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SERUM CYSTATIN C AS A PREDICTOR OF THE DEVELOPMENT OF ACUTE KIDNEY INJURY IN NEWBORNS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY SUBMITTED TO THERAPEUTIC COOLING

L. Stryzhak, I. Anikin

Zaporizhzhia State Medical University
(Zaporizhzhia, Ukraine)

Summary

Assessment of renal function in newborns is extremely important and at the same time challenging due to the unique body structure, increased vulnerability and rapid growth of the latter. However, for the early detection of acute kidney injury (AKI), rational dosing of drugs and safe drug therapy, the identification of early markers of renal dysfunction is essential.

The objective is to evaluate the prognostic value of serum biomarkers for the early diagnosis of AKI in newborns with hypoxic-ischemic encephalopathy against the background of therapeutic hypothermia and preventive use of methylxanthines.

Materials and Methods. *A single-center, prospective, randomized trial involving 44 neonates with AKI requiring therapeutic hypothermia and prophylactically receiving caffeine citrate or theophylline to prevent AKI progression was conducted in from 2019 to 2022 on the basis of the NICU of Zaporizhzhia Regional Clinical Children's Hospital.*

Laboratory analysis of blood serum samples was performed on day 1, day 3 and 5 from birth, creatinine (Cr) and cystatin C (CysC) levels and their associations with the development of AKI were determined according to the neonatal criteria of the 2012 KDIGO guideline.

Statistical analysis was performed using Statistica 13.0 program, TIBCO Software Inc. (license number JPZ8041382130ARCN10-J) and Microsoft Excel 2013 (license number 00331-10000-00001-AA404). The probability of the difference in absolute values of mean values was determined using non-parametric methods of statistical analysis: the Mann-Whitney U-Test for unrelated groups and the Wilcoxon signed-rank t test for related groups. Statistical significance was defined as $p < 0.0500$.

The study was performed in accordance with the moral and ethical standards established by the IGH / GCP guidelines, the World Medical Association Helsinki Declaration, adopted in 1964 and amended in 1975, 1983, 1989, 1996 and 2000, The European Convention of Human Rights and Biomedicine and the legislation of Ukraine. The protocol was approved by the Medical Ethics Commission at Zaporizhzhia State Medical University. The study was performed as part of the research project "Optimization of diagnostics and intensive care of polyetiologic lesions of the brain, gastrointestinal tract, and kidneys in newborns and older children" (State registration number O118U007142) of the Pediatric Surgery and Anesthesiology Department of the State Institution "Zaporizhzhia State Medical University of the Ministry of Health of Ukraine."

Results and their discussion. *In general, AKI according to KDIGO developed in 5.00 (11.36 %) neonates out of 44.00 (100.00 %), stage 0 was found in 39.00 (88.64 %). 4.00 (9.09%) newborns had stage I, and 1 (2.27%) developed stage II; the data obtained were similar: $p = 0.7872$; $U = 230.00$. None of the patients progressed to stage III.*

In the newborns with preserved renal function during the study there was a decrease in Cr and a predictable, by this marker, increase in GFR. A statistically significant increase in Cr level and decrease in GFR was found in the newborns with renal dysfunction on days 3 and 5 of the study. Cr level progressed from baseline 1.07 (0.87; 1.10) mg/dl to 1.13 (0.86; 1.25) mg/dl on day 3 and to 1.40 (1.15; 1.82) mg/dl on day 5, while GFR decreased from 19.76 (19.07; 22.90) ml/min/1.73m2 to 17.97 (13.84; 24.42) ml/min/1.73m2 on day 3 and was 12.38 (11.12; 17.54) ml/min/1.73m2 on day 5, with $p < 0.0500$.

CysC progressively decreased in the neonates without AKI from 2.50 (2.20; 2.60) ng/ml to 2.25 (2.08; 2.49) ng/ml, $p = 0.0095$; while in the neonates with AKI the level of this marker did not change and was 2.56 (2.41; 2.70) ng/ml on day 1 and 2.42 (1.89; 2.45) ng/ml on day 5, $p = 0.2963$. As this marker changed, eGFR (CysC) increased progressively in the cohort of patients without kidney damage but did not change in the other group.

The diuresis rates in the newborns of both groups did not differ, being ≥ 1.5 ml/kg/h, which is probably due to methylxanthine therapies, $p \geq 0.0500$.

Conclusions. *CysC assessment did not provide additional information on the development of acute kidney injury in neonates (nAKI) in the first 5 days of life, which would have allowed a quick decision to change the intensive care program. Further studies involving newborns who did not receive prophylactic therapy are needed.*

Key words: *Asphyxia; Hypoxic-ischemic Encephalopathy; Acute Kidney Injury; Creatinine; Cystatine C; Methylxanthines; Newborn; Glomerular Filtration Rate.*

Introduction

Acute kidney injury (AKI) is a frequent and threatening complication among critically ill patients and often requires renal replacement therapy (RRT) [1]. The progression of this complication has a negative outcome and increases the risk of mortality among ICU patients to 60-80% [2 - 3].

Among the neonatal cohort, AKI is one of the most frequent complications in critical conditions with a mortality rate of about 50%, increased risk of chronic disease among survivors and future disability [4].

Factors associated with a higher risk of developing AKI in newborns are perinatal asphyxia, prematurity, neonatal sepsis, congenital anomalies of the urinary or cardiac system, exposure to nephrotoxic drugs and oxygen therapy [5-6].

The incidence of AKI in newborns after asphyxia is more than 40%, while the rate of severe asphyxia even reaches 61-70% [7].

AKI is a sudden, within less than 48 hours,

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increase in serum Cr level by ≥ 1.5 times compared to baseline or an increase in its level by 0.3 mg/dL or more within 48 hours, and/or a decrease in urine output to ≤ 0.5 ml/kg/h in 6 hours [8].

Early AKI is a potentially reversible syndrome; however, in newborns it usually does not have specific clinical symptoms, and in cases where there is no relevant examination, is diagnosed late [9].

