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CONSIDERATIONS ON THE CLINICAL EVOLUTION OF AN ASPERGILLOSIS CASE IN CHILDREN

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Abstract

Aspergillosis is a group of diseases caused by microorganisms of the genus Aspergillus. Most pediatric cases are caused by Aspergillus fumigatus. Atopic asthma can be precipitated by inhalation of specific spores, leading to an immediate response by IgE and bronchospasm. Allergic bronchopulmonary aspergillosis joins in 7-10% of patients with corticodependent asthma.

The authors present the case of a child diagnosed with asthma, when an infant, with oscillating evolution. Controller therapy is associated with progressively increasing values of eosinophilia, and finally a syndrome "hyper IgE" (values >4000UI). Inflammatory syndrome is strongly positive. Finally, we excluded any autoimmune or neoplastic and infectious etiology and we confirmed allergic bronchopulmonary aspergillosis.

Keywords: *Aspergillosis, asthma*

Introduction

Aspergillosis is one of the most common causes of exacerbation of asthma. Exposure to colonies of Aspergillus has been described as a factor causing numerous respiratory diseases, including asthma, chronic eosinophilic pneumonia, hypersensitivity pneumonia and bronchopulmonary aspergillosis.[1,5] While bronchopulmonary aspergillosis complicates asthma in adults, the association with asthma in children is very rare.[8] Atopic asthma can be precipitated by inhalation of specific spores, leading to IgE-mediated response and bronchospasm.

Case report

We report the case of a 3-year-old girl who was diagnosed with asthma and eczema at 1 year of age. At onset, the clinical symptoms present an erythematous-maculo-papular eruption, and dyspepsia. The pacient was consulted and admitted to the Hospital for Infectious Diseases and Pneumology "Dr. Victor Babes" in Craiova for 13 days; laboratory findings revealed a 30% eosinophilia and he was recommended bone marrow biopsy, investigation which was refused by his mother who required an examination within the Fundeni Hospital in Bucharest. After performing a bone marrow puncture, the evaluation of the bone marrow aspirate smears showed slightly reduced cellularity for the patient's age, a left shift in the granulocyte lineage, normal maturation. Some cellular elements showed macrocytosis and cytoplasmic vacuoles, mild eosinophilia. Erythropoietic lineage showed mild hyperplasia, normoblastic, with normal maturation. The megakaryocytes were in normal percentage in all stages of maturation. After smear examination, we did not find atypical cells.

The child is sent to "Matei Bals" Hospital in Bucharest, where tests reveal Hyper IgE>4000UI, negative pediatric allergy panel, IgG negative for Ascaris, Toxocara serology, Toxoplasma, Trichinella negative, specific IgE Aspergillus = 0.88 (normal value <0.35).

We excluded HIV infection, adenovirus, parainfluenza virus, Chlamydia, pneumoniae, Mycoplasma pneumoniae, CMV, Epstein Barr virus, Echo virus, hepatitis with virus A, B, C, D.

We learned from the patient history about repeated exposure to mold in the family!

Laboratory findings: ALT = 50 U/L, AST = 88 U/L, Iron = 106 μg/dl γGT = 62U/L, LDH = 548 U/L, uric acid = 2.7 mg/dL, Total Calcium=10.4mg / dl

Eosinophils: November 2013-May 2014: 30%, 12%, 8.5%, 6%, 13% (Fig.1.)

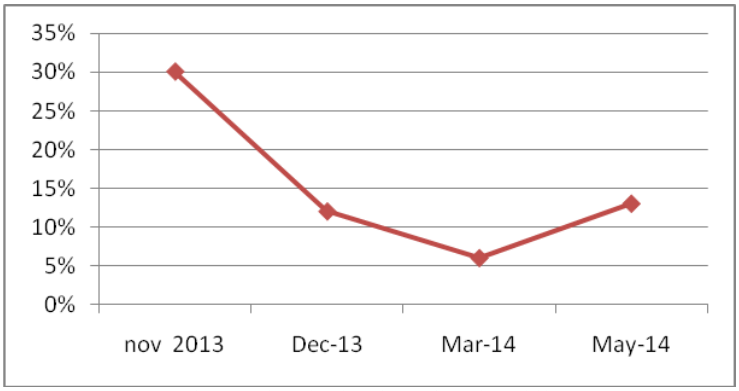

Fig. 1. The distribution of eosinophils

Total IgE values: 18 11 2013 > 4000 UI/ml, 02. 12. 2013: 961.6 UI/ml, 05.03. 2014: 324 UI/ml .(Fig. 2.)
IgE Aspergillus fumigatus: 02. 02. 2014: 0.95 kU/L, 18.11.2014: 7.92 kU/L. (Fig. 3.)

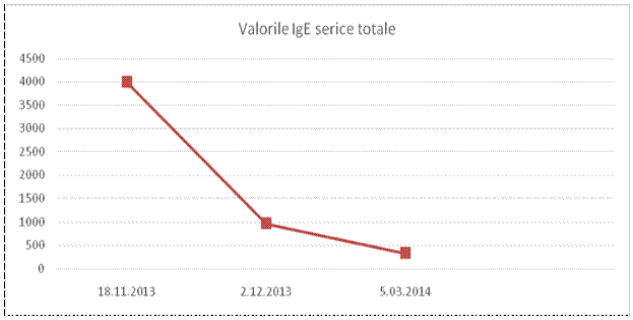

Fig. 2. The distribution of total IgE

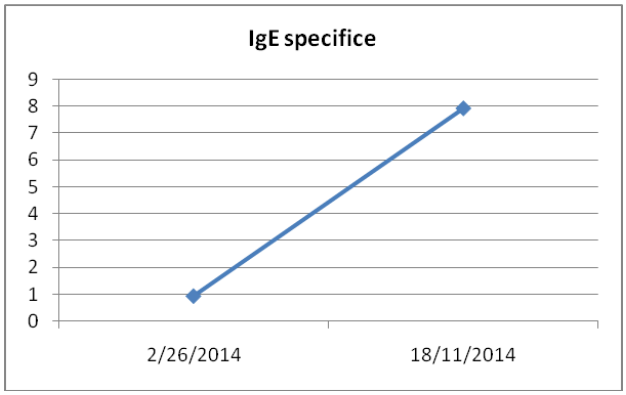

Fig. 3. The distribution of IgE Aspergillus fumigatus

Chest X-ray: reticular and micronodular opacities of different size, diffuse contour supra--, para- and infrahilar bilateral. (Fig 4, 5.)

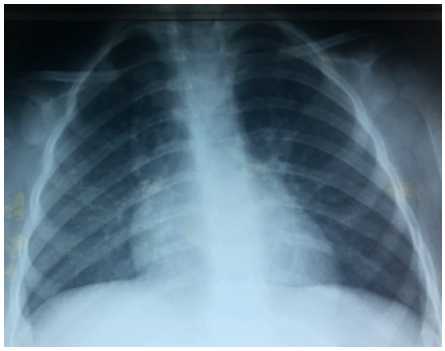

Fig. 4. Radiologic aspect

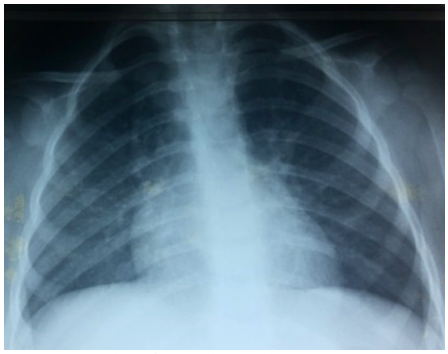

Fig. 5. Radiologic aspect

Bronchoscopy: 12.12.2013: muco-purulent secretions very abundant coming from all lobar orifices, predominantly right. The cultures were negative for microbial germs, negative for fungi, with rare lysed neutrophils; eosinophils, crystals Charcot were not noticed, AFB absent.

Given the historical data, the high values of total IgE, IgE to aspergillus value, extensive bilateral infiltrates radiological aspect; one can support the diagnosis of allergic bronchopulmonary aspergillosis.

This was followed by treatment with Ciprofloxacin 200 mg/day, 8 days, Amikacin 180 mg/day 8 days, Voriconazole 9 days, Ventolin inhaler, Flixotide, Singulair, Zantac, Azithromycin orally 3 days, Ophtamezole intranasally. The evolution was slow with progressive diminishing functional respiratory syndrome.

Discussions

Allergic bronchopulmonary aspergillosis pathogenesis involves an allergic reaction to aspergillus species. Patients with chronic lung disease (eg, asthma, cystic fibrosis) can retain the secretions aspergillus fumigatus, leading to an immune response that exacerbates respiratory symptoms. A chronic colonization of mucosal fumigatus produces elevated levels of immunoglobulin G (IgG) and immunoglobulin E (IgE), which lead to recurrent bronchospasm.

Allergic bronchopulmonary aspergillosis occurs in 1-2% of patients with asthma.[2]

The normal levels of total IgE in a patient with active pulmonary disease exclude the diagnosis of allergic bronchopulmonary aspergillosis.[4]

Serum levels of total and specific IgE are required for the differential diagnosis of allergic bronchopulmonary aspergillosis; the degree of activation correlates with the disease and this is useful to monitor the response to treatment.[7] A skin prick test and specific IgE (methods with high sensitivity and specificity) allow early diagnosis of the disease, being associated with a favorable prognosis, thus preventing progression to irreversible lung tissue changes (fibrosis).[3,12]

Conclusion

Allergic bronchopulmonary aspergillosis is an undervalued disease, the true prevalence of aspergillosis is not known. There is a similarity between the clinical manifestations of allergic bronchopulmonary aspergillosis and asthma with fungal sensitization.[9] The correct diagnostic can be difficult but very important for the prognostic.[11] The key therapy remains the antifungal treatment whose duration depends on the evolutionary peculiarities of the underlying disease, the stage screening and the variable risk of the bronchial colonization of Aspergillus spores, which requires special measures for prevention in these patients.[6,10,13]

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PECULIARITIES OF SEVERE ASTHMA IN CHILDHOOD

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Abstract

Asthma is the most common chronic disease in children and an important health problem worldwide. Asthma severity is the most important feature of asthma being related to its short and long term outcomes.

At first asthma severity was established according to the level of symptoms, the need for rescue medication and lung function tests.

Most recent guidelines for asthma management recommend that asthma severity should be determined according to the step of therapy needed to achieve asthma control.

Before deciding whether a patient has severe asthma or not, it is important to distinguish between severe asthma and difficult to treat asthma related to incorrect inhaler technique, improper adherence or incomplete controlled comorbidities.

Severe asthma in children has a series of distinctive features towards severe asthma in adults.

In children severe asthma is closely linked with atopy unlike severe asthma in adults.

Adolescents are at a highest prevalence of severe asthma and at the highest death risk through asthma do to poor treatment adherence and do to at risk behaviors (smoking).

In children with severe asthma lung function measurements are age dependent and might be between normal ranges despite the presence of symptoms at the time of the assessment.

Severe asthma in children has a multifactorial etiology. There are many molecular, genetic and epigenetic patterns related to severe asthma.

The main drivers of severe asthma management in children are therapy optimization and comorbidities treatment.

The perspectives in severe asthma management in children include individualized treatment and biological therapies.

Key words: *chronic respiratory disease, severe asthma, child*

Asthma represents an important health problem in the entire world. Asthma is thought to affect 350 millions people around the world and to cause 250.000 deaths each year [1]. At the same time asthma is the most common chronic disease in children[2].

Asthma prevalence among children is estimated to be around 9,3% in USA and between 5-27% in Europe and it follows an ascendant pattern in the entire world [3, 4, 5, 6]. Furthermore, approximate two thirds of asthma cases in adults are thought to emerge before the age of 18 years old [7].

Asthma is a very heterogeneous disease. The heterogeneity of the disease is the result of the interaction between genetic factors and environmental factors, pathological mechanism underlying the disease, health care access, the pattern of treatment response, asthma severity, asthma comorbidities, short and long term outcomes of the disease and other factors [1].

Asthma severity is the most important feature of asthma, being related to the short and long term outcomes of the disease.

In the early asthma guidelines asthma severity was established according to the level of symptoms, the need for rescue medication and lung function tests[1]. Recently it became more and more obvious that the assessment of asthma severity must include the assessment of asthma treatment responsiveness. That is due to the fact that a child who appear to have severe asthma based on his symptoms can gain a quick treatment response and, in reverse, a child who may appear to have mild or medium asthma may fail to achieve a good asthma control contrary to exception [1].

Asthma severity must be periodically reevaluated because asthma severity is not an unchangeable feature of the disease, but a feature that can vary over time [1].

Most recent guidelines for asthma management provide the recommendation that asthma severity must be determined according to the step of therapy needed to achieve asthma control [8]. According to Global Strategy for Asthma Management and Prevention, asthma is considered moderate to severe if requires step 4 medication (medium/high dose of inhaled corticosteroids + long acting beta agonist) or step 5 medication (step 4 + oral corticosteroids or anti IgE therapy) resulting controlled asthma or still uncontrolled asthma, despite therapeutical interventions with step 4 or 5 medication[8].

According to International ERS/ATS Guidelines on Definition, Evaluation and Treatment of Severe Asthma, severe asthma is asthma which requires treatment with high dose inhaled corticosteroids (ICS) plus a second controller (and/or systemic CS) to prevent it from becoming uncontrolled or which remains uncontrolled despite this therapy received for at least tree months [9].

World Health Organization included also the category of untreated severe asthma in the definition of severe asthma. Untreated severe asthma is an asthma that is not receiving a proper controller therapy due to poor socioeconomic resources[1].

It is commonly accepted that, before deciding whether a patient has severe asthma or not, it is important to distinguish between severe asthma and difficult to treat asthma related to incorrect inhaler technique, to improper adherence or to incomplete controlled comorbidities (obesity, chronic rhinitis, etc) [2].

It is also important to distinguish between severe asthma and uncontrolled asthma due to persistent environmental exposure, untreated comorbidities or psychosocial factors [2].

Uncontrolled asthma is more often responsible for persistent symptoms and severe exacerbation than severe asthma. At the same time uncontrolled asthma is much easier to treat than a severe asthma [2].

Difficult to treat asthma is asthma which fails to become controlled do to comorbidities, poor adherence or persistent environmental allergen exposure [2].

Treatment resistant asthma or refractory asthma is asthma which fails to maintain a complete control of symptoms and exacerbation despite treatment with high dose ICS (inhaled corticosteroids) and a second controller (LABA – long acting beta agonist or OCS – oral corticosteroids) [2].

Properly severe asthma refers to refractory asthma and to asthma in which the comorbidities are only partially treated [2].

Before establishing the diagnosis of severe asthma we must exclude: poor inhaler technique (encountered in up to 80% patients), poor treatment adherence, incorrect diagnosis of asthma (which explains lack of response to specific asthma therapy), comorbidities (obesity, rhinosinusities, gastroesophageal reflux, depression and obstructive sleep apnea), persistent environmental exposure to allergens and irritants [2].

Statistically, 12-50% of the patients considered to have severe asthma, in reality, have a completely different diagnosis [2]. The prevalence of severe asthma in children is estimated to be 0,5% among general pediatric population and 4,5% among children with asthma [10]. Severe asthma in children has a series of distinctive features towards severe asthma in adults. Severe asthma in childhood is closely linked with atopy, unlike severe asthma in adult. In children, severe asthma is associated with atopy in 93,5% of cases [10]. In children, as in adults, risk factors for severe asthma are obesity, air pollution, smoking, genetic and epigenetic factors [9]. Adolescents are at the highest prevalence of severe asthma and death risk due to poorly adherence to treatment and at risk behaviors (smoking) [11].

The onset of symptoms in children with severe asthma occurs often in the first 3 years of life [11].

Children with severe asthma have higher serum IgE levels, higher serum and sputum eosinophils levels, higher exhaled nitric oxide than the children with mild and moderate asthma [11].

Nevertheless the use of sputum eosinophils or exhaled nitric oxide for guiding asthma treatment in children is not yet accepted [9].

Unlike severe asthma in adults, which tends to follow a persistent pattern, in children, severe asthma often follows a pattern with rapid evolving, frequent and severe exacerbations triggered by viral infections or/and allergens. [11]

An important particularity of severe asthma in children is that they are often asymptomatic between exacerbations.[11] In children with severe asthma we can find a series of clinical and inflammatory phenotypes very different from those found in adults. Furthermore, these phenotypes change in time with age, unlike severe asthma in adults. [11] Also, in this category of children with severe asthma, lung function measurements are age dependent and might be between normal ranges despite the presence of symptoms at the time of assessment. [11]

In severe asthma the small distal airways are more affected than the large proximal airways. This fact explains why FEV1 (forced expiratory volume in one second) is often normal in children. For these reason, in children, FEV1/CV (vital capacity) may be better correlated with asthma severity than FEV1 alone. [11]

Four clinical phenotypes of severe asthma in children have been described: late onset with normal lung function, atopic early onset with normal lung function, atopic early onset with mild air flow limitation and early onset with important air flow limitation. [12]

Three inflammatory phenotypes of severe asthma in children have been described: eosinophilic inflammation (more corticosteroid responsive), paucigranulocytic inflammation and neutrophilic inflammation (poor corticosteroid response). [10] Severe asthma in children has a multifactorial etiology and there are many molecular, genetic and epigenetic pattern related to severe asthma (IL4 receptor polymorphism, IL6 receptor polymorphism, etc). [9]

The evaluation of an asthmatic patient suspected to have severe asthma must include: watching the patient using his inhaler, showing him the correct inhaler technique and recheck up to three times and again at each visit, discussing treatment adherence and the impediments of proper adherence, confirming the diagnosis of asthma, managing the comorbidities, identifying and exclusion of risk factors (environmental exposure, smoking, using of non steroidal anti inflammatory drugs), treatment stepping up and reevaluating after three to six months. [8]

Severe asthma in children requires regular medical check-up, but also a careful follow-up at home. This condition often requires long term peak expiratory flow (PEF) monitoring. [8]

The main drivers of severe asthma management in children are therapy optimization and comorbidities treatment.

Fortunately, only a very small group of patients with severe asthma is completely resistant to corticosteroid therapy, therefore ICS are the first line treatment for patients with severe asthma. [1]

Severe asthma management in children includes, as additional therapeutic options, optimization of ICS/LABA dose, addition of OCS, add-on treatments without phenotyping, sputum guided treatment, phenotype guided add-on treatment (anti IgE antibodies), non pharmacological interventions and comorbidities treatment. [8]

To minimize the risk of severe outcomes patients with severe asthma need to undergo influenza vaccination each year.[8] The perspectives of severe asthma treatment in children include individualized treatment based on the clinical and molecular phenotype pattern and biological therapies addressed to specific molecular particularities of each patient. [11]

Conclusions

Severe asthma treatment in children involves the optimization of ICS/LABA dose and association with other controller therapies. Severe asthma management in children must include comorbidities treatment. The additional use of approved biological therapy (anti IgE antibody) provides an improvement in severe asthma control. The perspectives of severe asthma treatment in children include individualized treatment and biological therapies.

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FACTORS INFLUENCING THE INCREASED FREQUENCY OF ACUTE OTITIS MEDIA

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Abstract

Background: Acute otitis media (AOM) is the condition with the highest degree of medical addressability during early childhood. In recent years our department has faced a growing number of cases with AOM. Objective: Analysis of the factors that can influence the growing numbers of children presenting with AOM. Methods: We studied retrospectively records of patients aged 1 to 24 months, admitted with AOM to the 2nd Pediatrics Clinic, between January 1st 2013 and December 31st 2014. Our group consisted of 59 patients for whom we analyzed the following parameters: gender, birth rank, gestational age, type of birth (vaginal versus caesarean section), birth weight, current vaccination status, and recent antibiotic use. Results: Most children diagnosed with AOM were born through caesarean section (33.9% vs 66.1%, p = 0.018). For most children with AOM antibiotics were used in recent history (27.12% vs 72.88%, p = 0.001). 83.5% of patients were immunized according to the Ministry of Health schedule, while none of the children were vaccinated against Streptococcus Pneumoniae. Conclusions: In our study, increased frequency of caesarean section, recent antibiotic therapy and the lack of pneumococcal vaccination seem to be responsible for the increasing number of AOM. Large-scale studies are needed to confirm these hypotheses.

Keywords: *acute otitis media, cesarean section, antibiotics, anti-pneumococcal vaccination*

Background

Acute otitis media (AOM) is the condition with the highest degree of medical addressability during early childhood.[1] In recent years our department has faced a growing number of cases of AOM. We aimed to analyze the factors that can influence the growing rates of AOM.

Material and method

We conducted a descriptive study that involved a retrospective analysis of the observation charts made for children hospitalized in the 2nd Pediatrics' Clinic, Timişoara with the diagnosis of AOM. The review period was: January 1st 2012 to December 31st 2014. Our group included 59 children. The study followed the international standards of medical ethics established by the Declaration of Helsinki, regarding confidentiality of patient data.

We analyzed the following variables: age (months), sex, birth weight (g), gestational age (weeks), child's rank (only child or younger brother) and the mode of birth (vaginal or cesarean), current weight (g), vaccination status (national vaccination program, anti-pneumococcal vaccination), recent antibiotic therapy (the month before hospitalization)

The results are presented as percentages or means and 95% confidence intervals for mean, depending on the type of the variable analyzed. The non-parametric binomial test (for the 95% confidence interval) was used to evaluate differences across the study group in terms of the mode of birth and recent antibiotics' status.

Results

Descriptive characteristics of the study group are shown in Table 1 and Table 2.

66.1% of the children were born through caesarean section (Fig. 1).

49 children received antibiotic treatment in the month before admission (Fig. 2).

Table 1 - Descriptive characteristics of the study group (I)

Variable	n= 59*	
	Mean	95% Confidance interval for mean
Age (months)	14.64	12.69 - 16.59

Gestational age (weeks)	38.63	38.12 - 39.13
Birth weight (g)	3244.82	3059.42 - 3430.22
Actual weight (g)	9864.29	9216.06 - 10512.51

*3 missing variables

Table 2 - Descriptive characteristics of the study group (II)

Variable		n= 59	
		No. cases	Percent (%)
Sex	Girls	24	59
	Boys	35	41
Rank	Only child	31	52.5
	Smaller sibling	28	47.5
National Vaccination Program	Yes	49	83.05
	No	10	16.95
Anti-pneumococcal vaccination	Yes	0	0
	No	59	100

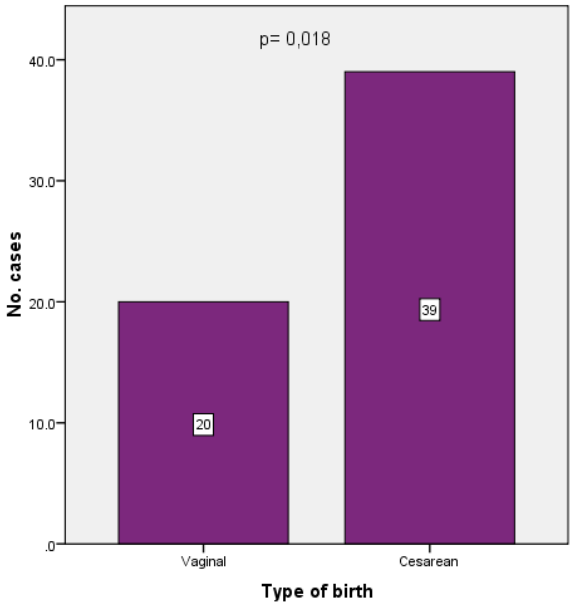

Fig. 1. Type of birth across the study group.
p denotes the statistical significance of the non-parametric binomial test.

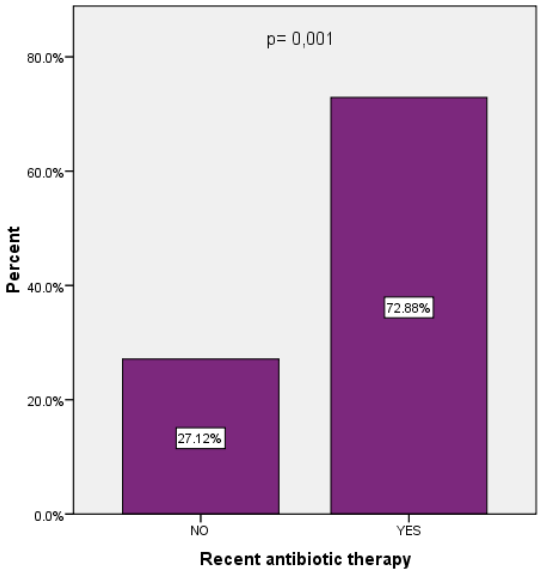

Fig. 2. Recent antibiotic therapy across the study group.
p denotes the statistical significance of the non-parametric binomial test.

Discussions

Our study shows with statistical significance that more than half of the children were born through caesarean section. It is well known that the manner of birth influences intestinal colonization of the newborn: for babies born vaginally, the gut flora is affected by the vaginal and intestinal flora of the mother, while birth by cesarean favors colonization with very different types of bacteria. Thus, their flora is substantially influenced by environmental factors. [2] Several studies have suggested a correlation between birth by caesarean section and obesity, asthma, allergies and various autoimmune diseases. Naso-pharyngeal colonization of the neonate may be influenced by the manner of birth, and a possible correlation with associated pathology requires further study.

This hypothesis is all the more important as the percentage of caesarean sections in Romania has gradually increased in recent years, with 38% of all births being cesarean sections.[3] A larger study is needed to verify our results.

The vast majority of children in our study were vaccinated as required by the National Program of Vaccinations, but none were vaccinated for pneumococcal diseases. Pneumococci are the most common etiologic agent of AOM in the pediatric population.[1] In countries where pneumococcal vaccination was introduced in the national program of vaccination, the incidence of AOM has significantly decreased. Since 2013, the pneumococcal vaccine is introduced into the national vaccination program in Romania, but so far no funds have been allocated for its acquisition.

In addition, significantly more children with AOM received antibiotics before admission to our clinic. This suggests an increased antibiotic resistance to the usual germs that cause AOM. A study performed in Braşov, Romania between 2009 and 2011 revealed a very high percentage of S. Pneumoniae isolates non-susceptible to penicillin (93.8%) and ceftriaxone (77.1%).[4] Another study conducted by "Matei Balş" Institute and published in 2014 showed an increased pneumococcal resistance profile: 72.5% of the S. Pneumoniae isolates were erythromycin resistant.[5]

Conclusion

In our study, increased frequency of caesarean section, recent antibiotic therapy and the lack of pneumococcal vaccination seem to be responsible for increasing the number of AOM. Large-scale studies are needed to confirm these hypotheses

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RARE CAUSES OF RECURRENT WHEEZE- NOT EVERY WHEEZING IS ASTHMA

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Abstract
Recurrent wheeze is a pathology frequently observed in daily pediatric practice. From asthma and tuberculosis to rare diseases like alpha 1 antitrypsin deficiency, bronchiectasis, it can be varied etiology and diagnosis extremely difficult sometimes. Methods: This paper aims to present two cases with recurrent wheezing: the case of a girl of 10 months, with 2 months long history, referenced for right upper lobe atelectasis. On admission, finds good general condition, wheezing unresponsive to bronchodilators, with radiologic evidence of left lung hyperinflation. During disease`s evolution, the wheezing persisted, accompanied by a strange transmission sound. Also cardiac ultrasound could not revealed the emergence of coronary trunk; Corroborating data a vascular compression was suspected and CT angiography confirmed the case of artery lusoria . Another 2 years old child suspected for asthma, shows, in the context of a hypotrophy weight, chronic cough and infection with *Pseudomonas aeruginosa*; sweat test was positive, in the context of a compound heterozygous genotype. Conclusion: Not all wheezing is asthma case, even if there is suspicion of allergic diseases; rare cause of wheezing should be considered when chronic evolution, nonresponsive to treatment is present and other complications associated.

Introduction

Wheezing is one of the most common clinical signs of respiratory disease in children, about 30% of children have at least one episode of wheezing during life. Increased frequency of wheezing episodes often suggest the onset of asthma, difficult to diagnose in childhood, but necessitating early treatment . Common causes of recurrent wheezing in infants, are represented by gastro-esophageal reflux, aspiration pneumonia, chronic pneumopathies like cystic fibrosis, alpha 1-antitrypsin deficiency, tuberculosis, tracheobronchial malformations, esophageal extern compression, bronchomalacia, bronchial stenosis and, in young children, frequently associated bronchial foreign body, tumors, broncho-pulmonary aspergillosis, interstial pneumonia. Methods: The paper present two cases of children with recurrent wheezing.

Results

Case 1

The first case is a 10 months old girl, with a history of wheezing of 2 months, addressed to our clinic with cystic fibrosis suspicion. On admission she was in good clinical condition, with wheezing. On lung auscultation bilateral symmetrical transmitted and disseminated sibilant rales and rhonchus, where heard and an inconstantly transmitted transmission. Cardio-vascular examination was normal, with rhythmic heart sounds, frequency of 90 beats / minute, without any pathological elements. Cardio-pulmonary radiography showed left lung hyperinflation with lateral deviation by rotating of the heart and tracheo-bronchial tree (fig. 1.), the profile picture was interpreted (fig. 2.) as a thickening of the main left fissure and a secondary interlobular fissure on the same left side.

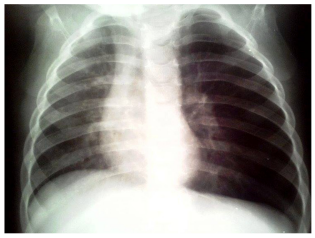

fig.1

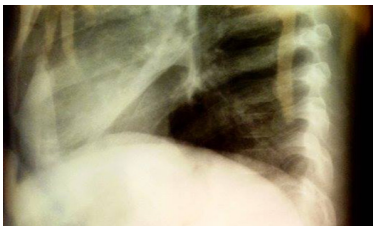

fig.2

Thoracic ultrasound showed no evidence of pulmonary condensation or pleural effusion.

Cardiac ultrasound reveals a structurally normal heart, great vessels apparently emerging from normal aortic arch. The right subclavian artery origin was not effectively evident on cardiac ultrasonography.

During hospitalization evolution has been favorable, but wheezing and a strange transmission sound, ameliorated in supine position persisted.

In those circumstances an extern compression process was suspected and a CT was performed which reveals the aberrant trajectory of the right subclavian artery, originating from the front side of the terminal segment of the aortic arch with imprinting esophagus. In the upper side of thoracic esophagus, the esophagus was is located to the left side of the trachea, and the aberrant right subclavian artery vessel passing to the right side of trachea.

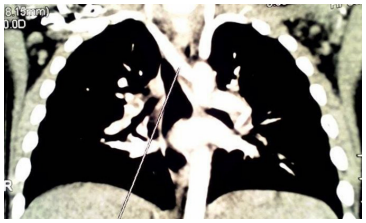

fig.3

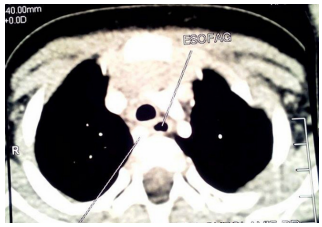

fig.4

Arteria lusoria – the right subclavian artery with anomalous trajectory is the most common abnormality of the embryonic aortic arch, with an incidence of 0.5 - 1.8 %, which manifests clinically in children with stridor, wheezing, repeated respiratory infections, failure to thrive and at older ages difficulty in swallowing, shortness of breath, chest pain, weight loss, upper limb numbness. Surgical treatment consists in endovascular closing of the aortic origin of the aberant artery with artery transposition and occlusion if the distal which seems to be the optimal surgical solution. In asymptomatic patients or those with minor symptoms, surgery is delayed and a " wait and see" situation is adopted alike in the case presented.

In evolution complications like : haematemesis, hemoptysis (arterio- oesophageal or arterio- trachea fistula) also formation of aneurysms with risk of rupture can occur.

Case 2

Another case presented is a 6 months infant, addressed to the clinic with suspected asthma, because of recurrent wheezing complicated with aspiration pneumonia. At admission he presented with wheezing, in a good clinical condition, but frequent regurgitation in the context of a weight deficit.

Biochemical investigations detected iron deficiency anemia, eosinophilia, elevated total IgE and the ultrasound revealed frequent gastroesophageal reflux, occult blood test positive. An allergy to cow milk protein was suspected , confirmed by serum antibody and established a hydrolyzed diet. Radiograph performed showed right upper lobe atelectasis (Fig. 5.), accompanied by inflammatory syndrome and hypo-pharyngeal aspirate was positive for *Pseudomonas aeruginosa.*

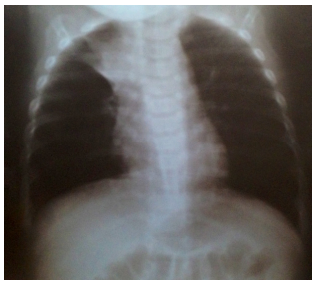

fig.5

Particularitatea cazului a constat in asocierea fibrozei chistice cu alergia la proteinele laptelui de vaca, ambele patologii capabile sa asocieze wheezing si pneumonie de aspiratie. Fibroza chistica este o boala complexa, cu manifestari respiratorii constand in tuse cronica, wheezing, atelectazia de lob superior fiind una din semnele de suspiciune, impreuna cu prezenta *Pseudomonas* aeruginosa in aspiratul hipofaringian.

Considering the case as a wheezy infant, with right upper lobe atelectasis and failure to thrive, a justified suspicion of cystic fibrosis was raised and the sweat test was positive, genetic test showed a compound heterozygous genotype. Evolution was favorable after treatment of *Pseudomonas* infection and exclusion of cow's milk allergy with an ascendent weight gain. The particularity of the case consisted in the association of cystic fibrosis with cow's milk protein allergy, both pathologies able to associate wheezing and aspiration pneumonia. Cystic fibrosis is a complex disease with respiratory signs

consisting in chronic cough, wheezing; the upper lobe atelectasis being one of the important signs of suspicion, together with *Pseudomonas aeruginosa* positive culture, wich should be more widely recognized and consider. The disease is polymorphic, manifested with steathorrea, nasal polyposis, male infertility, diabetes, pancreatitis, liver disease , digital clubbing, bine disease.

Conclusion

Not all wheezing cases are asthma, even if there is suspicion of allergic diseases and rare causes of wheezing should be considered as well. Suspicion of another cause should be raised in the context of persistent wheezing, unresponsive to bronchodilators and the associated complications suggestive.

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CONTROLLING SEVERE ALLERGIC ASTHMA WITH OMALIZUMAB (MONOCLONAL ANTI-IgE ANTIBODIES) IN CHILDREN

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Abstract

Allergic severe asthma is rare disease among young children. Most of these patients should be evaluated by an expert and treatment has to be tailored according to evolutive phenotype. One option for adult patients with such a condition is adding omalizumab [anti-IgE antibodies] to step IV GINA medication.

Authors present a small pediatric series of severe uncontrolled asthmatic patients treated with an average 12 months course of omalizumab. Demographics, clinical features and comorbid conditions are documented. Due to safety issues all patients were monitored during and after omalizumab injection in the resuscitation module of the Emergency Department from a tertiary referral pediatric hospital. Monitoring and protocol are presented. All children had a positive outcome, three with partial control and one with complete control. No serious side effects were observed. Low grade fever was easily controlled with trivial antipyretics.

Conclusion: anti-IgE monoclonal antibodies in severe allergic asthma patients are efficacious also in children and represent a safe and solid alternative for long-term oral corticosteroid treatment.

Keywords*: allergic asthma, anti IgE antibodies, child*

Background and aim

Asthma is a respiratory disease that affects approx. 300 million people worldwide and is associated with significant morbidity and mortality. Severe asthma (SA) in children is a rare and heterogonous condition, representing 2-10% of various asthma cohorts. [1, 2, 3], also in Romania.[4] In spite of being a rare condition it accounts for more than 1/3 of total costs of asthma patients.[2] Clinically, children with SA are different from other asthmatics by greater allergic sensitization, increased exhaled nitric oxide and significant bronchospasm that worsens as child grows older. These findings are generated by structural airway changes, abnormal and excessive airway inflammation which may explain the heterogeneity of treatment responsiveness of SA patients. [5, 6]

Guideline-based therapy of SA in childhood is based on extrapolated adult studies data. They should be treated with a similar step-wise tailored strategy: higher-dose inhaled or oral corticosteroids combined with long-acting β-agonists and other add-on therapies, such as antileukotrienes and methylxanthines. Crucial is to identify and approach influences that make asthma difficult to control: revisiting diagnosis, removing causal or aggravating factors, improving treatment compliance.

Progress in immunological methods used to evaluate human allergic diseases has led to identification of immunoglobulin E (IgE) as a diagnostic biomarker and a potential therapeutic target. Omalizumab (OZ) is the most advanced humanized anti-IgE monoclonal antibody that specifically binds serum-free IgE. OZ interrupts allergic cascade by preventing binding of IgE with FcεRI receptors on mast cells, basophils, antigen-presenting cells and other inflammatory cells. A 2013 update of National Institute for Health and Care Excellence (NICE) guidelines [7] recommends OZ for use as add-on therapy in adults and children over six years of age with non-controlled severe persistent allergic IgE-mediated asthma who require continuous or frequent treatment with oral corticosteroids. Because most children with SA have an inflammatory pattern OZ plays a central role in disease control [8, 9] or even can change natural course of disease - suggested by a one year course of OZ followed by 4 years of prospective survey. [10] OZ can decrease exacerbation rate in patients with moderate to severe asthma and is an efficient corticoid-sparing drug. [11] In special patients in whom treatment adherence does not improve in spite of strenuous efforts, OZ is an alternative, because it is provided in-office on a monthly [or bimonthly] basis. [12] OZ is the first of its class and many other biological agents and humanized monoclonal antibodies will be soon available. One such agent is mepolizumab that significantly reduces asthma exacerbations and improves markers of asthma control. [13]

The authors aim to describe a small group of children younger than 12 years treated with omalizumab for a relative long time.

Material and method

Retrospective analysis of a small group of severe asthma patients.

Inclusion criteria were age (6-12 years old), allergic asthma (ICD code J 45.0), uncontrolled disease (Asthma Control Test below 19), severe disease in spite of optimal treatment and rigorous compliance.

Exclusion criteria were non allergic asthma (ICD code J 45.1), moderate, mild-persistent or intermittent disease, controlled disease and uncompliant patient or family.

Results

From a group of 8 children with severe asthma previously published [4] we selected only allergic asthma patients with documented mechanism of disease: 3 boys and one girl. Increased eosinophilia, high IgE levels were present in all patients plus skin prick test in two children and elevated specific Ig E titers in the other two [mould and house dust mite respectively.

Average age at onset 7.81 years [extremes 6 – 9.75y]. All were previously treated with inhaled corticosteroids (ICS), medium or high dose, and intermittent systemic steroids. Three children were on combination therapy (ICS + LABA) and one child also with LTRA. Compliance was evaluated pre-treatment and was excellent. Inhalation technique was retrained with video and hands-on, both in children and parents, before treatment with omalizumab was started. Written action plan was provided and end extensive communication and adverse reaction reporting was explained.

Omalizumab has a relative large list of side-effects and most parents are reluctant in accepting anti-IgE treatment initially. In severe cases with a very low quality of life and a significant number of exacerbations in a given child, parental concern is shifted toward disease control than on potential side-effects of controller medication. In order to increase acceptance and to decrease parental anxiety all Omalizumab injections were performed according to a pre-specified protocol in the Resuscitation room of the Emergency Department (ED) of IOMC.

After patient file was recorded in hospital electronic file and informed-consent was signed, all patients had a complete evaluation and vital signs measurement. ECG, body temperature, pulse-oximetry, heart rate, respiratory rate and blood-pressure were continuously monitored with a Phillips triage monitor. Vacuum aspiration, advanced airway tools and devices, defibrillator and adequate amount of medication for the given weight were prepared. Omalizumab vial was slowly rewarmed at room temperature and homogenized according to in-file specifications. Only when child provided assent injection was performed in the deltoid area according to child preference. 75% of procedures were performed with topic analgesia (EMLA cream). All injections were administered by same physician. After continuous monitoring of at least one hour, each child had a complete clinical check-up and pulmonary function testing [PEF-metry of spirometry]. In some cases exhaled nitric oxide levels were measured. Children were discharged from ED in the same day after an average of 100 minutes monitoring. Longest stay were noted for first visit in all patients: 160-180 minutes.

A total of 51 sessions of omalizumab treatment were delivered. Average doze was 156 mg [extremes 75 – 300 mg] – fig. 1 *Omalizumab time line and doses in IOMC patients*.

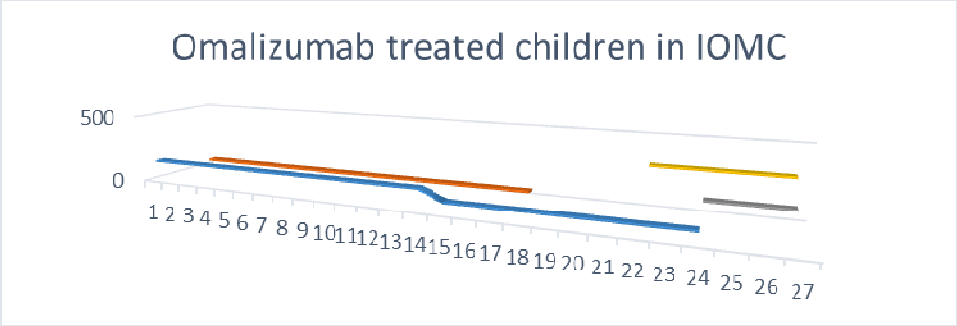

Fig. 1. Omalizumab time line and doses in IOMC patients.

No significant adverse events were documented. Fever was present in 3 children, never above 39◦C, lasting one up to three days. One child had intermittent fever post-injection for several months after onset and other two only 3 months. Fever was not associated with severe discomfort or pain and was easily controlled with regular fever-reducing agents [paracetamol or ibuprofen]. No severe allergic reactions were encountered, not local nor systemic. Only significant disease episode was varicella in patient number 4.

All patients had an improvement in ACT score and were able to perform after a while normal school activities – fig.2. *Asthma Control Test during treatment*. During this interval of 51 patient-months only 3 exacerbations were noted compared with a total or 11 episodes in the previous year before treatment [48 patient-months] (23% reduction in exacerbation rate, p=0.0061). – fig. 3. *Exacerbation rate*

Eosinophil count decreased and presented a rebound after stopping treatment [only one's child family decided to stop medication because of excellent control and fear of potential adverse events] - fig. 4. *Eosinophil count in IOMC patients*.

Discusion

Limited options are available for severe, treatment-resistant asthma pediatric patients. Because of parental corticophobia and because of severe limitations in quality of life in these patients alternative treatment approaches should be identified before significant airflow limitation occurs. One such option is add-on anti-IgE antibodies to step IV GINA treatment. Current indications for treatment with omalizumab in pediatric patients are clearly defined and are confined to moderate-to-severe uncontrolled allergic asthma and chronic spontaneous urticarial. [14] According to European Medical Agency omalizumab can be also used in selected asthma patients of 6 to 12 years old. [15]

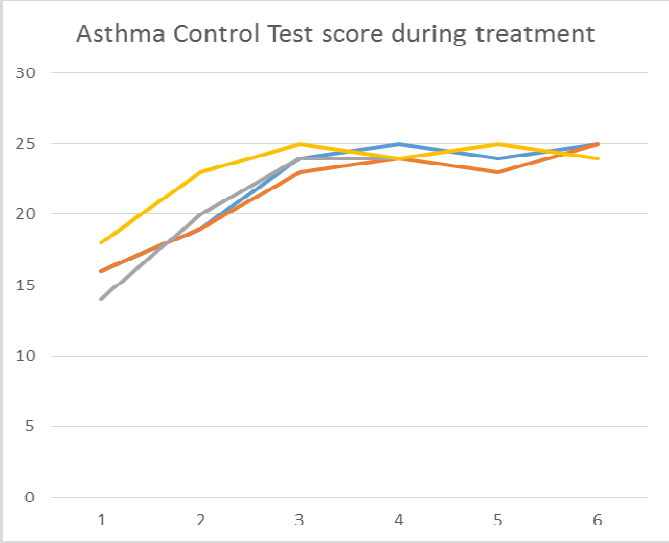

Fig. 2. Asthma Control Test during treatment [note – time intervals are not similar for all 4 patients and are not repetitive]

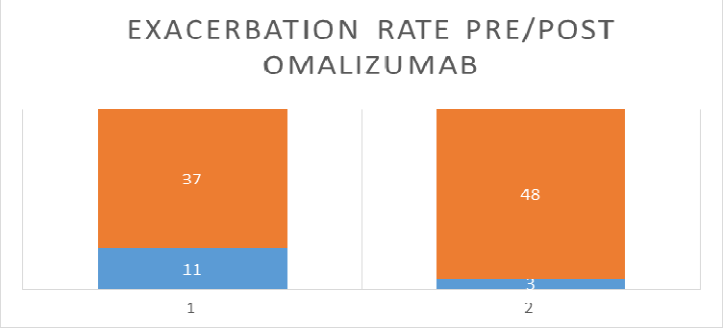

Fig. 3. Exacerbation rate in pre/post onset of omalizumab treatment

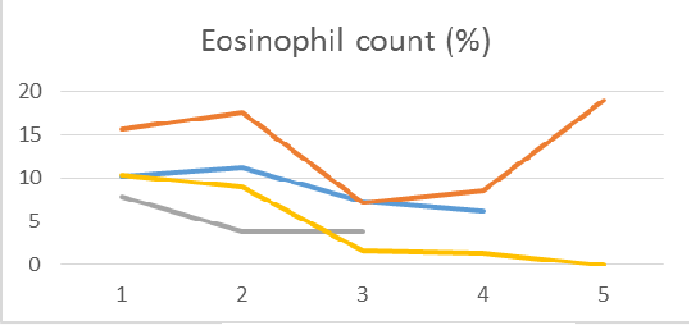

Fig. 4. Eosinophil count in IOMC patients

Authors present a limited experience of Omalizumab treatment in young children. To our knowledge this is the first such a paper regarding Romanian children with severe asthma treated with OZ. More patients should be included for a more robust result.

Conclusions

Omalizumab is an efficient and safe add-on treatment option in children with uncontrolled asthma. Significant reduction of exacerbation rate and sustained increase of quality of life are solid arguments to use anti-IgE medication in selected severe asthma children, even in the young age group (6-12 years).

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SEXUAL DEVELOPMENT DISORDERS – SMALL SOLUTION TO A BIG PROBLEM

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Abstract

Introduction: Sexual development disorders (DSD) are a group of relative rare diseases, induced by genetic or endocrine abnormalities that affect de endocrine and reproductive systems.

Material and Methods: Patients with DSD diagnosed in the Pediatric Endocrinology Department, Timisoara between 2010 and 2014 were included in this study. It consisted in a rigorous anamnesis, a complex physical examination including anthropometrical measurements and examination of the patient's genitalia. General and specific biological tests and karyotype were necessary and in some situations, the gene SRY was also determined. The paraclinic consisted in gynecological consult, an abdominal ultrasound or MRI.

Results: Out of 31 patients included in the study, 6 patients were identified as having congenital adrenal hyperplasia associating salt loss and dehydration, one patient was diagnosed with definitive urogenital sinus, while another patient had α reductase deficiency. Four patients were 46XY DSD and 20 patients 46XX DSD. Regarding the therapy prescribed, it was according to their genetic structure and their hormonal constellation.

Conclusions: 1. At this time sexual development disorders are underdiagnosed. 2. The implementation of a neonatal screening for 21-hydroxylase deficiency is a must. 3. The creation of a national and European register is necessary.
Key words: *disorders of sexual development, child.*

Introduction.

Sexual development disorders (DSD) are a group of relative rare diseases, induced by genetic or endocrine abnormalities that affect de endocrine and reproductive systems[i]. The terminology of *sexual development disorders* was introduced in the medical literature in the year 2006 after the Chicago Consensus and it replaced the old terminology of intersexuality (Table no.1). This change took place because it was considered that the term DSD is less incriminatory for patients than the old term *intersexuality.*

Table no. 1: The new terminology used in the medical literature after the Chicago Consensus.

Old terminology	New terminology
Female Pseudohermaphroditism	46XXDSD
Male Pseudohermaphroditism	46XYDSD
Real Pseudohermaphroditism	Ovotesticular DSD
Male XX	46 XX testicular DSD
XY Gender Uncertainty	46 XY gonadal dysgenesis

It is estimated that the prevalence is one in 4500-5000 in the general population or even higher.[ii] It can affect newborns usually with atypical genitalia or adolescents with atypical sexual development during the pubertal years. These clinical situations can often be difficult to manage, particularly in those cases where the sex of the newborn is uncertain.

Material and Methods:

Patients admitted to the Endocrinology Department of Children Emergency Hospital Timisoara and diagnosed with DSD were included in this study that took place between 2010 and 2014. The study protocol was complex and consisted in a rigorous patient history taking, including family history and past medical history. The physical examination included anthropometric measurements (height and weight, body mass index) compared with percentiles according to patient gender and age and a careful examination of the patient's genital area. The biological tests performed were blood ionogram, LH, FSH, estrogen, progesterone, testosterone, DHEA using immunochemistry, and in selected cases DHT, AMH (anti mullerian

hormone), inhibin B and plasmatic renin. AMH, DHEA-S, LH and FSH were tested using immunochemical detection through electrochemical luminescence, while DHT was determined using radioimmunological determination (RIA). Inhibin B was tested using the ELISA method. Plasmatic Renin was detected using immunochemical detection through chemiluminescence (CLIA); this is a "sandwich" test that detects active renin. The first type of anti-renin antibodies is chained to the solid phase (magnetic microparticles). The second type of anti-renin antibody is marked with a insoluminol derivate and binds to a region near the active situs of renin. The karyotype was determined in all patients analyzed. In special situations, the gene SRY was also evaluated. The gynecological examination took place after the patients or their parents gave their consent in the majority of cases. The abdominal ultrasound was performed in all cases and sometimes MRI or CT examination was imposed. All these took place according to the Helsinki Declaration and after the parents and patients older than 7 years signed an agreement for the procedure.

Results.

A number of 31 patients were included in the 5 years study. In the first 2 years of the study (2011-2013), a neonatal screening took place in the Endocrinology Department. During the screening time and using these complex methods (dry spot, ELISA and confirmed by tandem mass spectrometry), 6 patients were identified as having congenital adrenal hyperplasia associating salt loss and dehydration. Only one patient was diagnosed with definitive urogenital sinus, while another patient had α reductase deficiency (Fig. 1.).

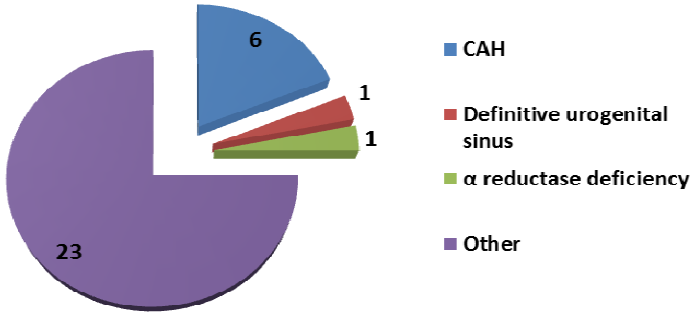

Fig. 1. The distribution of DSD in the study group.

