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Contents

Original Article

Research Article

Case Report

„Immortalis est ingenii memoria"

Seneca

Natalia Gheorghiu – *Pioneer of Pediatric Surgery in Moldova*

Original Article

Soave's transanal endorectal pull-through procedure for treatment children with Hirschsprung's disease

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Abstract

Procedeul endorectal tansanal Soave în tratamentul copiilor cu boala Hirschprung

Boala Hirschsprung (BH) are la bază malformațiile elementelor peretelui intestinal gros distal, a cărei esență este absența completă a ganglionilor neuronali intramurali. De-a lungul deceniilor au fost descries diferite tehnici chirurgicale de corecție a acestei maladii, actualmente cea mai răspândită abordare fiind procedeul endorectal transanal Soave (PETS).
Scopul lucării a fost în prezentarea propriei experiențe în managementul BH la copiii utilizând PETS cu analiza rezultatelor obținute.
Material și metode. În perioada 2014-2018, în clinica noastră au fost operați 15 pacienți cu BH utilizând PETS. Diagnosticul BH s-a bazat pe studierea atentă a anamnezei, metodele de examinare clinică obișnuite, inspecție manuală rectală, proctosigmoidoscopie, irigografie cu bariu, biopsia mucoasei intestinului gros cu examenul histologic și histochimic (activitatea acetilcolinesterazei), studierea microflorei intestinului gros.
Rezultate. De obicei, semnele și simptomele apar la scurt timp după naștere, dar uneori pot apăre și în perioade mai tardive. Constipațiile, meteorismul și vărsăturile au fost cele mai frecvente manifestări ale BH la copii. Clisma de contrast, care nu este o metodă specifică în diagnosticul BH, a fost efectuată în toate cazurile. Biopsia rectală cu examenul histopatologic au permis de a confirma diagnosticul. La toți cei 15 pacienți a fost constatat segmentul aganglionic de lungime standard - majoritatea pacienților prezentând segment de aganglioză localizat în regiunea recto-sigmoidă (66, 67%). În aceste cazuri a fost preferat procedeul Soave, realizat cu ajutorul tehnicii transanale într-o singură etapă la 11 (73,33%) pacienți și asistată laparoscopic într-o singură etapă la 4 (26,67%) pacienți. PETS asistată laparoscopic într-o etapă fără incizie abdominală a fost posibilă la pacienții cu BH cu prezentare tardivă și cu fecalom.
Concluzii:

1. PETS într-o singură etapă este tehnica chirurgicală sigură și eficientă în tratamentul pacienților cu BH cu afectarea segmentului rectal și rectosigmoid, rezultatele clinice fiind satisfăcătoare.
2. PETS asistat laparoscopic într-o singură etapă fără incizie abdominală a fost posibilă la pacienții cu BH diagnosticată tardive și cu fecalom.
3. PETS are principii anatomice optime pentru reconstrucția rectală în comparație cu alte tehnici chirurgicale utilizate în tratamentul BH la copii, fiind intervenția chirurgicală de elecție în patologia dată.
4. Complexul de examinare elaborate al pacienților cu BH permite nu numai un diagnostic precis al patologiei date, dar și depistarea precisă a cauzelor complicațiilor și tulburărilor funcționale intestinale în perioada postoperatorie.
5. Chiar și corectarea chirurgicală fără erori a BH la copii nu garantează succesul absolut fără un tratament de recuperare și reabilitare în perioada postoperatorie timpurie și târzie.

Cuvinte cheie: boala Hirschprung, diagnostic, tratament minim invaziv,

Abstract

Hirschsprung's disease is based on malformation of the distal large intestine wall's elements, which essence is either complete absence of intramural neural ganglia. Various surgical techniques have been described and employed over decades for the treatment of HD. The most popular approach for the children with HD today is the Soave's transanal endorectal pull – through (TEPT). *Aim* – to present own experience in the management of the HD in children with Soave's TETP method with the analysis of treatment results.

Material/methods. 15 patients were operated for HD with Soave's TEPT in our clinic between 2014-2018. HD diagnostics were based on careful studying of anamnesis, common clinical examination methods, manual rectal inspection, proctosigmoidoscopy, irrigography with barium, biopsy of a large intestine's mucous membrane for definition of histological and histochemical study (acetylcholineesterase activity), studying of large intestine's microflora.

Results. Usually signs and symptoms appear shortly after birth, but sometimes they're not apparent until later in life. Constipations, meteorism and vomiting were the most common sings of HD in children. Contrast enema is not a specific method for diagnosing diagnosis of HD was done in all the HD. Rectal biopsy and histopathology examination confirmed the patients. All 15 patient's hade standard length aganglionic segment – the majority of patients had segment of aganglionosis localized in the recto- sigmoid region (66, 67%). In cases used the prefered type of PT is the Soave approach, which is performed with the one – stage transanal technique by 11 (73,33%) patients and with one – stage laparoscopy - assisted by 4 (26,67%). One-stage laparoscopy – assisted TEPT by Soave without abdominal incision was feasible in patients with late-presenting HD and with fecaloma.

Conclusions.

1. One – stage Soave's TEPT is the safe and effective surgical technique for patients with rectal and rectosigmoid segment HD. The clinical outcome is satisfactory.
2. One-stage laparoscopy – assisted TEPT by Soave without abdominal incision was feasible in patients with late-presenting HD and with fecaloma.
3. Operation of endorectal pull-through by Soave method has optimum anatomic principles of a rectum reconstruction as compared to the other surgical techniques for the treatment of the HD in children and it is the operation of choice at the given pathology.
4. The developed examination complex of patients suffering from the HD allows not only to diagnose the abnormality itself, but also to reveal the reasons for complications and functional disorders of the brought down gut in the postoperative period in case of each child with big accuracy.
5. Even infallible surgical correction of HD in children does not guarantee absolute success in treatment without careful postoperative regenerative treatment and rehabilitation in the early and late postoperative period.

Keywords: Hirschprung disease, diagnosis, minimally invasive treatment

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Introduction

Hirschprung's disease (HD) is a condition that affects the large intestine (colon) and causes problems with passing stool. According to the modern concept based on clinical, functional, radiological and morphological data as well as on other research evaluations, Hirschsprung's disease is based on malformation of the distal large intestine wall's elements, which essence is either complete absence of intramural neural ganglia. The first description of this pathology dates back to 1886, when a Danish pediatrician Harald Hirschsprung presented the first portrayal of congenital megacolon in the Society of Pediatrics in Berlin [2]. Since then an abundant literature has given many controversial aspects of Hirschsprung's disease (HD) management. Various surgical techniques have been described and employed over decades for the treatment of HD, but all stem from the approaches described by Orvar Swenson in 1948 [5], Bernard Duhamel in 1956 [1] and Franco Soave in 1963 [4]. The most popular approach for the children with HD today is the Soave's transanal endorectal pull – through (TEPT).

The aim of this article – to present own experience in the management of the HD in children with Soave's TETP method with the analysis of treatment results.

Material and methods.

15 patients were operated for HD with Soave's TEPT in our clinic between 2014-2018. The age of patients was: beneath 1-year-old – 8 children (53,33%), 1-3 –years old – 4 children (26,67%), 4-5 years-old – 3 children (20%). HD is usually diagnosed in patients who are younger than 1 year, but in some individuals, it is found later, than 1 year old. HD diagnostics were based on careful studying of anamnesis, common clinical examination methods, manual rectal inspection, proctosigmoidoscopy, irrigography with barium, biopsy of a large intestine's mucous membrane for definition of histological and histochemical study (acetylcholine-esterase activity), studying of large intestine's microflora.

The majority of patients had rectosigmoid form of HD (10; 66,67%), rectal form of HD – in 5 children (33, 33%). Clinical outcome was assessed by interviews and questionnaires.

Table1. **Dynamics of HD clinical presentations**

Clinical presentations	Absolute number of patients	Percentage (%)
I. Early symptoms:		
- constipation with fecal impaction	15	100
- meteorism		
- anxiety	15	100
	11	73,33
II. Late symptoms:		
- anemia	11	73,33
- hypotrophy	11	73,33
- fecal bolus	4	26,67
- diarrhea	5	33,33
- trouble gaining weight	7	46,67
- growth delay	7	46,67
III. Complication symptoms:		
- vomiting	12	80
- abdominal pain	8	53,33
- malnutrition	11	73,33

Results and discussion

Dynamics of the clinical symptoms of HD in our patient's group has been presented in table 1. Usually signs and symptoms appear shortly after birth, but sometimes they're not apparent until later in life. Constipations, meteorism and vomiting were the most common sings of HD in children. A rectal examination may reveal a loss of muscle tone in the rectal muscles. This diagnostic clinical method we apply to all our patients. Contrast enema in patients with suspected HD is sill very popular, but it is not a specific method for diagnosing of HD was done in all the HD.

Rectal biopsy and histopathology examination confirmed the patients. We take the most distal biopsy at 2 cm or 3 cm from the dentate and used for HD diagnosis hematoxylin/eosin and acetylcholinesterase. Rectal biopsies are obtained using the open full-thickness in all patients (100%). To receive biopsy report it takes more than 3 days for all cases.

The timing of the pull – through operation is varies. We consider that the optimum time to perform a radical operation is the age of 3 – 6 months, which as we claim is due to:

- reduction of indications for prolonged preoperative preparation
- absence of the expressed secondary local and general character changes
- improvement of functional treatment results.

While waiting for surgery the bowel is maintained decompressed with rectal irrigation by 70% patients or with rectal dilatation by 30% children.

All patient's hade standard length aganglionic segment – the majority of patients had segment of aganglionosis localized in the recto- sigmoid region (66, 67%). In cases used the prefered type of PT is the Soave approach, which is performed with the one – stage transanal technique by 11 (73,33%) patients and with one – stage laparoscopy - assisted by 4 (26,67%).

The main feature of this operation is endorectal bringing down of a large intestine through demucosated rectal cylinder with a primary colo-rectal anastomosing at 1-1,5cm from anus. Endorectal bringing down with primary end -to – end anastomosis favorably differs when carried out in one stage, it is accessible to be performed in children in early age, simple in postoperative care and promotes early patient's activity. One-stage laparoscopy – assisted TEPT by Soave without abdominal incision was feasible in patients with late-presenting HD and with fecaloma. Rectal irrigation under general anesthesia and the use of laparoscopy and bipolar coagulator help to overcome the technical difficulties of his procedure.

There were no intraoperative or early postoperative complications. Patients started a diet a median of 3 days after the operation and were discharged a median of 12 days. All patients were followed up from 6 to 24 months. Clinical outcome was assessed by standardized interviews and questionnaires. During the first 6 months after Soave operation full normalization of function of the large bowel's reduced segment has occurred in 12 children which was 80% of the total. In children the mean stool times were 1 to 2 per day. Endorectal pull-through by Soave method has less impact on the sphincters. In 2

children large bowel's function disorders (grade I soiling) were still observed throughout the time of 2 years after operative intervention and they normalized after several courses of conservative regenerative treatment and rehabilitation had been carried out. 1 patient had stenosis of colo-rectal anastomosis. Colo-rectal stenosis anastomosis was subject for rectal dilatation in this patient.

Such postoperative complications as anastomotic leak, recurrence of constipation, recurrent enterocolitis have not been observed in our patients after SPTP.

Principal causes for coprogreasing (coproozing) are abnormalities of conditioned – reflex connections between the formed rectum and anus and also discoordination of the function of anal sphincters with reflex loss on defecation and increase of the endorectal pressure. In the presented group of the children correction of functional postoperative abnormalities was carried out by conservative treatment which included reflexotherapy, dietotherapy, exercise therapy, physiotherapeutic procedures, general and local pharmacotherapy with positive clinical effect. The organic reason for complication such as residual aganglionosis segment were subject for surgical elimination. Lynn's sphinctermyectomy was applied in this case where short aganglionotic areas – up to 5 cm long – was left [3].

Conclusions:

1. One – stage Soave's TEPT is the safe and effective surgical technique for patients with rectal and rectosigmoid segment HD. The clinical outcome is satisfactory.
2. One-stage laparoscopy – assisted TEPT by Soave without abdominal incision was feasible in patients with late-presenting HD and with fecaloma.
3. Operation of endorectal pull-through by Soave method has optimum anatomic principles of a rectum reconstruction as compared to the other surgical techniques for the treatment of the HD in children and it is the operation of choice at the given pathology.
4. The developed examination complex of patients suffering from the HD allows not only to diagnose the abnormality itself, but also to reveal the reasons for complications and functional disorders of the brought down gut in the postoperative period in case of each child with big accuracy.
5. Even infallible surgical correction of HD in children does not guarantee absolute success in treatment without careful postoperative regenerative treatment and rehabilitation in the early and late postoperative period.

REFERENCES

1. Duhamel B. Retrorectal and transanal pull-through procedure for the treatment of Hirschsprung's disease. Dis. Colon Rectum. 1964; 7:455-8.
2. Grosfeld J.L. Hirschsprung's disease: a historical perspective – 1691-2005. In: Holshneider A., Puri P. eds. Hirschsprung's Disease and Allied Disorders. Berlin Germany: Springer-Verlag; 2008: 1- 12.
3. Lynn H.B. Rectal myectomy a Hirschsprung's disease, a decade experience. Arch Surg 1975; 110: 991-4.
4. Soave F. A new original technique for treatment of Hirschsprung's disease. Surgery 1964; 56:1007-14.
5. Swenson O., Bill AH Jr. Resection of rectum and rectosigmoid with preservation of the sphincter for benign spastic lesions producing megacolon: an experimental study. Surgery. 1948; 24 (2): 212-20.

Conflicts of interest: *authors have no conflict of interest to declare*

The clinical-paraclinical profile and the spectrum of concomitant morbid states in congenital defects of the anterior abdominal wall (gastroschisis and omphalocele)

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Abstract

Profilul clinico-paraclinic și spectrul stărilor morbide concomitente în defectele congenitale ale peretelui abdominal anterior (gastroschizis și omfalocel)

Scopul studiului a fost evaluarea rezultatelor clinico-paraclinice ale afecțiunilor malformative ale peretelui abdominal cu identificarea factorilor de risc congenitali și dobândiți care au influențat prognosticul bolii.
MATERIAL ȘI METODE. Evaluarea clinico-epidemiologică a pacienților din lotul general de studiu și analiza rezultatelor tratamentului medico-chirurgical aplicat a fost efectuată în baza datelor extrase din fișele medicale de staționar ale copiilor cu defecte congenitale ale peretelui abdominal internați în staționar pe perioada anilor 2005-2016 în secția de chirurgie a nou-născutului a IMSP Institutul Mamei și Copilului, fiind selectate 64 cazuri clinice de gastroschizis și 59 cazuri de omfalocel.
REZULTATELE studiului au permis de a constata că defectele congenitale ale peretelui abdominal (gastroschizis și omfalocel) au fost întâlnite mai frecvent la nou-născuții de sex masculin, cazurile de gastroschizis fiind asociate predominant cu vârsta de gestație prematură și greutatea mică a nou-născuților, comparativ cu lotul de pacienți cu omfalocel unde au predominat nou-născuții la termen cu greutate normală. Au fost stabilite rate semnificativ sporite ale malformațiilor congenitale asociate atât în lotul bolnavilor cu gastroschizis (53,12%), cât și în cel cu omfalocel (61,02%), spectrul acestora fiind dominat de malformațiile tractului digestiv și cele *cardiovasculare.*
CONCLUZIE. Malformațiile congenitale concomitente împreună cu prematuritatea, greutatea mică la naștere, sindromul de compartiment și generalizarea procesului infecțios-septic au influiențat semnificativ evoluția și prognosticul nefavorabil al bolii.

Cuvinte cheie: gastroschizis, omfalocel, nou-născuți, malformații congenitale asociate, prognostic

Abstract

The aim of the study was to evaluate the clinical-paraclinical results of the abdominal wall malformations (gastroschisis and omfalocel) with the identification of congenital and acquired risk factors that influenced the prognosis of the disease.
MATERIAL AND METHODS. The clinico-epidemiological evaluation of the patients from the general study group and the analysis of the results of the applied medical-surgical treatment was performed based on the data extracted from the stationary medical files of the children with congenital defects of the abdominal wall hospitalized during the period 2005-2016 in the Newborn Surgery Department of the IMSP Institute of the Mother and Child, being selected 64 clinical cases of gastroschisis and 59 cases of omphalocele.
THE RESULTS of the study allowed us to find that congenital defects of the abdominal wall (gastroschisis and omphalocele) were more common in male neonates, gastroschisis being predominantly associated with premature gestation age and low weight of newborns compared to the group of patients with omphalocele where predominated term newborns with normal birth weight. There was established the significantly increased rates of congenital malformations associated with both the gastroschisis (53.12%) and the omphalocell (61.02%) patients, dominated by malformations of the digestive tract and cardiovascular ones.
CONCLUSION. Concomitant congenital malformations, which together with prematurity, low birth weight, compartment syndrome and generalization of the infectious-septic process, have significantly influenced the progression and unfavorable prognosis of the disease.
Keywords: gastroschisis, omphalocele, newborn, associated congenital malformations, prognosis

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Introduction

The interest of clinicians and pediatric surgeons worldwide in the congenital defects of the anterior abdominal wall in neonates is justified, these being some of the most serious pathologies, as the complications that accompany them present a serious, sometimes fatal danger to the child [22, 40]. The most common types of these congenital defects are laparoschisis (gastroschisis) and omphalocele.

Gastroschisis is a congenital structural defect of the anterior abdominal wall, located adjacent to and usually on the right of the insertion of the umbilical cord, through which abdominal viscera herniate [13, 31]. Although in most cases the parietal defect is located on the right side of the umbilicus [3, 9]. The abdominal parietal defect can rarely be found on the left side of the cord inserted normally [19, 31], sometimes casuistically supraombilical or infraombilical [25]. Eviscerated organs are not covered by any embryonic membrane or intrauterine sac, being exposed to amniotic fluid, which determines the development of morphopathological changes (edema, shortening of intestinal loops and so on) [25].

The incidence of gastroschisis varies from 0.5 to 7.0 cases per 15000 births, with an average of 1/2700 births [25, 30], the incidence of this malformation is increasing [12]. More commonly it appears as an isolated defect (83.3 - 93%), and in 12.2 - 35% of cases it may be part of some chromosomal syndromes and abnormalities (trisomy 13, 18, 21 etc.) [16, 30].

The incidence of omphalocele is 1-3 cases in 5000-10000 newborns [2, 26], the overall mortality rate in this pathology exceeding 25% [15].

The omphalocele can be found isolated or as a component part of some polimalformative syndromes, the syndromal omphalocele having a remarkable frequency in all the cases of omphalocel [38]. The associated malformations are found in 30-80% of the cases of omphalocele, their severity determining the prognosis of the disease [5, 32].

The aim of the study was to evaluate the clinical-paraclinical results of the abdominal wall malformations (gastroschisis and omfalocel) with the identification of congenital and acquired risk factors that influenced the prognosis of the disease.

Material and Methods

The clinico-epidemiological evaluation of the patients from the general study group and the analysis of the results of the applied medical-surgical treatment was performed based on the data extracted from the stationary medical files of the children with congenital defects of the abdominal wall hospitalized during the period 2005-2016 in the Newborn Surgery Department of the IMSP Institute of the Mother and Child, being selected 64 clinical cases of gastroschisis and 59 cases of omphalocele. Selection criteria: living newborns, who were diagnosed with various forms of gastroschisis, which were later transferred to the Newborn Surgery Department. The study did not include cases of gastroschisis with lethal end occurring during the early intranatal or postnatal periods, which did not benefit from medical-surgical treatment.

The research of the study group focused on the analysis of the following parameters: maternal age, gestational age at birth, body weight, prematurity.

The conventional laboratory examination included the hemoleucogram, the coagulogram, the biochemical analysis of the blood, the evaluation of the acid-basic balance, the microbiological investigations. Diagnostic methods included ultrasound of internal organs (fig. 1), ultrasound of the heart, neurosonography and radiological examination (fig. 2).

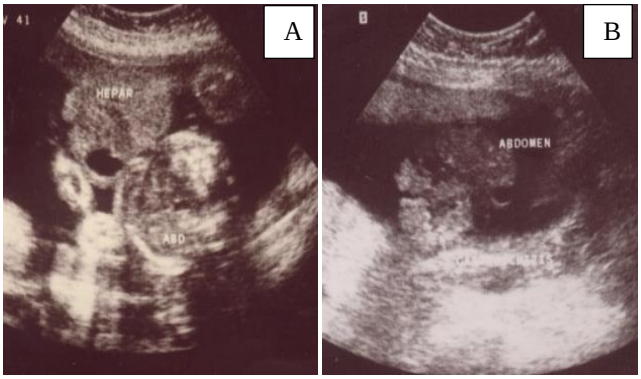

Fig. 1. USG of pregnancy: ultrasound appearance of omphalocele (A) and gastroschisis (B) at 18-19 weeks of gestation

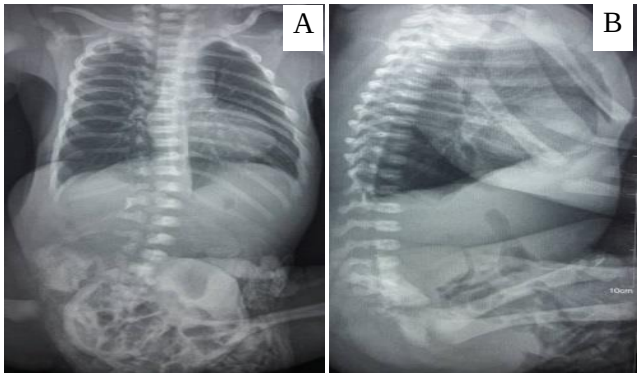

Fig. 2. Thoracic-abdominal panoramic radiography (A - anteroposterior; B - lateral to the right): appearance of aerated intestinal loops located in the embryonic sac in a newborn with omphalocele

The diagnosis of gastroschisis was established prenatally and confirmed immediately after birth. In most cases, after a quick evaluation, with the installation of a nasogastric tube and administration of intravenous fluids and antibiotics, the child was transferred quickly to the neonatal surgery service. Usually, the mothers of these children were monitored during the prenatal period with regular ultrasound examinations, paying attention to the dilation of the intestine, the thickness of the abdominal wall, the motility of the intestine, the amount of amniotic fluid during the gestation period. In 18 cases the birth occurred naturally, the rate of cesarean interventions constituting 4 cases.

The analysis of the medical files allowed to find that the average maternal age in the group of morphopathologically investigated patients was 23.06 + 0.91, the minimum age being 17 years, the maximum age - 32 years.

The results of the study were processed on the Pentium computer in the Microsoft Office Excel 7.0 program, using the statistical processing package according to the Student criterion, the differences p <0.05 being considered statistically conclusive.

Results

The study found that most cases of gastroschisis (10 cases) were recorded in 2006 and 2015, with the lowest number of patients (2 children) being recorded in 2012. The maximum number of cases of omphalocele was found in 2005 and 2008 (10 cases), while in 2007 only one child with this malformation was hospitalized, and in 2012 and 2014 - 2 cases (fig. 3).

The distribution by gender allowed us to determine that these malformations were more common in boys (gastroschisis - 59.37%, omphalocel - 64.41%) (table 1).

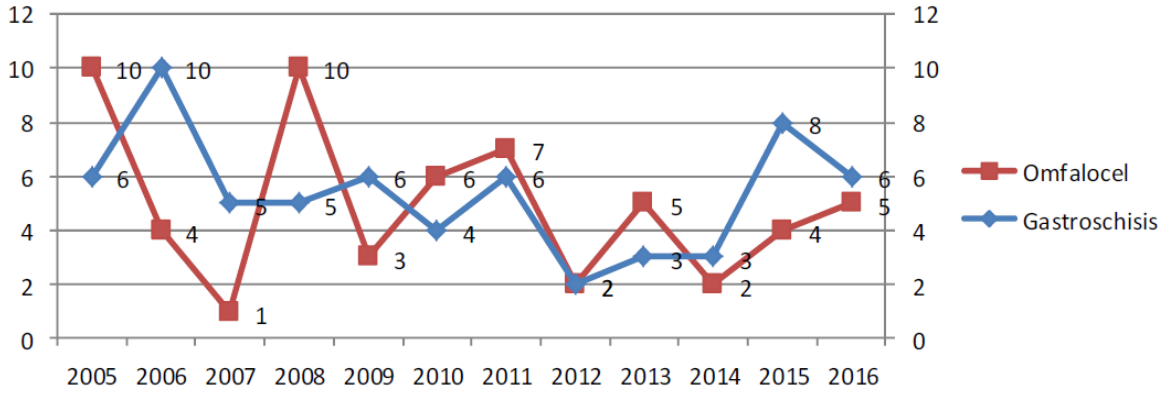

Fig. 3. Graphical representation of the annual frequency of cases of gastroschisis and omphalocele for the period 2005-2016

***Table 1.* Distribution of patients by gender**

	Gastroschisis		Omphalocele	
Gender	Nr.	%	Nr.	%
Male	38	59,37	38	64,41
Female	26	42,63	21	35,59

In this study, preterm infants with gastroschisis with a medium gestational age of 33.74 ± 0.43 weeks (40 cases - 62.50 %) and medium weight of 2022.45 ± 82.78 g predominated with a range from 720 g (26 weeks) to 3,200 g (36 weeks). The mean weight of term neonates (38.23 ± 0.19 weeks) with gastroschisis (26 cases) was significantly higher and consisted of 2673.04 ± 73.48 g (p <0.001), in 6 cases the weight was up to 2500 g, with an intrauterine growth retardation of the fetus. In 30 cases (45.45%) was determined gastroschisis with significant viscero-abdominal disproportion. In the group of patients with omphalocele predominated male neonates (64.41%), born in term (38.96 ± 0.15 weeks) - 45 children, mean weight (3241.00 ± 63.95 g) being significantly higher compared to the patients with gastroschisis born in term (p <0.001). The preterm group consisted of 14 children (34.64 ± 0.66 weeks) with an average weight of 2476.18 ± 207.27 g, significantly lower compared to term newborns (p <0.001), premature infants with omphalocele having a higher weight compared to those with gastroschisis (p <0.05). The number of patients with gastroschisis hospitalized in serious and extremely serious condition was 89.06% and 72.88% - in the group of children with omphalocele (tab. 2).

Depending on the size, the small size omphalocele was found in 33 (55.93%) newborns, medium omphalocele - 17 (28.81%) cases, the major and giant omphlocele in 9 (15.25 %) cases.

Complicated forms of malformation (inflammation, infection or rupture of the embryonic leaves) were determined in 25 (42.37%) cases, of which 20 with minor and medium omphalocele and 5 patients with major omphalocele.

The hemoleucogram of patients with congenital defects of the abdominal wall (tab. 2) did not find statistically significant differences of the selected indices between the group of children with gastroschisis and those with omphalocele (p> 0.05), the results of the average values obtained being within the conventionally normal limits. The mean values of the biochemical indices in the blood of infants with gastroschisis and omphalocele at the time of hospitalization, the evaluation of which showed that in the group of patients with gastroschisis the general protein values were actually lower (48.83 ± 1.27) compared to the values of the children with omfalocel (56.39 ± 2.04) (p <0.01), while the difference between the values of urea and creatinine level was insignificant (p> 0.05) (tab. 3).

