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CONTENTS

DIAGNOSTIC STAGES FOR FALLOT DISEASE IN NEWBORNS AND INFANTS

Ramona Olariu[1], Adrian Lăcătușu[1], Alina Agoston[2], Constantin Ilie[2]

Abstract

Introduction-Purpose of study. Fallot disease is a cyanotic heart malformation witch if diagnosed in an early stage can be optimally corrected trough surgery leading to a normal life style for the patient. Quantifying the clinical situation, systolic murmurs, low oxygenation, hemodynamic instability, fatigue, peripheral cyanosisand phenotypical dismorphism, need an: x-ray, echocardiography, cardiac MRI.Clinical signs, laboratory and imaging investigations establish the diagnosis and suggest the correct treatment. *Material and method*. The study was carried out using 16 newborn and infant diagnosed with Fallot in newborn Clinics in Timisoara, in 2015-2016. The diagnosis was suggested by the clinical evolution of the subjects and confirmed by echocardiography in most cases, some in utero. *Results.* Statistically most of the cases were diagnosed as newborns, when echocardiography was used to investigate a systolic murmur. The in utero diagnosis was useful in suggesting a place of birth in close proximity to a cardiovascular center in the cases of extreme Fallot disease. The genetic syndrome association with Fallot disease was present in 3 of the cases. *Conclusions.* 1. The neonatal screening is important in cardiac malformation cases witch require surgery. 2. The morphological variability translates in the clinical state of the patient and emergency degree, so the in utero diagnosis is very significant. 3. The morphological complexity and severity of the case was often associated with other lesions in the case of a genetic syndrome.

Key words: Tetralogy Fallot, cardio-vascular signs

Introduction - The purpose of the paper

Tetralogy of Fallot (TOF) is a cyanotic congenital heart malformation, is considered the prototype of cyanotic congenital heart malformations. The four classic morphological malformations are the following: ventricular septal defect, right-sided aortic arch, and pulmonary artery stenosis and right ventricular hypertrophy.[1,2]

In addition TOF may present other anatomical anomalies including: an atrioventricular septal defect, total or partial abnormal pulmonary venous return, coronary artery abnormalities, a patent foramen ovale or atrial septal defect, in which case the syndrome is sometimes called a pentalogy of Fallot.[3-6]

A severe variant of TOF is the type with complete obstruction (atresia) of the right ventricular outflow tract. In these individuals, blood flows from the right ventricle to the left where it is pumped only through the aorta. The lungs are perfused via extensive collaterals from the systemic arteries, and sometimes also via the ductus arteriosus.[3-5,7,9-12]

In the case of the infant who escapes the neonatal filter without being diagnosed, we can also observe sucking fatigue, varying degrees of shortness of breath, weight curve slowly upward or stagnant, syncopal episodes, fainting, repeatedear, nose and throat infections, brain abscess.[7,8,10]

Clinical signs and symptoms vary depending on the age of the patient at diagnosis and are nonspecific, but the presence of a systolic murmur raises the suspicion of congenital heart diseases. During the newborn stage different degrees of systolic murmurs and o2 desaturation can appear if the newborn is agitated or nursing, also hemodynamic instability , peripheral cyanosis , phenotypic dysmorphism absence of clinical symptoms or only present a systolic murmur can, prematurity or low weight for gestational age can be observed.[1,8,12]

Quantifying the clinical situation, systolic murmurs, low oxygenation, hemodynamic instability, fatigue, peripheral cyanosis and phenotypical dysmorphism, need an: x-ray, echocardiography, cardiac MRI. Clinical signs, laboratory and imaging investigations establish the diagnosis and suggest the correct treatment.[13-18]

Material and method

The study was carried out using 16 newborn and infant diagnosed with Fallot in newborn Clinics in Timisoara, in 2015-2016. The number of patients included in the extended retrospective doctoral study is 80 patients with TOF but the criteria for the present paper limited the number.

Patients diagnosed postpartum showed intense specific systolic murmur (pulmonary stenosis), and the echocardiography confirmed the diagnosis. We can say that 7 cases of typical Fallot were diagnosed in Maternity, post echocardiographic review of systolic murmurs. Patients were hemodynamically stable with varying degrees of cyanosis or sucking fatigue, with systolic murmurs of different intensity, they came from healthy parents and the pregnancies were monitored. Of the 7 cases, 4 were solved surgically, surgical correction being performed per primam between the ages of 4 months and 1.6 years, with good postoperative evolution without major problems and age-appropriate social insertion.

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We diagnosed echocardiographic a 5 months old infant with TOF and agenesis of eyeballs with systolic murmur, undiagnosed completely until this age, when the first hypoxic crisis occurred - in the context of a respiratory infection. Other associated malformations, maternal intrauterine infection or association with a genetic syndrome were excluded through imaging and laboratory means. Currently is 11 months old, it is stable and will soon benefit from surgery per primam.

We recruited two cases of tetralogy of Fallot with pulmonary atresia diagnosed in maternity with cyanotic heart malformation incompletely defined. The cases had loud systolic murmur, cyanosis, hypoxic seizures, and at 2 and 6 months were correctly diagnosed through imaging (echocardiography, Rx, angioCT), with Tetralogy of Fallot with pulmonary atresia (PA) and major aortopulmonary collateral arteries (or MAPCAs). These cases showed extensive collaterals and are suitable for surgery with aortal -pulmonary collateral focus and total correction.

TOF with PA is considered the extreme morphopatological variant. Pulmonary atresia may be limited to the valve (pulmonary atresia membranous) or infundibular subvalvular (muscular pulmonary atresia). [19-23]The lack of ante grade pulmonary blood flow in utero leads to a range of morphologic findings in the pulmonary artery vasculature such as, confluent true pulmonary arteries of variable sizes if the ductus arteriosus (DA) is present. Without flow through the DA, MAPCAs, fetal vessels derived from the splanchnic vascular plexus, may persist after birth. These vessels connect the systemic and pulmonary arterial vasculature, thereby supplying pulmonary blood flow. MAPCAs are tortuous vessels that arise directly from the aorta or its branches and vary in number and origin, follow routes to reach central, lobar, and segmental pulmonary arteries, and have variable areas and locations of stenosis. The morphology of the pulmonary vasculature and MAPCAs plays a critical role in determining management decision. [19,20]

We have included 3 cases of Fallot diagnosed intrauterine were fetal ultrasound raced a high suspicion of Fallot, these cases were confirmed by echocardiography postpartum, in the presence of systolic murmur and peripheral cyanosis. Because of hemodynamic stability, mild and rare hypoxic crisis, light/medium pulmonary stenosis, benefited from achieving total correction, optimally between 6 months and 1 year with very good postoperative adjustment.

We observed the association between DiGeorge syndrome and Fallot in 3 cases, in one case there was an antenatal diagnosis of congenital heart malformation. Patients had pulmonary atresia and multiple stenosis, in series of pulmonary branches. They presented systolic murmur, intense cyanosis, hemodynamic instability and PG therapy was established to keep the ductus arteriosus permeable, in order to find solutions for urgent surgical treatment. One patient received systemic-pulmonary shunt at 3 months old and then 3 pasty with stenting of pulmonary branches post cardiac catheterization approximately every 6 months / years, with complicated evolution and prognosis in the context of genetic syndrome and stenosis of multiple branches. The second patient with TOF associated genetic syndrome (who was diagnosed antepartum), presented a giant aneurysm of pulmonary trunk, pulmonary regurgitation grade III / IV. It has received a total correction at age 3 months. The third case is in the phase of investigation the genetic syndrome by karyotype and molecular techniques, is phenotypically classified in DiGeorge syndrome. It has benefited from prostaglandine (PG) therapy, total correction for Fallot the age of 4 months, later two pulmonary plasty post cardiac catheterization for stenosis of pulmonary branches. It is currently stable with good performance.[24-26]

Results and Discussions

Statistically most of the patients 56% have been diagnosed in maternity after the evaluation of systolic murmur. (Fig.1)

A total of 68% of the patients in study have been diagnosed with classical TOF, 3 cases (18%) with TOF with DiGeorge genetic syndrome and 2 cases (12.5%) with TOF with PA. (Fig.2)

11 patients (68%) from the study group had surgery, 10 patients (62%) benefitted of total correction per primam. 1 patient was initially stabilized by shunt systemic- pulmonary at age 3 months, and then received total correction surgery at 1 year old, as well as two plasty with stenting of pulmonary branches.

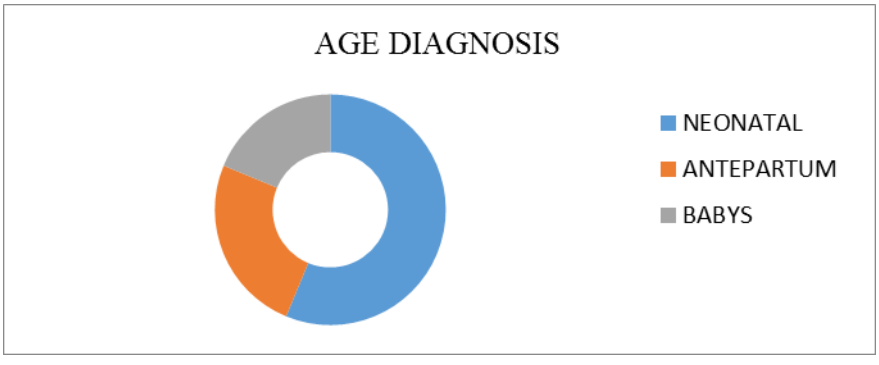

Fig. 1. Age of patients at the moment of diagnosis.

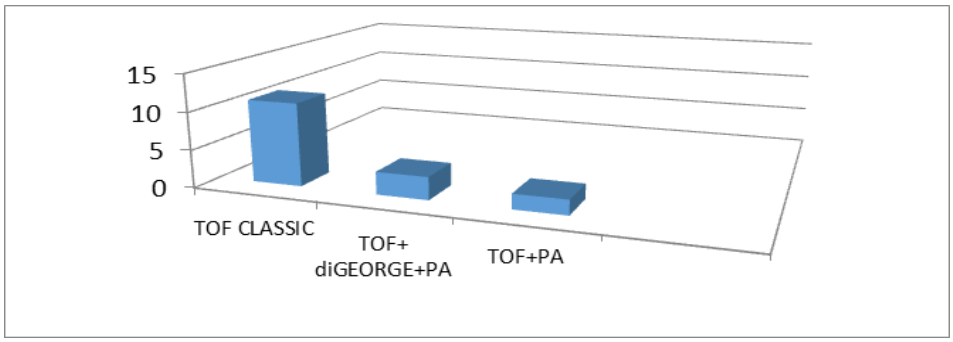

Fig. 2. The number of TOF (classic and extreme) and TOF with genetic syndrome association.

We could not perform surgical correction per premium due hemodynamic instability, severe hypoxic crises and malformation context.[2,28,29]

There were no deaths registered in the group of patients that received surgery or in the group that awaits correction surgery. (Fig.3)

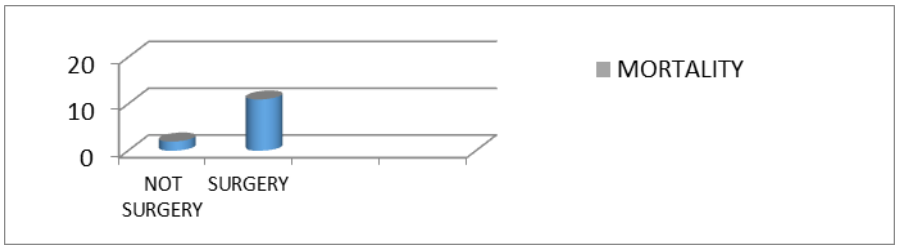

Fig. 3. The number of patients with and without surgical correction.

The in utero diagnosis was useful in suggesting a place of birth in close proximity to a cardiovascular center in the cases of the newborn with the extreme type of Fallot disease.

The prognosis is good for classic form TOF patients, because they have been diagnosed on time and have received or will receive timely total correction. In the case of the patient with TOF with PA the prognosis is reserved due to the many collateral arteries and surgery limitations. Patients with TOF and DiGeorge syndrome have a high-risk of developing complications and a reserved prognosis. (Fig.4)[2,30]

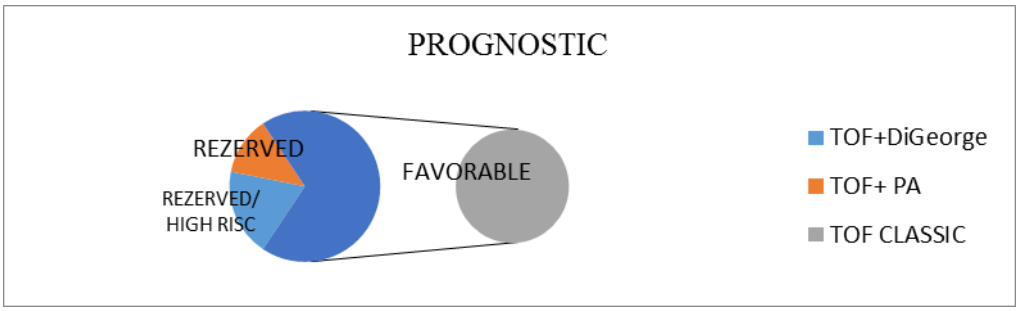

Fig. 4. Prognostic of TOF (classic, extreme, TOF with genetic syndromes).

Conclusions

1. The neonatal screening is very important in the case of cardiac malformations with surgical indication.

2. Echocardiography established the correct diagnosis for infants with cyanosis and systolic murmur and indicated the optimal surgery needed to correct the problem.

3. The morphological variability translates in the clinical state of the patient and emergency degree, so the in utero diagnosis is very significant.

4. The morphological complexity and severity of the case was often associated with other lesions in the case of a genetic syndrome.

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MORBUS ODELBERG-VAN NECK – A RARE ENTITY IN CHILDHOOD

Narcis Flavius Tepeneu[1,2]

Abstract

Morbus Odelberg-van Neck (van Neck disease, van Neck-Odelberg disease, ischiopubic "osteochondrosis", synchondrosis ischiopubic syndrome, assymetric ischiopubic syncondrosis syndrome, osteocondrosis of the ischiopubic syncondrosis) is a benign skeletal abnormality in children involving a hyperostosis of the ischiopubic syncondrosis seen on radiographs. Children complain of groin or buttocks pain and it's radiological features mimic osteomyelitis, fracture, tumors or posttraumatic osteolysis or even the normal skeletal development. This leads usually to unnecessary workup. It is often considered a diagnosis of exclusion, as laboratory values are usually normal and routine radiographic workup may be nonspecific.

The paper presents two cases of van Neck disease in which the correlation between the clinical, laboratory and imaging data enabled the diagnosis of Morbus Odelberg-van Neck.

Key words: Morbus Odelberg-van Neck, hip pain, children, ischiopubic "osteochondrosis", ischiopubic syncondrosis (IPS)

Introduction

The ischiopubic syncondrosis is a cartilaginous joint between the os pubis and os ischia. It is a temporary cartilaginous joint which is present at birth and undergoes complete ossification before puberty.

In 1923 Odelberg described 3 patients who had rarefying lesions of the ischium of doubtful etiology with pain in the hip, limping and limitation of hip movement [1]. All patients were operated and the histological investigation revealed a non-specific inflammation. In 1924 van Neck reported two similar patients upon whom he had operated and applied the term ischiopubic osteochondritis for the first time [2]. It was first considered a pathological fenomenon. Later, because of it's apparent benignity and spontaneous healing the terms of osteochondritis or osteochondrosis were used less and less. Nowadays, it seems to be clear, that widening of the ischiopubic junction is a process of normal skeletal growth, at least in asymptomatic individuals.

The development of the pubic bone begins in the fetus during the fifth or the sixth month, when the center of ossification is formed in the horizontal ramus at the edge of the obturator foramen. The ischium normally begins to ossify in the superior ramus in the fetus during the fifth month. As growth progresses, the cartilage between the ischial and the pubic rami is replaced by bone until fusion occurs. This area, called the "ischiopubic synchondrosis," (IPS) undergoes fusion relatively early in childhood, whereas in the acetabular region, synostosis does not occur until puberty.

In early childhood, enlargement of this synchondrosis is bilateral; however, in older children, it is commonly unilateral. Usually, the fusion of the ischial and pubic bones develops without any clinical symptoms. Recognition of the entity as a normal variant is important for radiologists when interpreting a pediatric pelvic radiograph[4,5]. One of the key questions to ascertain is wheather the region is painful or not.

Material and method

Two cases of Morbus Odelberg-van Neck are presented.

The *first case* is of a nine- year-old child from the rural area presenting with hip pain on the left side. The patient could walk, but his limp was obvious. He was a active football player, yet he denied traumatic events. From the patients history a insiduos appearance of the complaints is to be noted, the pain began about five weeks prior to presentation to the hospital. After sports the pain increased, so that the child developed a limp, which led to not being able to play football. The parents observed the limp but initially sought it came from a trauma in sports and it would go away. When the limp did not disappear after 2 weeks of observation at home they brought the child to the hospital. No medication was administered at home. There was no history of fever at home.

There was no palpable swelling of the IPS, the child complained about groin and hip pain, there was no limb length discrepancy, no local swelling or inflammatory signs, yet there was a contracture of the adductor muscles on the left side with restriction of external and internal rotation of the hip in comparison to the healthy right side.

Laboratory tests revealed a slight increase in the C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). Procalcitonin and Anti-streptolysin-O (ASO) titer was normal. The blood culture did not reveal any pathogenic germs. The Mantoux tuberculinic test was normal. Anteroposterior radiographs of the pelvis showed an enlarged left ischiopubic syncondrosis characterized by a focal area of osteolysis (Figure 1). A diagnosis of van Neck disease was made.

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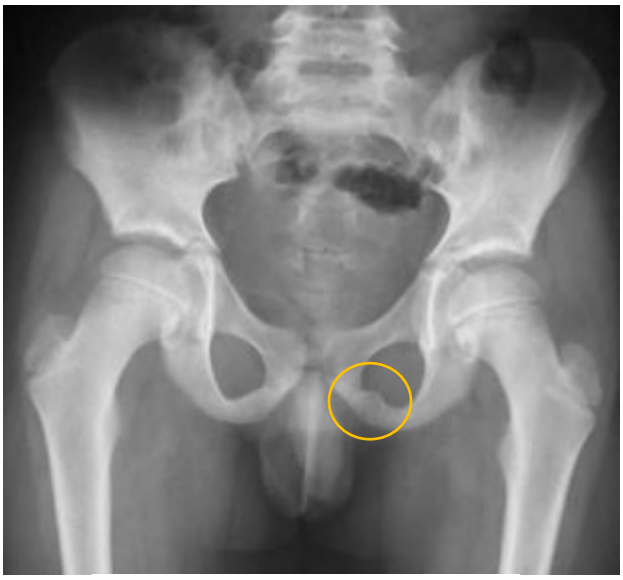

Figure 1. Radiography of the pelvis.

The child was treated anti-inflammatory therapy (NSAIDs) and bed rest for about 3 weeks. He was also prescribed crutches for ambulation and was advised to not be engaged in any sports. A clinical control of the patient 1 month later showed a remarkable improvement in symptoms, with an almost pain free patient. Laboratory tests revealed normal values. The ambulation with crutches was prescribed for a further month because of improvement of symptoms and the fact that the patient was very handy with them, despite of his relatively small age. At two month of treatment there was complete regression of symptoms. The patient was allowed to walk, but further sports interdiction was prescribed. A follow-up control at 4 months showed a virtually pain free patient, the laboratory tests revealed normal values so the patient was allowed to practice sports. He was eventually lost from our evidence, because the family moved to a foreign country.

The *second case* is of a 8 year old obese male patient presenting to the hospital with diffuse intermittent pain in the right groin region. The pain began about 3-4 weeks ago. The child was operated 6 years ago because of a undescended testes on the right side. 14 days before a foreign body (wooden piece) was removed by the family doctor from the scrotal skin. The mother of the child initially thougt it was scrotal pain, then a more detailed anamnesis revealed that the child was presumabily hit by another child with the foot in the pelvic region. There was no history of fever at home.

An ultrasound of the testis revealed no abnormalities. The ultrasound and radiographs of the pelvis and of the right hip (Figure 2,3) revealed also no abnormalities. Anteroposterior radiographs of the pelvis showed an enlarged bilateral ischiopubic syncondrosis, which was interpreted as the normal appearance of the ischiopubic syncondrosis.

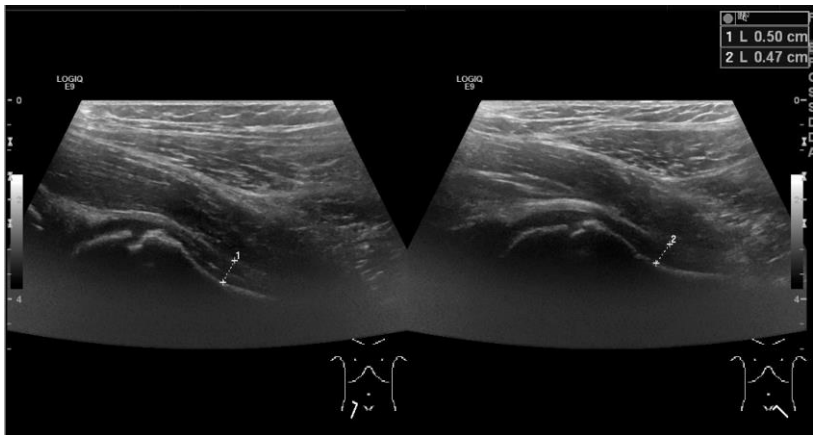

Figure 2 – Ultrasonography of the hips which shows no sign of intraarticular abnormalities.

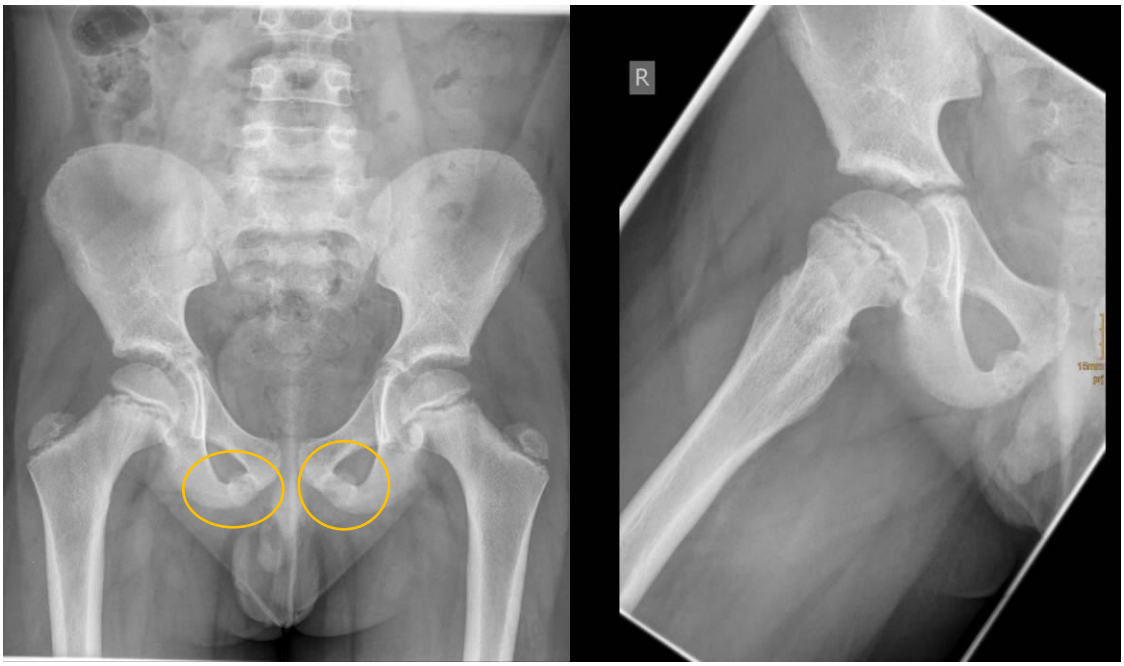

Figure 3 – Radiographs of the pelvis and the right hip.

Clinically both testes were descended, of normal shape and size. The was no sign of a inguinal hernia. The clinical examination revealed no limb length discrepancy, no local swelling or inflammatory signs, yet there was a contracture of the adductor muscles on the right side with restriction of external and internal rotation of the hip in comparison to the healthy left side. There was also a marked limp.

The child was initially treated with bed rest/ walking with with crutches and Ibuprofen p.o at home. There was no improvement of the pain, so a MRI of the pelvis was indicated.

The MRI images showed moderate oedema of the perilesional soft tissue and muscles and a fusiform enlargement of the right ischiopubic syncondrosis (Figure 4).

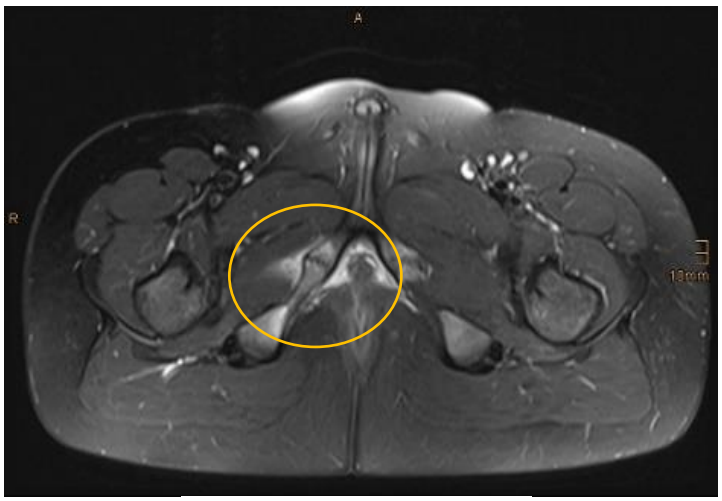

Figure 4 – MRI of the pelvis.

Laboratory values showed normal leucocites, ESR and a elevation of CRP of 1,5 mg/dl (normal value 0,5 mg/dl). Anti-streptolysin-O (ASO) titer was normal, also workup for rheumatoid diseases and Lyme disease. Under suspicion of a osteitis/osteomyelitis the pacient received a treatment with Clyndamicin 3x300 mg/day p.o for 3 weeks. The next week his CRP was in normal range and it continued to be with weekly followup. A follow-up radiography of the pelvis showed no dynamic of the image in comparison to Figure 4. Because the symptoms had a fluctuating character with

pain-free periods and then again impossibility to walk, a orthopaedic consultation was done. The pediatric orthopaedic surgeon recommened a bone scintigraphy. The bone scintigraphy showed no abnormalities. (Figure 5).

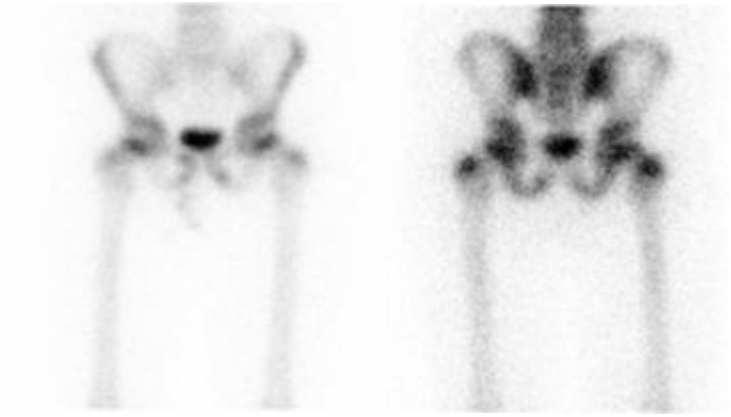

Figure 5 – Bone scintigraphy.

A neurologic exam by an pediatric neurologist was normal. Because the patient continued to have symptoms a biopsy under general anesthesia was proposed. The family refused.

As an alternative a five day i.v. therapy with Iloprost Trometamol (Ilomedin) 10 μg/0,5 ml was begun. The child was released in good condition from the hospital.

A follow-up MRI 6 weeks later showed that the local oedema was regredient and only discrete.

The soft tissues and muscles were normal. There were no other abnormalities (Figure 6).

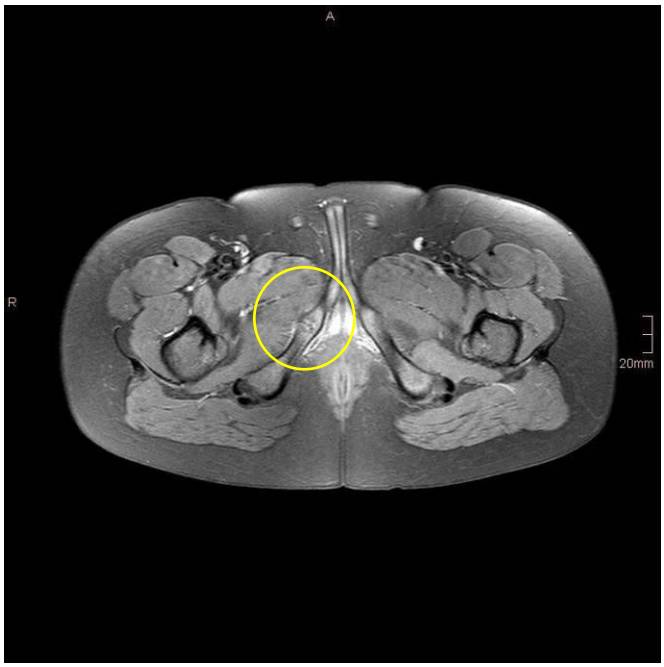

Figure 6 – Follow –up MRI of the pelvis.

Clinically the patient was also painfree and had a normal walking pattern .He was restrained from sports for another 4 weeks. At follow-up in 8 weeks the patient was painfree and had no complaints. He was again allowed to practice sports.

Discussion

In 1923 Odelberg [1] reported three patients (two boys aged eleven and fifteen years and a girl thirteen years) who had rarefying lesions of the ischium of doubtful etiology, with pain in the hip, a limp and limitation of movement. In each case the lesion in the ischium was scraped out. Bacteriological examinations for tuberculosis were negative, and histological investigation suggested non-specific inflammation. A fourth boy of eleven years who had been

operated on at another hospital was included in this report. In this child, and in the first of the other three children, a perineal fistula developed after an initial exploration. In retrospect it seems likely that these were cases of pain in the hip with altered ischio-pubic synchondroses. The descriptions of the findings at operation in these cases are very limited and the nature of the material submitted for histology was not recorded.

In 1924 Van Neck [2] reported two similar patients upon whom he had operated, and applied the term ischio-pubic osteochondritis for the first time. In one, a girl ofeight years, he found pain, limitation of hip movement, and a swollen labium majus. Radiographs showed the ischio-pubic synchondrosis to be enlarged and rarefied; and it was palpable per rectum. Suspecting osteomyelitis he incised the lesion but found only blood and friable bone, a small fragment of which was reported by one pathologist as a small-cell sarcoma, and by another as an osteochondritis. In the second child, a girl ofeleven, with similar clinical and radiological findings, operation revealed a hard bony swelling the size of a cherry; this was resected. Microscopically the cut surface of the swelling showed an irregular cartilage seeded with dark bony granules and islets of bone; no pus, sequestra or inflammatory tissue were present.

The histological report concluded that this was " osteochondritis, the bone and cartilage in the zone of endochondral bone formation showing inflammatory lesions of no specific character". Both patients recovered satisfactorily.

In 1956 Caffey and Ross [3] investigated the ischio-pubic synchondrosis in 549 radiographs of apparently normal children, with the object of estimating the time of closure of the synchondrosis and the incidence of swelling and uneven mineralisation. The ages of these children, of whom 48 % were boys, ranged from two to twelve years. They found that the fusion age was variable and extended from four to twelve years, but was commonest between nine and eleven years. Bilateral changes were more frequent than unilateral in a group of 246 children with swelling and uneven mineralisation; girls (134) were slightly more often affected than boys (112), and the greatest incidence was between five and ten years; 92 % of the changes occurred in this age range. They concluded that swelling of the ischio-pubic synchondrosis, with or without uneven mineralisation, is present at some time in almost all, or perhaps all, children.

Nowadays, it is well-known that the asymptomatic ischiopubic osteochondrosis is part of the normal fusion process. But when clinical symptoms are associated with these radiographic changes, must they be regarded as part of the normal growth process or as pathological?

Bernard et al [6] agree with the term "osteochondrosis" when clinical symptoms are associated with radiographic abnormalities and compare this entity with other osteochondroses such as Osgood-Schlatter's disease or Sinding-Larsen-Johansson's disease. Several authors [7,3] do not regard ischiopubic osteochondritis as a specific disease but suppose that the radiographic changes described are transitory stages in the normal fusion of the synchondrosis. Caffey and Ross found that more than 50% of asymptomatic children may present swelling and demineralization of the ischiopubic synchondrosis [3]. Neitzschman [8] considers this entity as part of the normal fusion process even though there is associated pain.

MRI was thought to be helpful in the differentiation of ischiopubic synchondrosis from other pathologic conditions because of its excellent tissue characterisation. However, most MRI findings in ischiopubic synchondrosis are non-specific and may add to the confusion concerning this physiological condition [9]. According to Herneth et al [10], typical MRI features of ischiopubic osteochondrosis involve signal alteration and contrast enhancement of the bone marrow, which is hyperintense on T2-weighted and STIR sequences and hypointense on T1-weighted sequences. Irregular swelling of the adjacent soft tissue is typically present and appears hyperintense on T2-weighted and STIR sequences. But only the fibrous "bridging" described by the same authors seems to be a characteristic MRI feature of the ischiopubic synchondrosis [8]. As described by Herneth et al, we also observed a band-like area in the center of the ischiopubic synchondrosis in the second case, which was hypointense on all sequences, consistent with a fibrous bridging. This finding, as well as the well defined margins of the ischiopubic bone on MRI, are reassuring to rule out a neoplastic lesion. The other MRI findings, however, were non specific.

Some differential diagnosis must be mentioned. Acute hematogenous ischiopubic osteomyelitis is rare. Symptoms include a limp, fever, pain with rotation of the hip. Laboratory tests show an elevated erythrocyte sedimentation rate, elevated C reactive protein and a positive blood culture.

MRI shows obvious myositis, abscess and free fluid surrounding the ischiopubic synchondrosis.

Stress fractures are common lesions in athletes and typically present with hyperintense bone marrow oedema on T2W images and a hypointense fracture line perpendicular to the long axis of the superior pubic ramus.

Tumors such as Ewing's sarcoma usually present with permeative bone distruction and extension into the soft tissue as well as neoplastic impregnation clinical symptoms.

Conclusions

Ischiopubic "osteochondrosis" is a well-known finding on conventional radiographs of both symptomatic and asymptomatic children. In this case report, the authors recall that the "atypical" radiologic appearance of the ischiopubic synchondrosis in children may be confused with other pathology, especially if it is discovered unilaterally. There is data that suggests that Morbus Odelberg-van Neck results from an excessive pull of the hamstring tendon on the ischial tuberosity – this pattern of oedema may suggest stress reaction and callus formation as a mechanism of ischiopubic hypertrophy[11].

MRI findings are strongly suggestive of oedema of the bone and adjacent soft tissue that may also be present in inflammation, tumour or trauma. Most MRI findings in ischiopubic synchondrosis are non-specific and may add to the confusion. Since MRI findings also seem to be non

specific, their interpretation warrants great care and a good knowledge of the physiological nature of ischiopubic synchondrosis.

Knowledge of this condition is essential to make sure it is not mistaken for stress fracture, infection or tumor in symptomatic children. "Cure" of the condition could be effected by bed rest alone or in association with anti-inflammatory drugs.

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DUODENAL STENOSIS IN INFANTS – CASE PRESENTATION

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Abstract

We present the case of a 7 ½ month-old male infant who was admitted to the 2nd Pediatric Clinic of the Emergency County Hospital Craiova for vomiting. The infant was repeatedly admitted to hospital, in Romania and in Italy, ever since he was 2 weeks old, for vomiting. The diagnosis problems are presented in order to establish the causes of the vomiting. In our clinic, we performed the barium transit examination which pointed out the duodenal stenosis. The infant was transferred to the Pediatric Surgery Clinic where he was subject to a surgical intervention and we discovered the presence of an intraluminal, incomplete diaphragm, at the level of duodenal section D1. The post-operatory evolution was fair.

Key words: infant, recurrent vomiting, duodenal stenosis

Introduction

Duodenal atresia or stenosis usually occurs in the first or second part of the duodenum, most often near the papilla of Vater. Congenital duodenal obstruction may be due to intrinsic or extrinsic lesions. Duodenal obstructions usually occur in the second part of the duodenum.

The duodenal obstructions can be caused by some intrinsic and/or extrinsic lesions. The intrinsic lesions, in the form of diaphragm, atresia, or stenosis usually occur in the second part of the duodenum, between the fifth and the tenth week of gestation, because of the vacuolization failure and the recanalling of the duodenum from its solid stage [1].

Case presentation

A 7 ½ month-old male infant, admitted to the 2nd Pediatric Clinic of the Emergency County Hospital Craiova, in July 2015, for vomiting.

Heredo-collateral antecedents: young, healthy parents, no chronic diseases within family.