Regarding the karyotypes, 4 patients were 46XY DSD and 20 patients 46XX DSD. Only 2 cases were diagnosed during the neonatal screening.

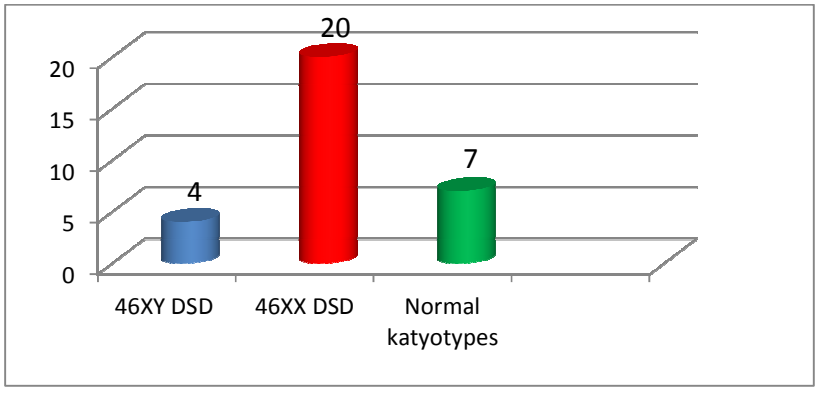

Fig. 2. Distribution of the karyotypes in the study group

Patients received therapy according to their genetic structure and their hormonal constellation. For instance, patients with 46XX received steroid medication such as etinilestradiol afterwards estro-progestative medication, while for patients diagnosed with CAH Hydrocortisone (15-20mg/m²/day) and Fludrocortisone (0,01mg/dose) were prescribed. This treatment was monitored through periodic anthropometric measurements (3 times per year) and bone age performed annually. The patients with genitalia abnormalities undertook surgical procedures in several clinics throughout all Europe. All patients and their families had psychological support.

Discussions:

DSD is a medical condition involving abnormalities of the development of the genital area induced by hormonal or genetic anomalies. There is underdiagnosed of these cases and that is why that the European DSD Work Group is working at a new approach for these conditions, from terminology to diagnosis.

The main reason for consulting the doctor in a case of patient with DSD was the abnormalities presented in the genital area. Various abnormalities can be detected by a careful examination. For example, the examination of the exterior genital organs of a girl with acute dehydration syndrome and hypernatremia can reveal a clitoris hypertrophy graded in the Prader Classification. The biological investigations can support the substitutive treatment with Hidrocortisone and Fludrocortisone.

For the 46XX patients, there was a great variety of karyotypes identified such as 46XO: 46XO/46XX , 46X rice 9 chromosome, or 46XO/46XY. In these patients, the sexualization therapy consisted in the prescribing of ½ of transdermal patch with estradiol once a week, starting with the age of 10-11 years[iii]. In 3 cases, menstrual cycles were induced and estro-progestative therapy followed in the therapy.

The reconstructive surgical treatment was done in one case in which the parents decided the child`s sex, which he later in his maturity contested. In 2 other similar cases such problems did not occur, but the patients aren`t yet mature. The optimal gender policy can reflect a kind of paternalism that is largely discouraged in modern health care delivery, because constitutes an ethically dubious practice. Clinicians have also suggested the notion of gender "neutrality at birth" .[iv]Psychological support was offered in all cases. The psychological input will allow patients with DSD to examine and understand their early emotional reactions as well as explore present and future worries and to pass through the period of uncertainty during the diagnosis process[v]

The diagnosis and treatment of DSD is complex, new advances have been made in the genetics, but also in the endocrinological, psychological and surgical fields. This is the reason why patients with DSD should be cared for in centers of excellence with a multidisciplinary team consistented in specialists in endocrinology, surgery and/or urology, clinical psychology/ psychiatry, radiology, nursing and neonatology. This team may exist as a clinical net-work with links between more than one specialist centers[vi].

The best approach to DSD is represented by the presence of a national neonatal screening in which chromosomal, genetically, hormonal and psychological investigations should be done. At this time, this approach is possible because Romania entered in a European project called "DSD net", that allows us to create a national and European register with a rational approach of the surgical treatment. It is recommended to apply surgical therapy at the age of 16, and the gender should be chosen by the child and not by the parents or doctors.

Conclusions:

1. At this time sexual development disorders are underdiagnosed.
2. The implementation of a neonatal screening for 21-hydroxylase deficiency is a must.
3. The creation of a national and European register is necessary.

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CORRELATION BETWEEN BODY MASS INDEX, BODY FAT PROPORTION AND LEPTIN LEVEL IN OBESE CHILDREN

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Abstract
Obesity is a disorder of the nutritional status characterized by excessive accumulation of fat in the subcutaneous tissue and / or other tissues as a result of energy imbalance. [1] Leptin, the most important anorexigenic hormone is secreted by the adipose tissue, acts directly on the satiety and hunger center localized in the anterior hypothalamus and arcuate nucleus. [2] Body mass index (BMI) is used to assess obesity and has variable values for different ages and sex.
The aim of the study was to establish the correlation between the body mass index, percentage/amount of fat and the serum leptin level in overweight children.
Material was represented by 126 children (the study group: 86 overweight children and the control group: 40 normal weight children), hospitalized during the last two years in the Medical Genetics Department of the Emergency Children Hospital Cluj-Napoca. Study methods included: anamnesis, clinical exam, anthropometric assessment (weight, height, BMI); body fat measurements by bio-impedance (OMRON); lipid profile, glucose, serum leptin.
Results. Obesity in predominates in female (58.6%) versus male (41.4%). Body composition analysis showed a higher percentage of body fat in women. The serum leptin correlated with BMI, body fat percentage, sex and age.
Conclusion. The high level of leptin in obese children contributes to early development of metabolic syndrome.
***Key words**: obesity, body fat percentage, leptin.*

Introduction

Obesity is a disorder of the nutritional status characterized by excessive accumulation of fat in the subcutaneous tissue and / or other tissues as a result of energy imbalance.[1] The fat tissue represents an active metabolic organ which secrets numerous cytokines with neuromodulator, immunomodulatory and proinflammatory effect.[2] Leptin and adiponectin are involved in energy homeostasis and food intake.[2] Leptin is the most important anorexigenic hormone, is secreted by the adipose tissue, acts directly on the satiety and hunger center localized in the anterior hypothalamus and arcuate nucleus.[3] Leptin is secreted by the ob gene, located on the chromosome 7.[4] Genetic deficiency of leptin leads to a severe form of obesity. In common obesity, although the level of leptin is elevated, it does not inhibit hunger and does not induce fat tissue wasting, which suggests leptin resistance.[5,6] Numerous studies revealed that leptin intervenes in lipid metabolism.[7] and carbohydrates metabolism regulation.[8,9] Leptin has also a proinflammatory effect by stimulating the synthesis of other cytokines (TNFα, IL6, prostaglandines), thus leptin contributes to the triggering and maintaining an low-grade inflammatory process which will initiate the atherogenesis process.[10,11] Obesity is an important risk factor for the metabolic syndrome.

The aim of the study was to establish the correlation between the body mass index, percentage/amount of fat and the serum leptin level in overweight children.

Material and methods.

Material was represented by 126 children, aged 6-19 years, 46 females and 40 males (the study group: 86 overweight children and the control group: 40 normal weight children), hospitalized during the last two years in the Genetic Diseases Department of the Emergency Children Hospital Cluj-Napoca.

Study methods included: anamnesis, clinical exam, anthropometric assessment (weight, height, BMI) – SECA 702 Hamburg measurement system; body composition was determined by bio-impedance using Body Analyzer Omron Body Fat Monitor 511 BF (OMRON). The control group included 40 subjects with sex and age appropriated to the studied group, with BMI between 25-75 percentile.

In all subjects (86 obese patients and 40 subjects with normal weight) were collected blood samples after a 12 hours fasting period. The following determinations were performed: leptin (immunoassay method, µg/L), total cholesterol (mg/dL),

HDL-cholesterol (mg/dL), triglycerides (mg/dL) and glucose (mg/dL) by colorimetric method. We calculated the following parameters: body fat mass (percentage and Z score) by reference to the normal range for age and sex, BMI (percentage and Z score) by reference to the normal range for age and sex, using the program www.cdc.gov. growthcharts/percentile data, LDL-cholesterol using the Friedewald formula: LDL cholesterol = total cholesterol - (HDL cholesterol + triglycerides/5).

Statistics.

Quantitative data were described using mean, standard deviation. To test the difference between sample pairs was used Student's test. Analysis of variance (ANOVA) was used to determine differences within groups. Pearson's correlation were used to establish correlations between the parameters in the obese group. For all statistical tests we used as significance level 0.05.

Results.

The anthropometric data are presented in table I. In obese children, it is noted statistically significantly higher values for overweight (percentage and Z score), BMI (percentiles and Z score) and percentage of body fat, with no significant differences for the waist. Laboratory parameters are presented in table II.

Table 1. Anthropometric parameters of obese and non-obese groups by gender.

Gender	Variable	Obese group		Non-obese group		p value
		n	Mean ± SD	n	Mean ± SD	
Females	Age	46	12,24±3,75	24	12.18±0.52	0.67
	Height (z score)	46	0,38±2,15	24	0,52±1,81	0.77
	Weight (excess %)	46	51,72±24,6	24	12,68±17,41	<0.001
	Weight (z score)	46	1,93±0.87	24	0,23±0,86	<0.001
	BMI percentile	46	96,11±3,40	24	32,06±22,78	<0.001
	BMI z score	46	2,00±0,54	24	0,52±0.43	<0.001
	FM%	46	37.25±5.94	24	19.24±8.27	<0.001
Males	Age	40	12,12±4,24	16	12.20±2.25	0.89
	Height (z score)	40	1,15±1,29	16	0,58±1,10	0.11
	Weight (excess %)	40	44,98±27,11	16	13,51±2,14	<0.001
	Weight (Z score)	40	2,01±0.34	16	0.35±0,26	<0.001
	BMI percentile	40	96,11±3,40	16	45,45±18,78	<0.001
	BMI z score	40	2,11±0,48	16	0.83±0.33	<0.001
	FM%	40	31.75±5.04	16	15.47±6.44	<0.001

SD = standard deviation, BMI = body mass index , FM = fat mass%

Table 2. Bioclinical parameters of obese and non-obese groups by gender.

Gender	Variable	Obese group		Non-obese group		p value
		n	Mean ± SD	n	Mean ± SD	
Females	Leptin (ng/ml)	46	28,02±16,17	24	5,16±4,50	<0.001
	Cholesterol total (mg/dl)	46	173,20±31,36	24	156.52±30,89	0.037
	HDL Cholesterol (mg/ml)	46	46,95±9,55	24	57,26±11,48	<0.001
	LDL Cholesterol (mg/ml)	46	97,77,±28.02	24	87,08±26,70	0.01
	Triglyceride (mg/ml)	46	100,01±44,86	24	71,57±28,70	<0.001
	Glycemic (mg/ml)	46	85,81±4,60	24	82,57±7,98	0.21
Males	Leptin (ng/ml)	40	15.84±4.02	16	4,03±2.57	<0.001
	Cholesterol total (mg/dl)	40	186,97±43,69	16	143,18±27.83	<0.001
	HDL Cholesterol (mg/dl)	40	48,47±10,94	16	59,45±11,83	<0.001
	LDL Cholesterol (mg/dl)	40	115,49±39,91	16	77,49±23,00	<0.001
	Triglyceride (mg/dl)	40	100,08±45,05	16	71,63±36,79	<0.001
	Glycemic (mg/dl)	40	85,67±7,45	16	88,09±7,44	0.27

Obese childrens shows statistically significantly higher values for leptin, total cholesterol, LDL cholesterol, triglycerides and low HDL cholesterol levels. The glucose had not statistically significant differences between the obese and normal weight children. Analysis of the gender and anthropometric data correlated with leptin is presented in Table III. In obese girls there are statistically significantly higher values for percentage of body fat and leptin compared to boys. In normal

weight children there are no statistically significantly differences. The correlation of leptin level with the percentage of fat tissue (Fig. 1.) showed a positive correlation but higher in obese children than in those with normal weight.

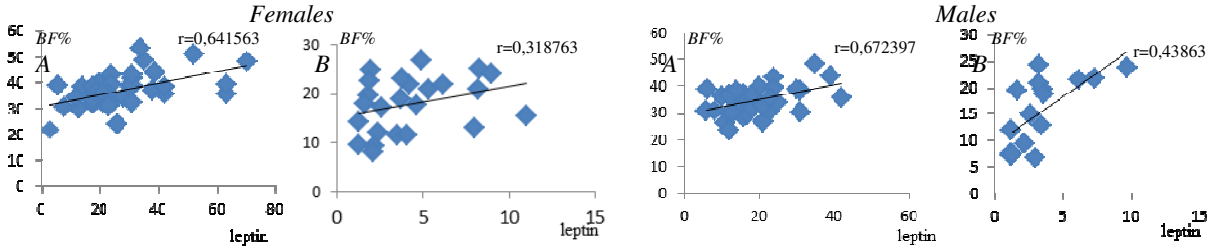

Fig. 1. Correlation between leptin level (ng/ml) and body fat % in obese (A) and non-obese (B) children

Table 3. Comparative anthropometric parameters and leptin of obese and non-obese groups by gender.

Variables	Obese group		P value	Non obese group		P value
	females	males		females	males	
Weight (excess%)	51,72±24,6	44,98±27,11	0.23	12,68±17,41	13,51±2,14	0,85
BMI z score	2,00±0,54	2,11±0,48	0.32	0,52±0.43	0.83±0.33	0.01
FM%	37.25±5.94	31.75±5.04	**<0,0001**	19.24±8.27	15.47±6.44	0.10
Leptin (ng/ml)	28,02±16,17	15.84±4.02	**<0,0001**	5,16±4,50	4,03±2.57	0.35

Discussion.

Obesity is the most common chronic metabolic disease that affects the overall world population. The recent decades reveals an increasing prevalence of obesity among children and adolescents.[1] In this study, obesity is most frequent in females (58.6%) than in males (41.4%). BMI determination represents a useful tool in overweight assessment, but does not offer accurate information about the body composition.[1] For more accurate characterization of overweight new methods are needed in order to permit the assessment of the percentage of body fat, muscle and bone, such as bio-impedance, MRI.[1] In our study, in obese subjects, although the overweight and Z score for BMI were similar in both sexes, body fat percentage was significantly increased in females, consistent with data presented by Garzia 1997.[12] The leptin level correlated with fat tissue percentage. In obese children the leptin was statistically significantly higher than in normal weight children, concordant with other studies. The level of leptin in obese girls was much higher than in obese boys compared with normal weight children where there are not significant differences between sexes. Leptin levels correlate positively with BMI and percentage of body fat, sex and pubertal development.[13,14] Leptin induces a number of changes in lipid metabolism (increased serum triglycerides, low LDL-cholesterol) and glucose (increased peripheral insulin resistance) and stimulates the proinflammatory cytokines which initiate an inflammatory subclinical process at the level of vascular endothelium leading to the metabolic syndrome.[11].

We found a statistically significant difference between total cholesterol, LDL cholesterol and triglyceride levels (increased), HDL-cholesterol (low) in obese children versus normal weight children (Table I), which indicates the presence of an atherogenic profile already associated with obesity in children. The increased levels of leptin and lipid metabolism changes represent risk factors for the development of metabolic syndrome in obese patients and require an appropriate therapeutic approach.

Conclusions.

Leptin reflects fat mass; its levels are influenced by gender and age, particularly at puberty when girls acquire more fat mass. The high level of leptin in obese children contributes to early development of metabolic syndrome

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DISRUPTION OF THE CYRCADIAN SYSTEM AND OBESITY

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Abstract

Disruption of the cyrcadian system is a relatively new concept incriminated as being responsible for obesity, cardiovascular involvement, cognitive impairment, premature aging and last but not least, cancer. Because obesity is undoubtedly assimilated today to the medical conditions related to the disruption of the normal chronobiology, this paper presents the pivotal role of chronodisruption in the neuroendocrine control of appetite among these patients.
Keywords*: sleep deprivation, circadian rhythms, chronobiology, chronodisruption, obesity.*

Introduction

The prevalence of obesity has risen significantly in the last decades[1] and the trend is alarming in children and adolescents as well. As childhood obesity is a crisis by any standard, so-called „facts on obesity" like: obesity is a complex polygenic disease, its related comorbidities (type 2 diabetes, metabolic and cardiovascular disorders, etc.) have become a major burden to public health system all over the world, obesity is a chronic inflammatory disease which in turn predisposes to cancer, disease susceptibility, risk of metabolic disease and response to a low-calorie diet are mainly determined by epigenetic variation, not to mention the interactions of genome and nutrient intake (nutrigenomics) on obesity, are now familiar to all clinicians in charge of these patients. [1,2,3]

As sleep has shown to exert profound modulatory effects both on the neuroendocrine control of appetite and the glucose regulation, new areas of research are opening both in terms of normal circadian rhythms (chronobiology) and their disruption (chronodisruption).[4] Furthermore, disruption of circadian rhythms (chronodisruption/CD) related to the new lifestyle in modern society marked by stress, sleep deprivation, night-time eating (NES), nocturnal "light pollution" was subsequently related to and finally incriminated for obesity, cardiovascular risk, cognitive impairment, premature aging and cancer. [5]

Starting to put the puzzle together

Genetic and neuroendocrine aspects of obesity

The discovery of neuropeptide Y (NPY) as an appetite stimulating factor, "*ob"* gene and its coded protein-leptin as an inhibitory peptide of NPY and Ghrelin with its orexigenic effect were the cornerstones of the emerging puzzle related to obesity.[6,7,8]

The "lipostat" and "glucostat' theories postulated in the early 1950s were finally validated. Leptin proved to be the missing piece of the "lipostat" theory and Ghrelin of the 'glucostat' one. As postulated by the "lipostat" theory, leptin is a peptide produced by the fat cells (in response to nutritional status, glucocorticoids and insulin) and acts similarly to a circulating hormone at the ventro-medial hypothalamic level regulating the food intake. Its receptor was found in choroid plexus, hypothalamus, kidneys and lungs. The choroid plexus receptor favors leptin's transport to the brain. Under normal situations, leptin suppresses food intake by decreasing the biosynthesis and secretion of the most potent stimulator of appetite- the neurotransmitter neuropeptide Y (NPY). Consistent with the previous "lipostat" theory, leptin is definitely the long-sought and expected "satiety hormone". The reverse is true when mutations occur in the "*ob"* gene, leptin's receptor or the post-receptor signaling pathway, as both nil/low leptinaemia and hyperleptinaemia (leptin resistance) are responsible for morbid obesity due to the same cascade of events: hyperphagia, hypercorticism, hyperinsulinaemia, etc (Fig. 1a and b).

When compared to the "lipostat" theory, the "glucostat" theory postulated hypoglicemia as the key factor for food intake. In fact, hypoglycemia proved to be the physiologic trigger for Ghrelin secretion. The so-called "hunger hormone", Ghrelin is also known as lenomorelin. The receptor for ghrelin, the ghrelin/growth hormone secretagogue receptor (GHSR), is located on the same cells in the brain as the receptor for leptin. Secreted during fasting (primarily from the stomach), Ghrelin acts in the central nervous system as an orexigenic neuropeptide (increases appetite and food intake) thus, with opposite effects when compared to leptin.

Circadian, ultradian and infradian rhythms

Recognised as a new field of science only in the middle of the 20th century, chronobiology studies the biological rhythms defined by the periodic and therefore predictable timing of different processes in living organisms.[9] This is also valid in humans, in which three types of patterns have been reported for the biological rhythms: circadian, ultradian and infradian.

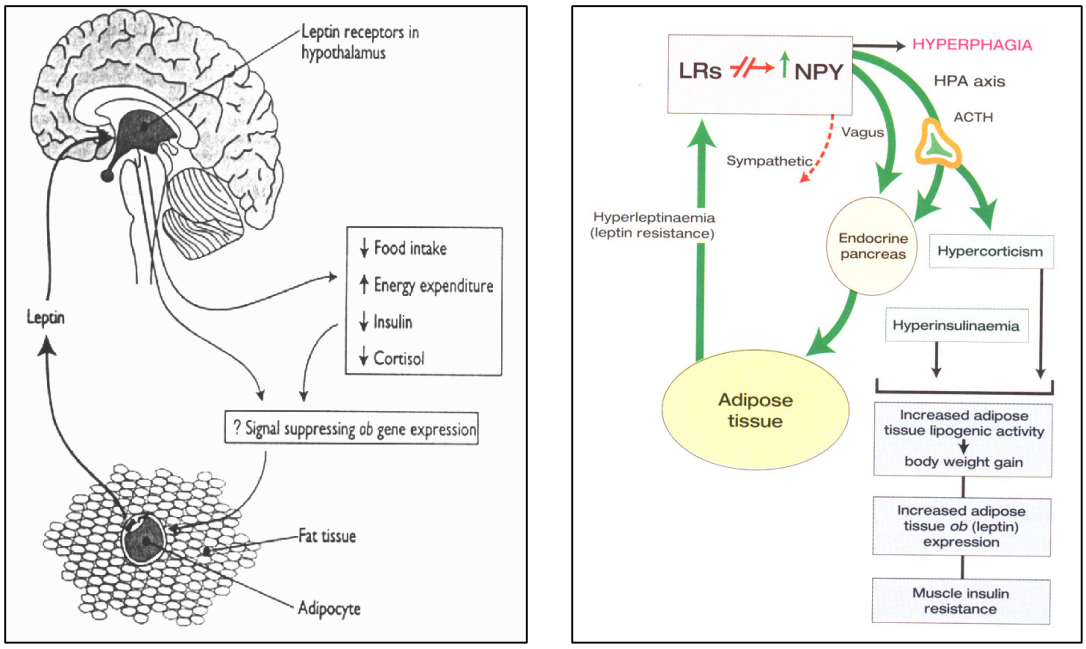

Fig. 1a. The loop system between NPY and leptin in normal rodents; b. The loop system between NPY and leptin in obese rodents. Rohner-Jeanrenaud F. *Horm Metab Res* (1996), 28: 642-648.

Circadian rhythms (from the Latin *circa diem* for „about a day") are those that occur on a 24-h scale, once a day and are related to and synchronized with sunlight.

Considered to be intrinsec to all living organisms, they are genetically induced and may persist in constant darkness (e.g. melatonin and cortisol secretion).[2,10,11] Ultradian rhythms occur with a higher frequency than one cycle per day, over a period of less than 20 hours (e.g. intestinal movements, heart beats, etc) while infradian rhythms are defined by a lower frequency than one cycle per day, over a period of more than 28 hours (e.g. the menstrual cycle in women, etc).[10] Sometimes, the same biological parameter may exhibit both ultradian pulsatility and a diurnal variation (e.g. adiponectin, etc).[12]

With respect to circadian rhythms, the one that dominates all our activities is the sleep and wake cycle (e.g. timing of sleep and wakefulness) but circadian rhytmicity is displayed by feeding and drinking pattern, body temperature, secretion of a large spectrum of hormones (cortisol, growth hormone, melatonin, noradrenaline, prolactin, luteinizing and follicle-stimulating hormones, thyrotropin, etc) and peptides and citokines related to adipose tissue (leptin, adiponectin, adipsin, resistin, etc).

Depending on the tissue in which they are expressed, 10-25% of the genes proved to have different and significant changes in their expression over a 24-h period of time.[13,14] This aspect highlighted the corresponding fact, that most of the changes in human's behaviour and/or physiology (all parameters) display such a circadian pattern in their expression. However, all these different variables should be maintained in coordination with one other within the organism for the healthy status to be achieved.

This timekeeping system or biological „clock" provides the internal fine tuned integration of all these variations. Furthermore, as already mentioned above, the biological clock allows the organism to prepare for and synchronize with the external changes induced by the variation in the day and night, dark and light cycles.[9] Under normal conditions, this difficult task is achieved by the circadian system.

The fine adjustment of the organism to both its internal and external environments is crucial for the well-being and survival of any subject. The other way around, desynchronization of the internal ordered rhythms leads to health problems such as those associated with sleep deprivation due to sleep time reduction or irregular day-to-day sleep-wake rhythm (jet lag, shift work and noctural bright light exposure), etc[10]

The circadian system

In humans and any other mammals, the circadian system is a complex network with structures involved both in the genesis of the circadian rhythms *per se* and in their permanent adjustment to both the internal and external environmental light-dark changes. The circadian system is represented by the central pacemaker located in the suprachiasmatic nucleus

(SCN) of the hypothalamus and the peripheral clocks outside the SNC. The activity (rhythm) of these peripheral clocks is synchronized by the SCN through both the cyclic, pulsatile hormone secretion (peak-trough) and the autonomous nervous system.

The Central Pacemaker/The Master Biological Clock/The Central Rhythm Generator

The most important structure is the central pacemaker located in the suprachiasmatic nucleus (SCN). It was discovered in 1972 by two groups of researchers, Stephan and Zucker[14] and Moore and Erlich.[15]

Its day-by-day fine synchronization with the environment is possible through the light input signal (light and dark cycle) based on a non-visual pathway from the melanopsin-containing ganglion cells of the retina and the retinohypothalamic tract. Even if light-dark cycle is definitely the most important circadian syncronizer to the SCN, it is not the only one, as periodic input signals like meal times (intake-fast) or scheduled exercise (activity-rest) may set the clock of the circadian system as well.[16,17]

SNC controls the expression of the circadian rhythms through a transcriptional-translational feedback loop between different types of so-called "clock genes" which may act as positive or negative elements. The positive-acting are the circadian locomotor output cycles out kaput (CLOCK) with its homolog-neuronal PAS domain protein 2 (NPAS2) and the brain and muscle aryl hydrocarbon nuclear receptor translocator-like protein 1/brain and muscle ARNT-like protein 1 (BMAL1). Heterodimerization of their coded transcriptional factors is responsible for the expression of the negative acting elements of the molecular circadian clock machinery such as periods (*Pers 1-3*), cryptochromes (*Cry 1-2*), *REV-ERB β* (reverse erythroblas-tosis virus α) and other genes controlled by the clock-controlled genes (CCGs) like *Pparα* (10,17). Then, the negative feedback loop is validated by the cytoplasmatic heteromerization of PER and CRY followed by nuclear translocation and inhibition of CLOCK/BML1 transcription. Currently, strong evidence has already confirmed the direct involvement of the circadian clock machinery in metabolism (e.g. BMAL1 and CLOCk proteins are involved in glucose metabolism, BMAL1 and PER2 proteins in appetite regulation and PPARα and REV-ERBα in lipid metabolism.[17]

SCN is able to act as the master circadian generator because each of its neurons has a molecular clock- the feedback loop between the positive (Clock and Bmal1) and negative (Per and Cry) elements and also, it is the beneficiary of the direct light information mediated by the melanopsin-containing ganglion cells of the retina.

As already mentioned before, the master biological clock represented by the SCN is then responsible for the consonant adjustment of the peripheral clocks rhythms through the fine tune between a wide range of hormones and other peptides, which are considered to represent the output signals.

Melatonin is the key output signal. Light message is passed indirectly from SCN to the pineal gland which in turn, secretes melatonin. In fact, melatonin secretion occurs from the pineal gland, due to the nocturnal activation by the SCN of the otherwise, limiting enzyme arylalkylamine N-acetyltransferase (AANAT). In healthy subjects, the peak of melatonin secretion occurs around 3am.[2] Considered "the sleep hormone", melatonin secretion rises during night time and declines during day time. Nocturnal pineal melatonin secretion is associated with the discharge of the indoleamine from the pinealocytes. Medical literature is replete with information regarding indoleamine's protective effect against all six hallmarks of cancer (e.g. against self-sufficiency in growth signals, insensitivity to the growth inhibitory signals, limitless replicative potential, sustained neo-angiogenesis, evasion of normal apoptosis, tissues invasion and the metastasis process).[18,19]

Melatonin secretion is not the only one with a recognized circadian cycle variation. Cortisol's morning peak (9 am) is also under SCN control via ACTH (adrenocorticotropic hormone) activation. Furthermore, the inverse relationship between cortisol and growth hormone (GH) 24-hour circadian cycles, including the preservation of this negative relationship per each pulse is a well known mandatory condition for maintaining the normal health status. (Fig. 2.)

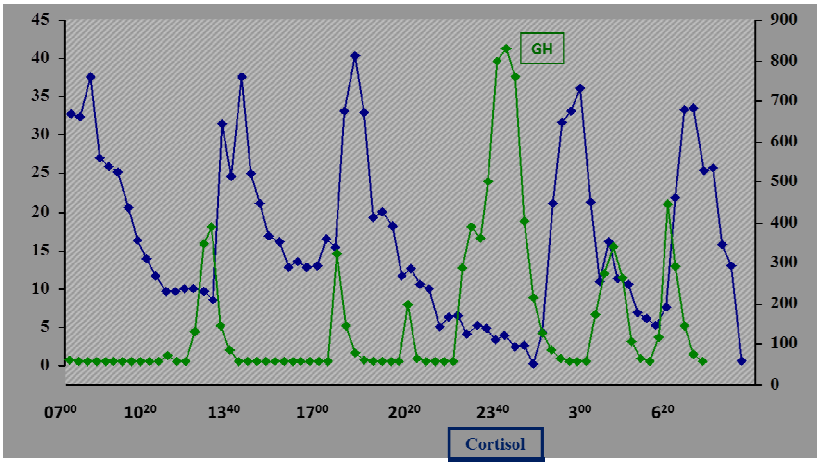

Fig. 2. Inverse relationship between GH and cortisol (24-h pattern of secretion) in normal subjects

It should be stressed out that midnight cortisol's nadir or trough occurs only during sleep. In addition, normal peak for GH which is set to 10pm regardless of sex, chronological age and stage of puberty, can also be achieved, only during deep sleep (REM IV).

To sum up, normal pattern and duration of sleep is one of the prerequisites of keeping different circadian rhythms in synchrony, which in turn is essential for optimal health. [20]

Sleep pattern, disruption of the cyrcadian system and obesity

In keeping with aforementioned considerations, it is obvious that disturbances in the photoperiodic environment might be responsible for disruption of normal circadian cycles.

As the light-dark input signal is the major circadian synchronizer to SCN, the current extension of the artificial light period after the normal darkness onset, was identified as a major cause of chronodisruption with marked deleterious effects on health.

The so-called "light pollution" of the modern life style (night shifts, jet lag, sleep deprivation, noctural bright light exposure, etc.) with concomitent nocturnal ingestion of food has been undoubtedly linked to increased weight gain and obesity.

In reference to the nocturnal pattern of secretion for melatonin, cortisol and GH, misinformation is sent to the SCN, wrong message is received by the pineal and pituitary gland. Melatonin and GH are dropping instead of increasing when compared to their normal timing of secretion, while cortisol level remains increased even during night time. The inverse relationship between GH and cortisol is lost and the 24-h pattern of secretion for cortisol mimics that of patients with Cushing's syndrome.

Furthermore, sleep deprived patients (less than 5 hours of sleep) and shift workers exhibit low levels of leptin, high levels of Ghrelin with high NPY and therefore, increased appetite, hyperinsulinaemia, high tryglicerides and low concentrations of HDL-cholesterol. According to these results, inadequate sleep with increased nocturnal food intake confirmed the link between sleep loss, obesity and the cardiometabolic syndrome.[21-25]

Clasic chronobiology supports the premise that circadian rhythms also control the eating pattern so that, feeding time is also important. *When* we eat could be just as important as *what* we eat.[26] As already mentioned, subjects with a nocturnal life-style (low melatonin and leptin and high Ghrelin) are prone to night-eating, obesity, diabetes, cardiovascular syndrome, not to mention the high carcinogenic risk.

Conclusions

Night-time eating, sleep deprivation, nocturnal "light pollution", are all responsible for severe disruption of the normal circadian rhythms. Chronodisruption is not a myth anymore. Chronodisruption along with its related neuroendocrine effects (low level of melatonin, hypoleptinemia, high Ghrelin with unsuppressed NPY, hyperinsulinemia, etc.) is a relatively new concept incriminated as being responsible for obesity, cardiovascular involvement, cognitive impairment, premature aging and last but not least, cancer. Accordingly, chronotherapy might be the best approach in the case of obese patients.

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BARRIERS IN GLYCEMIC CONTROL IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

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Abstract
Introduction. Although in recent years new technologies and new types of insulin have been used, no significant improvement has been observed in glycemic control of type 1 diabetes (T1D). This suggests that apart from the new methods of treatment and monitoring, there are also other factors that influence glycemic control.
Aim. In this research we have studied the existence of correlations between glycemic control and certain parameters in children and adolescents with T1D.
Material and method. The study was performed on a group of 41 children and adolescents with T1D, based on a questionnaire which was filled in by the parents. Optimal glycemic control was reached when the value of glycated hemoglobin (HbA1c) was below 7.5%.
Results. The main factors that were correlated with the deterioration of glycemic control in the children and adolescents with T1D who participated in the study were: pubertal age groups (72.73%), patients from rural areas (68.43%), the existence of permanent family conflicts (100%), patients who rarely exercise (92.86%), patients who do not weigh food (80%), patients whose parents have only secondary education (76.46%), patients in the first year after onset (60%), patients that had only one parent involved in diabetes care (61.54%), patients with less than 4 glycemic measurements per day (81.82%). Positive correlations have also been established between height, weight, body mass index and HbA1c.
Conclusions. At most children, an optimal glycemic control can be achieved through a better understanding of the disease and the factors influencing glycemic control.
Key words: *glycemic control, child and adolescent, diabetes*

Introduction

T1D is one of the most common endocrine and metabolic disorders in children and adolescents, with an increasing incidence worldwide. Maintaining a good glycemic control is a challenge for these children. According to the International Diabetes Federation[1], in 2013, 382 million people worldwide had diabetes. More than 79,000 children developed T1D in 2013. The highest incidence rates in the world are in northern Europe. In Romania the risk of T1D is low and remains relatively constant during adolescence, according to the study ONROCAD.[2]

Biochemical changes entailed by these disorders lead to functional cellular changes followed by irreversible anatomical lesions in many tissues and organs, especially the eyes, kidneys, nerves, heart and blood vessels, affecting quality of life and health expectancy[3,4]

In recent years, technological and pharmaceutical research have helped children with diabetes both in optimizing glycemic control and in preventing long-term complications.[5] These latest innovations include: new insulin analogs that are both very rapid acting and very slow acting, blood glucosemeters which require very small amounts of blood, continuous glucose monitoring systems (CGMS) which record the real-time fluctuations of glycemia and new insulin pumps that help patients to better adapt insulin administration to glycemic values.[6] In spite of all these innovations, it still proves difficult for some patients to reach glycemic control. Thus, there must be other factors influencing glycemic control besides the recent innovations.

Some authors indicate that the main barriers in optimizing glycemic control in children and adolescent are: factors that influence physical development, especially during puberty[7], psychological factors[8,9], family factors[10], the transition from pediatric diabetes care to adult diabetes care[11] and socioeconomic and cultural factors. [12,13]

It is thus a priority area of research because the main objectives of pediatric diabetes care are aimed at improving quality of life and long-term outcome. Therefore, it becomes important to assess the factors influencing glycemic control in children with T1D because a better understanding of these factors leads to a more adapted therapeutic attitude and helps provide a normal life for the child.

Materials and methods

The main purpose of this research was to study the correlations between the previously mentioned factors and glycemic control in children with T1D. The study included 41 patients, diagnosed with T1D, registered and monitored in Pediatrics Clinic, Emergency County Hospital Craiova. The study was based on a questionnaire that was completed by parents of children and adolescents with T1D after informed parental consent was obtained in accordance with the Ethics Committee of Emergency County Hospital Craiova.

We studied the following parameters: HbA1c level which served as an indicator of whether glycemic control was reached or not; age, gender, origin, pubertal status; history and family history; level of education of parents; quality of life of the patient; family members most involved in diabetes care; verification and interpretation of blood glucose; diet and physical activity; major mental stress (family conflict situation, accidents).

Metabolic control of diabetes is reflected by HbA1c value, which according to ISPAD 2013 Guideline should not exceed 7.5%.[1] Each parameter was correlated with HbA1c values, which allowed classification in steady state glycemic control if HbA1c was less or equal than 7.5%, or in a state of imbalanced glycemic control if HbA1c was higher than 7.5%

For statistical analysis the following software were used: Microsoft Office Excel 2007, Microsoft Office Access 2007 Epi Info 3.5.1/2008. For the analysis of statistical significance was used the Student's t-test and for correlation of analysis the Pearson coefficient was used.

Results and discussions

Patient age at the time of the study ranged from 4 to 18 years. In the study group, HbA1c after distribution was as follows: HbA1c ≤ 7.50 % characterized 16 patients (39.02%); HbA1c > 7.50 % characterized 25 patients (60.98%).

Incidence by age group was studied after the enrollment of 41 children and adolescents in the study group in one of the following categories: in age group 0-6 years were 3 patients (7.32%), in age group 7-12 years were 14 patients (34.14%), in age group 13 – 18 were 24 patients (58.54%). Analyzing the relationship between HbA1c and the age groups, it was found that in the age group 7-12 years, 71.43% of patients experienced glycemic imbalance, followed by the age group 0-6 years, with 66.67% of patients and the age group 13-18 years, 54.17% of patients had glucose imbalance. The greatest influence on glycemic control had the age group of 7-12 years old (p = 0.000798).

The study on the area of origin showed that in the rural areas glycemic imbalance was present in 68.43% versus 54.55% in the urban. Rural areas (p = 0.00066261) was a significant risk factor, a positive predictor for the occurrence of glycemic imbalance.

The group of 41 patients was divided into three categories according to pubertal stage. Thus, the prepubertal stage (Tanner 1) included 13 patients (31.71% of all patients), pubertal stage (Tanner 2-4) included 17 patients (41.46%) and the postpubertal stage (Tanner 5) included 11 patients (26.83%). According to HbA1c values, glycemic imbalance was found in 61.54% of the Tanner 1 patients, 52.95% of the Tanner 2-4 patients and 72.73% of the Tanner 5 patients. Our results show that there is a statistically significant direct correlation between the pubertal stage and changes in HbA1c (p = 0.000105).

Based on the height and weight values, we calculated body mass index (BMI) for each case. Minimum BMI was 14.17, the maximum value was 26.86, with an average of 19.12 and the median standard deviation of 2.87. Also, between BMI and HbA1c values a positive correlation was established.

Analyzing the relationship between HbA1c and the duration of the disease evolution, it was found that glycemic imbalance occurs in the first year of evolution (60.6%), then between the years 2-5 of evolution tends to decrease (55.56%), followed between 6-12 years of evolution to be again with predictive positive value (69.24%) of glycemic imbalance (p = 0.001135).

Glycemic imbalance is also positive predictive value for children whose parents had both secondary school education (p = 0.001969) and children whose parents have higher education (p = 0.00054). Analyzing the relationship between HbA1c and education level of the parents, the highest glycemic control was found in children whose parents have high school.

Analyzing the relationship between HbA1c and the family members most involved in the diabetes care, the highest glycemic imbalance occurs when only parents are involved, while the glycemic imbalance decreases if besides the parents the child is involved too.

It was also found that the method of calculation of carbohydrates by experience causes glycemic imbalance at 80% of the patients.

After observing the relationship between HbA1c values and the level of the physical activity performed by the patients, it was established that only 7.14% of the patients with rare physical activity are in glycemic control, while 55.56% of patients who are constantly engaged in physical activity are in glycemic control. Physical inactivity has high positive predictive value (p = 0.017846) for glycemic imbalance.

Analysing the relationship between HbA1c and family conflicts, it was found that in patients whose family have constant conflicts, glycemic imbalance is 100 %.

Analyzing the relationship between frequency of blood measurements and HbA1c value we found that 81.82% of the patients undergoing < 4 measurements/day were in glycemic imbalance, while of those exercising ≥ 4 determinations/day, only 53.33% were in glycemic disequilibrium.

Conclusions

Analyzing the factors that influence glycemic control at children with type 1 diabetes, we found that the following were more prone to have glycemic imbalance: the age group between 7 - 12 years (71.43%); children from rural areas (68.43%); children who always have family conflicts (100%); children who were doing physical activity sporadically (92.86%); children who did not weigh the food (80%); children whose parents had only secondary school (76.46%); children in the first year after onset of diabetes (60%).

In most children there are, at some point, difficulties in obtaining a metabolic balance. But good glycemic control can be achieved through a better understanding of the disease, of the factors influencing glycemic control and through a continuous family and entourage support.

Glycemic control in children with T1D is very important because better understanding leads to a more adapted therapeutic attitude and helps provide a normal life for the child.

Table 1. The correlations between HbA1c values and clinical parameters

HbA1c value / Parameters	Total cases		Cases with HbA1c ≤ 7.5%		Cases with HbA1c > 7.5%	
	No	**%**	**No**	**%**	**No**	**%**
Total cases	41	100%	16	39.02%	25	60.98%
Age groups						
0-6 years	3	7.32%	1	33.33%	2	66.67%
7-12 years	14	34.14%	4	28.57%	10	71.43%
13-18 years	24	58.54%	11	45.83%	13	54.17%
Rural/Urban						
Rural area	22	53.65%	7	31.57%	15	68.43%
Urban area	19	46.35%	9	45.45%	10	54.55%
Pubertal stage						
Tanner 1	13	31.71%	5	38.46%	8	61.54%
Tanner 2-4	17	41.46%	8	47.05%	9	52.95%
Tanner 5	11	26.83%	3	27.27%	8	72.73%
The evolution of disease						
<1 year	10	24.39%	4	40.00%	6	60.00%
2-5 years	18	43.90%	8	44.44%	10	55.56%
6-12 years	13	31.71%	4	30.76%	9	69.24%
Parents education level						
Secondary school	18	43.92%	4	23.54%	14	76.46%
High school	11	26.82%	8	72.72%	3	27.28%
University graduates	12	29.26%	4	33.34%	8	66.66%
Family members						
Parents and the patients	10	24.40%	4	40.00%	6	60.00%
Parents	18	43.90%	7	38.88%	11	61.12%
Mothers	13	31.70%	5	38.46%	8	61.54%
Carbohydrates calculation						
Experience	15	36.59%	3	20.00%	12	80.00%
Weigh	26	63.41%	13	50.00%	13	50.00%
Physical activity						
Rare	14	34.14%	1	7.14%	13	92.86%
Constant	27	65.86%	15	55.56%	12	44.44%
Family conflicts						
All the time	8	19.52%	0	0	8	100%
Sometimes	14	34.14%	5	35.71%	9	64.29%
Rarely	16	39.03%	9	56.25%	7	43.75%
No conflicts	3	7.31%	2	66.67%	1	33.33%
Frequency of blood glucose measurements						
< 4/day	11	26.82%	2	18.18%	9	81.82%
≥ 4/day	30	73.18%	14	46.67%	16	53.33%

Author contribution. All authors made equal contribution to the paper, to that of first authors.

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A STRANGE GLYCEMIC PROFILE IN A NON DIABETIC INFANT AS A DIAGNOSTIC CLUE

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Abstract

A 21 day old boy, on combined breastfeeding and standard formula, was admitted in the emergency care unit for poor weight gain and lack of appetite with clinical signs of dehydration, mild jaundice, marked hepatomegaly.

He presented a marked inflammatory syndrome, elevated transaminases, and cholestasis with mild unconjugated hyperbilirubinemia, elevated ureea and creatinine.

The glucose meter check showed a value of 72 mg%. The TORCH infections were ruled out and the cerebrospinal fluid (CSF) analysis, performed for subsequent bulging fontanel, was negative.

The breastfeeding was discontinued due the hipogalactia and the standard formula was replaced with a lactose-free formula, because of associated loose stools.

Under treatment, he had an improvement in feeding, growth and liver function tests.

He was discharged against medical advice, but a week later, he was readmitted for vomiting and dehydratation. Repeated glucose meter checks performed overnight were all greater than 200 mg/dl. The serum sample sent to the laboratory showed the glucose to be 60 mg%. The discordant glucose meter and serum sample glucose concentrations provided an important clue to the diagnosis of galactosemia.

The urine was strongly positive for reducing substances and negative for glucose. The red blood cell study of galactose-1-phosphate uridyl transferase (GALT) activity was confirmatory.

Keywords: *newborn, galactosemia, glucose meter check, hyperglycemia*

Introduction

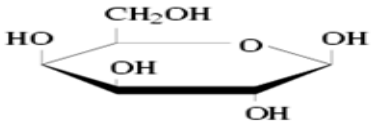
Galactosemia is an inherited autosomal recessive disorder of galactose metabolism that results from a defect in one of the following enzymes: galactokinase (GALK), GAL-1-phospate uridyltransferase (GALT) and uridine diphosphate-GAL-4-epimerase (GALE).[1] Dietary lactose is broken down by lactase into glucose and galactose and then, in a galactose is converted to glucose. (Fig. 1.)

This metabolic pathway is essential for the newborn, whose main carbohydrate resource is lactose.

The classical form of galactosemia, also called the "classical galactosemia" results from a defect in the GALT activity. Its estimated incidence is 1/40,000- 60,000 live births.[2] Patients with GALT deficiency appear normal at birth but soon after ingestion of galactose, develop jaundice, hepatosplenomegaly, hepatic insufficiency, hypoglycemia, renal tubular dysfunction, hypotonia, sepsis, .pseudotumor cerebri and cataract. Sepsis, frequently due to Escherichia coli is common in the neonatal inaugural clinical presentation of galactosemia. If not treated, some of the complications may lead to fatal outcome. The gold standard for diagnosis of classical galactosemia is the measurement of GALT activity in the red blood cells. The life saving treatment consists of severe restriction with removal of dietary galactose and lactose.[3]

The elimination of the galactose from the diet is not a 100% effective therapy. Long-term follow-up of patients with galactosemia has shown that, despite a scrupulous diet, most patients develop symptoms such as retarded mental development, motor abnormalities, developmental verbal dyspraxia, and hypergonadotrophic hypogonadism.[4]
The endogenous production of galactose has been suggested to be an important source of the late complications.[5]

The galactosaemia is part of the newborn screening programs of many European countries. In Romania, because o the lack of financial resources the extended newborn screening including galactosemia is not uniformly widespread.[6]



galacto- kinaza **gal-1P uridyl transferaza** **fosfogluco- mutaza**

gal --------> gal 1 P ----------> glc 1 P ----------> glc 6 P

^ \ / v

UDP-glc UDP-gal

^ /

_____/

epimeraza

Tip	Gena locus	Enzima	Numele bolii
1	GALT 9p13	galactozo-1-P uridyl transferaza	Galactozemia clasică
2	GALK1 17q24	galactokinaza	Deficit de galactokinază
3	GALE 1p36-p35	UDP galactozo-epimeraza	Deficit de UDP galactozo epimerază

Fig. 1.

Case presentation

A 21 day old boy, on combined breastfeeding and standard formula, was admitted in the emergency care unit for poor weight gain and lack of appetite. He presented clinical signs of dehydration, mild jaundice, marked hepatomegaly .The pregnancy and family history were unremarkable. He has three healthy siblings.

His weight on the day of admission was 2,1 kg, while his birth weight had been 3,1.kg. The boy was born at term with an Apgar score of 10.

In the second week of life, the breastmilk was supplemented with a standard formula. His mother noticed that he has poor weight gain, and was becoming increasingly less interested in eating. Initial laboratory findings were as follows: pH=7,33; pCO2=26,4mmHg, HCO3=15,3mmol/l, EB=-12,2mmol/l, white blood cell (WBC) count: 23,8 x 10^9 /L; hemoglobin (Hb): 13,1 g/dL; platelet (Plt) count: 308 x10^9 /L; C-reactive procalcitonin>10ng/mL; total bilirubin: 6,15 mg/dL: indirect bilirubin: 3,47 mg/dL; aspartate aminotransferase (AST): 273 U/L; alanine aminotransferase (ALT): 103 U/L; elevated gama glutamyl transferase GGT=320 U/L (normal value for age <231 U/L), cholinesterase= 1719U/L (VN: 2260-7550).

Serum sodium, potassium, ureea and creatinine were 150 mEq/L, 2,2 mEq/L, 101 mg/dL and 0.63 mg/dL, respectively. Microbiological work-up, including blood culture, was performed and the treatment with Meropenem plus Amikacin was empirically started. The patient received also supportive treatment.

He was transferred in the Pediatric Department. We noticed: weight 2100 g, no fever, slight sclera jaundice, signs of deshydratation, muscular hypotonia, the liver was palpable about 8 cm (Fig. 2.)

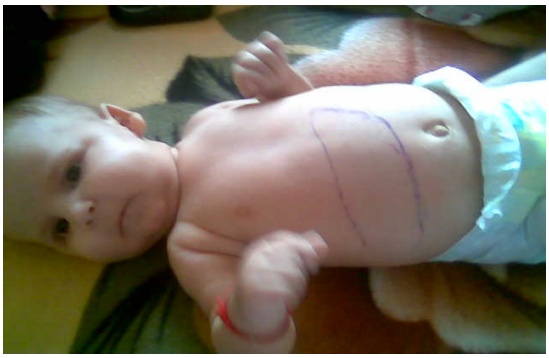


Fig. 2.

The abdominal ultrasound confirmed the hepatomegaly and revealed the increased dimensions of the kidneys. The transfontanellar ultrasound was normal.

The TORCH screening tests were negative: Epstein-Barr virus, Toxoplasma, Rubella, Cytomegalovirus, Herpes simplex virus, Human immunodeficiency virus, Treponema pallidum. The hepatitis A, B, and C were also ruled out. At admission, the bedside glycemia was 244 mg%, but we thought that it was a result of glucose infusion. We continued the broad specter antibiotic therapy, intravenous immunoglobulin and the supportive treatment for septicemia in a severe malnourished newborn.

The patient's clinical condition was stable, but in the second day of hospitalization he presented high fever (39 ^{0}C), a bulging fontanel and watery diarrhea. The cerebrospinal fluid CSF analysis was negative (elements = 0, CSF glucose= 40mg% for a glycemia of 74 mg%, CSF albumin = 0,5 g/L, soluble antigens for Neisseria Meningitidis, Haemophylus, Streptococcus pneumoniae, Streptococcus B negatives, CSF culture negative).

We replaced the standard formula witth a low lactose formula.

As a complication, he presented low natremia despite permanent perfusion, and obvious amelioration of the diarrhea. We suspected or a syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH), or a salt wasting syndrome. The serum cortisole vas normal =23 nmol/l (normal values 17 – 10 a.m.:171-536). He received Astonin 100μg/m^2/zi per os and intravenous Hidrocortisone hemisuccinate, than oral Prednisone.

The mother elicited the discharge despite medical advice.The patient weight at discharge was 3200 g, he presented no inflamatory syndrome, but the hepatomegaly and a mild hepatocytolisis were still present.

Two weeks after the child was hospitalised for vomiting and lethargy.

At admission he had no fever. ASAT= 69U/l, ALAT= 170 U/l, Na= 131,7mEq/l, K= 5,7 mEq/l, high glycemia, above 200 mg%.

