ESF
EIROPAS SOCIĀLAIS FONDS

doi:10.25143/prom-rsu_2011-18_dts

RĪGAS STRADIŅA UNIVERSITĀTE

Jana Pavare

Epidemiology, clinical signs and early diagnosis of systemic inflammatory response syndrome (SIRS) and sepsis in hospitalized children

Summary of doctoral thesis
Speciality – pediatrics

Scientific supervisor:
Dr. habil. med., professor, Latvian Academy of Sciences cor. mem. Dace Gardovska

Riga, 2011

RĪGAS STRADIŅA UNIVERSITĀTE

Jana Pavare

EPIDEMIOLOGY, CLINICAL SIGNS AND EARLY DIAGNOSIS OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) AND SEPSIS IN HOSPITALIZED CHILDREN

Summary

PhD Thesis in Pediatrics

Scientific supervisor:

Dr. habil. med., professor,

Latvian Academy of Sciences cor. mem. Dace Gardovska

Riga, 2011

PhD Thesis has been done in the Department of Pediatrics, Riga Stradins University.

Reviewers:

Dr. habil. med., professor, Latvian Academy of Sciences cor. mem.
Ludmila Viksna (Rīgas Stradins univeristy)

Dr.med., associated professor **Uga Dumpis** (University of Latvia)

MD Ph.D. DEAA EDIC **Maris Dubniks** (Lund University Hospital, Sweden)

Financing and Support of the Research Work:

ESF
EIROPAS SOCIĀLAIS FONDS

1. ESF National Program "Support for Implementation of Doctoral Programs and Postdoctoral Research in Medical Sciences" .
2. National Research Program *"Reduction of children's mortality by improving early diagnosis, treatment results and prophylaxis of life-threatening diseases in Latvia, employing methods of modern molecular biology, cytometry and immuno-genetics"*, within the project in 2007-2009.

Presentation of PhD Thesis will be held on the May 5th, 2011 at the open session of Promotional Council in Internal Medical Sciences, Riga Stradins University, Dzirciema str. 16, Riga, Hippocrates Auditorium.

PhD Thesis are available in the library of the Riga Stradins University.

Secretary of Promotional Council:

Dr. habil. med., professor Maija Eglīte

Content

Abbreviations

BKUS	– Children Clinical university hospital
RSU	– Riga Stradins university
SIRS	– systemic inflammatory response syndrome
IL-6	– interleukin 6
CRP	– C reactive protein
PCT	– procalcitonin
LPB	– lipopolysaccharide binding protein
HMGB 1	– high mobility group box 1 protein
ISI	– Information science institute
ROC	– receiver operator characteristics
AUC	– area under the curve
IQR	– interquartile range
SD	– standard deviation
CI	– confidence interval

Importance of the problem

Sepsis in children population is one of common causes of morbidity and mortality in the world as well as in Latvia. In the largest epidemiology study in children population which was carried out in 2003, the hospital mortality among children was 10.3%. In Latvia between 1995 and 2000 in Children Clinical university hospital mortality rate in children with sepsis was 24.4%. After almost ten year period the epidemiological situation in Children in Children Clinical university hospital has not improved – a repeated epidemiological survey demonstrated 21.7% of sepsis cases being fatal, only 22.9% children with sepsis were hospitalized on their first day of illness. The prevalence of sepsis detected in this study, was very low and out of proportion – only 0.16% which is inadequate considering that hospital is the only tertiary level hospital in the country, patients' flow in the emergency and intensive care units is significant and the usage of antibacterial therapy in hospital is broad. The detected low level of sepsis prevalence in children in Children Clinical university hospital may indicate under diagnosis of sepsis, evaluation of patients is often based on physician opinion less on evidence, as well as lack of common approved hospital algorithm for sepsis patients. Also, timely diagnosis of sepsis is difficult due to prolonged identification process of microorganisms in cultures.

The International Pediatric Sepsis Consensus Conference in 2002 became a milestone in clinical recognition of sepsis in children where specific clinical definitions of systemic inflammatory response syndrome (SIRS) and sepsis in children were defined. SIRS was defined as at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count: 1) Core temperature >38.0°C or < 36.0°C; 2) Tachycardia, defined as a mean heart rate >2SD above normal for age; or for children <1yr old, bradycardia, defined as a mean heart rate <10th percentile for age; 3)Mean respiratory rate >2SD above normal for age; 4) Leukocyte count elevated or depressed for age or >10% immature neutrophils. Sepsis was defined as systemic inflammatory response syndrome (SIRS) in the presence of suspected or proven infection.

The early diagnosis of sepsis with the laboratory methods is still valid in research worldwide and an intensive investigation work is conducted both in children and adult populations.

Taking into account the children sepsis definition accepted in the International Pediatric Sepsis Consensus and perceiving the necessity for qualitatively new approach at the clinical evaluation of children with sepsis as well laboratory diagnosis with the effective use of early hours of disease, the research work was started with the aim to reduce children sepsis mortality rates.

The research work is important as sepsis is not extensively studied in children population.

Objective of the work

To investigate the epidemiology, clinical signs and early diagnostic methods of systemic inflammatory response syndrome (SIRS) and sepsis in hospitalized children.

Terms of Reference

1. To perform a systematic literature review on SIRS and sepsis epidemiology, clinical signs, diagnostic methods and prognosis in children.
2. To detect SIRS and sepsis prevalence in hospitalized children with fever.
3. To do research on clinical signs of SIRS and sepsis in hospitalized children.
4. To investigate inflammatory markers (C reactive protein, interleukin-6, procalcitonin, lipopolysaccharide-binding protein, *high mobility group box 1 protein*) for early sepsis diagnosis in children and detect the most appropriate diagnostic markers for early identification of sepsis patients.
5. To develop the action algorithm for managing children with fever in Emergency departments of hospitals and ambulatory stage.

Ideas proposed for study

1. The prevalence of systemic inflammatory response syndrome in children population with fever is high - 72±9.2%, which gives strong evidence of development of life threatening illnesses in this patients population.

2. The early diagnosis of sepsis includes the combination of clinical evaluation of systemic inflammatory response syndrome together with inflammatory markers complex, which consists of C reactive protein, interleukin-6, and lipopolysaccharide-binding protein, taking into account the *cut-off* levels detected in the study.

Scientific Novelty of the Study

1. For the first time in Latvia the prevalence of systemic inflammatory response syndrome (SIRS) and sepsis was detected for hospitalized children with fever. A diagnosis of SIRS, in accordance with the definition of systemic inflammatory response syndrome in children, was mostly confirmed by the combination of fever with respiratory rate >2 SD above the age norm.

2. Low awareness in the evaluation process of children with sepsis in the outpatient setting was detected which correlates with the delayed hospitalization of these patients.

3. Statistically significant difference between median levels of inflammatory markers CRP, IL-6 and LBP in children with different severity of infections was detected. A strong correlation was found between CRP and LBP, good correlation between IL-6 and CRP and between LBP and IL-6. No correlations were found between HMGB1 and any of the other biomarkers.

4. The *cut-off* levels of inflammatory markers LBP, IL-6 and CRP were detected to identify children with bacteremia.

Practical Value of the Study

Based on study results for SIRS and sepsis prevalence, clinical signs and investigation of inflammatory markers, the algorithm for evaluation of children with fever was conducted for out-patient and hospital stages. The algorithm consists of clinical evaluation of children assessing signs of systemic inflammatory response syndrome and sequential use of modern testing systems which will allow physicians to base their treatment decisions on evidence.

The implementation of the algorithm will improve early clinical recognition of sepsis patients and will speed up the laboratory diagnosis and monitoring and will avoid using expensive and unnecessary diagnostic methods.

Structure and Extent of the Work

The PhD Thesis is written in the Latvian language, consists of 13 chapters: Introduction, Importance of the Problem, Goal of the Work, Terms of Reference, Questions of the Study, Novelty of the Study, Literary Description, Materials and Methods, Results, Discussion, Conclusions, References and Annex. Ph Thesis consists of 150 pages, including 21 tables, 21 picture. Reading list comprises 213 references.

Publications on the Theme

There are 42 publications regarding the present PhD Thesis, 3 of which published in internationally quoted medical research editions (all registered in PubMed database), 10 in Latvian scientific editions, 1 monograph, 1 oral presentation in the international scientific congress, 4 thesis of the study are published in international scientific congresses, 12 oral presentations in the domestic scientific congresses and 11 theses in domestic scientific congresses and conferences. 1 patent is issued on scientific work.

Materials and methods

Structure of the study

Research work "Epidemiology, clinical signs and early diagnosis of systemic inflammatory response syndrome (SIRS) and sepsis in hospitalized children" began in January 2007 and consists of 3 sections: A, B, C.

Section A - a systematic literature review on SIRS and sepsis epidemiology, clinical signs, diagnostic methods and prognosis in children.

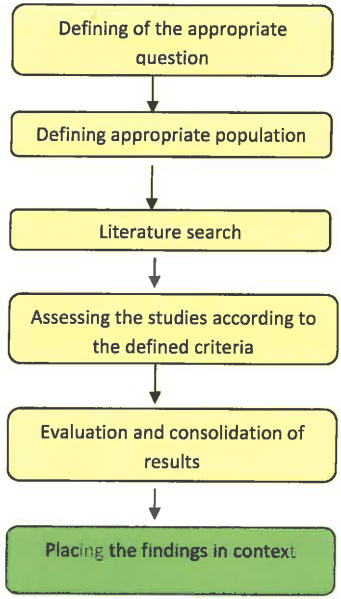
Section B - Prevalence of systemic inflammatory response syndrome (SIRS) in hospitalized children with fever: a point prevalence study.

Section C - Research on early sepsis and bacteraemia diagnostic markers in children.