To diagnose changes in serum Cr level, glomerular filtration rate and/or diuresis rate, several scales for grading the severity of AKI in children and newborns have been proposed: pRIFLE, nRIFLE, AKIN, KDIGO, etc., However, the determination of Cr, as well as the calculation of glomerular filtration rate (GFR), or the determination of decreased diuresis have their drawbacks in the neonatal cohort. The value of neonatal Cr is influenced by maternal Cr, which is evenly distributed across the placenta to the fetus and reflects the renal function of the mother. This marker is quite late for identifying renal dysfunction, as it increases 48-72 hours after the onset of injury, while > 50% of nephrons lose their function by this time. Moreover, the Cr level depends on the morpho-functional immaturity, the state of the muscular system and the water balance of the body. Elevated Cr in preterm neonates is indicative of the immaturity of the tubular apparatus, rather than renal damage [10-13].

Glomerular filtration rate (GFR) is widely recognized as the best overall index of kidney function. However, GFR is difficult to assess and is usually estimated from serum levels of endogenous filtration markers such as Cr. The calculation is based on proven formulas taking into account age, sex, race and serum Cr as a marker of filtration. Also, the period of GFR increase in full-term babies is about a week, when serum Cr gradually decreases, which demonstrates their own kidney function. In premature babies, due to their morphological and functional immaturity, normalization of serum Cr level takes several weeks [14].

Assessing diuresis rate in newborns during the first day of life is rather difficult because of low renal concentrating capacity and because the first urination in the delivery room may go unnoticed, and oliguria is physiological for the first day of life. Most often, AKI with decreased diuresis rate is detected on day 1-3 of life [15]. Besides, modified neonatal scales offer different thresholds for diuresis rate. For example, the mKDIGO (Modified Kidney Disease Improving Global Outcomes [16]) scale suggests < 0.5 mL/kg/h, while nRIFLE [17] < 1.5 mL/kg/h and the updated nRIFLE [18] < 1 mL/kg, with no consensus on this criterium.

In view of the above, neither Cr level, diuresis rate nor glomerular filtration rate can be considered as pathognomonic markers for the specific renal damage in neonates.

Modern research shows that the assessment of a biomarker such as CysC may be an improved alternative to Cr to be included in the GFR calculation equation. CysC is a protein that is freely filtered through the glomerular membrane, metabolized in the kidneys, but not secreted by proximal renal tubules. It is used as a highly sensitive and accurate marker of glomerular filtration rate, virtually unaffected by non-renal factors and unable to penetrate the placental barrier, which allows for a more accurate presentation of the renal function of a newborn. However, respiratory distress, asphyxia, concomitant use of aminoglycosides and sepsis can affect its value [19].

Increasing evidence suggests that AKI or milder kidney damage, or impaired renal function manifested by changes in urine output and blood chemistry, portends serious clinical consequences. There is hardly any consensus on the early diagnosis and treatment of neonatal AKI, so the identification of early markers of renal dysfunction and effective risk factors for early intervention is e of utmost importance for critically ill infants, given the high risk of mortality.

Materials and methods

All patients required treatment in the neonatal intensive care unit of Zaporizhzhya Regional Clinical Children's Hospital. The medical center provides tertiary care to infants delivered from medical institutions of Zaporizhzhya region or Zaporizhzhya city of the second level of care and is the clinical base of Zaporizhzhya State Medical University. All newborns after asphyxia with clinical signs of moderate or severe hypoxic-ischemic encephalopathy, which required a program of general therapeutic hypothermia, according to the guidelines of the Order of the Ministry of Health of Ukraine dated 28.03.2014 №225 (Unified clinical protocol "Initial, resuscitation and post-resuscitation care for newborns in Ukraine") were admitted to a prospective, randomized, controlled trial in the period between November 2019 and January 2022. Newborns who did not meet these criteria (n = 8) were excluded from the study. In total, 44 infants with gestational age ≥ 37 weeks were assigned to the final analysis.

The study protocol approval was issued by the Regional Bioethics Committee of Zaporizhzhya State Medical University. This study met the requirements of the 1964 Helsinki Declaration. Written informed consent was obtained from the patients' parents before them being enrolled in the study.

The study was performed as part of the research project "Optimization of diagnostics and intensive care of polyetiologic lesions of the brain, gastrointestinal tract, and kidneys in newborns and older children" (State registration number O118U007142) of the Pediatric Surgery and Anesthesiology Department of the State Institution "Zaporizhzhia State Medical University of the Ministry of Health of Ukraine."

Statistical analysis was performed using Statistica 13.0 program, TIBCO Software Inc. (license number JPZ804I382130ARCN10-J) and Microsoft Excel 2013 (license number 00331-10000-00001-AA404). The probability of the difference in absolute values of mean values was determined using non-parametric methods of statistical analysis: the Mann-Whitney U-Test for unrelated groups and the Wilcoxon signed-rank t test for related groups. Statistical significance was defined as $p < 0.0500$.

A special diagnostic approach for newborns is to determine the Cr level against the background of decreased diuresis rate. That is why the severity of the disease was assessed according to the criteria of

the modified neonatal scale mKDIGO (2012). The rate of diuresis was measured hourly, results being interpreted every 6 hours during the first 3 days.

Laboratory analysis included measurements of serum Cr, urea nitrogen, electrolytes, albumin and CysC. The level of plasma cystatin C (CysC) was determined by double-antibody enzyme-linked immunosorbent assay (ELISA) and calibration with a reference standard.

Glomerular filtration rate was calculated separately using the formulas for Cr and CysC. To determine the GFR by serum Cr level, we used the bedside Schwartz equation provided by the international KDIGO guidelines (2012): GFR (ml/min/1.73m2) = 41.3 x (height (m)/serum Cr (mg/dl) [20].

The GFR was calculated by the level of serum CysC using the equation obtained from the cohort of children with chronic kidney disease (Chronic Kidney Disease in Children (CKiD) 2012): GFR (ml/min/1.73m2) = 70.69 × (SCysC)-0.931 [21].

Given the high risk of acute kidney injury associated with asphyxia, theophylline was used to prevent this complication in patients in one group (n = 22), as recommended by the Kidney Disease International Expert Group: Improving Global Outcomes in 2012 with a level of evidence 2B, but without a specific dose and time of administration [22].

Therefore, the infants were treated according to the study protocol with theophylline (Eufilin-Darnitsa®) administered as an intravenous drip at a dose of 3mg/kg at 6-hour intervals during the first 3 days of life. Patients in the other group (n = 22) were alternatively treated with caffeine citrate (Peyona®) at a loading dose of 10mg/kg administered as an intravenous drip at 12-hour intervals on the first day of life, and at a maintenance dose of 5mg/kg at 12-hour intervals on days 2 and 3. Evaluation of the efficacy and safety of methylxanthines in full-term neonates for the prophylaxis and conservative therapy of AKI was published earlier [23].

