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Neutrophil-to-lymphocyte ratio in pediatric acute appendicitis

Neutrofilno-limfocitni odnos u akutnom apendicitisu kod dece

Nikola Stanković*, Ivan Stanojević[†‡], Dragan Djordjević[‡§], Zoran Kostić[‡‖], Ivo Udovičić[‡§], Maja Miličković[¶], Djordje Savić[¶], Blagoje Grujić, Slaviša Djuričić[†††‡‡], Maja Šurbatović[‡§]**

Mother And Child Health Care Institute of Serbia "Dr. Vukan Čupić", *Department of Anesthesiology and Intensive Therapy, [¶]Department of Abdominal Surgery, **Department of Neonatal Surgery, [†††]Department of Clinical Pathology, Belgrade, Serbia; Military Medical Academy, [†]Institute for Medical Research, [‖]Clinic for General Surgery, [§]Clinic for Anesthesiology and Intensive Therapy, Belgrade, Serbia; University of Defence, [‡]Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Banja Luka, [‡‡]Faculty of Medicine, Banja Luka, Bosnia and Hercegovina

Abstract

Background/Aim. Acute appendicitis (AA) is the most frequent emergency and appendectomy is the most frequent abdominal operation in the pediatric surgery. Diagnosis of AA in children is more difficult due to a lack of cooperation and poor clinical history data, leading to significant number of misdiagnostic cases. Our aim was to explore whether neutrophil to lymphocyte ratio (NLR) may be useful in diagnosis and follow-up of AA in children. **Methods.** Prospective investigation of NLR values in 129 consecutive patients admitted to the Mother and Child Healthcare Institute of Serbia and referred for surgery due to AA was performed. According to the pathohistological findings, patients were divided into 3 groups: normal/early, uncomplicated (phlegmonous) and complicated (gangrenous or/and perforated) AA. Laboratory analysis was done preoperativly and on the 1st and the 3rd postoperative days. **Results.** Statistically significant differences of NLR values were found in the different time points in total of patients and per groups. Some statistically significant differences of NLR values among histopathological groups were recorded. Investigations of correlation of NLR and other laboratory and clinical parameters showed strong positive correlation between NLR and C-reactive protein postoperatively and between NLR and Pediatric Appendicitis Score (PAS) preoperatively. Strong negative correlation with preoperative symptoms duration (PSD) were also present. Optimal cutoff NLR value between negative and positive appendectomies was 6.14. **Conclusion.** NLR provides well monitoring of progression of AA in children and, its cutoff values may help in distinguishing the phases of AA. Because of that, NLR should be used in diagnosis of AA in children.

Key words:
appendicitis; neutrophils; lymphocytes; diagnosis; diagnosis, differential; prognosis; child.

Apstrakt

Uvod/Cilj. Akutni apendicitis (AA) predstavlja najčešće hitno stanje, a apendektomija najčešću abdominalnu operaciju u pedijatrijskoj hirurgiji. Dijagnoza AA kod dece je otežana nedostatkom saradnje prilikom pregleda i nepreciznim anamnestičkim podacima, što dovodi do većeg broja nepravilno dijagnostikovanih slučajeva. Cilj rada je bio da se ispita da li neutrofilno-limfocitni odnos (NLR) može biti koristan u dijagnostici i praćenju AA kod dece. **Metode.** Sprovedeno je prospektivno ispitivanje vrednosti NLR kod 129 bolesnika primljenih u Institut za zdravstvenu zaštitu majke i deteta Srbije, planiranih za hirušku intervenciju zbog AA. Prema patohistološkom nalazu, bolesnici su podeljeni u tri grupe: normalni/rani, nekomplikovani (flegmonozni) i komplikovani (gangrenozni i/ili perforativni) AA. Laboratorijska ispitivanja su vršena preoperativno, kao i prvog i trećeg postoperativnog dana. **Rezultati.** Analizom je utvrđeno postojanje statistički značajnih razlika u vrednostima NLR preoperativno, prvog i trećeg postoperativnog dana kako na ukupnom uzorku tako i po formiranim grupama. Zabeležene su i statistički značajne razlike u vrednostima NLR između patohistoloških grupa. Ispitivanjem korelacija NLR sa drugim laboratorijskim i kliničkim parametrima, utvrđena je snažna korelacija sa C-reaktivnim proteinom postoperativno i snažna korelacija sa *Pediatric Appendicitis Score* (PAS) preoperativno. Takođe, preoperativno je utvrđena i snažna negativna korelacija sa preoperativnim trajanjem simptoma. Optimalna razgraničavajuća vrednost NLR za negativne i pozitivne apendektomije iznosila je 6.14. **Zaključak.** Parametar NLR omogućuje dobro praćenje progresije AA kod dece. Razgraničavajuće vrednosti NLR mogu biti od koristi u određivanju faza bolesti, zbog čega bi NLR trebalo koristiti u dijagnostici AA kod dece.

Ključne reči:
apendicitis; neutrofili; limfociti; dijagnoza; dijagnoza, diferencijalna; prognoza; deca.

Correspondence to: Nikola Stanković, Mother and Child Health Institute "Dr Vukan Čupić", Department of Anestesiology and Intensive Therapy, Radoja Dakića 6, 11 000 Belgrade, Serbia. Phone: +381 60 013 57 13. E-mail: kokanasta@gmail.com

Introduction

Acute appendicitis (AA) is the most frequent emergency and appendectomy is the most frequent abdominal operation in the pediatric surgery [1]. Despite new diagnostic methods [scoring systems, ultrasound, computed tomography (CT), nuclear magnetic resonance (NMR)], AA remains the most misdiagnosed surgical cause of pediatric acute abdomen [2].

Negative appendectomy is an operation done for suspected appendicitis with normal appendix on histological evaluation. A certain rate of these negative explorations for suspected appendicitis is accepted as a good surgical practice because of the devastating impact of perforated appendicitis. Especially in girls, negative appendectomy appears with incidence of up to 30% of all suspected appendicitis [3].

