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Rīgas Stradiņa universitāte

Astra Zviedre

CORRELATION OF ACUTE APPENDICITIS AND ACUTE MESENTERIC LYMPHADENITIS WITH THE CHANGES OF SERUM CYTOKINES IN CHILDREN

Summary of Doctoral Thesis
for obtaining the degree of a Doctor of Medicine
Speciality – Paediatric Surgery

Riga, 2016

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Doctoral Thesis were elaborated at the state tertiary healthcare institution – State Ltd Children's Clinical University Hospital Paediatric Surgery Clinic and Central Laboratory; Rīga Stradiņš University, Department of Paediatric Surgery, Department of Human Physiology and Biochemistry

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CONTENT

1 Introduction 4
Aim of the doctoral thesis: 5
Tasks of the doctoral thesis: 6
Hypotheses of doctoral thesis: 6
Scientific novelty of the doctoral thesis 6
Practical significance of the doctoral thesis 8
2 Materials and methods 9
2.1 Structure of the thesis 9
2.2 Selection of Subject data 9
2.3 Statistical analysis of data 11
3 Results 13
3.1 Incidence and changes of AA and AML in children in the period from 2010 to 2013 13
3.2 Description of first-time hospitalised patients with a suspicion of AA at State Ltd CCUH (2010 – 2013) 14
3.3 Results of data assessment regarding patient's subjective and objective state 15
3.4 Results of laboratory data assessment 16
3.5 SIMCG values in AA and AML patient groups 18
3.6 SIMCG un WBC cut-off values for early diagnostics of patients with AA and AML 20
3.7 SIMCG un CRP cut-off values for early diagnostics of patients with complicated and uncomplicated AA 21
3.8 Assessment of radiological examination results 23
3.9 Alvarado score criteria assessment data in AA and AML cases 24
4 Discussion 27
5 Conclusions 39
Practical recommendations 41
References 41
Publications and theses 45

1 INTRODUCTION

Despite of the established state tertiary healthcare system, widely available modern visual methods of diagnostics as well as the application of broad-spectrum antibiotics in treatment, the assessment of acute abdominal pain syndrome in paediatrics is really challenging. The cause of abdominal pain in children can have different roots, ranging from simple bowel movement problems to potentially dangerous conditions demanding for emergent paediatric surgery [*Bundy et al.,* 2007]. All over the world, including Latvia, some of the most common acute abdominal inflammation processes attributed to children are acute appendicitis (AA) and acute mesenteric lymphadenitis (AML). In the case of the abovementioned health problems, it is necessary to ensure timely diagnosis and appropriate treatment. AA and AML clinical presentation is often similar, but the tactics of treatment in both cases will probably differ. In the case of AML, treatment is conservative and does not require immediate hospitalization of the patient; however, the patient with AA most often has to be provided with emergent surgical intervention. Different scientific sources mention that on average 77 000 patients with suspicion of AA are hospitalised in paediatric departments in the United States (US) yearly, while in the UK an average of 40 000 children with acute abdominal pain and suspected AA are admitted to hospital every year [*Bundy et al.,* 2007; *Humes and Simpson*, 2006].

In Latvia, in the period from 2000 to 2009, an average of 1,162 children with AA until the age of 18 was hospitalized for the first time. In the same period of time State Ltd Children's Clinical University Hospital (State Ltd CCUH) had on average 225 patients with AA until the age of 18 years who underwent surgical treatment yearly [*Surna et al.,* 2011].

State Ltd CCUH patient data (2000 – 2009) show that on average from 18.8 % to 21 % of cases had a delayed start of treatment, which required not

only the treatment of the disease itself, but also the treatment of complications [*Surna et al.,* 2011]. Timely diagnostics of AA and AML improves patient's quality of life in future. It reduces the likelihood of negative appendectomy, rate of apendix perforation, shortens hospitalisation period, as well as speeds up the recovery process and reduces the risk of developing intestinal impenetrability at the late postoperative period due to complicated intra-abdominal infection [*Myers et al.,* 2012]. In future, the determination of serum inflammatory mediators of cytokine group (SIMCG) could serve as an indispensable part of a diagnostic marker of AA and AML in children aged 7 to 18 years that would shorten the differential time of diagnosis and facilitate the choice of appropriate treatment method.

The topicality of the research is supported by the fact that the available information on SIMCG analysis in relation to acute abdominal conditions in paediatrics is still incomplete, which emphasises the importance and need for a further and more detailed research. Therefore, the present thesis attempts to identify the most essential SIMCG diagnostic markers in AA and AML cases as well as to find out their correlation to other clinical diagnostic methods.

Aim of the doctoral thesis

To analyse changes in AA and AML morbidity in Latvia (2010 – 2013) and determine the correlation of admitting diagnosis to the discharged diagnosis; as well as to explore diagnostic significance of serum inflammatory mediators in cytokine groups differentially (epithelial growth factor (EGF), interleukin (IL)-10, IL-12(p70), IL-1β, IL-4, IL-6, IL-17, IL-8, monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor alfa (TNF-α)) and assess the link of biomarkers to other clinical diagnostic methods of AA and AML cases in children.

Tasks of the doctoral thesis

1. To determine AA and AML morbidity incidence and dynamic in Latvia and State Ltd CCUH in the period from 2010 to 2013.

2. To describe first-time hospitalisation cases of children (0–18 years old) with suspicion of AA in State Ltd CCUH and assess the correlation of the admitting diagnosis to the discharged diagnosis from 2010 to 2013.

3. To investigate the difference between clinical characteristics according to the Alvarado score criteria for the patients with AA and AML as well as children with complicated and uncomplicated AA.

4. To determine and analyse SIMCG (EGF, IL-10, IL-12(p70), IL-1β, IL-4, IL-6, IL-17, IL-8, MCP-1, TNF-α) concentration cut-off values and their difference between AA and AML patients in the age of 7 to 18.

5. To assess and compare clinical, laboratory and radiology data of children with AA and AML.

6. To develop AA prediction and action algorithm, adjusted to Latvian conditions, for children aged 7–18 with AA for the further application at hospital emergency medical service departments (EMSD) and ambulatory care stage.

Hypotheses of doctoral thesis

- SIMCG concentration differences are crucial in AA and AML differential diagnoses.
- Combination of SIMCG methodology with other clinical diagnostic methods can improve precision of AA diagnosis.

Scientific novelty of the doctoral thesis

For the first time AA and AML diagnostic data based on modern globally recognized, proven laboratory techniques, clinical criteria and simplified radiological examinations of children aged 7 to 18 years have been

analysed in Latvia. By identifying key SIMCG in serum of the cases with AA and AML in children, they can serve as additional markers for the differential diagnosis of these diseases.

So far, there have not been such varied case-control, prospective studies carried out in paediatric surgery of Latvia. The present research has used a complex approach for data analysis. The changes of AA and AML incidence were initially assessed in Latvia and State Ltd CCUH from 2010 to 2013. In addition, the diagnostic accuracy of AA performed by EMSD in surveyed population of State Ltd CCUH was assessed.

The epidemiological background of AA and AML cases provides an insight into key problems associated with the abovementioned disease diagnosis, registration system in the country and disease prognosis.

The research analysed the diagnostic accuracy of AA and AML methods in detail to assess their practical application. The research work provides SIMCG cut-off values for the early identification of AA patients.

The Alvarado score, being one of the AA diagnostic methods, has been previously used by State Ltd CCUH EMSD, but the results of the analysis have showed that it could be used as a sufficiently sensitive method together with laboratory values and ultrasonography (USG) examination for early identification of AA cases in children.

The data of doctoral thesis reflect significant differences in the results of various diagnostic methods of AA in comparison with AML in children aged 7 to 18 years. The incidence of AA complications and the number of negative appendectomy show the quality of national health care system for children with AA. Therefore, the information that could be useful in the development of various diagnostic algorithms for EMSD, paediatric surgeons, general practitioners and paediatricians in clinical practices of Latvia was analysed.

Practical significance of the doctoral thesis

The major scientific novelty of the study is the importance of SIMCG (EGF, IL-10, IL-12(p70), IL-1β, IL-4, IL-6, IL-17, IL-8, MCP-1, TNF-α) in differential diagnosis and the link of biomarkers with other methods of AA and AML case diagnosis in children of 7 to 18 years age group. There were statistically significant differences found between separate levels of inflammation markers C ractive proteine (CRP), IL-6, IL-10, IL-12(p70), IL-17, IL-1β, IL-4, EGF, MCP-1 and TNF-α in children with AA and AML, as well as between patients with complicated and uncomplicated AA and AML. There were defined cut-off values of laboratory markers concentration in serum for early AA patient identification. Taking into account the length of disease and analysed clinical, laboratory and radiological data of children with AA and AML, there has been developed AA prediction and action algorithm for children aged 7 – 18 with suspicion of AA (see Practical Recommendations).

2 MATERIALS AND METHODS

2.1 Structure of the thesis

The thesis "Correlation of Acute Appendicitis and Acute Mesenteric Lymphadenitis with the Changes of Serum Cytokines in Children" was started on October 1, 2010 and consists of three parts:

- Systematic literature review on AA and AML epidemiology, clinical manifestation, methods of diagnosis and prognosis in children.
- AA and AML incidence rates in children treated in Latvia and State Ltd CCUH.
- Determination and analysis of SIMCG diagnostic value and its correlation to other clinical diagnostic methods of AA and AML cases in children treated at State Ltd CCUH.
- The framework of the research is a mixed type prospective case-control study. The doctoral thesis was carried out at the state tertiary healthcare institution, State Ltd CCUH, Paediatric Surgery Clinic and Central Laboratory; Riga Stradiņš University, Departments of Paediatric Surgery, Human Physiology and Biochemistry in the period from 2010 to 2013.

The research was approved by the decision of State Ltd CCUH Medical Committee for Research Ethics (Reg. No. 40003457128). All children and parents/guardians have signed a written consent on their data inclusion in the study.