Aminoglycosides and amphotericin B preparations were not prescribed.

All patients in our study required vasopressor support to optimize hemodynamic status. To maintain adequate cerebral and renal perfusion, vasoactive drugs such as dopamine, phenylephrine, norepinephrine or epinephrine and hydrocortisone were administered. The results of studies of early impairment of renal perfusion and urgent therapeutic intervention aimed at restoring renal function have been published in previous studies [24].

According to the type of respiratory support, patients of both groups were identical - prolonged forced artificial ventilation with a gradual decrease in parameters, an increase in the interval of spontaneous breathing and complete weaning from assisted ventilation.

Clinical outcomes were assessed before discharge from the ICU, including the duration of mechanical ventilation (MV) and inotropic support, ICU stay, mortality, and complete or partial/absent recovery of renal function. After transferring patients from the ICU, renal function was monitored until discharge from the hospital.

Results

The infants enrolled in the study were representative in terms of age, sex, birth weight, gestational age, type of maternal delivery, with clinical and laboratory signs for perinatal asphyxia, which met the inclusion criteria.

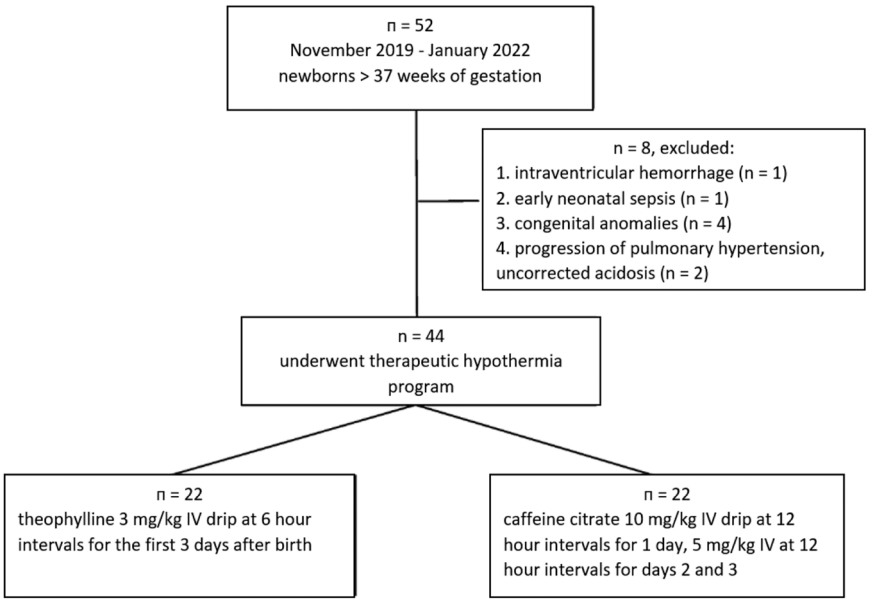

Figure 1. Scheme of the study

The following factors of complications of labor and delivery served as indicators of perinatal asphyxia: fetal distress (n = 26, 59.09%); unsuccessful attempt to stimulate labor with medications (n = 6, 13.64%); impaired labor force (n = 7, 15.91%); fetal malposition or presentation (n = 2, 4.55%); maternal pelvic anomalies; labour and delivery complicated by bleeding (n = 2, 4.55 %), pathological condition of the umbilical cord (n = 14, 31.82 %); unusually large fetus (n = 4, 9.09 %); use of vacuum extractor (n = 3, 6.82 %) and forceps (n = 1, 2.27 %); unsuccessful attempt to induce labor with the subsequent cesarean section (n = 21, 47.73 %).

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Table 1

General characteristics of the study groups

Indicator	Caffeine citrate (n = 22)	Theophylline (n = 22)	U	p-value
Gestational age, weeks	40,00 (38,00; 40,00)	40,00 (39,00; 41,00)	227,00	0,7336
Weight at birth, kg	3,30 (3,03; 3,54)	3,49 (3,29; 3,71)	180,50	0,1522
Height, cm	52,00 (51,00; 54,00)	53,50 (52,00; 56,00)	191,50	0,2405
Male sex, n (%)	16,00 (72,73 %)	12,00 (54,55 %)	198,00	0,3072
Female sex, n (%)	6,00 (27,27 %)	10,00 (45,45 %)		
Age at hospitalization, h	6,50 (4,00; 9,00)	7,50 (6,00; 8,00)	197,00	0,2962
Average age of mothers, years	27,50 (23,00; 31,00)	27,50 (24,00; 32,00)	240,00	0,9719
Pregnancy registration, n (%)	19,00 (86,36 %)	22,00 (100,00 %)	209,00	0,4456
Pregnancy registration, weeks	13,00 (10,00; 19,00) (n=19)	10,00 (9,00; 18,00)	168,50	0,2957
Births outside health care facilities, n (%)	2,00 (9,09 %)	0	220,00	0,5047
Caesarean section, n (%)	10,00 (45,45 %)	12,00 (54,55 %)	220,00	0,6138
Indirect heart massage, n (%)	4,00 (18,18 %)	2,00 (9,09 %)	220,00	0,6138
Primary resuscitation, n (%)	18,00 (81,82 %)	17,00 (77,27 %)	231,00	0,8053
1 min Apgar score, points	4,00 (3,00; 6,00) (N=20)	4,50 (3,00; 6,00)	205,50	0,7244
5 min Apgar score, points	6,00 (4,50; 7,00) (n=20)	7,00 (5,00; 7,00)	182,50	0,3514
Thompson scale score, points	14,00 (13,00; 17,00)	13,00 (12,00; 14,00)	170,00	0,0933
Abnormal aEEG pattern, n (%)	16,00 (72,73 %)	9,00 (40,91 %)	165,00	0,0726
Electroclinical convulsive seizures, n (%)	18,00 (81,82 %)	18,00 (81,82 %)	242,00	1,0000

Overall, AKI as defined by the mKDIGO (2012) criteria developed in 5.00 (11.36%) infants out of 44.00 (100.00%) subjected to therapeutic cooling for moderate or severe hypoxic-ischemic encephalopathy with prophylactic use of methylxanthines. Among the patients diagnosed with AKI as classified, stage 0 was found in 39.00 (88.64%) cases. 4.00 (9.09%) infants had stage I, and 1 (2.27%) infant developed stage II; the data obtained were similar for both groups: p = 0.7872; U = 230.00. None of the patients progressed to stage III as defined by KDIGO (2012), and renal replacement therapy was not performed. It is noteworthy that in the group of infants with AKI, this complication was more often manifested in patients with severe neonatal encephalopathy - 3.00 (60.00%) than with moderate - 2.00 (40.00%).

