PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B, Vol. 69 (2015), No. 6 (699), pp. 283–289.
DOI: 10.1515/prolas-2015-0044

DIFFERENT CYTOKINE PROFILES IN CHILDREN WITH ACUTE APPENDICITIS AND ACUTE MESENTERIC LYMPHADENITIS

Astra Zviedre[1#], Arnis Eņģelis[1, 2], Pēteris Tretjakovs[3], Antra Jurka[3], Irisa Zīle[4, 5], and Aigars Pētersons[1, 2]

[1] Department of Paediatric Surgery, Children's University Hospital, Vienības gatve 45, Rīga, LV-1004, LATVIA; astrazviedre@inbox.lv

[2] Department of Paediatric Surgery, Rīga Stradiņš University, Vienības gatve 45, Rīga, LV-1004, LATVIA

[3] Department of Physiology and Biochemistry, Rīga Stradiņš University, Dzirciema iela 16, Rīga, LV-1007, LATVIA

[4] Department of Research, Statistics and Health Promotion, Centre for Disease Prevention and Control of Latvia, Duntes iela 22, LV-1005, Rīga, LATVIA

[5] Department of Public Health and Epidemiology, Rīga Stradiņš University, Kronvalda bulv. 9, Rīga, LV-1010, LATVIA

[#] Corresponding author

Communicated by Dace Gardovska

The aim of this study was to investigate the role of serum cytokines in the diagnosis of acute appendicitis (AA) and acute mesenteric lymphadenitis (AML). Data were collected prospectively on 7 to 18 year old children (October 2010 – October 2013): 31 patients with AA, 26 patients with AML, and 17 patients with elective non-inflammatory surgical disease were selected as controls. Serum levels of IL-10, IL-12(p70), IL-1β, IL-4, IL-6, IL-8, IL-17, MCP-1, EGF, TNF-α were measured. Patients with AA had significantly increased serum levels of IL-6(1) ($z = -3.72$; $p = 0.0002$) and IL-10(1) ($z = -2.81$; $p = 0.005$) compared to AML before any treatment. The consecutive measurements of MCP-1 in serum demonstrated a significant difference within 72 hours in the AA group (Wilks' Lambda test 0.80; $F(2;29) = 3.5$; $p = 0.04$) and also in the AML group (Wilks' Lambda test 0.70; $F(2;24) = 5.0$; $p = 0.01$). The increased values of IL-6 and IL-10 were the most reliable cytokines one hour before surgical intervention for patients with AA. MCP-1 values changed significantly within 72 hours after patient hospitalisation but did not differ between the groups, and could not be a helpful serum biomarker in distinguishing patients with AA and AML.

Key words: *appendicitis, mesenteric lymphadenitis, cytokines, diagnosis.*

INTRODUCTION

Right lower quadrant pain is the most common condition necessitating surgical admission to a paediatric hospital (Hayes, 2004; Türkyilmaz *et al.*, 2006). The vast majority of cases in children are due to either acute appendicitis (AA) or acute mesenteric lymphadenitis (AML) (Khanna *et al.*, 1983; Macari *et al.*, 2002; Mandell *et al.*, 2009; Yoon *et al.*, 2002; Hayes, 2004). The incidence of AA is 25 cases per 10 000 paediatric patients per year between the ages of 10 and 17 years in the United States (Yoon *et al.*, 2002; Saliakellis *et al.*, 2013; Wray *et al.*, 2013). The discharge diagnosis was primary AML in 7–20% of paediatric patients with a clinical suspicion of AA (Macari *et al.*, 2002; Sicorska-Wiśneiewska *et al.*, 2006). The treatment tactics in these pathologies differ to a great degree. In the case of AML, treatment is more conservative and does not require hospitalisation (Khanna *et al.*, 1983; Macari *et al.*, 2002; Hayes, 2004; Toorenvliet *et al.*, 2011) while in the case of AA immediate hospitalisation is mandatory and with perhaps further surgery (Wray *et al.*, 2013). 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 (Yoon *et al.*, 2002; Chang *et al.*, 2010; Saliakellis *et al.*, 2013; Wray *et al.*, 2013). The rate of perforated appendicitis has been reported as high as 10–20% in children 10–17 years of age (Wray *et al.*, 2013). AA and AML in children remain the difficult differential diagnosis for physicians. One of the most significant factors affecting the diagnostic difficulties of these diseases in Latvia is the lack of equipment and specialists in radiological methods in emergency units to make the accurate diagnosis of AA and AML. Therefore, we need widely available and practicable laboratory tests to support diagnoses.

