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Neutrophil myeloperoxidase index in pediatric acute appendicitis

Neutrofilni mijeloperoksidazni indeks u akutnom apendicitisu kod dece

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Abstract

Background/Aim. Diagnosis of acute appendicitis (AA) remains the most common dilemma of pediatric surgical team. Our aim was to determine whether the neutrophil myeloperoxidase index (MPXI), in combination with other laboratory and clinical parameters, can be useful in diagnosis and follow-up of AA in children. **Methods.** A prospective investigation of MPXI values in 117 consecutive patients, planned for the surgical intervention due to AA, was performed. The patients were stratified into three groups according to the intraoperative finding: the normal/early, uncomplicated and complicated AA. Laboratory analyses were done preoperativly, on the 1st and on the 3rd postoperative days. **Results.** The statistically significant difference of MPXI values between the uncomplicated and complicated appendicitis before surgery and the positive correlations between the MPXI and C-reactive protein, as well as interleukin-6, before surgery were found. Postoperatively, in the group of uncomplicated, as well as complicated AA, a significant decrease of MPXI was recorded. **Conclusion.** The MPXI may be used as an informative biomarker in the follow-up of AA in children. A wide reference range for the MPXI and individual differences in the values of MPXI in the healthy children, generate difficulties for its use for the initial diagnosis of acute appendicitis. Usefulness of MPXI determination decreases with a delayed diagnosis.

Key words:
appendicitis; appendectomy; child; diagnosis, differential; peroxidase; neutrophils; c-reactive protein; interleukin-6.

Apstrakt

Uvod/Cilj. Dijagnoza akutnog apendicitisa (AA) i dalje ostaje jedna od najčešćih dilema u radu pedijatrijskog hirurškog tima. Naš cilj je bio da se ispita da li mijeloperoksidazni indeks (MPXI), u kombinaciji sa drugim laboratorijskim i kliničkim parametrima, može biti koristan u dijagnostici AA kod dece. **Metode.** Sprovedeno je prospektivno ispitivanje vrednosti MPXI kod 117 bolesnika, planiranih za hirušku intervenciju zbog AA. Bolesnici su bili podeljeni u tri grupe: normalni/rani, nekomplikovani (flegmonozni) i komplikovani (gangrenozni i/ili perforativni) AA. Laboratorijska ispitivanja vršena su preoperativno, kao i prvog i trećeg postoperativnog dana. **Rezultati.** Utvrđena je statistički značajna razlika u vrednosti MPXI između nekomplikovanog i komplikovanog apendicitisa, preoperativno. Ispitivanjem korelacije MPXI sa drugim laboratorijskim i kliničkim parametrima, utvrđena je korelacija sa C-reaktivnim proteinom i interleukinom 6, preoperativno. Postoperativno, i u grupi nekomplikovanog, kao i komplikovanog AA, zabeležen je značajan pad vrednosti MPXI. **Zaključak.** Širok opseg referentnih vrednosti i individualne razlike u vrednostima MPXI kod zdrave dece, ograničavaju upotrebu MPXI u dijagnostici akutnog apendicitisa kod dece. Odložena dijagnostika AA smanjuje upotrebnu vrednost MPXI.

Ključne reči:
apendicitis; apendektomija; deca; dijagnoza, diferencijalna; peroksidaze; neutrofili; c-reaktivni protein; interleukin-6.

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Introduction

Acute appendicitis (AA) is the most frequent emergency and appendectomy is the most frequent operation in the pediatric abdominal surgery [1]. Despite the new diagnostic methods [scoring systems, ultrasound, computed tomography (CT), nuclear magnetic resonance (NMR)], incidence of negative appendectomy as well as the rate of perforated disease, followed by many possible complications of this two misdiagnostic states, remains the same in some countries [2]. Negative appendectomy is the most commonly defined as the absence of inflammation, or pathology in the appendix after surgical intervention done for suspected appendicitis [3]. A certain rate of these negative explorations is accepted as good surgical practice because the devastating impact of perforated appendicitis. Epidemiological data in certain countries suggest that negative appendectomy could appear with incidence of up to 30% of all suspected appendicitis, especially in girls [4].

A diagnosis of AA in children is more challenging than in adults, due to a lack of cooperation and limited clinical history data. A missed or delayed diagnosis of appendicitis increases the possibility of perforation, which has the highest incidence in young children [5], and results in a fivefold increase in a postoperative complication rate [6]. So, distinguishing a complicated (gangrenous and perforated) from an uncomplicated (flegmonous), possibly conservatively treatable appendicitis, is of a great importance in clinical practice.

