ORIGINAL PAPERS

Adv Clin Exp Med 2013, **22**, 3, 377–386
ISSN 1899–5276

Monika Miklaszewska[1, A, B, D, F], Przemysław Korohoda[2, C, D, F], Katarzyna Zachwieja[1, A], Tomasz Mroczek[3, B], Dorota Drożdż[1, E], Krystyna Sztefko[4, B], Anna Moczulska[1, B], Jacek A. Pietrzyk[1, E, F]

Serum Interleukin 6 Levels as an Early Marker of Acute Kidney Injury in Children After Cardiac Surgery*

Znaczenie interleukiny 6 w surowicy jako wczesnego markera ostrego uszkodzenia nerek u dzieci po operacjach kardiochirurgicznych

[1] Department of Pediatric Nephrology, Jagiellonian University Medical College, Cracow, Poland
[2] University of Science and Technology (AGH), Faculty of Computer Science, Electronics and Telecommunications; Chair of Electronics, Cracow, Poland
[3] Department of Pediatric Cardiac Surgery, Jagiellonian University Medical College, Cracow, Poland
[4] Department of Clinical Biochemistry, University Children's Hospital of Cracow, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Cardiosurgical operations in cardiopulmonary bypass (CPB) constitute a risk of acute kidney injury (AKI).
Objectives. The aim of the study was an assessment of AKI risk in children within the first 24 hours after CPB cardiac surgery, evaluating serum interleukin 6 (sIL6).
Material and Methods. The study included 47 children with congenital heart disease operated in CPB. Blood samples were taken before the procedure (0 hour) as well as at 2, 6, 12, 18 and 24 hours after the operation.
Results. AKI was confirmed in 19 children. The mean sIL6 concentration in the AKI compared with non-AKI group was: 180.6 vs. 93.7; p = 0.0017. The maximum sIL6 in the AKI group was obtained at 2 hrs after CPB (350.36 pg/ml). Logistic regression analysis for AKI development depending on the value of sIL6 at 2 hrs after CPB proved that every rise of sIL6 by 100 pg/ml increased the chance of AKI development by 70% (p = 0.0161). With every circulatory arrest time prolongation by 10 minutes for a given sIL6 concentration, the chance of AKI development increased by 47% (p = 0.0407). AKI risk at 2 hrs after CPB, for a sIL6 cut-off point amounting to 185 pg/ml, increased more than 3-fold (AUROC – 68%).
Conclusions. Determining sIL6 in children after cardiosurgical operations at 2 hrs after the procedure constitutes a good, yet not a perfect marker of AKI risk development. Nomograms of the constant risk values of AKI were worked out presenting the ranges of values in relation to serum IL6 concentrations and the child's body mass, age and the time of circulatory arrest (**Adv Clin Exp Med 2013, 22, 3, 377–386**).

Key words: acute kidney injury, serum IL6, cardiopulmonary bypass, children.

Streszczenie

Wprowadzenie. Zabiegi kardiochirurgiczne w krążeniu pozaustrojowym (*cardiopulmonary bypass* – CPB) są obarczone ryzykiem ostrego uszkodzenia nerek.
Cel pracy. Ocena ryzyka AKI u dzieci w okresie pierwszych 24 godzin po zabiegu CPB z wykorzystaniem oceny stężenia IL6 w surowicy (sIL6).
Materiał i metody. Do badania włączono 47 dzieci z wrodzoną wadą serca, operowanych w CPB. Próbki krwi pobierano przed zabiegiem (godz. 0) oraz w 2., 6., 12., 18., 24. godzinie po zabiegu.
Wyniki. AKI stwierdzono u 19 dzieci. Średnie stężenie sIL6 w grupie z AKI w porównaniu do grupy bez AKI wynosiło: 180,6 *vs* AKI0: 93,7; p = 0,0017. Wartość maksymalna stężenia sIL6 dla AKI1 została osiągnięta w 2. godzinie

* The research project was sponsored by grant number: N 406 082 31/3061.

po CPB (350,36 pg/ml). Analiza regresji logistycznej dla wystąpienia AKI w zależności od wartości sIL6 w 2. godzinie po CPB wykazała, że każdy wzrost sIL6 o 100 pg/ml. zwiększył szansę na wystąpienie AKI o 70% (p = 0,0161). Każde wydłużenie czasu zatrzymania akcji serca o 10 minut dla danego stężenia sIL6 zwiększało szansę na wystąpienie AKI o 47% (0.0407). Ryzyko wystąpienia AKI w 2. godzinie po CPB dla punktu odcięcia sIL6 wynoszącego 185 pg/ml wzrastało ponad 3-krotnie (AUROC – 68%).

Wnioski. Oznaczanie sIL6 u dzieci po zabiegach kardiochirurgicznych w 2. godzinie po operacji jest dobrym, ale nie doskonałym markerem ryzyka wystąpienia AKI. Wyniki pracy pozwoliły na opracowanie diagramów przedstawiających przedziały określonej wartości stałej szansy oraz stałego ryzyka wystąpienia AKI w zależności od wartości stężenia sIL6 oraz masy ciała dziecka, wieku oraz czasu zatrzymania krążenia (**Adv Clin Exp Med 2013, 22, 3, 377–386**).

Słowa kluczowe: ostre uszkodzenie nerek, IL6 w surowicy, krążenie pozaustrojowe, dzieci.

The term "acute kidney injury" (AKI) was coined in order to define the state when kidney function deterioration is observed, which is defined by a change of serum creatinine concentration or urine output (UOP) decrease (hourly diuresis with regard to body mass).

