syndrome; ACR – urine albumin/creatinine ratio; eGFR – estimated glomerular filtration rate; * p < 0.05 girls after HUS vs boys after HUS; [#] p < 0.05 aHUS vs typical HUS.

Table 3. Mean concentration of examined markers in children after HUS and in control group

Parameter	Children after HUS (n = 29)	Girls after HUS (n = 29)	Boys after HUS (n = 29)	aHUS	Typical HUS	Control group (n = 21)
Serum IL-6 [ng/mL]	79.96 $\pm$ 26.68 (32.9–161.3)	65.7 $\pm$ 22.51 (32.9–93.9)	86.39 $\pm$ 26.38 (39.6–161.3)	82.18 $\pm$ 30.44 (32.9–161.3)	77.89 $\pm$ 23.55 (39.6–101.6)	7.34 $\pm$ 1.43* (4.77–9.61)
Urine IL-6 [ng/mL]	97.64 $\pm$ 15.46 (73.8–121.5)	97.62 $\pm$ 16.96 (73.8–117.6)	97.65 $\pm$ 15.2 (39.6–161.3)	102.3 $\pm$ 13.53 (75.9–121.5)	93.27 $\pm$ 16.29 (73.8–118.9)	36.87 $\pm$ 12.01* (18.6–55.7)
Serum L-FABP [ng/mL]	72.49 $\pm$ 18.53 (44.3–100.1)	75.61 $\pm$ 16.75 (49.9–93.8)	71.08 $\pm$ 19.52 (44.3–100.1)	74.66 $\pm$ 18.33 (48.5–96.6)	70.47 $\pm$ 19.11 (44.3–100.1)	2.65 $\pm$ 0.75* (1.1–3.75)
Urine L-FABP [ng/mL]	11.54 $\pm$ 4.32 (4.9–7.6)	11.80 $\pm$ 3.51 (5.8–15.7)	11.42 $\pm$ 4.72 (4.7–19.6)	11.62 $\pm$ 3.74 (5.9–16.7)	11.46 $\pm$ 4.93 (4.7–19.6)	2.76 $\pm$ 0.63* (1.9–4.1)

Data is presented as mean $\pm$ standard deviation (min–max); HUS – hemolytic-uremic syndrome; L-FABP – liver-type fatty acid binding protein;

* p < 0.05 children after HUS vs control group.

Table 4. Correlation between studied markers of chronic kidney disease and results of anthropometric measurements and biochemical parameters

Parameter	Examined children with HUS n = 29 (HUS)			
	IL-6 serum	IL-6 urine	L-FABP serum	L-FABP urine
Body weight [kg]	r = -0.009 p = 0.951	r = -0.04 p = 0.782	r = -0.05 p = 0.752	r = 0.0006 p = 0.997
Height [cm]	r = 0.034 p = 0.817	r = 0.009 p = 0.948	r = -0.029 p = 0.843	r = -0.004 p = 0.979
Age [years]	r = 0.112 p = 0.44	r = 0.073 p = 0.615	r = 0.022 p = 0.882	r = -0.079 p = 0.584
Age at HUS onset [years]	r = 0.07 p = 0.715	r = 0.168 p = 0.385	r = 0.029 p = 0.493	r = 0.189 p = 0.327
Time from HUS onset [years]	r = 0.095 p = 0.623	r = -0.17 p = 0.376	r = -0.133 p = 0.493	r = -0.12 p = 0.535
MAP [mm Hg]	r = -0.206 p = 0.283	r = -0.157 p = 0.416	r = 0.088 p = 0.65	r = -0.029 p = 0.882
GPT [U/L]	r = -0.007 p = 0.973	r = -0.036 p = 0.852	r = -0.103 p = 0.597	r = -0.057 p = 0.768
Serum albumin [g/L]	r = 0.299 p = 0.138	r = -0.08 p = 0.695	r = -0.132 p = 0.519	r = -0.0007 p = 0.997
Total proteins [g/L]	r = -0.21 p = 0.274	r = 0.147 p = 0.446	r = -0.011 p = 0.953	r = 0.219 p = 0.254
Total cholesterol [mmol/L]	r = -0.215 p = 0.262	r = -0.367 p = 0.05	r = -0.119 p = 0.54	r = -0.0005 p = 0.998
Triglycerides [mmol/L]	r = -0.138 p = 0.483	r = -0.211 p = 0.280	r = 0.215 p = 0.270	r = 0.205 p = 0.295
Creatinine [mmol/L]	r = 0.035 p = 0.858	r = -0.264 p = 0.166	r = 0.055 p = 0.777	r = 0.103 p = 0.593
Uric acid [mmol/L]	r = -0.046 p = 0.813	r = -0.134 p = 0.487	r = 0.058 p = 0.763	r = 0.057 p = 0.770
Urea [mmol/L]	r = -0.064 p = 0.741	r = -0.115 p = 0.553	r = 0.168 p = 0.385	r = 0.069 p = 0.720
ACR [mg/g]	r = -0.146 p = 0.515	r = 0.156 p = 0.487	r = -0.084 p = 0.712	r = -0.119 p = 0.598
eGFR [mL/min/1.73 m ²]	r = 0.01 p = 0.957	r = 0.187 p = 0.332	r = -0.245 p = 0.2	r = -0.261 p = 0.171
AKI time [days]	r = -0.1433 p = 0.458	r = -0.2717 p = 0.154	r = -0.0975 p = 0.615	r = -0.1604 p = 0.406
eGFR at the end of AKI [mL/min/1.73 m ²]	r = 0.4661* p = 0.011*	r = 0.242 p = 0.206	r = -0.3496 p = 0.063	r = -0.2419 p = 0.206
Renal recovery time [days]	r = -0.2842 p = 0.151	r = 0.0012 p = 0.995	r = -0.0344 p = 0.865	r = -0.043 p = 0.831
eGFR renal recovery [mL/min/1.73 m ²]	r = 0.3145 p = 0.110	r = 0.3642 p = 0.062	r = 0.2384 p = 0.231	r = 0.3503 p = 0.073