According to the baseline serum Cr level and GFR based on this marker, there was no statistically significant difference between the study groups on day 1, $p \geq 0.0500$. However, on day 3 there was a characteristic gradual statistically significant increase in serum Cr to 1.13 (0.86; 1.25) mg/dl in the newborns with AKI compared to the infants without this complication, in whom the index was 0.77 (0.64; 1.03) mg/dl, U = 38.00; p = 0.0291. There was a natural decrease in GFR derived from Cr to 17.97 (13.84; 24.42) ml/min/1.73m2 in the neonates with renal dysfunction compared to the group of patients with preserved renal function - 27.30 (21.71; 34.62), U = 31.00; p = 0.0147. In this cohort of infants, the serum Cr level gradually decreased during the study and was 0.78 (0.64; 0.93) mg/dl for day 5 vs. 1.40 (1.15; 1.82) mg/dl in the other group, U = 11.00; p = 0.0015; and Cr eGFR increased to 28.17 (23.78; 34.60) mL/min/1.73m2 compared to the newborns with AKI - 12.38 (11.12; 17.54) mL/min/1.73m2 , U = 8.00; p = 0.0010.

Plasma CysC in the patients with preserved renal function was identical to the level of this indicator determined in the infants with impaired renal function for day 1 of the study being 2.50 (2.20; 2.60) ng/ml and 2.56 (2.41; 2.70) ng/ml, respectively, U = 78.00; p = 0.4823. However, in the newborns without AKI on the day 5, the level of CysC progressively decreased compared to day 1 and was 2.25 (2.08; 2.49) ng/ml; the difference is significant, U = 327.00; p = 0.0095. Instead, in the infants with AKI, the level of this marker did not change during the study and was 2.42 (1.89; 2.45) ng/ml on day 5, U = 7.00; p = 0.2963. eGFR(cys) equivalent to changes in this marker, progressively increased in the cohort of patients without kidney damage from 30.12 (28.94; 33.93) mL/min/1.73m2 on day 1 to 33.23

(30.35; 35.75) mL/min/1.73m2 on day 5, U = 527.50; p = 0.0202; and did not change in the other group: 29.79 (29.46; 30.12) mL/min/1.73m2 and 28.33 (28.04; 39.08) mL/min/1.73m2, respectively, U = 7.00; p = 0.2963.