AKI incidence in hospitalized patients amounts to approximately 5%, whereas in Intensive Care Unit (ICU) patients, it even increases up to 90%, among which 6% of subjects require renal replacement therapy (RRT) with the mortality risk exceeding 60% [1, 2]. According to Devarajan, AKI incidence in all children hospitalized in ICU amounts to about 10%, of which 82% are in critical clinical condition [3].

AKI is a postoperative complication encountered in about 10% of cardiosurgical pediatric procedures [4].

The causes of this condition include: decreased renal blood flow, loss of its pulsation, hypothermia and the general systemic inflammatory response syndrome (SIRS) [5].

In clinical practice, the diagnosis of AKI is still made on the basis of serum creatinine concentration (SCr) changes despite the fact that this marker is hardly reliable, especially in the pediatric population [5–9]. In view of the SCr diagnostic limitations, the pRIFLE scale was created, which is used in early AKI diagnostic procedures; however, the parameters employed (i.e.: SCr and UOP) are not actually markers of the kidney tissue injury itself, but are only late consequences of that episode development in the past [10]. The UOP value is further influenced by many other factors, such as the intensity of the patient's hydration or administration of diuretics.

A chance for early AKI diagnostic management could help identify children who would benefit from early treatment implementation (at the RISK or INJURY stage), which would result in prompt nephroprotection or dialysis administration [11, 12].

The aim of the study was to assess the clinical applicability of serum IL6 determinations (sIL6) in early (within the first 24 hrs) AKI diagnostic management in children after cardiosurgical procedures, along with a determination of the specificity and sensitivity of that biomarker.

Material and Methods

The study was performed at Department of Pediatric Cardiac Surgery in 2006–2009. The study included children who underwent cardiosurgical operations in CPB. The exclusion criteria included: pre-existing AKI in the medical history data, urinary system defects, nephrotoxic medication administration (NSAID, aminoglycosides) within the period of the last 4 weeks, diabetes, inflammatory vasculopathies and current inflammatory condition of any kind.

The study included 47 children (24 boys, 23 girls) aged from 0.5 to 204 months. A detailed description of the study group including the division into children with and without AKI (AKI1, AKI0) is presented in Table 1. eGFR values were calculated by means of the method developed by Schwartz et al. according to the formula that involves the concentration of serum urea, creatinine and cystatin C [13]:

$$eGFR = 39.1 \times (H/SCr)^{0.516} \times (1.8/Cc^{0.294} \times \times (30/BUN)^{0.169} \times (1.099)^{G} \times (H/1.4)^{0.188} \quad (1),$$

where: H – height [m],
SCr – concentration of serum creatinine [mg/dL],
Cc – concentration of serum cystatin C [mg/L],
BUN – concentration of serum blood urea nitrogen [mg/dL],
G – gender: 0 – female, 1 – male.

AKI was diagnosed in 19 children (40.4%) on the basis of eGFR decline by more than 25% when related to the initial value within 24 hrs after the operation. Within this group, applying the pRIFLE criteria, the INJURY stage was diagnosed in 13 children and the RISK stage in 6 children. None of the children required renal replacement therapy within the period directly after the operation. Statistically significant factors that distinguished the groups of children with and

Table 1. The clinical data of the analyzed group of children, with the division into the AKI1 and AKI0 groups of patients
Tabela 1. Charakterystyka kliniczna dzieci, zakwalifikowanych do badania wraz z podziałem na grupę AKI1 i AKI0

	Total (Razem)	AKI1	AKI0	*p*
Number of children (Liczba dzieci)	47	19 (40.4)	28 (59.6)	
AKI1, RISK		13	–	
AKI1, INJURY		6	–	
Mean age, range – months (Średni wiek, zakres – miesiące)	28.55 (0.5–204)	12 (0.5–92)	39.8 (0.5–204)	0.0284
Mean body mass, range – kg (Średnia masa ciała, zakres – kg)	10.9 (3.2–53)	6.4 (3.2–18)	14 (3.6–53)	0.0186
Boys (Chłopcy): n (%)	24 (51)	9 (47)	15 (53.6)	
Girls (Dziewczęta): n (%)	23 (49)	10 (53)	13 (46.4)	
Course of surgery (Przebieg operacji)				
Circulatory arrest (Zatrzymanie krążenia): n (%)	36 (76.6)	16 (84)	20 (71.4)	
Circulatory arrest in deep hypothermia (Zatrzymanie krążenia w głębokiej hipotermii): n (%)	33 (70.2)	15 (79)	18 (64.3)	
Mean time and range of circulatory arrest in deep hypothermia – min (Średni czas i zakres zatrzymania krążenia w głębokiej hipotermii – min)	35.6 (2–62)	38.9 (2–58)	33 (7–62)	0.3752
Aortic clamping (Zaciskanie aorty): n (%)	40 (85)	17 (89.5)	23 (82)	
Mean time and range of aortic clamping – min (Średni czas i zakres zaciskania aorty – min)	43.2 (2–85)	51.1 (24–85)	37.4 (2–78)	0.0223
Mean time and range of perfusion – min (Średni czas i zakres perfuzji – min)	70.3 (30–153)	73.6 (36–153)	68 (30–131)	0.5510
Nephrological monitoring (Monitorowanie nefrologiczne)				
Mean UOP and range in the course of the operation – ml/kg/h (Średnia UOP i zakres w trakcie operacji – ml/kg/h)	11.96 (2.6–25.9)	10.1 (2.6–25.9)	13.2 (3.8–25.5)	0.3569
Mean UOP and range within 24 hours after the operation – ml/kg/h (Średnia UOP i zakres w ciągu 24 godzin po operacji – ml/kg/h)	2.89 (0.60–6.43)	2.33 (0.92–5.5)	3.1 (0.6–6.43)	0.0128
Mean eGFR value and range within 24 h after the operation – ml/min/1.73 m^2) (Średnia wartość eGFR i zakres w ciągu 24 h po operacji – ml/min/1,73 m^2	80.5 (38.6–151.74)	69 (38.6–118.53)	88.2 (49–151.74)	0.0235

