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RĪGAS STRADIŅA UNIVERSITĀTE

Olafs Volrāts

MORPHOFUNCTIONAL GROUNDS OF SPLENIC TISSUE REDUCING OPERATIONS AND ANALYSIS OF CLINICAL RESULTS IN CHILDREN WITH HYPERSPLENISM OF DIFFERENT AETHIOLOGY

Summary of the Promotional Work
Specialty – Pediatric Surgery

Rīga, 2011

RIGA STRADIŅŠ UNIVERSITY

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Abbreviations	
♀	Female rat
♂	Male rat
CT angiography	Computer tomography angiography
FEGS	Fibroesophagogastroscopy
g/dL	Grams per deciliter
HS	Hereditary spherocytosis
HβD – 2	Human β defensin – 2
i/v	Intravenous
IL-10	Interleukin – 10
IL-1β	Interleukin –1β
IL-6	Interleukin – 6
IMH	Immunohistochemistry
CGR	Control group rats
KMR angiography	Magnetic resonance angiography
PHS	Portal hypertension
PSE	Partial splenic embolization
PSR	Partially splenectomized rats
PSS	Postsplenectomy sepsis
SOR	Sham operated rats
SPR	Splenectomized rats
TNF	Tumor necrosis factor
TNFα	Tumor necrosis factor α
u/L	Mikroliter
UCH	University Children's Hospital
US	Ultrasonoscopy
μmol/L	Mikromol

INTRODUCTION

Despite the risk of postsplenectomy sepsis (PSS) in children, the only known method of treatment of hypersplenism in current clinical practice remains surgical reduction of splenic tissues (partial resections of splenic tissue, splenectomy). The younger the child, the greater risk to acquire PSS (1,45 to 24,8%). Mortality in case of PSS can be as high as 50% *(Price et al., 2007)*. According to available publications it is clear that the studies concluded in the world cannot give a full explanation about the rapid and, frequently fatal course of PSS. All of the above mentioned shows that spleen tissue has special function in the immunological processes of the body.

The necessary amount of splenic tissue that needs to be saved during an operation to simultaneously perform correction of hypersplenism and prophylaxis of PSS is not known. Thus more detailed studies are necessary to reveal the details of the significance of splenic tissue in the immune function of the body.

In Latvia partial splenic embolization (PSE) had been used for treatment of portal hypertension (PHS) and hereditary spherocytosis (HS) since 2000. The aim of the procedure is a simultaneous correction of hypersplenism and the optimal prophylaxis of PSS. It is not known whether the remaining splenic tissue amount after PSE will be sufficient to protect the patient from PSS. Thus experimental studies on laboratory animals are needed.

The treatment of portal hypertension syndrome in children is complex, and in most cases the cause of the illness is unknown, the treatment then is directed towards minimizing the consequences caused by the illness. Due to endoscopic sclerotherapy procedures of esophagus and stomach the tactics of treatment the patients with PHS had radically changed, thus reducing the number of life threatening cases of bleeding For more precise evaluation of these results about treatment the PHS's patients, more detailed studies are needed.

The study performed in the promotional paper will give deeper knowledge, and it will allow forming practical recommendations and inventing them in the practice of pediatric surgery.

AIM OF THE THESIS

The aim of the study was to evaluate the impact of splenic tissue reducing operations in development of sepsis in experimental animals, and to analyze the treatment results in children with hypersplenism of various etiologies to formulate practical recommendation, and implement them in the practice of pediatric surgery.

OBJECTIVES

1. To evaluate the techniques of PSE and the results of treatment in patients of PHS and HS with hypersplenism treated in UCH from the January 1st 2000 to the July 31st 2010.
2. To analyze the results of endoscopic treatment of patients with PHS in UCH in the time period from the January 1st 1998 to January 1st 2008.
3. To elaborate a method of general anesthesia in experimental animals (rats of *Wistar* population) for performing the operation of splenic reduction.
4. To perform splenectomy, splenic resection and laparotomy without reducing the amount of splenic tissue in experimental animals.
5. To cause sepsis of *Streptococcus pneumoniae* in experimental animals.
6. To determine survival and mortality rates in various groups of experimental animals, perform the statistical analysis of the data depending on the reducted splenic tissue amount, and compare data in different groups.
7. Determine the relative amounts of immunohistochemistry markers (IL-10, TNFα, HβD – 2, cell apoptosis) in the parenchymatous organs (spleen, lungs, liver and kidneys) of experimental animals, comparing the results in animals, that had undergone splenectomy, partial splenic resection, and the control group rats.
8. Based on the results of experimental and clinical studies, elaborate treatment guidelines:
   - in PHS patients with esophageal and stomach varicosis;
   - in PHS patients with hypersplenism.

PRESENTED IDEAS

1. PSE in the amount of 80 to 90% is effective in treatment of hypersplenism in children with PHS and HS.
2. The level of hypersplenism and PSE does not determine the severity of post-embolization syndrome.
3. In rats of SPR group with provoked sepsis of *Streptococcus pneumoniae* relative amounts of IL-10 in livers, lungs and kidneys is higher than in laboratory animals of PSR, SOR and CGR groups.

PERSONAL CONTRIBUTION

The author has treated the patients, enrolled in the study, and performed PSE. Also he has performed experimental operations in laboratory animals, injected *Streptococcus pneumoniae* in the tail vein, and also morphologically evaluated the preparations. Help of a certified laboratory specialist was used to prepare the specimens of parenchymatous organs for morphological examination.

SCIENTIFIC NOVELTY OF THE PROMOTIONAL WORK

1. The method of PSE has been introduced in treatment of hypersplenism in pediatric patients with PHS and HS, also the treatment results have been evaluated for the period of 9 years and 7 months.
2. The results of endoscopic sclerotherapy for treating varicose veins of stomach and esophagus have been evaluated for a time period of 10 years.
3. The data about quality of life in pediatric patients of PHS and HS with hypersplenism in Latvia after PSE have been collected.
4. For the first time in an experiment after splenic tissue reduction operation material of young rats of *Wistar* population after sepsis of *Streptococcus pneumoniae* has been analyzed, and also an original method of anesthesia has been developed.
5. Suvival was detected in laboratory rats of *Wistar* population in a situation of artificially created sepsis of *Streptococcus pneumoniae* after splenic tissue reduction operation.
6. For the first time complex analysis of IL-10, TNFα, HβD – 2 and relative amounts of cell apoptosis have been evaluated in the parenchymatous organs of rats of *Wistar* population in case of sepsis caused by *Streptococcus pneumoniae*.

ETHICAL CONSIDERATIONS

The study project had been accepted in Development Society's Clinical Study Committee of Ethics of Pauls Stradins Clinical University Hospital (Nr. 100609-14L; 10.06.2009) and the Food and Veterinary Service of the Republic of Latvia (Nr. 21-1-13/2099; 08.12.2006).

FINANSIAL SUPPORT

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ESF project „Support for doctorates in mastering the study program and acquiring the scientific degree", RSU, contract No2009/0147/1DP/1.1.2.1.2./09/IPIA/VIAA/009.

STRUCTURE AND AMOUNT OF THE PROMOTIONAL WORK

The doctorate thesis has been written in Latvian. The study consists of 23 chapters: abbreviations, introduction, aim of the thesis, objectives of the thesis, presented ideas, summary of literature, materials and methods, results, discussion, conclusions, clinical recommendations, algorithm for treatment and follow-up of the

patients, practical value of the thesis, scientific novelty, perspectives of the thesis, annotation, list of original publications, list of presentations connected with the thesis, acknowledgements, references and appendices. The total amount of the thesis is 254 pages, including 54 tables and 103 pictures. There are 330 references included in the thesis.

MATERIALS AND METHODS

MATERIALS AND METHODS OF THE CLINICAL PART

MATERIAL FOR EXAMINATION

40 patients of PHS and 11 patients with HS were included in the study.

There were total 580 FEGS performed in 40 patients with PHS in UCH from 1998 to 2008.

In 26 patients (15 with PHS and 11 with HS) 30 PSE were performed in total. Patients who had been performed PSE from 2000 to 2009 were included in the study, but the results of treatment were analyzed in the time period from January 1st, 2000 to July 31st, 2010. Parents of 23 patients were asked to fill in a questionnaire about the quality of life, 14 of those where PHS patients, and 9 HS patients, respectively. The data, necessary for the study were obtained from the medical documentation archive, using the case records of 351 patients.

Patients of PHS

In this study the patients were divided into 3 groups:

Group 1 consisted of 15 patients with PHS (♀ : ♂, 6 : 9) aged from 6 to 16 years (mean 10,5 years) with varices of esophagus and stomach, 18 PSE were performed for treatment of hypersplenism; Group 2 was formed of 21 patients with PHS (♀ : ♂ = 10 : 11) aged 1 to 15 years (mean 7 years) with varices of esophagus and stomach, which were treated with splenectomy; Group 3 had 4 patients with PHS (♀ : ♂ = 1 : 3) aged 2 to 16 years (mean 10 years) with varices of esophagus and stomach, whose hypersplenism had not been corrected.

Depending on the amount of PSE, the patients with PHS hypersplenism were divided in 3 groups: Group 1 – PSE performed in amount of 20 – 35%, in 5 patients aged 6 to 11 years (♀ : ♂ = 2 : 3); group 2 had PSE performed in amount of 60 – 80%, in 10 patients aged 8 to 16 years (♀ : ♂ = 5 : 5). 3 patients (No. 29, 39, 37) PSE were performed repeatedly (PSE for the first time was performed in amount of 20 – 35%); Group 3 consisted of 3 patients aged 6 to 13 years (♀ : ♂ = 1 : 2), whom PSE was performed in amount of 80 – 90%.

Patients with HS

Data about patients with HS, whose hypersplenism was treated with PSE from year 2000 to 2008 were analyzed. In 11 patients with HS hypersplenism 12 PSE had been performed. Patients (♀ : ♂, 7 : 4) were aged 4 to 17 years (mean age – 10,5 years).

HS hypersplenism patients were divided in 2 groups: group 1 had PSE performed in amount of 60 – 80%, those were 9 patients aged 5 to 18 years (♀ : ♂ = 6 : 3); group 2 had PSE performed in amount of 80 – 95%, those were 2 patients, 6 and 8 years old (♀ : ♂ = 1 : 1).

METHODS

Fibroesophagogastroscopy (FEGS)

FEGS was used for diagnosis and treatment of varicose veins of esophagus and stomach in acute and non-acute patients with PHS.

In treatment of varicose veins of esophagus and stomach 1% etoxysclerol solution was used; it was injected para- and intra-varicously. The administered dose of this preparation was adjusted individually, taking into account the size of the varices of esophagus and stomach, not exceeding the maximal dose 2 mg/kg.

Evaluation of signs of hypersplenism

Evaluation of signs of hypersplenism before PSE

In evaluations of signs of hypersplenism in patients with PHS the following inclusion criteria were used: 1. changes in platelet count below 150 000 uL in peripheral blood; 2. changes of leukocyte count below 3 000 uL in peripheral blood; 3. spleen dimensions – due to lack of experience with PSE and possible risks of complications, this procedure was performed in patients in who the longitudinal dimension of spleen in ultrasound (US) was not greater than 18,5 cm. The dimensions of spleen were determined by US specialist, measuring the distance between upper and lower pole of the spleen *(Rosenberg et al., 1991, Sivit et al., 2002)*.

The indications for treatment of hypersplenism in HS patients were determined by a hematologist.

For the basis of evaluating the total level of bilirubin the guidelines for treatment of HS patients, issued in year 2004 and 2008 were used; they state the indications for splenectomy and partial resection of splenic tissue, taking into account the severity of the disease *(Bolton-Maggs et al., 2004, Guitton et al., 2008)*. The patients included in the data used for the thesis, PSE was performed instead of splenectomy or partial splenic resection. The higher level of bilirubin, when there is no need in operation to reduce the splenic tissue, was set at 17 μmol/L; light form of HS – level of bilirubin from 17 to 34 μmol/L – operation for reducing splenic tissue are not performed; median severity of HS – level of bilirubin - 34 μmol/L to 51 μmol/L – the operation for reducing the splenic tissue is performed in school-aged children before puberty; severe form of HS – level of bilirubin is higher than 51 μmol/L – the reduction of splenic tissue is necessary after 6 years of age (if the general condition of the patients allows waiting that long).

Preparation of patients for PSE procedure

All patients (PHS, HS) received prophylactic vaccination 1 month before PSE. For *Streptococcus pneumoniae* prophylaxis PNEUMO 23 vaccine was used; for *Neisseria meningitidis* prophylaxis – meningococcal vaccine "A+C".

Procedure of PSE

After preparing the operation field, *a. femoralis* (by Seldinger) was punctured, through cannula in a control of digital angiography the catheter was inserted into *aorta abdominalis, a. lienalis*. Through the catheter the *a. lienalis* branches were filled with contrast-medium, and the anatomy of the spleen was evaluated. With help of microcatheter *(Microferret®-18 Zeta Infusion Catheter AQ® Hydrophilic Coating)* 25 patients were had the artery of the inferior pole of the spleen selectively catheterized, by introducing 300 – 500 μm polyvynilalcohol microspheres *(Contour® PVA Embolizalion Particles)*. After filling the lower branch, the middle branch of *a. lienalis* was catheterized, and it was filled with microspheres. In one case (patient No 11) during PSE the upper branch of *a. lienalis* was catheterized, and then the middle branch, which both were filled with microspheres. In all cases it was strictly monitored, and the reflux of microspheres in other branches of *a. lienalis* was prevented. The blood flow through the splenic tissue was reduced in the amount of 20% to 95%.

Pain relief therapy after PSE

For pain relief after PSE solution of Fentanyl was used, with starting dose 2 mcg/kg/hour, later that was reduced individually in every patient, by assessing the intensity of pain. The medication was administered intravenously (i/v), using infusion pump, continuously 24 hours. Additionally for pain relief sodium diclofenac was used (2-3 mg/kg/day), that was administered as i/v injection, dividing it in 2 to 4 injections per day. The pain relief therapy was administered by World Health Organization (WHO) suggested recommendation for analgesic therapy *(Twycross et al., 2009)*.

Evaluation of the results after PSE

All patients (PHS, HS) after PSE were evaluated for: duration of analgesic therapy (days), duration of hyperthermia (above 37,4°C), duration of hospitalization after PSE, complications and their causes.

