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# I. GENETICS

## CONTRIBUTIONS OF THE MOLECULAR CYTOGENETICS TO THE MANAGEMENT OF THE CHILDHOOD ALL

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### Abstract

The study of leukemia is a very present theme and it offers interest to many international research groups. In the last years remarkable progresses were made in the treatment of these affections. The accomplishments made in the children acute lymphoblastic leukemia treatment (ALL) are major aspects of the progress and the efficiency of the modern medical science in collaboration with medical genetics. One of the appropriate genetic techniques and very helpful for detecting ALL is the *in situ* hybridization (FISH). ALL is accepted nowadays as an exceptional case of curable cancer through a relatively cheap costs chemotherapy. It is considered as an stimulating example for obtaining same results in other cancer affections in children or adults. Despite the promising results, ALL still remains a heavy duty for the medical society around the world. It's been estimated that every year 50,000 new children ALL cases appear, aproximately 40,000 are in poor or insufficient appropriate medical support countries. In consequence, the curring rate is over 80% in rich countries but unfortunately it globally drops under 50%. The poor global results are not because the incapacity to defeat the abnormal leukemial behaviour but especially because of the unaccessability or wrong utilisaton of the nowadays' therapeutics.

**Keywords:** chromosomal rearrangements, ALL, FISH

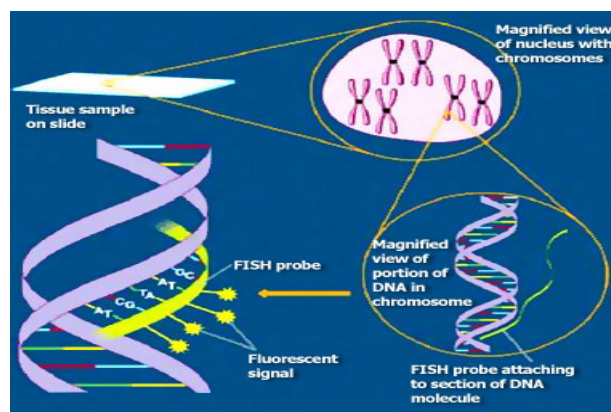
The detection of different nucleic acid sequences of the human chromosomes using the fluorescence *in situ* hybridization is providing the microscopic visualization of the region, making possible for the specialist to see wether there are rearrangements in that area or not. The method uses the fact of specific annealing of complemetary nucleic acid molecules through hydrogen bands between bases attached to the sugar-phosphate backbone as follows :

- adenine (A) anneals with thymine (T, in DNA) or uracil (U, in RNA)
- cytosine (C) anneals with guanine (G)

In this way, the base-pairing leads to the formation of the double-stranded DNA complex, in which the strands have opposite directions one to the other. In conclusion, any nucleic acid sequence can therefore be specifically detected by using the 'antisense' (complementary) sequence.

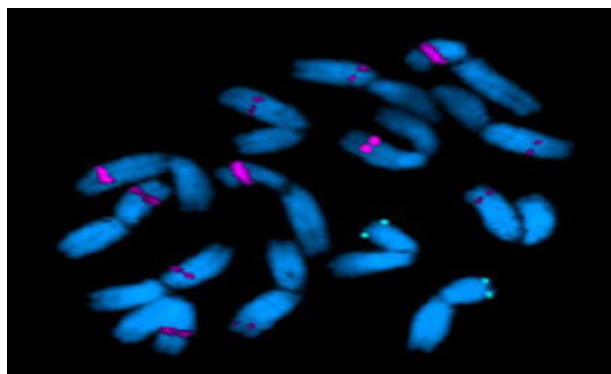
The main steps of the FISH protocol are :

- synthesis of a labelled antisense probe
- pretreatment of slides for increasing the accessibility of target and/or block non-specific sites

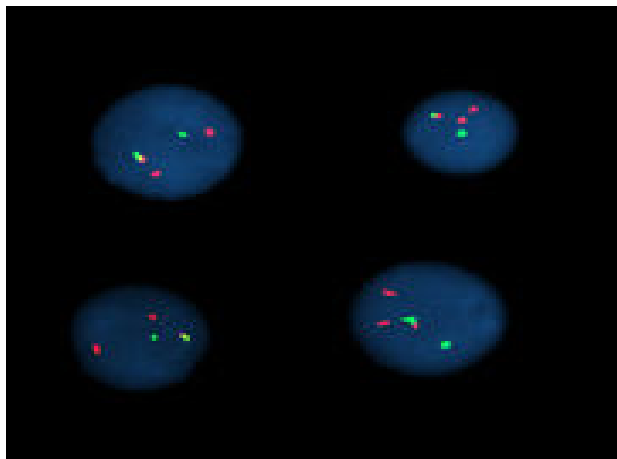


- denaturation of the double-stranded targets
- hybridization detecting fluorescence

Probes for localization by FISH are usually labelled with either biotin or digoxigenin. Deoxyribonucleotides are now also available conjugated to fluorochromes : FITC (fluorescein isothiocyanate), TRITC (tatrarmethylrhodamine isothiocyanate, and AMCA (aminomethyl coumarin acetic acid). The optimal size of labelled probe fragments is 300 bp. For detecting the labelled probe it is needed a fluorescence microscope with suitable fluorescence objectives and filter sets.