2.2 Selection of subject data

A complex approach was chosen in order to reach the set goal and prove the hypotheses put forward. To complete a retrospective data analysis the study used patient medical documentation and preservation database "Andromeda" of the State Ltd CCUH and information on AA and AML morbidity in Latvia in

the period of 2010 – 2013 provided by National Health Service. Following the primary enabling objective, there were 1228 data selected from State Ltd CCUH patient medical documentation and preservation database "Andromeda" (2010 – 2013) according to ICD-10 diagnosis classification in order to describe hospitalisation cases, the difference between the admitting and discharged diagnosis of patients suspected of AA. Only first-time hospitalisation cases were included in the study. The following patient selection criteria were determined: admitting diagnosis – suspicion of AA or AML and the age of a child from 0 to 18 years. Hospitalisation cases were classified according to ICD-10 diagnostic codes: acute appendicitis (K35), other type of appendicitis (K36), not specified appendicitis (K37), other type of caecal appendix diseases (K38), other functional colon diseases (K59), gastritis and duodenitis (K29), non-specific mesenteric lymphadenitis (I88.0), and other diseases: lymphocytic leukemia (C91.0), nerve root and plexus pathology (G54.8), salivary gland diseases (K11.2), gastroesophageal reflux disease (K21), acute lymphadenitis (L04) [*ICD-10*, 1992].

In order to achieve a prospective selection of data and meet the objectives of the study regarding the role of cytokines in AA and AML diagnosis in children, there were 57 patients prospectively selected according to the study inclusion criteria out of 178 study subjects, who were hospitalized at State Ltd CCUH Paediatric Surgery Department; 31 of them were patients with AA (AA group) and 26 patients with AML (AML group). To obtain a more detailed analysis, there were created uncomplicated and complicated AA groups out of 31 patients with AA. Patients with a diagnosis code K35.8 were included into uncomplicated AA group, while patients with principal diagnosis codes K35.2 and K35.3 were assigned to complicated AA group. Written informed consent forms from patients' parents or legal representatives were obtained in accordance with the protocol approved by the Medical Ethics Committee of State Ltd CCUH. All the patients suspected with AA were

checked-up by a paediatric surgeon at EMSD; blood samples were taken and examined and / or abdomen cavity organs were examined by one of radiological examination methods (ultrasonography (USG) or computed tomography (CT)). In all populations, venous blood samples were taken to determine SIMCG – an hour before surgery, at the 24th and 72th hour after the surgery. Control group (C group) samples were obtained using similar procedures. Patients with AML (AML group) gave their samples at the time of hospitalization after the diagnosis had been provided, at the 24th and 72th hour after conservative treatment.

2.3 Statistical analysis of data

The research data were analysed according to the standard methodology of biological data processing – applying descriptive, comparative and analytical statistics. The data necessary for the research were compiled and encoded with SPSS for Windows 20.0, statistical data processing program. According to the study objectives, new categories of variables related to the factors included in the analysis were established.

A measure of central tendency ratio was calculated for the studied variables – the arithmetic mean, the median and dispersion indicators - standard deviation (SD), features of a minimum and maximum value. The Shapiro - Wilk test was used to check data correlation to normal distribution; in cases which did not meet the normal distribution, the data were depicted with a median interval (25 and 75 percentile).

Chi square or χ^2 and Fisher's exact tests were used for data analysis of the studied population subgroups compared in 2×2 tables. The higher χ^2, the greater difference between observed characteristics in the groups is. The Kruskal - Wallis H test was applied for comparison of three or more groups that were independent. For the cases where the test indicated statistically significant

differences ($p < 0.05$), the Mann - Whitney U test, which shows the difference between the level of reliability in separate groups, was used for the further analysis of the data [*Peacok and Peacok et al.,* 2011].

In order to assess significance and accuracy of AA and AML cases diagnosed by the methods determined in the study (Alvarado score, laboratory and ultrasonography tests), sensitivity and specificity rates as well as in some cases positive predictive value (PPV) and negative predictive value (NPV) were calculated. PPV and NPV values are correlated with the spread of the disease, assuming that other factors are constant. PPV increases with the spread of the disease in population and NPV decreases accordingly [*Peacok and Peacok et al.,* 2011, *Parikh et al.,* 2008, *Fawcett,* 2009, *Erkel and Pattynama*, 1998].

Receiver Operating Characteristic (ROC) method was used to determine SIMCG, WBC (white blood count cells), CRP, ANC (absolute neutrophil count) concentration cut-off value one hour before the surgery and at the 24th hour after the surgery in the case of AA, as well as at the time of hospitalization after AML was diagnosed and at the 24th hour after the initiation of the treatment of AML cases. This method provides laboratory parameters concentration determined by the cut-off value of sensitivity and specificity. The area under the curve AUC (Area under the Curve) with the value from 0.5 to 1.0 – a perfect test, was used to compare the quality of cut-off value. Striving for accuracy, likelihood ratio (LR) was used to describe the diagnostic prediction level of a particular parameter (SIMCG, WBC, ANC) in AA cases.

3 RESULTS

3.1 Incidence and changes of AA and AML in children in the period from 2010 to 2013

In order to determine the overall incidence of AA and AML in the country, data from the National Health Service was additionally analysed, representing the number of first-time recorded cases (both outpatient and inpatient) of children with the aforementioned diagnoses.

The average reduction of the incidence with AA within the analysed period (2010 – 2013) was 1.8 cases per 10 000 population, a more rapid decrease of 5.3/10 000 was observed in adolescents (15–18 years), but the incidence reduction was not statistically significant. On the contrary, the overall incidence of AML showed a slight increase – 0.16 cases per 10 000 inhabitants in the same period, but it also was not considered statistically significant (see Table 3.1.1).

Table 3.1.1

Changing incidence of AA and AML in children from 2010 to 2013 (per 10 000 population)

	2010	2011	2012	2013	per 10 000 population*	p value
AA						
7–14 years	57.1	49.9	47.6	59.2	0.38	NS
15–18 years	68.9	72.9	63.4	54.3	−5.3	NS
Total	61.8	58.6	53.1	57.6	−1.8	NS
AML						
7–14 years	17.0	21.3	18.2	16.8	−0.34	NS
15–18 years	8.9	14.9	12.2	11.8	0.61	NS
Total	13.7	18.9	16.1	15.2	0.16	NS

Notes: *Average decrease/increase per year; NS – not statistically significant

3.2 Description of first-time hospitalised patients with a suspicion of AA at State Ltd CCUH (2010 – 2013)

There was a total of 1228 children, up to 18 years of age, with a suspicion of AA examined at State Ltd CCUH. There were 690 boys (56.2%) and 538 girls (43.8%) included into the study. Most often hospitalisation cases were observed for the age group of children from 10–14 years (37.9%). Within the analysed period of time, the morbidity of children with a suspicion of AA was registered in 307 (SD ± 146) cases on average. One of the most frequently stated diagnoses at the moment of hospitalisation was AA (K35) – 50.2% (95% CI 47.3–53.0) cases out of all analysed patients, but 0.8% (95% CI 0.4–1.4) cases had AML as the admitting diagnosis. In 42.2% (95% CI 39.4–45.0) of cases – the admitting diagnosis was not specified.

Having analysed admitting and discharging diagnoses of the patients suspected of AA in the period from 2010 – 2013, out of all analysed patients 76.2% (95% CI 73.8–78.5) of cases had incompatible admitting and discharging diagnoses. It was most frequently observed in patients with AML and other functional intestinal diseases that had the admitting diagnosis of AA (see Figure 3.2.1).

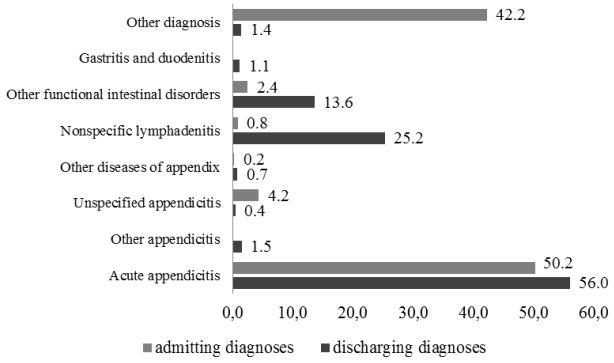


Fig. 3.2.1 **Compatibility of admitting and discharging diagnoses of hospitalised children at State Ltd CCUH, 2010 – 2013, %**

3.3 Results of data assessment regarding patient's subjective and objective state

Statistically significant differences in the incidence rates between AA and AML groups were observed in the following subjective symptoms: anorexia - (χ^2 = 7.7; p = 0.008) and vomiting (χ^2 = 8.1; p = 0.006) (see Table 3.3.1).

It can be clearly seen that on average anorexia and vomiting in AML group was observed by 36 percentage points less often than in AA group. The statistical significance was not observed in relation to the localization of the pain, migration of pain and dysuria. Practically all patients included in the study were reported to have abdominal pain in the lower right quadrant; dysuria was only found in one patient from AA group.

Table 3.3.1

Comparison of AA un AML subjective state assessment data

Symptoms	**AA group (n=31)**	**AML group (n=26)**	**p value**
Anorexia	21 (67.7%)	8 (30.8%)	0.008
Vomiting	16 (51.6%)	4 (15.4%)	0.006
LRQP (lower right quadrant pain)	31 (100%)	25 (96.2%)	NS
Migration of pain	5 (16.1%)	1 (3.8)	NS
Dysuria	1 (3.2%)	–	NS

Note: p value – according to Fisher's Exact test < 0,05; NS – not statistically significant

Having analysed the complaints of patients with uncomplicated (n = 21) and complicated (n = 10) AA, the following conclusion can be made that complaints of anorexia were less frequently observed in patients with uncomplicated AA than in patients with complicated AA, although the differences were not considered to be significant.

Having analysed the objective state of patients between groups the following symptoms were considered: abdominal wall rigidity, rebound tenderness, decreased bowel sounds and axillary temperature > 37.3°C.

Rebound tenderness were more frequently found to be statistically significant to AA patients (n = 21) or 67.7% than to the AML group (n = 6) or 23.1% patients (χ^2 = 11.3; p = 0.001). Altered intestinal motility in both groups of patients were observed in 25% of the cases on average, while the decreased bowel sounds were on average found in half of them, but slightly higher in AA patients. Altered passive abdominal wall rigidity was more frequently observed in AA cases (n = 25) or 80.6% (95% CI 63.7–90.8) compared with the cases of AML patients (n = 8) (30.8% (95% CI 16.5–50.0)). On average passive abdominal wall rigidity was three times more often observed in AA cases (χ^2 = 15.5; p = 0.001). The median length of symptoms before hospitalization as reported by the patient was 20 hours for AA and 42 hours for AML. The difference between these groups was not found.