In patients with PHS platelet and leukocyte count in peripheral blood was analyzed before PSE, 2 to 8 days, and 1 to 8 years after PSE. Also, recidives of hypersplenism after PSE, and insufficient efficacy of PSE in treatment of hypersplenism was evaluated. Recidive of hypersplenism was defined as elevation of platelets above 150 000 uL, elevation of leukocyte count above 3 000 uL with following decrease in platelet and leukocyte count below normal in any time interval after PSE. Insufficient effect of PSE was defined by platelet count not elevating to 150 000 uL, and leukocyte count not elevating up to 3 000 uL.

Analgesic therapy and duration of hyperthermia was evaluated according to longitudinal dimension of the spleen.

In patients with HS additionally levels of total bilirubin were analyzed before PSE, 2 to 8 days, and 1 to 8 years after PSE. The results of treatment were evaluated by a hematologist. The patients were also evaluated for the presence of hemolytic crisis (yes/no) which would require transfusion of erythrocytes. Evaluating the form of HS (light, medium, severe) in these patients, the latest levels of total bilirubin were taken into account.

Evaluation of the patients' quality of life

For assessing the quality of life of these patients, a questionnaire for children's parents were developed, that was approved in Development Society's Clinical Study Committee of Ethics of Pauls Stradins Clinical University Hospital (confirmation No 100609-14L). The questionary was filled in 2010. The questionarie was filled in for patients with PHS and HS, who had been performed PSE. Answers to questions from 1 to 10 provided with infromation about age, weight, height, frequency of visits at their general practitioners, frequently used medications. Answers to questions 11 to 19 provided with information about the quality of life of PHS and HS patients.

The questions for the parents of the patients with PHS were about vomiting with coffee-grounds like appearance, melaena, fatigue, heartburn, bitter taste in mouth, discomfort behind the breastbone, difficulties to swallow, pain in time of swallowing, fatigue, pain in the left side of epigastria, fatigue that developed after PSE.

The questions for parents of the patients with HS were about pain in the left epigastria region, fatigue that appeared after PSE.

MATERIALS AND METHODS OF THE EXPERIMENTAL PART

EXAMINED MATERIAL

For examining the role of the spleen in cases of *Streptococcus pneumoniae* sepsis, laboratory animals were performed splenic tissue resection operations.

Laboratory animals

We used 3 – 4 months old *Wistar* population rats, whose weight was 100 g.

The animals were divided in 4 groups, 10 animals each: rats of group 1 were performed splenectomy (♀:♂ = 5:5) (SPR); rats of group 2 – partial splenectomy, saving 1/3 of the splenic tissue (♀:♂ = 5:5) (PSR); group 3 were performed sham operations (opening and closure of abdominal cavity) (♀:♂ = 5:5) (SOR); group 4 was a control group (CGR).

Development of general anaesthesia method for laboratory animals – rats

Starting up the experimental part, high mortality of laboratory animals was seen after operation (10% ketamine and xylasine was used for anesthesia). It was necessary to develop another method of anesthesia.

Animals were divided in 2 groups. 14 animals of group 1 were administered 10% ketamine solution 0.015ml / 100g and xylasine solution 0.015ml / 100g intramuscularly, in the muscle of a lower leg. 30 animals of group 2 were administered 10% ketamine solution 0.015ml / 100g and domitore solution 0.015-0.02ml / 100g, and after operation antisedane solution 0.015-0.02ml / 100g was administered intramuscularly. All medications were administered undiluted.

10 rats of group 1 did not wake up from anesthesia, 4 animals survived, their waking-up period was from 50 minutes to 1 hour and 20 minutes, animals started eating after mean period of 24 hours.

All rats of group 2 were awake after anesthesia 3 – 7 minutes after intramuscular administration of antisedane solution, and animals started eating after mean period of 4 – 5 hours.

The above mentioned confirmed that young Wistar population rats, weighting 100 g does not tolerate the traditional method of anesthesia with ketamine and xylasine used in small animals in veterinary practice in Latvia.

Summary of operations performed on the animals of experimental study

Anesthesia

Ketamine 10% (0.015ml / 100g) and domitore (0.015-0.02ml / 100g) solutions were administered intramuscularly (before operation). After the operation antisedane solution 0.015-0.02ml / 100g was administered intramuscularly.

Techniques of operations

For investigating the anatomical properties of splenic tissue the following references were used: Braithwaite et al., (1957) and Kaufman et al., (2003).

After shaving the skin of the frontal abdominal wall, the operational field was covered with betadine solution. The region of the incision was lined with sterile linens.

During splenectomy the abdominal cavity was opened with oblique incision in the left epigastria region, parallel to rib cage. Spleen was located out of abdominal cavity. Splenectomy was performed with electrocoagulation, by cutting off the blood vessels, running to the spleen: *a. et v. lienalis, a. at v.gastricae breves.* Abdominal cavity was then closed layer by layer. In case of partial splenectomy the operation tactics was similar, only 1/3 of splenic tissue was saved from the upper pole of the spleen, together with *a. et v. lienalis, a. at v. gastricae breves*. Other blood vessels, coming to the spleen were coagulated. For splenic tissue resection also electrocoagulation was used (in region of demarcation line). The resected part of spleen weighted 0.40 ± 0.07 grams (the scales used were ВЛКП– 500 – M, V – 50 mg), which was approximately 1/3 of tissue mass of a healthy spleen (Fig. 1).

Sham operation was performed by opening abdominal cavity in the left epigastria region, parallel to the rib cage, and then closing it.

The blood loss during operation in all rats were compensated with 5% glucose solution (2,5ml /100g) that was administered subcutaneously in the region of the neck.

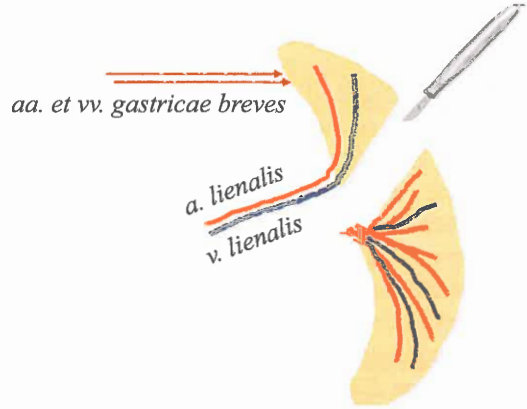

Fig. 1. **Sheme of partial splenic resection**

Induction of sepsis of *Streptococcus pneumonae.*

For inducing sepsis in experimental rats *Streptococcus pneumoniae* was chosen, as, according to different references, *Streptococcus pneumoniae* is responsible for PSS in 50% of cases *(Livingston et al., 1983, Offenbartl et al., 1986, Pratl et al., 2008)*. *Streptococcus pneumoniae* was cultured for 3 days in Columbiana blood agar culture media (the basis of Columbian agar with added 5% of de-fibrinated sheep blood) in anaerobic conditions.

Ten weeks after operations 0.1 ml *Streptococcus pneumoniae* MSCL 769 (ATCC 6305) 6×10^8 colony forming units, suspended in sterile NaCl 0,9% solution, were administered intravenously in the vein of the tail.

The administration of *Streptococcus pneumoniae* was performed in general anesthesia, it was done in one day for all animals, using the same series of *Streptococcus pneumoniae*. The animals were observed for twelve days, survival was determined, autopsy was performed for dead animals. The parenchymatous organs (lungs, liver, spleen and kidneys) were fixed in Stefanini solution: 2% formaldehyde and 0,2% pikrin acid 0,1 phosphate buffer (pH 7,2), for later determination of IL-10, TNFα, HβD-2 and cell apoptosis.

In 12 days after injection of *Streptococcus pneumoniae* the experiment was terminated, and remaining 21 experimental animals were euthanized, using overdose anesthesia. This experiment was approved in Food and Veterinary Service of the Republic of Latvia (No. 21-1-13/2099).

METHODS OF EXAMINATION

Routine histology method.

Method of biotin and streptavidine immunohistochemistry.

TUNEL method.

STATISTICAL ANALYSIS

STATISTICAL DATA PROCESSING OF CLINICAL PART

The aim of statistical analysis of the study data was to evaluate the clinical data obtained in experiments with animals, and from clinical measurements by processing those with adequate statistical methods.

During the study the data were registered and entered in MS Excel electronic tables Statistical processing of data was performed with software *SPSS for Windows* 12.0 (SPSS Inc., USA), and its newer version *PASW Statistics* 18.0.

For evaluating the frequency of endoscopic examinations, confidence interval analysis was used. The 95% confidence interval area upper and lower border was calculated (confidence interval 95%) *(Altman, 2000)*. The frequency of measurements (proportions), their comparison, and also for testing hypotheses chi-square (χ^2) test was used.

For different manipulations, and time period of complaints, changes in leukocyte and platelet counts descriptive statistics methods were used, i.e. central tendency rates (mean arithmetic, median and mode), and distribution rates (standard deviation, mean standard error and distribution amplitude).

As the number of data in the study was not large, in all cases before statistical analysis data were tested for normal probability distribution. This was done using Kolmogorov-Smirnov test. For data with normal distribution the hypothesis was tested by t test or dispersion analysis (*ANOVA*), depending on the count of comparable categories. For data that did not match normal probability distribution, non-parametrical methods of statistics were used. Non-parametric statistics (Mann-Whitney, Crascall-Wollis and Vilcockson) were used during statistical data processing, when evaluation and comparing used methods of sclerotherapy in children, who had performed PSE and splenectomy. The correlation of the acquired data was calculated, using appropriate methods of correlation analysis (Pearson or Spearman). In some cases, e.g. to evaluate the tendency for connection of dimensions of the spleen and period of hyperthermia, thus predicting the course of the illness, linear regression analysis was used. The mathematical and statistical processing of these data was done in Department of Physics of Riga Stradins University.

STATISTICAL PROCESSING OF THE DATA OF THE EXPERIMENTAL PART

Data was entered in MS Excel, and its processing was done with software PASW (SPSS) 18.0 (PASW, USA). For analyzing survival Caplan-Meyer survival statistical analysis method was used. Analysis of elative amounts of IL-10, TNFα and HβD-2 between groups (testing the hypotheses) non-parametric tests and Mann-Whitney test was used *(Altman DG, 1999, Riffenburgh, 2006)*.

For semi-quantitative counting of structures we used the following legends *(Pilmane et al., 1988)*: 1) 0 – IL-10, TNFα, HβD-2 and apoptotic cells cannot be seen in the field of view; 3) + - rare IL-10, TNFα, HβD-2 and apoptotic cells in the field of

view; 4) ++ - few of IL-10, TNFα, HβD-2 and apoptotic cells in the field of view; 5) +++ - many IL-10, TNFα, HβD-2 and apoptotic cells in the field of view; 6) ++++ - very many IL-10, TNFα, HβD-2 and apoptotic cells in the field of view.

RESULTS

RESULTS OF THE CLINICAL PART

Evaluation of treatment results in patients with PHS and varicous veins of esophagus and stomach

From 1998 to 2008 40 patients with PHS were treated in UCH (♀ : ♂, 17 : 23), 1 to 16 years old (mean age 7,5 years), they had performed in total 580 FEGS. In 386 cases sclerotherapy of varicose veins of esophagus and stomach was performed, in 194 cases sclerotherapy was not used during FEGS. To stop acute bleeding 30 (5,2%) 95% CI [3,7-7,2%] sclerotherapy procedures for 21 patients aged 1 to 14 years (mean 6,5 years) were performed. For prevention of acute bleeding sclerotherapy as a planned procedure was performed in 356 (61,8%) 95% CI [57,9-65,6%] cases. In all cases of acute bleeding from the varicose veins of esophagus and stomach endoscopic sclerotherapy was enough to achieve sufficient hemosthasis. There were 194 (33,0%) 95% CI [29,4-36,8%] diagnostic FEGS performed in 36 patients, no sclerotherapy was done during these procedures. During FEGS in 38 patients (95%) signs of gastropathy were seen. Positive urease test was detected in 11 PHS patients (27%).

40 patients with PHS were divided in 3 groups:

Group 1: 15 patients with PHS, whose hypersplenism was treated by performing PSE.

Altogether 210 FEGS were performed. For treatment varicose veins of esophagus and stomach 133 sclerotherapy procedures were done (in 14 patients), from which 10 sclerotherapy procedures were performed to stop acute bleeding (in 6 patients). For prevention of acute bleeding 123 sclerotherapy procedures were performed. For diagnostic purposes 77 FEGS in 14 patients were done (for evaluating the state of varicose veins of esophagus and stomach without sclerotherapy).

Group 2: 21 patient with PHS, which had been performed splenectomy for treatment of hypersplenism.

In total 336 FEGS were performed. For treatment of varicose veins of esophagus and stomach 231 sclerotherapy procedures (in 20 patients) were performed, from them 19 procedures were done for stopping acute bleeding from varicose veins of esophagus and stomach (in 14 patients). For prevention of acute bleeding 212 sclerotherapy procedures (in 20 patients) were performed. For diagnostic purposes 105 FEGS were done (for evaluation the state of varicose veins of esophagus and stomach without sclerotherapy) in 20 patients.

Group 3: 4 patients with PHS who had not been treated the signs of hypersplenism.

Altogether 34 FEGS were performed. 22 sclerotherapy procedures for treatment of

varicose veins of esophagus and stomach were performed (in 2 patients), out of those 1 procedure was done to stop acute bleeding from varices of esophagus ant stomach (in 1 patient). For prophylaxis of acute bleeding 21 procedures of sclerotherapy were performed. Diagnostics of varicose veins of esophagus and stomach was done by 12 FEGS (in 3 patients).

Results of treatment of patients of PHS with hypersplenism

15 patients with PHS hypersplenism were performed in total 18 PSE procedures (repeated PSE was performed in patients No 29, No 37, and no 39). Digital angiografies before and after PSE in the amount of 80-90% are shown in figure 2.