Physiologic personal antecedents: first born child, after a normal evolution pregnancy, on term, naturally born in a hospital in Italy, birth weight= 3000 g, height=50 cm, no sufferance, artificially fed with milk adapted formula when born, anti-reflux milk powder when he was 1 month old, diversified at 5 ½ months old, vaccinations performed in Italy. He was administered vitamin D3, prophylactically. With a corresponding psycho-motor development, he was brought in Romania by his parents when he was 5 ½ months old. He lives in a rural area, in a house with good conditions.

Pathologic personal antecedents:

- 3 admissions in Italy:
  - When he was 2 weeks old for vomiting occurred in the first days of life; he was administered an eso-gastroduodenal transit and he was diagnosed with gastro-esophageal reflux – he received anti-reflux milk powder and treatment with Gastrotuss oral suspension;
  - When he was 4 months old for vomiting and acute dehydration. There, he had a urine culture test, Doppler echocardiography, thorax and abdominal radiologic examination, biochemical tests which were normal;
  - When he was 5 months old, he was again admitted for vomiting, with an acute dehydration syndrome and metabolic alkalosis. Taking into account the recurrent vomiting episodes, the family was advised to go with their infant to a hospital specialized in metabolic diseases, but the family could not do it because of financial reasons;
- one admission in Romania, to a county hospital, when he was 6 ½ months old, for vomiting.

At admission to our clinic, the infant had an altered general state, without fever, weight=5000g, height= 69cm, PI= 0.66 (SD= -5); he was pale, with dark circles around his eyes, dry lips, abdominal skin fold with low elasticity, normal stetacustic pulmonary, rhythmic heart beats, HB=112/min, supple abdomen, normal stool, FA= 1/1 cm, slightly depressed, without meningeal symptoms.

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Investigations

Hemogram: Hb= 12.7g%, L= 15400/mm^3, NS= 64.8%, Ly=30%, Mo=5.2%, Tr.=285000/mm^3, VSH= 5/10 mm, urea= 41→20 mg%, creatinine= 0.45→0,46 mg%, GOT= 47→21 U/l, GPT= 38→21 U/l, γGT= 11 U/l, alkaline deposit= 17→23 mEq/l, calcemia=9 mg%, sideremy= 89 mg%, sanguine ionogram: Na=128 mEq/l, K= 3 mEq/l, total proteins= 6.1g%, negative uroculture, F.O. eye – normal aspect, chest X-ray – no pulmonary modifications.

We proceeded to an urgent PEV with glucose and electrolytes, Quamatel i.v., then we restarted feeding him, progressively, with anti-reflux milk powder.

He went on receiving food p.o., having a fair general state; on the third day from admission he vomited again, abundantly, followed by the rapid presence of the dehydration syndrome. The abdomen got excavated, with intestinal loops, which were visible to the wall.

Because the infant presented recurrent vomiting from his first days of life, which had required repeated admissions, being diagnosed with gastro-esophageal reflux when he was 2 weeks old, fact which determined his feeding with anti-reflux milk powder and Gastrotuss p.o., and he had a non-corresponding ponderal growth, PI=0.66 (SD -5), and because of the type of vomiting he presented in our clinic, we took into consideration the possibility of a pylorus stenosis or some other digestive tract malformation.

Although the infant was performed an eso-gastroduodenal transit in Italy, at his first admission, we repeated it with barium (fig. 1), which revealed: no gastro-esophageal reflux, slightly relaxed stomach, with its inferior pole lower than normal, having a permeable pylorus with rhythmic ejection; megaduoden with significant stasis on the first segments, the inferior pole passing the line of the iliac crests; slowly transit of the barium to the small intestine, one hour after its ingestion.

The infant was transferred, being diagnosed with duodenal stenosis, to the Pediatric Surgery Clinic, where he had a surgical intervention and we noticed, at the level of the duodenal section D1, the presence of an intraluminal, incomplete diaphragm (fig.2). The post-operatory evolution was fair. Discharge diagnosis: Duodenal stenosis due to an incomplete obstacle. Dystrophy degree II.

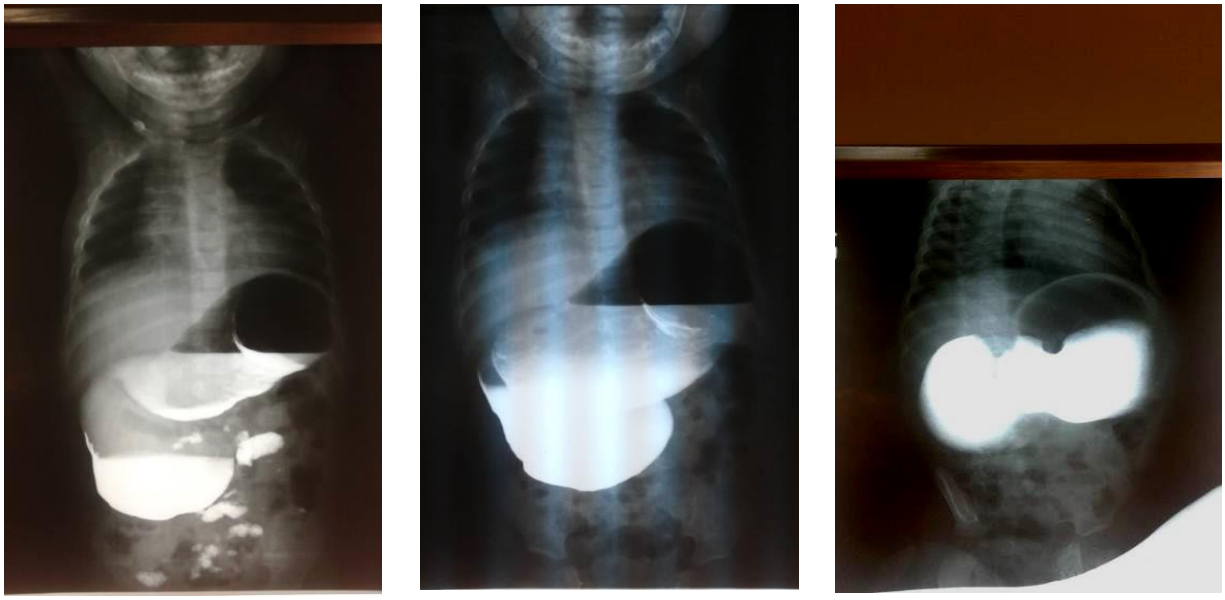

Fig. 1 Eso-gastroduodenal transit.

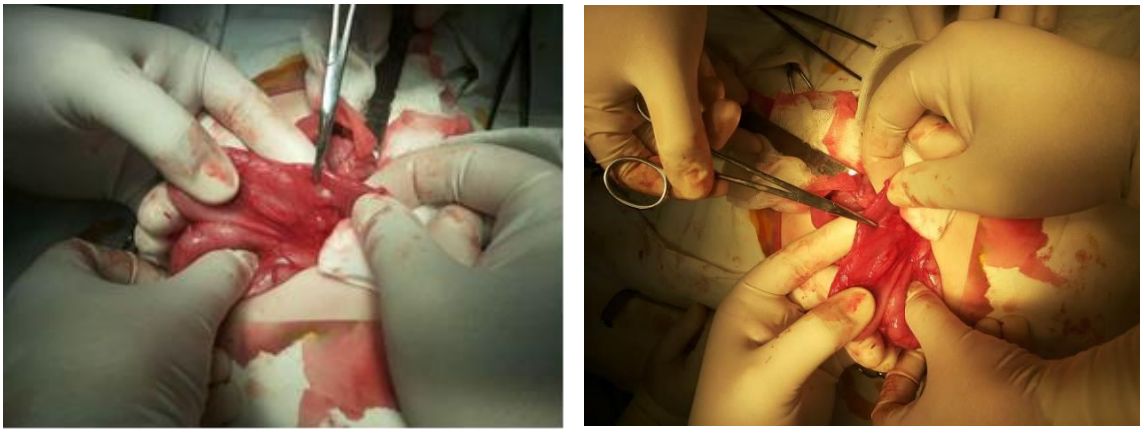

Fig. 2 Intra-operatory.

Discussions

Chronic vomiting in infants may have multiple causes, among which: organic vomiting, reflex vomiting and vomiting of neurologic sources [1]. The organic vomiting is present in: pylorus congenital atresia, duodenal or intestinal atresia/stenosis (there is a precocious onset of the vomiting, which has a bilious character; the X-ray examination tells exactly the location of the obstacle), cardia achalasia (the vomiting onset is within the first days of life and it disappears when positioning the infant in orthostatism), intestinal invagination (possible intestinal occlusion), pyloric spasm (precocious onset of vomiting even since the first days of life, frequent vomiting episodes, quantitatively reduced, during or after lunch; the X-ray examination revealed the absence of the gastric stasis liquid and a gastric plication (the vomiting has its onset several weeks after birth, it occurs during or after meals, and it is abundant; the postprandial Trendelenburg position prevents vomiting), annular pancreas (bilious vomiting) [1,2].

Vomiting represents the forceful expulsion of the gastric contents through the mouth, while the regurgitation means the effortless expulsion of the milk, usually accompanied by air. In the regurgitation caused by esophageal congenital atresia, the expelled liquid, at the first feed, does not have a gastric contents. In the atresia with tracheoesophageal fistula, the infant may present vomiting, cough, and cyanosis [3].

The reflex vomiting may be: infectious (gastroenteritis, urinary infections, ear infections, pneumonia, meningitis, septicemia, neonatal peritonitis – fever, meteorized and edematized abdomen), toxic (intoxications), metabolic (uremia, acidoketosis, ammoniemia, diabetes mellitus, congenital adrenal hyperplasia – Debre Fibiger: pseudo-hermaphroditism, vomiting starting the second week of life, hyperpigmentation, virilization, inapetence, hyperpotassemia, toxic state), food intolerance – intolerance to the cow milk proteins [4,5]. The vomiting episodes with neurologic causes are represented by: intracranial hemorrhages, tumors, abscesses, hematomas, meningitis; the vomiting may also be psychogenic [1,4]. The frequency of the duodenal stenosis: 1:20000-1:40000 births [6].

The duodenal obstructions can be caused by some intrinsic and/or extrinsic lesions. The intrinsic lesions, in the form of diaphragm, atresia, or stenosis usually occur in the second part of the duodenum, between the fifth and the tenth week of gestation, because of the vacuolization failure and the recanalling of the duodenum from its solid stage [7].

In the present case, the stenosis occurred because of an incomplete, intraluminal diaphragm which explains the intermittent character of the vomiting episodes and a diagnosis set when 7 ½ months and not during the neonatal period.

The extrinsic obstructions can be caused by an annular pancreas, malrotation or preduodenal portal vein [8]. The intrinsic lesions can appear periampullary in 20% of the cases or postampularry, and they are commonly accompanied by anomalies of the distal biliary tract and of the gallbladder. Other congenital anomalies are quite frequent, 30% of the cases being associated with Down syndrome and 50% of them with heart, genitourinary, anorectal malformations, esophageal atresia, and malrotations.

No other congenital malformations were diagnosed for this case.

Conclusions

We presented this case due to:

- its rich history, with repeated admissions for the vomiting episodes;
- the problems given by the etiologic diagnosis of recurrent vomiting episodes (gastro-esophageal reflux, metabolic disease, pylorus stenosis, other digestive tract malformations);

particularities of the duodenal stenosis.

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CUTANEOUS METASTASES IN CHILDREN. A HISTOPATHOLOGICAL CASE REPORT AND REVIEW

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Abstract

Cutaneous metastases are a rare finding in children, being diagnosed usually after primary tumor discovery, however it may be the first sign of a malignancy. Therefore, the origin tumor could be diagnosed trough the pathological findings, but in most cases this is impossible due to scant ammount of tissue involved in the metastasis. This article tries to approach, trough available scientific literature and procedures, a unidentified carcinomatous metastasis discovered in a 14 years old boy, proving to be a undifferentiated epithelial tumor. The importance of cytokeratines CK7 and CK20 is discussed, together with histological distinctive characteristics of other tumors that may metastasize and differential diagnosis with primary adnexal carcinomas that may mimic skin metastases.

Key words: cutaneous metastases, adnexal tumors, immunohistochemistry, children

Introduction

Metastasis is defined as a neoplastic lesion arising from another neoplasm that has no further continuity with it. Skin metastasis is defined as a spread of malignant cells from a primary malignant tumor to different regions of the skin. Cutaneous metastases (CM) have an increasing incidence in children nowadays, when histological detection is more accurate to lower ages, especially when differential diagnosis for benign conditions are taken in account. Skin secondary tumors appear as a consequence of primary tumor development, mostly soft tissue, being dependent on vascular or lymphatic spreading [1]. Many cases have been reported regarding all types of malignancies metastatic to skin, but few systematic attempts have been made to consider a pattern of apparition. No matter what the size and condition of the specimen might be, most carcinomatous metastases might be classified as adenocarcinoma, squamous cell or undifferentiated. Skin secondary malignancies with visceral origin are very important for dermatologists, pathologists and surgeons because of their multiform clinical presentation, while diagnosis delay might become dangerous for the child outcome [2]. Furthermore, CM might be the very first sign of a developing cancer or a recurrent tumor condition. The suspicion is raised by the pediatric doctor, while the surgeon and the pathologist might elaborate a final diagnosis. Therefore, rapid intervention becomes mandatory, while the survivability of a patient with a cutaneous metastasis is low being around 7.5 months [3]. The most frequent site of the CM are the abdomen skin (26%), followed by head and neck (25%), arms, legs, trunk, axillae, shoulder and anus. In females, 75% of cases are located on the anterior aspect of the abdomen and chest, while in men, the election region remains the anterior aspects of head and neck region. The back skin is a rare site for metastasis. The pathologists describe four main characteristics in CM: nodular, infiltrative, diffuse and intravascular [4].

Case report

A 14 years old boy presented to the Dermatologist office in our hospital for 3 melanocytic-like lesions: (a) first on posterior aspect of the right thorax, (b) the second at the right costal rebord and (c) the last located on the left internal maleolar region (fig. 1). The macroscopic appearance of the first lesion was that of an elevated, ovoid, brownish circumscribed lesion – 0,4/0,3/0,2 cm – while the second lesion (b) had larger dimensions – i.e, 0,7 / 0,7 / 0,3 cm – being almost spherical, bulging at the site. The third one (c) was also ovoid, but more irregular colored – brown and white alternant areas – with an gritty aspect, and intermediate dimensions, approximately 1,0 / 0,6 / 0,4 cm. The routine clinical and biological examination of the child revealed no abnormalities, having no paraclinical deviations (Fig 1). The young patient was reffered to the Surgical Department where resections have been made for the above mentioned lesions, within safety margins, and the obtained rhomboidal-shaped specimens were reffered to the Pathology Department. The tissue samples were fixed in buffered formalin 10%, and processed in our laboratory in succesive alcohool grades (70°, 80°, 96°), paraffin embedded, sectioned and stained trough standard procedure for haematoxilin and eosin. At microscopic examination, the second lesion showed a keratoachantoma-like appearance, jonctional melanocytic activity, with type B anc C melanocytes and rare melanosomes, arranged in compact groups, located between the rete ridges, with no mitotic activity or atypia. However, the third lesion proved more cytological activity, with slight melanocitic atypia and normal mitoses (0-3 / 10 HPF), bridging of adjacent, irregular, rete ridges, lentiginous proliferation of hyperchromatic, epithelioid and spindled melanocytes at dermoepidermal junction. Therefore, the final diagnosis for the second (b) and third (c) resected masses remains that of a (b) compound melanocytic naevus, respectively, (c) Clark or dysplastic naevus (fig 2).

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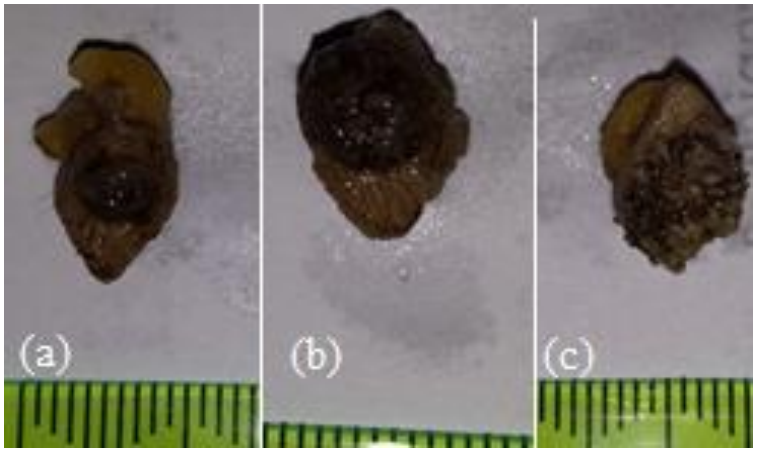

Fig 1. Macroscopical aspect of resected specimens for supposed naevi (buffered formalin 10%): (a) the lesion in matter - posterior aspect of the right thorax; (b) right costal rebord and (c) left internal maleolar region.

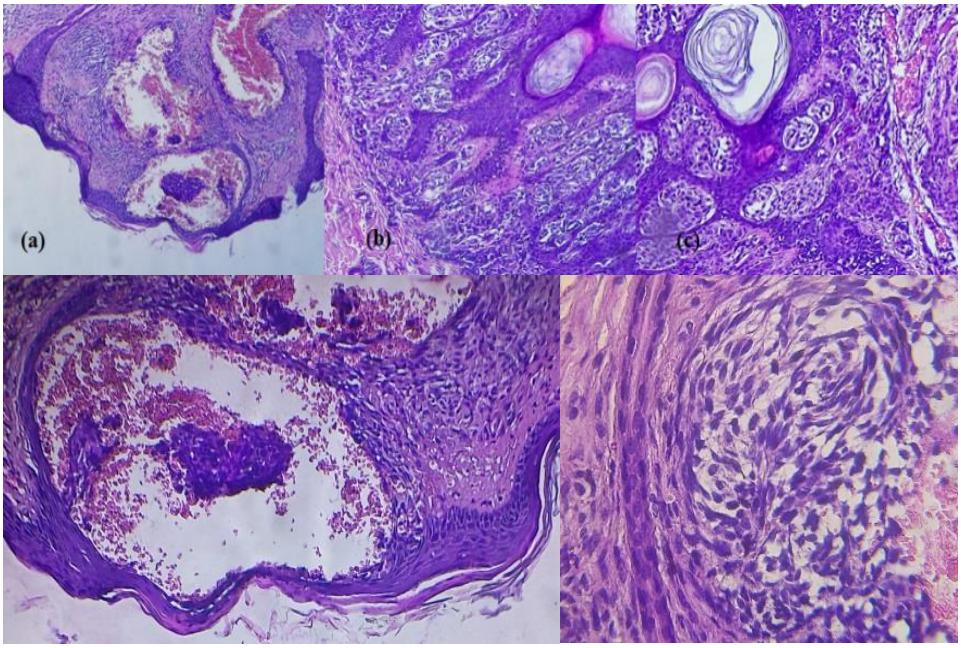

Fig 2. Histological aspects of resected specimens: (a) cutaneous metastase of unknown epithelial origin, ectatic vessels and spindle, clear, cell dermal perivascular, infiltrative pattern (4x10, HE); (b) histological aspect of a compund naevus with dermoepidermal jonctional melanocytic activity, arranged in compact groups (10x10, HE); (c) dysplastic melanocitic groups situated at the tip of connected rete ridges and architectural pleomorphism (10x10, HE); down - details from lesion (a): left – intravascular cell group, with clear cell-like distrophy, floating inside the lumen, surrounded by infiltrative pattern; right – infiltration of whorled, plexiform, spindle cell with clear cytoplasm, that delineates angiomatoid spaces (10x40, HE).

The first lesion (a) presented as a nodule, with ectatic vascular, angiomatoid, spaces developed within an capillary haemangioma, confined to the dermis, with epidermal hyperkeratosis and rete ridges effacement. These vessels showed no endothelial lining, being delinated by a plexiform, clear cell population, with a central, pyknotic and hypercomatic nuclei, with no atypia or mitoses, having a infiltrative, spindle shaped pattern and an epidermolitic activity. Within the vascular lumina, free floating glomeruloid, whorled, cellular groups were detected, having slight atypia and some intracitoplasmic vacuoles, probably containing lipids, that were consistent with an unspecifiable epithelial origin (Fig). The final diagnosis was that of a undifferentiated invasive carcinoma metastasis as the

infiltrative behaviour corresponds with this kind of tumor, i.e, spindle, plump rounded cells, focally with clear cell features suggesting a high grade pleomorphical neoplasm. Renal cell carcinoma, angiosarcoma and Kaposi sarcoma were ruled out, as no morphological and paraclinical criteria sustained these entities for the case in matter. Also atypical angiomatoid hyperplasia was in disscussion, however vascular spaces are not so ectatic and the presence of intraluminal metastasis ruled out benign conditions. The child was reffered to our local Oncological department for further investigation and treatment. Immunohistochemical analysis for the metastatic cells is out of discussion, as the necessary tissue volume is far insufficient for analysis.

Discussions

Clinically, most skin metastases appear as a rapid-growing solitary or mutliple mass, firm, flesh-coloured, round or oval, elevated lesion located in dermis or subcutaneous, that may have a stationary evolution. Sometimes, palpation might prove a local painful indurated tumor [5]. The variability of presentations might include nodules, papules, plaques and ulcers. Other frequent clinical expressions are alopecia, morphea-like lesions, dermatofibroma-like or pyogenic granuloma-like lesions, herpetic-like erruptions or even cellulitis [6]. Histologically, CM are more chaotic than primary tumors, as CM involve collagen disection and a rich vasculature with no epidermotrophic pattern. In children, renal cancer might be very frequent. Differential diagnosis with local evolving Kaposi sarcoma, pygenic granuloma or other vascular proliferations, should be made with a CM of a renal cell carcinoma. In these situations, CM might simulate an abscess on the head, neck or face. Histologically, proximal renal tubular cells might become visible showing a trabecular , papillary or tubulopapillary pattern, with a proeminent vascular pattern, extravasation of red cells and hemosiderin, while the cells contain abundant glycogen and lipids, sometimes showing plexiform appearance [7]. Also, gastrointestinal tumors might express a nodule located in middle or lower dermis, forming glands, clusters or strands of neoplastic cells together with an intense desmoplastic reaction. Colorectal carcinomas, however rare in children, should not be disregarded as a possible condition. The CM of this particular cancer appear on skin as a „Sister Mary Joseph's nodule", with forming glands and intense mucin production. In the above described situations, Alcian-blue, periodic acid-Schiff, CDX2, CK7 and CK20, together with carcinoembryonar antigen and EMA should clarify the diagnosis (5). Gynaecological malignancies rarely metastasize to skin, especially in children. However, cases have been cited of CM from ovarian tumors. Metastasis occur mostly in scars at the sites of paracentesis, and, present psammoma bodies, followed by a mucinous and endometrioid characteristic pattern. Breast carcinoma, although improbable in small children, becomes a serious condition in adolescents and young adults. In these situations, most CM have an undifferentiated pattern, while a well-formed duct pattern is less likely to be encountered. For instance, the pathologist may have difficulties in detecting breast lobular carcinoma CM. Although, CK7 and CK20 might help differentiating from a possible emergent digestive source, GCDFP-15 is a marker of appocrine differentiation in breast epithelium and might be used to confirm the mamar origin. In some cases, the breast malignant CM are further difficult to differentiate from a malignant melanoma, especially in epidermotropic metastases, as in-tumor melanocyte proliferation may occur. In heavy smoking adolescents, lung carcinoma may develop CM, located usually on the chest wall and posterior trunk, giving a clue for their origin. It is well known that lung small cell carcinoma is characteristically involving back skin. Differentials are made with primary Merkel cell carcinoma (CK20 positive and thyroid transcription factor 1 – TTF1 negative), squamous cell carcinoma and metastastic digestive carcinomas, while lung small cell cancer is CK20 negative and TTF1 positive [8]. Sarcomas might deliver CM as an inaugural disease manifestation, like in a femural osteosarcoma case, presented as two scalp nodular tumor [9]. Rare soft tissue tumors, like alveolar soft part sarcoma CM to scalp areas in a 21 years old man, were cited, but molecular analysis was required to identify the primary tumor, with RNA extraction and reverse transcriptase polimerase chain reaction in order to detect a specific genetic locus, called transcription factor 3, as this kind of neoplasm may have a close resemblance with renal cell cancer on standard stains [10]. Therefore, routine differentials for sarcomas or soft tissue neoplasms are almost impossible, requiring molecular biology techniques. In order to sustain a diagnosis, the pathologist may need to differentiate between primary cutaneos tumors and secondary metastases. Thus, primary adnexal carcinoma may mimic the pleomorphic multitude of cutaneous metastases, however these are very rare in children. Ductal eccrine carcinoma may mimic a ductal breast neoplasm metastatic to skin, while mucinous variant may, very well, resemble a gastrointestinal CM. However, some authors sustain that these lesions are easy recognaisable on routine stained sections. Although rare, high grade spindle cell sweat gland carcinoma, having a whorled, organoid, concentric pattern might be similar to nasopharyngeal CM [11].

Conclusions

Although any form of malignant tumor may metastasize to skin, in current practice there is a small number of neoplasms that are likely to do so. Immunohistochemical profiling is mandatory, especially regarding CK 7 and CK20, however, in undifferentiated carcinomas CM the antigen panel is almost impossible to apply as, in most cases, some like ours, there is no sufficient tumoral tissue for all molecular analysis possibilites. Therefore, interdepartamental approach – surgeon, pathologist, oncologist and radiologist – for this kind of situations must be accomplished for most accurate diagnosis.

Conflict of interests

The author declares no conflit of interests. We undersign, certificate that the procedures and the reports we have done respectthe ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law.

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CLINICAL MANIFESTATIONS IN ROTAVIRUS VERSUS NOROVIRUS INFECTIONS – OBSERVATIONS BASED ON 124 CASES OF VIRAL DIARHEEA IN CHILDREN

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Abstract

Background: Viral diarrhea is caused in children mainly by the rotavirus and norovirus. The aim of this study is to detect specific clinical elements to rapidly distinguish between the two types of digestive infections. *Methods:* This study has been prospectively elaborated by selecting two viral groups of rotavirus and norovirus respectively that have been confirmed by the Elisa Ridascreen test. Clinical parameters have been followed by filling a questionnaire by parents and a personalised medical record by the medical doctor treating the patient. Statistical processing of the obtained data has been performed using Student's t-test and the „Z-test" employing the Stata v12 statistical software. *Results:* For statistical processing of the data, the treshold value for the significance level (p) was set at 5%, (standard $\alpha = 0.05$ cutoff). The first symptom was for the rotavirus infection: vomiting (35.5%), diarrhea (35.5%) and fever (23.7%), compared to diarrhea (38.7%), fever (35.5%) and vomiting (25.8%) for the norovirus infection, the differences being statisticaly negligible ($p>0.05$). The fever in the first day was percentually lower in rotavirus infection in the 38.1-39^{0}C interval but higher in the norovirus infection between 39.1 - 40^{0}C (with no statistical significance). The norovirus infection shows persistent fever until day 5 whereas rotavirus infection remains until day 4. Vomiting preceeds diarrhea in most of the cases ($p>0.05$). Stools with bloody streaks are present mainly in the rotavirus infection (10.8% to 3.2%, $p>0.05$). In day 5 a higher number of cases show persistent diarrhea in the case of norovius infection compared to rotavirus infection. The only statistically significant data were for rhinorrhea ($p<0.05$), the rest of the neurological symptoms, coughs, myalgias being statistically negligible. *Conclusions:* The only difference statistically significant is rhinorrhea that is present most frequently in norovirus infections compared to rotavirus infections ($p<0.05$).

Key words: diarrhea, rotavirus, norovirus, clinical picture, child.

Introduction

Infectious diarrhea remains the main morbidity and mortality cause among children under five years (1). The rotavirus holds the chief position among the main pathogens that also include the norovirus, enteropathogenic and enterotoxigenic Escherichia coli, type 40/41 adenovirus, astrovirus and sapovirus. It was estimated that rotaviruses are responsible for about 453.000 deaths annually mainly in countries from Africa and Asia (2). The noroviruses are the second main cause for viral gastroenteritis in children under five and are estimated to cause about 200.000 deaths annually among the children from this age group in the developing countries (3).

Beside classic viruses, some new picornaviruses (Aichi virus, parechovirus, enterovirus) considered to be associated with the diarrhea in humans, have been identified in parallel with the evolution of molecular diagnostic methods (4).

The rotaviruses are transmitted by the oral-fecal route and are extremely contagious. This facilitates viral transmission especially in day care centres and hospitals. The rotavirus infection is the cause of acute gastroenteritis with diarrhea and different stages of dehydration. This results mainly from the destruction of the erythrocytes from the intestinal villi, affecting the ion transport and absorbtion. The highest incidence of rotavirus infection is during winter and summer in the countries with temperate climate. Many children have an asymptomatic infection that maintains the circulation of the rotavirus in the population (5).

The acute gastro-intestinal infections and diarrhea with vomiting are frequently encountered in ambulatory care. Due to a mainly auto limiting evolution, establishing the diagnosis is often useless. The viral infections caused by the noro- or rotaviruses are the most frequent infections. Bacterial infections are less frequent due to higher hygiene standards in the developed countries. In hospitals and after antibiotic treatments, Clostridium difficile is the main cause of diarrhea (6).

After an significant decrease in acute gastroenteritis caused by the rotavirus in children, due to the introduction of two vaccines (7), the norovirus has become the main cause of such disease in children below five in the US (8).

The incidence of viral diarrhea in Romania is unknown. The actual studies show limited groups for small geographical areas - București (9, 10), Cisnădie (11). The majority of these studies are related to the rotavirus.

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Studies concerning the norovirus have not been reported in Romania. Although, there are a few studies on clinically predictive factors in viral diarrhea (12, 13, 14), there are no studies regarding scoring sistems for the viral etiology.

In the literature, there are only two scoring scales used to establish the severity of the viral diarrhea: the modified Vesikari score (15) and the Clark score (16). However, these two scores cannot be used to predict the viral etiology of diarrhea (17,18, 19).

Both the rotavirus and norovirus infections are viral diseases of the digestive system with great impact upon the health of a child. The manifestations are considered more severe in rotavirus infections, affecting the younger age and are less severe in norvirus infection. Therefore, a rapid clinical differentiation between the two would be usefull for the approach and monitoring method.

The aim of this study is to differentiate between the clinical elements in rota- and norovirus infections in children**.**

Material and method:

A prospective longitudinal study was performed between October 2014 and January 2016 within the P1/4 internal UVVG grant frame.

The study was performed on children with diarrhea hospitalised the pediatric ward that screened positively for norovirus/rotavirus using the RIDA SCREEN test. These children were divided in two groups: ROTAVIRUS and NOROVIRUS respectively.

Group 1: ROTAVIRUS was made up of 93 children of which 46.2% were girls and 53.8% boys. The median age was 4.54 (0 – 17 years).

Group 2: NOROVIRUS was made up of 31 children of which 32.9% were girls and 67.7% boys. The median age was 3.71.

The informations from the questionnaire and personalised medical record coresponding to each child from the viral sub-group were introduced in an Excell database. The following parameters were monitored: first symptome, the fever in the first day, the fever during the first 5 days, the duration of the fever, number of the vomitings and stools during 5 days, duration of stools, the vomiting-stool chronology, aspect of the stools, duration of the diarrheic stools, presence of other symptoms: agitation, sleepiness, nasal secretions, myalgias, shivers and coughs.

The statistical processing of the collected data was done with the Stata v12 statistical software using Student's t-test and the „Z-test" to identify clinical parameters that are statistically significant to differentiate between the two groups.

Note: the study was approved by the Ethics Committee of UVVG Arad and the parents signed an agreement for this study.

Results

In this work, to determine the clinical parameters that are statistically significant to differentiate between the two groups under study, the treshold value for the significance level (p) was set at 5%, (standard $\alpha = 0.05$ cutoff).

Table 1 shows the gender and age distribution of the two groups. The median for females with norovirus is four years (1 year minimum and 16 maximum) and for rotavirus is 2.5 years (1 year minimum and 15 maximum). The median for males with rotavirus is 5 years (minimum 0 years and 17 maximum) and for norovirus is 2 years (minimum 0 years and 12 maximum). There is no significant difference between the median ages of the two groups (p>0,05).

Table 2 shows the distribution of the first symptom in the two groups under study. A higher frequency of the fever and vomiting can be observed in the rotavirus group compared with the norovirus group but without statistical significance.

Table1. Gender and age distribution of the groups.

		ROTAVIRUS							NOROVIRUS						
		#	%	Mean	Median	SD	Min	Max	#	%	Mean	Median	SD	Min	Max
Sex	F	43	46						10	32					
	M	50	54						21	68					
Age	F	43		4.72	4	3.5	1	16	10		4.1	2.5	4.5	1	15
(years)	M	50		4.36	5	3.3	0	17	21		3.33	2	3.4	0	12

Table 2. The distribution of the first symptom in the two groups.

	ROTAVIRUS	NOROVIRUS	z-value	p-value
Fever	22 (23.7%)	11 (35.5%)	1.29052	0.19687
Diarrhea	33 (35.5%)	12 (38.7%)	-0.32348	0.74633
Abdominal colic	3 (3.2%)	0	-1.01232	0.31138
Vomiting	33 (35.5%)	8 (25.8%)	0.99189	0.32125

Coughs	2 (2.2%)	0		

There were no significant differences regarding the febrile curve from the first 5 days or the intensity of the fever from day one (Figures 1 and 2).

The average duration of the vomiting is 1.7 ± 1.39 days for ROTAVIRUS group and 1.35 ± 1.45 days for NOROVIRUS group respectively. There is no significant difference in the duration between the two groups (p>0.05). (Figure 3)

The aspect of the stool was watery in 88.2% vs. 90.3% rotavirus/norovirus, mucus in 6.5% norovirus and with bloody streaks in 10.8% vs. 3.2% rotavirus/norovirus. Although percentually the rotavirus infection is characterised predominantly by invasive stools compared with norovirus infection, the difference is not significant statistically (Figure 4).

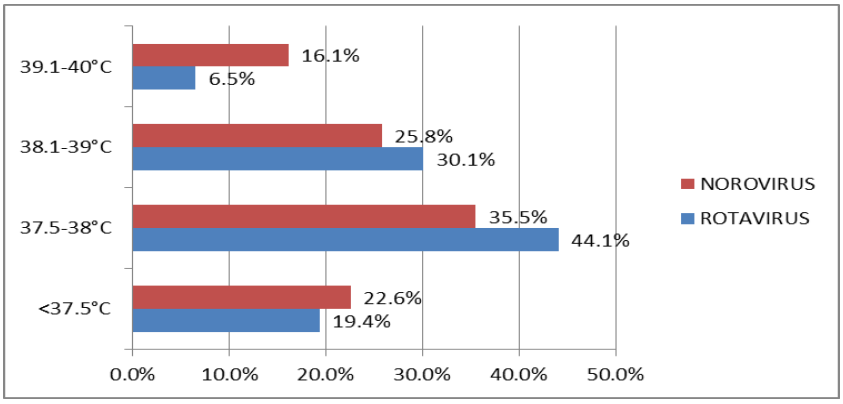

Figure 1. Fever in the first day.

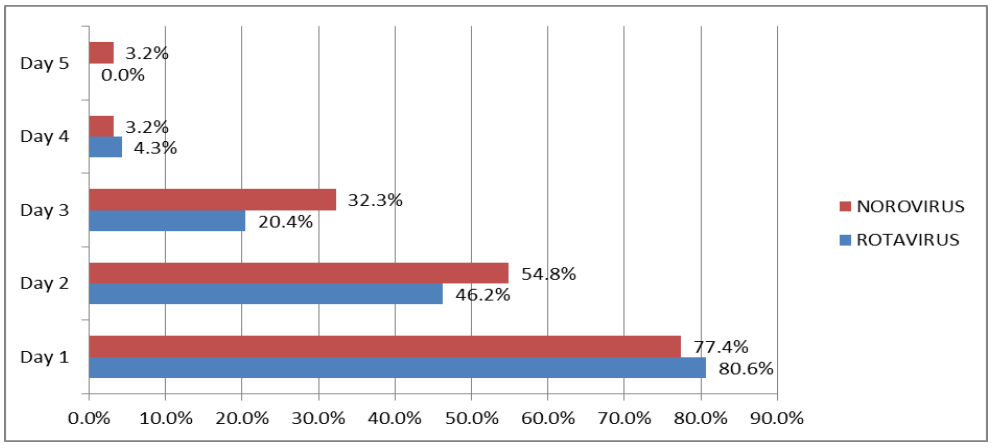

Figure 2. Fever during five days.