without AKI included: younger age, lower mean body mass, longer mean time of aortic clamping during the operation and lower mean UOP (normalized by kg of body weight) within 24 hrs after CPB. Blood samples were taken before the operation (0 hrs) and at 2, 6, 12, 18, 24 hrs postoperatively. Within 24 hrs after the procedure, UOP and blood pressure were monitored, as well as the administered medications and the circadian fluid balance, which for the first 24 hrs after the operation was maintained at the level of 80% of the patient's circadian requirement. The control group were healthy children admitted for same-day surgical procedures.

The project was approved by the Bioethical Committee of Jagiellonian University Medical College. Informed consent was obtained from parents or legal guardians.

Laboratory assessment of urea and creatinine was performed at the Department of Clinical Biochemistry by means of the VITROS system. Prior to the determination, the rule of single and gradual thawing of serum samples that were stored at a temperature of –70°C was observed. Laboratory assessment of IL6 was performed at the Department of Clinical Immunology. Laboratory determinations of cystatin C were made with the use of the N Latex Cystatin C kit by Siemens by means of a BNII-type nephelometer. Determinations of IL6 were made with the use of a Human IL6 kit by means of enzyme-linked immunosorbent assay (ELISA), by Invitrogen, Camarillo (CA), USA. Sample absorbance measuring was performed by means of the single-wave method with the use of the 450 nm filter in the Universal Microplate Reader El_x800_{NB} by BIO-TEK Instruments, INC.

Statistical Summary

Comparison of distributions was performed with the use of: mean value, standard deviation for the mean value (SEM), standard deviation from the sample, median, 25% and 75% quartiles, Wilcoxon's rank sum test for unpaired samples and Wilcoxon's signed-rank test for paired samples. Moreover, the ROC curves were determined, calculating the following at the selected cut-off points: sensitivity, specificity, LR (likelihood ratio), PPV (positive predictive value), NNV (negative predictive value), AUROC (area under ROC curve). Modeling of distributions was also made with the use of logistic regression of logit type, uni- and multivariable, together with an analysis based on the evaluation of odds ratio. The calculations were made with the use of the following programs: STATISTICA v.10, StatSoft, Inc., MatLab, v. 7.5 and Mathworks. "p" was assumed at the 0.05 level as the limiting value of statistical significance.

Results

The mean values and Wilcoxon's test (p) for sIL6 results in the study and control groups are presented in Table 2. Serum IL6 levels in both all the patients and the AKI1 patient group at the crucial study hours are presented in Table 3. As a statistically significant difference of sIL6 distributions for the AKI1 and AKI0 groups was found only at 2 hrs ($p = 0.0427$), the following evaluation was limited only to those results.

Logistic Regression Analysis

The results of logistic regression for AKI development depending on the values of sIL6 at 2 hrs (changes unit – 100 pg/ml) pointed to a moderately good adjustment of the model, according to which, with every 100 pg/ml increase of sIL6 measured at 2 hrs after CPB, the odds of AKI development increased by 70%. On the other hand, the results of logistic regression for AKI based on the logarithm of age or body mass revealed a moderately good adjustment of the model, according to which every duplication of the child's age decreased the odds of AKI by 24% and every duplication of body mass decreased the odds by 57% (Table 4).

Table 2. Mean values and Wilcoxon's test (p) for all sIL6 results in the study and control group

Tabela 2. Średnie i test Wilcoxona („p") wszystkich wyników sIL6 w grupie badanej i kontrolnej

sIL6 [pg/ml]	All patients (Wszyscy pacjenci)	Controls (Grupa kontrolna)	AKI1 group	AKI0 group
Mean value (SD)	129.54 (170.76)	0.28 (1.18)	180.6 (218.66)	93.70 (114.68)
p (Wilcoxon)	$p < 0.0001$		0.0017	

Table 3. sIL6 maximal values (2 hrs) after CPB in all patients recruited in the study and in the AKI1 group

Tabela 3. Wartości maksymalne sIL6 (2. godz.) po CPB u wszystkich dzieci zakwalifikowanych do badania oraz w grupie AKI1

[pg/ml]	0 hrs value: mean ± SD	Maximum 2 hrs value after CPB mean ± SD	Increase	p (Wilcoxon)
sIL6 All	35.29 (123.74)	216.93 (250.42)	6-fold	$p < 0.0001$
sIL6 AKI1	39.69 (86.08)	350.36 (342.56)	9-fold	$p = 0.0006$

Table 4. Results of logistic regression (1–3) for AKI depending on: 1) sIL6 value at 2 hrs (changes unit – 100 pg/ml); 2) logarithm of age; 3) logarithm of body mass; and multivariable logistic regression analysis (4–6) for sIL6 and 4) logarithm of age; 5) logarithm of body mass and 6) the time of circulatory arrest. All results are statistically significant

