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Role of serum cytokines in acute appendicitis and acute mesenteric lymphadenitis among children

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ABSTRACT

Background and objective: The diagnostic role of serum cytokines depends on the etiology and pathogenesis of acute appendicitis (AA) and acute mesenteric lymphadenitis (AML). The aim of this study was to evaluate differences in cytokine levels between AA and AML.

Materials and methods: Data of 7- to 18-year-old children were collected prospectively from October 2010 to October 2013. There were 31 patients with AA (AA group), 26 with AML (AML group), and 17 with elective non-inflammatory surgical disease (control group). Serum levels of IL-10, IL-12(p70), IL-1 β , IL-4, IL-6, IL-8, IL-17, MCP-1, EGF, TNF- α and white blood count (WBC) were measured three times consecutively in each group.

Results: The level of IL-6 and IL-10 was significantly higher in the AA group than the AML group at the first measurement (8 pg/mL vs. 3.2 pg/mL, $P = 0.000$; 6.1 pg/mL vs. 3.2 pg/mL, $P = 0.005$, respectively). There was a significant difference observed in time dynamics of concentration of IL-6 and MCP-1 for AA and AML. The area under the curve (AUC) was 0.77 (95% CI 0.64–0.89; $P = 0.001$) for IL-6 with a cut-off value of 4.3 pg/mL (67.7% sensitivity and 76.9% specificity) for AA 1 h before surgery. The AUC for WBC was 0.72 (95% CI 0.584–0.85; $P = 0.005$) with a cut-off value of $10.7 \times 10^3/\mu\text{L}$ (sensitivity 71.0% and specificity 46.2%).

Conclusions: Serum IL-6 with a cut-off value of 4.3 pg/mL and WBC with a cut-off value of $10.7 \times 10^3/\mu\text{L}$ assessed together will yield more sensitivity for AA.

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1. Introduction

The right lower quadrant pain is the most common condition necessitating surgical admission to a pediatric hospital [1,2]. The vast majority of cases are due to either AA or AML in children [1,3–6]. The incidence of AA is 25 cases per 10,000 pediatric patients per year between the ages of 10 and 17 in the United States [4,7,8]. 7%–20% of the discharge diagnosis in pediatric patients with a clinical suspicion of AA was primary AML [3,9]. Generally, the treatment tactics in both of these pathologies differ a lot. In the case of AML, treatment is more conservative and does not require hospitalization [1,3,6,10], while in the case of AA immediate hospitalization and perhaps further surgery can be mandatory [7]. Missing the diagnosis of AA in the emergency department may increase the probability of perforation of the appendix as well as the rate of other complications [4,7,8,11]. The rate of perforated appendicitis has been reported as high as 10%–20% in children 10–17 years of age [7]. AA and AML in children remain a difficult differential diagnosis for physicians.

The diagnostic role of some inflammatory variables depends on the etiology and pathogenesis of AA and AML [2,4,6]. AA arises from initial luminal obstruction of the appendix [1,12,13]. This results in local edema secondary to impaired blood and lymphatic flow. Soon the bacterial barrier function of the appendicular epithelium fails and bacterial invasion into the submucosal layers occurs [13]. The presence of bacteria in these areas results in the activation of immune defense and local infiltration by T cells, monocytes, and natural killer cells. Locally interleukins and chemokines are released to recruit these cells [4,8,14–17]. Cytokines are biological active substance of polypeptides and glycoproteins with a molecular weight of 8–30 kDa, which participate in the cellular immunity in response to specific inflammatory process in the body [14]. We hypothesize that the serum cytokine response of a patient with AA is different from that of a patient with AML.

The aim of this study was to evaluate the role of serum cytokines such as interleukin-10 (IL-10), interleukin-12(p70) (IL-12(p70)), interleukin-1 beta (IL-1 β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-17 (IL-17), monocyte chemoattractant protein-1 (MCP-1), epithelial growth factor (EGF), and tumor necrosis factor- α (TNF- α) in differentiating between AA and AML in children aged between 7 and 18 years.

2. Materials and methods

2.1. Subjects and study design

This study was approved by the local Ethics Review Board of the University Children's Hospital, Riga, Latvia (No. 40003457128). All of the patients and parents gave their informed consent to participate in the study. A prospective case-control study was conducted at Department of Pediatric Surgery, University Children's Hospital, Riga, Latvia, and carried out of 74 patients aged between 7 and 18 years from October 2010 to October 2013 admitted for surgical evaluation and treatment with a suspicion of AA.