Study scheme

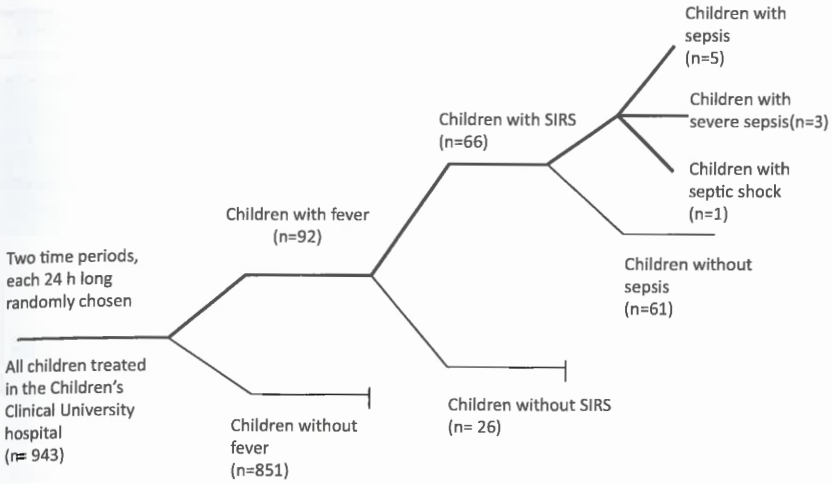
Section A - a systematic literature review on SIRS and sepsis epidemiology, clinical signs, diagnostic methods and prognosis in children.

In the systemic literature review the following methods were used to ensure the data consolidation and veracity of conclusions:

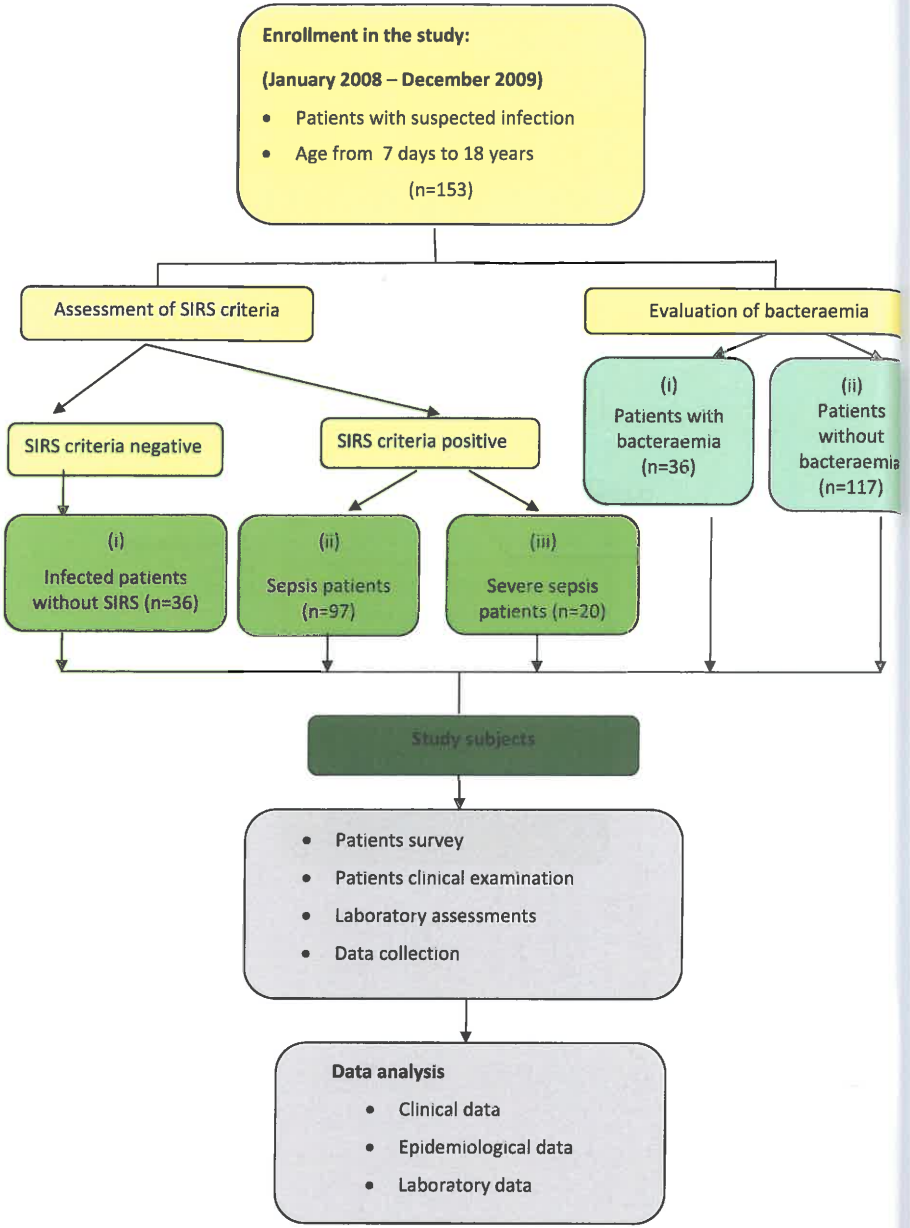


Section B - Prevalence of systemic inflammatory response syndrome (SIRS) in hospitalized children with fever: a point prevalence study.

Study design



Section C – research on early markers of sepsis and bacteraemia in children.



Materials

Study subjects

B section - Prevalence of systemic inflammatory response syndrome (SIRS) in hospitalized children with fever: a point prevalence study.

In part B two time periods, each 24 h, were randomly chosen in February 2007. All patients (n=943) treated in the hospital during these two time periods (n=456 and n=487 respectively) were screened, and all children with abnormal temperatures – temperature above 38.5 ^{0}C or below 36 ^{0}C were included.

Inclusion criteria:

1) Temperature above 38,5^{0}C
2) Temperature below 36 ^{0}C.

- All children with abnormal temperature (n=92) were included in study.
- For all children with abnormal temperatures, a questionnaire was completed recording heart rate, respiratory rate, core temperature and leukocyte count. SIRS criteria were evaluated for each child according to the criteria proposed by the Consensus conference definitions. The patients were divided into six age groups as proposed by the International consensus conference on pediatric sepsis: newborn, neonate, infant, toddler and preschool, school age, adolescent and young adult.

- Exclusion criteria:

1) At screening moment patient temperature in range from 36 ^{0}C to 38.5 ^{0}C.

C section - research on early markers of sepsis and bacteraemia in children.

- All patients with suspected or proven infection admitted to the Children's Clinical University Hospital between January 2008 and May 2009 and whose parents consented to participate were included in this study.

- Inclusion criteria:

1) Suspected or proven infection ;

2) Age from 7 days to 18 years.

Infection was defined according to International pediatric sepsis consensus conference as proven or suspected, caused by any pathogen, or clinical syndrome associated with high probability of infection.

- Overall 153 patients according to the inclusion criteria were enrolled in the study

- The questionnaire was completed and SIRS criteria were evaluated. Children with infection, but without SIRS (n=36) were classified in the separate group.

- Children with SIRS and infection according to International pediatric sepsis consensus conference were classified as sepsis patients. The second part of the questionnaire was completed and following evaluations of the severity of sepsis and organ dysfunction, children were classified in sepsis (n=97) and severe sepsis (n=20) groups.

- Patients were classified into three following groups according to severity of infection:

(I) Infected patients without SIRS (n=36)

(ii) Sepsis patients (n=97)

(iii) Severe sepsis patients (n=20).

- Age and inclusion time of the patient group "(i) patients with infection without SIRS" corresponded to the patients with SIRS from groups "(ii) patients with sepsis" and "(iii) patients with severe sepsis".

- Patients were furthermore classified according to bacteraemia into two groups:

  (i) patients with bacteremia and/or high possibility of bacteraemia (n=36)

  (ii) (ii) patients without bacteraemia (n=117).

Possible bacteraemia was defined based on the consensus definitions for bloodstream infections in children [22]. The definition includes the presence of SIRS and convincing location of bacterial infection (pulmonary infiltrates, soft tissue infections, pyelonephritis, osteomyelitis) given a negative blood culture.

- Exclusion criteria:

  1) antibacterial therapy within the last 48 h;
  2) immunodeficiency;
  3) chronic liver or kidney disease;
  4) vaccination within 5 days before the start of the illness;
  5) any chronic illness that alters levels of inflammatory markers;
  6) congenital metabolic defects;
  7) chromosomal anomalies;
  8) use of corticosteroids or immunosuppressant medications.

Methods

Section A - The preparation of a systematic literature review on SIRS and sepsis epidemiology, clinical signs, diagnostic methods and prognosis in children.

1) Definition of appropiate question

   To study the literature on SIRS and sepsis epidemiology, clinical signs, diagnostic methods and prognosis in children.

2) Defining the appropriate population:

   Initially in the literature review the studies carried out in children population were included. At the situation when studies number in children population are limited or the research is not carried out in children population at all – due to novelty – the sources of literature were expanded to adults population.

3) Definition of time period:

   In literature review the studies published from January 2000 to June 2010 were included.

4) Search of literature sources:

   Each literature source was evaluated according to the criteria for review and the full text articles were found where it was possible.

   Criteria for studies included in review:

   1. The type and *end-point* of study;
   2. The methodology of study;
   3. The size and age of population;
   4. The duration of study.

In the basic literature review the studies which were carried out to similar methodology and in children population were included.

In *PubMed* data base key words and *MeSH* terms were used.

Section B – Prevalence of systemic inflammatory response syndrome (SIRS) in hospitalized children with fever: a point prevalence study.

- The prevalence of SIRS among hospitalized children was detected with 95% confidence intervals.
- The prevalence of sepsis among children with SIRS was also determined.
- Patients' age-specific vital signs and laboratory values were determine for diagnosis of SIRS.
- Clinical and demographical data about SIRS and sepsis patients were analyzed.
- During the study period we followed the subject's disease process, analyzed the outcome of the disease and studied the final diagnosis, but we did not interfere with the treatment.

