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## I. GENETICS

# RARE DISEASES – A MAJOR PROBLEM OF PUBLIC HEALTH

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### Abstract

A rare disease is a disease that affects less than 1 of 2000 individuals. The term “orphan disease”, used especially in France, confers to rare diseases political and social dimensions, trying to transmit the need of patients to be taken into consideration their existence, as rare as it would be the disease. Today it is being reported that exists over 8000 of this kind of diseases. Numerous and complex, rare diseases are less known by the medical corps and by the authorities of the health care system. These diseases are connected with all medical specialties and there are of an extremely variable gravity in accordance with patient and disease. Since the rare diseases affects over 25 million individuals in Europe, the Health Care Committee of UE seems to become more and more preoccupied to rehabilitate the research in this domain, to create an educational climate of the medical corps, patients and general population, with the announced purpose of ensuring conditions for a correct and early diagnosis of these diseases. All these strategies must be adopted in our country as well with the sustained effort of National Alliance of Rare Disease from Romania (ANBRaRo), by creating and unrolling The National Plan for Rare Diseases.

**Key words:** rare diseases, orphan drugs, patient and parent organizations

### Definition

“A rare disease (RD) is a disease that occurs infrequently or rarely in the general population”. In Europe a disease is considered rare when it affects less than 5 in 10,000 citizens. This figure can also be expressed as 500 rare disease patients out of 1 million citizens. A RD is also called an “orphan disease”, a term that is usually used in France and it gives to RD political and social dimensions trying to transmit the solicitation of patients not to be forgotten even if their disease is very rare. Also, RD are orphan of treatment, recognition and proper care.

### Frequency

Despite the rarity of each RD, “about 30 million people have a RD in the 25 EU countries”, which means that 6% to 8% of the total EU population are RD patients. Considering that in Romania the frequency is similar to other EU countries we can appreciate that 1,3 million citizens are affected by RD and over 1,2 million do not

obtain an accurate diagnosis, so they do not benefit of adequate information about the disease, access to qualified specialists and proper treatment. For this reason RD represents a major public health problem as well in Romania as in all EU countries which made from this subject a priority in healthcare programs and research programs of the European Union.

Quoting from the Background Paper on Orphan Diseases for the “WHO Report on Priority Medicines for Europe and the World” (7 October 2004): “Unfortunately, the epidemiological data that are available are inadequate for most RD to give firm details on the number of patients with a specific RD. In general people with a RD are not registered in databases. Many RD are summed up as “other endocrine and metabolic disorders” and as a consequence, with few exceptions, it is difficult to register people with a RD on a national or international basis, and in a reliable, harmonized way”. In 1995 World Health Organization (WHO) reviewed about five thousand RD, but today 6000 – 7000 RD have been found and five new diseases are described every week in the medical literature.

### Common characteristics of RD

RD concern all medical specialties and their gravity differ widely in accordance with the disease and the patient. The disease can be discovered at birth or in childhood (for 50% of RD the onset of the disease occurs in childhood), but in many cases the first symptoms appear in adult age. In patients with RD are affected physical capabilities, mental abilities, behavior and sensorial capacities. Many disabilities can coexist, can be severe and it determines an important handicap. The impact on life expectancy varies greatly from one disease to the other: some RD cause death even before birth or at birth while others are compatible with a normal life if diagnosed in time and properly managed and/or treated. RD are severe to very severe, progressive, chronic, often degenerative and life-threatening and most of the time leads to the lifetime care necessity. The quality of life of RD patients is frequently compromised because of the loss of autonomy. It is also present a psychosocial burden for patients and their families due to lack of therapeutic hope. For most of these diseases there are no efficient treatments, but in some cases symptoms can be treated to improve quality of life and life expectancy. Fighting isolation and

despair the patients and their families need attention and emotional support.

### Etiology

80% of RD has identified genetic origins, involving one or several genes or chromosomal abnormalities. They are generally inherited and transmitted from one generation to another, but they can also be derived from de novo gene mutation or from a chromosomal abnormality. The other 20% of RD are caused by infections (bacterial or viral), allergies, or are due to degenerative, proliferative or teratogenic (chemicals, radiations, etc) causes. Some RD are also caused by a combination of genetic and environmental factors. But for most RD the etiological mechanism are still unknown due to lack of research in this domain.

### Prevention

Because most of the RD are genetically determined and have a great risk of recurrence it is very important for us to know if and how we can prevent these diseases. The concept of prevention is not new. The prevention can be collective by using general measures pointed to a certain disease (vaccine, control to detect breast or uterine cancers) and which is based on general criteria (age, sex, season, local or national epidemiological status). Unlike the collective prevention, in RD's pathology the prevention is frequently individual; the selection of risk patients and the prevention depends of the genetic features of each individual. The new possibilities of genetic diagnosis permitted the progressive implementation of some public health programs. These programs have started a new concept of predictive medicine which covers different situations the diagnosis being determined to subjects in good health. The most used prevention methods are connected with antenatal diagnosis, screening tests, in fact with the existence of a functional healthcare system that includes well trained specialists, diagnosis and counseling centers, an informed population, adequate sanitary laws, funds to support expensive investigations, quality of medical attendance, multidisciplinary supervision. The fight against genetic diseases must be based on an integrated and exhaustive strategy associating an optimum prevention and treatment, methods to aware the community, detection in population, genetic counseling and the possibility of an early diagnosis.

### Specialty drugs for RD ("orphan drugs")

Orphan drugs are medicinal products intended for the diagnosis, prevention or treatment of RD. People who have RD have not had research attention in past decades because the potential market for new drugs to treat RD is small. It is important to underline that many RD are transmitted along different generations and therefore investing in the fight against RD today may be a very profitable investment. In 1999 the European Union adopted a Regulation, based on experience in the United States, aimed at promoting the development of drugs for patients suffering from RD. Pharmaceutical and biotech companies that market an orphan drug received a variety of financial assistance, a guaranteed 10 year-monopoly on drug sales,

protocol assistance so they are constantly researching and developing new medications. Clinical evaluation of orphan drugs is restrained by the small number of patients available for clinical trials. In many cases, surrogate criteria are used instead of clinical endpoints. New drugs, with documented efficacy and safety, are now available for patients who previously had no effective treatment options. Access to these drugs varies greatly from one European Union Member State to another, mainly because of the high annual treatment costs so that patients in many European countries cannot benefit.

### What do patients with RD need?

- *Access to correct diagnosis:* it is very important to have an early and accurate diagnosis to prevent highly risk delays and inaccurate treatments due to wrong diagnosis
- *Basic information* about the disease and where to obtain help
- *Scientific knowledge* in order to develop therapeutic tools, therapeutic strategy and therapeutic products (medicinal products and appropriate medical devices)
- *Social integration:* patients with RD are often stigmatized, isolated, excluded from social community, discriminated for insurance subscription and professional opportunities
- *Quality healthcare:* RD patients need most of the time a multidisciplinary team: physiotherapist, nutritionist, psychologist, logoped, organ specialist, etc. Unfortunately, sometimes patients live for several years without competent medical attention or they are treated during many months or years for another more common disease. They remain excluded from the health care system even after the diagnosis is made.
- *Social benefits and reimbursement* so that the patients and their families can cover the high cost of the few existing drugs and care
- *Equities in availability of treatment and care:* new treatments are often unavailable even in the EU countries because of delays in price determination and/or reimbursement decision, lack of experience of the physicians and the absence of treatment protocols.

A delayed diagnosis has sometimes dramatic consequences:

- other children with the same disease in the family,
- parental splitting when a child with a RD is borne,
- lack of support from family members,
- clinical complications even leading to the death of the patient,
- distrust in the healthcare system.

The entire family of the patient is affected by the disease and becomes marginalized psychologically, socially, culturally and economically vulnerable. In order to help RD patients and their families face the future psychological support is greatly needed. This kind of support can be offered by family and friends, but also by specialists, support groups and electronic email discussion groups which link patients and also families and medical personnel.

### RD patient and parent organizations

The scientific community's insufficient knowledge of RD and the minimal attention given to them by national authorities and the pharmaceutical industry has led to the creation of associations of patients and parents. These were created as a result of experience gained by patients and their families who want to gather, produce and disseminate the existing information on their disease and to make patients and parents voices heard. These support groups for patients are the most motivated and have the most important achievements in the advocacy activities. They have succeeded to influence the policies and to stimulate the medical research because of the personal involvement. In the world there are various forms of organization of the patients, from very small groups to very large organizations, it can be focused to support patients affected by one disease or can include patients with different pathologies and have members from one country or there are organized at the European or international level. Certainly, the best results are obtained by patients that are organized in networks because they speak for a great number of patients influencing the legislation and sharing the experience of all smaller groups.

The association of patients, parents, specialists represents the most beneficial way to interfere efficiently in changing conceptions, in modifying the health care strategies, but the most important, in improving the life quality of these patients. The Health Care Committee of UE seems to become more and more preoccupied to rehabilitate the research in the RD domain, to create an educational climate of the medical corps, patients and general population, with the announced purpose of ensuring conditions for a correct and early diagnosis of these diseases. All these strategies must be adopted in our country as well. In 2005 Romanian Prader-Willi Association opens The Information Center for Rare Genetic Diseases in Zalau, which is the first center of this kind in Romania and its purpose is to be a resource center for patients with RD, their families and specialists involved in the diagnosis and management of these diseases. Some patient and parent organizations from Romania (Romanian Prader-Willi Association, Williams Association, PKU Life for Romania, Thalassemia Association, etc) together with specialists and volunteers founded in August 2007 The National Alliance of Rare Diseases from Romania which is affiliated to other European networks. This project was sustained from European funds and the main purpose for this Alliance is to create and unroll The National Plan for Rare Disease. The Ministry of Public Health from Romania proved to be interested for an efficient partnership in promoting this plan and developing the specific activities for its implementation. Some EU Member State (Denmark, France, Italy, Sweden, Spain, UK) have developed such a plan with specific public policies on RD.

### Access to information in RD

Today, patients, their families and medical personnel have access to many European information sources. Some of them are:

➤ **ORPHANET** – a database dedicated to information on RD and orphan drugs; access of this database is free of charge. Its aims are: to contribute to the improvement of the diagnosis, care and treatment of patients with RD, to optimize the correct use of clinical resources and to accelerate therapeutic development and research.

➤ **EURORDIS (The European Organization for Rare Diseases)** – a non-governmental patient-driven alliance of patient organizations and individuals active in the field of RD; represents more than 260 RD organizations in 29 different countries, covering more than 1.000 RD. It is dedicated to improve the quality of life of all people living with RD in all Europe. Its main priorities are: networking and empowering RD patient organizations, advocating and raising awareness, public health and health care policy.

➤ **RDTF (Rare Diseases Task Force)** – set up by the European Commission Public Health Directorate. Its aims are: to advise and assist this Commission in promoting the optimal prevention, diagnosis and treatment of RD in Europe and to provide a forum for discussion and exchange of views and experience on all issues related to RD.

➤ **EUROCAT (European Concerted Action on Congenital Anomalies and Twins)** - is a European network of population - based registries for the epidemiologic surveillance of congenital anomalies. Its objectives are: to provide essential epidemiologic information on congenital anomalies in Europe, to act as an information and resource center for the population, health professionals and managers regarding clusters or exposures or risk factors of concern, to provide a ready collaborative network and infrastructure for research related to the causes and prevention of congenital anomalies and the treatment and care of affected children.

➤ **NORD (National Organization for Rare Disorders)** - was created by a group of patients and their families involved in advocacy activities for the adoption of a regulation on research and development of orphan drugs, called the Orphan Drug Act. NORD's website includes two databases: one on rare diseases, the other on rare disease organizations. For each disease, a list of relevant patient organizations is available.

➤ **ORD (Office of Rare Diseases)** - was created by the National Institutes of Health (NIH) to stimulate and coordinate RD research in the United States. Its information centre, called Genetic and Rare Diseases Information Center (GARD), supplies reliable and valid information to the public, researchers and health care providers, about any one of the more than 6,000 rare diseases known today.

### Conclusions

The RD patient is the orphan of health systems, often without diagnosis, without treatment, without research. In this framework we must underline that there is always something useful that can be done in this domain. It is important to progress in all fields of activity: physiotherapy, nutrition, pain management, psychology, medical devices, advanced therapies and the main effort has to be done in order to stimulate research, to increase the existing knowledge.

## References

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| <ol style="list-style-type: none"><li>1. Cassidy S, Allanson J: Management of Genetic Syndromes, 2<sup>nd</sup> Edition, Wiley-Liss, 2005, p 385-397</li><li>2. Background Paper on Orphan Diseases for the “WHO Report on Priority Medicines for Europe and the World” – 7 oct 2004</li><li>3. “Rare Diseases : Understanding this Public Health Priority” Eurordis, November 2005 – <a href="http://www.eurordis.org">www.eurordis.org</a></li><li>4. <a href="http://www.rarediseases.org/">http://www.rarediseases.org/</a></li></ol> | <ol style="list-style-type: none"><li>5. Puiu M: Essential in 101 Rare Diseases, p 18</li><li>6. <a href="http://www.orpha.net/">http://www.orpha.net/</a></li><li>7. Jones KL: Smith’s Recognizable Patterns of Human Malformation, 6<sup>th</sup> Edition, Elsevier Saunders, p 124-127</li><li>8. Puiu M, Ursoniu S, Dan D: Tendencies and necessities in the education of population concerning rare genetic diseases, 2007, Timisoara Medical Journal, vol. 57, Supplement 2, pg. 59</li><li>9. <a href="http://www.geneclinics.org">http://www.geneclinics.org</a></li></ol> |
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# CONSIDERATIONS REGARDING THE IMPLICATION OF POLYMORPHIC VARIANTS AND CHROMOSOMAL INVERSIONS IN RECURRENT MISCARRIAGE

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## Abstract

Recurrent miscarriage becomes a problem that affect an increasing number of couples, almost 1% of the people who want to conceive. The aim of this study is to present the role of heterochromatic regions heteromorphism and chromosomal inversions in occurrence of recurrent miscarriage. In this study were included 354 couples with recurrent miscarriage, which presented at Medical Genetic Department of University of Medicine and Pharmacy Timisoara between octomber 2003 and november 2007. The most common findings were pericentric inversion of chromosome 9, heteromorphism of heterochromatic regions of chromosomes 1 and 16. Our date suggests that cytogeneticists should not ignore these variants that play an important role in reproduction failure.

**Key words:** heteromorphism, heterochromatin, recurrent miscarriage

## Introduction

Recurrent miscarriage becomes a problem that affect an increasing number of couples, almost 1% of the people who want to conceive. Causes of recurrent abortions are: chromosomal abnormalities of genitors, gynecological anomalies, antiphospholipid syndrome, polycystic ovary syndrome and different kind of infections.

In approximated 50% of cases, the cause of reproduction failure remains unknown. In a small number of cases, the abortions arise from transmission of structurally aberrant chromosomes from the parents.

Chromosome variants or polymorphisms are microscopically visible regions that vary in size, morphology and staining properties and have no apparent effect on the phenotype. They are inherited in a Mendelian fashion and are mostly found in the highly variable regions of chromosomes 1, 9, 16, the distal two thirds of the long arm of the Y chromosome and the short arms and satellites of the acrocentric chromosomes.

A number of findings revealed that chromosome inversions are more frequent than deduced from classical cytogenetic studies. Inversions in which a breakpoint is in heterochromatic regions (1qh, 9qh, 16qh, and Yq) are relatively frequent. We like to mention also the other

chromones inversions who are responsabile during the gametogenesis for aberant recombinations and in the same time will be the cause of aneuploid gametes. The role of constitutive heterochromatin is still unknown and the heteromorphism of constitutive heterochromatin was thought to cause no phenotypic alterations.

The aim of this study is to present the role of heterochromatic regions heteromorphism and inversions of chromosomes in occurrence of recurrent miscarriage. The reports regarding the heterochromatic regions found in literature are controversial; there were studies that suggest no correlation between this chromosomal rearrangements and abortions, but in the last years many scientists paid a special attention to this heteromorphism and chromosomal inversions and their implications in reproduction failure. In 2005 Madon reported a study including 842 individuals with primary infertility or repeated miscarriages and showed that polymorphic variants are involved in reproduction failure.

## Methods

In this study were included 354 couples with recurrent miscarriage, which presented at Genetic Department of University of Medicine and Pharmacy Timisoara between octomber 2003 and november 2007. The selection of the couples included in this study: one or more consecutive spontaneous abortions; both genitors with normal genitalia. The chromosomal preparations were analyzed after applying a trypsin G-band method. C banding was also used. For each individual, a minimum of 30 metaphase plates was counted and at least five cells were karyotyped.

## Results

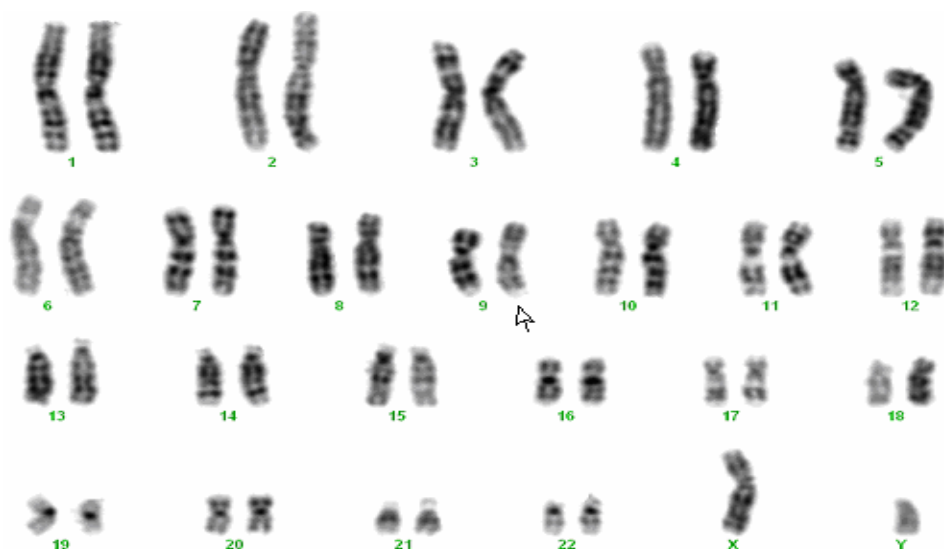
The chromosomal polymorphisms were classified as follows: obvious pericentric inversion of the constitutive heterochromatin; significantly enlarged heterochromatic region of the long arm; small Y (less than size of a G-group chromosome).

The following table presents the heteromorphism of heterochromatic regions and the chromosomal inversions in our study group.

Chromosome	Females	Males
<b>9</b>	46,XX, inv(9)(p12;q22.3)	46,XY, inv(9)(p24.3;q31.1)
	46,XX, inv(9)(p24.1;q31.1)	46,XY, inv(9)(p24.1;q31.1)
	46,XX, inv(9)(p24.3;q31.1)	46,XY, inv(9)(p21.1;q31.1)
	46,XX, inv(9)(p13.2;q21.11)	46,XY, inv(9)(p24.1;q31.1)
	46,XX,9qh+	46,XY, inv(9)(p24.3;q31.1)
	46,XX, inv(9)(p11;q13)	46,XY, inv(9)(p13;q13)
	46,XX, inv(9)(p22;q21.2)	
<b>1</b>	46,XX,1qh+, 16qh+	46,XY,1qh+
	46,XX,1qh+,	
	46,XX,dir dup (1)(q12)	
	46,XX,1qh+,	
<b>16</b>	46,XX,dir dup(16)(q11.2)	46,XY,16qh+
	46,XX,16 inv dup(q11.1;q11.2 )	
<b>8</b>	46,XX,inv(8)(p23.1;q21.2)	
<b>10</b>	46,XX,inv(10)(p11.2;q21)	
<b>15</b>	46,XX,inv(15)(p12;q12)	
<b>Y</b>		46,XY, del(y)( p11.2-p11.3)

The most frequent polymorphism found in this study lot was pericentric inversion of chromosome 9. The incidence of this variant is ranging from 1% to 1.65% in the general population. DNA sequence analysis of human

chromosome 9 has shown that it is highly structurally polymorphic, with much intrachromosomal and interchromosomal duplication, and contains the largest autosomal block of heterochromatin.



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**Cariotip: 46,XY,inv(9)(p24.1;q31.10)**

Figure 1 showing pericentric inversion of chromosome 9.

Heteromorphism of chromosomes 1 and 16 were also found in these patients, with prevalence in feminine subjects. This is according with the dates from literature where the incidence of chromosomal abnormalities is higher in females than that in males. A case of 46,XX,dir

dup(16)(q11.2) was diagnosed using prenatal diagnosis due to the suspicion based on echographic indicators of chromosomal aneuploidy and revealed the same chromosomal anomaly as the one found at her mother.