Among other laboratory parameters, some of which being used in diagnosing the AA in children, we routinely get the neutrophil myeloperoxidase index (MPXI), which is often unrecognized in clinical practice for this purpose. The MPXI represents quantity of myeloperoxidase (MPO) in the neutrophil population relative to the archetype (normal) population, and is calculated by the hematological autoanalyzer Advia 120/2120 (Siemens), as a standard output during a process of the white blood cell (WBC) differentials.

The objectives of this prospective trial were to investigate the possible relevance of MPXI in diagnosis and follow-up of AA in children as well as to investigate a possible correlation of MPXI trends with the recorded clinical parameters in the AA groups defined according to the intraoperative finding.

Methods

The patients, 3 to 16 years old, admitted to the Mother and Child Healthcare Institute of Serbia "Dr. Vukan Čupić"and referred for surgery after establishing the diagnosis of AA, were included into the prospective evaluation. Children younger than 3 years were not recruited. The patients with other acute diseases and the patients with intraoperative finding of other abdominal inflammation were excluded from the study. The study was approved by the Ethics Committee of the Mother and Child Healthcare Institute of Serbia (ref. number 8/8, from 08. Apr 2015), and run in line with the Good Clinical Practice and Declaration of Helsinki.

The baseline evaluation included medical history, duration of symptoms and blood sampling. The blood sampling evaluated the complete blood counts (CBC) and C-reactive protein (CRP). The serum obtained by the peripheral venous blood centrifugation was stored at -70 ºC for the later measurements of cytokines. The Pediatric Appendicitis Score (PAS) was calculated at the baseline for every patient (scoring from 1 to 10 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). Two additional blood samplings were performed at the 1st and the 3rd postoperative day for the same laboratory analysis.

The WBC differentials were measured using the hematological autoanalyzer Advia 120/2120 (Siemens AG, Eschborn, Germany). The measurement of MPO activity represented by the MPXI is displayed as an integral output/result. The test uses 4-chloro-1-naphthol as a substrate for the MPO in the granulocytes and black precipitates are formed in these cells. The neutrophils, monocytes and eosinophils are positive while lymphocytes and basophils are the MPO negative cells. The neutrophils are discriminated from the monocytes and eosinophils by the cell optical characteristics and peroxidase content. The MPXI is calculated as MPXI = [(Mean Neutrophil MPO Staining – Expected Staining Index) / Expected Staining Index] × 100, where the Mean Neutrophil MPO staining is the result of the absorbance measurement in the neutrophils. The Expected Staining Index is the expected MPO measurement result for an ideal standard neutrophil population which is maintained by the regular daily calibration [7]. The MPXI changes were analyzed before operation and during the postoperative period. The MPXI values were tested depending on the study groups and the time period that passed between the onset of symptoms and surgical intervention. Also, these values were correlated with the PAS, CRP and interleukin-6 (IL-6), and followed up over three postoperative days.

The determination of the cytokine concentrations in the sera of AA patients was performed on the Beckman Coulter FC500 cytometer using a commercial flow cytometric kit Human Inflammation 20 plex BMS 819, according to the manufacturer's instructions. Due to its role in a direct stimulation of CRP production – a reliable biomarker for cytokine-mediated response in the AA, the values of IL-6 were taken for a statistical analysis, while the values of other tested cytokines were beyond the scope of this article.

For the statistical analysis, we used the GraphPad Prism 5.01 (GraphPad Prism Software Inc. California, USA). Correlations (Spearman rho) and comparisons (Mann–Whitney *U*-test) were calculated for the comparative statistics (z-score and two-tailed *p*). Normality was assessed by using the Kolmogorov-Smirnov test.

Results

During the period May-October 2015, 117 consecutive patients were included in this prospective analysis. A total of 117 patients were stratified into three groups according to the intraoperative finding. The first group represented the patients with a normal appendix and an early stage of appendicitis (NEAA, n =

21), where normal appendix or mildly swollen (catarrhal appendicitis) was found; the second group consisted of patients with the phlegmonous or uncomplicated appendicitis (UAA, n = 45); the third group were the patients with gangrenous and/or perforated appendicitis noticed as complicated appendicitis (CAA, n = 51). There were 72 male and 45 female patients from 3 to 16 years of age (in average 10.28 ± 4.07 years). The baseline results and results obtained during analysis on the 1st and 3rd postoperative day are shown in Table 1.