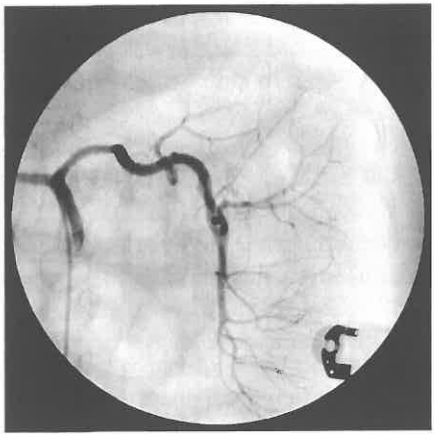

Fig. 2 (a). **Digital angiography before PSE**

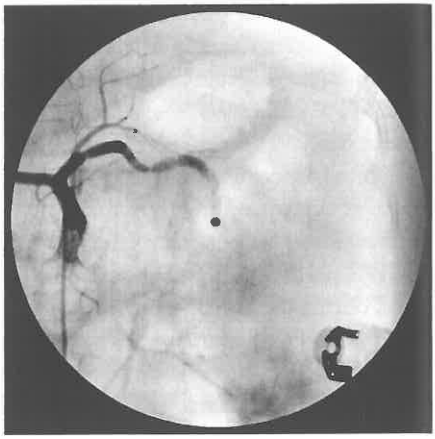

Fig. 2 (b). **Digital angiography after PSE in the amount of 80 – 90%**

Pain relief therapy – patients had complaints about pain in the left epigastria after PSE. Pain relief therapy was necessary 2 to 14 days after operation. Solution of Fentanyl was used 2 to 7 days after the procedure but Diclofenac Sodium was used 4 to 14 days after PSE.

Hyperthermia – all patients after PSE had hyperthermia (above 37,4 °C), which was observed from 3 to 28 day period after the operation. Not always the patients with larger amount of embolization had longer episodes of hyperthermia.

Period of in-patient treatment – patients after PSE were hospitalized from 6 to 38 days after the operation. The patient who spent 38 days in the hospital developed an abscess of the spleen after the PSE (in the amount of 20 – 35%).

Changes in the platelet count after PSE

Patients of group 1 (PSE in the amount of 20 – 35%) after PSE (Table 1)

Recidive of hypersplenism was seen in 2 patients – No 29 and No 39.

Insufficient elevation of platelet count was observed in 3 patients – No 17, No 37, and No 47.

The Patient No 17 had splenectomy performed three months after PSE due to septic complications – abscess of the spleen. The patient No 37 had been performed partial splenic resection four months after PSE (of 20%)

Patients of Group 2 (PSE in the amount of 60 – 80%) after PSE (Table 2)

Patients with normal platelet count – 7 patients- No 18; No 23; No 29; No 31; No 39; No 28.

A patient with a recidive of hypersplenism – No 44

Insufficient elevation of platelet count – 2 patients, No 37 and No 48 respectively.

Patients of Group 3 (PSE in the amount of 80 – 90%) after PSE (Table 3)

All patients had platelet count elevation above the lower normal limit

Changes in the leukocyte count after PSE

Patients of group 1 (PSE in the amount of 20 – 35%)

After PSE all patients showed increase in the total leukocyte count (Table 1).

Patients of Group 2 (PSE in the amount of 60 – 80%)

After PSE all patients showed increase in the total leukocyte count above the normal limit (Table 2).

Patients of Group 3 (PSE in the amount of 80 – 90%)

After PSE all patients showed increase in the total leukocyte count above the normal limit (Table 3).

Evaluation of the longitudinal dimension of the spleen and therapy for pain relief

The data were obtained from 2 patients of Group 1 (PSE of 20 – 35%); the Patient No 17 had the longitudinal dimension of the spleen 15,0 cm before PSE, and the pain relief therapy was necessary for 4 days. The patient No 39 had the longitudinal dimension of the spleen of 16,6 cm, and the pain relief therapy was continued for 6 days. Among the patients of Group 2 (PSE of 60 – 80%) it was observed that not always children with greater longitudinal dimensions of the spleen had longer duration of pain relief therapy; among the patients of the Group 3 (PSE of 80 – 90%) the duration of therapy for pain relief was the same (7 days) in both patients, No 27 and No 38, but the longitudinal dimensions of the spleen varied, they were 13,7 cm and 15,5 cm, respectively.

Evaluation of the longitudinal dimension of the spleen and duration of hyperthermia

From the Group 1 (PSE of 20 -35%) the patient No 17 with the longitudinal spleen dimension of 15,0 cm developed a splenic abscess, which explains the hyperthermia period of 28 days. The Patient No 39 had the longitudinal splenic dimension of 16,6 cm but the duration of hyperthermia after PSE was 5 days; among the patients of Group 2 (PSE of 60 – 80%) the longest period of hyperthermia (15 days) was seen in the patient No 44 with the longitudinal dimension of the spleen being 16,1 cm. Whereas the patient No 31 with the longitudinal dimension of the spleen 18,2 cm had hyperthermia for 6 days. The Patient No 29 with the spleen's longitudinal dimension of 11,5 cm had hyperthermia for 5 days after PSE. From the Group 3 (PSE of 80 –

Table 1

Comparison of changes in platelet (PLT) and leukocyte (WBC) counts in PHS patients of Group 1 before and after PSE in amount of 20–35% (2000 – 2008)

Patient No	PSE amount (%)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)
		before PSE		2–8 days after PSE		1 year after PSE	
29.	20 – 35 (1st time)	79	3,0	271	7,6	154	3,6
39.	20 – 35 (1st time)	73	2,2	400	9,2	129	4,0
17.	20 – 35	82	2,2	66	4,6	splenectomy	
37.	20 – 35 (1st time)	49	1,8	60	6,0	Partial splenectomy (20%)	
47.	20 – 35	69	4,1	121	4,1		

Patients with recidiving hypersplenism after PSE

Patients with insufficient correction of hypersplenism after PSE

Table 2

Comparison of changes in platelet (PLT) and leukocyte (WBC) counts in PHS patients of Group 2 before and after PSE in amount of 60–80% (2000 – 2008)

Patient No	PSE amount (%)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL))	WB (x 103
		Before PSE		2–8 days after PSE		1 year after PSE		2 year after PSE	
18.	60 – 80	87	3,3	334	11,6	250	5,5		
19.	60 – 80	120	3,5	229	10,5	225	5,9	197	4
23.	60 – 80	34	3,8	200	7,9	144	6,5	135	4
29.	60-80 (2nd time)	130	3,3	349	9,8	282	5,6	295	4
31.	60 – 80	74	1,6	360	8,1	277	5,2	200	
39.	60 – 80 (2nd time)	129	4,0	293	8,5	155	6,1		
28.	60 – 80	97	3,8	387	18,7	323	5,1	251	5
44.	60 – 80	81	5,5	181	10,2	148	8,3	127	6
37.	60 – 80 (2nd time)	138	4,5			123	4,3	122	5
48.	60 – 80	88	3,1	111	13,6	97	4,1		

Patients with resolved signs of hypersplenism after PSE

Patients with recidive of hypersplenism after PSE

Patients with insufficient correction of hypersplenism after PSE

PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)
2 years after PSE		3 years after PSE		4 years after PSE		5 years after PSE	
194	4,0	147	3,1	157	2,9	125	3,0
repeated PSE							

PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)
3 year after PSE		4 year after PSE		5 year after PSE		6 year after PSE		7 year after PSE	
					5,5			185	6,0
		192	3,9				4,0	169	
169	7,6								
335	4,1								
224	4,0	286							
266	4,1	267	4,5	258	5,6	250	5,6		
123	7,8							107	8,8

Table 3

Comparison of changes in platelet (PLT) and leukocyte (WBC) counts in PHS patients of Group 3 before and after PSE in amount of 80–90% (2000 – 2008)

Patient No	PSE amount (%)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)
		before PSE		2–8 days after PSE		1 year after PSE		2 years after PSE		3 years after PSE	
38.	80 – 90	58	3,3	227	15,1	376	11,2	326	11,9		
42.	80 – 90	81	3,6	302	5,8	323	7,4	297	13,7	259	7,4
27.	80 – 90	167	3,7	234	14,5	395	6,9	334	6,5		

Patient No	PSE amount (%)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)	PLT (x 103/uL)	WBC (x 103/uL)
		before PSE		2–8 days after PSE		1 year after PSE		2 years after PSE		3 years after PSE	
38.	80 – 90										
42.	80 – 90	274	8,4	300	6,5	260	7,1	246	7,7	184	5,9
27.	80 – 90	289	5,5								

Patients with resolved signs of hypersplenism after PSE

90%), the patient with the longitudinal spleen dimension of 13,7 cm had hyperthermia for 16 days; the patient with the spleen of 15,5 cm had hyperthermia for 6 days.

Complications after PSE (abscess of the spleen)

Abscess of the spleen was observed in one patient (No 17) after PSE. After the PSE procedure, despite the bacground of Benzylpenicillin, the patient had persistent hyperthermia (38,0 – 39,0°C) and pain in the left epigastria, which did not show tendency to resolve. The US scan revealed an abscess in the region of the lower pole of the spleen. The level of C reactive protein on the 6th day after the procedure was 133,7 mg/L. The blood cultures were negative. On the 22nd day after PSE a transcutaneous drainage procedure was performed and it revealed 3 – 4 ml of cloudy and sanguine liquid, there were no microbial pathogens detected. The patient's temperature went to normal only 1 day. On the 28th day after PSE a catheter was placed in *a. lienalis* and solution of Ciprofloxacin (200 mg twice a day for 9 days) was administered. On the 37th day after PSE the laboratory values showed remission of the inflammation process and patient was discharged. 3 months and 12 days after PSE the patient had pain in the left epigastria, the level of C reactive protein was 145 mg/L. The US scan revealed an abscess of the lower pole of the spleen 6,0 cm x 3,0 cm. CT scan showed an abscess of the spleen with diameter 7 – 8 cm. 3 months and 12 days after PSE this patient underwent splenectomy.

PSE was not performed

1 patient had not performed PSE as abnormal splenic tissue architectonic was detected in the digital angiography.

Results of treatment of patients with hereditary spherocytosis and hypersplenism

Pain relief therapy – all patients had complaints about pain in the left epigastria after PSE. Pain relief therapy (intravenous) was continued 1 to 19 days after PSE.

Duration of hyperthermia after PSE – all patients after PSE had hyperthermia from 2 to 9 day period after PSE.

Period of in-patient treatment – 8 to 19 days after PSE.

Changes of the level of total bilirubin after PSE

Group 1 (PSE in the amount of 60 – 80%)

Norma level of total bilirubin (up to 17 μmol/L) after PSE – was observed in 2 patients (No 4 and No 8) (Table 4).

Patients with light stage HS after PSE (level of total bilirubin – 17 to 34 μmol/L) – was detected in 5 patients (No 5, who had PSE performed for the 2nd time, No 6, No 7, No 9 and No 11).

Patients with medium stage HS after PSE (level of total bilirubin – 34 to 51 μmol/L) – was observed in 3 patients (No 3, No 5, when performing PSE for the 1st time, and No 10).

Group 2 (PSE in the amount of 80 – 95%)

The levels of total bilirubin decreased in both patients, and they corresponded with

Table 4

Changes in levels of total bilirubin in patients with normal total bilirubin in light and median severity ISA after PSE in amount of 60-80% (2000 – 2008)

Patient No	PSE amount %	Total bilirubin (μmol/l) before PSE	Total bilirubin (μmol/l) 2-8 days after PSE	Total bilirubin (μmol/l) 1 year after PSE	Total bilirubin (μmol/l) 2 years after PSE	Total bilirubin (μmol/l) 3 years after PSE	Total bilirubin (μmol/l) 4 years after PSE	Total bilirubin (μmol/l) 5 years after PSE	Total bilirubin (μmol/l) 6 years after PSE	Total bilirubin (μmol/l) 7 years after PSE	Total bilirubin (μmol/l) 8 years after PSE
4.	60–80	31,2	16,0		10,8						
8	60–80	50,2		7,3	8,3						
5.	60–80 (2nd time)	39,0	29,8		32,2	27,6					
6.	60–80	62,8	22,4							29,5	
7.	60–80	40,1	24,2							33,7	
9.	60–80	64,6	18,2								21,1
11.	60–80		17,5	Splenectomy							
3.	60–80	67,2	22,6	51,0	Splenectomy						
5.	60–80 (1nd time)	64,7	16,4	21,4	40,6	39,0					
10.	60–80	78,3	70,6	59,1	37,6						

Normal level of total bilirubin (up to 17 μmol/l)

Light form of ISA (total bilirubin 17 to 34 μmol/l)

Median severe form of ISA (total bilirubin 34 to 51 μmol/l)

Table 5

Levels of total bilirubin in ISA patients after PSE in amount of 80-95% (2000 – 2008)

Patient No	PSE amount (%)	Total bilirubin (μmol/l) before PSE	Total bilirubin (μmol/l) 2-8 days after PSE	Total bilirubin (μmol/l) 1 year after PSE	Total bilirubin (μmol/l) 2 years after PSE	Total bilirubin (μmol/l) 3 years after PSE
1.	80–95	84,3				19,5
2.	80–95	81,7	19,6			32,4

Light form of ISA (total bilirubin 17 to 34 μmol/l)

light stage HS (Table 5).

Complications after PSE of 60-80% – abscess of the spleen, fibrinous pleuritis

These complications were observed in a 5 year old patient (No 11). During the procedure of PSE *Contour* micro particles (250 – 355 mkm) were introduced first in the artery of the upper pole of the spleen, and then in the branches of the median artery. Hyperthermia was observed for 2 days. 9 days after PSE the patient was discharged. 44 days after PSE the child was repeatedly hospitalized in UCH due to periodic abdominal pain and hyperthermia up to 38,6°C. The antibacterial therapy was initiated with Ceftazidine (400 000 units 4 times a day for 8 days). The US scan revealed an abscess cavity in the spleen of diameter of 4 cm, there was also an abscess under the left diaphragm (9 x 5 cm). On the 45th day after PSE transcutaneous drainage of the splenic abscess was performed, 4 – 5 ml of pus was obtain, and it had positive culture for *Staphylococcus aureus*. The antimicrobial therapy was changed to Benzylpenicillin (500 000 units 4 times a day for 6 days) and Clarythromycin (300 mg 3 times a day for 6 days). The hyperthermia (up to 38°C) did not resolve. 55 days after PSE splenectomy was performed, 64 days after PSE the US scan showed exudates in the left pleural cavity. Computed tomography revealed a collection of exudates in the left pleural cavity that collapses the left lung. On the 70th day after PSE left side thoracotomy and decortications was performed. The child was discharged 20 days after thoracotomy (91 days after PSE).