Repeated glucose meter checks performed overnight were all greater than 200 mg/dl. In the morning, a simultaneous serum sample sent to the laboratory showed the glucose to be 60 mg%. The discordant glucose meter and serum sample glucose concentrations provided an important clue to the diagnosis of galactosemia [7, 8].

The urine was strongly positive for reducing substances and negative for glucose. The measurement of galactose and galactitol by gas chromatographic determination of urinary sugars and than by ELISA sustined our hypothesis of galactosemia.

The red blood cell study of galactose-1-phosphate uridyl transferase (GALT) activity (realized in Lyon -France) was confirmatory showing a zero activity of this enzyme.

As a surprise, the result of the neonatal screening confirming the galactosemia was available only 2 months after.

The boy received a pork collagen formula (Pregomin) until the age of one year, and a strict regimen with avoidance of lactose and galactose [9]. However, despite conscientious care, many foods (and medications) contain lactose and galactose that it is hard to avoid it

Nowadays he is 6 years old, presents a normal liver function, no hepatomegaly, no cataract, but a short stature and weight for his age (both under the 5 th percentile), an low IQ and troubles of language.

Conclusion

We presented the case of a newborn with classical galactosemia, where the right diagnosis was oriented by the "strange" glycemic profile, with high beside glycaemia measured by the bandelette, contrasting with the real blood glycaemia. The favorable initial outcome was facilitated by the regimen without lactose prescribed for the diarheea. Despite the pacient poverty and despite the fact that in Romania the special regimen without galactose for infants with galactosemia is not covered by a national program, the child had a good evolution, with no cataract, no liver disfunction, but with mild mental retardation and verbal dyspraxia..

The result of the neonatal screenig was available two months later after our diagnosis. In Romania the neonatal screening for newborns is not uniformly realized. The patient was born in a hospital were the screening for galactosemia is performed, but the results were communicated to the parents with a long delay.

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OXIDATIVE STRESS AND ANTIOXIDANT THERAPY IN CYSTIC FIBROSIS IN CHILDREN

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Abstract

Cystic fibrosis (CF) is caused by a defect of CFTR gene. The presence of defective CFTR appears to produce a redox imbalance in epithelial cells and extracellular fluids and causes an abnormal generation of reactive oxygen species (ROS). Airway surface liquid (ASL) in CF bronchi is characterized by increased concentration of ROS, lowered levels of glutathione and reduced nitric oxide. The increase of pro-oxidative species in ASL contributes to the progressive lung tissue damage and to the amplification of the inflammatory response in CF airways. Oxidative stress and inflammation can affect surfactant biophysical activity leading to early alterations of lung function and involving both lipid and protein components. Surfactant protein D becomes unable to agglutinate bacteria when it is modified by oxidation, which facilitates pathogen colonization in the lung. Antioxidant supplements might reduce the oxidative damage in the lungs. Different antioxidants as vitamin E, β-carotene and ω-3 fatty acids have observed to alleviate selected biochemical signs of oxidative stress. Glutathione - the major antioxidant shield in the epithelial lining fluid, in people with CF is not released into the lungs properly. Administration of glutathione improve lung function in some cases and lower oxidative stress. Some enzymes which help antioxidants work are dependent on selenium; so selenium supplements aim to stimulate antioxidant action. Vitamin C decreases with age in people with cystic fibrosis, so vitamin C supplements aim to rebuild these levels. Conclusion: antioxidant supplementation may prove beneficial in slowing the rate of deterioration in pulmonary function when combined with current therapies.

Key words: *cystic fibrosis, children, oxidative stress, antioxidants.*

Cystic fibrosis (CF) is the most common hereditary disease of the Caucasian population that involve multiple organs, especially the respiratory and the digestive systems. The defect in CF is the loss of function of the transmembrane conductance regulator (CFTR) protein. The disease severity is the consequence of environmental and genetic factors. Among them, the oxidative stress (OS) play an important role in the evolution of CF, with susceptibility to oxidative damage, decline of pulmonary function and impaired lung antioxidant defense.[1]

Oxidative stress represent an imbalance between oxidant production and antioxidant defense, resulting in an increase in the steady-state levels of oxidized cellular macromolecules.[2] Oxidants are derived from an NADPH-oxidase that reduces molecular oxygen to superoxide. Thr superoxide dismutates to hydrogen peroxide which is used by the heme enzyme myeloperoxidase to oxidize chloride and thyocyanate to hypochlorus acid and hypothiocynite.[3]

Patients with CF are exposed to chronic OS due to an overproduction of reactive oxygen species (ROS) as a result of chronic activation of neutrophils and macrophages and of impaired antioxidant status which is not confined to the fat soluble antioxidants vitamin E and carotenoids.[4]

The basic CF genetic defect itself is a source of OS. Class II CFTR mutation, such as ΔF508, cause accumulation of misfolded CFTR protein in the endoplasmic reticulum (ER) resulting in OS. CFTR transports the glutathione – the major antioxidant in the lung, and mutations in CFTR lead to low levels of this antioxidant in airway surface liquid. It was demonstrated that CFTR expression and function are modulated by OS. CFTR can impair cell volume and pH regulation, transepithelial transport, membrane conductance and the glutation (GSH)-related antioxidant and detoxication activity in the extracellular milieu.[5] Furthermore, CFTR dysfunction drives mitochondrial defects, alterations in oxidative phosphorilation, calcium homeostasis, OS, apoptosis and innate immune response.[1] The respiratory tract is protected from infection by ROS generated by phagocytic and epithelial cells. As a result of chronic pulmonary infections and digestive malabsorption, appear an imbalance between the production of ROS (superoxide and hydrogen peroxide) and their inactivation by protective systems.[6] An oxidative environment influences intracellular signaling events leading to apoptosis, increased synthesis and secretion of mucin and alterations in ion transport. The constitutive deffect of GSH metabolism with a lowered intake and absorption of fat-soluble antioxidant vitamins contribute to a defective antioxidant protection which exacerbate OS indices.[5]

Oxidative unbalance in CF is characterized by increased concentrations of ROS, lowered levels of glutathione (GSH) and reduced nitric oxid (NO).The increase of pro-oxidative species in airway surface liquid contributes to the progressive lung tissue damage and to the amplification of the inflamatory response in airways characterized by the release of chemokines and cytokines (IL-6, IL-8), NADPH oxidase, mieloperoxidase, lactoperoxidase (fig. 1.) [5]

Neutrophils are a major pro-inflammatory cell in CF. Activated neutrophils are a major source of free radicals and are considered a potent mechanism for killing organisms and damage the pulmonary epithelium. Activated neutrophils migrate into the airways to attack invading bacteria and release in their microenvironment ROS (superoxide anion, hydrogen peroxide, hydroxyl free radical), mainly by the activation of the NADPH oxidase.[5,7,8] Some bacteria such as P. aeruginosa

generate oxygen radicals though the release of pyocyanin and other phenazine pigments. Thus, the CF airway is exposed to oxidants derived from inflammatory and infectious processes.[8]

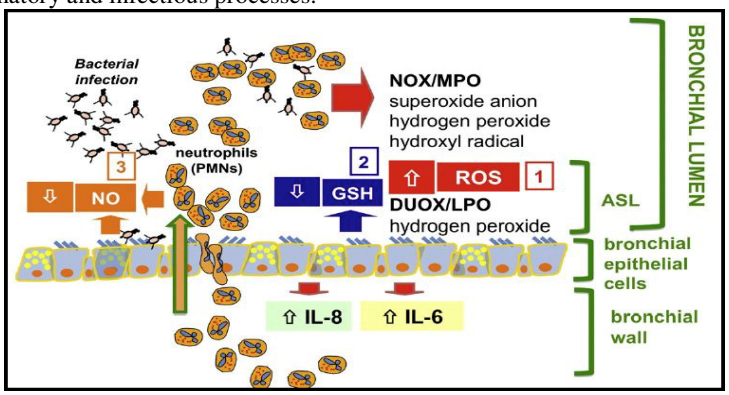

Fig.1. Oxidative unbalance in conductive airways of patients affected by cystic fibrosis.[5]

The cumulative effect of repeated infections is responsible for the decline of lung function in CF. In particular, the production in excess of oxygen free radicals overburdens the antioxidant defences and oxidises membrane components of lung cells, contribuiting to the decrease in lung function.[7] Other studies sugest that the increased OS is in response to inflammation associated with infection, rather than part of the primary defect.[9]

In the presence of chronic inflammatory syndrome, ROS may lose the physiological role in the killing of pathogen. ROS can modify the thiol homeostasis of extracellular fluids and epithelia and promote the activation of MAPK signaling pathways.[5] The markers of free radical-mediated damage in plasma of CF patients (lipid hydroperoxides, malondialdehyde, protein carbonyls) are present in patients with normal concentrations of circulating antioxidants, indicating that oxidative damage is most the result of elevated rates of free radical production. OS and inflammation in CF can affect surfactant biophysical activity, thus leading to early alteration of lung function. Oxidative damage of surfactant involve both protein and lipid components.[5]

An other explanation for increases of OS markers is mithocondrial generation of oxidants. Mitochondria are the primary source of intracellular OS and can produce intramithocondrial ROS. Increases of intracellular level of Ca^{2+} can stimulate mithocondrial generation of ROS / reactive nitrogen species (RNS) and via protein kinase C activation may increase NADPH oxidase-dependant generation of free radicals and thus induce OS and ultimately apoptosis.[10]

Measurement of the mitocondrial aconitase and fumarase activities is useful to identifying mitochondrial oxidative stress. A decrease in aconitase activity without a concomitant decrease of fumarase suggests the presence of OS. Measurement of oxidized deoxyguanosine in the mitochondrial genome is a second specific marker for mitocondrial OS. CFTR play a role in modulating mitochondrial GSH levels in lung epithelium and is a contributing factor of lung OS.[2]

Normal airways are able to cope with OS through a variety of mechanisms, including the absorption and/or biosynthesis of antioxidants.

Brown et al. demonstrated that the products of lipid and protein oxidation are present in CF patients with normal concentrations of circulating antioxidants, indicating that oxidative damage is most the result of elevated rates of free radical production.[7]

CF-related diabetes is the most common complication. Recent evidence has confirmed that CFTR is an important regulator of insulin secretion by islet β-cell. In diabetes, hyperglycemia and hyperlipidemia are emerging factors which through complex mechanisms lead to oxidative stress. The combination of increased OS and accumulation of misfolded CFTR proteins in the ER of the β-cell may lead to endoplasmic reticulum stress and eventual apoptosis of this cell lineage. This effect can be potentiated by the malabsoption of dietary antioxidant in CF patients. The expression of the endogenous antioxidants is low in β-cells and this situation sets them up as easy targets for free radical production and OS. GSH deficiency is known to cause oxidative damage in the pancreas and is associated with the onset of diabetes. Since increased OS and altered Ca^{2+} homeostasis are found in CF, it is believed that both elements could be involved in insulin deficiency observed in CFRD. As the ER plays an important role in the regulation of Ca^{2+} homeostasis, it is possible that the combination of OS with ER stress during the course of CF further decreases insulin secretion. The multiple-organ dysfunction of CFTR protein is directly associated with an increase in OS which can alter glucose tolerance by reducing insulin secretion or inhibiting its signalling pathways then leading to CFRD.[10] (fig. 2.)

Quantification of OS can be assessed by the detection of lipid peroxidation end-products deriving from the degradation of polyunsaturated fatty acids. Several markers for lipid peroxidation are isoprostanes and aldehydes (malonaldehyde, lipid-adducts 4-hydroxynonenal). Oxidative stress biomarkers have been detected in exhaled breath condensates, bronchoalveolar lavage fluid and blood from CF patients. These lipid peroxidation products were also found to be increased in peripheral blood, plasma and urine, suggesting that oxidative stress originating from lungs can shift through other organs.[10]

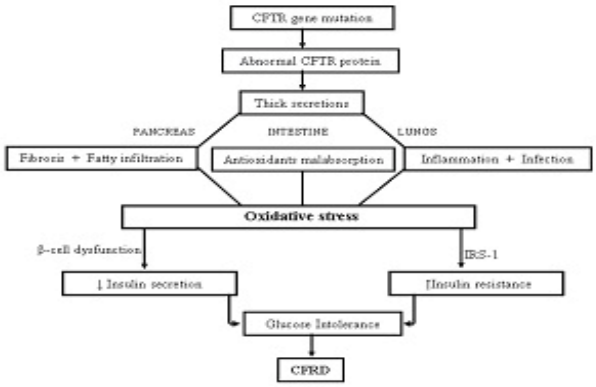

Fig. 2. The links between CFTR dysfunction, OS and occurence of CFRD in CF.[10]

Antioxidants in CF

Major antioxidants are:

-enzymatic: superoxide dismutase, catalase and glutathione peroxidase.

-nonenzymatic: ascorbate, urate, α-tocopherol, reduced glutathione (GSH), albumins and mucins.

In the normal lung, ascorbate and GSH are present in high concentrations in bronchoalveolar lavage fluid, but in the upper airways co8centrations are lower and mucin may represent the major antioxidant. α-tocopherol is present in low concentrations in the airway surface fluid.[7]

Glutathione – a tripeptide composed of L-cysteine, L-glutamic acid and glycine, is the major antioxidant in the epithelial lining fluid of the lung and protects this area from OS.[8,9] It is part of intracelular defense system which protects the epithelyum against the injuries and inflammation prsent in CF.[11] Cells can be protected against OS by extracellular glutathione through the degradation catalysed by the exo-enzyme γ-glutamyl transpeptidase and its de novo synthesis within the cytosol via the γ-glutamyl cycle.[9]

Oxidant-antioxidant imbalance in CF was confirmed in different studies.[6,12] A sub-optimal antioxidant protection represent an important contributor to OS and to the poor control of immuno-inflammatory pathways in patients with CF.

In CF there are low levels of total radical-trapping antioxidants parameters that are accompanied by low concentrations of vitamins A, E, β–carotene and oligoelements (selenium, zinc, copper) in plasma.[5,6] Pancreatic insufficiency also increase susceptibility to deficiencies in lipophilic antioxidants. The presence of malabsorption explains the high incidence of fat-soluble antioxidant deficiencies (vitamin, carotenoids) and essential fatty acid deficiency.[10]

Roum J.H. cited by Hector et al.[13] demonstrated that glutathione levels are decreased in patients with advanced CF lung disease, whereas in younger CF children was found only a tendency to lower levels. The analyses of broncheoalveolar lavages have revealed the presence of decreased levels of GSH in the alveolar epithelial lining fluid of CF patients.[5] CFTR has an important role in the extracellular transport of GSH. CFTR transports glutathione, this being the explanation for the low glutathione level in CF airways. Alternatively, activated neutrophils are capable of oxidising and disabling glutathione.[13] Different authors found a decrease of glutathione concentration in the epithelial lining fluid of CF patients with pulmonary inflammation. The glutathione deficiency is associated with changes in other antioxidants, increased OS with pancreatic and hepatic damage, increased lipid peroxidation and enhanced protein oxidation, denaturation and aggregation.[10] Administration of glutathione, orally or by inhalation, improves lung function and decreases OS.[14]

Vitamin E is an antioxidant that protects the lipid fraction of cell membrane from a free radical oxidative injury. Numerous patients with CF presents biochemical vitamin E deficiency defined as plasma concentrations below the mean -2 SD.

Lowered vitamin E level was found in CF in association with lowered levels of other liposoluble vitamins such as A and D. Increased number of exacerbations is correlated to lower plasma vitamins E and A.[15] The clinical setting of parenteral iron therapy demonstrates that only vitamin E status higher than normal protects against acute transition metal ion-induced oxidative-stress.[4]

Carotenoids (β-carotene, total lycopene) are lowered in CF and are associated with higher susceptibility to lipid peroxidation. Impaired vitamin E and β-carotene statuses are associated with enhanced susceptibility to oxidation of LDL and increased of lipid peroxidation.[4] Rust et al. cited by Galli[5] demonstrated that the long-term oral supplementation with 1 mg/kgBW/day restore the level of this carotenoid, confirming the need of high doses of this fat-soluble factor.

Co-enzyme Q10 (CoQ) or ubiquinone is a lipid-soluble component of the mitochondrial electron transport chain. Malebsorption or oxidation of CoQ may result in decreased circulating levels in patients with CF, possible resulting in decreased ATP production or protection against OS or both.[8]

Several antioxidants have been shown to have mucolytic and anti-inflammatory properties. Zinc and vitamin C help increase epithelial chloride secretion through CFTR-dependent and independent pathways. Zinc is important in antioxidant

defenses due to its association with superoxide dismutase (SOD) and a cytosolic copper-zinc SOD. Selenium has antioxidant function, but also other functions including neutrophil response to pathogens, cell cycling and metabolism of carcinogens.[8]

Patients with CF whose lungs are colonized by P. aeruginosa are at increased risk of iron-mediated ROS formation because elastase from the bacteria cleaves transferrin and lactoferin. Transferrin and lactoferin cleavage products appear in the epithelial lining fluid of patients with CF and P. aeruginosa. The iron released from these cleaved protein can catalyze hydroxyl radical formation.[8]

Conclusions

Oxidative stress characterizes CF patients from the pediatric age and it progressively increases over the years. Patients with CF and pancreatic insufficiency need a careful monitoring of redox balance due to the risk of OS.

The free radical-mediated damage may play a role in the increase in pulmonary dysfunction in patients with CF.

Normal levels of antioxidant defences in CF are insufficient to protect against the oxidative stress that the patients experience from repeated infections. So, additional antioxidant supplementation may prove beneficial in slowing the rate of deterioration of pulmonary function when is combined with current therapies and may help maintain an oxidant-antioxidant balance. The daily intake of antioxidants and the use of pharmacological inhibitors aims at suppresing chronic activation of stress-sensitive signalling pathways may be important in preventing the onset of CFRD.

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THE ROLE OF GLUTEN IN THE PATHOGENESIS OF TYPE 1 DIABETES MELLITUS AND CROHN DISEASE

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Abstract

The role of gluten is well known in the pathogenesis of coeliac disase,as its trigger factor. Gluten is characterized by its high content of prolin and glutamin, and after their digestion even by the normal small intestinal mucosa long polypeptides remain, which are able to increase the permeability of even normal intestinal mucosa through their ability to release zonulin. It can be stated, that gluten could have a dose-dependent detrimental effect on the gut barrier, primarily in the small intestine even without coeliac disease. This effect of gluten raises the possibility its pathogenetic role of other diseases in addition to coeliac disease, first of all in type 1 diabetes mellitus (T1DM) and Crohn disease (CD).

Our earlier observation that in the jejunal mucosa of children with T1DM signs of immune activation are observed could have been the consequence of increased intestinal permeability. In this study the patients had significantly more α4/β7 integrin positive cells in the lamina propria compared to controls. This observation is highly relevant, because T-cells from humans diabetic pancreas with T1DM express the gut-associated homing receptor α4/β7 integrin. The pathogenetic role of gluten in induction of T1DM is indicated also by the frequent association of T1DM and coeliac disease and it was observed that in the majority of cases the onset of T1DM is earlier than that of coeliac disease, when the patient is not on a gluten free diet.

According to several studies it is probable that beside characteristic genetic traits mostly the diet influences the development of CD. The prevalance of CD is on the rise in developed countries exposed to Western diet. This is also indicated by the very efficient therapeutical results of exclusive enteral nutrition (EEN) in CD. Pediatric studies confirmed that 70-90% of children fed an exclusive liquid formula and no exposure to other food, reach a complete remission. The effect of formula is not depend on its composition or the degree of hydrolysis of its protein (elemental or polymer formula). Therefore, the EEN may have its effect principally that it does not contain those compounds which are frequently occur in the normal diet, it is also gluten free. Increased gut permeability caused by gluten may be the primary factor triggering CD.

On the base of the above mentioned fact it is probable that gluten may play a decisive role in the pathogenesis of type 1 diabetes mellitus (T1DM) and of Crohn's disease (CD).

Introduction

The triggering role of gluten in the development and maintaining the pathological process in coeliac disease was proved more than sixty years ago by Dicke. His discovery made possible the treatment of this disorder with gluten free diet. Gluten is a storage protein with high viscoelastic properties and so it is an essential ingredient for making a high quality dough. Therefore gluten is one of the most commonly used proteins in the food industry. Its toxic component triggering coeliac disease is the ethanol soluble gliadin, which belongs to the prolamins. The related prolamins in rye and barley called secalin and hordein, which are also toxic in patients with coeliac disease (CeD).[1] These prolamins protein are different from other cereals' storage proteins insofar as they have a higher proportions of glutamine and proline, which make them resistant to enzymatic processing in the intestine.[1] It was showed that even beside prolonged in vitro digestion with gastric, pancreatic and brush border enzymes, a 33-mer peptide survived, which is toxic in coeliac disease.[2]

Fasano et al identified a human protein named zonulin which induces tight junction disassembly and consequently an increase in intestinal permeability. They also detected that the concentration of this protein is increased in the intestinal mucosa of patients with active CeD.[3] Later they also proved that gliadin induces a direct increase in small intestinal permeability by binding to the chemokine receptor CXCR3 expressed in the intetinal epithelium, which elicit MyD88-dependent zonulin release.[4] When biopsies taken from non-coeliac controls were exposed to gliadin a transient zonulin release was observed with an increase of intestinal permeability, which was less than in biopsy taken from active coeliac patients.[5]

It can be stated, that consumption of gluten could have a dose-dependent detrimental effect on the gut barrier, primarily in the small intestine even without coeliac disease.

On the base of undermentioned fact it is very probable that gluten may play a decisive role in the pathogenesis of type 1 diabetes mellitus (T1DM) and of Crohn's disease (CD).

T1DM

It has been recognised for decades that coeliac disease occurs with a greater than expected frequency in patients with T1DM. According to screening studies the prevalence of CeD ranges between 1,3% and 16,4%. In our previous study we found among 205 diabetic children 17 cases with CeD (8,3%).[6] The increased association of CeD and T1DM is probably due to a common genetic prediposition, approximately 95% ofpatients with CeD express HLA-DQ2, which is also apositive risk factor for T1DM.[7] In our study, patients with T1DM and those with CDand T1DM, the occurrence of HLA-DQ2/8 heterozygosity was significantly higher than in children with CD only and in control children.[8]

In the majority of cases in children with T1DM and CeD, CeD starts later than the diabetes. *In our series of 51 children with both T1DM and CeD in 45 T1DM started earlier than CeD.* The median of elapsed time from the onset of T1DM to CeD was 21 months, when T1DM started under 5 year, while with onset of T1DM over 5 years the median was 3 months.

Sapone et al detected that serum zonulin levels was higher in adult patients with T1DM, as compared with age-matched controls. **It was also observed in their study that the increased zonulin levels correlated with increased intestinal permeability** in vivo examined with lactulose/mannitol (L/M) test. In a pilot study it was also recognized that expression from the intestinal tight junction proteins claudin-1 was increased, while that of claudin-2 decreased. Zonulin levels were also moderately elevated in a group of individuals with risk of T1DM, who had elevated autoantibodies.[9] In this latter group no change in the expression of tight junction genes was observed, which indicate that zonulin upregulation precedes the onset of T1DM. In our study investigating claudin expression in the duodenal mucosa of patients with active CeD, increased expression of claudin 2 and 3 was observed compared to the controls.[12]

Our earlier observation that in the jejunal mucosa of children with T1DM signs of immune activation are observed could have been the consequence of increased intestinal permeability. In this study the patients had significantly more α4/β7 integrin positive cells in the lamina propria compared to controls.[10] This observation is highly relevant, because in T-cells from humans diabetic pancreas with T1DM T-cells express the gut-associated homing receptor α4/β7 integrin.[11] It was also observed that gliadin stimulated peripheral blood blood mononuclear cell of patients with T1DM without coeliac disease showed a mixed proinflammatory Th1 and Th17 activation.[13]

It is also known that in non-obese diabetic mice gluten-free diet significantly reduces the incidence of T1DM. Recently it was also observed that the gut microbial flora is also changed on gluten-free diet, Akkermansia species was increased, which suggests that dietary gluten could modulate the incidence of T1DM by changing the gut microbiome.[14] In isolated rat islets it was also detected that gliadin digest and the specific 33-mer protein from the gliadin increase the insulin secretion compared to the controls. Gliadin peptides are closing the K_{ATP} channels, hereby inducing insulin secretion and so gliadin may contribute to the beta-cell hyperactivity observed prior to the development of T1DM.[15]

A recently reported new observation indicates that gluten may contribute even to the development of diabetic nephropathy in children with T1DM. It was found that children with T1DM and CeD on a strict gluten free diet has slower progression in albuminuria over five years of follow-up than children with T1DM alone. It is possible to speculate that gluten-free diet confers renoprotection in T1DM.[16]

Crohn's disease

According to studies up to present it is probable that beside characteristic genetic traits mostly the diet influences the development of CD. It is obvious that the prevalance of CD is on the rise in developed countries exposed to Western diet.[17] This is also indicated by the very efficient therapeutical results of exclusive enteral nutrition (EEN) in CD. Several pediatric studies confirmed that 70-90% of children fed an exclusive liquid formula and no exposure to other food, reach a complete remission.[18,19] In a recent study Levine et al. prospectively evaluating 150 new-onset, untreated children with CD did not find difference between corticosteroid and EEN in achieving clinical remission and what is more EEN was significantly superior to corticosteroids for achieving mucosal healing (deep remission).[20] The effect of formula is not depend on its composition or the degree of hydrolysis of its protein (elemental or polymer formula).[21] Therefore, this diet may have its effect principally that it does not contain those compounds which are frequently occur in processed foods, which could damage the intestinal mucosa increasing the bacterial translocation. Avoiding such components in the diet may prevent the development of CD.[22] Such components in the regular diet are sodium caprate, carboxymethylcellulose, plysorbate 80 and gliadin. **As was mentioned earlier gluten can increase the intestinal permeability and one of the constant features in CD is an increase of intestinal permeability that can precede inflammatory lesions and trigger mucosal inflammation.**[23]

Impairment in the function of the intestinal barrier leads to increased permeability to luminal antigens and bacterial translocation. Pathogen associated molecular patterns of intestinal bacteria interact with pattern recognition receptors, including toll-like receptors (TLRs), which are important component of innate immunity.[24] In our previous study supported we demonstrated that TLR2 and TLR4 mRNA expression and protein levels were higher in the inflamed colonic mucosa of children with freshly diagnosed and relapsed CD compared to the controls, while in the non inflamed colonic mucosa TLR2 and TLR4 expression and protein levels were similar to controls.[25] TLR ligands promote epithelial regeneration in a lower dose, while in a higher dose they have growth-suppressive effects.TLRs in a higher dose promote also fibrogenesis and wound healing with scarring. Increased fibrosis leads to a faster wound closure which is advantageous in the short run, but it can cause long-term problems.[26]

Conclusions

Proving the role of gluten in the pathogenesis of T1DM would give the possibility the primary prevention of this disorder especially in the first degree relatives of the patients. Putting of these individuals on a gluten-free diet the development of T1DM could be prevented. As it was previously mentioned in our preliminary study we found that the onset of CeD is much more frequent after the onset of T1DM, as vice versa. This is an indirect evidence that gluten free diet may prevent the development of T1DM.

Establishing the role of gluten also in the development in diabetic nephropathy would give the possibility to prevent this complication with a gluten free diet even with patients with T1DM in whom coeliac disease is not detected.

Proving the role of gluten in the increase of intestinal permeability of Crohn disease may help to prevent the development of overt disease by introduction of a gluten free diet. Proving such an effect of gluten, we would get an explanation of the effect of EEN. As all formula used for EEN are gluten free, its effect could be explain that with this therapy the gluten exposition is avoided.

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COMPLEMENTARY FEEDING AND MICROBIOTA

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Abstract

This microbial ecosystem serves numerous important functions for its human host, including protection against pathogens, nutrient processing, stimulation of angiogenesis, and regulation of host fat storage. Microbial colonization runs in parallel with immune system maturation and plays a role in intestinal physiology and regulation. Increasing evidence on early microbial contact suggest that human intestinal microbiota is seeded before birth.

Two important points related to dietary drivers of the gut microbiome development in children were identified. First, breast-feeding, regardless of duration supports a specific bacterial state that is unique and markedly different from that observed in individuals consuming solid foods. Second, once solid foods are introduced, its role in shaping long-term gut microbiome profiles is so strong that individual's cluster based on diet type over other environmental and physiological factors. During infancy, the intestinal microbiota is less stable and more variable in its composition than in older children and adults.

The world is experiencing a progressive increase of metabolic and immune mediated diseases, with a dramatically high increase in infant populations. Allergic diseases comprise the most common chronic disease in childhood while obesity is the most prevalent nutritional problem in Western countries. As diet plays a major role in the development of microbiota during this pheriod, there is an opportunity for its manipulation.

Manipulation of the human microbiome may include microbial supplements (probiotics or synbiotics), foods or substrates (diet or prebiotics), and microbial suppression or elimination. Future studies may lead to improved health benefits for pediatric patients through the manipulation of the intestinal microbiota (antibiotics) strategies.

The adult human body typically comprises ten times more microbial cells than human cells, due largely to the extremely high density of microbes found in the human intestinal tract (typically 1011–1012 microbes/ml of luminal content) (Table 1).

Table 1. Predominant bacterial phyla in the human body.

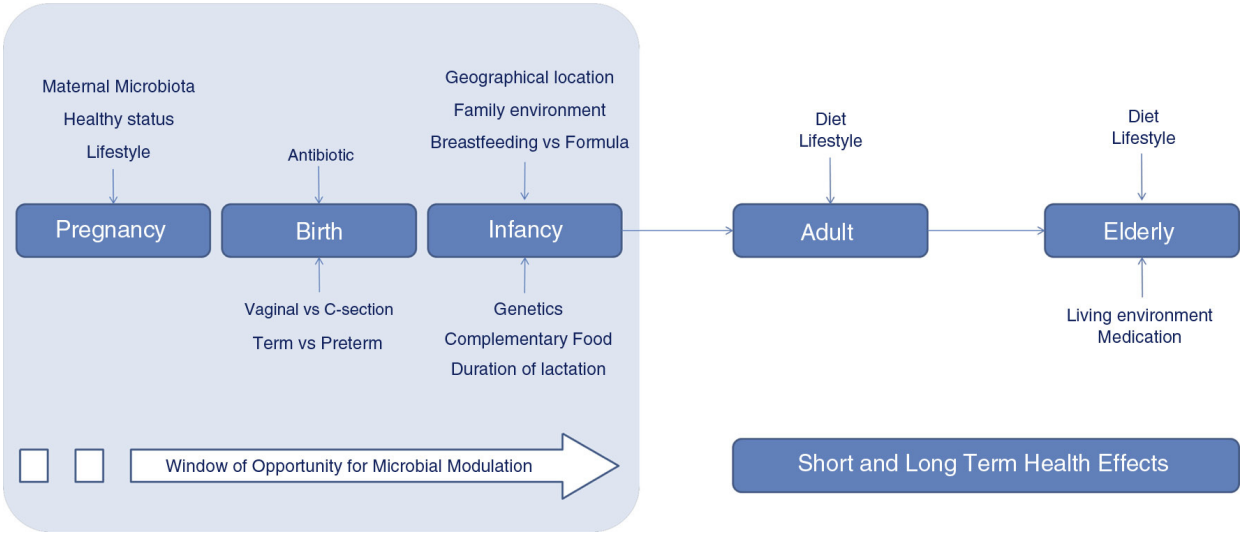
Phylum	Class	Characteristics	Examples
Firmicutes	Bacilli; Clostridia	Gram-positive; diverse in their morphology (rod, coccoid, spiral), physiology (anaerobic, aerobic); include commensal and beneficial bacteria	*Lactobacillus*; *Ruminococcus*; *Clostridium*; *Staphylococcus*; *Enterococcus*; *Faecalibacterium*
Bacteroidetes	Bacteroidetes	Gram-negative; composed of 3 large classes widely distributed in the environment, including soil, seawater, and guts of animals	*Bacteroides*; *Prevotella*
Proteobacteria	Gammaproteobacteria; Betaproteobacteria	Gram-negative; include a wide variety of pathogens	*Escherichia*; *Pseudomonas*
Actinobacteria	Actinobacteria	Gram-positive; diverse morphology; major antibiotic producers in the pharmaceutical industry	*Bifidobacterium*; *Streptomyces*; *Nocardia*

This microbial ecosystem serves numerous important functions for its human host, including protection against pathogens, nutrient processing, stimulation of angiogenesis, and regulation of host fat storage. [1–7] The intestinal microbiota has become a relevant aspect of human health. Microbial colonization runs in parallel with immune system maturation and plays a role in intestinal physiology and regulation. Increasing evidence on early microbial contact suggest that human intestinal microbiota is seeded before birth.

The infant gut is thought to be sterile at birth, although some new research characterizing the placental microbiome challenges that assumption. Meconium is not sterile, as was previously assumed, since it harbors a complex microbial community. A recent study characterized the microbiota of meconium and fecal samples obtained from preterm babies during the first 3 weeks of life using culture-dependent and culture-independent techniques. Firmicutes was the main phylum detected in meconium while Proteobacteria was abundant in feces. Proteobacteria was abundant in feces. Culture-based techniques showed that staphylococci predominated in meconium while enterococci and certain gram-negative bacteria, such as Escherichia coli, Klebsiella pneumoniae, or Serratia marcescens, were more abundant in fecal samples. In addition, 16S

rRNA gene-based microarrays revealed the high prevalence of bacteria related to Streptococcus mitis and Lactobacillus plantarum in meconium, whereas those related to E. coli, Enterococcus and K. pneumoniae predominated in the infant feces.[8] After birth, initial colonization and early establishment of the infant gut is influenced by whether delivery was vaginal or caesarean, feeding patterns, sanitary conditions, and antibiotic administration. By the end of the first year of life, infants possess an individually distinct microbial profile, converging toward the characteristic microbiota of an adult, such that by 2.5 years of age, the microbiota fully resembles that of an adult in terms of composition and diversity.[9,10] In Table 2 there are represented factors influencing the infant gut microbiota development and the adult and elderly microbiota. The first 3 years of life represents the most critical period for dietary interventions aimed at microbiota modulation to improve child growth and development and positively affect health.

Table 2. The gut microbiota throughout life, Citation: Microbial Ecology in Health & Disease 2015, 26: 26050- http://dx.doi.org/10.3402/mehd.v26.26050



A relevant and strong influence in the infant gut microbial development is the mode of feeding. In a single case study, the infant microbiota development was followed from birth to 2.5 years of age and results demonstrated a clear dietary influence on the microbiota composition.[9] In the table below (Table 3) there is a representation of the evolution of the infant gut microbiome development from birth to 3 years of age. By 3 years old, toddler's microbiomes are similar to that in adults and long-term dietary patterns are beginning to establish.

Table 3. Representation of the infant gut microbiome development from birth to 3 years of age. Adapted after Voreades et al., Diet and the development of the human intestinal microbiome, Frontiers in Microbiology Evolutionary and Genomic Microbiology, 2014 (5), Article 494

0-9 Months (Newborn)		9-18 Months (Infant-Pre-Toddler)	18-36 Months (Toddler)
Breast-Fed Characteristics (BF) • Low Species Diversity • Bacterial Composition Flux • Major Phyla: *Actinobacteria & Firmicutes*	**Formula-Fed Characteristics (FF)** • Low Species Diversity • Bacterial Composition Flux • Major Phyla: *Actinobacteria & Bacteriodetes*	**Introduction of Weaning & Solid Food** • Increased Species Diversity • Bacterial Composition Flux Persists • Increasing Butyrate Producing Bacteria • Major Phyla: *Bacteriodetes & Firmicutes*	**Diet-Influenced Microbiome Profile** • Stable Gut Microbiome Formation • Increased Species Diversity • Breast-Feeding History Ceases To Impact Gut Microbiome Profile • Increasing Butyrate Producing Bacteria Abundance • Dietary Intake Strongly Influences Abundances (*Prevotella* vs *Firmicutes*) • Major Phyla: *Bacteriodetes & Firmicutes*

Gut microbiota diversity increases following weaning and introduction of solid food, with enhanced colonization of butyrate producers, including Bacteroides and certain Clostridium species.[9,11] Two important points related to dietary drivers of the gut microbiome development in children were identified. First, breast-feeding, regardless of duration supports a specific bacterial state that is unique and markedly different from that observed in individuals consuming solid foods. Second, once solid foods are introduced, its role in shaping long-term gut microbiome profiles is so strong that individual's cluster

based on diet type over other environmental and physiological factors. The earlier that solid food is introduced into the diet, the more quickly the gut microbiome begins to resemble a stable adult-like microbiome. During infancy the intestinal microbiota is less stable and more variable in its composition than in older children and adults.

Studies performed in germ-free mice colonized with human microbial showed that they can be rapidly altered by diet.[12] Alterations of fiber and fat/protein content in the diets of a small cohort of children and adults also yielded changes in the composition of the microbiome within 24-hour period, which then remained stable over the duration of the study.[13] Longer-term changes in diet may be necessary to effect more substantial changes. Moreover, the Bacteroides enterotype was associated with consumption of animal protein and saturated fat, whereas the Prevotella enterotype was associated with a carbohydrate rich diet.[13] The nutrients identified to influence infant and child microbiota are: Iron is an essential nutrient for many gut microbes, but some beneficial barrier bacteria (e.g. Lactobacilli) do not require iron; for most enteric gram-negative bacteria (e.g., Salmonella, Shigella, or pathogenic Escherichia coli), iron acquisition plays an essential role in the virulence and colonization of most pathogenic strains. Fatty acids n-3 LCPUFA may modulate growth and adhesion of lactobacilli and may impair growth of Bacteroides thetaiotaomicron.

The world is experiencing a progressive increase of metabolic and immune mediated diseases, with a dramatically high increase in infant populations. Allergic diseases comprise the most common chronic disease in childhood while obesity is the most prevalent nutritional problem in Western countries. As diet plays a major role in the development of microbiota during this period, there is an opportunity for its manipulation.

The importance of the microbiota to many aspects of human health and the realization that its foundation is established in early infancy are becoming increasingly recognized. Manipulation of the human microbiome may include microbial supplements (probiotics or synbiotics), foods or substrates (diet or prebiotics), and microbial suppression or elimination. Future studies may lead to improved health benefits for pediatric patients through the manipulation of the intestinal microbiota (antibiotics) strategies.

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NUTRITION IN CHILD OBESITY

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Abstract

Child obesity is a complex, multifactorial, chronic medical pathology. There is no consensus in treating child obesity, so prevention remains still an ideal approach. If prevention did not succeed, then nutritional intervention is needed. In the last years, there is evidence that nutrition in early infancy and childhood might be used as a preventive tool in the management of obesity. Some factors in early life are important for the risk of overweight and obesity later in childhood. Among the postnatal factors, breastfeeding and complementary feeding are of interest. Breastfeeding has been shown to reduce the risk of later obesity. A high protein intake during complementary feeding is associated with a higher risk of obesity while, a high fat intake during this period does not seem to be a risk factor for later obesity. On the contrary, in older children and adults, a high fat intake is associated with higher risk of obesity. Rapid weight gain early postnatal (0 - 6 months), seem to be a good indicator for the risk of obesity later in life. If prevention does not succeed then, dietary intervention would be the second tool in management. Some standard general recommendations and also some special dietary models (low/medium carbohydrate diet, energy restricted diet, low glycaemic index diet) can be used to treat overweight/obese children. Conclusion. Nutrition even since early childhood is essential to prevent later obesity; nutritional intervention should be adapted to age and need to keep in mind that the child is continuously growing.

Key words: *child, obesity, nutrition*

Introduction

Obesity in children is defined as a chronic, complex, multifactorial disease. The prevalence of childhood overweight and obesity continues to be unacceptably high and, of public concern in Europe and some other parts of the world. And that is mostly because of its adverse health effect consequences, such as type 2 diabetes mellitus and the cardiovascular diseases. There is no consensus concerning the treatment of the obese children, that`s why, prevention remains the ideal approach. If prevention would not succeed, then nutritional intervention will be needed. The results of the studies ruled out in the last years revealed that nutrition during infancy and early childhood has important consequences on the risk of developing obesity later. Also, there is a tight connection between the growth velocity during this early period of life and the occurrence of obesity later. Keeping in mind all these arguments, we might say that nutrition during early infancy and, later, in early childhood, may represent the most important tool for the ***prevention of obesity***.

Prevention of obesity

During the early postnatal period, there are a few factors that are very important for the risk of developing overweight later in childhood. Among these, breastfeeding and complementary feeding are mainly involved. [1]

A large number of studies reported to date, have shown that breastfeeding reduces the risk of developing obesity in childhood. In a large meta-analysis including 28 studies and around 300.000 children it was shown that breastfeeding reduced the risk of obesity with 13 %.[2] In another meta-analysis that analysed the consequences of breastfeeding on the mean BMI, a minimum but significant decrease (of 0,04 units) was found in breastfed children. [3]

Other results revealed a 22 % decrease in the risk to develop obesity in breastfed children and a negative correlation between the duration of breastfeeding and the risk of obesity occurrence later. [4]

Complementary feeding is also important for preventing obesity. It is recommended that weaning should start around the age of 6 months, and, most of the studies reveal that the introduction of the complementary feeding between 4 and 6 months of age does not significantly influence the risk for developing obesity. [5] However, some data are showing an increased risk associated with early introduction of the complementary feeding, before the age of 4 months.[1,6]

On the other hand, some studies revealed that later introduction of the complementary feeding seem (s) to be associated with less overweight in the adult (around the fourth decade of life).[7]

Presently, there are numerous evidences that confirm the fact that high protein intake during infancy is associated with a higher risk of obesity later in life. Some authors that compared the weight for height and BMI, at age 1, in infants that have received high protein content formulas compared with those who received lower protein content formula. They have shown that the figures are significantly higher in the first group.[8] Furthermore these differences were still significant at the age of 2 years.[1]

Other data showed that protein intake both during the complementary feeding period and during the transition to the family diet (1-2 years) directly influences the weight gain. The excess of protein intake during this period is an independent predictor of an unfavourable body composition at age 7.[9]

A protein intake representing 15 % of the caloric intake of an infant 9-12 months, seems to be associated with a significantly higher BMI at 6 years of age.[10]

Opposed to the numerous studies concerning protein intake in infancy and its connection with the later overweight is the lipid intake during infancy. There are no studies at the moment, showing that high fat intake during infancy and the second year of life would cause an increased risk of obesity later.[1] On the contrary, in older child and (in) adult, an increased fat intake is associated with an increased risk of obesity later.

Rapid early weight gain from birth to six months is associated with an increased BMI, fat mass percent and obesity in the older child and adolescent.[1, 11]

Some studies that followed the association between weight gain during infancy and obesity in the older child and adolescent, found a positive association between weight gain in the first six months of life and later obesity, but no significant correlation with the weight gain during the introduction of the complementary feeding (6-12 months) was found.[12-14]

Concerning body composition, it was shown that breastfed infants had a higher fat accumulation during the first 6 months of life, compared with formula fed infants, while in the next 6 months (6 months to 1 year of age), breastfed infants accumulated more lean body mass than those fed with formula.[15] Further studies are needed to find the correlation between body composition and the risk for later obesity, as it is shown that breastfed infants have a lower risk for developing obesity later during life.[1]

If, during the early postnatal period, both excessive energy and protein intake have been related to later adiposity, during childhood, the dietary patterns are mainly important for the risk of obesity occurrence.[16]

Overweight appears when persistent positive energy imbalance occurs for long periods of time. In children, it has been shown that this imbalance should be around 100-200 kcal/day to allow obesity to occur. Other recent studies have shown that the energy gap should not exceed 46-72 kcal/day in order to prevent overweight in children.[17]

Once the child is already overweight or obese, the child requires evaluation and management that should be ensured by a team including a paediatric endocrinologist, nurse qualified in nutrition, psychologist and also a sports trainer. Sometimes a multidisciplinary team will be needed because of the associated comorbidities: paediatric cardiologist, ENT specialist, orthopaedist etc. The early the management of obesity is initiated, the better the results.

The Childhood Obesity Task Force (COTF) of the European Association for the Study of Obesity (EASO) recommended for growing children who are overweight or mildly obese, at least **weight maintenance** (and *not* weight loss) which would be sufficient to improve well-being and the metabolic profile.

The management of the overweight/obese child should include four groups of interventions: behavioural interventions, dietary management, physical activity and medication/ surgery (exceptionally).

The behavioural intervention consists in teaching the child and family about the healthy way of living. The family and child should be advised to reduce the time spent by the child in front of television or computer. Actually, it is recommended that in children younger than 2 years, the TV / PC screen exposure should be completely avoided, while in children older than 2, a maximum of 2 hours spent watching the TV or PC screen is accepted.

Dietary management consists in general recommendation on one hand, and specific diet plans, on the other.

The European guidelines (NICE and ESPGHAN) position paper recommended ***general measurements*** for obesity prevention and management.

General recommendations include: to eat minimum five regular meals, including breakfast, and, also, to eat in a pleasant, sociable environment without distractions (such as watching television). Parents and carers should eat with children – with all family members eating the same foods. A minimum of five portions of fruits and vegetables are recommended daily.

Healthy food options should be promoted for snacking (fruits, seeds, cereals). Food portion sizes should be appropriate for age and body size. Fast food meals, fried food and food rich in simple sugars and fat should be avoided as much as possible. Sugar-sweetened beverages, which are significant contributors to energy intake, should also be reduced to the minimum. Plain water should be promoted as the main source of fluids for children.

Some special dietary models have been designed for obese children and tried by different authors, their results being more or less satisfying. These dietary models include:

- Low carbohydrate diet (low CH diet) – CH <20 g/day or <10% of total caloric intake;
- Medium carbohydrate diet (CH= 45–50% of total daily calories);
- Low glycaemic index [medium carbohydrate (45–50% of total daily calories) with a low glycaemic index];
- Energy restricted, low calorie, including low fat (25–31% of total daily calories);
- The “Stoplight diet”

Table 1. - Standard population dietary recommendations [20]

Nutrient/food	Recommendation
Total fat	Reduce to no more than 35% food energy
Saturated fat	Reduce to no more than 11% food energy
Total carbohydrate	Increase to more than 50% food energy
Sugars (added)	Reduce to no more than 11% food energy
Dietary fibre	Increase non-starch polysaccharides to 18 g per day
Salt	Reduce to no more than 6 g salt per day[a]
Fruit and vegetables	Increase to at least five portions of a variety of fruit and vegetables per day

Low carbohydrate diets are not currently recommended for long-term weight loss in children. Few studies compared low-carbohydrate diets (<20 g/d or <10% of total daily calories) with the energy-restricted low-fat diets. All reported a significant ($P < 0.05$) reduction in weight or BMI in the low-carbohydrate group in the short – term (3 months). Another, 9 months study, which compared two similar energy-restricted medium- carbohydrate / high-protein diets (energy intake 54% protein / 15% CHO vs50% protein /19 % CHO), showed no significant difference between the two groups.

Although very-low-carbohydrate diets may have some beneficial effects on risk factors for cardiovascular disease and type 2 diabetes the overall effects of this approach on growth and development are unknown.

Two studies comparing an *ad libitum low-glycaemic index (GI) diet* with an *energy-restricted low-fat diet* showed statistically significant weight or BMI loss at 4 and 12 months, respectively.

In a larger, follow-up study, Ebbeling et al found no difference in weight loss between groups of young adults following a low GI diet and low fat diet. However, the participants with baseline increased markers of insulin resistance, that followed the low GI diet experienced a greater degree of weight loss.[21]

The American Diabetes Association, the North American Association for the Study of Obesity, and the American Society for Clinical Nutrition currently recommend *energy-restricted low-fat diets* (25–35% or less of total calories from fat) for the treatment of obesity, but there is little evidence to support this.

In a study comparing an energy-restricted diet and a less restrictive diet designed for pubertal obese children (the exact composition of these diets is not provided in the original paper), weight loss at 12 months was significantly greater in the energy-restricted group. Another study reported a significantly greater decline on the energy-restricted diet in the percentage of overweight children at 6 months ($P < 0.01$).

Developed in the 1970's by Leonard Epstein, the **Stoplight diet** is designed for younger children (ages 6–12 years) but can also be used as a basic starting point for older patients, as well. The energy goals for Epstein's Stoplight diet range from 900–1300 kcalories/day (ages 6–12) and daily recording/ journaling of all food and drink consumed is an integral component of this intervention.[22]

Green light foods consist of low calorie, high fiber content foods, with no restrictions placed on intake. (e*xamples*: non starchy vegetables, most fresh fruits, skim milk, lean meats, whole grain no sugar added cereal).

Yellow light foods viewed as essential to a healthful diet but due to higher nutrient density should be eaten in moderation. *(examples*: bananas, raisins, dried fruits, sugar added yogurt, sour cream, pasta and rice).

Red light foods are high in fat or simple sugars, limited to no more than one servings per week, should be eaten away from the home or not at all (e*xamples*: ice cream, French fries, candy).[22]

The vegetarian diet is not recommended in children. It is recommended that vegetables should be used as the main food contributors to a well- balanced diet in children, but not exclusively.[19]

When a vegetarian diet is practiced, appropriate planning (taking into account recommended macro- and micronutrient intakes) and monitoring (growth and potentially zinc, iron, vitamin B12, and vitamin D status) should be executed by a health care professional.

Dietary interventions combined with increasing physical activity are essential for weight loss.

Physical activity will be mild to moderate at first, and more vigorous, lately, as the child becomes more trained and has good tolerance to effort. The planned physical activity should last for at least 60 minutes daily.

Medication and surgery are not generally recommended for children younger than 12 years and 16 years, (post pubertal) respectively, but only in exceptional circumstances like severe life-threatening co-morbidities**.**

In order to successfully address the problem of childhood obesity, effective weight management programs must be established to treat the increasing numbers of overweight and obese children and adolescents. An effective program will identify children and adolescents with health risks related to excess fat, help families make permanent healthy lifestyle changes, and provide ongoing care to optimize long-term health. The management should include diet, physical activity and behavioral changes.

Conclusions.

Nutrition even since early childhood is essential to prevent later obesity; dietary interventions should be adapted to age and keeping in mind the fact that the child is continuously growing. Breastfeeding is associated with a lower risk of obesity. There is no evidence that the age of introduction of complementary feeding has an effect on later obesity. It seems that a low protein intake and high fat intake in the first year of life seem to protect against obesity. Energy restricted medium carbohydrate diet is the most efficient and, also healthy diet, that should be used in children. Stoplight diet is also efficient and healthy. Dietary interventions should be combined with physical activity for a better result.

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WHY IS CROHN'S DISEASE DIFFICULT TO TREAT IN PEDIATRICS?