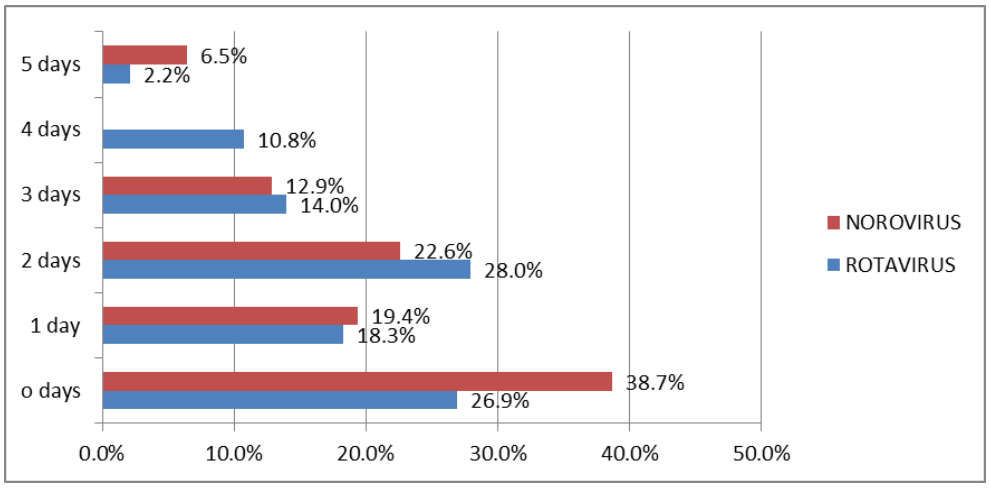

Figure 3. Duration of vomiting.

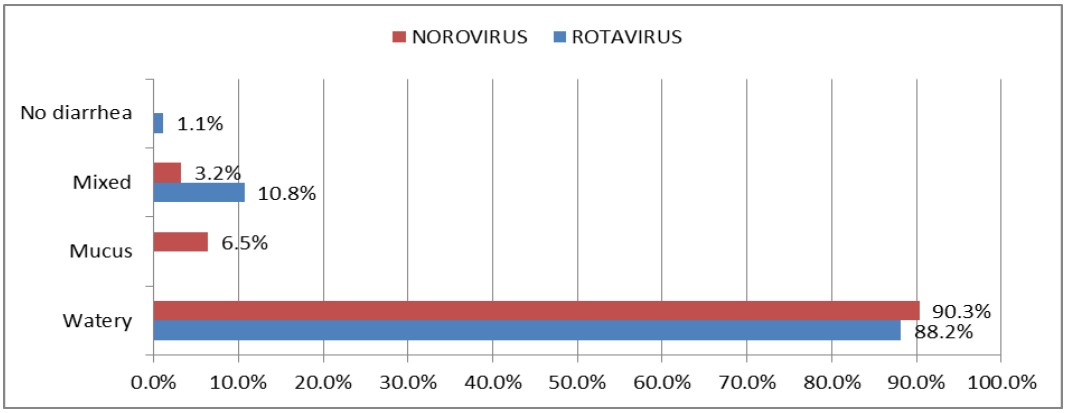

Figure 4. The aspect of stools.

The number of stools was 4.39 ± 2.20 for ROTAVIRUS group and 3.93 ± 1.74 for NOROVIRUS group respectively.

In the fifth day a higher number of cases of norovirus infection showed persistent diarrhea compared to rotavirus infection, without statistical significance for the two groups. (Figure 5)

Table 3 shows the associated symptoms present in the norovirus and rotavirus infections. It can be observed that among the associated clinical symptoms, only the rhinorrhea linked with the norovirus infection has a statistical significance.

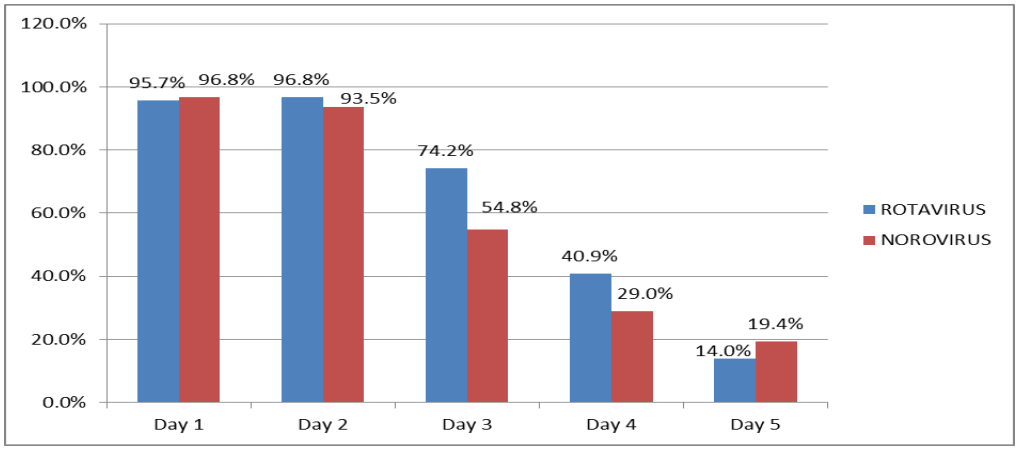

Figure 5. Persistence of diarrheic stools.

Table 3. Associated symptoms.

	LOT		
Associated symptoms	Rotavirus	Norovirus	p-val
Coughs	15	10	
	16.10%	32.30%	0.05257
Myalgias	16	1	
	17.20%	3.20%	0.05004
Nasal secretions	12	10	
	12.90%	32.30%	0.01457
Shivers	7	0	
	7.50%	0	0.11582
Convulsions	4	1	
	4.30%	3.20%	0.79211
Sleepiness	28	9	
	30.10%	29.00%	0.90978
Agitation	30	16	
	32.30%	51.60%	0.05337

TOTAL	93	31	

Discussions

Rotavirus and norovirus infections are the most frequent causes for the viral diarrhea in children.

The first symptom was for the rotavirus infection: vomiting (35,5%), diarrhea (35,5%) and fever (23,7%), compared to diarrhea (38,7%), fever (35,5%) and vomiting (25,8%) for the norovirus infection, the differences being statisticaly negligible (p>0,05).

The fever in the first day was percentually lower in rotavirus infection in the 38,1-39^{0}C, interval but higher in the norovirus infection in the 39,1-40^{0}C (with no statistical significance).

The norovirus infection shows persistent fever until day 5 whereas rotavirus infection remains until day 4.

Vomiting preceeds diarrhea in most of the cases, having no statistical significance (p>0,05). Stools with bloody streaks are present mainly in the rotavirus infection (10,8% to 3,2%) again without statistical significance (p>0,05).

In day 5 a higher number of cases show persistent diarrhea in the case of norovius infection compared to rotavirus infection.

The only statistically significant data were for rhinorrhea (p<0,05), the rest of the neurological symptoms, coughs, myalgias being statistically negligible.

O'Ryan (20), folloving a comparative study of rotavirus vs. norovirus showed that the rotavirus infection has a higher severity score than norovirus infection with a higher number of stools and more frequent fever. The duration of diarrhea, the shivers and intensity of vomiting was identical for the two groups. The severity of viral BDA in infants was the same.

It was noticed that the number of BDA with norovirus infection is on the rise, same as the moderate/severe forms which requires the use of a vaccine.

Narkeviciute (21), on a group comprised of 50 children with BDA with norovirus infection and 50 children with rotavirus infection, noticed that the rotavirus infection shows more frequently fever and diarrhea and in norovirus infection the vomiting is more frequent.

O'Ryan (22) highlights that each child shows an average of 1.4 BDA until 18 months of which 15% are rotavirus infections and 18% are norovirus infections. Rotavirus infection was more severe than the norovirus infection independent of vomiting. The re-infections with the norovirus are more frequent than with the rotavirus.

Abugalia (23) on 164/91 rotavirus/norovirus noticed that the rotavirus was identified in the hospitalised patients and the norovirus in those from ambulatory care.

Doll (24), following a study, believes that the patients with rotavirus show more often fever, intense dehydration, a higher number of stools and those with norovirus have higher number of vomitings within 24 hrs compared to non-specific gastroenteritis.

Our study suggests that the number of vomitings is percentually higher in norovirus infection than in norovirus infection but it doesn't have any statistical significance (p>0.05).

From the above, it can be observed the wide range of parameters followed by various authors in different associations without being able to achieve a net clinical differentiation of the etiology for the two types of groups.

Conclusions

1. The duration of the fever and stools was percentually longer in the norovirus infection vs. rotavirus infection
2. The norovirus infection is associated with a higher number of vomitings in the first day but of a shorter duration compared to rotavirus infection
3. The stools with bloody streaks are mainly present in rotavirus infection, without statistical significance
4. Rhinorrhea is present more frequently in norovirus infections compared to rotavirus infections, the differences being statistically significant (p<0.05).
5. Despite the many clinical parameters studied, only rhinorrhea is statistically significant in the differential diagnostic norovirus vs. rotavirus infection for the group under study.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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INFORMED CONSENT IN NEONATOLOGY

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Abstract

Neonatology is an unpredictable specialty with increased intrinsic potential for devastating situations. Progresses in obstetrics and neonatology dramatically decreased neonatal mortality in the last decades but the same technology that saves lives may cause severe co-morbidities and may have lifetime adverse effects. Ethical and moral debates about duties and responsibilities of both physicians and parents are, therefore, continuing. *Aim:* The authors aimed to review the data in the literature regarding problems and difficulties raised by informed consent in neonatology. *Material and methods:* The authors searched the medical literature for articles and official documents discussing informed consent and related ethical, moral, and legal issues in neonatology. *Results:* A short review of the history and content of the informed consent precedes the description of the problems and difficulties of the informed content in neonatology: emotional burden, intense stress, best interest of the child, emergency situations, assuming parental responsibilities, duties of the clinicians, multidisciplinarity, prenatal consent. Other issues discussed in the paper are: situations when the informed consent is needed, potential conflicting situations, possible solutions in limiting situations, and the role of the institutional ethical committees. *Conclusions:* Physicians are invested by the medical ethical code with important responsibilities towards the patient, a burden that, in front of a newborn, may be very complex. On the other side, parents have also responsibilities for their children. Together, physicians and parents, must take the best decisions for the child even though the process may be extremely difficult in many situations in the neonatal period. Today, the complexity of the ethical issues in medical practice is universally accepted, ethics became an important part of day-to-day medical practice in neonatology, and vital decisions for the neonate's survival are based on the informed consent.

Key words: neonatology, informed consent, ethics, moral, legal, parents

Introduction

Neonatology is an unpredictable specialty and an area where nobody, never intends to harm. But the neonatal period has an intrinsic potential for devastating situations and consequences that creates the sensation of a huge injustice. The neonates are the small, most vulnerable, most protected, and most celebrated members of a society, and for the family they are the future and an unlimited potential.

The essential goal of neonatology as a specialty is decreasing the neonatal mortality. In fair and just, democratic societies life must be saved at any cost and each individual is morally entitled to be as healthy as possible and to live as long as possible. The spectacular progress registered by neonatology in the latest decades meant a dramatic decrease of neonatal mortality. It also meant decreasing the viability threshold at lower and lower gestational ages. The survival of extremely low birth weight preterm infants and of the infants with severe perinatal conditions - implying multiple invasive and risky procedures - led to the occurrence of a new category of survivors that continue to be fragile even after the neonatal period, often long time dependent on medical technologies. The reality is that the same technology that saves lives may cause severe co-morbidities and may have lifetime adverse effects[1]. All these aspects sparked, already for some time, numerous ethical and moral debates about excess therapies, viability threshold, futile treatments, initiation and withdrawal of the vital support, the best interest of the child, child's rights, parental rights, the rights and responsibilities of the medical staff.

The ethical medical code invests the physician with important responsibilities towards the patient, a burden that, in front of a newborn, may be very complex. Together with the parents, the physician must take the best decisions for the child. The process may be extremely difficult and the informed consent is a synthesis of the information representing the grounds for the most important decisions taken for the child survival.

Short history

During 1930-1940, a period when most of the therapeutic interventions were still inefficient, no informed consent existed in neonatology. After 1954, the first miraculous drugs (Penicillin) and therapies (for example, ACTH therapy for retinopathy of prematurity) occurred and still parental approval wasn't considered necessary. If Penicillin was life-saving, ACTH therapy caused growth failure and other effects secondary to increased adrenal activity.

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The issue of controlled studies was raised at that moment even tough is was generally believed that the decision to enroll a patient in a study is the resolution and the responsibility of the physician. The study protocol was broken during the first such randomized controlled study and this led to the death of one patient. Although this study related to ACTH effect in retinopathy of the prematurity was illustrative as regards the need for moral justification of the controlled studies, the medical world continued to oppose to such studies and this resistance continued to increase during the 1960s, after the informed consent occurred in medicine[2].

Helsinki Declaration was adopted in 1964, underlining that individual needs must prevail in front of science and society needs, an important principle for medical research[3,4]. Baby Doe Regulation was elaborated in 1982 in USA, a bill that allowed withdrawal of the life support in extremely low birth weight infants and in children with increased risk for long term handicap. This bill was subsequently convicted in 1973 by another document - Rehabilitation Act -, being labeled as discriminatory and considered a violation of children's rights. A "hunting" period followed (Baby Doe Squads) for the cases in which the treatment was not offered and a storm of protests led, in 1986, to the abolishment of this act by the US Supreme Court. It was considered that the act violated the right of the US member states and it was not applied to the medical care of the handicapped children. Another bill was issued by US Congress in 1984 - Child Abuse and Treat Act -, a document that evaluated again Baby Doe Regulation guidelines and defined non initiation of needed medical treatment as child abuse and neglect and not as a discrimination[5,6].

In Europe, the first legislation referring to informed consent was issued in 2001 and was based on Helsinki Declaration (amended in 1996), United Nation Organization Declaration regarding children's rights, and The Convention for Protecting of the Human Beings Rights and Dignity Applied to Biology and Medicine (1997)[7].

Today, the complexity of the ethical issues in medical practice is universally accepted and ethics became a part of the medical practice. It is also accepted that natural rights are fundamental human rights and medical ethical principles are the ethical and moral basis of the informed consent.

What is informed consent?

Ethics implies understanding the nature of the conflicts occurring from moral imperatives and their management modalities, Medical ethics is not just a practical subject but also a branch of moral philosophy and an integrate part of a good medical practice. Ethics does not decide what is morally good and what is bad but how we should act better in the light of our duty and obligation as moral agents. Therefore, we must not forget that physicians have, towards the patients and the society, specific duties and in the center of the modern medical ethics lays the respect for patient autonomy and the fundamental principle of the informed consent derived form this autonomy[8,9]. The respect for patient's autonomy, the informed consent, and confidentiality are representing, according to some authors, key markers for an ethical medical practice and the circumstances in which these principles are not respected should be exceptional and well justified situations[9].

The informed consent represents a fundamental part of modern medical ethics implying that the patient agrees with the proposed treatment after being informed about this therapy in a fair and without restraints, including all the anticipated consequences of all the therapeutic options and their success chances: nature of the procedure/therapy, risks, benefits, alternative therapeutic options with their risks and benefits)[6,10]. Informed consent is a key element for protecting the patient well-being[9,11]. Validity of the informed consent is dependent on the quality of the presented information, understanding of the information by the patient, and the voluntary nature of this act[11].

Medical services - treatments, investigations - are ensured only if the patient or the parent/legal tutor (in the children's case) is informed and gives his informed consent. If the patient is not competent or in the absence of a legally authorized person to offer the informed consent, the medical service supplier (physicians, nurses) will offer medical services only if these are in the best interest of the patient and obeying the legal, professional, ethical and moral standards, according the manner in which they are necessary, minimizing the potential adverse reactions, and for optimizing the quality of the patient's life[7].

The informed consent may be interpreted also as a key factor for the judgment of an intervention or therapy: good/bad, proper/improper. Therefore, the informed consent must be specific to each individual patient.

There are two ways to approach informed consent:

- centered on the clinician, a modality that implies that the informed consent is of a legal type, taking into consideration what a clinician must say to the patient about the nature and risks of the therapy/intervention, a minimum necessary to protect the clinician against legal issues;
- centered on the patient, situation when all the aspects needed for an informed consent of the patient are taken into consideration; this is the real informed consent form the ethical point of view.

It is clear that informed consent has at least two aspects:

- ethical - based, as key element, on the reciprocal respect between the physician and patient, recognition of the value and integrity of the two implied parts;
- legal - with the role to exonerate the clinician of the legal consequences in some situations, representing, in fact, a defense against possible accusations.

As a communication, the informed consent can be written, verbal, or inherent (deductible form the patient's behavior)[8].

In the case of the informed refusal, the clinician must discuss the risks related to not initiating the proposed treatment/investigation, including the way the disease progresses in the absence of treatment. Knowing the consequences of the refusal gives a greater value of the informed refusal[10].

Ultimately, a correct informed consent, embracing the principles of modern medical ethics, has two goals:

- protection of the patient, offering complete information about the proposed therapy/intervention,
- protection of the physician (with some exceptions) of the legal and financial costs when correctly applying the therapy/intervention.

Informed consent in neonatology

The neonatal period is the period between birth and 28 days of life. Starting the half of the XXth century, the neonatal care received "a more and more ethical aspect" due to progresses noted in obstetrics and neonatology. The neonatal period is a special one, with extremely vulnerable population of patients, with specific problems, and patients that cannot defend themselves, cannot express their whishes and rights, and cannot offer informed consent. The anatomical and physiological aspects of the neonate correlated with the specific pathologies of this period are increasing the mortality risk and the risk for handicaps, affecting the future growth and development, including sensorial risks. The newborn and its care implies the emotional involvement of all the individuals participating in this care, both parents and medical staff, physicians and nurses. When the neonatal pathology and the technology necessary to treat the neonatal conditions (incubators, ventilators, monitors, etc.) intervene in the relationship between the child and its family the parental anxiety increases and the communication between clinicians and parents becomes more difficult.

In neonatology, the informed consent, even raising unique issues, aims to inform and imply the parents in taking the important decisions regarding the treatment of the child[12]. In neonatology, there are at least four situations that are ethically critical: obtaining the informed consent for treatment and investigations, the newborn's quality of life, the optimal palliative approach of the critical cases, and equitable distribution of limited resources of a society so that each newborn would benefit of these resources. For multiple reasons, the informed consent dogma has a limited applicability in neonatology.

Modern neonatology is marked by numerous changes, mostly by the progresses and performances of the neonatal intensive care. In the modern medical bioethics, the autonomy of the patient is a central element: the adult has the right to refuse the medical interventions and therapies offered by physicians[8]. In neonatology, the patient - the neonate - has not the ability to take decisions rendering the parents to take decisions in the best interest of the child. It is already well established the fact that, at birth, the parents have the right to be involved in decisions every time when optional therapies or investigations are necessary, so that informed consent of the legal caregivers became, in the latest years, a norm of conduct in neonatology[5,7]. Obviously, the patient's autonomy principle is not accepted by all the patients. In a survey conducted by Zupancic et al.[13], many patients preferred the medical advice against the decisional autonomy, only 27% of the patients wishing to take independent decisions regarding the informed consent.

In neonatology, problems and difficulties are occurring starting straight from the subject of informed consent, and, obviously, the problem of its fairness and validity raises. First of all, the newborn, the subject of the informed consent in neonatology, is not able to take decision for himself. The parental stress can be major in the case of the preterm infants and newborns admitted in the neonatal intensive care unit. Concerned, excited parents, frightened for the child's life may often feel physically and psychically powerless, incapable to take a decision. In this situation, asking the informed consent increases even more the stress level[8,14,15].

Another great difficulty encountered by physicians while obtaining a valid informed consent is the emergency. Neonatology is an emergency specialty, often the birth occurring after a stormy pregnancy or delivery history or the newborn's status may change abruptly, needing emergency diagnostic or therapeutic interventions. In such critical situations, the parents are forced to assume the responsibility of taking major decisions for another individual. According some authors[16], in such major stressful situations, the understanding of the situation by the caregivers is limited and informed consent cannot be practically obtained. The danger that parents will choose a path to follow based on their own preferences arises form this situations. These personal preferences may not reflect the best interest of the child and the informed consent is contradicting its own goal - the best interest of the child. Other parents, under the burden of the stress and need to hear good news about their child, let themselves to be overwhelmed by the situation, and, mostly when they lack previous experiences to lay on, are tempted to allow the physician to take the decision, being unable to process the offered information. As showed by Modi[17], the human response to disease if often very complex and not rarely less rational and in the case of the parents their capacity to take autonomous informed decisions is affected. Even more, most often the parents are not prepared for the emotional, moral and intellectual stress of the neonatal pathology and this may also be one of the reasons of the understanding and accepting difficulties of the situations[15]. In front of incertitude and risk, the term "objective" losses its value and meaning.

The moment when parents are approached for offering the informed consent is a moment of intense excitement and stress. The more intense is the maternal stress level, the more reduced is the real value of the informed consent. Zupancic et al [13] showed that a quarter of the participants in a survey about the informed consent in newborns would have preferred to be approached during pregnancy and not after birth. But even the prenatal informed consent is entailed to many question signs: in the case of the pregnant women asked for informed consent regarding peridural anesthesia during labor 33% of them did not remembered having this discussion at 48 hours after delivery irrespective if the informed consent was written or verbal[13]. A better rate of recall about informed consent discussions was obtained only in mothers approached during parental school lessons[13]. The value of informed consent obtained during labor is considerably diminished by the labor and delivery stress[3,5]. The authors are cautioning the antenatal informed consent is popular but may overwhelm the parents with information that are not necessary and in the case of

increased risk pregnancies may increase the pressure felt by parents[18-20]. Lack of the fetal legal rights is another major problem of the prenatal informed consent[3].

After birth, obtaining the informed consent from the mother may be hampered by particular situations as:

- postnatal transfer of the child to superior level units - raises legal problems mostly when the transfer was not done before delivery, in utero, even if a safe prenatal transfer could have been done[8]
- maternal postpartum complications: mother under anesthesia for cesarean section, or treated with psychotropic drugs or receiving medication affecting the decisional capacity, mother with major conditions, etc.[8]
- psychical and psychological maternal complications due to newborn's conditions - postpartum depression, feelings of guilt[15]
- changes of the familial relationships under stress pressure (between parents, between the parents and brothers, etc.)[15]
- limited technological, material, and human resources influencing the neonatal standards of care, creating inequities.

Obtaining the informed consent process must be based on sufficient, simple, easy to understand information, explaining concepts, potential risks, benefits, and implications of the decisions taken into informed consent. The validity of the informed consent may be subjected to discussions anytime the communication between clinicians and parents is poor due either to incorrect, incomplete, difficult to understand (medical slang), complicated (unexplained scientific concepts or too many information) offered by clinicians, or to difficulties of understanding secondary to language barrier, educational or intellectual deficiencies of the parents[8,14,15,21]. Therefore, it is important that clinicians take into account the results of the study developed by Zupancic[13] that showed that factors determining the parental preferences for a certain decision are related mostly to the process of the informed consent and not to the personal character of those participating in taking decisions.

Sometimes the parents don't even understand the concept of informed consent and other times they just don't want to completely assume the responsibilities of taking decisions[21,22]. In other situations, the physicians that want to impose what they consider as necessary treatment are assuming the decision irrespective if they ask or not the informed consent[8]. Such a paternalist behavior, a norm some decades ago, persistent as dominant up to nowadays especially in the case of futile medical interventions, situations when clinicians may override the informed consent, often with success[8]. Between futility and patient's autonomy there is a grey zone, a territory where the medical staff and the patient are discussing, negotiating, and compromising, the best model for solving potential conflicts. Such a grey zone is, in neonatology, resuscitation at the viability threshold, under 24 weeks of gestation[8].

Unrealistic parental expectations may also create difficulties of understanding and accepting the offered information during informed consent process and denial responses as a defense reaction in front of a very serious situation[15,21]. Another problem of the informed consent in neonatology is related to its content[9]. The correctitude and validity of informed consent may be influence by social, cultural, religious, gender (as in Roma population), familial norms or pressures[14,15,21]. All these norms may influence familial understanding about good/bad, correct/incorrect, possible/impossible.

Another problem of the informed consent is linked to the multidisciplinarity imposed by complicated cases, asking for solutions from a team of different specialists. Conflicting situations and misunderstandings between the team members are often arising in these situations, most often due to individual prestige, authority, priority of action, each individual role in the team, language, or even knowledge about the involved related specialties. Not rarely, the obstetricians opinion dominates in front of parents, among others because an older relationship between parents and the obstetrician and due to trust gained by the specialist in the eyes of the family. Also, in relation with other specialties, the neonatologist gains ground during postnatal period. Ideally, the relationship and communication between the members of the multidisciplinary team should not negatively affect the communication with the parents and with informed consent[15].

Many discussions existed in the latest years in the medical world due to numerous ethical, moral, and even legal issues raised by informed consent in neonatology. For instance, in situations where discordances between clinicians and parents that cannot be solved exist, the parents have the legal right to consent or not to the therapies applied to their child but this right is not an absolute one when it is judged in the light of the best interest of the child, including in situations where parents are unjustifiably asking for supporting the child's life[22]. United Nations Organization Convention for child rights clearly defines the society's responsibilities to support parents in applying their believes from the position of main persons responsible for the well-being of their child[23]. Legal courts may be solicited in conflicting situations in order to align the moral values of the professionals to those of the society. But the medical staff should have the right to decide when and where their professional knowledge is used and, in situations when these knowledge are causing unacceptable distress the medical staff should be allowed to withdraw their medical services. At least theoretically but also from a legal point of view, the parental right to decide for the child is not as strong as their right to decide for themselves. In the case of the minors, the standard of the best interest of the child dominates but the place for each decisional threshold is often subjective and the success of the treatment is defined in terms of survival or subsequent disabilities[6].

The informed consent in neonatology - when it is necessary

The informed consent means, in its essence, informing the patient or its legal representative about the risks and benefits of the proposed medical intervention or therapy and about alternative therapeutic options. But, especially in neonatology and in the case of a sick neonate and in preterm infants, very often, the therapeutic procedures are implying

also significant risks, sometimes on long time. Even more, any procedure, any therapy may have, sometimes, hundreds of associated risks. And, in case of the drugs some may consider the adverse effects as risks associated to that therapy. Therefore, the medical world agrees that into the informed consent only the relevant risks for the physician's specialty and those with the greatest severity and/or frequency must be described (for example, risks occurring with great incidence are those arising in more than 3% of the cases, while complications as death, palsy, cerebral lesions occurring in more than 1% of the cases are considered severe risks)[10]. Clinicians must pay attention to the risks presented in the informed consent process, mostly to the mode in which they communicate these risks to the patient. Informed consent must prepare the patient for adverse effects and also help him chose the best option for him, including the option to refuse the proposed therapy and the consequences derived from this refusal.

In newborns, the informed consent must encourage the parents to imply themselves in taking decisions regarding the child's health. Ideally, these discussions must take place before birth but, in most of the cases, this is rarely possible. Most of the times, the parents are approached immediately after delivery for explaining the routine care, immunizations, and screening tests for the neonates without special problems or for presenting a diagnosed condition and therapeutic options in preterm infants and sick newborns. Whenever changes are occurring in the child's condition, changes of the therapy may be needed and the parents may need to be approached again with new therapeutic options, ideally before applying them (for example, the need for catheterization, phototherapy, ultrasound scans, treatment of complications, etc.). In emergency situations the parents must be informed as soon as possible after applying urgent treatments.

Not all the procedures performed in newborns need parental informed consent but there are certain situations when informed consent must be obtained any time when the situation is not an emergency: type of milk for feeding, feeding modality, immunizations, vitamin K administration, screening tests, blood withdrawal, antibiotic therapy, administration of drugs, including infusions, phototherapy, vascular catheterizations - peripheral and central -, oxygen therapy, non-invasive and invasive respiratory support, ultrasound scans, special radiological procedures (as contrast agents administration, magnetic resonance imaging, computer-tomography, etc.), blood or blood-derived products transfusions, any surgical procedure, any innovative procedure or therapy, participations in studies or research, resuscitation at viability threshold, organ donation, autopsy[10,22,24].

When presenting the risks related to the proposed therapy/procedure, the clinician must avoid formulations as "among the described risks there are ..." or "the risks include but are not limited to ...", formulations that are in a way elusive an may conduct to patient refusal (scared by too many risks) or lack of attention form a patient refusing to know the risks. Some authors are recommending not only documentation of the informed consent but also documentation the attitude of the patient during discussion[10].

Informed in neonatology - potentially conflicting situations

Disagreements between clinicians and the newborn's caregivers - parents of legal tutors - may occur in various situations. Most often, such conflicts are seen in the following situations:

- clinicians and parents have different opinions regarding the continuation or cessation of life support in extreme situations;
- personal interpretations or influence of religious groups (for example opposing blood or blood-derived products transfusion in the case of Jehovah witnesses);
- different interpretation of the autonomy principle when parents may believe that they have a better perception compared to physicians as regards the best interest of the child (for example, immunizations);
- differences in evaluation the future status of the newborn on medium and long term, mostly as regards the quality of life, between the parents and physicians (for example, in conditions affecting the quality of life, such as Down syndrome).

Informed consent in neonatology - possible solutions

The informed consent is not valid if it does not respect the principles of modern medical ethics:

- the best interest of the patient (including life preservation, removal of suffering);
- "non malefience" principle (do not harm);
- patient's autonomy (the patient or his tutor are regarded as moral agents, with duties and obligations, able to understand and take ethical decisions);
- equity principle (fair allocation of medical resources)[5,8,9,25].

In neonatology, other extremely important principles are: respecting the child's rights (by the clinicians, parents, and society), medical knowledge of the clinicians, and professional deontology[15].

Actually, the informed consent occurs in the context of understanding and trust between clinicians and parents and is determined by three elements: a substantial informational process of the parents, ability of the parents to take correct decisions for their child, and the capacity of the parents to freely take decisions, without coercive pressure from the physicians.

Ethically, the informed consent aims to offer easy to understand information and choosing a therapeutic conduct by the patient or by his legal representatives. For the parents, their ability to make a real informed choice implies a systematic approach of the informed consent as regards the moment, the content, and the communication modality. After open discussions, the parents must be guided to decide the best for the child's health. Tripp et al[22] are distinguishing several types of relationships between clinicians and parents that may burden the informed consent (Table no 1.). Based on the parental typology, the physician must be prepared to use one of the attitudes described in the table to reach a common decision for the best interest of the

child. The principles of the modern bioethics are recommending physicians to avoid paternalist attitudes and to try to imply the parents in taking decisions[26]. Clinicians attitude in discussing with parents is important, empathy being a major element. Physicians must guide and support the parents with calm and empathy, avoiding to set themselves as child's tutors[15].

Clinicians have also to avoid sliding on the path of simplified ethical questions, situation that minimize the relationship with the patient to the principles derived from Hippocrates oath[22]. Older and more experienced physicians can be involved in the discussion as counselors, facilitators, or even negotiators[22].

Table no. 1. Types of relationships between physician and parent[22]

	Physician's style			
	Informative	*Interpretative*	*Deliberative*	*Paternalist*
Parental values	Well defined, fixed, known by the parent	Rudimentary, conflicting, needing clarification	Open to revision by moral discussions	Objective, originating from the physician
Duties of the physician	Offering relevant information and implementing the option chosen by the parent	Clarifying and interpreting the relevant values of the parent, informing the parent and applying the option chosen by the parent	Showing and convincing the parent about the most important values and applying the option chosen by the parent	Promotion of the best interest of the child independent of the parents
Parental concept about autonomy	Chose and control of the medical care	Good understanding of the medical care	Moral self development related to medical care	Consent to objective values
Physician's concept about his own role	Competent technical expert	Counselor or advisor	Friend or educator	Tutor

The open and honest character of the discussions between clinicians and parents represents a good prerequisite for avoiding conflicting situations during informed consent in neonatology[22]. Also, the principles of respecting individuals and of responsibility are other two essential points for a valid informed consent. Repeating information (if there is time) allows avoiding misunderstandings and prevents unrealistic expectations of the parents. It also prevents confusions, suspicions, and overt hostility of the parents. Repeated discussions offer a better knowledge of the dialog partners and a better evaluation of both parts as regards the physician's rationale and integrity, creating a basis for objective informed consent[26].

In situations known before birth, communication between obstetrician, anesthesiologist, neonatologist, and other specialists that may be involved in the care of the neonate is essential for avoiding overwhelming the parents with multiple and not always concordant information.

Documenting (written in the patient chart) the discussion about risks and the informed consent is legally helpful for the physician and parent. The parent may loner deliberate and may ask for more information when he/she reads the information verbally presented[10,24]. Brochures, flyers, algorithms, audio tapes, computerized programs, interactive videos, prognostic tables stratified on gestational age in the case of preterm infants are helpful and recommended[13,24]. Such standardized information may help parents to take the best decisions for their children, decisions that, in neonatology, during an intense emotional moment, are often a too heavy burden on the parents' shoulder.

Informed consent in neonatology - the possible role of ethical committees

The role of ethical/bioethical medical committees of the medical institutions is unsure as regards obtaining informed consent. In order to reduce the risk of unsafe and unreasonable parental decisions, the physicians may call these committees for their quality of ethical counselors and for solving ethical disputes with parents[5]. Ethical medical committees may be useful for training the medical staff and patients (including parents) on the relevant ethical principles, development of medical ethical policies and standards in medical institutions, and applying in practice of the recommendations issues. Also, these committees must represent a support forum for the medical staff facing difficult ethical decisions[26].

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SEDATION AND ANALGESIA IN THE MECHANICALLY VENTILATED PEDIATRIC PATIENT

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Abstract

Aim. The aim of this study was to evaluate the sedation and analgesia regiments in PICU patients undergoing mechanical ventilation in our clinic. *Material and methods.* A four years observational study (January 2010 – December 2013) was conducted in the First PICU of Emergency Hospital for Children "Louis Turcanu" Timisoara and included 108 children, aged 0-18 years, who required mechanical ventilation for more than 24 hours. Sedation was achieved using benzodiazepines (Midazolam), opioids (Fentanyl or Morphine) and Propofol, as continuous infusion sedation or as intermittent bolus sedation. The levels of sedation and analgesia were based on Ramsay Sedation Scale. Sedation was considered appropriate at a Ramsay Scale of 2-4. *Results.* We used a number of 4 protocols for sedation during mechanical ventilation: one protocol used intermittent bolus medication (Midazolam) and 3 protocols used one drug or combinations of at least 2 drugs in continuous infusion. Midazolam bolus protocol was used only in a limited number of newborns (3.7%), in a dose of 0.1 mg/kg/dose. The mean number of boluses administered in 24 hours was 6.12±1.04. Ramsay Scale had a mean of 4.14±0.24. Midazolam in continuous infusion protocol was used in 38.88% of patients. The mean infusion dose of Midazolam was 0.31±0.08 mg/kg/h. Ramsay Scale had a mean of 3.43±0.35. Midazolam and Morphine in continuous infusion protocol was used in 2.77% of patients. The mean dose for Midazolam was 0.27±0.035 mg/kg/h and for Morphine 0.027±0.003 mg/kg/h. Ramsay scale had a mean of 2.21±0.57. Midazolam and Fentanyl in continuous infusion protocol was used in 54.62% of patients. The mean dose of Midazolam was 0.24±0.04 mg/kg/h and 2.9±0.6 mg/kg/h for Fentanyl. Ramsay Scale had a mean of 2.52±0.15. *Conclusions.* We consider that the combination of Midazolam and Fentanyl in continuous infusion is the best option for children, and adequate analgesia and sedation are achieve in a relatively short period of time. The association of Morphine with Midazolam in continuously infused was abandoned due to observed adverse effects.

Key words: sedation, analgesia, mechanical ventilation, child

Introduction

Appropriate sedation and analgesia are important parts of critical ill patient care and was described as inducing a state of comfort, without inducing coma (1). Sedatives and analgesics reduce anxiety, pain and agitation; facilitate mechanical ventilation and invasive procedures used commonly in PICU. Sedatives administered to critically ill children should be titrated according to the desired effect, since both under- and over-sedation can have negative effects.

Sedation in mechanically ventilated patients is necessary because they are awareness about the gravity of the situation (2) and because of the lack of control and inability to communicate (3). Also ventilated patients require analgesia because this category of patients is the most vulnerable to pain, which is caused by various invasive procedures; the presence of the endotracheal tube; immobilization for long periods; maneuvers such as suction of secretions from the airways. Among these, most patients indicated the presence of endotracheal tube as a major cause of pain and distress (4).

A superficial sedation during mechanical ventilation may cause undesired incidents such as accidental extubation, pull on the catheters, tubes and other devices, desynchronization with the ventilator and secondary hypoxia. On the other hand, excessive sedation was associated with a longer duration of ventilation and hospitalization, delayed patient recovery and may predispose to the occurrence of withdrawal phenomena (5).

An important aspect of sedation and analgesia in mechanically ventilated patients is the occurrence of withdrawal phenomena after discontinuation of medication. The drug dependence is encountered at any age and is most commonly manifested by insomnia, nightmares, agitation and anxiety. Factors associated with this syndrome are dependent on high doses and prolonged administration of opiates and/or benzodiazepines (6).

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A wide variety of pharmacological agents are now available for sedation and analgesia. An ideal sedative agent would have rapid onset of action, provide adequate sedation, allow rapid recovery after discontinuation, be easy to administer, lack drug accumulation, have few adverse effects, and interact minimally with other drugs. Unfortunately, sedatives have adverse effects, the potential to prolong mechanical ventilation, and may increase ICU (intensive care unit) stay and health care costs (7).

Aim

The aim of this study was to evaluate the sedation and analgesia regiments in PICU (pediatric intense care unit) patients undergoing mechanical ventilation in our clinic.

Material and Methods

A four years observational study (January 2010 – December 2013) was conducted in the Pediatric Clinic I of Emergency Hospital for Children "Louis Turcanu" Timisoara, University of Medicine and Pharmacy "Victor Babes" Timisoara and included 108 children who required endotracheal intubation and mechanical ventilation.