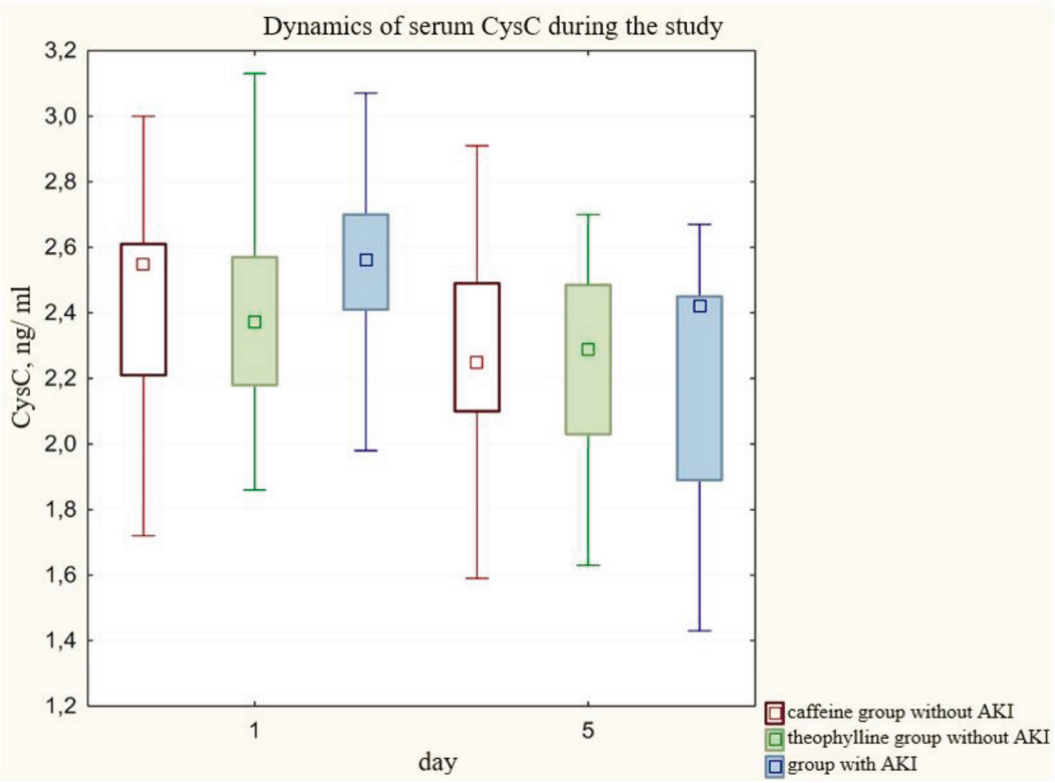

Figure 2. Dynamics of changes in serum creatinine level during the study

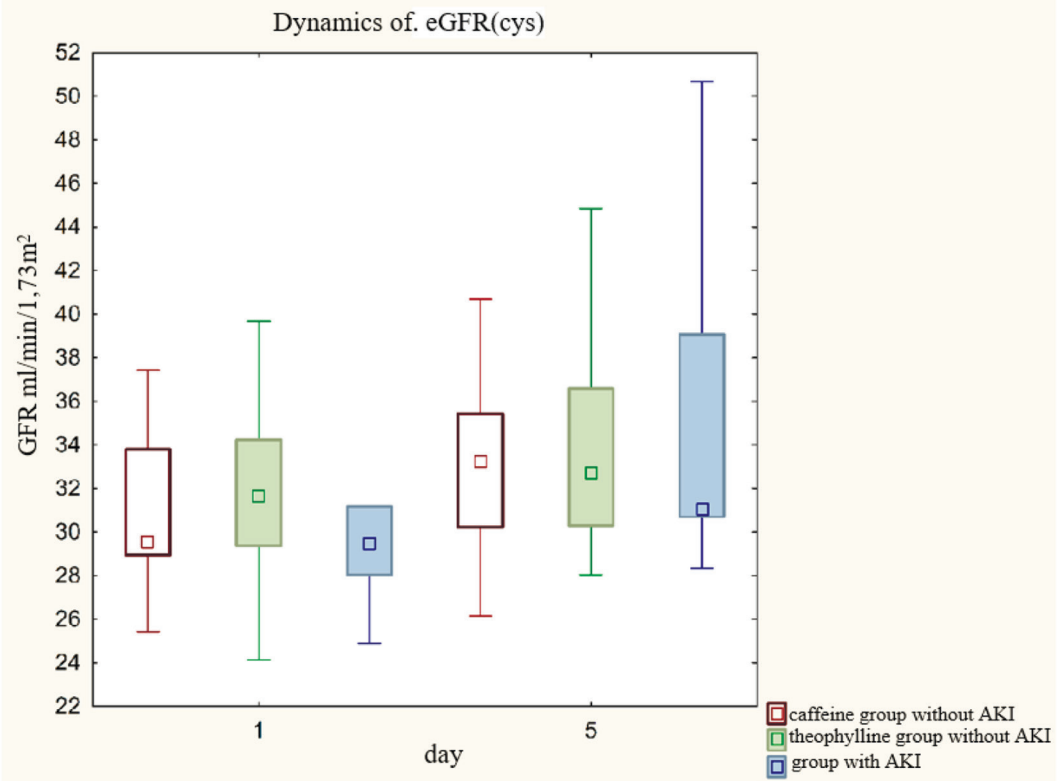

Figure 3. Dynamics of glomerular filtration rate by creatinine

It should be noted that the diuresis rate in the infants of both groups did not change during the study, the mean values obtained in the groups were ≥ 1.5 ml/kg/h, which is probably due to methylxanthine therapy; the data are similar, p ≥ 0.0500.