Tabela 4. Wyniki regresji logistycznej (1–3) dla wystąpienia AKI w zależności od: 1) wartości sIL6 w 2. godzinie pomiarowej (jednostka zmian – 100 pg/ml); 2) logarytmu z wieku; 3) logarytmu z masy ciała oraz wieloczynnikowej analizy regresji logistycznej (4–6) dla sIL6 oraz 4) logarytmu z wieku; 5) logarytmu z masy ciała i 6) czasu zatrzymania akcji serca, wszystkie wyniki są znamienne statystycznie

	Parameter (Wskaźnik)	OR	CI95%	*P*
1	sIL6(2h)	1.6998	1.0903– 2.6496	0.0161
2	Log2 (Age)	0.7571	0.5782–0.9914	0.0376
3	Log2 (Body Mass)	0.4280	0.1988–0.9217	0.0259
4	sIL6 (2h)	1.8343	1.0903–3.0855	0.0188
	Log2 (Age)	0.7166	0.5192–0.9891	0.0372
5	sIL6 (2h)	1.9760	1.0951–3.5647	0.0200
	Log2 (Body Mass)	0.3261	0.1193–0.8914	0.0247
6	sIL6 (2h)	1.9601	1.1452–3.3549	0.0116
	Circulatory arrest	1.4668	1.0058–2.1393	0.0407

Multivariable Logistic Regression Analysis

The number of collected data sets allowed for performing regression analysis with the use of two independent variables. The presented results were obtained for the models with the best adjustment. The child's gender did not influence the odds of AKI development. The results of multivariable logistic regression analysis for sIL6 at 2 hrs after CPB and for the logarithm of age and body mass revealed a good or very good adjustment of the model, according to which with every increase of sIL6 by 100 pg/ml for a given age or body mass, the odds of AKI increased by 83% or 98%, respectively; whereas for a given value of sIL6, every duplication of age or body mass decreased the odds of AKI by 28% or 67%, respectively. The comprehensive presentation of odds' changes depending on both parameters is presented in Table 4 and Figures 2 and 3.

The results of multivariable logistic regression analysis for sIL6 at 2 hrs after CPB and the time of

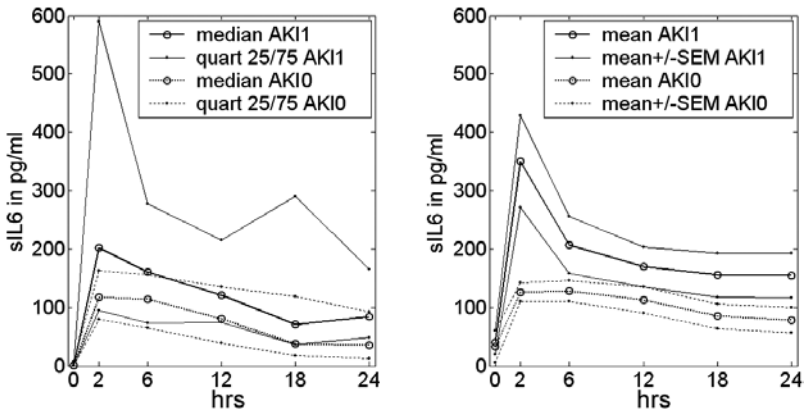

Fig. 1. Comparison of sIL6 values distributions for the AKI1 and AKI0 groups, depending on time. On the left – values of median and quartiles: 25, 75. On the right – mean values and ranges resulting from evaluation of standard deviation of that value. In the graphs, changes for these parameters in the AKI1 and AKI0 groups in relation to time are confronted

Ryc. 1. Porównanie rozkładów wartości sIL6 dla grupy AKI = 1 i AKI = 0, w zależności od czasu. Po lewej – wartości mediany oraz kwartyli: 25, 75. Po prawej – wartości średnie oraz przedziały wynikające z oszacowania odchylenia standardowego dla tej wartości. Na wykresach skonfrontowano zmiany zachodzące w czasie dla tych parametrów w grupie AKI1 oraz AKI0

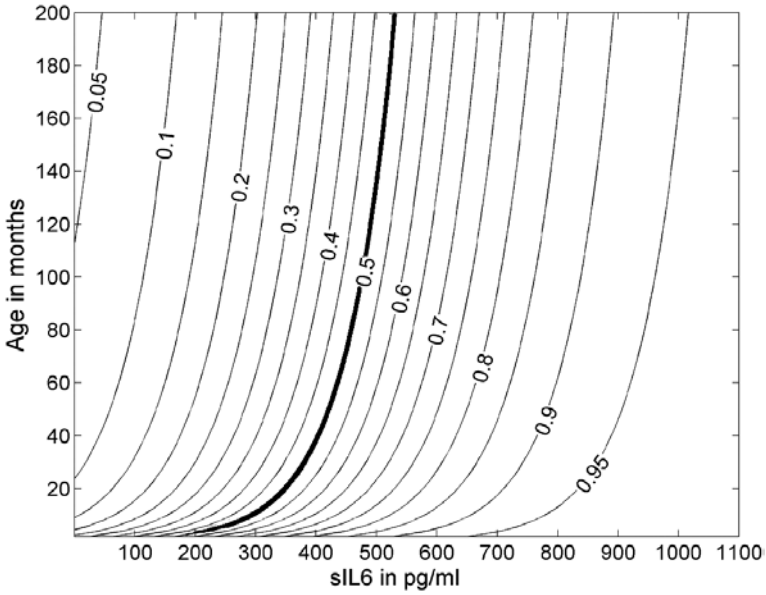

Fig. 2. The graph of the constant risk lines of AKI development resulting from the determined regression model both for sIL6 at 2 hrs after CPB and logarithm of age (Table 4)

Ryc. 2. Wykres linii stałego ryzyka rozwinięcia AKI wynikający z wyznaczonego modelu regresyjnego dla sIL6 w 2. godzinie po CPB oraz logarytmu z wieku (tab. 4)