* significant correlation coefficients $p < 0.05$; AKI – acute kidney injury; HUS – hemolytic-uremic syndrome; L-FABP – liver-type fatty acid binding protein; MAP – mean arterial pressure; GPT – glutamic pyruvic transaminase; ACR – urine albumin/creatinine ratio; eGFR – estimated glomerular filtration rate.

in which the time since the disease resolution was longer than 6 months ($r = 0.5534$; $p < 0.005$ and $r = 0.5194$; $p < 0.01$, respectively).

Discussion

A large number of reports published in recent years has proved that the concentration of L-FABP in urine could serve as a useful marker for the diagnosis of early stage

kidney damage, especially acute kidney injury (AKI).^{21,28} L-FABP is a protein found in the cytoplasm of the proximal tubule cells in the kidney, both healthy and injured.¹⁵ Various pathological conditions such as proteinuria, hyperglycemia, hypertension and toxin-induced injury to the proximal tubule cells may result (directly or through the regulation of gene expression) in the increase of the excretion of urine derived L-FABP.^{11,15} Recent studies have demonstrated that L-FABP can play an important role in injury and repair processes in the kidneys, and that

Fig. 1. Analysis of correlation results in examined children (> 6 months after HUS)

HUS – hemolytic-uremic syndrome; L-FABP – liver-type fatty acid binding protein; eGFR – estimated glomerular filtration rate.

Fig. 2. Analysis of correlation results in examined children (> 6 months after HUS)

HUS – hemolytic-uremic syndrome; L-FABP – liver-type fatty acid binding protein; ACR – urine albumin/creatinine ratio; eGFR – estimated glomerular filtration rate.

the monitoring of urine L-FABP concentration may make it possible to predict the occurrence and severity of various renal diseases.^{21,29} In the literature there is no data on the urine/serum concentration of L-FABP in children with HUS. In our retrospective study, we assessed in a single evaluation the concentration of L-FABP after the acute phase of HUS, and a significant increase in both plasma (30-fold) and urine (5-fold) concentrations was recorded. A negative correlation of L-FABP concentration and eGFR in the group of children with more than 6 month observation after HUS may confirm L-FABP participation in CKD. Single evaluation is a limitation of our study. Further studies are needed, with prospective assessment of the parameters in a few time intervals to confirm that patients with the highest concentration of L-FABP shortly after HUS should present decreasing eGFR in the longer follow-up. We also did not find a relationship between AKI or renal recovery time duration and FABP serum and

urine concentration, which also could be the result of only single measurement.

Parr et al. in their study evaluated biomarkers for early detection of AKI in 152 adult patients with known baseline serum creatinine concentration.²¹ They considered that, in the case of only a slight increase in serum creatinine, L-FABP concentration can help in determination of outcome, and could even predict the progression of kidney damage in patients at risk of AKI.²¹ The role of the concentration of L-FABP in urine was also examined as potentially useful for the assessment of prognosis in renal transplant recipients.^{12,20} Pajek et al., in a group of 71 adult patients after kidney transplantation, confirmed that the determination of L-FABP in urine may be helpful for the prediction of graft function within the 1st week, and especially after 48 h from transplantation surgery.²⁰ Acute kidney injury is a common and serious postoperative complication of cardiac surgery. In studies carried out in adult patients after cardiac surgery, the usefulness of L-FABP as a marker of AKI in the postoperative period was also assessed.^{19,30} Kokot et al. demonstrated that increased L-FABP levels may lead to the detection of very early stages of AKI, and thereby identify patients in imminent danger of complications after abdominal aortic aneurysm surgery.¹⁹ In a study carried out by Hwang et al. in 26 children after cardiac catheterization, the authors proved that urine concentration of L-FABP was useful in the diagnosis of a subclinical form of contrast induced nephropathy.³¹ In patients with CKD, some studies confirm that the concentration of L-FABP in urine accurately reflects the degree of kidney damage and is correlated with the rate of progression of renal disease.³² Xie et al. demonstrated this relationship in a group of 90 patients with obstructive nephropathy.³³ They also showed that the concentration of L-FABP in urine is more sensitive than albuminuria in predicting the progression of CKD.³⁴ However, in our study the relationship of urine L-FABP and albuminuria has not been proved. The interesting finding was that the concentration of L-FABP in serum and urine positively correlated with SDS for growth in children with a history of HUS. The girls in our study tended to be older and thus grew better than the younger boys, however without significant difference in SDSs, while renal function expressed as eGFR was similar.