3.4 Results of laboratory data assessment

There were no differences in all three measurements observed between groups for the following biological indicators: WBC, CRP and ANC. WBC median was of 11.1 × 10^3/μL (8.6–17.9) in AA patients at the first measurement or one hour before surgery, whereas in AML patients it was 9.6 × 10^3/μL (8.4–12.8) at the first-time of measurement or in other words at the hospitalization time right after the diagnosis was provided. One hour prior to surgery ANC median was 8.8 × 10^3/μL (6.2–15.5) in AA treated patients and 7.4 × 10^3/μL (6.2–10.4) in AML patient population at the time of hospitalization after the diagnosis was provided. One hour before surgery CRP median was 12.3 mg/L (3.1–45.5) in AA group and 15.9 mg/L (6.3–31.5) in AML group.

Having analysed the median values of WBC, CRP, and ANC between patients with uncomplicated and complicated AA, there were no statistically significant diferences found; however, the median values were slightly higher

for the patients with complicated AA. One hour before surgery, WBC median concentration was respectively 10.7 × 10^3/μL (8.4–17.8) in uncomplicated cases of AA, but in the case of complicated AA – 15.6 × 10^3/μL (9.4–19.3). Meanwhile, one hour prior to surgery the median CRP concentration in uncomplicated cases of AA was 11.5 mg/L (3.2–40.0), but for complicated cases of AA – 26.5 mg/L (1.5–50.0).

Having analysed laboratory markers (the first serum sample) by the duration of the symptoms (from the onset of symptoms to hospitalization time at EMSD) in both groups of patients in more detail, no significant difference was not found. However, we can conclude that CRP reaches its maximum from the 25th to 48th hour in both groups of patients; the highest concentration of ANC in AA case is observed from the 13th to 24th hour. WBC concentration dynamics decreases in AML case, but it does not change significantly within 48 hours in AA case (see Table 3.4.1).

3.4.1. Table

WBC, CRP and ANC concentration (median, 25 and 75 percentile) in AA and AML patients depending on the duration of the symptoms during the first measurement

Duration of disease, h	WBC × 10^3/μL	CRP, mg/L	ANC × 10^3/μL
AA			
0 – 12 h	13.7 (11.3–17.0)	6.6 (2.9–23.9)	8.4 (8.2–15.1)
13 – 24 h	13.5 (10.4–19.0)	12.2 (3.0–30.7)	12.8 (6.9–17.5)
25 – 48 h	13.8 (8.4–19.0)	28.9 (5.8–85.8)	6.2 (5.9–15.5)
AML			
0 – 12 h	11.4 (9.0–12.7)	8.5 (1.3–21.3)*	9.4 (8.4-18.3)
13 – 24 h	10.5 (8.4–12.2)	6.8 (6.4–20.9)	7.1 (5.8-8.3)
25 – 48 h	9.9 (7.7–13.2)	22.4 (11.0–33.8)*	7.0 (5.6-11.0)

Notes: h – hour; *p=0.01 comparison between 0 – 12 h and 25 – 48 h

The increase of CRP concentration was observed in only from the 13th hour in uncomplicated AA cases. The increase of WBC concentration is

observed up to the 12th hour of the disease in uncomplicated AA cases, and then its concentration gradually decreases.

3.5 SIMCG values in AA and AML patient groups

There was a statistically significant difference observed between AA and C groups within all three measurements in terms of the following cytokines concentration as IL-10 and IL-6: (IL-6(1) – 8 pg/mL vs. 3.2 pg/mL (p = 0.02), IL-6(2) – 9.3 pg/mL vs. 3.2 pg/mL (p = 0.006), IL- 6(3) – 3.4 pg/mL vs. 3.2 pg/mL (p = 0.05) IL-10(1) – 6.1 pg/mL vs. 3.2 pg/mL (p = 0.02) IL-10(2) – 4.0 pg/mL vs. 3.2 pg / mL (p = 0.000), IL-10(3) – 3.6 pg/mL vs. 3.2 pg/mL (p = 0.000). For AA patient group the concentration level of serum cytokines IL-6 and IL-10 was statistically significantly higher in comparison to the cytokine levels in AML patients at the first time of measurement (IL-6(1) – 8 pg/mL vs. 3.2 pg/mL (p = 0.005), IL-10(1) – 6.1 pg/mL vs. 3.2 pg/mL (p = 0.005) (see Table 3.5.1).

3.5.1. Table

SIMCG concentration difference between study groups at the first measurement

SIMCG (pg/mL)	**AA group (n=31)**	**AML group (n=26)**	**C group (n=17)**	***Kruskal-Wallis* test, p value**	***Mann-Whitney* U test, p value**
	Median (25 un 75 percentile)				
IL-10	6.1	3.2	3.2	0.005	0.02*
	(3.2–17.0)	(3.2–3.2)	(3.2–3.2)		0.005†
IL-6	8.0	3.2	3.2	0.0003	0.01*
	(3.2–97.6)	(3.2–3.2)	(3.2–11.7)		0.0002†

Notes: *p < 0.05 AA group compared to C group; †p < 0.05 AA group compared to AML 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 one hour before surgery to the 72nd hour 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 within the dynamics of time (from admission to hospital to the 72nd hour after conservative treatment): Wilks' Lambda test 0.70; F (2,24) = 5.0; p = 0.01.

In order to obtain a more thorough analysis of the results, SIMCG were analysed in AA patients. Cytokine median values taken one hour before surgery, at 24th and 72nd h after surgery were relatively higher in all samples of the patients with complicated AA than of the patients with uncomplicated AA. SIMCG median values also showed decreased dynamics in both groups of the patients. Meanwhile, the first cytokine sample or the sample obtained one hour before surgery provided statistically significant differences between uncomplicated and complicated AA patients as IL-6, IL-8 un MCP-1 (IL-6(1) – 5.4 pg/mL vs. 257.8 pg/mL, (p = 0.001); IL-8(1) – 9.3 pg/mL vs. 36.2 pg/mL, (p = 0.02); MCP-1(1) – 341.3 pg/mL vs. 653.9 pg/mL, (p = 0,03) (Table 3.5.2).

3.5.2. Table

Cytokines concentration in serum (median (25–75 percentile)) one hour before to surgery in patients with uncomplicated and complicated AA, pg/mL

CGSIM (pg/mL)	**Median (25 and 75 percentile)**		**p value**
	uncomplicated AA (n=21)	**complicated AA (n=10)**	***Mann-Whitney* U test**
IL-6	5.4 (3.2–15.6)	257.8 (67.5–343.3)	0.001
IL-8	8.0 (5.5–43.2)	36.2 (16.8–121.3)	0.02
MCP-1	341.3 (215.1–563.4)	653.9 (468.9–2165.8)	0.03

In order to assess how the changes of cytokines in serum are related to duration of symptoms, there were cytokine values analysed, which statistically differed between complicated and uncomplicated AA cases one hour before surgery or hospitalization time (see Table 3.5.3).

3.5.3. Table

Cytokines concentration in serum (median (25–75 percentile)) depending on the duration of symptoms of patients with uncomplicated and complicated AA, pg/mL

Disease duration, h	IL-6	IL-8	MCP-1
AA uncomplicated			
0 – 12 h	6.7 (3.2–75.8)	8.8 (6.8–39.4)	483.5 (276.3–842.6)
13 – 24 h	7.8 (3.2–57.9)	8.0 (5.6–40.4)	389.2 (204.9–548.2)
25 – 48 h	3.2 (3.2–6.5)	5.0 (2.6–64.0)	244.1 (182.7–335.5)
AA complicated			
0 – 12 h	205.3 (97.6–312.9)	186.2 (88.9–283.5)	1309.1 (496.7–1500.2)
13 – 24 h	389.4 (7.4–400.5)	17.3 (15.1–25.0)	811.1 (411.1–1120.3)
25 – 48 h	209.4 (45.4–317.0)	34.1 (14.2–109.4)	495.8 (255.8–3645.3)

Note: h – hour

The increase of IL-6, IL-8 and MCP-1 concentrations was observed within the first 12 hours in both AA groups, but a greater increase of concentration was found in AA complicated cases. IL-6 concentration peaked from the 12th to 24th hour in both AA cases.

3.6 SIMCG un WBC cut-off values for early diagnostics of patients with AA and AML

To determine the role of SIMCG in the diagnosis of AA, the ROC analysis was used to compare the cut-off value area under the curve or AUC values, taking ≥ 0.7 as the basis, which is the lowest value of the test that can be considered of high-quality as its sensitivity and specificity would be in optimal relations.

The results of ROC analysis showed that mostly all cytokines in serum had the same values in both groups of AA and AML patients, as the AUC values ranged from 0.4 to 0.6, which presents evidence of low sensitivity and specificity of a particular cytokine ratio. The exceptions were IL-6 and WBC concentration cut-off values applied to patients with AA, IL-6 AUC values

were 0.77 (95% CI 0.64–0.89; p = 0.001) and AUC values were WBC 0.72 (95% CI 0.58–0.85; p = 0.005). Having determined the concentration cut-off level for both indicators in serum to identify AA patients one hour before surgery, the optimal IL-6 cut-off level was ≥ 4.3 pg/mL with 67.7% sensitivity and 76.9% specificity, LR+ 2.93, LR− 0.42, but the WBC cut-off level was ≥ 10.7 × 10^3/μL with 74.2% sensitivity and 53.8% specificity, LR+ 1.6 and 0.5 LR− (see Figure 3.6.1).