Missed or delayed diagnosis of appendicitis increases the possibility of perforation, which has the highest incidence in small children [4] and results in a fivefold increase of complication rate in the postoperative period [5]. So, in the routine clinical practice it is important to find out whether AA is the cause of symptoms and if it is, to distinguish complicated (gangrenous and perforated) from uncomplicated AA.

Early (catarrhal) appendicitis is characterized by the presence of neutrophils in the lumen or focally in the mucosa, often with the absence of any clinical symptoms. The significance of this finding is controversial since it is often found in patients as an incidental finding [6].

Diagnosis of AA in children is more difficult due to a lack of cooperation and poor clinical history data. Routine laboratory parameters we used for diagnosis of AA in children are white blood cells (WBC) count with leukocyte formula and C-reactive protein (CRP). Neutrophil to lymphocyte ratio (NLR) is directly derived from the WBC, and its diagnostic relevance in AA was studied more in adults, although AA is more common in children. Expected differences in the results are based primarily on the diversity of immune mechanisms in children and adults.

In this prospective investigation we analyzed NLR during AA in children, before operation and during postoperative period. At the same time, other laboratory parametars, including CRP, were mesured and patients condition was estimated by Pediatric Appendicitis Score (PAS).

Methods

Patients admitted to the Mother and Child Healthcare Institute of Serbia in Belgrade and referred for surgery after established diagnosis of AA, were consented according to the good clinical practice (GCP). In total, 129 consecutive patients, 3 to 16 years old, were analyzed from May to November 2015. Children younger then 3 years were not recruited. Patients with other actual acute disease were excluded from the study as well as the patients with operative finding of some other abdominal inflammation. The study was approved by Ethics Committee of Mother and Child Healthcare Institute of Serbia.

After obtaining anamnesis data, including duration of symptoms, blood sampling was done for determining the WBC count and CRP, and PAS was calculated. Two additional blood samplings were performed at the 1st and at the 3rd postoperative day for the same laboratory analysis.

In the process of the WBC count determination, hematological autoanalyzer Advia 120/2120 (Simens) was used. Calculation of NLR was done automatically as the percentage of neutrophils divided by the percentage of lymphocytes, using the formula after entering data in Excel table.

For statistical analysis we used PRISM GraphPad softver version 5.01. Correlations (Spearman's rho) and Mann–Whitney U-test were calculated for comparative statistics (z-score and two-tailed P). The cutoff values were determined using the receiver operating characteristic (ROC) analysis. The optimal cutoff value was represented as the most prominent point on the ROC curve for sensitivity and specificity. A *p* value less then 0.05 was considered statistically significant.

Results

We analyzed 129 consecutive patients, 77 boys and 52 girls (male/female ratio 1,48), with average age of 10.43 ± 4.02 (boys: 10.03 ± 3.90; girls: 11.03 ± 4.16). Preoperative symptoms duration in days, in total of patients was 1.845 ± 1.61. Average preoperative pediatric PAS in total of patients was 7.31 ± 1.87, median 8 (2–10). Length of hospital stay in total of patients was 8.12 ± 4.09 days in average.

A total number of patients were separated into 3 groups. The first group represented patients with normal appendix and early stage of appendicitis as histopathological finding (NEAA); the second group, consisted of patients with phlegmonous or uncomplicated appendicitis (UAA); the third group were the patients with gangrenous and/or perforated appendicitis noticed as complicated appendicitis (CAA). Demographics and distribution of patients in the 3 formed groups are shown in the Table 1. Recorded preoperative parameters as well as those from the 1st and the 3rd postoperative days in the formed groups are listed in Table 2.

Table 1

Demographic characteristics and distributions of patients in the three formed groups

Group of patients	Patients' age (years)					
	(3–8)		(9–12)		(13–16)	
	male	female	male	female	male	female
NEAA (n = 23)	5	4	2	3	3	6
UAA (n = 50)	9	5	10	6	10	10
CAA (n = 56)	17	8	10	4	11	6
Total	31	17	22	13	24	22
	48		35		46	

NEAA – normal/early acute appendicitis; UAA – uncomplicated acute appendicitis; CAA – complicated acute appendicitis.

Table 2

Preoperative parameters and parameters from the 1st and the 3rd postoperative days in the formed grouops

Parameters	Group of patients		
	NEAA (n = 23)	UAA (n = 50)	CAA (n = 56)
PSD (days), $\bar{x} \pm SD$			
before surgery 1st postop. day 3rd postop. day	3.28 ± 2.66	1.24 ± 0.66	1.79 ± 1.26
PAS, $\bar{x} \pm SD$			
before surgery 1st postop. day 3rd postop. day	6.56 ± 2.02	6.76 ± 1.83	8.11 ± 1.56
WBC (10^9/L), $\bar{x} \pm SD$			
before surgery	12.31 ± 4.27	16.39 ± 5.82	19.31 ± 6.92
1st postop. day	11.62 ± 2.79	11.88 ± 3.44	13.39 ± 5.62
3rd postop. day	7.82 ± 2.04	7.76 ± 2.47	10.21 ± 6.52
Ne (%), $\bar{x} \pm SD$			
before surgery	69.42 ± 10.09	79.14 ± 12.33	83.26 ± 6.54
1st postop. day	77.59 ± 7.58	77.07 ± 7.61	78.74 ± 9.22
3rd postop. day	60.81 ± 6.14	59.35 ± 11.53	67.35 ± 12.74
Ly (%), $\bar{x} \pm SD$			
before surgery	20.34 ± 8.87	12.93 ± 9.54	8.49 ± 4.14
1st postop. day	14.49 ± 6.09	15.12 ± 5.89	12.83 ± 7.34
3rd postop. day	26.78 ± 5.95	27.96 ± 10.33	20.92 ± 10.60
NLR, $\bar{x} \pm SD$			
before surgery	4.79 ± 4.00	10.66 ± 8.13	14.10 ± 11.94
1st postop. day	6.73 ± 4.48	6.29 ± 3.56	8.61 ± 6.02
3rd postop. day	2.44 ± 0.86	2.65 ± 1.66	4.59 ± 3.32
CRP (mg/L), $\bar{x} \pm SD$			
before surgery	44.75 ± 48.9	61.88 ± 55.40	140.9 ± 90.83
1st postop. day	53.91 ± 42.58	61.88 ± 55.40	140.9 ± 90.83
3rd postop. day	37.88 ± 31.79	34.53 ± 32.53	89.14 ± 61.10
MPXI, $\bar{x} \pm SD$			
before surgery	-0.42 ± 5.46	-2.49 ± 5.92	-1.30 ± 5.57
1st postop. day	1.87 ± 3.34	-3.94 ± 6.95	-2.37 ± 7.07
3rd postop. day	0.21 ± 4.52	-5.66 ± 8.07	-3.43 ± 6.94
LOS (days), $\bar{x} \pm SD$			
before surgery 1st postop. day 3rd postop. day	8.91 ± 5.42	7.56 ± 3.38	8.27 ± 4.05