Part C - research on early markers of sepsis and bacteraemia in children.

A venous blood sample was drawn from each patient under local anesthesia induced by an EMLA patch. Blood culture was obtained from puncture of two peripheral veins.

- All analyses were performed immediately, excepted HMGB, where the samples were frozen at -80^{0}C within 30 min of sampling;
- Clinical and demographic data of the patients were assessed;
- Biochemical markers of inflammation (HMGB1, LBP, IL-6, PCT, CRP) were determined;
- Leukocyte count, CRP, PCT and IL6 levels were determined on 0th, 24th and 48th hour.
- LBP levels were determined on 0th, 24th hour.
- HMGB1 levels were determined on 24th and 48th hour.
- Blood culture was obtained on 0th hour.

Laboratory assays

HMGB1

- HMGB1 levels were measured with a commercially available enzyme-linked immunosorbent assay (HMGB1 ELISA kit; Shino-Test Corporation, Tokyo, Japan). The measuring range was 1 to 80 ng/ml, the coefficient of variation being <10%. Recovery of HMGB1 in this ELISA was 80-120%. HMGB 1 dynamic range 2.5 - 80 ng/ml.

LBP and IL-6

- IL6 and LBP were determined with a chemiluminescent immunometric assay Immulite® 2000 (Siemens Medical, Germany). The analytical sensitivity for IL6 was 2 pg/ml and 0.2 µg/ml for LBP. Manufacturer range for IL-6<10pg/ml, LBP<15 µg/ml.

PCT

- PCT levels were determined with BRAHMS PCT-Q immunochromatografic test (*Brahms - Diagnostica, Germany*). PCT reference card was used with the following ranges < 0,5 ng/ml, ≥ 0,5 ng/ml, ≥ 2 ng/ml un ≥ 10 ng/ml.

CRP

- CRP levels were measured by the latex method (COBAS INTEGRA; Roche professional Diagnostics), the lowest assay sensitivity being 0.085mg/L. CRP levels <20mg/L were accepted as normal.

Blood culture

- Blood culture was obtained from two separate vena punctures, before start of antibacterial treatment and immediately was carried to laboratory for testing in the automatic *Bactec* instrument.

All the laboratory analyses were carried out at the Children Clinical University Hospital (Latvia), except for HMGB 1 which was analyzed in the laboratory of Clinical Immunology and Immunogenetics, Riga Stradins University.

Ethical considerations

The study protocol was approved by the Central Medical Ethics Committee of Latvia. Each child's parents signed a written consent form. All patients received the standard of care according to hospital guidelines.

Statistical analyses

The data was analyzed using SPSS version 18.0 for Windows and Epi Info 2000. The results are presented as numbers (n), frequencies (%), means with respective standard deviation (SD) and as medians with their interquartile ranges (IQR). Differences in continuous variables between different groups of infections were performed using the Kruskal – Wallis test and Mann – Whitney tests as the continuous variables did not follow a normal distribution. Correlation analysis was carried out calculating the Spearman rank coefficient and the respective p-value.

To assess the performance of the selected biomarkers with respect to bacteraemia, receiver operating characteristics (ROC) curves, sensitivity and specificity values were calculated. The 95% confidence interval and p value were reported for the area under the curve (AUC) for the optimal cut-off levels. A p-value of less than 0.05 (two-tailed) was considered statistically significant for all tests.

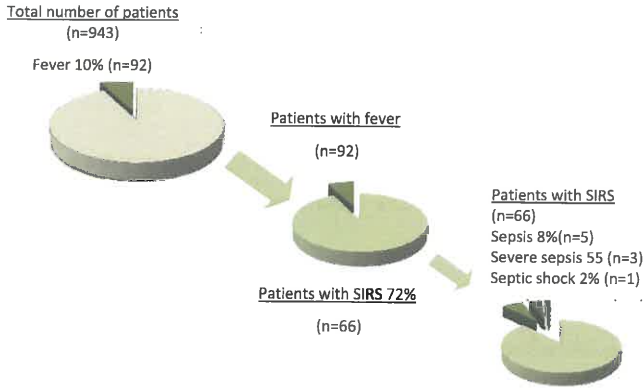
Results

Section B - Prevalence of systemic inflammatory response syndrome (SIRS) in hospitalized children with fever: a point prevalence study.

SIRS and sepsis prevalence

The study was performed in The Children's Clinical University Hospital, two time periods, each 24 h, were randomly chosen. All patients (n=943) treated in the hospital during these two time periods (n=456 and n=487 respectively) were screened, and all children with abnormal temperatures (n=92) were included in the study. All these children had fevers. No patient had hypothermia. Seventy-two percent (n=66) of the children with fever according to the definitions of International pediatric sepsis consensus conference had SIRS, 8% (n=5) of the SIRS patients in our study developed sepsis, 5% (n=3) severe sepsis and 2% (n=1) septic shock (Figure 1). The prevalence of SIRS among all the hospitalized children was 0.07±0.016, but among the children with fever it was 72±9.2%.

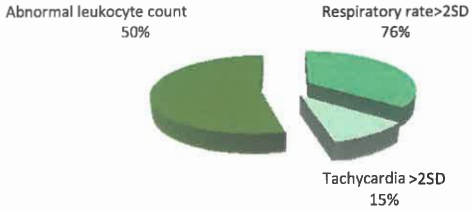
Figure 1 SIRS and sepsis prevalence in hospitalized children with abnormal temperature.



Confirmation of SIRS diagnosis

A diagnosis of SIRS, in accordance with the definition of systemic inflammatory response syndrome in children, was mostly confirmed by the combination of fever with respiratory rate >2 SD above normal for age (76%, n=50). Fever with abnormal leukocyte count was present in 50% (n=33), and fever with tachycardia >2SD above normal for age in 15% (n=10) (Figure 2).

Figure 2 Criteria confirming SIRS diagnosis (besides fever).



Clinical and demographical data of SIRS and sepsis patients

Thirty-nine percent (n=25) of the SIRS patients were in the 2-5 year age group, 25% (n=17) in the 1 month–1 year group, 21% (n=14) in the 13 to <18 year group and 15% (n=10) in the 6-12 year group. In neither time period did we identify any patients in the newborn and neonate age groups. Fifty-six percent (n=37) of the SIRS patients were treated in infectious diseases departments, 24% (n=16) in general pediatrics, 12% (n=8) in surgical and 8% (n=5) in ICU.

Sepsis, according to definition of the International consensus conference on pediatrics, was confirmed in 60% of cases by the combination of SIRS with suspected infection and in 40% of cases by the combination of SIRS with proven infection.

No gender difference was detected among the SIRS and sepsis patients (boy/girl ratio 36/30 in the SIRS group, 5/4 in the sepsis group). Statistically significant difference was not found between groups according the age of patients (77,6 months for SIRS patients and 98,6 months for sepsis patients, respectively). Mean length of hospitalization was 7,1 days for SIRS patients and 12 days for sepsis patients. Antibacterial therapy was used for 33% of SIRS patients (n=22) and 100% of sepsis patients. On average, antibacterial therapy was started on the 1,9nd day of hospitalization in SIRS patients and on the 1st day for all sepsis patients. The mean duration of antibacterial therapy was 5,6 days in SIRS patients and 10,8 days in sepsis patients which was statistically significant different. The results are presented in Table 1.

Despite the study findings that 8% of children had sepsis, 5% - severe sepsis and 2% septic shock, none of the sepsis cases were recognized by doctors and recorded on the patients' cards. Only 40% of sepsis patients were treated in Intensive care unit. Fatal cases were not established in this study.

Table 1 Clinical and demographical data of SIRS and sepsis patients.

	SIRS patients (n=66)	Sepsis patients (n=9)	p value
Boys(n)	36	5	
Girls(n)	30	4	
Age(months)	77,6±71,5[1]	98,6±69,9	0,17
Number of days of symtoms at hospital admission	3,4±2,6	2,9±1,8	0,73
Number of day of symptoms at study entry	4,4 ± 3,0	4,7 ± 2,6	0,52
Treatment time in the hospital(days)	7,1 ± 5,4	12 ± 7,5	0,01
Antibacterial therapy % (absolute value)	33% (22)	100%(9)	
Initialization day of antibacterial therapy	1,9±2,3	1±0	0,12
Duration of antibacterial therapy	5,6±3,2	10,8±5,1	0,0016

[1]mean ± standard deviation; P >0,05 not significant.

Section C - Research on early markers of sepsis and bacteraemia in children

A total of 153 patients fulfilled the entry criteria (infection) and were enrolled. Patients were classified at the time of inclusion into three following groups of severity of infection: (i) infected without SIRS (n=36), (ii) sepsis (n=97) and, (iii) severe sepsis (n=20). They were furthermore classified according to bacteraemia into (i) patients with bacteremia and/or high possibility of bacteraemia (n=36) and (ii) patients without bacteraemia (n=117).

I. Baseline and clinical characteristics of the study sample

1. Baseline and clinical characteristics of the study sample according to severity of infections

1.1. Baseline characteristics of the study sample according to severity of infections

The mean age of infected patients with SIRS was 84,6 months, 68,0 months for sepsis patients and 94,0 months for severe sepsis patients. Infected patients with SIRS were admitted to hospital at 3,8 day of their illness, sepsis patients at 3,3 of illness, but severe sepsis patients – at 2,8 day. Statistically significant difference was not found between patients groups according to the days of symptoms at hospital admission. Also, statistically significant difference was not found between patients groups according to a day of symptoms at the study entry. Statistically significant differences were detected in analysis of treatment time in hospital. Sepsis patients were treated in hospital 8,5 days which was statistically significant different ($p<0,05$) from treatment time of infected children without SIRS (mean 6.3 days). Severe sepsis patients mean treatment time in hospital was 17,1 days, which is statistically significant longer than treatment time of sepsis patients ($p<0,05$). The baseline characteristics of the study sample are presented in Table 2.