The diagnostic value of some inflammatory variables depends on the etiology and pathogenesis of AA and AML (Khanna *et al.*, 1983; Yoon *et al.*, 2002; Türkyilmaz *et al.*,

2006). Appendicitis arises from an initial luminal obstruction of the appendix (Hayes, 2004; Tsuji *et al.*, 1990; Lamps *et al.*, 2008). This results in local edema secondary to impaired blood and lymphatic flow. Soon the bacterial barrier function of the appendix epithelium fails and bacterial invasion into the submucosal layers occurs (Lamps *et al.*, 2008). The presence of bacteria in these areas results in the activation of immune defence and local infiltration by T cells, monocytes, and natural killer cells. Locally, interleukins and chemokines are released to recruit these cells (Hessle *et al.*, 1999; Yoon *et al.*, 2002; Dala *et al.*, 2005; Manuzak *et al.*, 2012; Arlt *et al.*, 2013; Saliakellis *et al.*, 2013). Cytokines, composed of polypeptides and glycoproteins, are biological active substances with molecular weight of 8 to 30 kDa, which participate in cellular immunity in response to specific inflammatory process in the body (Dala *et al.*, 2005). We hypothesise 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 investigate the role of serum cytokines (interleukin-10 (IL-10), interleukin-12(p70) (IL-12(p70)), interleukin-1 beta (IL-1β), 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), tumour necrosis factor-α (TNF-α)) as indicators in the early diagnosis of AA and AML in children aged between 7 and 18 years.

MATERIALS AND METHODS

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

The studied patients had to meet the following inclusion criteria: children aged between 7 and 18 years hospitalised in the Children's University Hospital of Latvia, able to obtain parental or legal guardian written informed consents as regulated by local laws and regulations, evidence of AA in the abdominal ultrasonography (appendix more than 6 mm diameter and is not compressible) or evidence of AML in the abdominal ultrasonography (three and more enlarged mesenteric lymph nodes, short axis 10 mm and more), and at least one of clinical symptom (abdominal pain, nausea, rebound tenderness, fever ≥37.3 °C, white blood cell (WBC) count >10 x 10^3/μL). If the patient was female of child- bearing potential she had 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, 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 seven days, and non-steroidal inflammatory drug and hormonal treatment within 30 days. To minimise selection bias, all patients meeting the inclusion criteria were evaluated by one of five consultant surgeons to establish eligibility and perform study enrolment.

The patients were divided into three groups according to their outcomes and inclusion and exclusion criteria. The first study group consisted of 31 patients who were taken to the operating theatre for appendectomy and AA confirmed by intraoperative findings and pathological examination of the excised appendix (AA group). All these subjects underwent 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 patients with evidence of gross perforation of appendix. Peritoneal culture specimens were tested at the local microbiology laboratory. The second study group consisted of 26 patients with AML confirmed by ultrasound examination and who did not undergo surgical intervention (AML group). Abdominal ultrasonography was performed by a 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).

The control group consisted of 17 healthy children admitted for elective non-inflammatory surgical disease (C group). They were selected to determine reference values of serum cytokines EGF, IL-10, IL-12(p70), IL-1β, IL-4, IL-6, IL-8, IL-17, MCP-1, TNF-α, for comparison with values of serum cytokines of the AA and AML groups. All characteristics of patients and the pre- and postoperative course were recorded prospectively using standardised assessment sheets filled out by one of the authors.

Cytokine measurements. Blood samples for the determination of cytokines were taken from patients with AA and patients with elective non-inflammatory surgical disease three times: one hour before surgical intervention (before the onset of incision), and 24 and 72 hours after the surgical treatment. For patients with AML, blood samples also were collected three times: at hospital admission (before any treatment was given), and 24 and 72 hours after patient hospitalisation. 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 to 30 min at room temperature. Serum was separated by centrifugation at 4 ^{o}C for 20 min at 1600×g. All specimens were immediately aliquoted, frozen and stored at –80 ^{o}C. Serum EGF, IL-10, IL-12(p70), IL-17, IL-1β, IL-4, IL-6, IL-8, MCP-1, and TNF-a levels were evaluated using a Milliplex Map kit (Human Cytokine / Chemokine Magnetic Bead Panel) for Luminex xMAP Technology (Luminex 200, Luminex Corporation, Austin,

Texas, United States). The minimum detectable level of the assay kit was 3.2 ng/mL for all cytokines.

Statistical analysis. The data were examined for normality of distribution by the Shapiro-Wilks test. In case of rejection of normality, a 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 a Mann-Whitney U test. Each serum sample of the AA group was compared with the corresponding sample of the C group. Categorical variables were analysed with a chi-square test. A probability p value of < 0.05 was considered as statistically significant. Statistical analyses were performed using SPSS Statistics for Windows version 20.0 package software (SPSS Inc., United States).

RESULTS

This report is based on 57 patients: 35 (61.4%) boys and 22 (38.4%) girls) with mean age 12.9 years (SD 3.2). Structured histopathological examination of the excised appendix showed that ten patients had complicated (gangrenous or perforated) AA and 21 had uncomplicated (acute or phlegmanous) AA. Of the 31 patients with AA, ten peritoneal cultures were obtained, of which six were positive. Escherichia coli, Bacteroides, Pseudomonas, Clostridia, Enterococci, Streptococcus and Staphylococcus were isolated from the specimens (data not shown). Abdominal ultrasound was performed for all study patients. Enlarged abdominal lymph nodes were detected simultaneously with inflamed appendix in three subjects with AA. Appendectomy was performed and phlegmonous inflammation of appendix was confirmed in all; therefore none were excluded from study. Comparison of demographic features of the study subjects are shown in Table 1. The median length of symptoms before hospitalisation as reported by the patient was 20 hours for AA and 42 hours for AML. A significant difference between these groups was not found.