***Table 2.* Parameters of hemoleucogram in neonates with congenital defects of the abdominal wall (M ± m)**

Parameters	Gastroschisis n=30	Omphalocele n=30
Hb, g/l	188,3 ± 5,74	191,33 ± 6,6
Er x 10^{12}/l	5,8 ± 0,17	5,8 ± 0,19
Le x10^{9}/l	10,4 ± 1,25	12,21 ± 0,81
ESR, mm/h	3,7 ± 0,8	4,27 ± 0,63

***Table 3.* Preoperative biochemical analysis of blood in children with congenital abdominal wall defects at the time of hospitalization (M ± m)**

Parameters	Gastroschisis n=30	Omphalocele n=30
Protein (g/l)	48,83±1,27	56,39±2,04**
Urea (mmol/l)	4,82±0,45	4,56±0,41
Creatinine (mcmol/l)	54,51±3,39	63,84±5,18
Total bilirubin (mcmol/l) Conjugated bilirubin (mcmol/l) Free bilirubin (mcmol/l)	39,41±6,72 1,69±0,64 30,76±6,52	101,33±13,92*** 4,4±2,34 84,11±22,28***
ALT	46,71±6,32	36,31±4,35
AST	59,32±6,42	54,94±7,75
Prothrombin index (%)	86,3±2,15***	81,54±2,08
Fibrinogen (g/l)	4,28±0,44	3,28±0,28
K^{+} (mmol/l)	4,95±0,25	5,25±0,12
Na^{+} (mmol/l)	136,88±4,55	139,04±1,56
$Ca2^{++}$ (mmol/l)	2,05±0,04	1,99±0,05

Note: * P <0.05; ** p <0.01; *** p <0.001 (as in all the tables)

In children with omfalocel the total bilirubin level was significantly higher with about 2.5 times compared to the group of children with gastroschisis ($p<0.001$), an insignificant increased level being characteristic for conjugated bilirubin ($p>0.05$) and veridically increased - for free bilirubin ($p<0.001$). We note that the study did not find statistically reliable differences in the blood levels of transaminases in these 2 groups of patients ($p>0.05$), although these values were slightly higher in patients with gastroschisis, who also had a comparatively higher level of prothrombin index ($p<0.001$), fibrinogen values being insignificantly high ($p>0.05$). The estimation of the serum level of potassium, sodium and calcium did not determine a statistically true difference of the values obtained between these 2 groups ($p>0.05$), the indices being within the range of normal conventional values (Na^+: 133-146 meq/L; K^+: 3.2-5.5 meq/L).

The average values of the basic indices of the acid-basic balance (tab. 4), the maintenance of which is a considerable challenge for the newborns with congenital defects of the abdominal wall. At the time of hospitalization, the average pH values in neonates with gastroschisis (7.27 $\pm$ 0.03) were insignificantly lower compared to the values recorded in cases of omphalocele (7.31 $\pm$ 0.03) ($p> 0.05$) , while the values of PCO_2 in the group of patients with gastroschisis (38.75 $\pm$ 3.53) really exceeded the values of the group with omphalocele (30.78 $\pm$ 1.74), the indices of both groups being in the normal range (35 - 45 mm Hg). The level of PO_2 in both groups did not significantly exceed the range of normal values (50 - 70 mm Hg) without finding a statistically significant difference ($p> 0.05$), the rule also valid for the O_2 level. The values of BE (excess of bases) in the group of children with gastroschisis constituted -10,62 $\pm$ 0,98, in those with omfalocel - they were of -11,1 $\pm$ 1,2 ($p<0,01$), whereas the serum indices of HCO_3^- in neonates with gastroschisis was 16.17 $\pm$ 1.31, and in those with omphalocele 15.89 $\pm$ 0.73, with no statistically significant difference ($p>0.05$), the results being suggestive for an acidosis of metabolic origin, characteristic for newborns in the first days of life.

We note that in the evaluated group the pH values <7.2 were found in 2 cases, and pH <7.0 were observed in 4 cases of gastroschisis, the range of values was 6.84 - 6.99 , whereas in the group of patients with omphalocele pH <7.2 was found only in one case (pH = 6.67).

In these cases, PCO_2 indices exceeded the conventional limits (<50 mmHg), in children with gastroschisis, a range of values of 50.6 - 88.3 mmHg was found, and in the only case of omphalocele, this index constituted 109.9 mmHg, at the same time the BE indices ranged from -7.2 to -23.6 mmol/l in children with gastroschisis and -26.2 mmol/l in the case of omphalocele. PH values >7.4 were found in 5 cases of gastroschisis with a range of values of 7.41-7.47 and in 4 cases of omphalocele with a range of values of 7.42-7.59. The incidence of congenital malformations in neonates with gastroschisis constituted 53.12% (34 patients), coexisting anomalies in neonates with omphalocele being found in 36 patients (61.02%) (tab. 5.).

Analysis of morbidity structure in neonates with gastroschisis allowed to highlight the predomination of digestive tract malformations (65.62%) and cardiovascular malformations (43.75%), followed by urogenital malformations (28.12%), bronchopulmonary malforma-tions (7.81%) and neurosurgical malformations (3.12%), 28 patients (43.75%) being diagnosed with 2 or more congenital malformations at the same time.

***Table 4.* Comparative values of acid-base balance indices in children with congenital abdominal wall defects at the time of hospitalization (M $\pm$ m)**

Parameters	Gastroschisis n=30	Omphalocele n=30
pH	7,27±0,03	7,31±0,03
pCO_2 mm Hg	38,75±3,53*	30,78±1,74
pO_2 mm Hg	72,66±10,19	73,22±6,6
O_2 %	80,08±4,43	87,25±2,35
BE mmol/l	-10,61±0,98	-11,1±1,2**
HCO_3- mmol/l	16,17±1,31	15.89±0,73

***Table 5.* Frequency of congenital malformations associated in neonates with gastroschisis and omphalocele**

Coexisting malformations	Gastroschisis		Omphalocele	
	Nr.	Incidence (%)	Nr.	Incidence (%)
1. Cardiovascular malformations	**28**	**43,75**	**18**	**30,51**
- atrial septal defect	5	7,81	3	5,08
- patent ductus arteriosus	8	12,5	6	10,17
- bicuspid aortic valve	1	1,56	-	-
- congenital aortic valve stenosis	3	4,69	-	-
- persistent foramen ovale	7	10,94	5	8,47
- ventricular septal defect	3	4,69	2	3,39
- coarctation of the aorta	1	1,56	-	-
- dextrocardia	-		2	3,39
2. Urogenital malformations	**18**	**28,12**	**7**	**11,86**
- Renal cystic dysplasia	7	10,94	2	3,39
- abdominal ectopia of the testis	1	1,56	-	-
- congenital hydronephrosis	3	4,69	1	1,69
- ureteral stenosis with ureteral geniculation	6	9,37	-	-
- vaginal atresia	1	1,56	-	-
- hypospadias	-	-	1	1,69
- persistence of the hurricane	-	-	1	1,69
- bladder extrusion	-	-	1	1,69
- cloaca extrusion	-	-	1	1,69
3. Malformations of the digestive tract	**42**	**65,62**	**30**	**50,85**
- severe intestinal and fixative disorders	20	31,25	6	10,17
- pancreatic cystic fibrosis	1	1,56	-	-
- atresia of the small intestine	2	3,12	-	-
- segmental stenosis of the small intestine	3	4,69	-	-
- colon atresia	4	6,25	-	-
- segmental colonic stenosis	2	3,12	-	-
- duplication of the gallbladder with stenosis of the colleague	1	1,56	-	-
- gallbladder hypoplasia	1	1,56	-	-
- congenital megaduodenum	1	1,56	-	-
- vascular malformations of the mesenter and intestine	1	1,56	-	-
- agenesis of the cecum, vermicular appendix	2	3,12	1	1,69
- anal atresia et recti	1	1,56	-	-
- incomplete annular pancreas	2	3,12	-	-
- duplication of the small intestine	1	1,56	18	30,51
- the Meckel diverticulum	-	-	2	3,39
			1	1,69
			2	3,39
4. Neurosurgical malformations	**2**	**3,12**	**1**	**1,69**
- microcephaly	1	1,56	-	-
- cerebral vascular malformations	1	1,56	-	-
- spina bifida	-	-	1	1,69
5. Bronchopulmonary malformations	**5**	**7,81**	**1**	**1,69**
- Lobar pulmonary agenesis	3	4,69	-	-
- Lobar pulmonary hypoplasia	2	3,12	1	1,69
6. Disembryogenetic stigmas	**2**	**3,12**	**2**	**3,39**

The coexisting congenital malformation spectrum in neonates with omphalocele was represented by malformations of the digestive tract (50.85%), congenital cardiovascular defects (30.51%), urogenital malformations (11.86%), neurosurgical and bronchopulmonary malformations found casuistically (fig. 4). In 24 cases (48.68%) of omphalocele, multiple malformations were detected. In 4 (6.78%) cases, syndromal omphalocele was diagnosed in neonates with arthro-griposis (2 cases), Cantrel pentas (1 case) and Beckwith-Wiedermann's syndrome (1 case).

According to the anatomopathological findings, together with the associated prematurity and malformations, the unfavorable evolution of the disease was influenced by other pathological conditions, including (table 6): congenital peritonitis (aseptic and septic-infectious), encountered in all cases of gastroschisis, sepsis (59,37%), hypoxic-discirculatory encephalopathy (35.94%), intrauterine infection with the generalization of the inflammatory-septic process (32.81%), amniotic fluid aspiration with the development of focal atelectasis and pneumonia (23.44%), the development of enterocolitis (25%), obstetrical trauma (7.81%).

These pathological conditions on the background of tissue immaturity of the internal organs, determined the development of polyorganic insufficiency with an unfavorable exodus of the disease. In most cases 2 and more of these nozological entities were found.

The analysis of the anatomopathological protocols allowed us to find that in the cases of omphalocele, along with the concomitant pathological conditions described above, the nozological entities that determined the unfavorable exodus of the omphalocele were: natal trauma with intraventricular haemorrhage and cerebral edema - 2 cases, hypoxic-discirculatory encephalopathy - 5 cases, intrauterine infection - 6 cases, aspiration of amniotic fluid with the development of atelectasis and pneumonia - 7 cases, enterocolitis - 6 cases, progressive peritonitis - 11 cases. In most cases with unfavorable development (12 cases), 2 or more of the listed pathologies were found.

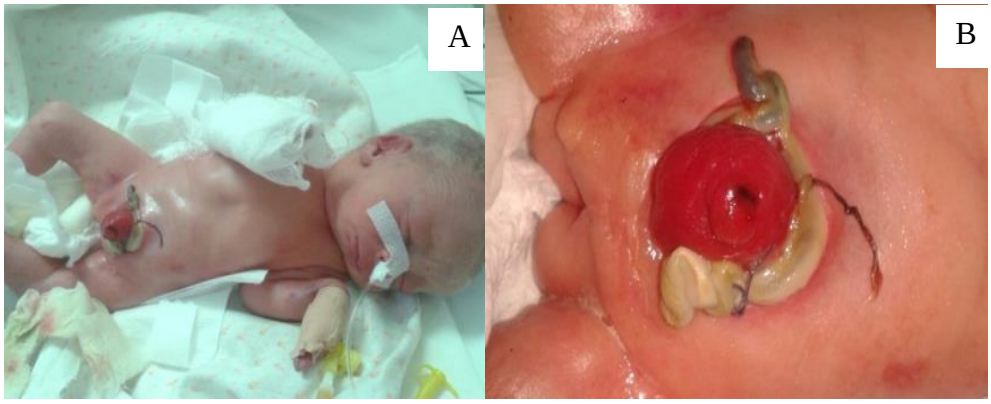

Fig. 4. The appearance of a newborn with omphalocele complicated with the rupture of the embryonic leaves associated with arthrogryposis, bladder extrusion and bladder.

***Table 6.* Frequency of pathological conditions that influenced significantly the evolution of the disease in the cases of gastroschisis**

Nozological entity	Nr.	%
Hypoxic-discirculatory encephalopathy	23	35,94
Amniotic fluid aspiration with the development of focal atelectasis and pneumonia	15	23,44
Enterocolitis	16	25
Intrauterine infection with the generalization of the inflammatory-septic process	21	32,81
Obstetrical trauma	5	7,81
Congenital peritonitis (aseptic and septic-infectious)	64	100
Sepsis	38	59,37

Discussion

Although the strategies of surgical correction of congenital defects of the anterior abdominal wall have evolved considerably in recent decades, their prognosis remains severe [33]. Hypothermia, fluid loss, sepsis, low birth weight, gestational age has a significant influence on the risk of death in case of congenital defects in the abdominal wall [27, 34]. Some authors reported an index of 5.9 – 23.4% of postnatal mortality in the case of congenital defects of the abdominal wall [1, 15], in some countries the values of this index reaching 28 % [17] or even 80%. A significant survival of 90-97% is recorded in the simple gastroschisis [16, 35], versus 10% in complex gastroschisis [29]. The incidence of congenital malformations in gastroschisis patients is about 15-35%, depending on the inclusion criteria [8, 16]. At the same time, some studies indicate that 48.1% - 68% of neonates with gastroschisis have one or more associated abnormalities, including intestinal atresia, cryptorhidia, persistence of fetal circulation, etc., multiple congenital malformations constituting about 32% from cases [5, 11]. Thus, intestinal malformations occur in 5-20% of cases, urinary tract malformations - in 6%, heart defects - in 1% [10]. It was determined that fetuses with gastroschisis have an increased risk of prematurity (22-40%), oligohydroamnios (36%), intrauterine retardation (38-77%) or miscarriage (7%) [1, 27]. Associations of gastroschisis with cardiac malformations have been reported, such as septal defects, tetralogy Fallot, Ebstein anomaly, aortic coarctation, major artery transposition, etc. [24], facial, musculoskeletal [28], chromosomal, including trisomies and Turner syndrome [28], neural tube defects [20], hydrocephalus [14], Hirschsprung disease [4], anorectal malformations [28], diaphragmatic hernia [21], cholecyst cyst [37].

The associated malformations are found in 30-80% of the cases of omphalocele, their severity determining the prognosis of the disease [5, 32]. Cardiac abnormalities were recorded in 50% of patients with omphalocele, gastrointestinal - in 40%, the chromosomal ones in 10-40%, the most frequent being trisomies 13, 14, 15, 18, 21, Turner syndrome (45, X), Klinefelter syndrome (47, XXY), triploidy (69, XXX). Genetic syndromes associated with omphalocele are the Beckwith-Wiedemann syndrome, the Goltz syndrome, the Marshall-Smith syndrome, the Cantrell pentalogy, bladder and cloacal extrofia, Toriello-Carey syndrome, Carpenter syndrome, etc. [7, 18, 23, 39].

Conclusions

1. The results of the study allowed us to find that congenital defects of the abdominal wall (gastroschisis and omphalocele) were more common in male neonates, gastroschisis being predominantly associated with premature gestation age and low weight of newborns compared to the group of patients with omphalocele where predominated term newborns with normal birth weight.
2. There was established significantly increased rates of congenital malformations associated with both the gastroschisis (53.12%) and the omphalocell (61.02%) patients, dominated by malformations of the digestive tract and cardiovascular ones.
3. Concomitant congenital malformations, which together with prematurity, low birth weight, compartment syndrome and generalization of the infectious-septic process have significantly influenced the progression and unfavorable prognosis of the disease.

REFERENCES

1. Akakpo-Numado G.K., Gnassingbe K., Boume M.A. et al. Emergency treatment of a ruptured huge omphalocele by simple suture of its membrane. Ann. Surg. Innov. and Research. 2012. 6:2.
2. Akhtar J., Skarsgard E.D. Associated malformations and the "hidden mortality" of gastroschisis. J. Pediatr. Surg. 2012; 47:911.
3. Aldridge B., Ladd A.P., Kepple J., Wingle T., Ring C., Kokoska E.R. Negative pressure wound therapy for initial management of giant omphalocele. Am. J. Surg. 2016; 211:605-9.
4. Allin B.S.R., Irvine A., Patni N., Knight M. Variability of outcome reporting in Hirschsprung's disease and gastroschisis: a systematic review. Sci. Rep. 2016; 6:38969. doi: 10.1038/srep38969.
5. Benjamin B., Wilson G.N. Anomalies associated with gastroschisis and omphalocele: Analysis of 2825 cases from the Texas Birth Defects Registry. J. Pediatr. Surg.2014; 49: 2014. 514-9.
6. Calcagnotto H., Muller A.L., Leite J.C. et al. Associated factors for perinatal mortality in gastroschisis. Rev. Bras. Ginecol. Obstet. 2013; 35(12):549-53.
7. Chen C.P. Syndromes and disorders associated with omphalocele (III): single gene disorders, neural tube defects, diaphragmatic defects and others. Taiwan J. Obstet. Gynecol. 2007; 46(2):111-20.
8. Christison-Lagay E.R., Kelleher C.M., Langer J.C. Neonatal abdominal wall defects. Semin. Fetal & Nenat. Med. 2011; 16:164.
9. Ciobanu O., Stoica A., Zavate A. ş.a. Laparoschisis – Consideraţii clinico-terapeutice. Craiova Medicală. 2007; 9(4):314-7.

10. Corey K.M., Hornik C.P., Laughon M.M., McHutchison K., Clark R.H., Smith P.B. Frequency of anomalies and hospital outcomes in infants with gastroschisis and omphalocele. Early Human Develop. 2014; 90:421-4.
11. Corona-Rivera J.R., Nieto-Garcia R., Lopez-Marure E. et al. Associated congenital anomalies in infants with isolated gastroschisis: a single-institutional experience. Am. J. Med. Geenet. A. 2016; 170(2):316-21.
12. Dalton B.G., Gonzalez K.W., Reddy S.R., Hendrickson R.J., Iqbal C.W. Improved outcomes for inborn babies with uncomplicated gastroschisis. J. Pediatr. Surg. 2017; 52:1132-4.
13. de Buys Roessingh A.S., Damphousse A., Ballabeni P. et al. Predictive factors at birth of the severity of gastroschisis. World J. Gastrointest. Pathophysiol. 2015; 6(4):228-34.
14. Dribin T., McAdams R.M. A neonate with gastroschisis and hydrocephalus complicated by central diabetes insipidus. J. Ped. Surg. Case Rep. 2014; 2:366-8.
15. Eamer G.J., Alfraih Y., Stein N., Bailey K. Splenic and pancreatic torsion after giant omphalocele repair. J. Ped. Surg. Case Rep. 2017; 25:40-3.
16. Feldkamp M.L., Botto L.D., Byrne J.L. et al. Clinical presentation and survival in a population-based cohort of infants with gastroschisis in Utah, 1997-2011. Am. J. Med. Genet. A. 2016; 170(2):306-15.
17. Feldkamp M.L., Bowles N.E., Botto L.D. AEBP1 gene variants in infants with gastroschisis. Berth Defects Res. A Clin. Mol. Teratol. 2012; 94(9):738-42.
18. Heider A.L., Strauss R.A., Kuller J.A. Omphalocele: Clinical outcomes in cases with normal karyotype. Am. J. Obst/ Gynecol. 2004; 190:135-41.
19. Hombalkar N.N., Rafe A., Prakash G.D. Left-sided gastroschisis with caecal agenesis: a rare case report. Afr. J. Paediatr. Surg. 2015; 12(1):74-5.
20. Hwang P.J., Kousseff B.G. Omphalocele and gastroschisis: an 18-year review study. Genet. Med. 2004; 6:232-6.
21. Inoue S., Odaka A., Muta Y. et al. Coexistence of congenital diaphragmatic hernia and abdominal wall closure defect with chromosomal abnormality: two case reports. J. Med. Case Rep. 2016; 10(1):19.
22. Ionescu S., Mocanu M., Andrei B. ș.a. Diagnosticul diferențial al defectelor de perete abdominal – omfalocel versus gastroschisis. Chirurgia (Bucur.). 2014; 109(1):7-14.
23. Karaman A., Aydin H., Goksu K. Concomitant omphalocele, anencephaly and arthrogryposis associated with trisomy 18. Genet. Couns. 2015; 26(1):77-9.
24. Kunz L.H., Gilbert W.M., Towner D.R. Increased incidence of cardiac anomalies in pregnancies complicated by gastroschisis. Am. J. Obstet. Gynecol. 2005; 193:1248-52.
25. Ledbetter D.J. Congenital abdominal wall defects and reconstruction in pediatric surgery: gastroschisis and omphalocele. Surg. Clin. North Am. 2012; 92:713-27.
26. Marshall J., Salemi J.L., Tanner J. P. et al. Prevalence, correlates, and outcomes of omphalocele in the United States, 1995-2005. Obstet. Gynecol. 2015; 126(2):284-93.
27. Marshall Niles S.G., Mitchell-Fearon K., Gill M. I., DeSouza C. J., Fearon I. C., Abel C. A. et al. Mortality-related factors in gastroschisis – a Jamaican perspective. J. Pediatr. Surg. 2017; 52:530-3.
28. Mastroiacovo P., Lisi A., Castilla E.E. et al. Gastroschisis and associated defects: an international study. Am. J. Med. Genet. A 2007; 143:660-71.
29. Masui D., Fukahori S., Asagiri K., Ishii S., Saikusa N. et al. Wandering spleen associated with omphalocele in a neonate: An unusual case with non-operative management. J. Pediatr. Surg. Case Rep. 2017; 24:8-11.
30. Materna-Kiryluk A., Wieckowska B., Wisniewska K. et al.Geospatial clustering of gastroschisis in Poland: data from the Polish Registry of congenital malformations (PRCM). Int. J. Occup. Med. Environ. Health. 2016; 29(3):461-70.
31. Nam N.F., Kaneko J.S., Intelizano P.M., Ferreira R.M., Oliveira N.M. et al. Left-sided gastroschisis associated with situs inversus totalis. J. Ped. Surg. Case Rep. 2018; 28:6-8.
32. Peters N.C.J., Vissert Hooft M.E., Ursem N.E., Eggink A.J., Wijnen R.M.H. et al. The relation between viscero-abdominal disproportion and type of omphalocele closure. Eur. J. Obstetr. Gynecol. Reprod. Biol. 2014; 181:294-9.
33. Puligandla P.S., Baird R., Skarsgard E.D., Emil S., Laberge J.M. Outcome prediction in gastroschisis – The gastroschisis prognostic score (GPS) revisited. J. Pediatr. Surg. 2017; 52:718-21.
34. Riggle R.M., Davis J.L., Drugas G.T., Riehle K.J. Fatal Clostridial necrotizing enterocolitis in a term infant with gastroschisis. J. Ped. Surg. Case Rep.2016; 14: 29-31.
35. Risby K., Husby S., Qvist N., Jakobsen M.S. High mortality among children with gastroschisis after the neonatal period: A long-term follow-up study. J. Pediatr. Surg. 2017; 52:431-6.
36. Sangkhathat S., Patrapinyokul S., Chiengkriwate P. et al. Infectious complications in infants with gastroschisis: an 11 year review from a referral hospital in southern Thailand. J. Pediatr. Surg. 2008; 43:473-8.
37. Serber J., Stranzinger E., Geiger J.D., Teitelbauma D.H. Association of gastroschisis and choledochal cyst. J. Pediatr. Surg. 2009; 44:E23-E26.
38. Țârcă E., Aprodu S.G., Straticiuc-Ciongradiu I., Goția D.G. Aspecte clinico-evolutive în cazul omfalocelului sindrom. Rev. Rom. Pediatr. 2013; 2:171-5.
39. van Eijck F.C., Wijnen R.M., van Goor H. The incidence and morbidity of adhesions after treatment of neonates with gastroschisis and omph.: a 30-year review. J. Pediatr. Surg. 2008; 43(3):479-83.
40. Watanabe S., Suzuki T., Hara F., Yasui T., Uga N., Naoe A. Omphalocele and gastroschisis in newborns: Over 16 years of experience from a single clinic. J. Neonatal. Surg. 2017; 5;6(2):27. doi:10.21699/jns.v6i2.530.

Research Article

Characteristic of the cellular spectrum of lymphocyte and macrophage populations in the fibrous capsule and pericystic pulmonary tissue in pulmonary hydatid cyst in children

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Abstract

Caracteristica spectrului celular al populaţiei limfocitare şi a macrofagelor în capsula fibroasă şi ţesutul pulmonar perichistic în chistul hidatic pulmonar la copii

În cazurile de chist hidatic relația gazdă-parazit este interactivă, evoluția bolii fiind dependentă de echilibrul între mecanismele sistemului imun ale gazdei și de strategiile complexe de evaziune ale agentului parazitar.
Scopul studiului a fost testarea imunohistochimică a populației limfocitare în componența infiltratului inflamator la nivelul stratului adventicial (perichist) și țesuturile perichistice în diferite forme clinico-morfologice ale chistului hidatic pulmonar la copii.
Investigaţiile morfopatologice au fost direcționate în testarea imunohistochimică a tipurilor de limfocite T implicate în imunitatea celulară locală, cât şi aprecierea limfocitelor B și a macrofagilor implicate în componenta celulară inflamatorie cu utilizarea anticorpilor monoclonali împotriva markerilor de suprafață CD3, CD4, CD8, CD20cy-pozitive și CD68. În calitate de material de studiu au servit probele tisulare prelevate intraoperator din capsula fibroasă și țesuturile adiacente la 20 pacienți cu hidatidoză pulmonară cu vârsta între 10-16 ani, grupați în 3 loturi: 1) chist hidatic pulmonar necomplicat (7 copii); 2) chist hidatic pulmonar cu semne de inflamație ăperichistică (7 copii) și 3) chist hidatic pulmonar complicat prin ruptură (6 copii).
Rezultatele studiului au permis de a conchide că:

1. Evoluția chistului hidatic pulmonar la copii este caracterizată de un infiltrat inflamator cronic al parenchimului perichistic cu predominarea limfocitelor T CD3 comparativ cu capsula fibroasă, unde predomină procesele necrolitice.
2. Numărul sporit de CD20 indică activarea semnificativă a sistemului imun adaptativ în agravarea proceselor inflamatorii în parenchimul perichistic în formele complicate ale maladiei, creșterea numărului de macrofage în aceste cazuri fiind nesemnificativă.
3. Numărul de macrofage CD68 din capsula fibroasă și la nivelul pleurei a fost semnificativ mai mic comparativ cu densitatea acestor celule depistate în infiltratele inflamatorii din parenchimul pulmonar subiacent capsulei fibroase, un număr nesemnificativ mai mare fiind constatat în formele complicate ale maladiei.

Cuvinte cheie: chist hidatic, hidatidoză pulmonară, process inflamator local

Abstract

In cases of hydatid cyst the host-parasite relationship is interactive, the evolution of the disease being dependent on the balance between the mechanisms of the host immune system and complex escape strategies of the parasitic agent.