Study patients had to meet following inclusion criteria: children aged between 7 and 18 years hospitalized in the University Children's Hospital of Latvia, able to obtain parental or legal guardian written informed consents as applicable by local laws and regulations, were to be supported with the evidence of AA in the abdominal ultrasonography (appendix more than 6 mm diameter and is not compressible) or the evidence of AML in the abdominal ultrasonography (3 and more enlarged mesenteric lymph nodes, short axis 10 mm and more), and at least one of the clinical symptom (abdominal pain, nausea, rebound tenderness, fever ≥ 37.3 °C, white blood cell (WBC) count $> 10 \times 10^3/\mu\text{L}$). If the patient is female of child-bearing potential she had to have a negative pregnancy test. Exclusion criteria were diabetes, septic shock, severe acute renal and liver impairment and chronic hepatic impairment, known immunosuppression, clinical manifestation of intestinal malabsorption, chronic inflammatory bowel diseases, infections originating from the female genital tract, a perforation of the upper gastrointestinal tract, presence of liver and spleen abscess, all pancreatic processes, gastrostomy, an indwelling peritoneal catheter, operated abdominal trauma in past, systemic antibacterial treatment within the previous 7 days, non-steroidal inflammatory drug and hormonal treatment within 30 days. To minimize selection bias all patients meeting the inclusion criteria were evaluated by 1 of 5 consultant surgeons to establish eligibility and perform study enrollment.

From the total number of 91 patients who entered the study, 17 patients were excluded from study 24 h after randomization due to following reasons: clinically or microbiologically diagnosed infection in 11 patients (acute tonsillopharyngitis in 5 patients, pneumonia in 2, urinary tract infection in 2, and enterocolitis in 2) and technical problems in blood sampling and storing in 6 patients. The remaining 57 patients aged 7–18 years were further analyzed and were divided into 2 groups according to their outcomes and inclusion and exclusion criteria. The first study group consisted of 31 patients who were included before the treatment with the evidence of AA in the abdominal ultrasonography and were taken to the operating theater for appendectomy and AA confirmed by intraoperative findings and pathological examination of the excised appendix (AA group). All these subjects were undergone conventional open appendectomy with right lower quadrant incision by the consultant surgeon. A pathologist blinded to other clinical data confirmed the diagnosis of AA. Cultures of the peritoneal fluid during appendectomy were obtained only for the patients with evidence of gross perforation of appendix. Peritoneal culture specimens were tested at the local microbiology laboratory. The second study group was constituted of 26 patients with AML confirmed by ultrasound examination at the time of hospitalization and who did not undergo for surgical intervention (AML group). Abdominal ultrasonography was performed by the certified radiologist to avoid operator-dependent differences. All patients were examined using an ATL HDI 5000 ultrasound system (Philips Medical Systems, Bothell, Washington, United States).

In order to determine the reference values of serum cytokines EGF, IL-10, IL-12(p70), IL-1 β , IL-4, IL-6, IL-8, IL-17,

MCP-1, TNF- α and to be compared with the serum cytokines of AA group and AML group, there were 17 healthy children (the control group) prospectively selected for elective non-inflammatory surgical disease (control group). All characteristics of patients and the pre- and postoperative course were recorded prospectively using standardized assessment sheets filled out by one of the authors.

2.2. Cytokine measurements

Blood samples for the determination of cytokines were taken from the patients with AA and patients with elective non-inflammatory surgical disease three times: 1 h before the surgical intervention (before the onset of incision), 24 and 72 h after the surgical treatment. For those with AML, blood samples also were collected three times: as soon as the patients had hospital admission (before any treatment was given), 24 and 72 h after the patient hospitalization. The peripheral venous blood samples were collected from an antecubital vein drawn with a sterile syringe, transferred to a centrifuge tube. The samples were allowed to clot for 20–30 min at room temperature. Serum was separated by centrifugation at 4 °C for 20 min at 1600 \times g. All specimens were immediately aliquoted, frozen and stored at -80°C . Serum EGF, IL-10, IL-12(p70), IL-17, IL-1 β , IL-4, IL-6, IL-8, MCP-1, TNF- α levels were evaluated by using Milliplex Map kit (Human Cytokine/Chemokine Magnetic Bead Panel) for Luminex xMAP Technology (Luminex 200, Luminex Corporation, Austin, Texas, United States). The minimum detectable levels of all cytokines for this assay kit were 3.2 pg/mL.