Evaluation of the questionaire of the patients' parents

Patients of PHS

The questionnaire was filled in by 14 parents of PHS patients. 8 parents noted, that they were followed-up by a general practitioner, 4 – by a pediatric surgeon and a general practitioner, 1 – by pediatric surgeon and another one was followed-up by another specialist (a hepatologist). 5 patients had regular visits to a doctor but they had no complaints, 4 parents did not reveal complaints, which make them to seek for doctors' appointment, parents of 2 patients noted abdominal pain, 1 patient had pan in

the left epigastria, and parents of 2 patients had prophylactic doctor's appointments.

7 patients have been continuously (1 to 3 years) taking medications; 1 is taking Omperazole and Propranolol; 3 are taking Anaprilin, 1 patient has been taking Ursofalk and Essentiale Forte; parents of 2 patients did not answer about the medications their children use. 4 patients have been taking medications for more than 3 years (Propranolol, Anaprilin, Omperazole, Essentiale Forte and Ursofalk); 2 patients have been using medication for a period of 1 to 3 years (Anaprilin), parents of 1 patient did not state the duration of medicaments' therapy. Parents of 9 patients had not observed signs of melaena. 4 patients have had signs of melaena. Parents of 10 patients had not observed coffee ground vomiting. 4 patients have had coffee ground vomiting. Bitter or acidy taste in the mouth had been detected in 2 patients, 12 patients denied these complaints. Complaints about heartburn or discomfort behind the sternum were noted by parents of 7 patients, the other 7 did not have them. Parents of 12 patients denied difficulties of swallowing in their children, they were seen by parents of 1 patient, and parents of another patient left this question unanswered. Remarkable fatigue was noted by parents of 2 patients. Abdominal pain (once a month) was observed by one patient. Abdominal pain at physical exercise had been seen in 3 children. Pain in the left epigastria after PSE was observed in 4 patients, parents of 2 patients denied it, and parents of another 2 patients did not answer. There were no other complaints detected in patients after PSE.

Patients of HS

The questionnaire was filled by parents of 9 patients with HS. Parents of 7 patients were followed-up by a general practitioner, parents of 1 patient were not followed-up but parents of 1 patient answered that their child was followed-up by a general practitioner, pediatric surgeon and a hematologist. None of the patients received continuous medicaments' therapy. 2 of the children do not have abdominal pain. 4 children have rare episodes of pain in the left epigastria, 1 child has left epigastria pain 2 – 3 times a month. Parents of 1 child noted rare episodes of pain at physical exercise, and parents of another child – rare abdominal pain episodes at physical exercise. Fatigue was noted in 2 patients. Other complaints that could have appeared after PSE were denied by parents of 7 patients. In one case sclera jaundice was detected at times of psycho emotional stress, and in one case headache was observed.

RESULTS OF THE EXPERIMENTAL PART

Survival of experimental animals after intravenous administration of *Streptococcus pneumoniae* and splenic reduction operations

Experimental rats with intact splenic tissues, and also with 1/3 of splenic tissue had greater survival rates than animas after splenectomy; a correlation was observed – the less amount of splenic tissue, the higher the mortality. In SPR group al rat died in the first 3 days, thus this time period was called "mortality zone" (Table 6).

Table 6

Survival and mortality rates of splenectomized, partially splenectomized and sham-operated experimental rats after *Streptococcus pneumoniae* challenge

	Mortality (in days and per cents)	Splenectomized rats (SPR)	Partially splenectomized rats (PSR)	Sham operated rats (SOR)	Control group rats (CGR)
Mortality zone †	1	6 (60%)	1 (10%)		
	2	2 (20%)			
	3	2 (20%)			
	4				
	5		6 (60%)		
	6				
	7		1 (10%)		
	8		1 (10%)	1 (10%)	
	9				
	10				
	11				
	12		1 (10%)	9 (90%)	10 (100%)
		Survival in average 1,6 ±0,8 days Mortality 100%	Survival in average 6,0 ± 2,5 days Mortality 90%	Survival in average 11,6 ± 1,3 days Mortality 10%	Survival in average 12 days Mortality 0%

SPR group – on the first day 6 animals died (60%), on the second day 2 rats (20%) died, on the 3rd day 2 rats (20%) died. The average survival in the SPR group was 1,6 ± 0,8 days but mortality – 100%. In PSR group 1 rat (10%) died on the first day, 6 rats (60%) died on the fifth day, on the seventh day 1 rat (10%) died, but on the eight day 1 rat (10%) died. The average survival was 6,0 ± 2,5 days, mortality – 90%. The survival rates of PSR and SPR groups had statistically significant differences (95% CI 2,7 to 6,1 days).

In SOR group one rat (10%) died on the eight day. The average survival was 11,6 ± 1,3 days, and mortality was 10%. During autopsies all PSR group animals were observed to have remains of the spleen with good blood flow.

RESULTS OF THE IMMUNOHISTOCHEMISTRY MARKERS' STUDY

Comparing the count of cells expressing IL-10 in parenchymatous organs it was observed that in all organs the cell count with IL-10 is significantly higher than the number of cells containing HβD – 2 and TNFα ($p < 0,05$) (Table 7). The greatest,

Table 7

Relative amount of IL-10, TNFα, HβD – 2 in parenchymatous organs of laboratory animals

Parenchymatous organs	Splenectomized rats (SPR)			Partially splenectomized rats (PSR)			Sham operated rats (SOR)			Control group rats (CGR)		
	HβD-2	IL-10	TNFα	HβD-2	IL-10	TNFα	HβD-2	IL-10	TNFα	HβD-2	IL-10	TNFα
Spleen	.....	.....	.....	++++ - +++	+++	++++	++ - ++++	+++	++++	++ - ++++	+++	+++
Lungs	++	++++	0/+ - +	0	+++	++ - +++	++	++ - +++	++ - +++	+++	++ - +++	0 – 0/+
Liver	0 – 0/+	++++	0/+	0	++ - +++	0/+	0	++ - +++	0/+ - +	0 - ++	+ - ++	0 - +
Kidneys	0	++++	+	0/+	+++	++	++	+++	++	+++	+ - +++	0 - +

0 – IL-10, there are no structures containing TNFα and HβD-2 in the field of view; 0/+ - rare IL-10, TNFα, and HβD-2 containing cells in the field of view; + - few IL-10, TNFα, and HβD-2 containing cells in the field of view; ++ medium amount of IL-10, TNFα, and HβD-2 containing cells in the field of view; +++ - many IL-10, TNFα, and HβD-2 containing cells in the field of view; ++++ - very many IL-10, TNFα, and HβD-2 containing cells in the field of view.

No statistically significant differences

No statistically significant differences

No statistically significant differences

No statistically significant differences

No statistically significant differences

No statistically significant differences

No statistically significant differences

In all parenchymatous organs the cell count with IL-10 is significantly higher than the HβD-2 and TNFα containing cells

statistically significant count of IL-10 containing cells was seen in SPR group ($p < 0,05$). It was statistically significant that the lowest number of IL-10 containing cells was detected in CGR liver ($p < 0,05$).

The lowest number of HβD – 2 expressing cells was seen in the liver of SPR, PSR, SOR, groups, kidneys of SPR and PSR groups, and in lungs of animals of PSR group. It had a statistically significant difference from the number of cells containing HβD – 2 in parenchymatous organs of other groups ($p < 0,05$).

Spleen – Analysis of splenic tissue indicated that the *Streptococcus pneumoniae* challenge did not influence the levels of defensin, or pro and anti-inflammatory cytokines in all groups where rats had a spleen or a larger amount of splenic tissue (PSR, SOR and CGR). Immunohistochemistry markers IL – 10, TNFα and HβD – 2 expressing cell count in the spleen (SPR, PSR, SOR and CGR) had no statistically significant differences ($z = 5,021$; $P < 0,01$).

In splenic tissue, the numbers of cells containing HβD – 2, IL-10 and TNF- α were higher than in other PSR, SOR, and CGR parenchymatous organs, and the difference was statistically significant. Mean HβD-2 levels in spleen were 94,3 units but only 48,9 in the lungs, liver, and kidneys ($z = 6,287$, $p < 0,001$). Mean IL-10 levels in spleen were 86,9 relative units but only 51,3 in other parenchymatous organs ($z = 5,171$, $p < 0,001$). Mean TNF- α levels in spleen were 104,8 relative units, but they were 45,6 in other parenchymatous organs 45,6 ($z = 8,167$, $p < 0,001$)).

Lungs – HβD – 2 amounts in CGR and PSR groups had statistically significant differences ($p < 0,05$). It was the highest in the CGR group but the lowest in the PSR group ($p < 0,05$). SPR and SOR group had the same number of HβD – 2 containing cells ($p > 0,05$). PSR, SOR and CGR had similar relative amounts of IL – 10, and there were no statistically significant differences ($z = 4,266$; $p < 0,01$), (relative amount of IL – 10 was slightly higher in PSR group but it was not statistically significant).

The count of TNFα expressing cells in the groups of PSR and SOR was greater. There were no significant differences ($p > 0,05$); it was higher than in SPR and CGR and the difference was statistically significant ($p < 0,05$).

Liver – HβD – 2 amounts in CGR and SOR, PSR, SPL groups did not show statistically significant differences ($p > 0,05$). Levels of IL – 10 in SOR and PSR groups did not have statistically significant differences ($p > 0,05$). TNFα amounts in the groups of CGR, SOR, PSR, and SPR animals had no statistically significant differences ($p > 0,05$).

Kidneys – The greatest amount of HβD – 2 containing cells was seen in CGR animals, it was slightly less in SOR group, but the least amount of HβD – 2 expressing cells was seen in SPR group ($p < 0,05$). Differences in HβD-2 levels in the kidney were statistically significant for all groups ($z = 2,916$, $p = 0,004$), with a gradual increase between groups as follows: 0 – SPR, 0,5 – PSR, 2 – SOR, 3 – CGR. The numbers of HβD-2 containing cells in the CGR kidney were greater than in rats of SPR, PSR, SOR groups kidney ($p < 0,05$). Statistically highest IL-10 containing cells was detected in SPR group ($p < 0,05$). Amount of IL-10 containing cells in CGR, SOR and PSR groups did not have statistically significant differences ($p > 0,05$). Comparing the

count expressing TNFα in CGR, SOR, PSR and SPR groups it was observed that TNFα containing cells in SOR and PSR groups were statistically significant ($p > 0,05$) and it was higher than in CGR group ($p < 0,05$).

IL-10 and TNFα (IL-10 / TNFα) ratio

In the spleen the ratio increased proportionally to the amount of splenic tissue; in PSR it was 0,7; in SOR 0,8, and in CGR animals it was 1,0. The greatest IL – 10/ TNFα ratio was observed in the lungs of SPR rats – 18,1, and it had statistically significant difference from the ratios in lungs of animals in other groups (PSR – 1,2; SOR – 1,0; CGR – 10,0) ($p < 0,05$). It was similar in the liver of SPR animals, the ratio IL – 10 / TNFα, comparing to other groups, was the greatest – 8,4 (PSR – 5,0; SOR – 3,3; CGR – 3,0), and it had statistically significant difference from other groups ($p < 0,05$). It is interesting, that the IL – 10 / TNFα ratio in kidneys of SPR and CGR animals did not have statistically significant differences (SPR – 3,9; CGR – 4,0) ($p > 0,05$). Rats of PSR and SOR groups had the same IL – 10 / TNFα ratio in kidneys, without statistical significance ($p > 0,05$). In the lungs of PSR animals the IL – 10 / TNFα ratio was 1,2, but in the lungs of SOR animals it was 1,0. and there were no statistical differences ($p > 0,05$). Comparing the ratio of IL – 10 / TNFα in the liver of PSR and SOR groups, it was concluded that IL – 10 / TNFα ratio was statistically ($p < 0,05$) greater in PSR rats – 5,0, than in SOR rats, which had it lower – 3,3. IL – 10 / TNFα ratio in the lungs of CGR animals was 10, and compared to PSR and SOR animals it was 10 times greater (PSR – 1,2; SOR – 1,0), in the same time it was almost ten times lower than in SPR animals (18,1).

Relative count of apoptotic cells in the parenchymatous organs of experimental animals

Greater count of apoptotic cells was found in the kidneys of SOR group (260,333 ± 73,230), it was less in the kidneys of CGR animals (227,500 ± 77,071), and kidneys of SPR (224,500 ± 58,439), and PSR animals (164,000 ± 100,786) (Table 8). The highest number of apoptotic cells in the spleen was found in the SOR group (182,267 ± 142,213). The count of apoptotic cells was rather similar in PSR (147,833 ± 128,313) and CGR animals (142,400 ± 91,386). The amount of apoptotic cells found in liver was the highest in the SPR group (127,500 ± 46,173), then SOR group (119,000 ± 66,822). Less amounts of apoptotic cells were seen in the PSR group (75,667 ± 42,622) and CGR (72,833 ± 47,153). In the lungs the highest number of apoptotic cells was seen in the PSR group (166,667 ± 52,978), then SPR group (132,500 ± 24,761). In CGR animals the count of apoptotic cells was (103,167 ± 92,817). The least amount of apoptotic cells was seen in the lungs of SOR group animals (87,167± 50,831).

Table 8

Relative amounts of apoptotic cells in splenectomized, partially splenectomized, sham-operated and control group rats

Groups	Lungs	Liver	Kidneys	Spleen
Splenectomized rats (SPR)	132,500 ±24,761	127,500 ± 46,173	224,500 ± 58,439	----------
Partially splenectomized rats (PSR)	166,667 ± 52,978	75,667 ± 42,622	164,000 ± 100,786	147,833 ± 128,313
Sham operated rats (SOR)	87,167 ± 50,831	119,000 ± 66,822	260,333 ± 73,230	182,267 ± 142,213
Control group rats (CGR)	103,167 ± 92,817	72,833 ± 47,153	227,500 ± 77,071	142,400 ± 91,386

DISCUSSION

The meaning of the spleen in immunological processes nowadays had not been fully understood. Several groups of investigators have described the splenic tissue role not only in the protective processes of the body (in the fight with infection) but also in the regulation of autoimmune processes *(Kimpel at al., 2003)*. Even with the possible risk of PSS in children and in adults the splenic tissue surgical reduction (splenectomy, partial splenic tissue resection) are the only known method for treatment of hypersplenism nowadays. The highest possibility for PSS is for children, especially up to 2 years of age (the incidence of PSS varies from 1,45% to 24,8%). That is the reason why university hospitals in many countries use splenic tissue saving operations for treatment of hypersplenism, and splenectomy is performed only in cases of urgency (bleeding in case of traumatic injury of the spleen if the splenic tissue saving operation is impossible to perform) *(Price at al., 2007)*. When detecting the antibody titers in patients after splenectomy *Livingston* and *Grikscheitet* noted that patients after splenectomy had almost 10 times lower antibody titers than patients without splenectomy *(Livingston et al., 1983; Grikscheitet al,. 2008)*. For treatment of hypersplenism several splenic tissue saving operations have been developed all over the world, their aim is to reduce the signs of hypersplenism syndrome and to save a patients form a possible PSS in the same time. PSE is used in the University Children's Hospital, the aim of that is to reduce the amount of splenic tissue hoping that the saved part of the organ will be able to function in the immunological processes of the body, and prevent the threat of PSS. It is important to find out how much from splenic tissue is necessary to save for keeping the balance of the immune system, in which the spleen takes a big part. Unfortunately the studies done until now do not

fully reveal the significance of immune processes in the spleen. It is thought that age, sex, heredity and even diet plays role in the immune processes. *(Torres et al.,2005; Pratl et al., 2008)*. *Shatz* et al and *Musher* et al describe elevated possibility of PSS in patients with little immune experience and immature immune system that does not allow full production of antibodies against microorganisms with capsules *(Shatz, 2005; Musher et al., 2005)*.