All patients mechanically ventilated for more than 24 hours, aged 0-18 years were eligible for inclusion.

Exclusion criteria was represented by: mechanical ventilation < 24 hours; patients in cerebral death, with Glasgow coma score < 6; noninvasive ventilation; and preterm babies, patients with congenital immunodeficiency disorders, malignant or surgical diseases were excluded from the study.

Criteria for intubation were: apnea, impaired alveolar ventilation ($PaCO_2$ > 55 mmHg), inadequate oxygenation despite FiO_2 > 60% (PaO_2 < 60 mmHg) and for airway protection.

Demographic data (gender, age, weight), reason for mechanical ventilation (MV), number of days on ventilator, hospital length of stay, outcome (discharge, transfer, death), and pediatric risk of mortality score (PRISM) III (8) were collected in all patients.

All patients were mechanically ventilated using pressure limit: SIMV (synchronized intermittent mandatory ventilation) or A/C (assist control). Vital parameters including respiratory rate, heart rate, and non-invasive blood pressure (NIBP) were documented. The oxygen saturation of each child was monitored continuously by pulse oximetry.

Sedation was achieved using benzodiazepines (Midazolam), opioids (Fentanyl or Morphine) and Propofol, as continuous infusion sedation or as intermittent bolus sedation.

The protocols for the infusion of sedatives (mode of administration, dosage) are shown in Table 1.

The levels of sedation and analgesia were based on Ramsay Sedation Scale (9). The scale determines the state of consciousness in 6 levels: level 1, where the patient is anxious or restless to level 6, in which the patient is completely unresponsive to stimuli (Table 2).

Table 1. Protocols for sedation and analgesia in the study patients.

Sedative and analgesic drug	***Protocol***
Midazolam	Intravenous bolus of 0.1-0.2 mg/kg every 15 min as needed Continuous infusion at 0.1-0.2 mg/kg/h; dosage to be increased at 0.3 mg/kg/h until adequate sedation is achieved
Fentanyl	Intravenous bolus of 1-2 mcg/kg every 15 min as needed Continuous infusion at 1-2 mcg/kg/h; dosage to be increased at 4 mg/kg/h
Morphine	Continuous infusion at 0.01 mg/kg/h; dosage to be increased at 0.03 mg/kg/h
Propofol	Intravenous bolus of 1-2 mg/kg every 1 hour as needed Continuous infusion at 1-2 mg/kg/h; dosage to be increased at 4-5 mg/kg/h

Table 2. Ramsay Sedation Scale.

Awake levels	1	Patient anxious, agitated or both
	2	Patient cooperative, orientated and tranquil
	3	Patient responds to commands only
Asleep levels	4	A brisk response to a light glabellar tap
	5	A sluggish response to a light glabellar tap
	6	No response

In our study, sedation was considered appropriate at a Ramsay Scale of 2-4. Sedation assessment was performed by intensive care nurses at hourly intervals. The presence of a score of 1 required supplementation of sedative medication to avoid the patient`s fight with the ventilator. A value of 5 or 6 at three successive determinations led to reduction of medication to avoid over sedation.

We used a number of 4 protocols for sedation during mechanical ventilation:

- one protocol used intermittent bolus medication (Midazolam) in 4 patients (3.7%) and
- 3 protocols used drugs in continuous infusion:
  - Midazolam continuous infusion in 42 patients (38.88%)
  - Midazolam and Morphine in 3 patients (2.77%)
  - Midazolam and Fentanyl in 59 patients (54.62%); and of these patients:
    - In 3 patients we associated continuous infusion with Propofol
    - In 4 patients we associated continuous infusion of neuromuscular blocking agents

This study was approved by the Hospital institutional review board and parents inform consent in writing was taken.

Statistical analysis was performed using Microsoft Excel 2007 software. Results are expressed as percent (%) and mean±standard deviation (M±SD).

Results

A total of 108 PICU patients receiving mechanical ventilation for a minimum of 24 hours met the inclusion criteria and were included in the study.

Study population characteristics are shown in Table 3. More than 50% of the patients were males. The mean age of the study group was 2.3 years; more than 50% were infants aged less than a year. The mean duration of mechanical ventilation was 9 days and the mean hospital length of stay was 25 days. The median value of PRISM III score on admission was 17, corresponding to a predicted death rate of 22%. Mortality rate for intubated patients was 31.48%.

Table 3. Study population characteristics.

	N=108
Age (M±SD) month (0-216)	27.65±51.00
Age, N (%):	
0-1 month	11 (10.18)
1 month-1 year	61 (56.48)
1-3 years	17 (15.74)
3-6 years	5 (4.62)
> 6 years	14 (12.96)
Sex, N (%)	
Male	74 (68.51)
Female	34 (31.48)
Ventilator days (M±SD)	9.36±8.52
Hospital length of stay (M±SD)	24.7±18.66
Outcome, N (%)	
Discharged	68 (62.96)
Death	34 (31.48)
Transferred to another hospital	6 (5.55)
PRISM III score (M±SD)	17±6.83

1. Midazolam bolus protocol

This protocol was used only in 4 newborns (3.7%).

The pathology of newborns who have received this type of intermittent sedation was: neonatal respiratory distress syndrome in 2 (1.85%) patients and the other two (1.85%) patients were with congenital cardiac malformations. We reported in this group 2 (1.85%) deaths.

The mean duration of mechanical ventilation was 11.25±7.36 days and the mean duration of hospitalization was 20.25±7.5 days.

Consciousness of these patients has been altered without the need to establish a protocol for continuous sedation. Midazolam was administered as intermittent sedation in dose of 0.1 mg/kg/dose, the mean number of boluses administered in 24 hours was 6.12±1.04.

Ramsay Scale had a mean of 4.14±0.24.

2. Midazolam in continuous infusion protocol

This protocol was used in 41 (38.88%) patients.

Analysis by age group reveals the use of this protocol mainly in the age group 1 month-1 year (45%) (Figure 1).

The main reasons for intubation and mechanical ventilation for this group were extremely various, but mostly being represented by acute respiratory failure (Figure 2).

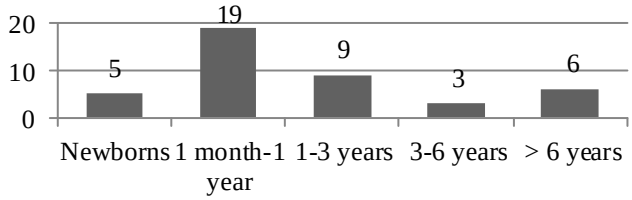

Figure 1. Age group distribution for Midazolam in continuous infusion protocol.

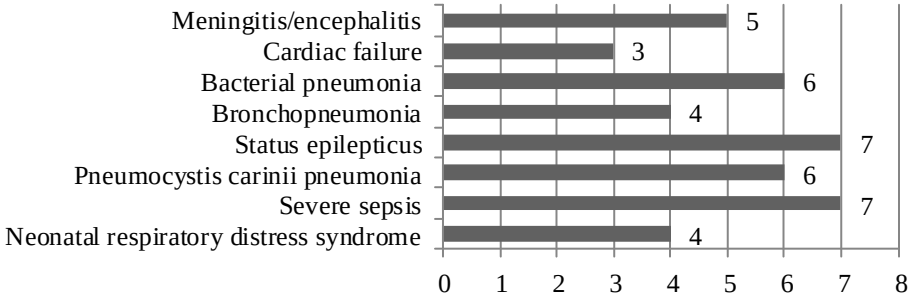

Figure 2. The main diagnosis on admission in PICU for Midazolam in continuous infusion protocol.

Survival in this group was 63.41%.

The mean duration of MV was 7.35±6.31 days and the mean duration of hospitalization was 22.4±22.37 days.

The starting dose of Midazolam infusion was 0.1 mg/kg/h, the dose being adjusted according to the Ramsay Scale. Increasing the dose of midazolam to prevent agitation was required in all cases after about 24 hours. The mean infusion dose of Midazolam was 0.31±0.08 mg/kg/hr.

Ramsay Scale had a mean of 3.43±0.35. Maintaining an adequate level of sedation was achieved by administration of additional boluses of Propofol (1 mg/kg) and Fentanyl (1 mcg/kg).

3. Midazolam and Morphine in continuous infusion protocol

This protocol was used in 3 (2.77%) patients. All patients had acute respiratory failure. One patient died.

The mean duration of MV was 5±4.35 days and the mean duration of hospitalization was 24.33±15.04 days.

The starting dose of infusion was 0.1 mg/kg/h for Midazolam and 0.01 mg/kg/h for Morphine. Dose escalation was necessary in all cases, the mean dose for Midazolam being 0.27±0.035 mg/kg/h and for Morphine 0.027±0.003 mg/kg/h.

Ramsay scale had a mean of 2.21±0.57. For episodes of agitation we administered boluses of Midazolam (0.1 mg/kg) or Popofol (1 mg/kg).

Regarding side effects, all patients had gastric stasis and bilious vomiting, necessitating discontinuation of enteral feeding and transition to total parenteral nutrition. These digestive disorders were the main reason that we stopped using Morphine to sedate mechanically ventilated patients.

4. Midazolam and Fentanyl in continuous infusion protocol

It was used in 59 (54.62%) patients, representing the most common protocol to facilitate mechanical ventilation in our study.

Distribution by age group reveals the use of this protocol mainly in the age group 1 month - 1 year (68%) (Figure 3).

The main pathology for the establishment of MV in this group was extremely variable, represented mainly by respiratory disorders in 67% of cases (Figure 4). Survival in this group had the highest rate (72.88%).

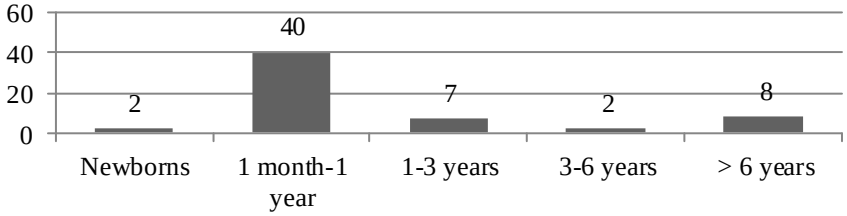

Figure 3. Age group distribution for Midazolam and Fentanyl in continuous infusion protocol.

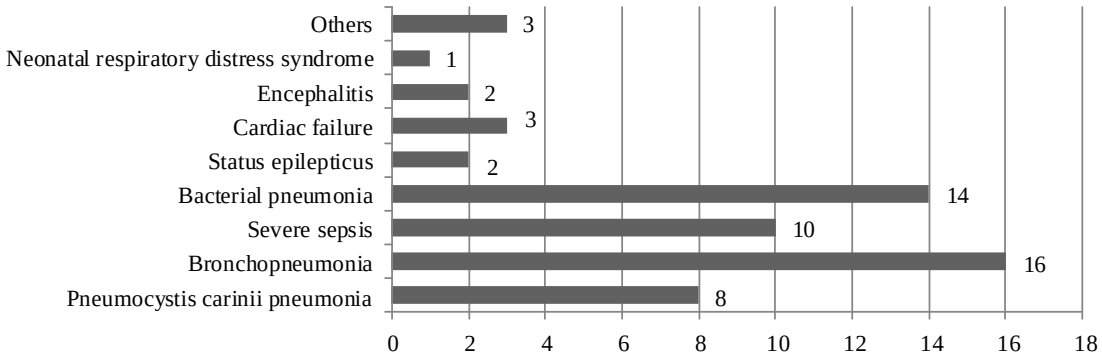

Figure 4. The main diagnosis on admission in PICU for Midazolam and Fentanyl in continuous infusion protocol.

The mean duration of MV was 11.03±9.7 days and the mean duration of hospitalization was 26.86±16.39 days. It can be noted that patients in this group required the greatest number of days of ventilation and hospitalization in the entire study group.

The starting dose of infusion was 0.1 mg/kg/h for Midazolam and 1 mg/kg/h for Fentanyl. Tolerance of medication was installed relatively quickly, in about 24 hours; so it was necessary to increase the rate of infusion. The mean dose of Midazolam was 0.24±0.04 mg/kg/h and the mean dose of Fentanyl was 2.9±0.6 mg/kg/h.

Ramsay Scale had a mean of 2.52±0.15. Supplemental boluses from this combination of Midazolam and Fentanyl equivalent with the dose per hour or Propofol (1 mg/kg) were needed.

Because the installation of tolerance to this protocol, with the need for high doses of opiates (which led to the installation of the chest wall stiffness), in 3 patients (2.77%) Propofol was associated in continuous infusion at a rate of 2 mg/kg/h. In 4 patients (3.7%) continuous infusion of neuromuscular blocking agents (Rocuronium) was associated at a dose of 0.3 mg/kg/h, thereby allowing to decrease the dose of opioid.

Discussions

Monitoring the level of sedation and analgesia in mechanically ventilated patients is essential, allowing an optimum patient comfort and good synchronization with the ventilator when given minimal doses of sedatives and analgesics.

Currently there are many sedation assessment scale, most commonly used in pediatric sedation are Ramsay Scale (9), COMFORT Scale (10), Richmond agitation and sedation Scale (RASS) (11) and Bispectral Index Score (BIS) (12) - score goal resulted from mathematical analysis of electroencephalogram.

In our study, the effectiveness of sedation and analgesia was based on the Ramsay scale, which was considered appropriate at a value of 2-4.

There are currently many drugs used for facilitation of mechanical ventilation, but without a uniform conduct of their choice. Despite their widespread use, analgo-sedative drugs still lack data supporting appropriate dosing, safety, and efficacy of combined therapies, and optimal drug regimens for sedation during mechanical ventilation (13). In many intensive care units, sedatives are infused continuously (14). As compared with intermittent bolus infusion, this approach provides a more constant level of sedation and may increase patient's comfort.

We analyzed a total of four protocols for sedation/analgesia, one using only bolus medication. The most common sedation protocol used was the combination of Midazolam and Fentanyl in continuous infusion.

The choice of agent and the way in which they are used varies widely between and within ICUs. Propofol is the preferred iv infusion sedation agent in most U.S. ICUs and is gaining in popularity compared with other sedatives (15). In the United Kingdom, the most common sedative agents used in PICUs continue to be Midazolam and Morphine (16). A prospective multicenter patient-based study in France shown that Midazolam is the agent most commonly drug used for sedation, and for analgesia Sufentanil and Fentanyl are the most frequently used opioids (17). Recent Italian guidelines for sedation shown that benzodiazepines are the most commonly used drugs to sedate mechanically ventilated patients (18).

A review published last year by Vet et al (19) highlights several specific aspects about the use of sedatives in mechanically ventilated pediatric patients. After exclusion criteria, the authors analyzed 25 studies, in total 1,163 mechanically ventilated children aged 0-18 years. The most common drugs used were benzodiazepines (Midazolam in 22 studies) and opioids (Morphine in 14 studies). Other drugs used were: fentanyl, ketamine, clonidine, propofol, barbiturates, and dexmedetomidine hydrochloride in various combinations. In all these studies a number of 12 different scales of sedation have been used, the most common being the COMFORT scale, followed by Ramsay scale. Regarding the degree of sedation in these patients, optimal sedation was achieved in 57.6% of patients, under-sedation in 10.6% and over-sedation in 31.8%. The authors concluded that over-sedation in mechanically ventilated children is more

common than under-sedation and highlight the importance of finding an optimal and unitary strategy for sedation in PICU.

Conclusions

In our study, we had better results using a continuous infusion sedation protocol. Our experience in the management of mechanically ventilated patients showed that the combination of Midazolam and Fentanyl in continuous infusion is the best option for children, and adequate analgesia and sedation are achieved in a relatively short period of time. This combination provides a lower Ramsay score, at lower doses of Midazolam.

The association of Morphine in continuously infused Midazolam was abandoned due to observed adverse effects. All patients had gastric stasis, bilious vomiting and ultimately paralytic ileus, requiring discontinuation of enteral feeding and transition to total parenteral nutrition.

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OVARIAN CYSTS IN INFANTS-OUR EXPERIENCE

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Abstract

Neonatal ovarian cysts (NOC) are being diagnosed more often now that routine ultrasonography is carried out antenatally and postnatally. A truly cystic abdominal mass in a newborn is most likely to be an ovarian cyst, although duplication cyst or mesenteric cyst should be considered in the differential diagnosis. There is controversy about the best treatment for these cysts, opinions ranging from oophorectomy to follow-up by ultrasonography alone. Material and methods: A retrospective study of patients with NOC, out of 73 asymptomatic female infants that underwent abdominal ultrasonography as part of a screening, in the Neonatal Ward of the Emergency Hospital for Children Timisoara "Louis Turcanu" between July 2013 – July 2014. Size and localization of the cyst, as well as age at which they were detected and the possible maternal etiologic factors were recorded. Results: 33 (45.20%) infants were diagnosed with NOC according to echographic criteria, out of 73 female infants that were evaluated. The majority of the cysts were unilateral and detected in the right ovary in 23 (69.7%) of patients. All cysts were small; mean size of the cysts was 15.0± 5.0 mm (range 10mm and 30mm). No complications were noted (torsion, haemorrhage, peritonitis, bowel obstruction or respiratory distress). Periodic ultrasound examinations revealed a tendency towards spontaneous regression of this cysts. Most of the cases resolved spontaneously by the age of one year. Conclusions: Ovarian cysts are seen more frequently than expected in the neonatal period. Ovarian cysts are the rule, not the exception in newborn infants.

Key words: ovarian cysts, infancy

Introduction

Neonatal ovarian cysts (NOC) are the most common type of benign tumors found in female newborns [1]. A case of NOC was first mention in literature in 1889 as an autopsy finding in a stillborn preterm infant. In 1942 Bulfamonte reported the first case of an ovarian cyst successfully treated during the newborn period [2]. Nowadays, the routine use of ultrasound allows the detection of NOC during the neonatal period. NOC with a diameter exceeding 2 cm are considered pathological. The incidence of ovarian cysts has been estimated at more than 30% (this estimate is based on an investigation of stillborns or infants who died within 28 days of birth) [3].The correlation of the diameter with the clinical symptoms and ultrasound appearance allows an optimal therapeutic approach [4].

The etiology of NOC remains unknown, but hormonal stimulation, advanced gestational age and increasing placental chorionic gonadotropin levels in complicated pregnancies with large placenta such as in diabetes, pre-eclampsia and Rh incompatibility are the most frequently mentioned assumptions [5,6,7]. Additionally, fetal hypothyroidism and congenital adrenal hyperplasia due to 21-hydroxylase deficiency or 11 beta-hydroxylase deficiency have also been reported to cause NOC.[1,17] NOC are classified according to their ultrasonographic features as "simple" or "complex", and according to their size as "small" or "large" cysts [8,9]. Most cysts are functional in origin and histologically simple and benign [10]. Complications that can occur include intracystic hemorrhage, rupture with possible intraabdominal hemorrhage, gastrointestinal or urinary tract obstruction, ovarian torsion and necrosis, incarcerated inguinal hernia, dystocia by excess of fetal abdominal part, and respiratory distress at birth from a mass effect on the diaphragm [11,12].

Spontaneous regression of both simple and complex cysts often occurs by six months of age, as the hormone concentrations fall and the stimulus for growth disappears, therefore management is usually expectant. The rate of malignancy is so low that it need not be considered in making therapeutic decisions. [13]

Material and method

Retrospective study of patients with NOC, out of 73 asymptomatic female infants that underwent abdominal ultrasonography as part of a screening, in the Neonatal Ward of the Emergency Hospital for Children Timisoara "Louis Turcanu" between July 2013 – July 2014. Size and localization of the cyst, as well as age at which they were detected and the possible maternal etiologic factors were recorded.

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The cysts were classified according to their size as "small" for cysts with diameter < 4 cm, and "large" for those > 4 cm. The Nussbaum criteria were used for discrimination between simple and complex cysts. When completely anechoic and with a thin wall, the cyst was defined simple [14]. Cysts, which presented themselves echogenic, with a fluid-debris level, a retraction clot or a septation were defined complex. Patients were followed-up with serial ultrasound examinations.

Results

33 (45.20%) infants were diagnosed with NOC according to echographic criteria, out of 73 female infants that were evaluated.

There was history of pre-eclampsia in 3 mothers and gestational diabetes in one. Rh incompatibility without hydrops was noted in two patients. Thyroid function tests were within normal ranges in all but one patient, that presented with elevated TSH. None of the patients had any congenital malformations.

The majority of the cysts were unilateral and detected in the right ovary in 23 (69.7%) of patients. (Fig.1) There were five cases with bilateral cysts. All cysts were small; mean size of the cysts was 15.0± 5.0 mm (range 10mm and 30mm). (Fig. 2) Ultrasonographic evaluation revealed simple cysts - completely anechoic, homogeneous, thin-walled, unilocular structures in the ovarian tissue - in 29 (87.8%) cases; the cyst wall was imperceptible with sonography in 7 of these cases. Thick-walled septated cyst which contained blood clots or debris were identified in 4 cases (12.2%).

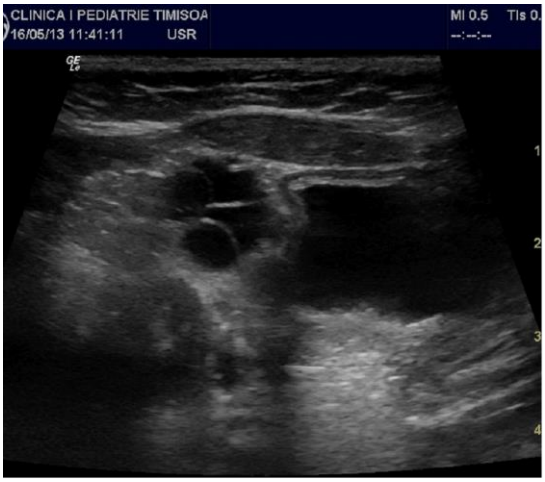

Fig. 1. Ovarian cysts in 2 months old girl.

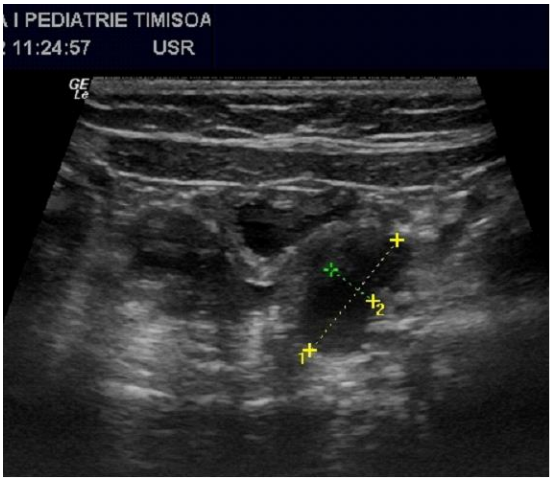

Fig. 2. Ovarian cyst under 2 cm in a 4 months old girl.

No complications were noted (torsion, haemorrhage, peritonitis, bowel obstruction or respiratory distress).

Periodic ultrasound examinations revealed a tendency towards spontaneous regression of the cysts. Most of the cases resolved spontaneously by the age of one year.

Discussions

NOC are being diagnosed more often now that routine ultrasonography is carried out antenatally and postnatally. A truly cystic abdominal mass in a female newborn is most likely to be an ovarian cyst, although duplication cyst or mesenteric cyst should be considered in the differential diagnosis. There is controversy about the best treatment for these cysts, opinions ranging from oophorectomy to follow up by ultrasonography alone.

Follicular ovarian cysts in fetuses and neonates are common, and increase in frequency with advancing gestational age and some maternal complications, such as diabetes mellitus, preeclampsia, and rhesus isoimmunization, in which the large placentas determine elevated placental chorionic gonadotropin levels. Under the hormonal influence, follicular cysts develop in the fetus, and can be seen on ultrasound by 28 to 32 weeks gestation [15]. In our case, 36% of the infants originated from pregnancies complicated by maternal diabetes, toxemia and Rh isoimmunization. Simple ovary cysts were detected by ultrasound in two cases diagnosed with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. (Fig.3)

Serum estrogen and HCG levels experience a postnatal decrease, as the maternally produced hormone levels fall. Fetal gonadotropins (FSH and LH) decrease during the last trimester of pregnancy, but increase subsequently after birth, reaching a peak at 3 to 4 months of age, probably as a response to the postnatal fall in estrogen. [16] The increase in gonadotropins after birth and persistence in the first few months of life is attributed to immaturity of the hypothalamic–pituitary–ovarian axis ("gonadostat"). Once the "gonadostat" matures, the hypothalamus and pituitary become sensitive to the negative feedback of low levels of sex steroids, resulting in the fall of FSH and LH to normal, prepubertal levels. [17,18]

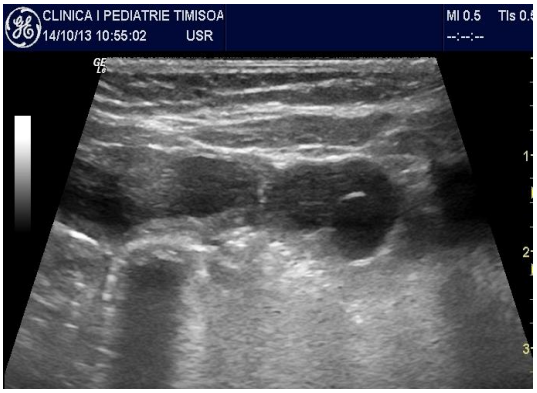

Fig. 3 Left ovarian cysts in a 5 months girl with congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

Simple cysts less than 2 cm in diameter are considered physiologic. Larger and complex cysts are more likely to be non-physiologic. Since the cysts usually result from hormonal stimulation, NOC patients do not associate chromosomal and congenital malformations. [19] None of the infants from our study presented any anomalies.

The differential diagnosis of a neonatal intraabdominal cystic mass includes: genitourinary tract disorders (eg, reproductive tract anomalies, urinary tract obstruction, urachal cyst), gastrointestinal tract disorders (eg, mesenteric or omental cyst, volvulus, colonic atresia, intestinal duplication), and miscellaneous disorders (eg, choledochal, splenic, or pancreatic cyst, lymphangioma).

The treatment of ovarian cysts is not standardised; it depends on the size and appearance of the cyst based on sonographic examination. Some authors report that complex but asymptomatic neonatal ovarian cysts have a natural tendency towards spontaneous resolution and, therefore, recommend conservative approach through clinical and sonographic monitoring. Postnatally asymptomatic ovarian cysts smaller than 5 cm in diameter, even exceeding 5 cm at initial diagnosis, with tendency to regress should be closely monitored until spontaneous resolution. If they regress spontaneously, no surgical intervention is necessary independent of their sonographic appearances. Symptomatic cysts or cysts with a diameter greater than 5 cm that do not regress or enlarge should be surgically treated [20].

Conclusions

Ovarian cysts are seen more frequently than expected in the neonatal period. Ovarian cysts are the rule, not the exception in newborn infants. In our case, due to the fact that all cysts were small (< 4 cm), and in the absence of complications, it has been possible to apply a wait-and-see policy, assessing the course of this condition by means of follow-up program - non-invasive periodic ultrasound monitoring.

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INTESTINAL INFARCTION THROUGH SECONDARY VOLVULUS – TWO CASES FROM PEDIATRIC SURGERY CLINIC CLUJ-NAPOCA

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Abstract

This article presents two case reports of intestinal infarction which were successfully managed in the Department of Pediatric Surgery, Emergency Hospital for Children Cluj-Napoca. In the first case a volvulus secondary to a congenital malrotation in a 15 days old neonate is described. The second case is focused on a segmental volvulus caused by adhesions in a 16 years old female patient.

Key words: malrotation, volvulus, intestinal infarction

Introduction

Intestinal ischemia consists of the interruption of the blood flow in the irrigation area of the superior or inferior mesenteric artery, that results in intestinal infarction – the hemorrhagic necrosis of the intestines [1]. Intestinal ischemia is a rare condition in neonatology and paediatrics.

Volvulus is a special form of mechanical intestinal and vascular obstruction which results from abnormal twisting of a loop of bowel around the axis of its own mesentery in malrotation [2]. Mesenteric rotation causes vascular insufficiency, and ischemia; infarction occurs in approximately 50% of cases [3]. Surgical intervention is necessary to avoid intestinal infarction necrosis of the bowel [4].

Volvulus can be primary, without any predisposing anatomic abnormalities and risk factors, or secondary, caused by anatomical anomalies (midgut malrotation, congenital fibrous bands [3,5]) or acquired lesions (postsurgical adhesions) [6-7].

Cases presentation

Case 1. Malrotation with volvulus

B.M. a two days old neonate was hospitalised in the Pediatric Department for repeated nonbilious, nonfeeding related vomiting. At this stage a clinical diagnosis of maternofetal infection was proposed, but the antibiotic treatment with Ampicilinum didn't lead to a favorable evolution. The patient kept on vomiting accompanied by mucosanguineous stools, so he was transferred to the Pediatric Surgery Department for further investigation and treatment.

At admission in the Surgical Department the clinical examination was suggestive for dehydration (dry skin and mucosa) with decreased blood pressure (70/40 mmHg), heart rate (40 beats/ minute) associated with distended abdomen and oliguria. Laboratory investigations revealed metabolic alkalosis, hypochloremia and hyponatremia. The radiological examination with contrast agent showed the opacification of the stomach until the distal part of the duodenum without a further passage (Fig. 1). The abdominal ultrasound showed a distended, fluid-filled jejunum with diminished or absent peristaltic.

The patient was rehydrated and was given a large spectrum antibiotic treatment with Meropenem and Teicoplanin.

After the initial fluid and electrolyte resuscitation the patient was taken to the operating room where jejunal atresia, a 360º intestinal volvulus on a common mesentery and secondary intestinal perforation with generalized peritonitis were discovered. The intestinal derotation, segmental resection of the necrotized jejuno-ileum part which included the atretic portion of 30 cm (Fig. 2) were performed followed by a lateral jejunostoma with the resection of adherences, the lavage and drainage of the peritoneal cavity. At a microscopic level the resection piece presented necrotic areas which involved all the width of the intestinal wall (Fig. 3).

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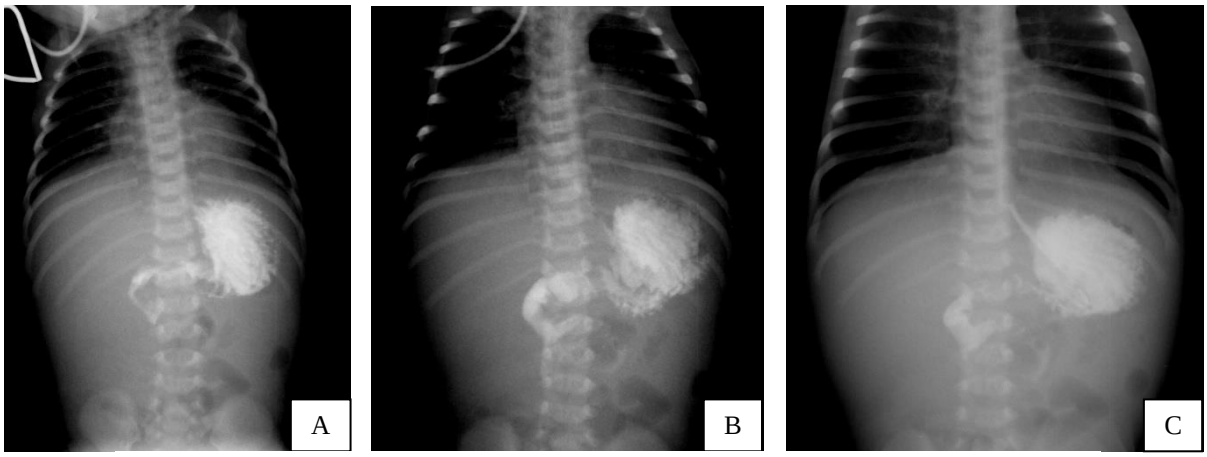

Fig. 1 Radiological examination with contrast agent: postduodenal digestive obstruction; A –30 seconds capture; B –10 minutes capture ; C – 20 minutes capture.

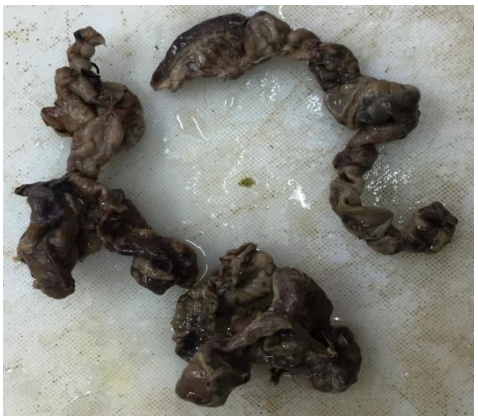

Fig. 2 Macroscopic aspect of the resection piece (at 24h after formol fixation): 3 jejuno-ileum segments of resection.

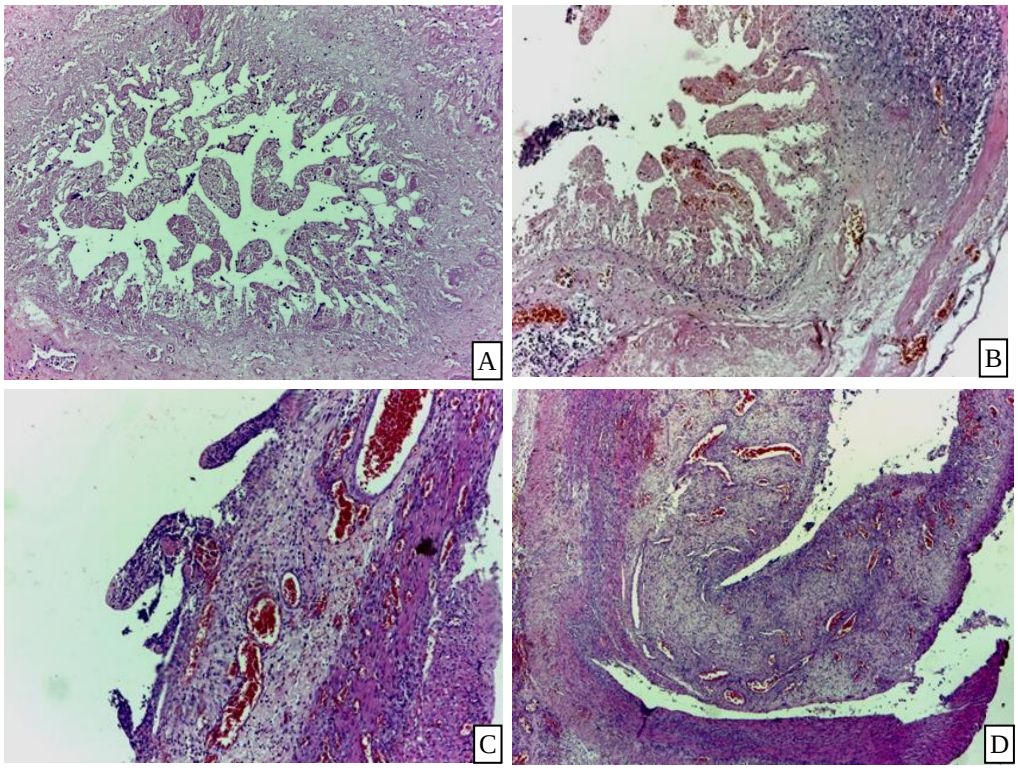

Fig. 3 Microscopic aspect (jejunum, HE, 40x HE, 40x): A – complete coagulation necrosis areas of mucosa (ischemia) of the intestinal wall: transmural infarction; B – necrotised mucosa and submucosa with a normal muscular layer; C – villosities necrosis; D – necrotised area, ulcerated and replaced by a granulation tissue.

After the surgical intervention, the patient was transferred to the neonate intensive care unit in order to continue monitoring. The postoperative course was uneventful with a relatively good digestive tolerance for delactosed milk-powder.

Case 2. Segmentary volvulus through postsurgical adhesions (band)

J.A., a 16 years old girl patient arrived in the pediatric emergency department with epigastric pain that actually started within one hour prior the arrival. Laboratory investigations were within normal range and the transabdominal ultrasound didn't show any pathological aspects. At this stage the clinical diagnosis of a dyspeptic syndrome was proposed and a treatment with omeprazolum and sucralfate was prescribed. In the following two days, the abdominal pain persisted, being accompanied by repeated emetic episodes. The patient was hospitalized in the III Pediatric Clinic Cluj-Napoca on 11/04/2013.

The patient was appendicectomizated 14 months prior to admission.

Her abdomen was asymmetrically distended with tenderness all over.

The transabdominal ultrasonography revealed at the right side of the abdomen, under the umbilical line, a distended ileum, without peristaltic movements, with both liquid and solid content that could not be compressed with the transducer (Fig. 4). The Doppler mode examination didn't point out any arterial blood flow signal in the intestinal wall. The abdominal plain radiograph revealed multiple hydroaeric levels.

The clinical, laboratory and imagistic findings were highly suggestive for acute abdomen associated to intestinal necrosis so the patient was transferred to the Department of Pediatric Surgery Cluj Napoca.

The patient was immediately taken to the operating room. The abdomen was accessed through a midline laparotomy. Intra-operatively, a segmental intestinal volvulus determined by a cecum adherence and terminal ileum secondary necrosis were found (Fig. 5). A segmental resection of the affected portion and a termino-lateral ileocolic anastomosis were performed followed by the lavage and drainage of the abdominal cavity.