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

The cytokines IL-10, IL-12(p70), IL-1β, IL-4, IL-6, IL-8, IL-17, MCP-1, EGF, and TNF-α levels obtained consecutively at the different time points in the AA and AML groups are presented together with the reference values of the control group in Table 2 and 3.

Consecutive measurements of IL-6 and MCP-1 in serum in the AA group demonstrated a significant difference between values one hour before the surgical intervention and 72 hours after the operation (Wilks' Lambda test 0.80;

Table 1

CHARACTERISTICS OF THE STUDY PATIENTS WITH AA AND AML

Characteristics, n (%)	Acute appendicitis (AA group) (n = 31)	Acute mesenteric lymphadenitis (AML group) (n = 26)	p value
Age, mean (SD), (years)	13.4(± 2.5)	11.0 (± 2.4)	NS
Gender, male	18 (58.1%)	17 (65.4%)	NS
Nausea	21 (67.7%)	8 (30.8%)	0.01
Vomiting	16 (51.6%)	4 (15.4%)	0.01
Temperature ≥ 37.3°C	22 (71.0%)	15 (57.7%)	NS
Rebound tenderness	31 (100.0%)	25 (96.2%)	NS
WBC > $10 \times 10^3/\mu L$	24 (77.6%)	14 (53.8%)	NS
CRP > 5 mg/L	23 (74.2%)	21 (80.8%)	NS
ANC > $6.75 \times 10^3/\mu L$	11 (68.8%)	10 (66.7%)	NS

Continuous data are expressed as means ± standard deviation (SD) and categorical data are expressed as percentages n (%); NS – not significant ($p \geq 0.05$ for all groups); WBC – white blood cell count; CRP – C reactive protein; ANC – absolute neutrophil count; Categorical data were compared by Chi square test and continuous data compared by the Mann-Whitney test.

Table 2

SERUM CYTOKINE CONCENTRATIONS OBTAINED PREOPERATIVELY AND 24 AND 72 HOURS AFTER START OF THE OPERATION IN THE AA GROUP AND THE REFRENCE VALUES OF CYTOKINES IN THE C GROUP

Serum cytokine	AA group (n = 31) 1 (pg/mL)	2 (pg/mL)	3 (pg/mL)	p value	C (n = 17) (pg/mL)
EGF	108.7 (41.1–108.7)	84.0 (44.4–128.0)	98.7 (63.1–150.4)	NS	87.0 (38.3–133.6)
IL-10	3.2 (3.2–17.0)	3.2 (3.2–6.4)	3.2 (3.2–3.6)	NS	3.2 (3.2–3.2)
IL-12(p70)	3.2 (3.2–4.9)	3.2 (3.2–4.7)	3.2 (3.2–4.4)	NS	3.2 (3.2–3.5)
IL-17	5.9 (3.2–38.0)	6.2 (3.2–56.1)	5.5 (3.2–22.7)	NS	5.8 (3.2–29.5)
IL-1β	3.2 (3.2–3.2)	3.2 (3.2–3.2)	3.2 (3.2–3.2)	NS	3.2 (3.2–8.6)
IL-4	3.2 (3.2–3.2)	3.2 (3.2–3.2)	3.2 (3.2–3.2)	NS	3.2 (3.2–3.2)
IL-6	8.0 (3.2–97.6)	9.3 (3.2–59.9)	3.2 (3.2–41.7)	0.04	3.2 (3.2–11.7)
IL-8	15.1 (5.7–53.8)	12.6 (7.1–43.0)	16.7 (6.0–25.8)	NS	18.3 (7.1–55.3)
MCP-1	411.1 (254.6–811.1)	319.5 (230.7–495.8)	394.6 (246.2–494.7)	0.04	352.9 (235.8–520.7)
TNF-α	12.8 (10.0–19.2)	11.3 (9.3–18.9)	13.3 (11.4–18.1)	NS	12.4 (9.9–13.4)

NS – not significant ($p \geq 0.05$ for all groups); data are presented as median and range (25th and 75th percentile); C – control group: reference values of cytokines; AA group – patients with acute appendicitis; 1 – cytokine concentrations one hour before the operation; 2 – cytokine concentrations 24 hours after start of the operation; 3 – cytokine concentrations 72 hours after start of the operation.