2.3. Statistical analysis

The data were examined for normality of distribution by the Shapiro–Wilks test. In case of rejection of normality, nonparametric test was used. Continuous data were expressed as median with range (25th and 75th percentile). The Kruskal–Wallis test was used for three group comparisons. The concentrations of cytokines in the first serum assay were compared among all study groups. In case of significance, individual differences were identified with the Mann–Whitney U test. Each serum sample of the AA group was compared with the corresponding sample of the control group. Categorical variables were analyzed with the chi-square test. A *P* value of <0.05 was considered as statistically significant. A receiver operating characteristic (ROC) curve was constructed to assess sensitivity and specificity as well as optimal cut points for each serum cytokine to diagnose AA and AML. The criterion value indicated the value corresponding with the highest accuracy (minimal false negative and false positive results). The ROC curve is the plot of the proportions of true positive vs. true negative for each value of the study variable. Receiver operating characteristic (ROC) curves and the related areas under curve (AUC) were calculated for each cytokine and represent discriminatory power of the variable. The area of 0.50 represents a variable with no discriminatory capacity, and the area of 1.00 indicates a perfect discriminator.

Statistical analyses were performed using SPSS Statistics for Windows version 20.0 package software (SPSS Inc., United States).

3. Results

3.1. Characteristics of subjects

This report is based on 57 patients: 35 (61.4%) boys and 22 (38.6%) girls with the mean age of 12.9 years (SD 3.2). Abdominal ultrasound was performed before any treatment for all patients enrolled in the study. Enlarged abdominal lymph nodes were detected simultaneously with the inflamed appendix in three study subjects. Appendectomy was performed and phlegmonous inflammation of appendix was confirmed, therefore, none of them was excluded from the study. After the structured histopathological examination of 31 excised appendixes, 10 patients had complicated (gangrenous or perforated) AA and 21 had uncomplicated (acute or phlegmonous) AA. Of these 57 patients, 9 peritoneal cultures were obtained and 6 of them were positive. *Escherichia coli*, *Bacteroides*, *Pseudomonas*, *Clostridia*, *Enterococci*, *Streptococcus* and *Staphylococcus* were isolated from all specimens (data not shown). During abdominal ultrasound, 26 patients with AML were identified with mesenteric lymph nodes spans ≥ 10 mm, as it was also one of the inclusion criteria and were received conservative treatment. The comparison of demographic and clinical features of the study subjects is shown in Table 1. It can be clearly seen that on average nausea and vomiting in AML group was observed by 36 percentage points less often than in AA group. There were no difference between diagnostic values by groups such as WBC, C reactive protein (CRP), absolute neutrophil count (ANC) and duration of symptoms.

The control group consisted of 17 healthy children: 9 (52.9%) boys and 8 (47.1%) girls with the mean age of 13.2 (SD 3.54). They underwent laparoscopic ovarian cystectomy, varicocele, open umbilical and inguinal hernia repair and circumcision procedure with the mean WBC count 6.3 (1 SD) $\times 10^3/\mu\text{L}$.

3.2. Analysis of the cytokine measurements

The values of cytokines IL-10, IL-12(p70), IL-1 β , IL-4, IL-6, IL-8, IL-17, MCP-1, EGF, TNF- α obtained in the AA group one hour before the operation and in the AML group on the day of patient admission to hospital are presented with the reference values of the control group in Table 2. The serum levels of IL-6 and IL-10 significantly increased in the AA group one hour before the surgical intervention compared to the corresponding values in the AML group on the day of patient admission to hospital (IL-6(1): $z = -3.72$; $P = 0.0002$; IL-10(1): $z = -2.81$; $P = 0.005$). For the AA group, the concentration of serum IL-6 and IL-10 was statistically significantly higher in comparison to those in AML patients at the first measurement (8 pg/mL vs. 3.2 pg/mL, $P = 0.000$; and 6.1 pg/mL vs. 3.2 pg/mL, $P = 0.005$, respectively).