Microscopically, the resection sample presented a haemorragic necrosis affecting all the width of the intestinal wall (Fig. 6). At the surface of the adjacent mesentery fibrin deposits could be seen.

The patient was discharged on postoperative day 8 without further complications.

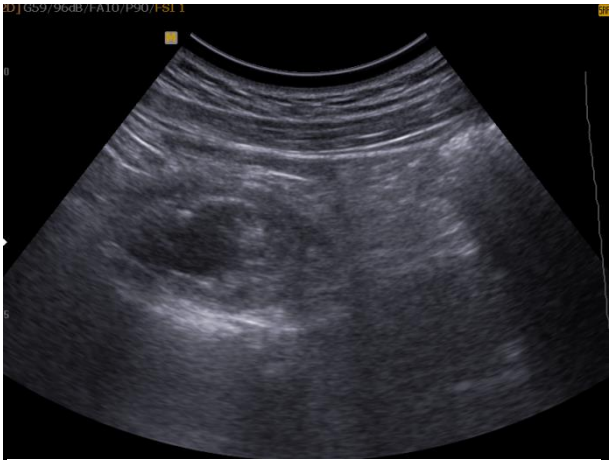

Fig. 4 Transabdominal ultrasonography: aperistaltic and distended ileum, with fluid and solid content.

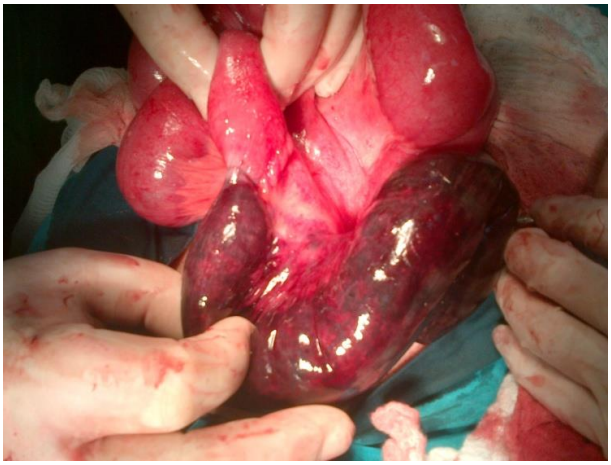

Fig. 5 Intraoperative image: adherence; terminal ileum necrosis.

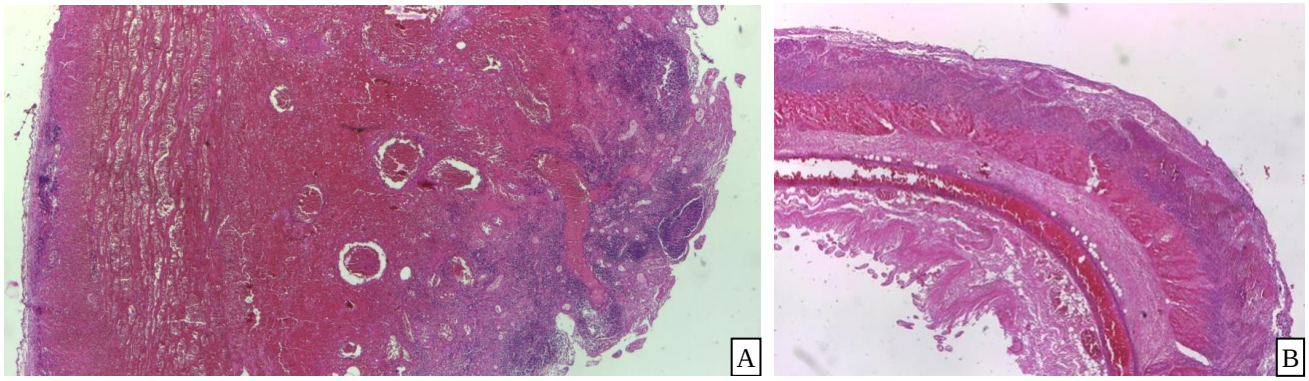

Fig. 6 Microscopic aspect (ileum, HE, 40x): A – intestinal infarction extending to the whole width of the intestinal wall; B – intestinal infarction with mucosal necrosis.

Discussions

Malrotation with volvulus is one of surgical emergencies of infancy and childhood [8].

The process of intestinal rotation begins during the fifth gestational week and involves a series of steps during which the bowel undergoes counter-clockwise rotation around the superior mesenteric artery. This physiologic fixation keeps the ascending and descending colons anchored in the right and left abdominal gutters: the ligament of Treitz in the upper left and the cecum in the lower right [9].

Disturbances in the normal rotational pattern of the midgut will result in a spectrum of malrotation possibilities. Intestinal malrotation is a developmental anomaly affecting the position and peritoneal attachments of the small and large bowels; it has been defined as absent or incomplete rotation and fixation of the embryonic gut around the superior mesenteric arteryand predisposes to midgut volvulus. In malrotation, the right colon can be abnormally positioned, resulting in aberrant attempts at fixation; these attempts cause the colon to develop attachments to the right side of the retroperitoneum that have become known as Ladd's bands [9-11].

Symptomatic malrotation is a diagnosis usually made in the newborn and infant; traditional teaching says that up to 75% of cases occur within the newborn period and up to 90% of cases occur within the first year of life [8]. However, intestinal malrotation can occur in patients of any age [12]. Patients with anorectal malformations and two or more VACTERL anomalies should undergo screening for malrotation [13].

The classic clinical presentation of *midgut volvulus* on malrotationis of aninfant discharged from the hospital after birth, only toreturn with bilious vomiting. However, the clinical picture is frequently ambiguous and the child might present with nonbilious vomiting, diarrhea, suspected sepsis, shock or gastrointestinal bleeding; symptoms can be long standing with signs of malabsorption and failure to thrive [14].

Proximal large bowel volvulus is considered as an extremely rare surgical emergency in children. A child with neurodevelopmental delay and a history of constipation presenting with an acute onset of colicky abdominal pain and progressive abdominal distension with vomiting should be suspected of having a cecal and proximal large bowel volvulus [15-16]. Also, in infants and children, *sigmoid volvulus*is exceedingly rare [17].

In acute obstruction through midgut volvulus, the simple radiographs most often show air in the stomach with little or no distal bowel gas. The radiographs may be useful in determining if there is a distal bowel obstruction or free intraperitoneal air [18].

The gold standard diagnostic investigation for intestinal malrotationis the upper gastrointestinal (UGI) contrast study. It may be performed via a nasogastric tube. Thus, barium can be injected into the stomach in a controlled fashion, avoiding overfilling of the stomach. The normal position of the duodenal-jejunal junction (DJJ) is a critical anatomic landmark at UGI imaging. The DJJ should be located to the left of the left vertical body pedicle at the level of the inferior margin of the duodenal bulb; it should also be located posteriorly on the true lateral view since it is a retroperitoneal structure [19]. There are variations that may cause the DJJ to be displaced either inferiorly or medially. Situations that may mimic malrotation with abnormal DJJ position include splenomegaly, liver transplant, gastric overdistention, small bowel obstruction, and spinal curvature. In children under the age of 4 years, the normal peritoneal ligaments are lax and so the DJJ may be manually displaced [20]. Therefore, UGI contrast study can occasionally be misleading. There is a significant rate of negative laparotomy following diagnosis of malrotation on UGI contrast study [19, 21].

The role of the enema therefore is a secondary one and may help to determine the position of the cecum and colon in indeterminant cases [18].

The surgical approach to malrotation with or without ("Ladd's procedure") consist of: (1) detorsion of the bowel when volvulus is present, (2) lysis of peritoneal bands, (3) broadening the mesentery to separate the duodenum and cecum as far away as possible, (4) placement of the small bowel to the right side of the abdomen, and (5) placement of the colon to the left side of the abdomen [22]. Historically, surgeons used to perform pexy of small bowel loops to the parietal peritoneum [23].

In case 1, the repeated emesis in a newborn should first of all suggest a congenital digestive malformation. The emesis in the first days could also point out a neonate with a maternofetal infection, the mother being the infected one or just a carrier.

Probably, at the arrival in the pediatric department the neonate was septic due to the peritonitis which appeared consequently to the intestinal perforation (less probably a congenital peritonitis). This case was interpreted as a maternofetal infection and was treated with antibiotics followed by fluid and electrolyte resuscitation.

The alternation of the symptoms with short periods of amelioration was due to the repeated episodes of volvulus (Fig. 3D) with subsequently intestinal ischemia and the excretion of necrotising mucosa as mucosanguineous stools. Consequently, the intestinal infarction lead to perforation and peritonitis. The septic exacerbation was hidden by the antibiotic treatment.

Clinical examination and laboratory investigations suggested medium to sever dehydration characterized by hypovolemic hypotonic hyponatremia with fluid loss in the third space, metabolic alkalosis due to the repeated loss of H^+ during each emetic episode and hypochloremia given to the loss of Cl^- within the HCO_3^-/Cl^- exchanger.

Taking into account the length of the jejuno-ileal portion, a short bowel syndrome should not be excluded.

Peritoneal adhesions may be classified as congenital (embryological anomaly in the development of the peritoneal cavity) or acquired (inflammatory or

postsurgical). Postsurgical adhesions, which constitute the majority of the peritoneal adhesions, develops as a result of the wound healing process (injured tissue surfaces following incision, cauterization, suturing or other means of mesothelial trauma) and can be associated with any kind of abdominal surgery [24-25]. Inflammatory response has a pivotal role in peritoneal adhesion formation through immune cells and mediators [26-28].

Intestinal obstructions, chronic pelvic pain and female infertility are associated postoperative problems with adhesion formation [29].

Adhesions are an important cause for long-term complications in both open and laparoscopic surgery; adhesiolysis during reoperations seems to impact adhesion-related morbidity most [30-31].

Complete adhesion prevention is an unsolved problem, and the search for an ideal antiadhesion agent is still ongoing [32].

In case 2, the adherences appeared consequently to the prior appendicitis surgery. The main complaint was the pain followed by emesis due to the occlusive syndrome. The presence of daily stools most probably originating from the subjacent portion of the volvulus area was misleading, but explanatory for the late diagnosis of the intestinal occlusion.

Conclusions

Midgut volvulus continue to represent a complex problem for surgeons and radiologists. Midgut volvulus can lead to necrosis of the midgut with significant mortality. An interdisciplinary collaboration is important, since patients are not initially evaluated by surgeons.

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THE ROLE OF PATHOLOGIST TO GUIDE THE DIAGNOSIS IN A CASE OF RECURRENT BLOODY DIARRHEA IN A YOUNG CHILD

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Abstract

Introduction: Primary eosinophilic gastro-intestinal disorders represent a specific group of inflammatory diseases characterized by the presence of eosinophilic infiltrates of the digestive wall. These disorders are classified into eosinophilic esophagitis, gastro-enteritis and colitis, the last one being the rarest described. *Objectives:* This paper presents a case with eosinophilic colitis in a 3 years old boy which was considered at the onset as Crohn's disease due to common clinical manifestations and similar endoscopic findings, along with an initial inconclusive histopatological interpretation of the biopsy sample. *Case presentation:* Young male, without significant postnatal medical history, at the age of 3 years old presented an episode of acute bloody diarrhea without fever. The investigations performed at the local county hospital, including colonoscopy, established the diagnosis of Crohn's disease. Oral systemic corticosteroids were initiated. After tapering the doses, the symptoms reappeared. The case was addressed to our University Hospital for further investigations. Upon admission, the child didn't present relevant changes at clinical examination. Inflammatory tests were negative, with moderate peripheral eosinophilia, increased total IgE serum level, increased level of specific IgE antibodies against casein/lactalbumin/beta-lactoglobulin. Fecal calprotectin was increased, colonoscopy showed disseminated aphtoid lesions separated by normal mucosa from descendent colon to cecum. Histopathological examination revealed inflammatory infiltrates composed by lymphocytes/eosinophils (~ 30 eosinophils/field). We sustained the diagnosis of eosinophilic colitis associated to cow's milk proteins allergy. After dairy exclusion, with systemic corticosteroids and leukotriene inhibitors, the evolution was favorable. *Conclusions:* A number of pediatric disorders may present similar clinical manifestations with Crohn's disease: infectious enterocolitis/cow's milk protein allergy/eosinophilic colitis. Fecal calprotectin and colonoscopy examination don't have maximum accuracy for differential diagnosis. Histopatological examination is the most specific tool for defining the diagnosis.

Key words: eosinophilic colitis, Crohn's disease, colonoscopy, biopsy

Introduction

During the last few years the incidence of inflammatory bowel diseases (IBD) has risen among the pediatric population. The negative impact upon the normal growth and development process increased the interest of pediatrics specialists towards this pathology. Many other chronic pediatric enteropathies can have similar clinical symptoms as Crohn's disease: cow's milk protein allergy, eosinophilic colitis, intestinal tuberculosis, celiac disease, cystic fibrosis, recto-sigmoid juvenile polyps.

Primary eosinophilic gastro-intestinal disorders represent a specific group of inflammatory diseases characterized by the presence of eosinophilic infiltrates of the digestive wall. These disorders are classified into eosinophilic esophagitis, gastro-enteritis and colitis, the last one being the rarest described. Eosinophilic colitis is a heterogeneous entity with a bimodal distribution of age of onset and can be presented as an acute diarrheal disease in infants and young children, or as a recurrent colitis in teenagers. Pathogenesis of primary eosinophilic colitis is partially elucidated, being incriminated IgE mediated food allergy to certain food proteins or delayed type allergy non IgE mediated and T lymphocyte associated. (1)

Given the nonspecific symptoms, most commonly abdominal pain, constipation/diarrhea and rectal bleeding that are associated with eosinophilic colitis, the lack of distinctive clinical findings and its relapsing-remitting course made the examination of colonic biopsy the golden standard for diagnosis. Eosinophils are easily visible in routine haematoxylin-eosin (HE) stained paraffin embedded sections and can be assessed semi-quantitatively. At present, no consensus has been reached on the histological criteria required to make the diagnosis of eosinophilic colitis.(2)

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There is no consensus over its diagnosis and management and uncertainty is compounded by the use of the same term to describe an idiopathic increase in colonic eosinophils and an eosinophilic inflammatory reaction to known aetiological agents such as parasites or drugs. In patients with histologically proven colonic eosinophilia, it is important to assess the underlying causes and to integrate the medical history of the patient with clinical and laboratory data.(3)

Eosinophils respond to stimuli, including trauma, infections and allergens, by degranulating to release inflammatory mediators including leukotrienes, vasoactive intestinal polypeptide, tumour necrosis factor and interleukins. Eosinophils density in the colon is increased in various disorders including food allergy, parasitic infections and IBD, but in some patients no underlying gastrointestinal pathology is identified and in these cases a diagnosis of primary eosinophilic colitis can be made.(4)

Since tissue eosinophils are increased in many chronic inflammatory conditions, there is a potential for misdiagnosis of early IBD as eosinophilic colitis. In contrast to IBD, the architecture of the colonic crypts in eosinophilic colitis is normally preserved. In doubtful cases with prominent eosinophils, especially if symptoms worsen, rebiopsy after several months may be necessary to exclude IBD.(5)

Case report

The authors present the case of a 3,5 years old child, male patient, who has been admitted for diarrhea and stools with blood streaks (3-4/day) without having fever.

The child comes from a young, healthy couple from rural environment. He was borne at term, weighting 3600 grams. The child was vaccinated according to the national programme, he has been breastfed until 2 years of age and, the complementary feeding has been properly initiated at the age of 6 months old.

As past medical history, at 3 years old, the child presented the first episode of acute diarrhoea with blood streaks, without fever and he was hospitalised at the local County Hospital, Paediatric Ward. After running a set of investigations, infectious enterocolitis was ruled out, Colonoscopy was performed and the endoscopist described redness and small ulcers disseminated form the sigmoid level until the cecum area.Ileo-cecal valve and terminal ileum were normal. Biopies were taken. The histopathological examination showed inflammatory infiltrate consisting predominantly of plasma cells, lymphocytes and several eosinophils, micro abscess with massive necrosis and frequent macrophages. The conclusion was: pancolic Crohn's disease. Oral systemic corticosteroids were initiated. After tapering the doses, the symptoms reappeared and the case was addressed to our University Hospital for further investigations.

Clinical examination upon admission showed normal somatic development for his age - weight 16 kg, height 97 cm. There weren't any relevant changes at clinical examination except the digestive system: painful abdomen at palpation, diarrhea with streaks of fresh blood. There was not liver or spleen enlargement.

The laboratory tests showed normal parameters for total blood count except moderate peripheral eosinophilia (12% eosinophils). Inflammatory tests were negative. Liver and renal tests were normal. Blood coagulation parameters were normal. Assessment of serum immunoglobulines levels IgA, IgG and IgM were within normal values for age. We found increased total serum IgE level, increased level of specific IgE antibodies against casein/lactalbumin/beta-lactoglobulin and egg white. Fecal calprotectin level was increased. We ruled out infectious causes of enterocolitis. Intestinal tuberculosis was also ruled out by performing Quantiferon test that was negative. We ruled out congenital immunodeficiency, celiac disease, cytomegalovirus (CMV) and human immune deficiency virus (HIV) infection.

The laboratory results are summarized in table 1.

Abdominal ultrasound showed normal aspect.

In case of a pediatric patient with recurrent bloody diarrhea considered as steriod dependend Crohn's diseases according to previous endoscopic assessment along with an initial inconclusive histopatological interpretation of the biopsy sample, endoscopic re-assessment become mandatory.

In such a case it is important to integrate clinical and medical history data with laboratory tests and histopathological results. Good clinical status with normal development, along with peripheral eosinophilia, increased total IgE serum level and elevated specific IgE against dietary proteins must drown attention to any clinician to the diagnosis of cow's milk proteins and egg white allergy that could mimic IBD in a young child. The association of IgE mediated alimentary allergy with eosinophilic colitis is well known and the histopathologic report must guide the diagnosis.

We performed upper endoscopy and we repeated the colonoscopy taking multiples biopsies samples from this patient.

Upper endoscopy showed esophagus, stomach and duodenum with normal endoscopic appearance. Colonoscopy showed rectum and sigmoid with normal mucosa. From descending colon to the cecum there were aphtoide lesions/small ulcerations, separated by normal mucosa and there was inflammation of ileo-cecal valve. Serial biopsies were taken.

Figure 1 shows endoscopic aspect of the descending colon.

Histopatological examination showed lymphocytic and eosinophilic infiltrate in the lamina propria (~ 30 eosinophils/field). Glandular architecture was preserved with normal appereance. PAS stain showed no microbial colonies/parasites.

Figures 2 and 3 show histopathological images with dense eosinophilic infiltrate especially in the descending colon.

Table 1: Laboratory results.

Hemoglobine Erytrocytes	12.5 g % 4327000/mmc	Inflammatory tests	ESR=10 mm/h CRP=0.40 mg/l Fibrinogen=2 g/l
Leukocytes Formula	7600/ mmc Sg=21% Ly=62% Eo=12% Mo=7%	Bleeding time Coagulation time INR APTT	2 min 3 min 15 sec 1 35 sec
Platelets	225000/mmc	Iron level	12 µmol/l
Blood gases level	pH = 7.37 pCO2 = 32.3 mmHg, BE = -2 mmol/l HCO3 = 22 mmol/l	Serum elektrolytes	Na=140, K=4.16, Ca= 2.75, Cl=102 mmol/l
Proteinemia	60,6 g/l	Glicemia	4 mmol/l
ELFO	Albumines = 64.3%, α1=2.1%, α2=9.9%, β=10%, γ=13.7%	Immuno-globulines serum level	IgA = 0,71 g/l IgG = 14,37 g/l IgM = 2,04 g/l
Liver function	GPT = 25 U/l GOT = 37 U/l Gamma GT = 42 U/l Alkaline phosphatase = 215 U/l	Stool analysis	Stool cultures: negative Rotavirus/Adenovirus Antigen: negative Clostridiun Difficile stool toxin A/B: negative Coproparasitologic exams: repeated negative Coprochemical exam: stool pH = 6, starch, muscle fibers, fat: absent Fecal calprotectin > 60 mg/g (Cal-Detect kit)
Renal function	Ureea =1.25 mmol/l Creatinine=62 µmol/l	Other tests:	IgA anti tissue transglutaminase and anti-endomisium antibodies = negative Quantiferon test = negative IgM/IgG CMV antibodies = negative HIV test = negative
Total serum IgE level	425 IU/ml (normal values < 60 IU/ml)	Elevated specific IgE against casein, alpha lactoglobulin, beta lactoglobulin and egg white (class 2 and 3).	

APTT: activated partial thromboplastin time, INR: international normalized ratio, CMV:cytomegalovirus, HIV: human immune deficiency virus.

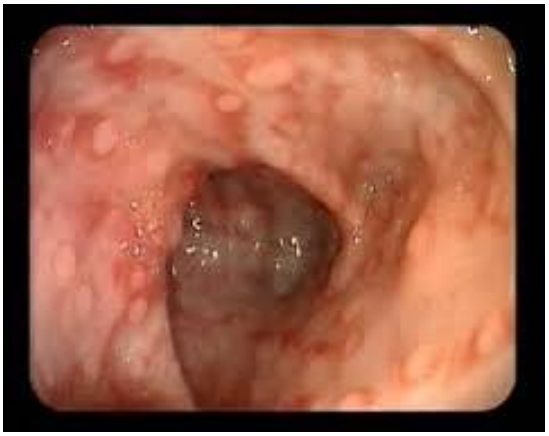

Figure 1: Endoscopic aspect of descending colon with multiple aphtoide lesions separated by normal mucosa.

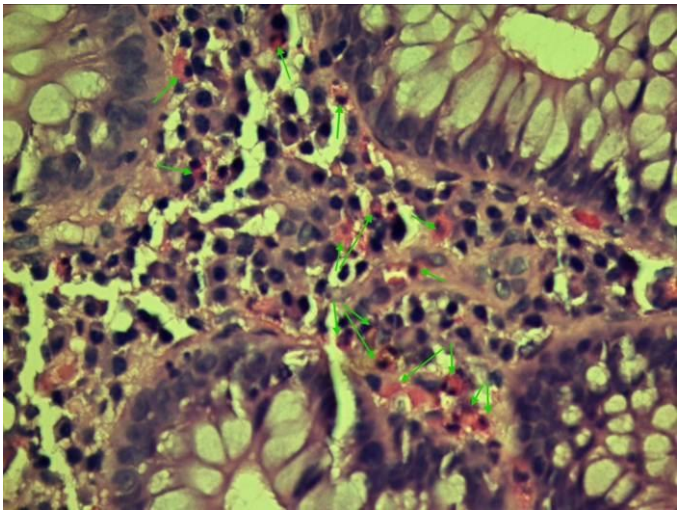

Figure 2. Descending colon biopsy, MOx200, HE staining. Lymphocytic and eosinophilic infiltrate in the lamina propria.

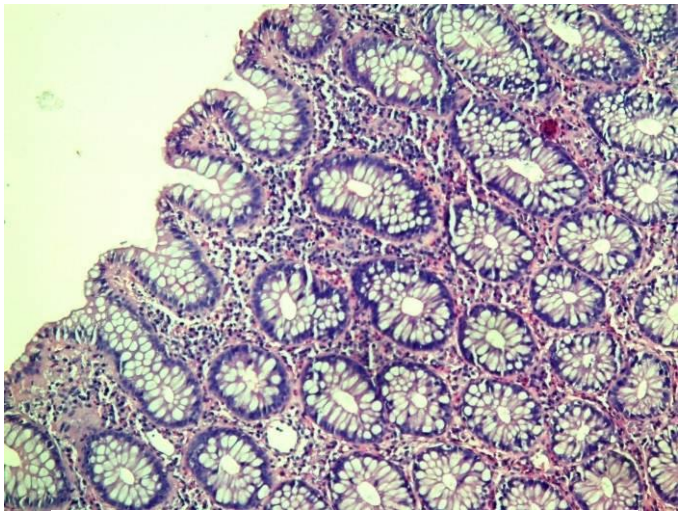

Figure 3: Transverse colon biopsy, MOx100, HE staining. Lymphocytic and eosinophilic infiltrate in the lamina propria. Normal glandular architecture.

The massive eosinophilic infiltrate in the colonic mucosa along with peripheral eosinophilia and increased total and specific IgE serum level guided the diagnosis to eosinophilic colitis associated to cow's milk proteins and egg's white allergy in this case.

In order to achieve symptoms resolution, an important part of the treatment consists in diet. In this case, we excluded dairy and egg's white from the child's diet and we recommended a semi-elemental formula based on extensive proteins hydrolysates. The medication consisted in systemic oral corticosteroids - Prednisone 1 mg/kg/day, 4 weeks, with progressive withdrawal, aminosalicylates - Mesalazine 50 mg/kg/day, leukotriens inhibitors: Montelukast 5 mg/day, antihistamines - Aerius 2.5 ml/day and probiotics.

During hospitalization, the evolution was favorable, with disappearance of rectal bleeding and normalization of stools. After corticosteroids tapering, the child remained in good clinical condition, the somatic development was within normal ranges. There were no relapses, under semi-elemental formula and exclusion diet, along with mesalazine and leukotriene inhibitors for 1 year as maintenance therapy. The child was complaint to the diet and medical treatment. The prognosis is favorable on long-term, with the possibility to recover tolerance to allergenic foods after the age of 5-6 years according to some authors. (6)

Discussions

Eosinophilic colitis was first described in 1936 and the term first appeared in the literature in English in 1959 (7), (8). In 1985 Naylor and Pollet reviewed 22 cases of eosinophilic colitis: no common aetiology was identified, though food allergies, drug reactions, and parasites were reported in several cases.(9)

Drugs reported to cause colonic eosinophilia include nonsteroidal anti-inflammatories, tacrolimus, rifampicin, carbamazepine. Particular attention should be paid to the temporal relationship between drug administration and symptoms and colonic eosinophilia should not be attributed to a drug reaction without adequate clinical-pathological correlation.(10)

In 1990, the publication of a series of thirteen cases of allergic colitis that had initially presented before two years of age, identified a specific subtype of eosinophilic colitis: a treatable disorder of early childhood that was caused by food allergy (usually to eggs, milk, or soya) and was of limited duration, typically remitting entirely with an appropriate exclusion diet. (11)

Eosinophilic colitis has been described in association with eosinophilia elsewhere in the gut using the term eosinophilic gastrointestinal disorder. However, evidence linking eosinophilic colitis with eosinophilic infiltration elsewhere in the gut is lacking: a recent review of eosinophilic gastrointestinal disorder concluded that eosinophilic colitis has a different pathophysiology and is probably best regarded as a separate entity. (12)

According to some reports, there is the possibility to diagnose an overlap syndrome between eosinophilic colitis and IBD in children. One study has described Crohn's colitis with a heavy eosinophilic infiltrate as eosinophilic-Crohn overlap colitis.(13) Pensabene et al found a higher overall colonic eosinophil density in children with IBD compared to those with food allergies (14), which suggests that it may not be possible reliably to diagnose eosinophilic colitis in the presence of inflammatory bowel disease, especially Crohn's disease, where eosinophils are typically more numerous than in ulcerative colitis. (15) Patients with eosinophilic colitis may show peripheral eosinophilia and there is a statistically significant association between colonic eosinophil density and elevated total serum IgE levels.(16) It has been proposed that gut eosinophilic disorders are IgE-

mediated through the high-affinity receptor FcepsilonRI. (17)

The onset of food protein-induced enterocolitis, especially cow's milk protein allergy, appears usually in the first year of life. In the case presented above, there are no details about symptoms during infancy regarding history of atopy or digestive manifestations of cow's milk protein allergy. From his medical past history, we found out that the child has been breastfed for 2 years. This fact could induce a degree of oral tolerance and could delay the onset of proteins' allergy in this case.

We rejected the first established diagnosis of Crohn's disease at this young patient due to histology result in the first place, along with missing clinical and laboratory findings characteristic to Crohn's disease as: failure to thrive, anemia, inflammatory syndrome. This patient presented in a good clinical state, with normal somatic development for his age, without anemia, signs of intestinal malabsorption or inflammatory syndrome. Peripheral eosinophilia and increased total serum IgE level along with elevated specific IgE against cow's milk proteins represent important markers for the diagnosis of eosinophilic colitis associated to cow's milk protein allergy. Moreover, the histopatological examination didn't reveal any granulomas and it showed normal glandular architecture that could ruled out Crohn's disease. In contrast to IBD, the architecture of the colonic crypts in eosinophilic colitis is normally preserved. Another argue that could rule out Crohn's disease in this case is the favorable evolution of this patient without relapsing after corticosteroids withdrawal, following exclusion diet, semi-elemental formula and leukotriene inhibitors as maintenance therapy.

Colonic eosinophilia may be a significant finding in some symptomatic individuals but there has been no consistency regarding the cut-off point above which eosinophil density should be regarded as increased, the region of colon to be assessed, or the number of microscopic fields examined. Several studies have reported geographic variation in eosinophil density in the normal colon. A detailed study on endoscopic material from normal colon carried out by DeBrosse found a gradient of eosinophil density from ascending colon to rectum from 20/field to 8/field, respectively. (18) As eosinophil density varies with site, it would be important to set a limit for each site or to use a mean value across the whole colon in order to diagnose eosinophilic colitis.

In addition to counts of total eosinophil density, some studies have assessed degranulation as an indicator of eosinophil activation. It is possible to observe degranulation

in routinely stained sections and to grade it semi-quantitatively; however, it is not known if the trauma of biopsy could provoke degranulation of otherwise inactive eosinophils. (19) The uncertainty over the diagnosis of eosinophilic colitis emphasises the need for proper clinical-pathological correlation.

Conclusions

A number of pediatric intestinal disorders may have similar clinical findings as Crohn's disease: infectious enterocolitis, cow's milk protein allergy, eosinophilic colitis, malabsorption syndromes, celiac disease etc.

Fecal calprotectin and colonoscopy don't have maximum accuracy for differential diagnosis, histopathology is defining for diagnosis.

Eosinophilic colitis is often under-diagnosed. Colonic eosinophilic infiltration can be parceled, imposing the need for serial biopsy sampling for diagnosis.

In patients with histologically proven colonic eosinophilia, it is important to search the underlying causes and to integrate clinical status of the patient with medical history data and laboratory tests.

The lack of distinctive clinical symptoms or laboratory anormalities associated with eosinophilic colitis imposed to establish the diagnosis by examination of colonic biopsies. There is no consensus over its diagnosis and management. In the future it will be necessary to standardize the diagnosis of eosinophilic colitis by establishing clear histological criteria, including a limit of colonic eosinophils above which the diagnosis will be made.

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PERSISTENT DUCTUS ARTERIOSUS – AN IMPORTANT RISK FACTOR FOR NEONATAL MORBIDITY AND MORTALITY IN VERY LOW BIRTH WEIGHT PRETERM INFANTS

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Abstract

Introduction: Ductus arteriosus (DA), an important vascular structure during fetal life, persists with increased incidence in preterm infants as gestational age (GA) and birth weight (BW) decreases and significantly alters the neonatal course. Severe perinatal complications of prematurity are occurring more often in association with persistent ductus arteriosus (PDA), increasing the neonatal mortality rate and negatively affecting the long-term outcome. *Aim:* To evaluate the impact on neonatal morbidity and mortality of PDA in very low birth weight preterm infants (VLBW) with GA ≤ 32 weeks. *Material and methods:* All VLBW infants with GA ≤ 32 weeks admitted to the neonatal intensive care unit (NICU) of the Clinical County Emergency Hospital Sibiu between 1 January 2010 and 31 December 2015 were included in the study. Epidemiological and clinical data were collected in the National Registry for Respiratory Distress Syndrome and comparatively analyzed using SPSS 10.0 for Windows; p was considered statistically significant if < 0,05 (confidence interval 95%). *Results:* 391 preterm infants with GA ≤ 32 weeks were admitted in the NICU, of whom 262 had BW ≤ 1500g. Of the 262 VLBW infants forming the study group 151 were diagnosed with PDA (57.3%). VLBW preterm infants with PDA had significantly lower GA and birth weights (p<0.05), were more often outborn (p=0.008, OR 2.26), and had significantly lower Apgar scores at 1, 5, and 10 minutes (p<0.05). Also, they needed more often surfactant (p=0.010, OR 1.92), mechanical ventilation (p=0.001, OR 2.59), longer oxygen therapy, and respiratory support (p<0.05). VLBW preterm infants with PDA had increased rates of bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), severe intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and severe retinopathy of prematurity (ROP) (p>0.05) but only the association with NEC was statistically significant (p=0.012, OR 5.57). Also, PDA was associated with increased risk of death in VLBW preterm infants (p=0.001, OR 3.40). Persistence of DA was associated with increased risk for unfavorable long term outcome as revealed by the association with a composite outcome comprising BPD, NEC, severe IVH, PVL, ROP, and death) - p=0.000, OR 2.81. No significant associations were found between PDA and neonatal sepsis. *Conclusion:* In accordance with data in the literature, PDA occurred in more than half of the VLBW infants and was associated with lower GA and BW, lower Apgar scores, and more severe respiratory distress syndrome. Also, PDA was associated with an increased incidence of neonatal mortality and increased the rates of the most severe complications of prematurity, increasing significantly the risks for unfavorable long-term outcome.

Key words: persistent ductus arteriosus, prematurity, very low birth weight infants, respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, neonatal mortality.

Introduction

Ductus arteriosus (DA) is an important vascular structure connecting the proximal ascending aorta with the root of the pulmonary artery, close to the left pulmonary artery origin[1]. Closure of DA the is an important process of the cardiovascular and pulmonary adaptation process after birth, influenced by many factors: increased arterial oxygen pressure, decreased pressure of the pulmonary blood flow, decreased concentrations of prostaglandin E2, and decreased number of prostaglandin E2 receptors are favoring the contraction of DA while hypoxia, acidosis, increased pulmonary resistance, increased sensibility to vasodilator effect of prostaglandin E2 and nitric oxide, down regulation of their receptors, and increased volume of fluids administered during the first days of life are hindering DA contraction[2-4]. Spontaneous functional closure of the DA occurs in 50% of the cases at 24 hours, in 90% of the cases at 48 hours, and in almost 100% of the cases at 72 hours of life in term infants[1,5], while anatomical closure is accomplished in about 2-3 weeks[1]. Failure of DA closure in preterm infants is attributed mostly to developmental immaturity[1,6].

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The term "patent ductus arteriosus" is assigned to describe both physiological and pathological situations when DA is open while the term "persistent ductus arteriosus" (PDA) defines a patent DA after the first 72 hours of life[7,8].

An incidence of 0.3-4/1000 live births at the end of the neonatal period was reported for PDA in infants delivered at term, representing 5-10% of all congenital heart defects[1,9]. A much higher incidence of PDA is reported in preterm infants, the rates increasing as GA and BW are decreasing[6], varying between 20-70% in very low birth weight infants (VLBW)[2,7,10-22].

In preterm infants, PDA is a multifactorial condition often affecting significantly the neonatal development, influencing hemodynamics by compromising the blood flow and oxygenation of all organs and systems. Decreased blood pressure with subsequent hypotension[7,23], renal dysfunction[24], cardiac congestive failure[17,18,25], pulmonary hemorrhage[7,21,26], apnea and prolonged duration of mechanical ventilation[25,27], feeding intolerance[6,9] are cited as effects of PDA during the first days of life, effects that are significantly contributing to increased rates of bronchopulmonary dysplasia (BPD)[7,19,22,25,26,28-34], necrotizing enterocolitis (NEC)[2,7-9,17-19,22,32,33,35-37], intraventricular hemorrhage (IVH)[17-20,22,32,33], periventricular leukomalacia (PVL)[38], retinopathy of prematurity (ROP)[39], and death[19,22,23,32,40-43]. On long-term, these severe conditions associated with prematurity are associated with increased risk for neurodevelopmental deficits and cerebral palsy[6,16,20,22,32,42] and delayed physical growth[9].

Unfortunately, currently there is no consensus as regards the management of PDA in preterm infants as most of the studies evaluating the influence of different therapeutic strategies - conservative management (including fluid restriction during the first days of life, waiting for spontaneous DA closure, and treatment of large PDA significantly influencing the respiratory support), early, presymptomatic or symptomatic pharmacological treatment, or surgical ligation[11] - showed that some therapeutic strategies may decrease the rate of conditions associated with PDA (BPD, NEC, IVH, ROP) and death but have no influence on long-term outcome of these infants[33,42,44-48]. Therefore, a better understanding of PDA influence on the preterm infants development is still needed in order to decrease its impact on short and long term prognosis.