Table 3

SERUM CYTOKINE CONCENTRATIONS OBTAINED ON THE DAY OF PATIENT ADMISSION TO HOSPITAL AND 24 AND 72 HOURS AFTER HOSPITALIZATION IN THE AML GROUP AND THE REFRENCE VALUES OF CYTOKINES IN THE C GROUP

Serum cytokine	AML group (n = 26)			*p* value	C (n = 17)
	1 (pg/mL)	2 (pg/mL)	3 (pg/mL)		(pg/mL)
EGF	106.8 (46.5–152.7)	113.1 (51.8–151.7)	123.7 (75.8–204.3)	NS	87.0 (38.3–133.6)
IL-10	3.2 (3.2–3.2)	3.2 (3.2–3.2)	3.2 (3.2–3.3)	NS	3.2 (3.2–3.2)
IL-12(p70)	3.2 (3.2–3.2)	3.2 (3.2–3.2)	3.2 (3.2–3.2)	NS	3.2 (3.2–3.5)
IL-17	3.3 (3.2–9.1)	3.3 (3.2–10.9)	3.2 (3.2–8.6)	NS	5.8 (3.2–29.5)
IL-1β	3.2 (3.2–3.2)	3.2 (3.2–3.2)	3.2 (3.2–3.2)	NS	3.2 (3.2–8.6)
IL-4	3.2 (3.2–3.7)	3.2 (3.2–3.2)	3.2 (3.2–3.2)	NS	3.2 (3.2–3.2)
IL-6	3.2 (3.2–3.2)	3.2 (3.2–3.2)	3.2 (3.2–3.2)	NS	3.2 (3.2–11.7)
IL-8	10.6 (7.0–19.2)	13.4 (7.4–20.9)	11.6 (7.3–22.1)	NS	18.3 (7.1–55.3)
MCP-1	333.9 (266.0–378.2)	387.3 (250.1–431.7)	405.8 (313.6–484.2)	0.01	352.9 (235.8–520.7)
TNF-α	14.2(11.0–19.0)	14.2(10.9–19.5)	16.4(11.5–18.8)	NS	12.4 (9.9–13.4)

NS – not significant ($p \geq 0.05$ for all groups); data are presented as median and range (25th and 75th percentile); C – control group: reference values of cytokines; AML group – patients with acute mesenteric lymphadenitis; 1 – cytokine concentrations on the day of patient admission to hospital; 2 – cytokine concentrations 24 hours after the hospitalization; 3 – cytokine concentrations 72 hours after the hospitalization.

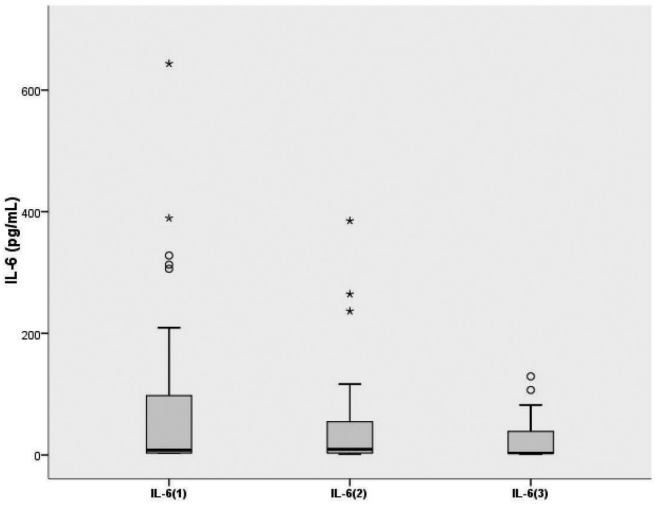

Fig. 1. Serum concentrations of IL-6 at different time points in the group of patients with AA. IL-6(1) – cytokine concentrations one hour before the operation; IL-6(2) – cytokine concentrations 24 hours after start of the operation; IL-6(3) – cytokine concentrations 72 hours after start of the operation.

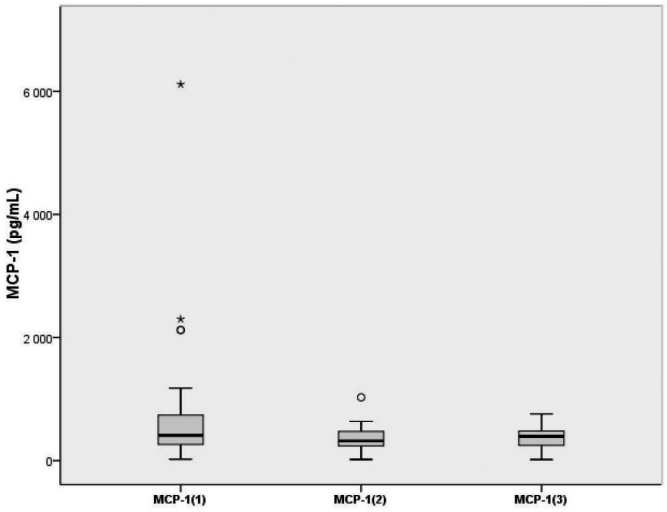

Fig. 2. Serum concentrations of MCP-1 at different time points in the group of patients with AA. MCP-1(1)– cytokine concentrations one hour before the operation; MCP-1(2) – cytokine concentrations 24 hours after start of the operation; MCP-1(3) – cytokine concentrations 72 hours after start of the operation.

F(2;29) = 3.5; $p = 0.04$) (Figs. 1 and 2). The serum levels of MCP-1 differed significantly from the day of patient hospitalization to those at 72 hours after hospitalisation also in the AML group (Wilks' Lambda test 0.70; F(2;24) = 5.0; $p = 0.01$) (Fig. 3).