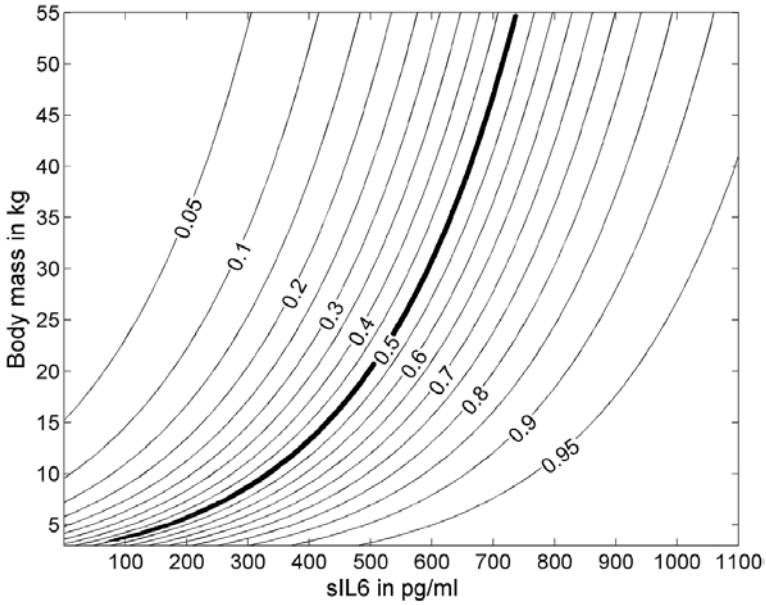

Fig. 3. The graph of constant risk lines of AKI development resulting from the determined regression model both for sIL6 at 2 hrs after CPB and the logarithm of body mass (Table 4)

Ryc. 3. Wykres linii stałego ryzyka rozwinięcia AKI wynikający z wyznaczonego modelu regresyjnego łącznie dla sIL6 w 2. godzinie po CPB oraz logarytmu z masy ciała (tab. 4)

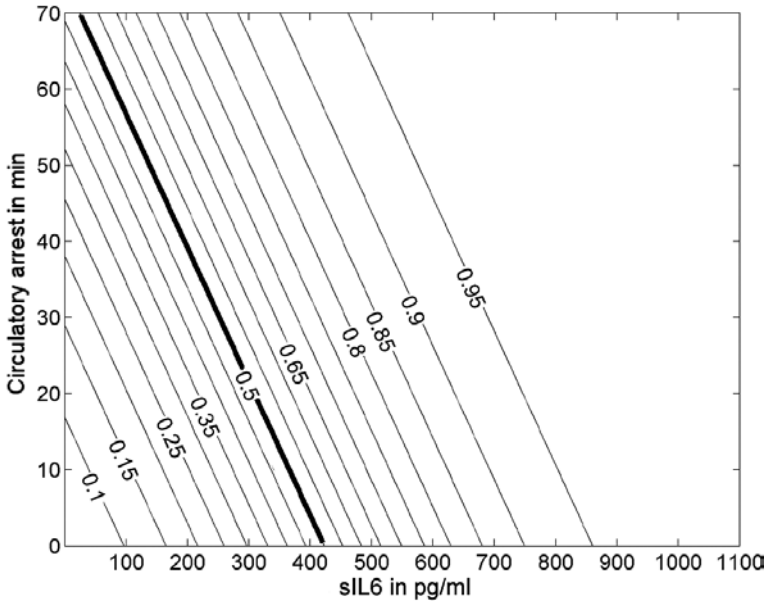

Fig. 4. The graph of constant risk lines of AKI development resulting from the determined regression model both for sIL6 at 2 hrs after CPB and the time of circulatory arrest (Table 4)

Ryc. 4. Wykres linii stałego ryzyka rozwinięcia AKI wynikający z wyznaczonego modelu regresyjnego łącznie dla sIL6 w 2. godzinie po CPB oraz czasu zatrzymania krążenia (tab. 4)

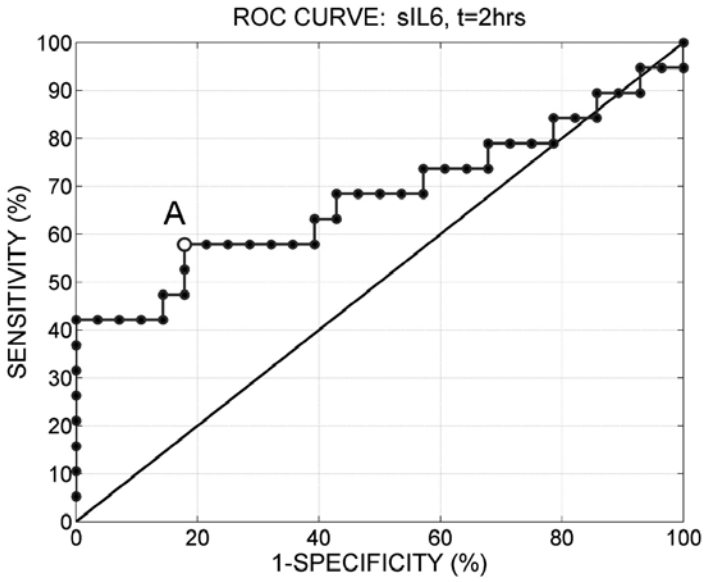

Fig. 5. sIL6 concentration ROC curve at 2 hrs after CPB for AKI development

Ryc. 5. Krzywa ROC stężenia sIL6 w 2. godzinie po CPB dla wystąpienia AKI

circulatory arrest (changes unit of sIL6 was 100 pg/ml, unit for circulatory arrest time – 10 minutes) revealed a good adjustment of the model, according to which, with every extension of circulatory arrest time by 10 minutes for a given sIL6 concentration, the odds of AKI increased by 47%. On the other hand, a sIL6 concentration increase by 100 pg/ml for the established circulatory arrest time increased the AKI odds by 96%. The comprehensive presentation of odds' changes depending on both parameters is presented in Table 4 and Figure 4.