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Abstract

Incidence of Crohn's disease (CD) continues to rise, especially in regions where previously it was not common. Particularly in children, therapy of this condition could be very problematic, since disease presentation and course are more severe than in adults. Nowadays, deep remission (with mucosal - and preferably - transmural healing) represents the major therapeutic goal. Despite enormous progress in development of new drugs, there is yet no cure for CD and no therapy with 100% success. Patients and families should be informed from the beginning about the unpredictability of CD course. Delayed diagnosis could impair a successful therapy, patients presenting with already severe forms of CD. Available medical tools [i.e., exclusive enteral nutrition - EEN, corticoids, anti-tumour-necrosis-factor (TNF) agents] may work well in induction of clinical remission in most patients, however mucosal healing could be obtained especially with EEN and anti-TNF. For prevention of relapses, immunomodulators and anti-TNF could be useful, but long-term deep remission is achieved in less than 50% of patients. Many patients still require surgery and there is no effective therapy to prevent post-surgical recurrence. Most therapies have adverse reactions and benefits and risks should be always discussed with patients and families. Other therapeutic issues that have to be taken into consideration are correction of nutritional deficits, with restoring growth (including bone growth and density) and development, improvement of quality of life and maximization of adherence to therapy. We discuss difficulties in treating CD children, based on the experience of the 2nd Department of Pediatrics, Cluj-Napoca, Romania (1998-2015).

Key words: *delayed diagnosis, deep remission, maintenance of remission, growth delay, quality of life*

Introduction

Crohn's disease (CD) represents a heterogeneous, chronic relapsing inflammatory condition of the gastrointestinal (GI) tract, which causes significant morbidity. Approximately 25% of CD patients are diagnosed under the age of 18 years.[1] There is an increasing incidence of pediatric CD all over the world (2.5 – 11.4%ooo),[1] with an estimated prevalence of 58%ooo.[2] A particular increase in incidence has been detected in developing countries (where previously it was uncommon), as they have Westernized.[3] This was also observed in our clinic, the 2nd Department of Pediatrics from Cluj-Napoca (Romania), first case of CD appearing in 2006 and 34 cases being diagnosed by the beginning of 2015 (with a frequency peak of 9 cases in 2014). Despite tremendous research work all over the world, there is yet no clear etiology and no cure for CD. Particularly in children, therapy of this condition could be very problematic, since disease presentation and course are more severe than in adults,[2,4,5] requiring more aggressive therapy, including surgery,[2,4] while the available therapeutic tools are less numerous than in adults. The actual goals of therapy in CD aim to: induce and maintain deep remission (with mucosal and – recently - transmural healing), avoid complications of the disease (including prevention of structural damage) and of the therapy (avoiding steroids, as much as possible), correct nutritional deficits, with restoring growth (⊃ bone growth and density) and development, improve quality of life and maximize adherence to therapy.[2] Therapy should rely on the recent ECCO/ESPGHAN Consensus Guidelines on management in pediatric CD[2] and should be personalized, according to the peculiarities of each patient, including risk factors.

Difficulties in treating pediatric Crohn's disease patients

There are major problems and pitfalls for both pediatric gastroenterologists (Ped GIs) and patient/families regarding CD therapy. Since all these issues are crucial, they could not be ranked by order of importance.

Before starting therapy, it is mandatory to establish a correct and complete diagnosis: positive diagnosis - CD (Revised Porto criteria),[6] location and behaviour (Paris classification),[7] severity (wPCDAI score)[8] and presence of extraintestinal manifestations and complications (including growth retardation). As there is no universal diagnostic marker yet, according to the Revised Porto Criteria, accurate diagnosis of CD should be based on a combination of history, physical and laboratory examination, esophagogastroduodenoscopy and ileocolonoscopy with histology, and imaging of the small bowel.[6] Ped GIs should be aware of these new criteria, inform patients/families and perform all the required tests. On the other hand, patients and families should agree with all necessary techniques, which is not always easy. Not performing all

tests could lead to misdiagnosis and wrong therapy, with severe consequences. One of our patients had only a partial colonoscopy in another service, being misdiagnosed with ulcerative colitis and treated with low dose of 5-aminosalycilates (5ASA). We diagnosed him with severe CD (including perianal fistula) and under nutrition. Therapy was very challenging, as he responded only to Infliximab (IFX) and repeated surgery. Of the 34 CD cases diagnosed in our clinic, 18% had been previously treated for IBD (9% by surgery), in other services, without any adequate investigation, which is unacceptable.

Delayed diagnosis is another important issue, patients presenting in our service with already severe forms of CD, increasing the risks of complicated course and surgery.[2] This delay impaired a successful therapy that could have been efficacious at the beginning, with severe consequences for both children health and healthcare costs. Of the 34 CD cases, 97% had been previously diagnosed and treated for other diseases, in different services. In our experience, the mean duration of disease until diagnosis was 13 months (mo) (vs. 8.7 in Slovenia)[9] and the median one was 5 mo [vs. 3 in Slovenia [9] and Belgium[10]]. Moreover, 23.5% were diagnosed 24 mo after disease onset, the maximum delay being 86 mo. This last patient was diagnosed only after surgery and found with multiple ileal severe stenoses, requiring extensive resection and, eventually, Adalimumab (ADA) therapy. Another patient had been treated for 4 years (y) with vitamins and other supplements, despite severe digestive symptoms and undernutrition, leading to school abandon. We diagnosed her with severe stenosis of the transverse colon, requiring endoscopic dilation and IFX therapy. Ignoring the possibility of CD by paediatricians could be attributable, in part, to the rarity of CD in our area years ago, but also to a lower age at onset and the absence of family history. There is a definite tendency towards lower onset ages [3,10,11], even if median and mean ages at diagnosis remain around 12-14 y, in our series (median 12, mean 12.4) and recent papers from Scotland [5], Belgium [10], Slovenia [12], France [4], and Poland[13] (with respective median ages of 11.5, 12.5, 13.6, 14 and 14) and from the EUROKIDS registry [11], Poland [13] and Hungary[14] (with respective mean ages of 12.5, 13, and 13.2). Children aged $\leq$ 10 y represented 29.4% in our series, 25.7% in Hungary[14], 20% in EUROKIDS Registry[11] and 13.8% in Slovenia[12]. Paediatricians should be aware of the absence of family history in our area, contrasting with recent data from North-Eastern Slovenia[12], Belgium[10], Central and Western Slovenia[9], EUROKIDS registry [11] and Hungary [14] (respectively, 18.5%, 11.4%, 11%, 11% and 10.1%).

Only a specialized/experienced IBD team (Ped GI, specialist in GI tract imaging, IBD nurse, nutritionist, surgeon, and psychologist) should treat CD children and this is another very tough task in our country. *Taking time and using appropriate language to communicate* with family and patient are essential. Physicians should explain carefully and extensively what the disease means, what impact it will have on the family/patient's life, how unpredictable it is, how it could be treated, what are the possible complications, and risks and benefits of any therapy. Patient and family need to be educated towards a new life and fears should be always taken into consideration. A real dialogue should exist at any time and physicians should listen and negotiate concerns.

The possibility of non-adherence (50-66% in teenagers) should be considered from the beginning, warning patients of the risks they would face. Every effort to enhance adherence should be made by physicians during all follow-up visits.

The actual concept of „treat to target" is another issue: treating to obtain mucosal healing - MH (ideally also with transmural healing - TH), measured by objective biomarkers (endoscopy, fecal calprotectin, C reactive protein, enteral imaging techniques).[15] Both physicians and patients should be aware that absence of symptoms is not enough to ensure a sustained remission and > 50% of patients in clinical remission - CR (10 y after diagnosis) are found with active endoscopic inflammation and structural damage (strictures and/or fistula, detected by imaging) (rev. in 15). Of 20 investigated patients in our clinic, 17 (85%) did not achieve and/or maintain MH and TH, despite clinical remission (wPCDAI < 12.5), requiring therapy escalation (including surgery). In pediatrics, non-invasive methods should be used.

Choosing the right therapy to correct nutritional deficits and restore growth should be a priority. Undernutrition, linear growth deficiency (including bone formation/osteopenia) and delayed puberty are detected in up to 85% of CD children[16].

Weight loss was diagnosed in 97% of our CD patients at presentation and 80% had a Z-score for Body Mass Index (BMI) $\leq$ - 2 standard deviations (SD), contrasting with other recent data (Z-score $\leq$ - 2SD in 25%, in Belgium).[10] Height Z-score $\leq$ - 2 SD was found in 6.5% in Hungary[14] and 8.7% in Belgium[10], contrasting with 26.5% in our series. Height velocity impairment represents the best indicator of a damaged growth.

The absence of a universal therapy, with 100% success represents also a major difficulty and available tools are less numerous than in adults. *Choosing the right therapy for the right patient to induce remission, to act fast and strong,* before severe structural damage appears is another major issue. Despite their wide use (Table 1), 5ASA alone are not able to induce CR (except in very mild colonic cases) and MH; therefore, 5ASA should not be used.[2] Moreover, the high number of pills to be taken decreases adherence. One of our patients had been diagnosed in another service with ileal CD and treated with only 5ASA, despite of severe lesions. We detected a severe ileal stenosis, finally requiring resection. Of the efficacious tools, EEN (Table 1) is recommended as first line therapy for active luminal CD, over 6 to 8 weeks, having a safety profile and many benefits, including restoring growth and bone health.[2] Recent studies, described in the pediatric ECCO/ESPGHAN guidelines have shown CR rates up to 85%, with MH in 19-75% of patients,[2] but complete TH in only 21%,[17] as in our cases. Efficacy of EEN tends to decrease with the second course.[2] Usually, similar rates of CR are induced with both EEN and corticosteroids (CS),[18] but MH is significant with EEN vs. steroids: 74 vs. 33% (rev. in 2). Moreover, CS should not be used in malnutrition, severe osteopenia, presence of fistulas and possibility of surgery in the near future (with high risk of severe complications). In a very recent US study, EEN vs. CS showed more positive effects over time via quicker induction of CR, significant changes in linear growth, and

avoidance of CS over a 3-y follow-up period.[19] Despite many severe adverse reactions (AR) (including reducing height and affecting body image), pill steroids are preferred by some children, who find it hard to follow an EEN (Table 1). In patients with mild or moderate active ileal (and/or ascending colonic) CD, budesonide may be an alternative to prednisone.[2] However, in one of our patients, budesonide worsened the ileal inflammation. If factors for poor outcome are present [i.e. pan-enteric disease, stenosis or fistulae at onset, severe perianal disease, deep colonic ulcerations on endoscopy, persistent severe disease despite adequate induction therapy (including need for steroids at diagnosis), marked growth retardation (> -2.5 height Z-scores) and severe osteoporosis] or there are severe extraintestinal manifestations, anti-TNF should be administered early [2] (Table 1). Anti-TNF should be used as primary induction therapy in active perianal fistulising disease in combination with appropriate surgical intervention.[2] Anti-TNF agents induce CR in about two thirds of patients. The major benefits of both IFX and ADA have been documented in a plethora of large trials[2], including: MH, steroid sparring effects, improvement of anthropometric parameters,[20] bone density and structure, and muscle area Z–scores, [21] improvement of quality of life and decrease in hospitalizations and surgery.[2] A top-down approach to medical therapy is increasingly being adopted for patients with risk factors for severe inflammation or an unfavourable disease course in an attempt to halt the inflammatory process as early as possible, prevent complications and induce MH. A recent study showed that fecal calprotectin ≤ 250 μg/g was achieved in 45% with EEN and 62% with anti-TNF, but only EEN significantly improved the quality of life in the body image subgroup.[22] AR that could appear during induction include infusion reactions and severe infections.[2] In mild to moderate luminal inflammatory CD, azithromycin and rifaximin may be of benefit.[2] Surgery (especially limited resections) could be useful in achieving CR and improving growth[2] (Table 1), however the post-surgical recurrence cannot be prevented, with cumulative clinical recurrence rates at 1, 5 and 10 y of 50%, 73% and 77%, respectively.[23]

Table 1. Treatment of children with Crohn's disease at diagnosis and during follow-up (%).

Author, Year, Country	EEN	5ASA	CS	ABi	IMD	Anti-TNF	Surgery	Time
Vernier-Marsouille, 2008, France[4]	*	93	85	*	61	IFX 24	44	F-u
De Greef, 2013, Belgium[10]	8	41	65	25	43	IFX and ADA 1	17	Dg
Muller, 2013, Hungary[14]	*	88	69	36	**42.5	*	*	Dg (< 3 mo)
	2.5	87	19	5.5	**55	IFX 14	11.3	F-u (1 y)
Urlep, 2014, Slovenia [12]	41.6	48	46.2	*	**47.7	0	*	Dg (< 1 mo)
	64.7	89	75.5	*	**80.1	IFX 36.9, ADA 13.9	29	F-u (> 2 y)
2nd Department of Pediatrics, 1998-2015, Cluj-Napoca	47	70.6	67.6	67.6	**70.6	IFX 5.9	20.6	Dg (< 3 mo)
	82.3	53	29.4	14.7	**70.6	IFX 5.9	38.2	F-u (2.2 y)

EEN – exclusive enteral nutrition; 5ASA – 5-aminosalicylates; CS – corticosteroids; ABi – antibiotics; IMD - azathioprine/6-mercaptoputine and/or methotrexate; IFX - infliximab; ADA - adalimumab; * - not reported; ** - only thiopurines; mo – month; y – year; Dg – diagnosis; F-u – follow-up;

Thinking of maintenance medication when starting induction is another important task, as there are not many options and none assures a sustained long-term MH in more than 50% of patients. Timing of introduction and choosing the right medication are crucial. Since maintaining remission with CS or 5ASA is not recommended, the main options are immunomodulators (IMD) [i.e. azathioprine (AZA) and methotrexate (MTX)] and anti-TNF. Again, benefits and risks of these therapies have to be carefully discussed. CD could lead to complications, cancer and death, without a pertinent therapy, however medication could lead to adverse reactions, also including cancer and death. Either AZA or subcutaneous MTX are recommended as an option for maintenance of steroid free remission in children at risk for adverse outcome.[2] AZA should be introduced from the beginning, given its long latency period and, ideally, only after testing thiopurine methyltransferase genotype or enzymatic activity. CR is maintained in 49-60% of children after 1 year, however dosing its metabolites (which is not available in our country) could aid in optimizing therapy and determine possible toxicity and compliance. Long-term severe risks with AZA are especially infections and cancers (4-times increased risks of non-Hodgkin's lymphoma and of non-melanoma skin cancer).[2] MTX does not carry clear risks for cancer, however its efficacy appears lower than AZA. In a very recent study in thiopurine-resistant CD, MTX maintained CR in 44%, also with MH, after a median use of 2.9 y.[24]. In a large US study, MTX used as a first IMD provided sustained CR ≥ 1 y in 27%, while as a second IMD – in 35%, with minimal toxicity.[25] AntiTNF are recommended in children failing IMD (step up approach) and in those with risk factors for poor outcome (top down therapy).[2] The main issue impairing their efficacy in maintaining remission is the secondary loss of response (LOR), appearing in over 50% of CD patients.[26] AR associated with long-term anti-TNF use include severe infections, lymphoma and melanoma. The fear of fatal hepatosplenic T-cell lymphoma (HSTCL), especially in young male, has precluded the use of anti-TNF, also based on the family non-acceptance. However, as of yet, of the 40 HSTCL cases (half treated only with long-term thiopurines and half of them with combined thiopurines and anti-TNF), there was no reported patient with anti-TNF monotherapy.[27] Even if adult studies clearly showed a better efficacy (by lowering immunogenicity) in patients treated with AZA and anti-TNF, in children this association remained controversial. However, the most recent US multicentre study proved that concomitant treatment with an IMD for > 6 months after starting IFX increases the chances that

patients will remain on IFX. In boys, MTX appears to increase the durability of IFX therapy compared with thiopurines.[28] Special strategies should be used in case of LOR. Dosing levels of anti-TNF and of antibodies to anti-TNF may facilitate decision-making whether to optimize, to switch to another agent or to stop therapy.[2] *Providing a sustained psychological support for these children* appears also mandatory, in order to improve their quality of life.[2]

In conclusion, treating a child with CD involves a specialized team and requires many skills. Objective markers will allow a personalized therapeutical approach, with a better control of the disease (identifying CD risk profile, factors to predict response, selection of optimal therapy and factors to predict risk of toxicity).

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DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF VERY EARLY ONSET INFLAMMATORY BOWEL DISEASE

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Abstract

The relation between the age at diagnosis and the evolution of pediatric inflammatory bowel disease (IBD) is not well known. However it is obvious that IBD has particular characteristics in pediatric patients. For the first time, Montreal classification delimited patients whose disease started under age 17, as a distinct group of patients – pediatric IBD. Recently there were proposed IBD subgroups according to age: very early onset IBD - (<6 years), infantile IBD (<2 years) and neonatal IBD (<28 days). An increasing incidence of IBD in pediatric patients was noticed; affected children are increasingly younger.

Over 163 associated genetic loci have been identified. This could be explained by a particular genetic predisposition to develop IBD, and also by changes in environmental factors that initiate the disease. There are at least 50 monogenic defects responsible for an IBD-like pathology.

Patient history, physical examination, endoscopic investigations, imaging, are required to establish the diagnosis of IBD. Gastrointestinal infections are frequent in pediatric patients and are excluded by stool culture and virology tests. Cow's milk protein allergy and celiac disease should be excluded. Several studies reported an increasing incidence of very early onset-IBD, frequent pancolonic ulcerative colitis, children presenting with severe ulcerative colitis disease activity

Very early onset IBD has a severe prognosis and often needs an aggressive therapeutic approach.

Keywords: *very early onset inflammatory bowel disease, ulcerative colitis, Crohn's disease, immunodeficiency, genetics, diagnosis, treatment*

Introduction

Inflammatory bowel disease (IBD) develops during childhood in 25% of patients. The relation between the age of onset and the phenotype of pediatric IBD is incompletely known. However it is obvious that IBD has particular characteristics in pediatric patients.

For the first time, Montreal classification delimited patients whose disease started under age 17, as a distinct group of patients – pediatric IBD. Paris classification has defined a group of patients with early onset IBD, under the age of 10. Recently there were proposed more IBD subgroups according to age: very early onset inflammatory bowel disease (VEOIBD) (<6 years), infantile (and toddler) IBD (<2 years) and neonatal IBD (<28 days).[1]

An increasing incidence of both ulcerative colitis (UC) and Crohn's disease (CD) in pediatric patients was noticed; affected children are increasingly younger.

A report which included 1370 patients from six centers in North America found that in 15% of the patients, IBD presented before the age of 6.[2] A population-based retrospective cohort study of the Canadian children diagnosed with IBD, showed that the incidence increased by 7.4% per year among children younger than 6 years old.[3]

Increasing incidence could be explained by a particular genetic predisposition to develop IBD, and also by changes in environmental factors that initiate the disease. Intestinal flora changes, resulting from the change in dietary habits associated with sterile living conditions, have increased the risk of IBD.[4]

Monogenic forms of IBD

The genetics of IBD is complex and incompletely elucidated. Over 163 associated genetic loci have been identified but most of them have a small genetic contribution;[5] these variants commonly implicated in IBD pathogenesis account for only 25% of the heritability.

A high-density Immunochip genotyping was performed on 1008 pediatric patients with IBD (801 children with CD, 121 children with UC and 86 children with IBD undetermined) and could not identify additional common variants for early onset IBD. The researchers concluded that with the exception of infantile IBD, no overt differences in genetic susceptibility have been identified.[6]

More than 50 monogenic defects responsible for IBD or IBD-like pathology were found. Three-fourths of these genetic defects are associated with primary immunodeficiency: neutrophil (and other phagocyte) dysfunctions, various defects of adaptive immunity (including regulatory T cells and anti-inflammatory cytokines) and innate immunity.[5]

Primary immunodeficiency is especially suspected in young children with IBD associated with high susceptibility to infections and the presence of other autoimmune and/or inflammatory disorder.

Congenital defects of phagocytes

Chronic granulomatous disease (CGD) is caused by defects of respiratory burst; it is characterized by the innability of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme (from patient's phagocytes) to produce superoxide. It is caused by mutations in genes that encode the subunits of the NADPH oxidase:

a. X-linked CGD - Mutation in *CYBB*
b. autosomal recessive CGD – p22 phox deficiency - Mutation in *CYBA*
c. autosomal recessive CGD – p47 phox deficiency - Mutation in *NCF1*
d. autosomal recessive CGD – p67 phox deficiency - Mutation in *NCF2*
e. autosomal recessive CGD – p40 phox deficiency - Mutation in *NCF4*

Nearly half of the patients had at least one episode of gastrointestinal symptoms. Clinical manifestations (diarrhea, perianal ulcerations, bowel obstruction, and malnutrition) are suggestive for CD. Histology reveals multiple granulomas which resemble CD but the presence of large pigment-laden histiocytes distinguishes CGD-associated enterocolitis from CD.

Defects in T- and B-cell function

IBD-like immunopathology was reported in patients with B-cell defects such as common variable immunodeficiency, hyper-immunoglobulin M syndrome, and agammaglobulinemia.[7]

VEOIBD has been described in children with atypical severe combined immunodeficiency (caused by hypomorphic mutations in multiple genes), such as Omenn syndrome –(hypomorphic mutations in the RAG1or the RAG2 gene).[5]

Wiskott– Aldrich syndrome is caused by the absence or abnormal expression of the WAS protein that regulates the actin cytoskeleton; it is characterized by microthrombocytopenia, immunodeficiency, autoimmunity, and susceptibility to malignancies. A study performed in France showed that 9% of the patients with Wiskott– Aldrich syndrome developed IBD.[8]

Patients with XIAP (X-linked inhibitor of apoptosis) deficiency have the risk to develop life-threatening hemophagocytic lymphohistiocytosis and also IBD. The XIAP protein has an important role in the proinflammatory response; XIAP deficiency was involved in colitis and CD-like illness.[9] Patients with XIAP deficiency develop a severe IBD, with poor response to treatment. This disorder can be tested by a flow cytometry-based assay that measures expression of the XIAP protein on white cells. An allogeneic hematopoietic progenitor cell transplant is required in order to prevent the development of life-threatening hemophagocytic lymphohistiocytosis.

Autoinflammatory disorders are characterized by abnormally increased inflammation, determined by the cells and molecules of the innate immune system.

Autoinflammatory disorders recognized as being associated with VEOIBD are:

- defects effecting the inflammasome include familial mediterranean fever - Mutations of MEFV (lead to gain of pyrin function, resulting in inappropriate IL-1β release)
- non inflammasome-related conditions include: mutations in interleukin-10 (IL-10) results in increase many proinflammatory cytokines, mutations in interleukin-10 receptor A (IL-10RA), mutations in interleukin-10 receptor B (IL-10RB).[10,11]

Mutations in IL-10RA was detected especially in infants and todlers with IBD; a recent study reported that half of the children with infantile-onset IBD had IL-10RA mutations.[12]

IL-10 is an anti-inflammatory cytokine and has a crucial role in maintaining immune homeostasis of the bowel. IL-10 signals through a receptor complex consisting of two α subunits (encoded by IL-10RA) and two β subunits (encoded by IL10RB). Mutations in genes encoding the IL-10RA and IL-10RB proteins abrogate IL-10–mediated immunomodulatory signaling and are responsible of the hyperinflammation of the intestine. IL-10 suppresses the production of pro-inflammatory cytokines by monocytes.[13]

Children with IBD and mutations affecting the IL-10 receptor have a high production of cytokines including TNFα and a severe disease course with perianal fistulas, growth failure. The disease is refractory to medical therapy. More intensive therapy is required, but there are frequent relapses.[14, 15]

Interleukin-21 deficiency was also diagnosed in children with VEOIBD and defects in B-cell development similar to those found in common variable immunodeficiency.[16]

IPEX is characterized by immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; it is caused by mutations in FOXP3 gene[17] which affect regulatory T cells and cause autoimmunity, immunodeficiency and also enteropathy. Recently Okou et al. [18] reported a family in whom a novel c.694A>C mutation in the *FOXP3* gene was idenfied. This novel mutation in FOXP3 abrogates the suppressive function of T regulatory cells and causes an atypical IBD-like phenotype.

Clinical characteristic and diagnosis of VEOIBD

UC is more frequent in VEOIBD [19, 22] and CD is often diagnosed in older children.

The study performed in Grigore Alexandrescu Emergency Children's Hospital, Bucharest included 41 patients diagnosed with IBD between January 2004 and December 2013. We found an increased frequency of cases with VEOIBD – 14 (34%) cases.[20] Most of the patients under 6 years (71%) were diagnosed with UC.

Several studies support the suggestion that IBD phenotype differs in very early-onset disease. Children with VEOIBD may have a mild disease with isolated colitis and bloody stools whether Crohn disease, ulcerative colitis, or indeterminate colitis. [21, 22]

The patients with very early onset CD have perianal disease and a severe inflammatory disease presentation. In a cohort of 1928 children with IBD from multiple centers of North America, 112 children with VEOIBD (<5 years) were identified: children with very early onset CD had a mild disease and presented with a colonic phenotype. The presenting colon phenotype changed to ileo-colonic phenotype at 6-10 years Five years post-diagnosis, disease activity was similar regardless of patient age at onset.[21] A more frequent isolated colonic and upper gastrointestinal involvement were noticed in a cohort of patients with CD diagnosed in Italy. [19]

Very early onset UC is characterized by an extensive disease; proctitis is rarely described. A report which included 30 Australian children diagnosed with VEOIBD revealed that UC was characterized by less abdominal pain at presentation, but an aggressive course with and a significant reduction in weight-for-age. [22]

If only consider infantile IBD, it is found that it has a poor prognosis. Cannioto et al. [23] describe o group of 16 patients under 2 years old with a very severe IBD course and high mortality (3 patients died). Almost all infants with IBD have chronic diarrhea; a high proportion of very young patients have hematochezia, perianal disease and more severe extensive disease.

A significant proportion of children with VEOIBD have malnutrition. Growth failure is more severe at diagnosis in CD patients than in UC patients. It is known that pre-pubertal onset of IBD (especially CD) is a risk for lower final height.

Medical history, physical examination, laboratory tests (for anemia and inflammatory markers), imaging, upper and lower gastrointestinal endoscopy with histological analysis should be performed in order to establish the diagnosis of IBD; it is also important to evaluate disease behavior and locations as well as disease activity. We have to classify the disease status according to the Paris Classification.[24]

Investigations are also done to rule out certain diseases that are more common in this age, such as gastrointestinal infections (caused by Salmonella, Shigella, Yersinia, Campylobacter, Cytomegalovirus, Clostridium difficile, HIV), cow's milk protein allergy, celiac disease.

The diagnosis of monogenic IBD is difficult in clinical practice. Functional screening is followed by genetic confirmation;[5] whole-exome sequencing is recommended.[25]

Therapeutic management

Children with VEOIBD often need aggresive treatment strategies. Frequently, VEOIBD is severe at dignosis and treatment with corticosteroids is required. In steroid dependent patients or in patients who fail to respond to corticosteroids, immunnossupressive agents are used and sometimes decrease the need for surgery.

The recently described North American cohort included a high percentage of children 1 to 5-years-old with CD who received corticosteroids and methotrexate and a high percentage of children 1 to 5-years-old with UC who received mesalamine and thiopurine immunomodulators. [21]

The group of children diagnosed with VEOIBD in Australia often required immunosuppresive treatment, and surgery. [22]

Cannioto et al have remarked that patients with infantile IBD needed multidrug, immunosuppressive approach; 25% received total parenteral nutrition and 12.5% received colectomy

A molecular diagnosis became an important stage in the diagnosis of all cases of therapy-refractory VEOIBD because it has a significant impact on patient management. Hematopoietic stem cell transplantation has been proposed as a curative treatment in patients with severe VEOIBD and IL-10 deficiency; it was performed in several cases and clinical remission was achieved in the majority of patients.[26]

Conclusion

More than 50 monogenic defects responsible for VEOIBD or IBD-like pathology were identified and the majority is associated with primary immunodeficiency.

IBD phenotype differs in very early-onset disease and children with VEOIBD often need aggresive treatment, early surgery. Hematopoietic stem cell transplantation has been performed in certain monogenic defects responsible for VEOIBD.

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FROM PNEUMONIA TO RETROCECAL APPENDICITIS: AN INTRICATE PATH TO THE CORRECT DIAGNOSIS

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Abstract

Introduction: Acute appendicitis is one of the most common surgical pathologies encountered in the pediatric field with complex clinical symptoms that can mimic a wide variety of diseases, so errors in diagnosis are common. Objective: To present the diagnostic challenges in the case of a 5 year old boy admitted to our hospital with a suspected right medio-basal pneumonia. Case Report: The patient didn't have a significant history. He presented with high fever for over a week, irritating cough mostly during the evening, apathy, adynamia and mid-abdominal pain. Biological investigations showed neutrophilic leukocytosis and marked inflammatory syndrome. Findings during the physical included: influenced general condition, left thoracic paravertebral muscle contracture, decreased right medio-basal vesicular murmur and left medio-basal crackles on auscultation. The chest X-Ray revealed an accentuated bilateral interstitial pattern, while the abdominal ultrasound identified a liver tumor. Computed abdominal tomography established the presence of a liver abscess, presenting a communication with the lumen of the ascending colon, which was highly distended. The presence of a calcified deposit is also noted at the level of the cecum. The patient was transferred to the pediatric surgery clinic where laparotomy, appendectomy and subhepatic abscess drainage were performed. The definitive diagnosis was: perforated acute retrocecal appendicitis and subhepatic abcess. The evolution was favorable and the patient was discharged 2 weeks after the surgery. Conclusions: The nonspecific onset (no vomiting, initial mid-abdominal pain, which became progressively generalized and the presence of intestinal transit) mad the final diagnosis truly challenging and could have negatively affected the final outcome.
Keywords: *hepatic abscess, retrocecal appendicitis*

Introduction

Acute appendicitis is the most common abdominal surgical emergency. The determining cause of acute appendicitis is microbial infection. The predominant mechanism triggering this condition is lumen obstruction. The causes of obstruction are usually coprolites, edema and lymphoid tissue hypertrophy, various foreign bodies, vegetable and fruit seeds, intestinal parasites.

Case report. L.Daniel, a 5 years old male is hospitalized in the Pediatric Clinic II between April 8th to 10th 2015.
The patient's history reveals high fever for about a week (T = 39^0 C), pallor, apathy, moderately irritating cough, diffuse abdominal pain. The personal pathological history was unremarkable. The personal physiological history - vaginal birth, gesta VI, para III, born prematurely, 28 weeks, BW - 2000g. During the physical examination we noted his weight=15kg. He was afebrile, with decreased appetite, had bilateral eyelid edema, bilateral angular cheilitis, global underrepresented subcutaneous tissue, cold extremities, bilateral cervical lymphadenopathy, left paravertebral muscle contracture. The lung examination showed a present vesicular murmur, decreased on the left side medial and basal. On auscultation crackles were found in the left medio-basal lung region. The abdomen was slightly distended, pliable diffusely sensitive with a present intestinal transit. The liver, spleen and kidneys were impalpable. The urine was normal in color. There were no signs of intra-cranial hypertension (ICH) or meningeal involvement. He showed no signs of ear or sinus were pain.

The biological investigations revealed leukocytosis with neutrophilia and marked inflammatory syndrome (Table 1).

Table 1. Biological investigations

8.04.2015	8.04.2015	9.04.2015	9.04.2015
WBC = 23.58/ul ↑ Ne = 75.7% ↑	ALT = 35 U/L AST = 31 U/L	WBC = 22.49/ul↑ Ne = 73.8% ↑	Urea = 11 mg/dl↓
Ly = 12.4% ↓	GGT = 123 U/L ↑	Ly = 12.4%	Creatinine = 0.5mg/dl↓
RBC= 3,13 mil/ul ↓	ALP = 233 U/L ↑	RBC = 3,35 mil/ul	Ferritin = 398mg/dl↓
Hb = 7.7g/dl ↓	SI = 8 ug/dl ↓	Hb = 8.2g/dl↓	PLT = 305.000/ul↑
Ht = 25.3% ↓	LDH=231U/L↑	Ht = 26.9%	ESR = 103mm/h

HEM = 24.6 pg ↓	Eo =2.7% ↓	HEM = 24.5 pg↓	AFP=0.0 ng/ml
CHEM = 30.4g/dl ↓	Urine Exam - normal	CHEM = 30.5g/dl↓	
PLT = 576.000/ul ↑	Urine culture – sterile		
ESR = 121mm/1h↑	CRP = 191mg/l↑		

The pulmonary X-ray showed bilateral interstitial inflammation.

On the second day of hospitalization a pneumology consult is requested, excluding a respiratory condition. On the same day an abdominal ultrasound is performed and a homogeneous mass is found in the liver (Fig. 1.). The oncological consult revealed: hepatomegaly; anemic syndrome; inflammatory syndrome; pneumonia. Recommandations: Vanilmandelic acid (VMA), alpha fetal protein (AFP), neuron-specific enolase (NSE), abdominal MRI and chest CT.

The evolution was not favorable: the abdominal pain had intensified becoming the main symptom. The patient also presented two febrile episodes without vomiting, with 1-2 loose stools/ day. On the third day of hospitalization a thoracic CT is performed, showing no mediastinal, pulmonary or parietal lesions. An abdominal CT was also performed, showing a highly compressive liver abscess (Fig. 2.). The patient was transferred to the Pediatric Surgery Clinic. A median supraombilical laparotomy was performed with appendectomy and evacuation of the subhepatic abscess. The postoperative evolution was favorable. Two weeks after surgery the patient was discharged.

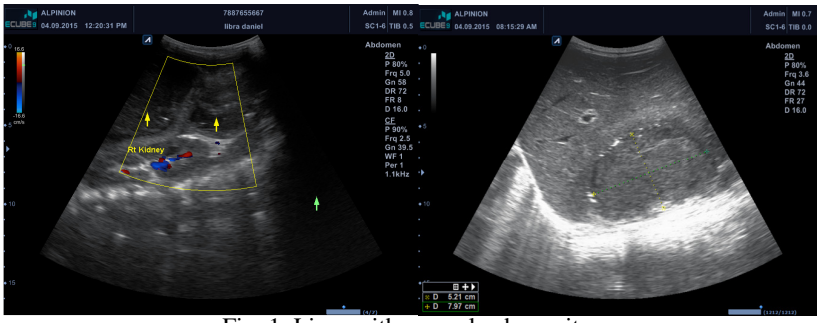

Fig. 1. Liver with normal echogenity.

Relatively well-defined, right hepatic lobe mass of around 5.7 / 5.2cm, maximum 5.2 / 7.97cm with homogeneous content. Portal vein= 0.7cm in the hilum, gallbladder – transonic, normal walls. Spleen -normal structure and dimensions (9.9 / 1cm). Normal urinary tract. Conclusion: Suspicion of hepatocellular carcinoma (HCC). Recommendations: ultrasound reevaluation, complex biological assay and oncological evaluation.

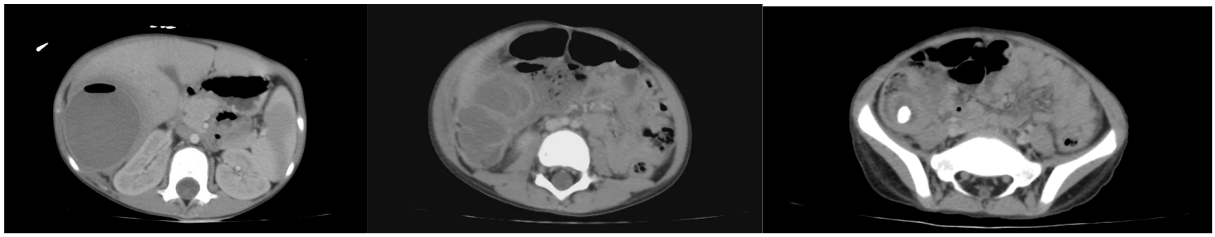

Fig. 2. Abdominal CT.

Highly compressive right liver abscess (8cm / 6cm / 8cm). The lesion is communicating with the lumen of the ascending colon forming a fistula with a complex path. A calcar formation (possibly an ingested foreign body) of 20 / 15mm is seen at the level of the caecum. The ascending colon is markedly distended (typhlitis).

Table 2. Differential Diagnosis of hepatic masses (selection)

Hepatic abcess	- insidious onset: chills, fever, nausea and vomiting, loss of appetite, weight loss, diarrhea and right upper abdominal pain / tenderness - increase of ALP and liver transaminases - radiological: ascending of the right hemidiaphragm due to liver purulent collection.
Hepatocarcinoma	- fatigue and weight loss, occurs in children under age 2 - increased transaminases, hepatomegaly - hepatitis B - right upper quadrant abdominal pain - modified alkaline phosphatase - increased AFP

Hepatoblastoma	- the most common malignant liver tumor in children - liver tumor formation - large tumor mass, well defined, multi nodular with areas of necrosis and cavities - AFP ↓↓/↑↑
Neuroblastoma	- increase in size of the abdomen: hard abdominal mass, firm surface with irregular edges, painless - cord compression → neurological manifestations, sphincter disorders - prolonged febrile syndrome - LDH, feritin- increased - NSE, VMA, - increased

Discussions

The retrocecal appendicitis diagnosis is elusive. The differential diagnosis may vary from mesenteric lymphadenitis to various renal and hepatic tumor formations (Table 2)

In 15% of cases the appendix is located in the retrocecal area and symptoms can mimic biliary and renal pathology. The pain is sometimes posterior, lumbar and the patient may present antalgic flexion of the thigh. In evolution, when an abscess is formed, it may disseminate to the subhepatic region.

The appendicolith found on the CT examination of the abdomen (20 / 15mm) is a calcified deposit of feces inside the appendix. It can also occur in asymptomatic patients and has an increased incidence in appendicitis with retrocecal localization. Perforation is more frequent in patients with appendicoliths.

Conclusions

The nonspecific onset (no vomiting, initial mid-abdominal pain, which became progressively generalized and the presence of intestinal transit) mad the final diagnosis truly challenging and could have negatively affected the final outcome.

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RECURRENT ACUTE HEPATITIS INDUCED BY ALBENDAZOL – CASE REPORT

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Abstract

Albendazole is a broad spectrum anthelmintic drug metabolized in the liver. It, like all drugs, can cause changes in liver function tests, envolving to severe acute liver failure. Albendazole induced toxic hepatitis is rarely reported in pediatric pathology. We present a 15 years old teenager, rural, interned in Pediatrics Clinic II Timisoara in March 2015, for a biological picture of acute liver failure of unknown etiology, with onset at 14 days after the first dose of Albendazole. The teenager had a history (2010) of cytolytic syndrome to the empirical administration of albendazole. Based on medical history, clinical and laboratory examinations, the normalization of liver biochemical profile after stopping albendazol and liver failure treatment, using Scala CIOMS / RUCAM (Roussel Uclaf Causality Asseement Method of the Council for International Organization of Medical Science) proposed to establish relationships causal link between drugs and liver damage (score = 13 - "defined or very likely" to score> 8) was diagnosed of acute hepatitis induced toxic albendazol. This case draws attention to the fact that we, as clinicians, need to be aware of these rare but significant side effects occurred after the administration of drugs often consumed empirically.

***Key words**: toxic hepatitis, child, albendazol*

Introduction

Albendazole binds to parasite's tubulin inhibiting its glucose absorption. Its common adverse effects are nausea, vomiting, constipation, thirst, dizziness, headache, hair loss and pruritus. Although mainly metabolized in the liver, abnormal liver function tests were a rare adverse effect during clinical trials.

Case report.

T.L.M., 15 years old , male, was transferred in March 2015 from Drobeta Turnu Severin Hospital to Pediatrics Clinic II Timisoara, for a biological picture of acute liver failure manifested by hepatic cytolysis with elevated TGO, TGP and impaired blood values, with onset at 14 days after single dose of Albendazole. On 2010 the teenager was interned in our clinic for another cytolytic syndrome (TGP = 1000 / L), after empirical administration of one dose of albendazol, which had resolved after stopping the drog. Family history is non significant for the underlying disease. The discharge from our hospital in 2010 conducted periodically (quarterly) analyzes that inspection can detect normal liver function. Last inspection carried out in February 2015 (TGO, TGP). *Physical examination*: At admission, the patient had influenced general condition, G = 58 kg, T = 159 cm, BMI = 22.9 kg / m2, the skin and mucous are normal color, the liver was palpable 2cm below the costal margin, spleen was not palpable, and he had dark urine.

Table 1. Temporal profile of liver biochemistry.

	Drobeta Turnu Severin (12.02.2015) – control	Drobeta Turnu Severin (08.03.2015)	Timisoara (09.03.2015)	Timisoara (13.03.2015)	Timisoara (30.03.2015)
TGP (U/L)	16	1626.3	**1809**	**867**	**150**
TGO (U/L)	11	2456.4	**3530**	**407**	29
PT (s)		25.6	**21.7**	**18**	11,6
INR		2.41	**1.85**	**1,5**	1,05
APTT(s)		45.3	30.8	30	31,1

BD		1.05	**2.2**	**0,5**	
BT		1.84	**3.8**	**3,09**	0,9
GGT		94.35	**174**	**140**	
FAL (U/L)		302	**270**	**189**	
LDH (U/L)			**1101**	177	152

Paraclinical exams reveals: *hepatic cytolysis* : TGO↑, TGP↑, LDH↑, *proteopriv syndrome*: albumin ↓, INR ↑(1.85), serum cholinesterase N (8216U/L), *excretion syndrome:* BT↑, BD↑, FAL↑, GGT↑, *mesenchymal inflammation syndrome*: gamaglobulines ↑, renal function: urea, creatinine normal, HAV - Ac IgM anti v. hepatitic A negative, HEV - Ac IgM anti v. hepatitic E negative , hepatitis B at onset - Ac IgM HBC – negative, Hepatitis C – Ac HCV negative ,v.Epstein Bar - Ac anti v. Epstein Bar VCA IgM – negative, citomegalovirus – IgM CMV – negative , IgG CMV – positive. During the episode of hepatitis his serum ceruloplasmin level, anti-transglutaminaza IgA, alfa 1 antitripsina, alfa feto-proteina level were normal. His ANA, Sm, LKM antibodies tests were negative Ultrasound, MRI : without structural changes of the liver and abdominal or pelvic lymphnodes.

Based on medical history, clinical and laboratory examinations, the normalization of liver biochemical profile after stopping albendazol and liver failure treatment, using Scala CIOMS / RUCAM (Roussel Uclaf Causality Asseement Method of the Council for International Organization of Medical Science) proposed to establish relationships causal link between drugs and liver damage (score = 13 - "defined or very likely" to score> 8) was diagnosed of acute hepatitis induced toxic albendazol.

Scala CIOMS / RUCAM (Roussel Uclaf Causality Asseement Method of the Council for International Organization of Medical Science) was proposed to establish a causal relationship between the drug and liver damage. Scala CIOMS / RUCAM involves a scoring system that classifies suspicion in: "Definite or likely" (score> 8),"Likely" (score 6-8), "Possible" (score 3-5), "Unlikely" (score 1-2),"Excluded" (score ≤ 0).

Notification shall be made according to the following parameters:

Table 2. Score CIOMS/RUCAM

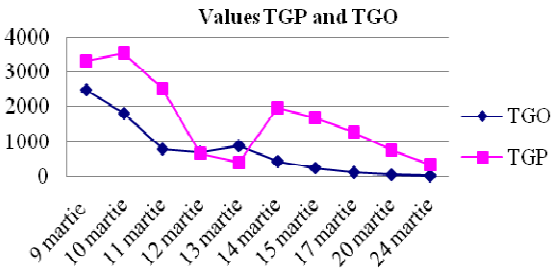
Type of liver injury	Hepatocellular		Cholestasis / Mixed		Score	Score
Time passed since the beginning of the event	First exposure	Second exposure	First exposure	Second exposure	-	-
Time passed since ingestion of the medicine to symptoms	5-90 days	1-5 days	5-90 days	1-90 days	+2	**+2**
	>5 and <90 days	>15 days	<=30 days	<= 30 days	+1	
Time passed since the stopping drug to relieve symptoms	<=15 days	<=15 days	<=30 days	<=30 days	+1	**+1**
Risk Factors	Alcohol		Alcohol or pregnancy		+1	
	Age > 55 years		Age >55 years		+1	
Evolution	>50% improvement in 8 days		-		+3	**+3**
	>50% improvement in 30 days		>50% improvement in 180 days		+2	**+2**
	-		<50% improvement in 180 days		+1	
	Stationary status		Stationary status		0	
	progress negative or improvement <50% in 30 days		-		-1	

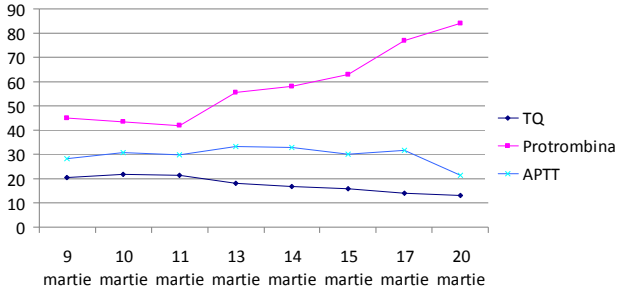
Treatement

- Bed rest, 24h / 24h
- Diet: stopping fat and protein in the first days, only intake of carbohydrates from fruits and vegetables, the scheme was subsequently adapted by evolution
- Medications: vitamin K, inj im 2x 1 f / day, Plasma, 2 units , Ranitidine (to prevent gastrointestinal bleeding), Aspatofort , inj iv 1f / day , Tiosen, Liv 52, 2x1cp / day,- Arginine sorbitol, 1 fl / day, corticosteroid (anti-inflammatory, immunosuppressive, nonspecific antitoxic): iv inj HSH 3x100mg / day with subsequent reduction of the dose.
    - In evolution the level of TGO, TGP were decreased, as well as INR, BT, BD.

Table 3. Score CIOMS/RUCAM

Concomitant Therapy	Time incompatible onset	0	
	Time of onset after ingestion of the drug with unknown side effects	-1	
	Time of onset after ingestion of the drug with known side effects	-2	**-2**
	The role shown in this case	-3	
	No information available	0	
Exclusion of other causes	Excluse	+2	**+2**
	Possible	-2	
	Probable	-3	
Information - known hepatotoxicity	Unknown side effects	0	
	Published side effects, but insignificant	+1	
	Side effects listed on the package leaflet	+2	**+2**
Response to repeated administration	Positive	+3	**+3**
	Compatible	+1	
	Negative	-2	





Discussion

Our patient had hepatitis recurring each time after a single dose of albendazole.

The exact mechanism of albendazole-induced hepatitis is not known. Our patient had symptom onset within 14 days of drug ingestion, hence a possibility of immune-mediated injury could be considered.

Drug-related hepatotoxicity is a common medical problem, with implications for health systems, and is also a cause of acute liver failure and, in many cases, responsible for the elimination of new pharmacological agents during efficacy and safety studies. Risk factors and pathogenesis of toxic hepatitis are poorly understood.

The diagnosis of toxic hepatitis is one of exclusion, because it is not easy to define a toxic etiology as the cause of liver disease due to the heterogeneity of the clinical presentation and evolution. It can range from transient elevations in liver enzymes to liver failure and even symptomatic chronic liver diseas.

Awareness about this condition is essential because anti-parasitic medication is taken frequently, often empirically.

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THE ROLE OF GUT MICROBIOTA IN THE PATHOGENESIS OF LIVER DISEASES

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Abstract

Microorganisms are present in number of million colonies in the gastrointestinal tract and it is known their beneficial role on the health of the host, and the quantitative and qualitative changes depend on nutrition, geographic and environmental factors, associated diseases, treatment. Until recently there were no methods for enterely identification of intestinal microflora and its compex role in health or disease was lnor completly understood. Recent literature data suggest that gut microbiota has a certain role in maintaining homeostasis. Qualitative changes (dysbiosis) or quantitative (overgrowth) of microbiota have a proven role in the pathogenesis of the gastrointestinal and liver diseases. The authors tried in this presentation, using literature data and published experimental studies, to synthesize and rank information about the pathogenic role of microbiota in the pathogenesis of the liver diseases such as hepatic steatosis associated or not with obesity, steatohepatitis, liver cirrhosis and its complications. Liver diseases were associated for long time, as pathophysiological possible mechanism, with the quantitive or qualitative variability of the microbiota. Extrinsic factors may cause dysbiosis, resulting in intestinal inflammation, change of the digestive tract barrier function, bacterial products translocation that may cause liver injury and inflammation. Microbial metabolites present in a intestinal tract with dysbiosis and host-related factors are equally involved in producing and progression of liver disease. The purpose of the studies is to prove the efficiency of using probiotics in therapy of hepatic steatosis, even as a therapeutic tool in cases of emergency steatohepatitis and cirrhosis.