More detailed analysis revealed that the concentrations of IL-6, and IL-10 were significantly higher in all serum samples obtained from patients with AA than in patients in the C group (IL-10(1): z = –2.29; $p = 0.02$; IL-10(2): z = –3.5; $p = 0.0004$; IL-10(3): z = 3.59; $p = 0.0003$; (IL-6(1): z = –2.55; $p = 0.01$; IL-6(2): z = –2.77; $p = 0.006$; IL-6(3): z = –1.96; $p = 0.05$). The serum levels of IL-6 and IL-10 were significantly higher one hour before surgical intervention in the AA group compared to the corresponding values on the day of patient admission to hospital in the AML group (IL-6(1): z = –3.72; $p = 0.0002$; IL-10(1): z = –2.81; $p = 0.005$). The concentration of IL-12(p70) was significantly higher in serum 24 hours after operation in the AA group than in the C group (IL-12(p70)(2): z = –2.15; $p = 0.031$). Further analysis demonstrated that 24 and 72 hours after operation the concentrations of IL-1β, IL-4, TNF-α and EGF were significantly different between the AA group and the C group. The concentrations of IL-8 and MCP-1 at different

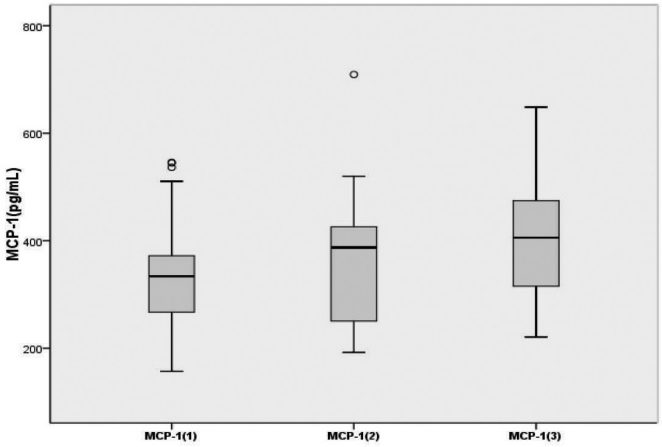

Fig. 3. Serum concentrations of MCP-1 at different time points in the group of patients with AML. MCP-1(1) – cytokine concentrations on the day of patient admission to hospital; MCP-1(2) – cytokine concentrations 24 hours after the hospitalization; MCP-1(3) – cytokine concentrations 72 hours after the hospitalization.

time points in these study subgroups did not differ significantly.

DISCUSSION

Patients with AML typically have a history of fever and abdominal pain, frequently with localisation in the right lower quadrant (Macari *et al.*, 2002; Hayes, 2004; Sicorska-Wiśneiewska *et al.*, 2006; Mandell *et al*., 2009; Toorenvliet *et al*., 2011). The clinical presentation is similar to that of acute AA (Khanna *et al.*, 1983; Yoon *et al.*, 2002; Toorenvliet *et al.*, 2011; Saliakellis *et al.*, 2013). Few data are available on the incidence of this syndrome, and it may vary with the geographic location. In one series of hospitalised patients, 50 of 651 (7.7%) admitted with a diagnosis of AA had a discharge diagnosis of AML (Mandell *et al.*, 2009).

In most cases, gut bacteria have a possible role in causing common clinical symptoms of both mentioned diseases. Bacteriologic studies of nonspecific AA using microbiologic culture techniques have revealed a wide variety of anaerobic and aerobic bacteria that are present in these diseases (Lamps, 2008; 2010; Arlt *et al.*, 2013;). Nonspecific AML organisms isolated in culture include *Escherichia coli, Bacteroides, Clostridia* species, *Enterococci*, beta-hemolytic *Streptococcus*, *Staphylococcus aureus* and *Yersinia* (Khanna *et al.*, 1983). These microbes were also observed in the present study, and *Pseudomonas* was isolated from some specimens of patients with AA. The character of bacteria is a major factor in determining their arrest in lymph nodes (Khanna *et al.*, 1983; Mandell *et al.*, 2009). Therefore, it is possible that different cytokines can appear in blood at the early stages of AML. It was observed (Li *et al.*, 2010) that *Staphylococcus aureus* infection was reduced by local MCP-1 therapy aimed at monocyte and macrophage recruitment in the first 48 hours at the site of infection. According to our study, MCP-1 levels in serum also significantly increased at 42 hours from start of symptoms to 72 hours after patient hospitalisation with AML.

