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INTERDISCIPLINARY APPROACH IN BRAIN TUMORS IN CHILDREN - RETROSPECTIVE STUDY IN CLINIC III PEDIATRICS

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Abstract

Brain tumors in children are the most common solid tumors in pediatrics, occupying the second place among neoplasia after acute lymphoblastic leukemia. In the past 20 years, the mortality of brain tumors (0.8 out of 100,000) exceeded the mortality due to acute lymphoblastic leukemia (0.4 out of 100 000) (1). This shows that tumor management with intracranial location is a challenge to the medical world and requires an interdisciplinary approach with the participation of pediatrician, neurosurgeon and radiotherapist. Our study wanted to be a retrospective analysis of 28 cases of brain tumors selected from the oncological pathology of Pediatric Clinics III of Children's Emergency Hospital, diagnosed and treated for a period of 10 years, seeking an interdisciplinary approach to improve quality of life and an increase in the survival rate of these children.

Keywords: brain tumor, child, interdisciplinary approach

Introduction

Brain tumors are neoplasms of central nervous system (CNS) cells, representing the second largest group (2). It is a heterogeneous group of diseases characterized by high histological variability, but their common elements are the intracranial location and difficulty of treatment due to this critical location. Data from the Surveillance, Epidemiology and Final Outcomes (SEFO) program from 1973 to 1989 showed an incidence of 2.8 cases per 100 000 children per year and a mortality rate of 45%. Epidemiological data suggest an increase in the incidence of brain tumors in children although they are not very clear and some specialists disagree with this. It is supposed to be a false increase in the incidence of intracranial tumors following the development of diagnostic imaging techniques. (1) They are associated with significant morbidity due to motor and intellectual deficits due to both the tumor itself and the treatment. Two incidence peaks were described: the first in children up to 10 years with an incidence of 2.5 cases per 100 000 children a year with a slightly male predominance (1.1: 1) and the second in decades three and four. Etiology for most tumors with CNS localization is unknown at present, but known risk factors are ionizing radiation, immunosuppression and association with genetic syndromes. (1, 2, 3) A study conducted in 2013 showed that there is a significant risk of developing a brain tumor after conventional radiation therapy. (4) Exogenous immunosuppression occurs in transplant patients and is associated with an increase in the incidence of SNL localized lymphomas. (5) Endogenous immunosuppression in patients with Wiscott-Aldrich syndrome or ataxia-telangiectasia is associated with an increased frequency of primary CNS lymphomas. (1,2,3). Genetic syndromes such as type I neurofibromatosis (NFI), type II Neurofibromatosis (NF II), Gorlin, Gardner, Li-Fraumeni, Turcot, von Hippel-Lindau syndrome, Tuberous sclerosis and type I endocrine neoplasia are most often associated with brain tumors .(3)

The classification of tumors with intracranial localization has been in continuous dynamics in recent decades due to advances in diagnosis and improvement of knowledge. It is important to have a more accurate fit for the correct treatment. The most commonly used is the World Health Organization (WHO) classification based on the histological appearance of the tumors, which is also the most important element in the choice of treatment. (8)

In terms of location, brain tumors may be: supratentorial localized in or in contact with the cerebral hemispheres, more common in adults; infratentorial located in the posterior cerebral fossa, in the cerebellum, in the cerebral trunk or in contact with these, more common in children and basal tumors that originate in the structures of the skull base (bone, meninges, nerve structures) or are invaded by tumor formations of the structures adjacent (rhino pharyngeal, mucous bone sinuses, vascular structures). (3)

The main objectives of the paper were to evaluate the need for an interdisciplinary approach from the moment of diagnosis by analyzing the clinical and par clinically aspects that allowed the framing and staging of diseases, on the one hand, and the choice of therapy and the pursuit of survival and evolution until the end of life, on the other.

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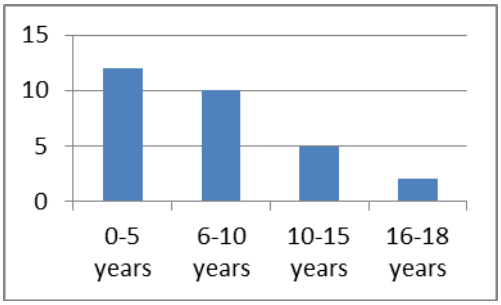

Fig.1. Distribution by age group

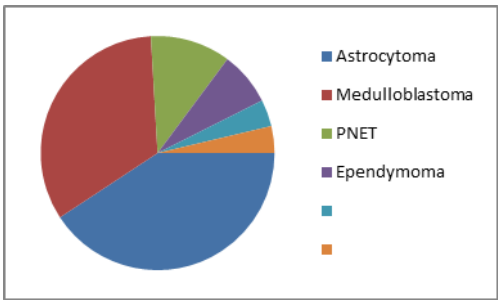

Fig.2 Distribution by histological type

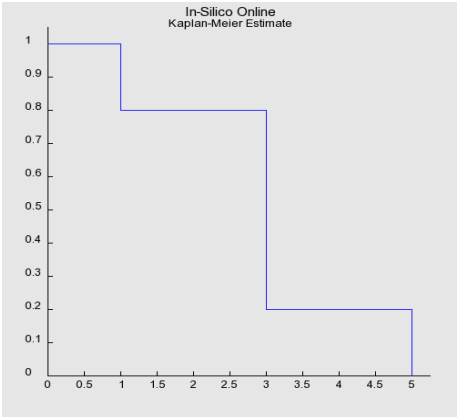

Fig. 3. Kaplan-Meier curve for general survival analysis

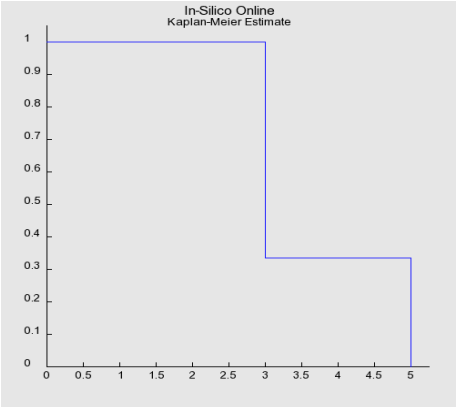

Fig. 4. Kaplan Meier Survival Curve for Astrocytoma

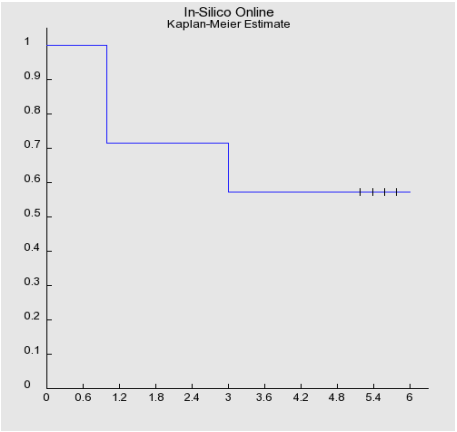

Figure 5. Kaplan-Meier survival curve for Medulloblastoma

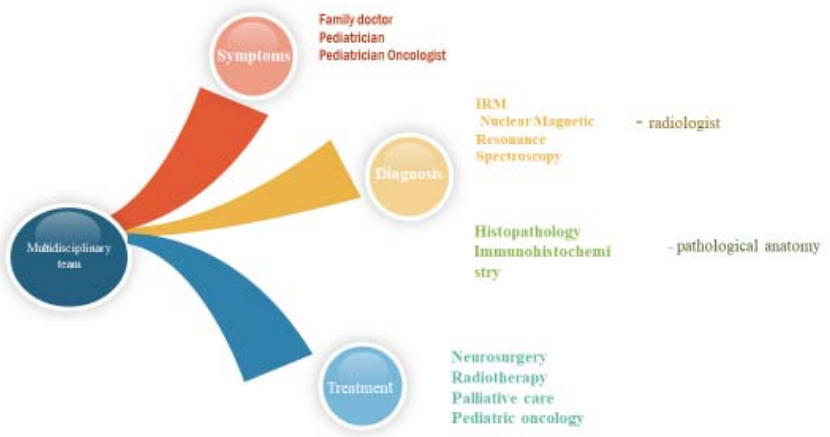

Fig. 6. The interdisciplinary approach

	Number of deaths	Number of survivors	Total
Subtotal ablation	5	6	11
Total ablation	2	14	16
Total	7	20	27

Table 1. Survival contingency chart at 3 years depending on the type of surgery

	Number of deaths	Number of survivors	Total
Subtotal ablation	6	5	11
Total ablation	4	12	16
Total	10	17	27

Table 2. Contingency Table of Survival at 5 Years Depending on Type of Surgery.

Material and method

The study group was comprised of 28 cases of children diagnosed with intracranial tumor selected from the total cases of pediatric neoplastic pathology admitted and treated on the Department of Pediatric Oncology of the Emergency Clinical Emergency Hospital for Children Louis Turcanu Timisoara during 2006-2016. Patients are aged between 1 and 17 years old with a ratio of boys: girls 1.33: 1. The criteria for inclusion in the study were: age under 18 years, tumor diagnosis with intracranial location, oncology treatment: surgical resection, chemotherapy and / or radiotherapy, possibility to follow up the patient for 5 years after diagnosis except for those deceased at less than 5 years after diagnosis. For each patient, we looked at the following issues: age, gender, background (rural or urban); histological type and tumor location (infratentorial or supratentorial), symptoms present at diagnosis; (total or subtotal) surgical ablation, radiotherapy or chemotherapy as well as evolution and survival The data obtained from the observation sheets (with the opinion of the ethics committee of the institute) were statistically analyzed by EpiInfo and the Kaplan-Meiner survival curves were calculated.

Results and discussions

Analyzing the gender distribution in the 28 patients study group, 16 are male and 12 female represented 57%, respectively, 43%. The boys / girls ratio is 1.33 to 1. As far as the environment of origin is concerned, 11 patients are urban and 17 rural, representing 39%, respectively, 61%. The median of the diagnostic age is 6 years old and the average diagnosis age is 6.9 years old. Most patients were diagnosed between 0-5 years (12 cases) and as we approach the age of 18, the number of diagnosed cases decreases progressively. In the age range of 16-18 years, only 2 cases were recorded (Fig. 1). Data from literature shows the highest incidence of SNC tumors to be in children aged 12 months to 6 years. (6)

Taking into account histological types of CNS neoplasm: Astrocytoma was the most commonly diagnosed tumor, accounting for 39% of the total of 28 cases, followed by Medulloblastoma by 32%. There were 3 cases of PNET (10%), 2 cases of Ependymoma (7.14%), 1 Glioblastoma and 1 case of Oligodendroglioma (Fig. 2). Our data are comparable to those in literature, pilocytic Astrocytoma being the most commonly diagnosed intracranial tumor in the pediatric population in the US. (6) In terms of location: 64% of the tumors have infratentorial localization and 36% supratentorial. These data overlap with those in literature, the most common localization described in pediatric patients being infratentorial. (3)

In 67% of the patients the most common symptom in diagnosis was headache, followed by nausea and vomiting 53%. In 75% of patients, there were signs and symptoms of intracranial hypertension, in 46% cognition and balance disorders were present, in 32% brain nerve damage, epileptic seizures in 32% and in 17% hemiplegia / hemiparesis. According to an outpatient study, in 75 patients aged 5 months to 16 years, the most common symptoms in diagnosis were headache (51% of cases), vomiting (51% of cases), seizures (24%), and personality changes (11% of cases). New imaging techniques are MRI and Positron emission CT (CT) and with agents such as fluorodeoxyglucose (FDG) that have a high sensitivity (92%) and specificity (69%) in the differentiation of radiation-induced necrosis tumor recurrence compared to conventional MRI and are required in current practice. (9, 10)

The first step in the treatment of tumors with intracranial location is surgery. Exceptions to this therapeutic strategy are infiltrative diffuse Glioma and globular chiasmatic Glioma in patients with Neurofibromatosis type 1 (NF1) because tumor resection does not influence prognosis and diagnosis can only be determined by MRI.

The purpose of surgery is total resection (if possible), being the main prognostic factor for Ependymoma, small grade Glyoma, Craniopharyngioma, Meduloblastoma, high grade Glioma. Nevertheless, aggressive resection increases the risk of short and long-term morbidity and mortality. (3)

Out of the 28 patients enrolled in the study, in 27 (96%) surgery was recommended and performed. The only case in which neurosurgical intervention was not performed was a case diagnosed at a very advanced stage in which surgery was not possible and would not have brought any additional benefit to progression and prognosis. Out of the 27 neurosurgical patients treated, in 11 (40%) there was subtotal ablation and the remaining 60% benefited from total macroscopic ablation. Total ablation is also the major prognostic factor for these patients. The limitations of surgical intervention related to localization and technique make prognosis in brain tumors to be unfavorable.

Overall systemic chemotherapy was performed according to the histological type in 82% of the patients, some of whom also had local chemotherapy administered via an Ommaya reservoir (25% of all patients). Most of the patients (46%) received maximum treatment consisting of surgical ablation with chemotherapy and radiotherapy. The second place as a therapeutic strategy is the combination of surgical ablation with chemotherapy performed in 32% of patients. Radiotherapy can be used for curative or palliative purposes. There are differences in the sensitivity of tumors to radiotherapy, for example Medulloblastomas are more sensitive than Glioblastomas, and the indication of radiotherapy is made considering histology results. (7)

Radiotherapy was performed in 57% of patients in combination with chemotherapy and / or surgical ablation. Alternatives to fractionated radiotherapy could be performing hyper-fractured radiotherapy that allows a higher total dose to be given in a larger number of smaller adverse reaction radiation sessions. Out of the 28 patients, only one performed hyper-directed radiotherapy outside of our country. Patients' access to hyper-fractionation radiotherapy should be considered, as this type of therapy could bring additional benefits in terms of both quality of life and progression and prognosis. (6)

The main complications of chemotherapy (22 patients who underwent chemotherapy) were: bacterial infections (95%), aplasia (90%), candidiasis (59%), toxic hepatitis (50%) and bleeding (31%). 13 patients (46%) were admitted to the department of anesthesia and intensive care. 92% of the patients survived 1 year, 3% lost to 71% and 5% survived only 53% of the patients. And of those who survived, of those who survived over 5 years there were 4 more deaths, which means that the survival rate has fallen to 39%.

Neurological tumors generally fall into high or low grade strata and generally higher grade tumors have poorer survival, however, patients with either high grade or low grade tumors suffer the adverse effects of chemotherapy, radiation, surgery, and direct disease sequelae, which may be ameliorated with palliative care. (12)

Of the total patients who underwent subtotal ablation (7), 6 survived at 3 years while the total of those who benefited from total ablation, 14 survived at 3 years (Table 1).

The death risk in patients who have undergone total ablation is 2/16 = 12.5% compared to the risk of death in patients who have undergone subtotal ablation that is 5/11 = 45.4%. The relative risk is 3.6, which means that the group of patients undergoing subtotal ablation has a 3.6-fold higher risk of death at 3 years than the group of patients

who have undergone total ablation. The same type of contingency table was used to calculate the risk of death at 5 years depending on the type of therapy performed: total ablation, subtotal ablation (Table 2).

The results show that the risk of death at 5 years for those who performed subtotal ablation is 54.5%, whereas in patients where total ablation was possible it is 25% with a 29% risk difference p = 0.07 with a confidence interval of 95.5%. These results shows that the values obtained have no statistical significance, probably due to the small number of cases included in the study.

For the survival analysis we used the Kaplan-Meier survival curve. The figure below is the time axis, meaning the years of survival from diagnosis (1, 3 and 5), and the percentage of deceased patients at 1.3 and 5 years, starting from a 100% survival, that is, the value 1 on the graphic.

Figure 3 shows that approximately 20% of patients died from the total of deaths in the study at one year. Three years after diagnosis, the highest death rate (approximately 60%) was recorded and 20% of all deaths were diagnosed 5 years after diagnosis. These results could be an alarm signal for the period in which we have the greatest risk of losing a patient with intracranial tumor.

Figure 4 shows the Kaplan Meier survival curve for Astrocytoma at 1, 3 and 5 years. Of all deaths by astrocytoma, no death is recorded at 1 year after diagnosis. Three years after diagnosis the highest death rate is recorded (over 60%), and at the age of 5 from the time of diagnosis, approximately 40% of all deaths by Astrocytoma are recorded.

Figure 5 shows the Kaplan Meier survival curve for Medulloblastoma. Unlike Astrocytoma, in the case of Medulloblastoma, deaths are also recorded one year after diagnosis (about 30% of the total) and at 3 years 20% of all Medulloblastoma patients die. The interdisciplinary approach in our study, from diagnosis to treatment, is illustrated in figure 6.

Palliative care was imposed at diagnosis to a case that was surgical unapproachable, in two cases that died in the first months of treatment and in all cases with unfavorable progression to death. Survivors of neoplasia with intracranial location had an increased rate of neurological squeal secondary to treatment including strokes, motor impairment, hearing loss, increased risk of stroke or the occurrence of Meningioma or other secondary therapies for chemotherapy and radiotherapy.(3)

A palliative consult for patients with brain tumors is associated with longer survival and better quality of life. In the ENABLE III study, patients who received early palliative oncology care had significantly longer 1 year survival rates than who received delayed palliative care. (11)

Conclusions

In our study, the most affected age group was 0-5 years totaling a number of 12 patients diagnosed with CNS tumors, followed by the age range of 6-10 years in which 10 patients were diagnosed. The histological type of CNS tumor most commonly diagnosed was Astrocytoma followed by Meduloblastoma and PNET. Surgical treatment was used in 27 of 28 patients with or without chemotherapy and radiotherapy. One of the prognostic factors identified in the study is the type of ablation (total or subtotal), subtotal ablation patients with a 3-year-risk death rate, 3.6 times higher than those who benefited from total macroscopic ablation. The limits of surgical intervention are related to localization and technique. Radiotherapy was performed in 57% of the patients in combination with surgery or chemotherapy. It has become necessary for the interdisciplinary approach to be formed by pediatrician, neurosurgeon, radiotherapist, intensive care physician and palliative care specialist.

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METABOLIC AND ENDOCRINE COMPLICATIONS OF CHRONIC KIDNEY DISEASE IN CHILDREN

TO Bizerea-Moga[1,2], A Crăciun[1,2], P Tegian[2], A Velcelean[2], O Mărginean[1,2]

Abstract

Introduction: Pediatric CKD and the long-term complications not only have an impact on the child's health, but are also transforming this pathology into a worldwide health problem. Aim: To evaluate the metabolic and endocrine complications described in patients with CKD. Material and method: A four-year retrospective observational study was conducted between January 2015 and the December 2018, at "Louis Turcanu" Children's Clinical and Emergency Hospital. 41 pediatric patients, diagnosed with chronic kidney disease (CKD) were included. According to the etiology of CKD, patients were divided in two study groups: 22 patients (53.7%) with congenital anomalies of the kidney and urinary tract (CAKUT) and 19 patients (46.3%) with glomerular disease. Metabolic and endocrine complications if documented were recorded in all patients. Results: Dyslipidemia (47.7%) and hypertension (36.8%) were more prevalent in patients with glomerular disease whereas CAKUT more often associated mineral-bone disease (31.8%) and anemia (27.3%). Patients from the CAKUT group showed higher complication rates in end-stage CKD, 18.8% for both bone mineral disorder and anemia. Patients with glomerular disease had complications in early stages of CKD, predominantly represented by dyslipidemia (73.7%) and hypertension (52.6%). The highest prevalence of short stature was noted among end-stage CAKUT-CKD patients (22.7%), followed by stage 3 CAKUT-CKD (13.6%) patients. Conclusions: The main cause of CKD in children is represented by CAKUT. Anemia and mineral and bone disorder are the main complications in CAKUT-CKD, whereas glomerular disease primarily associates hypertension and dyslipidemia. Complications appear in early stages of glomerular disease - CKD and advanced stages of CAKUT-CKD. Short stature is more prevalent in advanced CKD stages.

Keywords: chronic kidney disease, congenital anomalies of the kidney and urinary tract, children, metabolic and endocrine complications

Introduction

Kidney Disease Improving Global Outcomes (KDIGO) defines chronic kidney disease (CKD) as a chronic pathology, result of a gradual loss of kidney function developing for more than 3 months, manifested by the following features: GFR <60 mL/min/1.73 m2 or structural damage of the kidney and urinary tract, identified by blood or urine studies, imaging tests or kidney biopsy [1].

CKD has become a major health problem over the last decade, due to its increasing incidence and prevalence. Primary causes of CKD in young children are represented by congenital anomalies of kidney and urinary tract (CAKUT), while tubular and interstitial glomerulopathies are predominant in children over the age of 10 years [2-5].

CKD is often clinically asymptomatic, especially in earlier stages. The symptoms can be subtle and frequently unspecific, making the diagnosis a real challenge for the clinician.

Growth impairment is one of the most common and perhaps the most noticeable CKD complication. The degree of the growth impairment correlates with GFR, and its severity increases as the age of onset is decreased. The risk factors that contribute to growth impairment are: metabolic acidosis, anemia, mineral and bone disorders and electrolyte imbalances. However, especially after early childhood, growth failure is mainly due to decreased bioavailability of somatotropin in uremia [7-9].

The mineral and bone disorder of CKD is a systemic disorder defined by the presence of disturbances of biohumoral markers or pathological findings on imaging tests or bone histology. The term renal osteodystrophy is exclusively used to define alterations of bone morphology. This complication is present in about 60-80% of children with CKD and is manifest after the loss of approximately 50% of the nephron population. CKD leads to alterations of mineral homeostasis characterized by imbalances of serum calcium (Ca), phosphorus (P), parathormone (PTH) and 1,25- Dihydroxyvitamin D3 levels. GFR decrease under 60 ml/min/1, 73 m2 determines phosphate retention, an increase in PTH secretion and suppression of calcitriol synthesis in the kidney [4, 10].

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Common causes in children < 5 years old :	Common causes in children > 10 years old :
Malformative uropathies 25%	Glomerular nephropaties 33%
Hereditary nephrophaties 16%	• Focal segmental glomerulosclerosis • Proliferative endo- and exocapillary glomerulonephritis
• Cystinosis • Oxalosis • Alport Syndrome	Vascular nephrophaties 5%
Hypo-/ Dysplasia 17.6 %	• Hemolytic-uremic syndrome • Diabetes mellitus
• Cystic/ Noncystic • Segmental hypoplasia • Cystic kidney disease	• Hypertension • Renal vein thrombosis

Table 1. Etiolgy of CKD in children [6]

Study group	*Patient number*	*Endocrine and metabolic complications*	*Paraclinical investigations*
Congenital structural abnormalities of the kidney and urinary tract	22	➢ Growth impairment ➢ Systemic hypertension ➢ Anemia	➢ Anthropometric measurements, bone age ➢ Blood pressure ➢ Complete blood count, reticulocyte count, serum iron, ferritin, transferrin, serum erythropoietin
Glomerular disease	19	➢ Dyslipidemia ➢ Mineral and bone disorder	➢ Lipid panel ➢ Total calcium, ionized calcium, phosphate, intact parathyroid hormone from plasma

Table 2. Patient distribution in study groups according to the etiology of CKD

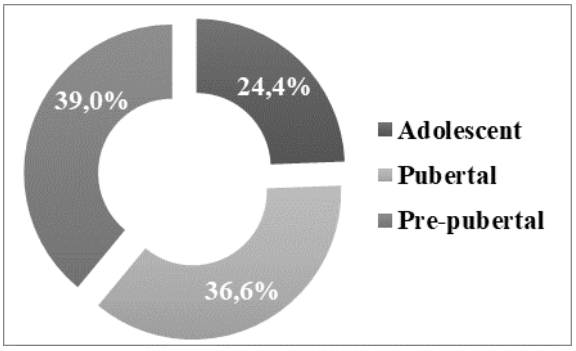

Fig. 1. Patient distribution according to age at time of study

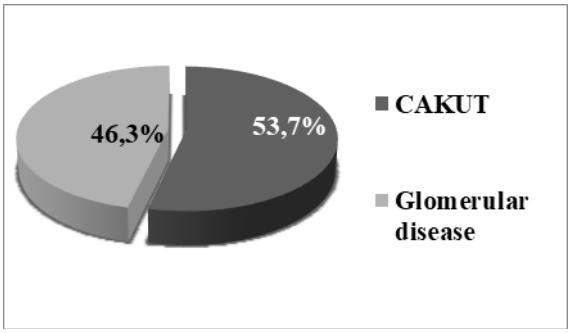

Fig. 2. Percentage distribution of patients according to etiology of CKD

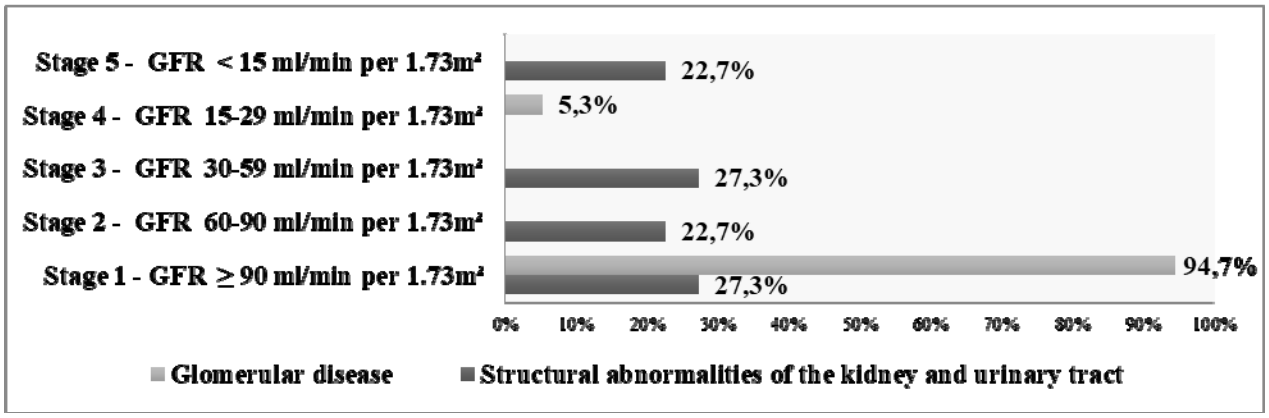

Fig. 3. Stages of CKD among studied patients according to GFR

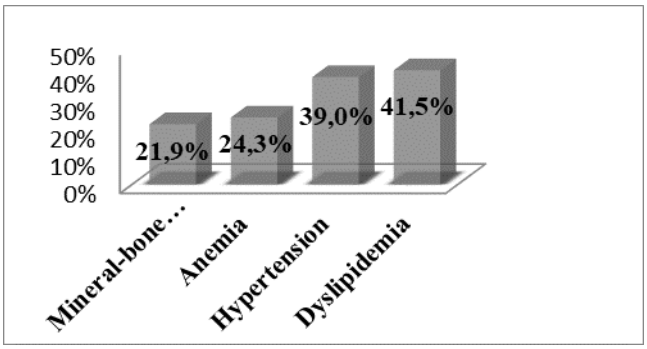

Fig. 4a. Endocrine and metabolic complications of CKD in the studied patients

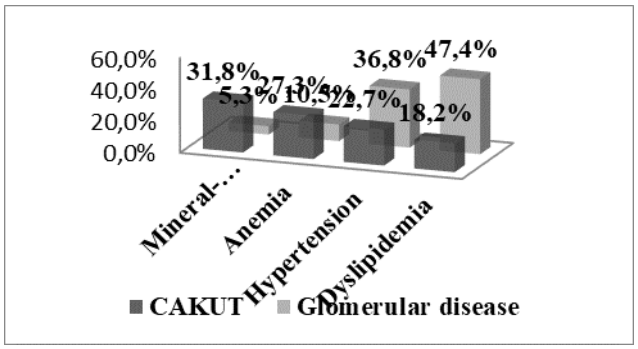

Fig. 4b. Percentage distribution of endocrine and metabolic complications in studied groups divided according to CKD etiology

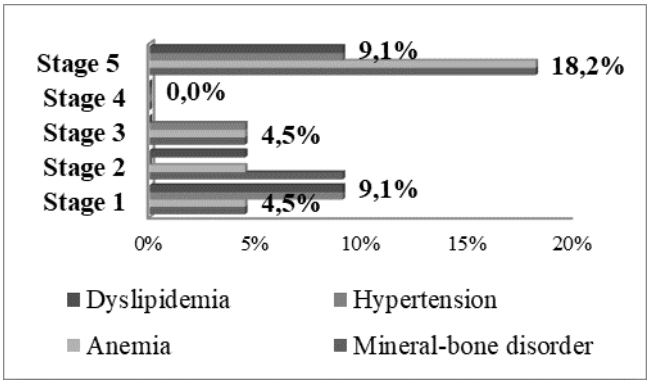

Fig.5a. Percentage distribution of complications according to CKD stages in patients with CAKUT

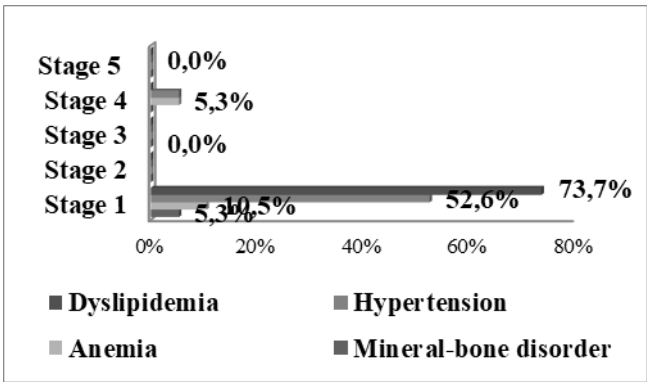

Fig.5b. Percentage distribution of complications according to CKD stages in patients with glomerular disease

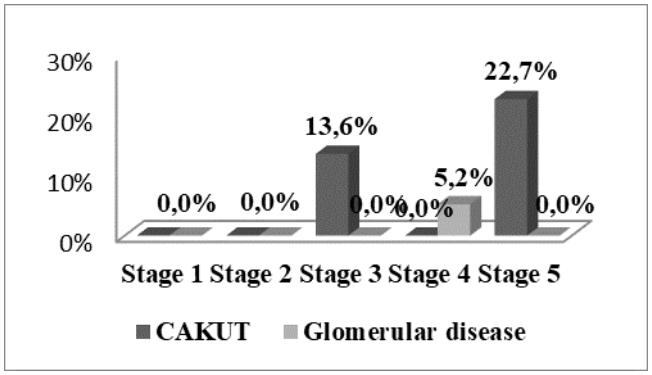

Fig. 6. Prevalence of short stature among studied patients

Anemia is a common complication in children with CKD and is defined as hemoglobin (Hb) levels under the 5th percentile relative to age and sex. The prevalence of anemia increases with CKD progression. Renal anemia is present in 73% of stage 3, and in over 93% stage 5 CKD cases [11]. Although anemia has a complex etiopathogenesis resulting from the interaction of multiple factors, the principal role is played by erythropoietin (EPO) deficiency, especially in advanced stages of CKD [12]. The decreased EPO synthesis at cellular level is caused by interstitial fibrosis that leads to irreversible loss of kidney function. Renal fibroblasts or EPO producing pericytes are transformed into myofibroblasts and lose synthesis function. The anemia of CKD is typically normocytic, normochromic, and hypoproliferative. Other causes include iron deficiency, chronic inflammation, malnutrition, secondary hyperparathyroidism and uremia [13].

The typical CKD dyslipidemia is hypertriglyceridemia. There is an inversely proportional relationship between GFR and triglyceride (TG) and total cholesterol levels, but a direct proportional one with high density lipoprotein (HDL) levels. The abnormally high TG levels are attributed to overproduction, due to increased hepatic VLDL synthesis associated with a decreased catabolism. Furthermore, the levels of apolipoprotein C-III, a direct inhibitor of lipase, are increased in uremia, causing a rise in TG values. Secondary hyperparathyroidism plays an additional role in the reduction of VLDL catabolism. Although, LDL levels are within normal range in CKD, the particles tend to be smaller, denser and more atherogenic [14, 15].

In children, 70% of hypertension cases are secondary and 50-80% of these are caused by renal parenchymal disease. Thus, arterial hypertension can be the first sign of CKD in children. Hypertension, defined as arterial blood pressure values over the 90th percentile relative to age, sex and height, can manifest even in early stages of CKD and is negatively correlated to GFR [16]. Recent randomized studies have shown that maintaining the tensional values below the 50th percentile, delays the progression of CKD [3, 17].

Aim of the study

The purpose of the study was to evaluate the metabolic and endocrine complications described in patients with CKD.

Materials and Methods

A retrospective observational study was conducted over a four-year period (1st of January 2015 and the 31th of December 2018), at the 1st Pediatric Clinic of "Louis Turcanu" Children's Clinical and Emergency Hospital. Medical records of pediatric patients aged 0-18 years diagnosed with CKD were analyzed. An electronic registry composed of anonymized patient data was created by searching individual patient files. 41 patients were divided into two study groups according to the cause of CKD, respectively 22 patients with CAKUT and 19 patients with glomerular disease. Anthropometric measurements, blood pressure and bone age were recorded. Blood studies including anemia, lipid and bone metabolism panel were documented in all subjects, as shown in Table 2.

Results

The distribution of patients from the study group according to age is shown in Figure 1. The highest

percentage was represented by pre-pubertal children (39 %) followed closely by pubertal patients (36.6%).

As shown in Figure 2, there are no significant differences between the study groups resulting from the distribution of patients according to the etiology of CKD. CAKUT was the underlying cause in 54% and glomerular disease in 46% of cases.

Figure 3 highlights the distribution of patients according to CKD stages based on GFR values. The study group consisting of patients with CAKUT had a more severe impairment of renal function translated by a higher percentage of patients with end-stage CKD (22%). In comparison, only 5% of the patients with glomerular disease have reached fourth stage and none end-stage CKD.

Figures 4a and 4b show the incidence of CKD complications among the patients included in the study. A specificity of the complications that have arisen depending on the etiology of CKD is noted.

In Figures 5a and 5b, the distribution of complications according to CKD stages is shown. According to the underlying cause for CKD, specific patterns can be noticed. Bone mineral disorder and anemia, both with the highest prevalence of 18.2% in end-stage CKD in patients with CAKUT. Dyslipidemia (73.7%) and hypertension (52.6%) are noted in stage 1 CKD in patients with glomerular disease.

Discussion

Although progress has been made in recent years in the diagnosis, monitoring, and treatment in children, CKD still represents a „silent epidemic" in this age-group. The initial stages of CKD are usually asymptomatic leading to late discovery and guarded prognosis of the disease.

This study shows a relatively balanced age-related distribution of subjects in three distinctive groups: 39% pre-pubertal, 36.6% pubertal and 24.4% adolescent patients.

Data form literature records a correlation between socio-economic status and the etiology of CKD. In a study carried out in France on a group of 127 children with CKD, 68.5% were shown to have underlying CAKUT, and 30.7% acquired nephropaties [18]. In a retrospective study conducted in Iran, the main cause of CKD in children was glomerular disease (34%), followed by reflux nephropathy (16.7%) [6]. Similar data was published in a review from Nigeria, which confirms glomerulopathies as the leading cause of CKD, in 53.3% of 45 end-stage cases evaluated along 15 years [19]. No significant difference between the two main causes of CKD were noted in this study. CAKUT was diagnosed in 53.7% and glomerular disease in 46.3% of children admitted for CKD, at the Emergency Hospital for Children "Louis Turcanu" Timisoara.