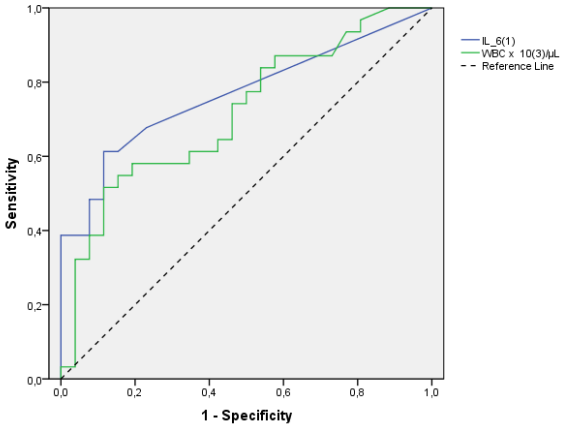


Figure 3.6.1. **ROC curve of cytokines in serum, IL-6 and WBC, one hour before surgery in AA patients**

3.7 SIMCG un CRP cut-off values for early diagnostics of patients with complicated and uncomplicated AA

ROC analysis revealed IL-6 and IL-8 as statistically significant cytokine serum concentrations (AUC ≥ 0.7) in patients with complicated AA one hour before surgery and at the 24th hour after surgery, as well as the concentration of MCP-1 before surgery and IL-10 concentration at the 24th hour after the

operation. Changes of CRP concentration in serum at the acute-phase significantly differed between uncomplicated and complicated AA.

One hour before surgery IL-6 concentration cut-off level was ≥ 39.2 pg/mL in complicated AA case with the AUC value of 0.86 (95% CI 0.69–1.0; p = 0.001), sensitivity of 80.0%, specificity of 81.0% and LR+ 4.2, LR− 0.3, while the IL-6 concentration cut-off level at the 24th hour after surgery was ≥ 9.4 pg/mL with the AUC value of 0.85 (95% CI 0.69–1.0; p = 0.002), sensitivity of 90.0%, specificity 76.2% and LR+ 3.8, LR− 0.1 (see Figure 3.7.1).

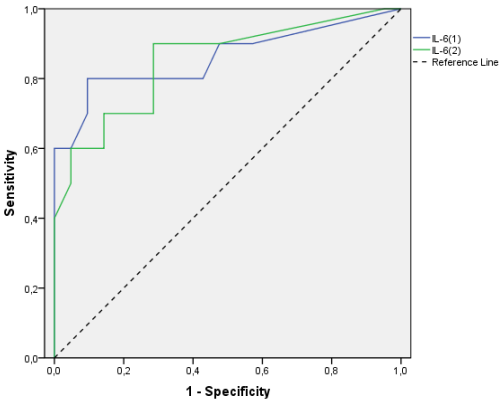


Figure 3.7.1. **ROC curve of IL-6 serum cytokine one hour before surgery (IL-6(1)) and at the 24th hour after surgery (IL-6(2)) in patients with complicated AA, pg / mL**

One hour before surgery IL-8 concentration with the cut-off level was ≥ 12.3 pg/mL in the case of complicated AA with AUC value of 0.76 (95% CI 0.57–0.95; p = 0.02), sensitivity of 90.0%, specificity 66.7 %, and LR+ 2.7, LR− 0.2, while IL-8 concentration cut-off level at the 24th hour after the operation was ≥ 11.6 pg/mL with AUC value of 0.78 (95% CI 0.61–0.94; p = 0.01), 90.0 % sensitivity and specificity of 61.9%, LR+ 2.4, LR− 0.2 (see Figure 3.7.2).

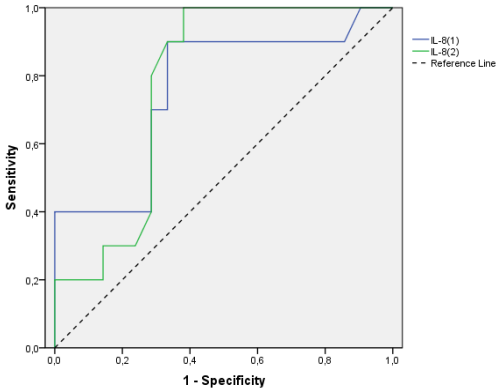


Figure 3.7.2. **ROC curve of IL-8 serum cytokine one hour before surgery (IL-8(1)) and at the 24th hour after surgery (IL-8(2)) in patients with complicated AA, pg/mL**

One hour before surgery MCP-1 concentration cut-off level was ≥ 400.2 pg/mL in complicated AA, with the AUC value of 0.75 (95% CI 0.55–0.96; p = 0.03), sensitivity of 90.0%, specificity of 66.7%, LR+ 2.7, LR− 0.2.

At the 24th hour after the operation the cut-off level of IL-10 concentration in serum was ≥ 4.7 pg/mL in the case of complicated AA, with AUC value of 0.76 (95% CI 0.58−0.94; p = 0.02), sensitivity of 80.0%, specificity of 76.2%, LR+ 3.4, LR− 0.3.

One hour before surgery CRP serum concentration cut-off level was ≥ 8.4 mg/L in complicated AA case, with the AUC value 0.71 (95% CI 0.48–0.94; p = 0.05), sensitivity of 80.0%, but specificity of 51.4% and LR+ 1.7, LR− 0.4.

3.8 Assessment of radiological examination results

USG examinations were performed to all study patients with AA and AML. During USG examination, all patients with AML were identified with mesenteric lymph nodes spans ≥ 10 mm, as it was also one of the inclusion

criteria of the study. Thus, for AA cases related to the same diagnostics, ultrasound method, one took into account the criteria which set appendix cross-section of ≥ 7 mm and a wall thickness ≥ 2 mm, sensitivity was 64.5 % (95% CI 46.9–78.9) and specificity – 76.9% (95% CI 56.4–90.9), PPV – 70.0% (95% CI 57.7–91.3), NPV – 64.5% (95% CI 46.9–78.9) and accuracy – 71.9 % (95% CI 59.2–81.9), p = 0.007. Having a more detailed analysis of the USG data relating to coprolith presence and patient AA discharging diagnosis, it was found more common to see coprolith of 30.0% (95% CI 10.8–60.3) in complicated AA than in uncomplicated AA cases, with only 19% (95% CI 7.7–40.0).

3.9 Alvarado score criteria assessment data in AA and AML cases

Statistically significant differences in the incidence rates between AA and AML groups were observed according to Alvarado score of subjective and objective criteria: anorexia, vomiting, and rebound tenderness (see Figure 3.9.1).

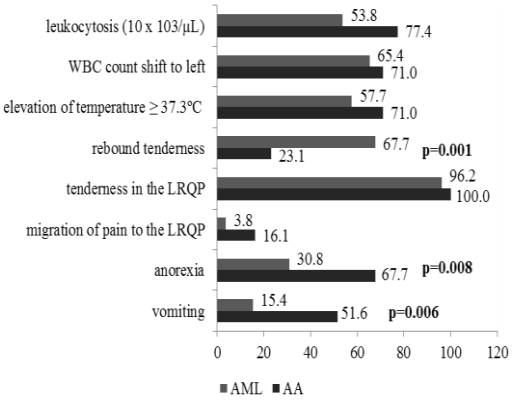


Figure 3.9.1 **Alvarado score criteria incidence rate in AA and AML patients, %**

Statistically significant differences in relation to the patient's discharging diagnosis were observed in the proportion of patients according to Alvarado score ≥ 7 points (χ^2 = 13.0; p = 0.001). In AA patients the value of ≥ 7 points – 71.0% (95 % CI 53.4–83.9) was most frequently observed in contrast to 23.1% (95% CI 11.0–42.1) in the case of AML by Alvarado score.

According to Alvarado score with values of ≤ 4 and of 9–10 points in the proportion of patients, there were found significant differences in relation to the patient's principal diagnosis (χ^2 = 6.3; p = 0.02 and χ^2 = 7.8; p = 0.006). The most common scale value of ≤ 4 points – 42.3 % (95 % CI 25.5–61.1) (n = 11) was observed in the case of AML, while in AA case the most often value was 7–8 points – 45.2% (95% CI 29.2–62.2) (n = 14). Meanwhile, Alvarado score with the value of 9–10 points was observed only in the case of AA – 25.8% (95% CI 13.7–43.2) (n = 8).

Analysing Alvarado score from the point of its precision as the diagnostic method of AA, the score value of ≥ 7 points, 71.0% of sensitivity (95% CI 53.4–83.9) was obtained, 76.9% of specificity (95% CI 57.9–89.0), PPV 78.6% (95% CI 60.5–89.8), 67.0% NPV (95% CI 50.8–82.7) and accuracy – 73.7% (95% CI 61.0-83.4) with LR+ 3.1, and LR− 3.8 (p = 0.002).

Analysing data in relation to the score points of the system, the AUC value was < 7 for all the cases; therefore, the group of ≥ 7 points was further on used in the calculation of data. Accordingly, up to four points (AUC = 0.35; p = 0.06), 5 – 6 points (AUC = 0.59; p = 0.08), 7 - 8 points (AUC = 0.39; p = 0.15), 9 – 10 points (AUC = 0.37; p = 0.09). Combining different features with Alvarado score of ≥ 7 points, the highest diagnostic accuracy of AA was provided by the set of features: Alvarado score of ≥ 7 points, WBC ≥ 10.7 × 10^3/μL and IL-6 ≥ 4.3 pg/mL (AUC = 0.89, LR+ 1.9 and LR− 0.1). Meanwhile, a more accurate detection of AA case can be provided by an additional ultrasound examination of abdominal organs if patients are identified according

to the criteria: Alvarado score of ≥ 7 points and IL-6 ≥ 4.3 pg/mL with 100% specificity and PPV (see Table 3.9.1).