sIL6 ROC Curves

In order to further verify the test applicability with the use of the studied marker and to determine the cut-off points, a ROC curve analysis was performed. The AUROC value [CI 95% (51%–85%)] for sIL6 concentration amounting to 68% revealed moderately good applicability of this cytokine as an AKI predictor in children at 2 hrs after CPB. AKI risk development at 2 hrs after CPB for the sIL6 cut-off point amounting to more than 185 pg/ml increased more than 3 times (sensitivity: 57.9%; specificity: 82.1%; PPV: 68.8%; NPV: 74.2%; LR: 3.24, Figure 5).

Discussion

Interleukin 6 is a 26 KDa glycoprotein. It is secreted by lymphocytes T and B, macrophages, fibroblasts, endothelium cells, mesangium and by renal tubule cells. It is the main cytokine secreted in the kidneys and this secretion is stimulated by TNF alpha [14]. Moreover, it is one of the main proinflammatory cytokines, the concentration of which rapidly increases in the serum of AKI patients [15]. In view of the above, sIL6 seems to be a valuable marker in predicting AKI episodes.

The present study included children who, due to their congenital heart disease, required CPB surgery. CPB cardiosurgical procedures are associated with a risk of multi-organ failure development, including renal failure. The more complicated the heart disease and the longer operation time, the greater the risk of low output syndrome and AKI directly after the surgery [16, 17].

The technical conditions of performing the surgery and the patient's initial clinical condition from the very beginning have a significant impact on AKI risk episode development [18, 19]. According to Devarajan, AKI diagnosis on the day of admitting the child to the pediatric intensive care unit (PICU) is associated with a 5-fold death risk increase, whereas AKI occurrence within this unit is with up to a 9-fold increase of that risk and a 4 times longer hospitalization period [3].

The AKI incidence in children in the studied group (40.4%) corresponds to the average frequency quoted in the literature (28–51%) [5, 20–24].

Analyzing the results obtained in this study, it was concluded that in the AKI1 group, the children were younger, with lower body mass and lower UOP during the surgery and within 24 hrs postoperatively as compared to children without AKI. Moreover, in children with AKI, circulatory arrest in deep hypothermia occurred more often and lasted longer, aortic clamping was more frequent and longer and the mean perfusion time duration was longer (Table 1). Similar results were obtained by Krawczewski et al., who measured NGAL concentration (Neutrophil Gelatinase Associated Lipocalin) as an early AKI

marker in the pediatric population after CPB procedures [25].

Liu et al. monitored, among others, serum IL6 in children undergoing cardiosurgical operations. The sIL6 level (similarly as in the present study) increased at 2 hrs after the operation, and its concentration at 2 and 12 hrs after the surgery was exactly correlated with the subsequent AKI episode and with the necessity of longer mechanical ventilation after the surgery. The values of sIL6 quoted by the authors were slightly lower than the ones obtained in the present study (the mean values of sIL6 at 2, 12 and 24 hrs after CPB amounted to 171.5, 72.5 and 45.7 pg/ml, respectively). On the other hand, circadian sIL6 profile secretion was similar in both studies [20].

It can be noted that in the present study there is an evident increase of mean values of sIL6 at 2 hrs after CPB not only in the AKI1 group, but also in all the children included in the study, increasing respectively in relation to the initial value 9 and 6 times (Table 3). The obtained results throw light on significant stimulation of the immune system, which occurs in every patient (both with and without AKI) subjected to CPB due to the bioincompatibility phenomenon of medical equipment and the ischemia of peripheral organs evoked by their decreased perfusion. During the study, because of the relatively short monitoring period (24 hrs) and routine qualification for CPB procedures strictly of children without any active infections, there were no infectious complications stated in either the AKI1 or AKI0 group.

IL6 is one of the fundamental effector cytokines taking part in AKI-CPB pathogenesis. In the course of CPB, systemic inflammatory response syndrome (SIRS) is mainly evoked by the contact of morphotic blood elements with the artificial surface of the cardiopulmonary bypass system. AKI-CPB is a complex pathophysiology which covers the phenomena that take place in the vascular endothelial cells, renal tubule cells and in numerous cells that are mediators of the inflammatory process. The essential role is played by a decreased renal perfusion pressure by about 30%, activation of proinflammatory cytokines with SIRS effect, hemolysis induced by CPB and complement system activation, as well as direct nephrotoxicity of the very system of cardiopulmonary bypass circulation [20, 26–28]. Relatively little is known about the direct impact that activates the tissues and cells, which is a result of deep hypothermia and subsequent reperfusion syndrome. The significance of CPB influence on the AKI risk can be observed when the AKI incidence in patients subjected to CPB cardiosurgical operations is compared to that noted in cardiosurgical patients without the need of this procedure [27].

Based on the analysis of sIL6 at 2 hrs ROC curves, it is concluded that the cut-off point value amounting to 185 pg/ml was characterized by 58% sensitivity and 82% specificity in AKI episode prediction. Lower sensitivity (along with high specificity) of the obtained results might be caused by the significant dispersion of the individual results obtained in particular measurements. sIL6 AUROC value amounted to 68%, which indicated a relatively low, yet still statistically significant applicability of this parameter to estimate the AKI risk. The likelihood ratio for the discussed cut-off point amounted to 3.24.