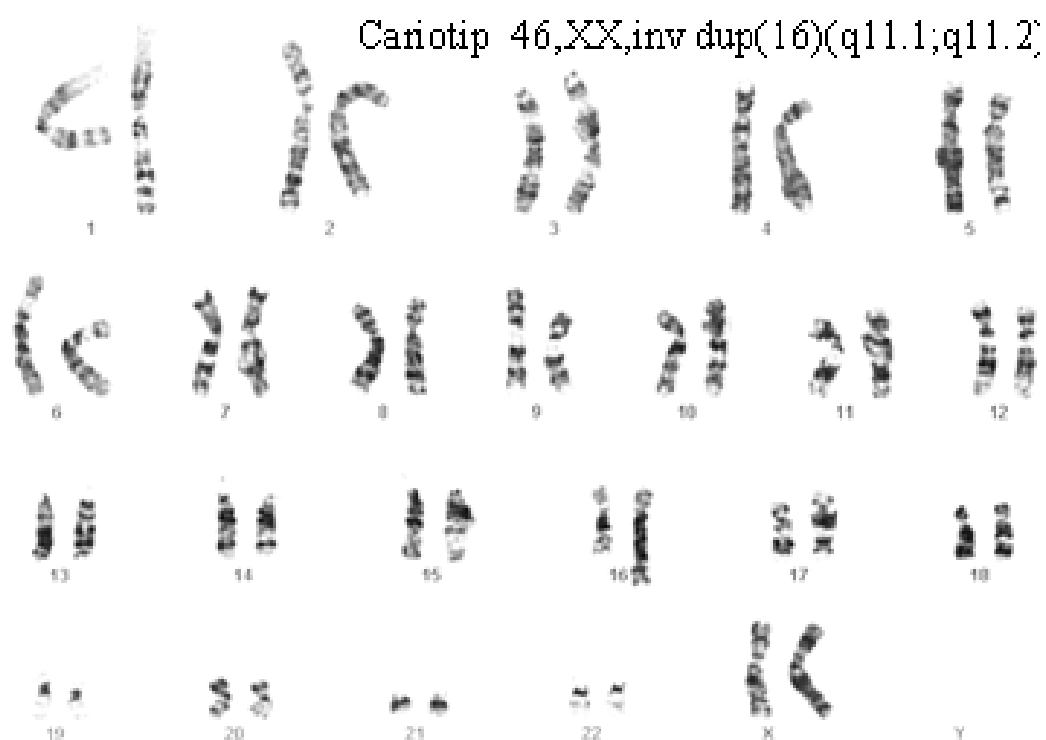


Figure 2 showing Karyotype 46,XX,inv dup(16)(q11.1;q11.2)

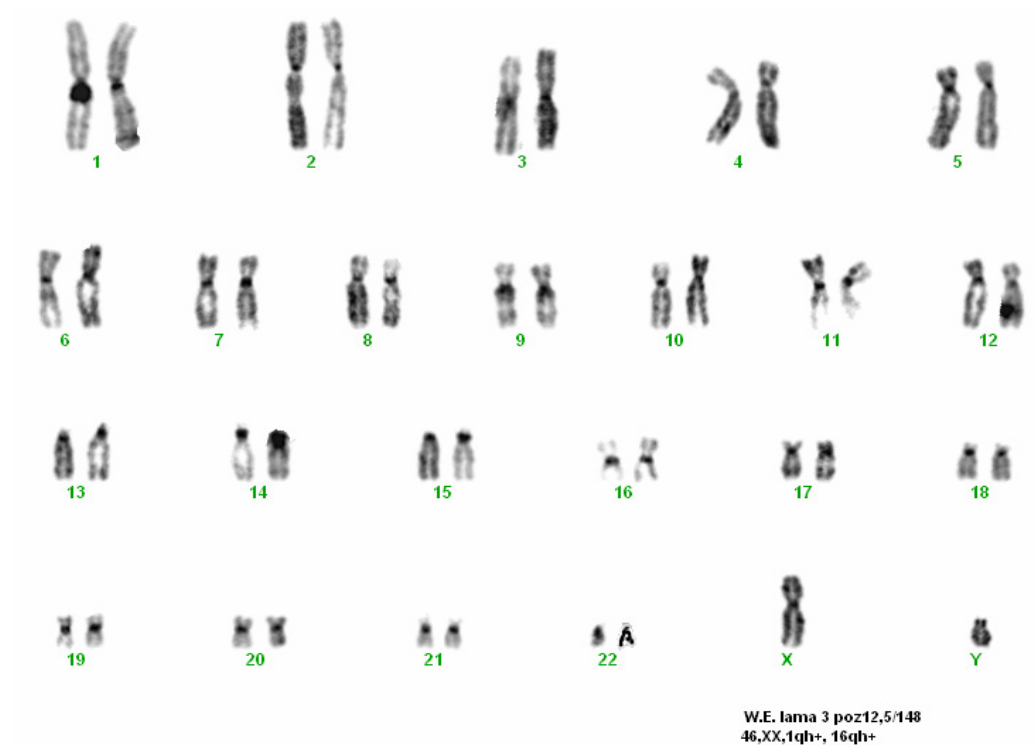


Figure 3 showing C banding

The Y chromosome has an abundance of low copy repeats which render this chromosome susceptible to a multitude of rearrangements that, when involving the long arm, are often the cause of spermatogenic failure. Deletions of short arms of Y chromosome that include a part of pseudoautosomal region have no phenotypical

manifestations due to the compensation of the pseudoautosomal region from X but the deletion of a part of SRY gene, found in this region, could explain the reproductive dysfunction.

Rarer were cases involving other autosomal chromosomes: 8, 10, and 15.

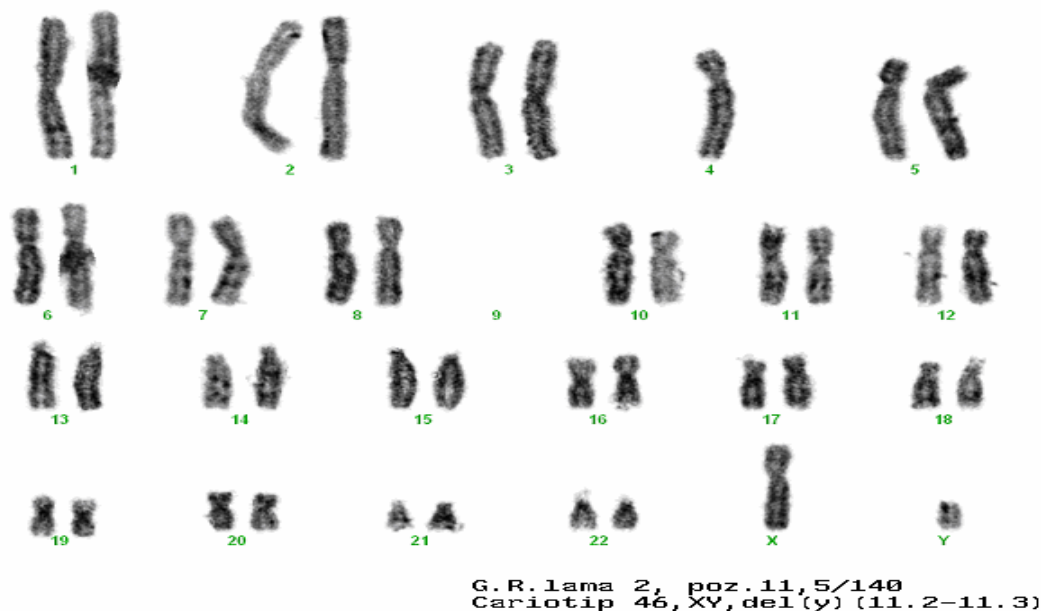


Figure 4 showing del(y)(p11.2-p11.3)

Cariotip: 46, XX, inv(10)(p11.2q21)

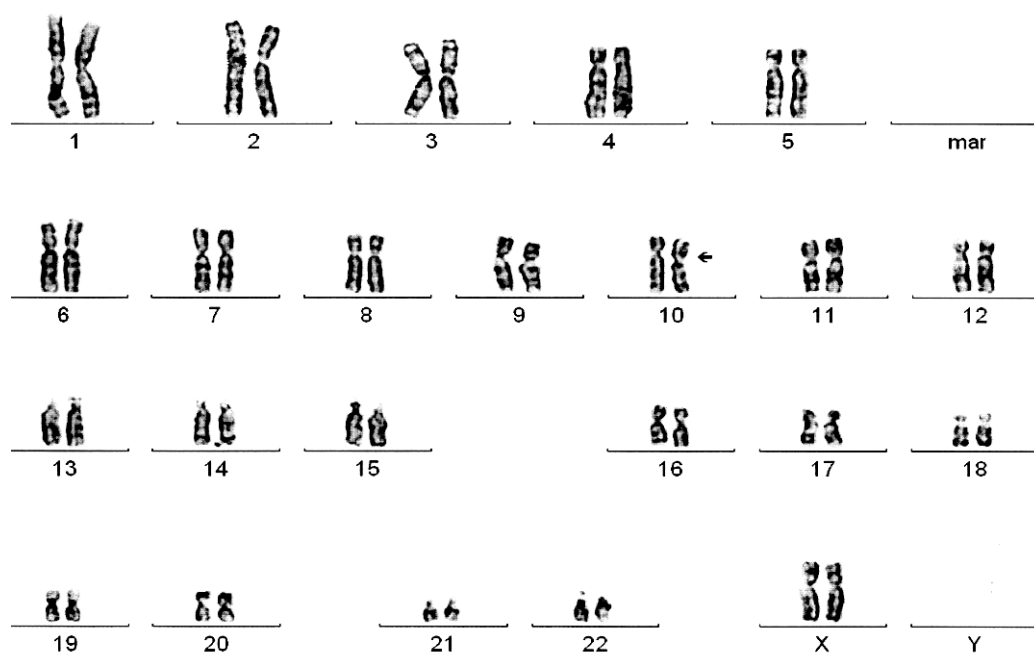


Figure 5 presenting 46,XX,inv(10)(p11.2q21).

## Discussions

The present study in which were included 708 individuals with primary infertility or repeated miscarriages, showed polymorphic variants in 3 males and 6 females. Inversions of chromosomes were observed in 10 females and 6 males.

The banding techniques and the high resolution banding permit the evidencing of more discrete chromosomal anomalies and revealed a great variety of heteromorphisms. In the cases of polymorphic inversions the different orientation of chromosomal segments may lead to misalignment between non-allelic segmental duplications.

The carriers of the inversion may have a risk of *de novo* deletion or other chromosomal rearrangement during meiosis. It is important to know if these variants are “normal” or may be “disease-causing” and it is now known that the contribution of structural variation to the overall heterogeneity of the human genome is considerable.

Due to the fact that the heterochromatin has no coding potential and contain genes for rARN, polymorphic variants on chromosomes were considered “normal”. Despite being categorized as a minor chromosomal rearrangement that does not correlate with abnormal phenotypes, many reports in the literature raised conflicting views regarding the association with recurrent abortions and abnormal clinical conditions. The associations of this “variants” and cases with infertility or recurrent abortions have been reported. Using refined molecular techniques, it is

now thought that genes for fertility and viability are resided in heterochromatin.

## Conclusions

For the carriers there is a risk of formation of a recombinant aneusomy and later the transformation of the inverted chromosome during gametogenesis. The chromosomal unbalance of gametes may produce spontaneous fetal death and malformed offspring. This suggests that cytogeneticists should not ignore these variants and that these play an important role in reproduction failure. Prenatal examination is also indicated. No treatment is available for patients diagnosed as carriers of an abnormal karyotype, and they should be thoroughly counseled to avoid unnecessary reproductive wastage.

The characterization of polymorphisms at the molecular level is not as yet systematic. Due to the fact that banding techniques increased the number of polymorphisms that could be detected microscopically, it is clear that molecular cytogenetics may increase this number even further, leading to the detection of new forms of polymorphisms in the human genome not detectable by previous methods. As we gain more insight into the human genome, the identification and eventual understanding of chromosome variation such as common population inversions and acrocentric short arm variants will probably receive new connotations.

## References

1. E. D. S. Borgaonkar, Chromosomal variation in man. A catalog of chromosomal variants and anomalies Wiley US, 1998.
2. H.E. Wyandt, V.S. Tonk, Kluwer Atlas of Human Chromosome Heteromorphisms. Ed. Acad. Press, Netherlands, 2004.
3. Feuk, L. *et al.* Discovery of human inversion polymorphisms by comparative analysis of human and chimpanzee DNA sequence assemblies. *PLoS Genet.* 1, e56 2005.
4. Iafrate, A. J. *et al.* Detection of large-scale variation in the human genome. *Nature Genet.* 36, 949–951 2004.
5. JIANG Jing, FU Manfen, WANG Defen. Cytogenetic analysis in 61 couples with spontaneous abortions. *Chinese Medical Journal*, 2001, vol. 114 No.2: 200-201.
6. Madon PF, Athalye AS, Parikh FR. Polymorphic variants on chromosomes probably play a significant role in infertility. *Reprod Biomed Online*. 2005. Dec; 11(6): 726-32. Review. PMID: 16417737 [PubMed - indexed for MEDLINE]
7. Samonte, R.V., Conte, R.A., Ramesh, K.H., Verma, R.S.: Molecular cytogenetic characterization of breakpoints involving pericentric inversions of human chromosome 9. *Human Genet*, 98: 576-580, 1996.
8. Sharp, A. J. *et al.* Segmental duplications and copy number variation in the human genome. *Am. J. Hum. Genet.* 77, 78–88 2005.
9. Tabet, A.C., Dupont, A., Lebbar, Couturier-Turpin M.H., Feldemann, D., Rabineau, D. Heteromorphism 18qh+: with or without reproductive consequences? *Ann. Genet.* 44, 139-142, 2001.
10. Teo SH, Tan M, Knight L, Yeo SH, Ng I. Pericentric inversion 9--incidence and clinical significance. *Ann Acad Med Singapore*. 1995 Mar; 24(2): 302-4

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## II. NEONATOLOGY

# HISTOLOGICAL MODIFICATIONS OF THE UMBILICAL CORD IN PREGNANCY INDUCED HYPERTENSION

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### Abstract

**Objective.** The main structural modifications of the umbilical cord in pregnancy induced hypertension (PIH) are presented versus the normotensive pregnancy.

**Material and method.** Over 160 histological sections were obtained from 42 umbilical cords, distributed into two equal monitored groups: one group (n= 21), from mothers with (PIH); and another group (n=21), from normotensive mothers, representing control-group. The histological sections were made from the placental and fetal side of the umbilical cord. The histological method for preparation and the colored stain was that for Hematoxylin-Eosine (H.E.); for the examination of the samples we used an optical microscopy.

**Results.** In the study, were registered the following structural modifications in the pregnancies with PIH versus normal pregnancies:

- diameter and volume reduction of the umbilical cord and umbilical vessels;
- numerical reduction and structural disorders of the smooth muscular fibers and fascicles, from the vascular (and especially, arterial) media;
- vascular endothelium thickening and vascular caliber reduction;
- numerical reduction and structural disorders of the collagen and elastic fibers (especially to the umbilical cord vein). It is a special interest in the constant association of these lesions to the patients with PIH, versus the normotensive cases, where they occur rarely and isolatedly.

**Conclusions.** The above described lesion complex has at least three physiopathological consequences:

- fetal blood stream reduction;
- fetal oxygenation and nutrition reduction, with an impact upon the general development;
- a fetal chronic hypoxemia, with a direct impact upon the fetal cerebral development.

**Key words:** Pregnancy Induced Hypertension (PIH); Umbilical cord

### Introduction

PIH is registered in various studies as an evolutive complication of 6-12% of the pregnancies. Although the etiology is not specified, the emergence of the disease is incontestably related to the presence of the placenta and the complex: placenta – umbilical cord (after the birth and the delivery of the placenta, the arterial hypertension disappears).<sup>1</sup> PIH represents one of the most important causes of: intrauterine growth limitation, premature birth, low birth weight, perinatal mortality. PIH is associated to the increase of the placental – uterine vascular resistance<sup>2</sup>. A lot of studies have shown the existence of some structural differences between the placenta and the umbilical cord of the normotensive and respectively, hypertensive, pregnant women. These differences refer to the thickness (diameter) of the umbilical cord. No relations of causality have been established by now, between the morphological modifications of the placenta, umbilical cord and the degree of the fetal ischemia/hypoxia.<sup>2</sup> Authors like Di Naro, Junex and others, have shown a significant global reduction of the umbilical cord and of its structures, during its entire length to the mothers with PIH versus the normotensive ones. At the level of the cord vessels, these differences are noticed especially in the media and intima, significantly contributing to the alteration of the hemodynamic conditions in the PIH.<sup>3</sup>

Even in a normal pregnancy (normotensive), the thickness of the umbilical cord undergoes an insignificant reduction, achieved mainly due to the Wharton’s jelly; the vascular modifications are quite rare, inconstant and do not realized long lasting hemodynamic alterations. Probably, a certain degree of fetal ischemia/hypoxia, not quantified yet, represents a trigger factor of the birth at the normal time of gestation.<sup>4</sup>

### Material and method

The study was carried out upon 42 umbilical cords sampled with the written consent of the mothers: 21 umbilical cords sampled from mothers with PIH and 21 normotensive mothers. Pieces of umbilical cord of about

2cm were achieved, both from the placental and fetal side, for the both groups of study; of each umbilical cord piece, at least two histological sections were carried out.

The main clinical characteristics of the two groups are presented in table 1.

Table 1. Clinical characteristics of normal pregnancy and PIH (mean values).

Clinical Characteristics	Without PIH (n =21)	With PIH (n=21)
Mother's age (years)	24,09	26,23
Parity (Nullipar/multipar)	13/8	15/6
Birth's type (Spontan/Cesarian section)	15/6	5/16
Gestational age (weeks)	38,09	36,38
Fetal weight (grams)	2928,57	2669,04
APGAR	8,04	7,66
Sistolic Blood Pressure (mmHg)	110,71	152,85
Diastolic Blood Pressure (mmHg)	69,76	99,52

The main factor of differentiation was the value of the blood pressure:

- For the group of normotensive pregnant women, the values of the systolic TA ranged between 100-135 mmHg, and of the diastolic TA, between 60-85 mmHg;

- The difference to the hypertensive pregnant women group was made only in the cases whose values of the systolic TA > 140mmHg and diastolic TA > 90 mmHg.

For the rest of the clinical parameters, significant differences between the two groups were registered also for the gestational age, birth weight, type of the birth and immediate neonatal adaptation.

Because during the study, only a limited number of cases (no. = 7) benefited from Doppler ultrasonography, the fact did not allow us to use this variable in our analysis. We mention that in all subjects to Doppler investigation, an important reduction of the vascular caliber and of the blood stream at the level of the umbilical arteries was registered; we are suggesting for the systematic utilization of this investigation in the PIH cases.

For both groups, the following cases were not included: those with essential hypertension, multiple pregnancy, diabetes mellitus, chronic renal diseases, epilepsy and hematological disorders.

The working method for all of the histological sections followed the usually procedure:

- fixation in a 10% formalin solution;
- dehydration in ethanol gradated series;
- sedimentation in xylene;
- paraffining;
- deparaffining;
- hydration and coloring with hematoxyline – eosine .

We mention that the samples and the measurements were carried out immediately after the birth.

### Results and discussions

The comparative analysis of the main morphometric and histological parameters is presented in table 2.

Table 2. Umbilical cord's morphological parameters.

PARAMETERS	Without PIH		With PIH	
	Placental side (n = 21)	Fetal side (n = 21)	Placental side (n = 21)	Fetal side (n = 21)
Cord's diameter (mm)	11,09	9,71	8,04	7,71
Cord's total area (mm <sup>2</sup> )	95,76	73,96	50,74	45,99
Reduction of the muscular area in arteries	2/21	2/21	17/21	19/21
Smooth muscular cells hypoplastic and discontinuous	1/21	2/21	18/21	18/21
Thickening of the vascular endothelium	0/21	1/21	17/21	20/21
Reduction and disorder of the collagen and elastic fibers	2/21	2/21	15/21	19/21
Reduction of the arterial caliber	2/21	3/21	18/21	19/21
Reduction of the venous caliber	1/21	2/21	19/21	19/21

The diameter and volume reduction of the umbilical cord, is significant in the group with PIH, and it is realized especially due to the Wharton's jelly. All the conditions which lead to the limitation of the uterine growth are characterized by a narrow umbilical cord and a Wharton's jelly very much reduced, until its complete disappearance.

In this sense, PIH represents a real natural model of fetal malnutrition and hypoxia. The histological lesions are registered almost constantly in the cases with PIH versus the normotensive group. The reduction of the vascular

dimensions is constantly accompanied by significant structural disorders which have an impact upon the vascular intima, media and fibrillary structures.

These structural modifications are associated quasi-constantly with the cases with PIH versus the normotensive cases, in which they appear quite rarely and isolatedly (Figure 1-4). There are some minimum structural modifications, quite rare and never associated with the normotensive cases, suggests processes of prenatal vascular senescence, common at the normal term of gestation.

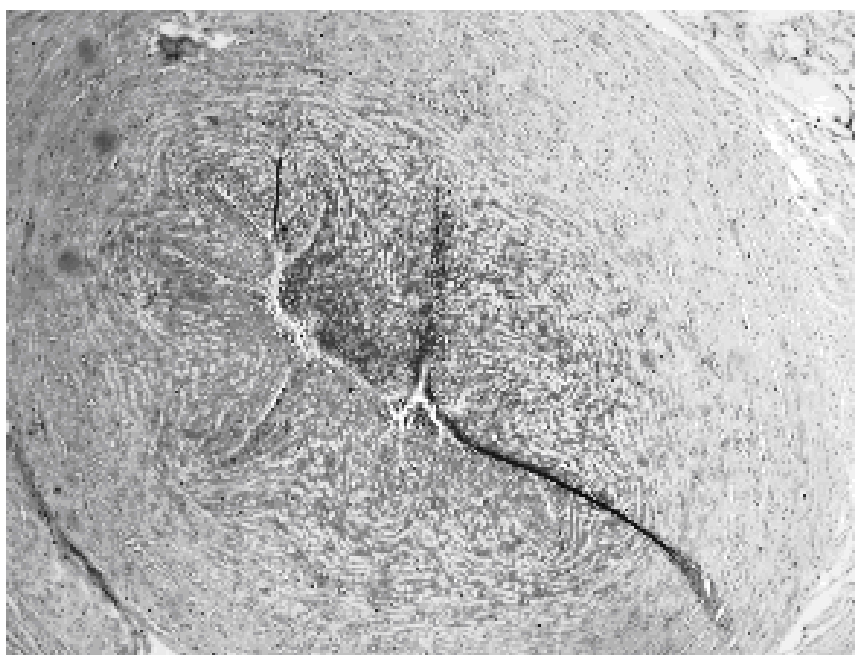


Fig. 1. H.E. X 10, General view of the umbilical cord artery, with significant narrowing lumen and muscular disorders.

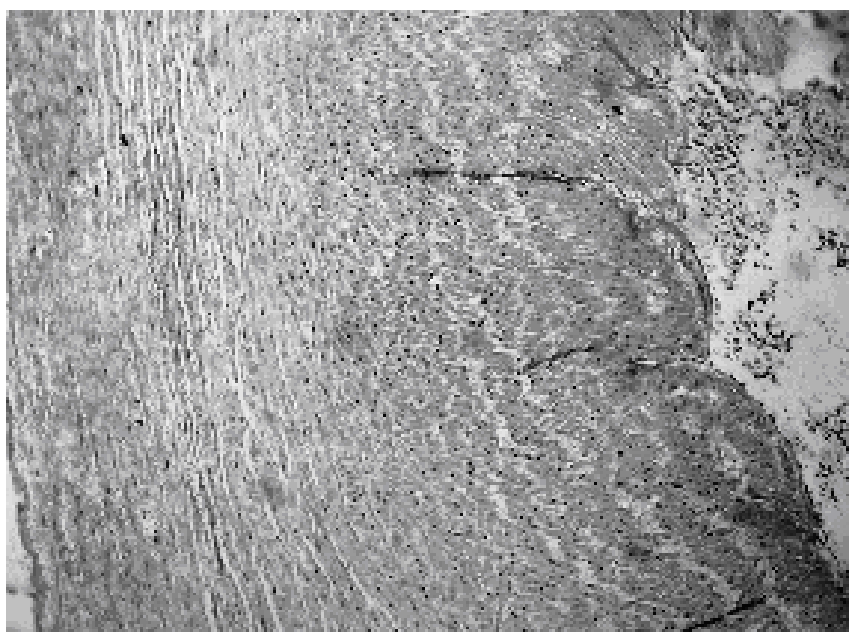


Fig. 2. H.E., x 40, the muscle area disposed in separated layers, due to the increase of the connective tissue and to the edema.

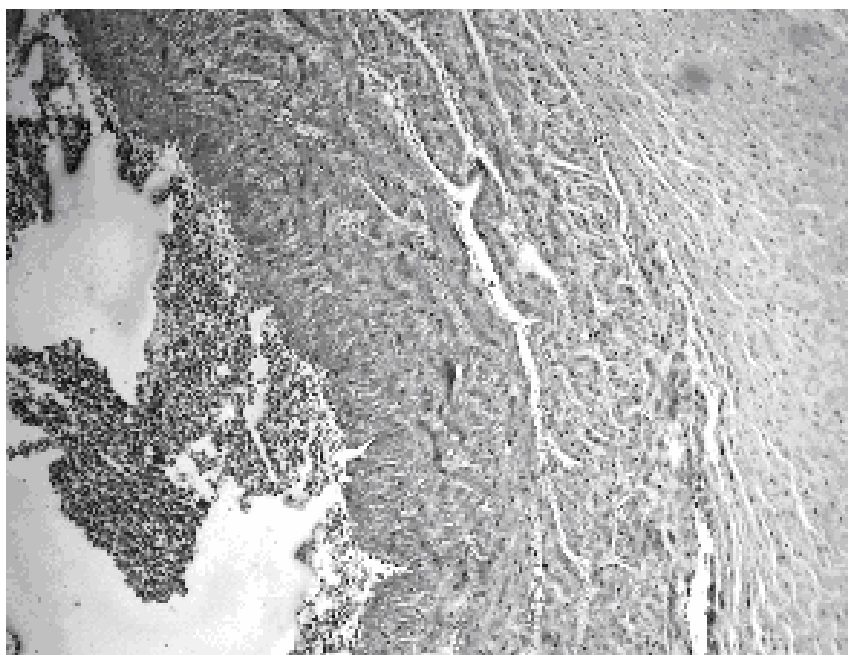


Fig. 3. H.E., x 100, detail of the umbilical vein, with smooth muscle cells contracted and separated from each other; endothelium, subendothelium and some muscle layers have join completely.