A more detailed analysis revealed that there was a statistically significant difference observed between the AA and control groups within all three measurements in terms of the following cytokines concentration as IL-10 and IL-6: 8 pg/mL vs. 3.2 pg/mL ($P = 0.02$) for IL-6(1); 9.3 pg/mL vs. 3.2 pg/mL ($P = 0.006$) for IL-6(2); 3.4 pg/mL vs. 3.2 pg/mL for IL-6(3) ($P = 0.05$); 6.1 pg/mL vs. 3.2 pg/mL ($P = 0.02$) for IL-10(1);

Table 1 – Characteristics of the study patients with AA and AML.

Characteristic	Acute appendicitis (AA group) (n = 31)	Acute mesenteric lymphadenitis (AML group) (n = 26)	P value
	Age, mean (SD), years	13.4 (2.5)	
Gender, male, n (%)	18 (58.1)	17 (65.4)	NS
Nausea, n (%)	21 (67.7)	8 (30.8)	0.01
Vomiting, n (%)	16 (51.6)	4 (15.4)	0.01
Duration of symptoms, h	20 (7.0–29.0)	42 (11.9–54.0)	NS
Temperature ≥ 37.3 °C, n (%)	22 (71.0)	15 (57.7)	NS
Rebound tenderness, n (%)	31 (100.0)	25 (96.2)	NS
WBC, $\times 10^3/\mu\text{L}$	11.1 (8.6–17.9)	9.6 (8.4–12.8)	NS
CRP, mg/L	12.3 (3.1–45.5)	7.4 (6.2–10.4)	NS
ANC, $\times 10^3/\mu\text{L}$	8.7 (6.2–15.4)	15.9 (6.3–31.5)	NS

Values are expressed as median (range) unless otherwise stated. NS, not significant; WBC, white blood cell count; CRP, C reactive protein; ANC, absolute neutrophil count.
Categorical data were compared by the Chi-square test and continuous data, by the Mann-Whitney test.

Table 2 – Serum cytokine levels preoperatively in the AA and control groups and on admission to the hospital in the AML group.

Serum cytokines (pg/mL)	AA (n = 31)		AML (n = 26)		Control group (n = 17)		Kruskal-Wallis test, P value
	Median	Range	Median	Range	Median	Range	
EGF	108.7	41.1–108.7	106.8	46.5–152.7	87	38.3–133.6	NS
IL-10	6.1	3.2–17.0	3.2	3.2–3.2	3.2	3.2–3.2	0.005
IL-12(p70)	3.2	3.2–4.9	3.2	3.2–3.2	3.2	3.2–3.5	NS
IL-17	5.9	3.2–38.0	3.3	3.2–9.1	5.8	3.2–29.5	NS
IL-1 β	3.2	3.2–3.2	3.2	3.2–3.2	3.2	3.2–8.6	NS
IL-4	3.2	3.2–3.3	3.2	3.2–3.7	3.2	3.2–3.2	NS
IL-6	8	3.2–97.6	3.2	3.2–3.2	3.2	3.2–11.7	0.000
IL-8	15.1	5.7–53.8	10.6	7.0–19.2	18.3	7.1–55.3	NS
MCP-1	411.1	254.6–811.1	333.9	266.0–378.2	352.9	235.8–520.7	NS
TNF- α	12.8	10.0–19.2	14.2	11.0–19.0	12.4	9.9–13.4	NS

NS, no significant difference; AA, acute appendicitis; AML, acute mesenteric lymphadenitis.

4.0 pg/mL vs. 3.2 pg/mL ($P = 0.000$) for IL-10(2); 3.6 pg/mL vs. 3.2 pg/mL ($P = 0.000$) for IL-10(3).

For the patients included in this study, some cytokines were found to undergo significant changes over the period of time within the same study group. There was a statistically significant difference observed in time dynamics of cytokine serum concentration of IL-6 and MCP-1 for AA patients from 1 h before surgery to 72 h after surgery (Wilks' Lambda test 0.80; $F(2, 29) = 3.5$; $P = 0.04$).

There was a statistically significant difference observed in AML patients in the case of MCP-1 serum concentrations of cytokines from admission to hospital to 72 h after conservative treatment (Wilks' Lambda test 0.70; $F(2, 24) = 5.0$; $P = 0.01$).