Key words: *microbiota, liver, pathology*

Introduction

Intestinal epithelium is the interface between the external environment and the host organism. Adult human bowel contains 10^{10-14} microorganisms/ml of intestinal contents, named "microbiota", a number of microorganisms at least 100 times more numerous than the number of genes of the human genome and 10 times more numerous than the number of body cells.[1] Normal intestinal flora comprises over 500 different microbial species, but only 10-20 are prevalent species (Bacteroides, Lactobacillus, Clostridium, Fusobacterium, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Escherichia coli). Some of these bacterial strains are pathogenic organism (e.g. proteolytic Clostridia), but most are beneficial microorganism, creating a favorable habitat for their development and prevent the growth of pathogenic bacteria (Lactobacilli and Bifidobacteria which represents over 85% of total intestinal bacteria). Qualitative and quantitative distribution of intestinal microflora is variable in different intestinal segments with a numerical increase from the esophagus to the colon, where diversity and the number of microorganism is the most numerous. [2,3]

Bacterial Colonization

Human fetus is developing until the birth in a sterile environment and after the delivery is exposed to a huge variety of organisms, the most of them from the mother intestinal flora making the intestiunal colonization. Colonization depends on the mother's diet in the last period of pregnancy, gestational age, type of delivery. Fetal intestine may be exposed to microbes and colonized by swallowing amniotic fluid. In vaginal delivery the mother's vaginal and intestinal flora inhabits the intestines of a newborn (Lactobacillus). In cesarean section flora present on the skin (Staphylococcus, Clostridium) performs colonization.[1,4] In the process of colonization of the intestinal tract play also role environmental factors (medication, stress, lifestyle), type of diet, geographic area, bacterial interactions, diseases, genetic and immunological particularities of the host. Microbiota composition is individual and can be stable for months, in the same individual it varies according to age, more in infant and child. The composition of the intestinal microflora differs significantly in naturally and artificially fed infants. In the breast fed infants, due to the presence of prebiotics oligosaccharides and molecules with antibacterial activity from human milk microbiota contains more bifidobacteria and lactobacilli species, while the artificially fed infant formula enterococci and Enterobacteriaceae predominate. Early colonization and microbiota composition of the intestinal flora are determinant factors involved in the normal development of permanent individual microbiota and in preventing of certain diseases such as colon cancer, inflammatory bowel disease, irritable bowel, allergic disorders, liver (nonalcoholic fatty liver disease, steatohepatitis and liver cirrhosis), diabetes, obesity.[1,5]

The role of the microbiota

Intestinal microbiota is called "an organ in an organ" or a "super-organism" because of its multiple and complex roles: the maturation of the intestinal epithelium, involvement in digestion and synthesis, angiogenesis, bowel immune system maturation and modulation, regulating of lipid up-take, intervention in feed-back of gut-brain axis, modulation of gut motility. The intestinal bacteria have multiple beneficial effects on the intestinal epithelium with some metabolic consequences: inhibit the growth of pathogens by producing antimicrobial substances, contributes to strengthening of tight junction barrier function and interact with dendritic cells and the immune system, interact bidirectional with the enteric nerves for secretion of immune mediators.[5] In metabolic process the microorganisms participate to the digestion in indigestible carbohydrates, influences the expression of genes involved in the absorption of nutrients, play a role in energy production and up-take, in modulating of the lipid absorption, in production of vitamins (K, B) and gluconeogenesis from short chain fatty acids, activate hormones that inhibit intestinal motility, increase nutrient absorption and stimulates appetite. Substances produced by bacteria pass into the blood: fatty acids produced from fermentation of polysaccharides regulate the lipogenesis gene expression in the liver. [5,6,7,8]

Microbiota interacts with gut-brain axis by emission/reception of signals. Microbiota influences enterochromaffin cells innervated by the vagus nerve and, through unclear mechanism, plays a role in abdominal pain. The brain can modulate microbiota indirectly (changes in motility, secretion, intestinal permeability) or directly through cytokines released into the intestinal lumen by enterochromaffin cells, neurons, lamina propria cells. The role of microbiota in maturation and modulating of the sensorial and motor gut function can be explained by: indirect effect-the receptor interaction with epithelial cells or neural cells or directly-stimulating effect of lamina propria neuronal cells with intestinal permeability increasing. [9,10] The microbiota - disease relationship is limited explained, but there was a link between altered intestinal microbiological balance (dysbiosis) and the presence of inflammatory, allergic, autoimmune disorders. [5,11]

The role of microbiota in the pathology of liver disease

The liver has a dual blood circulation: 70% of the blood comes from the portal vein and the rest from the hepatic artery. Although the intestinal mucosa barrier has a complex immunologic mechanism, small amounts of bacterial particles get into the portal circulation. The liver plays, in this process the role of a filter between contaminated gut and sterile blood circulation: cells with immune role from sinusoid capillaries (natural killer-NK, NKT-cells, Kupffer cells stellate cells) remove bacteria and bacterial products protecting the systemic circulation of endotoxemia. It maintains a balance between exogenous antigens and immune tolerance. Activation of immune cells, as a response to the exogenous antigens, can cause liver inflammation, autoimmune disorders, fibrosis, liver carcinogenesis. Qualitative or quantitative changes of intestinal microflora or bacterial translocations disrupt the balance at this level with the loss of the "liver tolerance" and produce liver injury secondary. Many recent studies tries to demonstrate the relationship microbioma- liver disease as alcoholic liver disease, nonalcoholic fatty liver disease, cirrhosis, hepatic encephalopathy, hepatocellular carcinoma. The pathogenesis of all hepatic diseases involves common findings: cells inflammatory infiltrates, increased liver enzymes (liver injury), increased levels of pro-inflammatory cytokines. Primary mechanisms involved are: altered composition of the microbiota, small intestine bacterial overgrowth, increased permeability of the gut epithelium, changes in systemic and intestinal immunity. This relationship microbiota-liver disease is influenced by many factors as the diet, exposure to toxic factors and, probably, individual genetic predisposition. Change in composition of microbiota influences on both the incidence of liver disease and rate of progression and complications.[12,13]

Microbiota- bile acids

Intestinal microflora, by intestinal enzymes, metabolizes many indigestible molecules including substances from two classes of steroids: cholesterol and bile acids. Cholesterol is predominantly converted in coprostanol, a non-absorbed sterol, excreted fecal. Primary bile acids (cholic and chenodeoxycholic acid) are converted into over 20 secondary bile acids through the intervention of microbiota. Reducing the activity of intestinal microflora by antibiotic therapy reduces drastically the synthesis and fecal excretion of bile acids. Conjugated bile acids in the intestine are hydrolyzed by enzymes produced by Bacteroides and Clostridium and form de-conjugated salts which can be absorbed through the cell membrane, or can be combined with insoluble fiber. From the metabolic process result carbon, nitrogen and sulfuric acid used as a nutritive source by Bacteroides and Clostridium strains. In patients with cirrhosis, the level of hydrolyzed bile salts is low, correlated with reduced population of Bacteroides and Clostridium.[14,15] On the other hand, bile acids represent a factor that regulates the composition of microbiota in cecum. It is recognized, for a long time, the anti-bacterial role of bile acids by its interaction with the phospholipid membrane and alteration of the microbial cell membrane. [16]

Micorbiota in HBV infection

Studies of intestinal microbiota in patients infected with HBV (chronic carriers, active hepatitis or cirrhosis) highlight important changing in the ratio of intestinal bacteria particularly for Faecalibacterium prausnitzii, Enterococcus faecalis, Enterobacteriaceae, bifidobacteria, lactic acid bacteria (Lactobacillus, Pediococcus, Leuconostoc, Weissella). The ratio Bifidobacteria/ Enterobacteriaceae (B/E) is 1.15 in controller patients, 0.99 in HBV carriers, 0.76 in patients with chronic hepatitis B and 0.64 in those with decompensated cirrhosis, suggesting that this report B/E may be a factor in assessing microflora disturbance during the progression of liver disease.[17]

Microbiota and cirrhosis

Reduction of intestinal blood flow, mesenteric ischemia, slowing of bowel movements (reduced levels of ghrelin-hormone of gastric regulation) in cirrhosis determine the changing of the normal intestinal environment, making it beneficial for the growing of pathogenic species Bacteroides and Clostridium and allowing to opportunistic Enterobacteriaceae and Veillonella to overgrowth in the colon despite to a reduced number of Lachnospiraceaecare species. It could be observed a correlation between the score Child Turcotte-Pugh and the number of colonies of Enterobacteriaceae, Streptococcaceae, Veillonella, but not for Bacteroides. In liver cirrhosis the metabolic processes of the gut microbiota consist in: increasing in metabolism of glutathione, branched amino acids, urea and lipids, aromatic amino acids and gluconeogenesis and reducing the level of bile acids. Veillonella has the ability to hydrolyze conjugated bile salts and disturbs the micelle formation. Changes in intestinal microflora in cirrhosis would negatively influence prognosis.[18,19] In patients with liver cirrhosis microbiota is involved in the development of hepatic encephalopathy, primitive peritonitis , esophageal bleeding . Urease–producing bacteria: as Klebsiella and Proteus are associated with excessive production of ammonia and bacterial lipopolysaccharide that may cause hepatic encephalopathy. Bacterial translocation from the gut into the peritoneal cavity through intestinal permeability may cause primitive bacterial peritonitis. The same mechanism could be involved in the production of oesophageal varices bleeding. Efficient modulation of intestinal flora has been extensively studied and positive effects on long-term prevention of hepatic encephalopathy have been observed for probiotics.[19]

Microbiota in autoimmune hepatitis

Intestinal dysbiosis of the microbime could be involved in the pathogenesis of autoimmune diseases, with ample evidence that some change of the microbiota profile is associated with some of these diseases.[20] Some recent observations pay attention to the involvement of the microbiota in immune pathogenesis of liver damage. In primary sclerosing cholangitis (PSC) has been identified as an trigger for pANCA the isotope b-tubulin, but pANCA antibodies cross-react with FtsZ antigen present in almost all intestinal bacteria, which reflect abnormal immune response to antigens of microbiota in PSC. The same concept of antigenic mimicry was proposed in primary biliary cirrhosis (PBC), antimitochondrial antibodies cross react with proteins of E. coli isolated in feces; in addition in 50% of PBC patients with type IgG3 antibodies react with the B-galactosidase produced by Lactobacillus delbrueckii, a probiotic from yoghurts and 25% of patients have antibodies in the blood which react with proteins of fecal Novosphingobium aromaticivorans.[21]

Microbiota in nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH)

The fatty liver is a manifestation of the metabolic syndrome that causes chronic liver damage in children and adults with a wide range of injuries from simple steatosis to steatohepatitis (NASH). In preschool children with overweight or obese is noticed the high prevalence of the gram negative Enterobacteriaceae and Bifidobacteria inversely correlated with the level of transaminases (a surrogate marker of hepatic injury in obese patients), the predominance of Firmicutes species and reducing of bacteroides species in patients with obesity and metabolic syndrome.[13,22,23] Liver metabolic changes related with intestinal microbiota, characteristic of early stages of NAFLD, are obesogene and dysmetabolic: hepatic lipogenesis (fatty acid synthesis, accumulation of triglycerides in adipocyte and liver), liver neoglucogenesis from short chain fatty acids. Overgrowth of small bowel microbiota (increasing the number and diversity of bacteria) is due to an alteration of at least one of immune defense mechanisms. In obese patients is noticed, recently, the increase of plasmatic level of tumor necrosis factor (TNF), bacterial endotoxins, endogenous alcohol, intestinal permeability.[24] Bacterial overgrowing is explained by the reduction of intestinal motility and increase of intestinal transit time in obese patients with NASH or cirrhosis.

Increase of the intestinal permeability leads to bacterial translocation and transport of bacterial lipopolisaccahrides related to specific proteins from the intestinal tract to target organs. This process is facilitated by chylomicrons secreted by enterocytes as response to high-fat diet, process called "metabolic endotoxinemia".[8] Intestinal permeability can be measured by differential absorption of lactose/mannitol and more accurate by studying of transepithelial electric resistance. The increase of serum levels of zonulin is associated with obesity and secondary liver injury. Regarding the elements that determine the progression of NAFLD to NASH, it was observed that hepatocytes with Toll receptors (TLR) 2, 4 and 5 recognize bacterial lipopolisaccahrides as triggers for the inflammatory cascade (by stimulating the interferon chemokine b) and produce liver damage.[23] Intestinal dysbiosis increases the production of lipopolisaccahridic endotoxins which interact with TLR, increase nuclear factor-kB synthesis (NF kB), stimulates the production of TNF and Interleukin-IL-1b, primary responsible of the pro-inflammatory response in obesity, insulin resistance (a key element in the pathogenesis of NAFLD and the progression of fibrosis). All these pro-inflammatory factors stimulate the Kupffer cells that secrete themselves other pro-inflammatory and pro-fibrogenetic mediators and also stellate cells are stimulated, responsible for fibrogenesis of the extracellular matrix. Moreover, cellular wall components of gram negative germ modulate this effect on stellate cells by growth factor b.[12,24] It also demonstrated the relationship fructose-microbiota-obesity, which makes that low-fructose diet, use of antibiotics and probiotics could reduce insulin resistance, accumulation of fat in the liver, the level of endotoxins and inhibitor plasminogen activators, improve intestinal permeability.[25] Recently it was shown that enzymatic activation of a macromolecular complex called inflammasome containing caspasa-1 download IL-1b and IL-18 involved in inflammation in obesity, insulin resistance and in the pathogenesis and progression of NAFLD/NASH, as well as in other processes metabolic modulated by intestinal microbiota. Genetic deficiency of Inflammasone determines intestinal dysbiosis with elevated levels of bacterial products in the portal blood and hepatic steatosis. Bacterial translocation takes part, in addition, to the regulation of the

synthesis of nitric oxide which may influence the adipogenesis through the activity of lipoproteinelipase, which increases the intake of free fatty acids in the adipocytes.[23,26]

Conclusions

Intestinal microbiota in humans has a complex role in regulating the metabolic balance, occurrence and progression of gastrointestinal and liver disease such as hepatic steatosis, steatohepatitis, liver cirrhosis and its complications. Many aspects of the relationship microbiota-liver damage, liver inflammation and fibrogenesis remain unknown: why relatively minor changes in microbiota translate into major metabolic changes, dysbiosis of the gut microflora is the cause or the effect of the obesity? Qualitative or quantitative perturbations in intestinal microflora or bacterial translocations disrupt the immune balance at intestinal and hepatic level with the loss of the "liver tolerance" and produce secondary liver injury. The data obtained in human studies on the interaction microbiota - liver disease are still insufficient to establish a cause-effect relationship.

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PROGNOSTIC FACTORS IN ACUTE AND CHRONIC LIVER DISEASES IN CHILDREN

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Abstract.
Liver diseases in children have variable clinical manifestations, from asymptomatic increase of transaminases to fulminant liver failure. The disease evolution could be very severe, even fatal without right treatment on right moment. The level of transaminases is not a very valuable for predicting the severity of the disease. In neonatal cholestasis, biliary atresia is very important as it could have a severe evolution to cirrhosis and death. The most important prognostic factors in biliary atresia are the precocity of the diagnostic, the age at the surgical intervention, the experience of the surgical team. In acute liver failure there are many prognostic scores that include level of transaminases, coagulation factors or albumin level, with some differences depending on the etiologies. One of the clinical onset forms in Wilson disease is the fulminant liver failure. The severity and the need of liver transplantation could be assessed using a dedicated prognostic score. Even considered irreversible, liver fibrosis is a dynamic process potential reversible. The quick diagnostic, correct evaluation and timely therapy could limit the unfavorable consequences of liver cirrhosis. Liver biopsy is the gold standard in fibrosis evaluation, but sometimes the risks could limit its value. Non-invasive evaluation of liver fibrosis is based on measuring the serum level of makers involved in fibrosis or on imagistic methods and tend to replace the liver biopsy. In liver cirrhosis, there are different scores used for evaluation of the severity. Among those scores, PELD (pediatric end-stage liver disease) is used to evaluate the need of liver transplantation and also the urge of it. In conclusion, knowing the prognostic factors in different liver diseases is very important in order to assess the need of high specialized care in a liver center including liver transplantation in order to increase the survival of those children.
Keywords: *prognostic scores, biliary atresia, liver failure, fibrosis, cirrhosis, Wilson disease, liver transplantation*

Liver diseases in children have different etiologies, with variable clinical manifestations from asymptomatic mild increase of serum level of transaminases to fulminant liver failure. The disease evolution could be very severe, even fatal without proper treatment measures taken in right time. The prognostic scores are very important and its use could help to interpret the liver disease evolution, mainly in diseases with high severity and mortality. Knowing the prognostic could give time to take the therapeutic measures that could help to assure the survival of the patient and a good evolution of the disease. It should be mentioned that the level of transaminases is not a very valuable as a factor to reflect the severity of liver disease or as a prognostic factor for disease evolution.

Despite the idea that the high level of serum transaminases could be a marker of severity in liver diseases, the experience proves the contrary. There are causes of liver diseases where the most important change in laboratory parameters is the increase of transaminases at values of hundred times the normal value but the prognosis is very good as is in acute viral hepatitis or toxic hepatitis. Contrary, in fulminant liver failure secondary to Wilson disease the increase of transaminases is moderate (could be also normal value of ALT and increase only in AST) compared to other acute liver failure causes. But the prognosis in Wilson disease with fulminant liver failure is fatal without liver transplantation (LT). In cirrhosis the elevation of transaminases could be very mild. ALT is more specific for liver disease, but its increase reflects badly the importance of liver lesions. So, the level of transaminases alone, without corroboration with other clinic or laboratory parameters, is not a good prognostic factor.[1]

Biliary atresia. In neonatal cholestasis, biliary atresia is very important as it could have a severe evolution to cirrhosis and death without proper and timely surgical intervention and then, without LT if the portoenterostomy (Kasai intervention) failed. Approximately 70-80% of patients with biliary atresia will require LT; thus biliary atresia represents more than 50% of the LT indication in children.[2] The most important prognostic factors in biliary atresia are the precocity of the diagnostic, the age at the surgical intervention, the degree of experience in surgical intervention of the surgeons from the liver center. Taking into account these parameters, improving the time of diagnosis and the experience of liver center could assure a better evolution for those children, even with the decrease of the need of LT in the infant age.

Outcome of biliary atresia without portoenterostomy is very bad as 50-80% of children will die by age of 1 year and 90-100% of children will die by age 3 years without LT. The evolution will be characterized by complications of cirrhosis and portal hypertension, malnutrition, fat-soluble vitamin deficiency, mainly coagulation deficiencies, pruritus and cholestasis.

Portoenterostomy should be performed before age of 60 to 90 days in order to assure a better evolution, with a better survival of native liver. The 10-year transplant free survival varies between 30-40% in North America and Europe and 57% in Japan if portoenterostomy is performed before age of 60 days, compared to 13% if portoenterostomy is performed after 90 days.[2]

In a French study, the prognostic factors predictive of overall 10-year survival (portoenterostomy and LT) were the performance and age at portoenterostomy, anatomic form of biliary atresia (100% survival for atresia of the common bile duct only), polisplenia syndrome (48.8% vs 69.9% without polisplenia) and experience of the center or surgeon (58.9% for center with <5 cases/year vs. 77.8% for more than 20 cases/year). [3]

The experience in biliary atresia management of the liver center have a great impact on results in United Kingdom or France (with better results if caseload is greater than 5 cases/year), but did not appear to account for differences in Japan and Taiwan (mean caseload 6,5 new patients/year).[3-5] The age at Kasai operation has an important impact on long-term survival and also on the presence of cirrhosis at initial biopsy. The restauration of bile flow and the disappearance of jaundice after Kasai intervention (bilirubin level < 20umol/l) are the earliest indicators of success (2-year survival 68.2% vs 30.4% and 10-year survival 47.1% vs 0%). Cholangitis is a very common complication after Kasai intervention and prevention with antibiotics is indicated. Repeated episodes of cholangitis (more than 2 episodes) will decrease the survival with native liver (2-year survival 35.5% vs 58.2% and 5-year survival 14.2% vs 40.4%).[5] Portal pressure measured at the time of portoenterostomy in patients with biliary atresia have a prognostic value, patients with high portal pressure will have lower chances of success of this procedure and a higher risk of portal hypertension even if bilirubin level normalized after procedure. There is a clear relationship between liver fibrosis at the time of intervention and the worse restoration of bile flow that will have deleterious effects on long-time prognosis.[6]

There were several research groups that attempted to develop prognostic scores to predict survival and time for LT in children with biliary atresia using parameters like serum level of hyaluronic acid, procollagen III peptide and type IV collagen (as markers of fibrosis), the hepatic artery resistance index, urinary secretion of D-glutaric acid, postoperative bile bilirubin excretion. These indices have not been validated prospectively in large series and are not used in clinical practice.[2]

Alpha-1-antitrypsin deficiency (A1ATD) is the most common metabolic disease as cause of LT in children. Only some A1ATD PiZZ patients develop liver cirrhosis and portal hypertension. Approximately 10-15% of the PiZZ patients develop liver disease and 5% of them will require LT within the first 4 years of life.[7]

The level of transaminases and prothrombin time at diagnostic may have prognostic value. During clinical follow-up the recurrence or persistence of hyperbilirubinemia along with deteriorating of coagulation parameters are prognostic factors for the need for LT because of imminent poor outcome.[8] Other study concluded that various anamnesis parameters (manifestation of neonatal cholestasis) showed no prognostic significance, but thrombocytes, bilirubin, prothrombin time, cholinesterase, gamma-GT and GOT are prognostic factors in correlation with LT and/or death. Prognosis is difficult to be determined at an early stage of this disease, and a regular follow-up is necessary for the children.[9] Francavilla confirmed that persistence of jaundice and biochemical abnormalities are predictors of outcome, but furthermore, the severity of histological features (severe bile duct reduplication, severe fibrosis with bridging septa and established cirrhosis) in the initial liver biopsy will predict the development of end-stage liver disease.[7]

Acute liver failure (ALF) is rare in children, but unfortunately is a very severe disease with high mortality in some cases without emergency LT. The most frequent causes in our region are toxic (mushroom poisoning and drugs), metabolic (Wilson disease) and viral infection (hepatitis A, B). Many prognostic scores are used in ALF cases, including many parameters such as the serum level of transaminases, coagulation factors or albumin. There are some differences between the ALF etiologies, as evolution in paracetamol intoxication could be different that the other causes (as autoimmune hepatitis, viral hepatitis, mushrooms intoxication or Wilson disease). The most used prognostic scores are King's College Criteria (KCHC), Clichy criteria and MELD score (Model End-stage Liver Disease), that was first imagined for chronic liver failure.

KCHC used to predict the severity in ALF secondary to paracetamol overdose groups the patients into two categories: pH <7.30 or INR >6.5 (PT >100 sec) and serum creatinine >300 µmol /L (>3.4 mg/dL) in patients with grade 3 or 4 encephalopathy. In patients with non-paracetamol associated ALF the following criteria are associated with poor prognosis: INR >6.5 (PT >100 sec), or any 3 of the following: age <10 or >40 years; etiology non-A, non-B hepatitis, or idiosyncratic drug reaction; duration of jaundice before hepatic encephalopathy >7 days; INR >3.5 (PT >50 sec); serum bilirubin >300 µmol/L (>17.6 mg/dL).[10] United States ALF Study Group revealed that KCHC has specificity of 85.7% and sensitivity of 48.3% for death prediction in 838 cases of ALF. In ALF secondary to paracetamol overdose the specificity was 92.4% in 374 cases.[11] Majority of the studies are done in adults, but a study done in children concluded that KCHC is not very efficient in mortality prediction in children with ALF. The positive predictive value (PPV) and negative predictive value (NPV) of KCHC for non-paracetamol induced ALF children was 33% and 88% respectively and twice as many participants who met KCHC recovered spontaneously than died, indicating that using KCHC may cause over utilization of LT.[12]

Clichy Criteria are also used for prediction of the need of emergency LT in ALF and include the presence of hepatic encephalopathy and the serum level of factor V under 20% in patients under age of 30 years or under 30% in patients over 30 years. Using the Clichy criteria the mortality could be predicted with PPV of 82% and NPV of 98%.[13]

Prognostic score for ALF in Wilson disease. One of the clinical onset forms in Wilson disease is the fulminant liver failure, with non-immune hemolytic anemia and renal failure. The prognosis of the disease evolution and the need of LT

could be assessed in Wilson disease using a dedicated prognostic score, including laboratory parameters as bilirubin, transaminases, albumin level, leukocytes and coagulation factors (Table I).[14]

Non-invasive evaluation of fibrosis. Fibrosis represents an unspecific response to a liver injury and involve the synthesis in excess of extracellular matrix.[15] Even considered irreversible, liver fibrosis is a dynamic process potential reversible. The quick diagnostic, correct evaluation and timely therapy could limit the unfavorable consequences of liver cirrhosis.[16] Liver biopsy is the gold standard in fibrosis evaluation, but sometimes its value is doubt due to technical limits and risks. Non-invasive evaluation of liver fibrosis is based on measuring the serum level of makers involved in fibrosis or on imagistic methods and tend to replace the liver biopsy.[17]

Table I. Prognostic score for ALF in Wilson's Disease[14]

Lab parameters		Score				
		0	1	2	3	4
Bilirubine	mg/dl	5,9	6-8,8	8,9-11,7	11,8-17,5	>17,5
	µmol/L	0-100	101-150	151-200	201-300	>301
INR		0-1,29	1,3-1,6	1,7-1,9	2,0-2,4	>2,5
Transaminase (µi/l)		<100	100-150	151-200	201-300	>300
Leucocytes (/mm3)		0-6.700	6.800-8.300	8.400-10.300	10.400-15.300	>15.400
Albumine (mg/dl)		>4,5	3,4-4,4	2,5-3,3	2,1-2,4	<2
Score >11 reflects the need of liver transplantation (without it the patient will certainly die in maximum 2 years)						

The scores for fibrosis were imagined in order to improve the performance of serum markers alone and represent algorithms that use different combinations of many direct or indirect markers involved in fibrosis. There were described many scores: APRI (ratio of AST expressed as multiple of normal value and number of thrombocytes), PGA (association of prothrombin time, γGT and apolipoprotein A1), Oberti (prothrombin time, hyaluronic acid and α2 macroglobulin).[18-19]

FibroTest score associates five indirect makers of fibrosis: α2 macroglobulin, haptoglobin, apolipoprotein A1, γGT, and total bilirubin, corrected function to age and sex. Adding ALT value the ActiTest score is obtained, predictive for necro-inflammatory activity. In order to use in clinical practice tables for correspondence between FibroTest, ActiTest and different histology score systems (METAVIR, Knodell, Ishak) were proposed. [20-23] Recently, many non-invasive imagistic methods were introduced for liver fibrosis evaluation. Unidimensional transitory elastography is a non-invasive method that uses the evaluation of liver tissue rigidity in order to evaluate the stage of fibrosis. (Fibroscan© - Echosens, Paris). ARFI (Acoustic Radiation Force Impulse) is a new non-invasive method that could be adapted to a conventional ultrasound system (Acuson S2000, Virtual Touch Tissue Quantification mode, Siemens Healthcare). MR elastography is an imagistic method that is under development and it allow the quantitative evaluation of the mechanical proprieties of liver tissue.[24-26]

Liver cirrhosis. There are different scores used for evaluation of the severity of liver cirrhosis. Among those scores, MELD and PELD (pediatric end-stage liver disease) are used by United Network for Organ Sharing (UNOS) and the Organ Procurement and Transplantation Network (OPTN) organization in order to list the patients for LT. Three-month survival of children listed for LT is predicted by five variables: serum total bilirubin, INR, albumin, growth failure, and age less than 1 year. Until 2002 the classification using Child-Turcotte-Pugh score was used for prediction of severity in cirrhosis. Three month mortality using Child-Turcotte-Pugh Classification was 4.3% in Class A, 11.2% in Class B and 40.1% in Class C.[27]

Table II. Child-Turcotte-Pugh Score for liver cirrhosis evaluation[28]

Clinical Lab Criteria	Points		
	1	2	3
Encephalopathy	None	Mild to moderate (grade 1 or 2)	Severe (grade 3 or 4)
Ascites	None	Mild to moderate (diuretic responsive)	Severe (diuretic refractory)
Bilirubin (mg/dL)	< 2	2 – 3	> 3
Albumin (g/dL)	> 3.5	2.8 – 3.5	< 2.8
Prothrombin time (Seconds prolonged) and INR	< 4 <1.7	4-6 1.7 – 2.3	> 6 > 2.3
Child-Turcotte-Pugh Class obtained by adding score for each parameter (total points): Class A = 5-6 points (least severe liver disease); Class B = 7-9 points (moderately to severe liver disease); Class C = 10-15 points (most severe liver disease)			

PELD = 4.36 (if age <1 YR) – 6.87 x Log_e (albumin g/dL) + 4.80 x Log_e(total bilirubin mg/dL) + 18.7 x Log_e (INR) + 6.67 (if growth failure <2SD).

In conclusion, there is very important to know in each different etiology of liver disease in children the prognostic factors that could help in evaluation of the evolution and the need of high specialized care in a liver center or even the need of LT in order to increase the survival of children with liver diseases.

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FALLOT UP MRI VERSUS ECHOCARDIOGRAPHY

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Abstract

Tetralogy of Fallot - cyanotic heart malformation - is known in the literature as the prototype of complex cyanotic heart malformations. Current developments in advanced imaging techniques (ultrasound parameters, cardiac CT and MRI), allow for diagnosis, prognosis, and optimal surgery time calculation as well as tracking long-term outcome after surgery.
We present the case of a 17 years old teenager, diagnosed with the severe form of Tetralogy of Fallot at birth (with right-sided aortic arch almost 100%, classed as right ventricular type with double track output). She underwent her first surgery at age 2 yrs. 15 years after the surgery the patient has significant tricuspid and pulmonary regurgitation and also marked deterioration of the right ventricular function witch contrast to the good clinical condition. The bizarre echocardiographic character witch revealed the strange alignment between the aorta and left ventricle as well as the complex morphology, made necessary cardiac MRI assessment. Corroborating clinical data, anamnesis, and imaging was established prognosis, therapeutic and surgical subsequent conduct.
Conclusions.
1. Cardiac MRI imaging is the gold standard for evaluating the right ventricle.
2. The before/after surgery prognosis in the Tetralogy of Fallot is established by using modern echocardiographic markers and cardiac MRI.
3. After the MRI revue the case has a strong indication for subsequent surgery for the reconstruction of the right ventricle outflow path.
Key wors: *Tetralogy Fallot, echocardiogaphy, cardiac MRI*

Introduction

Tetralogy of Fallot - cyanotic heart malformation - is known in the literature as the prototype of complex cyanotic heart malformation.[5,10] Current developments in advanced imaging techniques (ultrasound parameters, cardiac CT and MRI are very useful in congenital heart pathology. In Tetralogy of Fallot (TF) this advanced imaging technics are used for diagnosis, prognosis, and optimal surgery time calculation as well as tracking long-term outcome after surgery. Echocardiography remains the gold standard in the diagnosis of TF, but sometimes special techniques are needed for postoperative following.[10,11]

Assessment of the right ventricle (RV) is often neglected in medical practice. The two ventricles are in an interaction during systole and diastole, due to the septum and the pericardial sac. RV is more difficult to assess echocardiography. Apical 4 rooms incidence can be subjective and can underestimate RV function. In the morphological study and prognostic assessment of TF are implemented echo cardiac parameters such as tricuspid annulus systolic displacement amplitude (TAPSE – assessed in M mode) by Tisular Doppler TDI - for tricuspid annulus velocity, myocardial acceleration during IVC (isovolumetric contraction), the E/Ea parameter on the tricuspid sidewall, strain and strain-rate techniques also the maximum longitudinal strain derived from TDI.[11] Transesophageal ultrasound and cardiac catheterization are used in qualitative and qualitative myocardial revaluation both pre- and postoperatively. Cardiac MRI is the modern imaging technique that is non-irradiated, non-invasive which allows in TF the evaluation of Cono-truncal pulmonary malformations and complex abnormalities, description of segmental abnormalities, detection and quantification of postoperative results, the evaluation of pulmonary arteries and assessment of cardiac function: ejection fraction, end systolic and end-diastolic volume, valvular kinetics. All imaging techniques of refinement and performance in the follow-up of TF must take into consideration mainly the RV. In order to study the morphology of the RV are used echo cardiac parameters and cardiac MRI reconstruction.[14]

Case presentation

17 years old adolescent, diagnosed at birth with TF extreme form - with the right situation of the aorta almost 100% (considered from a morphological point as a right ventricle with double outflow path), with frequent hypoxic seizures, fatigue and failure of growth in infancy. She was treated surgically "per primam" at age 2 in the cardiovascular surgery center of

Timisoara.[8,13] Postoperatively she shows significant clinical improvement, upward weight curve, almost normal physical tolerance. In the periodic postoperative echocardiographic follow-up are detected some morphological abnormalities at the pulmonary infundibulum and a deviated left ventricle-aorta axis. The patient has an age appropriate lifestyle, does not require treatment, social insertion and comfort of life are normal, the follow-up includes bacterial endocarditis prophylaxis as needed and periodic clinical imaging reevaluation. At the 15 years post operatory, evaluation the ultrasound reveals pulmonary regurgitation considered as significant PHT = 180 ms, RV consecutive dilatation, and as a result tricuspid regurgitation. The left ventricle (LV) function is conserved EF=50%, despite of the bad alignment of the LV and the aorta trough the ejection path and a moderate aortic regurgitation (fig.1, 2, 3, 4.). The deterioration of the RV function with tricuspid annulus dilatation and the interventricular desynchronization with impact on LV, contrasting with the patients unchanged very good general state, imposed an MRI assessment.

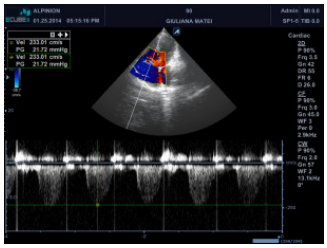

Fig. 1. Apical 4 rooms-Dopller

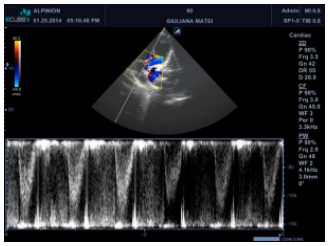

Fig. 2. Pulsating Dopller

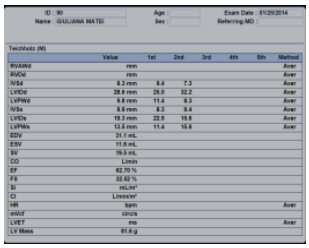

Fig. 3. Dopller record

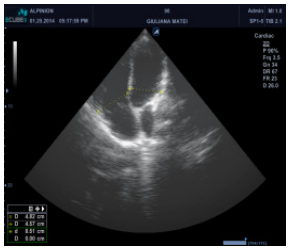

Fig. 4. Apical 4 rooms

MRI evaluation showed a heart with systolic-diastolic function of the left ventricle at the lower limit of normal: LVEF = 57.6%, LV TDV = 58.2 ml, TSV = 24.6 ml; RV much increased in volume, systolic-diastolic function moderately impaired: RVFE = 35.0%, RV TDV = 105.8 ml, TSV = 68.7 ml. the cine True FISP sequences showed the ventricular septum bulging to the left. Cardiac MRI also showed tricuspid regurgitation grade II / III, pulmonary artery trunk diameter of 3.32 cm (Fig. 5 - 7). The patient could not maintain apnea, the breathing artifacts are present and the degree of pulmonary regurgitation (which is considered significant) couldn't be quantified. Cardiac MRI in this case remains sovereign.[14,15]

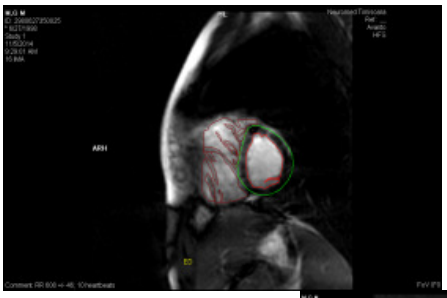

Fig. 5. Incidence cardiac MRI

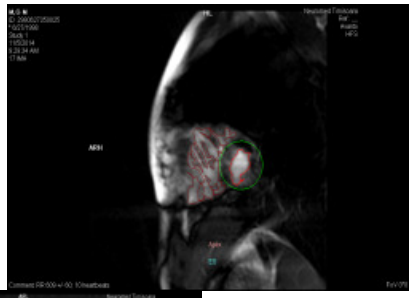

Fig. 6. Incidence cardiac MRI

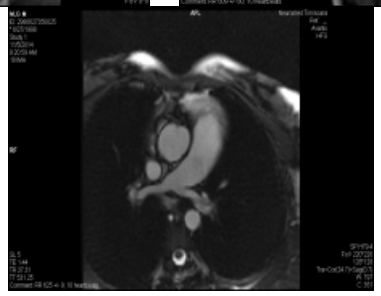

Fig.7. MRI cardiac

Corroborating clinical data, anamnesis, imaging, ultrasound and MRI, we believe the patient, despite a lengthy clinical stability and comfort of life equal to that of a young woman of the same age; shows progressive right ventricular inefficiency due to pulmonary valve malformation, symptoms of heart failure will become more evident. As a result the patient will require a correction, probably valvuloplasty +/- reconstruction of the tricuspid valve. [4,7]

Conclusions.

1. MRI cardiac imaging is the gold standard for evaluating the right ventricle.
2. Pre / post operatory prognosis in Tetralogy of Fallot is appreciated by using modern echocardiography markers and cardiac MRI.

3. After the MRI review the patient received indication for surgical intervention with reconstruction of the right ventricular outflow tract.

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SIMILAR SOUNDS, OPPOSITE MORPHOLOGICAL SITUATIONS

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Abstract

Cardiac malformations are often diagnosed by a routine pediatric exam, most of them passing undetected through the neonatal "filter". The detection of a heart murmur, with the added support of imaging and laboratory investigations, allows the condition to be classified as eider functional or organic. The clinical experience associated with a well conducted physical examination can guide the physician in respect to the lesion type, but many morphological variants can have the same sounds. For example, interventricular septal defect and pulmonary stenosis have similar sounds but are very different clinical conditions. We are presenting the cases of two patients 17 and 10 years old witch during the physical examination showed systolic-diastolic murmur grade IV / VI, suggesting a permeable arterial channel. The first patient was diagnosed in early infancy with valvular malformation, inappropriately followed for about 10 years; at 15 years of age she developed a endocarditic process witch compromised the hole valvular apparatus and resulted in marked heart failure. The second patient comes from difficult social environment (institutionalized) with suspected cardiac malformation unsolved until hospitalization in our clinic. Echocardiography was used to establish the real morphologic substrate, resulted in the classification of the malformation type quite different from the first case and indicated a diametrically oppose therapeutic approach.

Conclusions.

1. Sometimes, in the case of rare lesions- the sounds can suggest false pathology
2. The value of imaging in the diagnosis of heart malformations is sovereign
3. We are curious how it will these patients sound after surgical resolution ("still resemble?").

***Key words**: murmur, cardiac malformation, echocardiography*

Introduction

Cardiac malformations are often diagnosed by a routine pediatric exam, most of them passing undetected through the neonatal "filter". The detection of a heart murmur, with the added support of imaging and laboratory investigations, allows the condition to be classified as eider functional or organic. The clinical experience associated with a well conducted physical examination can guide the physician in respect to the lesion type, but many morphological variants can have the same sounds. For example, interventricular septal defect and pulmonary stenosis have similar sounds but are very different clinical conditions(1).

Case report

We are presenting the cases of two patients 17 and 10 years old witch during the physical examination showed systolic-diastolic murmur grade IV / VI, suggesting a permeable arterial channel (PAC).

The patient, C.A. 17 years old, was hospitalized in February and June 2015. She was diagnosed in infancy with subvalvular aortic stenosis and is consulted in several cardiovascular surgical centers in the country. At age 11yrs develops endocarditis process, which destabilizes the aortic valv. After the acute episode she develops severe aortic insufficiency by remodeling of the aortic valve leaflets and heart failure. The patient has episodes of fainting, fatigue on minimal effort, palpitations, restricted activity. Her heart noise is rhythmic, well beaten, with rare extra systolic beats, marked trill on palpation and the apex ample shock visible on the anterior axillary line, systolic murmur V/VI and diastolic murmur IV / VI with maximum intensity in the aortic foci with irradiation in all foci of auscultation and laterocervical bilateral, discrete free interval between diastolic and systolic murmur.

Given the conditions on admission, the differential diagnosis included besides the general PAC (with left ventricular damage) and congenital or acquired (postendocarditis) valvular damage with stenosis and regurgitation - between them, and also of primary or acquired cardiomyopathies, with long evolution.[2,3]

ECG shows signs of left ventricular hypertrophy and diastolic suffering.

Imaging investigations have established real morphological substrate.

Chest radiograph: significant cardiomegaly, cardio-thoracic index = 0.68, left arc stretching.

Cardiac ultrasound reveals left ventricular dilatation, TDDlv = 58mm, concentric hypertrophy mostly septal; at the subvalvular aortic level (about 1,3 cm) 10mm diaphragm stenosis, aortic valve with three leafs, highly reflective of the ultrasounds, with the leafs margins of granular aspect, 27 mm on annulus, P max anterograde = 85 mmHg, P medium = 50mmHg, PHTao = 210mmHg. The right ventricle and the pulmonary artery of normal aspect, minor mitral regurgitation with posterior jet, free aortic cross, no PAC (Fig. 1 - 4). Conclusion: Severe subvalvular aortic stenosis, severe aortic valve regurgitation, moderate LV insufficiency with EF 50%, extrasistole during the ultrasound examination.

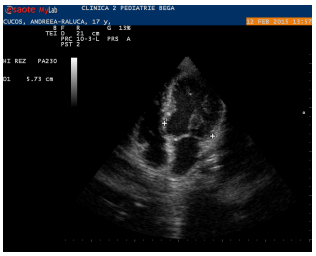
Fig.1. Apical 4 rooms

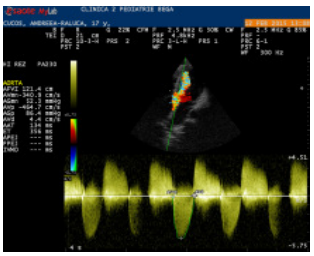
Fig. 2. Doppler

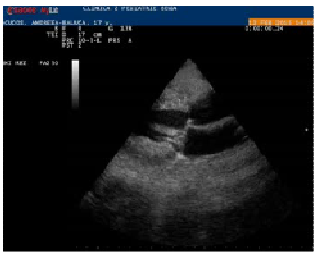
Fig. 3. Parasternal long axis

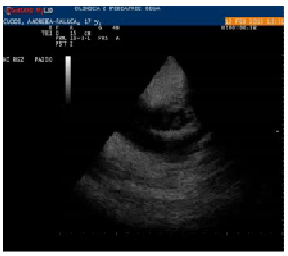
Fig.4. Parasternal ax short

In may 2015, the patient was operated (in "Herz- und Diabetes Zentrum Bad Oeynhausen cardiosurgery Center" Germany), with the resection of the diaphragm stenosis (incomplete in order not to destabilize the mitral valve) and one aortic leaf was repaired using a pericardial fragment.[13] The use of prosthesis was unnecessary and a long anticoagulant treatment was avoided.[4] Three weeks after the surgery the patient was admitted in our clinic, showing improving general health, superior quality of life, the systolic-diastolic murmur in precordium and aortic foci subsided.

The post operator ultrasound shows a mitral leafs prolaps with a small posterior regurgitation, dskinetic movements in the 1/3 apical septum, concentric left ventricle and septum hypertrophy, SIV=19mm, TDVlv = 52mm; subvalvular aortic at the insertion of the mitral valve it is still present an fibro-muscular spur, aortic valve 26mm on annulus, the left coronary leaf has an moderate discontractil aspect, commissure of the right coronary and noncoronary cusp discreetly enlarged, regurgitation at this level PHT 600msec, anterograde flux 12 mmHg, significant improvement of the hemodynamic function. (fig. 5-8 postoperator ultrasound).

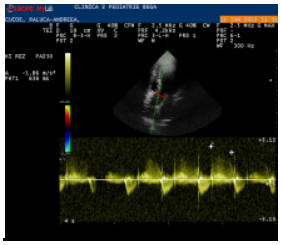
Fig. 5. Doppler

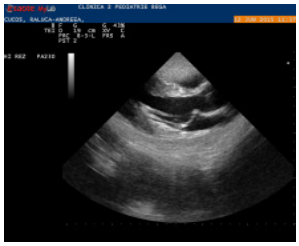
Fig. 6. Parasternal long axis

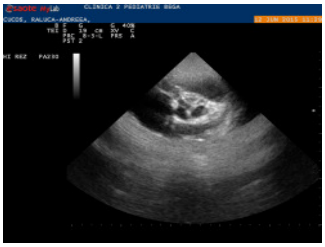
Fig. 7. Parasternal short axis

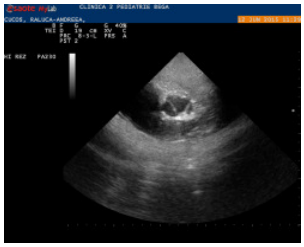
Fig. 8. Short axis

Patient P.G., 10 years old, from social care institution, with congenital heart disease that was not diagnosed earlier. Shows fatigue at medium efforts, coupled with poor communication (unaccompanied, no medical records, no anamnesis information). On physical examination shows improper development in procurement, microclamps, undescended testicles, intense systolic-diastolic murmur, rough, with a maximum in parasternal left, with irradiation in the axilla and left supraclavicular fossa, very marked trill, shock apex ample bulge visible parasternal left. Auscultation suggested a permeable arterial canal (PAC).

Chest X-ray shows a normal heart shape, interstitial drawing emphasized bilaterally. EKG sows left axis deviation. On ultrasound in the specific incidences for PAC (parasternal short axis and suprasternal), there is no aorta-pulmonary communication visible. Turbulent flow in the right ventricular infundibulum, which apparently came from the aorta (Fig. 9-11). It raises the suspicion of coronary artery fistula.

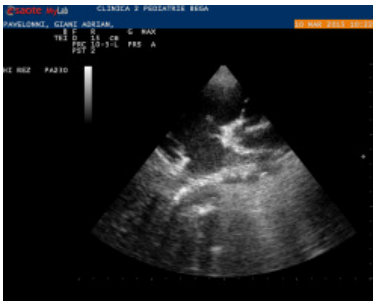
Fig. 9. Echocardiography

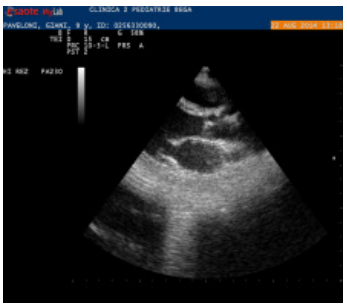
Fig. 10. Parasternal long axis

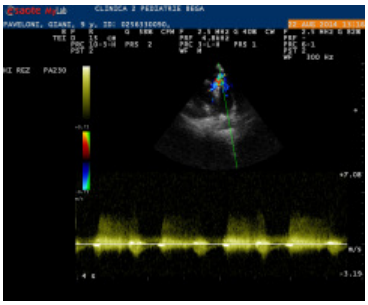
Fig.11. Dopller

Confirmation of the diagnosis by CT angiography (fig. 12 - 20) revealed normal coronary emerging, dominant right coronary system, permeable coronary artery, with discernable paths until medium segments; cranial from the Emerging right coronary is visualized a fistula with a diameter of 1,57cm, which creates a communication between the aorta and the right ventricle infundibulum; Global dilated heart cavities.

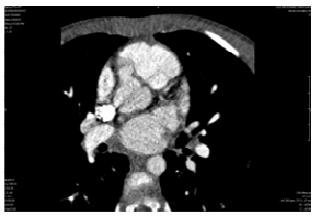
Fig. 12.

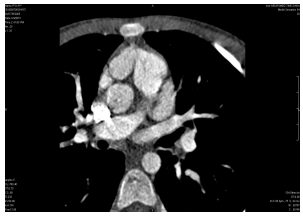
Fig. 13.

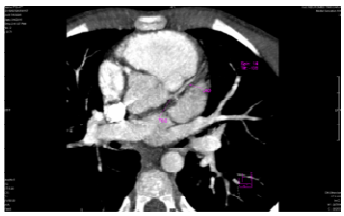
Fig. 14.

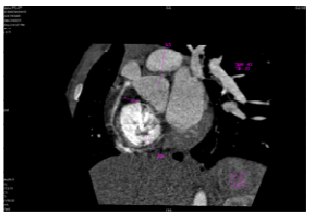
Fig. 15.

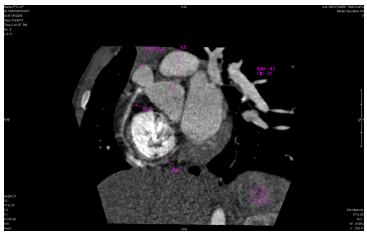
Fig. 16.

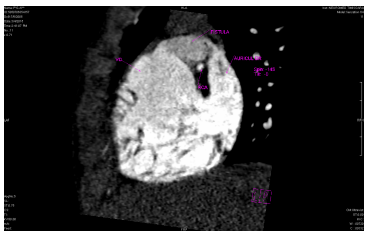
Fig. 17.

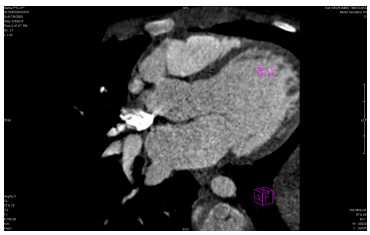
Fig. 18.

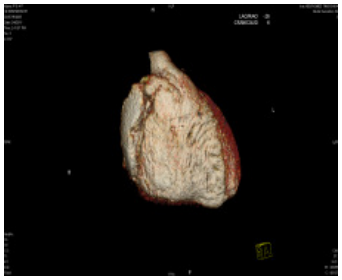
Fig. 19.

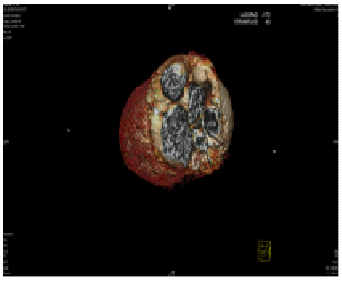
Fig. 20

Settling the diagnosis of right coronary artery fistula, the patient is directed to undergo surgery or interventional trans catheter embolization.[8,10,12] The goal of treatment is fistula obliteration with preservation of coronary flow, the devices sugested are type "coil" or ductoclude.[7,11] The alternative is surgery postcateterism cardiac (used in case of multiple fistulas.[5,6,7] The patient is currently stable, is waiting for the defect repair intervention, endocarditis prophylaxis was recommended, effort in the limit of toleration with no chronic medication.

Conclusions:

1. Sometimes, in the case of rare lesions- the sounds can suggest false pathology
2. The value of imaging in the diagnosis of heart malformations is sovereign.
3. The heart murmurs change depending on the natural history, possible complications and post-surgical or interventional solving of the congenital heart defects.

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DIAGNOSIS AND MANAGEMENT, MAJOR IMPACT PARAMETERS ON THE PROGNOSIS OF ATRIOVENTRICULAR SEPTAL DEFECT

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Abstract.

Introduction. Atrioventricular septal defect (AVSD) is a congenital heart defect, ranging from simple to complex, each form with different management and evolution. Our aim is to assess the diagnosis and management of children with AVSD as parameters with major impact on the prognosis. Materials and Methods. We realized a retrospective study including 31pacients of Clinic II Pediatrics Timisoara having AVSD, during follow-up echocardiographic examinations, between 2010-2015. Results. Both sexes were equally affected and 38,7% of all patients had Down syndrome, number comparable to literature. Most cases were diagnosed under age1, correlated with pregnancy follow-up, adequate equipment and well trained personnel. The majority of patients underwent total primary correction, some needed palliative surgery with subsequent correction and a small number benefited only of pulmonary artery banding. Some are yet to undergo surgery. Survival is 100% in the partial or transitional form, but it decreases in the complete form in direct proportion with the complexity of lesions, because severe impaired forms lead to surgical backlogs, some requiring reintervention.

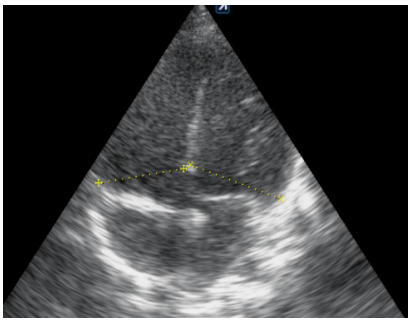
Conclusions.

1. The ideal diagnosis is the prenatal diagnosis.
2. Delay in diagnosis leads to delaying surgical treatment, thus altering the outcome due to complications.
3. The evolution depends on the type of defect: complex forms, are technically more difficult to correct and therefore with less favorable prognosis.

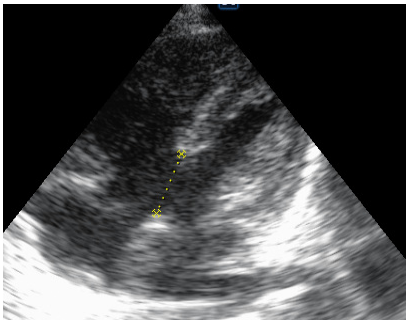
***Keys words**: atrio-ventricular septal defect, congenital heart defect, prognosis.*

Introduction

Atrioventricular septal defect (AVSD) is a congenital heart defect(CHD), due to an imperfect development of endocardial cushions during weeks 4-5 of pregnancy [10], which play an important role in the formation of the inferior part of atrial septum, superior part of ventricular septum, mitral valve and tricuspid valve[1,4,6]. This represents 3-7% of all CHD[2,4,6] and may range from simple to complex, each form with different management and evolution.[6]



types of CAVC



From the changeable risk factors for congenital heart defects in general and AVSD in particular we mention alcohol consumption, ineffective diabetes control, medication during pregnancy[7] and from the unchangeable risk factors we mention

the family history: 15% of mothers with AVSD give birth to children with the same defect, 45% of children with Down syndrome have CHD, of which 30-40% are AVSD.