Most of the epidemiological information on CKD and associated metabolic and endocrine complications, comes from registries of end-stage patients requiring renal replacement therapy (RRT). Data on early stages of CKD are still limited. Complications of CKD in both early and end stages were observed in the conducted study. Comparative analysis of GFR in the two study groups revealed more severe kidney damage, in patients with CAKUT. Thus, 22.7% of these patients had been diagnosed with end-stage CKD, and a cumulative percentage of over 70% of patients demonstrated impaired renal function, translated by decreased GFR. By comparison, 94.7% of patients with glomerular disease showed GFR within normal ranges at the time of study. A study conducted by Deleau and colleagues demonstrated that although initial GFR is better at onset in glomerular disease, the decrease of kidney function occurs more rapidly in these cases [18].

As can be seen in Figure 4a, the main complication of CKD was dyslipidemia (41.5%), followed by hypertension (39.0%), anemia (24.3%) and bone mineral disease (21.9%). Figure 4b highlights the distribution of complications according to the etiology of CKD. Thus, it can be noticed that dyslipidemia (47.7%) and hypertension (36.8%) are complications with the highest prevalence in the glomerulopathy group, totaling more than 80 % of cases, compared to anemia and mineral-bone disease, which add up to only 15.8 %. These data are consistent with recent clinical trials, such as the Taiwan Pediatric Renal Collaborative Study conducted in 2016 by the working group of Chou and collaborators [20]. In contrast, CAKUT more often associated mineral-bone disease (31.8%) and anemia (27.3%). In this study group, the complications were more evenly distributed. Hypertension and dyslipidemia had a lower prevalence of 21% and 17%, respectively. These findings demonstrate the importance of renal parenchyma integrity for endocrine function of the kidney.

In Figures 5a and 5b, the distribution of complications according to CKD stages is shown to follow specific model. Patients from the CAKUT group showed the highest complication rate in end-stage CKD. In this group there was a significantly higher prevalence of bone mineral disorder and anemia (18.2% in both cases). In the group of patients with glomerular disease, most complications appear as early as stage 1 and are predominantly represented by dyslipidemia (73.7%) followed by hypertension (52.6%). The data coincide with those of other studies conducted so far, in which hypertension and dyslipidemia have been recorded in early stages of CKD. In a study conducted by Wong and colleagues, 63% of the 366 included patients were diagnosed with hypertension in stage 1 CKD [21]. The inverse correlation between impairment of kidney function, and occurrence of complications in CAKUT-CKD in the pediatric population, has been demonstrated in other studies such as those conducted by Chou et. al in Taiwan, Bek and colleagues in Turkey and the working group of Ardissino in Italy [20, 22, 23].

Short stature, as illustrated in Figure 6 showed the highest prevalence among end-stage CAKUT-CKD patients (22.7%), followed by stage 3 CAKUT-CKD patients (13.6%). One stage 4 glomerular disease - CKD patient representing 5.2%, had growth failure. This is supported by evidence from literature that attributes growth failure to a complex multifactorial ethiopathogeny. Metabolic acidosis, anemia and phospho-calcic metabolism disorders as well as human growth hormone (hGH) resistance in uremia contribute to a decreased height in CKD patients [7-9, 24].

Conclusions

1. Congenital anomalies of the kidney and the urinary Tract (CAKUT) represent the main cause for CKD in children.

2. Similar to adults, the most common complications of CKD in children are represented by dyslipidemia, hypertension, anemia and mineral and bone disorder.

3. There is a correlation between the etiology of CKD and the complications it causes. CAKUT are associated with anemia and mineral and bone disorder, whereas glomerular disease causes hypertension and dyslipidemia.

4. Glomerular disease associates complications at onset of CKD with normal or slightly decreased GFR values. CAKUT require longer until the onset of complications, in more advanced stages.

5. Short stature due to decreased bioavailability of somatotropin in uremia, metabolic acidosis, anemia and phospho-calcic metabolism disorders is specific for the pediatric population. A higher prevalence is noted in advanced stages of CKD.

6. Screening and early diagnosis of CKD and its complications, as well as close monitoring and appropriate therapy, are crucial to prevent disease progression and complications.

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CROHN'S DISEASE PRESENTING AS ANOREXIA NERVOSA

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Abstract

Introduction: Crohn's disease (CD) is a chronic inflammatory bowel disease that can involve any part of the gastrointestinal tract. Anorexia nervosa (AN) is a severe mental disorder included in the category of eating disorders, characterized by maintaining inadequate small body weight, a distorted body associated with the fear of weight gain. CD and AN share common symptoms of weight loss and reduced oral intake. The prevalence of both pathologies has increased over time, symptoms may be similar, leading to a delayed diagnosis and requiring complex, multidisciplinary management. Aim: To present the case of a teenager with CD, initially diagnosed with AN. Case report: The 17-year-old patient was referred to our clinic for: involuntary weight loss (10 kg/3 months), nausea, asthenia, fatigue, constipation alternating with diarrhea. AN was suspected, which was supported by the pediatric psychiatrist. During the hospitalization in our clinic we noted painful perianal skin tags and anal fissures, as well as inflammation and high fecal calprotectin levels. Upper and lower digestive endoscopies were suggestive for CD. The histopathological examination confirmed the diagnosis of moderate CD. Conclusion: Although the two pathologies have relatively common symptomatology, in this case, anorexia is only a sign associated with Crohn's disease.

Keywords: anorexia nervosa, Crohn's disease, weight loss.

Introduction

Crohn's disease (CD) is a chronic inflammatory disorder that can involve any part of the gastrointestinal tract (1). Anorexia nervosa (AN) is a severe mental disorder included in the category of eating disorders, characterized by the maintenance of a small, inadequate body weight, a disturbed view on body image associated with the fear of weight gain (2). CD and AN share common symptoms of weight loss and reduced oral intake. The prevalence of both pathologies has increased over time, symptoms may be similar, leading to a delayed diagnosis and requiring complex, multidisciplinary management (3, 4).

Aim

We aimed to present the case of a teenager with CD, initially diagnosed with AN**.**

Case report

The 17-year-old patient was referred to our clinic for: involuntary weight loss (10 kg/3 months with a body mass index (BMI) of 15.4 kg/m2), nausea, asthenia, fatigue and constipation alternating with diarrhea. AN was suspected, which was supported by the pediatric psychiatrist. She was also diagnosed with anxiety, depressive disorder, recommending investigations and treatment in the Pediatric Psychiatry Clinic. During the hospitalization in our clinic we noted painful perianal skin tags and anal fissures (Figure 1). The blood work showed moderate inflammation, anemia and a significantly increased fecal calprotectin (1970 µg / g). Infections causes were excluded. We performed an upper (Figure 1) and lower digestive endoscopy (Figure 2), which was suggestive for moderate CD (Montreal Classification A2 L3 L4 B1, PCDAI score= 32.5). The histopathological examination confirms the diagnosis. We initiated induction of remission with exclusive enteral nutrition (1200 ml polymeric formula/day, 1 ml = 1 kcal). Concurrently, maintenance therapy with Azathioprine (2.5 mg / kg / day) was started. After 2 weeks, although the patient was in clinical remission, the blood work revealed increased inflammation (PCDAI score = 40, Figure 3). Thus, we decided to step up the induction therapy using Prednisone (1 mg/ kg/day). We associated partial enteral nutrition and continued Azathioprine therapy. The patient achieved remission two weeks later (PCDAI score = 10, Figure 3).

Discussion

CD is a chronic idiopathic intestinal inflammatory disease characterized by transmural inflammation and granulomatous lesions in the gut. Its prevalence is rising globally. Features in childhood include anorexia, weight loss, diarrhea, abdominal pain, perianal disease, pubertal and growth delay (4, 5).

AN is an eating disorder diagnosed by the following criteria: restriction of energy consumption in relation to the requirements, leading to a significantly lower body weight in the context of age and gender. AN prevalence is 0.3% in both male and female adolescents and is increasing in developed countries (7). The prevalence of AN subtypes that appear similar but do not meet the full diagnostic criteria is 1.5% in females versus 1% in males (8).

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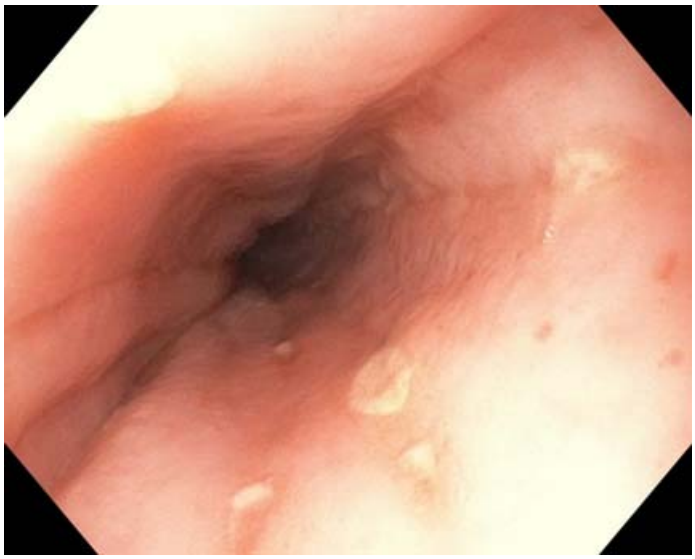

Fig. 1 Upper Digestive Endoscopy: Aphte of the esophagus

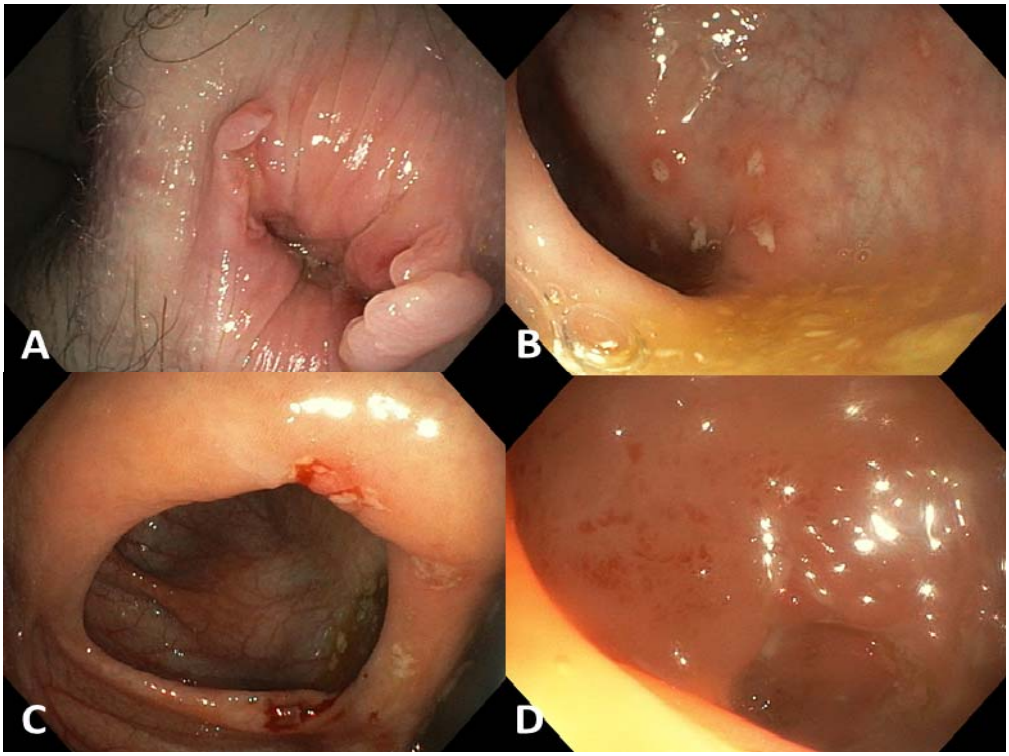

Fig. 2 Lower Digestive Endoscopy: A. Inflamated Crohn's Disease skin tags; B. Aphte of the left colon; C. Ulcerations of the ileo-coecal valve; D. Congestion and aphte of the ileum.

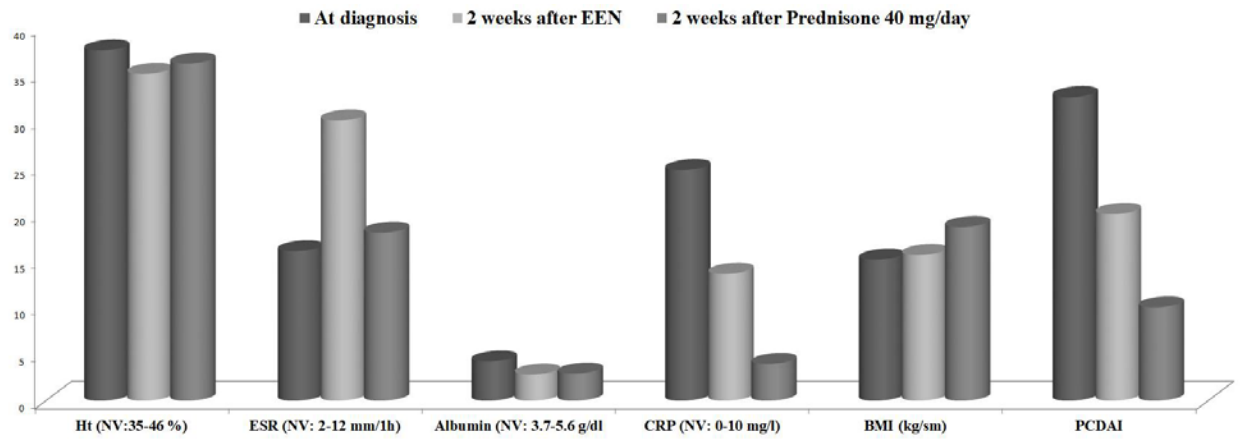

Fig. 2 Anthropometric and biological indices at diagnosis, after EEN and Prednisone, respectively. EEN denotes exclusive enteral nutrition; HT, hematocrit; NV, normal values; ESR, erythrocytes sedimentation rate; CRP, C reactive protein; BMI, Body Mass Index; PCDAI, Pediatric Chron's Disease Activity Index.

The incidence of CD in adolescents is 6-7.4:100.000 per year. CD should be considered in the differential diagnosis of AN, especially in younger patients. CD and AN share certain clinical features indistinguishable in diagnosis (4). A series of published reports highlights delays in diagnosing CD due to a presumed initial diagnosis of nervous anorexia (3). Current guidelines of the American Psychiatric Association recommend the examination and exclusion of gastrointestinal disorders in the diagnosis of anorexia nervosa. Gastroenterologists should maintain a high index of suspicion regarding the development of AN in patients with pediatric in inflammatory bowel disease (IBD).

Continuous weight loss in relation to disease activity is a clinical "red flag", especially among patients reporting weight satisfaction with the current weight, with normal food intake and good drug delivery. Plotting weight and height on appropriate growth charts at each visit, regardless of the reported clinical symptoms, is essential. Concerns about body image changes while on treatment should also be anticipated and explored. Access to multidisciplinary assessment of the potential factors to AN development is essential for pediatric gastroenterologists (8). Factors that include interpersonal relationships, aggression, family dynamics, and personality traits may influence the onset of AN. IBD are associated with other mental health disorders. The rates of depression and anxiety in CD patients are higher than in the reference population, even 5 years after the initial diagnosis. Children and adolescents with IBD have higher rates of depression, anxiety and phobias, than healthy children or patients with other chronic pediatric diseases. The multidisciplinary approach and support for infants and adolescents with IBD is vital in order to elucidate such complex presentations and provide patient support. CD is a complex and very variable disease. Multidisciplinary support for children with CD disease is necessary, even during remission (9). Concerns about non-compliance with treatments, abnormal eating habits or unexpected weight loss should trigger an early multidisciplinary approach.

Although the two pathologies have relatively common symptomatology, in this case work-up revealed that anorexia was only a sign associated with CD.

Conclusions

Mental health disorders are commonly associated with BC, therefore it is desirable to examine and exclude gastrointestinal disorders before diagnosing AN.

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MONITORING BRONCHIAL INFLAMMATION IN PEDIATRIC ATHLETES – A PROSPECTIVE STUDY

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Abstract

Fraction of the exhaled nitric oxide (FeNO) is used as a biomarker for eosinophilic inflammation, mostly on the respiratory tract. This tool may be used as a predictor of exercise-induced bronchospasm in children practicing various sports. The aim of our research was to study the pediatric athletes and to correlate the bronchial inflammation with the type of sport and the clinical features of the subjects. Method. We conducted a prospective study of children aged over 6, practicing outdoor and indoor sports, admitted to our pediatric department. After anamnesis and clinical examination, the patients performed FeNO measurements using the NioxVero analyzer (Aerocrine, Sweden). Results. Our study included 178 children (102 boys) practicing outdoor sports (football, track and field) and indoor sports (basketball, volleyball, handball, swimming). The FeNO levels were significantly higher in children practicing indoor vs. outdoor sports ($p = 0.0001$, t test). The prevalence of atopy was similar ($p = 0.55$, chi square) and the FeNO values in atopic children were slightly elevated but not statistically significant compared to the main sport groups. Data regarding gender, age and social status did not bring significant statistical differences. Conclusions. In our study FeNO was useful in differentiating the indoor vs the outdoor sports. The data regarding the atopic children or the demographic features were not significant.

Keywords: children, exhaled nitric oxide, athletes.

Introduction

Up to now, there is still a rather small number of systematic research to track the risk factors for broncho-obstructive pathology in children practicing various sports. In Romania there is also lacking structured data on the practice of sports in children (whether we are talking about recreational or mass sports), the small amount of data being a result of private initiatives. Bronchial asthma - the most common chronic disease in childhood - has an effort-induced component that can often be difficult to diagnose. Effort-induced bronchoconstriction may vary between 30 and 70% (1), while more recent studies (2, 3) claim that its prevalence in pediatric athletes is 15%.

Determination of bronchial inflammation is a desideratum we have approached in the last few years by the dosing of nitric oxide in exhaled air (FeNO), a reproducible marker and relatively easy to obtain, which makes it useful in the diagnosis of atopic pathology of the respiratory apparatus (4,5). Although FeNO does not yet have the importance of "classical" pulmonary function tests, the fraction of exhaled nitric oxide is an important adjunct to the diagnosis and management of atopic pathology, especially asthmatic, and may have a predictive role in the evolution and treatment of this chronic disease (6, 7, 8).

The purpose of our research was to evaluate bronchial inflammation in children practicing sports admitted to the Pediatrics Department of the Filantropia Municipal Hospital in Craiova and to try to establish a correlation between the value of FeNO, the clinical-biological status and the type of sport practiced by our subjects.

Material and methods

We conducted a prospective study of children practicing sports admitted in our clinic between January and December 2018. We included children who were able to perform pulmonary function tests (spirometry, peakflowmetry) and the determination of nitric oxide in the exhaled air using the NioxVero analyzer (Aerocrine, Sweden). The subjects admitted were over 6 years because this is the generally accepted minimum age for performing this kind of measurements (9, 10).

The analyzer used is the second generation of devices available in our clinic using a calibrated electrochemical sensor that measures the expired air composition and produces molecular results, expressed in "parts per billion" (ppB).

FeNO dosing was done after a training performed before either by the examiner using the dedicated device software or a human model, that is, another already trained child exemplifying how to use it. The results obtained after a maximum of 5 attempts, performed over a minimum of 3 minutes interval between de tests, were validated.

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Type of sport (urban/rural)	Football	Track and field	Indoor	Swimming	TOTAL
Boys	**57 (47/10)**	**14 (11/3)**	**23 (16/7)**	**8 (7/1)**	**102 (81/21)**
Girls	**6 (6/0)**	**13 (8/5)**	**41 (25/16)**	**16 (11/5)**	**76 (50/26)**
Total	**63 (53/10)**	**27 (19/8)**	**64 (41/23)**	**24 (18/6)**	**178 (131/47)**

Table 1. Athletes distribution

SPORT	Football	Track and field	Indoor	Swimming
Mean	18.58	18.77	29.57	33.29
SD	15.62	14.11	23.89	29.86
p value t test (2 tail)	Football vs. track and field		0.95	
	Football vs. indoor		**0.002**	
	Football vs. swimming		**0.003**	
	Track and field vs. indoor		**0.03**	
	Track and field vs. swimming		**0.02**	
	Indoor vs. swimming		0.65	
	All indoor vs. all outdoor		**0.0001**	

Table 2. FeNO levels vs. type of sport

SPORT	Football	Track and field	Indoor	Swimming	TOTAL
Asthma	**4**	**2**	**5**	**2**	**13**
Atopy (other)	**12**	**6**	**18**	**5**	**41**
Non-atopic, no. (%)	**47 (74)**	**19 (70)**	**41 (64)**	**17 (70)**	**124**
p value Chi square (2 tail)					
	Football vs. track and field			**0.67**	
	Football vs. indoor			**0.19**	
	Football vs. swimming			**0.72**	
	Track and field vs. indoor			**0.56**	
	Track and field vs. swimming			**0.97**	
	Indoor vs. swimming			**0.55**	
	All indoor vs. all outdoor			**0.55**	

Table 1. Distribution of atopic children vs. type of sport

Type of sport	Football	Mean	SD	t test
Football	**All athletes**	18.58	15.62	**0.02**
	Atopic	33.87	22.29	
Track and field	**All athletes**	18.77	14.11	0.24
	Atopic	26	18.6	
Indoor	**All athletes**	29.57	23.89	0.07
	Atopic	40.78	30.93	
Swimming	**All athletes**	33.29	29.86	0.11
	Atopic	56.14	41.6	

Table 2. Influence of atopy in FeNO levels

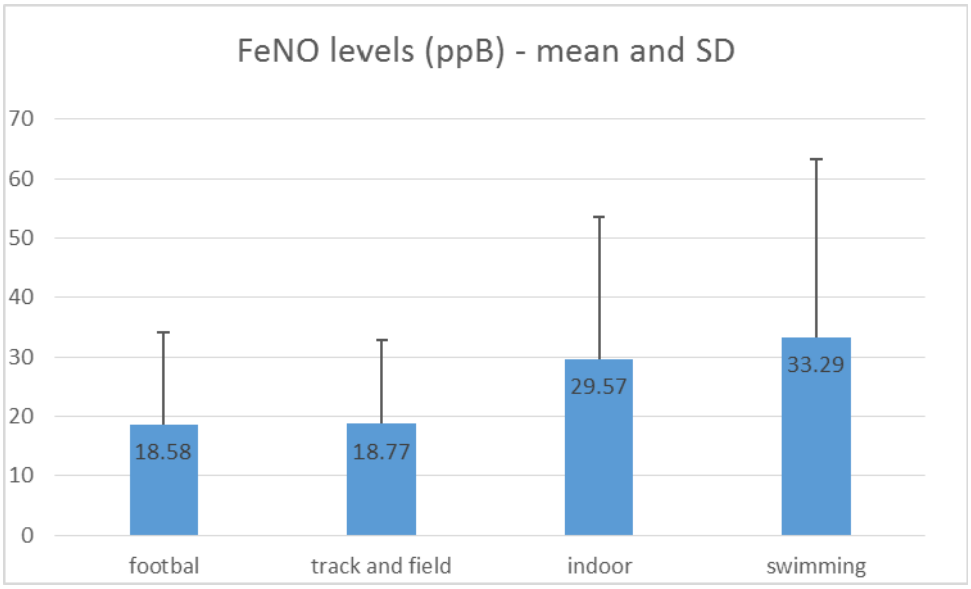

Fig. 1. Graph1. FeNO levels vs. type of sport

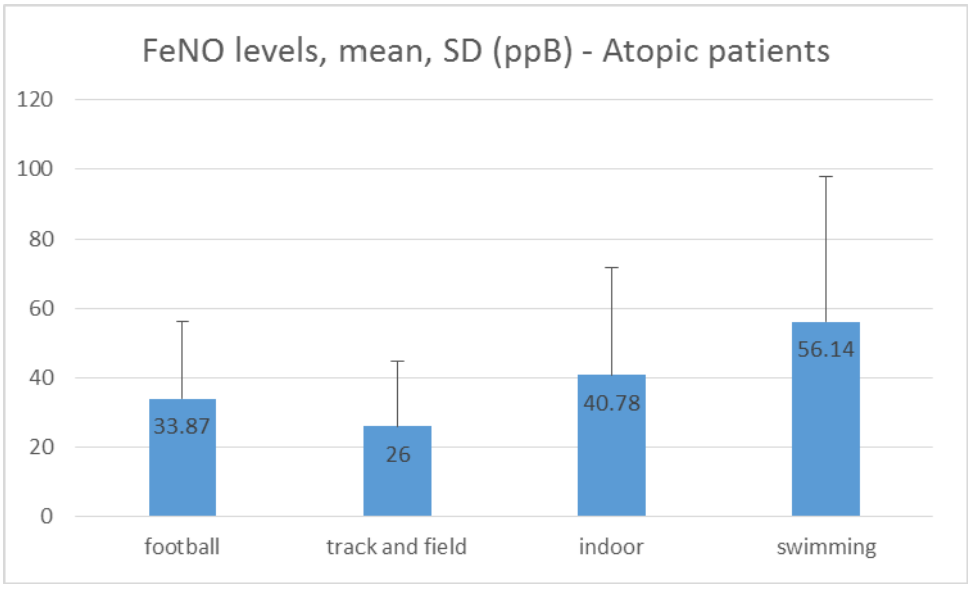

Fig. 2. Graph 2. FeNO levels in atopic patients

The determinations were performed before the functional spirometry or peak flow meters to avoid a false diminution of the values obtained - according to international guidelines (4, 5). The subjects excluded from the study were:

- ✓ children who have failed to get a correct measurement after the 5 attempts,
- ✓ those who consumed foods rich in nitrite before the determinations,
- ✓ children with extreme anxiety,
- ✓ adolescents with a well-founded suspicion of being exposed to cigarette smoke.

Statistical evaluation was performed using Excel (Microsoft Windows) and OpenEpi (Center for Disease Control, USA).

Results

178 children (102 boys) who were enrolled successively and underwent anamnesis, clinical examination, determination of nitric oxide in the exhaled air, and eventually functional ventilation samples (spirometry and/or peakflowmetry) were included in the study. Most of the children studied (131 cases) came from the urban environment, which is generally the profile of the patients admitted in our unit. The general structure of the study group is shown in Table 1

.

As expected, in boys, the "king of sports" is football, chosen by 55% of those surveyed, while in girls, 53% say they practice indoor sports (basketball, volleyball, handball, etc.). Another notable gender difference is observed in swimming where several girls seem to be interested in practicing this sport, the double number of cases being statistically significant: $p = 0.05$; OR = 0.31 (95% CI: 0.12-0.79). Regarding athletics, numerical values are approximately equal and have no statistical significance ($p = 0.26$). Basically, indoor sports (gymnastics and swimming) were favored by girls, while most boys are engaged in outdoor sports.

The distribution of the mean values of nitric oxide in the expired air (mean and standard deviation) according to the type of sport practiced by the children in the study is presented in figure 1.

As we can see, the values obtained in children who performed physical activity mostly outdoors are lower compared to those who trained mostly in closed buildings. The statistical evaluation was done in this case with a t test (2 tail) between the types of athletes, and the statistical significance was considered present when the values obtained were below 0.05, as is common in medical specialties. The results are shown in Table 2.

The comparison between football and athletics, both outdoor sports show similar values of FeNO, while comparing football with gymnastics and swimming as well as athletics with indoor sports and swimming brings statistically significant results, thus athletes who have carried out their activity mainly in the interior, have obviously higher levels of nitric oxide in the exhaled air. These athletes have a predisposition to eosinophilic bronchial inflammation ($p = 0.001$, t test), which at some point might result in an exercise induced bronchospasm.

The evaluation of the groups of athletes on general demographic criteria did not reveal significant differences related to sex ($p = 0.76$), origin (urban vs. rural: $p = 0.09$) or age (children under 12 years vs. over 12 years: $p = 0.21$).

The next phase of our research was to evaluate the impact of atopic pathology on our lots. Although some of the children were engaged in performance sports, many of them had a history of allergic diseases and obstructive respiratory pathology (bronchiolitis, bronchitis, obstructive pneumonia, or even bronchial asthma). 5 of the cases included in the study (3 boys and 2 girls) were already diagnosed with bronchial asthma at admission and another 8 children were diagnosed along the way.

The distribution of children with atopic pathology was evaluated according to the type of sport declared (see Table 3).

23% of the subjects (54 children) were diagnosed as atopic patients during the study. The stratified analysis of indoor sports versus outdoor sports did not reveal any significant data (chi square test, 2 tail). Although our lots were not very numerous, the proportion of children with atopy is relatively similar, regardless of the type of sport involved.

We further investigated the nitric oxide values in the exhaled air in the atopic children included in the study. The results are shown in graph 2.

Individual values were calculated in sub-lots with atopy and compared with the mean and standard deviation of the corresponding total number of athletes. So we tried to see if this biomarker is increased in allergic children. The comparison was made using t test (2 tail) results are shown in Table 4.

Although at first glance the mean values and the standard deviation on the 4 sub-lots seem different when compared to those obtained in atopic patients, however the statistical interpretation is significant only in a single case (football - t test = 0.02) and the results are not highly significant. Nitric oxide values are thus higher in children with a history of allergic pathology, but this change does not allow us to assert that this parameter can be widely used in prediction of atopy in pediatric patients performing regular physical exercise.

Discussion

Respiratory atopic pathology, especially the broncho-obstructive type, is based on two physio-pathological pillars: bronchial hyper reactivity and chronic inflammation. Functional ventilatory tests were mainly focused on the first component, and these determinations have already entered the standard of management of asthmatic pathology in children and adults (6). The emergence of an inflammatory mediator that is present in a large amount in the respiratory system and which can be relatively easy measured has opened new horizons over the past 20 years in assessing the inflammatory parameter in chronic respiratory pathology. Although a number of other medical specialties appear to be interested in the evaluation

of nitric oxide in the expired air (11,12,13,14) its utility is evident primarily in pneumology, especially in terms of reducing the rate of asthma exacerbations and mostly in children because the data are still unclear in adults (6, 7).

However, the link between nitric oxide and sports is less studied, the accessible data being disparate and with different methodologies, so we were unable to identify specific recommendations in the therapeutic guidelines. Sporting activity itself is a confounding factor for the amount of nitric oxide in the expired air because although technical recommendations say that repeated effort can lead to decreased values, there are studies demonstrating higher values after physical exercise in performance athletes (15). These increases in athletes may suggest suboptimal control of asthma (16), which means that FeNO could be used as a method of preventing asthma exacerbations in performance athletes.

There are authors (17) who say that practicing a certain type of sport can lead to changes in exhaled nitric oxide probably due to specific environmental conditions. This makes even more difficult to set cut off values with utility in medical practice.

One of the main drawbacks of FeNO dosing is the multitude of factors that may affect the measurement results, which is why this biomarker does not yet have an important place in the eosinophilic inflammation guidelines. Demographic factors such as race or ethnicity (18), but also age, sex, height - especially in older children (19), inflammatory factors of the rest of the respiratory system, or produced in other organs (11,12,13) are involved. Certain eating habits such as eating rich nitrite foods, water, caffeine or alcohol can alter the recorded values (20, 21).

Although rarer in pediatrics, exposure to cigarette smoke has a paradoxical and controversial effect, but it seems that most authors tend to accept the decrease of the concentration of nitric oxide in the exhaled air (22, 23). The presence of asthma did not influence the effect of smoking on fraction of nitric oxide (24).

Anti-inflammatory (corticoid or leukotriene inhibitor) therapy, used in most chronic respiratory diseases, also has a controversial relationship with FeNO values. The most likely association could be with inhaled corticosteroids and therefore nitric oxide was proposed as a method of monitoring this kind of therapy (6).

Conclusions

Our lot was set up in collaboration with the Sports Department of the Craiova Emergency Clinical Hospital, because only this way we managed to gather a fairly consistent number of children practicing sports. The lack of structured data does not allow us to say that our group is representative for the situation of pediatric sports in our city or at regional or national level. That is why our research continues and with the help of our pediatric colleagues from 2 other national centers, we are trying to complete a multicenter study with higher statistical significance. The most important data obtained so far could be systematized as follows:

- most of the included children were from urban areas because this was the profile of the patients common to the two medical institutions involved in the study. As expected, boys have had a greater attraction for football and so male sex has been involved mainly in outdoor sports, while girls have preferred indoor sports;

- FeNO values were significantly higher in children who practiced indoor sports than those who practiced outdoor exercise;

- the presence of allergic manifestations in children practicing sports cannot be ignored, with almost a quarter of our subjects being atopic; however, the FeNO value was not significantly higher in these subgroups than the total values for the corresponding groups of athletes;

- demographic data related to gender, age and social status did not bring significant statistical differences.

The final conclusion of our research is that the dosage of nitric oxide in the exhaled air appears to be modified especially in athletes practicing indoor sports without being able to tell whether this is the consequence of the sport itself or the environmental conditions in which it occurs. In literature, we have found quite a few papers to cover this type of patient, and the reported results are also insignificant in terms of both the predictability of asthmatic manifestations (24) and the differences between different types of sports (25, 26).

However, the performance athlete is an organism that is pushed to the limit of its physiological capabilities, and therefore any method that may prevent the occurrence of an acute severe manifestation such as an effort-induced bronchospasm is welcome. This is probably the best argument to continue evaluating inflammatory biomarkers in sports pathophysiology, especially in children.

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DIAGNOSIS AND CLINICAL CONSEQUENCES OF URINARY TRACT MALFORMATION IN CHILDREN

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Abstract

Congenital alterations of the kidney and urinary tract have been grouped under the name of CAKUT, although it includes a large spectrum of malformations. Early diagnosis of CAKUT is important. During pregnancy, the third trimester threshold value for the antero-posterior renal pelvis diameter measurement and should be followed by postnatal ultrasound. Children with CAKUT often have comorbidities, as CAKUT may lead in time to Chronic Kidney Disease (CKD) due to reduced nephron number at birth or progressive nephron loss, as well as recurrent urinary tract infections. We analyzed 42020 patient files between January 2015 and June 2017 admitted in "Louis Turcanu" Clinical Emergency Children Hospital in Timisoara, and we identified and analyzed 252 individual patients with CAKUT in a cross sectional study. Prevalence of CAKUT was 0.6%, with a male: female ratio of 1.35. 14% of cases had prenatal diagnosis determined by abdominal ultrasound performed in the third trimester of pregnancy, while 66 patients were diagnosed with congenital anomalies by accident, during a screening abdominal ultrasound. Unilateral kidney agenesis (URA) was found in 20 patients, with a male/female ratio = 1 and right/left ratio of 2.33. In 25% of cases, URA was associated with dilatation of the urinary tract on the contralateral kidney. Treatment options included medical/observational treatment and surgical options. Early diagnosis of CAKUT using a simple abdominal ultrasound screening allows early and proper treatment and reduces the risk of parenchymal complications and CKD.