The serum concentration of IL-12(p70), IL-1 β , IL-4, TNF- α , EGF, IL-8 and MCP-1 did not differ significantly between the AA group and AML group.

Diagnostic cut-off levels for cytokines IL-10, IL-12(p70), IL-1 β , IL-4, IL-8, IL-17, MCP-1, EGF, TNF- α with the low sensitivity and specificity derived from the area under the ROC curve (AUC) were 0.40–0.60 in the AA and AML group. In fact, the significant discriminator power of IL-6 for AA preoperatively and 24 h after operation was determined by ROC analysis. AUC was 0.77 (95% CI, 0.64–0.89) for IL-6 with the cut-off value of 4.3 pg/mL showed 67.7% sensitivity and 76.9%

specificity for AA ($P = 0.001$) 1 h before the operation. AUC for WBC was 0.72 (95% CI, 0.58–0.85; $P = 0.005$) with cutoff value $10.7 \times 10^3/\mu\text{L}$ (sensitivity 71.0% and specificity 46.2%). The results of comparisons of the AUC for the WBC and preoperative IL-6 in the AA group were summarized in Fig. 1.

AUC was 0.78 (95% CI 0.66–0.90) for the repeated measurement of IL-6 with the cut-off value of 6.1 pg/mL 24 h after the operation, showing 54.8% sensitivity and 96.2% specificity for AA ($P = 0.000$) (Fig. 2).

4. Discussion

Patients with AML typically have a history of fever and abdominal pain, frequently with localization in the right lower quadrant [1,3,5,9,10]. The clinical presentation is similar to acute AA [4,6,8,10]. A few data are available on the incidence of this syndrome, and it may vary with the geographic location. Within one series of hospitalized patients, 50 out of 651 (7.7%) admitted with the diagnosis of AA had a discharge diagnosis of AML [5].

In most cases, the gut bacteria have a possible role in causing common clinical symptoms of the both mentioned diseases. The bacteriological studies of nonspecific AA, using

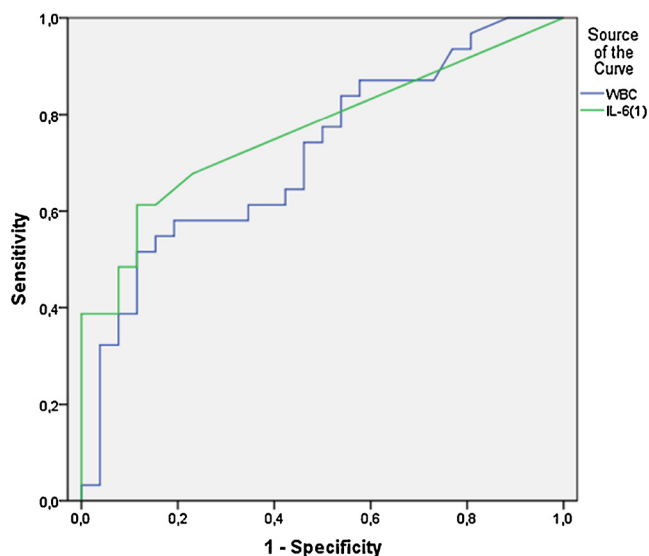


Fig. 1 – ROC curve for IL-6(1) level and WBC preoperatively in the AA group.

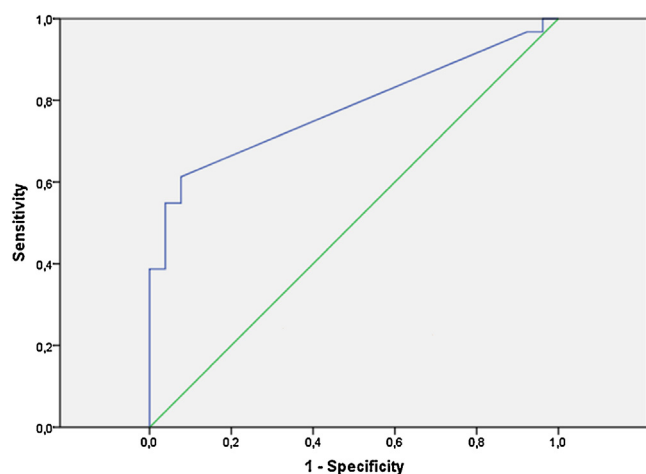


Fig. 2 – ROC curve for IL-6(2) level in the AA group 24 h after surgery.