A statistically significant difference in distributions of sIL6 in the AKI1 and AKI0 group at 2 hrs was revealed. Logistic regression analysis for AKI in relation to sIL6 revealed that with every increase of sIL6 by 100 pg/ml, the AKI odds increased by 70%.

The present study revealed that young age and lower patient body mass favored AKI development. The CPB procedure times strictly depend on congenital heart disease type, meaning that most complicated heart diseases, which require longer surgery times (thereby longer aortic clamping, which may impair heart function during the early post-surgical period and decrease renal blood flow) require earlier intervention – hence the younger age and lower body mass in those patients and probably higher AKI risk which reflects IL6 levels. This fact was also proved by multivariable logistic regression analysis, which revealed that every duplication of child's age or body mass significantly decreased AKI risk, whereas every increase of sIL6 for a given age or body mass substantially increased that risk. The cumulative analysis of sIL6 and the time of circulatory arrest revealed that every 10-minute prolongation of circulatory arrest time for a given cytokine level caused an increase of AKI odds almost by a half, whereas the increase of sIL6 by 100 pg/ml within the established time of circulatory arrest actually doubled the AKI odds.

In the present study, the mean eGFR value in the AKI1 in relation to AKI0 group was measured at the level of 69 *vs.* 88 ml/min/1.73 m^2 ($p = 0.0000$). It is worth emphasizing that on the basis of eGFR assessment, AKI could be found only at 12 hrs after the cardiosurgical operation in the studied group (AKI1: 58 ml/min *vs.* AKI0: 85 ml/min, $p = 0.0039$), whereas on the basis of sIL6 levels at 2 hrs after CPB, there was a chance to determine the increased AKI risk. Similar results were obtained by Xin et al., who estimated the AKI risk in adult patients undergoing CPB with the use of serum creatinine concentration. According to the authors, AKI can be defined only at 12–48 hrs after the operation [29].

The present research is a prospective study, with a carefully selected patient cohort undergoing CPB operations, with proper renal function before the operation, without impairing comorbid diseases. Blood samples were taken according to the protocol, at strictly defined time intervals, what allowed for creating a transparent and homogeneous study environment that allowed for kidney function evaluation without any unnecessary disturbing factors.

The present study offers a graphic presentation in the form of nomograms of risk distributions of AKI development in relation to two parameters: serum IL6 levels and other clinical parameters. In the nomograms, a clinically useful "point of reference" of AKI was presented, which corresponded to the risk value of 0.5. The risk equal to 0.5 meant that in a given child population with defined sIL6 levels and with the defined body mass, age or defined time of circulatory arrest, there would be the same number of subjects who would develop AKI as those who would not. The diagrams may turn out to be useful in the clinical assessment of relative AKI risk in children in relation to sIL6 concentration and to the child's age, body mass or circulatory arrest duration. In a clinical practice, it is relatively easy to assign defined patient populations to a given odds or risk range of AKI development.

However, the essential limitation of the present study was the small number of patients undergoing cardiosurgical operations, as well as the fact that the research was done only in one medical center. The results of the present study require confirmation and specification with regard to the cut-off points, sensitivity and specificity in relation to the results obtained in other projects performed in other centers. Moreover, in the present study, only ischemic AKI was assessed and the very observation lasted only 24 hrs, excluding the period of normalization of the studied marker concentrations.

Having early biomarkers of kidney injury at our disposal is particularly significant for the overall AKI diagnostic-therapeutic process and it is essential in limiting the risk of systemic complications. Nowadays, developing an AKI diagnostic panel is crucial, as the markers currently studied can independently introduce some additional information concerning the patient's clinical condition and credibly stratify the risk, which would be great progress in the diagnosis and treatment of AKI in children. Of note, the concentration of another inflammatory marker – C reactive protein (CRP) – increases as late as 4 hrs after the activation of any triggering factor and lasts in serum for 24–72 hrs, which constitutes the main reason why CRP cannot be used as a specific, sensitive and early marker of AKI in the studied population [30].

Summarizing the research results, the authors conclude that: 1) sIL6 concentration exceeding 185 pg/ml at 2 hrs after CBP increases more than 3-fold the odds of AKI development in children, whereas every increase of the marker by 100 pg/ml increases the odds almost twice. 2) A younger age, lower body mass and longer circulatory arrest time increase the odds of AKI development in children after CPB. 3) Nomograms of AKI constant values risk development were worked out, presenting the ranges of values in relation to sIL6 concentrations and the child's body mass, age and the time of circulatory arrest.

References

[1] **Mc Ilroy DR, Wagener G, Lee T:** Biomarkers of Acute Kidney Injury. An Evolving Domain. Anesthesiology 2010, 112, 998–1004.

[2] **Ramesh G, Krawczeski CD, Woo JG, et al.:** Urinary Netrin-1 Is an Early Predictive Biomarker of Acute Kidney Injury after Cardiac Surgery. Clin J Am Soc Nephrol 2010, 5, 395–401.

[3] **Devarajan P:** Biomarkers for the early detection of acute kidney injury. Curr Opin Ped 2011, 23, 194–200.

[4] **Sorof JM, Stromberg D, Brewer ED, et al.:** Early initiation of peritoneal dialysis after surgical repair of congenital heart disease. Pediatr Nephrol 1999, 13, 641–645.