Keywords: hydronephrosis, ureter, kidney disease, ultrasound, CAKUT

Introduction

Congenital alterations of the kidney and urinary tract have been grouped under the name of CAKUT, includes a large spectrum of malformations [1]. Given the complex development process of genitourinary system it is no surprise that CAKUT are among the most common congenital abnormalities in children [2], occurring in 1 in every 500 live births [3] and represent a major cause of CKD in children. Severity of CAKUT varies between incompatible with life malformations (bilateral renal agenesis) to minor abnormalities that are often asymptomatic for long periods of time [4].

Early diagnosis of CAKUT is important, during pregnancy, the third trimester threshold value for the antero-posterior renal pelvis diameter measurement and should be followed by postnatal ultrasound [5]. Prenatal urinary tract dilatation is common, present in 1-4% of pregnancies. Hydronephrosis is the most common anomaly identified on prenatal ultrasound, affecting 1–5 % of all pregnancies, but usually mild and resolves by itself [6, 7, 8]. Postnatal, the most common cause of pelvic and calyceal dilatation is ureteropelvic junction obstruction (PUJO) [9]. Prenatal diagnosis and supportive or corrective surgical inventions have improved survival rate of affected newborns. As a consequence, children with CAKUT often have comorbidities, as CAKUT may lead in time to Chronic Kidney Disease (CKD) due to reduced nephron number at birth or progressive nephron loss, as well as recurrent urinary tract infections (UTIs) [10,11]. Despite recent improvement in prenatal diagnosis and early surgical interventions, CAKUT remains the primary cause of kidney failure in infants. Almost 70% of pediatric patients with CKD progress to end stage renal disease (ESRD) before reaching adulthood. The care of patients with CKD focuses on interventions in order to preserve renal function [11]. In addition, adequately identifying, treating and preventing UTIs is another important goal to spare the remaining kidney.

Materials and Methods

We analyzed 42020 patient files between January 2015 and June 2017 admitted in "Louis Turcanu" Clinical Emergency Children Hospital in Timisoara, and we identified and analyzed 252 individual patients with CAKUT in a cross sectional study over a period of 30 months.

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	Male	Female		M:F ratio
Antenatal n=34	22 (64.7%)	12 (35.29%)		**1.83**
Neonatal n=48	28 (58.33%)	20 (41.66%)		**1.4**
UTI n=104	61 (58.65%)	43 (41.34%)		**1.41**
Ultrasound screening n=66	34 (51,51%)	32 (48.48%)		1.06

Tabel 1. Sex distribuiton correlated with moment of diagnosis

	Left	Right	Bilateral
Renal agenesis (n=20)	6 (30%)	14 **(70%)**	-
Renal hypoplasia (n=13)	7 (53.84%)	5 (38.46%)	1 (7.69%)
Renal ectopy/malrotation (n=14)	4 (28.57%)	10 **(71.42%)**	
Kidney fusion anomalies (n=7)	-	-	7
Displastic kidney (n=22)	7 (31.81%)	9 **(40.90%)**	6 (27.27%)
Total (n=76)	24 (31.57%)	**38 (50%)**	14 (18.42%)

Tabel 2. Uniterality of renal malformations

	Left	Right	Bilateral
Hydronephrosis PUJO (n=131)	59 **(45.03%)**	50 (38.16%)	22 (16.79%)
Ureterohydronephrosis (n=56)	20 **(35.71%)**	10 (17.95%)	26 (46.42%)
Uretheral duplicity (n=21)	7 (33.33%)	8 (38.09%)	6 (28.57%)
Total = 208	**86 (41.34%)**	68 (32.69%)	54 (25.96%)

Tabel 3. Unilaterality of ureteral malformations

Inclusion criteria were age between 1 month and 18 years and presence of any type of CAKUT by abdominal ultrasound examination. We excluded from our study children with urinary tract dilatations due to external compression or lithiasis (secondary hydronephrosis). We collected data about the age of the patient, age at the time of diagnosis, prenatal ultrasound, sex of the patient, type of diagnosis, clinical manifestations at the time of diagnosis and evolution, malformation type, severity and treatment options. For statistical analysis we used Microsoft Office Excel

Results

Prevalence of CAKUT in Children Pediatric Emergency Hospital Timisoara admissions over a 30 months survey time was 0.6%, with 252 patients presenting the required criteria to be included in our study out of 42020 hospital admissions, with a male: female ratio 1.35. About 14% of cases had prenatal diagnosis determined by abdominal ultrasound performed in third trimester of pregnancy, while 66 patients were diagnosed with congenital anomalies during a screening abdominal ultrasound.

Golden standard for structural malformations of kidney and urinary tract is abdominal ultrasound and it was performed for all patients, considered to be an inclusion criteria in our study. One third of patients, though, required further and more detailed imaging of the structural malformation of urinary tract including CT urography or retrograde cystography to evaluate the complexity, possible complications and comorbidities like as vesico-ureteral reflux (VUR). Over 40% of patients were diagnosed with CAKUT with the occasion of their first urinary tract infection.

Analyzing sex distribution correlated with moment of diagnosis we revealed that more boys are diagnosed with CAKUT in prenatal screening with a male (M): female (F) ratio of 1.83, as well in the neonatal period (M: F=1.4) and simultaneously to their first UTI (M: F=1.41). Gender equality was to be found in children diagnosed accidentally

(M: F=1.06) (Table 1). Age at the time of diagnosis within the group (252 patients) had two peaks of incidence: less than 1 year (81patients) and children over 6 years of age (99).

We divided CAKUT in three major groups based on anatomic level of the defect: renal malformations (76 patients), uretero-pelvic malformations (208patients) and vesical/subvesical malformations (20 patients). Out of all the patients, 20.23% had complex renal-ureteral malformations, affecting more than one anatomic level based of our classification.

Unilateral kidney agenesis (URA) was found in 20 patients, with a male/female ratio=1 and right/left ratio of 2.33. In 25% of cases, URA was associated with dilatation of urinary tract on the contralateral kidney. Compensatory hypertrophy (renal length greater than two standard deviations above the mean), is commonly observed in patients with a congenitally solitary kidney. In our study one quarter of patients were found with Hypertrophic contralateral kidney.

Other renal malformations included renal hypoplasia (13 patients), renal ectopy/malrotation, double kidney, renal fusion anomalies and dysplastic kidney (22patients) (Table 2). More than 80% of renal malformations were unilateral, predominantly affecting the right side, with a right/left ratio of 1.58.

Ureteropelvic malformations included patients with Hydronephrosis due to ureteropelvic junction obstruction (PUJO), Hydronephrosis associated with ureteral distension and ureteral duplicity/double collecting system (Table 3). Out of 208 patients with ureteropelvic defect, 154 (74.03%) had unilateral defect with a Left: Right ratio = 1.26. Severity of hydronephrosis was scored I-V grade depending on the dilatation of the renal pelvis and calyces, bilateral involvement, dilatation of the ureter and management of case was according to guidelines. Congenital obstruction in urine flow, referring to PUJO, obstructive megaureter, obstructive ureterocelle, ureteral stenosis were found in 140 patients out of 252 (55.55%). Reflux anomalies including primary or secondary vesicoureteral reflux (VUR) were found in 41 patients (16.26%). Double collecting urinary system consists of both obstructive and reflux anomaly as one or both ureters have modified structure leading to slow drainage of urine flow and incompetent ureterovesical valve and was found in 21 cases (8.3%). Lower urinary tract malformations involving the bladder and the urethra were found in 20 patients (7.93%).

Almost half of our patients were diagnosed with anomalies of urinary tract with the occasion of their first UTI. UTI has a high incidence among patients with abnormal urinary tract, especially when associating VUR or when UTI becomes recurrent. Half of our patients (56.34%) had at least one episode of UTI during our observation time, while several patients had more than one episode in the case of reflux anomalies. Each UTI episode received antibiotic treatment according to antibyogram. Antibiotic prophylaxis was recommended in 13 patients that had recurrent episodes of infection associated with high grade (III/IV) VUR.

Treatment options included medical/observational treatment and surgical options (Hynes-Anderson pyeloplasty, percutaneous ureterostomy, unilateral nephrectomy, endoscopic PUV removal, and endoscopic correction using Vantris. Surgical treatment is required in case of high grade VUR, progressive renal scarring associating any VUR grade, solitary kidney associating high grade VUR. Repeated renal scarring leads to Chronic Kidney Disease (CKD), as encounter in 12% of patients.

Discussion

Congenital anomalies of kidney and urinary tract are one of the most frequent malformations to be found in children, considered among the top 5 anomalies. In our study CAKUT prevalence was slightly lower (0.6%) compared to other studies Salento, Italy 0.96% (171 CAKUT out of 17783 children) and Beijing 1.67% incidence (489 CAKUT din 26989) [12], most probably due to our selection of patients. The patients were selected from our hospital admitted patients, not being able to take under consideration the asymptomatic ones, or diagnosed outside the hospital within the same area.

Significant maternal factors associated with CAKUT are being searched and proved to be linked, like maternal age (30–39 years), gestational diabetes, polyhydramnios/oligohydramnios, thalassemia/hemochromatosis and other illness. Infants with CAKUT are more likely to be boys and born at small gestational age (SGA) [13].

Renal agenesis may occur as an isolated finding or, commonly, in association with other anomalies or syndromes conditions. Unilateral renal agenesis is thought to occur in approximately 1 in 1000–3000 live births [14]. There is a slight predilection for the left side and almost twofold higher incidence in males than in females [14]. In our study, surprisingly, right side was more frequently affected with a right/left ratio of 2.33 and a male/female ratio of 1.

Unilateral renal agenesis may occur by itself or, commonly, in association with other congenital genitourinary and non-genitourinary anomalies. Anomalies of the contralateral kidney and collecting system have been reported to occur in 32–50 % of patients with unilateral renal agenesis. The most common abnormality affecting the contralateral kidney is vesicoureteral reflux, which occurs in approximately 24 % of patients with unilateral renal agenesis. Other less common associated urinary tract anomalies include ureteropelvic junction obstruction (6 %), megaureter (7 %), and duplicated collecting system (3 %) [14]. in our study, 25% of patients with unilateral renal agenesis had urinary tract dilatation (hydronephrosis or megaureter) on the contralateral kidney, and one had dysplastic contralateral kidney. Right kidney anomalies are more frequent in our group. Unilateral right kidney anomalies represented 50% of all renal malformations. Left unilateral hydronephrosis is the most frequent anomaly regarding the urinary collecting system (51.98%).

CAKUT is more frequent in symptomatic male children compared to symptomatic female children. Male

pediatric patients with UTI symptoms should be further investigated for CAKUT. The diagnosis of VUR often follows the diagnosis of febrile UTI. According to NICE guidelines, children with atypical UTI or under the age of 6 months at their first UTI should have ultrasound of the urinary tract during the acute infection in order to identify eventual malformations of the urinary tract [14]. In case of atypical or recurrent UTI in children, with the suspicion of an underlying vesicoureteral reflux, micturition cystourethrogram is recommended to be performed [15]. The procedure should be performed on the second day of a 3 day antibiotic prophylaxis [15]. In our study VUR was identified in 41 patients out of 52 cystography performed.

Conclusions

The proportion of children with CAKUT is high among pediatric population. Positive prenatal ultrasound should be followed by a postnatal abdominal ultrasound examination in order to diagnose CAKUT and reduce the risk of complications. Early diagnosis of CAKUT using a simple abdominal ultrasound screening allows early and proper treatment and reduces the risk of parenchymal complications and CKD. Male pediatric patients that have UTI should be further investigated. Crucial for diagnosing VUR in complete flow of urinary bladder.

Abbreviations

CAKUT – Congenital Anomalies of Kidney and Urinary Tract
VUR – Vesico-Ureteral reflux
UTI – Urinary tract infection
CKD – Chronic Kidney Disease
URA – Unilateral renal agenesis
UTD – urinary tract dilatatition

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CLOSTRIDIUM DIFFICILE – PATHOGEN OR NOT FOR NEWBORNS?

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Abstract

Clostridium difficile (CD), described initially as a commensal germ in infants under 1 year of age, is more and more often recognized as enteric pathogen in newborns and children, even in those without risk factors. Aim: The paper is shortly reviewing available data regarding CD colonization and infection in neonates and presents a case report. Material and method: The case of a preterm infant, positive for A and B toxins of CD, with gastro-intestinal complications during neonatal period is presented. Results: In the 9th day of life, the preterm infant develops symptoms of colitis, concomitant with maternal CD infection. Despite antibiotic treatment and intensive care, peritonitis developed during the 24th day of life. The clinical course was further complicated by an umbilical fistula, prolonging hospitalization. No other pathogen except CD has been isolated starting birth but the clinical course suggests for neonatal infection with CD. Conclusion: Given the increased incidence of CD in the latest period, we do have to reconsider the role of CD as a potential etiological agent in neonatal infections.

Keywords: Clostridium Difficile, newborn, necrotizing enterocolitis, colitis, peritonitis

Introduction

Clostridium difficile (CD) has been described initially as a commensal germ in infants under 1 year of age, is more and more often recognized as enteric pathogen in children, even in those without risk factors [1-3]. Clostridium difficile is more often encountered in infants admitted to neonatal intensive care unit (NICU) [4] and in preterm infants [5]. Most often, CD is colonizing preterm and term neonates and different factors are suggested as protectors against neonatal infections with CD [1, 4, 6]. Also, risk factors for neonatal colonization with CD were identified [1, 4], yet transmission from mother to child, irrespective the delivery mode, is debated [1, 6, 7]. Even though Clostridium Perfringens, C. Butyricum, and C. neonatale were clearly identified as etiologic agents in neonatal necrotizing enterocolitis (NEC), a causal link between CD and NEC is still controversial [8-11]. This paper briefly reviews the data in the literature regarding colonization and infections with CD during neonatal period and presents the case of a neonatal infection with CD.

Clostridium Epidemiology

Clostridium difficile is a gram positive, sporulated bacillus with toxic and non-toxic forms. A and B toxins, produced by CD, are mediating the pathogenesis of CD infections. Based on toxin production, there are three types of CD: toxin A producing CD (A+B-), toxin B producing CD (A-B+), and non-toxic CD (without virulence genes), non-pathogenic [12]. Transmission occurs by direct contact between individuals or with contaminated surfaces, and by aerosolized spores. The spores are resistant to heat, acidity, and many disinfectants [1]. After ingestion, spores are surviving in the stomach and germinate into vegetative forms inside colon. In newborns, CD transmission occurs mostly from the environment or from other children [1, 13] although identical strains of CD were also isolated per partum from mother and neonate pairs [6, 7]. Most of the studies showed no differences of the CD colonization rates according to the delivery mode [1, 6, 14], although some studies reported an increased colonization rate after cesarean section [4, 15]. Symptomatic and asymptomatic carriers of CD toxigenic strains are representing a reservoir [1].

Old epidemiological studies flagged asymptomatic CD colonization in infants [13] but during the last decade CD was recognized as potential enteric pathogen in children, even in those without risk factors [1-3]. Rates of neonatal CD colonization are variable: 25-30% of the newborns, over 33% in the NICU[4], 61-79% in preterm infants discharged from the NICU[5,13,16,17], 14-71% of infants under one year of age[1] (usually with non-toxigenic strains), decreasing to 4% between 12-18 months, rates similar to that of non-hospitalized adults[1]. Most of the toxigenic strains are found in preterm infants with intestinal conditions [17].

The commensal colonization of the neonatal gastro-intestinal tract, mainly with non-toxic strains, begins during birth and with the first feedings [1]. Factors increasing the risk for neonatal colonization and infection with CD are: antibiotic therapy (disrupting endogenous flora)(mainly due to Clindamycin, cephalosporin, penicillin)[1,4,18], increased hospitalization duration[1,4,6], significant chronic conditions[1], increased contamination of the neonatal environment[1,19], feeding method and mode, and use of acid suppressor agents[1].

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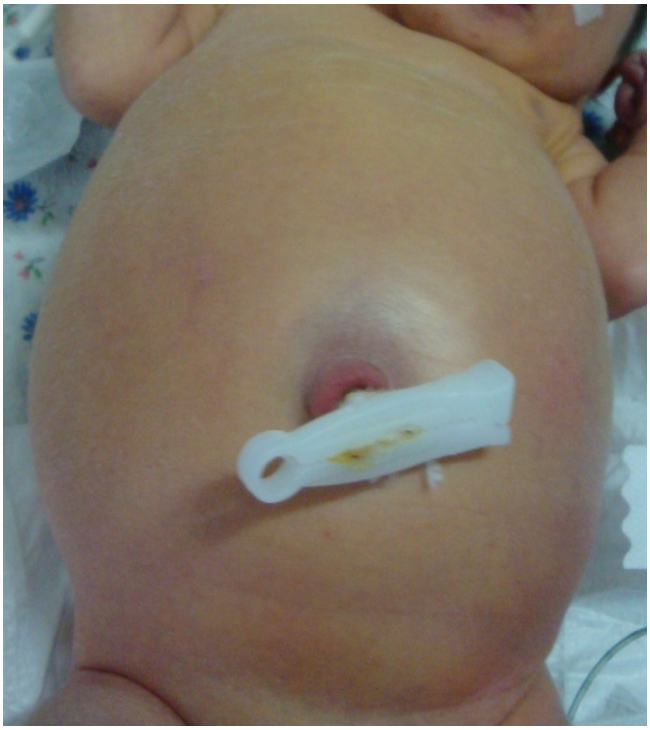

Fig. 1. Abdominal distension, mild edema of the abdominal wall

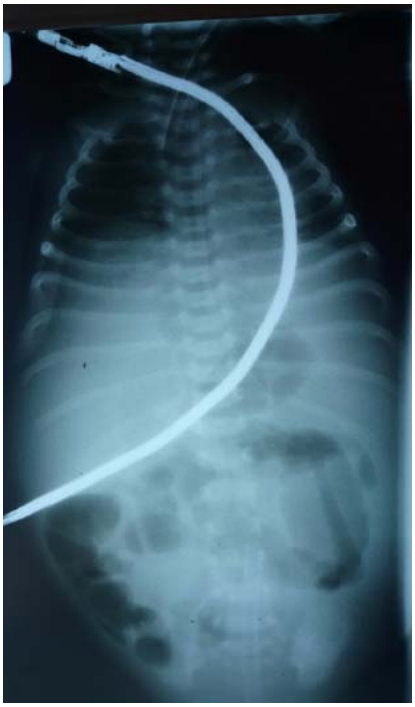

Fig. 2. Abdominal radiography – 9th day of life

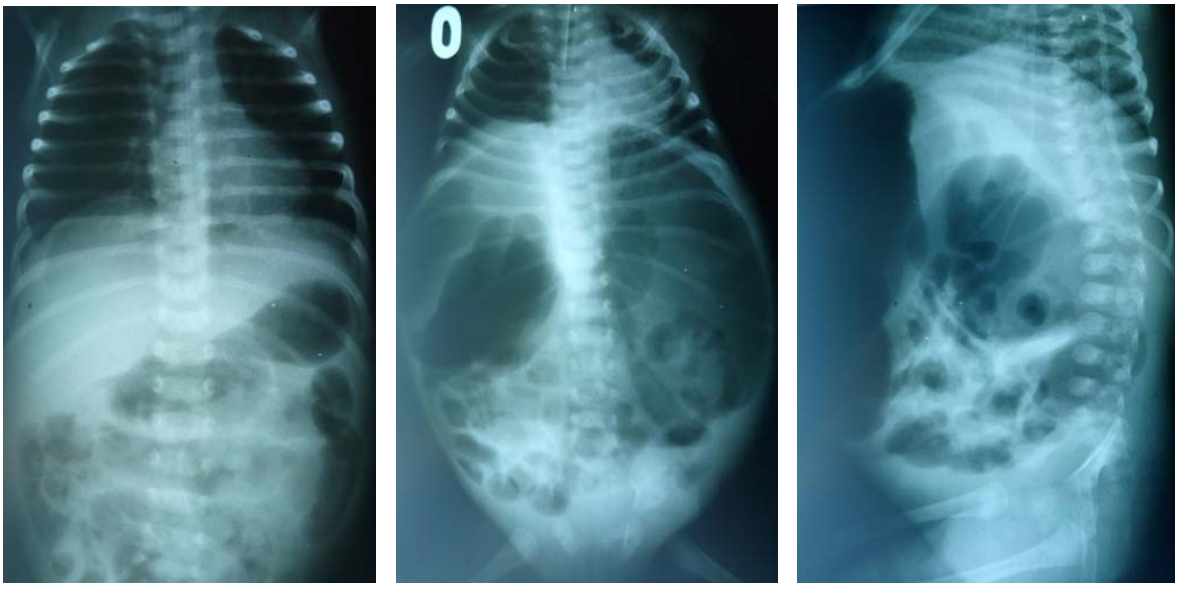

Fig. 3. Abdominal radiography survey after the 24th day of life

Clinical aspects. Laboratory diagnostic

In neonates, symptoms are varying from asymptomatic colonization to diarrhea or severe pseudo-membranous colitis. Bloody stools are the most frequent symptom seen in newborns [22, 23]. In infants, diarrhea with CD is usually mild, aqueous, accompanied by fever, anorexia, and abdominal cramps [1].

Infections with CD may be classified according to the severity – presence of complications, lab markers, and clinical signs – [1]. Pseudo-membranous colitis, toxic mega colon, gastro-intestinal perforation, intestinal pneumatics are all possible complications of neonatal CD infection, responsible both for NICU admission and surgical interventions. Laboratory tests are often showing leukocytosis or leukopenia, decreased albumin levels and increased creatinine levels [1].

Molecular testing is more and more used to identify CD. Due to the high rates of neonatal CD colonization, routine testing of the newborns is not recommended [1]. Enzyme immunoassay is a rapid, easy to perform, and very specific test for A and B toxins of CD, used currently by most of the laboratories [1]. Detection of glutamate dehydrogenase (an antigen present on toxigenic and non-toxigenic CD) has an increased negative predictive value and can be used in the diagnostic algorithm. Polymerase chain reaction (PCR) is more expensive but has increased sensibility and specificity for A and B toxins [1].

Prevention and treatment

Limiting unjustified antibiotic use, isolation of the contacts, decontamination (with agents capable to kill CD spores) of the surfaces, and hand washing are critical for prevention and limiting transmission of CD colonization and infections in the NICU [1]. Alcohol-based hand disinfectants are less efficient as compared to hand washing with warm water and soap since CD spores are resistant to alcohol [24]. A vaccine is in study [1].

Basic principles of the treatment are symptomatic care (intravenous fluids), cessation of useless antibiotic therapy (sometimes mild symptoms are vanishing [1]), and initiation of efficient antibiotics. Metronidazole (30 mg/kg/day in 4 doses, a maximum of 2 g/day) is recommended in moderate CD infections [1, 25]. Metronidazole has reduced oral absorption, rapid intestinal transit and diffusion through inflamed colonic mucosa [1]. Prolonged use is associated with increased risk for peripheral neuropathy [1, 6]. For children with severe CD infection an association of oral vancomycin (40 mg/kg/day in 4 doses, a maximum of 2 g/day) and intravenous metronidazole is recommended [25]. Vancomycin has also a reduced absorption rate therefore intestinal vancomycin levels are increased [1]. Enema with vancomycin is an alternative to oral administration [25]. The minimum duration of the treatment is 10 days [3]. A second course of treatment is recommended for the first recurrence (as in adults) [1]. Fidaxomycin is a new macrocyclic antibiotic approved in 2011 for adult treatment, in study for children [1]. Nitazoxanide and rifaxamin are other possible efficient agents, under study [6]. Restoration of the microbioma can be achieved using probiotics and is recommended mainly in children with recurrent CD infections [1].

Clostridium Difficile and necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is the most frequent gastro-intestinal emergency in premature infants, occurring in 7% of infants weighting under 1500 g at birth [8]. Prematurity, abnormal intestinal colonization, and formula feeding are cited as main risk factors for NEC [8, 26]. Recently, abnormal intestinal microbioma has been shown as a key factor in NEC pathogenesis, bacteria having an important role in the progression and severity of the intestinal lesions [26]. Clinical signs of NEC are very unspecific, varying form signs limited to gastro-intestinal tract – feeding intolerance, increased gastric residuals, abdominal distension, ileus, and bloody stools – to signs of severe disease with multiple organ failure – lethargy, apnea, metabolic acidosis, shock, intravascular disseminated coagulation, and even death [8]. Different types of Clostridium – C. Perfringens, C. Butyricum, C. neonatale – were cited in association with NEC both in animal studies and human newborns [8, 26, 27]. However, implication of CD in NEC pathogenesis is controversial: a causal connection could not be identified, toxigenic strains of CD are rarely described in NEC but outbreaks of CD infections associated with NEC were also reported [4, 8, 10, 11]. For example, CD was isolated in 12 in 13 infants with NEC versus 2 in 17 controls in an outbreak finished after oral vancomycin was administered in sick infants, contacts were isolated and strict control measures were implemented [10].

Case report

The female newborn was delivered vaginally, in cranial presentation, after 6 hours after amniotic membranes rupture, at 36 weeks gestation with a birth weight of 2140 g, height 46 cm, cranial circumference 29 cm. The mother, 23 years old, primigravida, primipara had an uneventful pregnancy except non-specific vaginitis and one episode of urinary tract infection one month before delivery, incompletely treated with antibiotics. Two hours after delivery, the premature infant developed signs of respiratory distress (tachypnea, intercostal retractions, low oxygen saturations), was admitted in the NICU and placed on Bubble CPAP on nasal cannula with 30% oxygen. An umbilical line was placed for partial parenteral nutrition and antibiotics (Penicillin and Amikacin, 7 days). Leukocytosis (37.420/mm3) and a shift to the left on the differential (1% myelocytes, 1% metamyelocytes, 4% immature granulocytes, 71% mature granulocytes) were seen on the blood count and C reactive protein was elevated – 13,6 mg/L (N<5 mg/L). Oxygen and pressure need decreased continuously, so that the CPAP support was removed after 3 days, also the umbilical line, CRP decreased to 5,13 mg/L, the infant had a good enteral tolerance and was transferred to the Premature Infants Department. Blood culture performed in the first hours of life came back negative, while yeasts grew in the gastric aspirate collected at birth and in the pharyngeal exudate in the 4th day of life. The infant was fed by gavage with maternal milk, freshly

expressed by the mother every 3 hours, supplemented by formula without lactose in the first 3 days and special formula for preterm infant afterwards.

The clinical course was uneventful until the 9th day of life when the preterm infant suddenly presented with altered general status, large gastric residuals, abdominal distension with mild edema of the abdominal wall (Figure 1), tachycardia, mild hypotonia, and fever. Enteral feedings were interrupted and total parenteral nutrition was started. Antibiotics (gentamycin and meronem) were started intravenously. Laboratory tests revealed elevated C reactive protein (250 mg/L), leukopenia (3470/mm3, 53.3% mature granulocytes), immature/total neutrophil ratio was 0.32, and abdominal radiography showed a tendency to hydroaeric levels in the abdominal flanks (Figure 2). Blood culture, repeated at this time, showed no bacterial growth. At the same time, the mother presented diarrhea and altered general status, being diagnosed with CD infection.

In the next 24-48 hours, the infant continued to show and altered general status, pallor, abdominal distension, bilious gastric residuals, intermittent fever, stools with mucus and blood. Stool tested positive for A and B CD toxins and negative for Shigella and Salmonella. Colistin, vancomycin and nystatin were added in the antibiotic therapy (replacing gentamycin) and total parenteral nutrition was continued.

Irritability and respiratory distress syndrome (mainly due to intense abdominal distension), sub-occlusion, and intermittent elevations of the temperature occurred in the next days so that in the 14th day of life the infant was intubated and mechanically ventilated. After infectious disease consult, review of the clinical, laboratory, and imagistic data, the preterm infant was diagnosed with toxic megacolon and septic shock, and the antibiotic treatment was changed again – meronem, teicoplanin and vancomycin -, intravenous immunoglobulin and pentoxifylline were added to therapeutic plan, total parenteral nutrition and mechanical ventilation were continued. C reactive protein remained at high values the next days while all other laboratory tests were in normal limits (creatinine, blood urea nitrogen, liver function tests, blood gas analysis). In the 24th day of life abdominal radiological survey showed gigantic colic distension and pending hydroaeric levels in the lower abdomen consistent with increased abdominal distension (with increased need of oxygen and pressure on the ventilator) (Figure 3), occurrence of collateral abdominal circulation, persistent sub occlusion.

With a diagnosis of pseudomembraneous colitis, possible secondary to CD infection, and suspected peritonitis, the infant was submitted to pediatric surgery. Laparotomy performed the same day revealed thickened peritoneum, loss of its shiny luster, air and fecaloid fluid in the peritoneal cavity, lavage and drainage were performed and repeated regularly. Complex antibiotic and antifungal therapy, mechanical ventilation and total parenteral nutrition were continued the next month. The infant was extubated after 2 weeks, the drainage tube was removed after one month, enteral nutrition was initiated and gradually increased after 26 days. Blood cultures, peripheral cultures, cultures from stools, and repeated cultures from the drainage fluid showed no bacterial growth.

The infant was submitted to Premature Infant Department after 44 days with 2350 g for nutritional recovery. The next 3 weeks were complicated by an umbilical fistula and an associated inflammatory syndrome but finally the fistula spontaneously closed, the infant grew up, and has been discharged on special preterm formula.

A final diagnosis of necrotizing enterocolitis with secondary peritonitis was formulated at discharge.

Discussion

The presented case is illustrative for difficulties encountered in neonatal gastro-intestinal tract conditions, as NEC. Difficulties had started with correct diagnosis and timely decision as regards the need for surgical intervention. Obviously, another major difficulty was to find the correct antibiotic therapy. Decisions regarding antibiotic therapy had been hampered at least by two things: failure to isolate an etiologic agent and concurrent infection of mother and infant with CD. As most of the literature shows that colonization with CD is frequent in the neonatal intensive care unit and in preterm infants and only very few cases of infection had been cited, it was the initial clinical course that suggested that CD may be the etiological agent in the presented case therefore vancomycin had been one of the antibiotic used. But evidence was not convincing for the neonatologist-pediatric infection disease specialist team, so other antibiotics were added and antibiotic strategy was changed many times resulting in antibiotic overuse..

Conclusions

The final correct diagnosis is still unclear since NEC rarely presents only with peritonitis. Fortunately, the infant survived and has a good clinical evolution up to one year. Given the increased incidence of CD in the latest period, we do have to reconsider the role of CD as a potential etiological agent in neonatal infections.

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LASER LABIAL FRENECTOMY IN A 12 YEARS-OLD PATIENT -A CASE REPORT

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Abstract

We present the case of a 12 years-old male patient with maxillary diastema induced and maintained by labial frenum in which we performed a laser-assisted labial frenectomy.

Keywords: frenectomy, diastema, diode laser

Introduction

Frenulum is an anatomical fibrous mucosal fold of congenital origin located on the midline, which connects the lips and cheeks to the alveolar mucosa or gingiva and the underlying periosteum (1). It is composed from connective, fibrous, muscular or fibromuscular tissues, covered with a mucosal membrane (2). Its main function is to modulate the labial movement, but when it grows beyond the normal limits and is located very close to the marginal gingiva, it acts like a limitation for the lip movements and as a local contributing factor in the appearance of teeth, diastemas, teeth deformities, dental plaque accumulation and speech problems (1, 2).

The upper lip frenulum extends from the internal surface of the upper lip to its insertion on the midline of the attached interincisal gingival tissue of the upper maxilla and in children, these frenula cause interincisal diastemas, which in turn require orthodontic treatment, entire-related problems, periodontal disease secondary to retained or impacted food, oral hygiene difficulties, and impairment of lip mobility and/or a short lip (2).

A radiological study is also required to discard other possible causes of diastemas, such as mesiodens, odontoma or root cysts, among other causes, and assess the characteristics of the interincisal bone (3).

Treatment of diastema varies and it requires correct diagnosis of its etiology and early intervention relevant to the specific etiology. No treatment is usually initiated if the diastema is physiological/transient as it spontaneously closes after the eruption of permanent maxillary canines (11-12 years) (4).

The conventional frenectomy techniques involve complete excision of the frenum, including its attachment to the alveolar bone, followed by the suture of the remaining wound edges. This type of soft tissue excision carries the routine risks of surgery like bleeding, postsurgical pain, discomfort and a longitudinal surgical incision and scarring, problem that may lead to periodontal involvement and an unaesthetic appearance (5).

Another approach of this pathology is represented by the laser-assisted surgery which can performed with various types of lasers: neodymium-doped yttrium aluminum garnet (Nd: YAG), carbon dioxide (CO2), erbium YAG (Er: YAG), erbium, chromium YSGG (Er, Cr: YSGG) (1, 2, 6-10).Many authors have described the use of different lasers in oral soft tissue surgery (1, 2, 6-10).

Case report

We present the case of a 12 years-old male patient with maxillary diastema maintained by upper lip frenulum. The patient's medical history did not reveal any systemic diseases. Intra-oral examination revealed presence of high frenum attachment, with strong connective tissue fibers (Figure 1) and midline spacing between maxillary central incisors, the latter also revealed at the X-ray exam (ortopantomography) (Figure 2).

After obtaining informed written consent from the parents, decision was made to remove high frenum attachment by a laser-assisted technique. Frenectomy was carried out under local anesthesia with Diode Laser Epic X® (BIOLASE, California, USA), 940nm. The optical fiber used was 400 μm diameter allowing a very fine soft tissue cut (Figure3) used in pulsed mode CP2 (comfort pulse) at a power of 2 W.

The patient was at the recall visit, after 3 weeks, the tissues healed completely (figure 4).

Discussion

In the conventional frenectomy procedure, the main accusations of the patients are postoperative discomfort, pain, and bleeding caused by the sutures (1). Remarkable aspects of laser surgery in pediatric dentistry are the minimum level of pain felt by the patient during (the local anesthesia is not mandatory) and after the procedure, the reduction of bleeding, swelling and scarring. Furthermore higher precision of the practitioner when compared to surgical tools represents a major feature which recommends this time-saving technique in the management of the pediatric cases (6).