The neutrophil myeloperoxidase index values are the lowest in children with uncomplicated acute appendicitis

Regarding the differences of MPXI values between the groups at the different time points from surgical intervention, the significantly lower MPXI values before surgery in the UAA group compared to the children in the CAA group were found [-2.83 ± 6.07 vs. -1.01 ± 5.73; $p = 0.0058$, Figure 1A)]. There were no statistically significant differences in the MPXI values between the UAA and NEAA groups nor between the NEAA and CAA groups in the samples taken before surgery.

On the 1st postoperative day, the lowest MPXI values were also in the UAA group with a very high significant difference in comparison with the NEAA group [-4.487 ± 7.125 vs. 2.405 ± 3.000; $p < 0.0001$ (Figure 1B), and a significantly lower one in comparison with the CAA group [-4.487 ± 7.125 vs. -2.10 ± 7.407; $p = 0.0472$ (Figure 1B)]. A statistically significant difference was found between the NEAA and CAA group on the 1st day after surgery as well [2.405 ± 3.000 vs. -2.10 ± 7.407; $p = 0.0101$ (Figure 1B)].

On the 3rd postoperative day, again, we found the lowest MPXI values in the UAA group with a very high significant difference in comparison with the NEAA group [-6.353 ± 8.329 vs 0.400 ± 4.677; $p = 0.0006$ (Figure 1C)], and a significantly lower one in comparison with the CAA group-[(6.353 ± 8.329 vs. -3.444 ± 7.125; $p = 0.0321$ (Figure 2C). There were no statistically significant differences in the MPXI values between the NEAA and CAA groups in the samples taken on the 3rd postoperative day.

The value of neutrophyl myeloperoxidase index determination changes with delayed diagnosis of acute appendicitis

In 65 out of 117 patients, the surgery was performed within 24 hours from onset of symptoms and in 52 of them after this period of time. A classification of patients regarding the time duration that elapsed between the onset of symptoms and surgical intervention, revealed the marked differences in comparison to the MPXI values among the NEAA, UAA and CAA groups.

In the patients with the symptoms duration less than 24 hours, in the samples taken before surgery, the lowest MPXI values in the UAA group were found, which were highly significantly lower compared to the NEAA group (-2.7 ± 6.7 vs. 4.8 ± 0.8; $p = 0.0040$) and significantly lower compared to the CAA group [-2.7 ± 6.7 vs. -0.6 ± 6.7; $p = 0.0449$ (Figure 2A)]. The MPXI values of the NEAA group were significantly higher in comparison with the CAA group [4.8 ± 0.8 vs. -0.6 ± 6.7; $p = 0.0224$ (Figure 2A)]. On the contrary, in the patients with symptoms duration more than 24 hours, there was no statistically significant differences in the MPXI values before surgery when the NEAA, UAA and CAA groups were compared (Figure 2A).

On the 1st postoperative day, in the patients with symptoms duration less than 24 hours, the significantly lower MPXI values in the UAA group compared to the NEAA group (-5.4 ± 7.9 vs. 3.5 ± 3.6; $p = 0.0018$) and in comparison with the CAA group was found [-5.4 ± 7.9 vs. -1.8 ± 7.9; $p = 0.0129$ (Figure 2B)]. In the patients with symptoms duration more than 24 hours, the statistically significant differences between the MPXI values emerged as well, and we found the lowest values in the CAA group, significantly lower compared to the NEAA group [-2.5 ± 7.0 vs. 1.9 ± 2.7; $p = 0.0382$ (Figures 2B)]. The MPXI values in the UAA group of patients with the symptoms duration more than 24 hours were lower in comparison to the NEAA group with a high statistical significance [-1.6 ± 2.2 vs. 1.9 ± 2.7; $p = 0.0076$ (Figure 2B)].

On the 3rd postoperative day, in the patients with symptoms duration less than 24 hours, the lowest MPXI values were again in the UAA group, with a very high statistical significance in comparison to the NEAA group [-6.2 ± 6.6 vs. 3.8 ± 3.4; $p = 0.0008$ (Figure 2C)] as well as in comparison to the CAA group [-6.2 ± 6.6 vs. -2.3 ± 7.2; $p = 0.0008$ (Figure 2C)]. In the patients with the symptoms duration more than 24 hours, on the 3rd postoperative day, there was no statistically significant differences in the MPXI values between the NEAA, UAA and CAA groups (Figure 2C).