Several study groups have reported insufficient efficacy of prophylactic vaccination and preventive antibacterial therapy in prophylaxis of PSS. Unfortunately both, prophylactic vaccination and antibacterial therapy are the only available preventive measures nowadays that can be offered to patients in cases of splenic tissue reducing operations *(Torres et al., 2005; Kuranga et al 2006)*.

For studying the immune function of splenic tissue in cases of *Streptococcus pneumoniae* infections, during the work on thesis an experiment with laboratory animals was performed. Usually grown-up experimental animals are used in animals' studies *(Deng et al., 2004; Simovart et al., 2006)*. For making the experiment as close to reality as possible young laboratory rats were chosen with their mean weight and age being 1/3 of a grown-up rat (the grown-up rats usually weight up to 350 grams, and they live up to 3 years). Evaluating the results of the study, it needs to be taken into account that experimental rats live in the laboratory in conditions, which are close to sterile, and they have limited immune experience, thus it is possible that antibody production in these animals against encapsulated microorganisms has not occurred. Perhaps only the age of the rats and the state of maturity of the immune system are important. It is possible that the results of the experiments performed during the thesis work would be different if we had used younger rats, e.g. 1 – 1,5 months old and approximately 50 grams heavy rat youngsters. It must be taken into account that working with so small experimental animals is extremely difficult. It is proved with the fact, that it was necessary to develop a special method of anesthesia for working with rats that weighted 100 g. It is possible that the method of general anesthesia for 50 gram animals would be more complex. It must be noted that during laparotomy the 100 gram heavy rats had tendency of lowering body temperature so they had to be continuously heated. Heating of experimental animals of smaller weight would require equipment unavailable in the vivaries of Latvia. Usage of rat youngsters in experiments was complicated also by the small diameter of their blood vessels, it was proved during the experiment when heating of a rat's tail was necessary to find a vein and administer *Streptococcus pneumoniae* in the vein of the tail. For intravenous injection the smallest intravenous catheter was used (the same as used for newborns in hospital's intensive care units). Starting the experimental study it was also evaluated which route of administration of *Streptococcus pneumoniae* culture to choose. Analyzing the available literature it was concluded that the infection penetration site in cases of PSS have not been fully understood. It is clear that the antigens from the intercellular spaces are being caught by regional lymph nodes but the ones that

penetrate through airways or gastrointestinal tract are being caught by local lymph nodes or lympho-epithelial organs. The antigens that circulate in the blood are being caught by the spleen, which also form the reaction of body's immune response (*Kimura et al., 2002; Pratl et al., 2008; Zhu et al., 2008)*. Because of this the intravenous route of infection was used for experimental animals that complicated the course of the experiment – to achieve the same results it is very important to administer the culture of *Streptococcus pneumoniae* right in the vein, not subcutaneously.

Analyzing the mortality rates of experimental animals it is clear that rats which underwent SPL had the lowest survival (during the first 3 days all rats died). In the group of PSR survival was higher – during the first 3 days only 10% animals died, the highest death rate (60%) was seen on the 5th day. Similar experimental results have been shown by many other authors who evaluated not only survival with different causing factors of sepsis (*Streptococcus pneumoniae, Escherichia coli)* intravenous or intranasal administration but also the bacterial clearance in peripheral blood. It was noted that the highest survival rates and the lowest bacterial clearance showed rats with bigger saved splenic tissue fragment *(Iinuma et al., 1992; Torres et al., 2005)*. Work on the thesis showed remarkably higher relative levels of defensine, IL-10 and TNFα in the splenic tissue rather than in other parenchymatous organs. It shows the significance of splenic tissue in the immune processes of the body. In several studies the high mortality rates of SPR rats after sepsis caused by *Streptococcus pneumoniae* were analyzed; it was concluded that phagocytic actions of reticuloendothelial cells of liver and spleen are different, and it could be the reason for immune dis-balance in animals after splenectomy *(Torres et al., 2005)*. Even if the survival rates were higher and mortality was lower in the PSR group than in the SPR group, still the PSR group mortality seems surprisingly high. When analyzing the available publications before experiment, it was thought that in experiment rats with 1/3 of splenic tissue survival rates would be higher. Unfortunately, the mortally in SPR and PSR groups differed unexpectedly little (100% in SPR, and 90% in PSR, respectively). It can be assumed that the mortality in PSR group would be different, if the animals received prophylactic vaccination and preventive antibacterial therapy before administration of *Streptococcus pneumonia.*

Studying the efficacy of antibacterial therapy in rats after splenectomy and auto transplantation of splenic tissue, *Eskitürk* presumes that liver function in reticuloendothelial system could compensate the missing splenic tissue but adequate antibacterial therapy would be necessary *(Eskitürk et al., 1995)*. Possibly, the time period of 10 weeks between the operation and the challenge with the *Streptococcus pneumonia* was too short for the splenic remnant to regenerate sufficiently, and that might have influenced the survival of PSR rats. *Scher* and *Muftuogly* et al studied the regeneration abilities of the spleen, and concluded that in several months after traumatic splenic injury or partial resection, regeneration of the spleen and resurgence of the filtering function was seen. *Anderson, Offenbartl* and *Scher* et al describes

administration of *Streptococcus pneumoniae* in experimental animals after 4 to 5 weeks after operation *(Scher et al., 1982; Offenbartl at al., 1986; Anderson et al., 1987; Muftuogly et al., 2000)*. When drafting the experimental part of the thesis it was decided to take this experience into account, and to administer the culture of *Streptococcus pneumoniae* in experimental rats 1 month after the operation. The *Streptococcus pneumoniae* culture intended to be used in the experiment was first tested with several random rats, as none of them developed signs of illness, the culture was found to be invalid. That is why the injection of *Streptococcus pneumoniae* in the tails of experimental animals was postponed. It was possible to perform only 10 weeks after the experimental operations. That definitely could have influenced the results of the experiment.

Anderson et al reports that splenic tissue after ligating *a. lienalis* in cases of abdominal trauma can regenerate in a period of 6 months *(Anderson et al., 1987)*. Regeneration of splenic tissue in animals in 4 months after the tissue auto transplantation in *omentum majus* has been described by *Markques* et al *(Markques et al., 2003)*. *Iinuma* et al reports regeneration experiments with splenic tissue in a rat 6, 8 and 16 weeks after splenic tissue auto transplantation in *omentum majus*. Authors conclude that 8 weeks after operation it is possible do differentiate the white pulp from the red pulp but on the 16th week after the operation the tissue of the auto graft are similar to healthy splenic tissue *(Iinuma et al., 1992)*.

The amount of saved splenic tissue is very important. The more splenic tissue is saved during operation, the more wholesome the regeneration is, and the less it histological differs from healthy splenic tissue *(Iinuma et al., 1992; Markques et al., 2003)*. Based on the abovementioned, the evaluation of efficacy of different amount PSE in PHS and HS hypersplenism patients was considered to be a very important part of the thesis. *Sato* et al and *Brandt* et al describe that better results of treatment of hypersplenism are obtained, if PSE is performed in the amount of at least 70% *(Brandt et al., 1989; Sato et al., 2000)*. Quite similar data were obtained in the performed study in this thesis. Analyzing the platelet count in patients with PHS after PSE it was seen that the results are better, when PSE was performed in the amount of 80 – 90% (there were no cases of hypersplenism recidive in 1 to 8 years). The results were slightly poorer in the group were PSE was performed in the amount of 60 – 80% (1 patient had recidive of hypersplenism, and 2 patients had insufficient correction of hypersplenism). Evaluating these results it must be noted that the 80 – 90% PSE group had only 3 patients, but the 60 – 80% PSE group had 10 patients. Similar results were seen in HS patients, where the best results were noted in the group that had been performed PSE in the amount of 80 – 95%. Knowing that splenic tissue regenerates better if during operation it is saved in larger amount, we consider that PSE of 95% could be too much.

Several authors of scientific publication notes that it is very important to save sufficient blood flow to the spleen. Only optimal arterial flow can assure the important blood filtering function of the spleen *(Scher et al., 1982; Muftuogly et al., 2000)*.

The advantage of PSE is the possibility to examine the architectonics of the blood vessels of the spleen by angiography before embolization. It allows evaluating the blood circulation of the remaining part of the spleen. Embolization is performed by gradually administering the embolizing material in the arterial branches of the spleen, thus making it possible to choose the amount of infarction precisely. Some authors point out that for efficient blood filter and immune function the remaining fragment of splenic tissue must be well vascularized, and its size should be at least 30 – 50% of the normal spleen size *(Scher et al., 1982)*. Unfortunately PSE in the amount of 20-35% did not show the expected results. Clinical practice shows that this amount of splenic tissue infarction is too small and there is high probability of recidives. Performing PSE of 80-90% the results are better but in this case we can only hope to achieve possible regeneration of splenic tissue to promote the immunological function. But there is a controversy, as the regeneration of the tissue of the spleen can cause recidive of hypersplenism syndrome. Because of this, the objectives of the experiments made for this thesis was to resect the maximal possible amount of splenic tissue, and to save the least possible fragment of the spleen that could maintain its own blood supply. One third was the least fragment (with its own circulation) of the spleen that had possibly been saved during the experimental operations. Unfortunately it was not possible to perform angiography in experimental animals, as it is not available in vivaries. That is why we can make indirect proofs about blood supply to the splenic tissue of experimental animals, only using the data from autopsies and IHM examinations. Autopsies revealed red and well supplied with blood fragments of spleen, but during immunohistochemistry examinations no statistically significant changes in amounts of IL–10, TNFα and HβD–2 in PSR and SOR splenic issue were seen.

The surgeries on experimental rats were performed as atraumatic as possible in sterile conditions by using single-use operation linens, materials and sterile surgical instruments. The suture material used in the operations was the same as used in Clinics of Surgery for operations on newborns. Rats were operated with caution, using electrocoagulation. It provided the expected result. None of the operated animals had postoperative complications – bleeding or infection of the operation incision. *Morales* et al reports high postoperative mortality in experimental animals after different amount splenic resection (25, 50, 75%) and splenic tissue autotransplantation in the great oment (*omentum majus)*. The author did not use electrocoagulation, but the resection of tissue of the spleen was performed by ligating the tissue by hand *(Morales et al., 1990)*. Even with the possible cautions in operation technique during experiment, the examination in the light microscope shows fibrous tissue ingrowths and practically diminishing white pulp of the spleen in rats after PSR. Similar histological picture in the tissue of the spleen after ligating a. lienalis, auto transplantation the splenic tissue in the great oment and partial splenic resection was seen by other authors *(Yoo et al., 1992; Marques et al., 2004)*. It is possible that the abovementioned microscopic view in the slices of splenic tissue is not connected with sepsis caused by *Streptococcus*

pneumonia, rather it is the trauma of the surgery of the spleen. The ingrowths of fibrous tissue could also be caused by electrocoagulation. Also the fact that in SOR group animals in review cuts of splenic tissue white pulp without signs of activation is seen is in favor of the presumption that the ingrowths of fibrous tissue is the result of operation trauma. By imitating a trauma of the spleen in a rat, after which the animal was infected with *Streptococcus pneumoniae* and *Haemophilus influenza, Karp* et al concluded that the presence of scar tissue in the spleen did not affect its filtration function *(Karp et al., 1989).*

Evaluating the results of IHM we concluded that IL–10, TNFα, HβD–2 and cell apoptosis differences between groups were not as great as we expected before the experiment. Taking into account the fact that in the available references we did not find any studies that analyzed IL–10, TNFα, HβD–2 and cell apoptosis in complexion in parenchymatous organs of 3 – 5 months old rats, the comparison and evaluation of these results are difficult.

Detecting the relative amount of IL-10 in the parenchymatous organs of experimental animals, we concluded that in the group of SPR it was greater than in other groups. In the statistical analysis of data it was seen that the lung, liver and kidney cell count, containing IL-10 is statistically higher than the cell count containing TNFα and HβD–2. It shows that lack of splenic tissue did not influence the secretion of IL-10 in the parenchymatous organs in the animals of SPR group. The possible elevation of IL-10 in the SPR group can be explained by compensatory immune response reaction to the inflammation caused by *Streptococcus pneumoniae* in case of lacking spleen. When studying the amounts of IL-10 in experimental animals *Carrillo-Vico* concluded that the spleen is not the most important centre of IL-10 production *(Carrillo-Vico et al., 2005).* Analyzing the available literature we can conclude that several authors note the special roe of Kupfer cells in both, IL-10 and TNFα synthesis. The author recognizes the deep interaction of Kupfer cells and splenic tissue in the regulation of inflammation process, the Kupfer cells' ability to change the production of IL-10 in the spleen, and also the special significance of IL-10 and TNFα ratio – the higher it is, the lower the survival rates *(Kurachi et al., 2003).* In the experimental study with animals included in the thesis, we observed a similar correlation: rats of SPR group had the IL-10 and TNFα ratio of 18.1 in the lung tissue, which was almost twice higher than in CGR (10,0), in the liver of the SOR group's animals it was only 3,3 but in PSR group rats - 5,0. Findings in the lung and in the kidney were similar. When analyzing the ratio of IL-10 and TNFα in SPL group animals, we detected that it has changed due to elevated relative amount of IL-10, and decreased relative amount of TNFα. Detecting the ratio of IL-10 and TNFα in the heart muscle, *Kaur* concluded that by normalizing the relations between IL–10 and TNFα the function of the heart muscle can be improved *(Kaur et al., 2008).* Other studies concluded that severely elevated cytokine IL-10 level can be harmful for the body. *Van der Sluijs et al. (2004)* describes the significance of IL–10 neutralization in secondary infection with *Streptococcus*

pneumonia, when survival of rats improved, when the levels of IL-10 were decreased. Similar conclusions are made by *van der Poll et al. (1996)*, who studied the role of IL-10 in the mechanisms of immune defense, and observed that the neutralization of IL-10 improved the survival of experimental animals in airway infections caused by *Streptococcus pneumonia*.