Purpose.

Our aim is to assess the diagnosis and management of children with AVSD as parameters with major impact on the short-tern, but also long-term prognosis.

Materials and Methods

We conducted a retrospective study including 31 pediatric patients with follow-up examination in Clinic II Pediatrics Timisoara between 2010 and 2015. Were counted in patients having AVSD, alone or in association with other lesions, operated or unoperated. Anamnestic data was colected regarding evolution from birth until diagnosis and from diagnosis until registration in clinic and noted afterwards evolution until present.

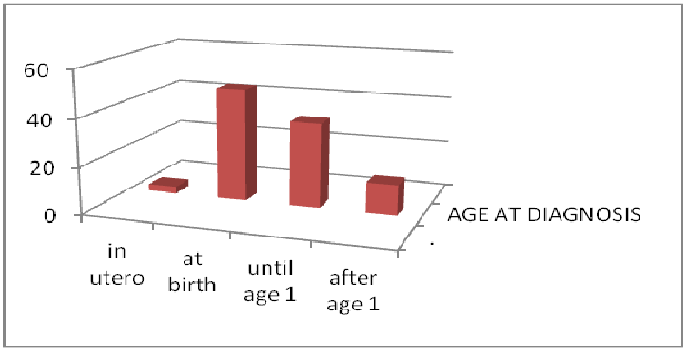
During neonatal period clinical findings (repeated respiratory tract infections, failure to thrive, heart failure, sistolic murmur) consistent with AVSD [6,7] confirmed the diagnosis through echocardiography.[11,12]

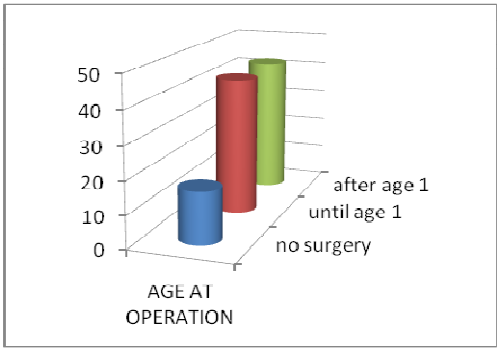
In one case the diagnosis was confirmed in utero through fetal echography. [7,8,12]

Simple AVSDs may go underdiagnosed due to few to no noticeable symptoms and are detected sometimes after years; these cases are discovered when complications such as arrhythmias, heart failure, pulmonary hypertension, infective endocarditis occur.[6]

Results.

AVSD had Tetralogy of Fallot associated in 3 cases and heterotaxy + great arteries transposition in another.[3,4,10,11,12] Both sexes were equally affected and 38,7% of all patients had Down syndrome,[4,5,6,10,12] number comparable to literature. Most cases were diagnosed under age 1 (84%), 13% after age 1 and only 3% in utero, fact correlated with pregnancy follow-up, adequate equipment and well trained personnel.

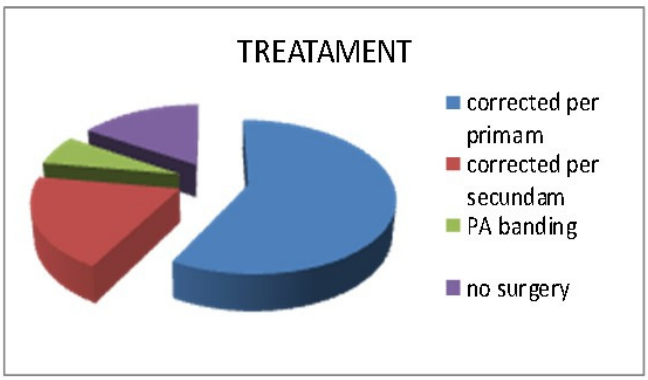


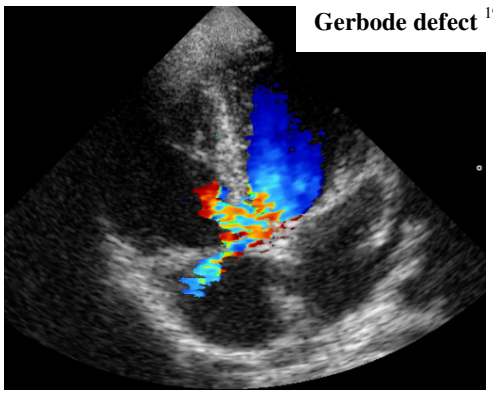


Medical treatment consists of heart failure medication[6,9,12] when needed and endocarditis prophylaxis.[11]

Elective treatment is surgery and is recommended between age 2 and 4 in asymptomatic children, but may be carried out in toddles with marked symptoms – this time with higher surgical risk.[7,9,12]

The majority of patients underwent total primary correction (69,5%), some needed palliative surgery with subsequent correction (23%) and a small number benefited yet only of pulmonary artery banding (7,5%). Some are yet to undergo surgery.





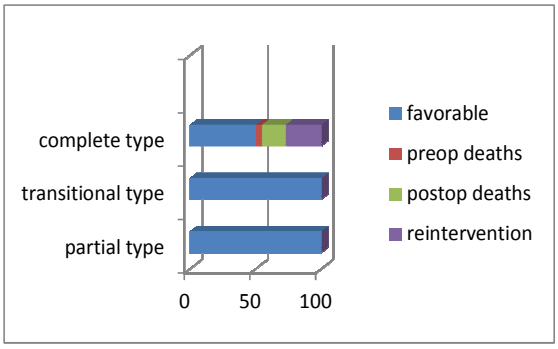
Gerbode defect [19]

From all operated patients (84%), 65% have surgical backlogs and only 35% have no surgical backlogs.

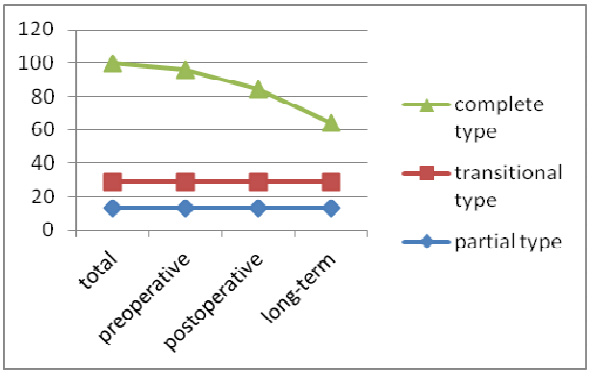
Complex types of AVSD remain with significant lesions after surgery because of severe impaired morphology, which is a challenge for the surgeon due to surgical technical difficulties to restore heart anatomy, 27% requiring reintervention. Only one patients developed pulmonary hypertension postoperative.

Survival is 100% in the partial or transitional type, but it decreases in the complete type (71%) in direct proportion with the complexity of lesions.

We recorded one deceased preoperative (during neonatal period), 2 patients died during surgery and 2 postoperative, one of which had noncardiac cause.



EVOLUTION



SURVIV

Conclusions

1. The ideal diagnosis is the prenatal diagnosis, but it represents only o small number of diagnoses in our country, hampered by a numerous pregnancies with no follow-up and many mothers with low socio-economic status and reduced addressability/medical education.
2. Delay in diagnosis leads to delayed surgical treatment, thus altering the outcome due to complications.
3. The evolution depends on the type of defect: simple AVSD benefits of relatively simple treatament with good prognosis; complex types, however, are technically more difficult to correct and imply less favorable prognosis.

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UNCOMMON PANCREATIC NEUROENDOCRINE TUMORS IN CHILDREN

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Abstract

Pancreatic neuroendocrine tumors (PNT) are uncommon type of pancreatic tumors in children. PNT arise from the pancreatic islets of Langerhans and present either as a functional or non-functional tumor. In functional tumors the symptoms are a result of the secretion of specific hormones such as insulin, glucagon, gastrin, and vasoactive intestinal peptide (VIP) or others. All these entities may be sporadic or associated with inherited neoplasia syndromes such as MEN1 and von Hippel-Lindau disease. Symptoms of both functioning and nonfunctioning tumors may include pain in the abdomen, diarrhea, stomach pain, a tired feeling all the time, fainting, weight loss, or weight gain without eating too much. Imaging is used to localize primary and metastatic lesions and to determine resectability or alternative palliative and curative treatment options. Current treatment options for pancreatic neuroendocrine tumors are surgery, combination chemotherapy, hormone therapy.
Keywords: *pancreatic neuroendocrine tumors, children.*

Introduction

Pancreatic neuroendocrine tumors are made up of endocrine cells that are usually found in islet cells throughout the pancreas. Pancreatic neuroendocrine tumors remain a rare primary pancreatic neoplasm described mostly in adults and in childhood appear in about 5% of primary pancreatic neoplasms described in adults.[1] The islet cells produce and release the following hormones into the blood*: insulin* reduces the amount of sugar (glucose) in the blood and stimulates the liver, muscles and fatty tissues to absorb and store the extra sugar; *glucagon* increases the amount of sugar in the blood and stimulates the liver and other body tissues to release stored sugar; *somatostatin* slows the release of other hormones produced by the pancreas; *gastrin* is a polypeptide hormone secreted by certain cells of the pyloric glands, which strongly stimulates secretion of gastric acid and pepsin, andweakly stimulates secretion of pancreatic enzymes and gallbladder. Pancreatic neuroendocrine tumors are a subset of gastroenteropancreatic neuroendocrine tumors and can be broadly divided into functioning and non-functioning tumors based on their physiologic activity. Functioning tumors can arise from any of the neuroendocrine system. These tumors are usually sporadic but can be associated with genetic syndromes such as MEN-1,[2] von Hippel-Lindau,[3] neurofibromatosis 1 and tuberous sclerosis. Whatever the etiology, pancreatic neuroendocrine tumors are rare and are reported to be less than 3% of all primary pancreatic neoplasms.[4]

Diagnostic

Clinical features of the syndrome depend on tumor cell type.

Insulinomas are characterized clinically by the Whipple triad (Presence of symptoms of hypoglycemia, documented low blood sugar at the time symptoms and reversal of symptoms by glucose administration). The biochemical diagnosis of insulinoma is established during prolonged fasting (up to 72 h) when the following results are found: Serum insulin levels of 10 µU/mL or more (normal < 6 µU/mL); Glucose levels of less than 40 mg/dL; C-peptide levels exceeding 2.5 ng/mL (normal < 2 ng/mL); Proinsulin levels greater than 25% (or up to 90%) of immunoreactive insulin levels; Screening for sulfonylurea negative.[5]

Gastrinoma are usually found in the duodenum wall or in pancreas. In addition to locations in the duodenum and pancreas, gastrinomas have been described in the lymph nodes, liver/biliary tree, gastric antrum, and jejunum. Gastrinomas are the second most common neuroendocrine tumors after insulinomas and may secrete not only high levels of gastrin, causing peptic ulcer disease (PUD) but also may secrete other hormones such as adrenocorticotropic hormone (ACTH), peptides such as insulin, pancreatic polypeptide, glucagon, chromogranin A, neuron-specific enolase, and the alpha and beta subunits of human chorionic gonadotropin (HCG). The typical presentation is severe abdominal pain, with or without diarrhea. Most children present with complications of peptic ulcer disease (PUD), such as bleeding from an ulcer or duodenal perforation. Laboratory studies to confirm the diagnosis of Zollinger-Ellison syndrome (ZES) measurements of the fasting serum and gastrin levels, secretin and calcium stimulation tests, and measurements of the basal acid output.[6]

The onset of *VIPoma* is insidious. Approximately 60-80% of VI Pomas are malignant and have metastasized at the time of diagnosis. Metastasis occurs most frequently in the liver but may also occur in the lymph nodes, lungs, or kidneys. Clinical diagnosis is based on a history of approximately 10 watery stools per day. The loss of water, sodium, and chloride may lead to volume depletion, dehydration, and exhaustion among patients who are unable to replace the lost fluid and electrolytes.[7] Weight loss and even renal failure have been reported in some patients. Plasma vasoactive intestinal peptide (VIP) levels are determined by radioimmunoassay and in cases of VIPoma, VIP levels are usually 2-10 times the normal range (20-30 pmol/L).

In *glucagonomas* the most common findings include severe dermatitis, necrolytic migratory erythema, mild diabetes, stomatitis, and weight loss.[8] The diagnosis of glucagonoma may be suggested by the clinical presentation and biopsy of the skin lesions, but is secured by the documentation of elevated levels of fasting serum glucagon. Most glucagonomas have been located in the body and tail of the pancreas, typically large.

Somatostatinomas are islet cell tumors that cause nonspecific symptoms like steatorrhea, diabetes, hypochlorhydria and cholelithiasis by releasing large quantities of somatostatin into the blood stream.[9] A fasting blood somatostatin level can be used to confirm the diagnosis of a somatostatinoma. The majority of somatostatinomas have been located in the head of the pancreas.

Functioning tumors usually are small at presentation, and localizing these tumors can be challenging to the radiologist. Hormonal and biochemical parameters are invaluable for skillful interpretation of the imaging and clinical features and to arrive at a specific diagnosis. Nonfunctioning tumors usually are larger and present because of their size or metastatic spread.

The diagnosis is facilitated by the development of specific plasma or serum assays for peptides and amines produced by PNT as well as the development of immunohistochemistry. Chromogranin A is a water-soluble acidic glycoprotein stored in the secretory granules of neuroendocrine cells, and its detection in plasma can be used as a general tumor marker for gastroenteropancreatic neuroendocrine tumors including 'nonfunctioning' tumors.[10] Chromogranin A is a sensitive (70–85%) marker of pancreatic neuroendocrine tumors, but nonspecific test because elevated levels may be find in other types of NENs as well as small-cell lung, and even some prostate carcinomas [10]. The rate of proliferation of a NEN can be quantified by counting the number of mitoses per high powered field on a hematoxylin- and eosin-stained slide, or by counting the percentage of cells that stain positive with the Ki-67 antibody. The Ki-67% has been widely accepted as the cardinal feature of tumor grading WHO NEN classification.[11]

Imagistic diagnosis

The evaluation of the tumor extent and the identification of the exact site of the primary and metastatic lesions are necessary to decide whether a curative surgical approach is possible. Standard abdominal ultrasound, EUS, CT scan, and MRI study are used to assess tumor extent and the possible location of the primary lesion.[12,13]

CT is an excellent modality to detect larger tumors. A hyperdense or isodense mass in the precontrast CT images may demonstrate insulinomas. After IV contrast administration, the tumor reveals intense enhancement with heterogeneity when necrotic areas are present within the mass and hypervascular may sugest glucagonoma. Another method with higher sensitivity than CT is MRI and may offering additional information that characterize islet cell tumors. MRI performed with gadolinium may be more sensitive to tumor vascularity. Plain radiographs have no role in the diagnosis or localization of islet cell tumors, but may reveal thickened mucosal folds in the small bowell from villous hypertrophy in patients with glucagonoma or calcification in a peripheral curvilinear pattern or a central dystrophic pattern in the region of the pancreas. Also, in Vipomas and glucagonomas may show delayed transit on barium studies. EUS is superior to single-slice spiral CT and should replace the latter for preoperative detection of pancreatic insulinomas. However, it is an invasive study and it should be reserved for patients in whom noninvasive modalities fail to localize the expected tumor. Also, EUS guide the biopsy, a well-described technique in the workup of pancreatic lesions in adults and has also been described in children.[11]

Classification

The PNTs are classified according to site, differentiation (well vs. poorly differentiated), a marker of cell proliferation e.g. Ki-67, grade and stage, the hormones or amines produced, and markers of behavior such as chromogranin A [11]. Tumor grade refers to the degree of biological aggressiveness. Tumor stage refers to the extent of spread of the tumor. The extent of invasion into the organ of origin and involvement of nodes or distant sites are critical factors. Prognostic factors include histological grading, tumor differentiation, and the tumor staging. According to the recent World Health Organization (WHO) 2010 classification, three classes of tumors are identified (G1, G2, and G3), Table 1 [11,14].

Table 1. WHO 2010 classification and grading of PNT evaluated in areas of highest mitotic density[11],[14]
(PNT – pancreatic neuroendocrine tumor, hpf - high power field; 10 HPF =2 mm^2, at least 40 fields (at 400× magnification)

Classification/grade	Nomenclature	Mitotic count (per 10 hpf)	Ki-67 index (%)
PNT – G1	Well-differentiated pancreatic endocrine tumor, low grade	**<2**	**<3**

PNT – G2	Well-differentiated pancreatic endocrine tumor, intermediate grade	**2 - 20**	**3 - 20**
PNT – G3	Poorly differentiated pancreatic endocrine tumor, small cell carcinoma or large cell neuroendocrine carcinoma	**>20**	**>20**

Treatment

The treatment and follow up of non-functioning pancreatic neuroendocrine tumors is poorly defined in children. Surgery is the first-line therapy for patients with disease amenable to surgical resection, formal pancreatectomy with conventional lymph node sampling.[15] In case of functioning well-differentiated neuroendocrine tumors, it is important to control the hormone hypersecretion, which determines symptoms, usually by the administration of somatostatin analogues.[12,13] In the Zollinger-Ellison syndrome, the administration of high-dose proton pump inhibitors is necessary, whereas the hypoglycemia typical of insulinoma can be controlled by frequent small feedings and the use of diazoxide.[13] Resection of liver metastasis is the recommended treatment for patients with multiple liver metastases. Besides surgery exists other therapeutic options for pancreatic neuroendocrine tumors like loco-regional therapies, medical therapy including chemotherapy, somatostatin analogs as well as streptozotocin and 5-FU.[16] These therapeutic options have been effective in palliating symptoms and slowing the progression of disease. Chemotherapy may be useful for treatment of localized or metastatic pancreatic carcinoma. The combination of cisplatin and doxorubicin has produced responses in pancreatoblastoma prior to tumor resection.[17,18] Postoperative treatment with cisplatin, doxorubicin, ifosfamide, and etoposide has also produced responses in patients with pancreatoblastoma, although surgery is the mainstay of therapy.[19,20] The palliative treatment may include radiofrequency ablation (RFA), transarterial chemoembolization (TACE).[21] Other promising systemic agents, including sunitinib and everolimus (mTOR inhibitor), have targeted critical PanNET in adults. Everolimus or sunitinib is not recommended in children. [22]

Conclusions

In summary, pancreatic neuroendocrine tumors are generally indolent neoplasms, even though the majority do present at an advanced stage. Once PETs is suspected based on the histologic features, immunohistochemistry plays a critical role to confirm the diagnosis. The 2010 WHO classification of tumors of the digestive system introduces grading and staging tools for pancreatic neuroendocrine neoplasms. Besides surgery and somatostatin analogues treatment, the emerging compounds including chemotherapeutic agents and target therapies may provide new hope for patients with PETs.

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HYPOGAMMAGLOBULINEMIA – MAJOR SIGN IN PRIMARY IMMUNODEFICIENCIES DIAGNOSIS

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Abstract

Hypogammaglobulinemia is defined by decreased gammaglobulins serum level less than 2 SD for age specific normal values, and it can be found at any age. After exclusion of secondary causes, hypogammaglobulinemia represents the major expression for humoral immunodeficiencies or for those with humoral component. These include the most frequent primary immunodeficiencies, being approximately 50% from all of them. The mechanisms are complex and diverse and the clinical picture consists of recurrent, persistent despite the correct treatment, with an unusual severity infections, with encapsulated germs, but also of autoimmune disorders and cancer, with a higher risk than in general population. Laboratory tests are multiple and sophisticate, and the treatment, on one hand, addresses to infectious, autoimmune, malignant complications and on the other hand, in function of the type of immunodeficiency, for infections prevention using regular immunoglobulin administration or for cure of the immune defect trough bone marrow transplantation. An earlier and accurate diagnosis and a correct treatment offer to these patients the chance for a normal life.

***Key words**: hypogammaglobulinemia, primary immunodeficiency, child*

Introduction

Hypogammaglobulinemia is defined by decreased gammaglobulins serum level less than 2 SD for age specific normal values and it can be found at any age. Causes are multiple, secondary (Table 1) or determined by a primary immunodeficiency, hypogammaglobulinemia representing the major expression for humoral immunodeficiencies or for those with humoral component (Table 2). These include the most frequent primary immunodeficiencies, being more than 50% from all of them.

Table 1. Secondary causes of hypogammaglobulinemia

Drug induced	Antimalaric drugs, Captopril, Carbamazepine, Corticosteroids, Cytostatics, Gold compound salts, Penicillamine, Phenitoin, Sulfasalazine
Genetic disorders	Some metabolic disorders, 21 trisomy, 8 trisomy, 22 monosomy, chromosome 18q- syndrome
Infectious disorders	HIV, EBV, congenital CMV infection, congenital rubella, congenital toxoplasmosis
Malignant disorders	Chronic lymphocytic leukemia, thymoma, non-Hodgkin lymphoma, B cell malignancies
Systemic disorders	Immunoglobulin hypercatabolism Nephrotic syndrome Intestinal lymphangiectasia Severe diarrhea

HIV=Human Immunodeficiency Virus; EBV=Epstein-Barr Virus, CMV=Cytomegalovirus

The mechanisms are complex and diverse represented by B lymphocyte (the main actor) maturation defects, in early stage (Bruton disease, some severe combined immunodeficiencies) or in terminal stage (common variable immunodeficiency), defects between B and T cell cooperation (some severe combined immunodeficiencies), immunoglobulin class switch recombination defects (Hyper IgM syndrome) or intrinsic B cell defects. (Table 2).

Clinical picture typical for predominantly antibody deficiencies consists of infections with some characteristics: they are recurrent, persistent despite the correct treatment, with an unusual severity, predominantly occurring in the respiratory and gastrointestinal tract. The infections are caused by pyogenic and incapsulated bacteria: Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginose, also Giardia lamblia and Cryptosporydia species being responsible for gastrointestinal infections.

Unlike patients who have only B cell defects, patients with B + T cell immunodeficiencies have increased susceptibility to opportunist, viral and fungal infections. Also these patients are in increased risk for autoimmune, inflammatory disorders and cancer.

Laboratory tests required for the type of immunodeficiency diagnosis are multiple and sophisticate, including immunogram, IgG subclasses, lymphocyte subpopulations, response to vaccination and even genetic testing obligatory for definitive diagnosis in some primary immunodeficiencies.

On one hand, the treatment addresses to infectious, autoimmune, malignant complications and on the other hand, in function of the type of immunodeficiency, for infections prevention using regular immunoglobulin administration or for cure of the immune defect trough bone marrow transplantation.

Table 2. The main humoral primary immunodeficiencies or those with humoral component and its mechanism of genesis.

Mechanism	Primary immunodeficiency	Indication of IVIg
Predominantly antibody deficiencies		
Defects in early stage of B cell maturation	X-linked agammaglobulinemia Bruton Autosomal recessive agammaglobulinemia	Yes
Defects in terminal development of B cell	Common variable immunodeficiency	Yes
	Selective IgA deficiency	Usually no;
	Isolated IgG subclass deficiency	Only in the case of recurrent infections
	Specific antibody deficiency	Only in the case of recurrent infections
Defects in immunoglobulin class switch recombination due to intrinsic B cell defects	AID deficiency UNG deficiency Other	Yes
Primary immunodeficiencies with humoral component		
Defects in early stage of B and T cell defects Defects in T and B cell cooperation	Severe Combined Immunodeficiency	Yes
Defects in immunoglobulin class switch recombination affecting CD40-CD40 ligand way	CD40 ligand deficiency CD40 deficiency	Yes
Thymus aplasia	Di George syndrome	Yes
Lymphocyte toxicity due to enzyme defects	Adenosine deaminase deficiency Purine nucleoside phosphorylase deficiency	Yes
DNA repair defects	Ataxia-telangiectasia Nijmegen breakage syndrome Bloom syndrome	Yes
CD27+ memory B cell reduction	WHIM syndrome	Yes

IVIg=intravenously immunoglobulin; AID=activation-induced cytidine deaminase; UNG=uracyl-DNA glycosylase

An earlier and accurate diagnosis and a correct treatment offer to these patients the chance for a normal life.

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NEW PERSPECTIVES AND THERAPEUTIC STRATEGIES IN PROTEINURIC CHRONIC STATUS IN CHILDREN

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Abstract

Proteinuria is not a small problem and represents a diagnosis challenge in pediatric pathology. Persistent proteinuria is a marker of chronic kidney disease (CKD), in adults and children equally.[1,2] In children, any condition predisposing to glomerulosclerosis (genetic mutations and podocyte injury, severe infections, tubular ischemic or toxic necrosis, metabolic diseases, congenital anomalies of kidney and urinary tract with consecutive intrarenal stasis, reflux nephropathy, systemic vasculitis, progressive glomerulonephritis, solitary kidney/renal agenesis, unilateral multicystic displastic kidney, etc), could be responsible of inadequately filtrated protein.[3] It is widely accepted that proteinuria reduction is an appropriate therapeutic goal in chronic proteinuric status. Based on large randomized clinical trials, ACE inhibitor (ACEI), and angiotensin receptor blocker (ARB) therapy, have emerged as the most important antiproteinuric and renal protective interventions.[4,5,6] I also introduce some other new clinical strategies and therapies that have been shown to be antiproteinuric and therefore renoprotective.

Keywords: *proteinuria, renourinary pathology, perspectives & therapeutic strategies, children*

Introduction

In pediatrics, proteinuria is a problem both diagnostic and therapeutic. In the last 20 years, there have been remarkable progress in identifying the genetic basis of congenital nephrotic syndrome (CNS) and focal segmental glomerulosclerosis (FSGS). Any condition predisposing to glomerulosclerosis could be responsible of inadequately filtrated proteins in children and in adults equally. Inflammation, reduced number of functional nephrons, any pathological increased of glomerular filtration rate, induces hemodynamic adaptable changes in functioning glomeruli and membrane (GMB) denudation.[7] These mechanisms are essential for formations of adhesions (synechiae) to the Bowman's capsule in glomerulosclerosis. The presence of proteinuria is a well-known risk factor for both the progression of renal disease and cardiovascular morbidity and mortality, and decreases in urine protein excretion level have been associated with a reduced decrease in renal function, and decreased risk of cardiovascular events. It is widely accepted that proteinuria reduction is an appropriate therapeutic goal in chronic proteinuric status.[8,9]

Purpose

In recent years, many specialized publications increasingly covers the pathophyisiological mechanisms responsible for proteinuria, and their management. In connection with this reality, I propose to present an overview of the clinical facts about proteinuria, renourinary pathology and therapeutic strategies in proteinuric chronic status in children.

Material and methods, results

Persistent proteinuria is a marker of chronic kidney disease. Blood pressure together with proteinuria represents one of the most important factors in the progression of chronic kidney failure. In children, any condition predisposing to glomerulosclerosis may be responsible of inadequately amount of protein: genetic mutations and podocyte injury, severe infections, tubular ischemic or toxic necrosis, metabolic diseases, congenital anomalies of kidney and urinary tract with consecutive intrarenal stasis, reflux nephropathy, systemic vasculitis, progressive glomerulonephritis, solitary kidney/renal agenesis, unilateral multiycistic displastic kidney, etc.

Mechanisms of proteinuria

(1) In any glomerular disease there is increased permeability of the glomerular basement to proteins; if filtered protein amount is over tubular handling (tubular reabsorption of albumin capacity), proteinuria is the result.
(2) The loss/lowering of the electrical glomerular barrier (loss of anionic charges) which would allow negatively charged protein to gain access to the urinary space as in nephrotic syndrome with minimal change disease, and congenital nephrotic syndrome of the finish type.
(3) Reducing in fonctional nephron number is responsible of the increasing of the glomerular basement permeability, proteinuria, and increased pressure in the rest healthy nephrons resulting hyperfiltration injury: congenital/surgical unique kidney, kidney donors and progressive kidney disease which associates loss of functional nephrons (cystic kidney diseases).

(4) Increasing in protein concentration at the lower extremities of glomerular capillaries over the limits induces glomerulosclerosis. Proteinuria was detected in patients with high blood levels of angiotensin and this is one of the reason why the treatment with angiotensin converting enzime inhibitors, or angiotensin inhibitors, is recommended in proteinuria. In any diagnosed CKD, in which glomerular filtration rate (GFR) is below 60mL/ min/1,73m^2, the disease obviously progress to end stage renal disease (ESRD).

The glomerulus is the principal responsible of plasma ultrafiltration process. The podocytes, high specialized structures of the glomerular basement, represents the final barrier in urinary protein loss.[10] Podocytes have a tridimensional configuration supported by a complex cytoskeleton Podocytes injury and loss are thought to be the initiating factor leading to glomerulosclerosis. The podocytes loss leads to glomerular hyperfiltration, and hypertrophy of the remaining podocytes. The result consists in additional podocytes stress, and, finally, in scar formation. The earliest genetic defects isolated in corticoresistant nephrotic syndrome are those that encoding the slit diaphragm protein, *nephrin* (NPHS1) and *podocin* (NPHS2). This proteins generate a "zipper-like" multi protein complex of the slit diaphragm, a key structural barrier to loss of protein in the urine (Fig. 1).

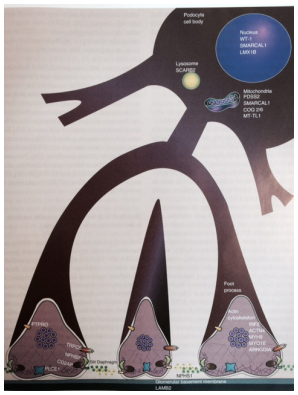

Fig. 1. The Podocite - the three dimensional structure of the podocyte cytoskeleton (Pediatr. Nephrol (2015) 30: 221 – 233.223)

CKD etiology in children differs mostly from those in adults. According to several records concerning CKD etiology in children (NAPRTCS, Italian, Belgian, ESPN/ERA-EDTA, UK Renal, Japanese registries and ANZDATA), the first cause of CKD in children are congenital anomalies of the kidney and urinary tract (CAKUT), hypodysplasia, reflux nephropathy and obstructive nephropathy followed by ,glomerulonephritis, hereditary nephropathy, CNS, metabolic disease, cystinosis, cystic kidney disease, ischemic renal failure, miscellaneous (Table 1).

Table 1 – Selected studies on the causes of chronic kidney disease in children (Pediatr. Nephrol (2012) 27: 363 - 373.364)

	Causes of CKD			Causes of ESRD			
Study [reference]	**NAPRTCS [12]**	**Italian Registry [5]**	**Belgian Registry [13]**	**ANZDATA [27]**	**ESPN/ERA-EDTA Registry [28]**	**UK Renal Registry [29]**	**Japanese Registry [30]**
Population	CKD (GFR < 75)	CKD (GFR < 75)	CKD (GFR < 60)	ESRD (RRT)	ESRD (RRT)	ESRD (RRT)	ESRD (RRT)
Age range	0–20	0–19	0–19	0–19	0–15	0–15	0–19
Patients	Registered 1994–2007	Incident 1990–2000	Incident 2001–2005	Incident 2003–2008	Incident 2008	Incident 2004–2008	Prevalent 1998
Number of cases	7,037	1,197	143	369	499	428	582
Etiology							
CAKUT	3,361 (48%)	689 (58%)	84 (59%)	127 (34%)	182 (36%)	184 (43%)	208 (36%)
Hypodysplasia ± reflux nephropathy	1,907	516	66	95		135	198
Obstructive uropathy	1,454	173	18	32		49	10
Glomerulonephritis	993 (14%)	55 (5%)	10 (7%)	108 (29%)	76 (15%)	78 (18%)	130 (22%)
HUS	141 (2%)	43 (4%)	9 (6%)	9 (2%)	29 (6%)		13 (2%)
Hereditary nephropathy	717 (10%)	186 (15%)	27 (19%)		112 (22%)		69 (12%)
Congenital NS	75	13	5	7		15	34
Metabolic disease			5		17	18	
Cystinosis	104	22	2	4			2
Cystic kidney disease	368 (5%)	101 (8%)	13 (9%)	25 (7%)	59 (12%)	49 (11%)	35 (6%)
Ischemic renal failure	158 (2%)	49 (4%)	3 (2%)	8 (2%)	11 (2%)		11 (2%)
Miscellaneous	1,485 (21%)	122 (10%)	10 (7%)	65 (18%)	52 (10%)	19 (4%)	83 (14%)
Missing/unknown	182 (3%)	40 (3%)		16 (4%)	37 (7%)	65 (15%)	34 (6%)

CKD, chronic kidney disease; ESRD, end-stage renal disease; RRT, renal replacement therapy; GFR, glomerular filtration rate (ml/min/1.73 m^2); CAKUT, congenital anomalies of the kidney and urinary tract; NS, nephrotic syndrome; HUS, hemolytic uremic syndrome; NAPRTCS, North American Pediatric Renal Trials and Collaborative Studies; ANZDATA, Australia and New Zealand Dialysis and Transplant Registry; ESPN/ERA-EDTA Registry, European Registry for Children on Renal Replacement Therapy.

It is widely accepted that proteinuria is an appropriate therapeutic goal in chronic proteinuric status in children. Based on large randomised clinical trials, angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blocker (ARB) therapy, have emerged as the most important antiproteinuric and renal protective interventions. ACE inhibition when started early in CKD is more renoprotective as compared to late CKD stages.[11,12]

There are three levels of recommendations according to clinical studies; the goal of antiproteinuric therapy is to reduce proteinuria ideally to < 300-500 mg/daily.[13]

Level I: Control blood pressure (BP); the goal is a sitting BP in the 120s, or less, if tolerated (usual goal 140/85 mmHg) the greater the proteinuria, the greater the benefit of the low goal . Restrict NaCl intake (in children 1- 2 g/daily), control protein intake (in children 1g/kg/daily) and ACEI therapy (captopril), or combination ACEI and ARB (lorasartan or inrbesartan) therapies. ACEI medication (captopril or enalapril) started as a low dose, and increased as tolerated (0,2-0,6 mg/kg to 1 mg/kg). ARB medication (lorasartan), 0,7-1,4 mg/kg , induces proteinuria reduction; used only in children over 6-7 years of age. Combination ACEI-ARB therapy is more antiproteinuric than ACEI or ARB alone; a good strategy could be, addition of ARB to maximum ACEI in severe proteinuric status as GSFS - steroid non responsive nephrotic syndrome, IgA nephropathy, or Alport syndrome.[14] ACE inhibitors and AT II receptor antagonists are lowering the intraglomerular pressure independent of any change in systemic BP by dilatation of efferent arteriole of the glomerulus.

Level II. Restrict NaCl intake (2g/daily in children). Control fluid intake (higher fluid intake was associated with GFR decline in ESRD) but only in edematous status in children. Control blood lipids: ACEI inhibitors & statins reduce proteinuria in children above 15 years of age suffering of associated dyslipdemia (nephrotic syndromes). Lisinopril therapy may contribute to lipid control in children above 15 years old. It seems to exist a close correlation between an increase in lisinopril dosage: 5,10,15,20 mg/daily, and the fall in urinary protein secretion. In humans, long –term treatment with statins may exert renoprotection that may be partly independent of lipid-lowering effects. FDA issued a statement that statin associated proteinuria does not suggest a toxic effect on the kidneys. Some investigators have found that statin use may be associated with reduced inflammation and tubulointerstitial fibrosis, thus protecting than threatening kidney function.[15] NDH CCB therapy: long–acting dihydropyridines (betablokers) includes *diltiazem&verapamil* are anti proteinuric and may be renoprotective. Aldosterone antagonists: spironolactone prevents the progressive proteinuria in combination with ACEI therapy. Reduce obesity: excessive exercise increase proteinuria but, sufficient exercise avoid thrombosis (low – dose aspirin therapy could be associated) particularly in nephrotic status.

***Level III*:** Antioxidant therapies (antioxidants, d-α tocoferol, vitamin C, α- lipoic acid, decrease proteinuria. Sodium bicarbonate orally administrated is not antiproteinuric but it prevent nonselective proteinuria according with the complement alternative pathway, and correct metabolic acidosis, therefore decreasing protein catabolism. NSAIDs both COX 1-2 (cyclooxygenase-inhibitors) may be administrated in untreatable severe nephrotic syndrome, they can evidently reduce proteinuria. Mycophenolate mofetil (MMF) used as immunosuppressive drug is also antiproteinuric and renoprotective.[16,17,18]

New molecules & new therapies

(1) *CD 20* (B - lymphocite antigen CD 20) is a glicosylated phosphoprotein expressed on the surface of all B-cells (*Wikipedi*a). CD 20 is the target of chimeric monoclonal antibody, *Rituximab*, used in the treatment of corticodependent/corticoresistent nephrotic syndrome and in others FSGS glomerulopathies. *Rituximab* kills the B cells.

(2) *CD 80* (B7-1) is a protein found on activated B cells and monocytes that provide a costimulatory signal necessary for T-cell activation and survival (*Wikipedia*). B7-1 could be found in the podocytes of the patients diagnosed with nephrotic syndrome, FSGS and proteinuria. *Abatacept* (CTLA- 4-Ig) is an inhibitor of T cells costimulatory CD 80 (B7-1) modifications; has beneficial effects on proteinuria, until its disappearance, in corticoresistant nephrotic syndromes and patients with Rituximab-resistant FSGS.[19]

(3) *Endotelin receptor antagonists*: The endotelin system (ET) controls vascular tone and regulate regional blood flow; three isoforms of the endotelin peptide have been described (endothelin-1,-2,-3). Most biological effects of endotelin are mediated by 2 endotelin receptors, ETA si ETB. Endothelin influences cell proliferation and extracellular matrix synthesis, and contributes to the homeostasis of water and electrolytes by direct effects on the kidney. ET system seems to play a pivotal role in hypertension and in proteinuric kidney disease, including the micro-and macro-vascular complications of diabetes.[20,21] *ET receptor antagonists* produce favorable hemodynamic changes that reduce proteinuria. ETA receptor antagonists have the ability to reduce the BP particularly in hypertensive patients with CKD; effect is synergistic to angiotensin –converting enzyme inhibitors ACEI and is abolished by significant concurent ETB receptor blocade.[22] Given the great promise of these drugs in pediatrics, the pharmaceutical industry and academia must work together to conduct long-term studies on cardiovascular and renal outcomes.[23,24,25]

Discutions & Conclusions.

1). ACEI and ARBs should be considered the first–line therapy in children with CKD, particularly those with concomitant proteinuria.

2). According to the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative Working Group guidelines, the goals of anthypertensive therapy have as targets: to decrease BP, to decrease the risk of cardiovascular disease and slow the progression of kidney disease especially in pediatrics.

3). Because proteinuria is associated with both risk of cardiovascular disease and progression to ESRD, and decreases of proteinuria correlate with decreases in cardiovascular morbidity and mortality, and preservation of kidney function, changes in urinary protein excretion level would best reflect the effect of antihypertensive treatment in these patients.

4). Increasing efforts are being made to prevent and treat CKD in children , before serious complications develop during adolescence and adulthood.

5). The final target of this complex subject is to design studies and strategies that directly engage in manipulation of modifiable factors as RAS interventions, new molecules antagonists, ET antagonists, diet, control BP, etc., to promote best retention of renal function in children with progressive renal disease and CKD.

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WHAT LIES BEHIND CHILDHOOD LIPOID NEPHROSIS?

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Abstract

Lipoid nephrosis or idiopathic nephrotic syndrome (INS) is a syndrome characterized by an increase in glomerular permeability followed by massive proteinuria, hypoproteinemia, hypogammaglobulinemia and dyslipidemia. Clinical expression of these biological changes is the installation of massive edema and oliguria. INS etiology is currently unknown but numerous studies are pointing out the connection between INS and atopic diatheses. Based on this finding some authors have tried a diet of exclusion which resulted in decreased proteinuria but not significantly reduced the number of relapses. The link between diet and altered intestinal permeability and the decisive role of diet in the regulation of intestinal microflora and the modulation of intestinal and systemic inflammatory response is currently demonstrated by many authors. The presence in serum of patients with INS of antibodies to food, either IgE, or IgG4 may be considered a marker of increased intestinal permeability, local inflammation and intestinal microbiota disturbance. In our experience, exclusion from the diet of patients with INS of gluten, dairy products and of food for which there is an immune response (sIgE or sIgG4) resulted in a rapid decrease of proteinuria, allowed us to reduce the total length of corticosteroid therapy and prevented relapse of the illness over a period of one year. Changing the treatment protocol from administration of anti-inflammatory medication (glucocorticoid drugs) to restoration of a balanced intestinal microbiota, improvement of intestinal permeability and modulation of the gut inflammatory response must be the primary objective in the management of the child INS.

Keywords: *atopy, gut microbiota, idiopatic nephrotic syndrome, IgG4-related disease, food allergy, podocyte*

Introduction.

Lipoid nephrosis or idiopathic nephrotic syndrome (INS) is a syndrome of unknown etiology characterized by an increase in glomerular permeability followed by massive proteinuria, hypoproteinemia, hypogammaglobulinemia and dyslipidemia. Clinical expression of these biological changes is the onset of massive edema and oliguria.

The link between nephrotic syndrome and atopy. INS pathogenesis is not currently known but is believed to be an immune-mediated process[1,2] with the involvement of LT dependent circulating factors that cause podocyte dysfunction and massive proteinuria.[3] Over time many authors have noticed the connection between INS and atopic diatheses (asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis, etc.) which led to assumptions about involvement of IgE allergy in the pathogenesis SNI, or the existence of possible common causes for both diseases[4,5,6,7,8,9,10]

The main arguments that associate INS with atopic diathesis can be summarized as follows:

1. The incidence of atopic disease is higher in patients with INS than in the control groups. In 1951, Fanconi highlighted that 43% of patients with INS also exhibited atopic disorders.[11] Since then other authors have subsequently confirmed this association, with or without the presence of elevated serum levels of IgE: Thomson (1976)[12], Sandberg (1977)[4], Trompeter (1980)[13], Meadow and Rebien (1981)[14,15], Yap (1983)[16], Lyn (1990)[17], and Yap (2001)[18], Tenbrock (2002)[19], Tain (2003)[20], Salsano (2007)[21], Maher (2009)[2], Youn (2012)[22], Wei (2014)[9].
2. IgE serum levels are much higher in patients with INS than in those with other glomerular diseases.[23] At the same time INS patients may have elevated levels of IgE in the absence of clinical signs of atopy.[21,15]
3. Many reports describe INS relapses after exposure to allergens (pollen, mold, bee stings, administration of vaccines, etc).[24,25,26,27]
4. There is an increase in serum levels of IgE during relapses of INS and a decrease in periods of remission.[28]

Food allergens and nephrotic syndrome. The potential role of food allergens in triggering INS and its relapses is frequently discussed in the literature. Cow's milk proteins, gliadin, ovalbumin, fish, chicken or pork meat are the most frequent allergens linked to INS. The published results are difficult to interpret because they include: patients with steroid-resistant INS, [8,29,30,31] patients with multiple relapsing INS,[4] patients under 1 year old where the diagnosis of INS is questionable,[2] patients in whom renal biopsies revealed other forms of nephrotic syndrome[2]. A number of exclusion diets have been used in patients with INS and food allergies such as elemental diets[4,7] oligo-antigenic diets[29] or diets that exclude food allergens (cow's milk products, gluten)[7,8,32,33]. The reduction of proteinuria after the implementation of a restrictive diet has been reported in most cases,

sometimes even without adding prednisone therapy, but long-term effects and efficiency of diet in preventing relapses are much less consistent. However, some patients with INS have not relapsed after removal of food allergens from the diet.

Common immunological aspects in INS and Atopy. The mechanism of IgE immune reaction is triggered by two main signals: the release of IL-4 and IL-13 by TH2 lymphocytes and the expression of the surface antigens (CD40) by B lymphocytes that are fixed by a ligand on activated T cells (CD28). Kimata[34], Garin[35] and Abdel-Hafa[2] highlights the increased production of IL-13, but not IL-4 in INS. Thus IL-13 levels are elevated in serum and urine of patients during INS decompensation and return to normal after remission occurs. Also serum levels of IL-13 correlates directly with serum IgE levels[28]. Thus the podocytes possess the ability to express receptors for IL-13 and furthermore, to express a binding membrane protein (CD80) with a role in T cell costimulation.[36,37,38] Reiser et al. highlights that the increase of proteinuria in mice correlated with the expression of CD80 membrane protein associated with lining of podocytes suggest that CD80 expression by the podocytes could be a possible mechanism in INS.[38,39] Such research shows that atopy does not play a direct role in the pathogenesis of INS but rather there is a pattern that predispose to both allergic and nephrological disorders. The increase of IL-13 on the one hand induces a shift in production of immunoglobulins from IgM to IgE from B lymphocytes, and on the other hand, induces the expression of CD 80 membrane proteins in the podocytes that is associated with proteinuria[2].

The role of gut and gut microbiota in modulating the host immune response. The human gut, with a total area of approximately 300m^2 in adults, is the place where more than 100 trillion organisms coexist, including bacteria, viruses, fungi, bacteriophages and archaea. Had been established a mutually beneficial relationship between the human body and the enteric microbial flora. Enteric environment supplies intestinal microbiota with nutrients. Furthermore, it contributes to functional balance and body health through degradation of some food components (complex carbohydrates, xenobiotic substances, etc), production of short chain fatty acids, synthesis of vitamins, and by influencing the metabolic profile. Above all gut microbiota plays a crucial role in the development, maturation and modulation the host immune response.[40,41,42,43,44] Microbial colonization of the digestive tract occurs during and immediately after birth with germs consists mainly of Bifidobacterium and Lactobacillus species (bacteria that metabolize lactose).[45,46] Due to the diversity of activities and food diversification the intestinal microbiota becames unstable among children between 1 to 4 years old and after this age it resembles the adult gut flora.[44,46] In healthy people the gut microbiome contains more than 200 types of bacteria belonging to about 100 different species, so the intestinal microbial genome exceeds 100 times that of the host[47,48,49] Gut microbiome of each individual is remarkable constant after the age of 2-4 years and its composition is influenced by genetic programs and a large number of epigenetic factors like: mode of infant delivery, diet, environment, antibiotic exposure, hygiene, stress and other factors.[44] Also it is characterized by resilience, namely spontaneous tendency to return to equilibrium after a period of initial disorganization (e.g. after antibiotic treatments, gastroenteritis, other viral or bacterial infection etc).[40,49,50,51] Thus the intestinal microbiota can be considered a virtual organ composed of 10 times more bacteria than the number of host cells[44].

During the evolutionary process host organisms have developed a complex system of immune tolerance related to intestinal microbiota. In parallel they also developed improved methods of keeping microbial population in the lumen of the gastrointestinal tract and prevention of its penetration in the body's internal environment.[40,44] Qualitative and quantitative balance of intestinal microbial flora can determine the type of local and systemic immune response. Research on animals that grow in germ-free conditions revealed that they have functional-deficient T and B lymphocytes, a low number of CD4 + lymphocytes, a decreased production of immunoglobulin A and deficient intestinal lymphatic tissue. These imbalances can be restored by repopulating the gut with commensal bacteria.[52,53] Involvement of commensal bacteria in modulating the immune response is extremely complex.

Current knowledge can be summarized as follows, without any pretense of completeness: role in the induction of immune tolerance phenomenon[40], role in adjusting the number of protective CD4+ T lymphocytes[43]. *Bifidobacterium spp*. enhance the maturation of the mucosal sIgA system[52]. Segmented filamentous bacteria are required for induction of intestinal TH17. TH17 play an important role in stimulating the production of mucus and antibacterial proteins (at the level of both intestinal mucosa and mucosa of the respiratory tract), enhance cell epithelial tight junctions and contribute to antifungal and antibacterial protective mechanism[40,54]. Early colonization with *Bacteroides fragilis* down-regulates lipopolysaccharide responsiveness in infancy[55]. *Bacteroides fragilis* activate CD4+ T helper cells and promote TH1/TH2 balance[56] through polysaccharide A. *Bacteroides fragilis* and some *Clostridium spp*. promote T_{reg} cells and increase the releasing of IL-10, an anti-inflammatory cytokine that protects against chemically induced colitis[57]. The presence of *Clostridium spp*. during the early life may play a significant role in resistance to allergy and autoimmune diseases[56]. *Clostridium coccoides* and *C. leptum* have a protective action against inflammator y bowel disease (IBD)[58] and are major producers of butyrate, a short chain fatty acid that represent a source of energy for colonocytes and a protection against damaging during local inflammatory response[59,60].

Diet, intestinal microbiota and human pathology. Intestinal microbiota is influenced in proportion of 57% by the diet while genetic factors account for only 12%[61]. If a balanced diet is the promoter of a healthy gut and a balanced microbiota, nutritional imbalances can cause a number of disorders by affecting intestinal homeostasis[60].
A large number of studies highlights diet-induced changes on intestinal microbiota[60]:

- High fat diet decreases the population of *Bifidobacterium*
- Diet high in fat and sugar increases population of *Clostridium* difficile, C. *perfringens* and *Enterococcus spp*. and while decreasing the population of *Bacteroidetes spp.*
- Vegetarian diet prevents the growth of pathogenic bacteria such as *E. coli* and other members of the *Enterobacteriaceae* family.
- Eating complex carbohydrates increases the number of beneficial *Bifidobacteria spp.*
- Caloric-restriction diets prevent the growth of *Clostridium coccoides*, *Lactobacillus spp*. and *Bifidobacteria spp*. - the main butyrate producers that play an essential role in the nutrition and homeostasis of enterocytes.

It is now known that "Western diet" (high in fat and sugars) induces intestinal dysbiosis with impaired gastrointestinal cell metabolism and disruption of immune homeostasis[62]. Also, gut microbiota in European children is lower in *Bacteroidetes* and higher in *Enterobacteriaceae* compared with African children, due to a low intake of fiber[63].

Unbalanced diet cause disruption of intestinal microbial flora and induce: an increased production of mucus, alteration of intestinal motility, enterocyte dysfunction (by reducing the intake of nutrients) and local inflammatory reactions. This results in the appearance of a leaky gut syndrome: an alteration of intestinal permeability and loss of functional barrier of the gut to intraluminal environment and triggers local and systemic inflammatory reactions. Altering of the TH1/TH2 ratio in allergies and associated disorders and mucosal TH17 overstimulation with increased mucosal permeability and podocytes dysfunction are examples of pathogenic mechanisms with intestinal starting point. Asthma and allergic inflammation, Type 1 diabetes, metabolic syndrome, IBD, celiac disease, acute pancreatitis, psoriasis, rheumatoid arthritis, ankylosing spondylitis, ADHD, multiple sclerosis, schizophrenia and mood disorders, some forms of autoimmune encephalitis or cancer (glioma, human lung squamous cell carcinoma, pancreatic carcinoma, hepatocellular carcinoma) are examples of diseases presently connected with microbiota disruption[40,44,60,64,65]. And the range of diseases is growing. The whole concept of the pathogenesis of autoimmune and inflammatory diseases is currently under review by identifying the crucial role of tight junctions in: regulating intestinal antigens traffic along the intestinal barrier, in the process of immune tolerance and by revealing the interaction between intestinal epithelium - neuroendocrine system – immune system - intestinal microbiota[65,66,67].