Table 3.9.1

Changes in Alvarado score according to additional factors in AA diagnosis

Variables	Sensitivity	Specificity	PPV	NPV	Accuracy	LR+	LR−
	%, (95% CI)						
2 features: Alvarado score ≥ 7 points and additional factors							
IL-6 ≥ 4.3 pg/mL	77.2 (23.7–76.3)	66.7 (60.0–92.3)	89.5 (26.7–81.1)	44.4 (18.9–73.3)	71.0 (53.4–83.9)	2.3	0.3
US	63.6 (43.0–80.3)	66.7 (30.0–90.3)	87.5 (64.0–96.5)	33.3 (13.8–60.9)	64.3 (45.8–79.3)	1.9	0.5
WBC ≥ 10.7 × 10^3/μL	9.9 (72.2–97.5)	16.7 (3.0–56.4)	80.0 (60.9–91.1)	33.3 (6.1–79.2)	75.0 (56.6–87.3)	1.1	0.5
CRP ≥ 8.4 mg/L	54.5 (34.7–73.1)	33.3 (9.7–70.0)	75.0 (50.5–89.8)	16.7 (4.7–44.8)	50.0 (32.6–67.4)	0.8	1.4
ANC ≥ 6.7 × 10^3/μL	90.0 (59.6–98.2)	25.0 (4.6–69.9)	75.0 (45.4–88.3)	50.0 (46.8–91.1)	71.4 (45.4–88.3)	1.2	0.4
3 features: Alvarado score ≥ 7 points and IL-6 ≥ 4.3 pg/mL and additional factors							
WBC ≥ 10.7 × 10^3/ μL	94.1 (73.0–99.0)	50.0 (9.5–90.5)	94.1 (73.0–99.0)	50.0 (9.5–90.5)	89.5 (68.6–97.1)	1.9	0.1
US	64.7 (41.3-82.7)	100 (34.2-100)	100 (74.1-100)	25.0 (7.1-59.1)	68.4 (46.0-84.6)	–	0.4
CRP ≥ 8.4 mg/L	47.1 (26.2–69.0)	50.0 (9.5–90.5)	88.9 (56.5–98.0)	10.0 (1.8–40.4)	47.6 (27.3–68.3)	0.9	1.1

4 DISCUSSION

Emergent abdominal inflammatory processes, which are characterized by abdominal pain syndrome are the most important and common surgical diagnostic problems of children at EMSD and outpatient medical facilities. There are several reasons for acute abdominal pain syndrome in children. Some authors have classified these reasons into nine major groups: the first group – urgent surgical conditions in the abdominal cavity, which require urgent surgical treatment; the second group - AA and AML; the third – partial or total impenetrability of intestinal tract; the fourth group – viral and bacterial gastroenteritis; the fifth group – ulcer disease and acute gastro-duodenitis; the sixth group – hepatobiliary and pancreatic disease; the seventh group – virus disease and infectious disease, which does not affect the gastrointestinal tract; the eighth group – functional gastrointestinal disorders; and the ninth group – non-specific acute abdominal pain [*Kim et al.,* 2014]. Throughout the world, including Latvia, AA and AML are the most domineering emergent abdominal inflammatory processes in children requiring urgent diagnosis and treatment [*Brătucu et al.,* 2013; *Dinu and Moraru,* 2011; *Šurna et al.,* 2011; *Sikorska-Wisniewski et al.,* 2006]. From the 1930s, epidemiological data in the US and Europe have displayed decreasing trends in the incidence of AA; nevertheless, AA is still one of the most common reasons for surgical acute abdominal pain in these regions [*Kang et al.,* 2003]. Referring to the retrospective analysis of the epidemiological situation of AA in Latvia from 2004 to 2009, a decline by 23.4% of patients with AA in the age group from 0 to 17 years was noticed [*Šurna et al.,* 2011]. In addition, AA incidence significantly decreased by 21.3% in 15 to 17 year-old group [*Šurna et al.,* 2011]. However, our study of children aged 7 to 18 showed that on average the total incidence of AA from 2010 to 2013 decreased by 2% in Latvia. The average reduction of the incidence with AA within the analysed period (2010–2013) was 1.8 cases

per 10 000 population, but it was not statistically significant. Latvian situation can be explained not only by common AA incidence trends in the world, but also by the demographic processes in the country. According to the data of Latvian National Health Service on the resident population changes during the period from 2010 to 2013 year in the age group from 15 to 19 years, observed rapid population decrease in absolute terms (from 136.2 to 99.6 thousand) than the population in the age group 10 to 14 years (from 91.9 to 89.8 thousands) [*CSP,* 2015]. A negative net migration in Latvia was observed in the period of 2000-2009 [*Indāns,* 2010]. The following migration trend was also observed in the period from 2010 to 2013 [*CSP,* 2015]. Also, forecasts of 2025 regarding the future changes in population predict depopulation at the national level – population (1900 or 2140 thousands) in 2025. [*Eglīte,* 2003].

The reverse trend in AML cases was observed within the present study – the incidence has increased by 5.8% in comparison with AA incidence rates for children aged 7 to 18. It can probably be explained by a better accessibility of USG methods, more accurate USG examinations data interpretation and more advanced electronic systems for patient records in the country. Studies, analysing the diagnostic accuracy of AA, as the main criteria for the evaluation of the situation tend to assess the decline of undiagnosed AA, negative appendectomy and perforation of appendix during the relevant period. In this work, the analysis of AA cases revealed that 32.3% of the cases had perforated appendicitis and 3.2% of cases showed negative appendectomy. Similar results were obtained by the USG studies, where patients younger than 19 years old, being operated with suspected AA, were detected with negative appendectomy in 3.6% of cases, and 36% of cases were identified as perforated appendicitis [*Bachur et al.,* 2012].

History and data of physical examination in AA and AML cases are very similar, so it is clinically difficult to distinguish these diseases [*Abbas et al.,* 2007]. The study has found that anorexia, vomiting, passive abdominal wall

rigidity and rebound tenderness were found statistically significantly less frequent in patients with AML in comparison to the AA patients. Similar observations were also identified in a prospective cohort study in the Netherlands from 2005 to 2006, which included a total of 289 patients with acute abdominal pain. As a result of this study, AML was found in 38 patients and 69 patients had AA. Analyzing clinical parameters between groups, anorexia, vomiting, pain and migration of pain to LRQP (lower right quadrant pain) as well as rebound tenderness were observed to be statistically significantly higher in AA group than in AML group. However, analyzing the clinical parameters of the overall diagnostic value, they were considered to correspond to the method with PPV of 62% in the case of AA and 42% in the case of AML, which indicates that if only a physical examination were used for the differential diagnosis of both diseases, one would have to face a lot of undiagnosed cases of the disease [*Toorenvliet et al.*, 2011]. However, such situations would not be tolerated for children with acute abdominal pain and suspicion of AA.

There are various clinical diagnostic scores of AA in children described and analysed by different researches [*Dingemann and Ure*, 2012]. Alvarado score and Paediatric Appendicitis Score (PAS) are the most widely used diagnostic scores of AA in children with proven high level of diagnostic accuracy [*Kulik et al.,* 2013]. However, analysing the data one should be aware of the fact that these two scores are based on retrospective studies, thus diagnostic accuracy might be different if prospective study is conducted [*Pogorelić et al.,* 2015]. Alvarado and PAS scores have not been analysed or applied in Latvia until the present the study was initiated at State Ltd CCUH; however, some clinical symptoms had been retrospectively analyzed for different age groups to assess the clinical course of AA characteristics. The prospective analysis has helped to determine that the following Alvarado score criteria as anorexia, vomiting, and rebound tenderness are statistically

significantly more often dominant in AA case (in more than half of the patients) in comparison to AML patients, but the statistical significance for other Alvarado score features, such as the migration of pain to the LRQ, tenderness in the LRQ, elevated temperature ≥ 37.3° C, leukocytosis (≥ $10 \times 10^3/\mu L$) and shift of WBC to the left is not observed.

Memon *et al.* have found that the subsequent treatment of a patient is dependent on the value of Alvarado score [*Memon et al.,* 2013]. The present research has showed that Alvarado score of ≥ 7 points is one of the criteria to distinguish patients with AA and AML with 73.7% of accuracy. The available literature used by the prospective study described the importance of Alvarado score in differential diagnosis of AA and AML, presenting data analysis of 69 patients with AA and 38 with AML. The data analysis showed that Alvarado score of ≥ 7 points was evaluated with 79% accuracy of the method, 70% sensitivity, 71% specificity, 81% PPV and 56% NPV [*Toorenvliet et al.,* 2011]. Meanwhile, our study has showed that the Alvarado score of ≥ 7 points displayed similar results of sensitivity, specificity, PPV and NPV, respectively, 71%, 76.9%, 78.6% and 21.4%. Referring to the diagnostic quality of Alvarado score analysis provided by both studies, one could conclude that the scale as the only diagnostic tool would not be sufficiently precise in choosing the future tactics for patients with AA and AML. If the Alvarado score with the value of ≥ 7 points is the only method used to differentiate AA patients from AML, it can lead to the increase of false-negative cases.

The present thesis has revealed a group of factors that can help to discriminate patients with AA from AML patients much earlier and more accurately. At the same time, the following three criteria should be fullfilled: Alvarado score of ≥ 7 points, WBC ≥ $10.7 \times 10^3/\mu L$ and IL-6 with a cut-off value ≥ 4.3 pg/mL, resulting in sensitivity of 94.1%, PPV 94.1%, specificity 50%, NPV 50% and overall diagnostic accuracy of parameters should be 89.5%. It explains the fact that having such a positive test result, AA can

be diagnosed in 94.1% of the cases. Meanwhile, if such a set of criteria is not met in patients with AA, then 50% of the cases would have to be considered with false-negative results. If for AA diagnosis one would select either IL-6, or WBC or Alvarado score, then its individual diagnostic accuracy would be lower than 89.5%. The practical part of the research on assessment of laboratory parameters for cut-off values of AA and AML in differential diagnosis showed that for IL-6 with AUC of 77% cut-off value was ≥ 4.3 pg/mL, achieving 67.7% of sensitivity and 76.9% of specificity. It illustrates that IL-6 with such a cut-off value is one of the earliest diagnostic indicators to be applied in order to differentiate AA patients from AML patients. However, individual measurement of IL-6 in patients with AA and AML has a lower diagnostic accuracy of the test in comparison to the amount of diagnostic accuracy of three abovementioned criteria taken altogether. The same picture is presented by the WBC concentration in blood with cut-off value ≥ 10.7 × 10^3/μL, 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. This study showed that the accuracy of each diagnostic criterion separately was lower than the diagnostic accuracy of the total number of criteria. The present results are partly consistent with the data from another study, where the analysis of 49 children with AA and 11 children with AML, determined IL-6 cut-off value ≥ 5.4 pg/mL with the AUC value of 77%, including 73.5% of sensitivity and 69.7 % of specificity, but the WBC cut-off value ≥ 11.6 × 10^3/μL with the AUC value of 68%, 73.5% of sensitivity and 65.6% of specificity [*Groselj-Grenc et al.*, 2007].