The neutrophil myeloperoxidase index do not correlate with the Pediatric Appendicitis Score before surgery

A significant correlation between the MPXI and PAS values before surgery was not found, neither with some single parameters of this score nor with the absolute number of leukocytes and the percentage of neutrophils.

The neutrophil myeloperoxidase index correlate positively with C-reactive protein

In the peripheral blood samples of the total number of patients (n = 117), taken before surgery, a significant positive correlation between the MPXI values and the CRP concentrations was found [Spearman's $r = 0.3014$; $p = 0.0082$ (Figure 3A)]. In the peripheral blood samples taken on the 1st postoperative day, no statistical significance was shown. On the 3rd postoperative day, in the total number of patients, a significant correlation between MPXI and CRP was present again [Spearman's $r = 0.2132$; $p = 0.0370$ (Figure 3B)].

By classifying the patients into three groups, a positive correlation between the MPXI and CRP before surgery was found only in the NEAA group of patients [Spearman's $r = 0.4667$; I = 0.0440 (Figure 3C)]. In the peripheral blood samples taken on the 1st and the 3rd postoperative days in the NEAA, as well as in all samples from the UAA and CAA groups, a statistical significance could not be reached.

Table 1

Parameters in the groups of patients with acute appendicitis on the different time points from surgery

Group of patients	Sampling time	PSD (days) mean ± SD	PAS mean ± SD	MPXI mean ± SD	WBC (10^9/L)	Ne (%)	Ly (%)	NLR	CRP (mg/L) mean ± SD
					mean ± SD				
NEAA (n = 21)	before surgery	3.41 ± 2.47	6.71 ± 2.05	-0.20 ± 5.58	12.49 ± 4.42	69.05 ± 10.45	20.42 ± 9.29	4.88 ± 4.18	48.91 ± 49.52
	1st postop. day			2.41 ± 3.00	11.55 ± 2.90	77.31 ± 7.86	14.62 ± 6.32	6.76 ± 4.67	57.80 ± 42.97
	3rd postop. day			0.40 ± 4.68	7.726 ± 2.11	60.03 ± 5.36	27.38 ± 5.71	2.33 ± 0.72	38.78 ± 33.37
UAA (n = 45)	before surgery	1.22 ± 0.67	6.89 ± 1.76	-2.83 ± 6.07	16.76 ± 5.61	80.27 ± 11.05	12.23 ± 8.96	10.83 ± 7.74	26.28 ± 33.89
	1st postop. day			-4.49 ± 7.12	11.88 ± 3.42	76.86 ± 7.65	15.31 ± 5.98	6.20 ± 3.55	66.99 ± 56.62
	3rd postop. day			-6.35 ± 8.32	7.79 ± 2.36	59.08 ± 10.98	28.06 ± 10.01	2.55 ± 1.45	37.01 ± 33.63
CAA (n =51)	before surgery	1.83 ± 1.31	8.02 ± 1.61	-1.01 ± 5.73	19.08 ± 6.99	83.10 ± 6.75	8.61 ± 4.26	14.20 ± 12.45	94.36 ± 74.40
	1st postop. day			-2.10 ± 5.84	13.36 ± 9.50	78.39 ± 7.57	13.21 ± 6.24	8.49 ± 89.95	137.10 ± 7.40
	3rd postop. day			-3.44 ± 7.12	10.42 ± 6.75	66.93 ± 12.67	21.42 ± 10.51	4.43 ± 3.33	86.25 ± 61.06

PSD – preoperative symptoms duration; PAS – Pediatric Appendicitis Score; MPXI – myeloperoxidase index; WBC – white blood cell; Ne – neutrophils; Ly – lymphocytes; NLR – neutrophil/lymphocyte ratio; CRP – C-reactive protein; NEAA – normal/early acute appendicitis; UAA – uncomplicated acute appendicitis; CAA – complicated acute appendicitis; SD – standard deviation.