Immune reactions to food. Immune reactions to food are of several types. They can be both immune and non-immune related. IgE immune reactions to food are well known and studied, however, regarding non-IgE mediated immune reactions. The latter may be associated with a large variety of glandular, digestive, renal, respiratory, skin and vascular symptoms. Sometimes the two types of immune responses may coexist, in conditions such as atopic dermatitis.

IgG4 immune reactions to food (also called food intolerance) is a controversial topic nowadays, most allergists considering that the presence of IgG4 represents no more than a marker for the installation of immune tolerance to a particular food. Therefore, *The European Academy of Allergy and Clinical Immunology* drew the following conclusion: „IgG4 against foods indicates that the organism has been repeatedly exposed to food components, recognized as foreign proteins by the immune system. Its presence should not be considered as a factor which induces hypersensitivity, but rather as an indicator for immunological tolerance, linked to the activity of regulatory T cells"[68]. A series of data however come to contradict this belief:

1. Results from IgG4 panels made in patients with various symptomatology highlight a growth in IgG4 antibodies titers only for a limited number of foods. In general these foods are about the same for all patients and are well-known for their potential of inducing food allergy. In our patients with INS the top foods that cause IgG4 type immune reactions are: bananas, cow's milk, wheat, rye and kiwi.
2. In some situations, the same patient shows an increase in both IgE and IgG4 antibodies.
3. Despite conventional opinion which considers IgG4 molecules as anti-inflammatory, this Ig is currently found to be involved in a number of immune-mediated disorders[69].
4. The production of both IgE and IgG4 is controlled by TH2 lymphocytes. IL-4 and IL-13 amplify the synthesis of both types of Ig, whereas IL-10 mediate the shift in the synthesis from IgE to IgG4[70].

It has been already recognized the involvement of IgG4 in diseases such as: atopic dermatitis, thrombotic thrombocytopenic purpura, pemphigus, glandular pathology (salivary, lacrimal, and thyroid), chronic inflammatory processes of the aorta and great vessels, respiratory tract, orbit, mediastinum, retroperitoneum, kidney, etc.[69]. In some circumstances IgG4 has also rheumatoid factor activity. In many pathologic circumstances IgG4 and IgE growth occurs simultaneously.

IgG4 and renal pathology. Although still in its infancy, several research already established a role of IgG4 in immune-related pathology. Regarding renal pathology it is currently known the involvement of IgG4 autoantibodies in membranous glomerulonephritis (MGN), where they act like auto-antibodies against phospholipase A2 M-type receptor found on the podocytes[60]. Tubulointerstitial nephritis (IgG4-TIN) with increased IgG4-positive plasma cells and fibrosis is another feature of IgG4 related kidney disease and may cause acute or chronic renal dysfunction[71]. In some patients IgG4-TIN might present concurrently with MGN or with other fibroinflammatory conditions. It is also known that the production of IgG4 is mediated by TH2 lymphocytes as well as the production of IgE, with IL-13 playing an essential role. Meanwhile IL-13 is involved in the pathogenesis of INS in children through binding to a podocytes membrane protein (CD80), followed by the appearance of

proteinuria[36,37,38,39]. The pathogenic triangle: IgE – IgG4 – podocytes dysfunction takes on this way new meanings. It remains to be determined whether there is a causal relationship between these conditions or they are based on a common cause.

We allow in this moment to issue the following hypothesis: *several genetic and epigenetic factors (individually or in association) lead to the disturbance of intestinal microbiota and alter the local and systemic immune response (among them a switch in the TH1/TH2 ratio and mucosal TH17 overstimulation). Alteration of the intestinal barrier creates conditions for entering into circulation of incompletely digested food structures that become antigens. These antigens stimulate TH2 production of pro-inflammatory cytokines IL-4, IL-13. They induce hyper IgE and IgG4 with related symptoms. In turn, IL-13 levels by binding to podocyte membrane receptor CD80 generate dysfunctions in the endothelial cells barrier and the appearance of proteinuria. INS may also represent not so much a dysfunction of podocytes, as an adaptive pathway to remove the excess of pro-inflammatory molecules.*

Discussions

Atopy is unlikely to have a role in the pathogenesis of INS, first, because allergic immune response is a type of immune reaction limited to the contact surfaces of the body with the outside environment, where the body comes in direct contact with allergens, in order to limit the penetration of the allergen into the body. This type of immune response is pointless in the kidney whose function is to remove endogenous substances. However frequent association between atopy and INS and the proof of efficiency of exclusion diets (even partially) in decreasing proteinuria and induction of remission lead towards a common cause of both types of disorders.

Intestinal microbial flora represents a "virtual organ" whose role is crucial in modulating the immune response. Altered intestinal microbiota is able to induce a local and systemic inflammatory response and to create hyperpermeability of the intestinal mucosa. Remotely, there are evidences of increased permeability of the podocyte lining in the Bowman's capsule, in the airway epithelium and even alterations of the blood-brain barrier.

The enhanced systemic inflammatory response is responsible for altering the TH1/TH2 balance with the production of IL-4 and IL-13 and overstimulation of TH17 lymphocytes with increased production of IL-17, IL-21 and IL-22. From here to the impaired function of podocytes in the renal glomeruli is just a step. In fact, we ask ourselves if in the conditions of a systemic inflammatory response INS is a pathological process or a defense mechanism intended to eliminate excess of circulating pro-inflammatory mediators thus protecting the rest of the organs and systems. From this pathogenic perspective, the limited success of nutritional interventions implemented so far in INS is understandable. Removing food immune aggression is followed naturally by a decrease in inflammatory response and decrease in proteinuria. But in the absence of restoring the local microbiota and the intestinal permeability, nutritional intervention become only partial and ineffective. Moreover, a number of studies show that administration of cortisone type medication increases intestinal permeability and amplify the host allergic immune response and the production of IgE. This may explain the existence of numerous cases of multiple-relapsing, corticodependent or corticoresistant INS.

Current studies about INS focus on IgE allergic reactions to food, ignoring other type of immune-reactions like IgG4 antibody production. This may be another explanation why exclusion diets have failed in some published studies.

In our patients with INS at onset we have achieved remission, early normalization of proteinuria and we have prevented relapse for a period exceeding one year by intervention especially on the digestive tract. Because we haven't found any correlation between IgE and IgG4 reaction to food in the same patient the exclusion diet targeted both IgE and IgG4. In addition, we focused therapy on restoring gut microbiota in order to reduce inflammation and intestinal permeability.

The current trend to extend corticosteroid therapy in hopes of obtaining more prolonged remissions can be dangerous and may paradoxically increase the duration, severity and number of relapses because: corticosteroid medication increases IgE serum levels in patients with atopy,[72] induce production of IgE and IL-4 in purified B cells from patients without atopy,[73] increase the gut permeability by affecting the epithelial lining of the gut.

Conclusion.

In conclusion, a shift from administration of steroid anti-inflammatory medication to restoration of intestinal microbiota, improvement of intestinal permeability and modulation of the inflammatory response at the intestinal level must be the primary objectives in the management of the child with INS. An initial diet of exclusion is mandatory but the emphasis should be on food for which there is food intolerance reactions (IgG4) and not IgE mediated ones. Exclusion of dairy and gluten should be part of the nutritional plan even if there are no allergies or food intolerances to these components. Both are irritants of the gut, stimulate excessive mucus production, favor the multiplication of pathogenic bacterial strains, maintain increased intestinal permeability, and reduce recovery of intestinal and systemic inflammatory processes. Adherence to a schedule of eating is also essential. Abundance and diversity of fresh and unprocessed natural products provides the necessary macro and micronutrients while the high content in prebiotic, fiber and antioxidants of fresh fruits and vegetables restore the intestinal microbiota, regulate intestinal motility and reduce local and systemic inflammatory processes.

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PRIMARY IDIOPATHIC POLYMYOSITIS. CASE REPORT

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Abstract

Introduction. Polymyositis is an inflammatory muscle disease, with autoimmune character.

Aim: To present the case of a 15 years adolescent with myopathy.

Case report: A 15 years old male patient was admitted to the hospital complaining of severe muscle pain in the arms and legs. Clinical exam revealed: warm teguments, muscular hypertonia, limited flexion of the legs on the thighs, limited extension of the arms and rising of the arms over the head. Laboratory findings: CK = 603 U/L, LDH = 999 U/L, ESR = 30 mm/h; CRP = 46.2 mg%. The neurological exam found: secondary inflammatory myopathy. EMG: hyper-voltage potentials with short duration, increased pattern of recruitment, suggestive for a myopathy. Muscle biopsy revealed: local atrophy of muscle fibers, endomysium inflammatory lymphocytic infiltrate and perivascular infiltration. Myositis-specific antibodies (Anti-Mi-2, Anti Jo-1, Anti SRP, Anti Pl-7, Anti Pl-12, Anti EJ, Anti OJ) and myositis-associated antibodies (Anti Ku, Anti Pm-Scl 100, Anti Pm-Scl 75, Anti Ro-52) were all negative. The results of the investigations correlated with the clinical context leaded to the diagnostic of Primary idiopathic polymyositis. Pulse corticosteroid therapy with intravenous Methylprednisolone was initiated (1 g per day for 3 days), and then orally corticosteroid therapy with Prednisone (80 mg per day, with progressive decrease of the dose). The symptoms remitted after therapy, the muscular enzymes normalised but the inflammatory syndrome persists and so does the EMG myogenic route.

Particularity of the case: male patient with negative specific antibodies and arthropathic onset.

Keys words: *polymyositis, teenager, arthralgia*

Introduction

Idiopathic inflammatory myopathies form a group of autoimmune muscular pathologies characterized by muscle inflammation and progressive muscular weakness. The cause of this myopathy it is still unclear, but it is general accepted the theory conform with there is an individual genetic predisposition while some trigger factors action upon it. The diagnostic it is based on EMG, clinical and biological findings, but the muscle biopsy confirms the final diagnostic.[1] Corticotherapy is the first line of therapy, although its side effects limit the possibility of using it. The additional treatment with immunosuppressant (Azathioprine, Methotrexate, Cyclosporine) is often used. The association between these two therapies may improve the therapeutic results and also allows the reducing of corticotherapy dose, helping so to prevent long-term complications. One of the main important side-effects is the myopathy induced by corticotherapy.[2,14]

Case report:

We present the case of a male patient, 15 years old, who complains of muscle fatigue when he initiates the active move, especially after long period of repose, associated with muscle pain and severe asthenia. Positively, six months ago, the patient presented edema at his left knee and muscular weakness. The treatment with antibiotics and topical anti-inflammatory cream was initiated. Initial, the symptomatology was ameliorated but after a week, the edema re-appears in the right knee this time, without local inflammatory signs. The edema remits itself spontaneously in 3 – 4 days. One month later, edema occurs in the tibio-tarsal joints.

At admission, the clinical exam revealed: warm teguments, signs of juvenile acne on the face and the back side, muscular hypertonia, limited flexion of the legs on the thighs and difficult extension of the arms, walking on stairs and raising the arms over the head.

The laboratory findings (directed on the muscle disease) revealed: elevated levels of muscular enzymes: creatine-kinase (603 U/L) and lactate dehydrogenase (999U/L), and also elevated level of CRP (46.2 mg %) and accelerated erythrocyte sedimentation rate (30 mm/h). Anti-nuclear antibodies and myositis-specific antibodies (3) (Anti-Mi-2, Anti Jo-1,

Anti SRP, Anti Pl-7, Anti Pl-12, Anti EJ, Anti OJ) and myositis-associated[3] (Anti Ku, Anti Pm-Scl 100, Anti Pm-Scl 75, Anti Ro-52) were all negative.

The neurological exam established the preliminary diagnosis of secondary inflammatory myopathy (walking on heels was difficult, the osteotendinous reflexes were hyporeflexive; the patient accused pain bilaterally in the triceps and quadriceps muscle and also pain when standing up. The Gowers sign was positive).

The electromyography revealed hyper-voltage potentials with short duration, with increased pattern of recruitment, suggestive for a myopathy.

The muscle biopsy was required. Using the hematoxylin-eosin stain (Fig. 1.) discreet endomysial and perivascular inflammatory lymphocytic infiltrate and, extended areas of degenerescence, discreet atrophy of muscular fibers, characteristic for a myopathy as a myositis-type. Using immunohistochemistry techniques: discrete lymphohistiocytic focal infiltrate, perivascular, endomysial and rare atrophic muscular fibers, rare CD 68 positive cells interstitial (Fig. 2.), rare LCA interstitial (Fig. 3.) (more numerous than CD 68 positive cells). The aspect was inconclusive because of the focal and discreet inflammatory infiltrate, but suggestive for an inflammatory myopathy (myositis).

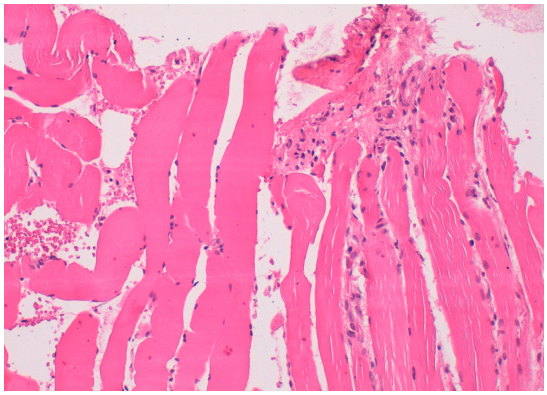

Fig. 1. Detail with rare inflammatory mononuclear cells infiltrate. Muscle fibers homogenization. HEx200

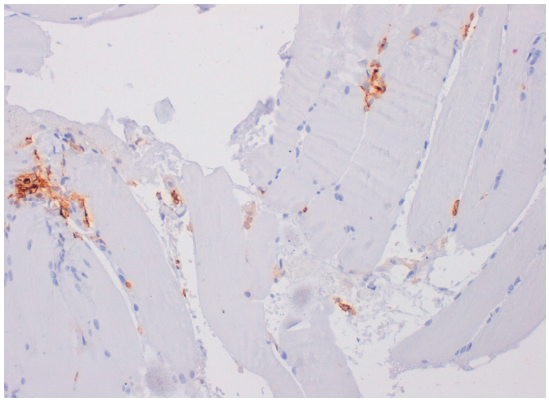

Fig. 2. Inflammatory cells marked with common antigen leucokite (LCA), anti-LCA. DAB+HE stain x 200

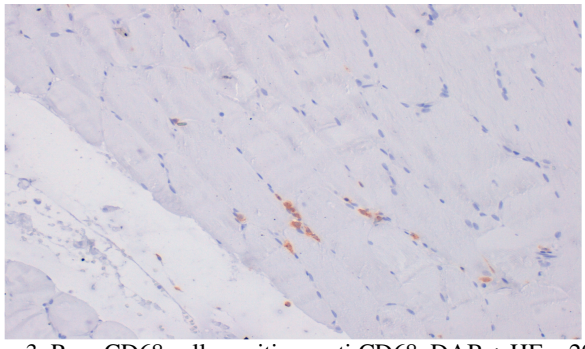

Fig. 3. Rare CD68 cells positive, anti CD68. DAB + HE x 200

Diagnosis. Treatment

The major pathologies that define the idiopathic inflammatory myopathies are: Polymyositis (PM), Dermatomyositis (DM) and inclusion body myositis (IBM).[4] Even if generally Primary idiopathic polymyositis is considered a diagnosis of exclusion, the results of the investigations correlated with the clinical context leaded to this diagnosis (Table 1).[5]

Table 1 – Bohan and Peter criteria for PM [5]

1.	Symmetrical weakness of the limb girdle muscle and anterior neck flexors, progressing over weeks to months, with or without dysphagia or respiratory muscle involvement
2.	Muscle biopsy evidence of necrosis of myofibers, phagocytosis, regeneration with basophils, large vesicular sarcolemmal nuclei, and prominent nucleoli, atrophy in a perifascicular distribution, variation in fiber size and an inflammatory exudate, often perivascular
3.	Elevation in serum of skeletal-muscle enzymes, particularly the CK and often aldolase, aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase
4.	EMG triad of short, small, polyphasic motor units, fibrillations, positive sharp waves and insertional irritability, and bizarre, high frequency repetitive discharges

Diagnosis: positive= 4/4; probable= 3/4; possible= 2/4

Early diagnosis of PM and aggressive treatment with corticotherapy are the key to obtaining remission.[6] In this context the goals of the therapy are: 1. To improve muscle weakness and 2. To avoid the development of extra-muscular disease of the vital organs.[7]

Pulse corticosteroid therapy with intravenous Methylprednisolone was initiated (1 g per day, 3 days) and then, corticosteroid therapy orally with Prednisone (80 mg per day, decreasing the dose with 5 mg every 5 days); in addition, Esomeprazole was given for the gastric protection. Adjuvant non-pharmaceutical therapy, is also very helpful. Physiotherapy is represented by exercises aimed to improve the muscle straight, measures to prevent the aspiration and also general supportive care.[8] With therapy the patient`s evolution was good. At discharge the patient was able to do the flexion of the leg on thighs and the extension of the arms, he was able to rais his arms in vertical position. The biological investigations (inflammatory syndrome and muscular enzymes) normalised.

After 2 weeks when he completed prednisone-therapy, the clinical symptoms dissappeared, the muscular enzymes normalized but the inflammatory syndrome was high (Table 2) and the EMG myogenic aspect persisted.

Table 2 – The evolution in time of skeletal-muscle enzymes and of the inflammatory process

	First admission (presumptive diagnosis)	Control (10 days after the onset)	Before starting the therapy	10 days with therapy	At the end of the therapy
LDH (u/l)	999	432	369	180	189
CK (u/l)	434	603	49	15	35
CKMB (u/l)	27	27	16	26	12
ASAT (u/l)	72	92	39	12	18
ESR (mm/h)	30	31	43	5	61
CRP (mg %)	46.2	29.5	35	2.9	52

Discussion

The case, posed some difficulties in the interpretation of clinical and biological findings and the diagnosis. Is it the case of an Idiopathic Inflammatory Myopathy or we ae facing a case of Primary Idiopathic Polymyositis?

No matter which of the classification/diagnosis criteria we consider, an important number of patients will defy, through their clinical and paraclinical finding, the protocol of specific diagnosis of some pathologies.[4]

With negative specific and associated antibodies,[3,12] with EMG test and muscle biopsy non-concludent, but suggestive for a myopathy and with an arthropathic onset, in our case the diagnose was established after we had excluded all other types of myopathies.[8] The infectious (viral, bacterium), endocrinology and metabolic causes were excluded by their specific biological values which were normal. The differential diagnosis between PM and muscular dystrophy, is difficult, both pathologies being characterized – in the incipient stages – by chronicle muscle weakness associated with inflammation and similar clinical manifestations. The imagistic tests (MRI, muscular Doppler echography) were not performed. The main important criteria, to differentiate between PM and muscular dystrophy, is the histopathological exam, which in muscular dystrophy are re[presented by large zones of muscular atrophy.[10, 11]

The patients with PM need to be monitored periodically when the corticosteroid doses are high. The creatine-kinase level needs to be determined in order to evaluate the answer to therapy. Initial, the discharged patients must be monitored every 3 weeks, and, once they are stable, the monitoring (mandatory) will be performed monthly.[9]

Case particularity. It is well known the fact that autoimmune diseases are more frequent in female patients. Our case, is a male patient with an autoimmune muscle disease, with specific and associated negative antibodies and with an onset that was suggested for an arthropathic disease.

Conclusions

This complex case, with many questions that need to be answered, reminds us of the importance to integrate the results of the investigations in the clinical context, especially when the laboratory data cannot identify with certitude the pathology involved. It is important to understand that in this cases the preliminary diagnose may not coincide with the final one. The continuous monitoring of the patient is very important, so we that therapy could be adjusted as needed for the good of the patient.

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RELATIONSHIP BETWEEN HERPES SIMPLEX VIRUS TYPE 1 AND EXTENSIVE CEREBRAL SINOVENOUS THROMBOSIS IN A CHILD WITH INHERITED HYPERCOAGULABLE STATES

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Abstract

Cerebral sinovenous thrombosis (CSVT) is a rare, but potentially serious condition among children which requires a multidisciplinary team approach. Clinical manifestations of CSVT include: headache, vomiting, photophobia, blurred vision, focal or generalized seizures, motor deficits, altered mental status and coma.

Objective: Exposure clinical data, laboratory and management in cerebral venous thrombosis in children- a clinical case report.

Material and methods. We present a case of 14 months - old boy, admitted in ICU National Institute for Infectious diseases "Prof. Matei Bals" for: fever, vomiting,focal and generalized seizures, right hemiparesis and coma. The child was diagnosed with acute herpetic gingivostomatitis one week before onset of neurological symptoms.

MRI brain and angiography revealed extensive thrombosis of the straight, transverse and occipital venous sinuses, bilateral thalamic infarction and left haemorrhagic transformation with intraventricular hemorrhage and univentricular obstructive hydrocephalus.

Thrombophilic screening was performed and a heterozygous mutations genes of C677T MTHFR and 4G/5G PAI was detected. He was treated with low molecular weight heparin (enoxaparine) followed by oral anticoagulant (acenocumarol) with good clinical outcome and complete neurological recovery.

Conclusions: CSVT in children can be fully reversible with early diagnosis and a prompt management. Brain MRI with angio MRI remains the gold standard for diagnosing CSVT.Thrombofilic screening should be considered in any child with stroke history and CSVT. Herpes simplex virus associated infections may precipitate thrombosis in individuals with inherited or acquired hypercoagulable states

Key Words: *anticoagulant, cerebral venous sinus thrombosis, magnetic resonance angiography, inherited hyper- coagulable state*

Introduction

Cerebral sinovenous thrombosis (CSVT) is a rare but potentially serious condition in children, involving a multidisciplinary team approach**.** CSVT is defined by thrombosis within the superficial (cortical veins, superior sagittal sinus, sigmoid sinus, transverse sinus and jugular vein) or deep (inferior sagittal sinus, straight sinus internal cerebral veins, vein of Galen) venous system.[2] Clinical manifestations of CSVT include: headache, vomiting, photophobia, blurred vision, focal or generalized seizures, motor deficits, altered mental status and coma. CSVT occurs in about one of 100,000 children per year including, neonates and is the most important and frequent cause of pediatric stroke, with a high rate of mortality (8-19%) and severe long-term neurological sequelae, that are reported in 38 up to 48 % of patients.[3]

CSVT in children is a multi - factorial disease, which, in the majority of cases, results from a combination of pro-thrombotic risk factors and underlying clinical conditions, that may consist of infections, anemia, dehydration, cranial trauma, systemic diseases, cardiac or renal diseases, malignancies and their treatment regiments (drugs and/or radiation associated procedures).[1] The diagnosis should be established as soon as possible after the symptoms onset in order to obtain good clinical progressions, with reduced mortality and complete/partial neurological recovery.

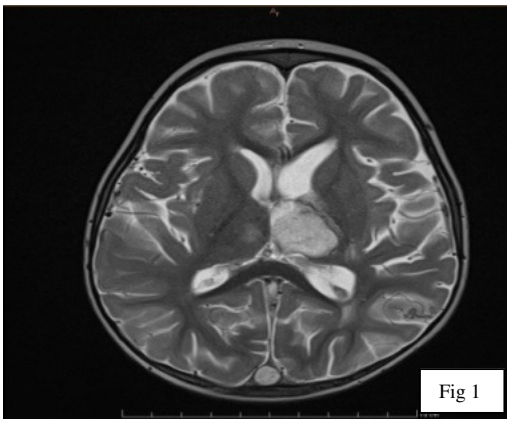
Clinical case

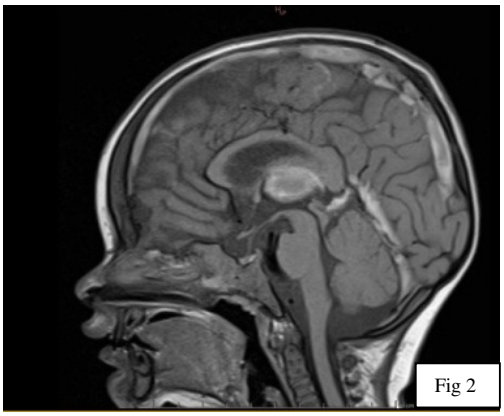
We present a case of a 14 months - old boy, admitted to the Pediatric Intensive Care Unit (pICU) at The National Institute for Infectious diseases "Prof. Matei Bals" - Bucharest in November 2014 for: fever, vomiting, focal and generalized seizures, right hemiparesis and coma.The child was diagnosed with acute herpetic gingivo - stomatitis one week before the onset of neurological symptoms. The patient was initially admitted to Curtea de Arges Hospital (Pediatric Compartment) where he received antibiotic therapy, intra-venous fluids therapy, antipyretics with unfavorable clinical and neurological evolution. The child experienced repeated vomiting, irritability and drowsiness, high fever, weakness, partial and generalized

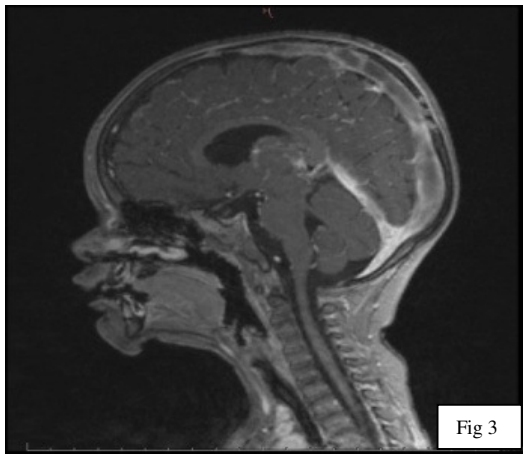
seizures with neurological deterioration and coma. He was transferred to the pICU at the National Institute for Infectious diseases "Prof Dr Matei Bals" - Bucharest. His family medical history revealed: maternal grandfather died of myocardial infarction, maternal grandmother died of stroke and mother has a history of miscarriage at 25 week gestational age.

On admission, the clinical examination revealed: altered general status, normal body temperature, comatose with GCS 10/15, the eye exam revealed pupils equal in diameter, round, reactive to light; anicteric sclera, pale skin, vesicular rash around the mouth, he presented spontaneous breathing, no rales were noted over the lung area, cardiac rhytm was regular, no extra-beats or murmurs were identified during the initial evaluation, heart rate - 120-130 beats/min, blood pressure - 90/58 mmHg; no cianosis; oxygen saturation measured by pulse oximetry was 97% in room atmosphere, multiples ulcerations on the gums, lips, tongue and oral mucosa were observed, with hyperemia and active hemorrhage of the gums, halitosis; the abdominal exam presented: symmetric, soft and non tender abdomen, no hepatosplenomegaly was discovered; physiological urination present; the neurological exam described: stiff neck, right hemiparesis, with reactivity to nocicepttive stimuli.
Laboratory blood tests showed increased leukocyte count, mycrocitic - hypocrome anemia, hypo-sideremia, no biological inflammatory syndrome associated, no coagulation disturbance.

Serological tests using Elisa immunosorbent assays (ELISA) came back positive for Herpes Virus for the acute (IgM) anti-body subclass (with rising antibody titers - 3 samples collected in dynamics Brain angio- MRI studies revealed extensive thrombosis of the straight, transverse and occipital venous sinuses, bilateral thalamic infarction and left hemorrhagic transformation with intra-ventricular hemorrhage and uni-ventricular obstructive hydrocephalus. (fig 1, 2, 3, 4)







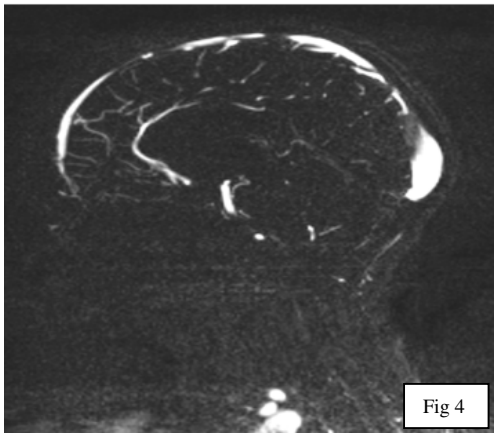


Fig. 1. Brain MRI showed bilateral thalamic infarction and left hemorrhagic transformation with intraventricular hemorrhage and uni-ventricular obstructive left hydrocephalus with transependymal resorption edema by obstruction foramina of Monro with mass effect

Fig. 2 and 3. Brain MRI showed extensive thrombosis (loss of normal signal intensity) of the straight, transverse, superior sagittal and occipital venous sinuses and intra-ventricular hemorrhage

Fig. 4. Magnetic resonance brain angiography showed extensive thrombosis of the transverse, occipital, superior sagittal and straight venous sinuse.

Anticardiolipin antibody, anti-thrombin III, protein S, protein C, lupus anticoagulant were in normal range. Thrombophilic screening was performed and a hetero-zygous mutations of the genes C677T MTHFR and 4G/5G PAI was detected. Due to focal neurological signs and active intraventricular homorhage the lumbar puncture was not performed.

He was treated with Aciclovir i.v, anti-convulsivant drugs in order to control seizures, anti cerebral-edema measures were implied - consisting of intravenous 20% mannitol and dexamethasone, i.v human non- specific immunoglobulins were administered. We decided to delay the initiation of anticoagulant treatment because of signs of active bleeding observed MRI evaluation and para – clinically supported by the constant drop of the hemoglobin and hematocrite values.

The clinical and neurological evolution was good, but subsequent brain angio - IRM reevaluations showed CVST progresion to superior sagittal sinus and related cortical veins. Anticoagulant therapy was started with low molecular weight heparin (Enoxaparine) followed by oral anticoagulant regimen (acenocumarol) with good clinical outcome and complete neurological recovery. Controle brain IRM showed repermeabilisation of all venous sinuses. The patient was discharged after 8 weeks, on oral anticoagulants, with the recommendation to weekly monitor the coagulation parameters and was addressed to a pediatric hematology department in order to stratify his residual risk factors and to receive long term recommendation due to his thrombophilic status.

Discusion:

We suggest that anticoagulation should be considered in all children with CSVT without active intracranial hemorrhage and that is should be used with caution in the presence of proven intracranial hemorrhage.[4] Early repeated brain angio - IRMs studies in order to screen for CSVT propagation, in children that are treated with a conservatory approach in regard to the anti-coagulation regimen is absolutely necessary for a successful and correct management. [4]

Still, it is difficult to affirm whether the herpes virus itself, the inflammatory process itself, the dehydration or the iron deficiency anemia in association with the inherited hypercoagulable states predisposed our young patient to cerebral venous thrombosis. A balanced clinical reasoning in association with brain angio-IRM studies are necessary for an accurate and early diagnosis of CSVT. On the other hand, the herpes virus infection can trigger CSVT in a patient presenting with an underling hypercoagulable states.

Conclusions:

1. CSVT in children can be fully reversible with early diagnosis and a prompt management
2. Brain MRI with angio MRI remains the gold standard for diagnosing CSVT.
3. Thrombofilic screening should be considered in any child with strokes and CSVT.
4. Herpes simplex virus may precipitate thrombosis in individuals with inherited or acquired hypercoagulable states

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INVASIVE PNEUMOCOCCAL DISEASE IN HIGH RISK PATIENTS

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Abstract

Introduction. The clinical and epidemiological burden of Invasive Pneumococcal Disease (IPD) is still very increased. In the pathogenesis of Invasive Pneumococcal Disease there are three essential elements involved: nasopharyngeal colonization, the inflammatory response of the host and also the effect of viral and bacterial co-infections on pneumococcus virulence.

The highest susceptibility for IPD is found in children less than 5 years of age, especially in those under 2 years of age. Concerning the risk factors for IPD, the most important are congenital malformations or trauma of the skull with rhinoliquorrhea and otoliquorrhea, along with immunodeficiency and other chronic conditions.

Material and method: This paper highlights skull malformations with corticospinal fluid (CSF) fistula as a risk factor in developing IPD, like sepsis or recurrent bacterial meningitis. During 2014-2015 in our clinic there have been 3 cases of sepsis with meningitis with Streptococcus pneumoniae in children with CSF fistula. We report the case of a 4-year-old patient with sepsis and recurrent meningitis with Streptococcus pneumoniae serotype 6B, resistant to Penicillin, with repeated surgical interventions for naso-frontal CSF fistula with rhinoliquorrhea.

Conclusions: The diagnosis and treatment of these affections need considerable effort from a multidisciplinary medical team with the ultimate goal of making a quick recovery of the patient and avoidance of recurrence. During 2010-2013, ACIP (Advisory Committee on Immunization Practices) published new recommendations for vaccination with PCV13 and PPSV 23 regarding patients with high risk factors for IPD, highlighting the ongoing clinical burden of this affection.

***Key words**: CSF fistula, child, invasive pneumococcal disease, rhinoliquorrhea*

Introduction

Streptococcus pneumoniae is a major cause of severe invasive infection like meningitis, bacteremia and pneumonia with bacteremia/ empyema. Children less than 5 years of age, and especially less than 2 years of age, are most susceptible to pneumococcal infections, due to the immaturity of their immune system, frequent exposure and colonization with Streptococcus pneumoniae.[1]

Amongst the 93 different serotypes of Streptococcus pneumoniae (grouped in 46 serogroups) identified based on antigenic differences in the structure of the polysaccharide capsule, 10 serogroups are responsible for the majority of cases of invasive pneumococcal disease . The last serotype described was 11E.[2,3,4] The most frequent serotypes involved in IPD in children are 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F.[1,5,6] The incidence of invasive infections, due to these serotypes, varies significantly according to age of the population, ethnicity, seasonal and geographic distribution.[1]

Factors that increase the risk for invasive pneumococcal disease are chronic conditions (chronic heart disease, chronic lung disease, diabetes mellitus, chronic renal disease, nephrotic syndrome), congenital or acquired immunodeficiency, functional or anatomic asplenia, cochlear implant, CSF fistula and some specific ethnic groups.[1]

Case presentation

We report the case of a 4 year-old boy admitted to our intensive care unit for high fever (39 C), vomiting, headache and meningeal syndrome, symptoms which started on the day of admission. His personal medical history revealed one episode of pneumococcal meningitis at the age of 1 year, idiopathic intracranial hypertension syndrome diagnosed at the age of 2 years and CSF fistula with rhinoliquorrhea diagnosed at the age of 3 years. One surgical intervention was performed for the closure of the CSF fistula, but after 1 month rhinoliquorrhea reappeared.

At the time of past clinical presentations with intracranial hypertension syndrome, a differential diagnosis of intracranial hypertension was performed. We excluded, based on clinical examination, cerebral imaging and laboratory blood parameters, acute meningitis and encephalitis, intracranial tumors, thrombosis of the intracranial venous sinuses, hydrocephaly, hypothyroidism, hypocalcaemia, high blood pressure.

On admission the child was febrile (38.5 C), pale, without cutaneous eruptions, with photophobia, somnolent but oriented to time and space, with a heart rate of 120 beats per minute and a normal respiratory rate and blood pressure. With the exception of meningeal syndrome, the rest of the clinical examination was within normal limits. Laboratory blood tests showed increased leukocyte count (32 000/mm^3) with 93 % neutrophils, increased inflammatory syndrome markers (C

reactive protein=42.6 mg/l, Procalcitonin= 29.2 ng/dl) and a positive blood culture for Streptococcus pneumoniae, serotype 6B, resistant to Penicillin. The lumbar puncture revealed a turbid, raised pressure CSF, with 6400/mm^3 cell count (out of which 87% Neutrophils), raised protein level and positive bacterial culture for S. pneumococcus serotype 6B.

The last brain magnetic resonance imaging, performed during this admission, showed asymmetry of the depth of the olfactory fossa (7mm on the right side and 4mm on the left side) and lateral right defect in the cribriforme plate of 4mm, partially covered with dura mater, with fluid signal that prolonged to the rhynopharynx. This image is specific for a CSF fistula, active at the time of examination, with origins most probably in the lateral right cribriforme plate defect. (Fig. 1.) The CT examination also revealed the ethmoidal asymmetry. (Fig. 2.)

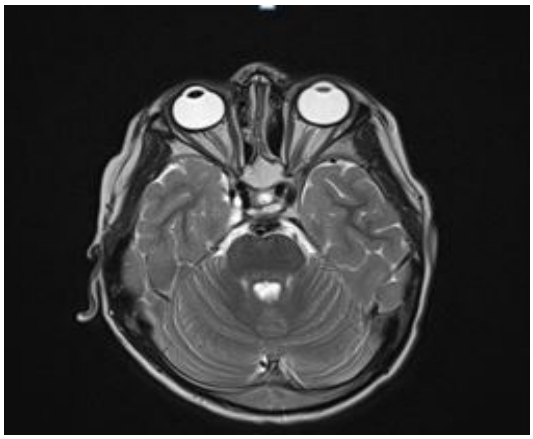

Fig. 1. IRM, T2 images, showing asymmetry of the olfactory fossa and lateral right defect in the cribriform plate

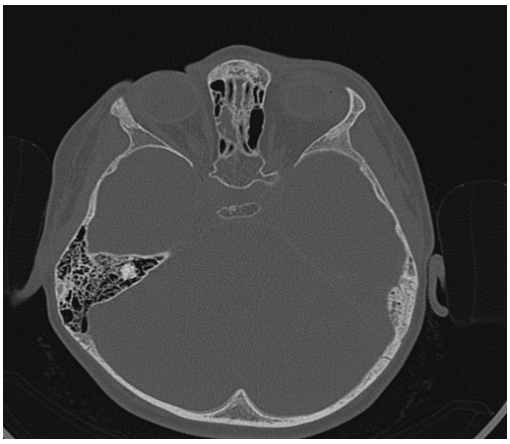

Fig. 2. CT scan- bone window, showing asymmetry of the ethmoidal bone

The patient received treatment with IV Meropenem 120mg/kg/day and Linezolid 30mg/kg/day for 21 days, IV mannitol and dexamethasone, acetazolamide orally, with favorable clinical and biological outcome. The patient underwent a second surgical intervention with resolution of the rhinoliquorrhea.

Discussions

CSF leakage commonly occurs secondary to head trauma or after skull base and endonasal sinus surgery. Spontaneous CSF leaks are caused mainly by occult malformations of the base of the skull, the majority of the sites of the fistulas being located at the cribriform plate and the ethmoidal roof, whereas the sphenoid bone and sinus being less involved.[7] Intracranial hypertension syndrome in also recognized to have an important role in developing a CSF fistula.[8]

In bacterial meningitis associated with cranial dural defects S. pneumoniae was found in 80% of cases, highlighting the fact that CSF fistula represents a high risk factor for invasive pneumococcal disease.[9]

The diagnosis and treatment of these affections need considerable effort from a multidisciplinary medical team with the ultimate goal of making a quick recovery of the patient and avoidance of recurrence.

During 2010-2013, ACIP (Advisory Committee on Immunization Practices) published new recommendations for vaccination with PCV13 and PPSV 23 regarding patients with high risk factors for IPD, including those with a CSF fistula.[10,11]

Conclusions

In this case, pneumococcal meningitis was associated with evident CSF fistula, clinically manifested with rhinoliquorrhea, in a child with idiopathic intracranial hypertension syndrome. Brain imaging showed clear evidence of recurrent rhinoliquorrhea and a second endonasal surgical repair was performed. The child received recommendation to vaccinate with both PCV 13 and pneumococcal polysaccharide 23-valent vaccine.

During 2014-2015 in our clinic there have been two other cases of sepsis with meningitis due to Streptococcus pneumoniae in children with CSF fistula; in one case we identified serotype 23F and the other was a nontypeable pneumococcus.

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CLOSTRIDIUM DIFFICILE COLITIS IN CHILDREN CLINICAL FORMS AND TREATMENT

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Abstract

Introduction: The pediatric cases of *Clostridium Difficile* infection (CDI), based on the host immune status, can present under a broad spectrum of clinical forms, from asymptomatic portage of the germ up to severe, life-threatening cases of pseudomembranous colitis.

Objective: In this work we aimed to analyze the clinical forms and treatment of *Clostridium Difficile* colitis in children.

Material and method: We conducted a clinic-based retrospective study in witch we analyzed the cases of confirmed CDI in infants and children that were treated and monitored at The National Institute for Infectious Diseases "Prof. Dr. Matei Bals", Bucharest during the period 2010 – 2014. For the selected cases the clinical and demographic patient features followed were: age, sex, immune status, severity of the disease and the treatment regimen. The etiological diagnosis was made by culturing the stool samples and by PCR testing for Toxigenic *Clostridium Difficile* strains.

Results. During the study period we registered 20 cases of CDI in infants and children. On subsets, 25 % of the cases were immunosuppressed. Based on the demographic features we observed female predominance and a pick of incidence in the 4-8 years subgroup. All the diagnosed patients were treated according to the international CDI treatment protocol. We registered a 15 % recurrences rate, with one or two relapsing episodes. One tenth of our cases pretended more than 3 CDI relapsing episodes and consequently received fecal microbiota transplantation with the transplanted material being donated by a healthy family member, with subsequent full recovery. The cases that underwent the stool transplant protocol did not registered relapsing episodes. No fatal cases were registered.

Conclusions: The CID in infants and children, especially in imunocompromised hosts, can present under sever clinical forms. In the case of multiple relapsing episodes the fecal microbiota transplantation procedure can be a successful treatment option.

Introduction.

The acute diarrheal diseases (ADD) represents, together with the acute respiratory disease the most frequent infectious pathologies encountered among the pediatric population. Even though the most common etiology of the pediatric ADD is a viral infection with digestive tropism, among the bacterial causes of acute gastrointestinal infections of children we encounter, more frequent in the last years, the *Clostridium Difficile Infection* (CDI). Nowadays the bacterial intestinal pathologies are changing and we are facing mare severe and complicated cases with a constant increased incidence, in the general population but also in the pediatric one. The differential diagnosis of CDI among infants and children remains a challenge for the medical teams, first of all due to the high percentage of healthy carriers of *Clostridium Difficile* strains, in this particular age group. The specialized literature reports different percentages in rapport with the clostridium carriers, but it is proved that the carriage rate is inversely proportional with the age, children in their first year of life being colonized in a proportion that can be as high as 70% in contrast with the adult population that reaches rates of maximum 6%. On the other hand we are faced with the lack of clear and standardized diagnostic criteria of CDIs in pediatrics, age group were the role of *Clostridium Difficile* is not clearly defined. In the actual epidemiological context we are faced with a constant rise in the CDI incidence but few data from the pediatric population are declared and analyzed, nationally and internationally, most probably in relation with the diagnostic controversely linked to the CDI pathology. The increased rates of CDIs are the direct result of the abusive antibiotic use which induces the pseudomembranous colitis. On the other hand the medical community becomes more aware of the *Clostridium Difficile* associated pathology and in consequence the cases are more accurately and rapidly diagnosed, with the intrinsic disadvantage of judicious and abusive testing for *Clostridium Difficile.*

Purpose.

Starting from the above mentioned facts we decided to analyse the clinical forms of CDI among children and to synthesize their evolution under different treatement regimens. Also we intended to compare data obtained in our study with the ones from the specialty literature in order to be able to adjust the international treatment protocols to the epidemiologic context of our country. For the CDI cases who present with multiple relapsing episodes we propose an innovative and modern treatment option: the intestinal recolonization with fecal microbiota (fecal matter transplant).This procedure is frequently an

option in the treatment of relapsing CDIs among adult patient in contrast with the pediatric population, where internationally few cases of fecal microbiota transplantation are reported; in consequence we adjusted the adult protocol for fecal matter transplantation in order to be suitable to treat recurrent episodes of CDI in infants and children. This treatment procedure is a national premiere in the pediatric population and we are aiming to standardize it and to use it in the suitable situations.

Material and methods.

We conducted a retrospective study on the pseudomembranous colitis that was submitted to the Pediatric Department of The National Institute for Infectious Disease "Prof. Dr. Matei Bals" – Bucharest in the period 2010-2015. On the selected cases we analyzed an array of parameters: age, sex, home environment and immune status. Also we focused our attention on the treatment regimens used and closely followed our patients evolution, in case of relapsing episodes we maintained the patients in our study. In the immunocompetent patients that presented with more than three relapsing CDIs we used intestinal fecal microbiota transplantation from a healthy, closely related donor.

The etiological diagnoses of the CDI associated colitis was based on specific diagnostic tests: culture for *Clostridium Difficile*, fecal screening for A/B toxin production, PCR (Polymerase Chain Reaction) identification of toxigenic *Clostridium Difficile* strains. In relation with the etiological diagnosis, in each particular case we used a wide array of biochemical tests that helped us, first to sub-categorize the form of the disease based on severity (CBC, inflammation tests, PCT-Q) and second to exclude differential diagnostics (rotavirus, adenovirus, norovirus, Salmonella spp., Shigella spp, Yersinia enterocolitica, E.Coli, Campylobacter jejunii, Candida spp. etc). Using laboratory tests and other paraclinic procedures we excluded the non-infectious etiologies of diarrheal diseases (intestinal inflammatory disease, intestinal malformations and metabolic pathologies).

The treatment regimens for the CDI associated colitis were chosen in concordance with the European Society for Clinical Microbiology and Infectious Disease Guidelines (ESCMID).

1. No treatment for asymptomatic forms.
2. Mild clinical forms - Metronidazole, orally 10-14 days. In case of intolerance we used as a first line of treatement Vancomycine in oral administration.
3. Severe clinical forms - Vancomycine. In some particular situations the association Vancomycine orally + Metronidazole i.v. was used next to non-specific Human Immunoglobulin.

The antibiotherapy choose according to the ESCMID guidelines was supported by hygiene measurement and by sustained disinfection, supportive care, which included iv. administration of fluids, symptomatic drugs (antalgics, antipyretics, antispastics and antiemetic) and by oral administrations of recolonizing flora - Saccharomices Boulardi +/- Rifaximina .

In case of initial relapsing CDI, if in the first infective episode the treatment was Metronidazole, then Vancomycine was administered orally for 14 days. In the second relapse we used as treatment Vancomycine in extended regimen: 14 days of regular oral administration (30 mg/kg in 3-4 doses) followed by decreased doses (5 days – 3 doses; then 5 days- 2 doses; then 5 days – 1 dose, then 1 dose every 2 days). In case of more than three relapsing episodes we proceeded to fecal microbiota transplantation.

The fecal microbiota transplant procedure, which has limited use in the pediatrics and an extremely limited experience around the world, was implemented in our clinic using an in-house developed protocol, elaborated based on the available literature data; the protocol was approved by The National Institute for Infectious Disease "Prof. Dr. Matei Bals" - Bucharest Ethic Commission and it started following the experience of our own infectious diseases specialists. This team successfully managed 25 cases of recurrent CDIs in adult patients. The fecal microbiota transplantation indication was made, to the pediatric patients that presented more than three recurrent CDIs, in the absence of therapeutic response to standard medical interventions and to the patients that associated a high risk of developing severe forms of pseudo-membranous colitis. Excluded from the procedure were the patients diagnosed with toxic-mega colon or with other subsequent acute infections, the ones that were under immunosuppressive therapies (corticoid therapy included) and the ones below the age of two. The transplant receiving patient preparation includes:

- No antibiotic administration 72 hours before.
- 12 hours of fasting and pre - procedural bowel emptying.
- Nasogastric catheterization (high level enema can be an option but in pediatrics is associated with lower success rates)

The donor is required to be a healthy related person (first degree relative – mother of father), that are passed through an detailed fecal screening (*Culture for Shigella, Salmonella, Yersinia, E. Coli O157:H7, Ziehl-Neelsen fecal Staining, Cryptosporidium, Cyclospora, Isospora, Clostridium Difficile A and B, fecal PCR testing for Costridium. Difficile, Coproparasitologic testing , Fecal Helicobacter pylori antigen detection*) and a serologic screening (HIV, HTLV, VHA, VHB, VHC, TPHA).The donor preparation starts 12 hours before the procedure and consists of mild laxatives administration, using for the procedure the first stool emission of the day after mixture, blending and triple filtration, steps that are completed in the microbiology laboratory under standard, level 2 biohazard, safety condition with immediate administration of the product. The fecal microbiota transfer was, in our study, made by nasogastric catheterization. We emphasize on the importance of the profound positioning of the nasogastric catheter in order to avoid the gastric inactivation of the product.

After the procedure, the nasogastric catheter has to be clamped for 2 hours and the patient will not receive any food products in the 12 following hours, after the post-procedural fasting episode normal diet, with no particular restrictions is recommended. The transplanted patient remains in our clinic monitoring schedule for the six following months after the procedure, with routine follow-up visits at one week, one month, three and six months – and if no relapsing episodes are registered, the patient is considered cured.

Results:

During the study period, from 2010 to 2015, we diagnosed, treated and monitored, in the Pediatric Department at the National Institute for Infectious Diseases "Prof. Dr. Matei Bals" – Bucharest, 20 pediatric cases of colitis induced by *Clostridium Difficile.* The cases predominated in the age group 4-8 years of age – 60% (12/20), 65% (13/20) of cases were diagnosed in female patients, up to 80% of the patients were residents in urban arias. Stratifying on the immune status 25% of the patients were immunocompromised children (13/20): 2 cases of HIV/AIDS, 3 patients previously diagnosed with malignancies. All the children diagnosed with CDI were treated according to the ESCMID protocol. No fatal cases were registered. The vast majority of the cases diagnosed with CDI evolved favorable (up to 70%), presenting with no relapsing episodes, in contrast with 30% of the patient from our cohort, who registered at least one relapsing episode.

Two of the relapses presented more than 3 recurrent CDIs, for these two particular patients, we used, as a treatment option, the intestinal fecal microbiota recolonization procedure (fecal matter transplant) using the mother as our donor in the first case, and the father in the second one.

The first fecal matter transplant was done in March 2014, on a female patient aged 5 who presented 4 relapsing episodes of CDI; the second one was done in December 2014, also on a female patient aged 6 who underwent her fecal matter transplant after 5 relapsing CDI.

In both cases the transplanted product was administered through nasogastric catheterization (1 ml of product for each kilogram), according with de presented protocol. Before the procedure both legal guardian of each child (mother and father) gave their written consent for the procedure. The procedures were successfully completed in both cases, with no procedural incidents encountered, the subjects being discharged after 48 hours.

Regarding the follow-up, in the first instance the patient presented with no relapsing CDIs at his annual follow-up visit; respectively at 6 months the second transplanted patients had no relapsing episodes. More than that, our first transplanted patient, came to our clinic, in May 2015, with an acute streptococcal infection that immediately required antibiotic therapy (oral penicillin). In this context we are happy to highlight the fact the she did not presented any gastro-intestinal complications.

Discussions and conclusions:

The specialized literature describes only some cases of *Clostridium Difficile* associated colitis and more than that very few cases of intestinal fecal microbiota recolonization procedures that were made on children. Facing the constant rise in incidence for this particular pediatric pathology, we consider that is extremely important to focus on CDIs, due to the possible severe course that can sometimes lead to fatal outcomes.