Evaluating the levels of IL–10, TNFα and HβD-2 in the spleen of animals of all groups (CGR, SOR, PSR), it was observed that they did not statistically differ, and that they are higher in the spleen than in other parenchymatous organs. These findings could be indicative for the activity of the splenic tissue in the processes of immune defense mechanisms. Similar proposition is expressed by *Kimpel at al. (2003)* after artificially created polyarthritis, by administering peptoglicane polysaccharide solution intraperitoneally in rats. The investigators unexpectedly revealed that in splenectomized rats the clinical and histological signs of polyarthritis were less remarkable than in the animals with intact spleen. Authors then concluded that the spleen is similar to a large reservoir, which extirpation causes changes in the activation of body's immune cells.

When evaluation the levels of HβD-2 in experimental animals was performed we concluded that the greatest count of cells expressing HβD-2 was seen in the spleen, although the number of cells expressing HβD-2 in the lungs, liver and kidneys of the CGR animals was higher than in other groups. It is possible that the regulation of HβD-2 was distorted because of sepsis but for confirmation of this additional research is necessary. *Lee et al. (2004)* describes the activity of HβD-2 against *Streptococcus pneumoniae.* The significance of secretion of HβD-2 in the mucous membranes in the stabilization of the whole immune system is described by other authors *(Sigh et al., 1998; Yuang et al., 2002). Chong*, and also *Mendez-Samperio* examining HβD-2, acknowledges that its regulation in immune processes is still little known *(Chong et al., 2008; Mendez-Samperio et al., 2008).* Performing experiments with animals and detecting the role of HβD-2 in the immune processes of the body, *Chong* et al anticipates that in the future it would be possible to create therapy of defensines that could act in two ways: 1) by stimulation the production of defensines on the surfaces of mucous membranes; and 2) by administering the preparations of defensines in the body exogenously *(Chong et al., 2008)*.

By analyzing apoptosis in the parenchymatous organs of the experimental animals, we detected that it was more remarkable in kidneys than in other organs. Quite similar apoptosis was seen in the liver of the SOR and PSR animals. Taking into account the available data from literature, it is difficult to fully explain these processes; yet it is known that the cytokine TNFα exhibits anti-apoptotic action, and is possibly involved in the regulation of the apoptosis process. Studies have revealed that the level of apoptosis in the intestinal epithelium is influenced by injections of ferrous dextrane; after those apoptosis was more remarkable in older rats. In the epithelium of younger rats the apoptosis was influenced less by the injections of ferrous dextrane.

Thus it is proven, that sepsis does not play a crucial role in promoting apoptosis in young animals, although apoptosis in older animals could be more influenced by sepsis *(Javadi et al., 2004)*. Performing experiments with animals other investigators concluded that after artificially caused septic complications the enhanced apoptosis can decrease survival *(Iwata et al 2003; Turnbull at al., 2004)*. *Iwata* describes that apoptosis in the splenic tissue is greatly dependent on the levels of secreted myeloid differentiation factor in the body *(Iwata et al., 2003)*. It proves once more that the course of the immune processes after sepsis in case of splenectomy is not clear. That makes it important to develop the technique of spleen-saving operations in children, and to analyze its possible complications.

In the clinical trial all the included patients were observed postembolization syndrome after PSE. Several groups of investigators describe that the severity of postembolization syndrome (pain the left epigastria, hyperthermia) mostly depends on the amount of splenic tissue exposed to embolization *(Shah et al., 1990; N'Kontchou et al., 2005)*. For relieving the general condition of the patient the investigators suggest performing PSE in the amount of 20–30%. The authors consider that if necessary, PSE can be performed repeatedly *(Hirai et al., 1987; Meral et al., 2000; Lee et al., 2007)*. Analyzing the data of the study included in the thesis, we concluded that PSE in the amount of 20–35% is not sufficient. Out of five PHS patients three did not show platelet count elevation to the normal limit, but in one patient the platelet count elevated to sub-normal level in 1 year after PSE. In patients of other groups, which had PSE performed in amounts of 60–80% and 80–90%, the results of this treatment were better. Unexpectedly we recognized that the severity of the postembolization syndrome not always depends on the amount of splenic tissue affected by infarction during PSE. In several patients with greater embolized amount of splenic tissue, the therapy duration for pain relief and hyperthermia was shorter than in patients, which had been performed PSE in smaller amount. For example, patient No 47 had PSE performed in the amount of 20–35%, the duration of pain relief was 14 days after PSE, but in patient No 19, whose splenic tissue had been embolized in twice the amount (60–80%), the pain relief therapy was necessary for only 5 days after PSE. When comparing the duration of hyperthermia, we detected that the patient No 47 with PSE in the amount of 20-35% had hyperthermia for 10 days, whereas the patient No 38 with PSE in the amount 80–90% had hyperthermia for only 6 days. Similar data were seen in analysis of the hospitalization time after PSE. For example, the patient No 37 with PSE in the amount of 20–35% spent 14 days in the hospital; but the patient No 29 with PSE in the amount of 60–80% had hospitalization time of only 8 days. Results of this study show that the severity of postembolization syndrome does not depend only on the amount of the splenic tissue with infarction. Several groups of investigators note that the spleen not only has the specific function of building an immune reaction, but it also is a reservoir. The spleen is storage of both, macrophages and cytokines *(Kimura et al., 2002; Zhu et al., 2008)*. Similar data is collected also in

the experimental part of the thesis, where the highest levels of IL-10, TNFα and HβD-2 in experimental animals of all groups were seen in the tissue of the spleen. Thus, possibly the basis of the postembolization syndrome is not only aseptic inflammation of the splenic tissue but also some kind of "shock" of the all immune system of the body. There are also other authors that point out the role of the spleen in the regulation of the autoimmune processes *(Kimpel at al., 2003)*. We could speculate that in cases of disturbed splenic reservoir function after PSE the levels of cytokines are elevated both, in blood and parenchymatous organs. Possibly, it could affect the intensity of hyperthermia after PSE. Additional research is necessary to confirm this. Several authors describe that right after PSE the splenic tissue is edematous, "paralyzed", and filled with blood. That explains the pain syndrome in the period after embolization *(Spigos et al., 1979; Yamauchi et al., 1994)*.

Before starting this thesis, it was believed in the Clinics of Pediatric Surgery that the intensity of postembolization syndrome mainly depends on the size of the spleen, and the amount of tissue being embolized. Thus, in several patients with remarkably enlarged spleen we declined PSE and performed splenectomy, for prevention of the consequences of large infarction of splenic tissue (prolonged hyperthermia, pain, rupture of splenic tissue). Now we consider this wrong.

Analyzing the results of this study (30 PSE performed in UCH during eight years), there were no cases of splenic rupture after PSE. Reports about this kind of possible complication are made by, for example *Spigos (Spigos et al., 1979)*. Several other authors, who used PSE in treatment of patients with hypersplenism, point out that PSE is a safe method, and, if necessary, it can be repeated *(Ohmoto et al., 2006; Lee et al., 2007)*. Even if several authors consider PSE as a safe method, during this procedure the same level of sterility must be obtained as in the operating room. *Johansson* et al suggest that for prevention of infectious complications the material used for embolization (particles of polyvynilalcohol) should be mixed with an antimicrobial i.e. Gentamicin before PSE *(Johansson et al,. 1985)*. But *Brandt* reports that the risk of complications after PSE is lower if the procedure is performed in a smaller amount (20–40%) *(Brandt et al., 1989)*. In the thesis study we concluded that the frequency of complications is not always connected to the amount of PSE. For example, the Patient No 17 with PHS (PSE of 20 – 35 %) had septic complication, e.g. abscess of splenic tissue. Whereas other PHS patients with hypersplenism and much greater amount of splenic tissue infarction (80 – 95%) did not have septic complications. The Patient No17 had prolonged hyperthermia and pain in the left epigastria when performing the deep palpation of the abdomen. The possible cause of these complications is the infection brought in during PSE, although the procedure is performed in sterile conditions. The other possible cause could be intolerance of the microspheres (0,1 mm - 0,125 mm diameter). In 2002, when patients were performed PSE the *Contour's* micro particles were not available in Latvia. Abscess of the splenic tissue with following signs of fibrinous pleuritis were observed also in the Patient No 11 with

HS (PSE of 60 – 80%). For reducing the possibility of recidiving hypersplenism (the spleen receives additional blood flow from *a.a. gastricae breves* that can branch off the artery in the upper pole of the spleen), the patient had PSE performed in the splenic artery in the upper pole and in the middle branches, leaving the lower pole *a.lienalis* intact. We consider that the PSE performed in the upper pole branch could promote the forming of the exudate in the pleural cavity. Several authors describe that the irritation of the diaphragm, causes by the PSE in the spleen, could promote exudation in the pleural cavity. Despite this several authors describe that the PSE in the upper pole could be more effective due to the anatomical properties of the splenic blood flow *(Carlos et al., 1989; Yuamauchi et al., 1994; Sato et al., 2000; Petersons et al., 2002)*. Doubts about the real cause of these complications in the Patient No 11 are caused also by the fact, that nine days after PSE the child was discharged in a good condition, and hyperthermia and complaints about pain in the left epigastria appeared only after 30 days. Possibly the complications were promoted by the disruption of the reservoir function of the spleen during PSE, which caused elevated levels of cytokines and defensines in other parenchymatous organs (lungs, liver, kidneys) and in blood, also disrupting their relationship. There are additional studies necessary to prove this conception. It is less credible, that the abscess of the spleen formed due to intolerance of the *Contour's* microspheres (250-355 mkm).

All patients with HS after PSE were consulted by a hematologist, who considered the level of bilirubin as acceptable for patients after PSE. In a part of the patients the elevated level of bilirubin could be explained with the hemolysis process, taking place in the remaining part of the spleen, which indirectly shows that the remains of the spleen, possibly, are very good vascularized, and that it takes part in immune processes. The fact that none of the HS patients had hemolytic crisis after PSE with necessity of erythrocyte mass transfusion should be considered as very important.

Comparing PSE with other splenic tissue reducing operations, it is relatively new method of treatment of hypersplenism, which had not been widely used in Europe. This procedure had been introduced in the clinical practice gradually, so the true results of the treatment could be estimated only after several years. *Pratl B, Benesch M, Lackner H* and other authors point out that PSE is not a widely used method for treatment patients with HS, but it is safe and effective. It is a good alternative for splenectomy or resection of the spleen. When analyzing the usefulness of PSE in cases of HS, several authors report that patients with HS are more prone to PSS than, for example, patients with PHS. These peculiarities of hematological patients (HS, chronic idiopathic thrombocytopenic purpura, beta talassemia) are explained with decelerated blood flow in the splenic tissue, and that decreases the filtering function of the spleen *(Wang et al., 2003; Norgaard et al., 2006). Pratl et al. (2007)* point out that PSE is a good alternative in treatment of hematological patients. Despite the abovementioned, several problems were encountered during the work on the thesis. For example, the Patient No 3 had elevated level of total bilirubin (51,0 μmol/l) one

year after the PSE. In the second year after PSE splenectomy was performed. Taking into account the experience gathered during the work on thesis, it would be more useful to perform PSE instead of extirpation of the spleen. In the Patient No 5 with HS, whose bilirubin level was slightly elevated above the upper limit (39,0 μmol/L; the splenic tissue reduction operations are not performed up to 34 μmol/L), a repeated PSE could possibly be postponed, the procedure could have been repeated, when the level of total bilirubin would rise.

Describing the usefulness of PSE in patients with PHS, *Chikamori* and *Yoshida et* al concluded that as a result of the procedure the portal tension is decreased by reducing the venous blood flow through *v. lienalis (Chikamori et al., 2004; Yoshida et al., 2008)*. Several investigators point out that the decrease in the portal tension is the key to successful treatment of varicose veins of the esophagus and the stomach *(Howard et al., 1988; Hirota et al., 1999; Takahashi et al., 2009)*. Some authors studying changes in hemodynamic in patients with PHS after PSE concluded that by reducing the arterial blood flow through *a. lienalis* as compensation the blood flow through *a. hepatica* and *a. mesenterica superior* increases. Thus promote liver function (the levels of cytokines from the gastrointestinal tract in the liver is elevated). It must me pointed out, that the mentioned hemodynamic changes can form in a course of several years after PSE *(Yoshida et al., 2008; Takahashi et al., 2009)*. Other authors have concluded that by reducing the venous blood circulation through the spleen the *vv. gastricae breves* receive less amount of blood, thus reducing the varices of the stomach *(Adams et al., 1990; Mcdermott et al., 1995)*.

One patient with PHS had angiography performed before PSE, and abnormal splenic blood flow was detected. Taking into account the patient's age (16 years), thus the risk of PSS is lower than in younger children, splenectomy was performed. Reconsidering this, we can say it was not right. The patient had to have PSE performed, which, possibly, would have to be repeated. Perhaps it would be more suitable to perform partial resection of the spleen.

The evaluation of the platelet count was performed from the 2nd to the 8th day, which is quite a big interval, so the results could be incorrect. The patients, in whom the platelet count was detected on the 8th day after PSE, it could have been higher than in patients who had it detected on the 2nd day, when possibly not enough time had passed after treatment for the platelet level to raise. Also, the fact that PSE is relatively new method of treatment should be taken into account. When initiating it, there were no available data about the optimal time point for performing blood tests to evaluate the level of platelets after the operation (the number of studies like this is still very limited). That is why the detection of platelet count was performed in various time intervals (from the 1st to the 8th day) after PSE. Additionally to this, when initiating the method of PSE, in the available literature in Latvia it was noted that the platelet count will definitely rise. As the main result of treatment of hypersplenism we adopted the elevation of platelet count in 2 – 3 and more years after PSE.