The *purpose* of the study was to carry out the immunohistochemical test of the lymphocyte population in the inflammatory infiltration at the adventitial layer (pericyst) and pericystic tissues in different clinico-morphological forms of the pulmonary hydatid cyst in children.

Morphopathological investigations were focused on the immunohistochemical testing of T lymphocytes involved in local cell immunity, as well as the assessment of B lymphocytes and macrophages involved in the inflammatory cell component with the use of monoclonal antibodies against surface markers CD3, CD4, CD8, CD20cy-positive and CD68. As a study material, the tissue samples taken intraoperatively from the fibrous capsule and adjacent tissues in 20 patients with pulmonary hydatidosis, aged 10-16 year,s were grouped into 3 groups: 1) uncomplicated pulmonary hydatid cyst (7 children); 2) pulmonary hydatid cyst with signs of pericystic inflammation (7 children), and 3) pulmonary hydatid cyst complicated by rupture (6 children).

The results of the study allowed us to conclude that:

1. The evolution of the pulmonary hydatid cyst in children is characterized by a chronic inflammatory infiltration of the pericystic parenchyma with the predominance of CD3 T lymphocytes compared to the fibrous capsule where the necrolytic processes predominate.
2. The increased number of CD20 indicates a significant activation of the adaptive immune system in the aggravation of inflammatory processes in the pericystic parenchyma in complicated forms of the disease, the increase of the number of macrophages in these cases being insignificant.
3. The number of CD68 macrophages in the fibrous capsule and within the pleura was significantly lower compared to the density of these cells detected in inflammatory infiltrates of the pulmonary parenchyma underlying the fibrous capsule, an insignificant number being found in complicated forms of the disease.

Keywords: gastroschisis, omphalocele, newborn, associated congenital malformations, prognosis

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Introduction

The hydatid cyst represents an endemic zoonotic parasitic disease caused by the *Echinococcus granulosus* metacestode (larval stage), in which the human is an accidental intermediate host, the disease being characterized by cystic lesions occurring in different organs and tissues, most commonly affecting the liver and lung [12, 26, 33]. Morphologically, the hydatid larval cyst is composed of three structural components: the acellular laminar membrane, germinal membrane and hydatid fluid with germinal elements, the metacestode being surrounded by a fibrous capsule (pericyst) or granulation tissue, including inflammatory infiltrates [6, 21]. The pericyst, also called the adventitial layer, is the outer zone of the hydatid cyst, which develops through the reaction of the host tissue to the parasite and consists almost entirely of host cells [10, 25].

In cases of hydatid cyst the host-parasite relationship is interactive, the evolution of the disease being dependent on the balance between the mechanisms of the host immune system and complex escape strategies of the parasitic agent [37], which include antigenic variation, suppression and modulation of T cells, inhibition of chemotaxis of effector cell, release of antigenic proteins, antioxidant defense, resistance to host proteolytic enzymes, etc. Understanding these biological events is of fundamental importance in defining the diagnosis and treatment tactics [19, 27, 39]. It is known that the infection induces an immune imbalance on the tissues of the affected organ with a severe destruction of the architecture, caused by inflammatory infiltrates and the development of fibrosis. This process is caused by the persistent activation of the immune system, which imposes unfavorable changes on normal homeostasis of the organ, at the same time the parasite evading the defense forces of the host organism with the subsequent chronicization of the infection. In this context, the developmental aspects of local immune responses in organs affected by the hydatid cyst remain largely unknown [34].

The marked and persistent antigenic action of this parasitic agent is characterized by a major potential causing an inflammatory reaction, the local reaction being quite varied from severe granulomatous changes, associated with cysts degeneration and their eventual death, up to a collagen capsule, derived from the inflammation resolution correlated with a stable host-parasite relationship, the particularities of inflammatory reactions in pericystic tissues being determinant in the pathogenesis, clinical evolution and development of complications of the disease [7]. The control of *E. granulosus* infection is a complex process, involving the humoral and cellular components of the immune system, the immune defense reactions against an extracellular pathogen being dependent on the interaction of macrophages with T lymphocytes [5, 39].

T lymphocytes are an important type of immunocompetent cells that can produce a series of

morphological and functional changes. These cells and their subtypes have mutual coordination and antagonism during an *in vivo* immune response to maintain balance. The assessment of T lymphocytes, especially the balance of CD4 and CD8 subtypes, has a central position in the evaluation of cellular immunity and the role of these cells in neutralizing the hydatid infection [39]. According to some studies, parasite-derived substances regulate the host immune response by inducing protection (Th1 expansion) or susceptibility to disease (Th2 expansion) [18, 23, 27]. The production of Th1-polarized cytokine in early stages can kill the metacestode in the early stages of development, subsequently passing to a response with Th2 cytokine predominance characteristic for the chronic stage of *E.granulosus* infection, considering that Th2 cytokines are responsible for the inhibition of parasite destruction due to the anti-inflammatory action of IL-10 [40].

Th2 cells express IL-4, IL-5, IL-6 and IL-10, in which Th1 cells probe IL-2 and IFN-γ, accepting the fact that Th2 cytokines participate in the regulation of Th1-derived cytokines and vice versa. It has been shown that in human hydatid disease these cytokines coexist, the Th2 response benefiting the parasite, and the Th1 response benefiting the host [2].

There are many studies that have reported the results of immunity indices changes in circulating blood in cases of human hydatidosis [13, 23], but few data on the local immune response are described, most of the studies being carried out in the hepatic forms of the parasitic disease [35] or in animals [38].

The *purpose* of the study was to carry out the immunohistochemical test of the lymphocyte population in the inflammatory infiltration at the adventitial layer (pericyst) and pericystic tissues in different clinico-morphological forms of the pulmonary hydatid cyst in children.

Material and Methods

Morphopathological investigations were focused on the immunohistochemical testing of T lymphocytes involved in local cell immunity, as well as the assessment of B lymphocytes and macrophages involved in the inflammatory cell component with the use of monoclonal antibodies against surface markers CD3, CD4, CD8, CD20cy-positive and CD68 (table 1). As a study material, the tissue samples taken intraoperatively from the fibrous capsule and adjacent tissues in 20 patients with pulmonary hydatidosis, aged 10-16 year,s were grouped into 3 groups: 1) uncomplicated pulmonary hydatid cyst (7 children); 2) pulmonary hydatid cyst with signs of pericystic inflammation (7 children), and 3) pulmonary hydatid cyst complicated by rupture (6 children).

After preventive fixation of tissue samples in buffered formalin solution (pH =7.2-7.4) for 19-22 hours, they were processed according to the standard protocol for immunohistochemical examination, carried out in accordance with a special methodology. Sections subjected to immunohistochemical staining were subjected to deparaffinization. In order to facilitate labeling of the desired antigen with a specific antibody, deparaffinization is an essential primary step in immunohistochemical method.

***Table 1.* Immunohistochemical methods used in the study**

Antibody	Clone	Manufacturer	Dilution	System	Incubation time	Antigen unmasking
CD3	Monoclonal mouse	DakoCytomation	1:100	EnVision™ FLEX	30 min	Water bath with Target Retrieval high pH, 20 min
CD4	FLEX Monoclonal mouse 4B12	DakoCytomation	RTU	EnVision™ FLEX	15 min	Water bath with Target Retrieval high pH, 20 min
CD8	FLEX Monoclonal mouse C8/144B	DakoCytomation	RTU	EnVision™ FLEX	15 min	Water bath with Target Retrieval high pH, 20 min
CD20cy	FLEX Monoclonal mouse L26	DakoCytomation	1:2	EnVision™ FLEX	15 min	Water bath with Target Retrieval high pH, 20 min
CD68	FLEX Monoclonal mouse PG-M1	DakoCytomation	RTU	EnVision™ FLEX	15 min	Water bath with Target Retrieval high pH, 20 min

It has been shown that the action of organic solvent at 57° C results in a more effective dilution of the inclusion material. For this, the histological slides were subject to 2 toluene baths. The first bath lasted 60 min. in the thermostat at 59° C. The next bath was carried out at room temperature for 5 min. After deparaffining, the solvent was removed by placing it in successive baths of toluene + ethyl alcohol in the ratio of 1:1 for 5 minutes, 96% ethyl alcohol in two sessions, each stage lasted 5 minutes and two washing sessions with distilled water for 10-15 minutes each. Subsequently, the preparations were subjected to antigen unmasking, in order to break disulfide bonds and to expose antigenic epitopes more accessible to the primary antibody. Antigen unmasking was performed by heating it in a hot water bath, the histological slides being included in a helindal, where the temperature of the high pH Target Retrieval (Dako Cytomation Denmark) unmasking solution reached 95-96°C, the unmasking time being 20 min, with an additional pre- and post-treatment time of 60 min. After unmasking, the preparations were washed in diluted Wash buffer (1:20 distilled water) from the kit for 5 min., removing the excess buffer from the blade edge. After this stage the immunohistochemical process itself began. In the researches carried out, the manual IHC method was used along with the standardized EnVision^{TM} FLEX visualization system in the laboratory. After removing the excess buffer from the slide edge, the primary antibody was applied with individual incubation time for each (table). When the exposure time expired, the antibody was washed in two sessions with Wash Buffer for 5 minutes, removing the excess of washing solution. To block endogenous peroxidase, the slides were processed with undiluted peroxidase from the kit for 5 minutes, then washed in distilled water and Wash buffer successively for 5 minutes. Subsequently, the secondary antibody (HRP) was applied for 20 min. with the subsequent washing in two Wash Buffer sessions for 5 minutes each. After the excess buffer was removed, chromogen 3',3-diaminobezidine (DAB, Dako Cytomation Denmark) was applied for 3 min. in order to identify by color reaction (brown) the localization of antibodies chains. After 3 minutes, the excess DAB was removed with distilled water in two sessions for 5 minutes each. Hematoxylin was used to carry out contrasting for 1.5 min. with the subsequent washing in tap water. Subsequently, the preparations were subjected to 2 successive 96% alcohol baths, alcohol + toluene bath (1:1 ratio), two toluene baths for 5 minutes for all baths and the last toluene bath for 10 min. for final clarifying, followed by fixation in Canada balm. The method described above was applied in the case of simple immunolabeling (application of a single primary antibody). The cell density was found in 5 different visual fields in 5 different sections in a patient.

Results. The results of the study allowed us to find that in group 1 the inflammatory infiltrates of the pericystic pulmonary parenchyma underlying the fibrous capsule are dominated by $CD3^+$ lymphocytes ($p<0.01$), T lymphocytes practically missing in the fibrous capsule. The density of $CD3^+$ lymphocytes constituted 239.8$\pm$ 29.7 cells in the visual field, these being dispersed scatteredly and in agglomerations of cellular micropseudonodules (fig. 1A), also forming perivascular agglomerations in the perivascular inflammatory infiltrate (fig. 1B). The number of $CD4^+$ subpopulations in the inflammatory infiltrate of the pericystic pulmonary parenchyma was comparatively smaller (114.0$\pm$11.3 cells/field), being spread in pseudofollicular (fig. 1C) or discretely disperse (fig. 1D) appearance. The density of $CD8^+$ subpopulation was lower (126.8 $\pm$ 4.1 cells/field) compared to the density of $CD3^+$ cells ($p <0.01$), insignificantly exceeding the density of $CD4^+$ cells ($p>0.05$), these cells being widespread in dispersed appearance (fig. 1E, F). In group 1, B lymphocytes, identified by $CD20^+$ expression, were present in a number of 128.00 $\pm$ 14.2 cells/field, usually being located in the inflammatory infiltrate of the pulmonary parenchyma underlying the fibrous capsule, having lymphocyte infiltrate appearance in plates, pseudofollicularly and dispersedly, these cells missing in the fibrous capsule (fig. 2). The density of CD68 cells in the parenchyma adjacent to the fibrous capsule was quite high (189$\pm$13.4 cells/field) (fig. 3A), these cells being observed in perivascular infiltrates (fig. 3B), and in a quite large number within the fibrous capsule limits (fig. 3C) and regional pleura (fig. 3D).

In study group 2, the density of $CD3^+$ lymphocytes from the inflammatory infiltrate in the parenchyma adjacent to the fibrous capsule (257.25 $\pm$ 54.7 cells/field) significantly exceeded the values of $CD4^+$ and $CD8^+$ subpopulations ($p>0.05$), without finding a veridical difference compared with study group 1 ($p>0.05$). In these cases, $CD3^+$ lymphocytes were diffusely dispersed in the pericystic parenchyma, a higher density being determined in peribronchial spaces, single cells being observed in the fibrous capsule area (fig. 4A, B). In this group of patients $CD4^+$ subpopulation had a higher density (228.5$\pm$34.8 cells/field) compared to the values of group 1 ($p<0.01$), these cells being spread as pseudofollicular structures, located closely to the fibrous capsule (fig. 4C), these structures being well expressed in peribronchial spaces (fig. 4D). The density values of $CD8^+$ subpopulation (171$\pm$16.4 cells/field) exceeded statistically veridically values found in study group 1 ($p<0.05$), the cells of this subpopulation being spread irregularly. There were sectors with subpopulation density of 267 cells in the field of view, while in other areas - only 51 cells. Usually, $CD8^+$ cells were observed around the fibrous capsule in the form of pseudofollicular structures, these structures being also found peribronchially (fig. 4E), while in the distant interstice these cells were dispersely spread (fig. 4F).

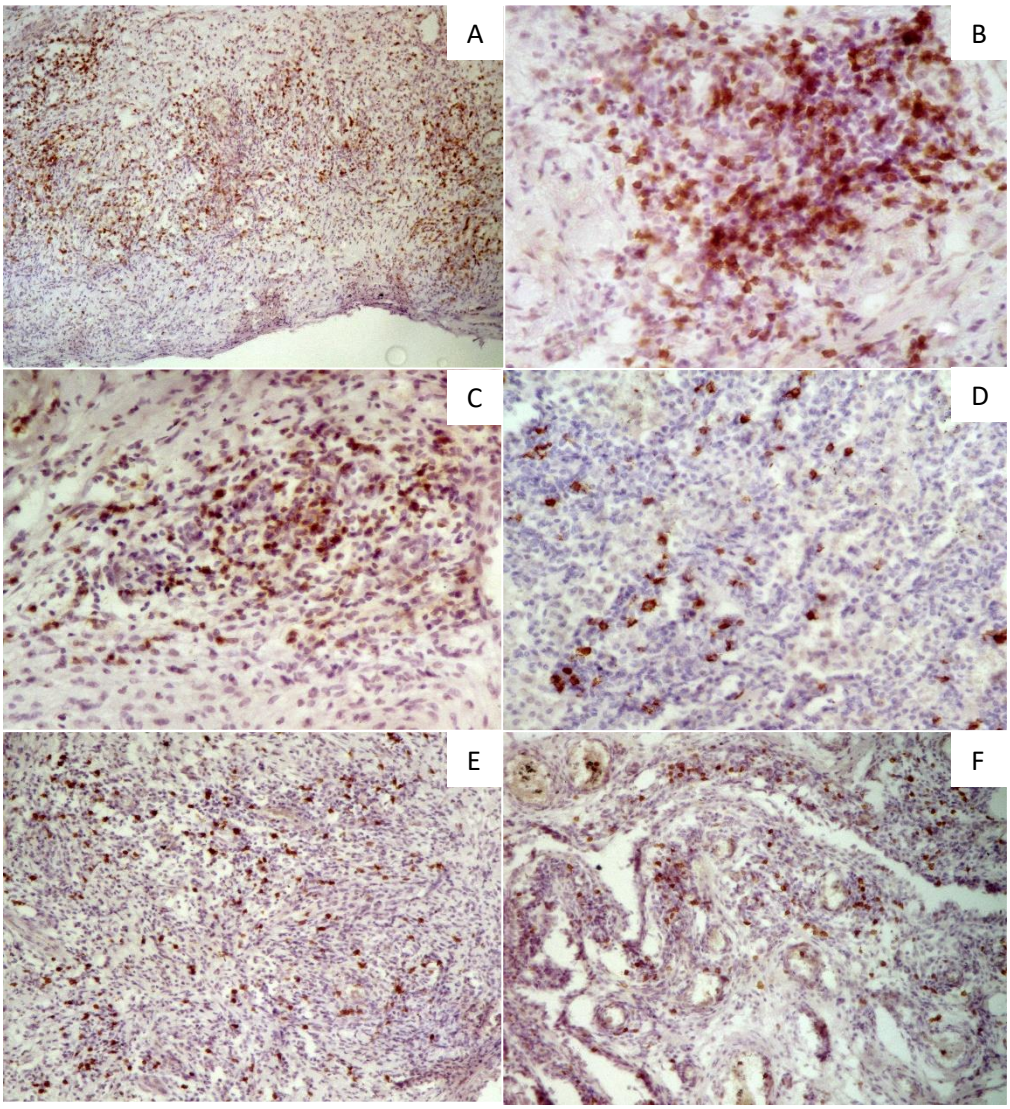


Fig. 1. Distribution of T lymphocytes in the fibrous capsule and adjacent parenchyma in study group 1: A - identification of CD3 lymphocytes in the inflammatory infiltrate of the pulmonary parenchyma underlying the capsule with recent necrolytic changes; B - perivascular inflammatory infiltrate of the pulmonary parenchyma underlying the capsule (explanations in the text); C, D - immunohistochemical identification of CD4 cells in the pulmonary parenchyma underlying the capsule (explanations in the text); E - identification of CD8 lymphocytes in the inflammatory infiltrate of the pulmonary parenchyma underlying the capsule and within the pleura (F). *Immunoreaction with anti-CD8*

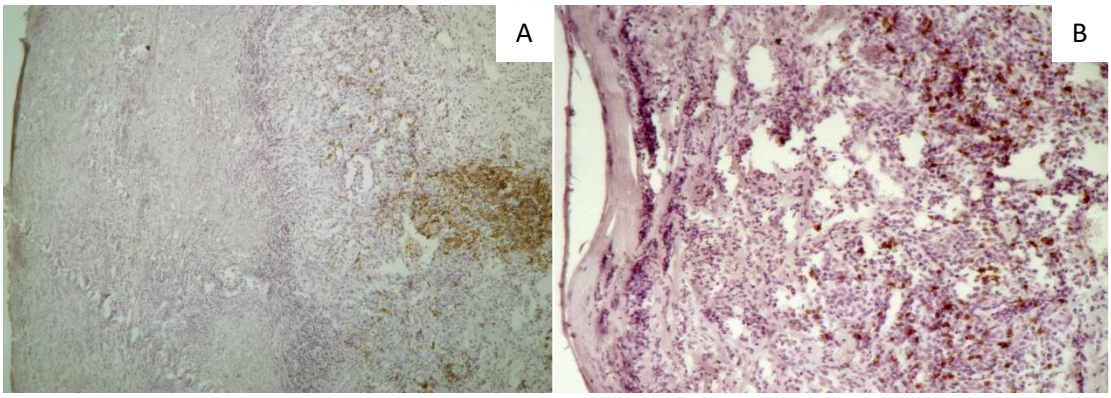


Fig. 2. Distribution of $CD20^+$ B lymphocytes in the adjacent parenchyma in study group 1. Identification of $CD20^+$ B lymphocytes in the inflammatory infiltrate of the pulmonary parenchyma underlying the capsule with recent necrolytic changes (A), and without necrolytic changes. *Immunoreaction with anti-CD20*.

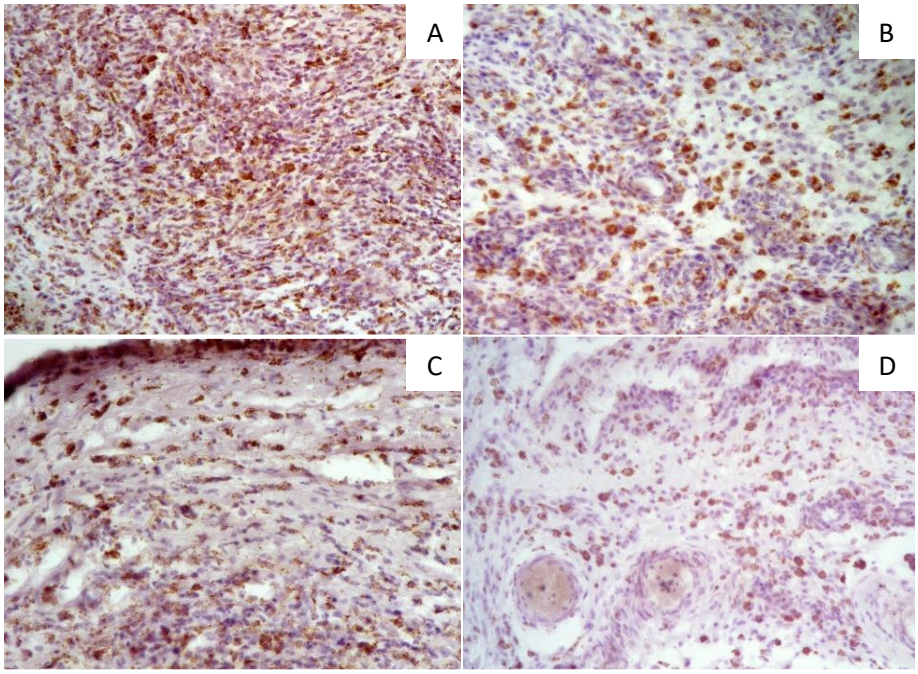


Fig. 3. . Distribution of $CD68^+$ cells in the adjacent parenchyma in study group 1: A - $CD68^+$ cells in the area of the pseudofollicular infiltrate underlying the capsule, some polynuclear cells; B - $CD68^+$ cells in the area of perivascular infiltrate with some polynucleated cells; C - $CD68^+$ cells in the area of connective capsule; D - low density $CD68^+$ cells with the presence of polynuclear cells in the pleura. *Immunoreaction with anti-CD68.*

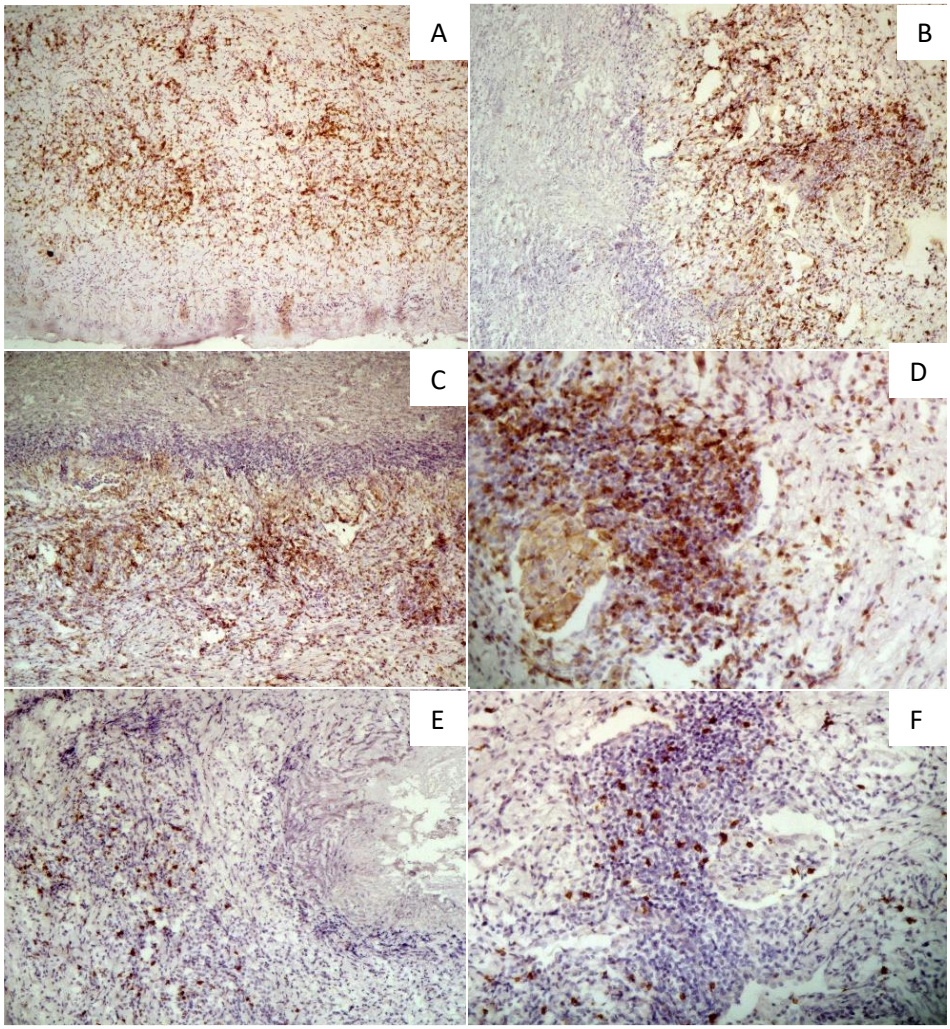


Fig. 4. The distribution of T lymphocytes in the pericystic parenchyma, group 2. A - $CD3^+$ T-lymphocytes diffusely dispersed adjacent to the capsule, solitary lymphocytes in the capsule area; B - $CD3^+$ T-lymphocytes diffusely dispersed adjacent to the capsule, peribronchially accentuated, solitary lymphocytes in the capsule area; *Immunoreaction with anti-CD3*. C - $CD4^+$ subpopulation in pseudofollicular structures in the capsule; D - $CD4^+$ subpopulation in peribronchial pseudofollicular structures; *Immunoreaction with anti-CD4*. E - subpopulation in pseudofollicular structures near the capsule with necrolytic modifications; $CD8^+$ subpopulation in peribronchial pseudofollicular structures and dispersed interstitially at distance; *Immunoreaction with anti-CD8.*

The $CD20^+$ B lymphocytes were much numerous (255.7±16.1 cells/field) compared to group 1 ($p<0.001$), being located in pericapsular inflammatory infiltrates in both dispersed and pseudofollicular aspect (fig. 5), numerous cells being observed in the fibrous capsule area.

The immunohistochemical testing of the $CD68^+$ macrophage cell component revealed that in group 2 the density of these cells (326.8±36.4 cells/field) veridically exceeded the values of group 1 ($p<0.01$). $CD68^+$ cells were predominantly concentrated in the border area between the fibrous capsule and pulmonary parenchyma, but they were found in a large number in the fibrous capsule area (fig. 6A). In the pericystic parenchyma, $CD68^+$ cells formed peribronchial pseudofollicular structures, in some places they being also observed in the bronchial lumen (fig. 6B).

In the group of patients with complicated forms of pulmonary hydatid cyst (group 3) there were increased values of CD3+ lymphocyte density (301.5±71.5 cells/field) without a statistically significant difference compared with group 1 and 2. Despite higher values, there was no real predominance of these cells in inflammatory infiltrates of the pericystic pulmonary parenchyma, these lymphocytes being unevenly spread. $CD3^+$ lymphocytes were predominantly concentrated in the sectors adjacent to the fibrous capsule, being diffusely spread in plates and dispersedly (fig. 7A). In the pericystic parenchyma, clusters of $CD3^+$ cells could be observed in peribronchial areas (fig. 7B), as well as in interalveolar septa (fig. 7C). The density of these T lymphocytes decreases while increasing the distance from the pathological focus (fig. 7D).