The most important criteria to be taken into account for the accurate diagnosis of AA is Alvarado score of ≥ 7 points, WBC ≥ 10.7 × 10^3/μL and

IL-6 with the value ≥ 4.3 pg/mL. In this situation, the patient would need a consultation of a paediatric surgeon and hospitalization for the further treatment of AA. At this diagnostic phase AA can be clinically accurately distinguished from AML without the use of additional visual diagnostics to clarify the diagnosis. An algorithm that includes a combination of these criteria has been developed based on the results of the study. The algorithm also anticipates the situations where AA diagnostic accuracy can be reduced if any of these three criteria changes its position in relation to the other two criteria. According to the present research results about half of the patients are not diagnosed with AA in the situations when the criteria do not match. Additional examination of patients with AML and AA could help reduce these false-negative interpreatations of AA. Therefore, the next step in the algorithm is to choose the diagnostic method with accuracy above 70%, making it possible to differentiate AML from AA. One of such methods used in our study was ultrasonography (USG) examination of abdominal organs with a compression test, showing 71.9% of diagnostic accuracy. A similar type of study has been conducted in Iran, where 67 patients were examined with LRQP and undergone appendectomy, having identical diagnostic accuracy of 72.4% after USG examination of abdominal organs with a compression test [*Nasiri et al.,* 2012]. Meanwhile, another prospective study using USG examination had the accuracy of 92.9%, establishing strict criteria of USG [*Toprak et al.*, 2014]. The present methodology determines the following key USG criteria for AA case: the external cross-section of caecal appendix should be of ≥ 7 mm, wall thickness of appendix should be of ≥ 2 mm and appendix cannot be pressed during the compression test. However, the current study found out that USG examination accuracy was lower. Thus, it can be concluded that USG examinations of abdominal organs at the State Ltd CCUH do not produce accurate results. Our study showed that sensitivity of USG method was only 67.7%, with specificity of 76.9% and accuracy of 71.9%. United States (US)

study, which had a collective comparison of the USG examination results in children with a suspicion of AA obtained from 10 pediatric tertiary care centers, found that the sensitivity of USG method in AA cases was dependent on the availability of the examination in the 24-h period. In hospitals where USG was provided within 24 hours the sensitivity of method reached 77.7%. In those hospitals where USG examination was available only during the day, the sensitivity of USG method decreased, showing only 51.6% [*Mittal et al.*, 2013]. Analysing the situation in Latvia, one may see that not all hospitals, including the State Ltd CCUH, have enough resources to provide 24/7 high quality USG programs and examinations that would provide more accurate diagnosis of AA. The changes in USG examination results also depend on the number of radiologists involved in the patient's USG data evaluation and how solid each individual specialist's qualification and experience in the USG methodology is. This study revealed the importance of the USG as an individual examination method in AA case not only due to 70% of PPV, but also as its diagnostic precision grew in combination with Alvarado score of $\geq$ 7 points and IL-6 with a cut-off value in serum $\geq$ 4.3 pg/mL, reaching 100% PPV.

There are not many scientific studies that have analyzed AA and AML differential diagnostic peculiarities in children, mostly because AA is compared with all other abdominal diseases characterized by acute LRQP. However, it is more challenging to differentiate AA from AML than to have AA cases compared to other emergency abdominal inflammatory processes. It should be emphasized that AA and AML are diseases that clinically simulate each other and at the same time also accompany one another. Thus, AML may be present as a separate disease, and also as a secondary cause of AA case; therefore, it is difficult to distinguish them accurately with the imaging diagnostics [*Macari et al.,* 2002; *Cobben et al.,* 2000]. Creating AA forecasting and action algorithm for Latvian children, situations where AA and AML relationships

may be different at the USG examination of abdominal organs, causing difficulties to provide a differential diagnosis of the disease, were taken into account. If during USG examination it is impossible to image caecal appendix, but increased mesenteric lymph nodes are found, one cannot convincingly claim the absence of AA. Another situation might occur when caecal appendix is imaged as unchanged by USG and enlarged mesenteric lymph nodes are additionally found; anyway, even in this case one cannot exclude the possibility that the patient will not have AA. Obtained USG results may depend on the time of performance; for instance, if they are made very early – at the early stage of AA. As clinical practice offers many different variations of USG examinations in patients with a combination of three changed clinical criteria, therefore, it is important to choose additional inflammatory markers to determine AA according to the disease duration.

Analyzing SIMCG differences in patients with AA and AML, it was found that IL-6 and IL-10 concentrations were statistically significantly higher in the case of AA than in the case of AML, but the ROC analysis showed statistically significant cut-off value set only for IL-6. Having performed the correlation analysis of laboratory values for the first serum sample, it was found that IL-6 correlated with IL-10 in patients with AA, but the correlation of the aforementioned inflammatory markers was not observed in patients with AML. This study found the median of IL-6 and IL-10 serum concentration of 9.4 pg/mL and 6.1 pg/mL in AA patients, with median of 3.2 pg/mL of IL-6 and IL-10 serum concentration in AML patients. Similar data have been found in other studies, which presented cut-off value of cytokines IL-6 in serum of patients with uncomplicated and complicated AA to be median of 14.4 pg/mL to 33 pg/mL, but in patients with non-specific abdominal pain IL-6 concentration median was 3 pg/mL [*Ozguner et al.*, 2014].

Providing a more detailed analysis of differences between various laboratory parameters of uncomplicated and complicated AA cases, one can

conclude that from all of the analyzed cytokines in the first serum sample, the concentration of IL-6, IL-8 and MCP-1 was statistically significantly higher in patients with complicated AA. Patients with uncomplicated AA had IL-6 and IL-8 median serum concentration of 5.4 pg/mL and 8.0 pg/ml, but patients with complicated AA had median concentrations of 257.8 pg/mL and 36.2 pg/mL. The abovementioned results are consistent with the data for IL-6 and IL-8 concentration changes in uncomplicated and complicated AA cases provided by other studies. IL-6 and IL-8 concentration in the median of 21 pg/mL and 13.5 pg/mL in uncomplicated AA group; and respectively 122.3 pg/mL and 25.2 pg/mL in complicated AA group [*Kharbanda et al.,* 2011]. Also, the present thesis has most often correlated IL-6 to IL-8 ($r = 0.6$; $p = 0.000$) and MCP-1 ($r = 0.5$; $p = 0.002$) from SIMCG group of cytokines to analyse obtained results. One of the publications mentions that the dynamics of MCP-1 concentrations increase at the postoperative phase in patients with uncomplicated AA after laparoscopic appendectomy rather than after conventional appendectomy, explaining this fact with carbon dioxide and irritation Gr− microbial effects on the peritoneal endothelial cells that secrete MCP-1 [*Serour et al.,* 2010]. Meanwhile, the results of this work showed that in the case of MCP-1 there was observed a statistically significant difference in serum cytokine concentration-time dynamics (from one hour before surgery to the 72th hour after surgery). Our study found statistically significant MCP-1 serum concentration only in the cases of AA, but clinical observations in other studies describe MCP-1 synthesis and its increasing concentration in the abdominal fluid [*Riese et al.,* 2004]. Local MCP-1, IL-6 and IL-8 synthesis of endothelial cells in the peritoneal abdominal fluid respond to the activity of Gr- microorganisms, promoting the formation of post-operative septic complications [*Riese et al.,* 2002; *Riese et al.,* 2004]. This is the explanation to the statistically significant increase of IL-6, IL-8 and MCP-1 concentrations in complicated cases of AA in comparison to uncomplicated AA in our study.

Taking into account the discovered importance of SIMCG in the diagnosis of AA, developed forecasting and action algorithm for AA by the study, it must be taken into account that the determination of inflammatory markers to clarify AA case depends on the patient's duration of symptoms and obtained concentration cut-off values that distinguish uncomplicated AA groups of patients from complicated AA patients. In this way one can forsee AA likelihood among children who do not meet the Alvarado score of ≥ 7 points, WBC ≥ 10.7 × 10^3/μL and IL-6 ≥ 4.3 pg/mL. Not only our study, but also other scientific researches have stated that IL-8 and MCP-1 concentrations increased in early AA cases (from 0 to 12th hour of disease) and continued to grow in complicated AA cases up to 48th hour of disease [*Kharbanda et al.,* 2011]. Serum IL-8 with a cut-off value ≥ 12.3 pg/mL and MCP-1 with a cut-off value ≥ 400.2 pg/mL showed the presence of complicated AA, regardless of disease duration. In its turn, IL-6 concentration in serum from the 13th hour of the disease with a cut-off value ≥ 39.2 pg/mL and CRP with ≥ 8.4 mg/L confirmed the diagnosis of complicated AA.

The hypothesis put forward that individual SIMCG concentration changes in AA and AML in children from 7 to 18 years were significant for the application in clinical practice of EMSD in hospitals and outpatient care stages has been confirmed. The findings are supported and verified by the designed forecasting and action algorithm for patients with AA and AML. The second hypothesis put forward has been partially confirmed due to the fact that by choosing the appropriate method of examination in combination with SIMCG it is possible to obtain a higher diagnostic accuracy than just by choosing an individual inflammatory marker for differential diagnosis of AA and AML.

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 74 patients was analyzed in the study. USG examination of abdominal organs

was necessary for 57 patients, but 74 patients needed a full SIMCG analysis. One of the limiting factors for patient inclusion in the study was the lack of accessibility to the abdominal USG in the examination period from eight o'clock in the evening until eight o'clock in the morning. The second factor was related to SIMCG determination in blood samples following the study methodology and completion of patient's consent form. Since the study was designed to obtain three additional venous blood samples from peripheral vein in order to determine SIMCG at different time intervals, then in some cases parents would change their mind about the participation in the study or did not agree at the very beginning. It should be noted that it was only possible to get venous blood samples for SIMCG 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 State Ltd CCUH. 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 check-up after acute treatment period. In this study, the group of AML patients was not invited to the check-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. Taking into account the abovementioned study results, we can propose that no changes in AML group would be identified by our study either.

Although having acknowledged the abovementioned limitations of the study in relation to the amount of analysed samples according to different inclusion criteria, the present study is essential for paediatric surgery in Latvia, as SIMCG and cut-off value serum have not been previously used in combination with other examination techniques of AA to improve its diagnostic accuracy in children.

Paediatric surgeons are interested in finding optimal methods to examine children with suspicion of AA in order to reduce undiagnosed cases of AA and their complications as well as reduce the number of negative appendectomy in Latvia.