Material and methods

All preterm infants with BW ≤ 1500 g (VLBW) and GA ≤ 32 weeks admitted to the neonatal intensive care unit (NICU) of the Clinical County Emergency Hospital Sibiu, a regional level III unit, between 1 January 2010 and 31 December 2015 were included in the study. Epidemiological and clinical data were collected retrospectively for 2010 and prospectively in 2011 in the National Registry for RDS. PDA was considered if ductal flow was visualized by color Doppler echocardiography after the 7th day of life, irrespective of its caliber and hemodynamic significance. The Romanian National Registry for RDS prospectively collects epidemiological data, information regarding birth, RDS severity and treatment, and on short term outcome in preterm infants with GA ≤ 32 weeks. For the present study we extracted and analyzed the following information: a) prenatal - maternal prenatal conditions, pregnancy complications, antenatal corticosteroid administration, preterm rupture of the membranes, pregnancy type, delivery mode, presentation; b) neonatal characteristics and data - GA, BW, ponderal index, SGA, gender, Apgar scores at 1 and 5 minutes, birth resuscitation and peripheral oxygen saturation during resuscitation at birth, surfactant administration, need and duration of oxygen therapy and respiratory support, neonatal sepsis, complications associated with prematurity (BPD, NEC, IVH, PVL, ROP, apnea of prematurity, neonatal sepsis), and death. All definitions used for neonatal conditions are based on the Vermont-Oxford trials network[49] except for BPD. BPD was diagnosed if positive-pressure respiratory support with any fraction of inspired oxygen (FiO_2) or supplemental oxygen were needed at 36 weeks corrected age. This definition was chosen since the protocol of our unit includes using continuous positive air (CPAP) pressure with room air as the method of choice for weaning from mechanical ventilation and CPAP is stopped when the patient achieves respiratory stability.

The VBLV preterm infants included in the study were divided into two groups: with and without PDA. Data are reported as values, mean values, standard deviations (SD), and percentages. SPSS 10.0 for Windows was used for data analysis. Independent t-test was used for scale variables while Fisher's exact test or chi square test (where appropriate) were used for the analysis of categorial variables. A $p < 0.05$ was considered statistically significant. Odds ratio were calculated using confidence intervals (CI) of 95%.

Results

During the 6 years study period, 391 preterm infants with GA ≤ 32 weeks were admitted in the neonatal intensive care unit of the Clinical County Emergency Hospital Sibiu, of whom 262 had BW ≤ 1500 g (VLBW) and comprised the final study group. The VLBW preterm infants in the study group had a mean GA of 28.8 ± 2.2 weeks (23-32 weeks gestation) and a mean BW of 1138.3 ± 243.7 g (500-1500 g). Of these 262 VLBW preterm infants 151 were diagnosed with PDA (57.3%) after the seventh day of life.

The mean GA of the preterm infants diagnosed with PDA was significantly lower than the mean GA of those without PDA - 28.5 ± 2.3 weeks versus 29.3 ± 1.9 weeks -, and significantly lower BW - 1085.4 ± 259.8 g versus 1210.2 ± 199.6 g - (Table 1). Presence of PDA was associated with decreased rate of prolonged rupture of the amniotic membranes (> 18 hours) and decreased gestational age at prenatal corticosteroid prophylaxis (Table 2). No significant differences were seen between infants with and without PDA as regards the gender, ponderal index, SGA status, presence and types of complications during

pregnancy, pregnancy type, delivery mode, and antenatal corticosteroid therapy (Table 2). A more than 2 fold increased risk for PDA was noted in association with delivery in lower grade hospitals and neonatal transfer to our level III unit after birth (Table 2). Lower Apgar scores at 1, 5 and 10 minutes were found in VLBW preterm infants with PDA but no significant difference was found between groups regarding the need for resuscitation at birth (Table 3). No differences were noted as regards the mean peripheral oxygen saturations and oxygen concentrations used during resuscitation at birth (Table 3). Severity of RDS was increased in very preterm infants with PDA, as revealed by increased need for surfactant administration and for mechanical ventilation, and prolonged length of CPAP respiratory support and oxygen therapy, although no difference in the need for oxygen therapy (Table 3). Severe conditions associated with prematurity as BPD, IVH grade III and IV, PVL grad II or III, ROP (requiring laser therapy) occurred more often in VLBW preterm infants but the stronger correlation was found between PDA and NEC and apnea of prematurity (Table 4). Also, presence of any of BPD, severe IVH, severe PVL, and severe ROP - conditions associated with unfavorable neurodevelopmental outcome - was associated with DA persistence (Table 4). No difference was found between the study group as regards neonatal sepsis, irrespective of the onset (Table no.4). PDA was also associated with a significantly increased rate of death (Table no. 4).

Table 1. Neonatal characteristics.

	No PDA		PDA		p	OR [95% CI]
	No.	Value	No.	Value		
Gestational age (weeks) (mean±SD)	111	29.3±1.9	151	28.5±2.3	0.003	-
Birth weight (g) (mean±SD)	111	1210.2±199.6	151	1085.4±259.8	0.000	-
Male gender (n/%)	111	55 (49.5)	151	86 (57.0)	0.236	1.35[0.82-2.20]
Ponderal index (mean±SD)	111	1.95±0.31	151	1.97±0.35	0.620	-
SGA (n/%)	111	64 (57.7)	151	84 (55.6)	0.745	0.92[0.56-1.51]

Legend: SD - standard deviation, SGA - small for gestational age.

Table 2. Maternal, pregnancy and delivery data.

	No PDA		PDA		p	OR [95% CI]
	No.	Value	No.	Value		
Corticosteroid therapy						
Prenatal corticosteroids (n/%)	111	63 (56.8)	151	72 (47.7)	0.148	0.69[0.42-1.14]
Complete course (n/%)	63	25 (39.7)	72	29 (40.3)	0.944	0.97[0.49-1.95]
Number of doses (mean±SD)	63	2.7±1.3	72	2.6±1.3	0.570	-
Gestational age at corticosteroid prophylaxis (weeks) (mean±SD)	63	29.4±1.6	72	28.1±2.2	0.000	-
Time elapsed from initiation of corticosteroid prophylaxis to delivery (hours) (mean±SD)	63	14.8±24.5	72	18.5±39.4	0.528	-
Complications during pregnancy						
Any complication (n/%)	111	23 (20.7)	151	30 (19.9)	0.866	0.95[0.52-1.74]
Diabetes mellitus (n/%)	111	1 (0.9)	151	0 (0)	-	-
Antenatal hemorrhage (n/%)	111	3 (2.7)	151	5 (3.3)	0.778	1.23[0.29-5.27]
Pregnancy-induced hypertension (n/%)	111	10 (9.0)	151	12 (7.9)	0.760	0.87[0.36-2.10]
Eclampsia (n/%)	111	3 (2.7)	151	1 (0.7)	0.185	0.24[0.02-2.34]
All types of maternal hypertension (n/%)	111	10 (2.7)	151	13 (10.2)	0.991	0.99[0.42-2.3]
Chorioamnionitis (n/%)	111	4 (3.6)	151	1 (0.7)	0.086	0.18[0.02-1.62]
Type of pregnancy						
Multiple pregnancy (n/%)	111	34 (30.6)	151	49 (32.5)	0.756	1.09[0.64-1.84]
Second twin (n/%)	34	18 (52.9)	49	28 (5.1)	0.709	1.18[0.49-2.86]
ART pregnancy (n/%)	111	5 (4.5)	151	5 (3.3)	0.620	0.73[0.20-2.57]
Delivery						
Outborn (n/%)	111	18 (16.2)	151	46 (30.5)	0.000	2.26[1.23-4.17]
Rupture of the amniotic membranes > 18 hours (n/%)	111	36 (32.4)	151	21 (13.9)	0.000	0.34[0.18-0.62]
Cesarean section (n/%)	111	35 (31.5)	151	41 (27.2)	0.442	0.81[0.47-1.38]

Legend: SD - standard deviation, ART - assisted reproductive techniques.

Table 3. Neonatal status at delivery and respiratory distress syndrome.

	No PDA		PDA		p	OR [95% CI]
	No.	Value	No.	Value		
Birth resuscitation						
Need for resuscitation at birth (n/%)	111	80 (72.1)	151	115 (76.2)	0.456	1.24[0.71-2.16]
FiO_2 during resuscitation (%) (mean±SD)	79	86.9±28.8	115	91.4±23.5	0.228	-
Peripheral oxygen saturation during resuscitation	68	87.5±9.5	106	85.2±8.5	0.105	-
Apgar score at 1 minute (mean±SD)	110	6.2±2.1	148	5.5±2.2	0.007	-
Apgar score at 5 minutes (mean±SD)	106	7.5±1.5	145	6.9±1.5	0.011	-
Apgar score at 10 minutes (mean±SD)	98	8.2±0.9	141	7.7±1.2	0.001	-
Apgar score at 20 minutes (mean±SD)	40	7.7±0.9	32	7. ±1.4	0.539	-
Respiratory distress syndrome management						
Need for surfactant administration (n/%)	111	46 (41.4)	151	87 (57.6)	0.010	1.92[1.17-3.16]
Surfactant dose (mg/kg)(mean±SD)	46	168.5±36.6	87	170.2±32.8	0.783	-
INSURE strategy (n/%)	111	33 (29.7)	151	62 (41.1)	0.060	1.65[0.98-2.77]
INSURE failure (n/%)	33	9 (27.3)	62	34 (54.8)	0.010	3.24[1.30-8.08]
Need for oxygen therapy (n/%)	111	107 (96.4)	151	144 (95.4)	0.682	0.77[0.22-2.69]
Oxygen therapy length (days) (mean±SD)	107	11.6±17.5	144	21.5±36.3	0.010	-
Need for CPAP (n/%)	111	106 (95.5)	151	142 (94.0)	0.606	0.74[0.24-2.28]
CPAP support duration (days) (mean±SD)	106	6.1±4.7	142	8.1±7.9	0.028	-
Maximum FiO2 on CPAP (%) (mean±SD)	106	45.6±23.4	142	48.0±22.8	0.406	-
Maximum PEEP on CPAP (mmHg) (mean±SD)	106	6.3±0.3	142	6.3±0.3	0.979	-
Need for mechanical ventilation (n/%)	111	22 (19.8)	151	59 (39.1)	0.001	2.59[1.47-4.59]
Duration of mechanical ventilation (days) (mean±SD)	22	11.3±15.2	59	15.1±21.2	0.439	-

Legend: FiO_2 - fraction of inspired oxygen, SD - standard deviation, INSURE - INtubate-SURfactant-Extubate, CPAP - continuous positive airway pressure, PEEP - positive end-expiratory pressure

Table 4. Complications during hospitalization.

	No PDA		PDA			
	No.	Value	No.	Value	p	OR [95% CI]
Bronchopulmonary dysplasia (n/%)	111	13 (11,7)	151	28 (18,5)	0.134	1,71[0,84-3,49]
Apnea of prematurity (n/%)	111	17 (15.3)	151	41 (27.2)	0.023	2.06[1.10-3.86]
Necrotizing enterocolitis (n/%)	111	2 (1.8)	151	14 (9.3)	0.012	5.57[1.24-25.03]
Intraventricular hemorrhage (n/%)	111	40 (36.0)	151	70 (46.4)	0.095	1.53[0.93-2.53]
Severe intraventricular hemorrhage (n/%)	111	4 (3.6)	151	13 (8.6)	0.105	2.52[0.80-7.95]
Periventricular leukomalacia (n/%)	111	3 (2.7)	151	7 (4.6)	0.422	1.75[0.44-6.92]
Severe retinopathy of prematurity (n/%)	111	0 (0)	151	3 (2)	-	-
Early onset sepsis (n/%)	111	16 (14.4)	151	30 (19.9)	0.253	1.47[0.76-2.86]
Late onset sepsis (n/%)	111	20 (18.0)	151	24 (15.9)	0.651	0.86[0.45-1.65]
Any neonatal sepsis (n/%)	111	35 (31.5)	151	50 (33.1)	0.788	1.07[0.64-1.82]
Hospitalization length (days) (mean±SD)	111	48.3±22.1	151	48.6±33.5	0.951	-
Combined severe complications of prematurity (n/%)	111	24 (21.6)	151	66 (43.7)	0.000	2.81[1.62-4.90]
Death (n/%)	111	10 (9.6)	151	38 (25.2)	0.001	3.40[1.61-7.16]

Legend: SD - standard deviation

Severe conditions associated with prematurity as BPD, IVH grade III and IV, PVL grad II or III, ROP (requiring laser therapy) occurred more often in VLBW preterm infants but the stronger correlation was found between PDA and NEC and apnea of prematurity (Table 4). Also, presence of any of BPD, severe IVH, severe PVL, and severe ROP - conditions associated with unfavorable neurodevelopmental outcome - was associated with DA persistence (Table 4). No

difference was found between the study group as regards neonatal sepsis, irrespective of the onset (Table no.4). PDA was also associated with a significantly increased rate of death (Table no. 4).

Discussions

Failure of DA closure is reported significantly more often in preterm infants than in term infants - 40-60% according to gestational age in preterm infants[14-16] versus 57/10.000 live births in term infants[19,50] - and is associated with considerably increased morbidity and mortality[11]. In preterm infants with GA ≥30 weeks gestation, functional closure of DA occurs towards the fourth day of life, while in those with GA <30 weeks or with significant RDS the incidence of PDA is about 65%[5,18,51]. We have chosen to define and analyze PDA only if the ductus was seen on echocardiography after the first week of life even though the classic definition states that PDA defines failed closure of DA after 72 hours of life[7,8] since in our study we did not evaluate the size and hemodynamic significance of the ductus. Therefore, the incidence reported in our study - 57.3% - in VLBW preterm infants includes all types of PDA, irrespective of size (small, moderate, or large) and hemodynamic significance (silent or with significant shunt). In a study on 272 VLBW infants with GA < 30 weeks surviving more than 28 days after birth, Al Nemri[3] reported a PDA incidence of 46% while other reported rates between 21.9 and 33%[7,13,15,18,35]. The difference between the rates reported in the literature and the rate found in our study group may be explained by definition criteria used in our study.

Persistence of DA is a multifactorial condition, but low GA and BW are by far the most often risk factors cited[2,6,9-13,18,19,28,29,35,52-54]. The direct relationship between GA and spontaneous closure of DA was evaluated by Koch et al.[18] who showed that for each gestational week after 23 weeks the odds for spontaneous closure of the ductus increases with a ratio of 1.5. A significantly lower mean BW and GA was associated with PDA in VLBW preterm infants in our study ($p<0.05$) (Table 1), in accordance with data published in the literature.

A higher occurrence rate of PDA in male infants was reported by some authors[11,41] but, in accordance with Nizarali et al.[13] we have found no difference between VLBW preterm infants with and without PDA. In other studies[16,55], SGA status was reported as a risk factor for PDA. Our analysis of the study groups did not revealed any difference between the proportions of SGA infants and the mean ponderal index (Table 1).

Antenatal corticosteroid prophylaxis decreases the risk and severity of RDS[56]. There also studies reporting a decreased risk for PDA after prenatal administration of corticosteroids[11,13,16,57,58]. A relatively small proportion of infants in our groups benefited from antenatal corticosteroid prophylaxis (56.8% of VLBW infants without PDA and 47.7% of infants with PDA) and even smaller proportion of the preterm infants received a complete course of steroids (39.7% of 63 VLBW infants without PDA and 40.3% of those with PDA) (Table 2). This may explain, together with the significantly lower mean GA when corticosteroid prophylaxis was initiated and relatively short interval between rupture of amniotic membranes and birth, and between steroid administration and delivery (Table 2), why antenatal corticosteroid prophylaxis had no influence on PDA occurrence in our study.

Maternal conditions as diabetes mellitus[11], chorioamnionitis[2,12,34,52,59,60], and antenatal hemorrhage[11] were reported in association with increased risk for PDA. Increased immaturity of all organs and systems may explain delayed cardiovascular adaptation with failed closure of DA in infants delivered by mothers with diabetes mellitus[61,62]. We weren't able to analyze such correlations since we had only one VLBW infant born from a pregnancy complicated by maternal diabetes in our study. No difference was found between groups as regards antenatal maternal hemorrhage (Table 2). In chorioamnionitis, inflammation increases cyclo-oxygenase activity and prostaglandin E2 production causing PDA[60] explaining the link found by many authors between chorioamnionitis and PDA[2,12,34,52,59,60]. The limited number of maternal chorioamnionitis registered in our groups and the fact that we considered only cases of clinical chorioamnionitis (since data about histological amnionitis were not available) may explain the lack of correlation between this condition and PDA in our VLBW preterm infants. But, a meta-analysis comprising 23 studies and 17.708 preterm infants done by Park et al.[52] have also shown a lack of association between PDA and clinical chorioamnionitis - OR 1.28 [95%CI 1.00-1.64]. On the contrary, pregnancy-induced hypertension and eclampsia were associated with decreased incidence of PDA[11,12,63], probably due to accelerated fetal pulmonary maturation in these conditions[11]. Even when counting together all types of maternal hypertension - pregnancy-induced or pre-existent - we have found no difference between VLBW infants with and without PDA (Table 2).

We have found in the literature only one study reporting and association between PDA and multiple pregnancy. Hammoud et al.[11] showed a four fold risk for PDA in infants born from multiple pregnancies (OR 3.8 [95%CI 1.5-12.4]). In our groups, no association was found between PDA and multiple pregnancy and birth rank (PDA occurred with similar incidence in the second twin, known to have a more complicated postnatal course than the first one) (Table 2).

No information was found in the literature as regards PDA incidence according to pregnancy type - naturally occurring or by assisted reproductive techniques - and to delivery mode - cesarean section versus vaginal delivery - in VLBW preterm infants. We weren't able to demonstrate that such correlations exists in VLBW preterm infants (Table 2). Delivery in a lower level neonatal unit and postnatal transfer to our unit significantly increased the risk for PDA in VLBW preterm infants - OR 2.26 [95% CI 1.23-4.17] - and this may be explained by a number of factors, including reduced access to modern equipment, lesser experience in carrying VLBW infants (births attended by obstetricians or midwives or pediatricians without neonatal training), lack or

insufficient training in neonatal stabilization and transport, delayed transfer, etc.

In a study of 318 VLBW infants less than 32 weeks gestation, Nizarali et al.[13] identified the need for resuscitation at birth as a risk factor for PDA - OR 13.1[95% CI 3.11-55.1]. As expected, a great proportion of VLBW infants in both our study groups - with and without PDA - required resuscitation procedures at birth (Table no. 3). Continuous adaptation of the resuscitation protocols to national and international guidelines during the study period - as use of lower oxygen concentrations during resuscitation, monitoring of peripheral oxygen saturation, acceptance of lower oxygen saturations in the first minutes of life, and more extensive use of positive pressure ventilation with T-piece resuscitator in very preterm infants in latest years - may explain our results.

Nevertheless, we noted that VLBW infants without PDA after the first week of life were resuscitated with slightly lower oxygen concentrations. This observation deserves a more detailed approach since currently FiO_2 of 21-30% are recommended for preterm infant's resuscitation[64,65].

An increased incidence of PDA was reported in association with hypoxia and low Apgar scores[13,66]. We have also found that VBLB infants with PDA had significantly lower mean Apgar scores at 1, 5, and 10 minutes ($p<0.05$) (Table 3).

Presence and severity of RDS is an important risk factor for PDA, PDA incidence of 80% being cited by Pegoli[41] in preterm infants with RDS. According to Smith[67], increased circulating prostaglandin E2 concentrations during RDS are responsible for ductus arteriosus persistence. The need for surfactant administration, reflecting in most of the cases the severity of RDS, was significantly increased in VLBW preterm infants with PDA compared to those without PDA ($p=0.010$, OR 1.92[95% CI 1.17-3.16] (Table 3), similar with data reported by other authors[13,16,68-70]. According to Clyman et al.[16], surfactant alters pulmonary vascular resistance, favoring early left-to-right shunting through DA. The administered dose of surfactant did not influenced PDA (Table 3). Compliance to national[71] and European guidelines for RDS treatment[72] - recommending non-invasive approach (INSURE strategy) in preterm infants that do not require assisted ventilation at birth - explains the proportions of VLBW infants treated using INSURE strategy (INtubate-SURfactant-Extubate on CPAP) but association between PDA and INSURE strategy failure ($p=0.010$, OR 3.24 [95% CI 1.30-8.08] may be due to both to a more severe or complicated RDS course or to an improper selection of cases for INSURE strategy. A strong tendency for non-invasive approach to RDS is also demonstrated by the high proportions of VLBW infants treated using CPAP in both study groups (Table 3), according to experts recommendations[72]. Also, biases due to CPAP management were excluded since no difference was found as regards FiO2 and positive end-expiratory pressure (PEEP) used on CPAP support in the study groups (Table 3). Similar with data reported by other authors[13,28,73], a significantly increased proportion of VLBW infants with PDA needed mechanical ventilation ($p=0.001$). Also, VLBW preterm infants had significantly increased duration of oxygen therapy, and CPAP support compared to those without PDA ($p<0.05$) (Table no. 3), all these data suggesting that increased severity of RDS was associated with increased risk for PDA after the first week of life in VLBW preterm infants.

Neonatal morbidity and mortality is significantly influence by PDA in preterm infants, according to numerous studies[6,9,22,32,35,74].During the first days of life, PDA is associated with arterial hypotension, myocardial dysfunction and systemic perfusion[5,7,75], renal functional disturbances[5,17,18], pulmonary hemorrhage[21,76,77], apnea and prolonged duration of mechanical ventilation[11,25,27], and feeding intolerance[10,11].

In preterm infants with RDS, PDA induces an interstitial and alveolar pulmonary edema, decreases pulmonary compliance, increases the need and length of mechanical ventilation and oxygen needs, thus increasing the risk for BPD[5,29]. Contrary to other studies[6,8,12,19,22,26,28,29-31,33,34,78], we have found no significant association between PDA and BPD ($p>0.05$) (Table no. 4) most probably because most of the cited studies used the classic definition of PDA and evaluated the influence of hemodynamically significant PDA while we included in the study all types of PDA, irrespective of size and hemodynamic significance, diagnosed after the first week of life.

An increased risk for apnea was signaled in preterm infants with PDA in some studies[11,25,27]. In our study, PDA doubled the risk for apnea of prematurity (OR 2.06 [95% CI 1.10-3.86]) (Table 4).

An increased risk for NEC in preterm infants with PDA was reported[9,22,28,37,79] due to diastolic blood stealing from the superior mesenteric artery and abdominal aorta through DA with secondary intestinal hypoperfusion[5,29,35]. Ductus arteriosus persistence was also associated with a 1.8 fold increased risk for NEC in very preterm infants in the study performed by Dollberg et al.[35]. In our batch of VLBW preterm infants, PDA increased the risk for NEC by 5.57 times (Table 4). Different approach to enteral feeding - minimal enteral nutrition, type of milk used, progression of feedings, etc. - may also be responsible for this increased risk for NEC.

Cerebral blood flow is also affected by significant PDA, mostly during diastole[29], a pathway considered responsible for IVH and PVL occurrence[20]. An increased incidence of IVH[19-22,33] and PVL[80] was reported in association with PDA. We have also found an increased rate of IVH and severe IVH (grade III and IV according to Papile[81]) and severe PVL (grade II and III according to deVries[82]) in VLBW preterm infants with PDA compared to those without PDA (Table no. 4) but the numbers failed to reach statistical significance, most probably due to low number of cases.

During the study period, only 3 cases of severe ROP (needing laser therapy) were registered in VLBW infants, all of them diagnosed with PDA and other severe complications

of prematurity, with gestational ages of 24 weeks (1 case) and 27 weeks (2 cases). We found only one study reporting a significant association between PDA and ROP - OR 2.41 [95% CI 1.08-5.38] but after adjusting for GA, the association was attributed only to GA[83]. It is most probably that occurrence of severe ROP in our cases was multifactorial, with a great contribution of GA and co-existent morbidities.

Same as in chorioamnionitis, an increased incidence of PDA was reported in preterm infants with early and late neonatal infections[2,34]. Our analysis showed no correlations between PDA and neonatal infections, analyzed separately or together ($p>0.05$). A trend for increased rate for early neonatal infections was observed in VLBW preterm infants with PDA compared to those without PDA (19.9% versus 14.4%) (Table no. 4).

Persistence of DA in VLBW preterm infants was associated, in our study, with increased risk for unfavorable neurodevelopmental long-term outcome since as revealed by the association with a combined outcome comprising BPD, NEC, severe IVH, PVL, ROP and death) ($p=0.000$, OR 2.81 [95% CI 1.62-4.90]) (Table 4).

Finally, we are reporting an 3.4 fold increased risk for death in VLBW preterm infants with PDA compared to those without PDA after the first week of life. Numerous studies are also demonstrating a significantly increased risk of death in preterm infants with PDA[6,16,22,32,42,84] - 4-8 times higher[22,40] -. Despite the significantly lower GA, BW, the more severe RDS, and the more frequent association with severe complications of prematurity - BPD, NEC, IVH, ROP, apnea of prematurity - no significant differences were seen in the hospitalization length, most because an important number of VLBW infants with PDA died before the first month of life.

Conclusions

Most of the studies in the literature evaluated risk factors only for hemodynamically significant PDA and its influence on neonatal morbidity and mortality, defining PDA as failed closure of DA after the first 72 hours of life. Our choice was to evaluate the impact of PDA on neonatal morbidity and mortality, when PDA was diagnosed after the first week of life, regardless of size and hemodynamic influence, in VLBW preterm infants (≤32 weeks gestation). Comparing the baseline maternal and neonatal characteristics we have found that PDA is associated with significantly lower GA and BW, Apgar scores, and with more severe RDS. Not surprisingly, in VBLW preterm infants, PDA was also associated with increased rates of conditions associated with prematurity - BPD, apnea of prematurity, NEC, IVH, PVL, severe ROP - and death. However, a significantly increased risk was demonstrated only for NEC, apnea of prematurity, and death. The relatively low incidence of BPD, severe IVH, severe PVL, and severe ROP is one plausible explanation for lack of association of PDA with these conditions along with defining PDA in our study.

Correlation of PDA with increased neonatal morbidity and mortality are still unclear: result of the left-to-right shunting, result of PDA treatment or simple consequences of prematurity[35]. Controversies still exists regarding the best management for PDA in preterm infants as most of the studies evaluating the influence of different therapeutic strategies - conservative management (including fluid restriction during the first days of life, waiting for spontaneous DA closure, and treatment of large PDA significantly influencing the respiratory support), early, presymptomatic or symptomatic pharmacological treatment, or surgical ligation[5] - failed to demonstrate an influence on long-term outcome of these infants[33,42,44-48]. More studies are needed to identify pathways for PDA involvement in the occurrence and development of comorbidities associated with prematurity. Also, studies of the most important risk factors, stratified on gestational age, are needed in order to develop more successful management strategies, to more clearly define who and when PDA treatment is needed, and which is the best therapeutic approach for a better long-term outcome. Improvement of the outcome of VLBW preterm infants with PDA must include also successful prophylaxis and therapy of the complications of prematurity.

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METHYLMALONIC ACIDEMIA IN CHILDREN – CASE PRESENTATION

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Abstract

We present the case of a one year and six months male infant, admitted to our Clinic in October 2014, for vomiting and hypotony. He is the fourth born child, full term, with a birth weight of 3080 g, naturally fed for 5 months, followed by an incorrectly diversified feeding, weaned at one year, with a normal psychomotor development, without significant AHC until the age of one year and 2 months. From the age of 1 year and two months, he was repeatedly admitted to hospital for vomiting associated with hypotony, dehydration syndrome up to coma, in September 2014, when he was admitted to ICU, the salt wasting syndrome being infirmed. At admission, he had no fever, was pale, with an altered general state, dark circles around his eyes, dry lips, lazy abdominal skin fold, hypotonic, lethargic, cardio-respiratory balanced, supple abdomen, without meningeal symptoms, his weight being 9000 g. We noticed that, every time he was admitted, the general state suddenly worsened the moment he started to vomit. The alkaline deposit, at admission, was 12 mEq/l and we corrected it after the administration of sodium bicarbonate i.v., p.o., maintaining it between 18 and 22 mEq/l – following this treatment the general state got stable with no more vomiting. We performed a series of biochemical seric and urinary tests, imagistic investigations, and an ophthalmologic examination and we considered that the vomiting occurred in the context of an organic acidemia. The infant was transferred to the Medical Genetic Department of the Hospital for Children in Cluj-Napoca, where specific tests were performed: the chromatography of the urinary organic acids pointed out a highly increased level of the methylmalonic acid and a moderate level of the uracil, results which suggested a methylmalonic acidemia. The patient remained in our clinic's records, being given a daily treatment with L-carnitine, Biocebral, dietetic regime, with a fair evolution.

Key words: methylmalonic acidemia, child

Introduction

The organic acidemias represent rare genetic diseases, characterized by the accumulation within the body of some organic acids which usually come from amino-acids or fat acids. The methylmalonic acidemia (described in 1967) is second only to the deficit of methylmalonyl-CoA mutase, necessary to transform the malonic acid into succinic acid. The enzyme requires, as a co-enzyme, adenosylcobalamine, a metabolite of vitamin B12. The gene of the enzyme is on the chromosome 6p; the enzyme deficiency (caused by 20 known mutations) can remain completely or partially unexpressed [1]. The disease is caused by the deficit of the specific mutase in approximately 50% of the patients and by the deficit of the co-enzyme (deficit or metabolism dysfunctions of vitamin B12) in the remaining 50%, the therapeutic answer to vitamin B12 being absent or present, respectively, in the two categories of patients [2].

Case presentation

Male-infant, aged 1year and 6 months, from rural area, was admitted to the 2nd Pediatric Clinic, Emergency County Hospital Craiova, in November 2014 (medical record 54657), for vomiting and hypotony.

Heredo-collateral antecedents: mother 32 years old - hypertiroidism, a healthy father - 38 years old, and 3 healthy brothers (16, 14, 7 years old).

Personal physiologic antecedents: 4th child, on term, normal birth with no sufferance, weight at birth 3010 g, 1 week physiologic jaundice, naturally fed for 4 months, incorrectly diversified at 5 months, weaned at one year, and fed with adult's food.

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He was treated against rickets with vitamin D3, and vaccinated according to the Health Ministry scheme. The psychomotor development was according to age stages.

Pathologic personal antecedents: repeated episodes of vomiting with hypotony and alteration of the general state in the last 4 months which required hospitalization. From 21.09 to 06.10.2014, the patient was admitted to ICU Clinic for incoercible vomiting followed by coma associated with hydro-electrolytic and acido-basic dysfunctions. We performed further investigations which revealed the absence of the salt wasting syndrome.

At admission: normal height for his age (79 cm), with a moderate deficit in weight (Weight= 9 Kg, PI= 0.79, NI= 0.85), no fever, an altered general state, pale, a discrete craniofacial dysmorphism, dark circles around his eyes, dry lips, lazy abdominal skin folds, bottom flared thorax, normal stetacustic pulmonary, rhythmic heart beats, HB= 112 b/min., supple abdomen, normal stool, hypotonic, lethargic, without meningeal symptoms.

Because the child was admitted once again with vomiting and a rapid alteration of his general state, presenting many episodes of the same symptomatology in the last four months (it started when the child was one year and six months old), and taking into account that his latest admission was to the ICU, in a coma, we decided that it was necessary to investigate him thoroughly in order to find the source of the vomiting.

Admission investigations: Hemogram: Hb= 8.8g%, Ht= 28%, HEM= 26pg, CHEM= 30g/dl, VEM= 87μ^3, L=6200/mm^3, NS= 30%, Ly= 61%, M=9%, Tr.= 323000/mm^3, VSH= 8/20 mm, urea= 15 mg%, creatinine= 0.36 mg%, GOT= 27 U/l, GPT= 18 U/l, cholesterol= 110 mg%, lipemia= 450 mg%, triglyceride= 112 mg%, total protein= 7.9 g%, total seric calcium= 8.4 mg%, ionic calcium= 3.85 mg%, sideremy= 21 μg/dl, ammoniac= 45.5 μmol/l (N=16-60), seric ionogram: Na= 129 mEq/l, K= 4.3 mEq/l, alkaline deposit= 12 mEq/l, glycemia= 64 mg, urine test: ketone bodies ++++, negative uroculture, normal ophthalmologic examination normal, normal abdominal ultrasound exam, eso-gastroduodenal transit – no modifications.

We excluded the possibility of urinary infections (negative urine culture), intracranial hypertension (normal ophthalmologic examination), malformations of the digestive tract (normal eso-gastroduodenal barium transit and abdominal ultrasound), and illnesses which could lead to recurrent vomiting.

The analyses we performed when admitted showed low values of hemoglobin and sideremy, the parameters of the lipidic metabolism, normal hepatic and renal functional tests.

At admission, we noticed very low values of the alkaline deposit (12 mEq/l), glycemia (64 mg%) and natremia (129 mEq/l) and the presence of ketone bodies in urine, things which made us monitor the values of the alkaline deposits, glycemia, seric and urinary ionogram, urinary and ketone body pH (table 1).

Table 1. Paraclinic Investigations in progress.

Parameters	*Date*										
	26.10	*30.10*	*31.10*	*1.11*	*2.11*	*3.11*	*4.11*	*5.11*	*6.11*	*13.11*	*16.11*
Alkaline Deposit (mEq/l)	12	14	21	14	15	20	18	19	23	31	22
Sanguine ionogram											
Na (mEq/l)	129	133	136	136	135	141	129	131	139	241	136
K (mEq/l)	4.3	3.7	3.8	4.3	4.3	3.2	4.4	3.9	3.7	4.6	3.7
Cl (mEq/l)	112	111	135	103	104	108	103	103	109	107	107
Glycemia (mg%)	64		67	88	67	83	64	67	86	65	70
Urinary Ph	5.5		3.5	5.5	6	6.5	6.5	6	6.5	8	7
Ketone bodies	++++	abs.	+	+++	abs	abs	abs	++	abs	abs	abs
Urinary ionogram											
Na (mEq/l)	116		253	116	179	208	208	231	146	152	171
K (mEq/l)	12.9		36	27.6	12.9	21	21.9	56.1	7.6	6.5	6.9
Cl (mEq/l)	63		130	105	6,3	170	167	156	136	125	104
Urinary ionogram – normal values *Na = 40-220 mEq/l* *K = 25-125 mEq/l* *Cl = 110-259 mEq/l*											

The patient received, as an emergency treatment, perfusion with physiological serum, then sodium bicarbonate, glucoses, and electrolytes, Quamatel i.v., eventually resulting in an improvement of the general state.

After returning to normal feeding, the child had a fair general state, but after several days, he presented nausea, then vomiting which was rapidly followed by hypotony and somnolence. Once the vomiting onset, we proceeded to emergency analyses which proved low levels of the alkaline deposits. Thus, we noticed that the cause of vomiting was the metabolic acidosis.

The repeated episodes of vomiting with hypertonia, the alteration of the general state associated with the onset of acute severe dehydration up to coma, hypoglycemia and metabolic acidosis were suggestive for an organic acidemia.

We contacted the Emergency Hospital for Children in Cluj-Napoca, the Medical Genetic Department, and following their recommendations the child received, daily, a solution of sodium bicarbonate p.o.; the vomiting episodes ceased and the general state was fair. The alkaline deposit was within normal limits.

Subsequently, the patient was transferred to the Medical Genetic Department of the Emergency Hospital for Children in Cluj-Napoca, where several specific tests were performed:

- The chromatography of the urinary organic acids which pointed out a significantly increased level of the methylmalonic acid (779 mmol/mol created, N< 20), and a moderately increased level of the uracil (99 mmol/mol created, N< 55)
- The chromatography of the plasmatic amino-acids did not highlight a deficit of the essential aminoacids, but it pointed out moderately increased values of glycine (475 μmol/l), glutamic acid (418 μmol/l), serine (228 μmol/l) and taurine (317μmol/l).

These results suggested a methylmalonic acidemia; a precise diagnosis can be set by determining the activity of the deficitary enzyme (methylmalonyl-CoA epimerase) at the level of fibroblasts and by identifying the mutation.

The patient remained in our clinic's records with the diagnosis:

- Methylmalonic acidemia
- 1st degree dystrophy
- Ferriprive anemia

The child received a hypoproteic diet (1-1.5g/kg/day), rich in carbohydrates, with regular meals, and avoiding the prolonged fasting.

He received a daily treatment with L-carnitine p.o. and Biocebral p.o., after quitting the alkalization treatment with sodium bicarbonate solution.

We determined the seric level of vitamin B12, which was within normal limits, fact that did not require the introduction of B12 within the treatment.

After discharge, he received Haussman Iron for the ferriprive anemia.

Discussions

The methylmalonic acidurias (MMA) are metabolic disorders resulting from deficient methylmalonyl-CoA mutase activity, a vitamin B_{12}-dependent enzyme. Genetic defects in the methylmalonyl-CoA mutase (MCM) gene result in methylmalonic acidemia which is inherited as an autosomal recessive disease [2].

This disorder can display a wide spectrum of clinical manifestations, spanning the prenatal period through late adulthood [3,4]. The incidence of methylmalonic acidurias ranges from 1:115,000 in Italy, to 1:169,000 in Germany, but remains unclear in China. However, its incidence appears to be higher than previous estimates when making a diagnosis of suspected cases using newborn screening studies or gas chromatography-mass spectrometry [3,5]. The pathophysiology of the complications observed in MMA patients is still not fully understood, although acute or chronic neurological signs may be caused by the accumulation of toxic compounds proximal to the metabolic block, including increased homocysteine concentrations and impaired methyl group metabolism, oxidative stress, and dysfunctional mitochondrial/ caspase pathway [3,6].

Methylmalonic acidemias are a group of inherited diseases characterized by lethargy, vomiting, developmental delays, hypotony, and enlargement of the liver. They involve defects in one of several proteins and enzymes that break down certain amino acids, fatty acids, and cholesterol in the body. Symptoms are due to the toxic build-up of these substances and their metabolites in organs and tissues [7].