The density of $CD4^+$ subpopulation constituted 162.3±22.8 cells in the field of view, with no veridical difference compared to the two study groups ($p>0.05$). $CD4^+$ cells were concentrated in large inflammatory infiltrates adjacent to the fibrous capsule, being spread in plates (fig. 8A) or pseudofollicular structures (fig. 8B). Frequently, $CD4^+$ lymphocytes could be observed in the infiltrates of interalveolar septa (fig. 8C) and as interstitial follicular structures (fig. 8D).

The subpopulation of $CD8^+$ cells, estimated in group 3 (228.8±17.6 cells/field), veridically exceeded the density of these cells in group 1 ($p<0.01$) and group 2 ($p<0.05$). $CD8^+$ lymphocytes were present in areas adjacent to the fibrous capsule in the form of dispersed pseudofollicular structures (fig. 9A), as well as in peribronchial spaces (fig. 9B), these lymphocytes having a moderate density in distant areas (fig. 9C), where they could also be observed as interstitial follicular structures (fig. 9D).

The examination results of group 3 showed an insignificantly higher density of $CD20^+$ B lymphocytes (180.3±62.02 cells/field) compared to group 1 ($p>0.05$), the recorded values being lower compared to the values of group 2 with no veridical difference ($p>0.05$). These lymphocytes were observed in the form of pseudofollicular structures, located adjacent to the fibrous capsule and in the distant pulmonary parenchyma (fig. 10A), as well as peribronchially (fig. 10B).

The density of $CD68^+$ cells (201.7±47.4 cells/field) in group 3 was insignificantly higher compared to group 1 ($p>0.05$) and insignificantly lower compared to group 2 ($p>0.05$). Despite the fact that in the areas bordering with the fibrous capsule $CD68^+$ lymphocytes had a significant density (fig. 11 B), they could be observed in the adjacent parenchyma and even in the fibrous capsule (fig. 11A). There were also CD68 cells intraalveolarly and interstitially, some being polynucleated (fig, 11C).

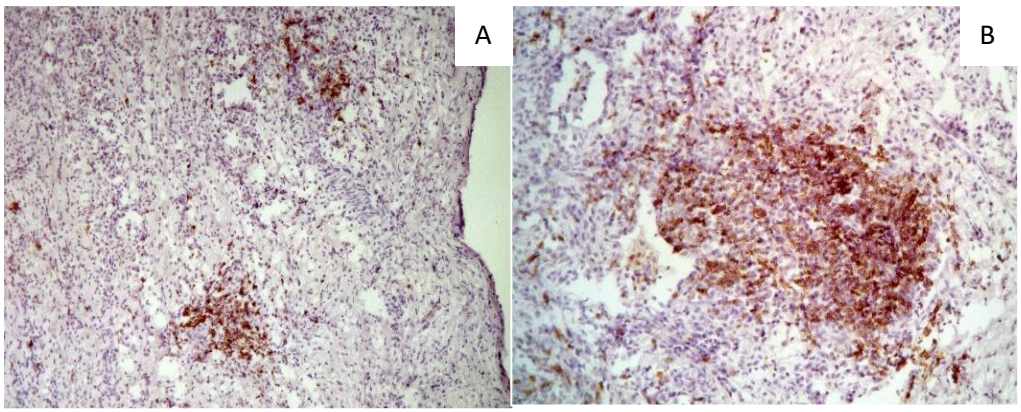
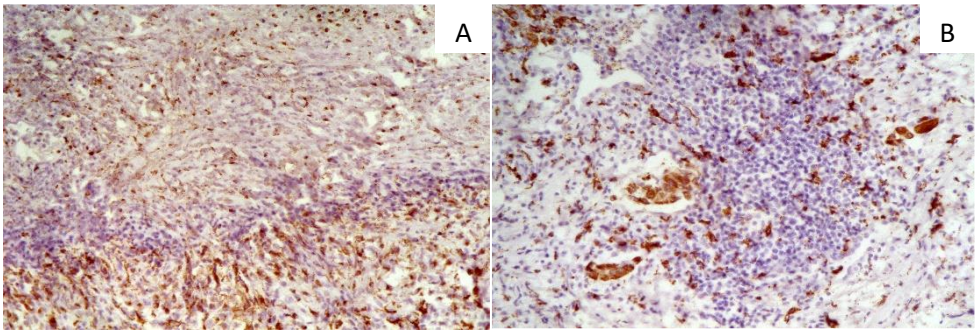


Fig. 5. Distribution of $CD20^+$ B lymphocytes in the pericystic parenchyma in group 2. A - $CD20^+$ B lymphocytes in dispersed and pseudofollicular appearance in the inflammatory infiltrate of the pulmonary parenchyma underlying the capsule with recent necrolytic changes; B - B $CD20^+$ lymphocytes in pseudofollicular structures near the capsule. *Immunoreaction with anti-CD20.*



Fic. 6. Distribution of $CD68^+$ cells in the pericystic parenchyma in group 2. A - $CD68^+$ cells in the border and conjunctival capsule areas; B - $CD68^+$ cells in peribronchial pseudofollicular structures and bronchial lumen, some having polynucleated appearance. *Immunoreaction with anti-CD68*

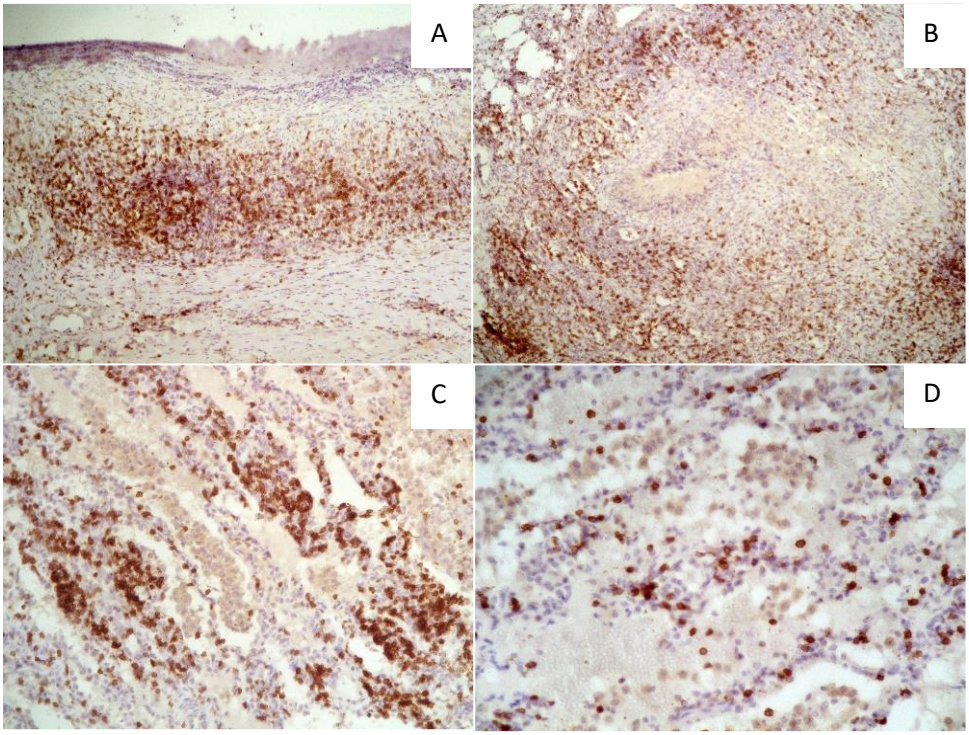


Fig. 7. Distribution of $CD3^+$ cells in the pericystic parenchyma in group 3. A - diffuse $CD3^+$ lymphocytes in plate-like and dispersed appearance adjacent to the capsule; B - peribronchially localized $CD3^+$ lymphocytes; C - $CD3^+$ lymphocytes of medium density in interalveolar septa; D - low-density $CD3^+$ lymphocytes in distant interalveolar septa; *Immunoreaction with anti-CD3.*

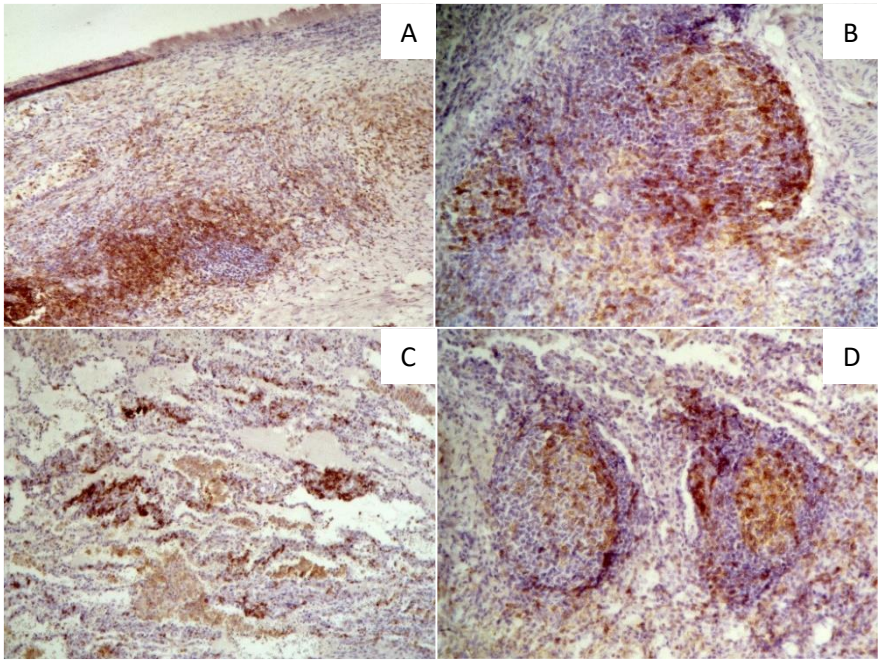


Fig. 8. Distribution of $CD4^+$ cells in the pericystic parenchyma in group 3. A - the subpopulation of $CD4^+$ in vast plate-like infiltrate around the capsule; B - $CD4^+$ lymphocytes in pseudofollicular structures around the capsule; C - $CD4^+$ lymphocytes in the infiltrate localized in interalveolar septa; D - $CD4^+$ lymphocytes in the form of interstitial follicular structures; *Immunoreaction with anti-CD4.*

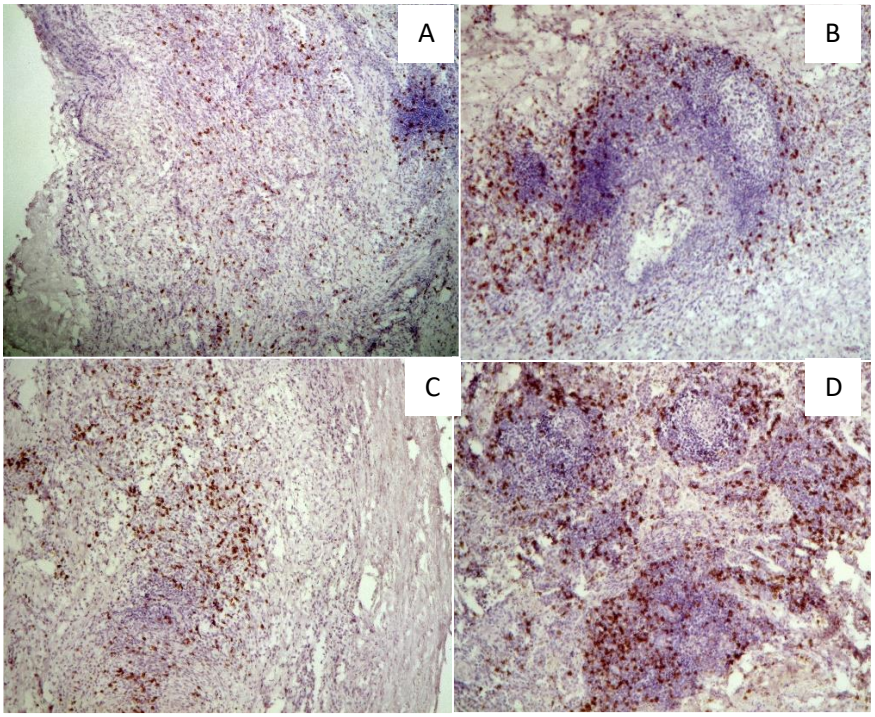


Fig. 9. Distribution of $CD8^+$ cells in the pericystic parenchyma in group 3. A - $CD8^+$ cells in pseudofollicular structures and interstitially dispersed adjacent to the capsule; B - $CD8^+$ cells in peribronchial pseudofollicular structures; C - $CD8^+$ cells of moderate density in pericapsular areas at various distance; D - $CD8^+$ cells in distant interstitial lymphoid follicular structures. *Immunoreaction with anti-CD8.*

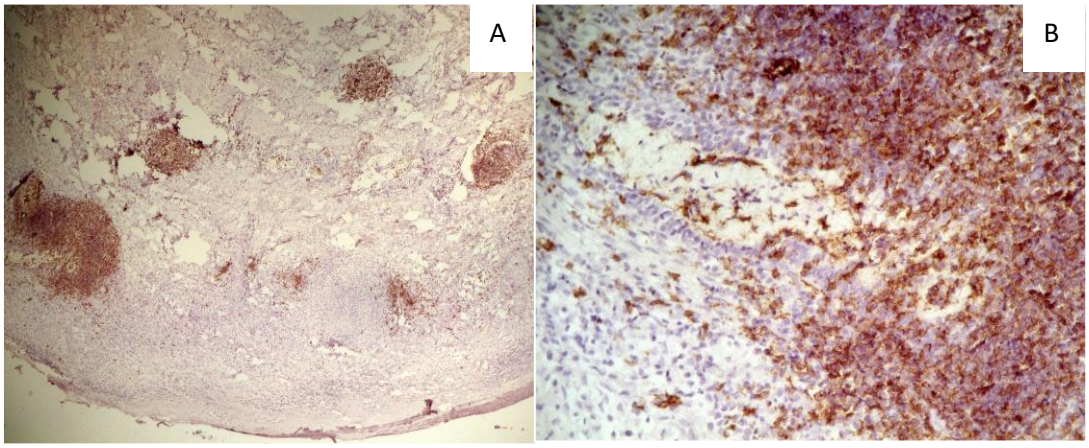


Fig. 10. Distribution of $CD20^+$ cells in the pericystic parenchyma in group 3. $CD20^+$ B lymphocytes in the form of pseudofollicular structures located around the capsule and distantly in the pulmonary parenchyma; B - $CD20^+$ B lymphocytes in peribronchial pseudofollicular structures; *Immunoreaction with anti-CD20*

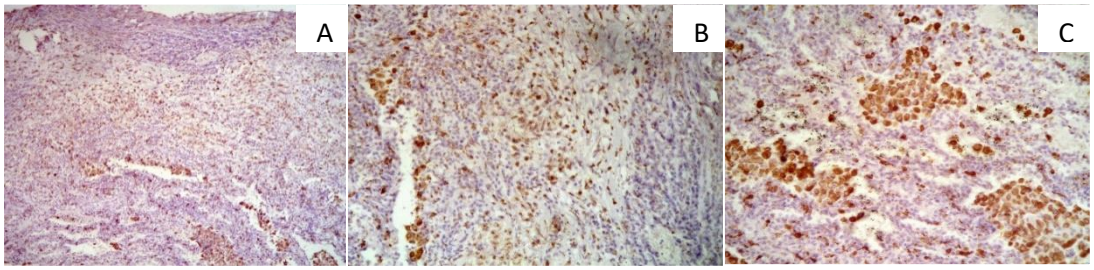


Fig. 11. Distribution of $CD68^+$ macrophages in the pericystic parenchyma in group 3. A - moderately diffuse CD68+ cells in the area of fibrous capsule and underlying parenchyma; B - $CD68^+$ cells in structures of marked density bordering with the fibrous capsule; C - $CD68^+$ cells in the intralveolar and interstitial solitary area, some polynuclear ones. *Immunoreaction with anti-CD68.*

Discussion

T lymphocytes constitute a relatively minor population in a normal lung, the number of these cells being increased, undergoing phenotypic changes in cases of the association of pulmonary inflammation and fibrosis. The role of T lymphocytes infiltrates in the development of fibrosis remains unclear, assuming that depending on the phenotypic characteristics of T lymphocytes in pulmonary infiltrates they may have a profibrotic or antifibrotic contribution [16].

Notwithstanding a significant immune response of the intermediate host [11], several mechanisms of a complex strategy have been found in the parasitic infection with Echinococcus granulosus, whereby this parasite modulates the host immune system response for long periods of time to ensure its continuous survival [8, 9]. These mechanisms can be classified into antigenic mimicry, antigenic exhaustion, antigenic variation, immunological indifference, immunological diversion, and immunological subversion [40]. It has been established that the hydatid content induces mitosis and proliferation of the subpopulations of T, B lymphocytes and macrophages [17], demonstrating a wide variety of immunomodulatory actions of the hydatid fluid, including cytotoxicity, polyclonal activation of lymphocytes T and B, influence of CD4 and CD8 cell expression, cytokine secretion, etc.[1, 14, 28, 29]. It has been found that the parasitic antigen AgB, factor EgEF-1 β/δ and other parasitic molecules have the ability to inhibit neutrophil recruitment and a number of immunomodulatory properties, inducing Th2 type of anti-inflammatory immune response, characterized by the production of IL-4, IL- 10, reduced level of IL-12 and IFN-c and IgG4 production [18, 29, 30].

Several studies have found that CD3 T lymphocytes predominate at the periphery of the hepatic hydatid cyst, followed by $CD20^+$ B lymphocytes, whereas $CD4^+$ and

$CD8^+$ cells have a weak presence around the pericyst, and the number of $CD68^+$ cells is highly variable [34]. Tissue scarring and fibrous tissue formation in chronic lesions are associated with phenotypic changes in T lymphocytes, which are involved in homeostasis disturbances of tissue. The long-lasting action of pathogen results in the differentiation of $CD4^+$ lymphocytes through which macrophages are activated with the subsequent development of fibrosis [22].

Some experimental immunohistochemical studies have highlighted the predominance of $CD8^+$ T lymphocytes at the periphery of progressive cysts [24]. It is assumed that $CD8^+$ lymphocytes are recruited to the injury site where, together with the activation of defense mechanisms, they may contribute to an increased tissue damage [40], this opinion being disputed by some authors [35].

The high density of $CD20^+$ B cells in pericystic tissue demonstrates a significant activity of the adaptive immune system, indicating the role of these cells in local immune functions against the parasite [35]. It should be taken into account that $CD4^+$ cells are essential in ensuring the microenvironment responsible for activating and differentiating B lymphocytes following exposure to antigen. It is considered that B lymphocytes located follicularly in peribronchiolar areas may contribute to the development of lymphoid tissue associated with bronchi, these cells contributing to the development of fibrosis by producing IL-6, which represents a pleiotropic cytokine, which in addition to its role in the acute phase reaction, it has various roles in the control of chronic inflammation, autoimmune processes, endothelial cell dysfunction and fibrogenesis [3, 4].

Pulmonary macrophages play a role in both acute and chronic pulmonary conditions, including cytotoxicity and fibrosis. Two phenotypically distinct subpopulations of macrophages are identified, being characterized as proinflammatory /cytotoxic M1 and M2 macrophages that participate in the regulation of inflammatory and wound repair processes, of which $CD68^+$ cells are also included [36]. M2 macrophages are also considered to be major regulators of fibrosis through the secretion of profibrotic mediators and subsequent activation of collagen-producing cells [20]. There are studies that show that $CD68^+$ cell expression testing is carried out to evaluate the inflammatory reaction in pericystic tissues of the hepatic hydatid cyst and to identify the involvement of macrophages against the parasitic agent [15, 36].

Conclusion

1. The evolution of the pulmonary hydatid cyst in children is characterized by a chronic inflammatory infiltration of the pericystic parenchyma with the predominance of CD3 T lymphocytes compared to the fibrous capsule where the necrolytic processes predominate.
2. The increased number of CD20 indicates a significant activation of the adaptive immune system in the aggravation of inflammatory processes in the pericystic parenchyma in complicated forms of the disease, the increase of the number of macrophages in these cases being insignificant.
3. The number of CD68 macrophages in the fibrous capsule and within the pleura was significantly lower compared to the density of these cells detected in inflammatory infiltrates of the pulmonary parenchyma underlying the fibrous capsule, an insignificant number being found in complicated forms of the disease.

REFERENCES

1. Ali W.R., Sadek A., Ghazi H.F. Evaluation of IL-2, IL-1 evaluation of IL-2, IL-10 and IFN-gamma immune expression in liver and spleen after treatment of experimental cystic echinococcosis.TOFIQ J. Med. Sci. 2016; 3(1):26-38.
2. Amri M., Mezioug D., Touil-Boukoffa C. Involvement of IL-10 and IL-4 in evasion strategies of Echinococcus granulosus to host immune response. Eur. Cytokine Netw. 2009; 20:63-8.
3. Atkins S.A., Turesson C., Myers J.L., Tazelaar H.D., Ryu J.H. et al. Morphologic and quantitative assessment of CD20B cell infiltrates in rheumatoid arthritis - associated nonspecific interstitial pneumonia and usual interstitial pneumonia. Arth. Rheum. 2006; 54(2):635-41.
4. Barnes T.C., Anderson M.E., Moots R.J. The many faces of interleukin-6: The role of IL-6 in inflammation, vasculopathy, and fibrosis in systemic sclerosis. HPC Int. J. Rheumatol. 2011. Art. ID 721608, 6 p. doi:10.1155/2011/721608.
5. Baz A., Ettlin G.M., Dematteis S. Complexity and function of cytokine responses in experimental infection by Echinococcus granulosus. Immunobiology. 2006; 211: 3-9.
6. Bektas S., Erdogan N.Y., Sahin G., Kir G., Adas G. Clinicopathological findings of hydatid cyst disease: A retrospective analysis. Ann. Clin. Pathol. 2016; 4(3):1071.
7. Breijo M., Anesetti G., Martinez L., Sim R.B., Ferreira A.M. Echinococcus granulosus: The establishment of the metacestode is associated with control of complement-mediated early inflammation. Exp. Parasitol. 2008; 118:188-96.
8. Cui S.J., Xu L.L., Zhang T., Xu M., Yao J. et al. Proteomic characterization of larval and adult development stages in Echinococcus granulosus reveals novel insight into host-parasite interactions. J. Proteom. 2013; 84:158-75.

9. Diaz A., Casaravilla C., Alen J.E., Sim R.B., Ferreira A.M. Understanding the laminated layer of larval Echinococcus II: immunology. Trends Parasitol. 2011; 27(6):263-72.
10. Golzari S.E.J., Sokouti M. Pericyst: The outermost layer of hydatid cyst. World J. Gastroenterol. 2014. 20(5): 1377-8.
11. Grubor N.M., Jovanova-Nesic K.D., Shoenfeld Y. Liver cystic echinococcosis and human host immune and autoimmune follow-up: A review. World J. Hepatol. 2017; 9(30):1176-89.
12. Halezeroglu S., Okur E., Tanyü O. Surgical Management for Hydatid Disease. Thorac. Surg. Clin. 2012; 22:375-85.
13. Hernandez A., O Connor J.E., Mir A. Phenotipic analysis of peripheral lymphocyte subpopulations in hydatid patients. Parasitol. Res. 1999; 85(11):948-50.
14. Kanan J.H.C., Chain B.M. Modulation od dendritic cell differention and cytokine secretion by the hydatid cyst fluid of Echinococcus granulosus. Immunology. 2006; 118:271-8.
15. Labsi M., Khelifi L., Mezioug D., Soufli I., Touil-Boukoff C. Antihydatid and immunomodulatory effects of Punica granatum peel aqueous extract in a murine model of echinococcosis. Asian Pacific J. Trop. Med. 2016; 9(3):211–20.
16. Luzina I.G., Todd N.W., Nacu N., Lockatell V., Choi J. et al. Regulation of pulmonary inflammation and fibrosis through expression of integrins V3 and V5 on pulmonary T lymphocytes. Arthritis & Rheumatism. 2009; 60(5):1530-9.
17. Macintyre A.R., Dixon J.B., Bleakley J.S., Green J.R. Echinococcus granulosus: assays for hydatid immunoregulatory factors using established lymphoid cell lines. Parasite Immunol. 2000; 22:475-85.
18. Malla N., Mewara A. Human cystic echinococcosis with special reference to India – An overview. Ann. Clin. Cytol. Pathol. 2016; 2(5):1038.
19. Monteiro K.M., de Carvalho M.O., Zaha A., Ferreira H.B. Proteomic analysis of the Echinococcus granulosus metacestode during infection of its intermediate host. Proteomics. 2010; 10:1985-99.
20. Murray P.J., Wynn T.A. Protective and pathogenic functions of macrophage subsets. Nat. Rev. Imm. 2011; 11(11):723-37.
21. Nabi M.S., Waseem T. Pulmonary hydatid disease: What is the optimal surgical strategy? Int. J. Surg. 2010. 8:612-61.
22. Pellicoro A., Ramachandran P., Iredale J.P., Fallowfield J.A. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat. Rev. Immunol. 2014; 13(3):181-94.
23. Rigano R., Buttari B., De Falco E., Profumo E., Ortona E. et al. Echinococcus granulosus-specific T-cell lines derived from patients at various clinical stages of cystic echinococcosis. Parasite Immunol. 2004; 26(1):45-52.
24. Sakamoto T., Cabrera P.A. Immunohistochemical observations on cellular response in unilocular hydatid lesions and lymp nodes of cattle. Acta Trop. 2003; 85(2):271-9.
25. Sarkar M., Pathania R., Jhobta A., Thakur B.R., Chopra R. Cystic pulmonary hydatidosis. Lung India. 2016; 33:179-91.
26. Sarkar S., Roy H., Sengupta M., Sarder K., Sengupta M. Cystic echinococcosis: A neglected disease at usual and unusual locations. Trop. Parasitol. 2017; 7:51-5.
27. Siracusano A., Delunardo F., Teggi A., Ortona E. Host-parasite relationship in cystic echinococcosis an evolving story. HPC. Clin. Develop. Immunol. 2012. Art. ID 639362. 12 pag. doi:10.1155/2012/639362.
28. Siracusano A., Margutti P., Delunardo F., Profumo E., Rigano R. et al. Molecular cross-talk in host-parasite relationships: The intriguing immunomodulatory role of Echinococcus antigen B in cystic echinococcosis. Int. J. Parasitol. 2008; 38:1371.
29. Siracusano A., Rigano R., Ortona E., Profumo E., Margutti P. et al. Immunomodulatory mechanisms during Echinococcus granulosus infection. Exp. Parasitol. 2008; 119:483-9.
30. SiracusanoA., Delunardo F., Teggi A., Ortona E. Cystic echinococcosis: Aspects of immune response, immunopathogenesis and immune evasion from the human host. Endocri., Metab.immune Dis. Drug Targets. 2012; 12:16-23.
31. Türkoğlu E., Demirtürk N., Tünay H., Akıcı M., Öz G., Embleton D.B. Evaluation of patients with cystic echinococcosis. Turkiye Parazitol. Derg. 2017; 41:28-33.
32. Vatankhah A., Halász J., Piurkó V., Barbai T., Rásó E., Tímár J. Characterization of the inflammatory cell infiltrate and expression of costimulatory molecules in chronic echinococcus granulosus infection of the human liver. BMC Inf. Dis. 2015; 15:530. DOI 10.1186/s12879-015-1252-x.
33. Vatankhan A. Immunopathology of hydatid infection in human liver. PhD Thesis. Budapest. 2016. 139 p.
34. Venosa A., Malaviya R., Choi H., Gow A.J., Laskin J.D., Laskin D.L. Characterization of distinct macrophage subpopulations during nitrogen mustard-inuced lung injury and fibrosis. Am. J. Respir. Cell Mol. Biol. 2016; 54(3):436-46.
35. Virginio V.G., Monteiroa K.M. Drumond F., de Carvalhoa M.O., Vargas D.M. et al. Excretory/secretory products from in vitro-cultured Echinococcus granulosus protoscoleces. Mol. Biochem. Parasitol. 2012; 183:15-22.
36. Vismarra A., Mangia C., Passeri B., Brundu D., Masala G. et al. Immuno-histochemical study of ovine cystic echinococcosis (Echinococcus granulosus) shows predominant T cell infiltration in established cysts. Vet. Parasitol. 2015. 209:285-8.
37. Wei X., Xu Q., Rexiti F.L., Zhu M., Lin R., Wen H. Dynamic changes of DC ant T cell subsets in mice during Echinococcus multilocularis infection. Cent. Eur. J. Immunol. 2014; 39(1):19-24.
38. Weiskirchen R., Tacke F. Cellular and molecular functions of hepatic stellate cells in inflammatory responces and liver immunology. Hepatobiliary Surg. Nutr. 2014; 3(6):344-63.
39. Zhang W., Ross A.G., McManus D.P. Mechanisms of immunity in hydatid disease: Implications for vaccine development. J. Immunol. 2008; 181:6679-85.
40. Zhang W., Wen H., Li J., Lin R., McManus D.P. Immunology and immunodiagnosis of cystic echinococcosis: An update. HPC Clin. Develop. Immunol. 2012. Art. ID 101895. 10 p. doi:10.1155/2012/101895.