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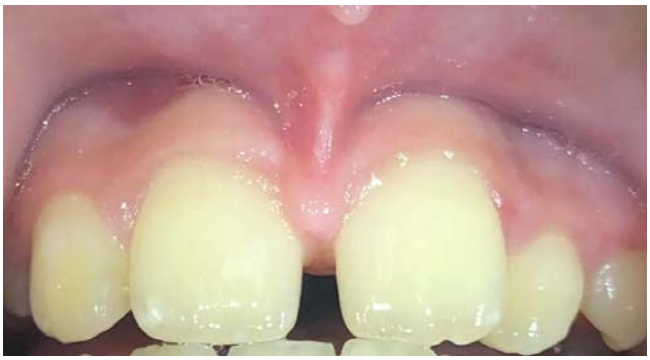

Fig.1. Intra-oral linical aspect of the upper-lip high frenal attachement

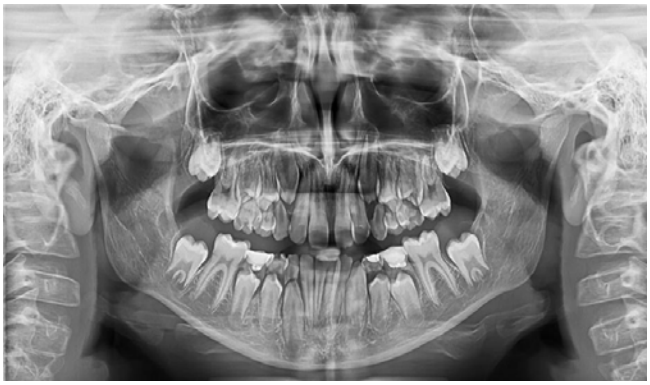

Fig. 2. Radiographic examination (ortopantomography) of the patient

Fig. 3. Diode Laser Epic X® (BIOLASE, California, USA)

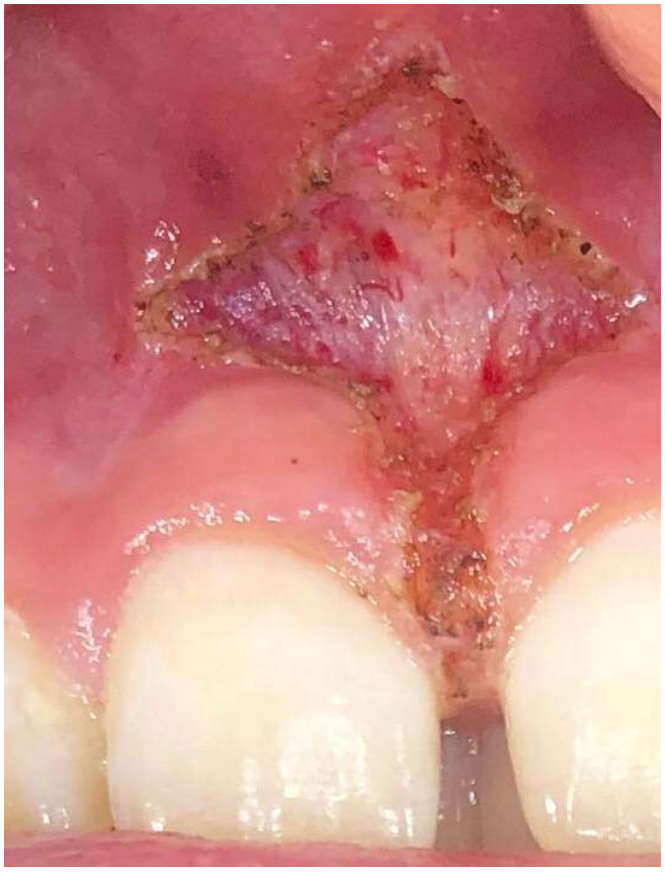

Fig.4. Intra-oral clinical aspect of the interventional site after frenulum removal

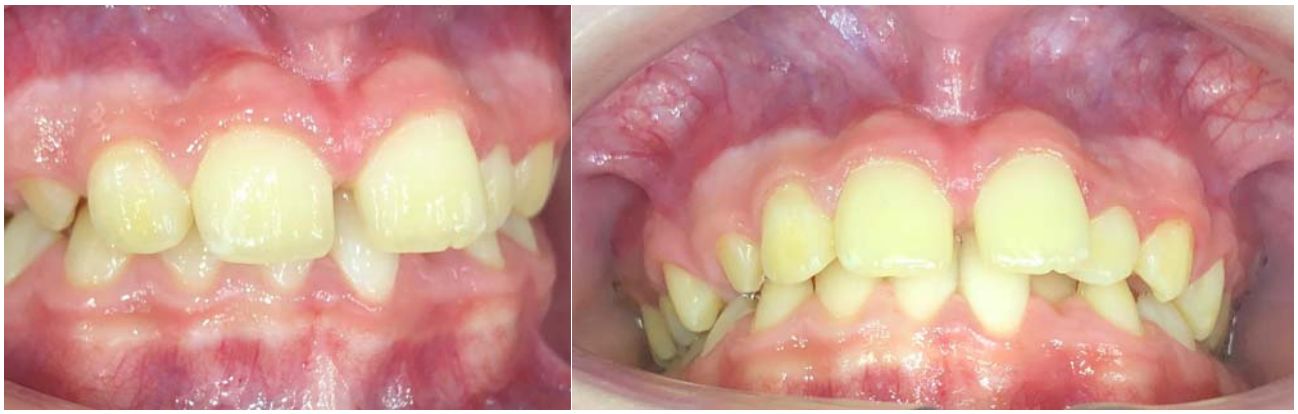

Fig. 5 a, b. Intra-oral clinical aspect of the interventional site after healing (3 weeks from the intervention).

Another benefit for the patient is the reduced risk of postoperative infection ensured by the lack of sutures needed (healing is by second intention) which usually accumulate dental plaque and by the fact that the laser beam provides a sterilization of the tissue during the process (1).

The CO2 laser offers a bloodless field and shorter surgical times compared with the Er, Cr: YSGG laser, the latter being considered for the post-op faster wound healing (2). The Nd: YAG laser surgeries have significant advantages over the conventional technique like minimal or no bleeding after treatment (1).

The great superiority of diode laser frenectomy in children is represented by the possibility to avoid needle-infiltrated anesthesia, when set at pain-free parameters. This might represent an advantage to the patient, but at the same time a prejudice for the clinician considering the time required for this procedure. The good compliance and cooperation with our case patient allowed us to use a higher power for this wavelength under local infiltration anesthesia.

Conclusions

This case report indicates that diode laser treatment used for frenectomy operations provides better patient perception in terms of postoperative time, pain and function than that obtained by the conventional scalpel technique.

Considering the above advantages, when used correctly, the use of diode laser offers a safe, efficient, acceptable, and impressive alternative for frenectomy operations.

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PEDIATRIC POISONINGS IN A ROMANIAN CHILDREN HOSPITAL: AN EPIDEMIOLOGICAL RETROSPECTIVE REPORT

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Abstract

Background and aim: Intoxications continue to present a challenge for clinicians in Romania, due to the extremely diverse range of toxins that can be involved, as well as the limited treatment options and specific antidotes available. Our objective was to perform an epidemiologic analysis of patients with intoxications from a Romanian emergency hospital. Methods: We analyzed the pediatric intoxications from The Regional Pediatric Toxicology Centre, part of The Emergency Clinical Children's "Louis Turcanu" Hospital from Timisoara, Romania. We focused on the Pediatric Emergency Department (PED) giving a longitudinal account of intoxication cases between the 1st of January 2008 and the 31st of December 2011. Results: In this time frame the Pediatric emergency department - PED had 76 223 visits, out of which 717 were acute intoxication cases. 60.4% were accidental. 89.4% of the voluntary intoxications were part of the 12-17 year olds group. While teenage females mainly ingested prescription drugs (67%), teenage males chose ethanol (71%) and illicit drugs (66.7%). The rural population is slightly more prone to accidental intoxication. Prescription drugs were the most common cause of voluntary intoxication observed in the PED, with n=247 (34.4%) cases reported in the 4 year period The second most common cause of this category was illegal substance use, with n=63 (8.8%) cases reported. An altered mental state was observed in n=46 (6.4%) of patients during the first medical evaluation. Discussions and conclusions: In spite of the steady 1% proportion of pediatric intoxications in relation with total PED visits, there has been a shift in substance preference, especially among teenagers. Alcohol remains popular, but it has seen a slowing trend, with the void being filled by new "legal highs".

Keywords: pediatric poisoning, children, intoxication.

Introduction

Intoxications continue to present a challenge for clinicians in Romania, due to the extremely diverse range of toxins that can be involved, as well as the limited treatment options and specific antidotes available. The potential threat posed to pediatric patients is amplified by the often unpredictable nature of the event, combined with the particular characteristics of children's health care and the limited information that can be gathered from a patient's history (1).

In the context of an increasing amount of new substances surrounding us (both natural and synthetic) due to industrial development and better market access, we decided to take a look at the pediatric intoxications from The Regional Pediatric Toxicology Centre, part of The Emergency Clinical Children's "Louis Turcanu" Hospital from Timisoara, Romania (2). Our objective was to perform an epidemiologic analysis of patients with intoxications from the local Emergency Pediatric Centre, in order to assistance both clinicians treating acute intoxications and hospital administration in regards to patient management and policy making (3, 4).

Material and methods

Hospital ethics committee approval was obtained before beginning the study: Informed consent clearly indicating that patients have given their informed consent for participation in the research study was obtained by signing the paperwork at admission in hospital.

Design: We performed a retrospective study assessing children with acute intoxications addressed to a university hospital.

We included all patients evaluated for acute intoxications in The Emergency Clinical Children's "Louis Turcanu" Hospital from Timisoara, Romania between the 1st of January 2008 and the 31st of December 2011.

Analysis of data: We analyzed the records of the patients evaluated in the two main areas of the hospital that deal with intoxications, the Pediatric Emergency Department (PED) as the point of first contact between a health-care provider and the patient, and the Pediatric Toxicology Ward (PTW) as the end point for the more demanding cases that require further medical treatment.

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Frequency		Type		Place		Patient's Sex		Coma		Admitted		Total
		A	V	R	U	F	M	No	Yes	No	Yes	
Year	2008	80	77	59	98	85	72	141	16	75	82	*157*
	2009	119	49	56	112	91	77	156	12	78	90	*168*
	2010	113	59	49	123	80	92	167	5	66	106	*172*
	2011	121	99	92	128	113	107	207	13	57	163	*220*
Total		*433*	*284*	*256*	*461*	*369*	*348*	*671*	*46*	*276*	*441*	*717*

A=accidental, V=voluntary, R=rural, U=urban, F=female, M=Male

Table 1. PED intoxications by year

Frequency		Type		Place		Patient's Sex		Coma		Admitted		Total
		A	V	R	U	F	M	No	Yes	No	Yes	
Age	0-30 days	8	0	6	2	5	3	8	0	0	*8*	*8*
	1-6 months	26	0	15	11	9	17	24	2	6	*20*	*26*
	7-12 months	25	0	10	15	15	10	25	0	11	*14*	*25*
	1-2 years	171	1	68	104	92	80	171	1	67	*105*	*172*
	3-5 years	107	4	35	76	51	60	107	4	47	*64*	*111*
	6-8 years	30	4	16	18	17	17	32	2	15	*19*	*34*
	9-11 years	25	15	16	24	21	19	38	2	20	*20*	*40*
	12-14 years	13	92	35	70	56	49	91	14	34	*71*	*105*
	15-17 years	18	162	53	127	96	84	160	20	65	*115*	*180*
	>18 years	10	4	2	12	7	7	14	0	10	*4*	*14*
	unknown	0	2	0	2	0	2	1	1	1	*1*	*2*
Total		*433*	*284*	*256*	*461*	*369*	*348*	*671*	*46*	*276*	*441*	*717*

A=accidental, V=voluntary, R=rural, U=urban, F=female, M=Male

Table 2. PED intoxications by age

Frequency	Type		Place		Patient's Sex		Coma		Admitted		Total
	A	V	R	U	F	M	No	Yes	No	Yes	
Drugs (prescription)	143	104	84	163	166	81	235	12	83	164	*247*
Alcohol	10	117	37	90	36	91	106	21	80	47	*127*
Drugs (illicit)	0	63	15	48	21	42	58	5	9	54	*63*
Volatile substances	56	3	26	33	30	29	59	0	17	42	*59*
Detergents & Soaps	49	0	16	33	26	23	49	0	23	26	*49*
Carbon Monoxide (CO)	37	2	15	24	21	18	36	3	15	24	*39*
Pesticides	35	1	17	19	20	16	34	2	2	34	*36*
Other substances	30	3	9	24	19	14	33	0	22	11	*33*
Nitrites	20	0	17	3	7	13	18	2	1	19	*20*
Inedible plants	15	0	3	12	6	9	15	0	6	9	*15*
Unknown substances	13	2	9	6	9	6	12	3	1	14	*15*
Caustics	10	0	6	4	2	8	10	0	5	5	*10*
Heavy Metals	10	0	5	5	2	8	10	0	6	4	*10*
Poisonous mushrooms	4	2	1	5	3	3	6	0	2	4	*6*
Nicotine	1	3	2	2	1	3	4	0	1	3	*4*

A=accidental, V=voluntary, R=rural, U=urban, F=female, M=Male

Table 3. PED intoxications by substance type

Frequency	Type		Place		Patient's Sex		Coma		Admitted		Total
	A	V	R	U	F	M	No	Yes	No	Yes	
Other prescription drugs	34	19	15	38	26	27	53	0	22	31	*53*
Salicylates & NSAIDs	17	12	8	21	20	9	28	1	9	20	*29*
Antibiotics	15	14	14	15	21	8	27	2	6	23	*29*
Sedatives & Hypnotics	8	19	9	18	23	4	24	3	6	21	*27*
Cardio-vascular (effect)	16	7	6	17	18	5	23	0	9	14	*23*
Anticonvulsants	8	14	10	12	18	4	16	6	1	21	*22*
Vitamin supplements	18	4	5	17	12	10	22	0	16	6	*22*
Metoclopramide	9	9	3	15	14	4	18	0	2	16	*18*
Acetaminophen	5	13	5	13	14	4	18	0	4	14	*18*
Unknown drugs	3	12	7	8	10	5	12	3	2	13	*15*
Mineral supplements	5	4	4	5	6	3	9	0	3	6	*9*
Metamizole sodium	1	8	2	7	8	1	9	0	1	8	*9*
Histamine antagonists	6	2	5	3	4	4	7	1	3	5	*8*
Antidepressants	3	4	1	6	7	0	6	1	3	4	*7*
Contraceptives	4	1	1	4	5	0	5	0	3	2	*5*
Theophylline	0	3	2	13	3	0	2	1	0	3	*3*

A=accidental, V=voluntary, R=rural, U=urban, F=female, M=Male
NSAID=non-steroidal anti-inflammatory drug

Table 4. PED prescription drug intoxications - detailed

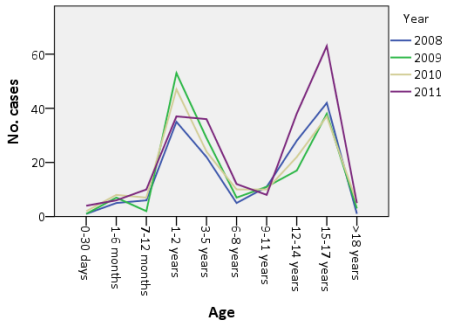

Fig. 1. PED intoxication cases by age & year

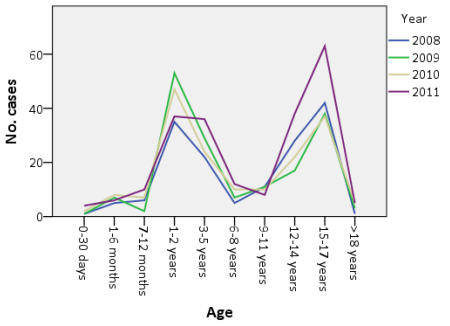

Fig. 1. PED intoxication cases by age & year

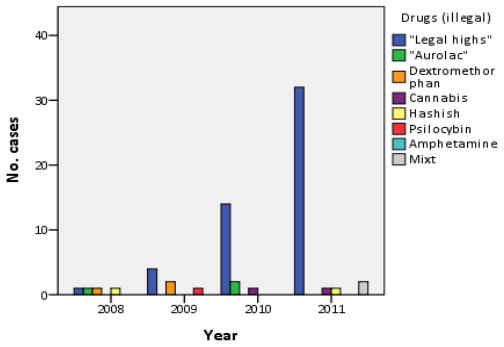

Fig. 3. PED illicit drug intoxications by year

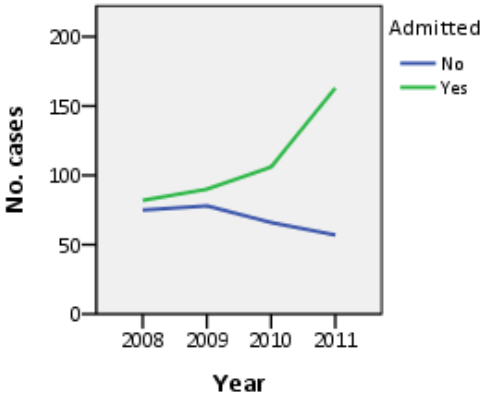

Fig. 4. PED cases admitted to toxicology ward

Analyzing the hospital's electronic database we collected data from all patients diagnosed with acute intoxications. We recorded clinical and demographic information as well as substance type from each patient included in the study.

In terms of demographics, we analyzed differences between patients coming from urban versus rural area. We used several age categories for analysis: 0-30 days, 1-6 months, 7-12 months, 1-2, 3-5, 6-8, 9-11, 12-14, 15-17, over 18 years. Distribution among males and females was also assessed.

The type of substance was recorded for every patient and whether it was accidentally or voluntary and assessed its relation with age. Subsequently we analyzed the seasonal trend related to certain types of intoxications. The admittance rate and length of in-hospital stay was recorded.

Statistical analysis: All data was collected in a Microsoft Excel Database. Statistical analysis, was performed the Statistical Package for Social Science (SPSS version 19, Chicago, Il, USA). A significance level of $\alpha=0.05$ has been chosen, with p values < 0.05 considered statistically significant.

Ethics approval was obtain from the local hospital ethic board.

Results

In this time frame the PED saw a total number of 76 223 visits, out of which 717 were acute intoxication cases.

The number of acute intoxications diagnosed in the PED has increased continuously during the study period, from 157 cases in 2008 to 220 cases in 2011. Although there has been an increase of 40.1% in acute intoxication cases, these absolute values strongly correlate with the total number of patient visits in the PED each year, with an average of 0.95% intoxication diagnosis per year ($\chi 2$ test, p=0.008).

Out of the 717 cases of acute intoxications diagnosed in the PED, 60.4% were accidental. The distribution by age (figure 1) shows a clear polar grouping around the 1-5 year olds group and the 12-17 year olds group. A similar distribution by age and type of intoxication (accidental vs. voluntary) shows a close resemblance to the overall age distribution (figure 2), with a strong statistical correlation between them ($\chi 2$ test, p=0.001). Out of the total 433 cases

of accidental intoxications, 64.2% were from the 1-5 year olds group. The ratio between females and males was 1.06. Both were just as likely to present with acute intoxication in the PED, the difference between sexes being the type of substance preferred. While teenage females mainly ingested prescription drugs (67%), teenage males chose ethanol (71%) and illicit drugs (66.7%).

Urban intoxications are 1.8 times more frequent than rural ones, with a total of n=461 (64.3%) intoxications taking place in urban areas and n=256 (35.7%) in rural areas.

Out of all the substance types registered, only Nitrites saw a larger rural-urban ratio, with 17 vs. 3 cases (table 3), explained by the nature of the substance and the main application in agricultural activities. The distribution by month highlighted some differences between the two populations, with peak activity in the months of March & September for the rural group and in the months of February – March, June & November for the urban group. The age distribution shows the same polar split as we observed on the global study population, but with different peak amplitudes according to area. The rural study population had more intoxications in the 1-5 years old group (103 vs. 88), while the urban population presented more cases from the 12-17 years old group (180 vs. 197). The rural population is slightly more prone to accidental intoxication, especially in the case of young children. Despite a slight dip in the number of cases from rural areas reported in 2008 – 2010, we observed a sharp increase of almost 53.3 % in intoxication cases from 2010 to 2011, following the upwards trend encountered in the urban areas.

An altered mental state was observed in n=46 (6.4%) of patients during the first medical evaluation, resulting in a mild to medium coma mostly due to voluntary intoxication with alcohol, prescription drugs and illicit drugs (60.52%). We observed two distinct peaks of activity.

The cold months, especially December and January, are also the ones that see the most Carbon Monoxide (CO) intoxications due to the fact that wood burning is still a popular method of heating the house among the low income population and some of the stoves are improvised installations. Out of the total n=39 cases of CO intoxication, only 3 (7.7%) resulted in coma and 24 (61.5%) were admitted to hospital for observation and treatment (table 3).

A total of n=441 (61.5%) patients presenting with intoxications to the PED were admitted to the PTW. The ratio has been growing steadily from 52.2% in 2008 to 70% in 2011 (table 1), with a sharper increase between 2010 – 2011 of 53.8% (figure 4). The mean hospital stay was 3.2 days, with 81.45% of admitted patients staying less than 5 days.

Prescription drugs were the most common cause of intoxication observed in the PED, with n=247 (34.4%) cases reported in the 4 year period of the study (table 3). Due to the diverse nature of substances covered by this generic class, it was further divided into drug types (table 4). After the reclassification, it was apparent that none of the prescription drug subclasses could rival the top most common toxic – alcohol, it being responsible for n=127 (17.7%) intoxications (122 ethanol & 5 rubbing alcohol). Ethanol overdoses were 2.43 times more common in urban areas compared to rural areas, and 2.53 times more frequent in the male population. It was also a leading cause of coma in patients presenting to the PED with altered mental status n=21 (45.65% of coma cases). Out of the 127 alcohol intoxications, n=117 were voluntary overdoses, with the majority being teenage males from urban areas. The surprising find was that ethanol's popularity has been in slow decline in the PED, the difference being filled by an increase in illicit drug use.

The second most common cause of intoxication was illegal substance use, with n=63 (8.8%) cases reported. This type of toxic has had an exponential increase in popularity over the years (figure 3). In particular, a subgroup known as "legal highs" or "ethno botanic", was the most popular recreational drug used n=50 (mainly by inhaling or smoking), followed at a long distance by natural cannabinoids n=4. The vast majority of cases n=60 (95.2%) were from the 12-17 year olds group. Illicit drug intoxications were 3.2 times more common in urban areas compared to rural ones, and 2 times more frequent in the male population. Teenage males from urban areas are more susceptible to this toxic, mostly due to increased accessibility on-line and in illegal shops. A total of n=5 patients presented with coma due to illicit drug overdose (10.87% of coma cases).

In contrast, teenage females were more prone to overdose on prescription drugs (67%). The vast majority of multi drug overdoses were part of the 12-17 year olds group. Intoxications due to medication were 1.95 times more common in urban areas, and 2 times more frequent in the female population. There were n=12 patients with altered mental status due to prescription drug overdose (26% of coma cases), with anticonvulsants and sedatives representing the major toxic responsible n=9. A detailed breakdown of the drug subgroups and tendencies can be found in table 4.

Detergents (from household products) were among the most common toxics to cause intoxications in younger children (1-5 years; n=46; 82.14%), showing a steady increase of 47.5% per year on average. All poisonings caused by detergents were accidental and 2 times more frequent in urban areas.

Volatile substances, such as chlorine based washing products (34.7%) and solvents (paint thinners) (38.8%) were equally popular among young children (1-5 years; n=41; 69.5%).

Discussion

This clearly shows that most voluntary pediatric poisonings are in fact overdoses, occurring in groups of teenagers with clear intent, for either recreational use or (para) suicidal purposes.

The urban high rate can be explained by Romania's national demographic, with 55.2% of the population living in urban areas (5), but also by the abundance and easier access to legal and illegal substances in larger, more populated communities.2

The first peak for alcohol is grouped the cold months of the year (November – February), which coincide with the winter holidays, New Year's celebrations and brief winter school vacations that provide ample opportunity for both young children and teenagers alike to explore their surrounding and ingest known or unknown substances readily available at winter fairs and parties. The second peak is in July, which coincides with the end of the school year and exam periods.

The "legal highs" packs probably contain synthetic cannabinoids and cathinones among other substances, but the exact composition is hard to track due to the uncertain origin of the products and intentional mislabeling.

The status of household substances has increased among young children in part due to the newer forms of presentation; liquid detergents can be easily confused as soft drinks and are particularly appealing to toddlers exploring their surroundings.

Along with detergents, nitrites, pesticides and ornamental flowers, volatile substances represent the most common substances children accidentally ingest. Similar results were reported by the WHO and other studies (6, 7).

The rise in the total number of pediatric intoxications presenting to the PED is attributed to an overall increase in patients and not a shift in the presenting pathology and is in line with the national average of 1%. (8) This result was lower than the average reported in other countries like France (3% of total medical emergencies) (9), Turkey (Eskisehir, 2.31% of overall emergency unit visits in 2009) (7).

According to The European School Project on Alcohol and other Drugs (ESPAD) Romania's youth is at or below the European average on cigarette use, alcohol use, cannabis and other illicit drug use, tranquilizer use (without prescription) and inhalant use (10). The slow downwards trend for alcohol observed in the study can also be found in the ESAPD report, remaining steady with little to no change over the years. Although alcohol's popularity is declining, it still is one of the most frequent toxics identified in pediatric intoxications due to its low price, abundance and ease of access. Our results were significantly lower than those reported in a Croatian study, in which 40.2% (vs. 17.7%) of all pediatric hospitalizations were due to alcohol overdose (11).

In contrast with alcohol, "legal highs" have been experiencing a surge in popularity among teenagers in Romania. Between 2005 and 2011 the European Monitoring Centre for Drugs and Drug Addiction has identified 164 new psychoactive substances, with 49 being reported in 2011 alone (2). The speed with which new substances are created is cause for concern, especially due to the fact that sales continue almost unhindered online, despite attempts to close down physical shops. In January 2012 there were at least 693 online shops offering to supply psychoactive substances or products likely to contain them. This number has increased constantly from 170 in 2010 to 314 in 2011 (2). The magnitude of the phenomenon is visible in our study as well. "Legal highs" had the fastest and strongest growth of all toxics identified in the study, increasing by 50% from 2008-2009, 183% from 2009-2010 and 111.8% from 2010-2011. It remains to be seen if local, national and international policies will slow or even reduce the expansions of such drugs.

The increasing number of patients presenting in the PED with acute intoxications are a clear sign that there is an increasing need for a comprehensive Pediatric Toxicology Ward to cater for a growing population coming in contact with an ever evolving array of new substances, some potentially harmful6. Health care quality can and should be constantly improved by creating new hospital-wide protocols to manage acute intoxications caused by the most frequently involved and toxic substances, as well as those caused by novel substances if new evidence based treatments emerge (12). All this should occur in conjunction with a continuous education for health-care providers in order to deliver the most efficient services possible.

Child injury prevention can also be improved by implementing better labelling regulations, improving storage and child resistant packaging (6). Alongside these wide-reaching measures, a better understanding of the relationship between caregiver and child with focus on prevention and care-giver education can reduce child mortality and morbidity due to accidental poisonings (13, 14).

Lastly, we would like to emphasize the importance of interactive, international poisoning surveillance and reporting tools, such as the upcoming Global Pediatric Emergency Poisoning Surveillance System or the Toxic registry (15).

Conclusions

As the county's population increases, the demand for quality health care will grow. The increasing number of pediatric intoxications is a clear sign that local Pediatric Emergency Departments and Toxicology Wards must keep pace with an evolving population. Hospital administrations must adapt protocols to fit current trends and provide health-care professionals with constant training in regards to novel psychoactive substances, as well as up to date evidence based treatments.

In spite of the steady 1% proportion of pediatric intoxications in relation with total PED visits, there has been a shift in substance preference, especially among teenagers. Alcohol remains a popular "legal" drug for male teens, but it has seen a slowing trend, with the void being filled by new "legal highs". A greater effort must be undertaken to provide urban areas with education, prevention (outdoor activities and group sports) and harm-reduction programs for children and parents alike, due to the higher frequency of poisoning situations.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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THE IMPACT OF RISK FACTORS ON THE EVOLUTION OF VLBW PRETERM INFANTS WITH NECROTIZING ENTEROCOLITIS

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Abstract

Necrotizing enterocolitis is the most common gastrointestinal complication of preterm infant. The incidence of NEC increase when two or more risk factors are associated to the preterm birth. Objective: A retrospective longitudinal study was conducted in a 3rd level neonatal intensive care unit in Romania. We evaluated the role of risk factors on NEC's severity and outcome. Material and methods: In the study group were enrolled preterm neonates with birth weight below 1500 g and gestational age less than 32 week with NEC. Were analyzed the influence of risk factors like maternal preeclampsia, enteral feeding type, pH gas values and blood transfusion on NEC' s severity and outcome. Quantitative and qualitative data were analyzed by using SPSS v. 25. Results: From the 596 VLBW neonates admitted in the study period 37 developed NEC, incidence of 6, 2% of NEC in the unit. We found a significant association of basal excess with NEC severity. This could have a predictive value on NEC's severity. 50% of preterm fed with formula had a severe outcome and were transferred to pediatric surgery. There was no statistically significant difference of transfusion counts between the patients who died and those who had good outcome (Mann-Whitney U test: p=0.61>0.05). Conclusion: Exposure of preterm infants to formula determined a higher rate of unfavorable evolution compared to ones fed with own mother milk. A significant link between NEC's severity and basal excess was found .No association between NEC and blood transfusion could be demonstrated.

Keywords: necrotizing enterocolitis, preterm, enteral feeding, metabolic acidosis

Introduction

Necrotizing enterocolitis (NEC) is the most often digestive tube pathology of preterm infants. The incidence of the disease among premature babies is 7-10%. The disease is more frequent as gestational age decreases. Onset typically occurs in infants aged between 2 weeks and 2 months [8, 13]. Growth restriction associated with prematurity is an important risk factor of morbidity. The incidence and the severity of the disease are higher if two risk factors are associated.

Mortality is high, reaching 30%. Mortality is higher among infants who develop severe forms of the disease that require surgical treatment. In the case of survivors, the risk of digestive sequelae – short gut syndrome, as well as neurological sequelae, is increased [2]. The etiology of the disease is not yet completely understood. What is certainly known at this point is its multifactorial etiology. NEC is accompanied by inflammation, ischemia and infection. In advanced stages, intestinal wall necrosis, perforation and peritonitis occur. Altered intestinal flora, pathogen colonization are an important link in NEC pathogenesis. Bacterial colonization has certain particularities in premature infants. Studies have demonstrated that before 33 weeks of gestation, bifid bacteria colonization is limited [3]. Elevated concentrations of gram-negative germs are an important risk factor in the pathogenesis of the disease. Enteral feeding of preterm infants with breast milk facilitates their colonization with bifid bacteria and significantly reduces the incidence of NEC among them. Breast milk exerts its protective effect through the presence of secretory IgA, fatty acids and antimicrobial proteins in its structure.

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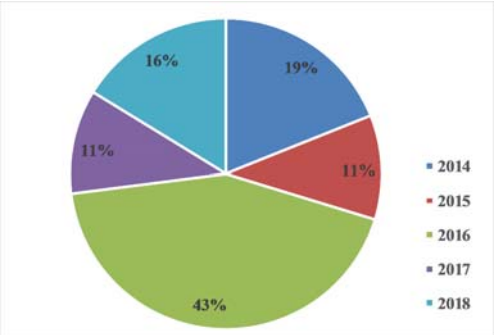

Fig. 1. Description of the patients' year of birth.

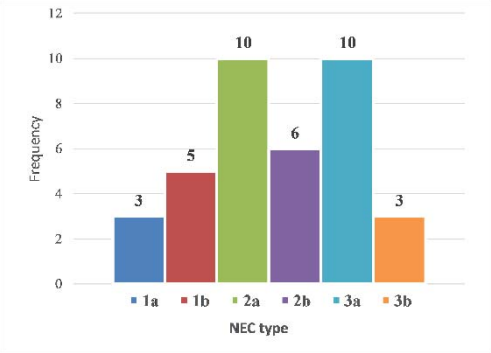

Fig. 2. Description of the patients' NEC type.

Stage	Form	Clinical findings	Abdomen examination	Radiological findings
I	Suspected	Temperature instability, apnea, bradycardia	Elevated gastric residuals, mild abdominal distention, occult blood in stool	Normal or mild ileus
IIA	Mild	Similar to stage I	Prominent abdominal distention ±tenderness, absent bowel sounds, grossly bloody stools	Ileus, dilated bowel loops, focal pneumatosis
IIB	Moderate	Mild acidosis, thrombocytopenia	Abdominal wall edema and tenderness, ±palpable mass	Extensive pneumatosis, early ascites, ±portal venous gas
IIIA	Advanced	Respiratory and metabolic acidosis, mechanical ventilation, hypotension, oliguria, disseminated coagulopathy	Worsening wall edema and erythema with induration	Prominent ascites, persistent bowel loop, no free air
IIIB	Advanced	Vital signs and laboratory evidence of deterioration, shock	Evidence of perforation	Pneumoperitoneum

Table 1. Bell classification of NEC[4]

Characteristic	All	F	M	Stat (p-value)
Gender [a]	37	19 (51.4%)	18 (48.6%)	0.232 (0.816)
Gestational age, weeks [b]	28 (26 to 30.5)	28.5 (26 to 31)	28 (25 to 30)	143.5 (0.399)
Weight, g [b]	920 (695 to 1255)	995 (697.5 to 1312.5)	800 (650 to 1200)	144.5 (0.420)
Apgar score [b]	7 (4.5 to 7.5)	6 (4 to 7.25)	7 (5 to 8)	151.5 (0.547)
pH [b]	7.28 (7.15 to 7.31)	7.29 (7.15 to 7.33)	7.24 (7.15 to 7.30)	133.5 (0.254)
NEC debut day [b]	14 (7 to 20)	13.5 (6 to 25.25)	15 (8 to 16)	163 (0.808)
[a] n (%), Z-test for proportion; [b] median (Q1 to Q3), Q=quartile, Mann-Whitney Test				

Table 2. Description of preterm infants with NEC

	Growth restriction N (%)	Antenatal corticoids n (%)	Preeclampsia n (%)	Surfactant n (%)	Transfer to pediatric surgery n (%)	Transfusion n (%)
Present	9 (24.33%)	20 (54.05%)	13 (35.14%)	21 (56.76%)	13 (35.14%)	28 (75.68%)
Absent	28 (75.67%)	17 (45.95%)	24 (64.86%)	16 (43.24%)	24 (64.86%)	9 (24.32%)
Total	37 (100%)	37 (100%)	37 (100%)	37 (100%)	37 (100%)	37 (100%)

Table 3. Frequency for the presence /absence of evaluated factors

Antenatal corticoids	Outcome		Total
	Death	Good state	
Yes	8	12	20
No	6	11	17
Total	**14**	**23**	**37**

Table 4. The relationship between corticoid treatment and the outcome

Preeclampsia	NEC type					Total
	1	2a	2b	3a	3b	
Preeclampsia -yes	2	4	2	4	1	13
Preeclampsia-no	6	6	4	6	2	24
Total	**8**	**10**	**6**	**10**	**3**	**37**

Table 5. The relationship between the Preeclampsia and NEC type

Enteral feeding start day	Enteral feeding type		Total n (%)
	OMM n (%)	Formula n (%)	
0	1 (4.76%)	0 (0%)	1 (2.7%)
1	0 (0%)	1 (6.25%)	1 (2.7%)
2	12 (57.14%)	6 (37.5%)	18 (48.65%)
3	3 (14.29%)	2 (12.5%)	5 (13.51%)
4	2 (9.52%)	4 (25%)	6 (16.22%)
6	1 (4.76%)	0 (0%)	1 (2.7%)
7	1 (4.76%)	1 (6.25%)	2 (5.41%)
10	0 (0%)	1 (100%)	1 (100%)
13	0 (0%)	1 (6.25%)	1 (2.7%)
14	1 (4.76%)	0 (0%)	1 (2.7%)
Total	**21 (100%)**	**16 (100%)**	**37 (100%)**

Table 6. The relationship between the start day and the type of enteral feeding

Exposure to formula (days)	Frequency	Percent
0	1	6.3
2	2	12.5
3	1	6.3
5	2	12.5
9	1	6.3
11	1	6.3
12	1	6.3
13	2	12.5
14	2	12.5
15	1	6.3
25	1	6.3
33	1	6.3
Total	16	100.0

Table 7. Frequency of exposure to formula milk

Enteral feeding	Initial outcome				Total
	death	good	poor	transfer to pediatric surgery	
Without formula	4	10	2	5	**21**
With formula	1	7	0	8	**16**
Total	**5**	**17**	**2**	**13**	**37**

Table 8. The relationship between the evolution of NEC and the type of feeding

BE < - 10	NEC type					Total
	1	2a	2b	3a	3b	
Severe acidosis	5	0	2	6	0	**13**
Mild/no acidosis	3	10	4	4	3	**24**
Total	**8**	**10**	**6**	**10**	**3**	**37**

Table 9. The relationship between NEC type and BE(<- 10)

Regarding the study of the NEC etiology, there are no specific animal models that reproduce the form of disease found among preterm infants. The inflammatory ischemic mechanism is complex, intricate with bacterial colonization. Under the action of TLR4-mediated bacterial stimulation, the intestinal mucosa is damaged, which will facilitate the entry of bacteria into circulation. In mesenteric circulation, bacteria through interaction with TLR4 will induce a decrease in the level of nitric oxide that will have a vasoconstrictor effect. [11, 18, 20]

The use of feeding protocols in clinical practice has led to a decrease in the incidence of the disease over the past years. Antenatal corticosteroids also contributes to the reduction of the disease incidence. [12, 17]

Aim

The aim of the study was to evaluate the role of risk factors on the incidence, severity and evolution of NEC, as well as to determine the factors causing an unfavorable evolution of NEC in the studied population. The study is relevant for the geographical region and the population in which it was conducted, given the presence of particularities regarding pregnancy follow-up and the

prophylaxis of pregnant women with imminent premature delivery.