Evaluating the level of platelets in the patient No 44 after PSE in the amount of 60 – 80% with recidive of hypersplenism, we concluded that platelets increased after the procedure (up to 107 000 uL in 7 years), and the count is not lower than it was before PSE (81 000 uL). The platelet count above 100 000 uL is not considered critical and dangerous *(Stanworth et al., 2010)*. Of course, the patient needs repeated PSE, but 3 years after the procedure when the platelet count was 123 000 uL, there were no guidelines that would note, when precisely PSE should be repeated. In the 4th, 5th and 6th year after the procedure the patient did not return for a follow-up, as he felt well. The low level of platelets was revealed during the study for this thesis, when the patients were telephonically asked to arrive at UCH for scheduled check-up. When analyzing the changes in platelet counts in the patient in 7 years after PSE, there is no reason to believe, that the platelet level will decrease rapidly, in a short period of time, so we believe it is best to repeat the procedure as a planned intervention.

When analyzing the platelet level in the patient No 48 (PSE of 60 – 80%), we concluded that the elevation of the platelet count is not sufficient, and the procedure must be repeated in the amount of 80 – 90%. Before the procedure the patient has to undergo a computed-tomography and angiography for diagnosing possible additional spleen, which could be the cause of the ineffective PSE. Several authors point out that in patients with recidive of hypersplenism or insufficient elevation of platelet count after PSE MRI is indicated, to rule out the possibility of additional spleen *(Gibson et al., 1986; Akwari et al., 1987; Schilling et al., 1997; Schiller et al., 1998; Pratl et al., 2007)*.

It must be pointed out, that evaluating the leukocyte count after PSE none of the patients with PHS with inadequate platelet count or recidive of hypersplenism with decrease of platelets after PSE, were not found to have decreased leukocyte count under the critical limit. There are further studies necessary to explain the abovementioned, but it is possible, that the leukocyte count does not play a crucial role in the clinical signs of hypersplenism.

Based on the research of several authors, who describe the ability of the splenic tissue to regenerate, and also the results of the study included in the thesis, it can be presumed that the optimal amount of PSE could be 80 – 90% (*Muftuogly et al., 2000; Pratl et al., 2008)*. There is some doubt about the concluded in the experimental part of the thesis, that one third of the splenic tissue in experimental animals is too small for protection of the artificially caused sepsis of *Streptococcus pneumoniae*. Perhaps the survival rates in the group of PSR rats would be higher, if the rats were given longer recovery time, and the remnants of the spleen could regenerate in the period of 4 – 6 months after the operation, and only then the challenge with *Streptococcus pneumoniae* was performed. It is possible, that the results of the experimental study must be evaluated critically, and they cannot be directly related to humans. Currently there are no other experimental models than experimental animals that could help in evaluation of the impact of various pharmaceuticals and operations to the human body.

When analyzing the changes in the longitudinal size of the spleen after PSE, there are some inaccuracies detected in the spleen size measuring before PSE. That is the

reason why the changes in the size of the spleen and their relation to the postembolization syndrome could not be explained in this study. There was a tendency observed that not always the patients with greater size of the spleen had more severe postembolization syndrome. In all children the spleen dimensions were detected by US. But it must be noted that until 2009 all procedures of PSE were performed in the department of Invasive radiology of the Pauls Stradins Clinical University Hospital. These patients were at first hospitalized in UCH, where all the necessary tests were performed and indications for PSE were established. In many cases PSE could not be performed straight away, thus the child was discharged from the UCH. Before performing PSE the US was not repeated. Due to this many children had not been performed detection of dimensions of the spleen, and the longitudinal dimension before PSE could not be included in the study. Also the fact that PSE is relatively new method should be taken into account, and the experience in performance and follow-up of the patients is not great even in the world. In the available literature the evaluation of the efficacy of PSE in patients with PHS is based mainly on the elevation of the platelet count *(Wang et al., 2006; Takahashi et al., 2009)*.

When creating the questionnaire for the parents and analyzing the available data from literature we did not find any publications with evaluation of quality of life in patients after PSE. That made the analysis of the parents' questionnaires complicated; there are no data from other authors for comparing the results. It is possible that these results would be more correct, if all parents would be interviewed after the same period of time after PSE, e.g. on the 1st and the 3rd year after the procedure. As during the work on the thesis the questionnaire was performed in different periods after PSE, possibly we have acquired more thorough range of answers. Before the questionnaire we prognosticated that the patients with shorter period of time after PSE (1 – 3 years) would have more remarkable complaints than the patients with PSE performed longer time age (4 – 7 years). Although by analyzing the answers we did not prove it. From questioned 14 PHS patients after PSE four had complaints of abdominal pain during physical exercise. Two patients had PSE performed 7 years ago, one – 2 years, and the fourth one – 6 years ago. Analyzing the complaints in connection with the amount of PSE there was no correlation; the patients with greater splenic infarction did not have more severe complaints. None of the patients with PHS noted that new complaints appeared after PSE. It means that before PSE these children had both, abdominal pain, and pain in the left epigastria, with or without physical exercise. It is possible that these complaints are due to hypersplenism, rather than PSE.

When questioning the parents of the HS patients about the possible complaints after PSE, one patient had headache, another one – scleral jaundice in cases of psycho emotional stress, which is more or less characteristic in all HS patients *(Szold et al., 2000)*. It must be stressed out, that in the second year after PSE this patient had a level of total bilirubin of 37,6 μmol/l, and it corresponds with the HS of medium severity. It is possible that in case of aggravation of these complaints the PSE might be repeated.

The patients with HS, just as the PHS patients, did not show any correlation in

the answers to the questions about abdominal pain – the shorter the time after PSE, the more severe complaints. Five patients pointed out rare episodes of pain in the left epigastria, but the time periods after PSE varied. Evaluating the complaints in children with various amounts of splenic infarction, the data were similar. By the analysis of the parents' questionnaire we can conclude that the minimal amount of new complaints, appearing after PSE, indirectly shows that none of the patient had symptoms that affected quality of life after PSE, comparing as it was before the procedure.

Goff and *Berner* et al describes the possible complications after sclerotherapy of varicose veins of esophagus and stomach, which are gastroesophageal reflux disease, esophagitis, gastritis, gastric ulcer and perforation of the esophagus *(Goff et al., 1988; Berner et al., 1994)*. Questioning the parents of the patients with PHS that were treated with the sclerotherapy, we concluded that 2 out of 14 patients marked bitter and acidy taste in the mouth. When answering questions about heartburn or discomfort behind sternum, four patients noted they had rare episodes of these complaints, two patients had regular episodes of those. That could indicate the disturbances of motility in the esophagus due to endoscopic sclerotherapy. It also must be considered that 38 out of 40 patients with PHS had PHS gastropathy, which could manifest with similar complaints *(Barakat et al., 2007; El-Rifai et al., 2007)*. That makes it difficult to conclude whether these complaints are due to gastropathy or as a sequel after the FEGS sclerotherapy. A hypothesis can be made that the complaints could be reduced by individually adjusted frequency and course of sclerotherapy procedures for treatment the varicose veins of esophagus and stomach. A hypothesis can be made that the complaints could be reduced by individually adjusted frequency and course of sclerotherapy procedures for treatment the varicose veins of esophagus and stomach. Presumably the attempts to detect the optimal count of FEGS sclerotherapy procedures in each patient were successful, as the patients were saved from additional (unnecessary) sclerotherapy, which could promote development of gastroesophageal reflux. Therapy with proton pump inhibitors in patients with PHS gastropathy is a very important tool for reducing complaints.

Several authors describe that gastropathy in patients with PHS can simulate bleeding from the varices of esophagus and stomach, which can be seen as hematemesis or melaena *(Barakat et al., 2007; El-Rifai et al., 2007)*. In the clinical study, and in the analysis of the case histories of the patients with PHS with bleeding from the gastrointestinal tract, we observed similar picture. Sometimes during FEGS it is not possible to diagnose the real cause of bleeding, it could be either, bleeding from varicose veins of the esophagus and the stomach, or in the background of esophageal and gastric varices the bleeding comes from the injured mucous membrane of the stomach because of gastropathy. In any case all these situations require sclerotherapy, and administration of proton pump inhibitors *(El-Rifai et al., 2007)*.

Analyzing the case histories of the patients from UCH we observed that in all patients in cases of bleeding varices of the esophagus and stomach endoscopic sclerotherapy was successful, and stable hemostasis was acquired. It must be pointed

out that blood vessel shunt operations were not performed in treatment of PHS patients in UCH. The analysis of the data about patients included in this study shows that the indications for shunt therapy must be set advisedly, and they are not always necessary for children. Several authors point out that as a result of sclerotherapy of varices of the esophagus and stomach the venous blood flow is reduced in esophageal and gastric walls and mucous membranes. Alternative venous collateral net is formed in the great oment. That reduces the portal tension, and in cases of prehepatic obstruction spontaneous improvement could be possible *(Poddar et al., 2000; El-hamid et al., 2008)*. Several authors also report that endoscopic sclerotherapy has become the leading method in the world for treating acute bleeding from varices of the esophagus and stomach, and it is successfully used also for prevention of acute bleeding *(Toubia et al., 2008; Monici et al., 2009)*.

PSE is not the only method of treatment for hypersplenism syndrome. In case of emergency splenectomy many investigators suggest auto transplantation of the spleen by suturing the splenic tissue in the great oment *(Resende et al. 2003)*. Animal studies show that rats after the spleen's auto transplantation can better survive artificially causes sepsis of *Streptococcus pneumoniae,* compared to the rats that had not had it. *Rice* and *Diesen* et al describes an operation of resection of the spleen, with 80 – 90% splenic tissue resection *(Rice et al 2003; Diesen et al., 2008)*. Patients with the syndrome of hypersplenism usually have severely enlarged spleen. In cases when 80 – 90% resection of the splenic tissue is necessary, it is difficult but possible to examine the architectonics of the blood vessels. Sometimes it is difficult to distinguish, which of the many branches of blood vessels in the fragment of the spleen is an artery, and which one is a vein. The disadvantage of the operation is poor cosmetic effect due to the large post-operative scar in the frontal abdominal wall. Some scientists believe that laparoscopic resection of the spleen is favorable alternative for treatment of hypersplenism. Authors also acknowledge that it is easier to examine the anatomy of the spleen by laparoscopy, and also the cosmetic outcome is better *(Meral et al., 2000; Tomikawa et al., 2009)*. In any event, none of the methods mentioned above is perfect for treating hypersplenism. The advantage of PSE is that it can be performed without general anesthesia in teenagers (10 – 18 years old), and it is possible to examine the architectonics of the blood vessels. The disadvantage of PSE is the postembolization syndrome, which can be detected in average 10 – 12 days after operation, and which requires medicaments' therapy. Despite the fact that PSE is performed with specific micro-catheters, traumatization of the arterial wall is still possible. But one of the main advantages of PSE is the possibility to repeat if necessary. In cases of laparoscopic resection of the spleen, repeated operation can be complicated by the possible adhesions in the abdominal cavity.

It is important to examine, whether the saved part of the spleen can function as the blood filter and perform its immune function. Unfortunately these possibilities are limited. *Moffett* et al describe the significance of *Howel–Jolly* bodies in the examining the immunological function of the spleen. The authors conclude: the less the *Howel–Jolly*

bodies in the peripheral blood smear, the better the filtering and immune function of the spleen. In this thesis we did not detect the *Howel–Jolly* bodies. The aim of the blood count control is to evaluate the risk of PSS in patients. It is possible that a special register should be formed for patients after splenic tissue reduction operations. Patients with detected *Howel–Jolly* bodies in the peripheral blood smear in cases of any inflammation must receive antimicrobial therapy *(Moffett et al., 2009)*.

Several investigators report that in 75%, and more often in children, prehepatic obstruction form of PHS are diagnosed. The etiology is often unclear, but if it has been revealed, the most frequently detected reasons are catheterization of the umbilical vein or omphalitis in newborns *(Guimaraes et al., 1998; Shah et al 2000; Orloff et al., 2002)*. The hepatic PHS is seen less frequently. It is explained by several factors: children do not have addictions and hepatic tissue damage from alcohol *(Ashcraft et al., 1997)*. The hepatic PHS is more characteristic in children with biliar atresia and congenital liver fibrosis. Often the condition of these patients is very severe, and their survival is only a couple of years, so the number of these patients is very limited *(Sigalet, 2000)*. *Orloff* et al report that in pediatrics mainly prehepatic obstruction PHS is observed, mainly due to *v.portae* obstruction (67% cases).

Analyzing the available literature we have to conclude that patients with prehepatic obstruction PHS can experience disturbances of liver function. It is explained by hemodynamic changes in the *v.portae* system. It is well known that the liver tissue receives 2/3 of the blood through the portal vein. When the blood flow in *v.portae* is compromised, the hepatic cells receive less necessary nutrient, which can cause formation of fibrosis or cirrhosis *(Dhiman et al., 2002; Abramowsky at al., 2003)*. *Poddar* and *Shettino* et al concluded that children with prehepatic obstruction PHS did not have such severe bleeding episodes from esophageal and gastric varices, and the hemostasis was easier achieved than in patients with hepatic PHS. The investigators have proved that most children with prehepatic obstruction PHS have chances of spontaneous improvement *(Poddar et al., 2000; Shettino et al., 2006)*.

Lebrec, when describing the role of beta blockers in treatment of PHS, reports that they reduce the constriction of intrahepatic fibroblasts, and activates the stellatae cells of the liver *(Lebrec, 1994)*. Thus, they are recommended for treatment in patients with PHS. Despite the fact that patients after discharge from the hospital are recommended to use beta-blockers, only two of the questioned patients received the medication as out-patients. It shows that the feedback between the consultant in the hospital, general practitioners and parents must be improved; perhaps informational leaflets must be created. *Ling* describes that the significance of beta-blockers in treatment of PHS is still not fully understood, and the dosage of the preparations, especially in children, must be adjusted individually *(Ling, 2005)*. Some investigators report that insufficient doses of beta-blockers will not provide the necessary effect for reducing the risk of bleeding *(Shashidhar et al., 1999; Bosch et al., 2009)*.

Taking into account the results of the study, and the available data from literature, we can conclude that the treatment of patients with PHS is still complicated, and

the basis of it is sclerotherapy of esophageal and gastric varices. PSE in the amount of 80 – 90% is safe not only in patients with PHS hypersplenism but also in HS patients. Further studies are necessary to explain the significance of the immune function of the spleen and its role in the origin of PSS.