Particularities of the regenerative processes in the reconstruction of diaphragmatic defects with decelularized grafts of porcine pericardium in experimental model

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Abstract

Particularitățile proceselor regenerative în reconstrucția defectelor diafragmatice cu alografe decelularizate de pericard porcin în model experimental

Actualmente se depun eforturi considerabile în elaborarea unor materiale biologice decelularizate ca alternativă de corecție chirurgicală a defectelor diafragmatice.
Scopul acestui studiu a fost evaluarea eficienței, siguranței și particularităților proceselor de regenerare și remodelare tisulară ale alografelor decelularizate de pericard porcin crioprezervate, utilizate în reconstrucția defectelor diafragmatice, create chirurgical în model experimental la porci.
Lotul de studiu a inclus 6 purcei cu greutatea de 10 kg, supuși laparotomiei subcostale stângi cu reconstrucția defectului diafragmatic, creat chirurgical, cu grefe decelularizate de pericard porcin (lotul 1 - 3 animale) și peritoneu porcin (lotul 2 - 3 animale).
Exameul radilogic efectuat în loul 1 la 15 zile postoperator a stabilit o configurație normală a neohemidiafragmului creat, la a 60 zi postoperator fiind documentată eventrația nesemnificativă a neohemidiafragmului. În lotul 2 la a 15 zi postoperator a fost observată o eventrație neînsemnată a neohemidiafragmului, ulterior, ambele animale acestui lot decedînd subit la a 54 și a 60 zi postoperator din cauza dehiscenței grefei.
În lotul I au fost observate zone cu o fibrilogeneză atipică (malformativă) care se evidențiau printr-un aspect multichistic sau lacunar-cavitar, cu suprafețe interne tapetate cu epiteliu mezotelial peritoneal unistratificat aplatizat fără aspecte de activitate mitotică. Formațiunea cavitar-chistică putea fi și o pseudotumoră chistică cu septuri mai subțiri, cu o carcasă redusă din fibre de colagen. La animalele lotului 2 în ambele cazuri s-a constatat un hemidiafragm cu defect persistent determinat de reabsorbția parțială a materialului biologic, necătînd că putea fi observat și zone de țesut neoformat.
Așadar, grefele decelularizate de peritoneu porcin utilizate în reconstrucția chirurgicală a diafragmului sunt caracterizate de o biorezistență redusă, care poate contribui la reabsorbția parțială a acestui material biologic cu dezvoltarea unor complicații grave. Grefele decelularizate de pericard au o biorezistență acceptabilă comparativ cu cele de peritoneu porcin, aceste date sugend necesitatea unor studii suplimentare pe termen lung, care ar avea ca scop obținerea unor rezultate mai durabile.

Cuvinte cheie: diaphragmatic defects, biological grafts, porcine pericardium, regenerative processes

Abstract

Considerable efforts are currently underway in the development of decellularized biologic materials as an alternative to surgical correction of diaphragmatic defects.

The aim of this study was to evaluate the efficiency, safety and particularities of tissue regeneration and remodeling processes of allograft decellularized cryopreserved porcine pericardium, used in the reconstruction of diaphragmatic defects, surgically created in experimental model in pigs.

The study group included 6 pigs weighing 10 kg, subjected to left subcostal laparotomy with surgical reconstruction of diaphragmatic defect, with decellularized grafts of porcine pericardium (1-3 animals) and porcine peritoneum (group 2- 3 animals).

The radilogic examination performed in group 1 at 15 days postoperatively a normal configuration of the neohemidiaphragm created, at 60 postoperative day being documented the insignificant eventration of neohemidiaphragm. In group 2 to 15 postoperatively, a slight incidence of neohemidiaphragm was observed, after which both animals of this group died suddenly at 54 and 60 days postoperatively due to graft dehiscence.

In 2 cases areas with atypical (malformative) fibrillogenesis were observed, which were evidenced by a multicystic or lacunar-cavity aspect, with internal surfaces covered with unistratified peritoneal mesothelial epithelium flattened without aspects of mitotic activity. The mesothelial cavity-cystic aspect has also been shown on border, in some cavities being present fibro-epithelial conjunctival micro-polyps. The cavity-cyst formation could also be a cystic pseudotumor with thinner septa, with a reduced collagen fiber housing. In the animals of lot 2, in both cases, there was a hemidiaphragm with persistent defect determined by the partial reabsorption of the biological material, although neoformed tissue areas could be observed.

Therefore, decellularized porcine peritoneum grafts used in surgical reconstruction of the diaphragm are characterized by a low bioresistence which can contribute to the partial reabsorption of this biological material with the development of serious complications. Decellularized grafts of porcine pericardium have an acceptable bioresistence compared to porcine peritoneum, and this data suggests the need for further long-term studies that would seek to achieve more sustainable outcomes.

Keywords: gastroschisis, omphalocele, newborn, associated congenital malformations, prognosis

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Introduction

Congenital diaphragmatic defects remain an important cause of morbidity and mortality in newborns, the size, anatomical details or their type directly influencing the prognosis [1, 36]. The surgical treatment of these defects significantly evaluated from emergency surgery to delayed interventions, with the elective plasty of the diaphragmatic defect, performed after stabilizing the general condition of the child and of the cardio-respiratory functions [27, 29]. Along with new modalities of diagnosis and treatment in neonatal intensive care [13, 35] including with the support of ECMO [15], were described several surgical techniques for repair and reconstruction of diaphragmatic defects [18, 19, 43], including minimally invasive [20, 38, 40, 51]. The indisputable advantages of contemporary methods of surgical treatment of diaphragmatic hernias have not reduced the early recurrence rates, which constitute 3-50% [10, 36]. To improve the situation, biological grafts with integration properties in the host tissues are used more frequently in the primary reconstruction of the diaphragmatic defects, and subsequently they are replaced [3, 4].Several biological grafts have been created, and some acellular tissues are approved in clinical practice without identifying the ideal material, including: human dermal acellular matrix [2, 9], porcine intestinal submucosa [31], collagenous material derived from porcine dermis [28], bovine pericardium [25]. These biological materials have been shown to be effective in repairing human tissue [5], some of them being used as an option in the reconstruction of diaphragmatic defects [17, 23, 47].

The aim of this study was to evaluate the efficiency, safety and particularities of tissue regeneration and remodeling processes of allograft decellularized cryopreserved porcine pericardium, used in the reconstruction of diaphragmatic defects, surgically created in experimental model in pigs.

Material and Methods

The study group included 6 Landrace pigs of 3 weeks of age and weighing 8.9-9.3 kg, undergoing general anesthesia and sacrificed over 60 (group I - 3 animals) and 90 respectively. days (group II - 3 animals), according to the requirements in force, the study being approved by Bioethics Committee.

The animals underwent left subcostal laparotomy (3 animals) and left postero-lateral thoracotomy (3 animals), with the surgical modeling of a circular defect of 4 cm in diameter in the left hemidiaphragm, after which it was closed with decellularized grafts of cryopreserved porcine pericardium (fig. 1). In the control group, the diaphragmatic defect was closed with decellularized porcine peritoneum (2 animals).

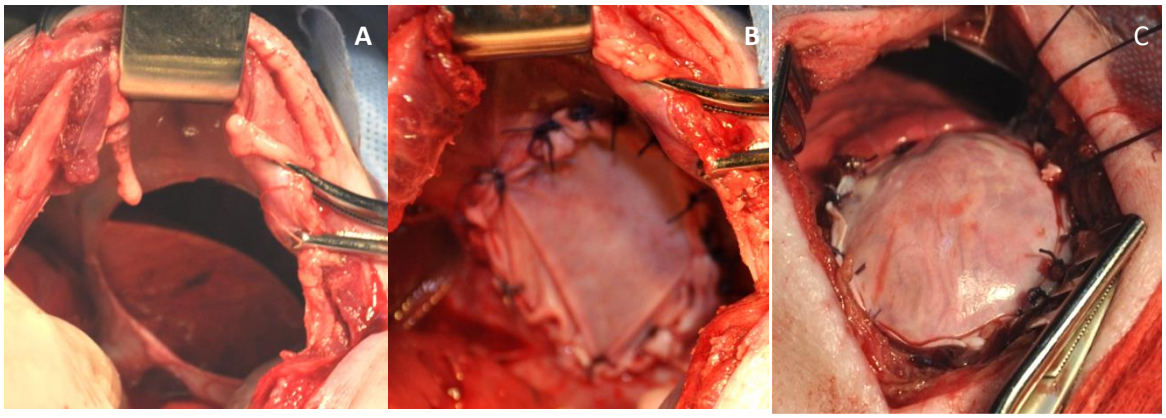

Fig. 1. The intraoperative aspect of the surgical modeling with decellularized biological graft of a diaphragmatic defect in animal experiment: A - the intraoperative aspect of the diaphragmatic defect created surgically by abdominal approach; B - the intraoperative aspect of closing the diaphragmatic defect with pericardial graft by abdominal approach; C - the intraoperative aspect of closing the diaphragmatic defect with pericardial graft through thoracic approach

Porcine pericardium and peritoneum were obtained immediately after slaughter of the animal at the slaughterhouse, and subsequently transported to the laboratory in 0.9% sodium chloride isotonic solution.
The processing of biological material included several stages:

- Decontamination: in antibiotic coctail (gentamicin, lincomycin, amphotericin B) for 6 hours;
- Decellularization: use of sterile 0.5% Sodium Duodecyl Sulfate solution, the solution being changed every 24 hours for 72 hours;
- Washing of the decellularization membrane: under sterile conditions with isotonic solution of 0.9% sodium chloride, in abundance;
- Repeated decontamination for 24 hours in the antibiotic cocktail; control of the efficiency of histologically confirmed decellularization.

As a control of the morphological particularities of hemidiaphragm tissue maturation in the pigs involved in the study, in 6 cases the contralateral hemidiaphragm from the same pigs and the same slaughter period was used. In order to assess the efficiency of the reconstruction of the diaphragmatic defect, the radiological examination was performed after 2 weeks, 2 months and 3 months from the time of the intervention.

For the histological investigations tissue samples (3 pieces with dimensions of 1.0 x 1.0 x 0.5 cm) were taken from the central, intermediate and peripheral areas sutured to the host tissues and at a distance of 1.5-2 cm. Once they have been fixed to the ground. Formol of 10% for 6-12 hours, were treated according to the standard protocol for histopathological investigations, using the network of histoprocessing and automated staining "Diapath". For the histological tests, at the SLEE MAINZ-CUT 6062 microtome microtome (Germany), 3-4 sections with a thickness of ≈ 3-4 μ, colored using the conventional hematoxylin-eosin (H&E) method were obtained.

Results and discussion

At the radiological examination, performed 15 days postoperatively, in the control group, where in the reconstruction of the diaphragmatic defect was used decellularized porcine peritoneum, an ascending position of the left hemidiaphragm was determined.

When using porcine pericardial grafts, the configuration of the hemidiaphragm undergoing surgery was normal and was maintained in this condition for 30 days after surgery (fig. 2).

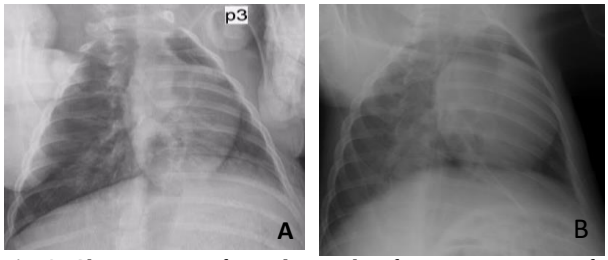

Fig. 2. Chest x-ray performed 2 weeks after reconstruction of the diaphragmatic defect with allograft of: a - peritoneum, b - pericardium.

Both animals in the control group died at the 54th and 60th day, respectively, after the intervention due to the recurrence of the diaphragmatic defect, complicated with strangulation, in both cases being observed, at necropsy, the subtotal resorption of the peritoneum graft (fig. 3).

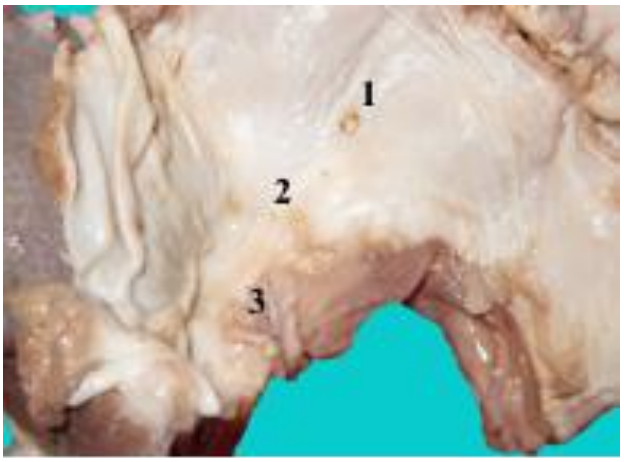

Fig. 3. The appearance of the hemidiaphragm undergoing reconstruction with decellularized graft of porcine peritoneum that has been resorbed subtotal, generating a persistent defect: 1 - fibro-muscular area; 2 - the limits of the plastic defect; 3 - unformed tissue reminiscent of graft

All animals in groups I and II remained alive, but showed signs of eventualization of the reconstructed hemidiaphragm (fig. 4).

Macroscopic exploration of the hemidiaphragm undergoing reconstruction showed that the development of the event, confirmed radiologically, is determined by the significant thinning of the graft following the regeneration and remodeling processes (fig. 5).

The histological examinations consisted of the evaluation of the process of neoformation of the connective tissue (fibrillogenesis), the presence of the inflammatory process and the reminiscences of the allografts according to the mentioned areas, as well as the evaluation of the activity of the fibroformed connective tissues.

In both lots, during the mentioned periods of sacrificing and examination, the presence of unformed connective tissue with insignificant deviations between lots of study was attested. The results obtained according to the evaluation indices are presented in tab.1.

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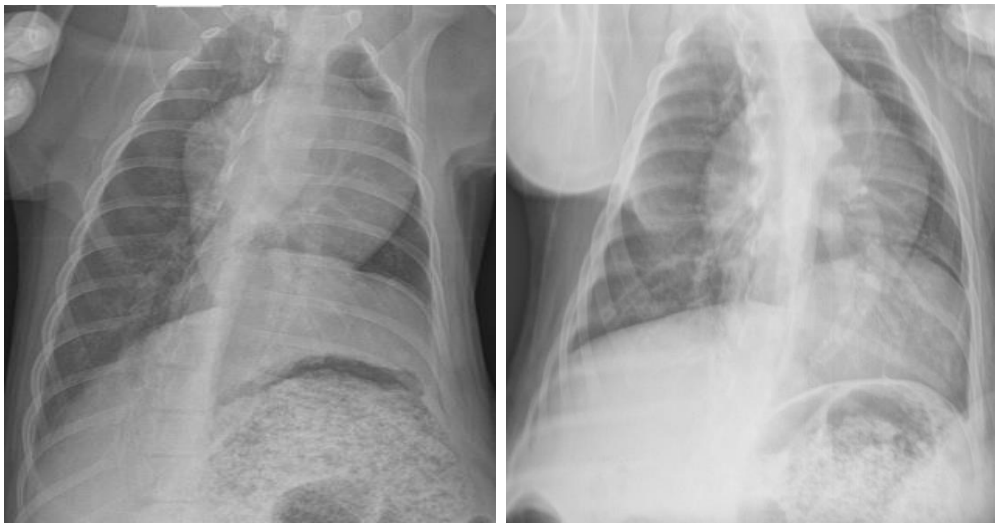

Fig. 4. Chest radiography of the laboratory animals from group II performed 60 days (A) and 90 days postoperatively (B). Viewing the event of the left hemidiaphragm

Table 1. Dynamism of normal and malformative fibrillogenesis

Indices	Depending on lots	
	Lot I (n=3)	Lot II (n=3)
Fibrillogenesis		
Mature	2	3
Intermediate (mature and imature)	2	-
Atypically (malformative)	1	1
Inflamation		
Polymorphonuclear (PMN)	2	-
Mononuclear (MN)	3	1
Granulomatous (GM)	2	1
Allograft residues		
In the contact area (graft-recipient)	1	-
In the new-formatted plate	3	-

In the morphological explorations, performed at the level of the hemidiaphragm after the use of pericardial grafted grafts, the presence of regenerative processes and neoformation of the connective tissue in the form of elastic bands, endowed with the vascular network, was found, the suture material persisting only at the border line. A more expressed and ordered differentiation of the aponevrotic conjunctival fascicular layers was observed in the animals in group II.

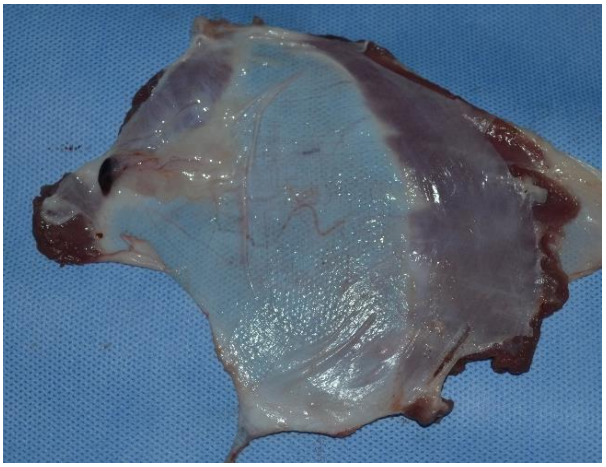

Fig. 5. Macroscopic appearance of the hemidiaphragm undergoing reconstruction with decellularized porcine pericardium.

The presence of the inflammatory process, discreetly marked by the polymorphonuclear inflammation adjacent to the allograft reminiscences, in association with mononuclear was observed, especially of the lymphocyte components which, in the border area with the graft, had the appearance of nodular or pseudofolicular structures. The reminiscences of the graft were circumscribed and of granulomatous inflammation. In the samples taken from the intermediate zone, with a predilection towards the center, in the uncomplicated cases, in both batches there were attested maturations of the newformed tissue in the form of strips with dense and lax collagen fibers, covered with mesothelial peritoneal lining. In areas with adhesive processes, the newformed tissue was much looser, in the form of adhesive bundles. In some areas towards the center, the newformed tissue showed only a conjunctival band with a reduced maximum density of collagen fibers, covered in lot I of focal granules, which occupied up to 95% of its thickness.

In some areas, there was a segmental disorder of the new-formed tissue with the presence of allograft segments, in the form of small islets and plaques, of the polymorphocellular active inflammatory processes and of the lymphocyte component in the nodules, from the host tissues, to the border between the graft and the host. In the presence of the inflammatory process there was a partial dehiscence of the border between the reminiscent graft and the host tissue. The presence of adhesion processes with the pleura and the capsule of the liver induced the disjunction from the beginning of the new-formed tissue plates lined with mesothelium. In 2 cases areas with atypical (malformative) fibrillogenesis were observed, which were evidenced by a multicystic or lacunar-cavity aspect, with internal surfaces covered with unistratified peritoneal mesothelial epithelium flattened without aspects of mitotic activity. The mesothelial cavity-cystic aspect has also been shown on border, in some cavities being present fibro-epithelial conjunctival micro-polyps. The cavity-cyst formation could also be a cystic pseudotumor with thinner septa, with a reduced collagen fiber housing.

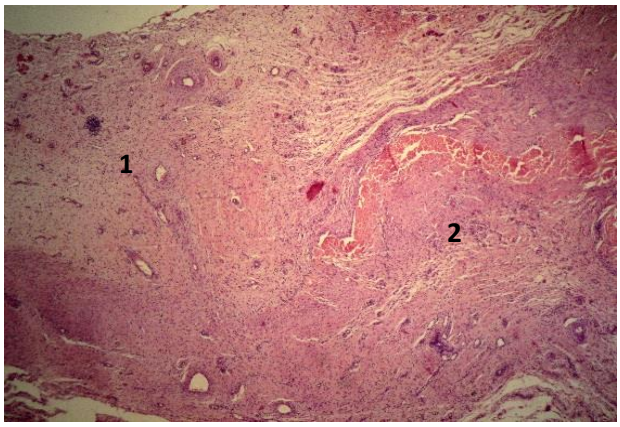

Fig. 6. Strips of newformed tissue of different density and fibroblastic cellular activity: 1 - of lax type; 2 - of dense type. Color. H-E

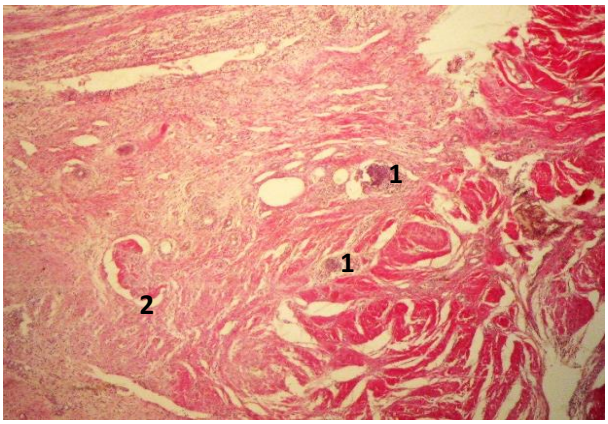

Fig. 7. The graft-host border area with lymphoid follicles (1) and graft reminiscences (2). Color. VG

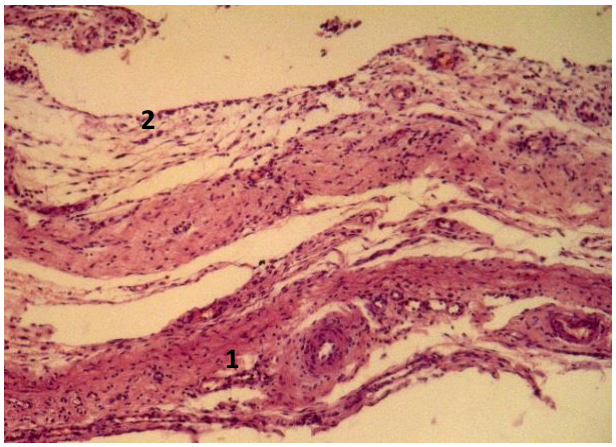

Fig. 8. Strips of fibrillar conjunctival newformed tissue (1) and loose tissue lined with peritoneal mesothelioma (2). Color.