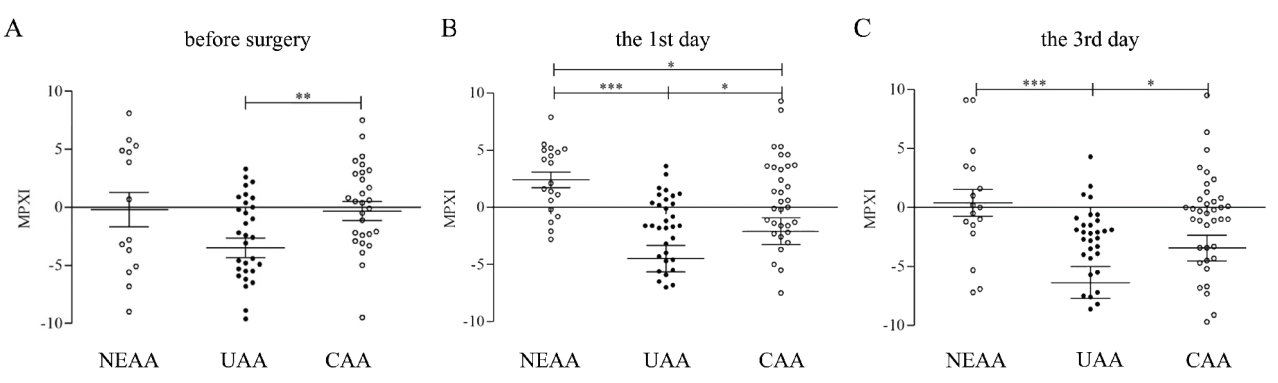

Fig. 1 – A) Comparison of MPXI among three groups of acute appendicitis (AA) patients before surgery, showing the significantly lower values in the UAA group when compared with the CAA group; B) Comparison of MPXI on the 1st postoperative day showing the lowest values in the UAA group when compared with the NEAA and CAA groups. The MPXI values of CAA group are significantly lower in comparison with the NEAA group; C) Comparison of MP XI on 3rd postoperative day showing the statistically lower values in the UAA group when compared with the NEAA and CAA groups.
The Mann-Whitney test (unpaired, two tailed) * – $p < 0.05$; ** – $p < 0.01$; * – $p < 0.0001$.**
For abbreviations see under Table 1.