PSD – preoperative symptoms duration; PAS – Pediatric Appendicitis Score; WBC – white blood cell; Ne – neutrophils; Ly – lymphocytes; NLR – neutrophil/lymphocyte ratio; CRP – C–reactive protein; LOS – length of stay; MPXI – mieloperoxidase index; NEAA – normal/early acute appendicitis; UAA – uncomplicated acute appendicitis; CAA – complicated acute appendicitis.

NLR values in the different time points samples in total of patients and per groups

In total of 129 patients, statistically difference was recorded between values of NLR preoperatively, at the 1st and at the 3rd postoperative day (Figure 1A). NLR decreased from preoperative time point to the 3rd postoperative day, showing high statistical difference between preoperative and NLR value on the 1st day (11.10 ± 10.03 *vs.* 7.38 ± 5.01, $p = 0.0021$) and very high statistically significant difference between preoperative and NLR value that on the 3rd day (11.10 ± 10.03 *vs.* 3.42 ± 2.60, $p < 0.0001$) as well as between NLR values on the 1st and the 3rd postoperative day (7.38 ± 5.01 *vs.* 3.42 ± 2.60, $p < 0.0001$).

A statistically significant differences in NLR values among preoperative, the 1st, and the 3rd postoperative day samples were also registered within each individual histopathological group but with different pattern in the NEAA group. Namely, in the NEAA group, NLR values were highest on the 1st postoperative day, with statistically significant difference when compared with preoperative NLR values (6.73 ± 4.48 *vs.* 4.79 ± 4.00, $p = 0.013$), and very high statistically significant difference when compared with NLR values on the 3rd postoperative day (6.73 ± 4.48 *vs.* 2.44 ± 0.86, $p < 0.0001$). Preoperative NLR values were higher than NLR values on the 3rd postoperative day with high statistically significant difference (4.79 ± 4.00 vs. 2.44 ± 0.86, $p = 0.0074$, Figure 1B). In the UAA group, the