Material and method

A longitudinal retrospective study was conducted at the Neonatology Department of the Gynecology Clinic I Cluj-Napoca, between 2014 and 2018. The clinic where the study was carried out is a third-level facility which serves an important part of the population in North-Western Romania (4 counties), preterm infants less than 32 weeks of gestation being admitted to this center. The current study included all preterm infants who were diagnosed with NEC in the mentioned period. Data were systematically extracted from the records of Neonatology department.

Modified Bell criteria were used for diagnosis. Diagnosis was based on clinical and laboratory criteria, according to the existing protocol (Table 1). Staging was performed for suspected disease, mild, moderate and advanced forms. (Table 1)

Abdominal X-ray was carried out at the time when clinical signs suggestive of NEC appeared: increased abdominal circumference, changes in abdominal skin color, bilious, bloody gastric residuals over 1 ml, enteral nutrition intolerance to more than 3 consecutive meals, positive Gregersen's reaction on stool examination. Radiological investigations were performed with the portable device available in the department.

pH gas value parameters

In parallel to the clinical and radiological elements, pH gas value were monitored in all infants with NEC. The blood gas values were determined from venous blood with the device available in the department. The aim was to find out if there was a significant link between the pH gas value, basal excess, lactate and NEC onset in order to detect the disease at an early stage. Thus, early initiation of conservative treatment allows limiting the unfavorable evolution and the need for surgical treatment.

Data processing

Qualitative data were described with the help of frequencies, percentages, frequency tables and Graphs (pie charts and column charts). Evaluating the existence of a link between two independent groups was done with Fisher exact test or Chi2 test. Proportions were compared with a test Z-proportions.

For quantitative data, the normality was tested using the Shapiro test. For data not following a normal distribution, frequencies, percentages, the median, the 25 %(Q1) and the 75 %(Q3) (IQR=interquartile range) were used for the statistical description. The Mann-Whitney Test was used to compare 2 independent groups.

The significance level was set at 0.05. Data analysis was made using SPSS v. 25.

Results

Characteristics of the group

Between 2014 and 2018, in the clinic were admitted to the intensive care unit 596 (460 inborn and 166 out born) preterm newborns having a gestational age of 32 weeks or less and birth weight below 1500 g, but only 37 (6.20%) of them were diagnosed with NEC and were included in this study. Most of the NEC cases were diagnosed in babies born in the maternity unit (inborn), just 35.14% (13/37) being NEC cases transferred from other lower-level units (outborn). (Table 2)

Most of the preterm with NEC from the study group were born in 2016 (43%) (Fig. 1).

The majority of the patients in the study group had a gestational age of less than 30.5 weeks and a weight less than 1200 g (table 2). Close to half of the preterm newborns, 45.95% (17/37), were delivered by cesarean section. (Table 2)

Diagnosis of NEC was made based on clinical and radiological findings, according to Bell criteria, the most frequently observed NEC types being 2a and 3a (Fig. 2).

The outcome of the preterm newborns was good in 62.16% (23/37) of the cases.

Analysis of risk factors

Several factor were considered for analysis and their presence was noted in Table 3.

Antenatal corticoids were applied to 20 of the preterm newborns (Table 4). There was no statistically significant link between the presence of corticoids and the patients' outcome (Chi2 test: $p=0.76>0.05$). (Table 4)

Next, we checked if there was a link between the presence of preeclampsia and patients' NEC type, but no statistically significance was found (Fisher exact test: $p=0.96>0.05$). (Table 5)

From the multiple factors have a role in NEC pathogenesis, in this study we have firstly considered those related to feeding: the time when enteral nutrition started (since day of birth, Table 6), the type of milk used for feeding own mother milk (OMM) or preterm formula (Table 6) and the length of exposure to formula (Table 7). The median for enteral feeding start day was 2, with an IQR between 2 and 4 days. Thus, for the majority of the infants with NEC enteral feeding started during the first 3 days of life. In one case, enteral feeding was not initiated before NEC onset. This case had an early NEC onset and developed the severe form of the disease, which required transfer to the service of pediatric surgery. (Table 6)

The exposure to formula had different length as presented in table 7.

Regarding the type of milk used at the onset of enteral nutrition, cases fed with OMM were predominant (21/37). Exposure to formula until NEC onset was present in 43.24% (16/37) of cases. The median for exposure to formula was 11.5 days, with an IQR between 3.5 and 14 days.

The initial evaluation of outcome was presented in table 8 with regard to the type of feeding. (Table 8)

Besides the feeding type, the transfusion was evaluated: for the patients who died the median number of transfusions was 2 (IQR 1-3.25), while the median for those who had a good outcome was 1 (IQR 0-5). There was no statistically significant difference of transfusion counts between the patients who died and those who had a good outcome (Mann-Whitney U test: $p=0.61>0.05$).

Next, the study aimed to check if there was a link between acidotic status and the development of NEC. We checked the link between the NEC type and pH value, basal excess (BE) and lactate. For BE we considered the value lower than -10 relevant for metabolic acidosis and results are presented below. (Table 9).

There was a statistically significant link between BE under -10 and patients' NEC type (Fisher exact test: P=0.007<0.05).

It was observed that the more severe the NEC type, the less favorable was the immediate prognosis (Table 10).

The NEC forms in the study group based on Bell criteria are shown in Table 10. Cases with stage 1-2 were predominant. There was a statistically significant link between the NEC type and outcome - final (Fisher exact test: p=0.0005<0.05). Death was observed more frequently in patients with more severe NEC (10 cases with 3a and 3b forms).

Discussion

Necrotizing enterocolitis is a gastrointestinal emergency with a complex pathogenic mechanism, more frequent in preterm infants. The incidence of the disease varies depending on gestational age. However, there are several risk factors that contribute to the increase in the disease incidence. [1, 2, 13]

In the study performed, we aimed to identify the risk factors that influence the incidence of NEC in the preterm population in our center. We analyzed the role of each factor on the severity of NEC, as well as on the evolution of the disease towards cure or death. Since the patients' data were collected from medical records, there were sources of errors. The analyzed risk factors are those described by other studies as being relevant for the incidence of NEC [1, 2].

During the study period we had an incidence of 6.20% of NEC. This finding is similar of our studies.[2,8] The incidence of NEC decreased in the past years due to the progresses on care of very low birth weight premature.[4]

Antenatal corticoids, an important prophylaxis for respiratory distress in preterm infants, periventricular hemorrhage, with a known influence on intestinal maturation as well.

In our study, this prophylactic measure was applied in a proportion of 54.1% of the studied sample. This percentage is lower than the ones of other studies. [15, 22] This situation is caused by the incomplete follow-up of pregnancies. There is a large number of preterm pregnancies in our area that are not followed up or are incompletely followed up and arrive late to our unit, administration of maternal corticoids being delayed. In the study group, 13 infants (35.13%) were outborn, of which 7 had a gestational age of less than 28 weeks. For these cases, in utero transfer was not possible, since they arrived to the units where they were born during the expulsion or advanced labor, which did not allow in utero transfer.

Preeclampsia has an impact on the fetus and newborn. Maternal hypertension, especially its severe form, is a factor that may influence mesenteric circulation, with an increase in the incidence of neonatal NEC. The incidence of preterm births and IUGR is higher in hypertensive compared to normotensive mothers. IUGR is an important risk factor for the development of NEC. In the studied group, preeclampsia had no effect on the severity of NEC (p>0.05) [6].

Growth restriction had 9 patient (24.33%) of the study group. We found no correlation of growth restriction with NEC's outcome. But it has to be considered that we had a small number of cases.

Enteral nutrition plays an important role in the pathogenesis of NEC. This can be an important risk factor in the genesis of the disease. Aggressive enteral nutrition will contribute to a significant increase in the incidence of NEC. Breast milk is the ideal solution for preventing NEC. [3, 5, 7]

In the studied group, most of the infants were enteral fed with own mothers milk starting from the second day of life, in progressively increasing doses. In the unit, an enteral nutrition protocol was adopted in 2016, based on which the enteral feeding volume is increased every 5 days in newborns with a weight of less than 1000 g, and every 3 days in those weighing less than 1250 g. The number of NEC cases has decreased after this protocol was adopted. Many studies have evidenced the fact that a slow increase in the volume of enteral feeding is an important method for preventing NEC [7, 12].In our country for preterm enteral feeding can be used only OMM and formula since there are no milk banks in Romania

Regarding the type of milk used and the time of initiation of enteral feeding, there were no differences in approach during the study period.

Exposure to preterm formula was present in 16 cases (43.2%) of the premature infants included in the study. Formula was used in 11 cases as initial enteral feeding, and in 5 cases it was used as a complement to breast milk. Formula is used in preterm newborns whose mothers have delivered by cesarean section, have no milk secretion on the third day of life, as well as in those who receive therapies contraindicating breast feeding, and also in the case of outborn babies admitted without their mothers. Preterm formula is used in these cases as the only alternative for enteral nutrition.

Although formula was used in 40% of the cases, no significant link was found between formula administration and NEC severity and evolution. However, the relatively small number of cases and the presence of multiple associated risk factors contributing to the development of the disease should be taken into consideration.

The benefits of feeding with breast milk cannot be replaced by formula. Although at this point the only enteral nutrition alternative for preterm infants in Romania is own mother's milk, in our center we attempt to limit as much as possible the use of formula in premature infants. Feeding with formula induces an alteration of intestinal microbiota, with bacterial multiplication and changes in the intestinal mucosa [1, 8, 9, 14, 21].

If there is a probability for feeding with breast milk, initiation of enteral feeding is delayed up to 36-48 hours,

especially if prematurity is associated with intrauterine growth restriction. It should be considered that excessive delay of enteral nutrition is not beneficial because it induces intestinal mucosal atrophy, altered absorption, changes in the exocrine secretory function and deviation of the inflammatory status towards a pro-inflammatory state mediated by cytokines and chemokine, which is not a desirable effect in the case of preterm infants. [21, 22, 23] Breast milk has a protective effect against NEC in the digestive tract of premature babies. Some studies show that this protective effect is dose dependent [1, 9, 13, 17].

Knowing the fact that anemia is associated with a pro-inflammatory state and transfusion for their treatment has an impact on NEC incidence, we analyzed the influence of blood transfusion on NEC in the study group. In 9 cases, blood transfusion was not needed, while 28 cases (75.7%) required at least one transfusion. However, transfusion administration did not prove to be significantly correlated with NEC severity and evolution (Table 6). Some studies report the occurrence of post-transfusion NEC within 36-48 hours. [10] In our case, we could not demonstrate a higher incidence of NEC after erythrocyte mass transfusion or a link between transfusion and NEC severity. However, these studies describe a higher incidence of NEC in preterm infants who, in addition to receiving blood transfusion prior to NEC, also had severe RDS that required long-term invasive respiratory support and treatment for PDA, respectively. [10, 19]

The analysis of risk factors for NEC in the study group showed that a factor with a significant predictive value for NEC was the modification of pH gas values. The value of excess bases was significantly correlated with NEC severity (Table 7). Metabolic acidosis, elevated lactate levels are predictive factors for the development of NEC in preterm infants. [19, 20]

Regarding the evolution of NEC cases in the study, 62, 2% of the cases showed a favorable evolution. The cases transferred to the service of pediatric surgery had an unfavorable evolution. The mortality rate among infants with NEC was over 37, 8% in the study period. Surgical cases have an increased mortality rate (69, 2%). Other studies report a mortality rate between 20% and 40% of NEC cases [15, 25, 26].

The limitations of the study were represented by the relatively small number of cases and also, by the retrospective nature of data collection for the study group. Multicenter studies in larger groups are required. Considering the implication of multiple risk factors in the pathogenesis of the disease, its prediction, early identification of risk and initiation of treatment are elements that allow reducing the disease incidence.

Conclusions

The analysis of risk factors in the study performed revealed a significant association of NEC with the acid-base status of the preterm infant, the study group showing a significant association of NEC with the value of excess bases.

Exposure of preterm infants to formula determined a higher rate of unfavorable evolution through NEC compared to preterm infants who were exclusively fed with breast milk. No link between the development of NEC and blood transfusion could be demonstrated in our study.

Despite the progress made in the care of very low birth weight infants, NEC remains a complication that has no specific etiological treatment. Due to the multiple pathogenic mechanisms involved, the possibility of specific therapy is limited.

It is important to identify specific diagnostic means and possibly, to detect specific biomarkers with a predictive value for NEC. Early NEC diagnosis and initiation of therapy limit the number of surgical cases that generally have an unfavorable evolution, resulting in long-term complications or death.

The protective role of feeding with breast milk is known, which is why this enteral nutrition modality should be promoted.

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BURDEN OF RARE GENETIC DISEASES –EXPERIENCE OF TIMIS REGIONAL CENTRE OF MEDICAL GENETICS, ROMANIA

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Abstract

Introduction. A rare disease is a low prevalence health condition, affecting a small number of people in general population, however raising specific issues in relation to their rarity. We aim to evaluate the diagnostic approach strategy in Timis Regional Centre of Medical Genetics (RCMGT), in collaboration with the Centre for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara. Material and method. Patients addressing the RCMGT have a comprehensive clinical assessment and different genetic testing, in accordance to their phenotype. Diagnostics yields were calculated for each test performed. Results. 1038 unique patients were evaluated in RCMGT between January 2015-November 2018, 567 males and 471 females, 74.3% from 4 surrounding counties and 25.6% from other counties. Most patients were between 1-7 years (32%), with median age 5.58 years (range 0–78). Most frequent disease suspicions were chromosomal anomalies, including micro-deletion/duplication syndromes (203 patients), followed by conditions with intellectual disability (195), and multiple developmental anomalies or syndromes (179). Diagnostic yield calculated for 820 patients addressing RCMGT was: karyotype-26.4%, SNP array (molecular karyotype)-20.5%, Next Generation Sequencing: Cardio panel-45% and"Clinical exome"-47%. RCMGT was successful to reach a diagnosis (sometimes using more than one type of test/per patient), with higher yields compared to those in literature, however with longer turnaround time due to limited human and financial resources. Conclusions. Rare diseases have become a health priority worldwide and in each community, aligning policies to improve strategies in the coordination of care, diagnosis and access to treatment in patients.

Keywords: Rare diseases, genetic testing, diagnosis, SNP array, NGS panel

Introduction

The state of the art in rare diseases around the world

A rare disease (RD), also named orphan disease, is a health condition with low prevalence affecting a small number of people in general population compared to common disorders, however raising specific issues in relation to their rarity [1,2]. Rare diseases term is relatively new, the first use of this term was introduced in relation to "orphan drugs" in the United States of America in the mid-1970s [3], and adopted by Europe and France between 1987-2000 [4,5,6]. The threshold that defines RD varies across countries worldwide, European Union accepting a prevalence inferior to 5 cases for every ten thousand inhabitants, USA 7.5 per 10.000 persons, Japan 1 per 2.500 and Russia and Australia 1 per 10.000 people [7, 8].

To date, there are approximately 6.000 to 8.000 described RD, with an estimation of 5 new entities every week, but 80% of patients are affected by approximately 350 RD [9]. Each affecting less than 0, 1% of the population, but collectively they affect a considerable proportion of the population in any country (between 6% and 10%), and about 350-400 million people around the world [11]. Data has shown that 80% of rare diseases have a genetic etiology, and the rest may occur as a result of viral or bacterial infections, allergies and other environmental causes [10]. Their clinical expression is different, persons having the same condition may express different signs and symptoms, or there may be subtypes of the same disease. Most of them appear at an early age and associate multiple disabilities (physical, sensorial or intellectual disability) being responsible for 35% of child mortality in children with less than one year of age, while others have a benign evolution and do not affect intellectual development, if diagnosed and treated at an early stage [11,12,13,14,15]. Some RD have onset in adulthood. This variety, along with their number, challenge healthcare practitioners and scientists alike in terms of being able to acquire experience of a given condition for the most appropriate and timely diagnosis and management [15].

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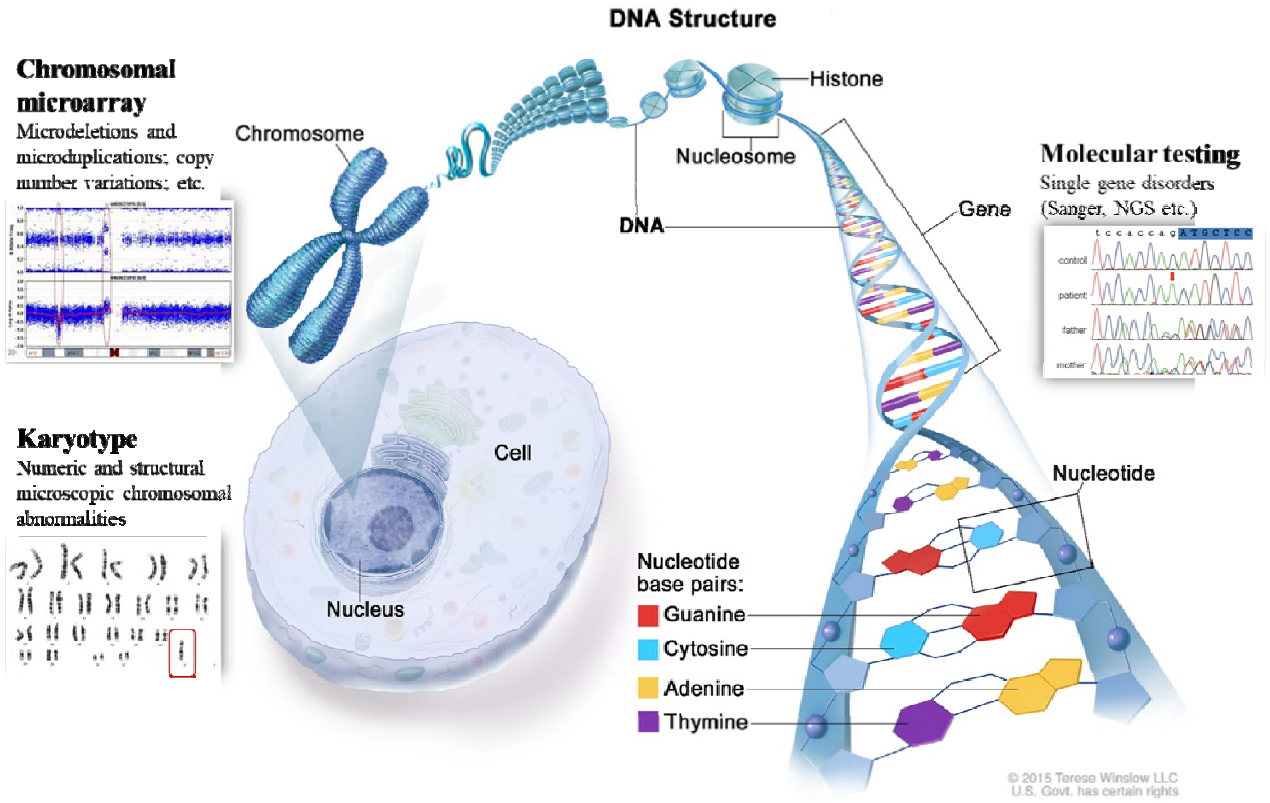

Fig. 1. Recommendations of different types of genetic testing based on clinical data of patients (adapted from: © 2015 Terese Winslow LLC, U.S. Govt. has certain rights)

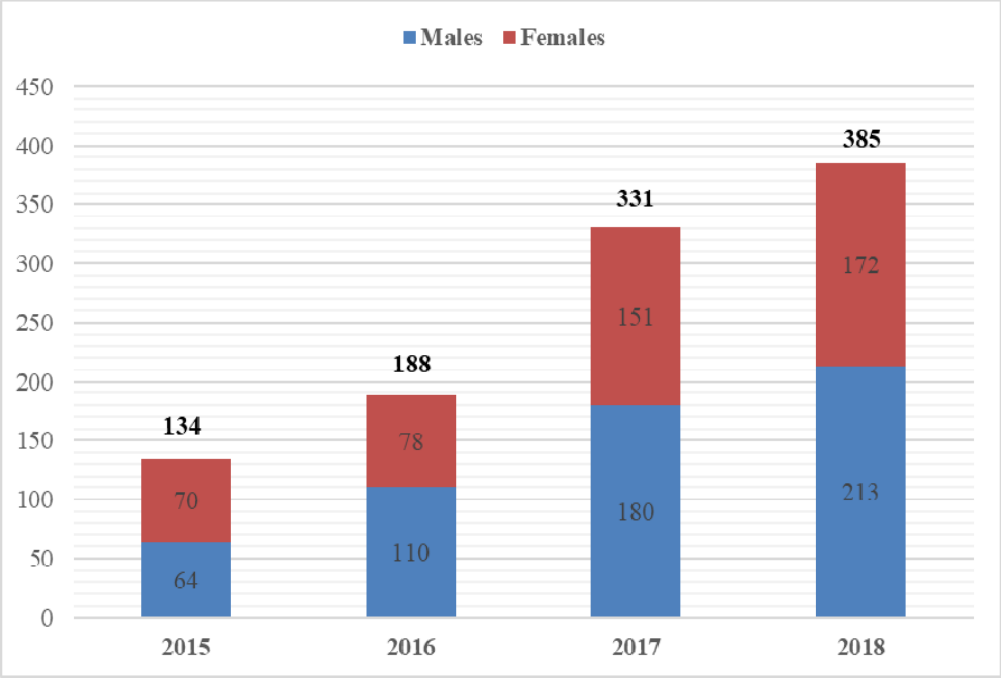

Fig. 2. Number of unique patients evaluated in RCMGT, distributed by the year of first presentation

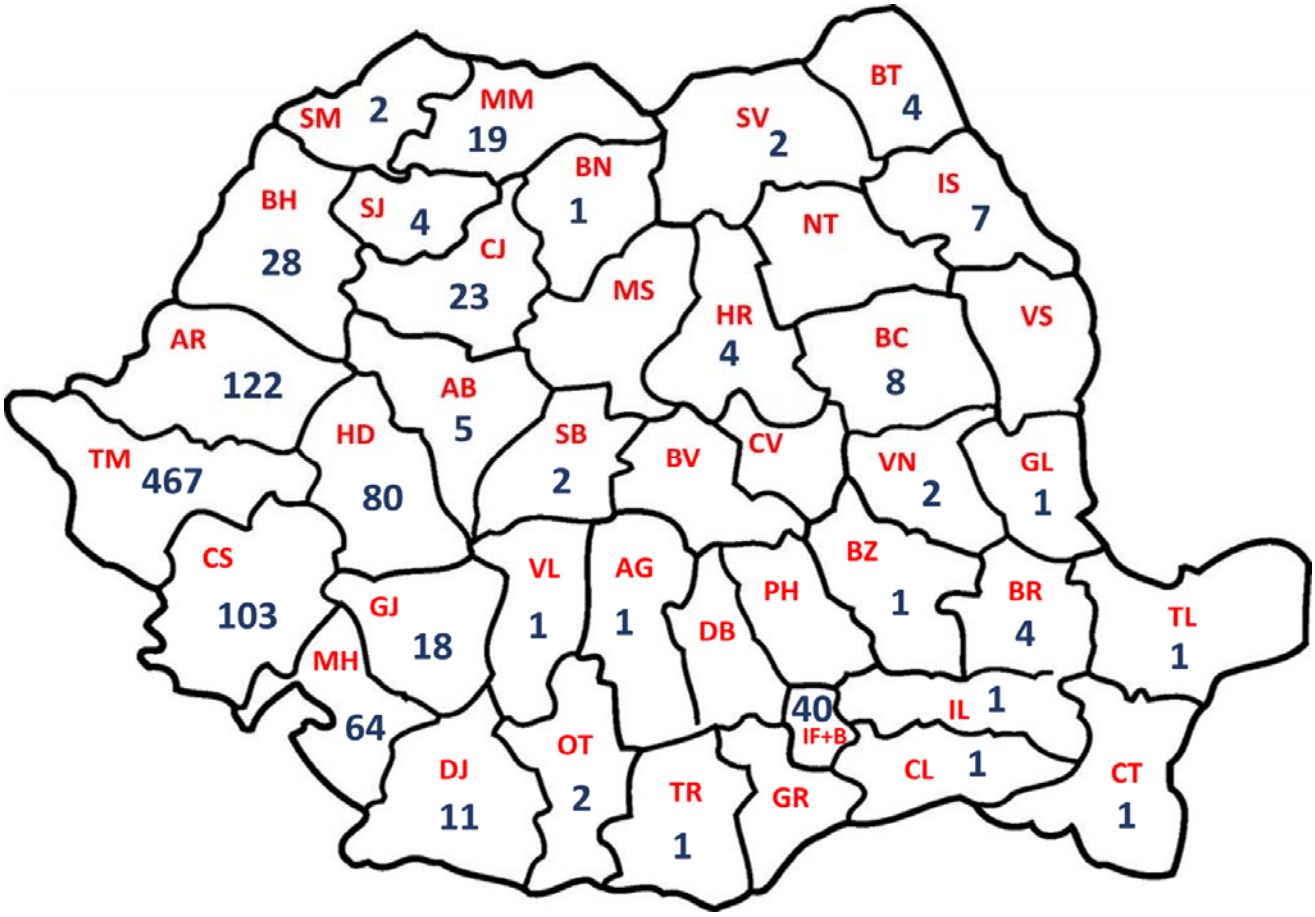

Fig. 3. Geographical repartition of our cohort of patients

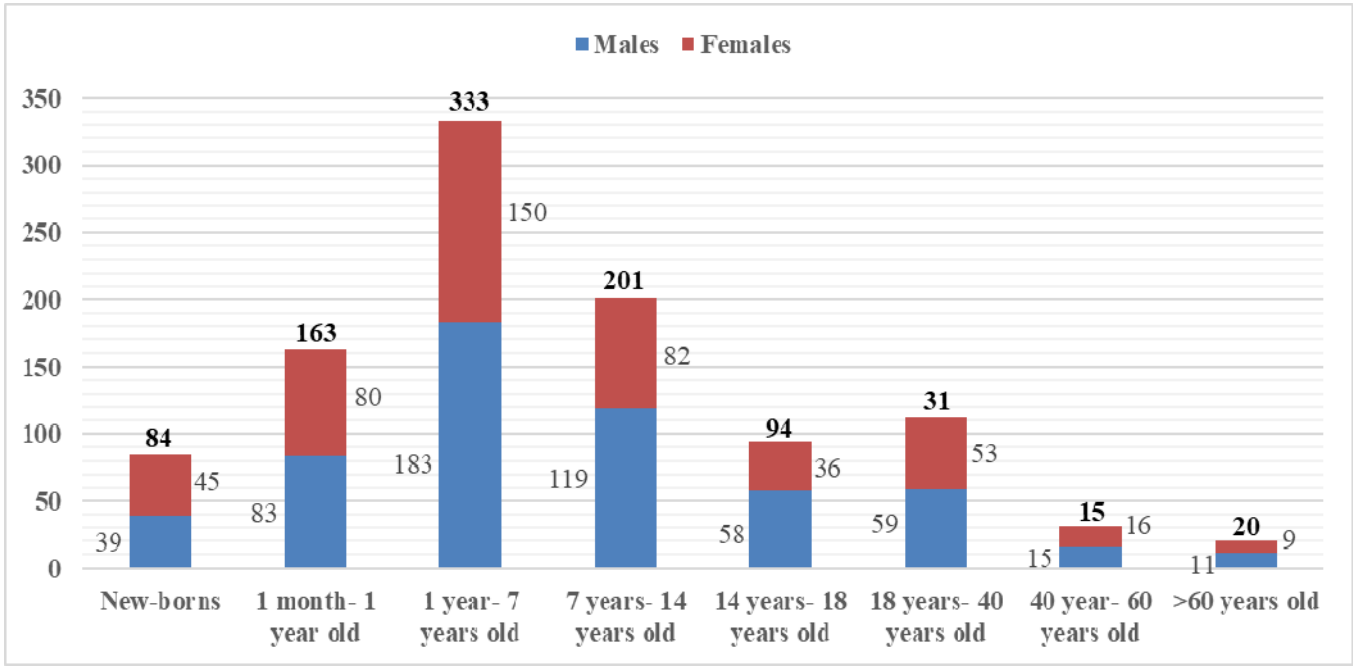

Fig. 4. Age repartition for both sexes in our patients

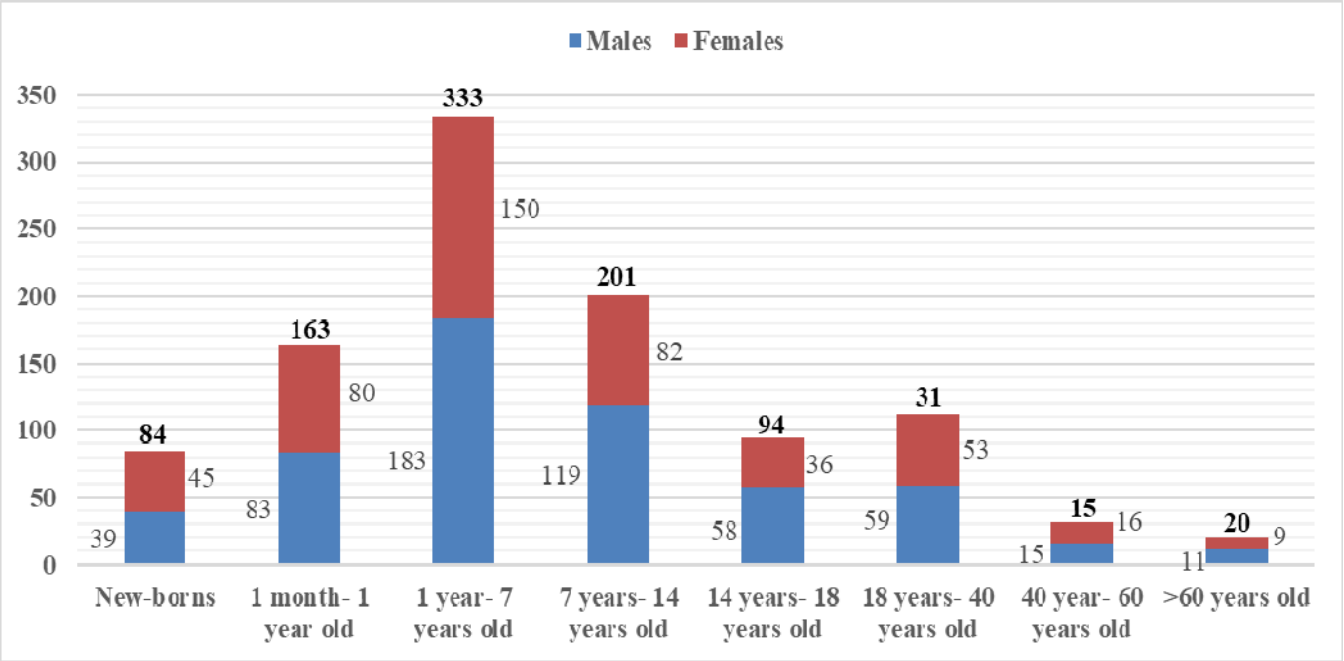

Fig. 4. Age repartition for both sexes in our patients

Genetic testing methods	Total number of tested patients/ test	Number of patients with pathogenic/ probably pathogenic variants/ findings	Diagnostic yield
Karyotype	242	64	26.4 %
SNP array	180	37	20.5 %
TruSightCardio Next Generation Sequencing panel	100	45	45 %
TruSightOne Next Generation Sequencing panel	92	51	47 %

Table 1. Diagnostic yields for each test

A patient's journey to diagnosis, also called "diagnostic odyssey", has a mean time of 7.6 years in the United States and 5.6 years in the United Kingdom and, during this period, patients receive several misdiagnoses after presenting to 8 physicians in average, inappropriate treatments or miss treatment opportunities, all these associating with increased morbidity and mortality [16, 17, 18]. Even more, rare diseases are further characterized by limited or non-existing treatment options (95% have no treatment option), lack of resources, significant disease burden and chronic course even when treatment is available. Healthcare systems and contributions of patient advocacy groups in moving forward implementation and inclusion of rare disease programs have diminished gaps across the policy landscape for different countries and are fighting the global burden of rare diseases by improving coordination of care, data registries, diagnostic resources, access to treatments, patient awareness and support, and promoting innovative research [19, 20, 21, 22, 23]. Therefore, the «EU Public Health Programme» identified Rare Diseases as a main priority for action. EURORDIS, the European Organization for Rare Diseases, in cooperation with national alliances rare disease patient organizations, and with previously described stakeholders, Member States developed National Commissions and put the basis of European Reference Networks [1, 7, 24].

Romanian landscape of rare diseases

With a total area of 238,397 square kilometers, Romania is the 12th largest country and also the 7th most populous member state of the European Union, having 20.121.641 inhabitants (2011) [25]. In Romania, the accepted definition of a rare is a health condition with less than 5 in 10,000 persons within the general population, 6-10% afflicted inhabitants [26]. These data allow to appreciate a number between 1.207.298 and 2.012.164 people in Romanian population presenting a RD.