[5] **Mishra J, Dent C, Tarabishi R, et al.:** Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005, 365, 1231–1238.

[6] **Haase M, Bellomo R, Devarajan P, et al.:** Accuracy of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Diagnosis and Prognosis in Acute Kidney Injury: A Systematic Review and Meta-analysis. Am J Kidney Dis 2009, 54, 1012–1024.

[7] [7] **Nguyen MT, Devarajan P:** Biomarkers for the early detection of acute kidney injury. Pediatr Nephrol 2007, 23, 2151–2157.

[8] **Bellomo R, Kellum JA, Ronco C:** Defining acute renal failure: physiological principles. Int Care Med 2004, 30, 33–37.

[9] **Devarajan P:** The future of pediatric AKI management biomarkers. Semin Nephrol 2008, 28, 493–498.

[10] **Askenazi DJ, Ambalavanan N, Goldstein SL:** Acute kidney injury in critically ill newborns: What do we know? What do we need to learn? Pediatr Nephrol 2009, 24, 265–274.

[11] **Goldstein SL, Somers MJ, Baum MA, et al.:** Pediatric patients with multi-organ dysfunction syndrome receiving continuous renal replacement therapy. Kidney Int 2005, 67, 653.

[12] **Mak R:** Acute kidney injury in children: the dawn of a new era. Pediatr Nephrol 2008, 23, 2147–2149.
[13] **Schwartz G, Munoz A, Schneider M, et al.:** New Equations to Estimate GFR in Children with CKD. J Am Soc Nephrol 2009, 20, 629.
[14] **Kwon O, Molitoris BA, Pescovitz M, et al.:** Urinary actin, interleukin-6, and interleukin-8 may predict sustained ARF after ischemic injury in renal allografts. Am J Kid Dis 2003, 41,1074.
[15] **Dennen P, Altmann C, Kaufman J, et al.:** Urine interleukin-6 is an early biomarker of acute kidney injury in children undergoing cardiac surgery. Crit Care 2010, 14, R1 81.
[16] **Dittrich S, Kurschat K, Dähnert I, et al.:** Renal function after cardiopulmonary bypass surgery in cyanotic congenital heart disease. Int J Cardiol 2000, 28, 73, 2, 173–179.
[17] **Dittrich S, Priesemann M, Fischer T, et al.:** Circulatory arrest and renal function in open-heart surgery on infants. Pediatr Cardiol 2002, 23, 1, 15–19.
[18] **Loef BG, Epema AH, Smilde TB, et al.:** Immediate postoperative renal function deterioration in cardiac surgical patients predicts in-hospital mortality and long-term survival. J Am Soc Nephrol 2005, 16, 195–200.
[19] **Tuttle KR, Worrall NK, Dahlstrom LR, et al.:** Predictors of ARF after cardiac surgical procedures. Am J Kidney Dis 2003, 41, 76–83.
[20] **Liu KD, Altmann C, Smits G, et al.:** Serum Interleukin-6 and interleukin-8 are early biomarkers of acute kidney injury and predict prolonged mechanical ventilation in children undergoing cardiac surgery: a case-control study. Crit Care 2009, 13, R1 04.
[21] **Parikh C, Mishra J, Thiessen-Philbrook H, et al.:** Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. Kidney Int 2006, 70, 199–203.
[22] **Bennett M, Dent C, Ma Q, et al.:** Urine NGAL Predicts Severity of Acute Kidney Injury After Cardiac Surgery: A Prospective Study. Clin J Am Soc Nephrol 2008, 3, 665–673.
[23] **Dent C, Ma Q, Dastrala S, et al.:** Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. Crit Care 2007, 11, R1 27.
[24] **Krawczeski CD, Vandevoorde R, Kathman T, et al.:** Serum Cystatin C Is an Early Predictive Biomarker of Acute Kidney Injury after Pediatric Cardiopulmonary Bypass. Clin J Am Soc Nephrol 2010, 9, 1552–1557.
[25] **Krawczeski CD, Woo JG, Wang Y, et al.:** Neutrophil Gelatinase-Associated Lipocalin Concentrations Predict Development of Acute Kidney Injury in Neonates and Children after Cardiopulmonary Bypass. J Ped 2011, 158, 1009–1015.
[26] **Hoke TS, Douglas IS, Klein CL, et al.:** Acute renal failure after bilateral nephrectomy is associated with cytokine mediated pulmonary injury. J Am Soc Nephrol 2007, 18, 155–164.
[27] **Kumar AB, Suneja M:** Cardiopulmonary Bypass-associated Acute Kidney Injury. Anesthesiology 2011, 114, 964–970.
[28] **Simmons EM, Himmelfarb J, Sezer MT, et al.:** Plasma cytokine levels predict mortality in patients with acute renal failure. Kidney Int 2004, 65, 1357–1365.
[29] **Xin C, Yulong X, Yu C, et al.:** Urine Neutrophil Gelatinase-Associated Lipocalin and Interleukin-18 Predict Acute Kidney Injury after Cardiac Surgery. Renal Failure 2008, 30, 904–913.
[30] **Panichi V, Migliori M, DePietro S, et al.:** The link of biocompatibility to cytokine production. Kidney Int 2000 (Suppl. 76), 58, S96.

Address for correspondence:

Monika Miklaszewska
Department of Pediatric Nephrology
Jagiellonian University Medical College
Wielicka 265
30-663 Kraków
Poland
Tel.: +48 606 262 484
E-mail: mmiklasz@wp.pl

Conflict of interest: None declared

Received: 8.08.2012
Revised: 23.04.2013
Accepted: 13.06.2013