Previously, studies examining the immunologic response to enteric bacteria within the intestinal lumen have shown specific cytokine production by certain bacteria, which might aid diagnosis of AA (Arlt *et al.*, 2013). Two important immunoregulatory cytokines produced by cells of the innate defense 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 (Hessle *et al.*, 1999). IL-10 was identified as a cytokine synthesis response inhibitory factor that is secreted as a part of Th2 response and that inhibits interleukin-2 and IFN-γ production by Th1 cells (Shaheen *et al.*, 2009). In the present study these same pro-inflammatory and anti-inflammatory cytokines were observed and their levels significantly differed at different time points of AA. The concentration of IL-12(p70) was significantly higher in serum 24 hours after the operation in the AA group, comparted to that healthy subjects. However, the IL-10 level was significantly higher in serum obtained 22 hours after the patient felt ill in the AA group, compared to the that in the C group. Previously it was observed that IL-6 and IL-10 had high concentration in the serum of patients with uncomplicated AA, while the proinflammatory cytokines (IL-1β, IL-12(p70), TNF-α) had low concentration in these patient (Rivera-Chavez *et al.*, 2003). Cytokine profiles in whole peripheral blood mononuclear cells was found to differ depending on the stimulating bacterial species: *Bacteroides fragilis* induced significantly more production of IL-12(p70) and interleukin-23 than *Escherichia coli* and *Enterococcus*, although *Escherichia coli* and *Enterococcus* induced levels of IL-10 that were significantly higher than the level induced by *Bacteroides fragile* (Manuzak *et al.*, 2012). These findings indicate that IL-10 and IL-12(p70) might be important diagnostic serum markers for AA arising due to commensal enteric bacteria. We emphasize that we only found significant increased secretion of IL-10 and IL-6 one hour before the surgical intervention in the cases of AA, while the corresponding values were observed on the day of AML patient admission to hospital. It is possible that AA and AML might be due to enteric bacteria. Consequently, enlarged abdominal lymph nodes can appear simultaneously with the inflamed appendix as a result of more severe local inflammation in appendix and bacterial translocation (Österberg *et al.,* 2004). In our study, enlarged abdominal lymph nodes were detected simultaneously with the inflamed appendix in three patients with AA. In an experimental study with mice where release of cytokines was stimulated by an innate immune regulatory factor, IL-10 began to appear after three hours, peaked at eight hours and returned to baseline by 36 hours, indicating that high concentrations in blood are achieved rapidly after the onset of bacterial invasion (Parker *et al.*, 2011). Increased levels of IL-10 and infiltration of polymorphonuclear leukocyte and plasma cell isotypes in the *lamina propria* of the appendix at 30 minutes were observed fol-

lowing experimental obstruction of the appendix lumen in 24 New Zealand-bred white rabbits (Tsuji *et al.*, 1990). Similarly, in our study the highest concentration of IL-10 occurred 22 h after AA patients felt ill.

IL-6 has a broad range of activity on multiple cells types and it induces a broad array of responses (Shaheen *et al.*, 2009). One of these is acute phase response followed by bacterial endotoxemia (Brănescu *et al.*, 2013; Saliakellis *et al.*, 2013). Brănescu *et al*. (2013) found preoperatively that IL-6 in serum exceeded a maximum value of 9.7 pg/mL in 93.7% of studied subjects with phlegmanous inflammation of appendix. This is consistent with our finding that preoperatively concentration of IL-6 was significantly higher (8.0 pg/mL) in patients with AA, compared to its concentration (3.2 pg/mL) on the day of AML patient admission to hospital. This can be explained also by the fact that there were more cases of uncomplicated AA in our study than complicated AA. Previous study (Gűrleik *et al.*, 2002) showed that measurement of IL-6 concentration did not benefit in increasing the accuracy of diagnosis of AA, as 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. We observed a significant difference between the levels of IL-6 cytokine at one hour before surgical intervention to 72 hours after the operation in the cases of AA. Sakamoto *et al.* (2003) found that serum IL-6 levels reached a maximum within the first postoperative day and decreased thereafter, which was explained by surgical trauma whereby IL-6 was produced in the operative field and entered the peripheral blood stream to induce elevation of serum IL-6. Examination of serum inflammatory markers in AA and AML cases might be helpful in differentiating the stage of inflammation, as most patients are healthy before developing AA and AML and since the length of symptoms before hospitalisation is typically less than 48 hours (Rivera-Chavez *et al.*, 2003).

A limitation of this study was the small number of patients. One of the reasons for this was due to the exclusion criteria of use of non-steroidal inflammatory and antibacterial treatment before the patient has admitted to hospital with AA and AML. The most significant factors affecting the diagnostic difficulties of both these diseases in Latvia is the lack of equipment and specialists in radiological methods in emergency units to make the accurate diagnose of AA and AML. Therefore, we need widely available and practicable laboratory tests to support diagnoses that would result from future research. In future, the study population should be increased and the additional analysis of clinical parameters is needed.

The increased cytokines serum concentrations of IL-6 and IL-10 were the most reliable indicators one hour before the surgical intervention (the median length of symptoms was 22 h) for patients with AA. MCP-1 values significantly differed 72 h after patient hospitalization compared to values at other time points, but did not differ between the AA and AML groups. Therefore, MCP-1 is not a useful serum biomarker in distinguishing patients with AA and AML.

ACKNOWLEDGEMENTS

The study was financially supported by a Grant No. 2009/0147/1DP/1.1.2.1.2/09/IPIA/VIAA/009 from the project Support for Doctoral and Post-doctoral Investigations Rīga Stradiņš University fellowship and a Grant No. 2010.10-4/VPP-4 of the framework of the Latvian National Programme and also by the National Research programme project Biomedicine for Public Health (BIOMEDICINE) No. 6.1 "Research on acute and chronic diseases in a wide age-range children to develop diagnostic and theraputic algorithms to reduce mortality, prolong survival and improve quality of life".