The first national initiative taken in relation to rare diseases was advocated by a patient association, the Romanian Prader Willi Association- President Dorica Dan [27], who established the fundaments of the Romanian National Alliance for Rare Diseases- ANBRaRo or RONARD [28] in August 2007, together with members of Romanian Society of Medical Genetics [29], other specialists and patient organizations. The first National Plan for Rare Diseases was adopted in 2013. These sustained efforts gained a place for RD in the National Public Health Strategy for 2014-2020 ("Prosperity of Health"), approved by Government Decision no. 1028/2014. The strategy established a regulatory and political framework which generated a system to integrate health and social services. A dedicated budget did not exist for the National Plan in Romania. The Government approved, by Decision, the national programmes for rare diseases to be carried out and funded in 2015 and 2016: Ministry of Health (dietary treatment of RD) and National Health Insurance House (curative treatment for rare diseases). Between 2014 and 2017, all the Romanian stakeholders (patients, health professionals, policymakers and academia) worked together and made notable progress towards improving the quality of life for RD patients. As a result of continuous activity and effort, the Ministry of Health acknowledged the six Regional Centers for Medical Genetics (Timis, Bihor, Dolj, Iasi, Bucuresti, Cluj) (1358/13.11.2014). Timis Regional Center of Medical Genetics along with other Regional centers were included in a European Reference Network – ITHACA on congenital malformations and rare intellectual disability [26, 28].

Herein, we present the experience and strategy for diagnosis of RD in the Timis Regional Centre of Medical Genetics (RCMGT), in collaboration with the Centre for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara [30].

Material and Method

Clinical assessment and investigations

Timis Regional Centre of Medical Genetics, affiliated to "Louis Turcanu" Emergency Hospital for Children offers medical services through inpatient and outpatient care. The cohort of patients includes a variety of people referred by different specialties, RCMGT having 4 assigned counties: Timis, Arad, Caraş-Severin, and Hunedoara, however available to evaluate patients from all the country.

Comprehensive clinical assessment is crucial for planning investigations in diagnosing a rare disease. A data set was collected for each patient, as requested in the medical genetics consultation chart of RCMGT including: 1) patient demographics and general information, 2) family history of diseases, 3) data about the antenatal and perinatal period, 4) personal physiological history, 5) symptoms and pathological medical history, 6) clinical findings in physical examination, 7) documentation of relevant investigation results, 8) medication, 9) other information. Patients presenting with dysmorphic features were asked to fill in a consent to allow photographs in order to facilitate diagnosis.

Investigation plan for each patient is personalized, following one of the five possible scenarios: 1) recommendation of additional tests and expert evaluations needed before genetic testing to sustain the suspicioned diagnosis, 2) when presenting with a specific phenotype for a genetic disease that may be confirmed by genetic testing, patients are asked to fill in the informed consent for genetic testing and a biological sample is taken, 3) when a genetic test is not available for the moment, patient's DNA may be stored for further research, with informed consent, 4) necessity of clinical genetics reevaluation in a defined period of time if suspected a disorder but with no sufficient features for undergoing the diagnosis process, 5) a genetic disease is excluded after comprehensive evaluation. Genetic counseling is offered for every situation.

Genetic testing

Patients underwent specific tests chosen by the clinical geneticist. Genetic testing services in Romania are commissioned and delivered in line with current national policy, free of charge for both children and adults enrolled in the National Program of Health of Women and Child, Subprogram VI.3 Prevention of congenital malformations by pre and postnatal diagnosis, [26]. Genetic testing was performed at the Centre for Genomic Medicine in UMF Timisoara, a partner research laboratory [30]. Test that were not available, were performed in collaboration with other Romanian Regional Centers for Medical Genetics (Dolj, Iasi, Bucuresti, Cluj).

A standard written informed consent was signed by children parents/ guardians or by the patients if over 18 years old. Most test were performed for children (and affected siblings if necessary) as first tier of investigation and further analysis of parent was recommended if needed.

The following tests are available in RCMGT: classic karyotype; FISH (10 specific regions), PCR (50 variants Single nucleotide base change), Fragile X Syndrome; SNP array (molecular karyotype); next generation sequencing (NGS) panels: TruSight Cardio (174 genes) and TruSight One panel (4813 genes).

The classic karyotype used the indirect method of studying human chromosomes, performed on a 72h cell culture and GTG banding technique (450 bands). Twenty metaphases are usually analyzed. Turnaround time varied between 10 days and 4 weeks.

The FISH (Fluorescent in situ hybridization) used the indirect method of analyzing targeted sequences stained with fluorescent dye. Visualization was performed by fluorescence microscopy and the hybridization signal was observed both in metaphase spreads and in interphase nuclei [31]. Turnaround time varied between 5 days and 2 months. Ten specific regions for the diagnosis of Prader Willi Syndrome, Angelman Syndrome, DiGeorge Syndrome, Williams Syndrome, Smith –Magenis Syndrome, Miller – Dieker Syndrome, Saethre-Chotzen Syndrome, Rubinstein – Taybi Syndrome, Neurofibromatosis, SRY deletion were available.

Genotyping analysis using RealTime-PCR for some frequent pathogenic variants in relation to Noonan Syndrome, 21-Hydroxylase deficiency, Galactosemia, Cystic fibrosis, Rett Syndrome, Bardet-Biedl Syndrome were performed. Turnaround time varied between 4 weeks and 6 months. Fragile X Syndrome was screened using the cut-off over 44 CGG repeats in FMR1 gene. Turnaround time varied between 4 weeks and 6 months.

SNP array was performed using specific kits with either 750k or 300k SNPs. Scanning was done with IScan Illumina and GenomeStudio v2.0 software was used for primary scanning of data generated. In data analysis the following databases: UCSC Genome Browser, DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources), OMIM (Online Mendelian Inheritance in Man), ISCA (International Standards for Cytogenomic Arrays), DGV (Database of Genomic Variants) were used. No genomic imbalances below the 100 kb limit, polymorphic variants CNV (copy number variation) were reported [32]. Turnaround time varied between 2 weeks and 6 months.

Next generation sequencing using panels of genes was performed with the MiSeq Illumina platform. For patients with cardiovascular diseases, a panel including 174 genes was used (Illumina TruSight Cardio). For patients with other type of disorders the next-generation sequencing was performed using the Illumina TruSight One Sequencing Panel kit, a broad panel including 4813 genes. End-to-end bioinformatics algorithms have been implemented and data analysis was conducted in concordance to current knowledge using several databases: UCSC Genome Browser, OMIM (Online Mendelian Inheritance in Man), DGV (Database of Genomic Variants). In interpretation of variants, all variants that can cause illness reported in HGMD®, ClinVar (class 1), and all minor allele frequency (MAF) variants less than 1%-2% in the ExAc database were considered. The evaluation was focused on exons. Only variants related to the phenotype for which the patient was sent were reported. Variants are interpreted according to the ACMG Guide [33]. Classification of Mendelian variants with ACMG: Class 1 - Pathogen/ Class 2 - Probably pathogenic/ Class 3 - Uncertainty of significance (VUS)/ Class 4 - Probably benign/ Class 5 - Benign. Turnaround time varied between 6 weeks and 1 year. An overview of main genetic testing performed at the Centre for Genomic Medicine in UMF Timisoara, are presented in Figure 1.

If a diagnosis is confirmed, the patient or his parent/guardians were asked to present for another consultation in the outpatient clinic to be informed about the global management of the disease, possible treatment approaches, complications prevention, about the initial needed clinical work-up and regular follow-up and genetic counselling.

Data analysis

Descriptive statistics for this retrospective cohort study included all individuals who had a genetic consultation in RCMGT and was performed using IBM SPSS Statistics v23. The age for each patient was calculated as date of presentation minus date of birth. The following age subgroups were used: new-born, infancy: 1 month- 1 year, early childhood: 1–7 years, middle childhood: 7-14 years, adolescence: 14-18 years, young adult: 18-40 years, middle-aged adult: 40-60 years, old adult: > 60 years. Geographical location was categorized as either urban (metropolitan) or rural and was determined by a person's last known postal code of residence. Diagnostics yields (positive predictive value for different genetic tests) were calculated as the proportion of positive findings in each test for all referred patients for that specific test.

Results

A total of new, unique, 1038 patients were examined in Timis Regional Centre of Medical Genetics between January 2015 and November 2018 and referred for testing to our diagnostic laboratory and partner national or international laboratories. For statistical analysis 6 patients were excluded because of missing data. 429 unique patients were admitted into the Medical Genetics Clinical Department, 411 were seen in the outpatient clinic, 140 were referred for consultation by other hospital units and 58 patients were addressed from Bucharest, Cluj-Napoca, Craiova, Iasi and Oradea hospital units, with a complete clinical work-up, for Next generation sequencing only.

The majority of evaluated patients were male, 567 (54.6%) vs. 471 females (45.4%). Increasing number of patients were evaluated in RCMGT in the 4 years of activity 2015-2018, as seen in Figure 2. In 2018, the number of new unique patients receiving a genetic consultation per month was in average 35.

Patients from whole Romania presented for genetic consultation, as seen in Figure 3, the majority from the 4 assigned counties (TM-45%, AR-11.8%, CS-9.9%, HD-7.7%), but also 6.2% of Mehedinti County and 19.5% of other 30 Romanian counties. 60% of patients are established in urban areas, while 40% in rural Romanian areas. 260 males and 207 females were examined from Timis county, 58% coming from urban areas and 42% from villages and communes.

Age repartition of patient's cohort examined in RCMGT is described in Figure 4. The median age was 5.58 years (range 0–78), average at 10.51, and the majority of patients from the 1 to 7 years old subgroup (32%). Majority of patients addressed for a genetic.

All patients having a genetic disease suspicion addressed to RCMGT are presented in Figure 5, classified in accordance to the global standard for diagnostic health information, International Classification of Diseases 11th Revision (ICD 11) [34], including developmental anomalies but also other categories of genetic diseases. The most frequent were chromosomal anomalies, including micro-deletion/duplication syndromes (203 patients, also with trisomy 21), followed by conditions with disorders of intellectual development as a relevant clinical feature (195 patients), multiple developmental anomalies or syndromes (179 patients), and unspecified developmental anomalies (172 patients).

Different genetic tests were performed for each patient according to suspected disorder. Diagnostic yields for each test were calculated for the first 820 patients addressed to RCMGT, from January 2015 to July 2018, as detailed in table 1.

Discussions

Rare diseases have become a worldwide health priority mostly in the last 10 years, aligning policies to improve coordination of care in patients by facilitating the provision of timely, equitable, and evidence-informed services. Dharssi et al. published in March 2017 a review of 11 national policies for rare diseases in the context of key needs of the RD community, and found five common dimensions: coordination of care, diagnosis, access to treatments, patient awareness and support, and research. This study also showed important differences in the status of RD across economic, political, healthcare national factors and examined the role of patient organizations in shaping national policy and programs, including rare disease legislation, national rare disease plans, and coordinated comprehensive services directed to rare diseases [5]. Our present work illustrates the intersection of these aspects in Romania, more specific, the activity in Timis Regional Centre of Medical Genetics (RCMGT), a part of the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERN ITHACA) in collaboration with the Centre for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara.

Concerning coordination of care, on a national level, Romania has gained the recognition of rare diseases as a priority for health care since the end of 2013 by adopting the Romanian National Plan for Rare Diseases [26], expanded the official Political Decision that supported the designation of Centers of Expertise using the EUCERD Recommendations on Quality Criteria, and ensured Centers of Expertise have adapted to the national status, and to ERN membership [25, 27]. Given that most rare diseases are genetic in origin, and beyond awareness, an important promoted strategy was to offer access for education in health care practitioners. Thereby, if in 2013 there were only 17 consultant geneticists, today Romania has around 60 doctors specialized in medical genetics (1 geneticist for 330.000 Romanians, and for 33.000 possible RD patients), increasing number of geneticists in training, but still no genetic counsellor profession recognition [35].

Patient registries, recognized as crucial tools for rare diseases national management, prevalence establishment, diagnosis and research [36], are implemented in our daily routine, but in need for standardization, coordination, and further development. RCMGT local patient's registry includes almost 1000 unique patients who received a genetic consultation in the last 4 years of activity, having a tripled number of patients in 2018, compared to 2015. Although RCMGT serves inhabitants from 4 counties, 25.6% of the addressed patients are from the rest of the country. This fact may be due to specific genetic testing performed in the Centre for Genomic Medicine in the University of Medicine and Pharmacy "Victor Babes" Timisoara (SNP array and NGS panels), but also to patient needs. Our cohort is dominated by male patients and urban area establishment, distributions maintained higher in all further characteristics. Male predominance could be due to a higher number of patients with intellectual disability in males due to X-linked mental retardation syndromes. Concerning age at first presentation for diagnosis, children and adolescents were the majority, most from the 1 to 7 years old subgroup (32%), followed by 7 to 14 years old subgroup and infants, but also 16% adult patients. These late presentations sustain the "diagnostic odyssey" widely recognized in the field of rare diseases [16, 17].

Diagnostic strategy for each case is particular: a clear phenotype allows rapid diagnosis suspicion, but unspecific ones require a thorough approach and further clinical investigations work-up in collaboration with different specialists, such as pediatricians, cardiologists, neurologists, metabolic specialists, nephrologists, gastroenterologists, endocrinologists, immunologists, oncologists, and others. For some cases, repeated clinical/dysmorphology and developmental assessments over time are more informative than one-off assessments in planning investigations and management. Also, online resources and access to them is an important tool for difficult phenotyping [37]. After this pathway towards a detailed clinical phenotyping, patients evaluated in RCMGT were mostly categorized as presenting unspecified developmental anomalies, followed by chromosomal anomalies, including micro-deletion/duplication syndromes, conditions with disorders of intellectual development as a relevant clinical feature, multiple developmental anomalies or syndromes and neuromuscular disorders. As such report publications are yet very rare, it was not possible to rely on comparisons between Romanian or international data. However, we found an extended population-based cohort study in Western Australia using ICD9 [38] describing highest rates in rare developmental defects during embryogenesis (19.1%), followed by rare neurological diseases (12.4%) and also a higher proportion of male population. The Italian National Rare Diseases Registry also reported disease-distribution in accordance to ICD9 and diseases of the central nervous system and sense organs were the most frequent (26%), followed by congenital anomalies (19.7%) [39].

In Romania, genetic testing is voluntary and by law written informed consent must be obtained prior to any form of genetic testing. Only medical geneticists are allowed to provide genetic testing, counselling and therapy for genetic diseases. Medical Laboratories may offer different types of genetic tests: new-born screening, diagnostic testing, and carrier testing, prenatal testing (RCMGT also has a Prenatal Diagnostic Department, data not included in this study), predictive and presymptomatic testing, forensic testing.

There are conventional stepwise strategies in choosing the right genetic testing for a given phenotype. This comes especially from each individual's unicity and the complex

interaction between their genetic background and environmental factors (phenotypic heterogeneity/ locus heterogeneity). Often, genetically heterogeneous phenotypes request costly and time-consuming investigations, a combination of chromosomal microarray analysis to detect copy number variation and targeted next-generation sequencing gene panels to detect single-nucleotide variants and small insertions and deletions [40, 41]. In our cohort what we firstly observed was a consistent decrease in karyotyping over years and increase of diagnostic yields (17.9% in 2016 and 26.4% in 2018), as SNP array offers better chances in diagnosing incomplete chromosomal deletions and duplications. Patients with intellectual disability, with or without malformations, had a diagnostic yield of 20.5% by SNP array analysis, compared to literature (8-12% [42]). As for NGS panels, molecular diagnostic yields were high for both Cardio and extended "Clinical exome" panels compared to literature (11.3% [43], 26% [44]), showing also an appropriate clinical assessment in guiding investigation. Novel disease-associated variants were also discovered (data not detailed in this work), needing supplementary investigations to be confirmed and to establish a better phenotype and management strategy for these patients.

Future priorities for RCMGT are to shorten the turnaround time by supplementing human and financial resources, to extend the tests offered to whole-exome whole genome sequencing (WES) and to improve research pipelines in rare disease in collaboration with ERN ITHACA.

Conclusions

Despite outstanding advances in policies, technology and bioinformatics, the burden of rare diseases is spread worldwide, raising specific issues in relation to their rarity. Nowadays, thorough clinical assessment is no longer the only available tool for diagnosis, but it is crucial in guiding towards different genetic investigations, restricting our focus to a specific organ, system or phenotype component.

RCMGT was successful to reach a diagnosis (sometimes using more than one type of test/per patient), with higher yields compared to those in literature, however with longer turnaround time due to limited human and financial resources. Further improvements are needed to bring forward the health care strategies for patients with genetic rare diseases in Romania, ultimately for improving their quality of life.

Conflict of interests

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

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Authors' contributions

All authors have contributed to either the assessment of subjects, phenotyping, data analysis, statistic interpretation, writing or final critical appraisal of the manuscript. JSIE, PM and CEA conceived the idea for the study and supervised the data collection, the research and the statistical analyses, taking responsibility for the integrity of the data and the accuracy of the data analysis. AN, MiA and TP performed genetic testing. ZC analyzed data from NGS and SNP array. CEA, AN, MiA, TP and DAI interpreted genetic testing data. JSIE drafted the manuscript and undertook data analysis. CEA and PAM researched data and contributed to the discussion and review and editing of the manuscript. PM, CEA and AN contributed

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MARFAN SYNDROME IN INFANCY – A RARE CONDITION WITH POOR PROGNOSIS

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Abstract

Our aim is to report a severe case of Infantile Marfan syndrome, which presented to the Emergency Department for shortness of breath, intense pallor and loss of appetite. Her particular physiognomy made us immediately think of Marfan syndrome. Marfan syndrome in infancy is a rare condition usually associated with poor prognosis. The child was admitted in the ICU for polypnea, failure to thrive and signs of heart failure. She was clinically evaluated, we performed lab tests, cardiology examination and AngioCT. The patient was referred to a surgical center because of a gigantic left atrium due to a severe mitral regurgitation. Unfortunately, she had a cardiac arrest during sleep. The genetic test confirmed Marfan syndrome. Clinical features for infantile Marfan syndrome have to be detected early after birth and genetically tested. Cardiac evaluation is mandatory. These patients have to be directed to an expert pediatric cardiovascular surgery center, because the heart involvement of the mitral valve is severe and hard to treat at this age.

Keywords: infantile Marfan syndrome, giant atrial dilatation, severe mitral regurgitation, dysplastic mitral valve.

Introduction

Marfan syndrome (MFS) is a hereditary disease involving chromosome 15, which affects connective tissue. The disease can cause a wide variety of issues. The main symptom of patients with Marfan syndrome is the excessive height, long arms and legs, flexible joints, which are more noticeable in adulthood. However, Marfan syndrome can be a de novo mutation, and in this situation, symptoms develop early in life, as early as infancy. Infantile Marfan syndrome is a rare phenotype of the disease and is the most severe form. Literature states that the majority of patients with infantile Marfan syndrome develop severe cardiac pathology with poor prognosis [1, 2, 3].

Case report

A 6 months old girl, presented into our Emergency Department for dyspnea, loss of appetite and intense pallor. Upon inquiry, we found out that the patient had prior admittances, after birth, being a premature baby with respiratory distress and another one at 3 months after birth, also for respiratory distress. We also found out that the child's aunt is with hyper stature (198 cm) and that her father has arachnodactyly and dilated cardiomyopathy.

At the first admittance, due to the particular physiognomy: excessive length, arachnodactyly, dolichostenomelia, muscle hypotonia, mix thoracic malformation with superior pectus carinatum and inferior pectus excavatum, lack of fatty tissue, the doctors in the neonatal department demanded a cardiology and genetic consult. From the cardiology point of view, the patient had a slight aortic bulb ectasia, but nothing else pathological and from the genetic point of view she was proposed to be tested for Marfan syndrome. Family did not respect the follow up program proposed by the cardiology doctor after the evaluation.

At the last admission, the general status was altered, the child's stature exceeded the 99th CDC percentile for age, and she had arachnodactyly and overlapping toe (Fig.1. A, B, C) that was impressive. She was also severely underweight, she had 5100 grams at 6 months of age. All physiological traits described above were present in the clinical evaluation. Furthermore, she presented polypnea and dyspnea, without pulmonary rales, hepatomegaly, severe hypotonia and loss of appetite.

At the cardiology clinical examination, the patient presented tachycardia 166 b/min, Oxygen saturation was low and grade IV-V/6 systolic cardiac murmur was detected in the apex, irradiating all over the precordium, with palpatory thrill.

The ECG showed sinus tachycardia, right axis deviation, mitral P wave and ischemia, with negative T waves almost to V6 (Fig.2.). The cardiac biomarkers value were extremely high, with an NTpro BNP of 26.964 pg/ml (normal value for age is 37-1000 pg/ml), which translates the fact that the myocardium is severely afflicted, with severe heart failure; Troponina T - 32, 83 pg/ml (normal value for age is 14 pg/ml) that confirms the myocardial ischemia. We did a chest X-ray (Fig.3.), which showed severe left cardiomegaly.

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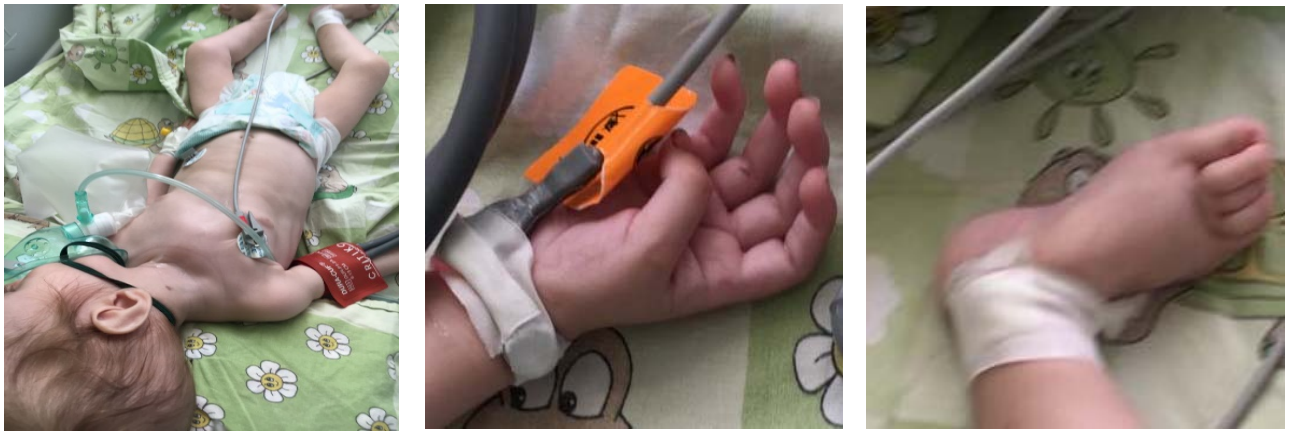

A **B** **C**

Fig.1. A: 6 months old infant girl with clear hyper stature. B: The patient's hand with arachnodactyly. C: The patient's foot with arachnodactyly and overlapping toes.

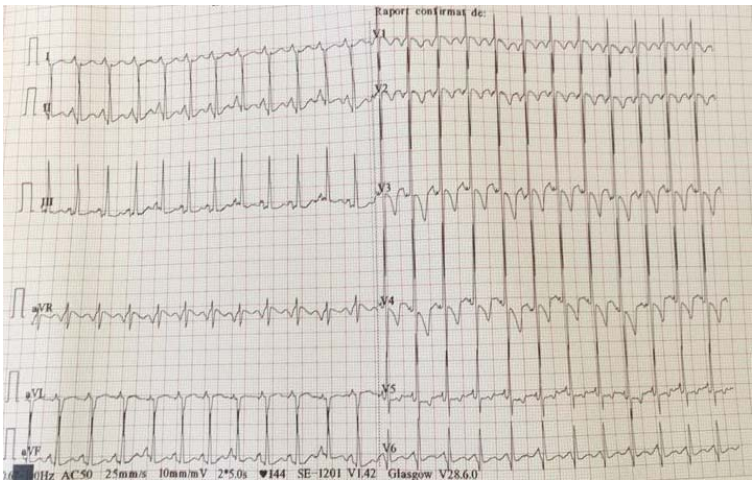

Fig.2. ECG with right axis deviation, mitral P wave and negative T waves in almost all precordial leads

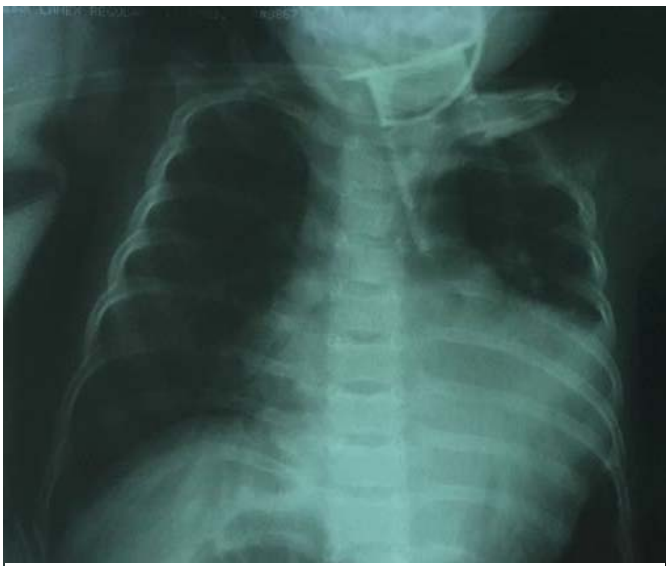

Fig.3. Chest X ray showing severe left cardiomegaly.

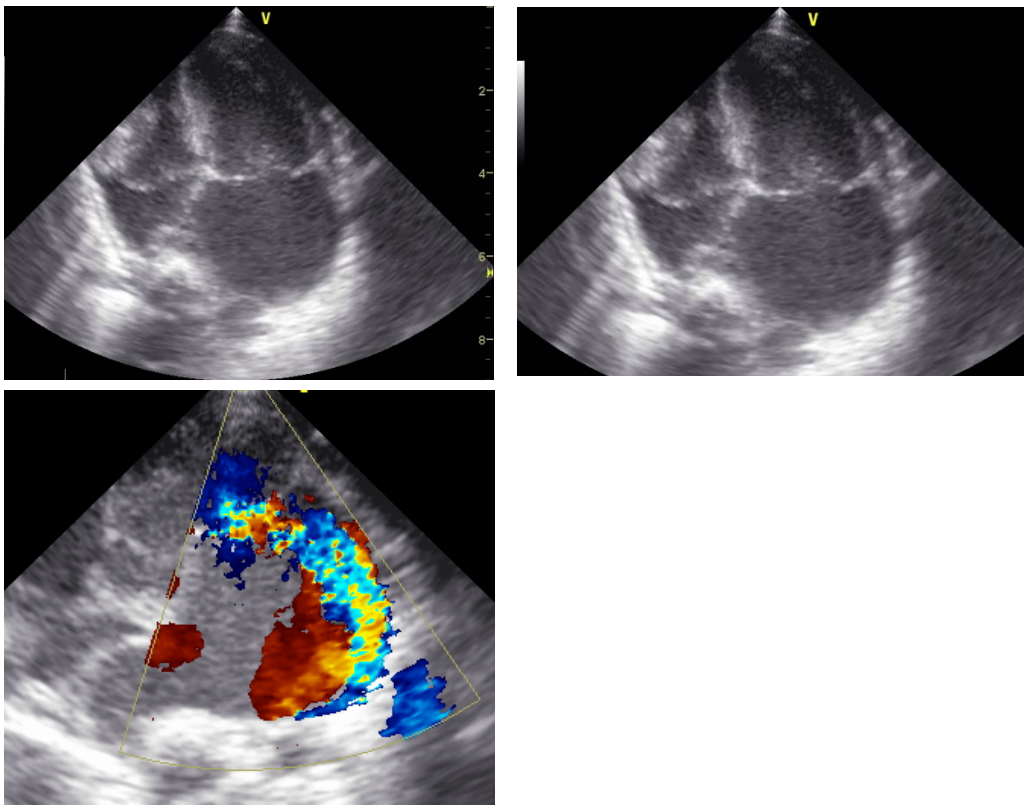

Fig.4. A: Echocardiography 4 chamber view showing severely dilated left atrium; B: Echocardiography modified 4 chamber view depicting a dysplastic mitral valve; C: Echocardiography color Doppler severe mitral regurgitation with Coanda effecT

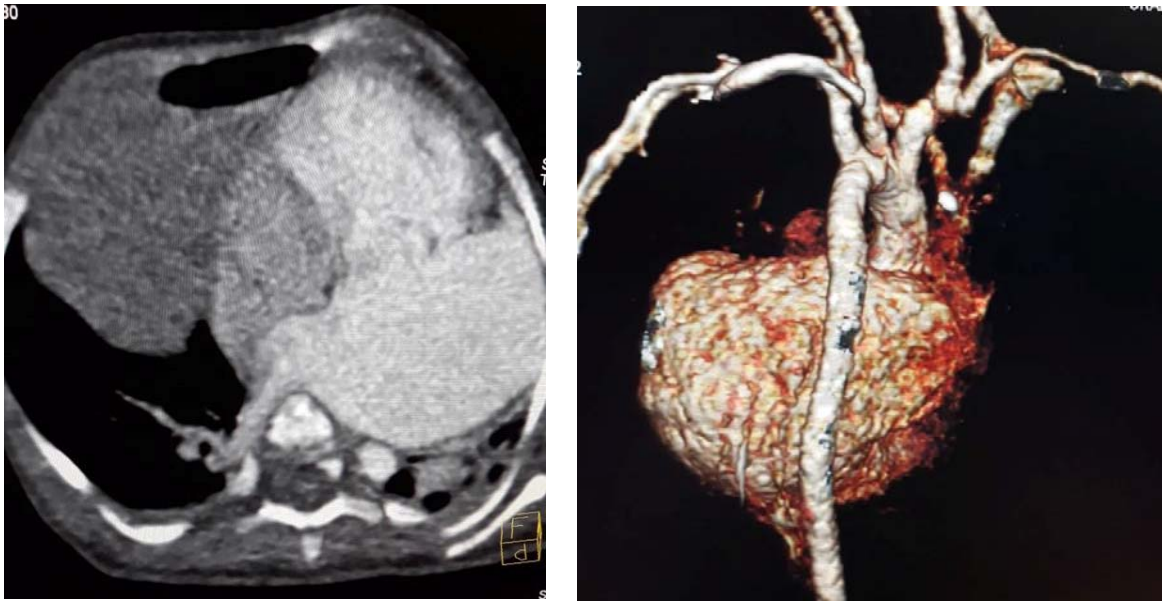

Fig.5. A: AngioCT showing enlarged left atrium; B: 3D reconstruction of the gigantic left atrium

Echocardiography revealed a severe left atrium dilatation (left atrium of 3.5 cm, maximum value admitted for age and weight is 1.6 cm), due to a severe mitral regurgitation (Coanda effect on color Doppler flow and vena contract of 8 mm) secondary to dysplastic mitral leaflets. Despite that, the cardiac function was within normal ranges (ejection fraction was 64%) (Fig.4. A, B, C). Aortic root was ectatic with Z score over 2. Pulmonary hypertension was also detected.

After echocardiography, Angio CT was performed which confirmed the severe left atrium dilatation, the left atrium occupied 50% of the left hemithorax (Fig. 5. A, B). Treatment with diuretics: Furosemide and Spironolactone, Captopril and cardiotonics: (Dobutamine) was initiated. Oxygen therapy was needed due to O2Sat of 88%, also we started the correction of electrolytes and IV supplement with amino acids.

After the complete evaluation, imagistic explorations, lab investigation and treatment initiation, the patient was immediately referred to an intensive care cardiovascular pediatric unit with pediatric cardiovascular surgery department. Unfortunately, the intervention was postponed due to the clinical status of the patient that could not be improved. The evolution was not favorable, she had a cardiac arrest during sleep

The Marfan syndrome was confirmed by the genetic test

Discussion

Marfan syndrome is an autosomal dominant disease, which is why a careful anamnesis and data collection is important in the diagnosis of early onset MFS [1, 3]. The diagnosis is mainly clinical, but genetic confirmation is mandatory. The main characteristics one must pay attention to are comprehensively summarized in the revised Ghent nosology for Marfan syndrome [4]. Unfortunately, infantile MFS is a different entity and the most severe form in the spectrum of Marfan, therefore the Ghent nosology is not applicable. The severity of infantile MFS is caused by cardiac and pulmonary afflictions with rapid and unforeseeable evolution [2, 3].

Although the most frequent cardiac modification is aortic root dilatation, a wide variety of diseases can appear, as we saw in our patient [5, 6, 7, 8].

When discussing the case of our patient, a timely diagnosis was attained, however the evolution was almost fulminant, as with most cases with infant MFS, literature states that 95% of patients with infant MFS die within the first year of life.

There was a slight aortic root ectasia in the neonatal department, which is why there was a follow up plan established with the mother, unfortunately it was not respected. Months later, an upper tract infection brought our patient to the hospital. A cardiological examination was not performed, but iv Furosemide was necessary for the treatment. Her health progressively worse, she refused alimentation, she lost weight, when signs of dyspnea appeared she was referred into our service. The cardiological examination showed that the left atrium was 50% of the left hemithorax because of severe mitral regurgitation due to a dysmorphic and incompetent mitral valve. Even with inotrope medicine and diuretics, and Captopril, her general state did not improve, so that Sildenafil and surgery was mandatory. Surgery was not timely performed due to the altered state of the patient, who unfortunately suddenly died in her sleep.

Infants with Marfan syndrome are the most severe group in this genetic disease, due to the cardiac abnormalities that are very difficult to be managed at this age. Even in most developed countries the management of the mitral valve involvement in these patients represent a challenge. The mitral valve surgery is very difficult and postoperative complications remain a great risk at all-time [3, 9].

Conclusions

In patients with infancy Marfan syndrome it is essential to establish a close monitoring system in order to prevent severe and irreparable complications. It is very important that the family understands the gravity of this disease and the patient should be monitored by a multidisciplinary team, including: neonatologist, pediatrician, geneticist, cardiologist, intensive care doctors and nurse team and cardiothoracic surgeons. Sometimes invasive cardiac surgery is in the benefit of the patient, but sometimes it is not. This complex team has to decide together with the family what is the best treatment of choice in the interest of the infant caring the gene of Marfan syndrome that expressed so early in life.