5 CONCLUSIONS

1. Within three-year period 0.6% out of total cases (n = 688) of treated patients at State Ltd CCUH were patients with AA and 0.3% of cases (n = 310) with AML. During this period the incidence of AA decreased by 2% on average, but the incidence of AML has increased on average by 5.8% in Latvia.

2. The analysis of first-time hospitalization of patients (0-18 years) with suspected AA (2010 – 2013) at State Ltd CCUH showed a mismatch of admitting and discharging diagnoses in 76.2% of cases.

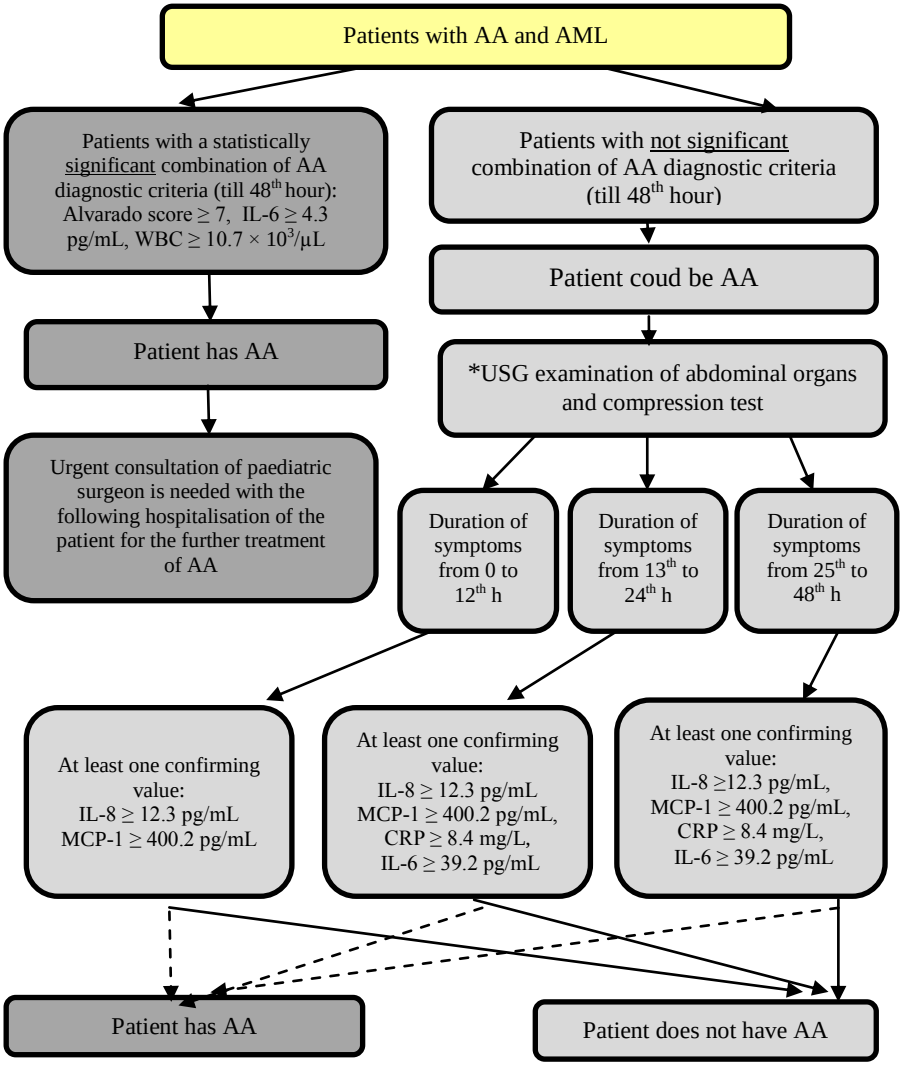
3. Alvarado score of ≥ 7 points is important in differential diagnosis of AA and AML in children aged 7 to 18 years, a diagnostic score is recommended for the application at hospital EMSD and outpatient care stage.

4. IL-6 with the cut-off value of ≥ 4.3 pg/mL is the most significant SIMCG to distinguish AA from AML. Higher concentration of SIMCG is observed in complicated AA cases in comparison to uncomplicated AA cases; thus, such SIMCG as IL-6 with the cut-off value ≥ 36.2 pg/mL, IL-8 with the cut-off value ≥ 12.3 pg/mL and MCP-1 with the cut-off value ≥ 400.2 pg/mL indicate complicated AA cases.

5. The following symptoms as anorexia, vomiting, passive abdominal wall rigidity and rebound tenderness are more often observed in children with AA than in children with AML. Patients with Alvarado score of ≥ 7 points should have the laboratory results assessed altogether, taking into account the cut-off values – WBC $\geq 10 \times 10^3/\mu L$ and IL-6 ≥ 4.3 pg/mL. Patients who have not met the three reliable diagnostic criteria combination of AA (Alvarado scale with a score of ≥ 7 points, WBC $\geq 10 \times 10^3/\mu L$ and IL-6 ≥ 4.3 pg/mL) should proceed with the USG examination of abdominal organs and a compression test.

6. AA prediction and action algorithm has been devised based on the results of the study. It has been adjusted to the conditions of Latvia with an aim to be applied at hospital emergency medical service departments and ambulatory care stage for the examination of children aged 7 – 18 with the suspicion of AA.

PRACTICAL RECOMMENDATIONS



Comments: AA – acute appendicitis; AML – acute mesenteric lymphadenitis; duration of disease – from the onset of the complaint up to the first-time examination at a medical institution; * USG examination of the abdominal cavity with the appropriate AA criteria: caecum appendix cross-section of ≥ 7 mm , wall thickness ≥ 2 mm, worm-like appendix is not compressible through the abdomen

⇢ patient has AA, in the case that at least one or more markers of inflammation is in the determined cut-off value;

⟶ a patient has no AA if no affirmative marker of inflammation in the cut-off value is determined.

REFERENCES

1. Abbas SM, Smithers T, Truter E. What clinical and laboratory parameters determine significant intra abdominal pathology for patients assessed in hospital with acute abdominal pain? World J Emerg Surg 2007;2:26.
2. Bachur RG, Hennelly K, Callahan MJ, Chen C, Monuteaux MC. Diagnostic imaging and negative appendectomy rates in children: effects of age and gender. Pediatrics 2012;129(5):877-884.
3. Brătucu E, Lazar A, Marincaş M, Daha C, Zurac S. Aseptic mesenteric lymph node abscesses. In search of an answer. A new entity? Chirurgia (Bucur) 2013;108(2):152-160.
4. Bundy DG, Byerley JS, Liles EA, Perrin EM, Katznelson J, Rice HE. Does this child have appendicitis? JAMA 2007;298(4):438-451.
5. Centrālā statistikas pārvaldes datu bāzes: Pastāvīgo iedzīvotāju skaits un vecuma struktūra gada sākumā (pa 5 gadu vecuma grupām) // http://data.csb.gov.lv/pxweb/lv/Sociala/Sociala__ikgad__iedz__iedzskaits/IS0022.px/table/tableViewLayout1/?rxid=cdcb978c-22b0-416a-aacc-aa650d3e2ce0 (sk. 15.04.2015.).
6. Centrālā statistikas pārvaldes datu bāzes: Iedzīvotāju ilgtermiņa migrācija //http://data.csb.gov.lv/pxweb/lv/Sociala/Sociala__ikgad__iedz__migr/IB0010.px/table/tableViewLayout1/?rxid=cdcb978c-22b0-416a-aacc-aa650d3e2ce0 (sk.15.04.2015.).
7. Cobben LP, Otterloo AM, Puylaert JB. Spontaneously Resolving Appendicitis: Frequency and Natural History in 60 Patients. Radiology 2000; 215(2):349–352.
8. Dingemann J, Ure B. Imaging and the use of scores for the diagnosis of appendicitis in children. Eur J Pediatr Surg 2012;22(3):195-200.
9. Dinu CA, Moraru D. The etiological aspects of acute abdominal pain in children. Rev Med Chir Soc Med Nat Iasi 2011;115(4):1018-1023.
10. Eglīte P. Demogrāfiskā situācija un attīstības prognoze. Rīgas attīstības plāna 2006.-2018. gadam apakšprojekts. – Rīga: LZA Ekonomikas institūts, 2003. - 9.-12., 115.-121. lpp.
11. Groselj-Grenc M, Repse S, Dolenc-Strazar Z, Hojker S, Derganc M. Interleukin-6 and lipopolysaccharide-binding protein in acute appendicitis in children. Scand J Clin Lab Invest 2007;67(2):197-206.
12. Groselj-Grenc M, Repse S, Vidmar D, Derganc M. Clinical and laboratory methods in diagnosis of acute appendicitis in children. Croat Med J 2007;48(3):353-361.
13. Humes DJ, Simpson J. Acute appendicitis. BMJ 2006;333:530-534.
14. Indāns I. Trešo valstu augsti kvalificēts darbaspēks. Starptautiskā pieredze un Latvijas situācija. – Rīga: Latvijas Universitāte, 2010. - 3.-7. lpp.
15. Kang JY, Hoare J, Majeed A, Williamson RC, Maxwell JD. Decline in admission rates for acute appendicitis in England. Br J Surg 2003;90(12):1586-1592.