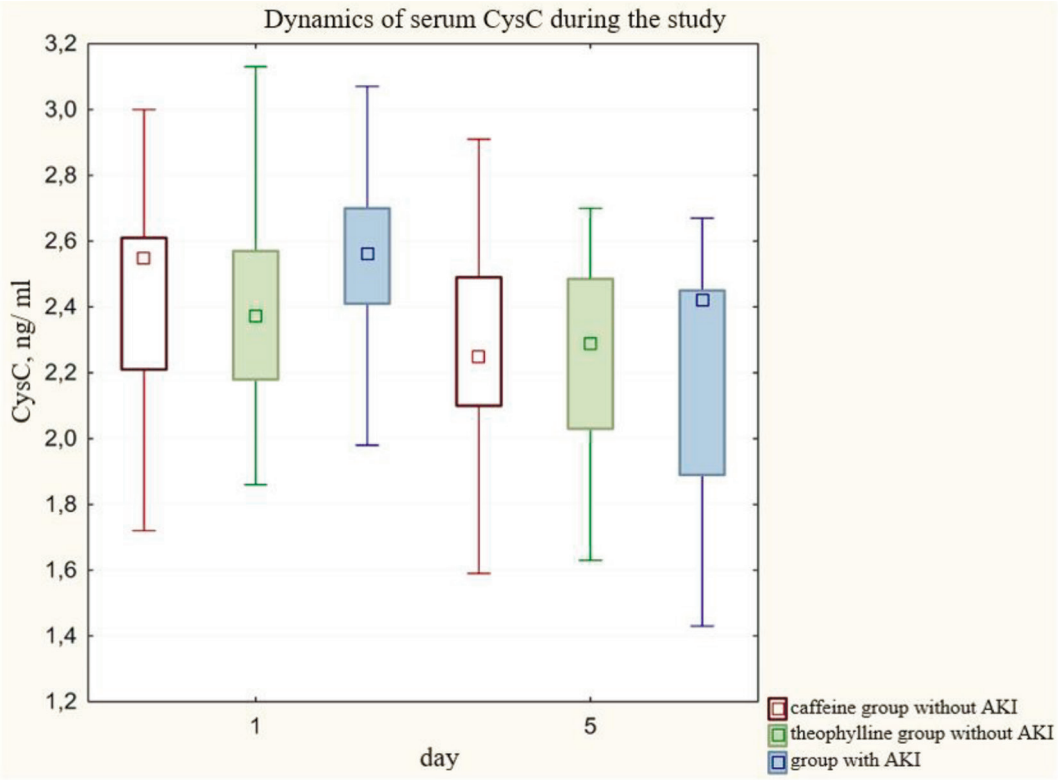

Figure 4. Dynamics of changes in serum cystatin C level during the study

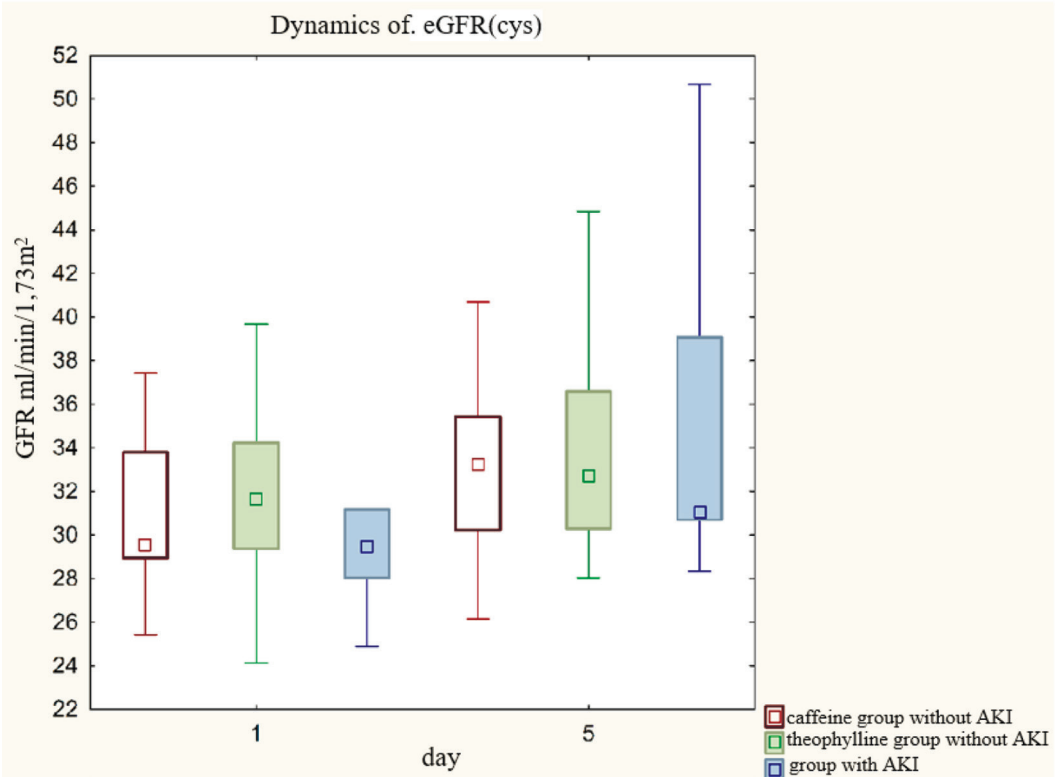

Figure 5. Dynamics of glomerular filtration rate based on cystatin C

In general, the results of treatment in the groups of critically ill newborns with asphyxia during therapy with caffeine citrate or theophylline indicate the effectiveness of these drugs in preventing and treating AKI in this cohort of patients. Methylxanthine therapy combined with therapeutic cooling helps to reduce Cr and CysC levels, increase GFR and diuresis rate.

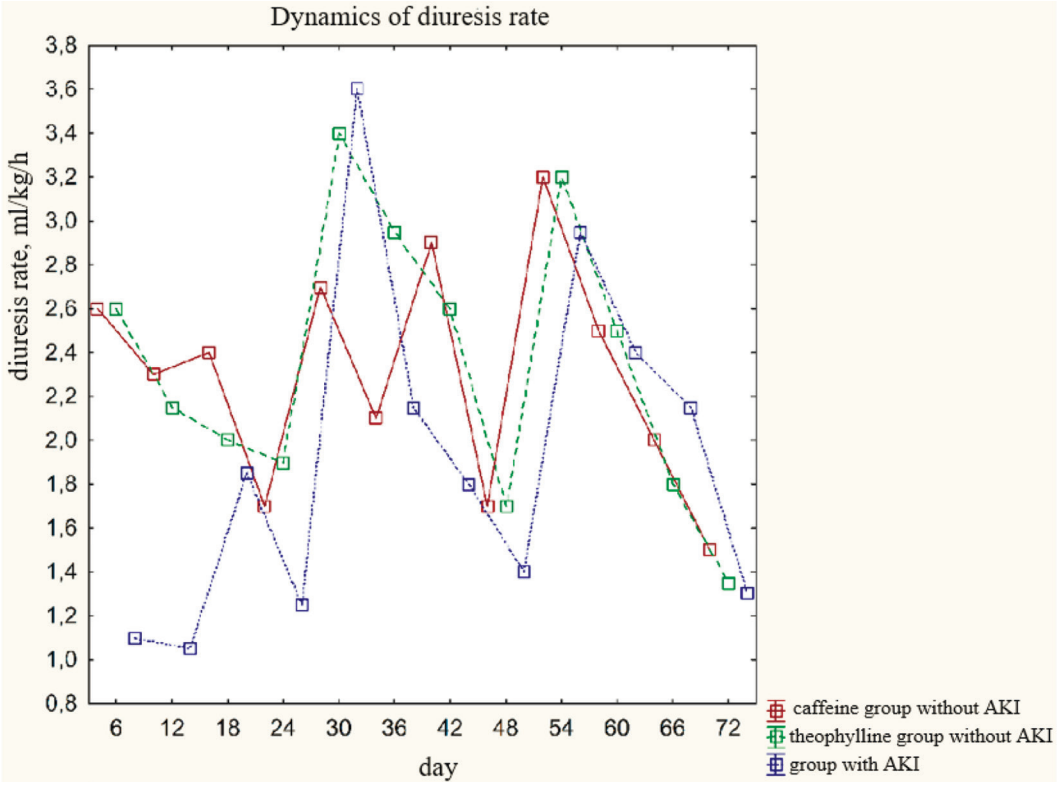

Figure 6. Dynamics of diuresis rate in the study groups

Table 1

Dynamics of indicators before and after the use of caffeine citrate

Indicator, measurement units	Day 1a	Day 5	T	p-value
Cr, mg/dl	1,04 (0,82; 1,25)	0,84 (0,64; 1,10)	60,00	0,0309
CysC, ng/ml	2,56 (2,29; 2,70)	2,26 (2,10; 2,49)	40,50	0,0052
eGFR(crea), ml/min/1.73m^2	22,03 (17,44; 25,35)	25,31 (19,52; 33,52)	56,00	0,0221
eGFR(cys), ml/min/1.73m^2	29,52 (28,04; 32,69)	33,16 (30,23; 35,43)	35,00	0,0030

Table 2

Dynamics of indicators before and after the use of caffeine citrate

Indicator, measurement units	Day 1a	Day 5	T	p-value
Cr, mg/dl	1,04 (0,82; 1,25)	0,84 (0,64; 1,10)	60,00	0,0309
CysC, ng/ml	2,56 (2,29; 2,70)	2,26 (2,10; 2,49)	40,50	0,0052
eGFR(crea), ml/min/1.73m^2	22,03 (17,44; 25,35)	25,31 (19,52; 33,52)	56,00	0,0221
eGFR(cys), ml/min/1.73m^2	29,52 (28,04; 32,69)	33,16 (30,23; 35,43)	35,00	0,0030

Table 3

Dynamics of indicators before and after the use of caffeine citrate

Dynamics of indicators before and after the use of theophylline	Day 1a	Day 5	T	p-value
Cr, mg/dl	0,89 (0,79; 1,05)	0,79 (0,66; 0,90)	72,00	0,0768
CysC, ng/ml	2,37 (2,16; 2,54)	2,29 (1,98; 2,48)	60,00	0,0309
eGFR(crea), ml/min/1.73m^2	24,59 (21,35; 29,87)	28,61 (23,78; 34,44)	63,00	0,0393
eGFR(cys), ml/min/1.73m^2	31,66 (29,68; 34,51)	32,71 (30,35; 37,43)	56,00	0,0221

Having analyzed the results of the patients' treatment, we concluded that the infants with AKI had a longer stay in the ICU (p = 0.0096), a longer need for mechanical ventilation and hospital stay in general.