Methylmalonic acidemia is a disease that varies in age of onset, severity, and responsiveness to vitamin B_{12} treatment. The most severe form of the disease is the most common, has onset in early infancy, and is least responsive to vitamin B_{12} treatment. Symptoms may include: vomiting, hypotony, lethargy, failure to thrive, hepatomegaly, hypothermia, coma or death, even with aggressive intervention [7]. Some individuals have onset in early childhood or later, and symptoms are often triggered by fasting, illness, or eating large amounts of protein. Symptoms are similar to the severe form; however, these individuals are typically more responsive to vitamin B_{12} treatment [8].

There is no cure for methylmalonic acidemia. Treatment typically includes a low protein diet, nutrition supplements, and vitamin B_{12}. Despite treatment, as many as 50% of individuals diagnosed in infancy die in early childhood. Methylmalonic acidemia is included on all newborn screening panels in the United States [9].

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HISTOLOGICAL EVALUATION OF THE EFFICIENCY OF HUMAN AMNIOTIC MEMBRANE USED IN EXPERIMENTAL RECONSTRUCTION OF THE ANTERIOR ABDOMINAL WALL DEFECTS

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Abstract

The purpose of the study was to conduct a morphopathological evaluation of the efficiency of human amniotic membrane used in experimental reconstruction of abdominal wall defects. The study group included 20 Californian rabbits, of both sexes, with the body weight ranging from 2300 to 2500 g. The abdominal wall defect was performed surgically under aseptic conditions resecting a fragment, 10cm x 5cm in size, involving the muscular-aponeurotic layer and parietal peritoneum. Animals subjected to the intervention of reconstruction of the anterior abdominal wall defect were divided into 4 groups consisting of 5 animals, depending on the method of implant processing and application. Unabsorbable polyester mesh of *Erceokaque* type was used in the abdominal wall reconstruction in the control group. The mesh was fixed to the abdominal wall layers but it was not covered by skin. In group 1 there was used the amniotic membrane treated with 0.1% glutaraldehyde protected externally with *Stypro* preparation. In group 2 there was used the amniotic membrane treated with 0.5% formaldehyde. In lot 3 the cryopreserved amniotic membrane and biological implants were protected by suturing the skin and subcutaneous layer. The study results allowed to conclude that the use of the amniotic membrane as implant, treated with glutaraldehyde and formalin, does not provide a long-term stability, the cryopreserved amniotic membrane having some advantages, namely, implant elasticity and stability of fixation sutures, as well as a marked reparative-regenerative activity. The amniotic membrane may be considered a useful temporary substitute for the peritoneum and a promising non-adherent adjuvant in reconstructive interventions of the abdominal wall defects with a viscero-abdominal disproportion. The obtained results justify the need to continue clinical trials to evaluate the efficacy and safety of the application of this biological material.

Key words: abdominal wall defects, amniotic membrane, graft, peritoneal adhesions.

Introduction

The correction of abdominal wall defects in children is a real challenge for paediatric surgeons, several reconstructive methods being proposed, including the use of protein synthetic and biological materials (3, 4). their characteristic varying by strength, biodegradability, resistance to infection and formation of adhesions (10).

The synthetic grafts, first introduced by Usher F.C. (1958) (23), are quite widely spread in surgery due to their acceptable biocompatibility and mechanical stability, but as nonresorbable material, they are quite frequently associated with various adverse postoperative effects, including persistent pain, hematomas, wound erosions, enterocutaneous fistulas, development of a significant intra-abdominal adhesive process, susceptibility to infection. The synthetic material does not increase in size as the child (6, 9, 13) is growing. In this context, the use of biological grafts, derived from animal and human sources, in the reconstruction of the anterior abdominal wall defects, including congenital defects, have certain advantages over synthetic prosthetic materials (2, 17).

Adhesive disease is a serious complication after corrective operations of congenital defects of the anterior abdominal wall (24). Several studies have found that amniotic membrane has several beneficial features. Some authors suggest using the amniotic membrane to cover the abdominal cavity organs in cases when there is no peritoneum (1), it having marked anti-adherent properties (12, 25). At the same time, some authors put into question the anti-adhesive effect of the amniotic membrane after interventions of closing the abdominal wall defect (15).

Purpose

The purpose of the study was to carry out a morphopathological evaluation of the efficiency of the human amniotic membrane used in experimental reconstruction of abdominal wall defects.

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Material and methods

The study group included 20 Californian rabbits, of both sexes, with the body weight of 2300 to 2500 g. The abdominal wall defect was performed surgically in aseptic conditions, under general anesthesia, resecting a fragment of 10cm x 5cm in size, involving the muscular-aponeurotic layer and parietal peritoneum (14, 18).

Animals subjected to the intervention of reconstruction of the anterior abdominal wall defect were divided into 4 groups consisting of 5 animals, depending on the method of implant processing and application. In the control group, the nonresorbable polyester mesh of *Erceokaque* type, (Fig. 1A) was used in the abdominal wall reconstruction, the mesh being fixed to the abdominal wall layers without being covered by skin. In group 1, there was used the amniotic membrane treated with 0.1% glutaraldehyde, protected on the outside with *Stypro* preparation (Fig. 1 B). In lot 2 there was used the amniotic membrane treated with 0.5% formaldehyde (Fig. 1C) and, in lot 3 – the cryopreserved amniotic membrane was used (fig. 1D), biological implants being protected by suturing the skin and the subcutaneous layer.

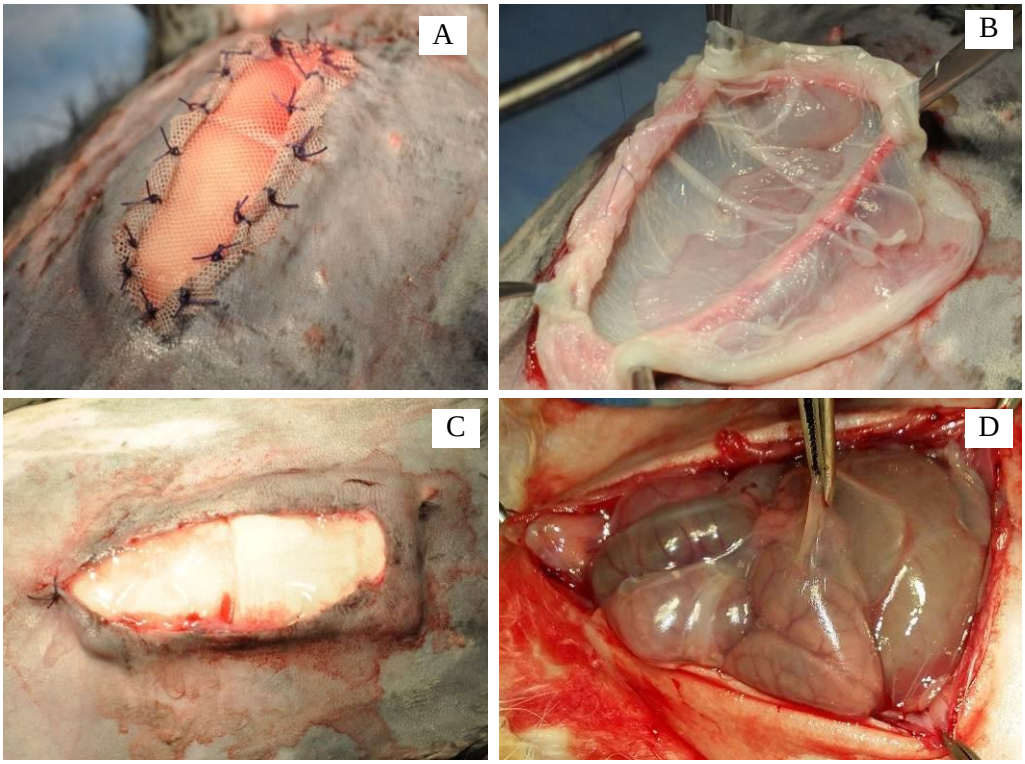

Fig. 1. Macroscopic appearance of the reconstruction of the abdominal wall defect with nonresorbable polyester mesh, *Erceokaque* type (A), amniotic membrane treated with 0.1% glutaraldehyde (B), amniotic membrane protected with Stypro (C), cryopreserved amniotic membrane (D).

In our research the grafts were treated at *Tissue Engineering and Cell Culture Laboratory*, State University of Medicine and Pharmacy "Nicolae Testemitanu". From graft harvesting to graft transplantation a series of systematized SOPs stages (standard operating procedures) are followed, structured according to the source used for the graft preparation.

The harvesting of the human amniotic membrane was performed immediately after birth by Caesarean section, to avoid contamination of the umbilical-placental complex through the birth pathways, from adult donors aged 25-35 years, after obtaining consent for harvesting, in compliance with the legislation in force (Law no. 42 of 06.03.2008 on transplantation of organs, tissues and cells).

After separation, the amniotic membrane was placed in decontamination medium comprising: RPMI (HiMedia), lincomycin, vancomycin (World Medicine), gentamicin (KRKA), hepes (HiMedia) 1 ml, is incubated at $+4^0$C for 12 hours, after which it is cut into strips of required size and placed in a double container in 50% of glycerol solution (Alchimia) with RPMI (HiMedia) and kept in the freezer at -80^0C.

Another method of the amniotic membrane preparation was to preserve the grafts in weak glutaraldehyde solution (0.1%) and formalin (0.5%). The graft is preserved in 0.5% neutralized formaldehyde solution, 7.3-7.4 pH, prepared in isotonic sodium chloride solution at $+4^0$C. The solution is daily changed for 10 days, then once a week up to 30 days. The first 30 days are the period of quarantine, as well as

sterilization and decrease of immunogenic properties. They were used after the serological and bacteriological validation of the grafts. The preparation of the amniotic membrane in glutaraldehyde and formalin allows to stabilize the implant, enhancing the biomechanical and enzymatic resistance (Spollensak G. et al., 2004).

The serological control is performed for the following tests: -Ac HIV 1+2, Ac HCV, HBV: (Ag HBs),(Ac anti HBs), syphilis.

Before use, during harvesting and after preparation some bacteriological tests are performed to establish the sterility, using 2 ml of transport liquid and 3-4 small portions of tissue, 5 x 5 mm in size, in various graft sectors in Thioglycolate Medium (HiMedia) and Sabouraud Dextrose Broth (HiMedia). The incubation is performed for 7-10 days to get final results.

The histological pieces were obtained by sectioning (four of each sample), using the semi-automatic microtome SLEE MAINZ-CUT 6062, 2.5 - 3µ thick. At the staining stage, the following methods were used: *hematoxylin-eosin* (H&E) and *Van Gieson* (VG).

Results and discussions

The animals were inspected daily to find the development of any complications. Animal sacrifice was made on the 7th day after surgery.

In spite of the postoperative wound infection in the control group, found in 4 animals, the dehiscence of sutures retaining the synthetic mesh was found in a case of animal`s death. Infection and wound dehiscence with eventration of the intestinal loops were found in 2 animals in group 1, and an animal died. Partial wound dehiscence with no signs of infection and development of incisional hernia were revealed in 3 animals in group 1, and in 2 animals in lot 2. In lot 3 partial dehiscence of the postoperative wound was recorded in a case (Fig. 2). The postoperative wound dehiscence was mostly caused by the implant rupture at the level of fixation sutures or less in central regions.

During the macroscopic evaluation of the peritoneal cavity, the development of an adherence process of various intensity was found in all cases of the control group, most often the synthetic mesh adhering to the omentum and intestinal loops, being observed the presence of some hard removable adhesions (3 cases), in four cases relatively easily removable inter-intestinal adhesions being detected with no signs of intestinal obstruction. In case of dead animals there were found purulent interintestinal collections and signs of diffuse peritonitis. There was revealed the development of an intestinal fistula in the control group. In a case in group 1 there was found the omentum adhering to the line of contact between the implant and the defect edge. In groups 2 and 3, even in cases of partial dehiscence of the fixation implant sutures no significant changes were observed along with the development of an adhesive process (Fig. 3).

The histological investigations of the samples taken in the study groups proved common and particular changes of the morphogenesis of reparative-regenerative processes at the tissue level and a correlation between the animal tissues and biological implant. The latter manifests asynchronously by the neoformation of granulations of various degree of maturity, expressed by fibroblastic elements, and of angiogenesis with persisting inflammatory response at the implant border, characterized by a various ratio of the cellular extrinsic and intrinsic component, with the predominance of polymorphonuclear cells (PMN).

The examination of tissue samples harvested from the contact line with the synthetic mesh (control group) did not reveal the presence of detersive reactions or formation of polynuclear giant symplasts, the latter being present in sutures. In these cases, an active regeneration was marked at the contact line with the synthetic mesh, which manifested by granulations rich in fibroblastic and capillary cells.

The changes in the intestinal visceral peritoneum in control animals showed reactive or proliferative processes.

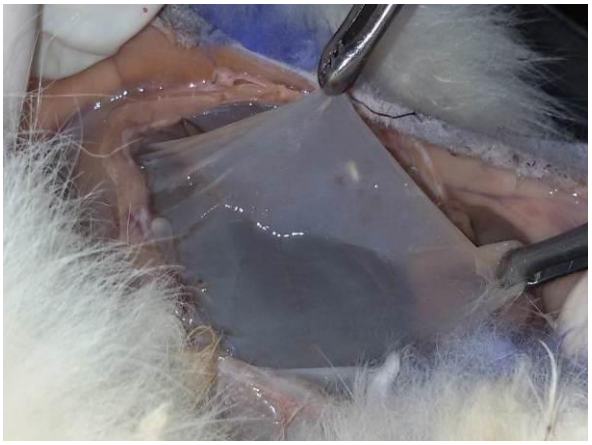

Fig. 2. Macroscopic appearance of the cryopreserved amniotic membrane fixed to the musculofascial layer with the preservation of the integrity of fixation sutures on the 7th day after surgery.

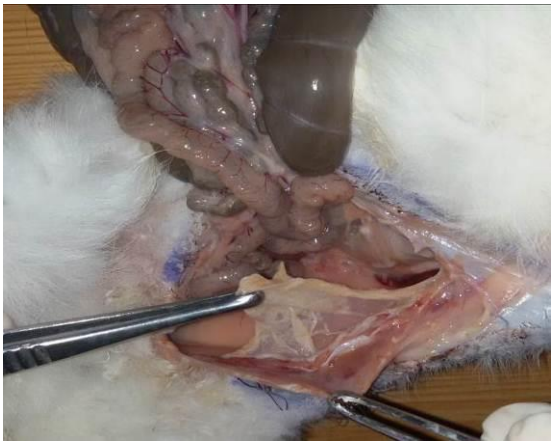

Fig. 3. Amniotic membrane treated with formalin on the 7th day. There is partial membrane rupture at the level of fixation sutures. The organs of the peritoneal cavity with no adhesions.

The animals in group 1, in large areas in the suture line between the musculo-peritoneal plane of the abdominal wall and amniotic membrane there was revealed the neoformation of granulation tissue in plateaus with various degree of maturity, with reduced PMN presence, the latter being more marked at the boundary with the connective layer of the amniotic membrane (fig. 4 A). It alternated with areas characterized by the proliferative mesenchymal-cellular anchoring features penetrating the connective matrix of the amniotic membrane, reaching the limit of the compact layer, sometimes its fasciculation being observed (Figure 4 B).

In those areas, at the level of the connective matrix of the amniotic membrane there was also found a hypercellularization on the account of fibroblasts, with the presence of some endothelial buds which emphasizes fibrillogenesis and angiogenesis activity (Fig. 4 C). A feature, found in the correlations between the granulation tissues and amniotic membrane, is active generalization of granulations on its surface, thus encompassing the amniotic membrane in various ratio. There were observed small areas of penetration and disjunction of the amniotic epithelium, most often bypassing the epithelium and forming small fissures (fig. 4 D).

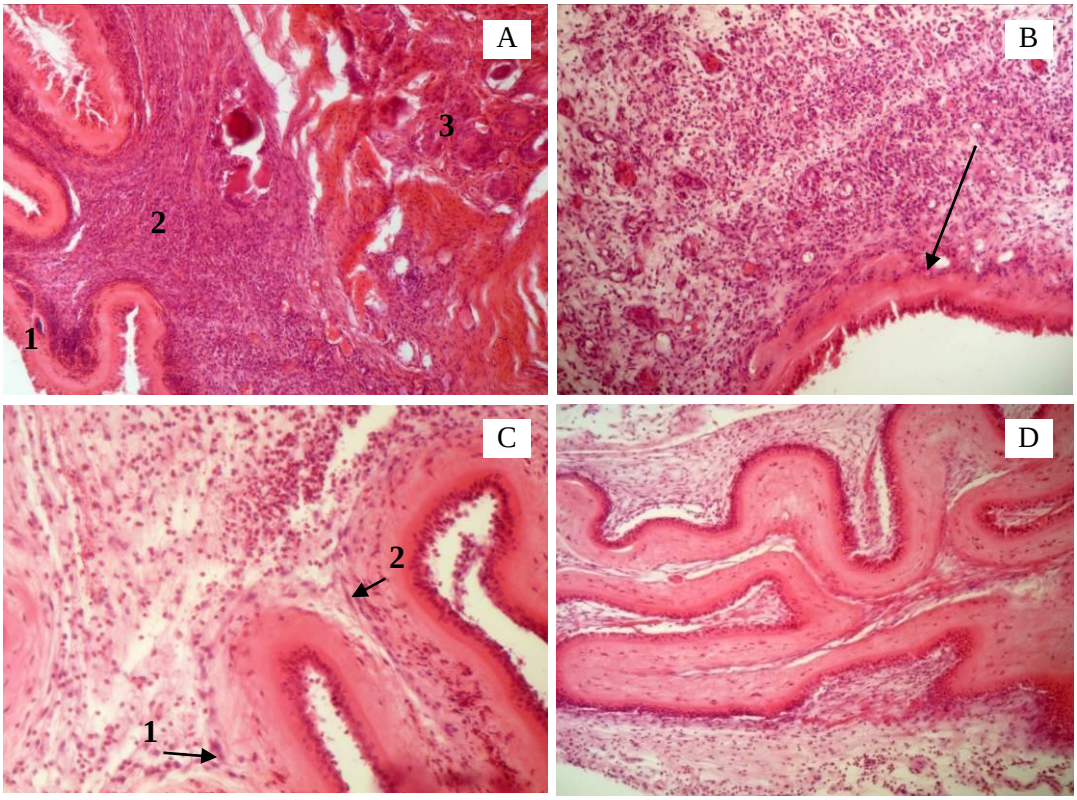

Fig. 4. A) Immature granulations in the plateau at the contact line: 1 - amnion; 2 - immature hypercellularized granulation tissue; 3 - abdominal muscular zone. x 75; B) Aspects of proliferative mesenchymal-cellular anchoring of the granulation with the amnion. x 125; C) Cellular-tissue elements in the cellular-mesenchymal anchoring area 1-endothelial buds; 2 fibroblasts. x 125; D) Aspect of granulations in strips embedding the amnion: 1-amnion; 2-granulations with reduced number of PMN. x100. *Color. H-E.*

At the border with the amnion, the granulation tissue had variable thickness, on some areas it being excessive and deformed. In close proximity there was often found the persistence of active inflammatory processes, characterized by PMN elements forming a fold (layer) with an abundant eosinophilic content that penetrated the area of the compact connective layer, with degeneration and disjunction phenomena with no detachemnt Fig. 5 A, 5 B). Thus, on some areas the loose connective zone of the amnion was invaded by immature granulations (Figure 5 B). At a higher magnification, a conventional cascade stratification of the cellular mesenchymal components of the immature granulation can be observed at the contact line, in which the angio-fibroblastic layer was followed by proliferative processes of fibroblasts and endothelial buds with the invasion of the loose connective (matrix) layer of the amnion, followed by the cellular PMN component which migrated in the dense connective layer of the amnion.

Examination and testing of the connective tissue revealed small precipitations and threadlike deposits of collagen at the level of granulations (fig. 5 C). There were also revealed reparative-regenerative processes of the

muscular and interstitial connective tissues, the regeneration of the connective tissue being predominant. Simultaneously with the changes described, the presence of microbial colonies was observed in some samples with the PMN predominance (fig. 5 D), which in fact accounts for polymorphism.

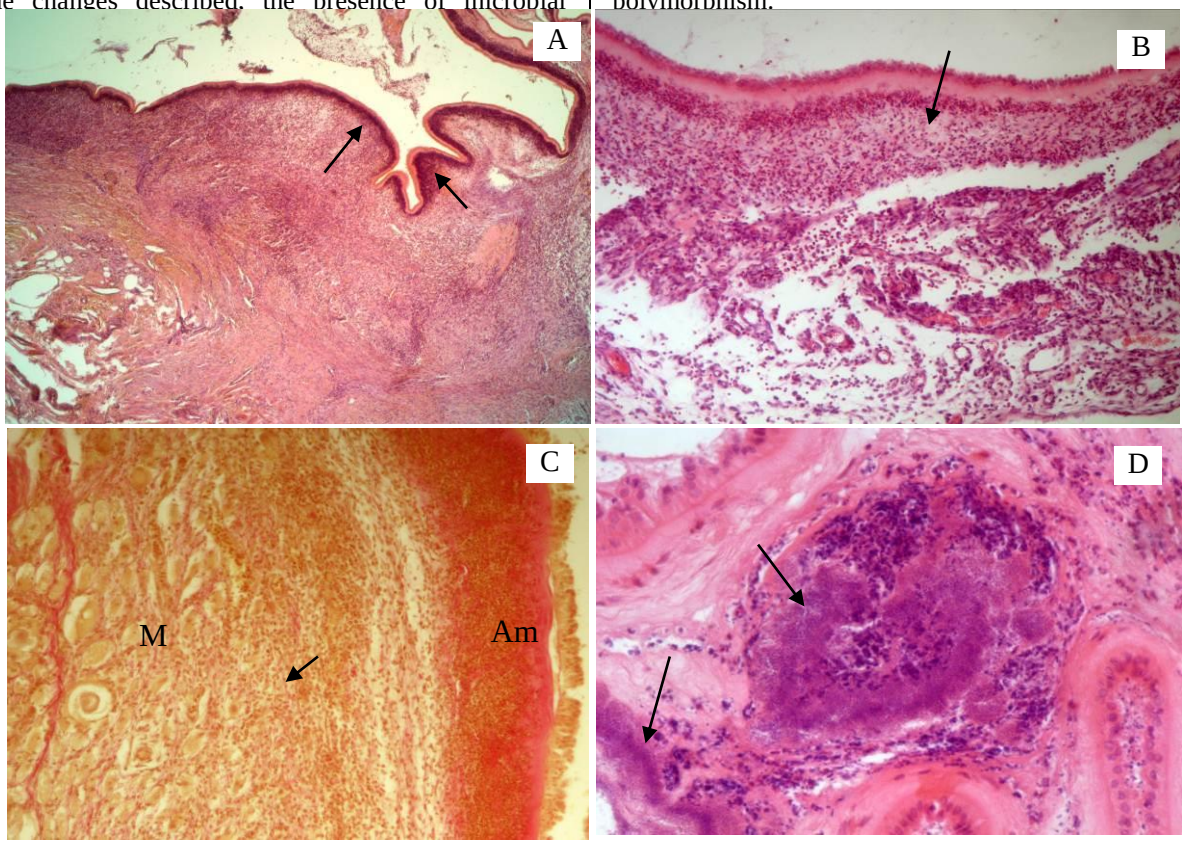

Fig. 5. A) Excessive granulations associated with the inflammatory process actively expressed by the PMN at the border with the amnion. x 25; B) Imature eosinophilic granulations in the loose connective layer (matrix) of the amnion. x 100; C) Precipitates and collagen filiform deposits in the area of granulations between the muscle area (M) and amnion (Am) x 100; D) Bacterial microcolonization with PMN and lymphocytic reaction at the level of the connective matrix of the amnion x 200. *Color. H-E*

In the area of the newly formed granulation tissue there were observed polynuclear cellular symplasts frequently adjacent to the host muscle area, characterized by deformed hypertrophic myocyte elements, dystrophy and polynuclear myogenic giant cells, reflecting the regenerative processes of the muscle area (Fig. 6 A). Giant cellular symplasts were also found adjacent to sutures, associated with the foreign-body type reaction (Fig. 6 B). In areas with the partial dehiscence of sutures fixing the amnion, the peritoneum showed an exudative inflammatory polymorphocellular serous-macrophagic reaction associated with active proliferative fibroblastic processes (Fig. 6 C). The inflammatory peritoneal process in the presence of microbial colonies revealed the presence of visceral focal proliferative- fibroblastic peritonitis (Fig. 6 D).

At the contact line of the amniotic membrane with the peritoneal stroma, the regenerative granulation processes manifested an activity analogous to that described in tha host muscle area, in 2 cases the proliferative-fibroblastic mesenchymal ones being predominant, and the PMN component being much lower. The correlations with the connective (matrix) layer of the amniotic membrane revealed a neoformation of the intimately anchored granulation tissue, also concrescent to it up to the dense connective membrane (fig. 7 A, B). In areas opposed to the epithelial layer of the implant there were revealed amniotic microcalcifications along with microfissures of its detachment; polynuclear giant symplasts being revealed at the level of granulations. In those areas, the amniotic element was intact or with some modifications of intumescence, circumscribed within the granulation of cellular reaction in polynuclear symplasts (Fig. 7 B). In these cases, when testing the connective tissue in the granulation area, the presence of threadlike collagen deposits was revealed on the 7th day, some resembling fine connective fibers.

Another phenomenon observed in the correlations of the newly formed granulation tissue with the amniotic membrane, particularly in the presence of the degenerescence of the amniotic layer, was the presence of pronounced cells at the level of the compact connective layer of the implant (Fig. 7 C), which became more pronounced, with an appearance of free or chain migration. On some areas fibroblast penetration from the granulation

region was observed, some fissures being present at this level, through which a trend of the cellular component penetration could be seen. Simultaneously, in areas with active regenerative processes, in the area of granulations, there were found only their residues as dense connective bundles comprised by spiral fibroblasts (Fig. 7 D).

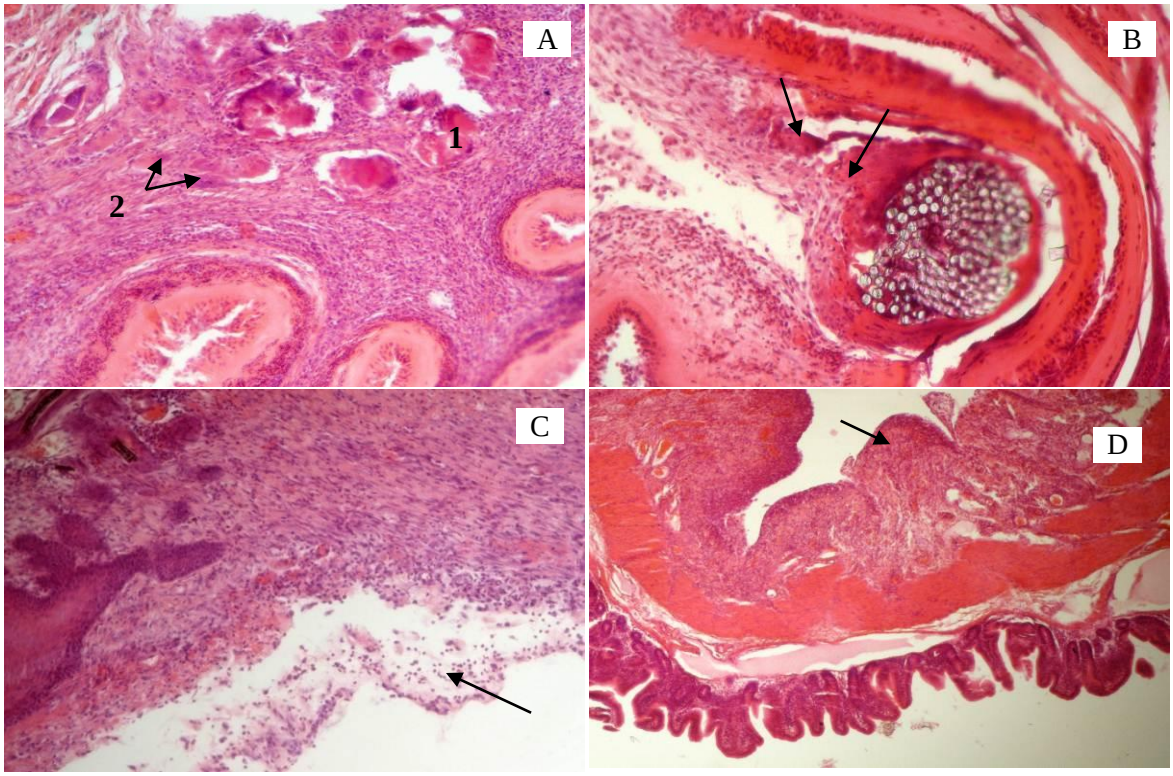

Fig. 6. A) Dystrophic and regenerative processes of the host muscular area 1 - hypertrophic myocyte cells with dystrophy; 2 - polynuclear giant cells of muscular origin in formation x 100; B) Foreign-body reaction with polynuclear symplasts around the suture wires. x 125; C) Area of the peritoneum contact line. Proliferative fibroblastic and exudative serous processes. x 75; D) Proliferative visceral fibroblastic peritonitis *Color. H-E.*

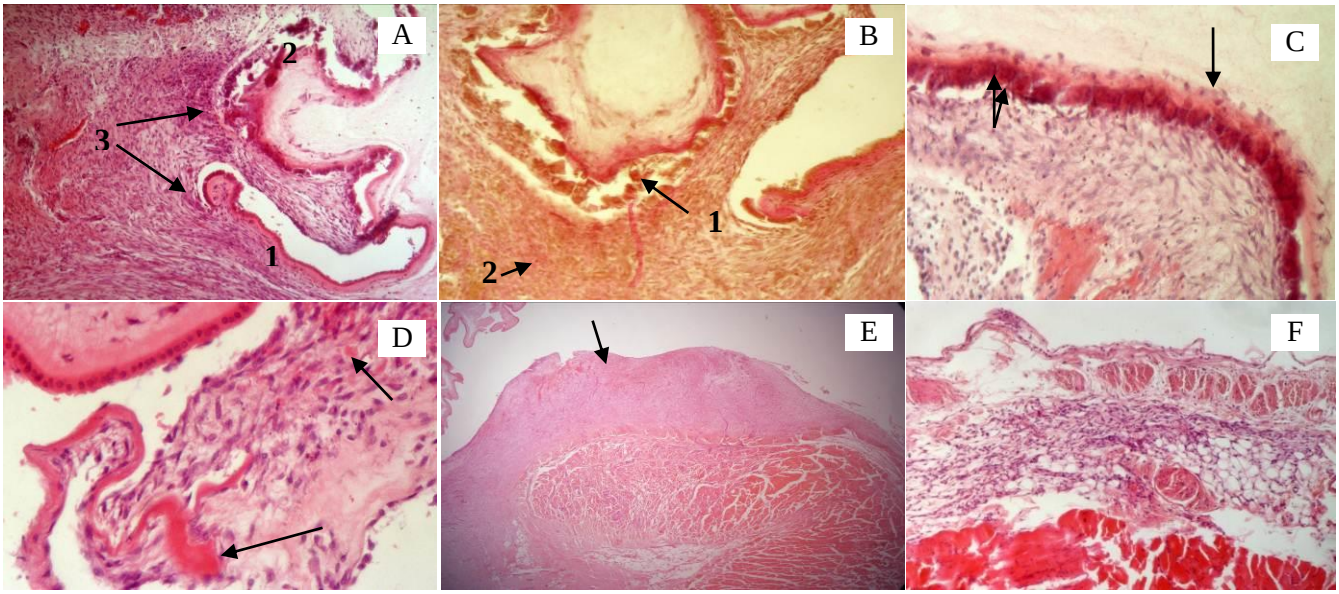

Fig. 7. A) Granulations in the peritoneal-mesenchymal zone-: 1 - intimate consolidation with matrix layer of the amnion; 2 – focal tension relieving of the amnion in the amniotic area; 3 - cellular polynuclear symplasts x 75; 4 - Amniotic membrane partially embedded in the granulation tissue B) Picture preceding amplification: 1- reaction in polynuclear giant cellular symplasts; 2 - threadlike deposits of collagen and fine connective fibers. x 120; C) Transepithelial migration of fibroblasts at the border with the amnion x 100; D) Bundles of the dense connective amnion in the granulation area embedded by spiral fibroblasts x 120; E) Processes of excessive and proliferative-fibroblastic peritoneal granulations nearby the contact line x 75; F) Distant view of the reactive peritoneal edema and cellular-adipose tissue, x 75. *Color. H-E.*

In samples taken in cases of the dehiscence of fixation sutures of the amniotic membrane, at the level of the adjacent peritoneum, including cases with the amniotic membrane detachment there were detected monstrous massive granulations with active proliferative fibroblastic processes (Fig. 7 E). The remote parietal peritoneum had no inflammatory changes, only a moderate reactive edema being present (Fig. 7 F).

In group 2, in the correlation with the contact surface of the amnion, there were revealed reparative-regenerative processes and correlation of tissues with the implant analogous to group 1. In the area of granulation plates, in various ratio, there were present the PMN elements with marked eosinophilia, mainly in areas overlapping with the amniotic epithelium. In some areas the granulations interposed between the very muscle area and the amnion were excessive. In group 2, compared to group 1, the amniotic component of the amnion manifested degenerescence, and sometimes it was absent (Fig. 8 A). In various areas nearby the very muscle area could be seen polynuclear muscular giant symplasts differentiated in myoblastic bud cords. When overlapping with the epithelial-cellular surface, as in previous cases, there were revealed signs of detachment of the amnion, in some areas there were focal calcifications of the epithelium; areas of partial detachment of the amnion and embedding of granulations being common (Fig. 8 B).

Eosinophilic cellular component manifested mosaic and disorderly character in the contact areas, which was confirmed by the absence of polymorphonuclear elements and eosinophils, including cases with overlapping epithelial cellular layer of the amniotic membrane, the proliferative fibroblastic cellular tissue manifesting phenomena of invading the epithelium area (fig. 8 C). These phenomena were marked with the decrease of the inflammatory process and the prevalence of the proliferative fibroblastic process. Compared with group 1, in group 2 there were more frequently observed polynuclear muscular cellular elements, sometimes in plateaus (Fig. 8 D). Frequently, the eosinophilic component was found perivascularly, including the boundary between the muscular tissue and granulations (fig. 8 E, F), being more frequent in overlapping areas with the epithelial-cellular surface. We should note that in study group 2 the polynuclear symplasts were frequently present in the area of sutures.

From the adjacent peritoneum there were found proliferative fibroblastic processes with the presence of polymorphonuclear cells and eosinophilic component. At the level of mesenterium, the presence of edema and polymorphonuclear cells are revealed regionally.

In cases with no dehiscence of sutures fixing the amniotic membrane, from the visceral peritoneum of the small and large intestines no inflammatory changes or vascular cellular reactions were observed in this study group.

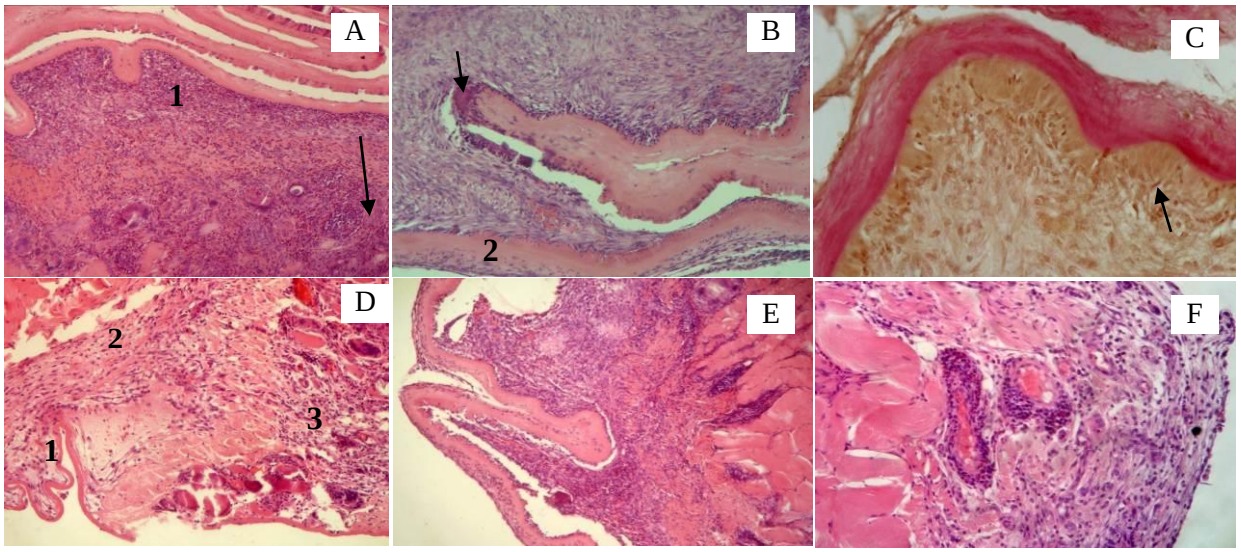

Fig. 8. A) Plate of immature granulations interposed between the muscle area and the amnion: 1- hipercellularization with PMN - eosinophils; 2- polynuclear muscular symplasts arranged (→) in small cords.x 25; B) Aspects of animal tissue correlation with the implant: 1- amnion with epithelial calcification and partial detachment; 2 - amnion lacking amniotic component embedded in granulation x 75 Color. H-E; C) Contact area - granulation plateau with phenomenon of PMN penetration and fibroblast implantation in amniotic component. x 200, Color. VG; D) Processes of regeneration of the connective-muscular tissue: 1 - amnion; 2 -granulations; 3- muscle tissue regeneration and angiogenesis. x 100; E) detachment phenomenon of the amnion when overlapping with the amniotic surface, immature granulations with PMN associated with perifocal vasculitis at the level of muscle layer, x 75; F) Polymorphocellular perivasculitis with predominance of eosinophilic component on the border with the musculo-fascial layer x 100. *Color. H-E*

The histological investigations in lot 3 have not confirmed any significant deviations of the reparative-regenerative processes of the tissue components and correlations between the tissues and amnion implant. There were frequently found morphological processes analogous to those found in lots 1 and 2, namely, a more marked activity of the regenerative processes and cellular-eosinophilic component. As in previous groups, the foreign-body type reaction with the presence of polynuclear giant cellular symplasts are frequently observed in the area of sutures, sometimes the degeneration of muscle fibers included in the area of monstrous massive granulations. Compared with the previous lots, in group 3 the PMN elements were found not only in the matrix or connective dense layer of the amnion, but dispersed throughout the area, often invading the epithelial cellular layer, which reveals the migration capacity of the cellular component in the implant area.