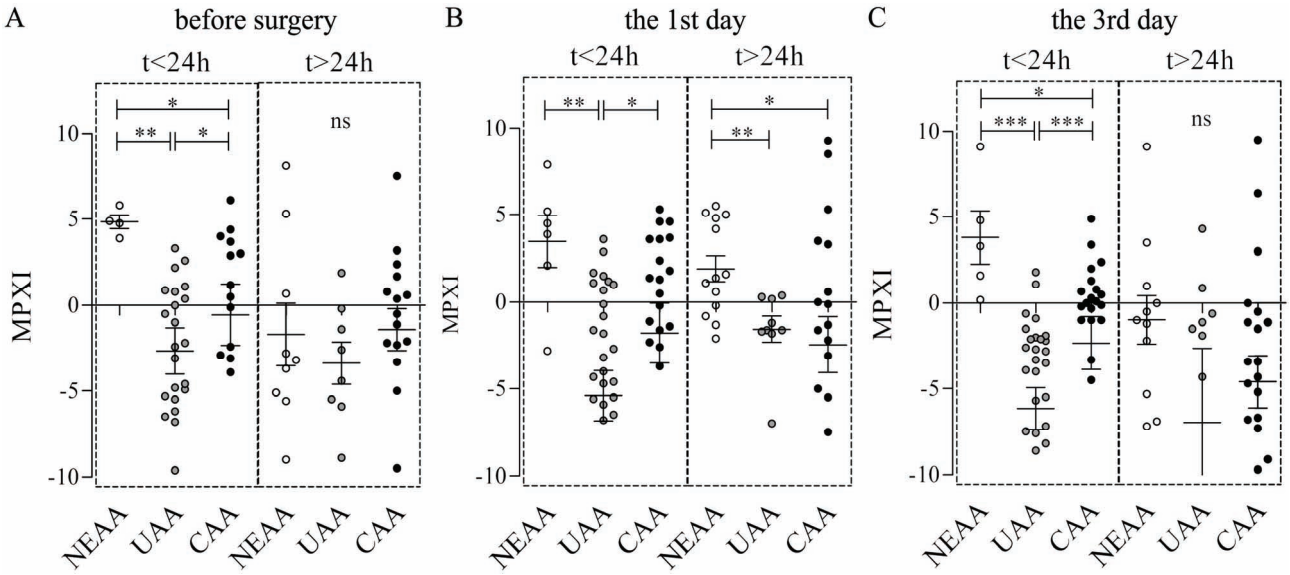

Fig. 2 – A) Comparison of MPXI values among the NEAA, UAA and CAA groups before surgery, showing the statistically significant differences between all tested groups in the patients who undergone surgery within 24 hours of symptoms' onset (t < 24h), with the lowest values in the UAA group. On the contrary, in the patients with surgical intervention after more than 24 hours of symptoms' onset (t > 24), the differences were insignificant before surgery; B) On the first postoperative day, among the patients who undergone surgery within 24 hours of symptoms' onset, the MPXI values are lowest in the UAA group, with a statistical significance in comparison with the NEAA and the CAA groups. In the group of patients who undergone surgery after more than 24 hours, the MPXI values are highest in the NEAA group, a significantly higher in comparison to the UAA and the CAA groups, with the lowest values in the CAA group; C) On the third postoperative day, in the patients who undergone surgery within 24 hours of symptoms' onset, the MPXI values in the UAA group are lower in comparison with the NEAA and CAA groups with a very high statistical significance. As in measurements before surgery, the MPXI values of NEAA group are significantly higher in comparison to the CAA group on the third postoperative day among the patients with surgery within 24 hours of symptoms' onset. On the contrary, in the patients which undergone surgery after more than 24 hours, the statistically significant differences between all tested groups are lost on the third postoperative day.
The Mann-Whitney test (unpaired, two-tailed): * - $p < 0.05$; ** - $p < 0.01$; * - $p < 0.0001$; ns – not significant.**
For abbreviations see under Table 1.

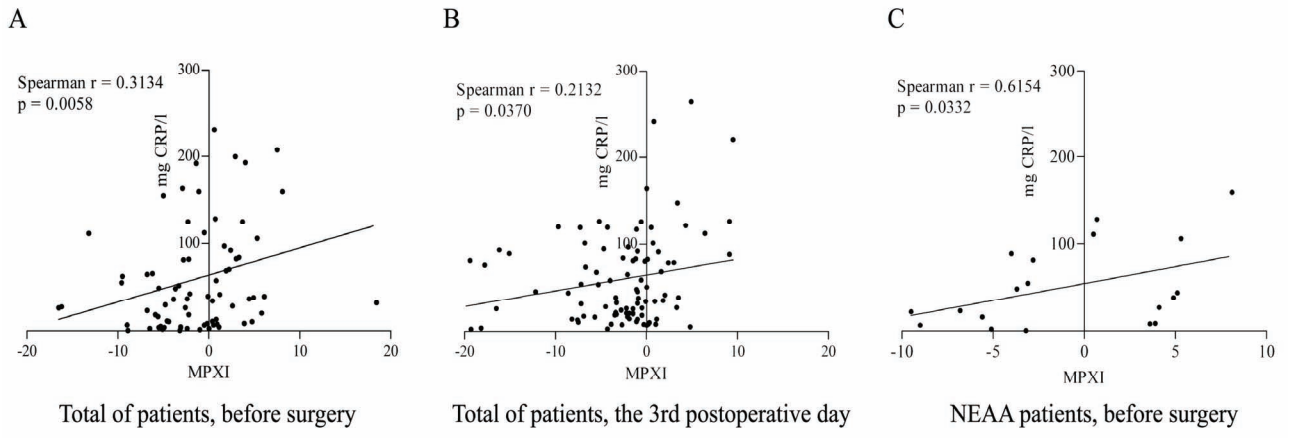

Fig. 3 – A) Correlation between MPXI and CRP before surgery in the total AA patients regardless of intraoperative finding, showing a highly significant positive relationship; B) On the 3rd day after surgery, a statistically positive significance is present again in the total AA patients regardless of intraoperative finding; C) A statistically significant correlation between the MPXI and CRP before surgery in the NEAA group.
For abbreviations see under Table 1.