16. Kharbanda AB, Cosme Y, Liu K, Spitalnik SL, Dayan PS. Discriminative accuracy of novel and traditional biomarkers in children with suspected appendicitis adjusted for duration of abdominal pain. Acad Emerg Med 2011;18(6):567–574.
17. Kim JH, Kang HS, Han KH, Kim SH, Shin KS, Lee MS, Jeong IH, Kim YS, Kang KS. Systemic classification for a new diagnostic approach to acute abdominal pain in children. Pediatr Gastroenterol Hepatol Nutr 2014;17(4):223-231.
18. Kulik DM, Uleryk EM, Maguire JL. Does this child have appendicitis? A systematic review of clinical prediction rules for children with acute abdominal pain. J Clin Epidemiol 2013;66(1):95-104.
19. 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 Am J Roentgenol 2002;178(4):853-858.
20. Memon ZA, Irfan S, Fatima K, Iqbal MS, Sami W. Acute appendicitis: diagnostic accuracy of Alvarado scoring system. Asian J Surg 2013;36:144-149.
21. Mittal MK, Dayan PS, Macias CG, Bachur RG, Bennett J, Dudley NC, Bajaj L, Sinclair K, Stevenson MD, Kharbanda AB. Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics. Performance of ultrasound in the diagnosis of appendicitis in children in a multicenter cohort. Acad Emerg Med 2013;20 (7):697-702.
22. Myers AL, Williams RF, Giles K, Waters TM, Eubanks JW 3rd, Hixson SD, Huang EY, Langham MR Jr, Blakely ML.Hospital cost analysis of a prospective, randomized trial of early vs interval appendectomy for perforated appendicitis in children. J Am Coll Surg 2012;214(4):427-34.
23. Nasiri S, Mohebbi F, Sodagari N, Hedayat A. Diagnostic value of ultrasound and the modified Alvarado scoring system in acute appendicitis. International Journal of Emergency Medicine 2012;5:26-30.
24. Ozguner İ, Kızılgun M, Karaman A, Cavusoğlu YH, Erdoğan D, Karaman İ, Afşarlar Ç, Yılmaz E. Are neutrophil CD64 expression and interleukin-6 early useful markers for diagnosis of acute appendicitis? Eur J Pediatr Surg 2014;24(2):179-183.
25. Pogorelić Z, Rak S, Mrklić I, Jurić I. Prospective validation of Alvarado score and Pediatric Appendicitis Score for the diagnosis of acute appendicitis in children. Pediatr Emerg Care 2015;31(3):164-168.
26. Riese J, Niedobitek G, Lisner R, Jung A, Hohenberger W, Haupt W. Expression of interleukin-6 and monocyte chemoattractant protein-1 by peritoneal sub-mesothelial cells during abdominal operations. J Pathol 2004;202(1):34-40.
27. Riese J, Schoolmann S, Denzel C, Herrmann O, Hohenberger W, Haupt W. Effect of abdominal infections on peritoneal and systemic production of interleukin 6 and monocyte chemoattractant protein-1. Shock 2002;17:361–364.
28. Serour F, Herman A, Babai I, Gorenstein A, Gershon N, Somekh E, Dalal I. Evaluation of a possible inflammatory response after appendectomy for non-perforated appendicitis in children. Eur J Pediatr Surg 2010;20(1):29–34.
29. Sikorska-Wiśniewska G, Liberek A, Góra-Gebka M, Bako W, Marek A, Szlagatys-Sidorkiewicz A, Jankowska A. Mesenteric lymphadenopathy - a valid health problem in children. Med Wieku Rozwoj 2006;10(2):453-462.

30. Šurna D, Eņģelis A, Pētersons A. Akūts apendicīts bērniem Latvijā laika posmā no 2000. līdz 2009. gadam: epidemioloģiskās situācijas izmaiņu statistiskā analīze. RSU Zinātniskie raksti. 2. sējums. Rīga:Rīgas Stradiņa universitāte, 2011. - 96.-106. lpp.
31. Toorenvliet B, Vellekoop A, Bakker R, Wiersma F, Mertens B, Merkus J, Breslau P, Hamming J. Clinical differentiation between acute appendicitis and acute mesenteric lymphadenitis in children. Eur J Pediatr Surg 2011;21(2):120-123.
32. Toprak H, Kilincaslan H, Ahmad IC, Yildiz S, Bilgin M, Sharifov R, Acar M. Integration of ultrasound findings with Alvarado score in children with suspected appendicitis. Pediatr Int 2014;56(1):95-99.

PUBLICATIONS AND THESES

Publications

1. **Zviedre A**, Engelis A, Tretjakovs P, Jurka A, Zile I, Petersons A. Role of Serum Cytokines Between Acute Appendicitis and Acute mesenteric Lymphadenitis in Children. Medicina (Kaunas), (accepted for publication 2015; 51)

2. **Zviedre A**, Engelis A, Tretjakovs P, Jurka A, Zīle I, Pētersons A. Different cytokine profiles in children with acute appendicitis and acute mesenteric lymphadenitis. Proceedings of the Latvian Academy of Sciences, Section B, (accepted for publication 2015).

3. **A. Zviedre**, A. Eņģelis, P. Tretjakovs, A. Pētersons, M. Kakars. Citokīnu grupas seruma iekaisuma mediatoru (CGSIM) dinamiskās svārstības un to nozīme bērniem ar akūtu apendicītu (AAp) un akūtu mezenteriālu limfadenītu (AMLa). RSU Zinātniskie raksti. - Rīga: RSU, 2012. - 38–44. lpp.

4. **Zviedre A**, Engelis A, Kakars M, Petersons A. Potential role of cytokines in children with acute appendicitis and acute mesenteric lymphadenitis in children. Acta Chirurgica Latviensis, 2011(11):130-133.

5. Engelis A, **Zviedre A,** Pilmane M, Petersons A. Torsion of the Diverticulum of the appendix. Acta Chirurgica Latviensis, 2011(11):178–179.

Oral Reports

1. **A. Zviedre**, A. Engelis, A. Petersons. Diagnostic values of Alvarado scoring system, ultrasound and C – reactive protein in pediatric acute appendicitis. The 5th Congress of European Academy of Pediatric Societies, Barselona, Spain, October 17–21, 2014

2. **A. Zviedre**, A. Engelis, P. Tretjakovs, M. Kakars, A. Petersons. Significance of cytokines in acute appendicitis and acute mesenteric

lymphadenitis in children. The 13th EUPSA and 59th BAPS Joint Congress, Rome, Italia, June 13–16, 2012.

Theses presented at International Conferences

1. **A. Zviedre,** A. Engelis, P. Tretjakovs, A. Vīksne, V. Titans, A. Petersons. Laboratory markers in the diagnosis of acute appendicitis in children. Abstract Book – The 16th European Congress of Paediatric Surgery. Slovenia, Ljublijana, June 17–20, 2015. – p. 101.

2. A. Vīksne, Z. Abola, A. Engelis, **A. Zviedre**, A. Petersons. Nonsurgical management of acute nonperforated appendicitis: are the selection criteria evidence based? Abstract Book – The 16th European Congress of Paediatric Surgery. Slovenia, Ljublijana, June 17–20, 2015. – p. 76.

3. **A. Zviedre**, M. Kakar, A. Engelis, A. Petersons. Alvorado scoring system, ultrasound and c-reactive protein in diagnosis of acute appendicitis. Abstract Book – The 18th European Congress of Paediatric Surgery. Dublin, Ireland, June 18–21, 2014. – p. 278.

4. **A. Zviedre**, A. Engelis, A. Petersons. Acute appendicitis and acute mesenteric lymphadenitis in children. Abstract Book – The 13th Conference of the Baltic Association of Paediatric Surgeons. Vilna, Lithuania, September 25-28, 2014. – Pp. 28–29.

5. **A. Zviedre**, A. Engelis, P. Tretjakovs, M.Kakar, A. Petersons. Serum level of cytokines allows to differentiate acute appendicitis and acute mesenteric lymphadenitis in children. Abstract Book – The 14th congress of the European Pediatric Surgeons' Association. Leipzig, Germany, June 5–8, 2013. – p. 351.

6. **A. Zviedre**, A. Engelis, P. Tretjkovs, M. Kakars, A. Petersons. Significance of cytokines in acute appendicitis and acute mesenteric

lymphadenitis in children. Abstract Book – The 13th EUPSA and 59th BAPS Joint Congress. Rome, Italy, June 13–16, 2012. – p. 267.

7. **A. Zviedre**, M. Pegasova, A. Engelis, A. Petersons. Epidemiology, Diagnosis and Tretment aspects of Acute Appendicitis (AP). Abstract Book – XI Conference of the Baltic Association of Pediatric Surgeons. Tallinn, Estonia, May 12–14, 2010. – p. 32.

Theses presented at Latvian conferences

1. **A. Zviedre**, A. Eņģelis, A. Pētersons. Citokīnu robežvērtības serumā akūta apendicīta un akūta mezenteriāla limfadenīta diagnostikā bērniem. RSU zinātniskā konference, tēzes, Rīga, 2015, 219. lpp.

2. A. Vīksne, Z. Ābola, A. Eņģelis, A. Zviedre, A. Pētersons. Nekomplicēta akūta apendicīta neķirurģiska ārstēšana bērniem. RSU zinātniskā konference, tēzes, Rīga, 2015, 254. lpp.

3. **A. Zviedre**, A. Eņģelis, A. Pētersons, Ilze Ose, Agnese Kāposte. Hospitalizācijas gadījumu raksturojums pacientiem ar akūtām aklās zarnas piedēkļa slimībām un akūtu nespecifisku mezenteriālu limfademītu VSIA Bērnu klīniskā universitātes slimnīcā (2010-2013). VSIA BKUS, RSU un LĀB rīkotā zinātniskajā konference, tēzes, Rīga, 2014, 60. lpp.

4. **A. Zviedre**, A. Eņģelis, P. Tretjakovs, A. Pētersons, M. Kakars. Citokīnu koncentrācijas izmaiņas serumā akūta apendicīta un akūta mezenteriāla limfadenīta diagnostikā bērniem. RSU zinātniskā konference, tēzes, Rīga, 2013, 232. lpp.

5. **A. Zviedre**, A. Eņģelis, A. Pētersons, M. Kakars, E. Zarembo, P. Tretjakovs. Citokīnu grupas seruma iekaisuma mediatoru dinamisko svārstību nozīme bērniem ar akūtu apendicītu un akūtu mezenteriālu limfadenītu. RSU zinātniskā konference, tēzes, Rīga, 2012, 237. lpp.

6. **A. Zviedre**, A. Eņģelis, M. Kakars, A. Pētersons. Hronisku vēdersāpju diagnostikas un ārstēšanas algoritms bērniem. RSU zinātniskā konference, tēzes, Rīga, 2011, 247. lpp.

7. **A. Zviedre**, A.Eņģelis, M. Pegasova, A. Pētersons. Akūts apendicīts: epidemioloģijas, diagnostikas un ārstēšanas datu analīze. RSU zinātniskā konference, tēzes, Rīga, RSU, 2010, 234. lpp.

Patent

Zviedre A., Eņģelis, A. Pētersons. Acute apendicitis prediction methodology for children 7 – 16 years with abdominal pain. (submitted to the Latvian Patent Board, pressmark: P–14–98; 03 Dec 2014).

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