REFERENCES

Arlt, A., Bharti, R., Ilves, I., Häsler, R., Miettinen, P., Paajanen, H. (2013). Characteristic changes in microbial community composition and expression of innate immune genes in acute appendicitis. *Innate Immun.*, **21** (1), 30–40.

Brănescu, C., Şerban, D., Dascălu, A. M., Oprescu, S. M., Şavlovschi, C. (2013). Interleukin 6 and lipopolysaccharide binding protein — markers of inflammation in acute appendicitis. *Chirurgia*, **108** (2), 206–214.

Chang, Y. J., Chao, H. C., Kong, M. S., Hsia, S.-H., Yan, D. C. (2010). Misdiagnosed acute appendicitis in children in emergency department. *Chang Gung Med. J.*, **33** (9), 551–556.

Dala, I., Somekh, E., Ilker-Reich, A., Boaz, M., Gorenstein, A., Serour, F. (2005). Serum and peritoneal inflammatory mediators in children with suspected acute appendicitis. *Arch. Surg.*, **140** (2), 169–173.

Gürleik, G., Gürleik, E., Çetinkaya, F., Unalmiser, S. (2002). Serum interleukin-6 measurement in the diagnosis of acute appendicitis. *Austral. New Zeal. J. Surg.)*, **72** (9), 665–667.

Hayes, R. (2004). Abdominal pain: general imaging strategies. *Eur. Radiol.*, **14,** 123–137.

Hessle, C., Hanson, Å., Wold, A. E. (1999). Lactobacilli from human gastrointestinal mucosa are strong stimulators of IL-12 production. *Clin. Exp. Immunol.*, **116**, 276–282.

Khanna, S. K., Jain, S. K. (1983). Staphylococcal suppurative mesenteric lymphadenitis. *Postgrad. Med. J.*, **59**, 191–193.

Lamps, L. W. (2008). Beyond acute inflammation: review of appendicitis and infections of appendix. *Diagn. Histopathol.*, **14** (2), 68–77.

Lamps, L. W. (2010). Infectious causes of appendicitis. *Infect. Dis. Clin. North. Amer.*, **24** (4), 995–1018.

Li, B., Jiang, B., Dietz, M. J., Smith, E. S., Clovis, N. B., Rao, K. (2010). Evaluation of local MCP-1 and IL-12 nanocoatings for infection prevention in open fractures. *J. Orthop. Res.*, **28** (1), 1–17.

Macari, M., Hines, J., Balthazar, E., Megibow, A. (2002). Mesenteric adenitis: CT diagnosis of primary versus secondary causes, incidence, and clinical significance in pediatric and adult patients. *Amer. J. Roentg.,* **178**, 853–858.

Mandell, G. L., Bennett, J. E., Dolin, R. (2009) Mesenteric Adenitis. In: Mandell, G. L. (ed.). *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, 7th edition*. WB Saunders, Philadelphia, p. 978.

Manuzak, J., Dilon, S., Wilson, C. (2012). 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.*, **19** (8), 1207–1217.

Österberg, J., Ljungdahal, M., Lundholm, M., Engstrand, L., Haglund, U. (2004). Microbial translocation and inflammatory response in patients with acute peritonitis. *Scand. J. Gastroenterol.*, **7**, 657–664.

Parker, T. A., Cheng, H., Willeford, K. O., Wu, S. (2011). Interleukin-6 expression in response to innate immune regulatory factor stimulation. *Biomed. Pharmacother.*, **65** (2), 90–94.

Rivera-Chavez, F. A., Wheeler, H., Lindberg, G., Munford, R. S., O'Keefe, G. E. (2003). Regional and systemic cytokines responses to acute inflammation of the vermiform appendix. *Ann. Surg.*, **237** (3), 408–416.

Saliakellis, E., Thapar, N. (2013). Paediatric GI emergencies. *Best Pract. Res. Clin. Gastroenterol.*, **27**, 799–817.

Sakamoto, K., Hisano, S., Kamohara, H., Ishiko, T., Mita, S., Ogawa, M. (2003). Changes of interleukin 6 and soluble IL-6 receptor levels after surgery. *Int. Congr. Ser.*, **1255**, 135–142.

Sicorska-Wiśneiewska, G., Liberek, A., Góra-Gebka, M., Bako, W., Marek, A., Szlagatys-Sidorkiewicz, A., Jankowska, A. (2006). Mesenteric lymphadenopathy — a valid health problem in children. *Med. Wieku. Rozwoj.* [Developmental Period Medicine], **10** (2), 453–462 (in Polish).

Shaheen, M., Broxmeyer, H. E. (2009). The humoral regulation of hematopoiesis. In: Hoffman, R., Benz, E. J., Shattil, S. J. (ed.). *Hematology: Basic Principles and Practice, 5th edition*. Elsevier Churchill Livingston, Philadelphia, p. 253.