In terms of CKD, data from the literature has documented the usefulness of L-FABP as a marker for diabetic kidney disease. Kamijo-Ikemori et al., in animal experiments, showed that expression of L-FABP increased significantly in the group of diabetic mice compared to control mice group.³⁵ Also, in studies in humans it was shown that L-FABP is an indicator of development of diabetic kidney disease, regardless of its stage.³⁶ Mou et al. confirmed the correlation between the level of L-FABP in urine and development of renal impairment in patients with chronic glomerulonephritis, and showed that L-FABP excreted in the urine may be a good marker of progression of chronic glomerulonephritis.³⁷ In Japan, the determination of L-FABP

in urine has been recognized as a novel biomarker for renal damage and in that country the use of a rapid kit for measuring the concentration of L-FABP in urine has been introduced for clinical use.³⁸

The second marker investigated in our study was IL-6, which is considered as one of the major pro-inflammatory cytokines.¹⁷ We have confirmed high serum and urine levels of IL-6 in the group of children after HUS. As already mentioned above, CKD is accompanied by local inflammation, regardless of CKD cause and the stage, as well as the age of the patient. The intensity of the inflammation negatively correlates with current kidney function.²⁶ A fundamental role in the development and progression of chronic nephropathy is attributed to inflammation mediators (including cytokines), which are released as a result of the action of kidney-damaging agents. Pro-inflammatory cytokines potentiate the activity of adhesion molecules in endothelial cells of capillaries. In turn, adhesion molecules bind to the receptors of activated T cells, which lead to the formation of inflammatory infiltrates in the interstitium of the kidneys and stimulation of fibroblasts, fibrosis development and progression of CKD.³⁹ Research studies in recent years have confirmed the elevated IL-6 levels in serum of CKD patients compared to healthy controls and a strong correlation with reduced eGFR.^{23–25} In a population-based study with 4926 Caucasian adults enrolled and followed over 15 years, Shankar et al. found that IL-6 levels were positively related to the presence of CKD in both the baseline and long term observation.²⁴ Gupta et al. in a group of 3939 adult patients with CKD showed higher levels of IL-6 in the serum of patients with lower eGFR.²³ We have also confirmed higher serum IL-6 levels in children after HUS with positive correlation with final eGFR at the end of AKI. Repeated examinations of serum IL-6 concentration in these patients during follow-up would be needed to validate this finding. Sikorska et al., in a cross-sectional study involving 96 patients diagnosed with CKD stages 1–5 of various etiology, found that an increased proportion of patients with elevated IL-6 levels was observed from the earliest stages of CKD, which highlighted the role of severity of inflammation.²⁵ Perlman et al. evaluated diabetic patients at different stages of development of diabetic kidney disease and found that IL-6 concentration in the serum increased with the progression of CKD.⁴⁰ Rodriguez et al., in a study of 32 children, have shown that patients who have developed renal scarring after suffering from urinary tract infection (UTI) in childhood have a higher concentration of pro-inflammatory IL-6 in the blood during a recurrence of an acute episode of UTI. Moreover, it can be concluded that children with a high concentration of IL-6 coexisting with high CRP level during infection are at higher risk of kidney scarring, and consequently the development of CKD.⁴¹ There are also many studies demonstrating the role of IL-6 in the pathogenesis of AKI.^{42,43} Greenberg et al., in a group of 106 children undergoing cardiac surgery, demonstrated

that children with high levels of IL-6 in the serum before surgery have a higher risk of postoperative AKI.⁴³ Zhang et al., in studies involving 960 adult patients after cardiac surgery, have shown that the concentration of serum IL-6 preoperative was not significantly associated with an increased risk of AKI, in contrast to the 1st postoperative concentrations of the interleukins.⁴⁴ The role of IL-6 in the development of acute renal failure and its predictive value in the diagnosis of AKI is not fully understood. Most of the studies assume IL-6 as a good, but imperfect marker for AKI development.⁴⁵

Conclusions

The results indicate that the higher concentration of L-FABP in the serum and urine of children with a history of HUS can be the result of protracted injury initiated during the acute phase of the disease. Lack of correlation of L-FABP concentration with ACR may be associated with a short (less than 6 months) observation after acute renal failure or merely temporary renal tubular damage in the acute phase of the disease. In contrast, higher levels of IL-6 in the serum and urine in children after HUS compared to healthy children, and the negative correlation of L-FABP concentration and eGFR in the group of children after 6-month observation after HUS may confirm their participation in CKD. Thus, L-FABP and IL-6 seem to be good biomarkers of chronic kidney damage in survivors of the acute phase of HUS.

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