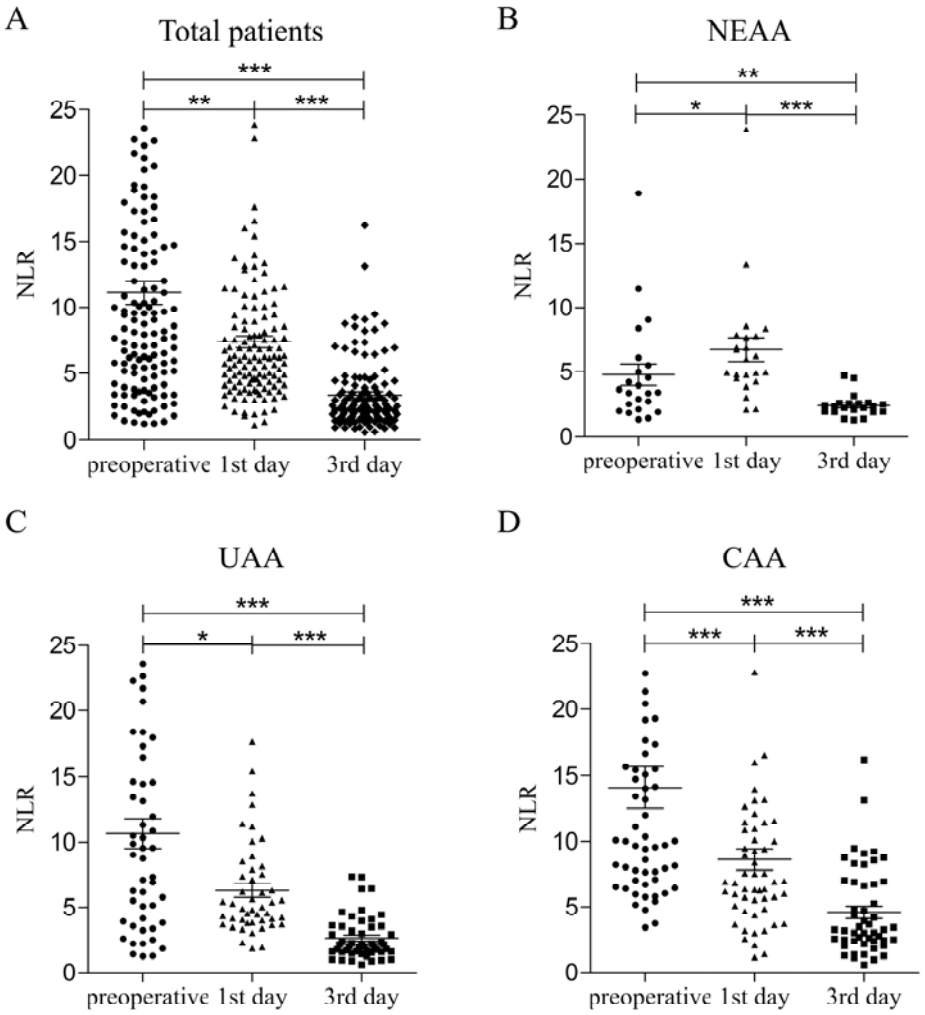

Fig. 1 – Comparison of the neutrophil to lymphocyte ratio (NLR) values measured in three different time points regarding to surgery: A) In total acute appendicitis (AA) patients, the highest NLR values were observed in the preoperative samples, with significant decrease toward the 3rd postoperative day; B) In the normal/early acute appendicitis (NEAA) group, the highest values were registered on the 1st postoperative day, with subsequent significant decrease toward the 3rd postoperative day; C) The NLR values in the uncomplicated acute appendicitis (UAA) and D) the complicated acute appendicitis (CAA) group showed similar pattern, being the highest in the preoperative samples with significant difference when compared with the 1st and the 3rd postoperative day samples. [mean ± standard error of mean (SEM), Mann-Withney test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)].

NLR values were highest in the preoperative samples, significantly higher when compared with samples on the 1st postoperative day (10.66 ± 8.13 *vs.* 6.29 ± 3.56, $p = 0.0148$), and higher than NLR values on the 3rd postoperative day with very highly statistically significant difference (10.66 ± 8.13 *vs.* 2.65 ± 1.70, $p < 0.0001$). The differences between preoperative NLR values and NLR values on the 3rd postoperative day were also very highly statistically significant in this group (6.29 ± 3.56 *vs.* 2.65 ± 1.70, $p < 0.0001$, Figure 1C). In the CAA group, again, NLR values were the highest in preoperative samples, with very high statistically significant difference when compared with NLR values on the 1st day postoperatively (14.10 ± 11.94 *vs.* 8.61 ± 6.02, $p = 0.0005$) and on the 3rd postoperative day (14.10 ± 11.94 *vs.* 4.59 ± 3.32, $p < 0.0001$). The NLR values the 1st postoperative day were higher than NLR values on the 3rd postoperative day with very high statistically significant difference (8.61 ± 6.02 vs. 4.59 ± 3.32, $p < 0.0001$, Figure 1D).

In both gender groups, statistically significant differences were also present among NLR values of the 3 blood sampling time points (Figure 2). In boys, significant difference was recorded between preoperative and NLR values on the 1st postoperative day (11.27 ± 9.57 vs. 7.53 ± 4.77, $p = 0.0101$), while the differences between preoperative and NLR values on the 3rd postoperative day as well as between

NLR values on the 1st and the 3rd postoperative day were very highly statistically significant (11.27 ± 9.57 vs. 3.44 ± 2.42, $p < 0.0001$; and 7.53 ± 4.77 vs. 3.44 ± 2.42, $p < 0.0001$, respectively, Figure 2A). In girls, very high statistically significant differences were found between NLR values on the preoperative time point and those on the 3rd postoperative day and between these values on the 1st and the 3rd postoperative day (10.86 ± 10.75 vs. 3.39 ± 2.87, $p < 0.0001$; and 7.17 ± 5.37 vs. 3.39 ± 2.87, $p < 0.0001$, respectively, Figure 2B).

NLR values between different histopathological groups in the 3 time points

In the preoperative samples, the lowest NLR values were found in the NEAA group, highly significantly lower in comparison with the UAA group (4.79 ± 4.00 vs. 10.66 ± 8.13, $p = 0.001$) and very highly significantly lower in comparison with the CAA group (4.79 ± 4.00 vs. 14.10 ± 11.94, $p < 0.0001$). Statistically significant difference was not reached in comparison with the preoperative NLR values between the UAA and CAA groups (Figure 3A). However, on the 1st postoperative day, the NLR values were statistically significant higher in the CAA group when compared with the UAA group (8.61 ± 6.02 *vs.* 6.29 ± 3.56, $p = 0.0175$, Figure 3B). Statistically significant difference in NLR values between the NEAA and UAA and between NEAA and CAA groups on the 1st postoperative day was not found. On the 3rd postoperative day, the highest NLR values were still in the CAA group, with statistically high significant difference when compared with the NEAA group (4.59 ± 3.32 vs. 2.44 ± 0.86, $p = 0.0036$) and with very high statistically significant difference in comparison with NLR values the UAA group (4.59 ± 3.32 vs. 2.65 ± 1.66, $p = 0.0006$). Statistically significant difference was not found in NLR values between the NEAA and UAA groups on the 3rd postoperative day (Figure 3C).

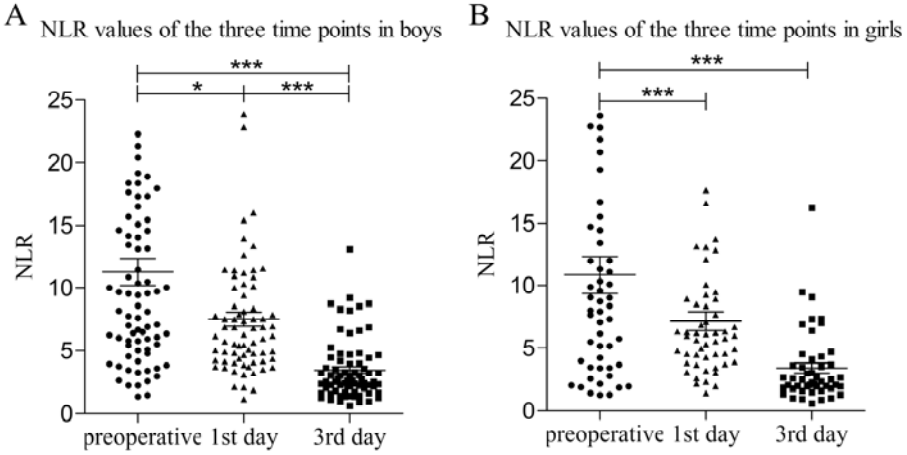

Fig. 2 – Comparison of the neutrophil to lymphocyte ratio (NLR) values measured in three different time points regarding to surgery; A) showing the highest values in the preoperative samples, with significant decrease toward the 3rd postoperative day in boys, as well as, in girls B) [(mean ± SEM, Mann-Withney test, *$p < 0.05$, ***$p < 0.0001$)].