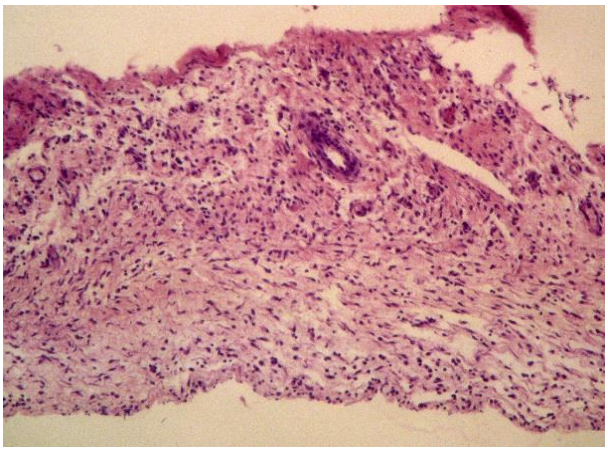

Fig. 9. New-formed tissue in the central area. Color. H-E

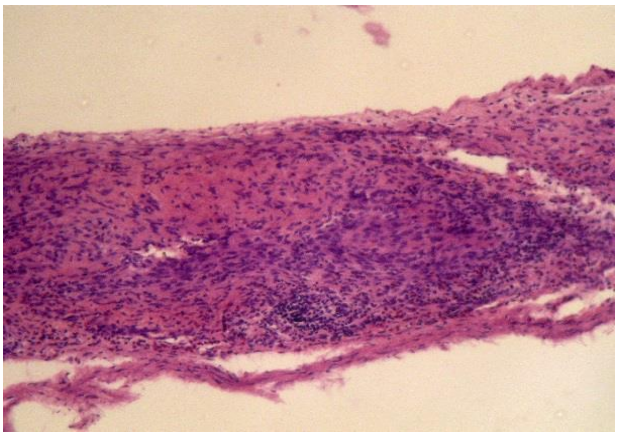

Fig. 10. New-formed tissue with focal granulomatous infiltrates. Color. H-E

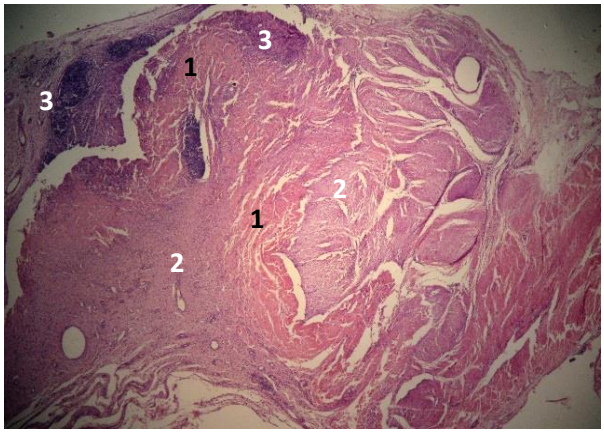

Fig. 11. Allograft residues (1) embedded in new-formed tissue (2) with the persistence of an active polymorphocellular inflammatory process (3) with a pseudofollicular lymphocyte component. Color. H-E

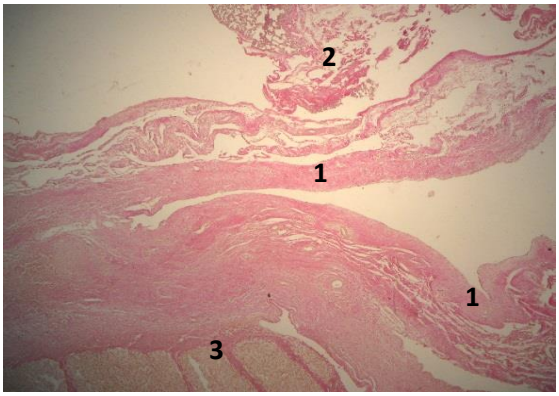

Fig. 12. Dehiscence in 2 connective plates of newformed tissue (1) by retractile-scar aspects with pleura (2) and liver (3). Color. H-E

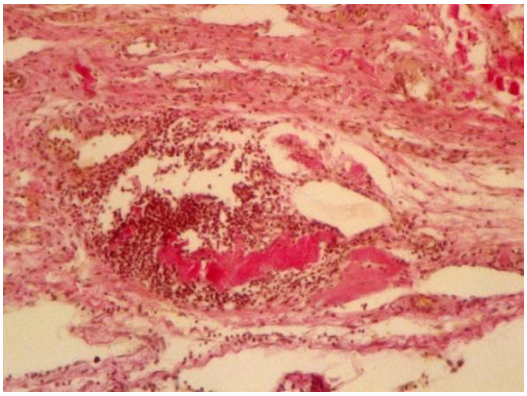

Fig. 13. Reminiscences of allograft with active polymorphocellular inflammatory reaction in the area of newformed tissue. Color. VG

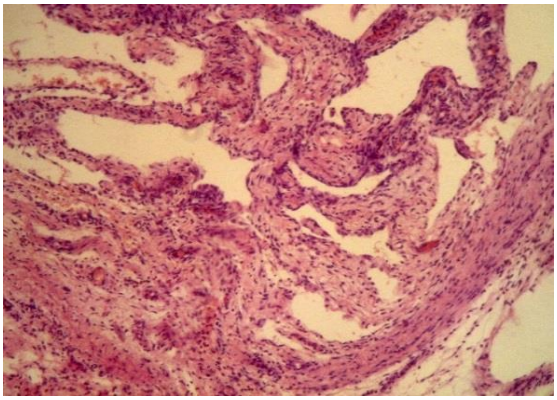

Fig. 14. Atypical-malformative appearance of fibrillogenesis in mesothelium-lined cavity-cystic structures. Color. H-E

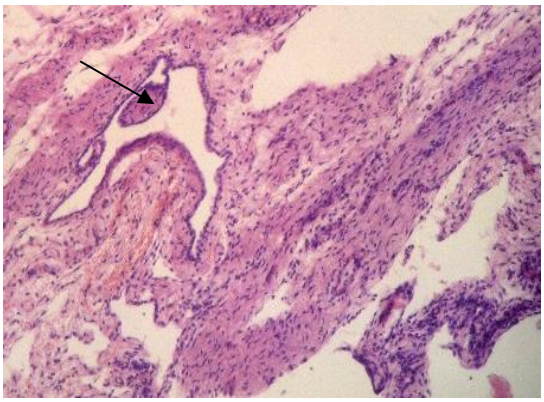

Fig. 15. Cystic structures covered with flaking or viable mesothelium with the presence of micropolyps. Color. H-E

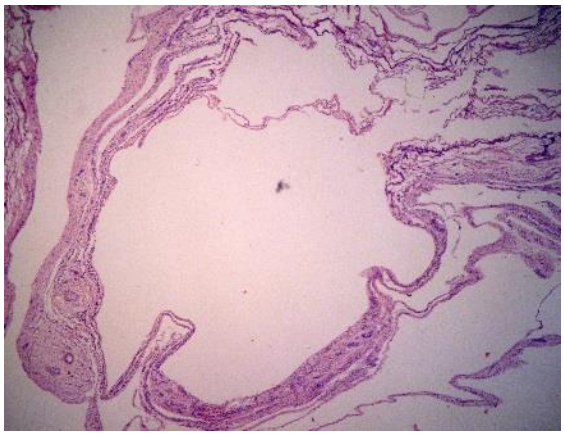

Fig. 16. Multicyctic cavity process with thin conjunctival septum. Color. H-E

Lymphocyte mononuclear cellular elements were present throughout the septa.

The extracellular matrix, derived from decellularized tissue, is increasingly used in regenerative medicine [8]. Decellularized pericardium, by endogenous cell repopulation, has been shown to be an optimal biological material for tissue regenerative processes [30].

The potential benefits of using bovine or porcine pericardial tissue are determined by the accessibility of this biological material, the structural and functional similarity with human tissue, the acceptable immunogenicity and biocompatibility provided by the decellularization process [7, 12]. Recent studies have suggested that porcine pericardium, due to its collagen bundle structure and regenerative properties, may be considered a suitable alternative to bovine pericardium in the treatment of cardiovascular disease [22, 46]. There are experimental studies to test the safety and efficacy of porcine pericardium in duraplasty [41].

In reconstructive operations of diaphragmatic defects, xenopericard grafts are rarely used, with unique cases of bovine pericardium being used in adult or elderly patients [39, 42, 50].

Conclusion

1. Cryopreserved decellularized grafts of porcine pericardium, used in the experimental reconstruction of diaphragmatic defects, have acceptable tissue biocompatibility and greater durability, compared to decellularized bovine peritoneum grafts, but a low bioresistence, after certain periods of time, with mechanical degradation properties and graft formation.
2. The result of this study revealed some morphological aspects of the regenerative-adaptive processes that take place using the porcine pericardial graft and the specific resorption capacities of this biological material, with the non-formation of a plate in which fibrilogenesis processes predominate, at different stages. of maturation.
3. When using the porcine pericardium in the reconstruction of the diaphragmatic defects, a very differentiated fibrilogenesis has been found, in some areas with small deviations, including the presence of a dematurity attested by the variety of collagen fibers and of the cellular fibroblastic component, the latter showing different fibriloblastic activity.
4. The atypical-malformative peculiarities of fibrilogenesis, attested in the mentioned cases, are, in our opinion, a consequence of the fibrilogenesis disorder that could have evolved, following mechanical actions, through dehiscences, gradual ruptures, later with epithelialization through the peritoneal mesothelioma invasion. epithelialization process, accumulation of serous fluids with evolution in hygroma.
5. The obtained results suggest the need for further long-term studies, which would aim to improve the methods of processing this biological material and to obtain more sustainable results.

REFERENCES

1. Ackerman K.G., Vargas S.O., Wilson J.A. et al. Congenital diaphragmatic defects: proposal for a new classification based on observations in 234 patients. Pediatr. Develop. Pathol. 2012; 15:265-74.
2. Alaish S.M., Strauch E.D. The use of Alloderm in the closure of giant omphalocele. J. Pediatr. Surg. 2006; 41(3):e37-e39.
3. Bassuner J.K., Rice D.C., Antonoff M.B., Correa A.M., Walsh G.L. et al. Polytetrafluorethylene or acellular dermal matrix for diaphragmatic reconstruction? Ann. Thorac. Surg. 2017; 103:1710-4.
4. Bekdash B., Singh B., Lakhoo K. Recurrent late complications following congenital diaphragmatic hernia repair with prosthetic patches: a case series. J. Med. Case Rep. 2009; 3:7237.doi: 10.1186/1752-1947-3-7237.
5. Binyamin G., Shafi B.M., Mery C.M. Biomaterials: A primer for surgeons. Semin. Pediatr. Surg. 2006; 15:276-83.
6. Chan E, Wayne C, Nasr A. Minimally invasive versus open repair of Bochdalek hernia: a meta-analysis. J. Pediatr. Surg. 2014; 49(5):694-9.
7. Choe J.A., Jana S., Tefft B.J., et al. Biomaterial characterization of off-the-shelf decellularized porcine pericardial tissue for use in prosthetic valvular applications. J. Tissue Eng. Regen. Med. 2018; 12:1608-20. https://doi.org/10.1002/term.2686.
8. Dong J., Li Y., Mo X. The study of a new detergent (octyl-glucopyranoside) for decellularizing porcine pericardium as tissue engineering scaffold. J. Surg. Res. 2013; 183(1):56-67.
9. Fosnot J., Kovach S.J., Serletti J.M. Acellular dermal matrix: general principles for the plastic surgeon. Aesth. Surg. J. 2011; 31(7S):5S-12S.
10. Gander J.W., Fisher J.C., Gross E.R., Reichstein A.R., Cowles R.A. et al. Early recurrence of congenital diaphragmatic hernia is higher after thoracoscopic than open repair: a single institutional study. J.Pediatr. Surg. 2011; 46(7):1303-8.

11. Gasior A.C., St. Peter S.D. A review of patch options in the repair of congenital diaphragm defects. Pediatr. Surg. Int. 2012; 28(4):3233.
12. Gauvin R., Marinov G., Mehri Y. A comparative study of bovine andporcine pericardium to highlight their potential advantages to manufacture percutaneous cardiovascular implants. J. Biomater. Appl. 2012; 28(4):552-65.
13. Georgescu R., Chiuțu L., Nemeș R., Georgescu I., Stoica A., Georgescu E. Possibilities and limits in the treatment of congenital diaphragmatic hernia. J. Med. Life. 2014; 7(3):433-9.
14. Grethel E.J., Cortes R.A., Wagner A.J., Clifton M.S., Lee H. et al. Prosthetic patches for congenital diaphragmatic hernia repair: Surgisis vs Gore-Tex. J. Pediatr. Surg. 2006; 41:29- 33.
15. Grover T.R., Rintoul N.E., Hedrick H.L. Extracorporeal membrane oxygenation in infants with congenital diaphragmatic hernia. Semin. Perinatol. 2018; 42(2):96-103.
16. Hilbert S.L., Ferrans V. J., McAllister H.A., and Cooley D. A., "Ionescu-Shiley bovine pericardial bio- prostheses: Histological and ultrastructural stud- ies," Am. Pathol. 1992; 140:1195-1204.
17. Holcomb G.W., Ostlie D.J., Miller K.A. Laparoscopic patch repair of diaphragmatic hernias with Surgisis. J. Pediatr. Surg. 2005; 40(8):E1-5.
18. Hyvarinen A., Sankilampi U., Tyvainen E., Vanam K. Abdominal muscle flap for repair of large congenital diaphragmatic hernia: Ultrasound evidence for retained motor muscle function. J. Pediatr. Surg. Case Rep. 2019; 45:101199. https:// doi.org/10.1016/j.epsc.2019.101199.
19. Joshi S.B., Sen S., Chacko J., Thomas G., Karl S. Abdominal muscle flap repair for large defects of the diaphragm. Pediatr. Surg. Int. 2005; 21(8):677-80.
20. Kawano T., Muensterer O.J. A new gentle reduction technique for patients with congenital diaphragmatic hernia - A case report. Int. J. Surg. Case Rep. 2019; 57:5-8.
21. Khor E. Methods for the treatment of collagenous tissues for bioprostheses. Biomaterials. 1992; 18:95-105.
22. Khorramirouz R., Go J.L., Noble C., Morse D., Lerman A. In vivo response of acellular porcine pericardial for tissue engineered transcatheter aortic valves. Sci. rep. 2019; 9:1094. https://doi.org/10.1038/s41598-018-37550-2.
23. Laituri C.A., Garey C.L., Valusek P.A., Fike F.B., Kaye A.J. et al. Outcome of congenital diaphragmatic hernia repair depending on patch type.Eur. J.Pediatr. Surg. 2010; 20:363-5.
24. Lemperle G, Morhenn V, Charrier U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. Aesthetic Plast Surg. 2003; 27(5):354-66.
25. Li X., Guo Y., Ziegler K., Model L., Sammy D.D. et al. Current usage and future directions for the bovine pericardial patch. Ann. Vasc. Surg. 2011; 25(4):561-8.
26. Lop L., Palmosi T., Dal Sasso E., Gerosa G. Bioengineered tissue solutions for repair, correction and reconstruction in cardiovascular surgery. J Thorac Dis. 2018; 10(Suppl 20): S2390–S2411
27. Maxwell D., Baird R., Puligandla P. Abdominal wall closure in neonates after congenital diaphragmatic hernia repair. J. Pediat. Surg. 2013; 48:930-4.
28. Mitchell I.C., Garcia N.M., Barber R., Ahmad N., Hicks B.A., Fischer A.C. Permacol: a potential biologic patch alternative in congenital diaphragmatic hernia repair. J. Pediatr. Surg. 2008; 43:2161-4.
29. Morini F., Lally K.P., Lally P.A., Crisafulli R.M., Capolupo I. et al. Treatment strategies for congenital diaphragmatic hernia: change sometimes comes bearing gifts. Front. Pediatr. 2017; 5:195. doi: 10.3389/fped.2017.00195.
30. Morticelli L., Thomas D., Roberts N., Ingham E., Korossis S. Investigation of the suitability of decellularized porcine pericardium in mitral valve reconstruction. J. Heart Valve Dis. 2013; 22(3):340-53.
31. Neto N.B., Jorge L.F., Francisco J.C., Erbano B.O., Barboza B.E.G. et al. Regeneration of tracheal tissue in partial defects using porcine small intestinal submucosa. Hindawi Stem Cells Int. 2018; Art. ID 5102630, 8 pag. https://doi.org/10.1155/2018/5102630
32. Olmos Z.R., Jasso V.R., Sotres V.A., Cedillo L.I., Arreola R.J.L., Gaxiola G.M. Utilidad del perica ´rdiobovinotratado com glutaraldeidoem la ˜s reseccionespulmonares no anato ˆmicas emperros. Ver. Inst. Nal. Enf. Resp. Mex. 1997; 10:155-9.
33. Paolantonio M, Scarano A, Di Placido G, Tumini V, D'Archivio D, Piattelli A. Periodontal healing in humans using anorganic bovine bone and bovine peritoneum-derived collagen membrane: a clinical and histologic case report. Int. J. Periodontics Restorative Dent. 2001; 21(5):505-15.23.
34. Piccoli M., Urbani L., Alvarez-Fallas M.E., Franzin C., Dedja A. et al. Improvement of diaphragmatic performance through orthotopic application of decellularized extracellular matrix patch. Biomaterials. 2016; 74:245-55.
35. Pires A.C., Saporito W.F., Leao L.E.V. Perica ´rdiobovinoutilizadocomoremendo no sistema cardiovascular. Ver Bra´s Cir. Cardivasc. 1997. 12:176-87.
36. Prayer F., Metzelder M., Krois W., Brugger P.C., Gruber G.M. et al. Three-dimensional reconstruction of defects in congenital diaphragmatic hernia: a fetal MRI study F. Ultrasound Obstet. Gynecol. 2019. 53:816-26.
37. Putnam L.R., Gupta V., Tsao K., Davis C.F., LallyP.A. et al. Factors associated with early recurrence after congenital diaphragmatic hernia repair. J. Pediatr. Surg. 2017. 52:928-32.
38. Putnam L.R., Harting M.T., Tsao K., Morini F., Yoder B.A. et al. Congenital diaphragmatic hernia defect size and infant morbidity at discharge. Pediatrics. 2016. 138(5):e20162043.DOI: 10.1542/peds.2016-2043.
39. Qin J., Ren Y., Ma D. A comparative study of thoracoscopic and open surgery of congenital diaphragmatic of congenital diaphragmatic hernia in neonates. J. Cardiothorac. Surg. 2019; 14(1):118. Doi:10.1186/s13019-019-0938-3.
40. Ricci K.B., Higgins R., Daniels V.C., Kilic A. Bovine pericardial reconstruction of the diaphragm after a heart transplant. Exp. Clin. Transplant. 2014; 2(3):277-8.

41. Schneider A., Becmeur F. Pediatric thoracoscopic repair of congenital diaphragmatic hernias. J. Vis. Surg. 2018; 4:43. doi: 10.21037/jovs.2018.02.03.
42. Seo Y., Kim J.W., Dho Y.S., Chowdhury T., Kim S., Park C.K. Evaluation of the safety and effectiveness of an alternative dural substitute using porcine pericardium for duraplasty in large animal model. J. Clin. Neurosci. 2018; 58:187-91.
43. Spiliotopoulos K., dela Cruz K.I., Gkotsis G., Preventza O., Coselli J.S. Repair of intrapericardial diaphragmatic hernia during aortic surgery in a 78-year-old woman. Tex. Heart Inst. J. 2017; 44(2):150-2.
44. Sugiyama A., Fukumoto K., Fukuzawa H., Watanabe K., Mitsunaga M. et al. Free fascia lata repair for a second recurrent congenital diaphragmatic hernia. J. Pediatr. Surg. 2011; 46:1838-41.
45. Thapar S., Ahuja A., Rastogi A. Rare diaphragmatic tumor mimicking liver mass. World J. Gastrointest. Surg. 2014; 6(2):33-7.
46. The Congenital Diaphragmatic Hernia Study Group. Congenital diaphragmatic hernia: Defect size correlates with developmental defect. J. Pediatr. Surg. 2013; 48:1177-82.
47. Tran H.L.B., Dinh T.T.H., Nguyen M.T.N., To Q.M., Pham A.T.T. Preparation and characterization of acellular porcine pericardium for cardiovascular surgery. Turk. J.Biol. 2016; 40:1243-50.
48. Tsao K., Lally P.A., Lally K.P. Minimally invasive repair of congenital diaphragmatic hernia. J. Pediatr. Surg. 2011; 46:1158-64.
49. Unsal B, Kurtis B, Ozcan G, Ozdemir A, Karaöz E. An investigation of resorption and tissue reaction after subcutaneous implantation of collagen based membrane materials in rats. J Marmara Univ Dent Fac. 1997; 2(4):609-15.
50. Wang L.C., Clark M.E., Edward M.J. Use of composite polyester/collagen mesh in the repair of recurrent congenital diaphragmatic hernias. J. Ped. Surg. Case Rep. 2015; 3:377-81.
51. Zardo P., Zhang R., Wiegmann B., Haverich A., Fischer S. Biological materials for diaphragmatic repair: initial experiences with the PeriGuard Repair Patch®.Thorac. Cardiovasc. Surg. 2011; 59(1):40-4.
52. Zhu Y., Wu Y., Pu Q., Ma L., Liao H., Liu L. Minimally invasive surgery for congenital diaphragmatic hernia: a meta-analysis. Hernia. 2016; 20:297-302.

Muscular (contracture) spasm of the bladder and urodynamic abnormalities in newborn and infants with refluxing megaureter

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Abstract

Spasmul (contractura) al mușchilor vezicii urinare și dereglările urodinamice la nou-născuți și sugari cu megaureter refluxant

Scopul lucrării a fost în aprecierea dereglărilor urodinamice cauzate de spasmul muscular al vezicii urinare la nou-născuți și sugari cu megaureter refluxant.
Din 71 copii de vârstă mică cu megaureter refluxant la renoscintigrafie dinamică în 99% curbele de evacuare sunt obstructive. Uneori cauzele refluxului nu se încadrează în cele cunoscute până în prezent, impunându-se mai multe întrebări, inclusiv de ce de rând cu stenoza ureterului în joncțiunea uretero-vezicală, confirmată intraoperator, se depistează și reflux sau de ce în lipsa stenozei constatate intraoperator ureterul dilatat se revarsă direct în vezică?
De rând cu cauzele cunoscute mai este o patologie neurologică – spasm (contractura) muscular al organelor pelviene – ureterul distal (segmentul Waldeyer), vezica urinară, colul vezical, sfincterul uretral extern la băieți și meatal la fetițe ceea ce duce la afectarea gravă al tractului urinar adiacent.
Diagnosticul a inclus: ecografia, electromiografia mușchilor abdominali suprapubieni sau perineali, cistouretrografia micțională, cistometria, renoscintigrafia dinamică. Toți pacienții au fost consultați de neurolog.
Tratamentul începe cât mai precoce – sondă vezicală, spasmolitici, proceduri fizioterapeutice, masaj, hidro- și kinetoterapie pentru a micșora spasmul și presiunea intravezicală. Apoi se aplică operații antireflux intravezicale de tip Cohen, Leadbetter-Politano, endoscopic contemporane cu injectare subureterală.
În cazul stenozei – rezecția segmentului stenozat cu neoimplantarea, ca și în cazul fără stenoze, pentru că în așa caz segmentul intramural este stenozat și fibrozat de mușchii vezicii urinare și presiunea intravezicală vădit mărită. După operație – monitorizare și tratament de lungă durată cu uroseptici, spasmolitici, (presiunea intravezicală greu se normalizează) sub controlul ecografiei și cistometriei.

Cuvinte cheie: megaureter, stenoza ureterului, reflux vezico-ureteral, diagnostic, tratament

Abstract

Purpose. To assess urodynamic abnormalities caused by the bladder muscle spasm in newborns and infants with refluxing megaureter.

In 71 cases of children with reflux megaureter at dynamic renoscintigraphy, 99% of the escape curves are obstructive. Sometimes the causes of reflux do not fit into the known ones so far, with ureter stenosis in the uretero-bladder junction, confirmed intraoperatively, and reflux is detected? Why, in the absence of stenosis found intraoperatively, does the dilated ureter flow directly into the bladder?

Common with the known causes is a neurological pathology - muscle spasm (contracture) of the pelvic organs - the distal ureter (Waldeyer segment), the urinary bladder, the bladder, the external urethral sphincter in boys and the flesh in girls, which leads to severe tract damage. adjacent urine.

The diagnosis includes: ultrasound, electromyography of the suprapubian or perineal abdominal muscles, micturition cystourethrography, cystometry, dynamic renoscintigraphy, consultation of the neurologist.

The treatment starts as early as possible - bladder probe, spasmolytics, physiotherapeutic procedures, massage, hydro- and kinetic therapy to reduce spasms and intravesical pressure. Then, Cohen, Leadbetter-Politano, contemporary endoscopic suburethral injection anti-reflux surgery is applied. In the case of stenosis - resection of the stenosis segment with neoimplantation, as in the case without stenoses, because in this case the intramural segment is stenosis and fibrosis of the bladder muscles and the clearly increased intravesical pressure. After surgery - a long-term monitoring and treatment with uroseptics, spasmolytics, (intravesical pressure is hard to normalize) under the control of ultrasound and cystometry.

Keywords: megaureter, ureteral stenosis, vesicoureteral reflux, diagnostic, treatment

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Studying the *vesicoureteral* reflux (VUR) for several years has contributed to the early detection of the cause and treatment optimization [3, 5, 7, 18].

When clinicians often do not know the cause of the disorders, they treat them as a neurogenic disorder. In case of the bladder, when clinicians do not find the cause of a serious disorder, it is considered to be a neurogenic bladder. But for what reason, what the clinicians have to treat is a problem. Until recently patients with micturition disorders, related to bladder *abnormalities*, have been treated as hypertonic and neurogenic bladder, the last few years – as overactive bladder [14, 15]. But what happens in the bladder, how should patients be treated? What should the treatment include? This group of patients is also treated by neurologists and urologists [2, 10] , but unfortunately without great success.

The effectiveness of the *vesicoureteral* junction in preventing vesicoureteral reflux, but also the functional mechanism are described by Bell (1812) and Young in 1897. In 1903, Sampson and Young described the functional mechanism of the vesicoureteral valve created through the oblique ureteral passage in the intramural portion, which urologists rely on to date. In 1992 Gruber noted that the incidence of VUR varies depending on the length of the intravesical ureter and the condition of the detrusor muscles [1].

Our clinic has been studying VUR since the `60s of the last century [2, 4, 6, 9]. We have tried to implement the literature data and our clinic experience. We have obtained satisfactory results both in diagnosis and treatment, but there are some issues that are not included in the reflux causes known today [11].

Known causes of the VUR etiology [3, 12, 17].

1. Shortened intramural portion of the ureter.
2. Lateralization of the ureteral orifice.
3. Recurrent urinary tract infection leads to the ureteral ostium dysfunction, morphological changes lead to intramural ureteral sclerosis, periureteritis with the disturbance of the *vesicoureteral* segment, muscular fibrosis of the Waldeyer`s sheath.
4. Infravesicular obstruction.

But there are still a number of questions not included in these cases - the dynamic renal scintigraphy performed in 71 children with reflux revealed that in 99% there are obstructed curves in the juxtavesical segment:

- Why do not these children stand the Nelaton catheter?
- Why at MCUG with reflux at urination the dilated ureters are full and tense with contrast agent or radionuclides.
- Why is urethral stenosis in the juxtavesical segment visualized and confirmed intraoperatively along with reflux at MCUG. Along the ureter only in this segment there are additional muscles - the Waldeyer sheath.

The question is why along with the *vesicoureteral* segment obstruction, there is also reflux. An increased intravesical pressure is required for the bladder contents to pass through the stenosed segment. Therefore the pressure increases, being often found in many children.

Why is not the reflux found after the antireflux operation? But the ureter dilatation is maintained, the reflux and nephropathy progress as well as the renal parenchyma healing occurs [8, 13, 16]. Initially we considered that this phenomenon is related to errors during the operation, inadequate application of sutures.

Another situation - when during the operation the stenosed segment is missing, but the dilated ureter directly pours into the bladder

We considered that dilation is the result of the hypotonic ureter. Although during the operation, after its sectioning the urine is poured in jet, the scintigraphy recorded contractions, showing that the ureter contracts to expel the bladder contents.

Therefore, besides the causes described above there is a neurogenic dysfunction: spasm, general and selective muscular contracture of the pelvic organs - distal ureter, bladder, bladder neck, urethra with marked spasm, which leads to urodynamic disorders of the lower urinary tract with the severe involvement of the kidneys, ureters, and bladder.

Muscle contracture is considered an excess of muscle tone, by maintaining an increased intramuscular tension which permanently presses on the intramuscular vessels and capillaries, causing an intramuscular circulatory deficiency, which in turn gives rise to muscular hypoxia.

In these conditions the muscles do not relax and are in contracture. This area, depending on the location, remains rigid and non-functional.

The most common muscle contractures occur at the level of the vertebral column at different regions - cervical, thoracic, lumbar-sacral.