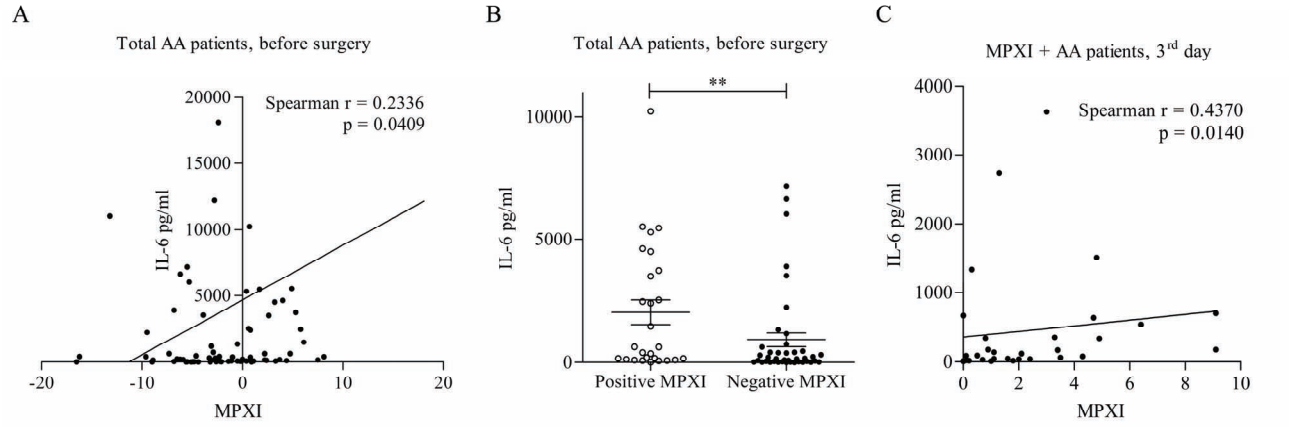

Fig. 4 – A) Correlation between the MPXI and IL-6 in the total acute appendicitis (AA) patients before surgery, showing a statistically significant positive relationship; B) Comparison between the patients with the positive and the patients with the negative MPXI in the total AA patients, showing a significantly higher IL-6 values in the patients with the positive MPXI, before surgery; C) Correlation between the MPXI and IL-6 in the AA patients with the positive MPXI values, showing a statistically significant positive relationship in the samples taken on 3rd postoperative day. For abbreviations see under Table 1.

The neutrophil myeloperoxidase index correlate positively with serum interleukin-6 values

The MPXI showed a significant positive correlation with the serum IL-6 values in the total of AA patients in similar manner as with the CRP values. Namely, the MPXI showed a significant positive relationship with the serum IL-6 values in the total of AA patients in the samples taken before surgery [Spearman's $r = 0.2336$; $p = 0.0409$ (Figure 4A)]. Next, regarding the MPXI, we stratified the patients into two groups: the group of patients with the positive MPXI and the group of patients with the negative MPXI values. When compared, the group of patients with the positive MPXI showed the significantly higher IL-6 values than the group of patients with the negative MPXI values [2041 ± 2563 vs. 917.5±1842; $p = 0.0088$ (Figure 4B)]. When we tested the samples obtained on the 1st as well as on the 3rd postoperative day, we did not find neither a significant correlation between the MPXI and serum IL-6 nor the statistical differences of IL-6 mean values between the MPXI positive and MPXI negative groups. However, when we analyzed the patients with the positive and negative MPXI separately, we found a significant positive correlation between the MPXI and IL-6 values in the samples taken on the 3rd postoperative day [Spearman's $r = 0.4370$; $p = 0.0140$ (Figure 4C)].

Discussion

In this prospective study, which included a total of 117 of AA patients, the MPXI values were analyzed in regard to both, the intraoperative finding and that from the time elapsed since the onset of symptoms to surgical intervention. The correlations of MPXI values with the PAS, CRP and IL-6 were evaluated as well. Consecutive measurements were performed at three time points: the baseline measurement (before surgery), at the 1st and at the 3rd day after surgery. In regard to the intraoperative finding and subsequent stratification of patients into the three mentioned groups, the lowest MPXI values were recorded in the UAA group at all three time points of measurement. Further stratification of patients in regard to the duration of symptoms before surgery, revealed a very interesting observation. Namely, in the group of patients with the symptoms duration less than 24h, in the samples taken before surgery the MPXI values were lowest in the UAA group again, and that trend was maintained in the subsequent measurements. On the contrary, when the symptoms lasted longer than 24 hours, the differences between the groups were lost in samples taken before surgery as well as in the samples on the 3rd postoperative day. The study also showed the significant positive correlation of MPXI with both, CRP and IL-6, but not with the PAS.