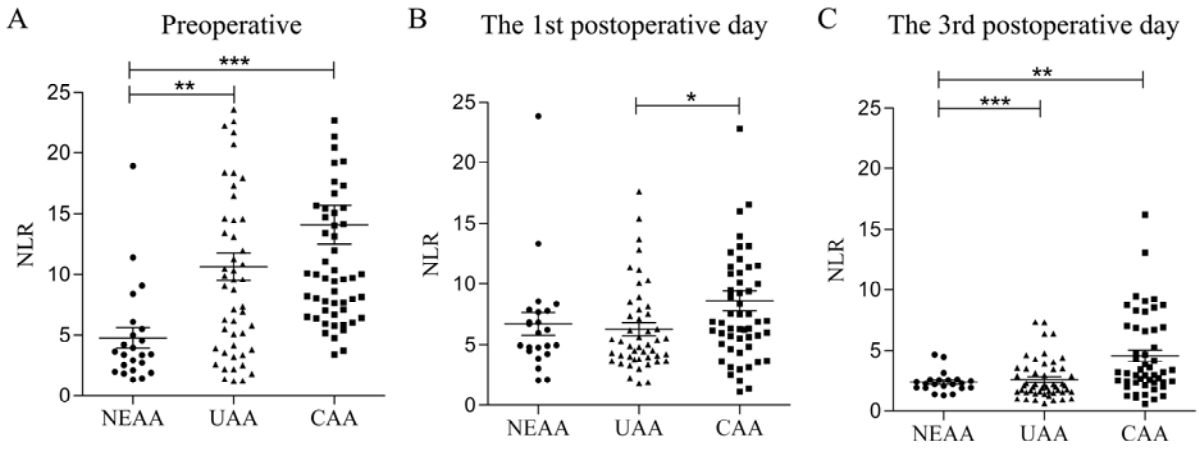

Fig. 3 – Differences in the neutrophil to lymphocyte (NLR) values between different histopathological groups at the three time points: A) showing the highest values in the CAA group in the preoperative samples, B) in the 1st day samples, and C) the 3rd day samples [mean ± standard error of mean (SEM), Mann-Withney test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$)]. Explanation for NEAA, UAA and CAA see under Table 1.

NLR correlates inversely with PSD and positively with PAS and CRP

In total of 129 patients, the NLR values showed significant inverse correlation with the preoperative symptoms duration (PSD) measured in days (Spearman's r = -0.3190, p = 0.0002; Figure 4A). At the same time, duration of symptoms was significantly different among the different histopathological groups (not shown).

On the other hand, NLR showed a significant positive correlation with PAS in total AA patients (Spearman r = 0.4899, $p < 0.0001$; Figure 4B), as well as in the NEAA group, the UAA group, male and female groups, separately (not shown). Preoperatively, the mean PAS values were different between the NEAA and the UAA group as well as between the UAA and the CAA group (not shown).

In total AA patients, NLR showed a strong correlation with CRP on the 1st (Spearma'n r = 0.4082, $p < 0.0001$, Figure 5A) and the 3rd postoperative day (Spearman's r = 0.5814, $p < 0.0001$, Figure 5B). Statistically significant correlation between NLR and CRP was not recorded in the preoperative samples.

Within the histopathological groups significant correlation between NLR and CRP was found in the UAA group on the 3rd day (Spearman's r = 0.0336, p = 0.3175, Figure 6A), in the CAA group on the 1st postoperative day (Spearman's r = 0.6040, $p <$ 0.0001, Figure 6B) and again in the CAA group on the 3rd postoperative day (Spearman's r = 0.7063, $p < 0.0001$, Figure 6C).

By gender, correlation between NLR and CRP showed significant positive relationship on the 1st and the 3rd postoperative day in both, boys (Spearman's r = 0.3546, p = 0.0026; and Spearman's r = 0.6622, $p < 0.0001$, Figure 7A and 7B, respectively) and girls (Spearman r = 0.4743, p = 0.0006; and Spearman's r = 0.4463, $p < 0.0017$, Figure 7C and 7D, respectively). There was no significant correlation between NLR and CRP in the preoperative samples within both gender groups.

NLR did not show correlation with milloperoxidase index (MPXI) and length of hospital stay neither in the total sample nor by gender and histopathological groups.

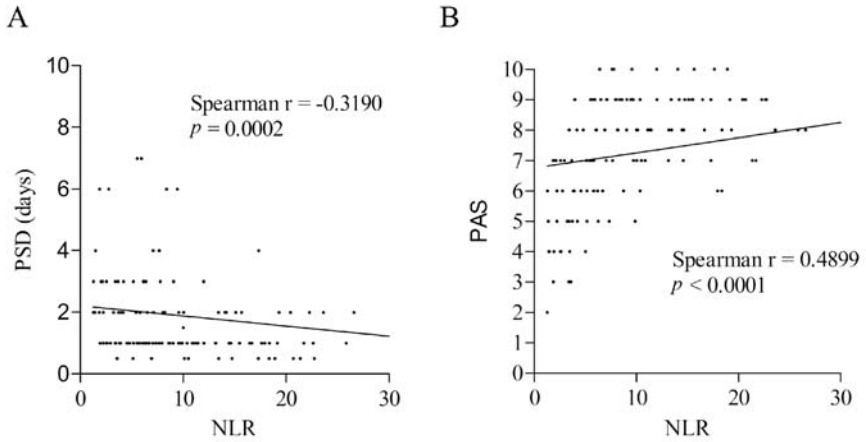

Fig. 4 – A) Correlation between neutrophil to lymphocyte ratio (NLR) and preoperative symptoms duration (PSD) in total acute appendicitis (AA) patients, showing significant inverse relationship; B) Correlation between NLR and pediatric appendicitis score (PAS) in total AA patients, showing significant positive relationship. (Spearman's correlation test).