Fig. 4. H.E., x 40, umbilical vein lumen narrowed, with separations between the muscle cells and layers; muscle cells contracted with a waved like aspect of the nucleus; muscle area separated from the connective tissue.

It is recognized the fact that the key-factor, which contributes to the growth and development of the vascular tree on the axis: placenta → umbilical cord → foetus, is the progressive growth of the blood stream. In PIH cases, a placental vascular disorder is initially produced, which is accompanied by the growth of the placental resistance and the reduction of the umbilical blood stream, with a fetal hypo-perfusion. The maintenance of these hemodynamic conditions leads to the stabilization of the vascular and umbilical cord structural pathological modifications and to their constant association and extension, while they are following the above mentioned vascular vector.<sup>5</sup>

The following significant morphological modifications were registered in the cases with PIH (table 2):

- the significant diameter reduction of the umbilical cord and its vessels; the most important reduction was registered for the diameter of the umbilical cord and it was realized due to the Wharton's jelly reduction;<sup>6</sup>

- there is a reduction of the smooth muscular fibers and fascicles number in the media of the umbilical arteries;<sup>7</sup> under the arterial epithelium, among the muscular layers extended acellular spaces occur, probably due to the interstitial edema; the contraction of the

muscular cells cause a “wave”-like shaped orientation of the nuclei ;

- the muscular area, separated by a conjunctive tissue tends to become narrower and thus, contributing to the diameter reduction of the lumen, more noticeable in arteries;

- the smooth muscular cells seem to be diminished or hypoplastic; in many areas, these seem to be discontinuous;<sup>8</sup>

- the thickening of the vascular endothelium and the significant reduction of the vascular caliber, both for arteries and for the umbilical cord; because the vascular endothelium is the first layer which undergoes to the hemodynamic modifications, it is possible that the reaction to be produced precociously, even at the beginning of the PIH;<sup>9</sup>

- the numerical reduction and the structural disorder of the collagen and elastic fibers are more noticeable in the umbilical vein where, under normal conditions, they are better represented.

The above mentioned morphological modifications are suggestive for a predominantly hypoplastic mechanism at a vascular level. The first reaction to hypoxemia is the vasoconstriction. If the hypoxemia continues, it shall produce in time the above mentioned hypoplastic modifications, with immediate and late hemodynamic

consequences. The morphological modifications of the umbilical vein wall and its caliber directly influence the fetal blood stream, which has an impact upon the fetal vascular system; the modifications of the fetal vascular system may represent a main factor, for vascular affections of the future adult.<sup>10</sup>

### Conclusions

The morphological modifications of the umbilical cord in the PIH represent a marker of some important postnatal and fetal hemodynamic deficiencies.

The hemodynamic status of the foetus and of the new-born baby with mothers suffering from PIH are characterized by hypoxia/ischemia with an immediate and late impact upon their cerebral development.

A good quantification of the morphological modifications of the umbilical cord in PIH provides an informational support to the practitioner concerning the baby's neurological future.

A systematically prenatal monitoring of the hemodynamic of the feto-placental circulation (including Doppler) may reduce the incidence of the severe forms of the intrauterine development and growth in the new-born babies with mothers suffering of PIH.

### References

1. Sweeney M, Jones C.J, Greenwood S.L, Baker P.N, and Taggart M.J. Ultrastructural features of smooth muscle and endothelial cells of isolated isobaric human placental and maternal arteries. *Placenta* 2006; 27: 635-647.
2. Mitra S.C, Seshan S.V, and Riachi L.E. Placental vessel morphometry in growth retardation and increased resistance of the umbilical artery Doppler flow. *J Matern Fetal Med* 2000; 9: 282-286.
3. Di Naro E, Ghezzi F, Raio L, Franchi M, and D'Addario V. Umbilical cord morphology and pregnancy outcome. *Eur J Obstet Gynecol Reprod Biol* 2001; 96: 150-157.
4. Junek T, Baum O, lauter H, Matejevic D, And Graf R. Pre-eclampsia associated alterations of the elastic fibre system in umbilical cord vessels. *Anat Embryol* 2000; 201: 291-303.
5. Sevinc I, Muzaffer S, Deniz C, Seda V, Ozgur O, and Sivekar T. Comparative morphological differences between umbilical cords from chronic Hypertensive and preeclamptic pregnancies. *Acta Medica Okayama* 2002; 4: 177-186.
6. Romanovicz L, And Sobolewski K. Extracellular matrix components of the wall of umbilical cord vein and their alterations in pre-eclampsia. *J. Perinat Med* 2000; 28: 140-146.
7. Dadak C, Ulrich W, and Sinzinger H. Morphological changes in the umbilical arteries of babies born to pre-eclamptic mothers: an ultrastructural study. *Placenta* 1984; 5: 419-426.
8. Cetin A, Kukner A, and Ozturk F. Ultra structure of human umbilical vessels in pre-eclampsia. *J Matern Fetal Neonatal Med* 2002; 12: 178-184.
9. Sulbaran T.A, Castellano A, Chacin L, Vergel C, Portillo M.A, Urbina E, Silva E.R, and Castejon O.J. Electron microscopy of umbilical cord endothelial cells in preeclampsia. *J Submicrosc Cytol Pathol* 2002; 34: 389-395.
10. Stehbens W.E. Wakefield J.S, Gilbert-Barness E, and Zuccollo J.M. Histopathology and ultrastructure of human umbilical blood vessels. *Fetal Pediatr Pathol* 2005; 24: 297-315.

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### III. PEDIATRICS

## CONSIDERATIONS ON A CASE OF SYSTEMIC SCLERODERMA IN CHILD

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#### Abstract

The authors present a patient who was diagnosed with scleroderma at the age of 7 years, sustained by the typical aspect of the cutaneous modifications (hard edema at the level of the face, neck and limbs), difficulties in opening the mouth and histological modifications (the atrophy of the epidermis and the increase in thickness of the derm through the proliferation of the dermic conjunctive). In evolution, until the age of 16 years, the cutaneous lesions became general and complications appeared: oesophagitis of reflux, articular modifications, hypacusia, muscular tiredness and systolic breath in the mitral focus. The case was appointed in scleroderma, systemic clinical form. Comments were made on the differential diagnosis, on the treatment, the evolution and prediction. The presentation of the case is motivated by the rarity of scleroderma and especially of the systemic clinical form at child. The hypacusia was considered as a particularity of the case.

**Key words:** scleroderma, systemic clinical form, child.

#### Introduction

Systemic scleroderma (Ss) is a chronic, multi – systemic disease, characterized by the hardening, increasing in thickness and the rigidity of the teguments and by modifications of fibrotic type, inflammatory and vascular of certain organs. (1, 10) The etiology is unknown. It is however sustained the role of heredity and of certain environment factors. (2, 10) The pathogenesis of this disease is not clear. (1) From the pathogenic point of view it is characterized by anomalies of the metabolism of the collagen (excessive proliferation of mesenchymatous cells, fibroblasts, myocytes, endothelial cells, which, activated by unknown factors determine an increased synthesis of collagen I, III, X and other components of the conjunctive tissue with excessive deposit inside the skin, systems and organs), associated with vascular anomalies (hyperplasia of intimate, of small arteries and vasospasm). (2, 10) A vascular lesion, possibly caused by deposition of immune complexes or by release of citotoxic factors, seems to be at the origin of the disease. As a consequence, platelet adhesion and activation might occur in sclerodermic patients. The observation that platelet might release, upon aggregation, a potent mitogenic factor, named Platelet

Derived Growth Factor has focused interest on platelets as the potential mediators of the fibrotic process characteristic of SS. (1) The skin of patients with SD is characterised by an excess accumulation of collagen in the extracellular matrix of the fibrotic reticular derms. (4) Abnormalities in newly formed collagen structures as well as splitting of newly formed collagen fibrillae into microfibrillae were observed. (8) Elastic fibers are also disrupted in this disease, however, in contrast to collagen relatively few studies have provided information concerning the changes that occur to elastic fiber components in SD. (4, 9) Eosinophilia as a possible heart damaging factor in Ss in children. It was discovered that associated Ss and eosinophilia ran a course marked by more well – defined exudative reactions, with the heart being injured more frequently and gravely. A correlation was noted between the “sclerodermic” heart and the eosinophil count in the peripheral blood. (5) The disease is accompanied by immunologic cellular and humoral anomalies. (2, 10) As spreading, the SD is met all over the world, but the frequency of the disease is relatively small. (10) At the child the frequency is smaller than at the adult. (2, 3) At the girls the frequency is more than at the boys. (3)

#### Clinical observation

The patient M.C. is presented, who was hospitalized for the first time in January 1998, at the age of 7 years, for edemas and discrete myalgias. At the clinical examination was noticed a hard edema at the level of the face, neck and limbs and a slight difficulty in speaking. The diagnosis of SD was suspected which was confirmed by cutaneous biopsy (made at IOMC Bucharest) which showed: hyperkeratosis, formation of infundibulate corneous corks, the epidermis moderately atrophic, the derm increased in thickness with approximately 3 times the normal thickness, the dermic conjunctive increased in thickness disposed with horizontal stripes, fragmented, for the rest, the clinical examination on apparatuses and the paraclinical one did not show any pathologic modifications. For 7 years the child was lost from evidence. At the age of 14 years, in March 2005, she is hospitalized again for epigastric pains, dysphagia, pyrosis, hematemesis and melaena. The barium transit showed hypokinesia of the esophagus. (figure 1) For the rest, the digestive tube was normal.

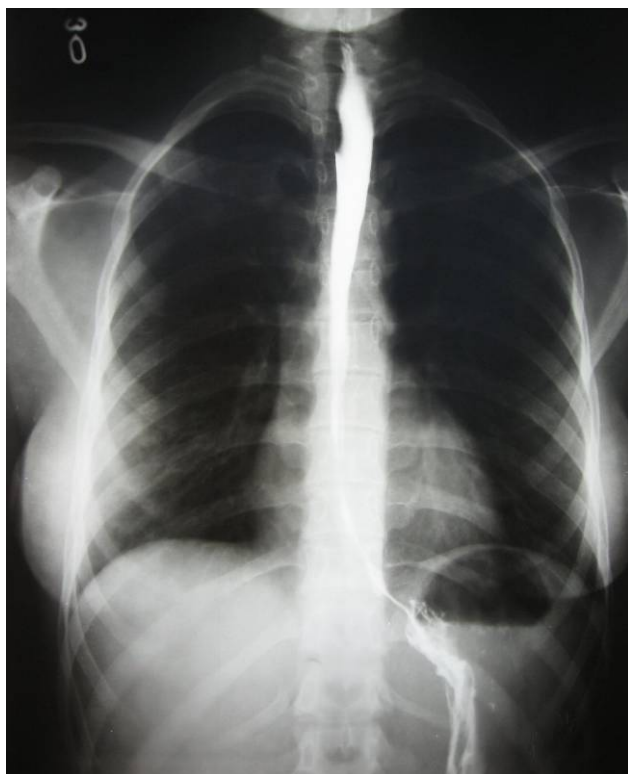


Fig. 1 SD. Barium radiography.  
Hypokinesia of the esophagus.

The esophagoscopy distinguished oesophagitis of reflux and esophageal cankers in the inferior third. After a year, in January 2006, she returns for: vertigo, headache, hypacusia. In January 2007 she is hospitalized again (F.O. 2452 / 15.01.2007) for the clinical and paraclinical revaluation accusing headache and tiredness to effort. The clinical examination finds a 16 year old teenager, with a

weight of 41 Kg, waist 150 cm, with hardened, rigid, infiltrated teguments. At the face level the teguments were glossy, spread, with the cutaneous pleats erased and lack of expression of the mimics. (figure 2) She has difficulties in moving her mandible and opening the mouth.

At the level of the hands, a slight fixing in semiflexion of the fingers was ascertained. (figure 3).



Fig. 2 Ss. Edemas and the rigidity  
of the teguments of the face.



Fig. 3 Ss. Fixation in semiflexion of the fingers of the hand by increasing the thickness and by sclerosis of the teguments.

More than the previous hospitalizations a systolic breath is noticed, degree II at the top of the heart.

Paraclinical explorations: HLG, serum iron, the inflammatory tests (VSH, fibrinogen, CRP), summary urine exam, urea, creatinine, uric acid, glycaemia, calcemia, blood ionogram, electrophoresis of proteins, transaminase, CP-K, aldolase were normal. FR and ANA absent. Immunelectrophoresis showed a raise of IgM (300ui/ml, N=100-200 ui/ml), FO normal, IDR 2u PPD = 0 mm.

Functional explorations : EEG, EKG, EMG – normal range; Normal breathing functional tests .

Imagistic explorations: abdominal echography – normal, cardiac lung radiography without pathologic modifications, hands radiography – normal, sinuses radiography – bi-jaw sinusitis.

Corroborating the case history data with the actual clinical and paraclinical exam the following diagnosis was reached: Systemic scleroderma (diffuse cutaneous form with late visceral affection). Oesophagitis. Hypacusia. Jaw sinusitis. Cardiopathy?

### Discussions

The positive diagnosis was sustained by the typical aspect for SD of the cutaneous modifications (confirmed by histological examination), to which the visceral affection was added in time (oesophagitis, muscular affection expressed by tiredness to effort and possibly heart affection). Lung and renal sufferance was not distinguished, Raynaud syndrome (met at 95% of the ill) (10) and neither visceral touches more rare (Sicca syndrome). From the point of view of the classification, the case was framed in Ss with diffuse cutaneous fibrosis and particularly with late visceral affection (non characteristic Ss diffuse which presents precocious visceral affection). (10) The differential diagnosis was made with:

- a) The localized sclerodermias (in plates and in band) which are also frequent at the child and present

cutaneous lesions limited to the skin and subcutaneous tissue. (2, 3, 10)

- b) Eosinophilic fasciitis (Schulmann syndrom) similar SD from which differs through characteristic eosinophilia in the blood and in the inflamed areas (at the bioptic) and good response to cortisone.
- c) Mixed disease of the conjunctive tissue (Scharp syndrome), characterized by common elements LES, DM and SD and good response to cortisone
- d) Secondary forms of SD (after medications, chemical substances, medulla transplant).
- e) Pseudosclerodermias (sclerema, scleromixedem, cutaneous amyloidosis).

The treatment is deceptive (7) and it has not been definitively standardized. (6, 11) The administration of immunodepressives, agents that diminish the collagen production or factor XIII do not have noticeable therapeutic results. (2, 3, 7) The presented case was initially treated for the cutaneous affection in the stage of edema with prednisone having an unsatisfying effect. Subsequently, the treatment of oesophagitis was made with rest with the bed raised at the head, diet regime, Ranitidine, Metoclopramid and Dicarbocalm, with a very good evolution. A recovery program was applied for the increase of the elasticity of the tegument and articular mobilities, by hydrothermal treatment and kinetotherapy. At the same time, we tried to diminish the hardening of the teguments by administration of vitamin E and application of ointments for the prevention of skin dryness and for the anti-inflammatory effect (ointment with hydrocortisone and emollient substances) in the areas more affected with modest benefits.

SD has a chronic evolution with progression more or less rapid. The systemic forms have a severe evolution, potentially fatal by renal, heart and lung lesions and not validating by cutaneous lesions. (6) The duration of survival after the age of 5 years is met in 50-70% of cases and of 10 years in 40-60% of cases. (10) The case presented, after the

last revaluation was framed in the forms with a less severe evolution (not characteristic for Ss), motivated by the fact that 9 years after the diagnosis was put, it surely presents only esophagial and articular affection and possibly heart and muscular affection, but the evolution is not validating by the generalized cutaneous lesions and hypacusia.

### Conclusions

The case was presented due to the very small frequency of SD at child (20 cases at 1000000 general population of which less than 8-10% at child) (2) and also

because of the fact that the clinical form of Ss described in that particular case is not characteristic to the child.

Taking into consideration the continuous evolution of the disease, it is imposed that the ill person to be periodically revaluated and the medicine and recovery treatment should be adapted to the functional modifications found.

### Particularity of the case

Is constituted by the signalling of the hypacusia as a complication not registered in the specialty literature studied.

### References

- Altomare GF, Polenghi MM, Pigatto PD et al – Growth factors in the pathogenesis of progressive systemic sclerosis. *G Ital Dermatol Venereol*, 1989, 124 (5), 187-192.
- Arion C, Dinu B – Boli inflamatorii sistemice. În: Ciofu E, Ciofu C (ed.): *Pediatria Tratat*, 965-967, Ed. Medicală, București, 2001.
- Bodemer C, Belon M, Hamel-Teillac D et al – Scleroderma in children: a retrospective study of 70 cases. *Ann Dermatol Venereol*, 1999, 126 (10), 691-694.
- Davis EC, Blattel SA, Mecham RP – Remodeling of elastic fiber components in scleroderma skin. *Connect tissue Res*. 1999, 40 (2), 113-121.
- Deliagin VM, Uvarova NN, Covaleva VN – Eosinophilia as a possible heart damaging factor in systemic scleroderma in children. *Pediatria*, 1991, (3), 38-42.
- Duport G, Gayet LE, Bascou V, Larreque M – Lesions of the hand and upper limb in children with linear scleroderma. Therapeutic approach in two cases. *Ann Chir Main Memb Super*, 1996, 15 (1), 5-10.
- Frați Munari AC, Culebro Nieves G, Velazquez E et al – Scleroderma in children. *Boll Med Hosp Infant Mex*, 1979, 36 (2), 201-214.
- Iacovleva GI, Gritsmann NN – Formation and destruction of the skin collagen structures in systemic scleroderma. *Arkh Patol*, 1978, 40 (11), 28-35.
- Quaglino D Jr., Bergamini G, Boraldi F et al – Connective tissue in skin biopsies from patients suffering systemic sclerosis. *J Submicrosc Cytol Pathol*, 1996, 28 (2), 287-296.
- Șuțeanu ȘT – Scleroderma sistemică. În: Păun R (ed.): *Tratat de Medicină Internă. Reumatologie*, vol. 2, 911-925, Ed. Medicală București, 1999.
- Teleche N – Recuperarea medicală a bolilor reumatismale. În: Păun R (ed.): *Tratat de Medicină Internă. Reumatologie*, vol. 2, 1860, Ed. Medicală, București, 1999.

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# ADOLESCENT WITH CYSTIC FIBROSIS ASSOCIATED LIVER DISEASE, DIABETES MELLITUS AND POOR COMPLIANCE TO TREATMENT- CASE REPORT

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## Abstract

The clinical outcome of cystic fibrosis patients seemed to be defined by the pulmonary condition, but, in the recent years, the liver disease became an important feature with potential impact on clinical outcome and life expectancy. Liver disease associated with cystic fibrosis is the second cause of death among CF patients. The aim of the paper is to present the case of an 15 years old boy, followed up in our CF Center with cystic fibrosis (CF) associated liver disease (LD).

The patient diagnosed with CF at the age of 3 month, presented for the first time in our clinic at 12 years old. He was registered in our centre with the following diagnosis: Cystic fibrosis case, complete form,  $\Delta F508$  homozygous, complicated with: *Pseudomonas aeruginosa* respiratory infection, medium obstructive pulmonary syndrome, bronchiectasis, associated liver disease, clubbing and failure to thrive. After starting the treatment with ursodeoxycholic acid, hepatomegaly decreased and liver tests normalized in 6 months. Two years after, patient was admitted in our clinic a change of mood and irritability associated with refusal of therapy, presenting visible abdominal circulation, hepato- and splenomegaly. The investigations confirmed portal encephalopathy, impaired glucose tolerance, modified liver texture on ultrasound examination, and magnetic resonance investigation confirmed the multilobular cirrhosis. He was discharged from hospital with dietary and therapy recommendation. In evolution, the case complicated with: portal hypertension and portal encephalopathy. Diabetes mellitus developed, with conspicuous hyperglycemia, but the adolescent refused starting the insulinotherapy. Lung function deteriorated, obstructive syndrome accentuated and frequent exacerbations of pulmonary disease occurred. Psychological issues expressed in refusal of therapy, irritability. Besides the matters concerning the medical management of a case with LD and diabetes, the psychological issues related to age or hepatic encephalopathy occur, making more difficult the manner of patient's life and worsening the course of disease.

**Key words:** cystic fibrosis, liver disease, diabetes, psychological issue, children

## Introduction

Improved life expectancy and prolonged follow up of patients with cystic fibrosis have allowed direct observation

of an increasing number of liver-related events. A broad spectrum of hepatobiliary manifestations have been recognized that include specific alterations ascribable to the underlying cystic fibrosis transmembrane regulator (CFTR) defect as well as lesions of iatrogenic origin or that reflect the effects of a disease process occurring outside the liver.

The aim of the paper is to present the case of an 16 years old boy, followed up in our CF Center with cystic fibrosis (CF) associated liver disease (LD).

## Case presentation

The patient was diagnosed with CF at the age of 3 month, presented for the first time in our clinic at 12 years old.

Clinical status at the first admittance was characterized by: moderate failure to thrive, clubbing, medium pulmonary condition and hepatomegaly.

## Laboratory investigations

Biochemical findings consisted in abnormal values of liver test (AST, ALT,  $\gamma$ -GT). Bilirubinemia, albuminemia and coagulation parameters were normal. Also investigation for hepatitis B virus, hepatitis C virus, cytomegalovirus, Epstein Barr and  $\alpha$ fetoprotein were negative. Concerning the pulmonary disease, tests showed medium obstructive respiratory disease, bronchiectasis and *Pseudomonas aeruginosa* present in sputum culture. The genetic test performed revealed homozygous genotype  $\Delta F508$ . Ultrasound examination showed increased echogenicity liver texture, without signs for vascular decompensation, like portal hypertension.

## Clinical course

After starting the treatment with ursodeoxycholic acid in dose 20 mg/ kilo/day, liver function tests normalized in 6 months and a decrease of hepatomegaly was registered. He was released at home with recommendation for treatment consisting in oral antibiotics for infection associated with aerosols therapy and physiotherapy for the pulmonary condition and liposoluble vitamins supplements.

Two years after, patient was admitted in our clinic presenting visible abdominal circulation, hepatomegaly and splenomegaly (fig.1).