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THE IMPORTANCE OF PERITONEAL CULTURES IN THE TREATMENT OF CHILDREN WITH RUPTURED APPENDICITIS

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Abstract

Introduction: The management of ruptured appendicitis in children is of great importance and still controversial. Acute appendicitis is one of the most common conditions treated by pediatric surgeons. Around 30% of the acute appendicitis treated in childhood involve a ruptured appendix. Objective: The purpose of this study was to establish if identification of both aerobic and anaerobic bacteria and their susceptibility to antibiotics, leads to better outcomes when treating perforated appendicitis. Material and Methods: This study was conducted at the Pediatric Surgery Department of Emergency Children's Hospital, Cluj Napoca, Romania. A total of 330 patients (aged 0 to 18 years) admitted between January 2007 and December 2014 were included in the study. Data collected from review of medical charts included age, gender, demographic data, and duration of hospitalization, surgical and medical treatment, initial presentation and investigations. The total number of patients was divided in two groups. Group A (153 patients) had no peritoneal fluid samples prelevated and Group B (177 patients)) in which peritoneal fluid samples were sent to the lab for determination of both aerobic and anaerobic bacteria. Results: Postoperative infective complications of group B were found in 20/177(11, 29%) patients. From a total of 24 complications we found 3 intraabdominal collections, 6 deep wound infections and 15 superficial wound infections. A significant decrease in number of complications can be noticed in group B which can result from better surgical technique but also from better antibiotic coverage. Discussion: The combination of cefuroxime/ gentamicin/ metronidazole can be appropriate until definitive culture results are done but in more severe cases of perforated appendicitis we recommend the use of a single broad-spectrum agent. This may help reduce the incidence of postoperative infectious complications associated with amoxicillin resistant E. coli in appendicitis related peritonitis. Conclusion: Based on the study results, a triple antibiotic combination of cefuroxime, gentamicin and metronidazole is reasonable empiric basis for treatment of perforated appendicitis in selected cases but when dealing with cases that have late diagnosis, are clinically and biologically impaired or show no improvement, the best option is a broad spectrum single agent, like ertapenem or piperacillin/tazobactam.

Keywords: antibiotic resistance, perforated appendicitis, peritonitis

Introduction

The management of ruptured appendicitis in children is of great importance and still controversial. Acute appendicitis is one of the most common conditions treated by pediatric surgeons. Around 30% of the acute appendicitis treated in childhood involve a ruptured appendix.

The classic description of appendicitis includes the onset of periumbilical pain followed by nausea, then migration of pain to the right lower quadrant (RLQ) and finally, vomiting and fever. However, this progression of symptoms is less common in children than adults [1]. Absence of classic symptoms leads to a higher rate of appendicular perforation in children. Pathogenesis of appendicitis is still uncertain, its significance in septic complications of appendicitis is well established [2, 3]. The exact cause remains unclear, but luminal obstruction, diet, and familial factors have been suggested, and the etiology may be multifactorial in some cases [4, 5]. Some bacteria and parasites were found in histopathological evaluations of the appendices [6]. Inflammation of the appendix ranges from minor, simple acute inflammation to necrosis and perforation, but in some appendectomies patients it could be histologically classified as normal appendices.

Peritoneal cavity swab during surgical treatment of perforated appendicitis is not a routine procedure in all pediatric surgery units. Cultures from inflamed appendices usually revealed that the most common organisms are a mixture of Escherichia coli (85%), enterococci (30%), non-hemolytic streptococci, anaerobic streptococci together with clostridium welchii (30%) and bacteroides [7, 8].

Microbiological investigation of intra-abdominal infections in children has been limited [9, 10, and 11]. Historically, empirical antibiotic therapy with clindamycin or metronidazole, gentamicin, and ampicillin has been used [12, 13], but the bacteriology and antibiotic susceptibility of specific pathogens involved in peritonitis requires epidemiological monitoring.

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This comparative study analyzed the outcome in treating perforated appendicitis in two groups of patients, the first (group A), without taking peritoneal cultures and the second (group B) with peritoneal fluid samples to establish the etiology and antimicrobial susceptibility of isolates (over a 8-year period)

Objectives

The purpose of this study was to establish if identification of both aerobic and anaerobic bacteria and their susceptibility to antibiotics, leads to better outcomes when treating perforated appendicitis.

Materials and Methods

This study was conducted at the Pediatric Surgery Department of Emergency Children's Hospital, Cluj Napoca, Romania. A total of 330 patients (aged 0 to 18 years) admitted between January 2007 and December 2014 were included in the study. Data collected from review of medical charts included age, gender, demographic data, and duration of hospitalization, surgical and medical treatment, initial presentation and investigations. The children diagnosed with acute appendicitis, gangrenous appendicitis and appendicular mass were excluded. The diagnosis of appendicular perforation was considered by clinical evaluation, blood tests, and ultrasonography and was confirmed at laparotomy. The study included 141 (42.72%) females and 189(57.27%) males. (Fig. 1)

The surgical approach to control the source of infection was appendectomy and irrigation in 107(32, 42%) patients and appendectomy, irrigation and drainage in 223(67.57%) patients. (Fig.2).117(35.45%) cases were considered generalized peritonitis and 213(64.54%) were considered localized peritonitis (Fig.3).

The age of the children included in the study ranged from 3 days to 18 years (median age was 9).The median hospital stay was 7 days (the minimum hospital stay was 3 days and the maximum 26 days).

The total number of patients was divided in two groups. Group A (153 patients) had no peritoneal fluid samples prelevated and Group B (177 patients)) in which peritoneal fluid samples were sent to the lab for determination of both aerobic and anaerobic bacteria.

Peritoneal fluid specimens in the group B were sent directly to the laboratory or kept at 4 °C until the next day if they were collected after hours. For aerobic culture, the fluid specimens were inoculated onto Columbia blood agar and MacConkey agar without salt. The plates were incubated at 37 °C in air atmosphere and were examined 24 and 48 h after incubation. For anaerobic culture, the fluid specimens were plated onto Columbia blood agar, neomycin blood agar, and nalidixic acid agar and each plated agar further impregnated with metronidazole discs so as to guide sensitivity analysis. All plates were incubated in an anaerobic gas jar with O2 levels<1 % and CO2 levels between 9 and 13 % and examined for growth at 24, 48, 96 and 120 h after incubation. All aerobic isolates were fully identified. Specimens with anaerobic isolates having more than one anaerobe identified were classified as mixed anaerobe. Sensitivity analysis was conducted with the aid of a rapid and automated VITEC-2 compact system (Biomerieux, France). [14]

Data recorded included: demographic data, microbiological data (peritoneal fluid specimens and susceptibility to antibiotics), antibiotic management (initial therapy, changes in therapy, and duration of treatment) and outcomes. Infectious complications were defined as those occurring within 30 days of surgery and included intra-abdominal abscess and wound infection. The intra-abdominal abscesses were confirmed by imaging and microbiological samples. Wound infection was confirmed clinically and by microbiological samples.

Adequate empirical antibiotic treatment was defined as resolution of disease with initial antibiotic treatment after primary surgery. Empirical antibiotic treatment was inadequate if the infection was no resolving and additional antibiotics were commenced postoperatively based on intraperitoneal fluid culture results [14].

Results

The group A, which was studied retrospectively, included 68 females(44,44%) and 85males(55,55%).The surgical treatment was appendectomy and irrigation in 42 patients(27,45%) and appendectomy , irrigation and drainage in 111 patients(72,54%). 51(33,33%) patients had generalized peritonitis and 102(66,66%) had localized peritonitis.

There was no consensus regarding the antibiotic treatment for perforated appendicitis, as a result there is a lot of variation in the medical approach. The most used antibiotics depending on the surgeon options and the intraoperative findings, were the associations of ampicillin/gentamicin and metronidazole, cefuroxime/ gentamicin and metronidazole and piperacillin-tazobactam as a single agent (Fig.4).

The age in group A was from 2 months to 18 years old with a median of 9, 5.The hospital stay ranged from 4 days to 26 days with a median of 8 days. Postoperative infective complications were found in 37/153(24.18%) patients. There were 49 infective complications (15 intra-abdominal collections, 13 deep wound infections and 21 superficial wound infection) Fig.5. The complications occurred more frequent in patients treated with ampicillin, gentamicin, metronidazole association.13 (8.49%) patients required change in antimicrobial therapy before clinical improvement and prolonged duration of hospital stay. Treatment failure was considered to be caused by inadequate antibiotic treatment or source control.

The patients in group B were studied prospectively whit the purpose of using the best suited antibiotics and avoiding the abuse of medication, but also getting the best antibiotic coverage depending on the peritoneal fluid bacteriology. This group included 73 females (41.24%) and 104 males (58.75%). Appendectomy and irrigation was done in 75(42.37%) patients while appendectomy, irrigation and drainage was performed in 102 patients (57.62%).66 (37.28%) patients had generalized peritonitis and 111(62.71%) had localized peritonitis.

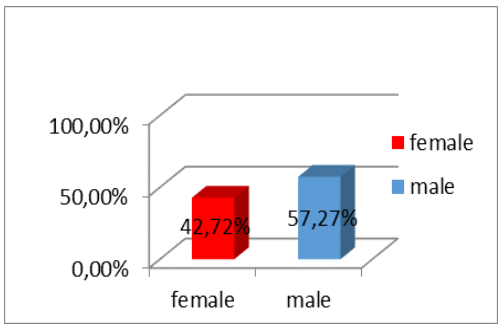

Fig. 1. Sex ratio

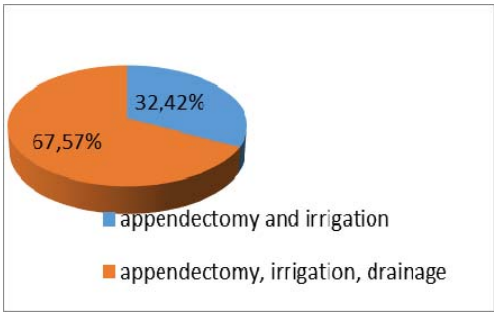

Fig. 2. Surgical approach

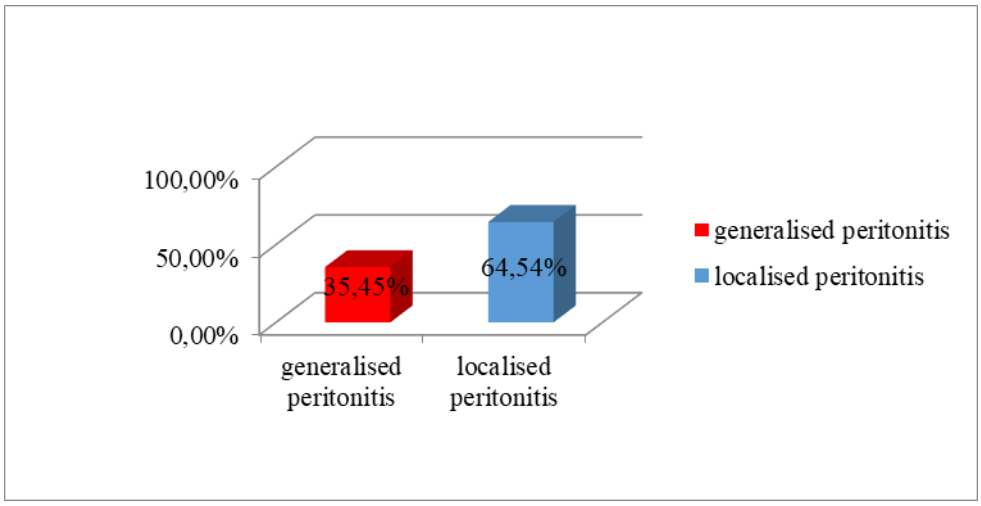

Fig. 3. Percentage of localised vs. generalised peritonitis

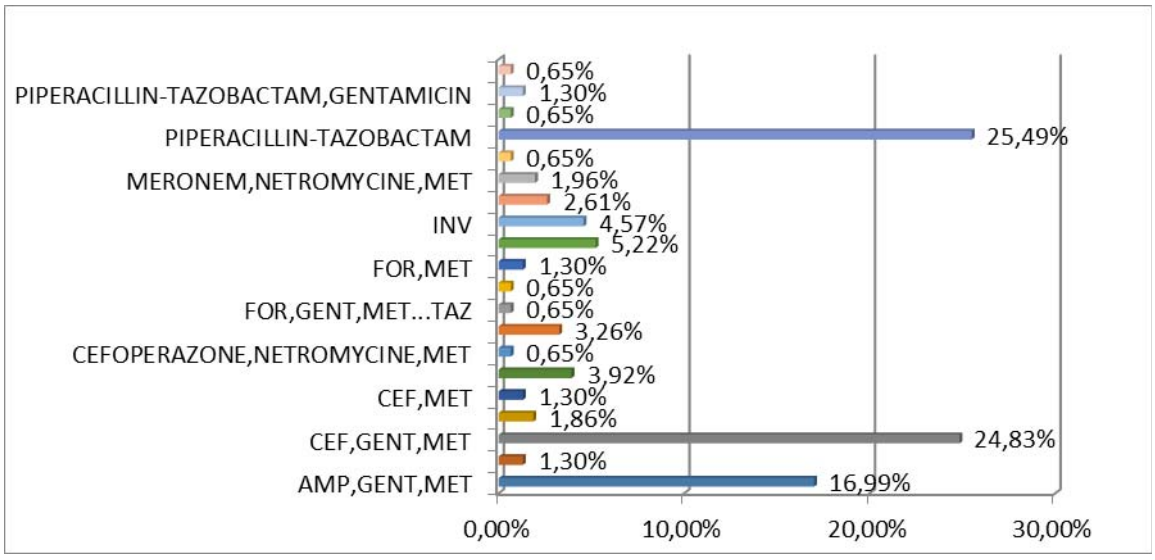

Fig.4. Antibiotic treatment for perforated appendicitis, retrospective group

We can easily observe that the sex ratio remained closed as values, but drainage was less used as surgical approach in group B despite the increase in generalized peritonitis proportion, which is statistically important (two tailed Z-test , p=0,0047).

The age of the patients ranged from 6 months to 18 years with a median of 10.The median hospital stay was 6(3 to 17 days).The antibiotic treatment was decided depending on biological status, paraclinical findings and intraoperative aspect (Fig. 5).

Postoperative infective complications were found in 20/177(11.29%) patients. From a total of 24 complications we found 3 intraabdominal collections, 6 deep wound infections and 15 superficial wound infections. A significant decrease in number of complications can be noticed in group B which can result from better surgical technique but also from better antibiotic coverage.

A total of 177 specimens were obtained from 177 children operated. From 361 isolates, 252 were aerobes and 109 were anaerobes. Anaerobes were less isolated probably because of technical difficulties. Single isolates were identified in 73/177patients (41.24%) and multiple isolate were identified in 104 patients (58.75%).The predominant aerobic bacteria was Escherichia coli and was found in 135/177(76.27%).101(57.06%) E coli isolates were resistant to ampicillin and 14(7.90%) of isolates were resistant to amocillin-clavulanate. Pseudomonas aeruginosa was found in 25/177 patients (14.12%), followed by Streptococcus, Klebsiella and Staphylococcus.The most encountered anaerobes were Bacteroides spp. followed by Peptostreptococcus and Clostridium perfringens (Table 1). In 13 out of 20 patients with no clinical improvement the antibiotic treatment was switched from cefuroxime/ gentamicin/ metronidazole to piperacillin/ tazobactam or ertapenem, after receiving the lab results, which led to prolonged hospital stay.

Antibiotic susceptibility pattern for aerobic and anaerobic bacteria which were isolated from peritoneal fluid specimens is presented in table 2.

Discussion

In our study the most encountered aerobic bacteria was E. coli while Bacteroides was the most frequent anaerobe, similar to previous reports [9, 15, 16, 17, 18]. Despite the increased number of generalized peritonitis in group B and the decreased percentage of children in which peritoneal drainage was used, the results were better regarding postoperative infectious complications. (24,18% in group A vs. 11,29%in group B).We assumed that this is a consequence of changing the standard treatment by eliminating the ampicillin and using more piperacillin/tazobactam and ertapenem, especially in children with delayed diagnosis and altered biological status. Better surgical technique (a more careful inspection and cleansing of peritoneal cavity) may also contribute to better results.

Isolation of E. coli resistant to ampicillin and amoxicillin–clavulanate may be associated with post-operative peritonitis [16].Appropriate initial antimicrobial therapy may predict successful treatment of peritonitis [19].The presence of resistant bacteroides and the isolation of P. aeruginosa in peritoneal specimens may be associated with post-appendectomy surgical infections in the absence of appropriate primary antibiotics [20, 21]. Although enterococci are frequently isolated as part of a polymicrobial intra-abdominal infection, their role as pathogens and the need for antibiotic coverage specifically toward this organism remains unclear in a review of several trials [22].

When looking at table 2 we found it justified to reduce the antibiotic regimens and to lean towards single regimen broad-spectrum antibiotic when treating perforated appendicitis in children. According to the Surgical Infection Society, monotherapy with broad-spectrum agents in perforated appendicitis is equally effective, possibly even more cost-effective. In addition, a retrospective study demonstrated that single broad-spectrum antibiotic in the treatment of PA used with increasing frequency might offer improvements in terms of length of stay, pharmacy charges and hospital charges[23].This is supported by the 13 children that improved only after changing the antimicrobial therapy from cefuroxime/ gentamicin/ metronidazole to ertapenem or piperacillin/tazobactam.

Medical studies illustrated that single-agent therapy with Carbapenems (Imipenem, Ertapenem) or penicillin plus a beta lactamase inhibitor (Ticarcillin-Clavulanic acid) were at least as effective as combination therapies [24, 25, 26]. These drugs have single or double daily dose administration schedule and are generally better tolerated by children. The median length of hospital stay was 8 days in group A and 6 days in group B which also suggests that the treatment was more efficient in group B. Routine use of peritoneal culture has been described as redundant [27], cost ineffective [28], and without any clinical advantage [29] or value [30].It has also been noted that culture results do not result in significant changes in antimicrobial management when empiric broad-spectrum antibiotics are utilized [31]. However, routine use of routine cultures may be useful epidemiologically, as it could allow early recognition of changing susceptibility patterns among intra-abdominal pathogens. This could potentially ensure use of the most appropriate empiric antimicrobial regimen, not only for those children with healthcare-associated intra-abdominal infections, but potentially even for those with community-acquired intraabdominal infections [7].

Krobot et al. [32], in a multicenter study of 162 patients with perforated appendicitis, found that appropriateness of initial parenteral antibiotic therapy was a predictor of clinical success and length of stay. Similarly, they demonstrated a high risk of postoperative infections in patients with inadequate empirical treatment.

Knowing the microbial and antibiotic resistance profile is critical in an attempt to provide the best empirical antibiotic treatment for secondary peritonitis arising from appendicitis in children [16]. There is no single empirical antibiotic known to reduce post-appendectomy infectious complications in patients with complicated appendicitis [9, 13, 32, 33].

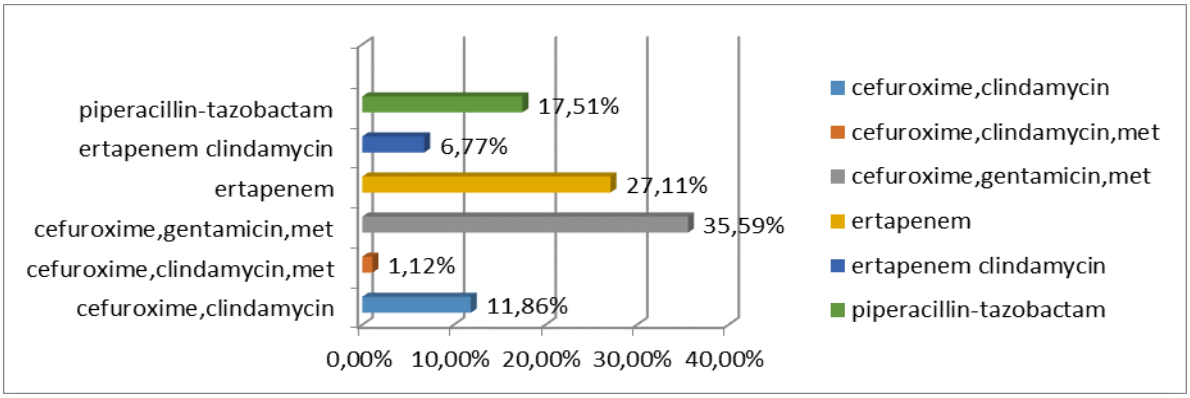

Fig.5. Antibiotic treatment for perforated appendicitis, prospective group

Bacterial species	Total(361)
Anaerobes	
Bacteroides spp.	90
Fusobacterium spp.	2
Clostridium perfringens	6
Peptococcus spp.	3
Peptostreptococcus spp.	8
Total	109
Aerobes	
Escherichia coli	135
Klebsiella pneumoniae	12
Enterobacter	8
Proteus spp.	2
Pseudomonas spp.	25
Streptococcus spp.	60
Staphylococcus spp.	10
Total	252

Table 1. Bacterial spectrum

Antibiotics	Susceptible (%)	Resistance (%)
Aerobic isolates		
Ampicilin	19%	81%
Amocillin-clavulanate	46%	54%
Piperacillin-tazobactam	95%	5%
Gentamicin	65%	35%
Amikacin	84%	16%
Cefuroxime	85%	15%
Ceftriaxone	85%	15%
Ertapenem	100%	Zero
Clindamicyn	90%	10%
Vancomycin	90%	10%
Anaerobic isolates		
Carbenicillin	90%	10%
Cefoxitin	60%	40%
Cefuroxime	65%	35%
Clindamycin	70%	30%
Chloramphenicol	100	Zero
Metronidazole	83%	17%
Tetracycline	35%	65%

Table 2. Antibiotic susceptibility pattern

Despite the changes of antimicrobial therapy and the better results in group B, other factors such as attention to basic infection control strategies, the surgeon's experience and technique, the duration of the procedure, hospital and operating room environment, instrument sterilization techniques, preoperative preparation and management of any underlying medical condition of the patient should also be considered[33].

All the studies that focused on the use of intraperitoneal fluid cultures were open, non-randomized, and retrospective with incompletely matched control groups, non-standardized swab collection techniques, and consequently lacked power to inform surgical practice. They concluded that an appropriately powered randomized, blinded, prospective, controlled clinical trial is needed to test for absolute efficacy in the use of peritoneal cultures in patient man- agreement.

The combination of cefuroxime/ gentamicin/ metronidazole can be appropriate until definitive culture results are done but in more severe cases of perforated appendicitis we recommend the use of a single broad-spectrum agent. This may help reduce the incidence of postoperative infectious complications associated with amoxicillin resistant E. coli in appendicitis related peritonitis.

Conclusions

Although, only 13 of the patients had changes in the antimicrobial regimens after peritoneal cultures results, taking peritoneal fluid samples for microbiological tests proves to be of great importance. Perforation of the appendix inevitably leads to significant bacterial contamination and morbidity. E.coli and mixed anaerobes are the predominant organisms involved in the resulting peritonitis. No single antimicrobial treatment is effective and antibiotic resistance is common. Inadequate initial empirical antibiotic and amoxicillin–clavulanate resistant E. coli as well as resistant Bacteroides and Pseudomonas may contribute to increased postoperative infectious complications. Based on the study results, a triple antibiotic combination of cefuroxime, gentamicin and metronidazole is reasonable empiric basis for treatment of perforated appendicitis in selected cases but when dealing with cases that have late diagnosis, are clinically and biologically impaired or show no improvement, the best option is a broad spectrum single agent, like ertapenem or piperacillin/tazobactam.

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„SCREENING" CHILDREN FOR SCREEN TIME – HOW CONCERNED SHOULD WE BE?

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Abstract

Today's children grow up immersed in digital media. The experts' recommendations are: no screen time <18 months, for children 2-5 years limited to one hour/day, for children ≥6 years there should be limits on the time spent using media and the types of media. The authors aimed to evaluate how much screen time the children are exposed to during the day. We conducted a prospective study, February-May 2019 in the Pediatrics Department of "Grigore Alexandrescu" Hospital. Parents were asked to fill in a questionnaire containing screen time, type of screen, behavior related to screens. 200 patients were enrolled, mean age 8 years 9 months, sex ratio M/F=1.1/1, 82% from urban areas. Parents declared the children started using screens at an average age of 4 years 1 month (minimum 6 months). 3% were exposed to screens below the age of one. The devices used were: smartphone, TV, tablet, laptop, computer and gameboard in 81%, 59.5%, 42.5%, 36.5%, 22. % and 22.5% respectively. Children used screens alone on average 2.8 hours/day, 5.6 days/week and alongside their parents on average 2.2 hours/day, 4.5 days/week. Devices were used during meal time in 41%. 22% of parents used screen time as reward and 48% felt retrieving the device to be an effective punishment. 20% of children were falling asleep with the TV on. Conclusions: Screen time for children is progressively increasing as the age of exposure decreases. The most frequently used devices are smartphones, laptops. The screen related behavior is "educated" in the family.

Keywords: screen time, media device, child

Introduction

In the past decade, media has become an important factor that influences children's physical and psychological development. Whether we are talking about traditional media (television) or "new" media (cell phones, iPads and social media) there is evidence on the negative impact that digital media and screen viewing has on general health and cognitive development of children.

The American Academy of Pediatrics (AAP) encourages health care providers, parents and teachers to take action on diminishing harmful media use. The latest statements recommend pediatrician to take "a media history" on a routine visit and ask at least 2 questions: "How much recreational screen time does your child or teenager consume daily?" and „Is there a television set or Internet-connected device in the child's bedroom?" (1). Some authors are concluding that media usage is one of the leading activities in young people other than sleeping (2, 3).

Prolonged screen viewing in infants and young children has been linked in several studies with neurodevelopmental problems such as delayed cognitive and language development (4,5), behavior issues such as violence and aggression (6-8) and low social interaction with peers and parents (9,10). A prospective study on a Canadian cohort of 2241 children evaluated using a developmental scale revealed that the higher the levels of screen time exposure, the poorer were the developmental test's performance (11).

Moreover, high screen view exposure has been correlated with vision (12-14) and overweight problems in late childhood and adolescence (15, 16).

Excessive screen media exposure has been associated to low quality of sleep by limiting sleep duration (17-20), increasing night awakenings and inducing an intermittent sleep pattern (21). The bright screens of electronic devices induce a state of hyper arousal by activating different automatic pathways of the nervous system (22). Also, recent research is suggesting that a good quality of sleep is mandatory for the processes of active learning and memory build up (23).

On the other hand, media use can promote social interactions, especially in teenagers. Social media platforms enhance communication with peers, create opportunities for engaging in community programs and promotes creativity through blogging and podcast production (24). Another advantage of social media is that it can improve the quality of learning by using platforms in which students can collaborate outside the class and exchange ideas and knowledge (25). Moreover, studies have shown positive impact for interactive technologies, such as smartphones and tablets, in supporting active learning beyond the formal way of education.

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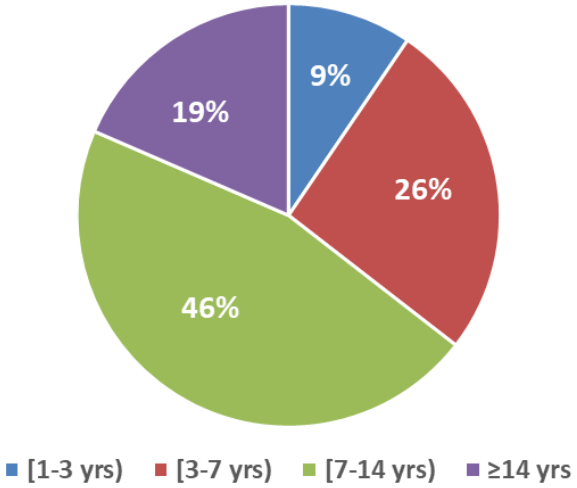
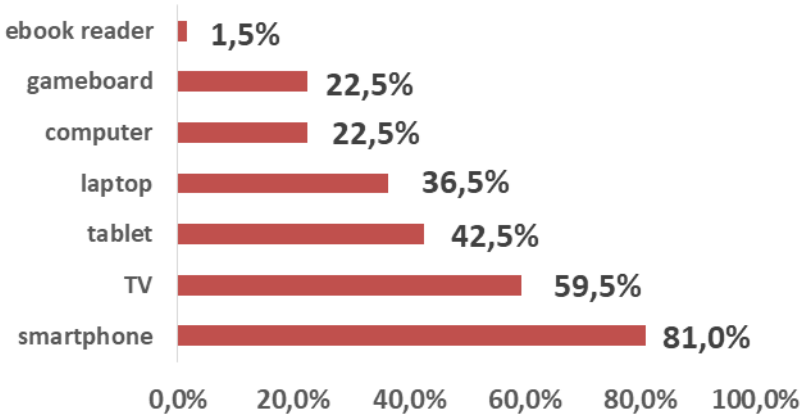


Fig.1. Age distribution



Taken into account all of the facts exposed, a balance must be kept in children of all ages between the time of screen viewing, media usage and quality of contents.

Material and methods

The authors aimed to evaluate how much screen time the children are exposed to during the day. We conducted a prospective study, February-May 2019. The patients were enrolled in the Pediatrics Department of "Grigore Alexandrescu" Emergency Children's Hospital, Bucharest, where they were admitted for unrelated pathology. Parents were asked to fill in a questionnaire containing brief personal data (age, sex of the child, socio-economic status, education level of the family), screen time, type of screen and behavior related to screens.

Results

200 patients were enrolled in the study. The mean age was 8 years 9 months (ranging from 12 months to 17 years). The distribution of by age is depicted in fig.1. The patients were equally distributed among genders. Sex ratio was M/F=1.1/1.

The majority of patients (82%) came from urban areas. 27.5% of children were in kindergarten, 51% in primary school and 13.5% in high school. The parents were in most cases (70%) university graduates. Socioeconomic level of the family as declared by the parents was above 1000 euro/month in half the cases. 11% of children came from monoparental families. The main caregivers of the child were the parents in 78.5%, followed by the grandparents in 18.5%.

Parents declared the children started using screens at an average age of 4 years 1 month (minimum 6 months). 3% were exposed to screens below the age of one. This age did not differ according to parents' level of education, primary caregiver, nor type of family (mono/biparental).

The devices available in the home were: smartphone, laptop, TV, tablet, computer, gameboard, eBook reader in 88%, 71%, 67.5%, 56%, 35%, 26% and 4.5% of cases. More than 3 devices were present in 81% of homes. The most frequently used devices by children were: smartphone, TV, tablet and laptop in 162, 119, 85, 73 patients (fig.2).

80% of children used at least two devices and one third more than four.

The most frequently used devise by children aged 1-3 years was by far the smartphone (78.9%), followed by the TV (42.1%) and tablet (15.7%). The percentages of children watching TV or using a laptop progressively increased with age. Boys played with game boards more than girls (31.1% vs. 12.7%). Children in rural areas used smartphones, tablets and TV less than city children: 67.5 vs. 84%, 32.4 vs. 44.7% and 48.6 vs. 61.9% respectively; nevertheless for all the other devices percentages were similar.

Children used screens alone on average 2.8 hours/day, 5.6 days/week and alongside their parents on average 2.2 hours/day, 4.5 days/week. Screen time did not differ according to age groups, gender, nor living area.

The declared main purposes for media device usage were: you tube (49%), game playing (46%), watching movies (45%) and listening to music (44%). Only 11% of children were using devices to read books online.

In 80% of cases at least one device was available in the child's room; 15% had more than 3 devices. The time period during the day the child was most likely to accumulate screen time is between 6 and 8 p.m. Only a little more than half of the parents (58%) declared they control the type of media content the child watches. The decision to get the media device was in most cases made by the parents (61%).

Devices were used during meal time in 41%. 22% of parents used screen time as reward and 48% felt retrieving the device to be an effective punishment. The device the child was most reluctant to be apart from was by far the smartphone (56.5%), distantly followed by the tablet (12%). Almost a quarter of children (20%) were falling asleep with the TV on.

Discussion

According to the American Academy of Pediatrics, the experts' recommendations are: no screen time <18 months, for children 2-5 years limited to one hour/day and as for children ≥6 years, there should be limits on the time spent using media and the types of media allowed (1). In our study parents declared the children started using screens at a relatively low average age (4 years 1 month). We consider the fact that 3% of children were exposed to screens below the age of one to be at least concerning, not to mention that the lowest age of exposure was 6 months.

Regardless of AAP recommendations, the majority of children are still spending most of their spare time in front of screens. Studies in the United States have shown that 98% of the aged 0-8 year-olds are spending an average time of 2 hours a day using screens (26), the average 8-10 year-old spends approximately 8 hours a day using different media sources, while teenagers spend more than 11 hours per day (2). The children in our group used screens alone on average 2.8 hours/day, 5.6 days/week and alongside their parents on average 2.2 hours/day, 4.5 days/week. Unlike literature data, in the present study, screen time did not differ according to age groups, nor according to gender or living area. The statistics on media usage and screen viewing are similar in other countries as well. In Japan, 86% of children by 18 months old spend > 1 hour watching TV daily (27). In Australia, children under 4 years of age watch TV more than 2 hours per day (28).

TV is still the leading type of used media (>4 hours daily), but with the development of new technology, about one third of TV programs are watched on alternative screens (computers, cell phones, iPads) (1). Our data placed the TV on the second place (59.5%), outranked by the smartphone (81%) and followed by the tablet (42.5%). Published data show that 71% of children and teenagers have a TV set in their bedrooms (2). This research revealed that in 80% of cases at least one device was available in the child's room; furthermore 15% had more than 3 devices. As regarding the use of internet, 98% of the children aged 0-8 years in the United States are living in houses with access to high speed internet (26), and about one third of all aged children have internet access in their bedrooms (1). Time spent using computers accounts for nearly 1.5 hours/day, half of it being used for videogames and social networking (1).

Although in most cases the decision to get the media device was made by the parents, a very concerning fact resulting from our study was that only a little more than half of the parents had control over the type of media content the child watches. The authors also identified other potentially harmful screen related behavior in parents: usage of media device to reward or punish the child, screen viewing during meal time or to induce sleep.

Conclusions

Screen time for children is progressively increasing as the age of exposure decreases. Today's children grow up immersed in digital media. The most frequently used devices are smartphones, TV and tablets. The screen related behavior is "educated" in the family and unhealthy conduct should be actively discouraged.

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SEVERE OBSTRUCTIVE SLEEP APNEA IN CHILDREN: A CASE REPORT

T Marcovici[1, 2], A Albu[2], D Zabos[3], R Horhat[1, 3], OBelei[1, 2], L Olariu[1, 2], O Marginean[1, 2]

Abstract

Adenotonsillar hypertrophy is a common cause of obstructive sleep apnea in children. Affecting ventilation and sleep architecture, recurrent respiratory obstruction can lead to metabolic, cardiovascular, neurobehavioral and somatic consequences. Purpose. To present the etiology and management of nocturnal and diurnal respiratory disorders in a pre-school patient coming from rural area. Materials and Methods. A female patient aged 4 years and 9 months was assessed by an interdisciplinary team. The evaluation included: case history, physical examination, laboratory tests and specialty consults. Case presentation. The patient presented multiple sleeping disorders (apnea, noisy and difficult breathing, intense snoring, orthopnea, frequent awakening) and diurnal symptoms (hypersomnia, attention deficit, nasal obstruction, diminished appetite, dysphagia, nasal voice). At admission, the clinical examination showed failure to thrive, adenoid face, sternal depression, noisy and difficult mouth breathing, severe substernal and intercostal retractions. Important adenotonsillar hypertrophy (grade IV) was confirmed by otolaryngology examination. Sleep polygraph showed very severe obstructive sleep apnea. Extracapsular tonsillectomy and adenoidectomy were performed under high anesthetic and surgical risk. Intra and postoperative outcome was positive, with the remission of the upper respiratory airway obstruction symptoms. Conclusions. Despite suggestive symptoms the patient presentation was late. Neurobehavioral manifestations and somatic disorders suggest the long-term evolution of upper respiratory airway obstruction. The extreme severity of sleep apnea was revealed by polygraph study. The sleep functional evaluation has contributed decisively to the therapeutic management and the favorable evolution of the case.