Children in the first months of life have a more marked muscle tone than older children. Being in the womb, the fetuses every day grow and develop, the space inside getting smaller and smaller. At birth the newborn's muscles are tense. Certain muscle groups may be in hypertonia, this being a norm.

What is normal in newborns, at an older age may be a pathological sign. The muscle tone of a 2-year-old child should be about the same as in adults. But there are many intrauterine factors, which at birth can cause different conditions of the muscle tone.

There are some common muscle disorders like: decreased muscle tone - hypotonia, increased muscle tone - hypertonia, incorrect distribution of the muscle tone and muscle groups - muscle dystonia.

In muscular hypertonia, the child does not relax even after 30 minutes, having a restless sleep. He cries, his chin trembles and he vomits.

Hypertonia can be increased symmetrically or asymmetrically on one side of the body. This can be a sign of trauma at birth, meningitis or internal *hemorrhage*. In late treatment (not found) it can lead to serious posture deviations: walking, scoliosis, torticolis, it can even cause incurable cerebral palsy.

In the case of hypotonia, the situation is reverse, when the tone is less than normal, hands and feet are open, the hands being parallel to the body.

In the face-down position on the adult`s hand with the child`s head and limbs hanging down the child is quiet, but with bad eating habits.

The most common change of the mixed muscle tone is when it is raised in one muscle group and reduced in another, thus initial hypotonia leads to hypertonia. This condition is called muscular dystonia.

Thus, one part of the body may be larger than the other. This may influence the baby's motor functions, namely, the ability of rolling from the back on the belly only at 5-6 months, sitting down after 7 months, walking only after 12 months.

Muscle spasm leads to severe spasm of the bladder muscles with (hypertonia) increased intravesical pressure, distal ureter - Waldeyer, muscular contraction of the bladder neck, external sphincter in boys and distal (meatal) part in girls, which in turn leads to ureteral reflux with constant sectioning (fig. 5, 6, 7).

The bladder muscles also obstruct the intramural ureteral segment. The bladder spasm leads to high intravesical pressure and thus children do not stand the bladder catheter.

Radionuclide voiding cystourethrography greatly improves the diagnosis of the functional status of the bladder and urethra. Reduced irradiation allowed the repeated use of the method for treatment assessment.

In girls urethral muscles can also be spasmed causing voiding dysfunction. The size of the *uroflowgrams* waves depends on the disorder degree and the passage of the urine below the obstruction, assessing the passage of the laminar flow into the turbulent current that cannot be detected by other examination methods. The wider the suprastenotic urethra region, the more pronounced the flow of the turbulent fluid is.

In parallel with the parietal movement, in girls the urine brings the urethral flora from the distal urethra, which is colonized 100% with pathogenic microbes. It rises to the bladder, which often leads to recurrent infections, more often than in boys, where also the turbulent and parietal movement is present. But the posterior urethra is rarely infected and recurrences are rare (fig. 5, 6).

This is the way stasis forms in the ureter. Any stasis sooner or later becomes infected and if the cause is not removed the infection recurs despite any intensive treatment.

The ureteral dilatation depends not only on the refluxing force, but also on the degree of obstruction and the ureteral contents return into the bladder (fig. 2, 3, 8).

The diagnosis of muscle contracture is improved by:

1. Neurological examination, that often detects pathological neurological signs. Both the doctors and boys` parents notice that they frequently urinate with a strong jet up to the chin.
2. Ultrasound of the urinary tract. Ultrasound reveals the bladder with a double contour with thickened walls, clear borders, in the dorsal part of the bladder the ureters are dilated (fig.12).

3. Electromyography of the suprapubic muscles - are signs of hypertonia (fig. 15, 16).
4. Plain and radionuclide *voiding* cystouretrography, reflecting a bilateral refluxing megaureterhydronephrosis, more often asymmetrical one, a more severe one, the opposite side - less pronounced, can be a marked unilateral reflux, which after antireflux operation also appears on the opposite side (fig. 8, 13, 19).

Unilateral vesicoureteral reflux, which can serve as a valve, protecting the contralateral kidney, can be caused by intravesicular hyperpressure. In this way the reflux does not disappear after the surgical correction.

- Dynamic renal scintigraphy - the filtering function is identical in both kidneys likewise the voiding function, the left and right obstructive curves differ (fig. 14, 19, 20);
- Cystomanometry – a high intravesical pressure up to 375 with H_2O (norm 140-180 cv H_2O);
- Morphology of the vesicoureteral segment (fig. 9, 10).

At first an organic obstruction is determined. At surgery the resection of the morphologically confirmed stenosed segment is performed. But it is not clear why along with stenosis in most cases the reflux is found. Stenosis of the distal fibrous-muscle Waldeyer sheath with stronger muscles and the distal ureter is stenosed (fig. 3).

In order to evaluate the activity of the perineal muscles, the electromyography of the anal sphincter is performed, because it is considered that the perineal muscles of the anal sphincter, the urethra and the bladder neck have a common innervation.

In newborns with a marked reflux, there are serious changes in urodynamics caused generally by the muscle spasm and the spasm of selective muscles of the lower urinary tract of the bladder.

In general the muscle spasm leads to intravesical hypertonia (confirmed by cystomanometry), intramural ureter obstruction, the Waldeyer urethral muscle spasm, with obstructive curves revealed at dynamic scintigraphy (fig. 11). The spasm is triggered at the intrauterine stage and is detected at ultrasound. In newborns there are already signs of fibrosis of the spasmed segments and dilatation of the ureters, which is impossible to prevent. Morphologically, the connective tissue fibrosis of the stenosed segments is often found (fig. 9, 10). Thus, the distal segment of the fibrosed and intramural ureter with a constant sectioning with a clear intravesical pressure leads to *vesicoureteral* reflux, which also under pressure as a spray pushes urine into the ureter. But the ureter has no force to return the contents into the bladder and to go through the distal ureter stenosis; the bladder muscles spasm, increased intravesical pressure (fig. 11).

Thus, it is a hypertonic bladder without dysuria because there is a megaureter with bladder neck spasm and external urethral sphincter in boys and meatal spasm in girls.

Clinical case: Child J. 20 days, hospitalized with bilateral megaureterohydronephrosis. The child had food intolerance, signs of endointoxication, hypotrophy and vomiting. Ultrasound revealed the bladder with double contour, bilaterally dilated ureters. At MCUG - marked reflux gr.V on the left, on the right - the reflux is not found (fig. 13). The Foley catheter was inserted. Dynamic renal scintigraphy - the filtering function is diminished, the voiding function is decreased - S≥D.

Urea, creatinine – in norm. General blood and urine analysis - no deviations. Electromyography of suprapubic muscles revealed a marked excitation (fig.15).

There was found a very high intravesical pressure - 375mm H_2O (norm - approx. 140-180) (fig. 17). There were administered uroseptics and spasmolytics. After 2 weeks, the electromyography revealed an improved hypertonus of the suprapubic muscles. The cystomanometry revealed a high pressure - 350.

The patient's condition improved. The patient was active. The patient`s pelvis on the left increased from 13 to 22 cm and on 02.07.19 there was performed uretero-cystoneoanastomosis on the left. The distal stenosed segment was resected, the condition being severe, but with a gradual improvement. On the 7th day, a urinary fistula opened, and the general condition improved. On 17.07.19 the patient was discharged. Shortly after, the fistula disappeared.The ultrasound performed over 2 months found that the ureters were bilaterally dilated (practically like before the operation), there was not fever, and the urine analysis was normal.

In these patients, the intravesical pressure is very high, which leads to VUR through the intramural and distal ureter, with a constant sectioning as a spray, because at examination the ureters were full of contrast agent. The refluxing ureteral contents are not able to return.

If the urinary tract infection is associated and there are urination disorders, the treatment is indicated only after the spasm improvement (intravesical pressure).

It should not be excluded that with age the spasm and fibrosis improve and the nervous system maturation occurs. The urodynamic abnormalities in newborns and infants in modern reflux classification not only depend on the reflux strength, but also on the degree of obstruction and return into the ureterovesical segment and increased intravesical pressure.

Clinical case (5 years ago): The more advanced (pronounced) the reflux is, the more obvious the stasis signs are, the spasm (fibrosis) of the distal ureter protects the kidneys from high bladder pressure.

Thus, this is a hypertonic bladder marked by the muscle spasm of the muscles of the lower urinary tract - distal ureter (Waldeyer sheath), bladder, bladder neck,

urethra, which leads to serious urodynamic abnormalities of the entire urinary tract and requires a complex treatment: spasm removal, surgery, intravesical pressure regulation, and fight with the urinary tract infection. In all the disorders described above there are some fibrosis features, but after the administration of spasmolytics the spasm

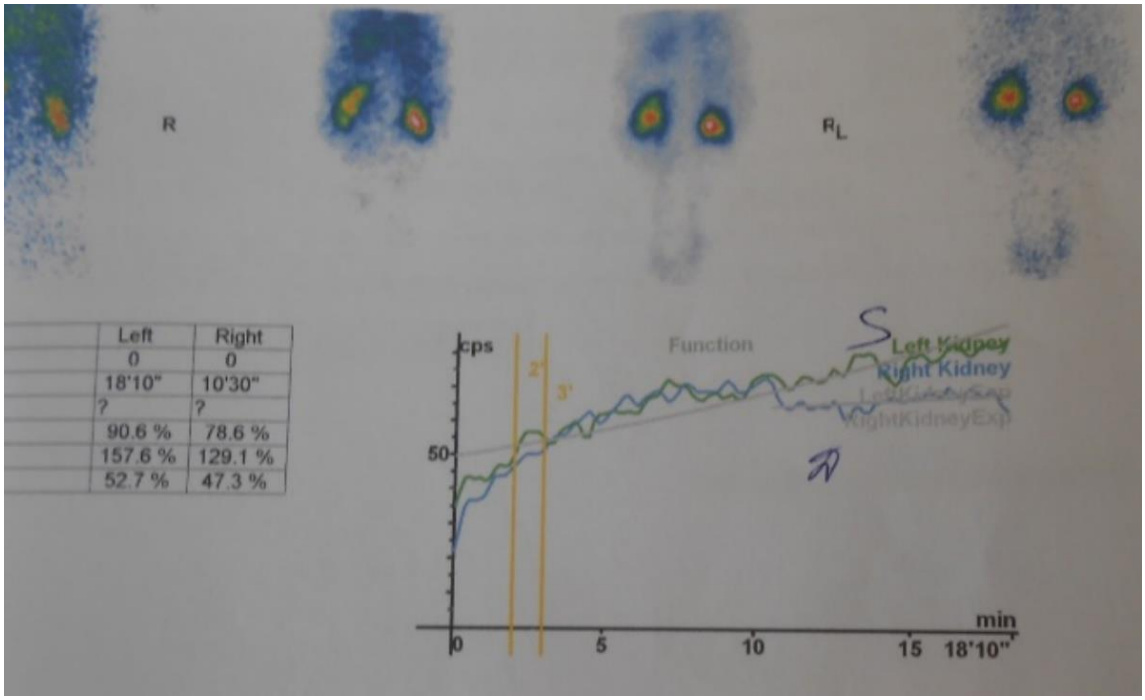

Fig.1. Dynamic renal scintigraphy, marked voiding *dysfunction* in a child with bilateral reflux.

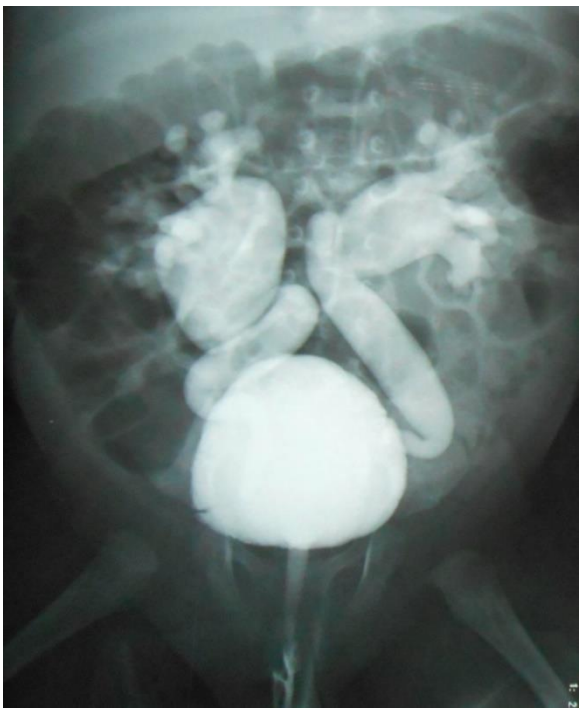

Fig. 2. MCU. Bilateral refluxing megaureter hydronephrosis

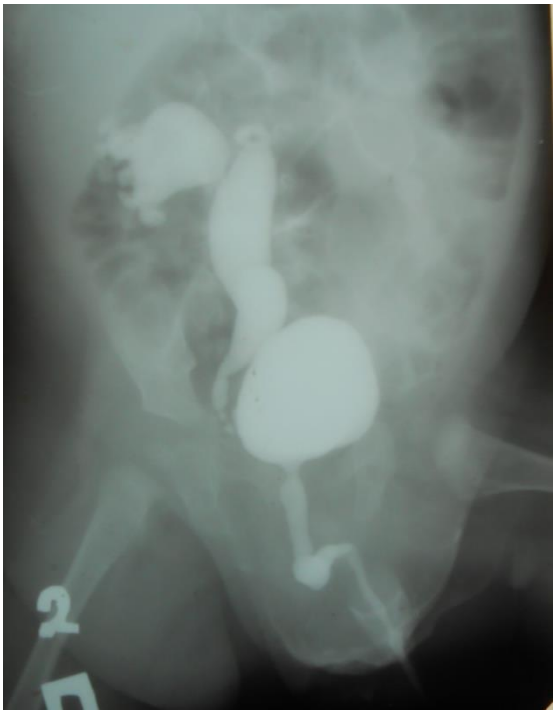

Fig. 3. MCUG - VUR on the right with stenosis in the juxtavesical region

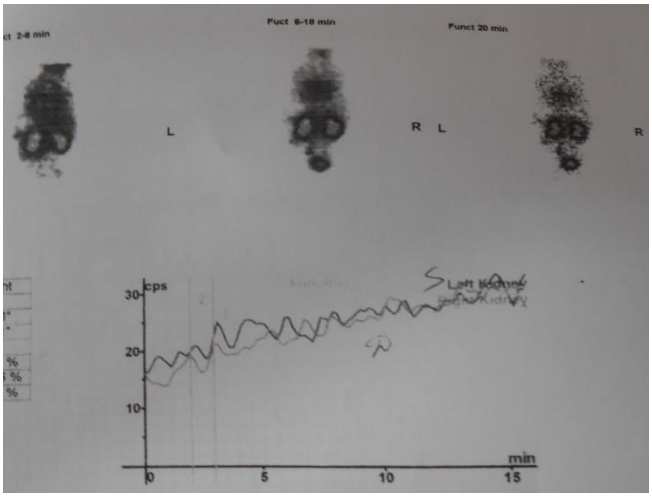

Fig.4. Dynamic renal scintigraphy: dilated ureters with peristaltic waves, obstructive emptying curves.

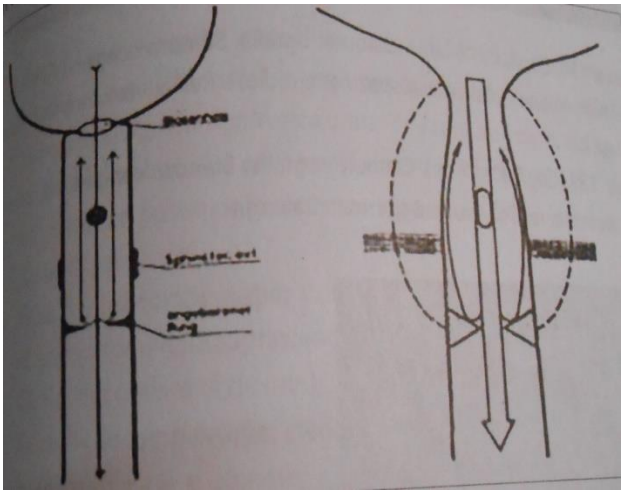

Fig.5. Parietal movement of urine in boys: a - without obstruction; b - obstruction of the posterior urethra

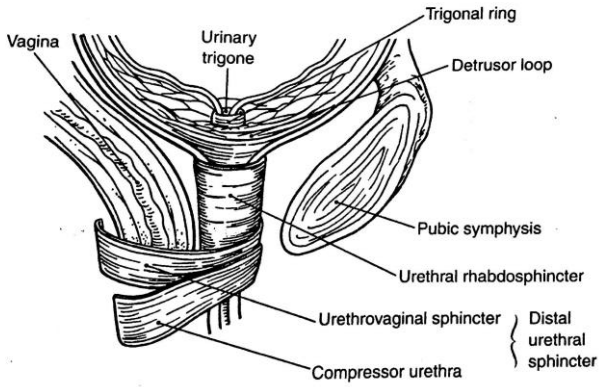

Fig.6. Schematic – urethral muscles in girls can also be spasmed, with voiding dysfunction (Plzak L., Staskin D., 2002)

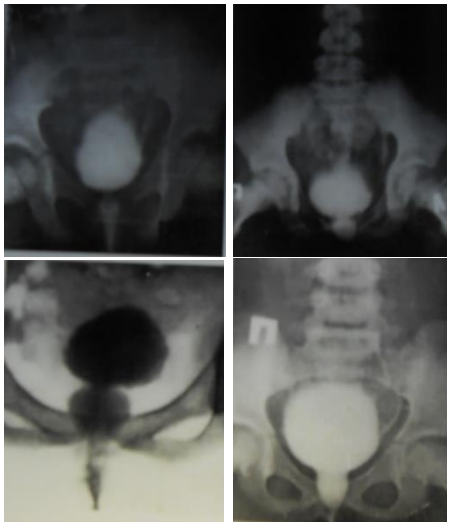

Fig.7 CUGM. Spasm of the distal muscles of the urethra in girls of various forms

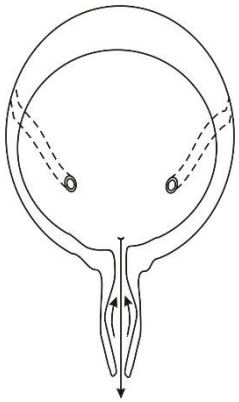

Fig. 8. Schematic - distal urethra spasm in girls with dilation of the proximal segment. Parietal movement of urine

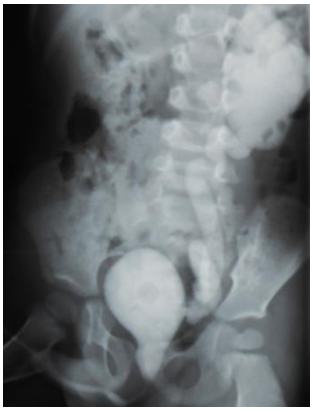

Fig. 9. MCU. Refluxing megaureter-hydronephrosis on the left. Spasm of external sphincter

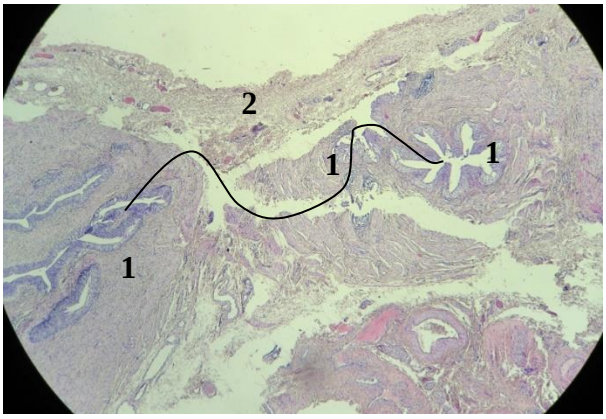

Fig. 10. Sinusoidal wavy path of the ureter adjacent to the urocyst implantation area: 1 - stenosed ureteral segment; 2 - peritoneum

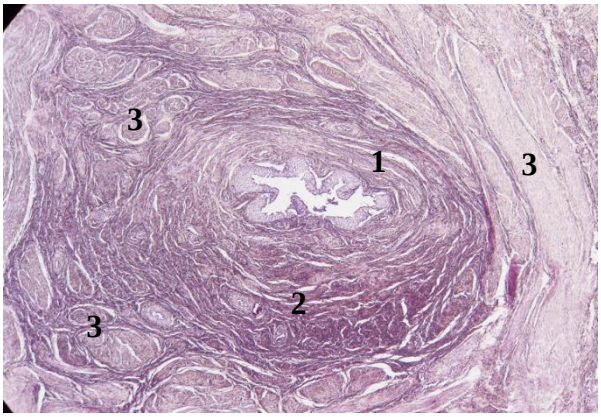

Fig. 11. Intramural ureteral segment: 1 - aplasia of ureteral musculature; 2 - abundant lax connective tissue with a sclerogenic reaction; 3 - musculature, muscle bundles of varying thickness due to aplasia and fibrosis. The lumen is preserved

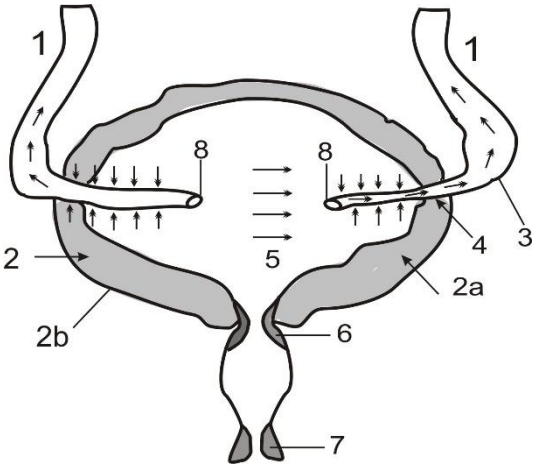

Fig.12 Schematic: etipathogenesis of reflux megaureter: 1-megaureter; 2 - spasmed bladder muscles; 2a - with stenosis in the uretero-bladder segment; 2b - without stenosis of the ureter; 3 - stenosis (spasm) of the distal ureter, Waldeyer area; 4 - obstruction of the ureter of the bladder muscles; 5 - obstruction of the increased intravesical pressure; 6 - spasm of the bladder neck; 7. - spasm of the sphincter external urethral; 8 - fibrosed ureter with constant section

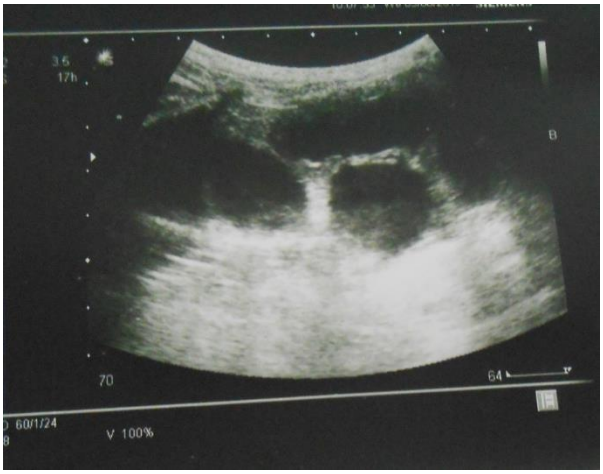

Fig. 13. Dilatation of ureters and renal cavities

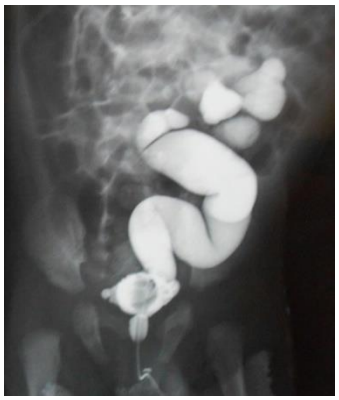

Fig. 14. At MCU - gr.V reflux on the left, posterior urethral stenosis

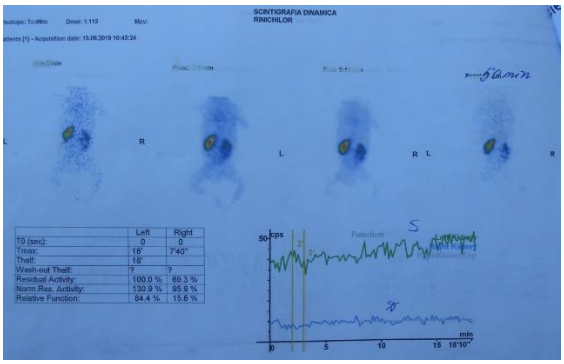

Fig. 15. Dynamic renal scintigraphy. On the left – significantly diminished voiding, decreased function on the right

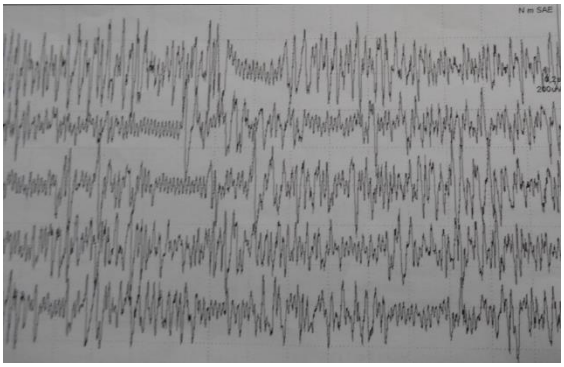

Fig. 16. Increased size and M-response amplitude frequencies. Suprapubic electromyography: before treatment

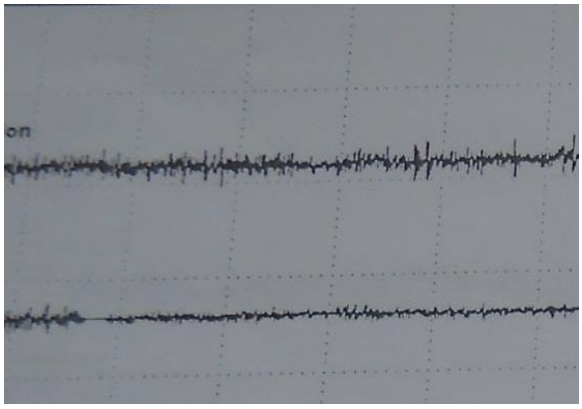

Fig. 17. 2 weeks after treatment

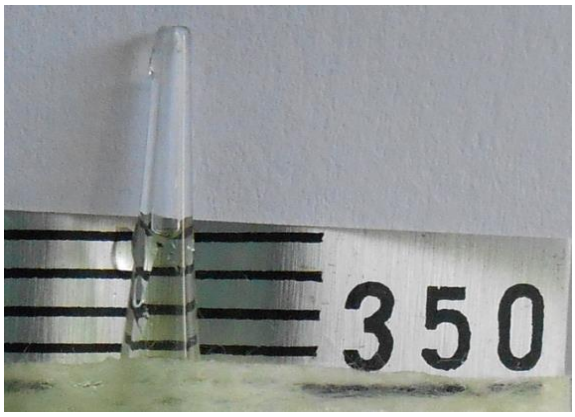

Fig. 18. Cystometry data

Fig. 19. The child during examination

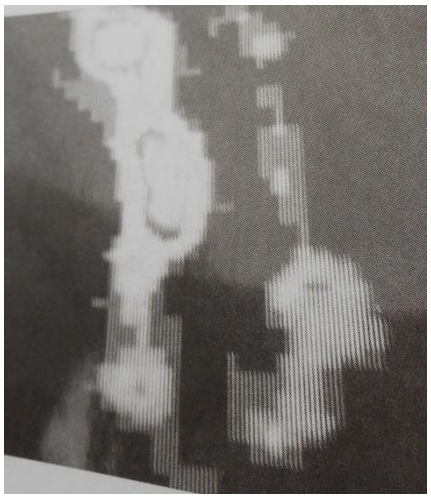

Fig. 20. CUG with radionuclides. Bilateral RVR, more marked on the right

diminishes. Their evolution must be studied more deeply. It is not excluded that they are reversible. Therefore it is necessary to study what is to be done to regulate their evolution.