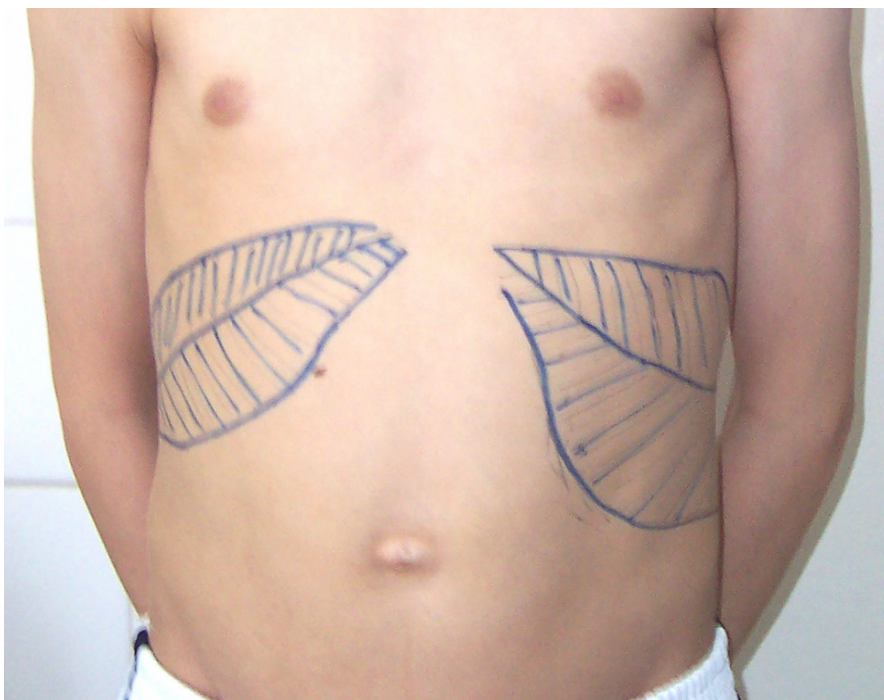


Fig.1.Spleno-hepatomegaly.

His mother observed a change of mood and irritability associated with refusal of any therapy. Investigations confirmed hepatic encephalopathy, with suppression of electric rhythm, impaired glucose tolerance, modified liver texture on ultrasound examination (fig.2), and

magnetic resonance examination (MRI) confirmed the multilobular cirrhosis and splenomegaly with portal hypertension (fig.3). Functional changes of liver was remarked at scintigraphy, with unhomogenous captation of reactive substance.(fig.4).

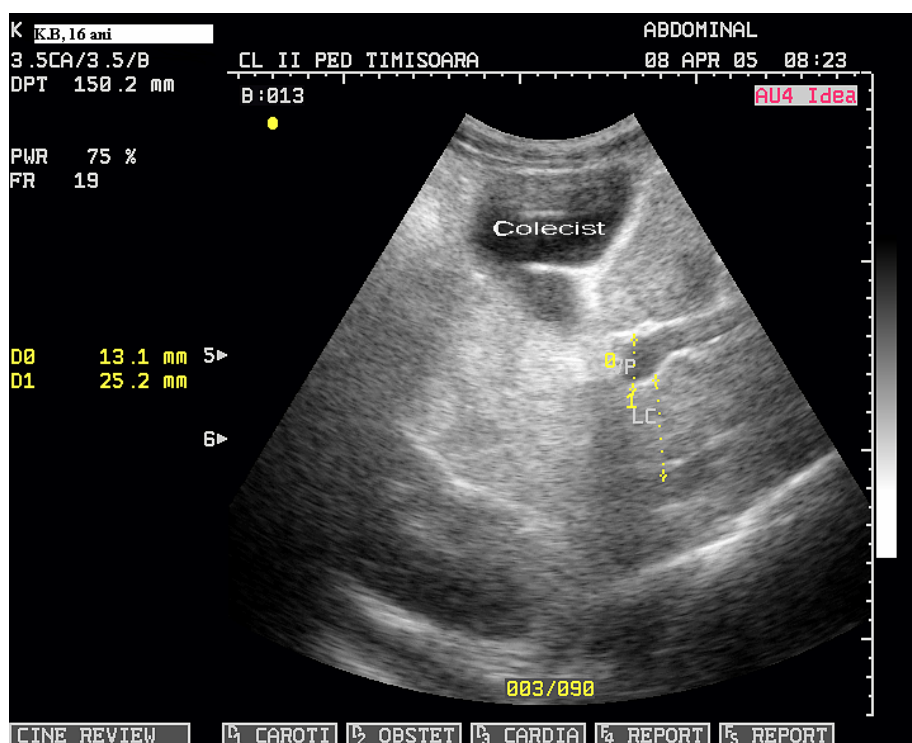


Fig.2-Ultrasound examination.

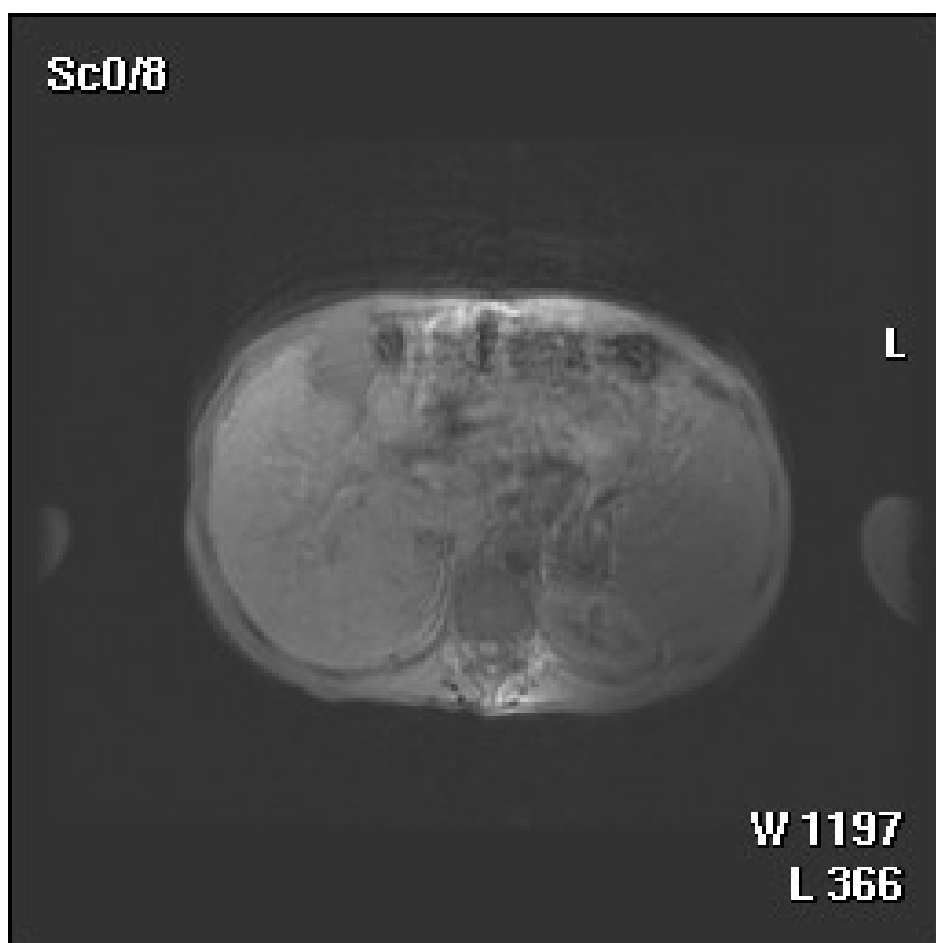


Fig.3 MRI scan.

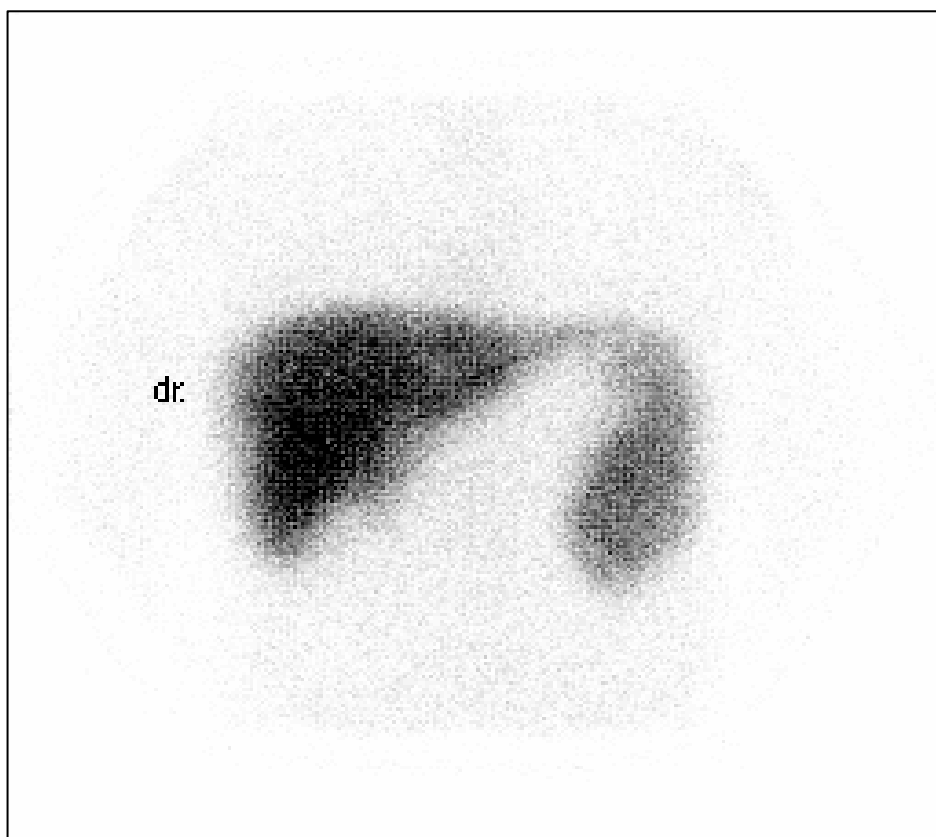


Fig.4-Hepato-biliary scintigraphy.



In evolution, the case complicated: concerning the liver disease, multilobular cirrhosis occurred with portal hypertension and neuropsychiatric symptoms became more evident. Diabetes mellitus developed, with conspicuous hyperglycemia, but the adolescent refused starting the insulinotherapy. The lung function deteriorated, the obstructive syndrome accentuated and frequent exacerbations of pulmonary disease occurred.

Psychological issues were accentuated by the occurrence of encephalopathy, beside the insulin injections necessary for control of diabetes in to a very difficult period of his life, the adolescence.

Finally he was convinced to accept the treatment with insulin, ursodeoxycholic acid, inconstantly diet and aerosol therapy and, in pulmonary exacerbation - intravenous antibiotherapy.

In conclusion, liver disease is a relatively frequent and early complication of cystic fibrosis. The pathogenesis is apparently multifactorial, with contributions of environmental and genetic determinants.

Its impact on quality of life and survival will increase in future years, the early detection and treatment will become an important issues. Ursodeoxycholic acid is the only treatment currently available, but novel therapeutic options are being evaluated.

Beside the matters concerning the medical management of a CF case with liver disease and diabetes, the psychological issues related to age or hepatic encephalopathy raise, making more difficult the manner of patient's life and worsening the course of disease.

## References

- Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. *N Engl J Med* 2005; 352:1992-2001.
- Colombo C, Battezzati PM. Liver involvement in cystic fibrosis: primary organ damage or innocent bystander? *J Hepatol* 2004; 41:1041-1044.
- Sokol RJ, Durie PR. Recommendations for management of liver and biliary tract disease in cystic fibrosis. Cystic Fibrosis Foundation Hepatobiliary Disease Consensus Group. *J Pediatr Gastroenterol Nutr* 1999; 28(Suppl 1):1-13.
- Cystic Fibrosis Foundation. Patient Registry 2003: Annual Report to the Center Directors. Bethesda, Maryland: Cystic Fibrosis Foundation; 2004.
- Lindblad A, Hultcrantz R, Strandvik B. Bile duct destruction and collagen deposition: a prominent ultrastructural feature of the liver in cystic fibrosis. *Hepatology* 1992; 16:372-381.
- Mehta A. CFTR: more than just a chloride channel. *Pediatr Pulmonol* 2005; 39:292-298.
- Oppenheimer EH, Esterly JR. Hepatic changes in young infants with cystic fibrosis: possible relation to focal biliary cirrhosis. *J Pediatr* 1975; 86:683-689.
- Colombo C, Apostolo MG, Ferrari M, *et al.* Analysis of risk factors for the development of liver disease associated with cystic fibrosis. *J Pediatr* 1994; 124:393-399.
- Wilschanski M, Rivlin J, Cohen S, *et al.* Clinical and genetic risk factors for CF-related liver disease. *Pediatrics* 1999; 103:52-57.
- Lindblad A, Glaumann H, Strandvik B. Natural history of liver disease in cystic fibrosis. *Hepatology* 1999; 30:1151-1158.
- Colombo C, Battezzati PM, Crosignani A, *et al.* Liver disease in cystic fibrosis: a prospective study on incidence, risk factors and outcome. *Hepatology* 2002; 36:1374-1382.
- Lamireau T, Monnereau S, Martin S, *et al.* Epidemiology of liver disease in cystic fibrosis: a longitudinal study. *J Hepatol* 2004; 41:920-925.
- Corbett K, Kelleher S, Rowland M, *et al.* Cystic fibrosis-associated liver disease: a population-based study. *J Pediatr* 2004; 145:327-332.
- Debray D, Lykavieris P, Gauthier F, *et al.* Outcome of cystic fibrosis-associated liver cirrhosis: management of portal hypertension. *J Hepatol* 1999; 31:77-83.
- Ling SC, Wilkinson JD, Hollman AS, *et al.* The evolution of liver disease in cystic fibrosis. *Arch Dis Child* 1999; 81:129-132.
- Stewart L. The role of abdominal ultrasound in the diagnosis, staging and management of cystic fibrosis liver disease. *J R Soc Med* 2005; 98:17-27.
- Colombo C, Battezzati PM, Podda M, *et al.* Ursodeoxycholic acid for liver disease associated with cystic fibrosis: a double-blind multicenter trial. *Hepatology* 1996; 23:1484-1490.
- Lindblad A, Glaumann H, Strandvik B. A two-year prospective study of the effect of ursodeoxycholic acid on urinary bile acid excretion and liver morphology in cystic fibrosis. *Hepatology* 1998; 23:166-174.

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# INTERVENTION OF ALPHA DORNASE (PULMOYZME) ON IMPROVEMENT OF RESPIRATORY PARAMETERS IN CYSTIC FIBROSIS

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## Abstract

In cystic fibrosis (CF), respiratory disease has leading role on disease's progression (1,7). Two aspect are very important in management of respiratory disease (4,8,9) – infection control and improvement of pulmonary secretion clearance (IPSC). Aerosol therapy include: anti-inflammatory medication, bronchodilators, antibiotics and mucolytic drugs. About mucolytic drugs, there are two means - with N-acetylcysteine and alpha dornase (Pulmozyme).

**Key words:** cystic fibrosis, infection control, pulmonary secretion clearance, aerosol therapy.

## Study background

In cystic fibrosis (CF), respiratory disease has leading role on disease's progression (1,7). From this point of view, two aspect are very important in management of respiratory disease (4,8,9) – infection control and improvement of pulmonary secretion clearance (IPSC). IPSC could be achieved through clearance technique (CT) and aerosols therapy (AT). Aerosol therapy include: anti-inflammatory medication, bronchodilators, antibiotics and mucolytic drugs. In fact, mucolytic drugs and CT signify one of the most important share of management in CF (9).

About mucolytic drugs, there are two means - with N-acetylcysteine and alpha dornase (Pulmozyme). N-acetylcysteine is a classical mucolytic agent. Opinions are different about efficacy (2,3,5). Alfa dornase is a new mucolytic agent, already with a large usefully. During the immunological local conflict from bronchial tree, is delivered a huge leukocyte DNA. Leukocyte DNA increase the thick of bronchial secretions. Alfa dornase cleavage the leukocyte DNA and clearance the sputum (6). However, there are any controversys about relation price/efficiency (3).

## Aim study

The aim study is to compare the respiratory parameters of alpha dornase therapy versus acetylcysteine.

## Study group and methods

• We performed two homegenous groups, each group formed by 6 patients with chronic pulmonary infection (3 with *Staph. aureus* and 3 with *Pseudomonas aeruginosa*). The including criterions:

- pulmonary stable condition
- aged over 12 years
- FEV1  $\geq$  45%
- FVC  $\geq$  50%
- Whithout mixed infection

• First evaluation was performed after 2 month and second evaluation after 6 month.

• All patients followed the same physiotherapy programme

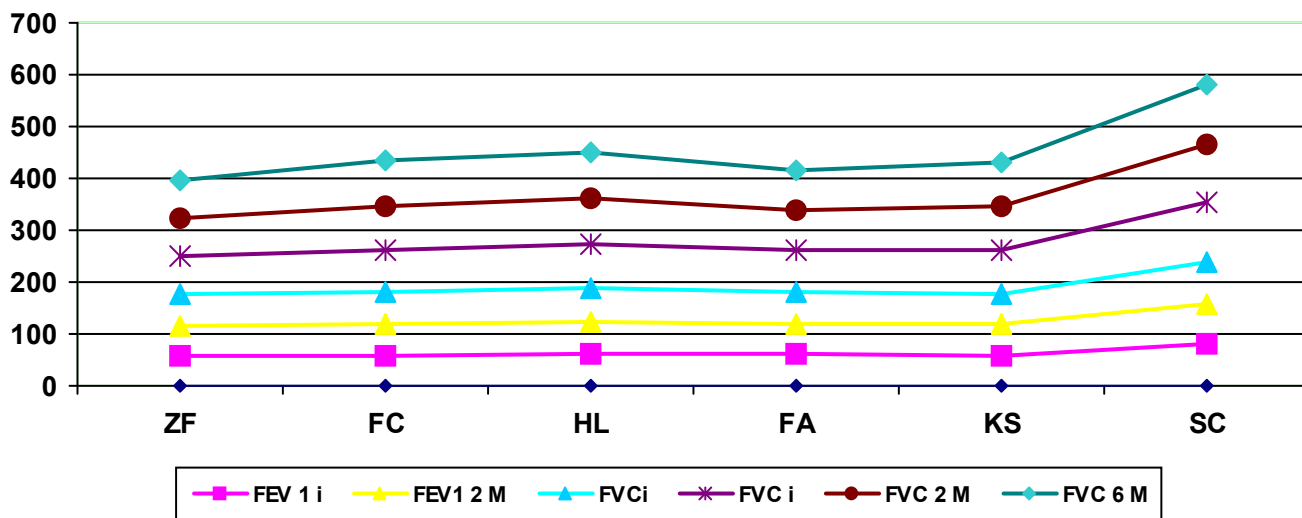
• Aerosols therapy consist of N-acetylcysteina (group 1) and alpha dornase (group 2) through jet nebuliser (Pary Boy device).

## Results

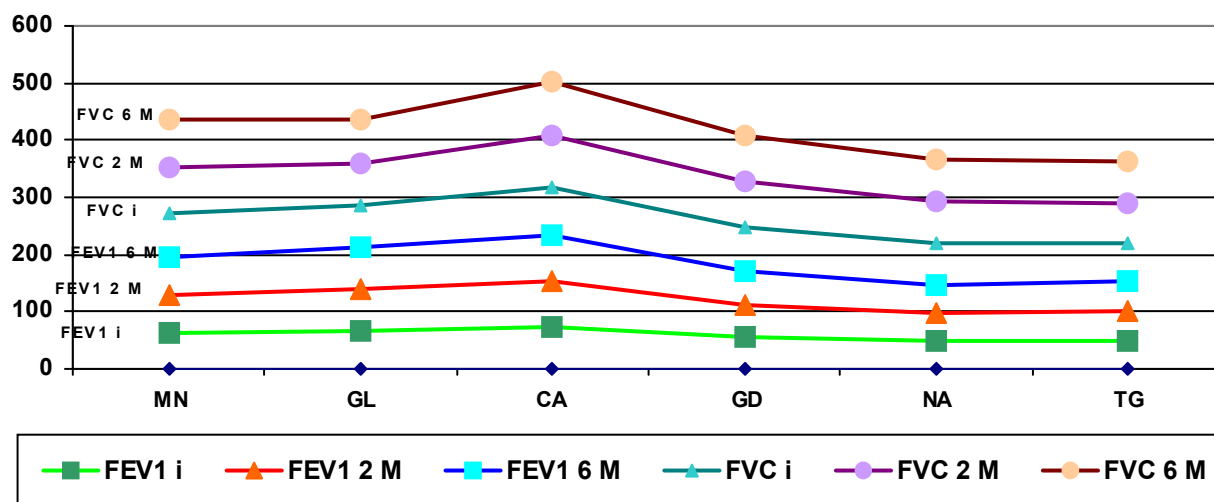
• At group 1 (N-acetylcysteina) increase of respiratory parameters was more significant during the first 2 month, with subsequent slowly increase afterwards. Values final were: FEV1 increse with 2,08% and FVC increse with 2,13 % (Fig.1)

• At group 2 (alpha dornase) respiratory parameters increse al 2 mouth with 4,8% (FEV1), respectively 4,1% (CFV). At 6 mouth FEV1 increased with 6,1% and CFV with 5,85% (Fig. 2). Answer to therapy was quit similar in patients infected with *Staf. aureus*, comparing to *Pseudomonas aeruginosa*

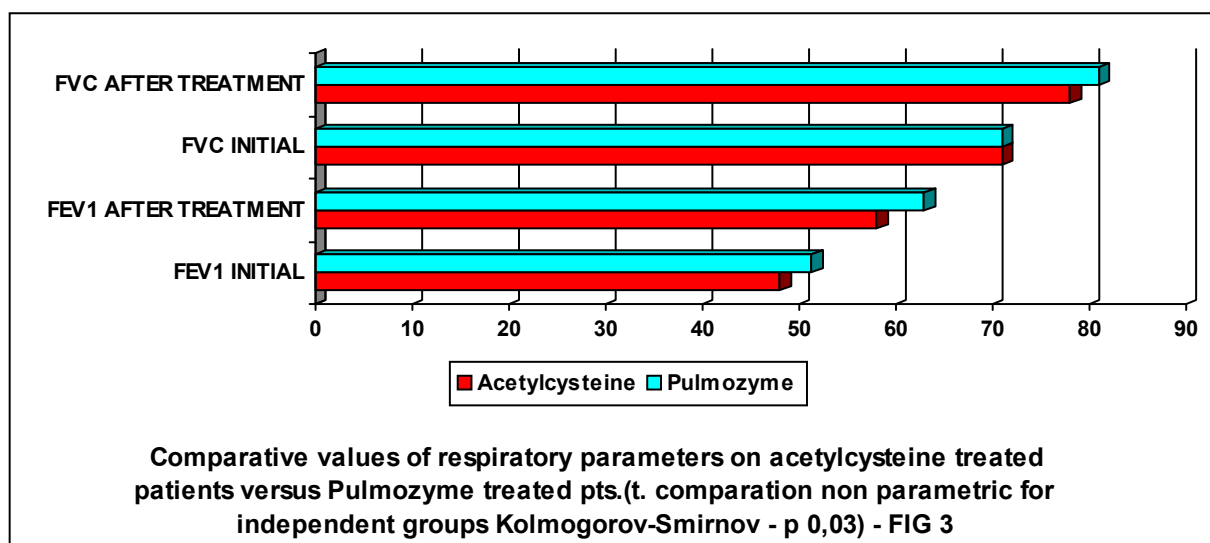
• The comparative values of respiratory parameters at the two groups (non parametric test for independent groups – Kolmogorov-Smirnov) showed a semnificative correlation (p 0,03)(Fig. 3).



Evolution of respiratory parameters -acetylcysteine aerosols (Fig 1)



Evolution of respiratory parameters - Pulmozyme aerosols (Fig. 2)



**Conclusions**

Alpha dornase aerosols therapy is distinctly superior to acetylcysteine aerosol treatment.