Table 2 Baseline characteristics of the study sample according to severity of infections

	Infected patients without SIRS (n=36)	Sepsis patients (n=97)	Severe sepsis patients (n=20)	p value[4]
Boys(n)	20	52	11	
Girls (n)	16	45	9	
p[1]	0,346	0,351	0,11	
p[2]		0,841	0,178	
Age (months)	84,6±76,9[1]	68,0±40,8	94,0±76,6	0,242
p[3]		0,245	0,143	
Number of days of symtoms at hospital admission	3,8±2,4	3,3±2,4	2,8±1,5	0,312
p[3]		0,320	0,379	
Number of day of symptoms at study entry	3,5 ± 3,2	4,5 ± 2,9	3,5 ± 1,7	0,033
p[3]		0,077	0,141	
Treatment time in the hospital(days)	6,3 ± 4,2	8,5 ± 5,8	17,1 ± 13,1	0,002
p[3]		0,011	0,038	

mean ± standard deviation;
p[1,2] p value calculated with *Chi square* method, *compared with the previous group,*
p[3] – T tests for two independent groups;
p[4] – all three groups are compared, ANOVA (*Analysis Of Variances*) test; p>0,05 not significant.

The severity of infections according to age-groups can be found in table 3.

Table 3 Age groups and severity of infection

Age group	Infected patients without SIRS (n=36)	Sepsis patients (n=97)	Severe sepsis patients (n=20)
0 days – 1 week	0	0	0
1 week- 1 month	0	8	2
1 month- 1 year	6	13	2
2 - 5 years	13	40	5
6 - 12 years	5	16	3
13 - 18 years	12	20	8

1.2. Clinical characteristics of the study sample according to severity of infections.

The number of infections according to age-groups and infection focus can be found in table 4. The most common infections were upper (40% (n=39)) and lower (32% (n=31)) respiratory tract infections as well as gastroenteritis (15% (n=15)). In severe sepsis patients the most common were lower respiratory tract infections 55% (n=11)and skin/soft tissue infections 55% (n=11).

Table 4 Infection focus among children without SIRS and sepsis patients

	Infected patients without SIRS (n=36)	Sepsis patients (n=97)	Severe sepsis patients (n=20)
Infection focus			
Upper respiratory tract	13	39	-
Lower respiratory tract	8	31	11
Gastroenteritis	11	15	-
Pyelonefritis	-	4	-
Skin/soft tissue	-	4	5
Osteomyelitis	-	2	2
Meningitis	-	-	2
Ocult bacteremia	-	2	-
Cistitis	4	-	-
Total	**36**	**97**	**20**

2. Baseline and clinical characteristics of children with and without bacteraemia

2.1. Baseline characteristics of children with and without bacteraemia

The mean age of children without bacteraemia was 67 month, but the mean age of children with bacteraemia 101,6 months, the statistically significant difference was not found between these groups. Children without bacteraemia were admitted to hospital on their 3,5 day of illness, but patients with bacteraemia – on 3th day of illness (p=0,271). There was a significant difference (p=0.0110) in evaluation of treatment time in hospital; patients with bacteraemia were treated longer (mean 14,6 days) than patients without bactraemia (mean 7,5 days).

Age groups of children with and without bacteremia

More frequently bacteraemia was detected in age 13 to 18 years (36% (n=13)), followed by 2 to 5 years old children – 27% (n=10).

2.2. Clinical characteristics of children with and without bacteremia

Bacteraemia was confirmed by 2 separate positive blood cultures. Gram-positive bacteria were identified in 5 patients – 3 patients had *Staphylococcus aureus*, one patient *Streptococcus agalactiae* and another one *Streptococcus pneumoniae*. Gram- negative bacteria were identified in 3 patients – two patients had *Escherichia coli*, one - *Acinobacter Baumanii*. Patients with strongly suspected bacteraemia without microbiological confirmation had pneumonia (15 children), skin/soft tissue infections (7children), pyelonephritis (4 children), osteomyelitis (2 children).

II. Characteristics of inflammatory markers in study sample

1. Characteristics of inflammatory markers in patients with different severity of infections

At the inclusion time in study the leukocyte count and inflammatory markers levels were detected in each child. Changes in leukocyte count (elevated or depressed) were defined according to International children sepsis conference. The level from which the inflammatory markers were evaluated as increased, was defined according to manufacturer rates and literature review. CRP concentration level was increased from 20 µg/ml; PCT level - above 0,5 ng/ml, IL-6 level above 10 µg/ml, LBP - above 15 µg/ml, but HMGB1 - above2 ng/ml.

Leukocyte count and inflammatory markers levels could be found in Table 5. Sepsis patients' age group and changes in leucocytes count and levels of inflammatory markers are represented in Table 6.

Table 5 Leucocytes count and levels of inflammatory markers in children with different severity of infections.

Patients group	Leukocyte count % (n)	CRP >20μg/ml % (n)	PCT >0.5ng/ml % (n)	IL6 > 10μg/ml % (n)	LBP 15μg/ml % (n)	HMGB1 >2ng/ml % (n)
Infected without SIRS (n = 36)	11,1% (n=4)	36,1% (n=13)	30,6% (n=11)	47,2% (n=17)	69,4% (n=25)	58,3% (n=21)
Sepsis (n = 97)	34% (n=33)	80,4% (n=78)	45,4% (n=44)	79,4% (n=77)	91,8% (n=89)	56,7% (n=55)
p vērtība	0,01	< 0,001	0,12	<0,001	0,001	0,87
Severe sepsis (n = 20)	60% (n=12)	100% (n=20)	90% (n=18)	100% (n=20)	100% (n=20)	50% (n=10)
p value	0,03	0,03	< 0,001	0,025	0,18	0,58

P>0,05 not significant, calculated with *Chi square* method, *compared with the previous group*

Table 6 Sepsis patients' age group and changes in leucocytes count and levels of inflammatory markers in 0 hour.

Patients group	Leukocyte count changed	CRP >20 μg/ml	PCT >0,5 ng/ml	IL6 >10μg/ml	LBP > 15 μg/ml	HMGB1 >2ng/ml
1 week- 1 month	25% (n=2)	75% (n=6)	25% (n=2)	87.5% (n=7)	100% (n=8)	62.5% (n=5)
1 month- 1 year	15,4% (n=2)	69,2% (n=9)	38,5% (n=2)	76,9% (n=10)	84,6% (n=11)	46,2% (n=6)
2 - 5 years	30% (n=12)	75% (n=30)	50% (n=2)	75% (n=30)	87.5% (n=35)	52.5% (n=21)
6 - 12 years	50% (n=8)	93,8% (n=15)	68,8% (n=2)	93,8% (n=15)	100% (n=16)	56,3% (n=9)
13 - 18 years	45% (n=9)	90% (n=18)	30% (n=6)	75% (n=15)	95% (n=19)	70% (n=14)

1.1. *High mobility group box 1* protein (HMGB1) between groups of different severity of infection

HMGB1 levels in part C were detected on 24th and 48th hour. HMGB1 levels in infected patients without SIRS did not statistically significantly differ from those with sepsis or sever sepsis. In addition there was no statistically significant difference observed in HMGB1 levels between children with sepsis and those with severe sepsis (Table 7). Median HMGB1 levels were not statistically different on study 48 h from 24 h in infected patients without SIRS and severe sepsis patients. HMGB1 levels statistically significant decreased in sepsis patients on 48th study hour compared with 24 th hour. (Table 7).

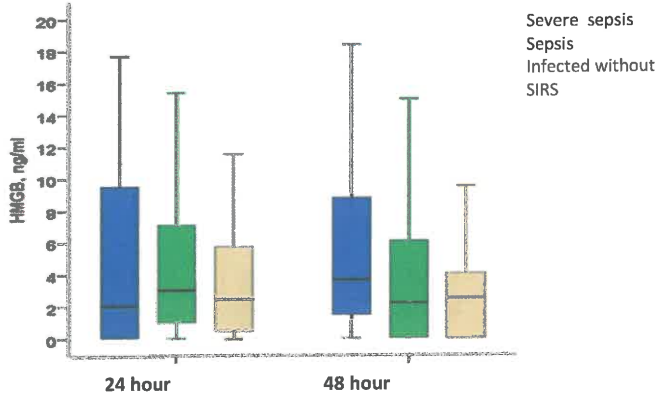
Table 7 Levels of HMGB1 in infected children without SIRS, with sepsis and with severe sepsis in 24 and 48 hour.

Inflammatory marker	Infected patients without SIRS (n=36)	Sepsis patients (n=97)	Severe sepsis patients (n=20)	p value[a]
HMGB1 (ng/ml)				
Study 24 hour				
Median	2,5	3,0	2,0	0,841
Interquartile range	0,3-5,9	0,5-7,2	0-9,9	
p value[b]		0,698	0,607	
HMGB1 (ng/ml)				
Study 48 hour				
Median	2,5	2.2	3.7	0,187
Interquartile range	0-4,3	0-6,4	1,1-9,2	
p value[b]		0,918	0,093	
p value[c]	0,082	0,005	0,372	
Decreased (n)	22	52	7	
Increased (n)	12	27	11	
Constant(n)	2	18	2	

Data presented as median and interquartile range (IQR), P>0,05 not significant. [a] Kruskal-Wallis test . [b] Mann Whitney two independent sample tests, compared with the previous group in the table.[c] Wilcoxon test, marker levels are compared in different hours of study.

Box plot of HMGB1 levels on 24 and 48 hour of study in patients with different severity of infections are represented in Figure 3.

Figure 3 HMGB1 *Box plot* according to severity of infections in different study hours.



1.2. Lipopolysaccharide-binding proteine (LBP) between groups of different severity of infection

LBP levels in part C were detected on 0^{th} and 24^{th} hour of study. LBP levels on 0^{th} hour were significantly higher among sepsis patients compared with infected children without SIRS (median 24,6 μg/ml and 14,7 μg/ml respectively, p<0,001) and were significantly higher in the severe sepsis group compared with the less severe sepsis group (90,6 μg/ml and 24,6 μg/ml respectively, p<0,001) (Table 8, Figure 4).