Table 4

Dynamics of indicators before and after the use of caffeine citrate

Indicator , measurement units	Without AKI (n = 39)	AKI (n = 5)	U	p-value
Length of MV, days	5,85 (4,94; 6,88)	6,55 (6,45; 8,51)	68,00	0,2835
Length of the ICU stay , bed-days	11,00 (10,00; 14,00)	16,00 (15,00; 17,00)	27,00	0,0096
Length of hospital stay, bed-days	22,00 (19,00; 32,00)	28,00 (25,00; 30,00)	65,00	0,2367
Mortality	0	0	97,50	1,0000
Length of hospital stay, bed-days	22,00 (19,00; 32,00)	28,00 (25,00; 30,00)	65,00	0,2367
Mortality	0	0	97,50	1,0000

Discussion

Despite the improvement and standardization of the criteria for AKI, clinical and laboratory diagnosis is still based on elevated serum creatinine levels and decreased diuresis rate. However, these parameters do not specifically reflect tubular function or damage.

Among the patients in our study presenting AKI, the diagnosis was made according to the criteria of creatinine level in 60.00%, a combination of creatinine rise with a decrease in diuresis rate in 20.00%, and another 20.00% of the newborns showed an isolated decrease in diuresis rate without an increase in creatinine. This is probably due to fluid overload and dilution of serum creatinine, which leads to a false-negative determination of AKI.

It is the determination of diuresis rate that can provide more timely information about renal dysfunction, as even minor changes in renal function can be manifested by oliguria long before creatinine levels rise, and patients with anuria have zero GFR. However, diuresis rate may not be sensitive, given the development of nonoliguric AKI. In a retrospective study of 115 premature infants with very low birth weight (< 1500 g), Daga A. (2017) reported that none of the patients who developed AKI (n = 26) had a decrease in diuresis rate otherwise of nRIFLE (< 1.5 ml/kg/h), and therefore, oliguric form of kidney injury did not develop [25].

Another study conducted for a cohort of critically ill newborns by V., H., Nesargi (2020) showed that the mKDIGO appeared to be more sensitive in the infants with impaired renal function because it identified 94% compared to nRIFLE (49%). However, the authors report that the latter scale allows identify infants with mild AKI more often (29% vs. 16% of mKDIGO) and can be used for screening newborns in resource-limited settings [26].

Most studies of AKI in critically ill infants have used CysC level as the main diagnostic criterion. However, given the limitations in the interpretation of this marker in the neonatal cohort, there is an urgent need to find new sensitive markers and introduce new criteria for AKI in newborns.

In a clinical study Babintseva A. (2018) studied the suitability of markers of tubular (a1-microglobulin urine and P2-microglobulin urine) and glomerular (Cr and CysC) renal dysfunction for detecting AKI in 95 critically ill full-term newborns using the nAKIN scale. The results of measurement of serum biochemical markers demonstrate significantly higher levels of Cr and CysC in the group of babies with AKI. However, Cr is a late and nonspecific marker of glomerular filtration rate decline. It is insensitive to acute changes in renal function and does not reflect the cause, location or degree of renal damage. The diagnostic model with Cr level did not demonstrate high discriminatory ability with a threshold level > 81.0 umol/L (AUROC 0.74, $p < 0.05$). The study showed a low sensitivity (48.4%) with a high specificity (97.2%) [27].

The study of the diagnostic value of CysC as an endogenous biomarker of AKI in the neonatal cohort of patients in the intensive care unit was carried out by Hidayati, E. L (2020), where a threshold value of CysC > 1.59 mg/dL was established at which high sensitivity and specificity were achieved. This level of CysC can be considered as a tool for screening for AKI in critically ill infants [28].

In our study, in babies who had signs of AKI according to the gold standard, an increase in the level of both Cr and CysC, respectively, was observed during the first 5 days of life, with a slow decrease. Thus, the method of determination of CysC as a marker of AKI in newborns did not have significant advantages over the standard. Perhaps this is explained by transient conditions and kidney adaptation in the first day of life. Besides, to determine serum cystatin C is more expensive than to determine creatinine.

According to a modern meta-analysis by Yang, H (2022) of 12 published articles, the diagnostic value of CysC in newborns as well as its sensitivity (0.84) and specificity levels (0.81). In this study, the value of DOR (diagnostic odds ratio) was 22.58 and AUC (area under curve) was 0.88, which proves the overall accuracy of CysC level in the diagnosis of nAKI [29].

We calculated eGFR(cys) using the most common formula obtained in a cohort of pediatric patients aged 1 to 16 years with chronic kidney disease. However, this equation has not been tested in the neonatal population, and other formulas have limitations. Thus, Treiber M. (2015) derived a new formula for calculating eGFR(cys) in neonates, but it includes 3D renal volumetry, which is not routinely performed [30].

Conclusions

In general, the measurement of CysC did not provide additional information on the development of AKI in neonates of the first 5 days of life, which would allow to make a quick decision to change the intensive care program. This may be due to the prophylactic use of methylxanthines (caffeine citrate and theophylline), which demonstrated

equal efficacy in preventing the progression of AKI. Further studies are needed, possibly involving children who have not received prophylactic therapy. Overall, this study highlights the advantages and limitations of the diagnostic criteria, and given the risk factor of perinatal asphyxia, emphasizes the need for the use of methylxanthines in combination with therapeutic cooling as indicated for this cohort of critically ill patients.

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СИРОВАТКОВИЙ ЦИСТАТИН С ЯК МАРКЕР ГОСТРОГО ПОШКОДЖЕННЯ НИРОК У НОВОНАРОДЖЕНИХ ІЗ ГІПОКСИЧНО-ІШЕМІЧНОЮ ЕНЦЕФАЛОПАТІЄЮ, ПІДДАНИХ ТЕРАПЕВТИЧНОМУ ОХОЛОДЖЕННЮ

Л.С. Стрижак, І.О. Анікін

Запорізький державний медичний університет
(м. Запоріжжя, Україна)

Резюме

Оцінка функції нирок у новонароджених є надзвичайно важливим і водночас складним завданням зважаючи на унікальну будову тіла, підвищену вразливість та швидкий ріст. Проте, для раннього виявлення гострого пошкодження, раціонального дозування препаратів та безпечної медикаментозної терапії виявлення ранніх маркерів ниркової дисфункції є досить важливим.