Efficiency is more implify as is early set up, enviable before the occurrence of respiratory infection.

It must be correlated with physiotherapy clearance techniques.

**References**

1. \*\*\* Cystic Fibrosis Trust – Report of the UK Cystic Fibrosis Trust Infection Control Group – sugestion for prevention and infection control, 2004
2. Henke MO; Ratjen F: Mucolytics in cystic fibrosis, Paediatr Respir Rev, 2007, 8,1, 24-29.
3. Jones A.P.: Recombinant human deoxyribonuclease for cystic fibrosis, Cochrane Rev Abstract. 2006.
4. Kerem E, Conway S., Elborn H, Heijerman H.: Standard of care patients with cystic fibrosis: a European consensus, Journal of Cystic Fibrosis, 2005, 4,1, 7-26
5. Littlewood J.M., European cystic fibrosis society consensus on standard – a roadmap to „best care”, Journal of Cystic Fibrosis, 2005, 4,1, 1-5.
6. Paul K., Rietschel E., Ballmann M et al: Effect of treatment with dornase alpha on airway inflammation in patients with cystic fibrosis, Am J Resp Crit care med, 2004, 169, 6, 719-725
7. Popa I., Pop L., Popa Z: Fibroza chistică (Mucoviscidoza), Ed. Viața Medicală Românească, București, 1998.
8. Pop L, Popa Z (sub red Popa I): Ghid de management în mucoviscidoză (fibroză chistică), Ed Brumar, Timisoara, 2006.
9. Popa Z., Pop L. (sub red Popa I.): Fizioterapia în mucoviscidoză (fibroză chistică), Ed. Mirton, Timisoara, 2003.

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## DIAGNOSIS OF TUBERCULOSIS ADENITIS IN CHILDREN

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### Abstract

In the last decade, tuberculosis has become a major health problem, due to the increase of the disease frequency, both in adults and in children. Tuberculosis adenitis diagnosis was made according to the clinical and paraclinical manifestations and it was confirmed by a histopathological exam or cultures for *Mycobacterium tuberculosis*. Tuberculosis adenitis was a frequently encountered adenopathy (87 cases), representing 7.8% of all the adenopathies under study and 12.5% of the infectious adenitis. TB adenitis was found in all the age groups, with a higher frequency at the 10-16 years group (39.1%). The most frequent localization of adenopathy was anterior cervical in 43 children (49.4%). In 6 children (7%), the evolution was towards opening the abscess and fistulization. Diagnosis was set according to the following criteria: positive cutaneous test at tuberculin (87.3%), notion of TB contact (82.7%), ganglionic biopsy (64.4%) and the presence of the characteristic changes on the pulmonary x ray (21.8%).

**Key words:** Tuberculosis adenitis, diagnosis, children.

### Introduction

Tuberculosis adenitis is a disorder which was known ever since antiquity under the name of “scrofula”, a term which comes from Latin and was used for the tuberculosis infection of the lymphatic ganglions in the cervical region. The first record of this disease dates from

400 BC, when Herodot suggested that persons suffering from it should be isolated (Lewis, 2003).

### Material and method

The group of the children with superficial tuberculosis adenitis was made up of 87 cases admitted in the Infectious Disease Hospital in Craiova, between 1996 and 2005. Tuberculosis adenitis diagnosis was made according to the clinical and paraclinical manifestations and it was confirmed by a histopathological exam or cultures for *Mycobacterium tuberculosis*, on a Loewenstein environment. An important role for setting the diagnosis was given by the cutaneous test at tuberculin with 2 or 10 units, being considered positive when the local induration was more than 10 mm, and the anamnesis notion of TBC contact.

In studying the group, we looked for: the reported frequency for the rest of adenopathies; particularities related to anamnesis; case distribution according to age groups, sex, origin; local and general clinical exam; specific (PPD test, pulmonary x ray, ganglionic biopsy, cultures) and unspecific paraclinical examinations (ESR, leukocytosis, lymphocytosis).

### Results

Tuberculosis adenitis held an important place in the studied case-book record (87 cases), representing 7.8% of all the adenopathies under study and 12.5% of the infectious adenitis. The frequency of infection with *M. tuberculosis* as reported to all the infections with microbacteria was 87.8%, the rest of 12.2% being diagnosed as infections with atypical microbacteria. (Table1).

Table 1. Tuberculosis adenitis frequency.

Type of adenopathy	No.	Tuberculosis adenitis (N=87)
Total adenopathies	1112	7.8%
Total infectious adenitis	694	12.5%
Total adenitis with microbacteria	99	87.8%

The increased frequency of the tuberculosis adenitis reflects a high incidence of the disease in our country. TB adenitis was found in almost all the age groups, with a higher frequency at the 10-16 years group (39.1%); during this period of life, there is a maximum receptivity to the disease, with a low natural resistance of the body.

The distribution of the cases according to sex reveals a slight predominance of females (51.6%), as compared to males (48.4%); according to environment, we have noticed a higher frequency for the children coming from rural areas (62%) as compared to those from the urban ones (38%).

The characteristics of the group according to age, sex, and environment are given in Table 2.

Table 2. Clinical characteristics of children with tuberculosis adenitis (N = 87).

Age group			Sex				Environment			
			Male		Female		Urban		Rural	
	No.	%	No.	%	No.	%	No.	%	No.	%
6 months-1 year	4	4.6	3	3.4	1	1.1	2	2.3	2	2.3
1-3 years	14	16.1	6	7	8	9.2	5	5.7	9	10.3
3-6 years	13	15	6	7	7	8	6	7	7	8
6-10 years	22	25.2	10	11.5	12	13.8	7	8	15	17.3
10-16 years	34	39.1	17	19.5	17	19.5	13	15	21	24.1
Total	87	100	42	48.4	45	51.6	33	38	54	62

The incubation period of the disease, from the moment of infection until the clinical appearance of adenopathy, was difficult to tell.

In all cases, the major clinical sign which determined seeing the doctor was the occurrence of adenopathy. The period of time between the adenopathy

onset and the moment of going to the doctor varied between 15 days and 6 months (on average - 30 days).

The clinical characteristics, localization of adenopathy and the systemic signs associated to adenopathy are given in table 3.

Table 3. Localization of adenopathy and the association of systemic signs.

Localization of adenopathy			Present systemic signs		Absent systemic signs	
	No.	%	No.	%	No.	%
<i>Cervical</i>	43	49.4	34	39.1	9	10.3
Submandibular	11	12.6	9	10.3	2	2.3
Submentonier	4	4.6	3	3.4	1	1.2
Supraclavicular	2	2.3	2	2.3	0	0
Axillary	6	7	4	4.6	2	2.3
Inguinal	5	5.7	2	2.3	3	3.44
Multiple	16	18.4	15	17.3	1	1.2
<b>Total</b>	87	100	69	79.3	18	20.7

The most frequent localization of adenopathy was anterior cervical in 43 children (49.4%), followed by a submandibular localization in 11 children (12.6%), submentonier in 4 children (4.6%), subclavicular in 2 children (2.3%), axillary in 6 children (7%) and inguinal in 5 children (5.7%). The multiple ganglionic affection was found in 16 children (18.4%).

The affected ganglions were moderately increased in volume, painless, of a firm/tough consistency.

In evolution, they increased their volume, becoming more or less coalescent and adherent to the neighboring plane. In 6 children (7%), the evolution was towards opening the abscess and fistulization.

The patients with tuberculous adenopathy are classically described, showing systemic clinical signs, such as: fever, weight loss, physical asthenia, nocturnal

perspiration. In our study, 69 children (79.3%) showed weight loss and tiredness, while in 18 children (20.7%) the adenopathy was not accompanied by signs of affecting the general state.

The primary pulmonary tuberculosis, which was radiologically confirmed, was present in 19 children (21.8%) with peripheral tuberculous adenitis: 12 children showed a primary complex, 4 children also had pleurisy, and 3 children had an infiltrated right upper lobe.

In all those children we noticed the presence of general signs (fever, asthenia, adynamy, weight loss, nocturnal perspiration) and of specifically pulmonary signs.

Among the biological investigations, the peripheral hematological examination revealed a moderate leukocytosis (8000 - 14000/mm<sup>3</sup>) in 24 children (27.6%), moderate lymphocytosis (40 - 60%) in 64 children (73.5%) and ESR >

30mm at 1h in 74 children (85%). Moderate anemia (Hb 8 - 11g%) was found in 48 children (55.2%).

The positive diagnosis was set according to the following criteria:

- positive results at the histopathological examination of the affected lymphatic ganglions, in 56 cases (64.4%);
- positive cutaneous test at tuberculin in 76 cases (87.3%);
- abnormal pulmonary x ray in 19 cases (21.8%): hilary adenopathy in 12 cases (13.8%), concentration in the upper lobe in 3 cases (3.4%), pleurisy in 4 cases (4.6%);
- familial or close contact with an adult presenting active tuberculosis was admitted in 72 cases (82.7%);
- positive cultures for *Mycobacterium tuberculosis* coming from the gastric aspiration in 5 cases (5.7%).

### Discussions

Tuberculosis represents the main cause of death in the world. Extra-pulmonary tuberculosis represents 1/3 of all cases of tuberculosis, children showing an increased predisposition to develop this kind of disease.

Superficial tuberculous adenitis represents the main manifestation within the extra-pulmonary tuberculosis, being the most frequent form of chronic cervical adenopathy

in children. The sucklings are more susceptible to severe forms of tuberculosis, with milliary tuberculosis prevalence; in preschool children there is a prevalence of superficial adenitis and tuberculous meningitis, while in school children the pleurisy and the bone tuberculosis.

In Romania, the tuberculosis incidence raised from 58.8/100.000 in 1985, to 141.3/100.000 in 2002 (4). In a study carried out by Didilescu and co. in 2002, 86.3% of the total localizations were pulmonary ones, 9.5% pleural and 4.2% extra-respiratory. According to the distribution on localization of extra-respiratory tuberculosis cases registered in Romania in 2002, we found out that tuberculous adenitis was present in 28.8% of the cases, followed by osteoarticular disorder 20.7%, meningoencephalitis 13.1%, disorders of urinary apparatus 9.7%, pericarditis 7.8%, digestive disorders 6.8%, nodose erythema 4.8%, genital 4.4% and ocular disorders 1.7%.

In our study, in most cases, the certainty diagnosis was established according to the histopathological aspect which was characteristic to that of ganglionic biopsy, in correlation with the PPD cutaneous test, the notion of TB contact and the association of changes on the pulmonary x ray.

In table 4 there is a brief description of some clinical studies on tuberculous adenopathy.

Table 4. Clinical studies in literature regarding tuberculous adenopathy.

Authors	Place and date of activity	No. patients	Sex M/F	Average age	Systemic signs %	Associated Rx changes %	Positive PPD %
Polesky and co.	San Jose 2005	106	-	Children and adults	-	38	94
Jhaa and co.	India 1997 - 1998	60	1/1,3	9 months - 62 years (23 years)	18	16	95
Dandapat and co.	India 1987 - 1988	83	1/1,2	1 - 65 years (21 years)	85	35 (8% TB active)	74
Castro and co.	UCLA 1973 - 1983	23	1/1,3	2 -56 years (23 years)	16	18	100

Fine needle aspiration – FNA - was used in the last 10 years as an initial diagnose test, with few risks, being a less invasive procedure as compared to ganglionic biopsy and having better results (5,9). The tests were evaluated by means of a cytological microscopic examination, which revealed a resistance of bacilli to acids and alcohol; we also carried out culture for microbacteria.

The studies demonstrated that the role of FNA in diagnosing tuberculous adenitis is comparable to that of excision biopsy, FNA allowing the setting of the tuberculous etiology in 80-85% of the cases, through a cytological, microscopic and cultures examination (5,9,10).

FNA was not performed for our group of children – we preferred a ganglionic biopsy, because, in children, FNA can favor the appearance of fistulous courses.

Extra-pulmonary tuberculosis is often difficult to diagnose, due to the small number; the reduced increase rate of Koch bacillus in cultures limits its finding through conventional techniques.

In TB adenitis, the demonstration of the Koch bacillus existence on the smear or on the products which were obtained after a biopsy puncture can sometimes be uncertain. We recommend finding out the mycobacterial DNA by using PCR (8).

PCR (Polymerase Chain Reaction) is a DNA amplifying technique which uses specific DNA sequences as a marker for microorganisms. Theoretically, by using this method, we can detect even one microorganism from biological products, such as: sputum, gastric aspiration, pleural liquid or blood.

In adults, the sensitivity and specificity of the test is considered to be over 90% in detecting pulmonary tuberculosis. In children, the use of PCR for detecting *M. tuberculosis* has not been extensively evaluated yet.

In children, performing PCR is recommended in the following situations: in case of serious pulmonary disease; when the diagnosis is difficult to set on the basis of epidemiologic findings and the current laboratory investigations; for the evaluation of the children with pulmonary disease and for establishing the diagnosis of extra-pulmonary tuberculosis and immunosuppression.

Many recent studies used ELISA test for detecting the antibodies to various antigens of *M. tuberculosis* in children. The detection of the mycobacterial antigens (counting the tuberculostearic acid and the mycobacterium mycolic acid) was evaluated more in adults and less in children.

However, these diagnosis methods require special equipments which are still unavailable in countries where tuberculosis is frequent.

## Conclusions

1. Tuberculous adenitis held an important place in the studied case-book record, representing 7.8% of all the adenopathies under study and 12.5% of the infectious adenitis which reflects the increased incidence of tuberculosis in children.
2. TB adenitis was found in all the age groups, but most frequent in teenagers (39.1%). The adenopathy with a cervical localization was most frequently found (49.4%), other localizations being submandibular and submentonier (17.2%), axillary (7%), inguinal (5.7%), supraclavicular (2.3%).
3. Diagnosis was set according to the following criteria: positive cutaneous test at tuberculin (87.3%), notion of TB contact (82.7%), ganglionic biopsy (64.4%) and the presence of the characteristic changes on the pulmonary x ray (21.8%).

## References

1. Baskota DK, Prasad R, Kumar Sinha B, Amatya RC. Distribution of lymph nodes in the neck in cases of tuberculous cervical. *Acta otolaryngol.* 2004 Nov; 124(9): 1095-8.
2. Castro DJ, Hoover L, et al. Cervical mycobacterial lymphadenitis. *Arch. Otolaryngol* 1985; 111: 816 - 9.
3. Dandapat MC, Mishra BM, Dash SP, et al. Peripheral lymph node tuberculosis: a review of 80 cases. *Br J Surg* 1990; 77: 911 - 912.
4. Didilescu C, Corlan E, Ploeanu D. Studiul actual al tuberculozelor extrapulmonare în România, *Pneumologia* 2003; 52 (2): 90 – 92
5. Hussain M, Rizvi N. Clinical and morphological evaluation of tuberculous peripheral Lymphadenopathy. *J Coll Physicians Surg Pak.* 2003 Dec; 13(12): 694-6.
6. Ilgazli A, Boyaci H, Basyigit I, Yildiz F. Extrapulmonary tuberculosis: clinical and epidemiologic spectrum of 636 cases. *Arch med Res.* 2004 Sep-Oct; 35 (5):435-41.
7. Jhaa BC, Dass A, et al. Cervical tuberculous lymphadenopathy: changing clinical pattern and concepts in management. *Postgrad Med J,* 2001; 77: 185 - 7.
8. Kidane D, Olobo JO, Habte A, et al. Identification of the Causative Organism of Tuberculous Lymphadenitis in Ethiopia by PCR, *Journal of Clinical Microbiology* 2002; 40 (11): 4230 - 4.
9. Marais BJ, Wright CA, Schaaf HS, Gie RP, Hesseling AC, Enarson DA, Beyers N. Tuberculous lymphadenitis as a cause of persistent cervical Lymphadenopathy in children from a tuberculosis endemic area. *Pediatr Infect Dis J.* 2006 Feb; 25(2): 142-6.
10. Polesky A, Grove W, Bhatia G. Peripheral tuberculous lymphadenitis: epidemiology, diagnosis, treatment, and outcome. *Medicina (Baltimore).* 2005 Nov; 84(6):350-62.

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## 15 YEARS-OLD GIRL WITH PARESTESIA, HEADACHE AND ABDOMINAL PAIN

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### Abstract

Neuronal migration disorders are the conditions for multiple heterogeneous neurological manifestations, difficult to diagnose because of atypical and various clinical expressions. Continuous development of imagistic investigation opened new study possibilities. Paper aim is to present the case of an adolescent, 15 years old girl, admitted in Clinic II Pediatrics for abdominal pain, parestesia/pareses and headaches.

**Key words:** neuronal migration disorders, children, partial vegetative seizures.

### Background

Neuronal migration disorders represent the substratum of numerous neurological manifestations (seizures, psychomotor retardation, dimorphism) that are hard to elucidate in the absence of neuroimaging investigations. The continuous development of new imaging techniques (computed tomography CT scanning, MR imaging, positron emission tomography, the study of cerebral metabolism) opened a new road in the study of abnormal neuronal development, differentiation and migration.

The alteration of the normal process of neuronal migration takes to cerebral malformations that are included under the terminology of ‘neuronal migration disorders’. The causes of inhibition of the neuronal migration process are various: infectious, vascular, (ischemic lesions that produce damage of the radial glioma fibers, of the molecular layer), toxic (exogenous and endogenous) and genetic causes.

For a better understanding diagnosis we will present a ethiopathogenic review. The neuronal migration represents the process in which thousands of nervous cells ‘walk’ from their place of origin (ventricular and sub ventricular areas), to their definitive places in the CNS, where they will stay for the rest of their life.

The neuronal migration disorders represent the alteration of the neuronal migration process; it happens during life in the uterus (the 3<sup>rd</sup>- 5<sup>th</sup> month of gestation).

The possibilities of alteration of neuronal migration are various: from very severe, with important alteration of the cerebral cortex, to minimal lesions. Neuronal heterotopias can be:

-Periventricular heterotopias

-Sub cortical heterotopias

-Superficial cortical-leptomeningeal heterotopias

-Schizencephalia

-Localised neuronal migration disorder

-Focal cortical dysplasia

-Microdysgenesis of cerebral cortex

### Case presentation

The adolescent girl diagnosed with anxious-depressive disorder, in the context of a particular psychological structure, perfectionist type, with clinical subjective phenomenon, presented with multiples symptoms as: parestesia/pareses of the extremities, episodes of headaches, subjective sensations of warm/ cold, under treatment with sedatives, anti-depressives, with almost 2 months before the moment of this admission in the hospital to the present clinical symptoms are associated also persistent abdominal pain, pyrosis, vomiting, for which the patient is following a symptomatic treatment.

In the context of the digestive symptomatology that is an acute one at the moment, the patient was admitted in our service a month and a half before and was diagnosed with chronic gastritis with *Helicobacter Pylori*, anxious-depressive disorder, and hypocalcaemia. She was treated for the *H.Pylori* infection and in this time the sedative medication was totally excluded.

### Evolution

After an initial improvement of the clinical status with 14 days before the actual admission she accuses the apparition of parestesia/pareses but this time organised on the left side of the body, on the left side of the skull, these being the reasons of the actual admission.

Ad admittance-subjective: anxiety, headache on left head part, parestesia/pareses on the left side of the body, objective: moderate abdominal pain in the upper abdominal area, bilateral positive Chwostek sign grade 2. The neurological examination was normal.

We were in front of a case with anxious-depressive disorders underlined by a specialist physician but the patient had a real subjective symptomatology, such as headache and parestesia/pareses on the left side of the body. In this context and having in mind the provided data a few questions were raised:



-Is it about an acute episode of the digestive disorder associated with subjective symptoms less representative?

-It represents a particular way of manifestation of hypocalcemia?

-Is the symptomatology strictly related to the anxious-depressive phenomenon?

-Is it a problem of a neurologic organic pathology?

**Laboratory investigations** revealed hypocalcaemia.

First neuropsychological examination revealed a very high emotional sensibility, immature state of affectivity, anxiety with vegetative hyperexcitability,

suffocating/choking sensation, sleep disorders, bodily discomfort.

An electroencephalography (EEG) was recommended and it was interpreted as normal electrical. The ophthalmologic evaluation and retinal vessels evaluation were in normal ranges. In evolution, the initial subjective symptomatology is maintained with the same characteristics, even with episodes of greater headaches than the initial ones.

A supplementary electrophysiological investigation is made-mapping EEG which revealed a normal route with synchronic peaks of discharging in the right temporal derivations with maximum in T4, P4 (fig.1).

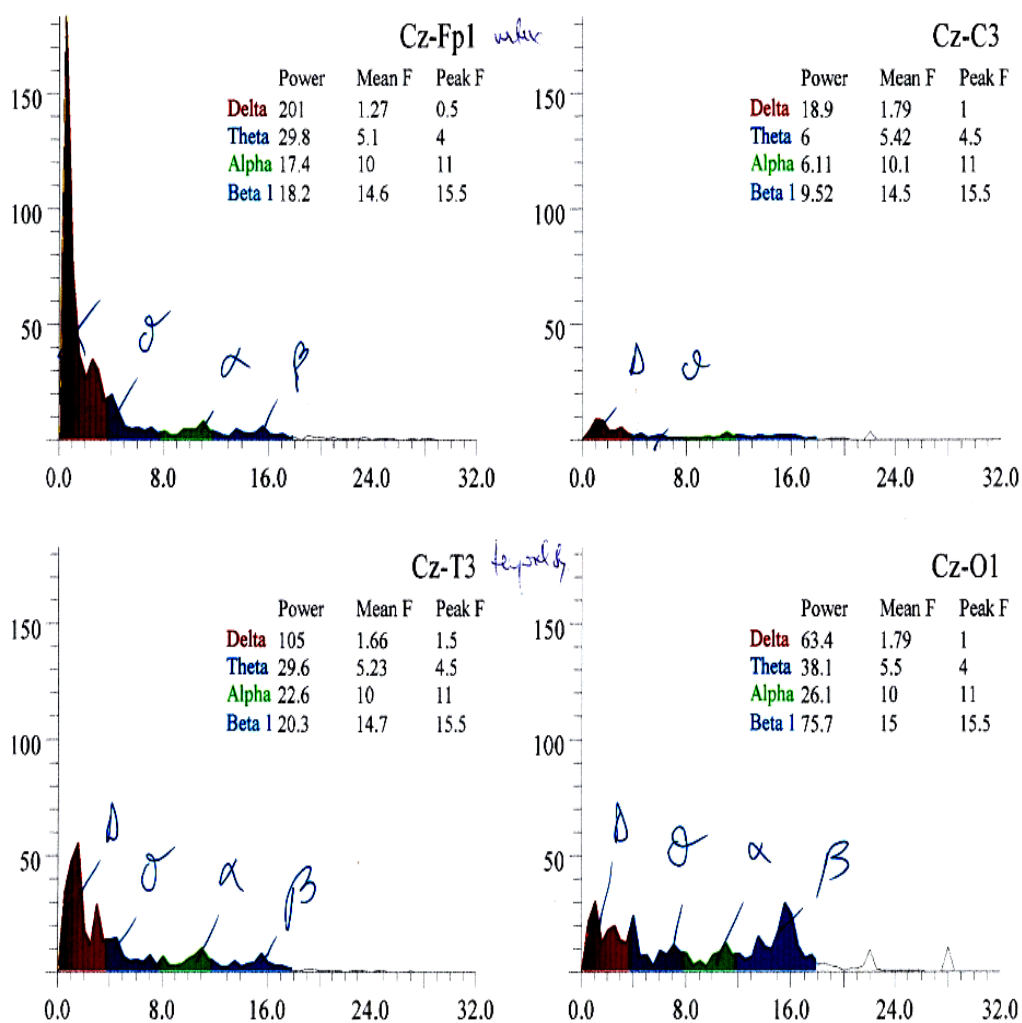


Fig.1 EEG mapping.