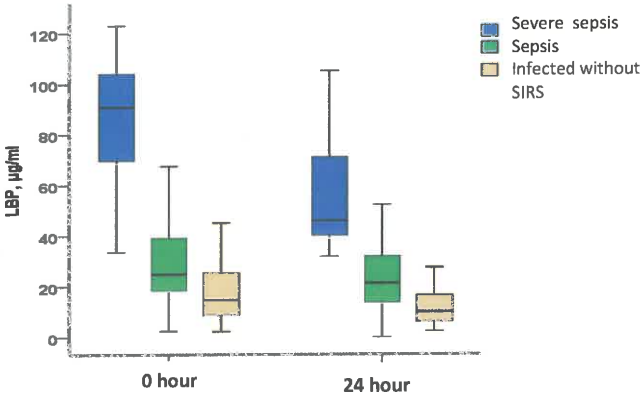
LBP levels decreased statistically significant on 24^{th} study hour compared with 0^{th} hour in all three patients groups, furthermore the most marked decrease was in severe sepsis patients (from 90,6 μg/ml on 0^{th} hour to 45,7 μg/ml on 24^{th} hour or on 49,5%). The decrease of biomarker levels was observed in 95% (n=19) of severe sepsis patients (Table 8).

Table 8 Levels of LBP in infected children without SIRS, with sepsis and with severe sepsis in 0 and 24 hour.

Inflammatory marker	Infected patients without SIRS (n=36)	Sepsis patients (n=97)	Severe sepsis patients (n=20)	p value[a]
Lipopolysaccharide-binding proteine (μg/ml), study 0 hour				
Median	14,7	24,6	90,6	
Interquartile range	8,7-26,0	17,9-39,2	68,0-106,6	<0,001
p value[b]		<0,001	<0,001	
Lipopolysaccharide-binding proteine (μg/ml), study 24 hour				
Median	9,9	21,0	45,7	
Interquartile range	5,6-17,1	13,3-31,7	39,6-74,4	<0,001
p value[b]		<0,001	<0,001	
p value[c]	0,001	<0,001	<0,001	
Decreased (n)	28	74	19	
Increased (n)	8	22	1	
Constant(n)	0	1	0	

Data presented as median and interquartile range (IQR), P >0,05 not significant. [a] Kruskal-Wallis test . [b] Mann Whitney two independent sample tests, compared with the previous group in the table.[c] Wilcoxon test, marker levels are compared in different hours of study.

Figure 4 LBP *Box plot* according to severity of infections in different study hours.



1.3. Interleukine-6 (IL-6) between groups of different severity of infection.

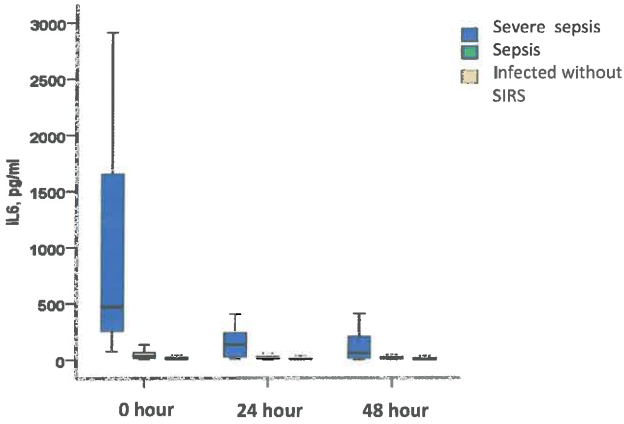
IL-6 levels in part C were detected on 0^{th}, 24^{th} and 48^{th} hour of study. IL-6 levels on 0^{th} hour were significantly higher in severe sepsis patients compared with patients in sepsis group (median 472,5 pg/ml and 32,1 pg/ml respectively, p<0,001). The statistically significant difference is detected between IL-6 levels in infected patients without SIRS and sepsis patients (median 32,1 pg/ml un 8,9 pg/ml, respectively, p<0,001)(Table 9 , Figure5). IL-6 levels significantly decreased in severe sepsis patients on 24^{th} study hour compared with 0^{th} hour (median from 472,5 pg/ml on 0^{th} hour to 136,5 pg/ml on 24^{th} hour; or on 71%) and this decrease is detected for all patients in severe sepsis group. IL-6 level significantly decreased in sepsis patients as well on 24^{th} hour (from 32,1 pg/ml to 14,3 pg/ml respectively). On 48^{th} study hour, compared with the 24^{th} hour, significant decrease of Il-6 levels is detected in all three patients' groups (Table 9, Figure5).

Table 9 Levels of IL-6 in infected children without SIRS, with sepsis and with severe sepsis in 0, 24 and 48 hour.

Inflammatory marker	Infected patients without SIRS (n=36)	Sepsis patients (n=97)	Severe sepsis patients (n=20)	p value[a]
Interleukine-6 (pg/ml)				
Study 0 hour				
Median	8,9	32,1	472,5	<0,001
Interquartile range	4,0–18,8	14,0-65,0	242,1-1738,5	
p value[b]		<0,001	<0,001	
Interleukine-6 (pg/ml)				
Study 24 hour				
Median	9,1	14,3	136,5	<0,001
Interquartile range	0,6-17,0	8,6-30,0	25,8-250,3	
p value[b]		<0,001	<0,001	
p value[c]	0,481	<0,001	<0,001	
Decreased (n)	18	71	20	
Increased (n)	13	26	0	
Constant(n)	4	0	0	
Interleukine-6 (pg/ml)				
Study 48 hour				
Median	2,9	10,9	59,7	<0,001
Interquartile range	0-13,1	4,7-21,1	12,7-214,0	
p value[b]		<0,001	<0,001	
p value[c]	0,039	<0,001	<0,001	
Decreased (n)	21	70	16	
Increased (n)	8	24	4	
Constant(n)	7	3	0	

Data presented as median and interquartile range (IQR), P>0,05 not significant. [a] Kruskal-Wallis test . [b] Mann Whitney two independent sample tests, compared with the previous group in the table.[c] Wilcoxon test, marker levels are compared in different hours of study.

Figure 5 IL-6 *Box plot* according to severity of infections in different study hours.



1.4. C-reactive protein (CRP) between groups of different severity of infection.

IL-6 levels were detected on 0^{th}, 24^{th} and 48^{th} hour of study. IL-6 levels on 0^{th} hour were significantly higher among sepsis patients compared with infected children without SIRS (median 62,7 mg/l and 12,0 mg/l respectively, p<0,001) and were significantly higher in the severe sepsis group compared with sepsis group (234,4 mg/l and 62,7 mg/l respectively, p<0,001) (Table 10, Figure 6).

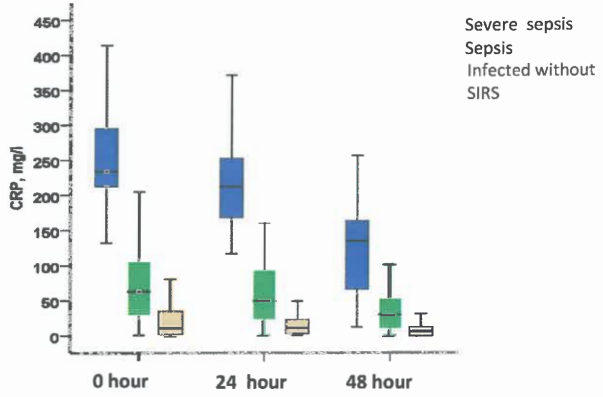
CRP levels on study 24^{th} hour statistically significant decreased compared with 0^{th} hour in all three patients groups. Compared the 48^{th} hour with the 24^{th} hour, the significant decrease of CRP levels was noticed in all three patients groups (p<0,001)(Table 10, Figure 6).

Table 10 Levels of CRP in infected children without SIRS, with sepsis and with severe sepsis in 0, 24 and 48 hour.

Inflammatory marker	Infected patients without SIRS (n=36)	Sepsis patients (n=97)	Severe sepsis patients (n=20)	p value[a]
CRP (mg/l)				
Study 0 hour				
Median	12,0	62,7	234,4	<0,001
Interquartile range	3,1-36,6	28,0-107,7	212,3-298,9	
p value[b]		<0,001	<0,001	
CRP (mg/l)				
Study 24 hour				
Median	11,0	49,3	212,3	<0,001
Interquartile range	2,8-24,5	22,7-93,8	167,5-257,4	
p value[b]		<0,001	<0,001	
p value[c]	0,015	<0,001	<0,012	
Decreased (n)	27	69	14	
Increased (n)	9	27	6	
Constant(n)	0	1	0	
CRP (mg/l)				
Study 48 hour				
Median	6,5	29,9	135,5	<0,001
Interquartile range	0,6-13,3	9,7-53,8	65,9-165,6	
p value[b]		<0,001	<0,001	
p value[c]	<0,001	<0,001	<0,001	
Decreased (n)	30	85	20	
Increased (n)	4	11	4	
Constant(n)	2	1	0	

Data presented as median and interquartile range (IQR), P >0,05 not significant. [a] Kruskal-Wallis test . [b] Mann Whitney two independent sample tests, compared with the previous group in the table.[c] Wilcoxon test, marker levels are compared in different hours of study.

Figure 6 CRP *Box plot* according to severity of infections in different study hours.



1.5. PCT levels between groups of different severity of infection.

On 0th study hour 55% (n=11) severe sepsis patients had the highest PCT concentration – above 10 ng/ml, 30% (n=6) severe sepsis patients PCT concentration was between range 0,5 - 2 ng/ml. In sepsis group most of all patients - 55% (n=53) had PCT concentration below 0,5 ng/ml. 69% (n=25) of infected patients without SIRS PCT concentration was below 0.5 ng/ml (Figure 7). On 24th study hour PCT levels were not statistically significant decrease (p=0,55) in severe sepsis patients, compared with the 0th hour (respectively 55% (n=11) patients on 0th hour and 45% patients (n=9) on 24th hour PCT concentration was above 10 ng/ml) (Table 11).