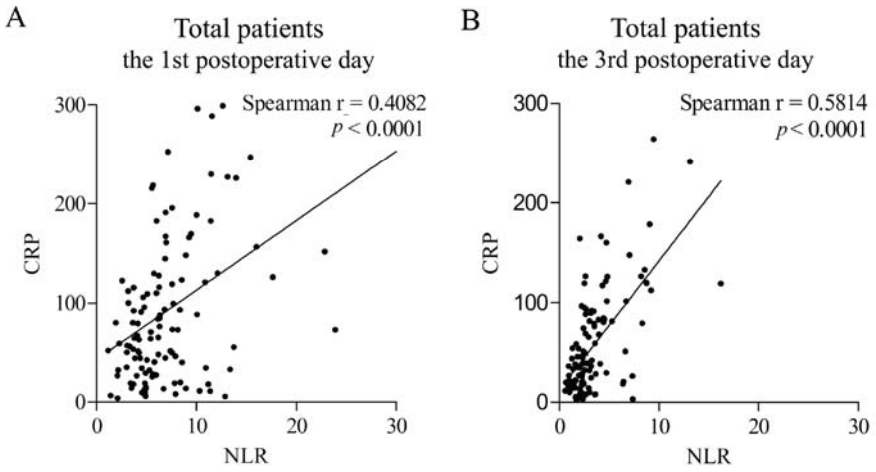

Fig. 5 – Correlation between neutrophil to lymphocyte ratio (NLR) and C-reactive protein (CRP) in total acute appendicitis (AA) patients, showing significant positive relationship in: A) the samples taken on the 1st postoperative day, as well as in B) the samples taken on the 3rd postoperative day (Spearman's correlation test).

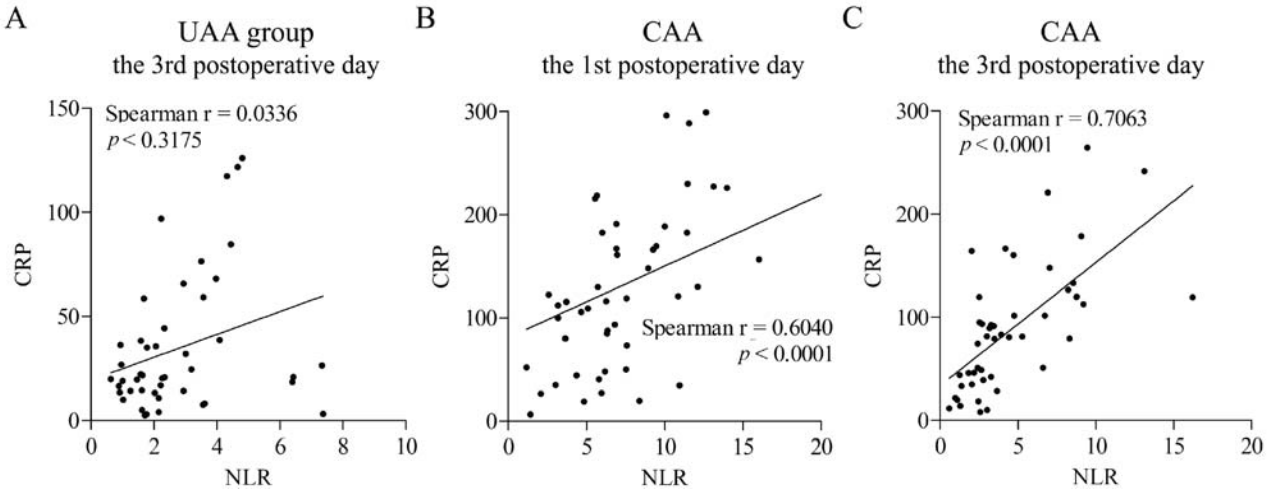

Fig. 6 – Correlation between neutrophil to lymphocyte ratio (NLR) and C-reactive protein (CRP) within different histopathological groups, showing significant positive relationship in: A) the samples from the uncomplicated acute appendicitis (UAA) group taken on the 3rd postoperative day; B) the samples from the complicated acute appendicitis (CAA) group taken on the 1st postoperative day and C) the samples from the CAA group taken on the 3rd postoperative day (Spearman's correlation test).