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Another neuropsychiatric children examination recommended a magnetic resonance procedure (MRI) which revealed an image in T1T2 weighed with grey substance in

the parietal-frontal area, anterior F at the level of the left semi-oval center-grey substance fig.2+ fig.3.

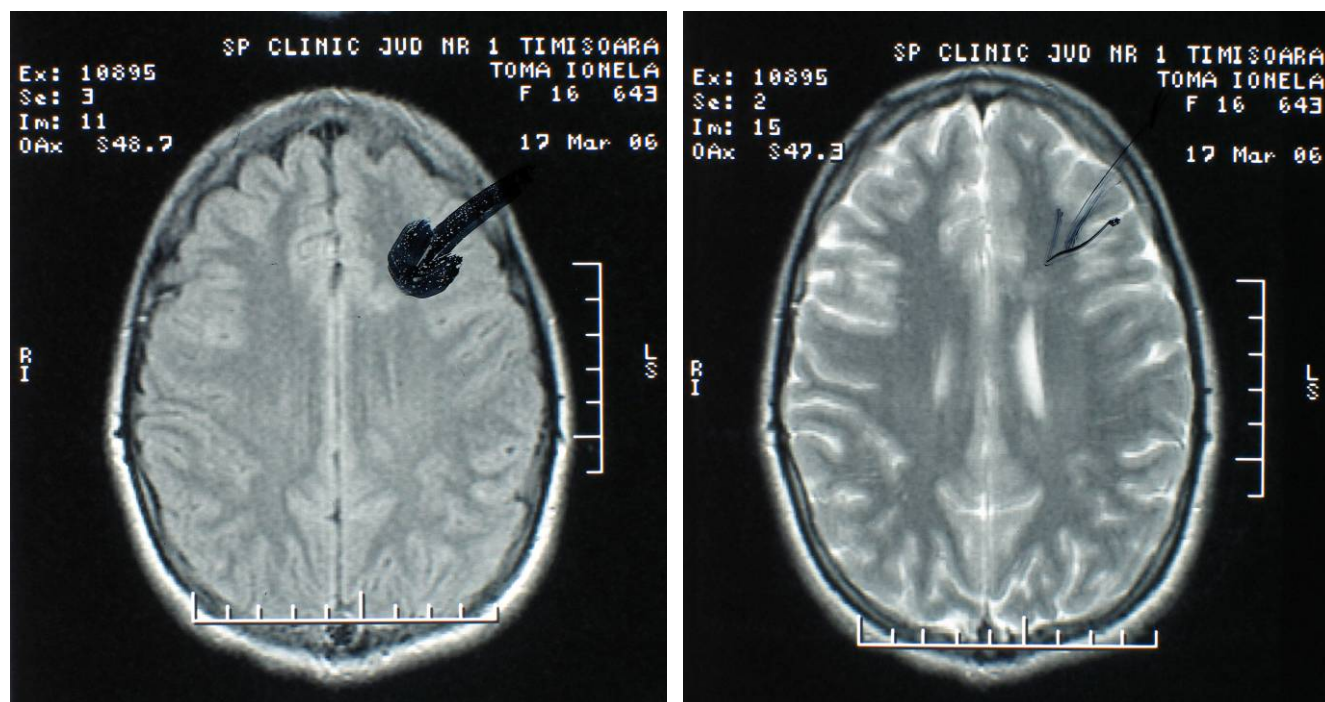


Fig 2 and 3. Magnetic resonance procedure (MRI).

The established diagnosis were:

-Partial comital crises through congenital neuronal migration disorders

-Hypocalcaemia

-Anxious-depressive disorders

The treatment applied was: Valproic acid-50mg/kg, 2x1tb/day of 300mg/day and calcium therapy.

After the clinical examination and the modification on the MRI very well defined and limited as extension we included this case in cortical focal dysplasia type I, in which there is evidence of a thickening of the brain in the affected area. The anatomic-pathologic examination is the one that confirm the exact diagnosis. The differential diagnosis of the partial seizures trough neuronal migration disorders could be made with:

1. First of all with type II of cortical focal dysplasia

2. Secondly with heterotopias with minor lesions, and from these with the neuronal migration disorders that are localised.

3. Other causes of partial convulsions have in their clinical aspect sings and symptoms that are present also at our patient:

-Partial convulsions with sensorial symptoms: transitory sensation of stinging, itchiness, numbness of the left side of the body. It reflects an epileptic discharge in the parietal cortex.

-Smell related convulsions- they can begin as an unpleasant odour felt by the patient, then they become generalised seizure, being an early manifestation of a temporal lobe tumour.

Partial convulsions with vegetative symptoms: they include paleness, redness of the skin, headaches, tachycardia, pupil dilatation, abdominal pain, loss of urinal bladder control.

-Complex partial convulsions that appear because of the convulsive discharges in the temporal lobes, the patient accusing a state of dreaming, illusions, complex visual hallucinations, impossible or fractionated talking, affective emotional disorders, anxiety. .

4. We also considered the intracerebral expansive processes (astrocytoma, ependimoma, ganglioma, meningioma), because of the various symptomatology that is present with simple partial epileptic crises or complex ones, progressive hemiplegia, intracranial hypertension. But in these cases, the neurological examination reveals sings of focus correlated with the tumors localization. The CT scanning reveals the location and aspect (calcification, chystic cavities) of the modifications. Neuronal ectopies can also bring up in discussion, like ectopic pinealoma.

Regarding hypocalcaemia could be secondary to decrease of calcium level or hypomagnesaemia.

The differential diagnosis of the anxious-depressive disorders raises the problem if they are primary in the context of a particular psychological structure of perfectionist type of the patient, being stressed out by the disease or they are secondary to the disease. Time will help to tell the delimitation of the exact ethiology.

The case evolution was favourable, with the disappearance of seizures. It still remains to discuss the

potential complications, in the absence of treatment and the secondary effects of medication, being well known that the valproic acid can accentuate the hypocalcaemia.

### Conclusions

Extremely common symptoms like: abdominal pain, headaches could “hide” less frequent diagnosis, but with important outcome.

Even if there is an explanation for a clinical sign, like parestesia, differential diagnosis should be performed and the follow up of the patient is necessary.

We must consider every symptom and sign, especially if is sustained by paraclinical investigation. Interdisciplinary team approach is the most correct manner of management for complex cases.

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### References

1. Cotter D.R., Honavar M.-Focal Cortical Dysplasia: a neuropathological and developmental perspective, *Epilepsy Res*, Sep; 36(2-3);p.155-164,1999.
2. Guerrini R., Canapicchi R, Dobyns W.B. -Epilepsy and malformations of cerebral cortex, *Neurologgia*, May, 14 Suppl.3, p.32-47,1999.
3. Doina Anca Plesca, Raluca Ioana Teleanu, Dimitrie Dragomir-Migrarea neuronală, Editura Academiei Române, Bucuresti, 2005
4. Lequin M.H., Barkovich A.J.- Current Concepts of cerebral malformation Syndromes, *Curr. Opin. Pediatr.*, Dec;11(6); p.492-496, 1999.
5. Volpe J.J-Neuronal Proliferation, Migration and Myelinisation în Volpe J.J.(ed): *Neurology of the newborn*, Third Edition, cap.2; p.43-95;W.B Saunders Company, 1995.

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# INVESTIGATIONAL LIMITS AND MULTI-DRUG RESISTANCE AMONG CHILDREN'S URINARY TRACT INFECTIONS – OUR RECOMMENDATIONS

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## Abstract

**Introduction:** It has been estimated that approximately 1% of boys and 3% of girls will experience a symptomatic urinary tract infections (UTI) before their 11th year of life. Infants and young children with UTI are of particular concern because the risk for renal damage is greatest in this age group and because the diagnosis is frequently challenging: the clinical presentation tends to be non-specific and valid urine specimens cannot be obtained without invasive methods - suprapubic aspiration (SPA) and transurethral catheterisation. Renal scarring, hypertension, and even renal failure can develop after recurrent pyelonephritis which is the most-common preventable cause of end-stage renal disease.

**Objective:** Our aim is to identify proper investigation methods and antibiotic regimens for UTI in children from the Timis County, by comparing two studies on UTI, recurrent and non-recurrent, with the results available in the international literature.

**Material:** We compared the result of two studies on patients admitted in the III<sup>rd</sup> Pediatric Clinic Timisoara with UTI between 2004-2005 and 2006-2007 (the first six months) and the data available in the international literature. The first group included 36 patients admitted in the III<sup>rd</sup> Pediatric Clinic UMF Timisoara in 2004 and 2005. The second study included a number of 81 patients admitted in the III<sup>rd</sup> Pediatric Clinic UMF Timisoara in 2006 and the first six months of 2007.

**Results of our studies:** Our patients continued to present recurrence under treatment in comparison with literature data. Only 15,12% of the E. Coli strains were sensible to ampicillin and trimethoprim-sulfomethoxazole. Male patients were most frequently affected (65,85%) in the age group of 0- 3 years; female patients were most frequently affected (75%) in patients above 6 years.

The strains responsible for the community acquired infections were initially sensible to ciprofloxacin, ampicillin, gentamicin, and colistin, but most of the strains developed resistance to these antibiotics; thus the % of successful empiric treatment with these antibiotics is reduced (<30%).

**Conclusions:** In case of infections due to more than one micro organism, it is essential to use multi-drug therapy, because the organisms are usually high-resistant and they have a selective sensibility.

Long term therapy must have high sensibility to pathogens and interfere as little as possible with non pathogenic flora, and reconsideration of fluoroquinolones group interdiction is mandatory. The study proved that organisms developed resistance, even to the late generation antibiotics, probably due to the irrational use of antibiotics. The antibiotics should be used in Romania according to our guidelines, on the basis of the sensibility/resistance and frequency of the organisms in our territory.

**Key words:** urinary tract infections, investigation methods, antibiotics

## Introduction

The urinary tract is a relatively common site of infection in infants and young children. UTI are important because they cause acute morbidity and may result in long-term medical problems, including secondary hypertension and reduced renal function. Management of children with UTI involves repeated medical check-ups, use of antimicrobial drugs, exposure to radiation, and cost. Accurate diagnosis is extremely important for two reasons:

- it allows identification, treatment, and evaluation of the children who are at risk for kidney damage
- it avoids unnecessary treatment and evaluation of children who are not at risk, for whom interventions are costly, potentially harmful and provide no benefit.

It has been estimated that approximately 1% of boys and 3% of girls will experience a symptomatic UTI before their 11th year of life (6). The prevalence of UTI in boys is higher in the first 3 months of life (7). Another study showed that both febrile UTI and APN were more prevalent in males under 1 year and females over 1 year (8).

Infants and young children with UTI are of particular concern because the risk for renal damage is greatest in this age group and because the diagnosis is frequently challenging: the clinical presentation tends to be non-specific and valid urine specimens cannot be obtained without invasive methods - suprapubic aspiration and transurethral catheterization. Considerable variation in the methods of diagnosis, treatment, and evaluation of children with UTI was documented 3 decades ago (21). Since then, various changes have been proposed to aid the diagnosis, treatment, and evaluation, but no data is available to suggest that such innovations have resulted in reduced variation in practice.

The aim of the clinical guidelines on management of acute UTI in childhood is to improve diagnosis and treatment of pyelonephritis and the conditions predisposing to UTI, such as congenital malformations of the urinary tract (1).

Renal scarring, hypertension, and even renal failure can develop after recurrent pyelonephritis (2,3) which is the most-common preventable cause of end-stage renal disease (ESRD). Guidelines from England and Wales (1991) (4) and Finland (1992) (5) recommend that all children, regardless of age and gender, should have renal tract imaging after the first episode of confirmed acute UTI. Failure to diagnose UTI could be reflected in subsequent increased occurrence of ESRD and a large number of kidney transplants due to recurrent pyelonephritis. Overdiagnosis of UTI, on the other hand, causes considerable extra cost for the healthcare system, mainly due to unnecessary imaging examinations and follow-up. Knowledge of the variable occurrence of acute UTI in children will help us to increase awareness and to improve the treatment of acute UTI as well as giving valuable clues to possible etiological factors.

This article is intended for use by clinicians who treat infants and young children in a variety of clinical settings (e.g., private practices, emergency departments, other departments of hospitals).

### Objectives

Our aim is identifying proper investigation methods and antibiotic regimens for UTI in children from the Timis County by comparing two studies about UTI, recurrent and non-recurrent, with the results available in the international literature. In the first study we included children aged 3 months to 2 years, which presented more than one UTI and which didn't had any renal tract malformations. We discussed the regimens for relapsed UTI in children using the antibiogram data and then finding the suitable treatment. In the second study we included a number of 81 patients, presented UTI, associated or not with renal tract malformations.

### Material and method

We compared the results of two studies on patients admitted in the IIIrd Pediatric Clinic Timisoara with UTI between 2004-2005 and 2006-2007 (the first six months) and the data available in the international literature.

The first group included 36 patients admitted in the IIIrd Pediatric Clinic Timisoara in 2004 and 2005, selected according to the following criteria:

- age from 3 months to 2 years
- all the patients presented recurrent UTI, but none of the patients had any renal tract malformations
- 6 children were admitted for more than 2 episodes of UTI during this study.
- all patients were required to provide urine samples for uroculture.
- positive urocultures were investigated for in vitro response to antibiotics
- in specific cases, other investigations such as ultrasound-echo, urography, scintigraphy and voiding cystography were performed.

Exclusion criteria:

- patients with obvious neurological or anatomic abnormalities known to be associated with recurrent UTI
- children older than 2 years who experience their first UTI, because they are more likely than younger children to have symptoms referable to the urinary tract, are less likely to have predisposing factors for renal damage, and are at lower risk of developing renal damage.

The second study included a number of 81 patients admitted in the IIIrd Pediatric Clinic Timisoara in 2006 and the first six months of 2007, selected by the following criteria:

- age from 3 months to 18 years
- all the patients presented UTI, associated or not with renal tract malformations
- all patients were required to provide urine samples for uroculture, no invasive methods (SPA) were performed
- positive urocultures were investigated for in vitro response to antibiotics.
- in specific cases other investigations such as ultrasound-echo, urography, scintigraphy and voiding cystography were performed.

### Results

*Of the first study:*

1. The recurrence rate of UTI had a low correlation with the reflux grade in all the cases.
2. Our patients continued to present recurrence under treatment in comparison with literature data (Greenfield 2001, Brumariu 2005).
3. Urocultures during re-admissions revealed:
  - Girls 0-6 y: 7 cases (5 - E. Coli, 2 - Proteus)
  - Girls over 6y: 13 cases (11 - E. Coli, 2 - Proteus)
  - Boys 0-6 y: 12 cases (7 - Proteus, 4 - E. Coli, 1 - Klebsiella)
  - Boys over 6y: 4 cases (E. Coli)
4. Best responses were obtained with cephalosporin group antibiotics (Ceftazidime, Ceftriaxone) and with Ciprofloxacin.
5. Less than 50% of the E. Coli and Proteus strains were resistant to Nitrofurantoin.

*Regarding the second study:*

1. The most frequent organism responsible for UTIs was Escherichia Coli (40,74%), followed by Proteus spp. (12,31%), Klebsiella spp. (2,46%), Pseudomonas Aeruginosa (2,46%), Citrobacter Koseri
2. (1,23%), Enterobacter spp. (1,23%) and fungus (1,23%). Infections with more than one organism were found in 4,93% of the cases (Fig 2).
3. Intermediary resistant E. Coli strains:
  - 54,54% of the UTIs with E. Coli
  - Resistant to Ampicillin and Trimethoprim-sulfomethoxazole

4. High resistant E.Coli strains:
  - 30,33% of the UTIs with E. Coli
  - Resistant to: Ampicillin, Gentamicin, Trimethoprim-sulfomethoxazole, Colistin and Ciprofloxacin.
5. Only 15,12 of the E. Coli strains were sensible to Ampicillin and Trimethoprim-sulfomethoxazole.
6. Proteus spp. was resistant to: Ampicillin, aminoglycozides (Gentamicin, Amikacin, and Netilmicin), Kanamicin, Trimethoprim-

sulfomethoxazole, Colistin, and Ciprofloxacin/Nalidixic acid.

7. Klebsiella spp. was proven to be resistant to Ampicillin, Gentamicin, Amikacin, Trimethoprim-sulphomethoxazole, Kanamicin and Nalidixic acid.
8. Pseudomonas Aeruginosa was resistant to: Ampicillin, Gentamicin, Trimethoprim-sulphomethoxazole, Kanamicin, Nalidixic acid.
9. Acute pyelonephritis (APN) only presented an incidence of 23,45%.

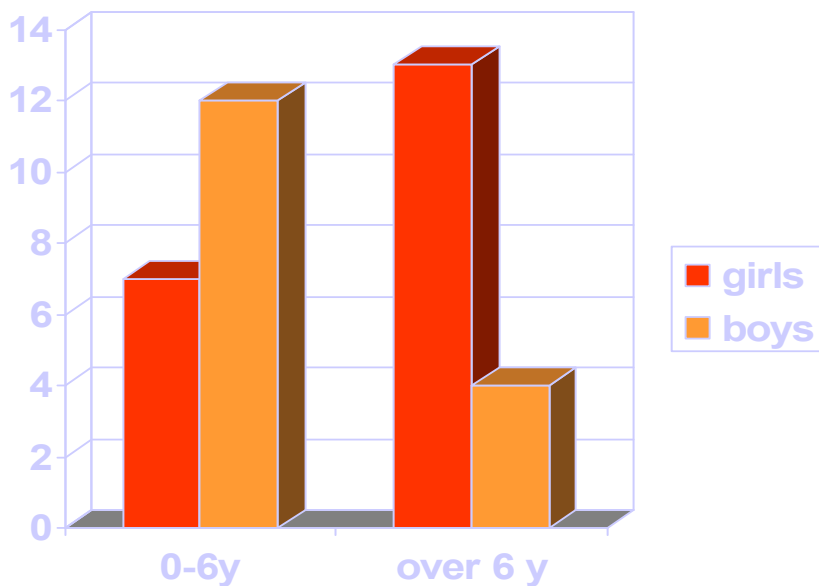


Fig 1: The distribution of recurrent of UTI according to age and sex.

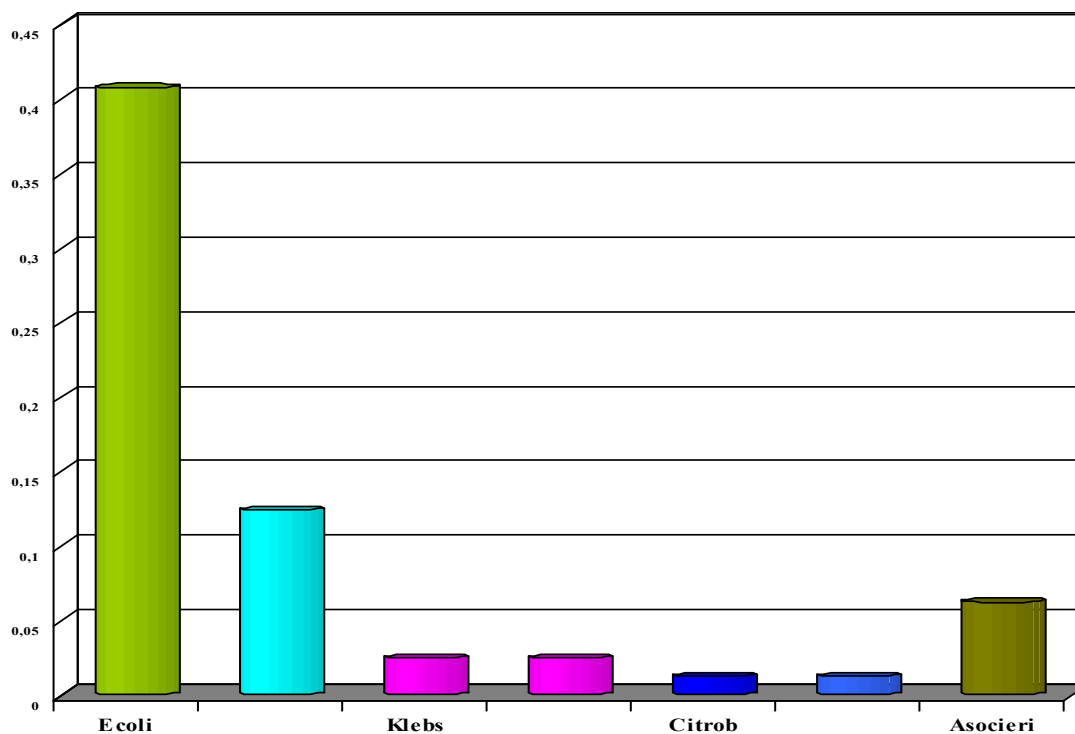


Fig. 2: The frequency of microorganisms causing UTI.

### Our study conclusions

#### 1. The study on recurrent UTI:

- Long term therapy must have high sensibility to pathogens and interfere as little as possible with non pathogenic flora, and reconsideration of fluoroquinolones group interdiction is mandatory.
- The understanding and awareness of the resistance pattern of pathogens in our region is essential in order to provide the best antibiotic regimen to our patients.
- Up-to-date antibacterial treatment must take into account the modification of pathogenic and non pathogenic colon flora as a result of past antibiotic administrations.

#### 2. The second study:

- The study confirmed the previous statistics according to which the congenital malformations predispose to UTI.
- Male patients were most frequently affected (65,85%) in the age group of 0- 3 years; female patients were most frequently affected (75%) in the group above 6 years.
- The strains responsible for the community acquired infections were initially sensible to ciprofloxacin, ampicillin, gentamicin, and colistin, but most of the strains developed resistance to these antibiotics; thus the percentage of successful empiric treatment with these antibiotics is reduced (<30%).
- The study proved that organisms developed resistance, even to the late generation antibiotics, probably due to the irrational use of antibiotics. The antibiotics should be used according to the guidelines, on the basis of the sensibility/resistance and frequency of the organisms.
- In case of infections due to more than one microorganism, it is essential to use multi-drug therapy, because the organisms are usually high-resistant and they have a selective sensibility.
- The antibiotics should be used in Romania according to our guidelines, on the basis of the sensibility/resistance and frequency of the organisms in our territory.

### Discussions

The prevalence of UTI, from international literature, in infants and young children 2 months to 2 years of age who have no obvious source of fever from history or physical examination is high - 5% (22,23,24). The genders are not affected equally - the prevalence of UTI in febrile girls aged 2 months to 2 years is more than double than that in boys (in girls younger than 1 year it is 6.5%; in boys, it is 3.3%; in girls between 1 and 2 years of age is 8.1%; in boys it is 1.9%). The rate in circumcised boys is low, 0.2% to 0.4% (25,26,27).