microbiologic culture techniques, reveal a wide variety of anaerobic and aerobic bacteria [13,17,18]. According to Khanna, in nonspecific AML organisms isolated in culture include *E. coli*, *Bacteroides*, *Clostridia* species, *Enterococci*, beta-haemolytic *Streptococcus*, *Staphylococcus aureus* and *Yersinia* [6]. The present study also revealed that these microbes and *Pseudomonas* were isolated from some specimens of the patients with AA. The character of bacteria was a major factor in determining their arrest in lymph nodes [5,6]. Therefore, it is possible that different cytokines could appear in blood of the patients with AML. According to the above mentioned statement, Li Bingyun and his co-workers found that *S. aureus* infection was reduced by local MCP-1 therapy which is essential for monocyte and macrophage recruitment in the first 48 h at the site of infection [19]. In our study we noticed that there was a significant difference in time dynamics of

serum levels of MCP-1 for AA patients and also for AML patients. In fact, using the analysis of ROC curves for MCP-1 cut-off values in discriminating between AML and AA, the AUC was 0.40–0.60 for initial and repeated measurements. Therefore, it could not be a helpful serum biomarker to distinguish the patients with AA and AML. However, further studies and additional statistical analysis are needed to estimate whether MCP-1 can predict AML on patients' admission to the hospital.

Several previous studies examining the immunologic response to enteric bacteria within the intestinal lumen have shown specific cytokine production by certain bacteria which help in the diagnosis of AA [17]. Two important immunoregulatory cytokines produced by cells of the innate defence system in response to bacteria are IL-12(p70) and IL-10. IL-12 (p70) stimulates interferon-gamma (IFN- γ) production from T cells and natural killer (NK) cells, and increases their cytotoxicity [15]. Although, IL-10 level was demonstrated to be significantly higher in serum obtained in the AA group initially within 20 h after the patient felt ill than of the patients in the AML group. It could be explained by the study report of Rivera-Chavez et al., in which IL-6 and IL-10 were found in high concentration in the serum of patients with uncomplicated AA, while the proinflammatory ones (IL-1 β , IL-12(p70), TNF- α) were found in low concentration [20]. Manuzak et al. reported that cytokine profiles in whole peripheral blood mononuclear cells differed depending on the stimulating bacterial species: *Bacteroides fragilis* induced significantly more production of IL-12(p70) and interleukin-23 than *E. coli* and *Enterococcus*, although *E. coli* and *Enterococcus* induced levels of IL-10 that were significantly higher than the level induced by *B. fragilis* [16]. These findings reveal that IL-10 and IL-12(p70) could be the important diagnostic serum markers for AA as a result of implication of commensal enteric bacteria. It should be emphasized that we only found a significantly increased secretion of IL-10 and IL-6 1 h before the surgical intervention in the cases of AA compared to the corresponding values on the day of patient admission to hospital with AML. Moreover, it might be that AA and AML are the result of implication of enteric bacteria.

IL-6 has a broad range of activity on multiple cells types and it induces a wide array of responses [21]. One of these is the acute phase response followed by bacterial endotoxemia [8,22]. Brănescu et al. found preoperatively that IL-6 in serum exceeded the maximum value of 9.7 pg/mL in 93.7% of the study subjects with phlegmonous inflammation of appendix [22]. This reasoning is consistent with our data finding that preoperatively concentration of IL-6 was significantly higher (8.0 pg/mL) in patients with AA as compared to its concentration (3.2 pg/mL) on the day of patient admission to hospital in the AML group. In our study ROC analysis revealed that AUC was 0.77 for IL-6 with the cut-off value of 4.3 pg/mL 1 h before the operation for AA with a sensitivity of 67.7% and a specificity of 76.9% ($P = 0.001$) in discriminating between AA and AML. It showed that less false positive rate would appear using IL-6 concentration (equal or more than 4.3 pg/mL) individually as a diagnostic parameter for AA. The same picture is presented by the WBC concentration in blood with cut-off value $\geq 10.7 \times 10^3/\mu\text{L}$ one hour before the operation for AA, reflecting the AUC value of 72% in ROC analysis, sensitivity of 74.2% and specificity of 53.8%. Having compared the

diagnostic accuracy of cut-off values between IL-6 and WBC in the cases of AA and AML, one must conclude that IL-6 is more specific for the diagnostics of AA than WBC because it reduces the possibility of false-positive case detection. Therefore, our study results do not support the use of IL-6 measurement alone as a substitute for clinical, routine laboratory and radiological examinations in patients with suspicion for AA. The similar results showed by Gürleyik et al., where the measurement of IL-6 concentration was not of benefit in increasing the accuracy of diagnosis of AA because the false positive rate of the test was 54% in normal appendectomy cases, and the false negative rate was 19% in patients with non-perforated AA [23].