The antibiotics abuse, the medically induced immunosuppression or some particular chronic diseases can be important causes of increased CDI incidence rates among the pediatric population, but here it is compulsory to add the modern diagnostic procedures that are nowadays available.

The treatment measurements require immediate implementation in the case of a positive diagnosis of *Clostridium Difficile* colitis since the cases can rapidly evolve towards toxic, severe forms, with reserved final outcome, even in the context of correct treatment regimens.

Based on our analyzed data we can state the fact that fecal matter transplantation represents an efficient and safe medical procedure for children, but under strict indication and for the immunocompetent patient. By the time we conducted our first fecal microbiota transplant (March 2014), on a pediatric patient being confronted with recurrent CDIs, the procedure was the only one described nationally and one of the few practiced on an international level, up to our knowledge, but we can now state that the fecal matter transplant represents a successful medical premiere, one that still represents a backup solution, for well established and protocol-defined situations.

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THE PLACE OF THE BORDETELLA SPP. INFECTION IN THE RESPIRATORY SYNDROMS DOMINATED BY COUGH IN INFANTS AND CHILDREN, A 5 YEARS SURVEY

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Abstract

Introduction: Cough is a common indication of respiratory illness and is one of the more common symptoms of children seeking medical attention.

Material and Method: We conducted a clinic-based retrospective surveillance witch analyzed the cases managed at “The National Institute for Infectious Diseases "Prof. Dr Matei Bals" – Pediatric Department, Bucharest during the period of January 2010 up to December 2014 who presented accusing cough episodes from over a week and who associated one of the following symptoms: paroxysms of coughing, inspiratory "whoop," posttussive vomiting or apnea. We selected 790 suitable cases for whom we analyzed: age, sex, vaccinal status, severity of the disease and the complications. The etiological diagnosis was made by serologic testing for Bordetella, Mycoplasma, Chlamydia, Adenovirus and by rapid testing for Sincitial Respiratory Virus (RSV). Results and findings. Based on the etiological stratification 108 patients (13,8 %) were diagnosed with Bordetella Spp infections, 62,4% of them being completely unvaccinated against Pertusis, representing 11% of the national reported cases of Whooping Cough during the 5 years of survey. With decresing frequencies the rest of the cases (682) were caused by: RSV (39,7%), Adenovirus (21,5%), Mycoplasma (18,3%), Chlamydia (6,7%). The majority of the cases evolved favorable, no fatal cases were registered but 279 presented with initial altered status and required, on average, 3 days of Intensive Care Unit management. The average hospitalization period registered is 6,9 days. All the severe complications were registered in the < 6 months age group. Conclusion: Whooping cough remains endemic in Romania and Bordetells Spp. infection is associated with substantial morbidity and mortality rates among children.

Key words: *whooping cough, Bordetella, coqueluchoid syndrome*

Introduction

The primary role of Bordetella Spp infection is causing lower respiratory tract disease among children and adults. The substantial morbidity and mortality rate associated with the whooping cough diagnosis has made its control a worldwide priority.

However, in Romania, the whooping cough is substantially under-diagnosed, especially due to the lack of awareness and the total burden of Bordetella Spp infection in the respiratory syndromes dominated by cough remains poorly defined, particularly in the rural settings and especially among our nomad minority.

Data reported by the Romanian Ministry of Health show that the vaccination rate against Bordetella Pertussis is substantially under the targeted 95% of immunized population with an important decrease (88.2) registered from the cohort of 2011.[1]

The aim of our study is to characterize the Romanian whooping cough endemicity, from the point of view of one national infectious disease center and to determine the population based burden of Bordetella Spp. associated infection among hospitalized children and primary care settings (our day clinic). We further sought to describe the effect and potential risks associated with the constant decrease of European and national immunization coverage.

Material and method:

We conducted a clinic-based retrospective surveillance witch analyzed the cases of pediatric respiratory syndromes dominated by cough and associated with infectious agents managed at “The National Institute for Infectious Diseases "Prof. Dr Matei Bals" Bucharest – Pediatric Department and Pediatric Intensive Care Unit, during the period of January 2010 up to December 2014.

Eligible children were under 18 years of age, presented with a history of cough episodes from over a week and who had received a diagnosis of acute respiratory infection, which was defined as an illness presenting with one or more of the

following symptoms: fever, nasal congestion, rhinorrhea, sore throat, vomiting after coughing, wheezing and labored, rapid or shallow breathing.

Excluded from our study were children who had respiratory symptoms dominated by cough but no infectious context or children with medical history of chronic cough. We obtained children's demographic *(age, sex, home environment)*, medical features *(severity of the disease and the complications)* and social histories *(especially previous immunizations status)* by questioning our hospital general database and by analyzing the standardized interviews of parents and legal guardians that were associated within the submission form and treatment sheets of all selected cases. For the risk factors stratification we recorded associated medical conditions which included prematurity history and chronic pulmonary, cardiac, renal, or immunodeficiency disease. Discharge diagnoses were based on clinical and laboratory information.

For the ethological diagnosis we obtained nasal and throat swabs for viral detection (inpatient specimens were tested by reverse-transcriptase polymerase chain reaction (RT-PCR) or by using the MariPOC point of care multivariate analyzer for influenza A, influenza B and Parainfluenza viruses 1, 2, and 3, Respiratory sincitial virus and Adenovirus). Serological testing was used for the diagnosis of Mycoplasma Pneumonie and Chlamydia Pneumoniae (*using Elisa immunosorbent assays)* and of Bordetella Spp. associated infections *(testing in dynamic and using the serological agglutination tests for Bordetella. pertussis or determining the IgM, IgG and IgA titers of Bordetella Pertussis. using Elisa immunosorbent assay).*

The choice of clinical management, including antibiotic regimens – if needed, hospitalization periods and intensive care stays – if needed, were determined by the child's affiliated team of physicians, who also monitored the evolution and introduced the patient data in the central database.

Results.

During the studied period we identified 790 cases of acute respiratory infections with intense cough episodes as the main clinical aspect (*suggestive presentation for "coqueluchoid" syndrome*), which stand for 13.5% of the total respiratory infection cases addressed to our department.

Regarding the initial clinical form 279 patiens presented with initial altered status and required, on average, 3 days of Intensive Care Unit management (see Fig. 1 repartition of cases on age groups).

The average hospitalization period registered is 6,9 days, from a total of 2971 hospitalisation days/cohort. All the severe complications were registered in the < 6 months age group all the patients evolved favorable, no fatal cases were registered, and all the emerging complications were reversible, the patients being discharged without important sequelae.

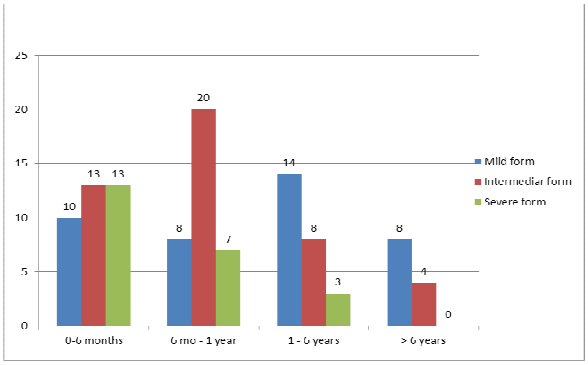

Fig. 1. Cases severity on age groups.

Based on the etiological stratification 108 patients *(13,8 %)* were diagnosed with Bordetella Specie infections *(106 patients diagnosed with Bordetella Pertussis infection and only 2 diagnosed with Bordetella Parapertussis infection)* and representing 11% of the national reported cases of Whooping Cough during the 5 years of survey; the Romanian Ministry of Health's listed in March 2015 the total number of 1010 confirmed cases of Whooping cough,[2] with 2 picks of incidence in 2012 and 2014, years that registered a maximum of Bordetella Spp associate infections diagnosed in our clinic too (33 respectively 25 cases – see Fig. 2, Fig. 3 for correlation).

Within decreasing frequencies the rest of the cases *(682 patients)* were caused by: RSV *(39,7%, which counts for 314 cases)*, Adenovirus *(21,5% which counts for 170 cases*), Mycoplasma *(18,3% which counts for 139 cases*), Chlamydia (*6,7% which counts for 51*).

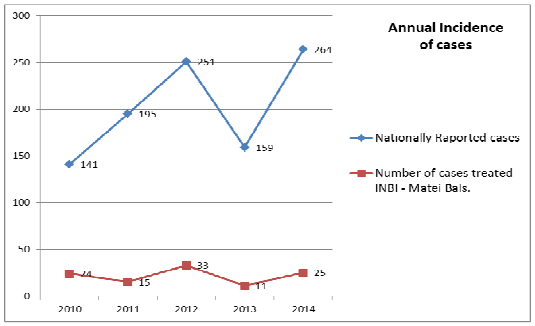

Fig. 2. Annual incidence of cases.

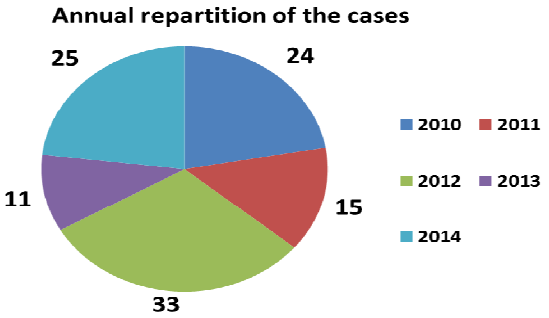

Fig. 3. Annual incidence of cases

The highest rate of complications was registered in infants, especially those under 6 months of age and accounted, in a decreasing frequency rate, of apnea or slowed breathing 65 cases *(60,1%)*, associate/secondary pneumonia 43 cases *(39,8%)*, dehydration and/or weight loss due to feeding difficulties 42 (38,7%), sub-conjunctiva hemorrhages 48cases *(44,4%)*, epitasis 12 *(11,1%)* seizures 4 *(3,7%), rib incomplete fracture* 1 case *(0,9$),* and syncope and brain damage 2 cases *(1,8 %).*

In our work cohort 62,4 % of the patients diagnosed with Whooping cough were found to be completely unimmunized; in absolute value – 67 children were found to be completely unvaccinated against Pertussis, 9 of them being infants under the age of 2 months at the time of the diagnoses, so too young to be vaccinated, and 3 of them were documentary linked to a family cluster of Whooping cough.

Sub-classifying the unvaccinated children we registered a higher rate of unimmunized children among the rural population (with a ratio of rural: urban cases of 69:39).

Significantly more family clusters *(from a total of 17)* of linked cases with Bordetella Persussis infections were identified in the rural regions of Romania, at least one case from the considered clusters was clinically and serologically diagnosed with Whooping Cough in our clinic.

Only 19 unvaccinated children were the result of an imperative refusal of the national immunization programs – declared and assumed anti-vaxxers - the rest of the unimmunized children were either social cases, either too young to be vaccinated or the victims of unawareness regarding the national immunizations programs.

Based on the age distribution the most affected age group was the 0-1 year group, 71 patients being under the age of one at the time of the diagnosis, 51% of them being younger than 6 month and in consequence too young to be completely immunized against Pertussis (see table 1 for age details).

The smallest prevalence of the infection registered among pupils (age > 6) with only 12 cases affiliated to the last mentioned age group. Analyzing the cases on the sex distribution bases we observed a male predominance (60, 7%) and a seasonal incidence of the cases, with higher rates of Whooping cough diagnosis during the Autumn-Winter months, seasonality that is known to be characteristic for the respiratory tract infections.

Table 1 – Complications rates.

Complications	Number of cases
Apnea	65 *(60,1%)*
Seizures	4 *(3,7%)*
Subconjunctival Hemorrhages	48 *(44,4%)*
Epitasis	12 *(11,1%)*
Secondary/Associated Pneumonia	43 *(39,8%)*
Sincope	2 *(1,8 %)*
Sleep disturbances	84 *(77,7%)*
Incontinence (la children>4 years)	6 *(5,55 %)*
Leukocitosis >100.000 elem/mmc	12 *(11,2%)*
Rib incomplete fractures	1 *(0,9%)*

Discussions and conclusions

Our findings from one national center for infectious diseases, which addresses to diverse populations groups, highlights once again that the Bordetella Spp. associated infection remain endemic in Romania and that the diagnosis of Whooping cough is associated with important rates of morbidity and mortality with a general burden rate that is greater among children within the first 6 years of life; young age imposed a significantly greater risk of severe illness.

Characteristics that were most frequently associated with Bordetella Spp. associated illness requiring hospitalization include lower socioeconomic status, male sex, chronic coexisting medical conditions, contact with other children and also lack or incomplete vaccination, which in our group proved to be the consequence of poverty and unawareness, and only in a small amount of cases the direct result of the anti- vaccines movement.

Historically controlled infections like Whooping cough have the potential to make a real and aggressive comeback not only in Romania but in the whole world.

The facts and data that we are now facing represent only the "tip of the ice-berg" since every day, more and more children are missing their vaccine doses, the heard immunity is fading and the medical community fears become real life experiences.

There is a certain amount of complacence today toward diseases that are thought to be largely a thing of the past. However we are now experiencing a resurgence of some of these diseases like Whooping cough and while the causes are complex and need to be teased apart, they are worsened by vaccine refusal which in turn is spawned by an anti-vaccine movement that is spreading misinformation and unwarranted fears.

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OUTCOME PATTERNS IN CYSTIC FIBROSIS - A DISEASE WITH MANY FACES

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Abstract

The classic picture of cystic fibrosis (CF) includes, in addition to chronic lung disease and steathoreea, a multitude of other clinical features like: CF associated liver disease, CF related diabetes, nutritional deficiencies, which can be way onset of the disease. Polymorphism and different outcome of patients with same genotype, in a monogenic disease as CF raises questions of diagnosis and monitoring in daily practice. The purpose of this paper is to present few ways of diseases onset and evolution among CF patients.

Methods. Since the CF gene discovery the concept of genotype/ phenotype correlation evolved, but different evolution of patients with the same genotype denied, over time, this correlation. Except respiratory infections and exocrine pancreatic insufficiency, cirrhosis or osteopathy may be ways of CF onset.

Results. This paper presents the evolution of patients with identic genotype and various types of onset and more or less particular.

Conclusions: Individuality is strongly manifested in patients with CF, there isn't only "disease" but patient. It is increasingly likely that other factors, than genotype and environment, influence the evolution and prognosis of children with cystic fibrosis.

Introduction

Cystic fibrosis (fibrosis) is the most common monogenic disease, potentially lethal, of Caucasian population, with an incidence of 1: 2000 newborns (1: 2054 in Romania). Pathophysiological alteration occurs in the membrane transport of chlorides through the cells membrane, with increased mucous viscosity of their secretions. Clinical polymorphism and different evolution of patients with a monogenic disease like cystic fibrosis (CF) raises issues in the CF diagnosis and monitoring. The disease is manifested with repeated respiratory infections, cystic fibrosis being an obstructive pneumopathy, and can associate nasal polyposis, chronic sinusitis, or digestive manifestations like steatorrhea secondary to exocrine pancreatic insufficiency, with impaired growth. Cystic fibrosis manifestations of the hepatobiliary system includes varying degrees of liver disease, from steatosis to liver cirrhosis, because of secondary to the obstructive cholangiopathy. Classic clinical picture of cystic fibrosis, includes, besides chronic lung disease and steathorrea, also a multitude of other events as diabetes, nutritional deficiency, hepatopathy, which can be o form of onset of the disease. Meconium ileus could be also a manifestation, besides male infertility, secondary osteopathy, salty taste of sweat, prolonged neonatal jaundice. Although clinical manifestations are extremely varied, respiratory status determines the prognosis of the disease, associated pathologies may aggravate and influence the evolution of the patient being often milestones in patient management with FC.

Since the discovery of CF gene mutations, the concept of genotype phenotype relation was raised but the evolution of different patients with the same genotype denied, over time, a direct correlation, suggesting the influence of other environmental causes. It remains possible that other factors, like genetic modifiers that could trigger the disease and the expression of related pathology.

Methods.

This paper proposes several ways debut exposure and evolution of FC in children by reviewing some particular cases of cystic fibrosis.

Results

Case 1.

The first case presented is a girl of 15 years old, with respiratory issues since 3 years of age, evoluating with frequent respiratory issues resistant to treatment. At 5 years, she was admitted in our center; clinical examination reveals hypotrophy, with a BMI =16, she presented dyspnea, productive cough; chest hyperinflation, crackles, digital clubbing, and a marked inflammatory syndrome. Differential diagnoses included chronic pneumopathies like: alpha 1- antitrypsin deficiency, primary cilliary dyskinesia, tuberculosis, common variable immunodeficiency syndromes that have been excluded; sweat test

was negative, chloride value was 36 mmol / l. Radiography showed emphysema, condensation areas located centro-lobular and basal bilateral (fig. 1.), as confirmed by CT scans showing areas of air trapping (fig. 2.) and condensation, with mosaic appearance, saccular and tubular bronchiectasis with mucus plugging (fig. 3.).

The evolution was marked by frequent respiratory exacerbations, *Stenotrophomonas maltophilia* infections, while there was colonization with *Pseudomonas aeruginosa*. The genetic test performed at that time (1998) detected a single allele ΔF508. It seemed to be a CF case but without the sweat test and/or genetic confirmation. This confirmation came afterwards, when DNA sequencing was performed, in a specialized laboratory when another more "gentle" mutation, 3489 + 10 kb AG, was found, the child having compound heterozygous genotype ΔF508 / 3489 + 10 kb AG. All sweat tests were within normal limits, expressing the case as atypical, without chronic diarrhea and with normal sweat test, phenotype due most likely to the "protective" effect of non ΔF mutation. Subsequent reports showed that the combination of mutations associated milder mutation, moderate the disease`s outcome, as in the presented case. Eventually she developed a cor pulmonale and died during an exacerbation.

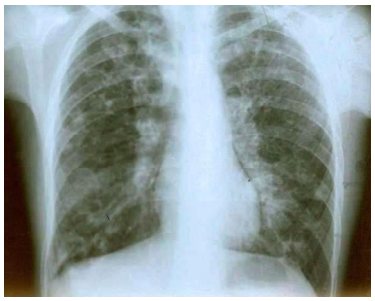

Fig. 1.

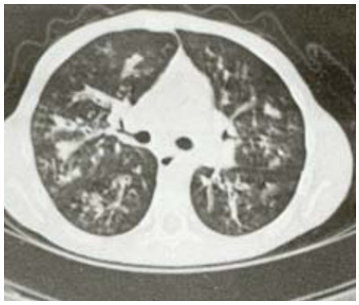

Fig. 2.

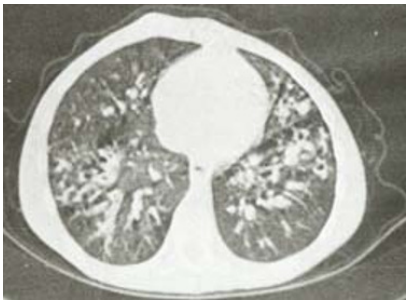

Fig. 3.

Case 2.

Another case is of a girl who presented in our clinic at age 5, with failure to thrive, hepato-splenomegaly and collateral circulation in “caput medusae".

The onset of the disease occurred in infancy, with upper gastrointestinal bleeding, in the context of acute liver failure. During early childhood, the girl had poor growth, respiratory tract infections and consistently hepatomegaly. She was referred to our CF center due to salty taste of sweat. Sweat test was positive, and genotype homozygous for ΔF508 mutation, indicating a genetic classical form, but without associating a corresponding phenotype with repeated pulmonary exacerbations and steatorrhea.

In this case the CF associated liver disease prevailed other manifestation, the biliary cirrhosis evoluated from hyperechoic ultrasound aspect to the development of liver fibrosis with portal hypertension (fig.4).

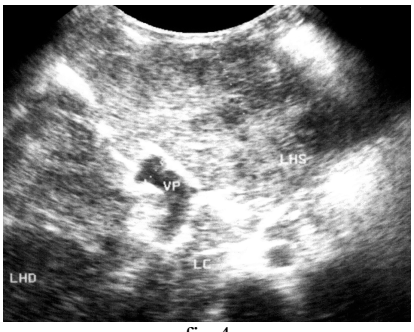

fig.4

Case 3 .

A third situation presents two brothers, a girl of 11 years, diagnosed with CF as toddler, with frequent exacerbations, steatorrhea and homozygous genotype ΔF508 / ΔF508. It`s evolution was marked by frequent exacerbations, early colonization with *Pseudomonas aeruginosa*, medium distal obstruction, tubular and saccular bronchiectasis, digital clubbing, failure to thrive. In this context, his brother was born with a normal birth weight (3700 g), but at the age of one month was hospitalized for: generalized edema and steathorrea, hypoalbuminemia, anemia. The boy outcome was very good with satisfactory growth, good pulmonary function, and structural pulmonary preservation, compared with his sister. Around the age of 3 years he was colonized with *Pseudomonas aeruginosa* , having a direct contact with his sister; the evolution was

stationary until 7 years of age, when it began to have frequent exacerbations and develop multiple allergies to antibiotics, associating bronchiectasis, emphysema, condensation, mucus plugging (fig. 5, fig.6).

At age 8, the boy dies in respiratory exacerbation despite correct treatment instituted, while his 13 years sister with the same genotype, has a stationary outcome. Factors that influenced the different evolutions of these children, with identical genotype, who lived in this same environmental factors are unknown; is possible that the boy's evolution would have been influenced by early colonization from a direct contact with *Pseudomonas* and the development of drug allergies.

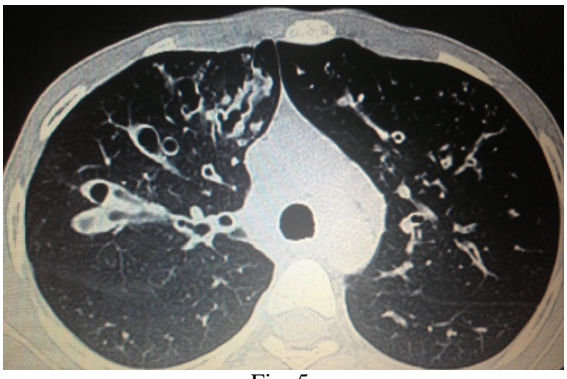

Fig. 5.

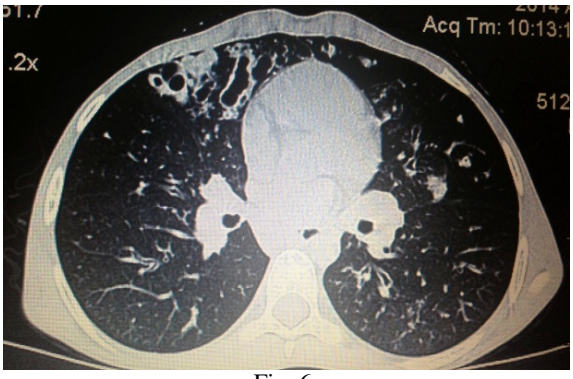

Fig. 6.

Conclusions

Individuality manifests itself strongly in patients with cystic fibrosis, we cannot talk about the “disease" but patient. It is increasingly likely that other factors different from genotype and environment, influence the evolution and prognosis of children with cystic fibrosis.

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CMV INFECTION, DIFFICULTY IN DIAGNOSIS AND TREATMENT IN THE NEWBORN AND IMMUNOCOMPETENT INFANT

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Abstract

Introduction. Cytomegalovirus (CMV) discovered in the early twentieth century has a ubiquitous distribution, being able to infect both human and several animal species. According to the literature, congenital infection prevalence is 0.2 to 2.4% and its subsequent fetal pathology ranks first in the maternal-fetal infections. Polymorphism manifestations of congenital infection is characterized by a wide range of asymptomatic starting point to various clinical symptoms. Regarding the treatment, scientific literature recommended "refraining" from the administering of antiviral therapy in mild and moderate clinical forms of CMV disease.

Methods. The study group was represented by a total of 186 children aged 0- 18 acutely infected with CMV, hospitalized in Pediatrics II Clinic and Infectious Diseases Hospital "Victor Babes", Timisoara. The diagnosis was confirmed by serological determination of specific antibodies IgG and IgM type.

Results and conclusions. From the epidemiological point of view, the incidence of CMV disease, prevailed in infants, with a slight predominance in males, no significant difference on the environment of origin (rural / urban).

The findings on clinical symptoms, laboratory investigations and, particularly the development of cases, determined the authors assert that, CMV infection is a major public health problem in the mother-child couple.

Keywords: *CMV, infection, children*

Introduction:

Cytomegalovirus (CMV) is a ubiquitous distribution, being able to infect both human and several animal species.

The name of CMV, and its origins belongs to the family of herpes viruses which was proposed by Weller and Colab in 1960, is widely accepted in literature.[1,2]

Human herpes virus type 5 (HHV 5) or Cytomegalovirus belongs to the subfamily Beta-herpesviride, it has a spherical shape and a size between 160-200 nm, and it is considered the largest of human pathogenic viruses.

Due to the peculiarities of replication and cytopathogenic they possess, cytomegalovirus has the capacity to generate two types of infections namely: primary and recurrent.

The prevalence of congenital infection is 0.2 to 2.4%, with a higher rate in developing countries and those with weak socioeconomic level compared with developed ones. Thereby, according to studies from the literature, prevalence in developed countries is about 40% versus 100% in developing countries where the infection is acquired early in childhood.[1,3]

Clinical symptoms of cytomegalovirus infection varies by age, mode of transmission, infection type (primary or recurrent) and immunological status of the body (with special reference to the pediatric age).[1,2,3]

1. **Congenital infection.** It is produced by vertical contamination, transplacentar. It includes three forms:
   - *The generalized form* (septicemic, severe) appears at less than 5% of infected newborns and has clinical manifestations of a generalized infection.
   - *The localized form* (incomplete, average) usually occurs 6-8 weeks old or later, up to 4 months old. The most common form is neonatal hepatitis followed by neurological form.
   - *The latent form* (asymptomatic)it is asymptomatic, being diagnosed using serological investigations.
2. **The perinatal infection.**
   - It is produced by horizontal contamination of newborn and infant. Most cases are asymptomatic and symptomatic forms are the localized ones (pneumonia, hepatosplenomegaly syndrome, syndrome purple haemolytic anemia) or disabling (microcephaly, chorioretinitis, deafness).[4]
3. **Postpartum infection.**
   - It appears by horizontal contamination being asymptomatic in 95% of cases. When there are symptoms, they can manifest as prolonged febrile syndrome or mononucleosis-like syndrome.
   - Regarding the laboratory diagnosis of CMV infection in the last decade we have made remarkable progress. The infection`s laboratory diagnosis has 3 components: histopathological diagnosis, virological and serological.[1,3]

Histopathological diagnosis consists in highlighting the tissue biopsy of giant cells with large intranuclear inclusions, owl-like eyes.

Virologic diagnosis consists in isolating the virus in urine, blood, saliva, biopsy fragments. The most commonly used methods are: cultivation cytomegalovirus DEAFE (Detection of early antigen fluorescent foci), viral DNA by PCR and protein pp65.

Serological diagnosis consists in determination of antibodies specific for IgM or IgG, IgA anti-CMV using ELISA method.[3]

IgM antibodies appear in about 3-4 weeks after the exposure to infection and persists in the body for 3-4 months. Thereby, the presence of IgM antibodies in a newborn means congenital infection.

IgG antibodies show an upward titer during active infection, which then stabilizes. Thereby, a person who has been exposed to CMV infection will remain, for the rest of the life, with stable IgG titers, which means that the virus has become inactive.

Etiological therapy for CMV infection is the use of antiviral medication. Severe forms "life-threatening" and those targeting eye damage (chorioretinitis) will benefit from this therapy represented by Ganciclovir, Valganciclovir, Cidofovir and Foscarnet.

Regarding infection prophylaxis, anti-CMV therapy has not proved useful in preventing CMV infection in the fetus and neither to decrease the frequency or severity of any fetal visceral damage. Currently there is no effective anti-CMV vaccine nor other specific and efficient methods for the prophylaxis of CMV infection in pregnant women.[5]

Material and Methods

The study group was represented by a total of 186 children - 115 boys and 71 girls - aged between 0-18 years old hospitalized in Pediatrics II Clinic and Infectious Diseases Hospital "Victor Babes", Timisoara during January 2009 - January 2012. The study method used was actively investigation of the selected persons. Demographic, clinical and paraclinical parameters were registered.

The infection was confirmed by serological determination of specific antibodies IgG and IgM type. The study included all patients tested positive for IgM antibodies to cytomegalovirus, regardless of age, gender, home.

Results and conclusions:

In the studied group was a slight predominance of males versus females in all years of study.(fig.1) Knowing that in the general population there is a predominance of females, it is likely that this reversal of the relations between the genders indicate either a male predisposition for contracting infections or more likely that they will develop more severe clinical forms that require hospitalization.

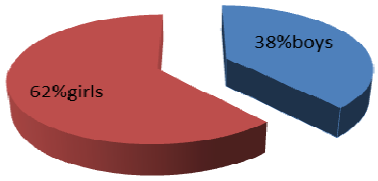

Fig. 1. Sex distribution

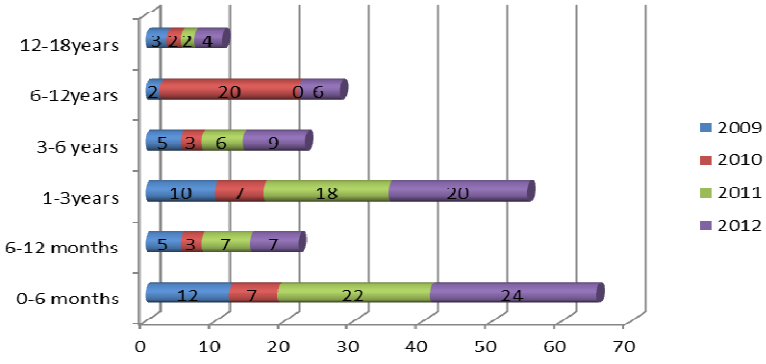

Fig 2. Age group

The obtained data in this study are overlapping with those specified in the literature, mentioning a slight prevalence of males in acquiring cytomegalovirus infection

By age group it is noted that a percentage between 30 and 40% of patients treated for CMV disease represents children under 6 months. Over 80% of each year's illnesses are children aged under 6 years.

Another parameter taken into consideration was the area of origin.

During those 4 years the percentage distribution is relatively similar, with the exception of 2010 when it was higher proportion of patients in urban areas. With all these variations, the differences are not statistically significant ($\chi^2 = 2.75$, $p = 0.431$), so we can not say the lack of homogeneity of the lot on this feature.

Clinical forms found in patients enrolled in this study were polymorphic, dependent on their age. We note that the most common clinical form encountered is hepatitis (almost 50% of cases), followed by mononucleosis (22% of cases) and

febrile syndrome (16% of cases). (Fig. 3) It is noted a predominance of hepatic form at patients under 1 year old; in other age groups this form decreases as frequency to increase the forms manifested as mononucleosis, prolonged febrile syndrome.

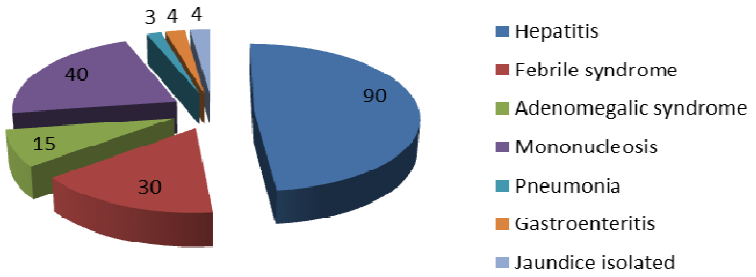

Fig 3. Clinical forms in CMV infection

In acute infections, the number of cases that presented complications are relatively few, the complication rate was of 9.14%. It is noted that hearing loss is the most common complication, followed by periventricular calcifications.

From the studied lot, 49 cases (26.44% of cases) received antiviral treatment, represented by Ganciclovir, mainly orally administered for 7 to 30 days. The significant factors that influenced the decision to adopt antiviral therapy were represented by clinical form of the disease and age. The antiviral treatment was given especially to very young patients (0-6 months old and 6-12 months old). The clinical forms which have been treated with antiviral therapy are: hepatitis (44.44% of cases), pneumonia (100% of cases) and gastroenteritis (75% of cases).

Conclusions:

1. Most likely, males have a higher vulnerability to develop more severe forms of infection with cytomegalovirus.
2. The most common clinical forms of CMV disease in hospitalized patients, in descending order, are: hepatitis (50%), mononucleosis (21%) and prolonged febrile syndrome (16%).
3. The most common complication is the hearing loss, followed by periventricular calcifications.
4. Antiviral treatment is indicated especially at very young age groups (1-12 months) and severe clinical forms (hepatitis, pneumonia and gastroenteritis).
5. There is no difference in prevalence between gender and residence.
6. In terms of age groups, there is an increased prevalence of infection in the age group of 0-6 years and 6-12 years.

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PARENTAL AND FAMILY FACTORS INVOLVED IN THE ETIOPATHOGENESIS OF ANOREXIA NERVOSA IN ADOLESCENTS

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Abstract

This paper aims to identify the family and parental factors encounter in clinical practice to teens diagnosed with anorexia nervosa, focusing on therapeutic intervention within the multidisciplinary team consisting of a paediatrician, child psychiatrist and psychologist. Dynamics and patterns of interaction in family proved to be the most important factors involved in the aetiology of eating disorders in adolescence. Numerous studies have confirmed the efficiency of family and systemic therapy in the treatment of anorexia nervosa in adolescents diagnosed with the disorder. Have been described three family typologies encountered in adolescents diagnosed with anorexia, namely: the perfect family with high expectations from parents and a teenager with a strong personality and sense of perfection; the chaotic family, unstable, abusive and the teen with an impulsive and tidy personality; overprotected family, symbiotic, denying the anorexic patient needs, with a hypercritical, eventually anorectic mother. Will be analyzed several clinical cases in that were identified parental and family factors involved in the occurrence of symptoms and the therapeutic intervention lines in terms of family and systemic therapy.

Key words: *anorexia nervosa, adolescents, family, family therapy.*

Introduction

Anorexia nervosa is a severe psychiatric disorder, its onset occurs mostly during adolescence, and is associated with social disabilities, psychological comorbidities, physical complications and a 10% rate of mortality, with a reserved prognosis when cases need hospitalisation.[1] The key manifestations are characterised by the patient's morbid preoccupation regarding the weight and shape of the body, with severe disturbance of eating behaviour. This is actually about a modified perception regarding body's image and weight. While most of the people evaluate themselves based on their performances in many life areas, in patients with anorexia nervosa self-image and auto evaluation is mostly or exclusively related to the body's shape and weight as well as the ability to control them. These patients' behaviour is centred on weight loss with self induced starvation, refusal of maintaining the weight at minimal value of normality corresponding to age and height, accompanied by an intense fear of gaining weight.[2,3] Consequently, there may occur numerous somatic manifestations and metabolic, hydro-electrolytic, hormonal changes which make the therapeutic approach of these patients to be made in multidisciplinary team consisting of paediatrician/nutritionist, psychiatrist and psychologist.

The etyology of anorexia nervosa is a pluri-factorial one,[2] the involved etiopathogenic factors could be grouped into:

- socio-cultural factors which involve cultural attitude that promote an ideal beauty type characterised by a weight below average, model type with a strong impact on adolescences through different mass-media means;
- biological factors comprising genetic (hereditary) factors and neurobiological factors which imply the hypothalamus and serotoninergic hyperactivity;
- individual psychological factors, parental and family.

In anorexia cases, three distinct family types were described:[4]

- perfect family with high expectances imposed by threat, the adolescent having a competitive personality of group leader, performance;
- chaotic, unstable, abusive family, the adolescent raise in this kind of family being impulsive with ordinate personality and organization bearing;
- hyper-protective, symbiotic family with identity confusion, the adolescent being obedient and subdued to implied constrains.

This paper propose itself an overview of family and parental factors involved in anorexia nervosa's etiopathogeny of adolescences and identifying these factors in two cases of adolescences diagnosed with this impairment and treated by a multidisciplinary team.

Interaction models within the family of the adolescent with anorexia nervosa

The first family approaches in the case of mental anorexia adolescents date back to 1963 when Mara Selvini Palazzoli, italian therapist with initial psychoanalytical orientation, afterwards centred on family, structural and strategic approaches.[5]

In her papers, Mara Selvini claims that anorexia's roots are found in mother-daughter relationships. Family therapy pioneers have already postulated that where there is a dysfunctional child there is also a dysfunctional family in which the child can or can't be involved in the dysfunctional relationship between the parents. In the case of anorexia adolescent, the therapist discovers a certain organising type of parental couple and a certain type of adolescent which will take the side of one of the parents at a certain point and the anorexia symptoms become the expression of adolescent's involvement in this game between the parents.

While exploring the couple's organising type, the therapist discovers an unsatisfied mother, displeased by her husband, which sees herself as his victim and as a victim of her origin family. She often gives up her goals and expressing her own personality to become a homemaker and a mother. But she is also an intrusive, dominating, controlling mother.[6]

When it comes to the father, we place ourselves in front of two different games: based on Marei Selvini's casuistry, 85-90% of the husbands are "collectors". They seem nice, hard-workers, stronger than their wife, most of the times more intelligent than she is. They often have numerous professional accomplishments but are completely incapable to reason down their wife's intrusive and unbearable behaviour. They don't defend themselves or their children. This type of husbands are in the same time the ones that block the communication, when the wife has the tendency to start a fight their answer is simply silence. On the other hand, there is a type of husband that chooses to be authoritarian, he does not allow his wife to speak and he reduces her to silence in a dictatorial manner when she starts exposing her complaints and demands. But looking at this husband more closely turn out that he often conforms to his wife's demands, when she claims them with a sort of tactic that he can't combat.[5]

In this kind of situation, when the adolescent becomes anorexic, he already feels in the middle of this conflict between parents and is very interested in this conflict. At the same time, he discovers the power he has over his parents because he doesn't eat, the symptomatology progressing little by little towards a hunger strike as a triumph over them, but at the same time as a hidden complaint against them.[5]

Another approach was made by Salvador Minuchin, the founder of structural family therapy, who highlighted four common features of the families with one psychosomatic illness member:[7, 8]

- enmeshment – an extreme intensity and proximity form within family interactions. The barriers of subsystems are very fragile, easy to pass trough and the interpersonal differences stay poor, so that the members of these families have the tendency to invade other's thoughts and feelings. At the same time, the connexions with exterior are absent;
- overprotection – manifested trough excessive care over the other's wellness. All the members of these families are very sensitive to distress symptoms indicating the approach of dangerous or threatening conflict tension;
- rigidity – these families search at all cost the maintenance of an intangible status quo because they live along with the difficulty of the periods that require a change or a maturing process. Even if they accept participating in therapy, they declare themselves "normal" and are affected only by the child's medical matter.
- The absence of conflicts settlement – the conflicts sensibility limen is very low, these families tend to avoid any dispute and relate to a very strict religious or moral code. This way, the problems remain unsolved and in distress of reappearing. The members of these family systems mobilize themselves quickly to maintain an easy to handle conflict level and their incapacity of tolerating differences may suppress any negotiation or disagreement solving possibility.

In these situations, any symptom receives a certain signification with the purpose to adjust the family system. The key of family organising seems to be the child's implication in parental conflict. The effectiveness of the symptom carrier as an adjustment agent of the family's intern stability reinforces the symptom's persistence on one hand and the dysfunctional family organising on the other hand.

This analysis modality established the base of a very precise array of the family with one anorexic adolescent. According to Minuchin,[7,8] the exterior boundaries of these families are solid and well defined, maintaining mutual over-engagement of the family members and reinforcing their isolation from the rest of the world; within the system, the boundaries are fragile and diffuse; the boundaries between the nuclear family and the origin family are very thin, both spouses or at least one of them keeps a strong affiliation with his/her origin family which can contribute to maintaining the conjugal conflicts to within the condition in which the trans-generational coalitions develop in detriment of mutual accommodation process of the spouses. This coalition type is sometimes reproduced within the nuclear family; when one of the spouses remains very close to his parents, he can transfer this relationship type to the child in order to fight against the other parent's coalition with a member of his origin family.

Clinical case I

Eleven years old boy coming from urban environment is hospitalized within the paediatric section, at his mother request, for a massive weight loss (20 kg in 2 months), refusal of eating, he is eating only 2-3 fruits a day for two weeks, accentuated fear of gaining weight, still sees himself as being fat, frequently weighs himself, after every meal studies his body in the mirror, has anger crisis when someone enforce him to eat. A few months before hospitalisation the patient started a weight loss diet after the girl he liked told him that she likes thinner boys. Initially, he stopped eating sweets, meat and dairy; later on he refused to eat.

Objective somatic exam: T = 159 cm, G = 43 kg (IMC = 15.8). Colourless, dried teguments, ashen-brown colouring of periumbilical tegument, dried lips, commissural fissures; ringed facies, friable appendages; hypodermal cellular tissue perished at the torso and extremities level; hypotrophy muscular system; FC = 108 b/min. Laboratory exams reveal thrombocytopenia and hypercholesterolemia. The psychiatric consult was made at the beginning of hospitalization, establishing the diagnosis of Anorexia nervosa restrictive form. Moderate depressive episode. Psyche exam: neat outfit patient, corporal hygiene maintained, wide outfit, with the attempt to hide his corporal aspect, tense posture, expressive facies, sad, hypermobile facial expression and gesture, is in constant movement and distress; psych contact is made relatively easy, visual contact is maintained intermittently during the interview, tendency to avoid the sight of the examiner, often looking aside as he is searching for something; clear actual conscience field; temporospatial oriented, auto and allopsychic; coherent ideational flux, numerous concerns regarding body weight and physical aspect; coherent speech, normal tone voice, sometimes lowered, sometimes precipitated speaking; corporal scheme disturbances, sees himself as being fat, studies and verifies certain parts of the body to see if he gained weight; concentration deficit and attention persistence; without distress in retrieval sphere; sad disposition with irritable episodes, intrapsychic marked tension, anxiety marked with nervous states when he has to eat; affective immaturity; concerned behaviour, low frustration tolerance, physical and verbal hetero-aggressiveness towards his school colleagues (his mother affirmed), difficult relation with parent, verbal aggressiveness towards his mother with tendency to insult and humiliate her; eating refusal; without sleeping disturbances. Psychological exam reveals: QI = 103 WISC with cognitive disharmony; immaturity of visual-driving function – MBL indexes; psych-affective immaturity; intense angst related to maternal image; self-image is unclear, disintegrated – intense anxiety, irrationality; predominant defence mechanisms – Ego regression, denial, dissociation; low frustration tolerance; low capacity of emotional self-adjustment; pleasure principle is predominant – avoids discomforting situations by immediate unsatisfying the needs.

Family history and interaction modalities within family system

He comes from a broken family through his parents' divorce about an year ago, the father being physical and verbal aggressive with both the patient and his mother. Nowadays, his relationship with his father is distant, tensioned. His father always encouraged his aggressive manifestation wanting to make him a man but in the same time alerted and humiliated him because of his weight ("you inflated again"). His current relationship with his mother is tensioned regarding his eating behaviour.

Until a year ago the patient lived with both parents, there were numerous family conflicts within parental couple; the mother having strong connections with her origin family, always visiting her parents that live in another locality, the patient being raised more by his mother's side grandparents until starting the fifth grade. Family system boundaries were diffuse, the patient was always engaged in his parents conflicts; he often became an ally of his father against his mother, even using the same humiliating expressions in reference to his mother; the mother, an active woman professional involved, also allied numerous times with her son and with her parents against her husband; lately, the mother tried to estrange her son from his father's influence, in this context the adolescent's symptomatology initiated. In this case are identified family interaction models described by Minuchin where the adolescent is placed in the middle of parental conflict.

Clinical case II

Fifteen years old adolescent coming from urban environment is hospitalized within the paediatric section for weight loss (11 kg in one year), symptomatology began one year ago after an observation made by a classmate on her weight, the patient started a weight loss diet adopting a vegetarian diet, with weight losses followed by adopting purging type behaviours (induced vomit) and exaggerated physical exercises with the purpose of maintaining corporal weight way above adequate level for her age and size.

Paediatric and neuropsychiatric consult establishes the diagnosis of Anorexia nervosa purging type. Secondary pancreatic sufferance. Objective somatic exam: T = 160 cm, G = 34 kg (IMC = 13.3). Colourless, dried teguments, ringed facies, friable appendages, hypodermal cellular tissue poorly represented; FC = 50 b/min. Laboratory exams: serum amylase = 110 U/L (25-101 U/L), serum lipase = 60 U/L (4-39 U/L), FT4 = 0.84 ng/dl (0.89 – 1.37 ng/dl). Densitometry: Osteopenia. High fracture risk. Z score = -1.3.

Psychic exam: neat outfit patient, corporal hygiene maintained, adequate outfit, hypermobile facial expression and gesture, expressive facies, sad, but sometimes sing language is hyper-expressive; psych contact is easy to be made, visual contact is maintained during the interview; clear current conscience field; temporospatial oriented, auto and allopsychic; coherent ideational flux, concerns regarding body weight and physical aspect, unsatisfied with the way that she looks,

numerous corporal schema disturbances although she claims to see herself as thin, she still thinks she has big hips, claims she isn't pretty; obsessive elements (exaggerated concern regarding food quality which have to be bio, pure, organic, certificated), coherent speech, low tonality voice; without retrieval-attention disturbances; sad disposition, low self-esteem, guilt feelings, affective immaturity; social retreat tendency, emotional lability with transitions from sadness states to irritability states. Food appetite kept although she feels anxiety and fear when she eats which leads to purging behaviours persistency (she has periods when she eats compulsively what she likes and afterwards she induces vomiting); sleeping insomnia.

Psychological exam: asthenic disposition with depressive elements, difficulties to fall asleep, affective immaturity, distorted conception of corporal scheme, low self-esteem widely influenced by corporal perception, obsessive elements (unwanted thoughts which never leave her mind, the need to repeat, verify and re-verify) and hypersensitiveness (discomfort sensations when she is watched, when she needs to eat or to drink in public).

Family history and interaction modalities within family system

She comes from a legal established family, only child. Affirmative, the adolescent has a good relationship with her parents, especially with her mother who she sees as her best friend. The father is less involved in the family live, working more. Affirmative, the mother of the patient follows weight loss diets for many years. The adolescent is being described by her mother as a model child with very good scholar results, that haven't brought up any problems until now. Although considered by the adolescent as best friend, the mother was always pretentious, critical with the patient and also preoccupied by maintaining corporal weight. The mother is also critical towards the husband, she sees himself as a hard-working but not knowing how to do anything and is absent from family live. The critical attitude of the mother towards the husband is sometimes induced towards the teenager who although resembles with her father, she says he doesn't matter. Thus, within the family system there are identified the interaction patterns described by Mara Selvini, where the teenage "sacrificed" by adopting an anorexiant behaviour in order to improve the couple relationship.

In both cases described above, the initial therapeutic approach was a multidisciplinary type one: initially the patients were somatic rebalanced and nourished by the paediatrician-nutritionist intervention; at the same time the adolescents received appropriate psychotropic medication prescribed by the psychiatrist. After stabilizing the patients individual psychotherapy sessions and family-type approaches were initiated, which improved the somatic and psychic symptomatology along with medication and helped improve family functioning.

Discussions

Anorexia has an obvious impact on the family and the symptom plays a central role in its life. After a while, the nutrition, food, and body weight are constant concerns for both the patient and for those who take care of her. Family members feel helpless in front of the symptom that is hard to explain and control and that increasingly restrict the repertoire of interaction patterns. Focusing attention on anorexia interferes with the accomplishment of the family life-cycle tasks.[9 -12] Sometimes the therapeutic modalities involve paradoxical attitudes where each family member's behaviour or the anorexia adolescent's behaviour is regarded as an act of kindness, generosity or sacrifice relating to the entire family or to one family member.

By following the family approach, the anorexic patient which had a lot of doctors or staff that were concerned about his nutrition until then, faces a team of therapists that are no longer concerned with the symptoms but with the family relationships and who consider the symptoms as an act of sacrifice, that doesn't correspond with the patient's original "intentions". For severe cases which require hospitalization (as the cases described above), it is recommended to organize lunch meetings that allow the observation of family's rigid configurations that maintain the symptom, on one hand, and to choose a strategy adapted to the configuration of the family system, on the other hand. In this context, the therapist may interpret the adolescent's anorexic behaviour as an act of disobedience and encourage in the same time the parent to firmly control this expression of rebellion which will lead to asking the child to eat. This is often useful at the beginning of the treatment to make the anorexic teenager eat, the focus of the therapy on nutrition can be abandoned afterwards and then be centred on a classical family approach, designed for family functioning.

More recently, in literature[11, 13] have been distinguished some phases of family type approach for adolescents with anorexia.

First phase of the treatment is mainly supporting parents in their efforts to restore the adolescent's weight. In order to achieve this objective, the therapist encourages the parents to make a common front toward restoring the weight. At first, the adolescent's food intake must be made under parental control, the parents should monitor the meals and snacks while restricting physical activity if necessary and limiting purging or other behaviours that can potentially lead to weight loss. Engaging the family in this task requires the therapist's ability to tell the parents that although it seems an impossible task, they will eventually succeed. Meanwhile, the therapist must assure the adolescent that he understands his fears regarding possible weight gain, but these fears and resistances should not impede his parents' effort to help him. It is necessary for the therapist to inform the parents about the disorder, its evolution and seriousness and that it is not allowed to have an inadequate attitude towards restoring the weight.

The therapist will not impose a particular course of action for the parents, but he will explore with them how the family functioned outside the disease context, he identifies the specific strengths of each parent and how these could be used to explore strategies for weight restoration.

The first phase of treatment focuses almost exclusively on weight restoration and a return to healthy eating patterns. Therefore, the therapist points out that this objective has priority over almost any other issue within the family up to the adolescent self-starvation.

The second stage of therapy begins when the patient has recovered a great proportion of the body weight, he eats without much resistance and family mood is more optimistic. In this phase, eating responsibility is again transferred to the adolescent, gradually and relating to his age. If the patient is 11-12 years old, normally the nutrition at this age is still under parental supervision. For an 17 years old teenager he will be trusted with confidence and independence. In this phase he can begin exploring topics and behaviours put on hold until then. The teenager will be allowed to go out with friends or at camps but only to the extent that the teen will continue to eat healthy.

The third phase of treatment usually begins around the time when the teenager has a healthy weight for his age and height. This part of the treatment focuses on general issues related to adolescence and on the possible mechanisms of family interaction that led to the symptoms and on building strategies to prevent relapse.

Conclusion.

Anorexia therapy is organized in compliance with certain principles:[9] teamwork is essential to combine psychiatric treatment, diet therapy, individual psychotherapy and family therapy. The most important elements of the intervention at a family-level are: assisting parents in assuming the role of authority and encouraging nutrition; encouraging an constructive communication style; exploring aspects of family life cycle; supporting family members who care for the patient; multi-family group therapy where several families with a member that is anorexic will attend, enabling learning and mutual support.

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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.