Figure 7 PCT concentration levels in infected children without SIRS, with sepsis and with severe sepsis.

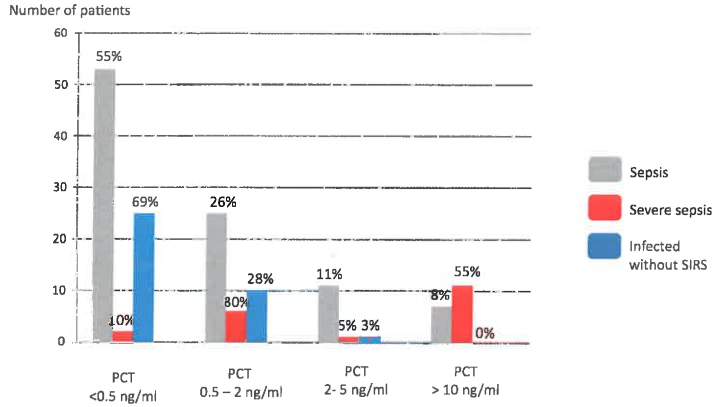


Table 11 PCT concentration levels in infected children without SIRS, with sepsis and with severe sepsis in study hour 0, 24 and 48.

Patients group/ PCT concentration		0 study hour/ number of patients % (n)	24 study hour/ number of patients % (n)	48 study hour/ number of patients % (n)
Severe sepsis	<0.5 ng/ml	10% (2)	5% (1)	20% (4)
	0.5 – 2 ng/ml	30% (6)	25% (5)	30% (6)
	2-10 ng/ml	5% (1)	25% (5)	30% (6)
	>10ng/ml	55%(11)	45%(9)	20%(4)
Sepsis	<0.5 ng/ml	55% (53)	59% (57)	76% (73)
	0.5 – 2 ng/ml	26% (25)	26% (25)	12% (12)
	2-10 ng/ml	11% (11)	8% (8)	8% (8)
	>10ng/ml	8%(8)	7%(7)	4%(4)
Infected	<0.5ng/ml	69% (25)	83% (30)	88% (32)
without SIRS	0.5 – 2 ng/ml	22% (8)	11% (4)	6% (2)
	2-10 ng/ml	3% (1)	0% (0)	6% (2)
	>10ng/ml	6%(2)	6%(2)	0%(0)

1.6. Correlation between HMGB1, LPB, IL6 and CRP in children

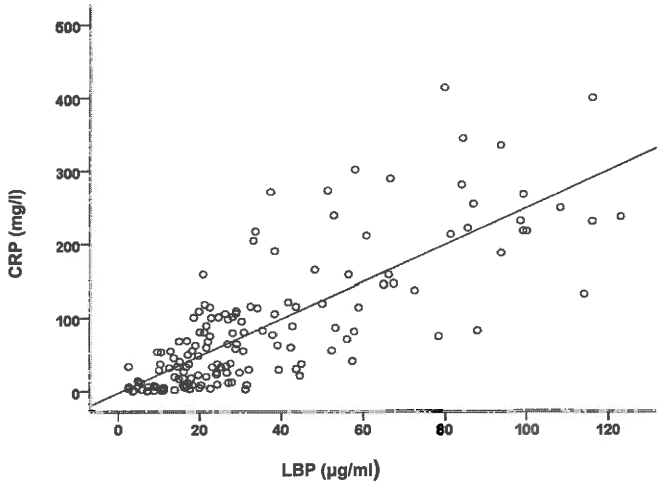
No correlations were found between HMGB1 and any of the other three biomarkers (correlation coefficient $r<0,25$, $p>0,05$) (Table 5, Figure 8). LBP correlated well with IL-6 (r=0,702, p<0,001) and correlated very strongly with CRP (r=0,783, p<0,001). In addition, a good correlation was found between IL-6 and CRP (r=0,691, p<0,001).

Table 5 Correlation between HMGB1, LPB, IL-6 un CRP in children with infections

HMGB1 versus marker	Spear men's	*P* value	LBP versus marker	Spear men's	*P* value	IL6 versus marker	Spear men's	*P* value
LBP	0,071	0,383[1]	HMGB1	0,071	0,383	HMGB1	0,033	0,685
IL6	0,033	0,685[1]	IL6	0,702	<0,001	LBP	0,702	<0,001
CRP	0,046	0,572[1]	CRP	0,783	<0,001	CRP	0,691	<0,001

[1] p>0,05 not significant

Figure 8 Correlation between LBP un CRP in study population



2. Characteristics of inflammatory markers in patients with and without bacteraemia.

2.1. Differences in levels of HMGB1, LBP, IL6 and CRP between children with and without bacteraemia

There was no statistically significant difference in HMGB1 levels between children patients with and without bacteremia (Table 13). However, LBP, IL-6 and CRP levels were statistically significantly higher in bacteremic patients compared to those without bacteraemia ($p<0.001$) (Table 13).

Table 13 Levels of HMGB1, LBP, IL-6 and CRP in children with and without bacteraemia.

Biomarker	Infection without bacteraemia (n=117)	Infection with bacteraemia (n = 36)	p-value[1]
HMGB1 (ng/ml)	2,5 (0 – 6,7)[6]	3,0 (10 –9,9)	0,66
LBP (μg/ml)	20,8 (13,2 – 31,4)	69,5 (33,2 – 97,1)	< 0,001
IL-6 (pg/ml)	18,6 (7,3 – 41,0)	244,0 (69,2 – 539,8)	< 0,001
CRP (mg/l)	34,0 (8,7 – 80,3)	217,7 (116,0 – 270,5)	< 0,001

Data presented as median and interquartile range (IQR), P >0,05 not significant.

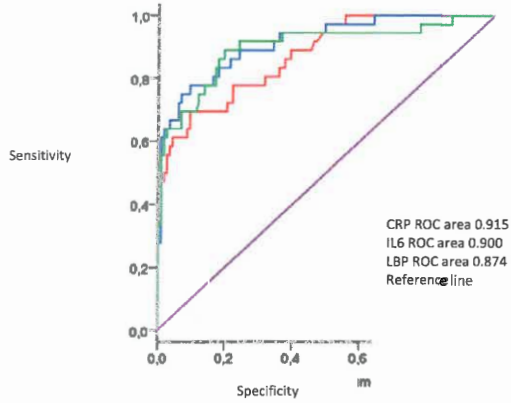
PCT levels in children with and without bacteraemia.

In patients with bacteraemia mainly the highest PCT concentration – above 10 ng/ml was observed (39%(n=14)patients), followed by PCT concentration in range from 0,5 to 2 ng/ml (33% (n=12) patients). The largest part of patients without bacteraemia - 65% (n=76), PCT concentration was below 0,5 ng/ml, 23% (n=27) patients PCT concentration was in range from 0,5 to 2 ng/ml.

2.2. Diagnostic abilities of HMGB1, LBP, IL6 and CRP in detecting children with bacteremia

In receiver operating curve (ROC) analysis for detecting bacteraemic patients, IL6 had area under the curve (AUC) of 0,900 (95% CI 0,83 – 0,97) and CRP had area under the curve (AUC) of 0,915 (95% CI 0,86 – 0,97) (Figure 1). LBP had an AUC of 0,874 (95% CI 0,81 – 0,94) (Figure 9).

Figure 9 ROC (*receiver operator characteristics*) in detecting bacteraemia in children.



The sensitivity using a cut-off level of 27,0 µg/ml (LBP), 61,4 pg/ml (IL6) and 99,5 mg/l (CRP) were 80,6 % in all three markers (Table 6). The corresponding false-positive rates were 64,1% (LBP), 82,9% (IL6) and 82,1% (CRP) for detecting children with bacteraemia (Table 14).

Table 14 Sensitivity and specificity of LBP, IL-6 and CRP according to the optimal cut-off levels in detecting children with bacteraemia.

Biomarker	Cut-off level	Sensitivity (%)	Specificity (%)
LBP	27,0 µg/ml	80,6	64,1
IL-6	61,4 pg/ml	80,6	82,9
CRP	99,5 mg/l	80,6	82,1

The results of the study demonstrate that initiation of novel and expensive investigation methods in practice could be done only after scrupulous investigations. For example the inflammatory markers HMGB1 and PCT, detected with immuno-chromotografic method, at the current evidence level are not suggested for early diagnosis of sepsis.

Considering that study was carried out in children population where till now the number of studies are limited (in *PubMed* data base, with key word *sepsis* and limit *adult* 1327 abstracts were found, but with key word *children sepsis* only 276 abstracts were found), furthermore some of inflammatory markers are not investigated in children population at all.

Based on study results the algorithm for evaluation of children with fever was conducted where besides clinical evaluation of children by signs of systemic inflammatory response syndrome sequentially the modern testing systems are used allowing to based the treatment on evidence.

The study has some limitations - difficulties to establish the control group – to receive the agreement from parents to take intravenous blood samples from healthy children was complicated; difficulties to include newborn children in study due to local health-care system (newborn treatment is mainly organized in specialized centers at maternity departments).

Conclusions

1. The performed literature review demonstrates that early clinical and laboratory diagnosis of SIRS and sepsis is still important in research context. There are 85% less studies in children population compared to adult population; the studies in children population are with small number of patients and only some markers are investigated.

2. The prevalence of SIRS in hospitalized children population was 7±1,6%, but prevalence of SIRS in children with fever was 72±9,2%, which indicates the high risk of development of life threatening diseases in this population.

3. Children with sepsis were hospitalized only on 2,9th day of illness, none of children with sepsis included in the study, were hospitalized on the 1st day of illness which demonstrates low awareness in the evaluation process of children in the outpatient setting.