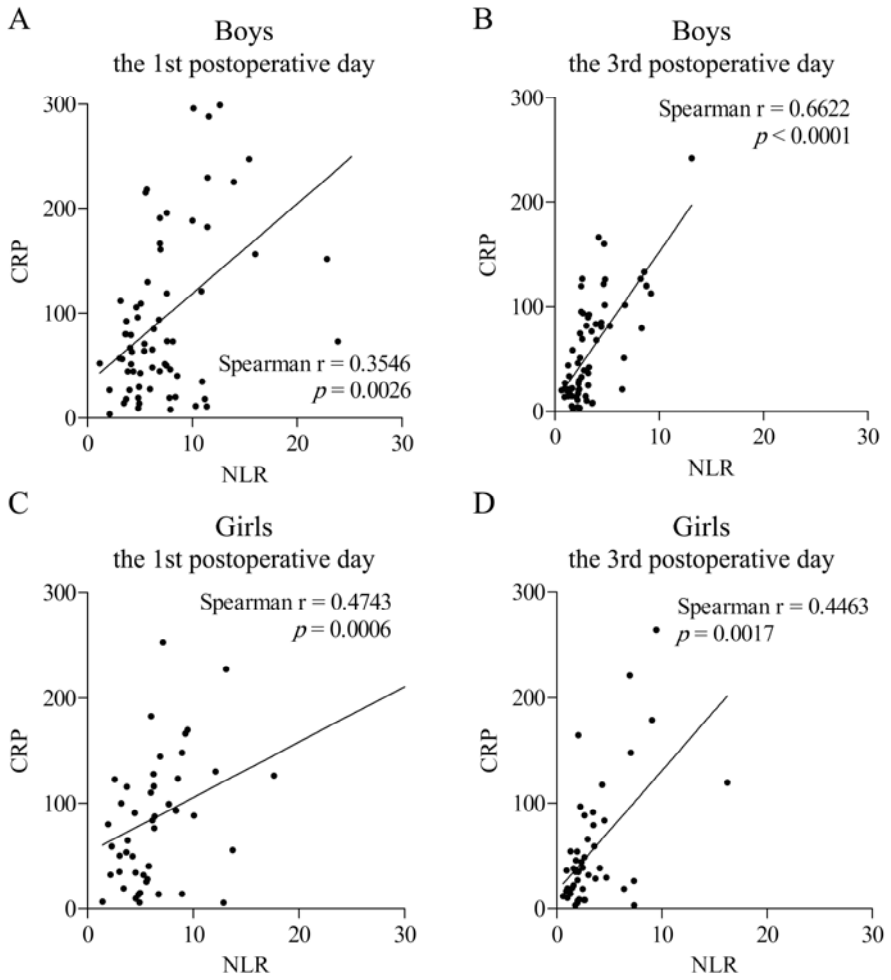

Fig. 7 – Correlation between neutrophil to lymphocyte ratio (NLR) and C-reactive protein (CRP) within gender groups, showing significant positive relationship in boys: A) on the first, as well as B) on the third postoperative day. Correlation between NLR and CRP in girls, also showed significant positive relationship C) on the first, as well as D) on the third postoperative day (Spearman's correlation test).

NLR cutoff values among histopathological groups

Optimal cutoff NLR value between the NEAA and the UAA group was 5.06, with sensitivity of 71.43% and specificity of 73.91%, which represents test with moderate accuracy (AUC = 0.7409, p = 0.001011; Figure 8A). Between the UAA and the CAA group, optimal cutoff NLR value was 6.325, with sensitivity of 83.93% and specificity of 40.82%, which represents test with poor accuracy (AUC = 0.6023, p = 0.06986; Figure 8B). Optimal cutoff NLR value between the NEAA and the IAA (acute inflamed appendix; UAA + CAA) group was 6.14, with sensitivity of 74.53% and specificity of 82.61%, which represents test with good accuracy (AUC = 0.8175, $p < 0.0001$; Figure 8C).

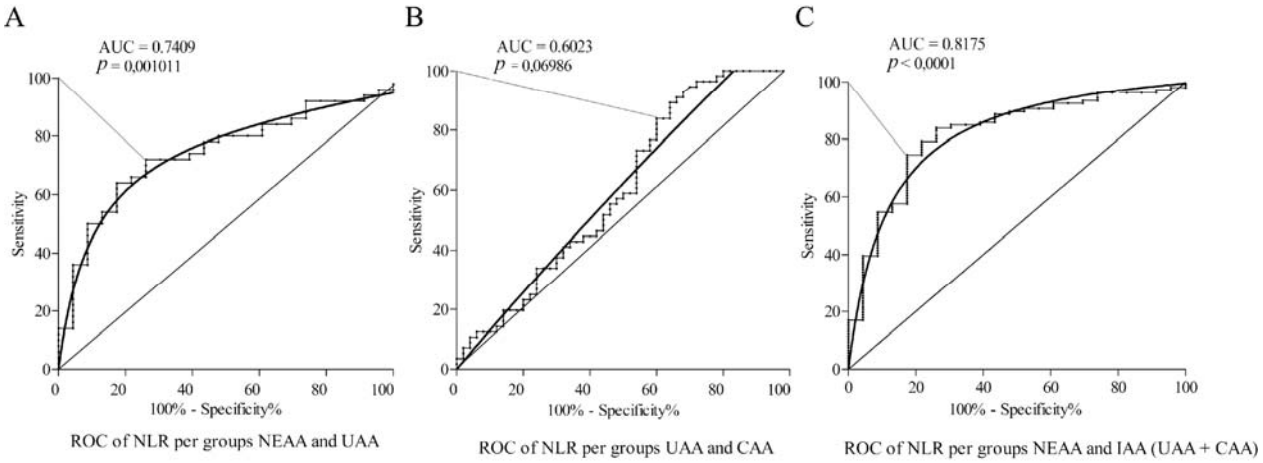

Fig. 8 – Receiver Operating Characteristic (ROC) curves for formed histopathological groups: A) ROC curve for the normal/early acute appendicitis (NEAA) group and the uncomplicated acute appendicitis (UAA) group (sensitivity 71.43%, specificity 73.91%); B) ROC curve for UAA group and the complicated acute appendicitis (CAA) group (sensitivity 83.93%, specificity 40.82%); C) ROC curve for the group NEAA and IAA (UAA+CAA) group (sensitivity 74.53%, specificity 82.61%).

Discussion

Reliable diagnosis of AA and proper indication for surgical intervention remain a challenge in everyday pediatric medical practice. Still, there is no clear boundary between conservative and surgical treatment. As prevention of advanced inflammation and perforation, operative approach of early appendicitis is accepted as a routine surgical practice. In this manner, patients are introduced into the risk for perioperative and postoperative complications.

Reliability in diagnosis of AA is improved by combining clinical, laboratory and imaging methods. However, imaging methods are time consuming and related to radiation while the echosonography is usually insufficiently informative [7]. So, physical examination with a combination of laboratory tests is the basis for diagnosis. Leukocytes, neutrophils, and CRP are commonly used, although none of these laboratory parameters show sufficient sensitivity and specificity [8, 9].