UTI may be limited to the bladder (cystitis) or may involve the renal parenchyma (pyelonephritis). APN can result in irreversible renal damage and in the well-recognized sequelae of scarring, including hypertension,

proteinuria, and chronic renal failure (13,14). Infants and young children are at higher risk than are older children for developing acute renal injury with UTI. The incidence of vesico-ureteral reflux (VUR) is higher in this age group than in older children, and the severity of VUR is greater, with the most severe form (with intrarenal reflux or pyelotubular backflow) limited to infants. Infants and young children with UTI need special attention because of the possibility to prevent kidney damage. Firstly, the UTI may indicate a child with an obstructive anomaly or severe VUR, and secondly, because infants and young children with UTI may have a febrile illness and no localizing findings, there may be a delay in diagnosis and treatment.

The presence of fever has long been considered a finding of special importance in infants and young children with UTI, because it has been accepted as a clinical marker of renal parenchyma involvement (pyelonephritis). The concept that fever in a child with UTI indicates renal parenchyma involvement is based on the comparison of children with high fever (>39°C) and the clinical diagnosis of APN with those with low fever (<38°C) and a clinical diagnosis of cystitis (28). Indirect tests for localization of the site of UTI, such as the presence of a reversible defect in renal concentrating ability, and non-specific tests of inflammation, such as elevated white blood cell (WBC) count, C-reactive protein, or erythrocyte sedimentation rate, are encountered more frequently in children with clinical pyelonephritis than in those with clinical cystitis. However, the indirect tests for localization of the site of infection and the non-specific indicators of inflammation do not provide confirmatory evidence that the febrile infant or young child with UTI has pyelonephritis. Cortical imaging studies using technetium 99 m Tc-dimercaptosuccinic acid (DMSA) may prove useful in determining whether the presence of high fever does identify children with pyelonephritis and distinguishes them from those with cystitis. The likelihood that UTI is the cause of the fever may be increased if there is a history of crying on urination or of foul-smelling urine. An altered voiding pattern may be recognized as a symptom of UTI as early as the second year after birth in some children. Dysuria, urgency, frequency, or hesitancy may be present but are difficult to discern in this age group. Non-specific signs and symptoms, such as irritability, vomiting, diarrhoea, and failure to thrive, also may reflect the presence of UTI, but data are not available to assess the sensitivity, specificity, and predictive value of these clinical manifestations.

Current studies that assess fever as a marker of pyelonephritis, defined by a positive scan, provide a wide range of sensitivity (53% to 84%) and specificity (44% to 92%) (29,30,31).

The incidence of APN in infants - around 60% - is similar in the studies (9). Several studies (10,11) have reported that the susceptibility of the renal parenchyma to infection varies with age, with those under 1 year at greatest risk. Both the host's immunological defence and microbiological virulence factors are critical co-factors for renal scarring (12).

Although high-grade VUR allows migration of bacteria into the parenchyma, APN and renal scarring can



still occur in the absence of reflux. The relationship between the extent of kidney inflammation in the acute phase and the late outcome in the presence or absence of VUR is still insufficiently explored. We hypothesize that VUR is one of the multiple risk factors for APN and renal scar formation, along with host susceptibility, urinary tract obstruction, inflammatory response and therapeutic delay.

Experimental studies in animals have validated dimercaptosuccinic acid (DMSA) scintigraphy as an accurate technique for the detection of acute infection (15,16).

Clinical, biological, and ultrasound parameters do not accurately identify children with acute renal infections documented by DMSA scan. Thus, the distinction between cystitis and pyelonephritis in children with acute UTI is not accurate and some (17) recommend that the DMSA scan should be added to the initial work-up of children with UTI. In our country we cannot performed this scan in the last two years!

Cortical scintigraphy with DMSA in the acute phase of a UTI allows detection of kidney involvement, thus presenting two advantages. The first advantage is the possibility of estimating the severity of the disease in each kidney and the presence of bilateral disease. The second advantage is represented by the possibility of evaluating the patient's risk for developing renal sequelae from the very onset of the acute disease. The available data support the hypothesis that the risk of developing renal sequelae is low when the early scintigraphy is normal and high in the presence of abnormalities during the acute phase of infection (18,19,20).

In the Kuang study the DMSA scan in most children with renal scarring (the incidence of APN in children with first febrile UTI was of 70%, and nearly half developed a renal scar) showed a small unifocal area. The long-term clinical significance of these unifocal areas of renal scarring is unknown. The incidence of febrile UTI or APN was higher in males than females under the age of 1 year, and the reverse was true thereafter. Since VUR was present in a third of our patients with APN, half of whom showed scar formation; other risk factors should be evaluated in infants with APN and renal scar formation (8).

Micturating cysto-urethrography (MCUG) gives information about the presence and degree of VUR, the bladder and the urethra. VUR may also be detected using mercapto-acetyl-triglycine renography (MAG3) renography (34).

Establishing the diagnosis of UTI requires a strategy that minimizes false-negative and false-positive results. Urine obtained by SPA is the least likely to be contaminated; urine obtained by transurethral bladder catheterization is next best (Table 1). Either SPA or transurethral bladder catheterization should be used to establish the diagnosis of UTI, definitive option in all the North-America guidelines. Cultures of urine specimens collected in a bag applied to the perineum have an unacceptably high false-positive rate; the combination of a 5% prevalence of UTI and a high rate of false-positive results (specificity: 70%) results in a positive culture of urine collected in a bag to be a false-positive result 85% of the time.

The probability of infection according to various methods of collection of urine is illustrated in Table 1.

Method of Collection	Colony Count (Pure Culture)	Probability of Infection (%)
Suprapubic aspiration	Gram-negative bacilli: any number Gram-positive cocci: more than a few thousand	>99%
Transurethral catheterization	$>10^5$	95%
	$10^4 - 10^5$	Infection likely
	$10^3 - 10^4$	Suspicious; repeat
	$<10^3$	Infection likely
Clean void		
Boy	$>10^4$	Infection likely
Girl	3 specimens $\geq 10^5$	95%
	2 specimens $\geq 10^5$	90%
	1 specimen $\geq 10^5$	80%
	$5 \times 10^4 - 10^5$	Suspicious; repeat
	$10^4 - 5 \times 10^4$	Symptomatic: suspicious; repeat Asymptomatic: infection unlikely
	$<10^4$	Infection unlikely

Table 1: The probability of infection according to various methods of collection of urine.



If antimicrobial therapy is initiated before obtaining a specimen of urine for culture that is unlikely to be contaminated, the opportunity may be lost to confirm the presence or establish the absence of UTI. Therefore, in the situation in which antimicrobial therapy will be initiated, SPA or catheterization is required to establish the diagnosis of UTI. SPA has been considered the “gold standard” for obtaining urine for detecting bacteria in bladder urine accurately and the technique has limited risks. Using other methods, variable success rates for obtaining urine have been reported (23% to 90%) (6,32).

Technical expertise and experience are required, and many parents and physicians perceive the procedure as unacceptably invasive compared with catheterization.

A recent randomized controlled trial of 218 children with acute pyelonephritis (age 3 months to 18 years) demonstrated no benefit of antibiotic prophylaxis for preventing recurrent UTI, pyelonephritis, or scarring in children with or without reflux (grades I – III only) after one year of follow up. The overall incidence of UTI recurrence after pyelonephritis was 20.1% with no statistically significant differences between those with or without VUR or those with or without prophylaxis. Most cases of recurrence were cystitis. Twelve patients had recurrence of pyelonephritis. Thirteen of the 218 patients developed renal scars, including 7 with VUR and 6 without. Most of the patients with scarring and VUR had grade III reflux (35).

The follow-up of patients is only necessary in the group of high-risk children (34), which are those with:

- Recurrent infections;
- Clinical signs such as poor urinary stream or palpable kidney;
- Unusual organisms (those which are not *E. Coli*);
- Bacteraemia or septicaemia;
- Prolonged clinical course with failure to respond fully to antibiotic treatment within 2-3 days;
- Unusual clinical presentation such as an older boy;
- Known dilatation or abnormality on antenatal screening of the urinary tract.

The first randomized controlled study that shows the role of probiotic *L. acidophilus* for preventing recurrent UTI in children with persistent primary VUR was finished last year. However, this study has the limitation of lacking a treatment arm and a low calculated power (78%). Further clinical trials are necessary, to compare antibiotic prophylaxis with probiotics and no prophylaxis in children with or without VUR (33).

#### Recommendations:

1. The presence of UTI should be considered in infants and young children 2 months to 2 years of age with unexplained fever. If an infant or young child 2 months to 2 years of age with unexplained fever is assessed as being sufficiently ill to warrant immediate antimicrobial therapy, a urine specimen should be

obtained by SPA or transurethral bladder catheterisation; the antimicrobials commonly prescribed in such situations will be effective against the usual urinary pathogens. Some clinicians may choose to obtain a specimen by non-invasive means (e.g., in a collection bag attached to the perineum). The false-positive rate with such specimens dictates that before diagnosing UTI, all positive results must be confirmed with culture of a urine specimen unlikely to be contaminated.

2. If the diagnosis of UTI is established, the general practitioner and the paediatrician should NOT prescribe empirical therapy without an accurate knowledge of the local resistance, susceptibility and frequency of the infecting organisms.
3. Once the diagnosis of UTI is confirmed (by culture), the next step should be an effort to investigate renal involvement (by renal ultrasound, scan, MRI or, if not possible, by urography or a micturating one - MGUG).
4. If renal involvement is present, appropriate therapeutical measures should be taken. The possibility of renal function reduction should be always taken into account when starting the antimicrobial treatment.
5. After the first UTI the decision regarding the usefulness of prophylactic antibiotics should be taken after an evaluation of:
  - individual risk of recurrence of UTI, especially of pyelonephritis
  - individual risk of scarring or other renal damage
  - presence of voiding dysfunction
  - adverse effects of long-term use of antibiotics
  - individual patient/family adherence/compliance behavior (35).
6. The follow-up of patients is essential, since recurrence of pyelonephritis can lead to severe kidney damage. The clinician should decide whether or not the patient is a high-risk child. If so, the investigations required are:
  - Renal tract ultrasound during acute infection
  - All children under 3 years of age with normal renal ultrasound findings (or abnormal ultrasound findings without dilatation) require a <sup>99m</sup>Tc- DMSA scan (if this result is abnormal they will also require a MCUG).
  - All children 3 years of age or older, who have normal renal ultrasound findings (or abnormal ultrasound findings without dilatation) require MAG3 renography and an indirect radioisotope cystogram. This is because normal renal ultrasound findings in a high-risk child do not exclude the presence of focal renal damage nor do they exclude bladder dysfunction in high-risk children. For children over 3 years of age and toilet-trained, MAG-3 and indirect radioisotope cystograms will provide information on renal function, drainage in the supine position and the

effect of a change of posture. Observing the entire urinary tract before, during and after normal micturition gives valuable information on bladder dynamics and avoids the catheterisation required for MCUG. The early MAG3 images give valuable information on renal parenchymal function and have

an 80% sensitivity in the detection of focal renal damage (34).

7. The antibiotics should be used in Romania according to our guidelines, on the basis of the sensibility/resistance and frequency of the organisms in our territory.

## References

- Matti Nuutinen, Matti Uhari, Michael F.G. Murphy, Kate Hey, Clinical guidelines and hospital discharges of children with acute urinary tract infections, *Pediatr Nephrol* (1999) 13:45–49.
- Jacobsson B, Berg U, Svensson L (1994) Renal scarring after acute pyelonephritis. *Arch Dis Child* 70:111–115.
- Rushton HG, Majd M, Jantusch B, Wiedermann BL, Belman AB (1992) Renal scarring following reflux and nonreflux pyelonephritis in children: evaluation with 99mtechnetium-dimercaptosuccinic acid scintigraphy. *J Urol* 147:1327–1332.
- Report of a working group of the Research Unit, Royal College of Physicians (1991) Guidelines for the management of acute urinary tract infection in childhood. *J R Coll Physicians Lond* 25:36–42.
- Lautala P, Uhari M, Huttunen N-P, Koskimies O, Jalanko H, Holmberg C, Ruuskanen O, Ala-Houhala M, Renko R, Lanning P (1992) Diagnostics and treatment of urinary tract infection in children. *Duodecim* 108:1344–1350.
- Jodal U (1987) The natural history of bacteriuria in childhood. *Infect Dis Clin North Am* 1:713–729.
- Stull TL, LiPuma JJ (1991) Epidemiology and natural history of urinary tract infections in children. *Med Clin North Am* 75:287–297.
- Kuang-Yen Lin, Nan-Tsing Chiu et al, Acute pyelonephritis and sequelae of renal scar in pediatric first febrile urinary tract infection *Pediatr Nephrol* (2003) 18:362–365.
- Goldraich NP, Goldraich IH (1995) Update on dimercaptosuccinic acid renal scanning in children with urinary tract infection. *Pediatr Nephrol* 9:221–226.
- Martinell J, Claesson I, Lidin-Janson G, Jodal U (1995) Urinary infection, reflux and renal scarring in females continuously followed for 13–38 years. *Pediatr Nephrol* 9:131–136 21.
- Gleeson FV, Gordon I (1991) Imaging in urinary tract infection. *Arch Dis Child* 66:1282–1283.
- Smellie JM, Edwards D, Normand IC, Prescod N (1981) Effect of vesicoureteric reflux on renal growth in children with urinary tract infection. *Arch Dis Child* 56:593–600.
- Bailey RR (1981) End-stage reflux nephropathy. *Nephron* 27:302–306
- Jacobson SH, Eklöf O, Eriksson CG (1989) Development of hypertension and uremia after pyelonephritis in childhood: 27 year follow up. *BMJ* 299:703–706.
- Parkhouse HF, Godley ML, Cooper J, Risdon RA, Ransley PG (1989) Renal imaging with 99mTc-labelled DMSA in the detection of acute pyelonephritis: an experimental study in the pig. *Nucl Med Commun* 10:63–70
- Rushton HG, Majd M, Chandra R, Yim D (1998) Evaluation of 99mTc-dimercaptosuccinic acid renal scans in experimental acute pyelonephritis in piglets. *J Urol* 140:1169–1174.
- Alberto Biggi et al, Acute renal cortical scintigraphy in children with a first urinary tract infection, *Pediatr Nephrol* (2001) 16:733–738.
- Merrick MV, Notghi A, Chalmers N (1995) Long term followup to determine the prognostic value of imaging after urinarytract infection. 2. Scarring. *Arch Dis Child* 72:393–396.
- Vernon SI, Coulthard MG, Lambert HJ (1997) New renal scarring in children who at age 3 and 4 years had had normal scans with DMSA: follow up study. *BMJ* 315:905–908.
- Stokland E, Hellström M, Jacobsson B (1996) Early Tc-99m DMSA scintigraphy in symptomatic first-time urinary tract infection. *Acta Paediatr* 85:430–436.
- Dolan TF Jr, Meyers A. A survey of office management of urinary tract infections in childhood. *Pediatrics*. 1973;52:21–24.
- Hoberman A, Chao HP, Keller DM, et al. Prevalence of urinary tract infection in febrile infants. *J Pediatr*. 1993;123:17–23
- Roberts KB, Charney E, Sweren RJ, et al. Urinary tract infection in infants with unexplained fever: a collaborative study. *J Pediatr*. 1983;103:864–867.
- Bauchner H, Philipp B, Dahefsky G, Klein JO. Prevalence of bacteriuria in febrile children. *Pediatr Infect Dis J*. 1987;6:239–242.
- Wiswell TE, Smith FR, Bass JW. Decreased incidence of urinary tract infections in circumcised male infants. *Pediatrics*. 1985;75:901–903
- Wiswell TE, Hachey WE. Urinary tract infections and the uncircumcised state: an update. *Clin Pediatr*. 1993;32:130–134
- Craig JC, Knight JF, Suresh Kumar P, Mantz E, Roy LP. Effect of circumcision on incidence of urinary tract infection in preschool boys. *J Pediatr*. 1996;128:23–27.
- Winberg J, Andersen HJ, Bergstrom T, et al. Epidemiology of symptomatic urinary tract infection in childhood. *Acta Paediatr Scand*. 1974;252: 1–20. Supplement.

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| <p>29. Tappin DM, Murphy AV, Mocan H, et al. A prospective study of children with first acute symptomatic <i>E coli</i> urinary tract infection: early 99 m technetium dimercaptosuccinic acid scan appearances. <i>Acta Paediatr Scand</i>. 1989;78:923–929</p> <p>30. Verboven M, Ingels M, Delree M, Piepsz A. 99 mTc-DMSA scintigraphy in acute urinary tract infection in children. <i>Pediatr Radiol</i>. 1990;20:540–542</p> <p>31. Rosenberg AR, Rossleigh MA, Brydon MP, Bass SJ, Leighton DM, Farnsworth RH. Evaluation of acute urinary tract infection in children by dimercaptosuccinic acid scintigraphy: a prospective study. <i>J Urol</i>. 1992;148:1746–1749.</p> | <p>32. Leong YY, Tan KW. Bladder aspiration for diagnosis of urinary tract infection in infants and young children. <i>J Singapore Paediatr Soc</i>. 1976; 18:43–47.</p> <p>33. Seung Joo Lee et al, Probiotics prophylaxis in children with persistent primary vesicoureteral reflux, <i>Pediatr Nephrol</i> (2007) 22:1315–1320</p> <p>34. Stephen D. Marks &amp; Isky Gordon &amp; Kjell Tullus, Imaging in childhood urinary tract infections: time to reduce investigations, <i>Pediatr Nephrol</i> (2007)</p> <p>35. Cincinnati Children's Hospital Medical Center, Evidence-Based Care Guideline for Medical Management of First Urinary Tract Infection in Children 12 Years of Age or Less, Changes to the guideline made in November, 2006.</p> |
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## IV. PEDIATRIC SURGERY

### MINIMALLY INVASIVE REPAIR OF PECTUS EXCAVATUM – CASE REPORT

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#### Abstract

The Nuss procedure is a minimally invasive technique using a retrosternal bar to repair pectus excavatum. Although its technical simplicity and cosmetic advantages are remarkable, early applications have been limited to children with symmetrical pectus excavatum. We report a 13 year old boy presented with pectus excavatum to witch we applied operative treatment, using principles of minimal-access surgery and thoracoscopy combined with placement of a Lorenz bar. The surgical intervention was accoplishied in the colaboration with our colleagues from Department of Thoracical Surgery Emergency Clinical City Hospital Timisoara witch provides us the logistical support.

**Key words:** Pectus excavatum, Nuss procedure, MIRPE

#### Introduction

Pectus excavatum is a congenital chest wall deformity in which several ribs and the sternum grow abnormally, producing a concave appearance in the anterior chest wall.

Pectus excavatum is the most common type of congenital chest wall abnormality (90%), followed by pectus carinatum (5-7%), cleft sternum, pentalogy of Cantrell, asphyxiating thoracic dystrophy, and spondylothoracic dysplasia. Pectus excavatum occurs in an estimated 1 in 300-400 births, with male predominance (male-to-female ratio of 3:1) [1,2]. The condition is typically noticed at birth, and more than 90% of cases are diagnosed within the first year of life. Worsening of the chest’s appearance and the onset of symptoms are usually reported during rapid bone growth in the early teenage years. Many patients are not brought to the attention of a pediatric surgeon until the patient and the family notice such changes. Most patients are asymptomatic and present for esthetic correction [3]. Cardiac function is usually normal, but mitral valve prolapse has been reported in 20-60% of cases. Echocardiography reveals an improved cardiac index upon exertion after operative repair of the deformity [4]. The long-term health risks of patients who are managed without surgery are not known [5,6].

The standard operative treatment of pectus excavatum has been the Ravitch’s technique that requires the

exposition of the thorax’s anterior region with resection of the costal cartilages affected bilaterally, the performance of a cross sternal osteotomy with the placing of a stabiliser, and the development of muscular flaps [7,8]. The Nuss Technique, also known as the “Minimally Invasive Repair of Pectus Excavatum” (MIRPE), uses principles of minimal-access surgery and thoracoscopy combined with the proper placement of a Lorenz Pectus Bar to achieve correction of Pectus Excavatum. The MIRPE procedure was developed by Dr Nuss in the 1980’s and was initially performed for children and adolescents [9]. This is a minimally invasive technique which places one or two curved steel bars - Lorenz pectus bar - behind the sternum and forces it back into a more normal shape. With this minimally invasive technique, 2 small incisions are made on the lateral chest wall, and a convex steel bar, contoured to the patient’s chest, is inserted under the sternum, with the convex surface facing posteriorly. The bar is rotated 180° so that the convex surface elevates the sternum and corrects the pectus deformity. The bar is removed after 2 years, when permanent remodeling has occurred [10,11]. The results have been good to excellent. The procedure offers several advantages over pectus repair in which cartilage is removed, with or without strut support. It is easier to perform, avoids having to make an anterior chest incision, returns the patient to full activity sooner, preserves elasticity of the chest, and does not retard chest wall growth. Currently, the ease of the Nuss technique makes it the procedure of choice for surgical repair of pectus excavatum [12]. Furthermore, its long-term benefits may be even greater. By preserving the costal cartilages, the Nuss procedure maintains chest elasticity and chest wall growth and it avoids the restrictive effects associated with costochondrectomy and has the potential to improve both cardiac and pulmonary functions [10,13,14].

The operation for correction starts with general anesthesia and the placement of an epidural catheter for the management of pain after the operation. Two lateral incisions are made on either side of the chest for insertion of a curved steel bar under the sternum. A separate, small lateral incision is made to allow for a thoroscope for direct visualization as the bar is passed under the sternum [11,15,16,17].



The bar is individually curved for each patient. The bar is used to pop out the depression. It is then fixed to the ribs on either side and the incisions are closed and dressed. A small steel, grooved plate may be used at the end of the bar to help stabilize and fix the bar to the rib. The bar is not visible from the outside and stays in place for a minimum of two years. When it is time, the bar is removed as an outpatient procedure<sup>[18,19]</sup>.

### Case Report

A 13 year old boy presented with an abnormal chest wall deformity in which several ribs and the sternum grow abnormally, producing a concave, or caved-in, appearance in the anterior chest wall. The deformity had been increasing for the past two year. The patient posture it was with significant anterior curvature of the thoracic spine with the shoulders slumped forward. Clinical aspect is shown on the following pictures [Figure 1,2].



Figure 1 - Clinical aspect.



Figure 2 - Clinical aspect.

There was no mediastinal shift clinically. There was no scoliosis or any other spinal deformity. Lateral view of the chest radiograph showed an abnormal protrusion of the second to eighth costal cartilages in the parasternal

region. A CT scan of the chest confirmed that the deformity was involving the costal cartilages as well as the costochondral junctions and with anterior indentation of the right ventricle. [Figure – 3,4,5].

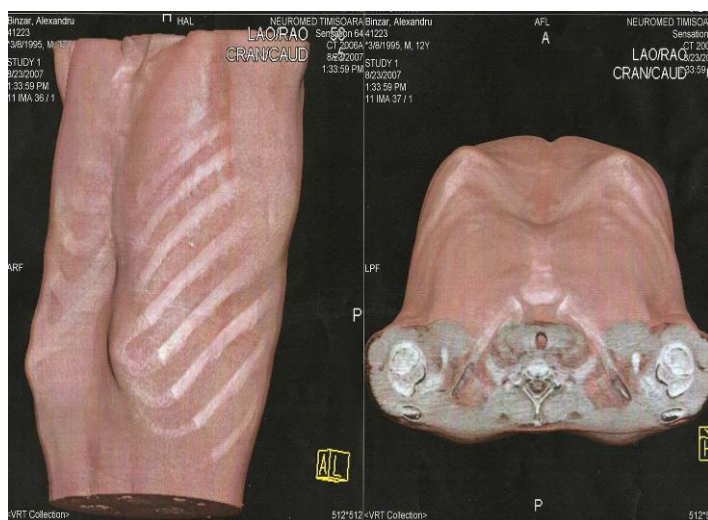


Figure 3 - CT scan of the chest confirmed the deformity.