4. Tachypnoe above 2SD together with fever (76, n=50) and tachycardia (15% (n=10)) were the most frequent SIRS criteria noticed in children and which should be considered when a child with an infection disease is investigated in a hospital or outpatient setting.

5. In the evaluation of children with fever and when preparing further investigations and therapy plan, the appropriate precaution level should be adjusted, SIRS criteria should be evaluated together with inflammatory marker at the following *cut-off* levels – LBP - 27,0 µg/ml, IL-6 - 61,4 pg/ml un CRP - 99,5 mg/l.

6. The levels of inflammatory markers IHMGB1 and PCT (detected with qualitative method) were not significantly different between patients groups and the inclusion of these markers in the algorithm of evaluation of children with fever are not justified.

Supplement

Algorithm for evaluation of children with fever

As a result of research an algorithm was developed. It consists of the following sections:

1) Definition of precaution level (high, medium, low);
2) Tactics in ambulatory stage;
3) Tactics in hospital;
4) Recommendations for children's care at home.

1. Definition of precaution level

The precaution level is defined by 5 criteria:

1) Systemic inflammatory response syndrome signs in children (SIRS) according to age specific vital and laboratory levels;
2) The patient's activity level;
3) Skin color and skin elements, toxic appearance of child;
4) Level of hydration ;
5) Results of laboratory investigations if performed.

The child is evaluated according to above mentioned criteria and the appropriate precaution level is determined and the further tactics is recommended. For better visualization a color scheme is designed for each level (high precaution level is red (*alarm*), low precaution level - blue (*the situation is under control*), medium level - grey (*careful follow-up is essential, situation unclear*).

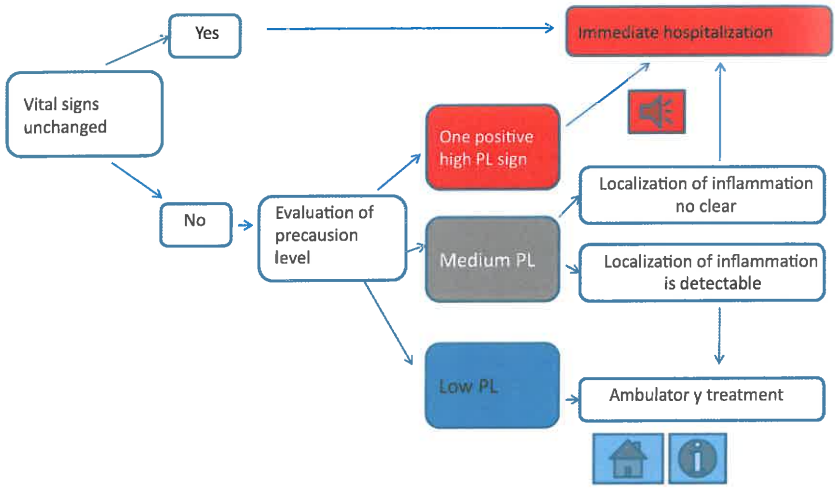
Table 1 Precautions' levels and appropriate tactic.

Precautions level (PL)	Symptoms	Tactic
High	1. SIRS + 2. Not able to stand up, groaning, monotone crying, not able to answer to questions. Disturbed cognition. Grey skin colour, cyanotic, hemoragic elements. Toxic appearance. 3. Decreased diuresis/no diuresis, reduced skin turgor. 4. CRO >100 mg/l.	If there is only one sign of five, an immediate hospitalization is required. 
Medium	1. SIRS - 2. Less active as usually, slower awakening. 3. Pale skin. 4. Decreased diuresis, reduced skin turgor. 5. CRP below 100 mg/dl.	If there is no clear source of infection the hospitalization is required.
Low	1. SIRS -. 2. Active, cheerful. 3. No changes in skin color, no any skin elements. 4. Good hydration.	Treatment at home. 

2. Tactics in ambulatory stage

1) If there is only one sign from mentioned five is expressed, immediate hospitalization is required.
2) If the clinical status corresponds to medium level, but the localization of inflammation is not clear, hospitalization is required.
3) If the clinical status corresponds to medium level, but the focus of inflammation is detectable, the child can stay at home with supervision of physician.
4) If the clinical status corresponds to medium level, the child could stay at home (Table 1).

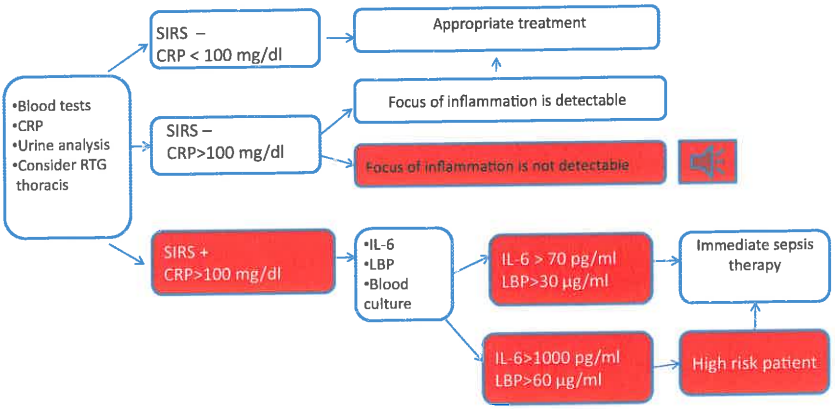
Figure 1 Tactics in ambulatory stage.



3. Tactics in hospital

1) Perform blood test, C reactive protein, urine analysis, consider chest x-ray. Evaluation of SIRS criteria.
2) If SIRS – and CRP <100 mg/dl, the appropriate treatment should be started.
3) If SIRS - , but CRP >100 mg/dl and the focus of inflammation is detectable, appropriate treatment should be started;
4) If SIRS - , CRP >100 mg/dl and the focus of inflammation is not detectable, the child should evaluate as high precaution level patient, careful monitoring is required;
4) If SIRS + or SIRS positive and CRP>100 mg/dl:
   - The detection of interleukin 6 (IL-6) un lipopysaccharide binding protein (LBP) is required;
   - Blood culture from two peripheral veins;
   - If IL-6 >70 pg/ml and LBP >30 µg/ml, essential to start immediate sepsis therapy.
   - If >1000 pg/ml and LBP >60 µg/ml, the child is evaluate as high risk patient and immediate sepsis therapy is required with monitoring of vital functions (Figure 2).

Figure 2 Tactics in hospital.



Additionally high level always corresponds to:

I. Every child below 3 months age if there is one sign of: 1) temperature above > 38 ^{0}C; 2) poor feeding; 3) ir izspīlēts lielais avotiņš; 4) hemorrhagic skin elements; 5) toxic appearance.

II. High level correspond to each child with elevated body temperature longer than 1 week and diagnosis is not clear after anamnesis and physical evaluation.

4. Recommendations for parents in home situation

1) The child must be hydrated according to physiological requirements;

2) The child must have appropriate clothes;

3) The dehydration signs must be checked;

4) The skin elements must be checked;

5) The child must be viewed at night;

7) The child can't attend school or kindergarten.

Medical help must be sought:

1) The child's status is worsening;

2) Child groans, poor contact with parents, disturbed cognition;

3) Child does not drink, reduced urination;

4) There are skin elements which do not disappear after pressing;

5) Fever is longer than 5 days;

6) Parents are worried and they evaluate their child as sick.

Publications on the study theme

International publications:

1. Jana Pavare, Ilze Grope, Imants Kalnins, Dace Gardovska: High-mobility group box-1 protein, lipopolysaccharide-binding protein, interleukin-6 and C-reactive protein in children with community acquired infections and bacteraemia – a prospective study. *BMC Infectious Diseases* 2010; 16:10-28. PMID: 20158885 [PubMed - indexed for MEDLINE].

2. Jana Pavare, Ilze Grope, Dace Gardovska: Prevalence of systemic inflammatory response syndrome (SIRS) in hospitalized children: a point prevalence study, *BMC Pediatrics* 2009; 3, 9-25. PMID: 19344519 [PubMed - indexed for MEDLINE].

3. Jana Pavare, Ilze Grope, Dace Gardovska: Diagnostic markers for sepsis identification in patients with systemic inflammatory response syndrome (SIRS): a prospective study, *Open Pediatric Medicine Journal* 2009; 3, 1-7. Open access journals (Bentham OPEN doi: 10.2174/1874309900903010001).

Publications in Latvian Council of Science editions:

1. Jana Pavare, Ilze Grope, Imants Kalnins, Dace Gardovska: Diagnostic markers for early sepsis diagnosis in children with systemic inflammatory response syndrome (SIRS). *Proceedings of the Latvian Academy of Science*, Section B, 2009; Vol. 63 No 4/5 (663/664), 197-203.

Monography

1. D. Gardovska, I. Grope, J. Pavāre, E. Miklaševičs, S. Ņikuļšins, Ž. Kovaļova, D. Grāvele, Ņ. Pugačova, L. Eihvalde, D. Zavadska, L. Čupāne, S. Polukarova, Z. Pučuka, M. Grūtupa, A. Nagle, K. Aksenoka, T. Lopatina, O. Šakele, E. Eglīte-Mahotkina, E. Hagina, A. Sočņevs, O. Rasnačs, I. Kalniņš: Bērnu mirstības samazināšana, uzlabojot dzīvībai bīstamu infekcijas slimību agrīnu diagnostiku. *Monogrāfija "Latvijas iedzīvotāju dzīvildzi un dzīves kvalitāti apdraudošās slimības, zinātniskā analīze un galvenās rekomendācijas"2009;* 95 – 108.

Publications in the Riga Stradins university Collection of Scientific papers,

Acta Chirurgica

1. Dace Gardovska, Jana Pavare, Ilze Grope, Aigars Petersons, Daila Pugacevska: A point prevalence study of systemic inflammatory response syndrome (SIRS) in different pediatric patients of children clinical university hospital in Latvia. *Acta Chirurgica Latviensis* 2007; (7) 28 – 32.