Another point of discussion of our study results is IL-6 a significant difference between the levels of cytokine in the cases of AA 1 h before the surgical intervention and 72 h after the operation. Sakamoto and colleagues revealed that serum IL-6 levels reached maximum within the first postoperative day and decreased thereafter. That phenomenon is explained by surgical trauma where IL-6 is produced in the operative field and enters the peripheral blood stream to induce the elevation of serum IL-6 [24]. The importance of studying serum inflammatory markers in AA and AML, could be helpful to differentiate the stage of inflammation because most patients are healthy before developing AA and AML, and the length of symptoms before hospitalization is typically less than 48 h [20].

One of the main factors hindering or limiting the study is related to the sample presentation or, in other words, the inclusion of appropriate number of cases according to the studied frequency of disease pathology. A total of 91 patients was analyzed in the study USG examination of abdominal organs was necessary for 74 patients, but 74 patients needed a full cytokine analysis. One of the limiting factors for patient inclusion in the study was the lack of accessibility to the abdominal ultrasound in the examination period from eight o'clock in the evening until eight o'clock in the morning. The second factor was related to get venous blood samples for cytokine determination during the work day, from eight in the morning until five o'clock in the afternoon, as during the rest of the hours and weekends it was impossible to adequately prepare and store collected blood samples according to the study protocol due to the high workload of the central laboratory of the Children's University Hospital of Latvia. At the onset of the study it was observed that most of the samples were unfit for further analysis because of blood haemolysis and elevated storage temperatures of refrigeration equipment. Therefore, subsequent blood samples were collected in originally specified working hours.

Another key limiting factor that should be mentioned was the possibilities for patient follow-up after acute treatment period. In this study, the group of AML patients was not invited to the follow-up visits after being discharged from the hospital to completely exclude any AA case possibility in that period of time. Consequently, it is difficult to draw any conclusions about AML as of an isolated disease for all AML patients. However, referring to a similar study in the Netherlands, where they compared AA and AML patients; having AML patients additionally invited for a check-up 3 years later after their discharge from the hospital, it was observed that 91.4% of

the cases confirmed diagnosis of AML, while the remaining cases documented recurrent abdominal pain without any treatment to be prescribed [10]. Taking into account the abovementioned study results, we can propose that no changes in the AML group would be identified by our study either. In future, the study population should be increased and the additional analysis of clinical parameters should be done.

5. Conclusions

Serum IL-6 with the cut-off value of 4.3 pg/mL and WBC with cut-off value of $10.7 \times 10^3/\mu\text{L}$ assessed altogether will yield more sensitivity for AA and could be useful diagnostic markers to differentiate AA from AML.

Conflict of interest

The authors declare no conflicts of interests.

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REFERENCES

- [1] Hayes R. Abdominal pain: general imaging strategies. *Eur Radiol* 2004;14:123-37.
- [2] Türkyılmaz Z, Sönmez K, Karabulut R, Elbeğ Ş, Moralioglu S, Demirtola A, et al. Sequential cytokine levels in the diagnosis of appendicitis. *Scand J Clin Lab Invest* 2006;66:723-32.
- [3] Macari M, Hines J, Balthazar E, Megibow A. Mesenteric adenitis: CT diagnosis of primary versus secondary causes, incidence, and clinical significance in pediatric and adult patients. *AJR* 2002;178:853-8.
- [4] Yoon DY, Chu J, Chandler C, Hiyama S, Thompson JE, Hines OJ. Human cytokine levels in nonperforated versus perforated appendicitis: molecular serum markers for extent of disease? *Am Surg* 2002;68(12):1033-7.
- [5] Mandell GL, Bennett JE, Dolin R. Mesenteric adenitis. In: Mandell GL, editor. *Mandell, Douglas and Bennett's principles and practice of infectious diseases*. 7th ed. Philadelphia: WB Saunders; 2009. 978 pp.
- [6] Khanna SK, Jain SK. Staphylococcal suppurative mesenteric lymphadenitis. *Postgrad Med J* 1983;59:191-3.
- [7] Wray CJ, Kao LS, Millas SG, Millas SG, Tsao K, Ko TC. Acute appendicitis: controversies in diagnosis and management. *Curr Probl Surg* 2013;50:54-86.