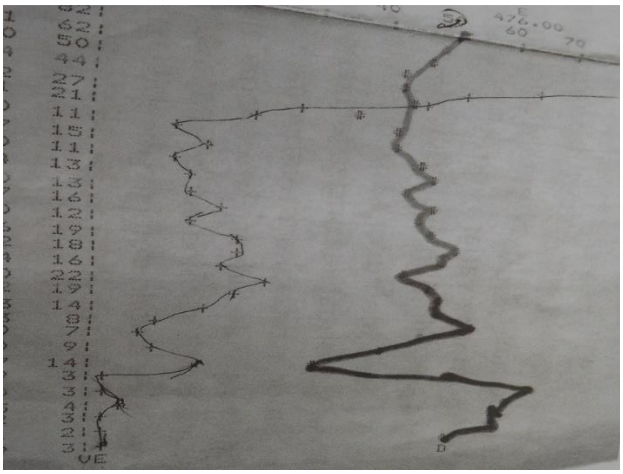

Fig. 21. The same child; curves collected from both ureters. On the right the ureteral voiding was severely diminished. On the left - complete voiding, but still a slow one

In fact, until now the attention has been drawn to the intramural ureter condition, trying to get it back to the normal condition. But our preventive studies account for a much more complicated and profound polyetiological condition in VUR. The treatment requires the restoration, the enlargement of the submucosal ureteral tract, that is prevalent today.

We have no experience in the treatment of this condition, but we recommend using a urinary catheter for a longer time, as well as fighting the infection and surgery. In stenosis the resection of the stenosed segment is performed. If stenosis is absent, the dilated ureter with signs of obstruction is also resected, because the intramural ureteral segment is stenosed and fibrosed by the bladder muscles and the intravesical pressure is increased.

The transurethral drainage through a catheter or via the suprapubic way contributes to an improved renal function. If the patient`s general condition does not allow radical treatment, a vesicostomy or nephrostomy is indicated. If the renal function does not improve which indicates a ureterovesical obstruction, the upper external derivation - pyelonephrostomy is indicated. Along with these methods, spasmolytics are administered, to eliminate, if possible, the spasm of the bladder muscles.

We recommend normalizing the intravesical pressure, preventing and combating the urinary tract infection in order to avoid irreversible complications of the upper urinary tract, reducing the number of chronic patients and patients requiring dialysis. Pediatric urologists, as well as adult urologists treat neurological symptoms, especially urination disorders, but they do not study the morphological and urodynamic abnormalities of the pelvic organs, especially due to the lack of appropriate equipment.

We recommend using frequently radionuclides in diagnosis, more often than urodynamic testing, which is noninvasive and informative. If the infection relapses the intravesical pressure is reduced by at least 50%, and intravesical anti-reflux operations should be carried out - the Politano–Leadbetter Cohen operation, in order to use the intravesical pressure and strengthen the antireflux mechanism. If we perform extravesical operations through the dilated open ureter**,** the intravesical pressure at each urination directly attacks the kidneys.

Treatment of the muscle spasm in newborns and infants is a rather serious problem. Only a few spasmolytic preparations are available – atropine and papaverine. The rest of drugs are recommended for adults.

The common efforts of physiotherapists and neurologists can help solve this issue.

Conclusions

1. The reflux megaureter develops intrauterine on the Waldeyer segment of muscle spasm, bladder muscles, bladder neck, external urethral sphincter in boys, and meatal in girls and substantially increased intravesical pressure of the open ureter with constant section.
2. The degree of the megaureter depends not only on the refluxing force but also on the degree of obstacle of the uretero-bladder segment when reintroducing the ureteral content.
3. Muscle spasm leads to disorders of the urodynamics with severe impairment of the upper urinary tract, renal parenchyma, in newborns already signs of renal failure with severe impairment of renal parenchyma - its deformation and fibrosis.
4. The treatment starts as early as the bladder, spasmolytics, physioprocedures, hydro massage and kinetotherapy, etc. to reduce the increased intravesical pressure after applying antireflux surgery, Cohen Leadbetter-Politano procedure, contemporary endoscopic methods to strengthen the antireflux mechanism.
5. After surgery, prolonged monitoring and treatment is recommended under the control of urine, cystometry, uroseptic, spasmolytic analysis because the intravesical pressure is difficult to normalize, it can only decrease.

REFERENCES

1. Bălgrădean M. Refluxul vezico-ureteral, în Pediatrie vol.II coord. Conf. Dr. F.Iordăchescu. Ed. Naț., București, 1998; pp. 895-901.
2. Buza A., Tănase A., Saptefrati L. Tratamentul endoscopic a refluxului vezico-renal cu „Deflux". Conferința II de nefrologie renală, dializă și transplant renal din Republica Moldova. Chisinau, 2002; pp. 30-1.
3. Curajos B. Diagnosticul și tratamentul chirurgical diferențiat al stenozei congenitale a uretrei posterioare și consecințelor la copii. Autoreferatul tezei de doctor habilitat în științe medicale, Chișinau, 1997. 28 p.
4. Curajos B., Dzero V., Zaharia I., Roller V. et al. Megaureter la copii. Diagnosticul și tactica de tratament/ Al X Congres al Asociației chirurgilor N.Anestiadi, I Congres de endoscopie din Republica Moldova, 10-17 septembrie 2003.
5. Curajos B., Dzero V., Curajos A., Zaharia I., Roller V. et al. Megaureter la copii. 2002; p.159-162.
6. Curajos B., Bernic J., Dzero V., Celac V., Curajos A. et al. Cistouretrografie și urofloumetrie radionucleidă. Al VI Congres de urologie, dializă și transplant renal din Republica Moldova. Chisinau; 21-23 octombrie 2015. p.97-9.
7. Curajos B., Bernic J., Curajos A., Dzero V., Celac V., et. al. Complicațiile operațiilor plastice pe ureter și prevenirea lor. Al VI Congres de Urologie, Dializă și Transplant renal. Chisinau, 21-23 octombrie.
8. Curajos B., Dzero V., Munteanu A., Zaharia I., Roller V. et al. Strictura congenitală a uretrei posterioare la copii. XVIII Rezumatul lucrărilor X-lea Congres Național de chirurgie. Chisinau, 22-25 mai 1995.
9. Curajos B., Petrovici V., Curajos A., Bernic J., et al. Un nou concept privind morfologia chirurgicală a stenozei și vascularizării ureterului în normă la copii. Ibidem. 2003. p.93-5.
10. Ghicavîi V. Tratament endoscopic diferențiat în patologiile urologice obstructive infravezicale. Autoreferatul tezei de doctor habilitat în științe medicale, Chișinău, 2018. 32 p.
11. Lowe L.H., Patel M.N., Gatti M.N. Utility of follow-up renal sonography in children with vezicoureteral reflux and normal initial sonogram. Pediatric. 2004; 13:548-50.
12. Matoo T.K., Carpenter M.A., Moxei-Mims M. et al. The RIUR trial: a factual interpretation of our data. Pediatr. Nephrol., 2015; 30:707-12.
13. Neveus T. Can postpyelonephritic renal scarring be prevented? Pediatr. Nephrol. 2013; 28:187-90.
14. Curajos A., Curajos B., Bernic J., et al. Megaureter refluxant la băieți. Al IV Congres de Urologie, Dializă și Transplant renal din rep. Moldova. Chișinău, 12-13 octombrie 2006.
15. Roberts K.B. Subcometee on Urinary Tract infections: clinical practice guideline for the diagnosis and management of the initial UTU in febrile infants and children 2 to 24 month. Pediatrics. 2011; 128: 595-610.
16. Roihuvuo H., Lahdes-Vasama T., Niskanen K., Ronhoim K. The association of adult kidney size with childhood vesicoureteral reflux. Pediatr Nephrol, 2013; 28:77-82.
17. Sjostrom S. Longitudinal study of infants with high grade vesicoureteral reflux. Göteborg. 2009; 68 p.
18. Пугачев А. Детская урология, Москва, 2009.

We sincerely thank Mr. Victor Poporcea (specialist in hydrodynamics) for his theoretical help and innovative ideas during this study.

Case Report

Gastric trichobezoars in children: A clinical case report

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Abstract

Trichobezoarele gastrice la copii: prezentare de caz clinic

Trichobezoarul reprezintă o concrețiune a părului ingerat depistat la un anumit nivel în tractul digestiv, care apare cel mai frecvent la pacienții cu tulburări psihice, care mestecă și înghit propriul păr (trichotilomanie și trichofagie).
Existând riscul dezvoltării unor potențiale complicații grave, se impune necesitatea unui diagnostic promt și rapid, care va determina alegerea unei opțiuni adecvate de tratament.
Autorii prezintă un caz clinic de trichobezoar gastric diagnosticat la o pacientă în vârstă de 13 ani cu trichotilomanie. Diagnosticul a fost stabilit în baza examenului radiologic, inclusiv tranzitul baritat și tomografia computerizată, examenul endoscopic punând în evidență o masă cenușie de dimensiuni impresionante, de culoare neagră, localizată în stomac.
Cazul dat a fost rezolvat prin îndepărtarea chirurgicală a unui trichobezoar uriaș de dimensiuni 20 cm x 6 cm. Postoperator, pacienta a fost supusă unui tratament de recuperare cu realimentare treptată, beneficind și de consiliere pisihiatrică.
Autorii conchid, că în pofida faptului că trichobezoarele de dimensiuni mici pot fi extrase endoscopic după un tratament conservativ preventiv, tratamentul de elecție în trichobezoarele de dimensiuni majore este cel chirurgical, în perioada postoperatorie pacienții necesitând un tratament de recuperare treptată și o evaluare psihoemotivă cu scop de a preveni reapariția acestora.

Cuvinte cheie: trichobezoar, sindromul Rapunzel, ocluzie intestinală, tratament chirurgical

Abstract

Trichobezoar is a concretion of ingested hair found at a certain level in the digestive tract, which occurs most commonly in patients with mental disorders, who chew and swallow their own hair (trichotillomania and trihophagia).
Given the risk of developing potential serious complications, the need for a prompt and rapid diagnosis is required, which will determine the choice of an appropriate treatment option.
The authors present a clinical case of gastric trichobezoar diagnosed in a patient 13 years of age with trichotillomalania. The diagnosis was established on the basis of the radiological examination, including barite transit and computer tomography, the endoscopic examination highlighting a gray mass of impressive size located in the stomach.
This case was solved by the surgical removal of a huge trichobezoar of dimensions 20 cm x 6 cm. Postoperatively, the patient underwent a recovery treatment with gradual refeeding, benefiting from psychiatric counseling.
The authors conclude that despite the fact that small trichobesores may be endoscopically removed after a preventive conservative treatment, the treatment of choice in the trichobezores of major dimensions is the surgical one, in the postoperative period the patients needing a gradual recovery treatment and a psycho-emotional evaluation, in order to prevent their recurrence.

Keywords: Trichobezoar, Rapunzel syndrome, intestinal obstruction, surgical treatment

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Background

The bezoar is a tumor formation containing swallowed substances, which are most commonly located in the stomach and small intestine. Based on their components, they are classified into phytobezoars that are made up of undigested fruit and vegetable fibers, trichobezoar -made up of hair and mixed bezoars consisting of gauze, paper, fungi, etc. [4, 12].

The most common type of bezoars are the trichobezoars mostly found in children with mental disorders. Specialized literature has described only a few cases of trichobezoars among children. The clinical picture onset is usually asymptomatic (eventually being detected during a routine investigation), whereas the clinical signs may develop in complicated cases. The most common complications involve intestinal obstruction (Rapunzel syndrome), intussusception, acute appendicitis, intestinal perforation and hemorrhage [1, 5]. The diagnosis is often challenging requiring differential diagnosis. Most cases of trichobezoars are treated surgically, whereas in Rapunzel syndrome, the treatment of the underlying disease is necessary to prevent recurrences. The bezoar disease ("morbus bezoaris") remains a rare pathology, which has so far been little studied by specialists. Thus, this is a major reason for a delayed diagnosis once the severe complications have already developed. Further basic investigations will include abdominal ultrasonography and radiography, fibrogastroduodenoscopy. However, CT (computed tomography) and NMR (nuclear magnetic resonance) are required for a more accurate topical diagnosis [2, 12].

Trichobezoars are commonly treated with spasmolytic, analgesic drugs and vegetable oil, which can be effectively in small trichobezoars. Endoscopic attempts may be performed to retrieve the trichobezoars via fibrogastroscopy. In case of huge trichobezoars and their complications (perforation or hemorrhage), a surgical intervention is required [2, 7, 11].

Throughout the last five years, 4 children from the Department of Pediatric Surgery were diagnosed with GI trichobezoars (2 children with gastric trichobezoars, of which one child exhibited a huge type and was operated on, whereas another child had a small-sized trichobezoar and self-induced vomiting). 2 children revealed small bowel trichobezoars, who underwent a conservative therapy. In order to alert the importance of an early diagnosis and treatment, we would like to report a clinical case of a 10-year-old girl with gastric trichobezoar, who showed specific symptoms and was hospitalized in our clinic for a period of several months.

Clinical case report

A 13-year-old girl was found to have intra-abdominal tumor formation, following an additional investigation for recurrent abdominal algic syndrome. Over the last month, the patient often experienced upper abdominal pain, vomiting, loss of appetite and nausea. During a detailed discussion, her parents stated that the child has a habit of pulling and eating hair, resulting in considerable weight loss within the last year. The clinical examination revealed a mobile, hard-elastic and slightly tender to touch tumor mass within the mesogastric region with a size of 20 x 6 cm and without signs of peritoneal irritation.

The barium swallow showed an enlarged, dilated and non-homogeneously opaque stomach with partial gastric emptying and contrast medium retention at intervals up to 12 hours after the ingestion (fig. 1).

Fibrogastroscopy revealed a mass of hair particles that occupied the entire gastric lumen and extended to the pylorus. The CT detected the presence of a gastric trichobezoar formation (fig. 2).

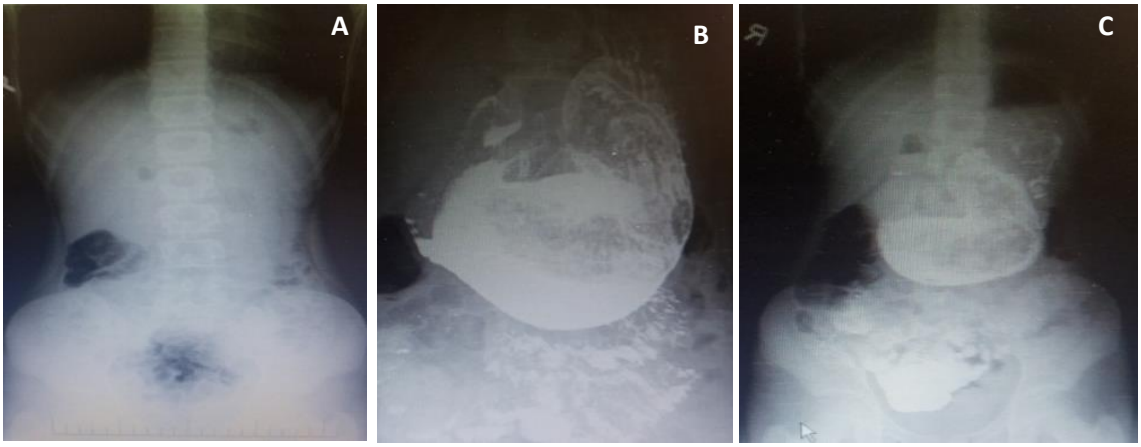

Fig.1. Abdominal X-ray: A - on an empty stomach; B - more than one hour after barium medium swallow ; C - over 12-hour interval

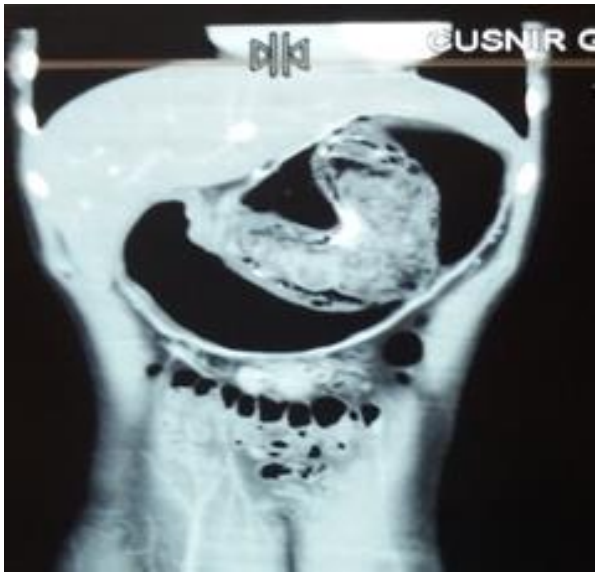

Fig. 2. The abdominal CT detected a homogeneous mass within the stomach, which occupied almost the entire lumen with signs of partial intestinal occlusion.

The preoperative children were administered symptomatic and parenteral nutrition therapy. Following an improvement in general condition, the children were subjected to a surgical intervention under general anesthesia via endotracheal intubation. The upper midline laparotomy and the inspection of the abdominal cavity revealed an enlarged stomach as well as collapsed small bowel loops ("hungry gut"). The laparotomy and the gastrostomy were made along the longitudinal axis, which revealed a giant hair mass, occupying almost the entire gastric lumen with a short tail of hair extending to the pylorus and some hairs present in the duodenum). The gastric trichobezoar was removed and the gastric incision was sutured.

The extracted trichobezoar was examined. It resembled the shape of the stomach, having dimensions of 20x6x2 cm and weighing 150gr (fig. 3).

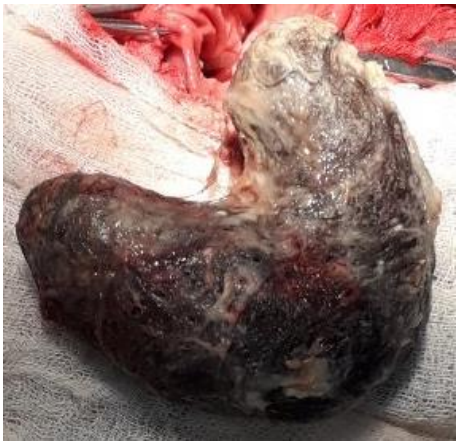

Fig. 3. Intraoperative piece. Giant trichobezoar.

The postoperative course was favorable, without complications. After the improvement of the child's overall condition and removal of the sutures, a psychologist and psychiatrist consulted the child in order to undergo a specific treatment. Subsequently, the child's condition has improved, followed by an increase in appetite and weight gain, as well as missing abdominal algic syndrome. The child was discharged 14 days postoperatively, having a good health condition.

Discussions

Trichobezoars particularly occur in girls with long hair, most commonly in teenagers [5, 8]. It commonly occurs due to the hair that is trapped in the gastric mucosal folds. Swallowed hairs gather and form a large hair bolus covered with gastric mucosa, which impairs the gastric peristalsis. The trichobezoar then gets too large to leave the stomach and that might result in gastric atony [2, 4, 11].

Rapunzel syndrome is a specific form of trichobezoar [1, 9, 10]. There have been used a number of methods and criteria to specify the diagnosis of Rapunzel syndrome. The researchers have found the following identical characteristics for Rapunzel syndrome: gastric trichobezoar of any size with a tail extending through the pylorus into the small intestine, the presence of symptoms suggestive of intestinal occlusion (a partial or a complete one) and mental impairment: trichophagia – compulsive eating of hair and trichotillomania [8, 10].

There are no pathognomonic or characteristic signs for bezoars. The most common clinical features include anorexia, weight loss, vomiting syndrome, recurrent abdominal pain, and detection of abdominal "pseudo-tumors" [2, 5]. The long-term evolution of the disease might lead to various complications, such as anemia, erosive gastritis, intestinal obstruction, malabsorption syndrome, intussusception and appendicitis [3, 10, 11].

The diagnosis is based on the patient's history taking, physical examination and imaging studies. The diagnostic standard includes: abdominal ultrasonography, fibrogastroscopy and computed tomography, as well as upper GT X-ray [2, 4, 5, 12]. The major treatment approach of bezoars is to remove the tumor mass and prevent its recurrence. The treatment of choice depends on the bezoar's consistency, size and location, thus it can be removed conservatively, by endoscopy or by a surgical intervention [1, 4, 7]. The treatment of bezoars can be initiated by using vegetable oil and spasmolytic drugs. In case of trichobezoars, a combined therapy of pancreatic enzymes and papain syrup (from Papaya fruit) can be administered [5, 6].

An attempt of endoscopic extraction via a rigid fibrogastroscopy may be used in small and medium sized bezoars; however, it is associated with various complications such as iatrogenic esophageal perforation and hemorrhage [2, 4]. At present, the emergence of mini-invasive surgery enabled the use of the laparoscopic

techniques for extraction of small and medium bezoars. Laparoscopy might be used to remove gastric trichobezoars if these are not large and can be fragmented by using special endoscopic bags. Nevertheless, it proves ineffective when extracting large-sized trichobezoars (> 20 cm). Thus, the surgical intervention is the treatment of choice in complicated cases [2, 3, 7, 11].

Conclusions

1. Small-sized trichobezoars might be extracted by fibrogastroscopy following a conservative and fragmentary therapy.
2. The endoscopic extraction of gastric bezoars may allow avoiding surgery or might be an alternative treatment to surgical intervention.
3. The treatment of choice in giant trichobezoars is the surgical intervention via the upper laparotomy and gastrostomy. A psychologist should council children postoperatively in order to avoid recurrences.

REFERENCES

1. Eryilmaz R., Sahin M., Alimoglu O., Yildiz M.K. A case of Rapunzel syndrome. Ulus Travma Acil Cerrahi Derg. 2004; 10:260-3.
2. Holcomb III G.H., Murphy J.P., Ostile D.J. Ashcraft's Pediatric Surgery, Sixth Ed. 2014.
3. Jensen A.R., Trankiem C.T., Steve Lebovitch S. et al. Gastric outlet obstruction secondary to a large trichobezoar. JPS. 2005; 40:8.
4. Lee J. Bezoars and foreign bodies of the stomach. Gastrointest. Endosc. 1996; 6: 605-19.
5. Naik S., Gupta V., Naik S. et al., Rapunzel syndrome reviewed and redefined. Dig. Surg. 2007; 24(3):157-61.
6. Nichol P.F., Rollins M.D., Muratore C.S. Fundamentals of Pediatric Surgery. Second Ed. 2017.
7. Prasad A.J., Rizvon K.M., Angus G. et al. A giant trichobezoar presenting as an abdominal mass. Gastrointest Endosc. 2011; 73(5):1052-4.
8. Santos T., Nuno M., Joao A. et al. Trichophagia and trichobezoar: Case report. Clin. Pract. Epidemiol. Ment. Health. 2012; 8:43-5.
9. Singla S.L., Rattan K.N., Kaushik N., Pandit S.K. Rapunzel syndrome: A case report. Am. J. Gastroenterol. 1999; 94(7):1970-1.
10. Zent R.M., Cothren C.C., Moore E.E. et al. Gastric trichobezoar and Rapunzel syndrome. Am. Coll. Surg. 2004; 990.
11. Веселый С.В., Сопов Г.А., Латышов К.В. и др. Инородные тела желудочно-кишечного тракта у ребенка на фоне частичной обтурационной кишечной непроходимости. Дет. Хирургия. 2012; 1:50-1.
12. Гиткевич А.Э., Шмаков А.П., Зуев Н.Н., Зуев Н.И. Трихобезоар желудка и тонкой кишки у ребенка. Дет. Хирургия. 2010; 1:48-9.

In Memoriam

Constantin Tica

With immeasurable sadness and deep pain in the soul, we announce the premature death of the renowned Romanian pediatric surgeon Constantin Tica, nicknamed "the angel of the children".

I knew about the suffering our good friend. I hoped with all my soul him to win and remain among us. But death was stronger, the soul leaving the tired body of the disease. On November 29, 2019, Professor Constantin Tica died, leaving behind a huge emptiness, sadness and pain.

Constantin Tica was born on January 6, 1953 in Cluj. He graduated the theoretical high school Calistrat Hogaş from Piatra Neamţ, then the University of Medicine and Pharmacy "Grigore T. Popa" from Iași. In 1978 he was employed, by competition, at the Constanta County Hospital, the specialty of Pediatric Surgery, going through all the stages of professional ascension, having as mentors Alexandru Pesamosca, Constantin Pavlovici and Mircea Petrescu. In 1993 he was chief of the Clinic of Pediatric Surgery and Orthopedics at the Constanța County Hospital.

In the 1990s, Constantin Tica supported the establishment of the "Ovidius" University and founded the Pediatric Surgery discipline. In 2002 he became a university lecturer, the discipline of surgery and pediatric orthopedics, and in 2006 - a university professor. Since 2016 he became the position of Dean of the Faculty of Medicine of the "Ovidius" University. Being actively involved in the activity of professional organizations and specialized commissions (member of the National Committee of the Romanian Society of Pediatric Surgery, member of the Disciplinary Committee of the National Society of Pediatric Surgery, chairman of the specialized committee of the Romanian Medical College, Chairman of the Pediatric Surgery Commission of the Ministry of Health, member of the *European Association of Pediatric Surgery*) he made significant contribution outstanding to increase of the performance of the Romanian pediatric surgery.

Professor Constantin Tica was loved and appreciated as a perfect surgeon and a good teacher for students, masters, doctoral students and residents, an excellent coordinator of professional and scientific activity. He was also a good friend of the pediatric surgeons from the Republic of Moldova, Russia and the countries of the European Union.

We express our gratitude for everything Professor Constantin Tica has done for "Ovidius" University and pediatric surgery in Romania. We extend sincere condolences to the grieving family and to all those who have known him.

God rest in peace.

Academic community of "Ovidius" University
(Constanţa city, Romania)
National Society of Pediatric Surgery of the Republic of Moldova

Dear Colleagues!

The journal is focused on the publication of the latest scientific performances and the results of the evidence-based clinical research in the field of pediatric surgical specialties and other related fields, being also a means of continuous medical education. The journal publishes two issues per year. The following categories of papers are published:

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