Figure 4 - CT scan of the chest.

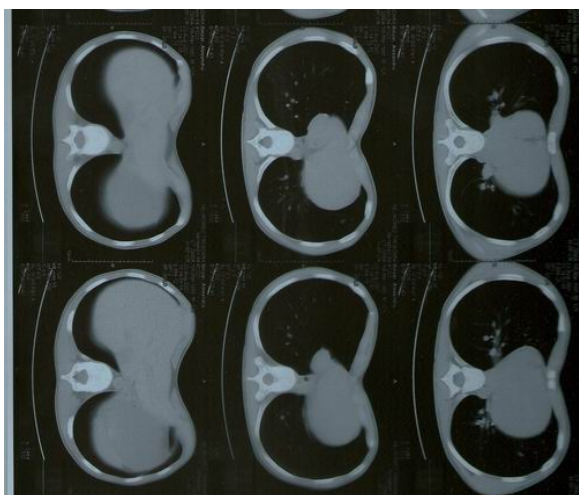


Figure 5-CT scan - anterior indentation of the right ventricle.

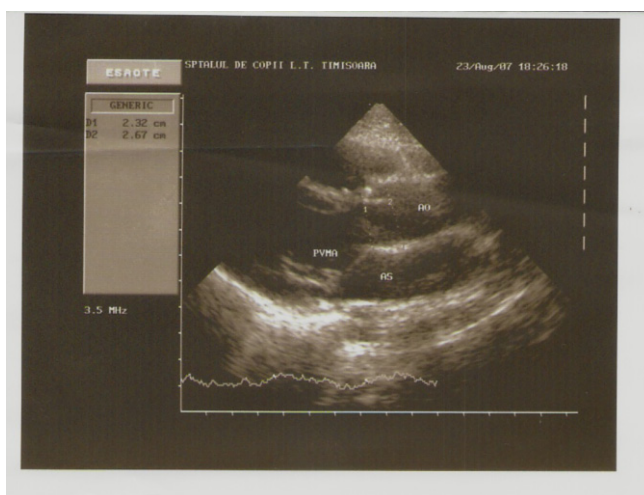


Figure 6 - Echographically aspect of mitral valve prolapse.

He has been diagnosed echographically with mitral valve prolapse [figure 6] and isolated atrial extrasistholic. He did not have any shortness of breath or recurrent respiratory tract infections.

After these completed investigations, included blood analyses and ventilatory evaluations we decided that this patient fulfilled conditions for MIRPE. Prior to surgery, the chest is measured from the midaxillary line to midaxillary line to determine the approximate length of the bar required. The surgical procedure was made under general endotracheal anesthesia.

Lorenz pectus introducer was advanced such that the tunnel space created is enlarged. The bar was inserted with the convexity facing posteriorly. In the next time the bar was turned over so that the concave part now faces posteriorly to the mediastinum [fig 7,8]. The ends of the bars was placed in the subcutaneous tissue and it was fixed with two dispositives for stabilization and limitation rotation of the pectus bar. Stabilizers were sutured around the bar and to the muscle.

Because the residual pneumothorax was significant drainage of chest was necessary. The subcutaneous tissue and skin was closed with absorbable sutures.

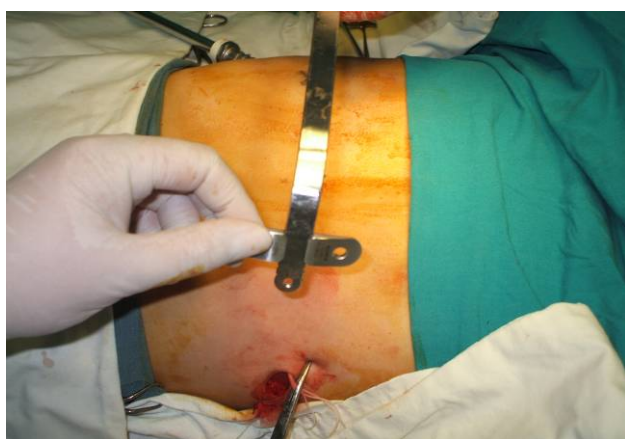


Figure 7 - Intraoperator details.



Figure 8 - Intraoperator details.

Follow-up postoperative repair of pectus excavatum involves outpatient visits with the pediatric surgeon 2-3 weeks after surgery and at regular intervals after that for the next 2 years. We recommend monitoring patient at least every 3-6 months to ensure that he is not developing an anterior protrusion of the chest due to too much pressure from the pectus bar.

## Conclusions

Advantages of the Nuss procedure in comparison with the open approach are decreased operative times, minimal blood loss, and improved cosmetics results. The Nuss procedure has already been shown to have a positive impact on both the physical and psychosocial well-being of

children who are suffering from pectus excavatum deformity.

The minimally invasive repair of pectus excavatum procedure is a safe and effective method of pectus excavatum repair.

## References

1. Tsirikos AI, McMaster MJ. Congenital anomalies of the ribs and chest wall associated with congenital deformities of the spine. *J Bone Joint Surg Am.* Nov 2005;87(11):2523-36.
2. Goretsky MJ, Kelly RE, Croitoru D, Nuss D. Chest wall anomalies: pectus excavatum and pectus carinatum. *Adolesc Med Clin.* Oct 2004;15(3):455-71. [Medline].
3. Cahill JL, Lees GM, Robertson HT. A summary of preoperative and postoperative cardiorespiratory performance in patients undergoing pectus excavatum and carinatum repair. *J Pediatr Surg.* Aug 1984;19(4):430-3. [Medline].
4. Quigley PM, Haller JA, Jelus KL, et al. Cardiorespiratory function before and after corrective surgery in pectus excavatum. *J Pediatr.* May 1996;128(5 Pt 1):638-43. [Medline].
5. Peterson RJ, Young WG, Godwin JD, et al. Noninvasive assessment of exercise cardiac function before and after pectus excavatum repair. *J Thorac Cardiovasc Surg.* Aug 1985;90(2):251-60.
6. Daunt SW, Cohen JH, Miller SF. Age-related normal ranges for the Haller index in children. *Pediatr Radiol.* Apr 2004;34(4):326-30.
7. Ohno K, Morotomi Y, Nakahira M, et al. Indications for surgical repair of funnel chest based on indices of chest wall deformity and psychological state. *Surg Today.* 2003;33(9):662-5. [Medline].
8. Ravitch MM. The operative treatment of pectus excavatum. *Ann Surg.* 1949;122:429-444.
9. Ravitch MM. Pectus excavatum. In: *Congenital Deformities of the Chest Wall and Their Operative Correction.* Philadelphia, Pa: WB Saunders Co; 1977.
10. Nuss D, Kelly RE Jr, Croitoru DP. A 10-year review of a minimally invasive technique for the correction of pectus excavatum. *J Pediatr Surg.* Apr 1998;33(4):545-52. [Medline].
11. Nuss D. Recent experiences with minimally invasive pectus excavatum repair "Nuss procedure". *Jpn J Thorac Cardiovasc Surg.* Jul 2005;53(7):338-44. [Medline].
12. Hebra A. Minimally invasive pectus surgery. *Chest Surg Clin N Am.* May 2000;10(2):329-39, vii. [Medline].
13. Hebra A, Jacobs JP, Feliz A, Arenas J. Minimally invasive repair of pectus excavatum in adult patients. *Am Surg.* Sep 2006;72(9):837-842. [Medline].
14. Fefferman NR, Pinkney LP. Imaging evaluation of chest wall disorders in children. *Radiol Clin North Am.* Mar 2005;43(2):355-70. [Medline].
15. Haller JA Jr, Kramer SS, Lietman SA. Use of CT scans in selection of patients for pectus excavatum surgery: a preliminary report. *J Pediatr Surg.* Oct 1987;22(10):904-6. [Medline].
16. Haller JA, Scherer LR, Turner CS, Colombani PM. Evolving management of pectus excavatum based on a single institutional experience of 664 patients. *Ann Surg.* May 1989;209(5):578-82; discussion 582-3.
17. Hebra A, Swoveland B, Egbert M, et al. Outcome analysis of minimally invasive repair of pectus excavatum: review of 251 cases. *J Pediatr Surg.* Feb 2000;35(2):252-7; discussion 257-8. [Medline].
18. Robicsek F. Surgical treatment of pectus excavatum. *Chest Surg Clin N Am.* May 2000;10(2):277-96. [Medline].
19. Marusch F, Gastinger I. [Life-threatening complication of the Nuss-procedure for funnel chest. A case report]. *Zentralbl Chir.* Nov 2003;128(11):981-4. [Medline].

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## GANGLIONEUROMA

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### Abstract

Ganglioneuroma is a tumor of the peripheral nervous system. Along with neuroblastomas, ganglioneuromas and ganglioneuroblastomas are collectively known as neuroblastic tumors. They originate from neural crest sympathogonia, which are completely undifferentiated cells of the sympathetic nervous system.

These tumors can grow wherever sympathetic nervous tissue is found, but they most frequently occur in the abdomen. Common location includes the adrenal gland, paraspinal retroperitoneum (sympathetic ganglia), posterior mediastinum, head and neck. Location such as the urinary bladder, bowel wall, abdominal wall and gallbladder are considered unusual.

**Key words:** ganglioneuroma, ganglioneuroblastoma, neuroblastoma

### Pathophysiology

The histologic difference between ganglioneuroma, ganglioneuroblastoma and neuroblastoma is their stage of neuroblast maturation. (Shimada, 1999). A tumor composed primarily of neuroblasts is referred to as neuroblastoma (NB), a tumor composed entirely of mature ganglion cells and other mature tissue is a ganglioneuroma (GN) and a tumor with both immature and mature cell types is a ganglioneuroblastoma (GNB). Therefore, ganglioneuroma is considered as a benign tumor. In rare cases, von Recklinghausen disease, Hirschprung disease, central failure of ventilation and DiGeorge syndrome are associated with ganglioneuroma and ganglioneuroblastoma. (Lonergan, 2002). Ganglioneuromas are rare, benign, fully differentiated tumors that contain mature Schwann cells, ganglion cells, fibrous tissue and nerve fibers. These tumors have no immature elements (such as neuroblasts), atypia, mitotic figures, intermediate cells or necrosis. The presence of any these tissue characteristics excludes the diagnosis of ganglioneuroma.

These tumors can arise de novo and result from the maturation of a ganglioneuroblastoma or neuroblastoma into a ganglioneuroma. They may also develop within a neuroblastoma treated with chemotherapy. Metastases in these tumors are exceedingly rare and are thought to be the end result of matured ganglioneuroblastoma or neuroblastoma metastases rather than true ganglioneuroma metastases. As many as 37% of ganglioneuromas secrete catecholamines.

**Frequency:** ganglioneuroma are rare tumors than occur in approximately 1 in 100,000 children. In the US about 30-50% of ganglioneuromas are retroperitoneal and

approximately 40% occur in the posterior mediastinum. Roughly 20% of these tumors occur in the adrenal medulla and cervical lesions constitute only 10% of these cases. Uncommon locations such as heart, bone and intestine also have been described. Central nervous system location is rare.

**Sex:** ganglioneuroma occur slightly more often in girls than boys, with a female-to-male ratio of about 1,5:1.

**Age:** ganglioneuromas are tumors of adolescents and young adults (40-60%), but individuals of all ages can be affected. The mean age of occurrence is 7 years.

### Gross anatomic features:

Ganglioneuroma are considered to be mature tumors and do not have immature elements. Ganglioneuroma average 8cm in size and have a pseudocapsule. They are firm to the touch and have a light color ranging from white to yellow. Internally, the tumor may have a whorled appearance with trabeculae.

**Risk groups:** two histologic classification systems are commonly used in the United States to stratify neuroblastic tumors into risk groups: the Shimada classification and the Pediatric Oncology Group (POG) classification. Both systems assess histologic features such as cellular differentiation, to arrive at a prognostic classification.

The POG system is based on the degree of differentiation of the different histologic elements. GN shows completely differentiated stromal and cellular components. NB contains less than 50% differentiated elements and GNB is intermediate. NB may be further subclassified as undifferentiated (the most immature NB), poorly differentiated or differentiating (the most mature NB). The Shimada classification combines histologic features and patient age at diagnosis. The histologic features consist of stroma, grade and architecture of

The age groups of the Shimada classification system are less than 1,5 years, 1,5-5 years and more than 5 years age. Children with favorable histologic characteristics are either less than 1,5 years of age with a low or intermediate MKI and differentiating or partially differentiating tumor or 1,5-5 years old with a low MKI and differentiating tumor. All other combinations are considered unfavorable histologic characteristics.

**Staging** -In 1986, an international consensus group devised the International Neuroblastoma Staging System (INSS), based on clinical, radiologic and surgical feature.



## Histopathology Age-linked Grading System of Shimada:

Type	Favorable Histologic Characteristics	Unfavorable Histologic Characteristics
Stroma-rich	Well differentiated intermixed	Nodular
Stroma-poor		
Age <18 mo	MKI <200/5,000	MKI >200/5,000
Age 18–60 mo	MKI <100/5,000 and differentiating	MKI >100/5,000 or undifferentiated
Age >5 y	None	All

Stage	Description
1	Localized tumor confined to the area of origin; complete gross excision, with or without microscopic residual disease; identifiable ipsilateral and contralateral lymph nodes negative microscopically
2A	Unilateral tumor with incomplete gross excision; identifiable ipsilateral nonadherent lymph nodes negative microscopically
2B	Unilateral tumor with complete or incomplete gross excision; positive ipsilateral nonadherent lymph nodes; identifiable contralateral lymph nodes negative microscopically
3	Tumor infiltrating across the midline (vertebral column) with or without regional lymph node involvement; or unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral regional lymph node involvement or extension by infiltration
4	Dissemination of tumor to distant lymph nodes, bone, bone marrow, liver, or other organs (except as defined in stage 4S)
4S	Localized primary tumor as defined for stage 1 or 2 with dissemination limited to liver, skin and/or bone marrow (<10% tumor) in infants younger than 1 y

**Biologic behavior**

In rare cases, GN secretes sufficient quantities of VMA (vanillylmandelic acid) or HVA (homovanillic acid). Catecholamine production by GN was previously believed to be unusual, because it was theorized that more mature tumors have more mature biologic behavior. However, in the largest series of GNs to date (49cases), 37% of the patients has elevated VMA or HVA levels.

**Clinic**

Ganglioneuromas are usually asymptomatic regardless of their size, and they are typically discovered on a routine radiograph. If the tumor is in the mediastinum, it may cause chest pain, cough, difficulty breathing or compression of the trachea.

If the tumor is in the retroperitoneal space, it may result in abdominal pain and distension. If the tumor is in near the spinal cord, it may cause spine deformity and possible compression of the spinal cord.

These tumors may be hormonally active and hypertension, diarrhea, flushing and virilization may occur as a result the secretion of catecholamine, vasoactive intestinal polypeptide, or androgenic hormone.

**Differential diagnosis**

- Adrenal adenoma
- Adrenal carcinoma
- Neuroblastoma, ganglioneuroblastoma
- Pheochromocytoma

**Radiography**

Although GN tends to be relatively homogeneous, the imaging characteristics of GN are similar to those of GNB and NB; hence, they cannot be discriminated at imaging evaluation save for the presence of metastases, which are quite rare in GN. Plain radiographs show a posterior mediastinal mass, which may cause rib spreading and foraminal erosion. A mass may also be noted in the retroperitoneumpelvis or neck.

**CT- Scanning** is the imaging modality that is commonly used to evaluate neuroblastic tumors. It is the superior imaging technique when it comes to identifying tumor size,organ of origin, tissue invasion, adenopathy and calcification. Newly diagnosed cases are evaluated with standard chest, abdominal and pelvic CT.

Retroperitoneal and adrenal GNs appear well defined. Their shape ranges from round to lobulated, they

show discrete and punctate calcification in 42-60% of cases, and they tend to grow around major blood vessels, which are not compressed by the tumor.

**MRI** creates images with better tissue discrimination than CT. Ganglioneuromas appear homogeneous on MRI and have relatively low signal intensity on T1-weighted images. On T2-weighted MRIs, the signal intensity is proportional to the ratio of myxoid stroma to cellularity and also to amount of collagen present in the tumor. Tumors with intermediate to high signal intensity on T2-weighted images have a higher degree of cellularity and more collagen. Markedly high T2 intensity signifies a high myxoid stroma component and low cellularity and collagen amount (Rha, 2003).

**Ultrasound** of GN shows a homogeneous, hypoechoic, well circumscribed mass.

**Scintigraphy** - Iodine-tagged metaiodobenzylguanidine (MIBG), a catecholamine analog, is used to identify catecholamine-producing tumors. MIBG has 88% sensitivity and 99% specificity for tumors containing sympathetic tissue such as GNB, GN, NB, pheochromocytomas and carcinoids. The disadvantage is that there is no way to discriminate the type of tumor in which the uptake occurs.

#### Treatment

GN are staged by using the INSS. Treatment is usually in the form of surgical excision, if the tumors are localized. Complete surgical resection is important because it allows for good tissue sampling and a thorough pathology examination of the specimen to ensure correct diagnosis of

ganglioneuroma. In rare cases, these tumors recur, so periodic radiologic surveillance is performed after resection.

**Prognosis** is usually good, because this tumor is generally benign. Rarely, a GN may become malignant and metastasize or recur.

**Complications** – may occur as a result of surgery. If the tumor has been present for a long time and is causing symptoms (such as spinal cord compression), removal of the tumor may not necessarily reverse the deficit.

#### CASE REPORT

We present a case of an 11 year-old boy was diagnosed with hepatic hidatic cyst, 4 months ago. Now, he was admitted in our clinic with abdominal pain and failure to thrive. On physical examination, his vital signs were normal. He looked pallor. Chest was clinically clear. Bowel sounds were existing and normal. On investigation, hemoglobin was 13.3, other blood parameters were within normal limit. Liver function tests and renal function tests were normal. Chest X-ray revealed nothing.

Ultrasound has shows a round, homogeneous, mixed(hypo-, iso- and hiperechoic), well circumscribed mass, situated in the right hepatic lobe, near to upper right renal pole.

The abdominal CT-scan has showed a 6,5/5,7/4 cm solid, heterogeneous mass, with microcalcification, situated at the right suprarenal gland. It was located between the diaphragm superior, the right renal artery inferior and the inferior vena cava medially. The right kidney was displaced caudal and posterior. Other abdominal viscera looked normal. This tumoral mass was thought to be a neuroblastoma.

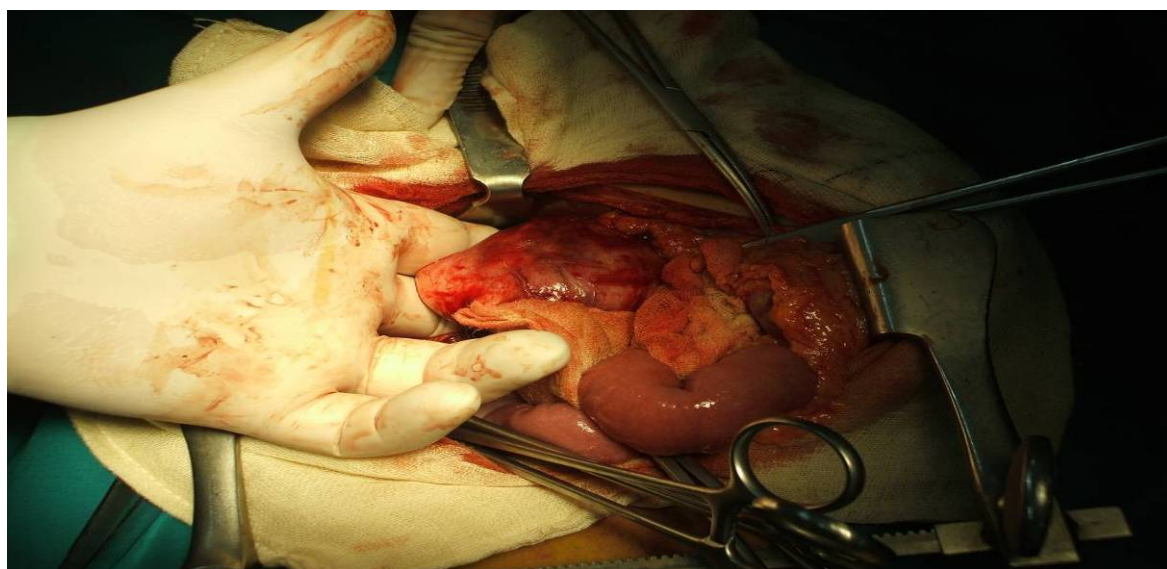


Fig.1. Intraoperative aspect. The tumor is in contact with upper pole of the right adrenal gland.

Based on above examination, after a previously preoperative prepare, laparotomy was done for exploration: the lateral attachment of the superior ascending colon and

hepatic flexure are divided and the colon retracted medially and inferiorly. The division of the peritoneum is extended up to the right of the duodenum which is then mobilized

after the fashion of Kocher. This allows access to the upper kidney, part of the adrenal. Perinephric fascia (Gerota) is divided over the kidney and it revealed a 7/5/4 cm, firm, solid, mass, who was arising from retroperitoneum, in contact with upper pole of the right adrenal gland. Grossly, the tumor appeared as a grayish, white, solid mass, slightly lobulated, with a pseudocapsule. The plane of cleavage between the tumor and the adjacent structures was established and the tumor was completely excised. No peritoneal seedling of tumor was there. No intraoperative bleeding occurred. A drain tube was left in the tumor site.

Postoperative period was uneventful, except a hemorrhage from the tumoral layer, during the first day, who was treated conservatively. Patient was discharged on 11<sup>th</sup> postoperative day. He had a uneventful recovery and is maintaining good health at 3 month after surgery at this moment.

Microscopic, the tumor containing mature ganglion cells, supported by a connective tissue network containing Schwann cells; perivascular calcification, focal necrosis and thrombosis. The diagnosis of ganglioneuroma was made.



Fig. 2. The tumor after resection appeared as a grayish, white, solid mass, slightly lobulated, with a pseudocapsule.

## References

1. Abramson SJ: Adrenal neoplasms in children. Radiol. Clin North Am 1997Nov; 35(6) 1415-5
2. Brossard J, Bernstein ML, Lemieux B: Neuroblastoma: an enigmatic disease. British Medical Bulletin 1996;52:787-801
3. Geoerger B, Hero B, Harms D, et al: Metabolic activity and clinical features of primary ganglioneuromas. Cancer 2001May 15; 91(10):1905-13
4. Ichikawa T, Ohtomo K, Araki T, et al: CT and magnetic resonance features.Br J Radiol 1996 Feb; 69(818):114-21.
5. Joshi VV: Peripheral neuroblastic tumors: pathologic classification based on recommendations of international neuroblastoma pathology committee(Modification of Shimada classification).Pediatr Dev Pathol 2000 Mar-Apr;3(2):184-99
6. Rha SE, Byun JY, Jung SE, et al: Neurogenic tumors in the abdomen: tumor types and imaging characteristics. Radiographics 2003 Jan-Feb;
7. Zhang Y, Nishimura H, Kato S, et al: MRI of ganglioneuroma: histologic correlation study. J Comput Assist Tomogr 2001 Jul-Aug;25(4):617-23.

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### **MANUSCRIPT REQUIREMENTS**

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

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