- [8] Saliakellis E, Thapar N. Paediatric GI emergencies. *Best Pract Res Clin Gastroenterol* 2013;27:799–817.
- [9] Sicorska-Wiśniewska G, Liberek A, Góra-Gebka M, Bako W, Marek A, Szlagatys-Sidorkiewicz A, et al. Mesenteric lymphadenopathy – a valid health problem in children. *Med Wieku Rozwoj* 2006;10(2):453–62.
- [10] Toorenvliet B, Vellekoop A, Bakker R, Wiersma F, Mertens B, Merkus J. Clinical differentiation between acute appendicitis and acute mesenteric lymphadenitis in children. *Eur J Pediatr Surg* 2011;21:120–3.
- [11] Chang YJ, Chao HC, Kong MS, Hsia S-H, Yan DC. Misdiagnosed acute appendicitis in children in emergency department. *Chang Gung Med J* 2010;33(9):551–6.
- [12] Tsuji M, McMahan G, Reen D, Puri P. New insights into the pathogenesis of appendicitis based on immunocytochemical analysis of early immune response. *J Pediatr Surg* 1990;25(4):449–52.
- [13] Lamps LW. Beyond acute inflammation: review of appendicitis and infections of appendix. *Diagn Histopathol* 2008;14(2):68–77.
- [14] Dala I, Somekh E, Ilker-Reich A, Boaz M, Gorenstein A, Serour F. Serum and peritoneal inflammatory mediators in children with suspected acute appendicitis. *Arch Surg* 2005;140(2):169–73.
- [15] Hessle C, Hanson Å, Wold AE. Lactobacilli from human gastrointestinal mucosa are strong stimulators of IL-12 production. *Clin Exp Immunol* 1999;116:276–82.
- [16] Manuzak J, Dilon S, Wilson C. Differential interleukin-10 (IL-10) and IL-23 production by human blood monocytes and dendritic cells in response to commensal enteric bacteria. *Clin Vaccine Immunol* 2012;19(8):1207–17.
- [17] Arlt A, Bharti R, Ilves I, Häsler R, Miettinen P, Paajanen H. Characteristic changes in microbial community composition and expression of innate immune genes in acute appendicitis. *Innate Immun* 2015;21(1):30–41.
- [18] Lamps LW. Infectious causes of appendicitis. *Infect Dis Clin North Am* 2010;24(4):995–1018.
- [19] Li B, Jiang B, Dietz MJ, Smith ES, Clovis NB, Rao K. Evaluation of local MCP-1 and IL-12 nanocoatings for infection prevention in open fractures. *J Orthop Res* 2010;28(1):1–17.
- [20] Rivera-Chavez FA, Wheeler H, Lindberg G, Munford RS, O'Keefe GE. Regional and systemic cytokines responses to acute inflammation of the vermiform appendix. *Ann Surg* 2003;237(3):408–16.
- [21] Shaheen M, Broxmeyer HE. The humoral regulation of hematopoiesis. In: Hoffman R, Benz EJ, Shattil SJ, editors. *Hematology: basic principles and practice*. 5th ed. Philadelphia: Elsevier Churchill Livingstone; 2009. 253 pp.
- [22] Brănescu C, Șerban D, Dascălu AM, Oprescu SM, Șavlovschi C. Interleukin 6 and lipopolysaccharide binding protein – markers of inflammation in acute appendicitis. *Chirurgia* 2013;108(2):206–14.
- [23] Gürleik G, Gürleik E, Çetinkaya F, Unalmiser S. Serum interleukin-6 measurement in the diagnosis of acute appendicitis. *ANZ J Surg* 2002;72(9):665–7.
- [24] Sakamoto K, Hisano S, Kamohara H, Ishiko T, Mita S, Ogawa M. Changes of interleukin 6 and soluble IL-6 receptor levels after surgery. *Int Congr Ser* 2003;1255:135–42.