Neutrophils are important types of cells in appendicitis, which is confirmed by histopathological reports. They represent one of the first lines of defense against penetrating agents such as bacteria. These cells are capable of phagocytosis, secretion of lytic enzymes and production of free oxygen radicals with high antimicrobial potential. Activation of neutrophils is a two-stage process triggered by bacteria and their products on cytokines and chemokines. The number of neutrophils in the blood during the inflammatory process growing by mobilization of marginal pool and then from the bone marrow, what is proportional to the extent of inflammation. Lymphocytes are immunocompetent cells that coordinate defensive responses and assist in neutrophil activation. In gangrenous form of AA, significant lymphopenia may occur. Its pathophysiological mechanism is not enough understood [10, 11]. In accordance with this, the increase in the value of NLR in developed form of AA occurs as a result of the increase in the number of neutrophils as well as of reducion of the lymphocytes number.

In our study, a statistically significant difference was recorded among NLR in three samples of blood taken preoperatively and on 1st and 3rd postoperative values, in the total of patients as well as in histopathological gender groups. Except in the NEAA group, all records showed continuous decline of NLR values. Increase of NLR value in the NEAA group on the 1st postoperative day could be result of surgical trauma. The most prominent decline of NLR values was in the CAA group, what may be explained by the highest extent of inflamation in this group and its attenuation after surgery.

Preoperative difference in NLR values among the groups was higher than on the 1st and 3rd postoperative day. This difference was most pronounced between the NEAA group and the other two groups, while smaller between the UAA and CAA group. This is probably due to developing inflammatory process in preoperative phase in the UAA group and the CAA group, while it was in the phase of recovery on the 1st and the 3rd day after surgery, this difference was

smaller due to stabiliyation of immunological condition. These preoperative differences could be of additional diagnostic importance in AA. On the 3rd postoperative day, difference between NLR values in the NEAA group and the UAA group was lost, while in the difference between CAA and the other 2 groups retained statistical significance, probably because of longer period needed for inflammation reduction in the CAA group where the inflammation was intense.

According to the original publication, PAS as a diagnostic tool had a sensitivity of 100% and specificity of 92% for the diagnosis of AA. PAS may have a value from 1 to 10, scoring as the following: migration of pain – 1, anorexia – 1, nausea/emesis – 1, tenderness in right lower quadrant – 2, cough/percussion and hop tenderness – 2, pyrexia – 1, leukocytosis – 1 and polymorphonuclear neutrophilia – 1 [12]. After the subsequently conducted trials, diagnostic value of PAS has been significantly lower [13–15]. NLR in our sample correlated with PAS on the total sample and by histology and gender groups. However, the sensitivity and specificity of NLR for differentiating negative and positive AA in our study were better than PAS. The parameters of PAS related to pain are subjective and unfavorable for assessment due to poor cooperation of younger children during physical examination, what reduces accuracy of PAS.

The clinical presentation of AA is individual and usually depends on the child's age, location and degree of inflammation of the appendix. Different PSD in histopathological groups in our study registered in all three groups, unlike in the previous studies where the PSD was similar in the UAA and the CAA group and clear distinct in the NEAA group [15]. In our study, a strong negative correlation between NLR and PSD was obtained. This could be explained by the fact that advanced forms of the disease quickly lead to the full clinical presentation of AA and decision for surgical treatment. This clinical presentation includes a pronounced neutrophilia sometimes followed by lymphopenia and resulting with increase of NLR.

In our investigation, there was the correlation between NLR and CRP on the 1st and the 3rd postoperative day, but not preoperatively. CRP is protein of acute inflamatory phase and laboratory parameter that along with positive physical findings and radiological findings may have good diagnostic value in AA. However, as an isolated parameter it is not useful because of low specificity [8]. Laboratory parameters which include the value of neutrophils, such as absolute neutrophil count, percentage of neutrophils in the leukocyte formula and NLR, are considered as even better diagnostic parameters in AA because neutrophils rise faster than CRP which needs time for synthesis in the liver [16]. Some studies showed that NLR appears to have greater diagnostic accuracy then the WBC count [17]. The first increase in CRP in blood records 12 hours after the occurrence of inflammation while peak plasma concentration reaches between 24 to 48 hours [18]. Another studies suggest that CRP is an important diagnostic agent for perforated AA but not for AA in general [19]. Our findings confirm a faster NLR increase and delayed increase of CRP in pediatric AA. In this way, increase of NLR may suggest earlier phases of AA which can be successfully treated with antibiotics.

Cutoff value of NLR distinguishing the NEAA from the UAA may be a good clinical indicator for the introduction of antibiotic therapy [20]. In our group of patients, this value was 5.06 what is significantly higher than the values obtained in other studies conducted in children [9, 15] and in adults or mixed groups of patients [21, 22]. At the same time, our cutoff value has much better sensitivity and specificity. On the other hand, according to our results, NLR could not be so reliable diagnostic tool for distinguishing the UAA from the CAA, what should be of great value for making a decision on an urgent surgery of AA. The value we received for distinguishing these two groups was 6.325, but with a lower sensitivity and specificity. This is not in accordance with the results of some studies in which the cutoff value to distinguish the UAA and the CAA had significantly higher sensitivity and specificity [21]. Combining the UAA and the CAA group we got a new group of acute inflamed appendix. Cutoff value of NLR distinguishing the NEAA group from this new group, which means between negative and positive appendectomy was 6.14 with high sensitivity and specificity. This value is very similar to that distinguishing the UAA from the CAA, taking into account limitation that we had small number of patients in the NEAA group comparing to newly defined group of acute inflamed appendix.

Conclusion

NLR well monitors and reflects inflammatory process progression in AA in children and could be useful in the clinical stage differentiation of the disease. We found strong positive correlation of NLR with CRP as well as with PAS, while NLR negatively correlates with PSD. Values of NLR are changing earlier then the CRP values, which means its better reactivity and, therefore, usefulness of NLR in early diagnosis of AA is better than that of CRP. Values of NLR for distinguishing the disease phases are much higher in our study then in the earlier condacted invastigations, with cutoff of 5.06 for the UAA and 6.325 for the CAA.

Considering good specificity and sensitivity, easy availability and use in practice, NLR should be used with other diagnostic methods in the diagnosis of acute appendicitis in children.

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