Toorenvliet, B., Vellekoop, A., Bakker, R., Wiersma, F., Mertens, B., Merkus, J. (2011). Clinical differentiation between acute appendicitis and acute mesenteric lymphadenitis in children. *Eur. J. Pediatr. Surg.*, **21**, 120–123.

Tsuji, M., McMahon, G., Reen, D., Puri, P. (1990). New insights into the pathogenesis of appendicitis based on immunocytochemical analysis of early immune response. *J. Pediatr. Surg.*, **25** (4), 449–452.

Türkyilmaz, Z., Sönmez, K., Karabulut, R., Elbeğ, Ş., Moralioğlu, S., Demirtola, A., Demiroğullari, B., Özen, I. O., Başaklar, A. C., Kale, N. (2006). Sequential cytokine levels in the diagnosis of appendicitis. *Scand. J. Clin. Lab. Invest*., **66**, 723–732.

Wray, C. J., Kao, L. S., Millas, S. G., Millas, S. G., Tsao, K., Ko, T. C. (2013). Acute appendicitis: Controversies in diagnosis and management. *Curr. Probl. Surg.*, **50**, 54–86.

Yoon, D., Chu, J., Chandler, C., Hiyama, S., Thompson, J. E., Hines, O. J. (2002) Human cytokine levels in nonperforated versus perforated appendicitis: Molecular serum markers for extent of disease? *Amer. Surg.*, **68** (12), 1033–1037.

Received 20 January 2015

ATŠĶIRĪGA CITOKĪNU GRUPAS SERUMA IEKAISUMA MEDIATORU IZPAUSME AKŪTA APENDICĪTA UN AKŪTA MEZENTERIĀLA LIMFADENĪTA GADĪJUMĀ BĒRNIEM

Citokīnu grupas seruma iekaisuma mediatoru nozīme atkarīga no akūta apendicīta (AA) un akūta mezenteriāla limfadenīta (AML) etioloģijas un patoģenēzes. Pētījuma mērķis bija atklāt dažādu seruma citokīnu nozīmi AA un AML diagnostikā bērniem. Pētījumā tika iekļauti 74 pacienti (7–18 gadi), kuri ārstēti VSIA Bērnu klīniskajā universitātes slimnīcā (BKUS), bērnu ķirurģijas nodaļā (2010–2013). Atbilstoši pētījuma mērķim bērni tika sadalīti divās grupās: 31 pacienti AA grupā un 26 pacienti AML grupā. Pētījuma kontroles grupā tika iekļauti 17 pacienti ar plānveidā operētām ķirurģiskām saslimšanām bez iekaisuma klātbūtnes organismā, kuri arī stacionēti VSIA BKUS pētījuma veikšanas laika posmā. Kontroles grupa tika izveidota ar mērķi noteikt citokīnu referentās vērtības un tās salīdzināt ar AA un AML pacientu rezultātiem. Visās grupās tika paņemti asins paraugi un noteikti šādi seruma citokīni: EGF, IL-10, IL-12(p70), IL-1β, IL-4, IL-6, IL-8, MCP-1, TNF-α. Kontroles grupai un pacientiem ar AA venozie asins paraugi tika ievākti stundu pirms operācijas, 24 un 72 stundas pēc operācijas, bet AML gadījumā: stacionēšanas brīdī, 24 un 72 stundas pēc iestāšanās stacionārā. Pētījumā iesaistīto pacientu ar AA un AML vidējais vecums 12,9 gadi (SD 3,2), no kuriem 61,4% (n = 35) bija zēni un 38,4% (n = 22) meitenes. Vidējais vecums AA pacientu grupā 13,4 gadi (SD 2,5) un AML pacientiem 11,0 gadi (SD 2,4). IL-6 un MCP-1 gadījumā tika novērota statistiski ticama atšķirība citokīnu koncentrācijai serumā laika dinamikā AA pacientiem (no stundu pirms operācijas līdz 72 stundām pēc ķirurģiskas iejaukšanās): Wilks' Lambda tests 0,80; F(2;29) = 3,5; p = 0,04. AML pacientiem MCP-1 citokīna koncentrācijai plazmā tika novērota statistiski ticama atšķirība laika dinamikā (stacionēšanas brīdī līdz 72 stundām pēc stacionēšanas): Wilks' Lambda tests 0,70; F(2;24) = 5,0; p = 0,01. IL-6 un IL-10 koncentrācija pirmajā seruma paraugā (t.i., pirms ārstēšanas uzsākšanas) paaugstinās pacientiem ar AA, salīdzinot ar AML pacientiem ((IL-6(1): z = –3.72; p = 0.0002; IL-10(1): z = –2.81; p = 0.005)). Pētījuma rezultātā būtiska IL-6 un IL-10 citokīnu koncentrācijas paaugstināšanās serumā tika novērojama stundu pirms operācijas, kas vidēji atbilst 20 stundu slimības ilgumam AA gadījumā. MCP-1 gadījumā tika novērota statistiski ticama atšķirība citokīnu koncentrācijai serumā laika dinamikā abu pacientu grupu iekšienē, bet ticamas atšķirības starp abām šim grupām netika atklātas.