In group 3, between the granulation tissue with proliferative fibroblastic activity and epithelial-cellular surface there was found a more pronounced intimate relationship with more active angiogenesis peculiarities, such as areas of granulations penetration in the connective dense layer of the amnion with more pronounced adhesion and anchoring.

From the connective matrix of the amniotic membrane, in the contact areas there were found proliferative angio-fibroblastic processes supplemented by polymorphonuclear elements, penetrating in depth, partially involving the dense connective membrane, the latter having dystrophic changes. In this group the proliferative fibroblastic and angiogenetic processes with the capillary neoformation were comparatively more obvious and active. Also, in this lot the amniotic epithelium had degenerative alterations with no calcifications or foreign-body type reactions, the granulation tissues being frequently less huge or monstrous.

In most cases (4 cases) in group 3, the intestinal loops showed no pathological changes, except for a case, where some insignificant inflammatory changes were observed in the mesenteric region.

On the 7th day, in the nearby ares of the musculo-fascial layer involved in suture and in areas adjacent to the contact area with no necrotic, exudative or detersive changes, there was recorded the development of myocytes in myosymplasts, then in polynuclear myoblasts, followed by the appearance of cellular cords improperly oriented towards the granulation tissue of the contact line. It can be considered a remultiplication of the muscle fiber myocytes being significant in the plasty and regeneration processes of the muscle tissues.

Thus, the study results allowed to reveal some features of the morphogenesis of reparative-regenerative processes of the animal muscular-fibrillar tissues and correlations with the human amniotic membrane used as implant. The histogenesis of regenerative processes was found to be comparable to the normal histogenesis, characterized by the connective tissue formation manifested on the 7th day by the neoformation of the proliferative-cellular immature and mature fibrovascular granulations. In the same period, the histogenesis of regenerative processes in correlation with the implant had a mosaic appearance, being regionally retarded, characterized by an active persistence of exudative inflammatory processes, cellular degeneration and the presence of infiltrating neutrophils, more pronounced in groups 1 and 2 compared to group 3. In our opinion, this phenomenon can be influenced both by the processing method of the implant and the physiological individual peculiarities of animals. As seen in this study, the regeneration by substitution with the connective tissue evolves synchronously with the regeneration of fibrillar-muscular elements.

The PMN persistence in various areas is a reaction of the inflammatory process directed towards the implant degenerescence due to their proteolytic peculiarities, expressed in lot 3 by their more accessible migration to the implant structure. The tendency to form generalized granulations on the implant surface, as well as its anchoring and embedding was another feature found between tissues and implant.

The formation of more excessive granulations with an ugly aspect, found in some areas, is not a pathological reparative-regenerative organization but rather the result of small detachments caused by physiological animal behavior and mechanical factors. Thus, they mark the reparative-regenerative activity of *per secundam* healing, which is more pronounced in cases of dehiscence. The regenerative pathological processes as foreign-body type reactions with polynuclear giant symplasts were frequently found around sutures, as well as perifocally in the very degenerative muscle tissues as a result of the prevalent activity of both the neoformation of granulations, and the lack of exudative-detersive residues removal, they remaining embedded in the granulations. In the same context the foreign-body type reactions to amniotic epithelial elements were seen in the first lot. Adherent processes found at the peritoneal level in implant cases are conventionally pathological being determined not by implant itself, but by intraperitoneal eliminations of exudate or necrotic-detersive masses in the regeneration area.

The amniotic membrane is known in transplantology more than 100 years, when it was proposed by Davis J.M. (1910) as skin transplant (5). During the 40s of the last century the anti-adherent property of the amniotic membrane was discovered, contributing to significant advances in many surgical fields (11, 21). Subsequently, this fetal membrane was used in ocular lesions, burns and

chronic skin ulcers, in the treatment of Stevens-Johnson syndrome, as well as in reconstructive surgery of tendons, nerves, bladder and vagina, in order to prevent adhesions (7,20,22). The use of amniotic membrane in the reconstruction of congenital abdominal wall defects in newborn was proposed in 1971, which led to a decreased mortality in cases of gastroschisis (8). In 1975, J.H. Seashore and coauthors proposed swine skin grafts and human amniotic membrane as biological dressings in the treatment of gastroschisis and omphalocele, these biological grafts being considered useful in the adjuvant management of these cases (19).

The increased interest in the amniotic membrane is caused by reduced antigenicity, antimicrobial, anti-inflammatory and anti-adherent properties, this biological material representing a substrate for the growth of tissues etc. Given these properties, several authors tried to use the amniotic membrane in combination with other synthetic materials in the treatment of abdominal wall defects, these studies being on the experimental stage (1, 16).

Conclusions

1. The use of the amniotic membrane as implant, treated with glutaraldehyde and formalin, in spite of its durability, biomechanical strength and acceptable biocompatibility, does not provide long term stability, its application resulting in the dehiscence of the post-operative wound particularly within the fixation sutures. In this context, the cryopreserved amniotic membrane has some advantages, namely, implant elasticity and stability of fixation sutures, as well as marked reparative-regenerative activity.

2. The detachment phenomenon of amniotic membrane is determined by epithelial-cellular overlapping, this indicating the incorrectness of the implant application.

3. The amniotic membrane may be considered a useful temporary substitute for the peritoneum and a promising anti-adherent adjuvant in reconstructive interventions of the abdominal wall defects with viscero-abdominal disproportion, the results justifying the need to continue clinical trials in order to assess the efficacy and safety of this biological material application.

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ROTAVIRUS ENTERITIS IN INFANTS, CLINICAL AND THERAPEUTICAL ASPECT

Corina Frecus[1]

Abstract

The following study aims are to establish the incidence of the rotavirus enteritis in infants, clinical manifestation and the main therapeutic measures in the Pediatric Department of Constanta County Emergency Hospital over a period of two years, on a group of 166 infants diagnosed with rotavirus acute enterocolitis. The incidence of rotavirus acute enterocolitis is significant in our study- 38% of cases hospitalized for acute diarrheal disease. In 8,7% of patients we found associations betwen rotavirus and adenovirus .The peak incidence was recorded in the winter months (71 cases - 42.7%). All infants had watery diarrhea and the most frequently signs and symptoms were: vomiting (76.5%), fever (47.5%) and anorexia (42.1 The hospital treatment has made rebalancing hydroelectrolytic predominantly orally (66%). Evolution was good for all infants studied. Only 14 infants (9%) of the study were vaccinated against rotavirus. It is important to introduce rotavirus vaccine in infants as a prophylactic measure.

Key words: acute enterocolitis, rotavirus, infants.

Introduction

Rotavirus infections are the most common cause of severe diarrhea in infants and children worldwide. The incidence of rotavirus wordwide is estimated to cause more than 125 million cases of infantile diarrhea annually [1,2]. Rotavirus is the foremost cause of childhood dehydrating gastroenteritis worldwide [2,3]. Symptoms of rotavirus infection include the following: anorexia, low-grade fever, watery, bloodless diarrhea, vomiting and abdominal cramps. Before the introduction of the newer rotavirus vaccines, rotavirus was estimated to cause 20-60 deaths annually in the United States in children younger than 5 years [4].

A significant proportion (20-40%) of infections are asymptomatic, which contributes to the spread of the virus and might reduce the efficiency of prevention measures given as they are implemented too late. Throughout most of the world, rotavirus is present all the year round, which suggests that low-level transmission could maintain the chain of infection. Prevention of RV infection by mass vaccination could have a positive impact on the incidence of NRV by reducing the number of children hospitalized for gastroenteritis, therefore reducing the number of hospital cross-infections and associated costs. Vaccines also contribute to reducing resource utilisation by preventing nosocomial infections, such as rotavirus gastroenteritis, which can increase hospital stays by 4-12 days [5,6,7].

Aims of the study

In this study we aimed to analyze the following data: incidence of rotavirus infection in the admissions at Pediatric Clinic Emergency Hospital Constanta, the main clinical manifestations, dehydration degree, study of acid-base and electrolyte balance in cases of dehydration; the main therapeutic measures and evolution, status of vaccination against rotavirus.

Material and methods

The trial was conducted on a total of 166 patients aged in the range of 0-1, admitted to the Pediatric Clinic Emergency Hospital Constanta with a diagnosis of acute enterocolitis immunochromatographic rapid test for rotavirus by viral etiology. The study was conducted between 1 January 2011 – 31 December 2013.

Patients studied were performed by an observation sheet where noted: age, sex, food type (natural-artifical-mixed), area of origin, education level of the mother and condition of life, reason and period af admission, previous medical history, general pshical examination, treatment and evolution.

Results and discussions

Rotavirus infection accounted for 35% of cases hospitalized for acute diarrheal disease. Rotavirus was isolated from 166 patients (35%) of 412 cases. The remaining cases had another cause: viral - adenovirus (20 %); bacterial: Klebsiella (10 %), E. Coli (7,2 %), Staph. Aureus (4 %). Noted that 26.9% were cases undiagnosed or other etiology. Rotavirus was the highest percentage of cases hospitalized for diarrhea.

76 studies from 16 countries were identified and the mean percentage of acute gastroenteritis (AGE) cases caused by rotavirus ranged from 25.3%-63.5% in children < 5 years of age, peaking during winter [8]. In Japan, from January 2001 through December 2002, a total of 443 children<5 years of age were hospitalized for acute gastroenteritis. Of 422 stool specimens collected, 244 (58%) tested positive for rotavirus [9].

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From the group of 166 infants diagnosed with rotavirus enterocolitis, 91 were male and 77 were female.

76 (46%) were from urban areas and 90 (54%) children come from rural areas.

Distribution of cases according to season and month: in our study the majority of rotavirus disease occurred in winter (42.7%) with a peak in February (32 cases), confirming the literature noted. In the temperate zone rotavirus infections is a winter disease while peaks in the autumn or spring are common in other parts of the world. In America the study found that their incidence peaked was in winter primarily [10,11].

During the year were diagnosed sporadic cases (Spring - 31.3% Autumn - 18.6% in the Summer - 7.2%). The most frequent causes of Rotavirus infections appeared in December (25), January(14) and February(32).

Distribution by age shows that rotavirus diarrhea incidence was highest in infants in the age group 3-6 Month(42%) (Figure 1).

The age of 3 month is low immunity (receive transplantal antoibody level decreased, while its antibodies are just beginnig to form); between 3 to 6 month, most infants begin to be feed artificially or mixed; early and improper diversification favors the appearance of acute diarrheal disease and dehydration default.

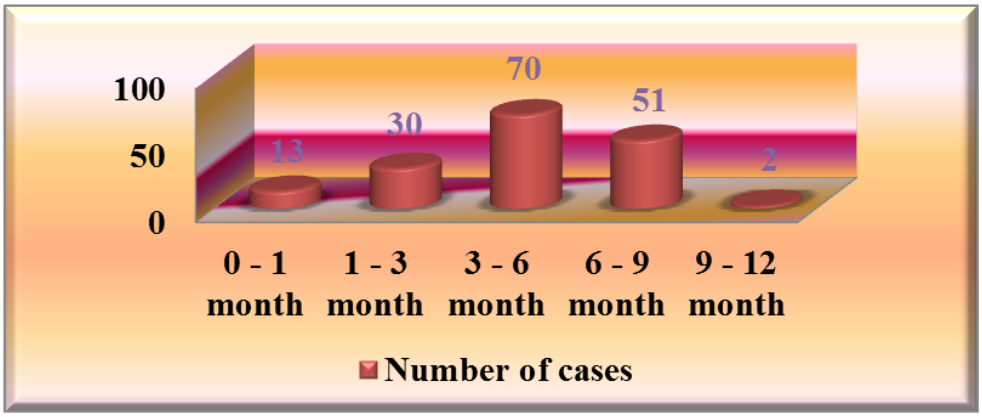

Figure 1. Patients distribution by age group.

Distribution of cases according tp type of feeding: from the 166 infants with rotavirus infection diagnosted by us, 36 infants were brest feeding, 21 infants mixed feed, artificial feed only 9 and majority – 80 infants with diversified feeded.

Distribution of cases according to education level of mother : 25 were illiterate (15,6%), 16 finished four classes (9,6%), 47 finished 8th grade middle school (28,3%), 60 (36,1%) complete high school and 18 (10,8%) finished college.

Nutritional Status of Patients: most infants, 95 in number, were normal weight (57%), the rest being underweight and divide thus:

- 48 infant (29 %) with dystrophy de grade I ;
- 18 infant (11 %) with dystrophy de grade II ;
- 6 infant (3 %) with dystrophy de grade III.

Early symptoms in the group of infants studied were : diarrhea, watery stools in all patients, vomiting in 127 patients and fever in 79 patients. There were no reported bloody stools (table 1).

Table 1 Clinical Manifestation of Patients.

Symptom	Number of Case	Percentage %
Diarrea	166	%100
Vomiting	127	76.5%
Fever	79	47.5%
Anorexia	70	42.1%
Full Skin inactivity	47	28.3%
Sunken Eyes	31	18.6%
Oliguria	6	3.6%
Extremely marbled	6	3.6%
Cough and Coryza	28	16.8%

Maculopapular rash	1	0.6%

90 patients who had signs of dehydration were divided as follows:

- 42 cases (0.4%) with slight dehydration of approximately 5% loss of liquid;
- 40 cases (0.5%) with an average drying loss of around 8% liquid;
- 8 patients (0.1%) had severe dehydration with loss of> 10% liquid;

Distribution of cases according to result of ionogram : imposed performance ionogram in 60 of the patients and found:

Serum Na :

- 39 cases had hyponatremia (<120 mEq/l);
- 17 cases had normal serum sodium levels (120 – 135 mEq/l);
- 4 cases had hypernatremia (>150 mEq/l).

Serum K:

- 43 cases had normal potassium level (3,5 – 5,5 mEq/l);
- 12 cases had hypokalemia (< 3,5 mEq/l);
- 5 cases presented hyperkalemia (> 5,5 mEq/l).

Balance of Acid-Base - metabolic acidosis is excessive loss of base expression and increased fluid loss through vomiting and diarrhea. Thus alkaline reserve was determined at 60 patients of which 40 (66%) had low values and the remaining 20 patients (34%) were within normal limits.

Therapy for children with acute diarrhea rotavirus in our study was determined by the degree of dehydration. They received oral re-hydration 110 children (66%) and the remaining 56 children (34%) received parenteral rehydration hydro and acid-base rebalancing.

From the 166 patient severe forms of dehydration syndrome has number of 98 cases treated with antibiotics was established from the beginning (regarding a possible infection enteral and / or parenteral) to obtain the result of FOBT (Fecal Occult Blood Test). The antibiotics used were ampicillin in combination with gentamicin or generation cephalosporin-III (ceftriaxone).

The most frequently prescribed were: ampicillin, ampicillin + gentamicin association, cephalosporin and cephalosporin + gentamicin (table 2).

Table 2. Distribution by antibiotics used.

Antibiotics used	Number of treated cases
Ampicillin	48
Gentamicin	22
Cephalosporin	28

Duration of hospitalization infectious diarrheal episode was divided as follows:

- 56 infants (34%) hospitalized from 3 -7 days
- 98 infants (59%) hospitalized between 8-12 days
- 12 infants (7%) hospitalized between 12 to 16 days.

Vaccination of Rotavirus infections: From the 166 infants diagnosed with Rotavirus Acute Enterocolitis only 14 of them (9%) were vaccinated and the remaining 152 patients (91%) were not vaccinated.

Conclusions

In our study rotavirus was the most common cause of acute diarrhea in children.(38%) of cases hospitalized for acute diarrheal disease.Were notice rotavirus and adenovirus associations to 8.7% of patients.

The peak incidence was recorded in the winter months (71 cases - 42.7%) with a peak in February (32 cases - 20.5%). The gender distribution was approximately uniform: 54% - male, 46% - female. Rotavirus diarrhea incidence is highest in the age group between 3-6 Month (70 cases - 42%).

Infants which feeding naturally showed a lower incidence cause of rotavirus infection (25%).

Data analysis indicates that the low level of education of the mother is a contributory factor for the occurrence of acute rotavirus enterocolitis.

All infants had watery diarrhea and hospitalization of the signs and symptoms most frequently encountered were: vomiting (76.5%), fever (47.5%) and anorexia (42.1%). Note that no patient had bloody stools.

From the 90 patients who had signs of dehydration, 42 cases (0.4%) had slight dehydration degree of 40 cases (0.5%) dewatering the medium and only 8 patients (0.1%) developed severe dehydration requiring parenteral rebalancing.

In moderate to severe dehydration forms were recorded electrolyte and acid-base disorders: hyponatremia (65%), hypernatremia (7%), hypokalemia (20%), hyperkalemia (9%) and metabolic acidosis in 66% of cases.

Mostly infants not receiving outpatient treatment (50%), only some were treated with antibiotics (22.8%), anti-diarrheal (19.2%) and very few attempted treatment by diet (8.4%). The hospital has made rebalancing hydroelectrolytic predominantly orally (66%).

Duration of infectious diarrheal episode was most of the time on average, between 8-12 days (59%). Evolution was good for all infants studied.

Only 14 infants (9%) of the study were vaccinated against rotavirus. The large number of rotavirus disease virus makes this a public health problem that requires the introduction of rotavirus vaccine in infants as a prophylactic measure.

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PROGNOSTIC FACTORS FOR CONGENITAL ABDOMINAL WALL DEFECTS

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Abstract

Omphalocele and laparoschisis are one of the most common congenital diseases with serious consequences upon the life and health of the population. Multiple studies, published in international important medical journals have analyzed and presented the factors that may influence the therapeutic outcomes of both diseases. The aim of this study is to assess the main medical, familial, socio-economic prognosis factors with influence over the diagnosis, treatment and post therapeutic outcomes of congenital abdominal wall defects in children. During this period a total of 51 patients with congenital abdominal defects were admitted to our hospital. Postoperative evolution was favorable for 31 patients. Complications occurred in 26 patients, most of them infection related, 3 local and 17 extended. Prolonged bowel obstruction was developed by 13 patients. 20 patients deceased, 15 with laparoschisis (45%) and 5 with omphalocele (28%). The overall mortality rate was 39.2% for both diseases. Predictive factors for complications are: males, low APGAR score, incongruence between the peritoneal cavity and herniated organs, postoperative anemia and/ or thrombocytopenia, renal failure. Unfortunately the mortality is high as well. Predictive factors for an unfavorable outcome being: males, low birth weight, low APGAR score, associated malformations, large defects, renal failure, bronchopulmonary infection and sepsis.

Key words: omphalocele, laparoschisis, prognostic factors, congenital abdominal wall defects

Introduction

Despite of the evident progresses of diagnosis and treatment of congenital lesions in children, omphalocele and laparoschisis remain one of the most common congenital diseases with serious consequences upon the life and health of the population. During the last decades important progress has been made in the field of prenatal diagnosis, progress that allowed us to make a better judgement over the indication for abortion or labor continuing and prepare for the postnatal treatment (1,2). On the other hand the introduction of prosthetic materials in the treatment of these diseases represented a huge step forward to reducing the complications and decreasing mortality (3). Not least the advancement in the field of the new-born intensive care and resuscitation had major influence over the outcome of the treatment. However, the mortality is still high for both these diseases, approximately 10% for laparoschisis and from 20 to 50% for omphalocele (1-3).

Multiple studies, published in international important medical journals have analyzed and presented the factors that may influence the therapeutic outcomes of both diseases. To my knowledge, there has been no such study in Romania for 2 decades. So, we consider that a study like this in the 21th century Romania is necessary and may contribute to the improvement of the treatment of these diseases in our country.

Our intention is to assess the main medical, familial, socio-economic prognosis factors with influence over the diagnosis, treatment and post therapeutic outcomes of congenital abdominal wall defects in children.

Material and method

This study has been carried out at the „Louis Turcanu" Emergency Clinical Hospital for Children in Timisoara. We have reviewed the medical recordings of the patients with congenital abdominal wall defects admitted to our hospital from the 1st of January 1999 until the 31 of August 2015.

We have recorded and reviewed the parameters shown in table 1. We assessed these parameters in relationship with the evolution of each individual case and the outcome of the treatment.

For statistical analysis we used IBM SPSS Statistics v20 for Microsoft Windows. We used t-Student test for numeric variables and Mann-Whitney test for non-numeric variables; p value was set at 0.05.

Results

During this period a total of 51 patients, 29 males and 22 females with congenital abdominal defects were admitted to our hospital. The gestational age ranged from 31 to 41 weeks, mean 36.73 weeks. 21 patients were born premature (less than 36 weeks).

Birth weight ranged from 1250 g to 4060g, meaning 2400g. Birth height ranged from 33cm to 60cm, meaning 46 cm. A total number of 31 patients had the birth weight under 2500 g, and 8 of them under 1500 g.

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Table 1. Assessed parameters.

Pregnancy and birth	Gestational age	APGAR Score
	Weight at delivery	Delivery rank
	Height at delivery	Pregnancy surveillance
	Sex	Type of delivery
Demographic data	Maternal age	Medical history
	Urban/ Rural	
Disease	Type of disease	Size of the defect
	Clinical form	Age on admitted
	Prenatal diagnosis/ on delivery diagnosis	
Associated pathology	Cardiac malformation	Skeletal malformations
	Intestinal malformation	Renal malformations
	Chromosomal anomalies	
Treatment	Concomitant surgical procedures	Re-interventions
	Surgical procedure	Other surgical procedures
	Age at Surgery	
Evolution/ Complications	Intestinal occlusion	Renal failure
	Respiratory infections	Survival rate/ age of decease
	Sepsis	

Prenatal diagnostic was made in 9 cases, in 3 cases there was no medical follow up during the pregnancy. There were 2 breech presentations and C-section was performed in 11 cases.

Out of the total of 51 cases, 33 were of laparoschisis and 18 of omphalocele. The size of the defect ranged from 2-12 cm, meaning 4,7 cm; 2-7 cm, meaning 3,5 cm for laparoschisis and 3-12 cm, meaning 5,6 for omphalocele. The herniated organ was the bowel alone in 39 out of the 51 cases. The liver was out in 3 cases of omphalocele. More than 3 concomitant organs were herniated in 4 cases of omphalocele.

Maternal age varied from 13 to 45 years, the average age being 22.9 years; 14 were under 20, 34 of them were between 20-35 years old and 3 were over 35 years old. Laparoschisis was more common in children from younger mothers and omphalocele is more frequently associated with older mothers (p=0,036).

Associated malformations were present in 27 out of the 51 patients. Chromosomal anomalies had been found in 1 patient, 9 had cardiac malformations, 2 skeletal malformations, 7 with intestinal atresia and 2 with renal malformations. Multiple malformations were present in 2 patients. The omphalocele was associated more frequently with multiple malformations and skeletal malformations. The rest of the malformations were divided equally between the 2 groups of diseases.

The age of the patients at admission into the hospital was between < 1 hour and 72, meaning 6.2 hours. Only in three cases the admission in a neonatal surgical department was not made in the first 24 hours. The age at surgery varied from 5 to 26 hours, meaning 9.4 hours. In only one case the surgical intervention was performed after 24 hours after admission.

Surgery was the first treatment option in 50 out of the 51 patients. Schuster procedure has been performed in 9 cases, Gross procedure in 2 laparoschisis and Fufezan procedure in other 3 omphalocele cases. In the past few years it was possible the use of silastic bags for the progressive reduction of the bowels in the abdominal cavity in 3 cases of laparoschisis and 1 of omphalocele (Table 2).

Table 2. Surgical procedure.

		Disease		
		LAPAROSCHISIS	OMPHALOCELE	Total
SURGICAL PROCEDURE	PRIMAR CLOSE	27	13	40
	SCHUSTER	2	0	2
	GROSS	1	0	1
	FUFEZAN	0	3	3
	SILICON BAG	3	1	4
Total		33	17	50

Concomitant surgical procedures were necessary in 8 patients, 5 intestinal resections and anastomosis for intestinal atresia, 1 resection of Meckel diverticulum and 1 repairing of the bladder exstrophy and 1 omental resection. Reinterventions were necessary for 19 patients, 10 of these had programed for second step Schuster procedure, Fufezan procedure and silastic bag; 2 for the programed delayed repairing of the intestinal atresia. In all there were 51 reinterventions.

Postoperative evolution was considered favorable for 31 patients. Complications occurred in 26 patients, most of them infection related, 3 local and 17 extended. Prolonged bowel obstruction developed in 13 patients. 20 patients deceased, 15 with laparoschisis (45%) and 5 with omphalocele (28%). The overall mortality rate was 39.2% for both diseases.

The statistical analyzes of the main factor influencing the evolution of abdominal wall defects are presented in table 3 and 4 (statistical significant ones with bold).

Table 3. Prognostic factors for complications.

	Mann-Whitney U	Wilcoxon W	Z	p
Sex	894,000	1674,000	-1,884	0,050
Gestational age	813,500	2088,500	-,554	0,579
Birth weight	878,000	2418,000	-1,306	0,192
APGAR	444,500	1572,500	-2,578	0,010
Follow up during pregnancy	273,000	769,000	-,151	0,880
Delivery	1089,000	2742,000	-,234	0,815
Presentation	1110,000	1890,000	-,029	0,977
Mother's age	683,500	1673,500	-,745	0,456
Type of defect	814,500	2467,500	-2,675	0,007
Size of defect	778,500	1306,500	-,358	0,720
Herniated organs	1056,000	1836,000	-,478	0,632
Chromosomal anomalies	1075,500	2728,500	-,561	0,575
Cardiac malformations	981,000	1761,000	-1,411	0,158
Intestinal atresia	1105,500	2758,500	-,078	0,938
Skeletal malformations	1069,500	1849,500	-,547	0,584
Renal malformations	1093,500	2746,500	-,388	0,698
Multiple malformations	1047,000	2700,000	-,953	0,341
Surgical treatment	1090,500	1870,500	-,374	0,709
Age at the surgery	264,000	792,000	-1,058	0,290
Surgical procedure	794,000	1497,000	-1,941	0,052
Surgical re-interventions	818,500	1521,500	-1,500	0,134
Concomitant surgery	908,000	2183,000	-,216	0,829

Discussions

Laparoschisis and omphalocele are of the most serious congenital diseases in terms of distress for the neonate, high mortality and morbidity rate altogether with a high incidence for both diseases. During the period of 12.6 years we have treated 51 cases of congenital abdominal wall defects, of which only 17 originated form Timis County. Of these 7 cases of omphalocele were from Timisoara and 10 patients of laparoschisis were from the rural area of Timis County. Corroborating this data with the general birth rate in our county of 7000/ year (4) we have found an incidence of 1:8400 for laparoschisis and 1:12000 for omphalocele. Our results are slightly different from those in the literature, 1:4000 for laparoschisis respectively 1:5000-10000 for omphalocele (5). The gender distribution of 1:1 for laparoschisis and 1.6:1 for omphalocele is similar with the previous reports (5) and we found a higher rate of mortality and complications in male patients (p=0,04, respectively p=0,050).

Periodic medical visits and appropriate follow-up during pregnancy represent an essential method for prevention and efficient treatment of congenital diseases. The prenatal diagnosis is an imperative condition for planning a good therapeutic conduct. The levels of alpha-fetoprotein and the transvaginal echography may establish the diagnosis since the 10th week of gestation (6). In case of laparoschisis this is of extremely importance since the majority of the specialist recommend that the baby should be delivered prematurely as soon as the fetus becomes viable in order to shorten the exposure of the bowels to the amniotic fluid (7). Unfortunately in our study the prenatal diagnosis was set in less than 20% of the cases. Moreover in 3 cases no medical follow up was carried on during pregnancy. This is a clear indication that there is still a lot to do for the primary care medical assistance in some remote rural areas.

Table 4. Prognostic factors for mortality.

	Mann-Whitney U	Wilcoxon W	Z	p
Sex	846,000	1512,000	-2,056	0,040
Gestational age	660,000	2145,000	-1,631	0,103
Birth weight	586,000	2297,000	-3,404	0,001
APGAR	307,000	1633,000	-3,718	0,000
Follow up during pregnancy	183,500	963,500	-,346	0,729
Delivery	1038,000	1704,000	-,444	0,657
Presentation	1074,000	2904,000	-,118	0,906
Maternal age	555,500	1731,500	-1,936	0,053
Type of defect	876,000	2706,000	-1,864	0,062
Size of defect	760,000	2245,000	-,224	0,822
Herniated organs	873,000	1539,000	-1,810	0,070
Chromosomal anomalies	1080,000	2910,000	,000	1,000
Cardiac malformations	882,000	1548,000	-2,171	0,030
Intestinal atresia	1056,000	2886,000	-,317	0,751
Skeletal malformations	912,000	1578,000	-2,219	0,026
Renal malformations	1056,000	2886,000	-,525	0,600
Multiple malformations	966,000	1632,000	-1,709	0,087
Surgical treatment	972,000	1638,000	-1,949	0,051
Age at the surgery	178,000	958,000	-1,603	0,109
Surgical procedure	705,500	1371,500	-2,786	0,005
Surgical re-interventions	883,500	2368,500	-,649	0,517
Concomitant surgery	853,500	2284,500	-,612	0,540
Intestinal occlusion	986,000	2526,000	-,045	0,964
Renal failure	810,000	1476,000	-2,764	0,006
Sepsis	642,000	1308,000	-3,848	0,000
Wound infection	1074,000	2904,000	-,118	0,906
Total complications	630,000	1296,000	-4,003	0,000

In our study the gestational age varied between 29 and 41 weeks, less than a third (28%) of children being delivered premature. We cannot talk about premature programmed delivery in laparoschisis because the prenatal diagnosis was set in only 17% of these, meaning 9 cases. So the majority of these cases were natural occurred premature birth meaning that they were rather an additional factor for the disease itself. Our statistical analysis did not revealed that. Instead we found out that the low birth weight is an important factor which influences the mortality not only in the case of neonates with congenital wall defects.

APGAR score is the indicator of health in neonates and offers an image upon the future evolution of the patient. Although is not necessary directly related to the underlying disease, a low APGAR score is a strong predictor for a baleful evolution (p=0,010) or for the complications (p=0,010).

The main factors in influencing the therapeutic conduct of congenital abdominal are: the size of the defect and the presence or absence of the associated diseases. Generally the size of the abdominal wall defect is lower in laparoschisis than in omphalocele (7). A possible predictive value and in direct relation with the size of the defect is the number and the type of organs herniated. In most of the cases (62%) the herniated organ was the bowel, followed by the liver. Associated disorders were present in approximately half of the patient and they were a predicating factor for unfavorable outcome. The cardiac malformations are preponderant 11 (32%) for omphalocele and 8 (12%) for laparoschisis. Intestinal atresia was presented in 10 cases of laparoschisis (16%) and 2 cases of omphalocele (5.8%), the percentage being similar to those from literature (1, 2, 3). Statistically speaking we found that the associated malformations in general (p=0.05) and cardiac malformations (p=0.03) had significant influence upon the mortality while the occurrence of postoperative complications had not been influenced by these. So we conclude that the associated malformations, especially the cardiac ones are an important factor in the patient survival with congenital wall defect.

One of the most important factors influencing the outcome of a congenital wall defect is the quality and the precocity of treatment (6). Specific medical measures and treatment planning should begin while the patient with abdominal wall defect is still in utero. After delivery the quality primary care of the neonate appropriate transport condition and the immediately as possible surgical closure of the defect are essential to obtain favorable results. Surgical treatment is the only curative way for these diseases. The choice of surgical procedure depends on the size of the defect, but also on the surgeon's ability and the available options for prosthetic materials. The small number of patients treated by surgical augmentation and/ or staged closure of the abdominal; wall defect does not allow us to

conclude upon the benefits of one procedure. Yet we want to make a special reference to Fufezan procedure which represents in our opinion a cheap and simple way of gradual reintegration of the abdominal organs in the case of patients with omphalocele (8). However, neither the type, nor the number of the surgical procedures influenced significantly the outcomes of the treatment in our study.

The postoperative course of the patients with abdominal wall defect, particularly for those with big defects or associated malformations is marked by high morbidity and mortality rates (9). In our study 60% of the patients had postoperative complications the most prevalent being the infectious ones. The presence of sepsis represented one of the main death causes, 20 out of the 26 patients with sepsis had unfavorable course (p=0.000). Renal failure is the most feared immediately after surgical closure of the defect and is directly related to the capacity of the surgeon to estimate the balance between the capacity of the abdominal cavity and the volume of the herniated organs. Fortunately this complication occurred in a limited number of cases but, also it had a significant influence over the unfavorable course of the case.

Eventually all of the parameters which we found to have predictive value for the course and the outcome of abdominal wall defects can be adjustable and the first step to solve the problem is to find out where it is.

Conclusions

Even though smaller than that reported in literature, both laparoschisis and omphalocele still have a high incidence in the western part of Romania. Primary or delayed closure of the defect shell being the first option, nowadays it is possible in most of the cases. Complication rate is still high with most frequent complications being infection related. Predictive factors for complications are: males, low APGAR score, incongruence between the peritoneal cavity and herniated organs, postoperative anemia and/ or thrombocytopenia, renal failure. Unfortunately the mortality is high as well. Predictive factors for an unfavorable outcome being: males, low birth weight, low APGAR score, associated malformations, large defects, renal failure, bronchopulmonary infection and sepsis.

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ERRATUM

Retraction note: *"Thyroid disorders in children, adolescents and young adults in Mures county (Romania): a 25 years retrospective study"*

The article *"Thyroid disorders in children, adolescents and young adults in Mures county (Romania): a 25 years retrospective study"*, published in Jurnalul Pediatrului, Year XVIII, Vol. XVIII, Nr. 71-72, july-december 2015, pages 3-9, was retracted on demand by the corresponding author and with the written consent of all the other authors of the manuscript, due to an issue reported by the 3 rd coauthor Angela Borda, regarding a conflict of interests.

To redaction of Jurnalul Pediatrului,

The authors of tha paper *Thyroid disorders in children, adolescents and young adults in Mureş county (Romania): a 25 years retrospective study* published in Jurnalul Pediatrului 2015, volume XVIII, Nr. 71-72, pages 3-9, kindly ask the redaction of Jurnalul Pediatrului to retract the manuscript with is title (both HTML and PDF version). This removel procedure is in compliance with the journaey publishing politics.

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În atenţia domnului Prof. Univ. Dr. Eugen Sorin Boia, Redactor Şef al Jurnalului Pediatrului

Stimate Domnule Redactor Şef

În Volumul XVIII, numărul 71-72, July-December 2015 a Jurnalului Pediatrului, a apărut articolul intitulat: "Thyroid disorders in children, adolescents and young adults in Mures county (Romania): 25 years retrospective study". În acest articol figurez şi eu ca al treilea co-autor (Borda Angela). Având în vedere faptul că acest articol a fost publicat FĂRĂ ştirea mea (fără să mi se ceară acordul), vă rog frumos ca în numărul următor al jurnalului să publicaţi în loc vizibil o erată prin care să anunţaţi că am fost scoasă din lista de autori, la cererea mea.
Vă solicit acest lucru, cu atât mai mult cu cât, la finalul articolului apare o frază în care se afirmă că **autorii declară că nu au conflicte de interese**!!! ceea ce este un lucru grav şi fals, având în vedere că articolul a fost publicat fără să fiu măcar anunţată că voi fi printre autori!!

Tîrgu Mureş
23.08.2016

Cu stimă
Prof. Dr. Angela Borda
Prorector, UMF Tîrgu Mureş

MANUSCRIPT REQUIREMENTS

The manuscript must be in English, typed single space, one column on A4 paper, with margins: top – 3 cm, bottom – 2,26 cm, left – 1,5 cm, right – 1,7cm. A 10-point font Times New Roman is required.

The article should be organized in the following format: Title, Names of all authors (first name initial, surname), Names of institutions in which work was done (use the Arabic numerals, superscript), Abstract, Keywords, Text (Introduction, Purpose, Materials and Methods, Results, Discussions and/or Conclusions), References, and first author's correspondence address.