Мета – оцінити прогностичне значення сироваткових біомаркерів для ранньої діагностики гострого пошкодження нирок (ГПН) у новонароджених з гіпоксично-ішемічною енцефалопатією (ГІЕ) на тлі проведення терапевтичної гіпотермії та превентивного застосування метилксантинів.

Матеріали і методи дослідження. Одноцентрове проспективне рандомізоване дослідження із залученням 44 немовлят із ГІЕ, що потребують проведення лікувальної гіпотермії та профілактично отримують кофеїну цитрат або теофілін для попередження прогресування ГПН, виконано на базі ВІТН Запорізької обласної клінічної дитячої лікарні з 2019 до 2022 року.

Лабораторний аналіз зразків сироватки крові проводили на 1, 3 та 5 добу від народження, визначали рівень креатиніну, цистатину С та їх асоціації з розвитком ГПН відповідно до неонатальних критеріїв шкали KDIGO (2012).

Статистичні аналізи проводилися з використанням програмного забезпечення Statistica 13.0, TIBCO SoftwareInc. (№ ліцензії JPZ804I382130ARCN10-J) та Microsoft Excel 2013 (№ ліцензії 00331-10000-00001-AA404). Визначення вірогідності різниці абсолютних значень середніх величин проводили, використовуючи непараметричні методи статистичного аналізу: критерій Манна – Вітні (U) для непов'язаних груп і критерій знаків Вілкоксона (Т) для пов'язаних груп. Статистична значущість визначалася як $p < 0,0500$.

Дослідження виконано із дотриманням морально-етичних норм згідно правил IGH/GCP, Гельсінської декларації (1964 з доповненнями 1975, 1983, 1989, 1996, 2000 р.р.), Конвенції Ради Європи про права людини і біомедицини та законодавства України. Протокол затверджено Комісією з медичної етики при Запорізькому державному медичному університеті.

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Результати та їх обговорення. Загалом, ГПН за KDIGO розвинулося у 5,00 (11,36 %) немовлят із 44,00 (100,00 %), стадію 0 виявили у 39,00 (88,64 %). 4,00 (9,09 %) немовлят мали стадію І, а у 1 (2,27 %) розвинулася ІІ стадія; отримані дані збіжні – $p = 0,7872$; $U = 230,00$. У жодного з пацієнтів не прогресувало ураження в стадію ІІІ.

У новонароджених зі збереженою функцією нирок протягом дослідження відбувається зниження креатиніну та закономірне підвищення швидкості клубочкової фільтрації (ШКФ) за цим маркером. Статистично вірогідне підвищення рівню креатиніну та зниження ШКФ виявлено у новонароджених з дисфункцією нирок на 3 та 5 добу дослідження. Рівень креатиніну прогресував з базового 1,07 (0,87; 1,10) мг/дл до 1,13 (0,86; 1,25) мг/дл на 3 добу та 1,40 (1,15; 1,82) мг/дл на 5 добу, а ШКФ знижувалась з 19,76 (19,07; 22,90) мл/хв/1,73м2 до 17,97 (13,84; 24,42) мл/хв/1,73м2 для 3 дня та становила 12,38 (11,12; 17,54) мл/хв/1,73м2 для 5 дня, $p < 0,0500$.

Цистатин С прогресивно знижувався у дітей без ГПН з 2,50 (2,20; 2,60) нг/мл до 2,25 (2,08; 2,49) нг/мл, $p = 0,0095$; натомість у немовлят з ГПН рівень даного маркеру не змінювався і склав 2,56 (2,41; 2,70) нг/мл на 1 добу та 2,42 (1,89; 2,45) нг/мл на 5-ту добу, $p = 0,2963$. ШКФ визначена за цистатином С, еквівалентно змінам даного маркеру, прогресивно підвищується у когорті пацієнтів без пошкодження нирок та не змінюється в іншій групі.

Темп діурезу у немовлят обох груп не різнився, склав $\geq 1,5$ мл/кг/год, що, ймовірно, пов'язано із терапією метилксантинами, $p \geq 0,0500$.

Висновки: визначення цистатину С не давало додаткової інформації щодо розвитку гострого пошкодження нирок у новонароджених перших 5 діб життя, які б дозволили швидко приймати рішення щодо зміни програми інтенсивної терапії. Необхідні подальші дослідження із залученням дітей, які не отримували профілактичну терапію.

Ключові слова: асфіксія; гіпоксично-ішемічна енцефалопатія; гостре пошкодження нирок; креатинін; цистатин С; метилксантини; новонароджений, швидкість клубочкової фільтрації.

Contact Information:
Liliia Stryzhak – PhD student of the Department of Pediatric Surgery and Anesthesiology Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine.
Contact Address: Maiakovskyi Avenue, 24A, Zaporizhzhia, 69035, Ukraine.
Contact Phone: +38(096)2836676
e-mail: liliia_stryzhak@ukr.net
ORCID: https://orcid.org/0000-0002-4204-2159

Ivan Anikin – PhD, Associate Professor, Department of Pediatric Surgery and Anesthesiology, Higher State Educational Establishment of Ukraine «Zaporizhzhia State Medical University» (Zaporizhzhia, Ukraine).
e-mail: anikin1974@ukr.net
ORCID ID: https://orcid.org/0000-0002-1125-0123

Контактна інформація:
Стрижак Лілія Сергіївна – аспірант кафедри дитячої хірургії та анестезіології Запорізького державного медичного університету, м. Запоріжжя, Україна.
Контактна адреса: проспект Маяковського, 24А, м. Запоріжжя, 69035, Україна.
Контактний телефон: +38(096)2836676
e-mail: liliia_stryzhak@ukr.net
ORCID:https://orcid.org/0000-0002-4204-2159

Анікін Іван Олександрович – к.мед.н., доцент кафедри дитячої хірургії та анестезіології, Вищий державний навчальний заклад України «Запорізький державний медичний університет» (м. Запоріжжя, Україна).
e-mail: anikin1974@ukr.net
ORCID ID: https://orcid.org/0000-0002-1125-0123

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