CONCLUSIONS

1. PSE is safe and effective method for treatment of hypersplenism in patients with PHS and HS. The optimal amount of PSE is 80 to 90%.
2. Insufficient efficacy of treatment of hypersplenism, and also recidiving hypersplenism requires repeated PSE.
3. Endoscopic sclerotherapy is effective and safe for treating and preventing acute bleeding from the varicose veins of the esophagus and the stomach in patients with PHS.
4. The stage of hypersplenism and amount of PSE does not determine the severity of postembolization syndrome.
5. The survival of partially splenectomised rats after *Streptococcus pneumoniae* sepsis is greater than in splenectomised rats. Sepsis of *Streptococcus pneumonia* causes death in 100% of splenectomised rats, and in 90% of partially splenectomised rats.
6. 1/3 of the splenic tissue cannot fully protect experimental rats from *Streptococcus pneumoniae* sepsis, but it functions as a factor for sepsis prevention. It is based on the similar expression of IL – 10, TNFα and HβD-2 in the spleen of partially splenectomised and control group rats.
7. Relative amount if IL-10 in the parenchymatous organs (lungs, liver and kidneys) of SPR rats is statistically significantly higher than in the parenchymatous organs of PSR, SOR and CGR rats.
8. Remarkable apoptosis in the kidneys and lungs of the experimental animals show that the programmed cell death in the spleen is not the most significant molecular shift in cases of partial splenectomy. The great amount of apoptotic cells in kidneys and lungs could be explained with dealing with the consequences after partial splenectomy in those parenchymatous organs, as a compensatory response reaction to the operation and sepsis.

CLINICAL RECOMMENDATIONS

1. Patients with acute upper gastrointestinal bleeding must be hospitalized in the intensive care unit of the hospital, where continuous hemodynamic (pulse, blood pressure) monitoring and stabilization should be initiated. Erythrocyte mass transfusion is indicated if the level of hemoglobin falls below 8 g/dL, and hematocrit is lower than 26.

2. Patients with upper gastrointestinal bleeding must have emergency FEGS performed. When bleeding varicose veins of esophagus and stomach are found, sclerotherapy must be performed.
3. After sclerotherapy the patient must be monitored in the intensive care unit for 24 hours, with controlling hemodynamic parameters and anemia.
4. Sclerotherapy must be continued until all varices of the esophagus and stomach have been treated. The endoscopic control of the esophageal and gastric varices must be performed no less than once per year.
5. PHS patients with hypersplenism require PSE, if the platelet count in peripheral blood is less than 150 000 uL, leukocyte count is less than 3 000 uL, and enlarged spleen is detected. HS patient require PSE if the level of total bilirubin is higher than 34 μmol/L.
6. Duration of pain relief therapy and antipyretics use after PSE must be adjusted to each patient individually.
7. In patients with PHS after PSE platelet and leukocyte count in peripheral blood, levels of inflammatory markers (C reactive protein) must be controlled on the 1st, 2nd, 3rd and 7th day after the procedure, also the size of the spleen must be controlled by ultrasound.
8. In patients with HS after PSE bilirubin and levels of inflammatory markers (C reactive protein) must be controlled on the 1st, 2nd, 3rd and 7th day after the procedure, also the size of the spleen must be controlled by ultrasound.
9. After PSE the follow-up of the patient must be performed no less than once per year: 1) patients with PHS require control of platelet and leukocyte count in the peripheral blood, and detection the changes in dimensions of the spleen; 2) patients with HS require control of bilirubin level, and detection the changes in the dimensions of the spleen.

ALGORITHM FOR TREATMENT AND FOLOW-UP OF PATIENTS WITH PORTAL HYPERTENSION SYNDROME

Treatment and follow-up of PHS patients with varicose esophageal and gastric veins

Patients are divided in 3 groups:

Group 1 – primary patients with splenomegaly of unknown etiology, without history of bleeding; Group 2 – patients with acute bleeding from esophageal and gastric varicose veins; Group 3 – patients with varicose veins of esophagus and stomach, with or without history of bleeding

Algorithm for treatment and follow-up of the patients of Group 1

1. FEGS is performed to evaluate the varices of the esophagus and the stomach. If necessary, sclerotherapy of the varicose veins of esophagus and stomach is initiated.
2. Platelet and leukocyte count is evaluated in peripheral blood smear. Liver tests (total and direct bilirubin, alaninaminotransferase, aspartataminotransferase).

3. In suspicious cases about hepatic PHS form markers of hepatitides should be examined, and liver needle biopsy performed.
4. The longitudinal dimension of the spleen is detected by US. Blood flow in the liver and in the spleen is measured by Doppler US. For evaluating the portal blood circulation CT angiography (KMR angiography) should be performed.
5. When hypersplenism syndrome is diagnosed, PSE in the amount of 80 – 90% should be performed.

Algorithm for treatment and follow-up of the patients of Group 2

1. In cases of massive bleeding, and if FEGS and sclerotherapy are not available, *Sengstaken-Blakemore* tube must be introduced (patient has indications for endotracheal intubation).
2. Endoscopic evaluation of the esophageal and gastric veins should be performed (FEGS), and sclerotherapy of the bleeding varices initiated.
3. Systemic pharmacological therapy is started (Somatostatin) for reducing the blood pressure in the portal system.
4. If the bleeding is stopped, planned course of sclerotherapy procedures is necessary until all varices of the esophagus and stomach are treated. Further endoscopic treatment is similar as in patients of Group 3. Parallel to sclerotherapy continuous medicaments' therapy is started (beta-blockers, e.g., propranolol). Therapy with proton pump inhibitors should be continued for 3 – 4 weeks.
5. If the bleeding from the esophageal and gastric varices continues after initiating of sclerotherapy, *Sengstaken-Blakemore* tube must be introduced repeatedly. Sclerotherapy is re-initiated in 24 hours, and continued once every 24 hours, until stable hemostasis is achieved. Transfusion of erythrocyte mass is indicated if the level of hemoglobin falls below 8 g/dL, and hematocrit is lower than 26.
6. Pharmacological therapy with proton pump inhibitors for suppressing gastric secretion should be started.

Algorithm for treatment and follow-up of the patients of Group 3

1. FEGS is performed, if it is necessary to perform sclerotherapy, the frequency is determined on individual basis, depending of the severity of the esophageal and gastric varices. In children with esophageal and gastric varices of Grade 3 sclerotherapy must be performed once a month. In patients with varices of the Grade 2 – 3 sclerotherapy is performed once every 2 months. In patients with Grade 1 – 2 varices, sclerotherapy is performed once every 3 months. If the sclerotherapy is not necessary, repeated FEGS are performed twice a year.
2. Pharmacological therapy is started with beta-blockers (propranolol).
3. When PHS gastropathy is diagnosed, proton pump inhibitors should be initiated.
4. Control or peripheral blood count (platelet and leukocyte count), liver tests (every time before FEGS).
5. The longitudinal dimension of the spleen is detected by US. Blood flow in the liver and in the spleen is measured by Doppler US. For evaluating the portal

blood circulation CT angiography (KMR angiography) should be performed.
6. When hypersplenism syndrome is diagnosed, PSE in the amount of 80 – 90% should be performed.
7. In suspicious cases about hepatic PHS form markers of hepatitides should be examined, and liver needle biopsy performed.

Treatment and follow-up of the hypersplenism syndrome in PHS patients

1. PSE in the amount of 80 – 90% should be performed.
2. After PSE no less than once every 6 months platelet and leukocyte count should be controlled in peripheral blood. No less than once a year ultrasound scan for abdominal cavity (evaluation of the longitudinal dimension of the spleen) needs to be performed.
3. Patients with recidiving hypersplenism syndrome or insufficient correction of hypersplenism after PSE must have repeated PSE performed in the amount of 80 – 90%.

PUBLICATIONS AND PRESENTATIONS CONNECTED WITH THE PROMOTIONAL WORK

PubMed publications:

1. A.Petersons, **O.Volrats**, A.Bernsteins. The first experience with non-operative treatment of hypersplenism in children with portal hypertension. Eur J Pediatr Surg, 2002; 12, p.299-303.
2. **O.Volrats**, M.Pilmane, A.Petersons. Postsplenecomy sepsis in splenectomized, partially splenectomized, and non-splenectomized rats after Streptococcus pneumoniae challenge. Eur J Pediatr Surg 2011; 21, p.65-69.

Publications in peer-reviewed medical and scientific journals of Latvia:

1. **O.Volrats**, M.Liepina, J.Krasts, U.Ligers, A.Bernsteins, A.Dombrovskis. Is partial splenic embolization the alternative to splenectomy for treating the hypersplenism? Latvijas Ķirurģijas žurnāls, 2002; Nr.2, lpp.50-54.
2. **O.Volrats**, A.Petersons, M.Pilmane. Spleen saving procedure necessity in children with portal hypertension and hematological disease to avoid overwhelming postsplenecomy sepsis infection. Latvijas Ķirurģijas žurnāls. 2007; Nr.7, lpp.53-56.
3. **O.Volrats**, M.Pilmane, A.Petersons, V.Nikolajeva, A.Ribakovs. Changes in parenhimatous organs after *Streptococcus pneumoniae* challenge in the splenectomized, partially splenectomized and non-splenectomized rats. Rīga Stradiņš University, Collection of scientific papers 2008; p.131-140.

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1. J.Krasts, U.Ligers, **O.Volrats**, I.Rikša. Barības vada varikozo vēnu endosklerotizācijas iespējas bērniem ar portālās hipertensijas sindromu (PHS). 1st Congress of the Surgeons of Latvia. 2000; p.104.
2. **O.Volrats**, A.Bernsteins, U.Ligers, M.Liepina, J.Krasts, I.Svekle. Is partial splenic embolization the method of choice of the treatment of hypersplenism for patients with portal hypertension? 6-th Conference of the Baltic Association of Pediatric Surgeons. Riga, 2000; p.121.
3. **O.Volrats**, A.Bernsteins, U.Ligers, M.Liepina, J.Krasts, I.Svekle, V.Ozolins. The first experience of non-operative treatment of hypersplenism in children with portal hypertension. 4-th European Congress of Pediatric Surgery. Budapest, 2001; p.15-16.
4. **O.Volrāts**, M.Liepiņa, J.Krasts, U.Ligers, A.Bernšteins, A.Dombrovskis. Portālās hipertensijas sindroma (PHS) izsaukta hipersplenisma ārstēšana bērniem. Latvijas Medicīnas akadēmija / Rīgas Stradiņa universitāte. RSU Scientific Conference 2002; lpp.97.
5. **O.Volrats**, M.Liepina, J.Krasts, U.Ligers, V.Ozolins, I.Svekle. Analysis of Partial Splenic Embolization (PSE) in Hypersplenism Patients. 7-th Conference of the Baltic Association of Pediatric Surgeons. Kaunas, 2002; p.28.
6. **O.Volrats**, J.Krasts, U.Ligers, M.Liepina, A.Bernsteins, V.Ozolins. Partial splenic embolization – the treatment of hypersplenism in portal hypertension patients. Baltic States Congress on Hepatology. Riga, 2002; p.40-41.
7. **O.Volrats**, A.Dombrovskis, K.Kupčs, M.Liepiņa, U.Ligers, J.Krasts, Z.Ābola. Vai bērniem ar portālās hipertensijas sindroma (PHS) izsauktu hipersplenismu (HSP) ir indicēta splenektomija un maģistrālo asinsvadu šuntēšana? Rīgas Stradiņa universitāte. RSU Scientific Conference 2004; lpp.124.
8. **O.Volrats**, J.Krasts, U.Ligers, A.Dombrovskis, K.Kupcs, V.Ozolins. Partial splenic embolization in hypersplenic portal hypertension patients and variceal bleeding. 8th Conference of the Baltic Association of Pediatrics Surgeons. Tartu, 2004; p.13.
9. **O.Volrats**, M.Liepina, J.Krasts, U.Ligers, V.Ozolins, K.Kupcs, A.Dombrovskis. Non-operative treatment of hypersplenism (three year follow-up). World Congress of Pediatric Surgery. Zagreb, Croatia, 2004. Abstracts (CD – Nr. 451).
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11. **O.Volrats**, A.Petersons, M.Liepina, J.Krasts, I.Rikša, V.Dulbinska, A.Ribakovs. The significance of shunting procedure today in the treatment of portal hypertension in pediatric patient. 9th Conference of the Baltic Association of Pediatric Surgeons. Riga, Latvia, 2006.

12. **O. Volrats**, A.Leontīne, E.Poppela, J.Petļa, G.Innus, M.Osipova, N.Osipova, S.Semjonovs, H. Gailišs, A. Ribakovs, A. Pētersons. Eksperimenta laboratorijas žurku vispārējās anestēzijas metode. RSU Scientific Conference 2007; lpp.63.
13. **O.Volrats,** M.Pilmane, A.Petersons. S*treptococcus pneumoniae* challenge in the splenectomized, partially splenectomized and non-splenectomized rats. RSU Scientific Conference 2009; p.224.
14. **O.Volrāts**, A.Petersons, V.Ozoliņš. Daļēja liesas embolizācija portālās hipertensijas sindroma un iedzimtas sferocitāras anēmijas hipersplenisma slimnieku ārstēšanā. RSU Scientific Conference 2011; lpp.264.

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1. **O.Volrats**, A.Bernsteins, U.Ligers, M.Liepina, J.Krasts, I.Svekle, V.Ozolins. The first experience of non-operative treatment of hypersplenism in children with portal hypertension. 4-th European Congress of Pediatric Surgery. Budapest, 2001. (oral presentation)
2. **O.Volrats**, M.Liepina, J.Krasts, U.Ligers, V.Ozolins, K.Kupcs, A.Dombrovskis. Non-operative treatment of hypersplenism (three year follow-up). World Congress of Pediatric Surgery. Zagreb, Croatia, 2004. (oral presentation)
3. **O.Volrats**, A.Bernsteins, U.Ligers, M.Liepina, J.Krasts, I.Svekle. Is partial splenic embolization the method of choice of the treatment of hypersplenism for patients with portal hypertension? 6-th Conference of the Baltic Association of Pediatric Surgeons. Riga, 2000. (oral presentation)
4. **O.Volrats**, M.Liepina, J.Krasts, U.Ligers, V.Ozolins, I.Svekle. Analysis of Partial Splenic Embolization (PSE) in Hypersplenism Patients. 7-th Conference of the Baltic Association of Pediatric Surgeons. Kaunas, 2002. (oral presentation)
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