2. Jana Pavāre, Ilze Grope, Dace Gardovska, Eva Platkāja: Iekaisuma indikatori bērniem ar sistēmiskā iekaisuma atbildes sindromu (SIRS). *Riga Stradins university, Collection of Scientific Papers* 2008; 11-18.

3. Dace Gardovska, Jana Pavare, Ilze Grope: Sistēmiskā iekaisuma atbildes sindroma (SIRS) prevalence slimnīcā ārstētiem bērniem ar drudzi. *Riga Stradins university, Collection of Scientific Papers* 2007, 92- 97.

4. Zanda Pučuka, Ilze Grope, Dace Gardovska, Jana Pavāre: SIRS un sepses prevalence BKUS Intensīvajā terapijā ārstētiem bērniem. *Riga Stradins university, Collection of Scientific Papers* 2008, 67 - 74.

5. Linda Eihvalde, Jana Pavāre, Ilze Grope, Oskars Rasnačs, Dace Gardovska: Sistēmiskā iekaisuma atbildes sindroma (SIRS) un sepses prevalence BKUS neatliekamās palīdzības nodaļā. *Riga Stradins university, Collection of Scientific Papers* 2009.

6. Sergey Nikulshin, Jana Pavare, Ilze Grope, Oskars Rasnachs, Dace Gardovska: CD64 expression on neutrophil leukocytes correlates with inflammation markers in pediatric patients with SIRS. *Riga Stradins university, Collection of Scientific Papers 2009*, 19-26.

7. Marika Grūtupa, Ilze Grope, Jana Pavāre, Žanna Kovaļova, Dace Gardovska, Oskars Rasnačs: CRP, IL6, PCT un LBP diagnostiskā nozīme sepses attīstības un neitropenijas ilguma prognozēšanai pacientiem ar ļaundabīgiem audzējiem un febrilu neitropeniju, *Riga Stradins university, Collection of Scientific Papers* 2009.

8. Svetlana Polukarova, Ilze Grope, Jana Pavāre, Oskars Rasnačs, Dace Gardovska "Iekaisuma marķieru īpatnības un sepses prevalence bērniem ar sistēmiskā iekaisuma atbildes sindromu pirmajā dzīves gadā". *Riga Stradins university, Collection of Scientific Papers* 2010.

Publications in Latvia physicians professional press (Latvijas Ārsts, Doctus u.c.)

1. D. Gardovska, J. Pavāre, I.Grope: Sistēmiskā iekaisuma atbildes sindroms un sepses definīcijas bērniem. *Latvijas Ārsts*, 2007; XII, 21 – 26.

Patent

„Sistēmiskā iekaisuma atbildes sindroma (SIRS) attīstības riska prognozēšanas paņēmiens jaundzimušajiem un bērniem", Dace Gardovska (LV), Artūrs Sočņevs (LV), Jeļena Eglīte (LV), Jana Pavāre (LV), Ilze Grope (LV); Nr 14066, from 24.03.2010.

Oral lecture on the study theme in the international congresses.

1. Lecture „Inflammatory markers HMGB1, LBP, IL-6, and CRP for identifying sepsis in children", 5th International Baltic Congress of Anaesthesiology and Intensive Care, 21. -23. 10. 2010, Tartu.

Conference theses on the study theme in the international congresses.

1. Jana Pavare, Ilze Grope, Dace Gardovska: Recognition of SIRS – imperative for early sepsis diagnosis in children. *5 th Word congress of Society for Pediatric Infectious diseases* 2007; Bangkok, Thailand.

2. Jana Pavare, Ilze Grope, Dace Gardovska: Recognition of SIRS For Early Sepsis Diagnosis In Pediatric Hospital. *26th European Meeting of the European Society of Pediatric Infectious diseases* 2009; Grac, Austria.

3. Jana Pavare, Ilze Grope, Dace Gardovska: Diagnostic markers for identifying sepsis in patients with systemic inflammatory responsed syndrome (SIRS). *27th European Meeting of the European Society of Pediatric Infectious diseases* 2009; Brussels, Belgium.

4. Jana Pavāre, Ilze Grope, Dace Gardovska: High-mobility group box-1 protein, lipopolysaccharide-binding protein, interleukin-6 and c-reactive protein for identifying sepsis in children: a prospective study. *27th European Meeting of the European Society of Pediatric Infectious diseases* 2010; Nice, France.

Presentations at the local scientific conferences.

1. Jana Pavare „SIRS prevalence hospitalizētiem bērniem ar drudzi", 1th place in the RSU TIF X residents scientific conference 2007, Riga.
2. Jana Pavāre presentation „Sistēmiskā iekaisuma atbildes sindroma prevalence slimnīcā ārstētiem bērniem ar drudzi", RSU Scientific conference, 2007.
3. Jana Pavāre, Oskars Rasnačs, Arina Lazareva, Ilze Grope, Dace Gardovska presentation „Iekaisuma marķieri agrīnai sepses diagnostikai bērniem ar sistēmiskā iekaisuma atbildes sindromu (SIRS)", RSU Scientific conference, 2009.
4. Jana Pavare, Ilze Grope, Dace Gardovska presentation „Bērns ar drudzi - rīcības algoritms ambulatorā un stacionārā etapā", RSU Scientific conference, 2010.
5. Jana Pavare presentation „SIRS prevalence VSIA BKUS „Torņkalns" ārstētiem bērniem ar drudzi", Association of Latvian Children infectionists, 29.02.2008.
6. Jana Pavare presentation „Jaunās SIRS un sepses ārstēšanas vadlīnijas bērniem", Association of Latvian Neonatologists, 12.02.2009.
7. Jana Pavare presentation "Iekaisuma marķieri agrīnai sepses diagnostika bērniem", Association of Latvian laboratory specialists 03.12.2009.
8. Jana Pavāre, Ilze Grope, Dace Gardovska, presenation „Bērns ar drudzi – ārsta piesardzīga rīcība" 1 st Interdisciplinary Conference of Children Clinical university hospital, 01.10.2009
9. Jana Pavāre, Dace Gardovska lectures cycle „Sepse bērniem", postgraduates' courses of Riga Stradins university, 01.04.2009.
10. Jana Pavare, Dace Gardovska lectures cycle „Sepse bērniem. Klīniskie gadījumi", postgraduates' courses of Riga Stradins university, 03.03.2010.
11. Jana Pavāre lecture „Enterovīrusu izraisītas saslimšanas bērniem", Association of Latvian Pediatricians, 08.10.2010.
12. Jana Pavāre, Ilze Grope, Dace Gardovska lecture „Bērna ar drudzi. Rīcības algoritms ambulatorā un stacionārā etapā", Association of Latvian Pediatricians, 23.10.2010.

Conference theses on the study theme in the local congresses.

1. Jana Pavāre, Ilze Grope, Edvīns Miklaševičs, Dace Gardovska: Asins kultūru un 16S Rdna PCR efektivitātes salīdzinājums agrīnai sepses diagnostikai bērniem. RSU Scientific conference 2009.

2. Jana Pavāre, Ilze Grope, Imants Kalniņš, Dace Gardovska: Iekaisuma marķieru HMGB1, LPB, IL6 un CRP līmeņi bērniem ar bakterēmiju. RSU Scientific conference 2010.

3. Jana Pavāre, Ilze Grope, Artūrs Sočņevs, Elvīra Hagina, Imants Kalniņš, Dace Gardovska: High-mobility group box-1 protein (HMGB1) bērniem ar sadzīvē iegūtām infekcijām. RSU Scientific conference, 2010.

4. Jana Pavāre, Ilze Grope, Imants Kalniņš, Dace Gardovska: Iekaisuma marķieru optimālie *cut-off* līmeņi bakterēmijas noteikšanai bērniem. RSU Scientific conference 2010.

5. Elena Eglite, Jana Pavare, Ilze Grope, Dace Gardovska, Arthur Sochnevs: Genetic polymorphisms HLA II classes in SIRS and sepsis. RSU Scientific conference, 2009.

6. Elvira Hagina, Jana Pavare, Ilze Grope, Arturs Sochnevs, Dace Gardovska: High mobility box-1 protein (HMGB 1) in infected children. RSU Scientific conference, 2009.

7. Linda Eihvalde, Ilze Grope, Jana Pavāre, Dace Gardovska: Sistēmiskā iekaisuma atbildes sindroma (SIRS) un sepses prevalence bērniem ar drudzi Bērnu klīniskās universitātes slimnīcas (BKUS) Neatliekamās palīdzības nodaļā. RSU Scientific conference, 2009.

8. Svetlana Polukarova, Ilze Grope, Jana Pavāre, Marika Grūtupa, Anda Nagle, Dace Gardovska: SIRS un sepses prevalence stacionārā ārstētiem neonatālā vecuma pacientiem. RSU Scientific conference, 2009.

9. Marika Grūtupa, Ilze Grope, Jana Pavāre, Žanna Kovaļova, Dace Gardovska: CRP, IL6 un PCT diagnostiskā nozīme sepses attīstības un neitropenijas ilguma

prognozēšanai pacientiem ar jaundabīgiem audzējiem un febrilu neitropeniju. RSU Scientific conference, 2009.

10. Olga Šakele, Ilze Grope, Jana Pavāre, Dace Gardovska: Sepses epidemioloģiskās un klīniskās īpatnības BKUS ārstētajiem pacientiem laika periodā no 2006.01. – 2007.12. RSU Scientific conference, 2009.

11. Elena Eglite, Elvira Hagina, Jana Pavare, Ilze Grope, Dace Gardovska, Arthur Sochnevs: Polymorphisms of HLA - DRB1* locus and the associations with HMGB1 protein in children with SIRS and sepsis. RSU Scientific conference, 2009.

Aprobation of research results

Approbation of the PhD thesis took place in extended session of RSU Paediatrics Department and Department of Infectology and Dermatology, on 19th June, 2010.