In the diagnosis of AA, different biomarkers are used together with clinical examination and clinical history data, especially in case of diffucult diagnosis, as in children [8]. No sufficiently specific and sensitive biomarker for the AA has been found so far [9, 10]. The present study aimed to investigate a possible relationship of MPXI – a parameter that can be quickly and inexpensively assesed – with the diagnosis and clinical course of AA, as well as with some of accepted AA biomarkers and scoring systems, such as the CRP and PAS respectively.

MPO is synthesized during the myeloblast and promyelocyte stage of neutrophils maturation and its production ceases at the promyelocyte to myelocyte stage.

Degranulation decreases intracellular MPO content and activated neutrophils have lower level of MPO in comparison with non-activated or immature ones [11–13]. Some specific patterns of MPXI change were reported, such as MPXI increase in myeloid leukemia [14, 15], megaloblastic anemia [16, 17] and some bacterial infections, the low MPXI in bacterial sepsis and unchanged in viral infection and tuberculosis [18]. In addition, the MPXI is useful as independent biomarker for diagnosis and follow-up of ischemic heart diseases [19]. However, there are limited data in the literature regarding the MPXI changes in the AA patients. In the study which in-

cluded 105 patients with the AA, Kim et al. [20] did not find a significant difference in the MPXI values between the complicated and uncomplicated AA . Our data showed the significantly lower MPXI values in the patients with the uncomplicated AA when compared with the patients with complicated and the patients without and/or the early AA. In addition, the significant differences in the MPXI values recorded between the groups in the first 24 hours of onset of symptoms, may suggest a greater usefulness of MPXI as a biomarker in the early established suspicion of AA. In our patients, the lower MPXI values in the uncomplicated appendicitis were interpreted as a greater rate of activation and degranulation of mature neutrophils (less MPO content). Absence of gangrene and perforation in these cases could be explained by and effective inflammatory process still kept under control by the immune system.

The PAS is sensitive and specific in the clinical assessment of AA [21]. However, in our study, there was no significant correlation between the MPXI and PAS. We did not find a significant correlation of MPXI neither with the overall PAS nor with the single parameters of this score, including the absolute number of leukocytes and the percentage of neutrophils. Additionaly, in our previous article we showed the lack of significant correlation between the MPXI and neutrophil-to-lymphocyte ratio (NLR) [22].

CRP is very reliable biomarker for cytokine-mediated response in AA [23]. A significantly positive correlation between the MPXI and CRP, which was demonstrated in this study, implied an observation which was contradictory to a certain extent – meaning that the patients with the lowest MPXI (presumably, the patients with the highest degree of neutrophils degranulation), at the same time had the lowest CRP values (considered as a reliable marker of inflamation). The synthesis of CRP was under control of IL-6, secreted mainly by mononuclear phagocytes, vascular endothelium or T-lymphocytes [24]. When we tested the relationship between the MPXI and IL-6, we found a significant, positive correlation between them as well as the significantly higher values of IL-6 in the patients with the positive MPXI. Taken together, we speculate that the appropriate neutrophil's degranulation had efficiently controlled and limited local inflammation, and prevented early systemic response in the AA patients (i.e., the secretion of IL-6 and subsequent synthesis of CRP). In addition, the lowest MPXI values in the UAA group with a statistical significance in comparison with the NEAA and CAA groups, could suggest, on the one hand, that the unnecesary neutrophils degranulation did not happend without need (the NEAA group), and, on the other hand, that the appropriate neutrophil's reaction gave rise to the favorable clinical course (in comparison with the CAA group). There are the data which suggest that the gangrenous and phlegmonous appendicitis are different entities with divergent immune regulation and that skewing of the immune response toward Th-17 type could result in the development of gangrenous – complicated AA [25]. Possible influence of MPO on the immune response skewing should be tested in the AA patients, since the immunomodulatory properties of this enzyme was described [26].

Conclusion

In this study, the statistically significant differences in the MPXI values between uncomplicated and complicated AA before and after surgery were found, suggesting that the MPXI may be used as an informative biomarker in the follow-up of AA in children, especially in the early phase of AA. However, a wide reference range for the MPXI and individual differences in the values of MPXI in the healthy children, generate difficulties for its use for the initial diagnosis. The described relationship between the MPXI, CRP and IL-6 speaks in favor of tight and balanced connection between the local inflamation and systemic acute phase response in AA. The evaluation of MPXI in a combination with other parameters for assessing the development and immune response during AA, should be tested by further investigations.

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