Keywords: obstructive apnea, sleep, child

Introduction

Obstructive sleep apnea (OSA) is a potentially lethal pathology that remains underdiagnosed in children. [1, 2, 3] Its etiology is polymorphic and should be systematically sought in order to initiate appropriate therapy. [2, 4, 5, 6, 7] Adenotonsillar hypertrophy is a common cause of obstructive sleep apnea in children. [1, 3, 4, 8] Affecting ventilation and sleep architecture, recurrent respiratory obstruction can lead to metabolic, cardiovascular, neurobehavioral and somatic consequences. [4, 9, 10, 11, 12, 13, 14]

Materials and Methods

A female patient aged 4 years and 9 months, coming from a rural area, has been evaluated in our clinic by: history, physical examination, paraclinical explorations: biological (hematological, biochemical, immunological, bacteriological); imaging (cardiopulmonary radiography, cardiac ultrasound); functional (electrocardiogram, sleep polygraph); specialty consults (otorhinolaryngology, pediatric cardiology, pediatric pneumology, pediatric orthopedics, somnology).

Case presentation

The girl was hospitalized in august 2018 for sleep apnea, restless sleep, nasal obstruction, constant noisy and difficult mouth breathing, excessive daytime sleepiness, attention deficit, diminished appetite, and difficulties in swallowing solid foods, failure to thrive. She is the third child of an affirmative healthy couple, from a physiological pregnancy carried to full term, born by caesarean section, with a birth weight of 4000g, birth length = 52 cm and an Apgar score 9. She was artificially fed with goat milk since birth and diversified incorrectly. Rickets prevention and vaccination were performed according to the national immunization schedule. The patient's personal history reveals recurrent upper airway infections and adenoidectomy performed in august 2017.

At admission, the patient was afebrile, with a relatively influenced general condition and failure to thrive (weight = 15 kg, height =111 cm, BMI = 12.2 kg/m2, less than the 5th percentile) (Figure 1), adenoid faces, pale skin, poor subcutaneous cellular tissue, noisy mouth breathing, nasal voice, speech difficulties using short sentences and excessive sleepiness. She presented inter and subcostal retractions and important depression of the stern during inspiration.

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Respiratory frequency was normal (22 breaths per minute), oxygen saturation around 96-97% and increased heart rate (128 beats per minute). Cardiopulmonary auscultation was normal. Blood pressure range was normal for age (105/60 mmHg). Oropharyngeal examination revealed important adenotonsillar hypertrophy, with large cryptic tonsils extended to the midline

The ENT exam described grade IV tonsillar hypertrophy (with the absence of the oropharyngeal isthmus); cryptic tonsils, hyperplasia of the rinopharyngeal lymphoid tissue with the complete obstruction of choanae; small bilateral auditory conduct for the biological age, retracted tympanic membranes.

The biological evaluation revealed atopic status (peripheral blood eosinophilia and elevated serum immunoglobulin E levels and mild allergic response to mites), inflammation, hyper-immunoglobulin G and hypergammaglobulinemia, post streptococcal inflammatory syndrome and vitamin D deficiency (Table 1, 2 and 3). Chest X-ray interpretation showed the sunken appearance of the chest (pectus excavatum) (Figure 2). The physical examination and paraclinical cardiac evaluation (electrocardiogram test – ECG, cardiac ultrasound) showed normal heart function. The somnological evaluation was achieved by using the sleep-related breathing disorder scale from the questionnaire on sleeping in children (22 questions) and polygraph sleep study The score calculated on the basis of the questionnaire was suggestive for a sleep-related breathing disorder. The sleep study has shown an extremely severe obstructive sleep apnea, with an apnea-hypopnea index = 112.9/h, desaturation index = 58.4/h, 74.5 obstructive apnea/h, 23.7 mixt apnea/h, 8 central apnea/h and 6.8 hypopnea/h (Figure 3). Respiratory events occurred in all positions adopted during sleep. The patient has persistently snored throughout the evaluation, adopted the seating position repeatedly and has woken frequently throughout the assessed period. She presented several abnormalities of the periphery oxygen saturation during sleep (average SaO2 = 90%, maximum SaO2 = 100%, minimum SaO2= 51%). In 16.7% of the time spent in bed, her oxygen saturation was less than 90%.

ENT consult recommended adenotonsillectomy. The Extracapsular tonsillectomy and adenoidectomy was performed the 5th day after admission, under high anesthetic and surgical risk. The postoperative care plan was complex: parenteral nutrition + oral; antibiotics, steroid anti-inflammatories, anti-hemorrhagic agents, analgesics, probiotics and roborants.

The intra and postoperative outcome was positive. The child regained appetite and became cheerful, the general condition improved, sleep became peaceful, without any respiratory events. Antistreptolysin O (ASO) titer decreased (1000 UI/mL). The hospitalization period lasted for 13 days.

Discharge recommendations included hygienic-dietary treatment (adequate nutrition, sleep hygiene, avoiding dorsal decubitus during sleep, avoiding exposure to environmental emissions), medication (antibiotics, nebulization with hypertonic saline, analgesics if needed, probiotics, oral vitamin D supplementation), physiotherapy (breathing exercises and Valsalva maneuver), heliotherapy and thalassotherapy.

Multidisciplinary team follow-up, motorization of nutritional status and sleep polygraph reevaluation after 6-8 weeks were recommended, but the patient did not return in the clinic as scheduled.

Discussions

Pathophysiological factors involved in obstructive sleep apnea (OSA) can be divided into anatomical factors that reduce airway caliber and those that promote increased upper airway collapsibility. [4, 6, 7, 15] Children with OSA experience partial or complete obstruction of the upper airway during sleep and they achieve to reopen them through short awakenings or hypercapnic ventilatory response. [15] Increased respiratory effort, alveolar hypoventilation, intermittent hypoxia and hypercapnic cause sleep fragmentation. [1, 12] The altered architecture can lead to dysfunction of the prefrontal cortex, affecting cognitive behavior, executive function and learning ability. [1, 2] The type of pediatric OSA is influenced by individual susceptibility, environmental and lifestyle conditions (diet, physical/intellectual activity). According to phenotype, OSA in children can be classified as: type I (adeno-tonsillar hypertrophy + OSA); type II (obesity + OSA); type III (craniofacial or neuromuscular disorders + OSA). [4, 16]

Genetic predisposition, inflammation and atopic status may be implicated in the etiopathogenesis of OSA in children.[2,8] Recurrent viral and bacterial infections as well as environmental exposure to irritants (cigarette smoke, allergens) would favor excessive adeno-tonsillar tissue proliferation and OSA worsening.[8]

The major consequences of pediatric OSA are neurobehavioral and metabolic disorders, cardiovascular pathology and somatic growth impairment. [1, 10, 11, 14, 16] The complications of OSA results from the interaction between intermittent hypoxia, hypercapnic, repeated variations of intrathoracic pressure and episodic awakenings. [12, 16]

Sleep fragmentation, associated with intermittent hypoxia and hypercapnic has negative effects on the prefrontal cortex and affects the executive functions in children with OSA. [1]. Intermittent hypoxia exacerbates neuronal apoptosis and affects the brain regions responsible for memory and learning. Academic performance can be affected in the long term. Excessive daytime sleepiness is the consequence of sleep fragmentation. [1, 2]

Some children with OSA (≤ 5%) fail to thrive as a consequence of anorexia, dysphagia, decreased nocturnal secretion of growth hormone, intermittent hypoxia and increased respiratory effort during sleep. [1, 2, 16, 17, 18] A sunken chest in children with OSA could be a consequence of chronic upper airway obstruction and difficult breathing, under the conditions of a compliant and immature chest. [1]

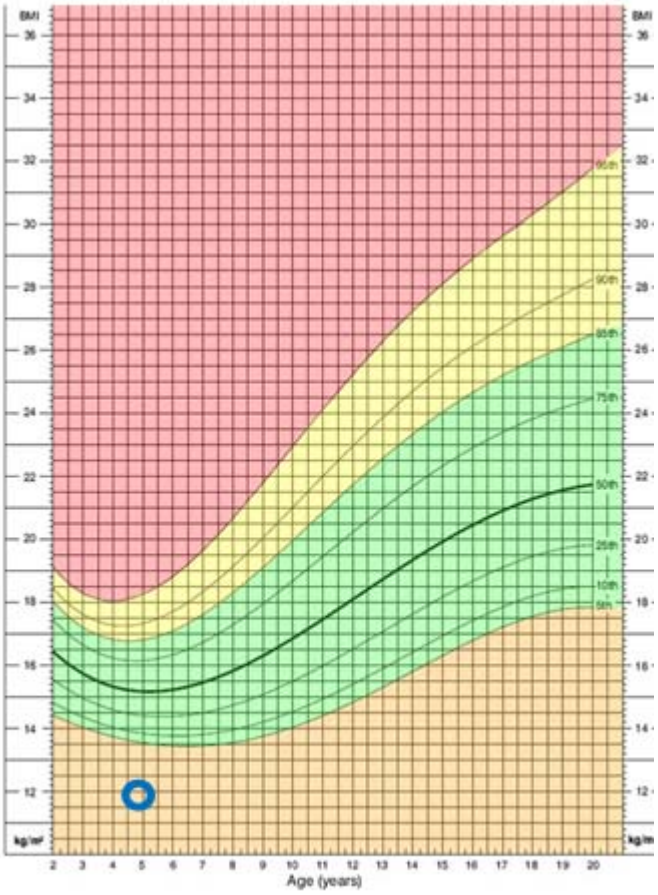

Figure 1. Evaluation of the nutritional status of the subject

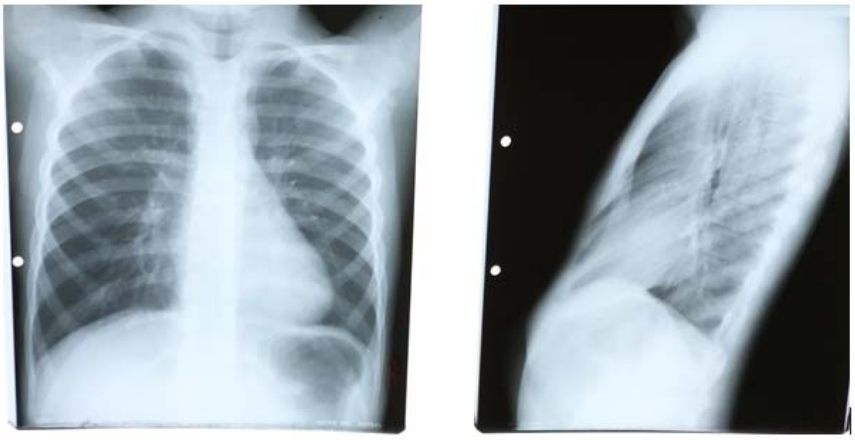

Figure 2. Chest x-ray (posteroanterior, lateral) of the presented case

WBC - Leukocytes	10,11	4 - 12	10^3/ul
RBC - Erythrocytes	4,17	3,9 - 5,3	10^6/ul
HGB - Hemoglobin	12,0	10,7 - 14,1	g/dL
HCT - Hematocrit	35,6	30 - 43	%
MCV - Mean corpuscular volume	85,4	72 - 88	fL
MCH-mean corpuscular hemoglobin	28,8	23 - 31	pg
MCHC - mean corpuscular hemoglobin concentration	33,7	32 - 36	g/dL
PLT – Thrombocytes	439	150 - 450	10^3/ul
Neutrophils	4,65	1,5 - 8	10^3/ul
Lymphocytes	3,59	1,5 - 10	10^3/ul
Monocytes	1,16	0,1 - 1	10^3/ul
Eosinophils	**0,58**	0,08 - 0,48	10^3/ul
Basophils	0,13	0 - 0,1	10^3/ul
Percent of neutrophils	46,0	18 - 44	%
Percent of lymphocytes	35,5	42 - 61	%
Percent of monocytes	11,5	2 - 8	%
Percent of eosinophils	**5,7**	2 - 4	%
Percent of basophils	1,3	0 - 1	%

Table 1. Full blood count – pacient case report results

ALT - alanine transaminase	16	<39	U/L
AST - aspartate transaminase	22	< 52	U/L
Creatinine	28	25 - 55	umol/L
C-Reactiva Protein	0,44	0 - 5	mg/L
ESR - Erythrocyte Sedimentation Rate	**29**	2-13	mm/h
ASO - Antistreptolysin O	**3204**	0 - 200	iU/mL
Total Protein	75,2	60 - 80	g/L
Albumin	57,4	59,8 - 72,4	%
Alpha1-globulin	2,6	1 - 3,2	%
Alpha 2-globulin	9,6	7,4 - 12,6	%
Beta-globulin	9,4	7,5 - 12,9	%
Gamma-globulin	**21,0**	8 - 15,8	%
IgA-Immunoglobulin A	1,99	0,27 - 1,95	g/L
IgG-Immunoglobulin G	**19,79**	5,04 - 14,65	g/L
IgM- Immunoglobulin M	1,24	0,24 - 2,1	g/L
Vitamin D (25-hydroxi)	**23,92**	30 - 100	ng/ml
Ionic Calcium	1.09	1,05 - 1,3	mmol/L
Total Calcium	2,46	2,3 - 2,75	mmol/L
Alkaline Phosphatase	132	< 269	U/L
Phosphor	1,27	1,1 - 2	mmol/L
Magnezium	0,78	0,7 - 1,05	mmol/L
Nasal secretion	negative culture result		
Pharyngeal exudate	negative culture result		

Table 2. Biological, biochemical, imunological and bacteriological evaluation of the patient

Allergen-Specific Immunoglobuline E (IgE)	
Herb pollen	0 - Undetectable
Birch pollen	0 - Undetectable
Black wormwood	0 - Undetectable
Derm. pteronyssinus	0 - Undetectable
Derm. farinae	**1 – Extremely low**
Cat hair/epithelium	0 - Undetectable
Dog hair/epithelium	0 - Undetectable
Horse hair/epithelium	0 - Undetectable
Cladospo. herbarum	0 - Undetectable
Aspergilius fumigatus	0 - Undetectable
Alternaria alternata	0 - Undetectable
Egg White	0 - Undetectable
Egg Yolk	0 - Undetectable
Milk	0 - Undetectable
Cod Fish	0 - Undetectable
Alpha lactalbumin	0 - Undetectable
Beta lactoglobulin	0 - Undetectable
Casein	0 - Undetectable
Bovine albumin	0 - Undetectable
Wheat	0 - Undetectable
Rice	0 - Undetectable
Soya	0 - Undetectable
Peanuts	0 - Undetectable
Almonds	0 - Undetectable
Carrot	0 - Undetectable
Potato	0 - Undetectable
Apple	0 - Undetectable
Total Immunoglobulin E 0 – 60 UI/ml	**715,6**

Table 3. Atopic status evaluation of the presented patient

The question are is used frequently to assess for OSA risk in children aged 2 to 18 years. [10, 14, 19] Preoperative sleep study (polysomnography/ polygraph) diagnoses sleep apnea and identifies severe OSA that amplifies risks of perioperative adenotonsillectomy complications. [2, 14, 20]

Characterized by adenotonsillar hypertrophy, case presented belongs to OSA type I. The important morbidity due OSA is somatic (failure to thrive, adenoid faces, sunken chest) and neurobehavioral (hyperactivity, attention deficit, fragmented and restless sleep, diurnal hypersomnia) [1, 16].

In children with moderate / severe OSA and adeno-tonsillar hypertrophy, ablation of adenoid vegetation and tonsillectomy is the first line therapy. [2, 4, 8, 18] In severe OSA the anesthetic and operative risk is important, co-morbidities increasing intra and perioperative risk. Recurrent hypoxemia enhances the sensibility to opioids directly proportional with the severity of OSA. Failure to thrive and severe OSA are risk factors for postoperative complications. [18] Pulmonary edema with severe respiratory obstruction can occur during the first postoperative hours and orotracheal intubation or non-invasive ventilation might be needed.

Up to 75% of operated patients continue having residual OSA. The longer OSA remains untreated, the less is probable the complete resolution of symptoms. [1, 16, 21]

Late diagnosis of OSA involves higher risks of developing cardiovascular, metabolic and somatic complications, as well as cognitive disorders with decreased scholar abilities. Often the quality of life of both child and family is affected. [4, 22].

Due to the persistence of OSA in a significant number of children, post-operative re-assessment through questionnaire and sleep study is mandatory. [14, 18]

Conclusions

Patient presentation and diagnosis were late despite suggestive symptoms. Neurobehavioral manifestations and somatic disorders revealed the long-term evolution of upper respiratory airway obstruction.

Specifying the extreme severity of sleep apnea by polygraph study has contributed decisively to the therapeutic management and the favorable evolution of the case.

Preventing the complications of obstructive sleep apnea on the growth and development of the child is imperiously needed and is based on early diagnosis and appropriate therapy of the disease.

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MULTIDISCIPLINARY APPROACH TO A COMPLEX CASE OF ACUTE CHILD PNEUMONIA

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Abstract

We present a non-vaccinated 2 years and 3 months old female patient with anti-convulsive therapy for Bourneville tuberous sclerosis (TSC) who was hospitalized for fever, polypnea, paroxysmal coughing episodes and post-tussive vomiting, respiratory failure and convulsive status. Basic paraclinical investigations revealed bilateral paramediohilar interstitio-alveolar infiltration, normal liver and renal parameters, elevated CRP and procalcitonin, but the presence of very high white blood cell count with lymphocytosis raised suspicion of leukemia. It was excluded by bone marrow aspirate examination and the diagnosis of convulsive cough was confirmed based on the clinical and laboratory parameters. Molecular genetic test confirmed the diagnosis of tuberous sclerosis type 2. Taking in account that the child had partially controlled seizures and cerebral, cardiac and renal characteristic lesions, this case raised a lot of questions regarding the infectious contact and the possibility of other potentially severe infectious diseases prevention by vaccination. A hope in this case is the new approved in children drug everolimus. In conclusion, pneumonia, a common disease in pediatric age, can raise many diagnostic and therapeutic problems mainly in patients with chronic pathology, requiring multidisciplinary collaboration for successful management of the case.

Keywords: pneumonia, pertussis, seizures, tuberous sclerosis, child

Introduction

Pneumonia is a common cause of morbidity in childhood. It can manifest as a severe form of the disease in patients with associated chronic pathology, especially in non-vaccination conditions, requiring complex investigations and multidisciplinary intervention.

Case report

A 2 years and 3 months old female patient with anti-convulsive therapy with carbamazepine retard and vigabatrin for Bourneville tuberous sclerosis (TSC) are hospitalized for fever, polypnea, and paroxysmal coughing episodes with post-tussive vomiting, and generalized tonic-clonic seizures. The onset of disease was one week before admission, with cough, followed 1 day after by fever up to 38.8° C. In spite of the anti-tussive treatment, the cough is aggravated, the patient exhibiting emetic paroxysms, thoraco-abdominal balancing, respiratory groaning noise, and, in the day of admission, generalized tonic-clonic seizures which did not respond to the intrarectal diazepam treatment. At the presentation in the emergency unit she was febrile, with generalized tonic-clonic seizures, loss of consciousness, with 70% SpO2 which is being corrected up to 97% under oxygen mask therapy. After exclusion of Influenza A and B infection by rapid test, it was admitted in the intensive care unit (ICU) with diagnosis of acute pneumonia with respiratory failure, convulsive status and TSC.

From the family history, we retain type II diabetes mellitus (paternal grandfather) and acute kidney failure due to renal lithiasis requiring nephrectomy (paternal grandmother). An important fact is that the patient's 10-year-old brother, who was vaccinated according to Health Minister's schedule, presented a prolonged cough for 1 month.

The physiological personal history reveals that the patient comes from a second followed pregnancy, born trough cesarean section with a birth weight of 2970 g, high of 50 cm and Apgar score of 10. She was breastfed for 8 months, then with formula milk, diversified correctly at 6 months of age, with correctly prophylaxis of rickets and was vaccinated only at birth for B hepatitis and tuberculosis.

Pathological personal history reveals the onset at the age of 5 months, of afebrile focal ± generalized and atypical absence type seizures.

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Test		Patient	Normal value
Haemoglobin	g/dl	11.3	10.7-14.1
Leucocyte	/mm^3	103 840	5 500-15 500
Neutrophil	%	23.6	18-44
Lymphocyte	%	71	42-61
CRP	mg/l	103.23	0-5
Procalcitonin	ng/ml	0.599	< 0.5

Table 1. Basic laboratory tests

	Clinical exam	Paraclinical investigations
Infectious mononucleosis	Absence of lymphadenopathy, pharyngitis, hepato/splenomegaly	IgM anti-VCA Epstein-Barr antibodies: negative
Scarlet fever	Absence of typical pharyngitis and exanthema	Pharyngeal culture negative for *Group A beta-hemolytic Streptococcus*
Rubella	Absence of lymphadenopathy, typical exanthema	
Varicella	Absence of contact and typical exanthema	
Tuberculosis	Absence of contact	Absence of typical radiological characteristics QuantiFERON TB gold test: negative
Pertussis	Not vaccinated Paroxysmal coughing episodes with post-tussive vomiting	IgM anti-*B. pertussis* antibodies: positive IgA anti-*B. parapertussis* = negative

IgA=immunoglobulin A; IgM=immunoglobulin M; TB=tuberculosis; VCA=virus capsid antigen
Table 2. Differential diagnosis of leukemoid reaction with lymphocytosis

Pneumonia	Respiratory failure	Convulsive status
-tachypnea -bilateral fine crackles rales -chest roentgenogram -leucocytosis -increased CRP	-cyanosis -SpO2 of 70% - thoraco-abdominal balance, -intercostal and subcostal retraction -respiratory groaning noise	- succession of tonic-clonic seizures without recovery of consciousness between individual attacks

Table 3. Positive diagnosis criteria of pneumonia, respiratory failure and convulsive status

Major criteria	Minor criteria
1. Hypomelanotic macules (≥ 3, at least 5mm diameter) 2. Angiofibromas (≥ 3) or fibrous cephalic plaque 3. Ungual fibromas (≥ 2) 4. Shagreen patch 5. Multiple retinal hamartomas 6. Cortical dysplasias* **7. Subependymal nodules** 8. Subependymal giant cell astrocytoma **9. Cardiac rhabdomyoma** 10. Lymphangioleiomyomatosis (LAM)# 11. Angiomyolipomas (≥ 2)#	1. "Confetti" skin lesions 2. Dental enamel pits (>3) 3. Intraoral fibromas (≥ 2) 4. Retinal achromic patch **5. Multiple renal cysts** **6. Nonrenal hamartomas**
Definite diagnosis: Two major features or one major feature with ≥ 2 minor features Possible diagnosis: Either one major feature or ≥ 2 minor features	
* Includes tubers and cerebral white matter radial migration lines. # A combination of the two major clinical features (LAM and angiomyolipomas) without other features does not meet criteria for a definite diagnosis.	

Table 4. Diagnostic criteria for TSC

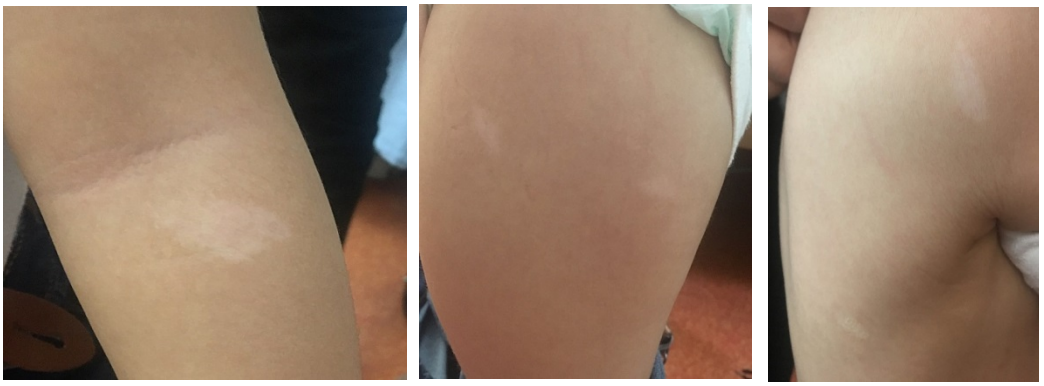

Fig. 1. White spots at the level of both upper and lower limbs and at the level of the posterior thorax

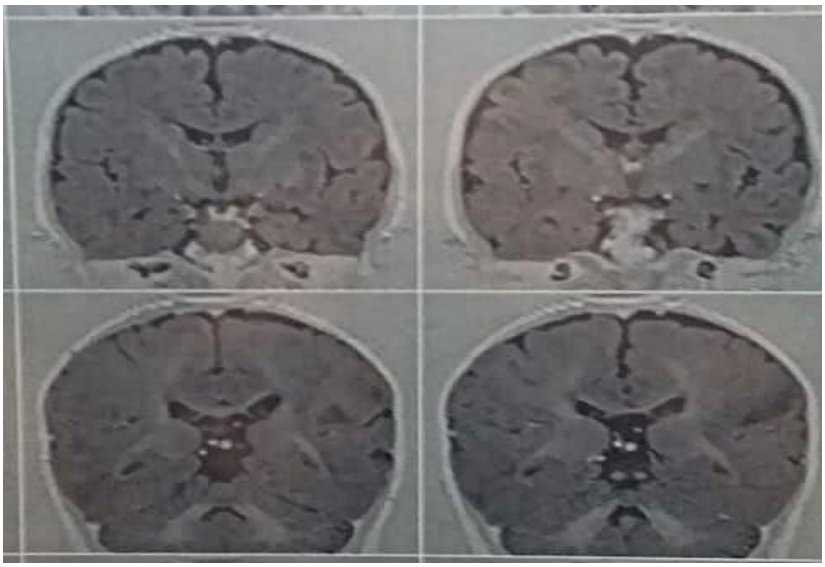

Fig. 2. Cerebral MRI: hamartomatous nodules

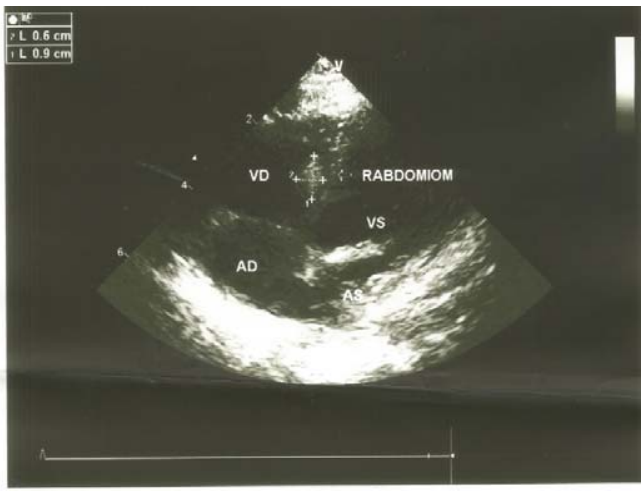

Fig. 3. Cardiac rhabdomyoma

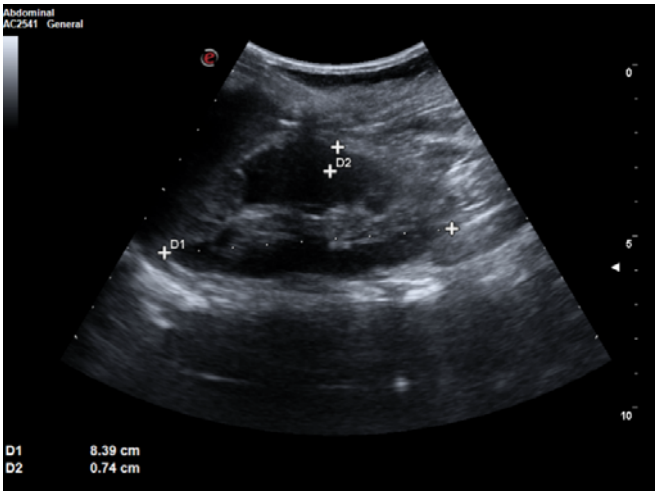

Fig. 4. Renal cysts

The clinical examination at admission showed a general influenced status, discrete pallor, white spots at the level of both upper and lower limbs and at the level of the posterior thorax (Figure 1), cyanosis, tachypnea (52 breaths/min), thoraco-abdominal balance, intercostal and subcostal retraction, presence of a symmetrical, bilateral pulmonary murmur, fine crackling rales, tachycardia, liver and spleen in normal range, loss of consciousness and no clinical signs of meningeal irritation.

Basic paraclinical investigations revealed bilateral paramediohilar interstitio-alveolar infiltration without pleurisy at chest roentgenogram, normal liver and renal parameters, elevated C reactive protein (CRP) and procalcitonin, but the presence of very high white blood cell count with lymphocytosis raised suspicion of leukemia (Table 1). The bone marrow aspirate examination showed erythroblast hypoplasia, reactive granulocyte series, lymphocytosis, 1% blasts, thus excluding leukemia and having the diagnosis of leukemoid reaction defined as leukocytes over 50 000 / mm3, with lymphocyte predominance [1].

Infectious mononucleosis, scarlet fever, rubella, varicella, tuberculosis and Bordetella parapertussis infection as a cause of lymphocytic leukemoid reaction are excluded based on clinical examination, chest roentgenogram, culture of pharyngeal exudate, serological investigations and QuantiFERON TB gold test (Table 2) [1, 2].

The neuropsychiatric examination revealed febrile infectious convulsive status.

Taking into account the presence of paroxysmal coughing episodes with post-tussive vomiting, absence of anti-pertussis vaccination and positive serology for Bordetella pertussis (B. pertussis), the diagnosis of pertussis infection complicated with pneumonia, acute respiratory failure and convulsive status was confirmed (Tables 2 and 3) [2,3].

The diagnosis of TSC requires 2 major criteria or 1 major criterion and at least 2 minor criteria mentioned in Table 4 [4]. To highlight them, cerebral MRI, cardiac and abdominal ultrasonography were performed. Cerebral MRI revealed supratentorial subcortical hamartomatous nodules and bilateral subependimal nodules suggestive for TSC (Figure 2). Cardiac ultrasonography showed the presence of cardiac rhabdomyoma with a maximum size of 1cm / 0.5cm (Figure 3), and abdominal ultrasound revealed a hypoechogenic lesion with a diameter of 0.5 / 0.5 cm in the median region of the right kidney medulla and bilateral cortical multiple small hypoechogenic lesions (Figure 4). Thus, with 3 major criteria and 2 minor criteria, the diagnosis of Bourneville syndrome can be supported. Since TSC is an autosomal dominant (AD) genetic disorder and the parents are clinically healthy, genetic testing was performed by sequencing for the TSC1 and TSC2 gene panel showing a pathogenic heterozygous mutation c.5138-5139del; p. (Arg1713Profs * 15) at the TSC2 gene level confirming definitively the diagnosis of TSC type 2.

Under treatment with intravenous meropenem, linezolid and clarithromycin, evolution was favorable, with CRP normalization after 14 days of treatment. Convulsions were immediately controlled with midazolam. Cough was present for a period of 7 weeks from onset and the number of leukocytes normalized after 8 weeks of evolution

Discussion

Pneumonia is a major cause of morbidity and in children it is the single largest infectious cause of death worldwide under the age of 5, accounting for 15% of all deaths [5]. In Romania, in 2010, the incidence of pneumonia at age 0-4 was 30 cases / 1 000 children, 26.7% of them being severe forms of illness, with a mortality of 9.3% [6]. B. pertussis pneumonia represents approximately 1.3% of cases of severe pneumonia at age 0-5 years [7]. Also several studies showed that about 9.5% of children with convulsive cough presented pneumonia as a complication [2]. This may be caused by B.pertussis or secondary bacterial invaders. The World Health Organization (WHO) estimated that in 2013, B. pertussis caused approximately 60 257 deaths in children <5 years of age [8]. In our country, in 2017 the incidence of convulsive cough was 0.5 / 100 000 inhabitants, lower than 2008 (2.4 / 100 000 inhabitants) when the last peak of incidence was registered [9].

Suggestive for the B. pertussis etiology in a child with acute respiratory failure is the presence of paroxysmal coughing episodes with post-tussive vomiting. Also very suggestive for diagnosis is very high leukocytosis with lymphocytosis, as in the present case. Leukocytosis is caused by the pertussis toxin and it is known that the severity of disease and the risk of death correlate directly with the white blood cell count and, in particular, the number of lymphocytes [2]. Considering that the patient is not vaccinated, does not attend the community and apparently has no infectious contact the question regarding who was the source of infection still remain. A number of studies showed that usually the source of infection with B.pertussis in infants and small children is a family member [10, 11, 12].

Both the disease and anti-B.pertussis vaccination do not provide lifetime immunity [2]. This explains why sporadic infection in the adolescent and adult reservoir is the major source of B. pertussis infections in nonimmune children. Studies of prolonged cough illnesses in adult and adolescents showed that 13-20% of the diseases are caused by B. pertussis infection (13, 14, 15). In our patient's case, the 10-year-old brother, considering that he had cough for 1 month before his sister's illness, may be the source of the infection even if he was vaccinated.

1.4-2.3% of patients with pertussis develop seizures and they are mainly caused by hypoxia [2]. In the presented case, seizures probably have a more complex etiology, caused by brain lesions from TSC, fever and hypoxia.

Bourneville's TSC is an AD genetic disorder characterized by skin, neurological (mainly seizures) manifestations and predisposition for tumors developing in any location. Although the diagnosis is based only on clinical criteria (Table 4), genetic confirmation is recommended, if possible, allowing for a proper genetic

counseling, prenatal diagnosis and therefore primary prophylaxis of the disease. TSC is a potentially progressive disease. This fact and the partially responsive to antiepileptic treatment seizures were the main justifiable causes of vaccination contraindication in our patient.

It is proved that the age-appropriate immunization coverage rate among children with TSC is low. In 72 children with TSC, the rate of adverse events or suspected adverse events after vaccination was 17% (12 cases), which was higher than the normal control children (2 cases, 3%) ($P<0.05$). The main side effects after immunization were seizure events, which accounted for 92% (11 cases). The high incidence of adverse events may be associated with the fact that there are nervous system abnormalities in patients with TSC. Despite of these, TSC children vaccination is considered relatively safe, with no serious adverse events [16]. Another study including 106 children with TSC showed that DTP immunization before seizure onset wasn't found to be a risk factor or predictor for mental retardation in children with TSC [17].

Since 2018, Food and Drug Administration (FDA) and European Medicine Agency (EMA) have approved the use of everolimus, a mechanistic target of rapamycin (m-TOR) inhibitor in TSC patients at least 2 years old presenting partial seizures, subependimal astrocytoma with giant cell and renal angiomyolipoma [18, 19]. This is a viable therapeutic option for our patient, because if the seizures and tumor progression are controlled, revaccination and thus the prevention of potentially fatal diseases could be performed.

Conclusions

In conclusion, pneumonia, a common disease in pediatric age, can raise many diagnostic and therapeutic problems mainly in patients with chronic pathology, requiring multidisciplinary collaboration for successful management of the case.

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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.