For example, a method of simultaneous hybridization and detection of 2 probes can be achieved using one probe labelled with biotin (detected with Texas red gives a red signal) and the second probe with digoxigenin (detected with FITC is giving a green signal).



Despite these scientific and technological progresses which concretized in medical successes, ALL still represents a challenge for medicine. Still a lot of patients are dying today in the world and lots of the survivors have all kind of physical and psycho-social marks. Nowadays it is known that the access to the treatment and diagnosis resources must represent a priority to everyone involved in the sanitary sistem and the chance to be cured must be a basic right for all the children in the world. All these are justifying the interest of the medical and scientific society for ALL in the purpose of a better and more precise identification of the biological bases of the treatment resistance and the risk of falling again or the therapy abortion.

The prognosis of ALL in children has changed positively in the last four decades, owing to the conjugated efforts of diverse study groups that used standard criteria for diagnosis and treatment, and which has also permitted result analysis in a short period of time, with the modifications in therapeutical protocols absed on new dicoveries in the leukemic malignant cellular biology. The current treatment is based on a better adaptation of the risk grades and the introduction of medications that acts on the target molecule (eg Introduction of imatinib-mesylatului in ALL BCR-ABL)

The german study group for ALL (BFM) recognized as having a significant result in the treatment of ALL in children, initially uses a risk group inclusion based on clinical criteria, hematological, immunophenotypic, cytogenetic, molecular biology and the response to treatment. According to these criteria, there are three groups:

- low risk : with the leucocyte count  $<20.000/\text{mmc}$  at diagnosis, age 2-6 yrs, immunophenotype with precursor B, absence of t(9;22)(BCR-ABL) or t(4;11)(MLL-AF4), favorable reponse ro cortizone in day+ 8 of treatment, complete morphologic remission at day +33, residual minimal disease negative in day +33 and +78 evaluated with a high sensibility technique. ( $10^{-5}/10^{-6}$ )

- intermediate risk : favorable reponse ro cortizone in day+ 8 of treatment, complete morphologic remission at day +33, absence of t(9;22)(BCR-ABL) or t(4;11)(MLL-AF4)

- high risk: leukocyte count increased at diagnosis ( $>100.000/\text{mmc}$ , age  $> 10$  yrs, presence of t(9;22)(BCR-ABL) or t(4;11)(MLL-AF4), non responsive to cortisone treatment at day +8 , absence of complete remission in day +33, minimal residual disease  $>> 10^{-3}$  in day +78.

Patients with high risk have indications for intense therapeutical programs which includes even hematopoietic stem cell transplant- a procedure which is now available even in Romania.

The German study group CCG currently identifies another category of children with “very low” risk : female sex, white race, age 1-9 yr, immunophenotype with precursor B cells, hyperploids, leukocytes  $< 50000/\text{mmc}$ , without CNS involvement at diagnosis, with early response to the treatment ( day 8, day14), minimal residual disease  $< 10^{-2}$  day +28; for which they propose a less intense chemotherapy- Children's Leukemia & Cancer Research Foundation (Inc), Children's Cancer Group (CCG).

**In spite of all these progress in the understanding of the characteristics of the malignant cells, some patients are still over treated and some are under treated. The approach to the leucomogenesis process, which is no more considered secondary to a certain translocation but as a result of complex modifications at the genic level, has changed with the introduction of the genic expression profile analysis.**

Using this technology we can analyse over 40.000 genes. We identified 6 risk subgroups of LAL named after criteria mentioned above – LAL-T, E2A-PBX1, TEL-AML1, BCR-ABL, MILL, hyperdiploia. All of these categories of AL are associated with the abnormal expression of a very large number of genes, their functions in the biology of a normal cell or malign cells is more or less known. This type of studies has evolved during the last 5 years, the first results being published at the end of 2003. In this context, our studies are being aliened with the present preoccupations of the International Science Community.

Nowadays a large number of research groups are studying the AL in children based on the BMR study: European BIOMED-1 Concerted Action “Investigation of minimal residual disease in acute leukemia: international standardization and clinical evaluation” with the participation of 14 diferent laboratories from 8 European countries (ES, NL, PT, IT, DE, FR, SE and AT). Another study undertaken by European Study Group on MRD Detection in ALL (ESG-MRD-ALL)” include 23 laboratories from 10 different European countries (NL, DE, FR, GB, AT, IT, ES, SE, DK, and CZ). Intercontinental-BFM 2002 Protocol (ALL IC BFM 2002) is a larger project produced by American researchers, including BFM group (ALL BFM/AIEOP 2000, Germany, Austria, Italy and Holand), laboratories from Argentina, Chile, Croatia, the Czeck Republic, Hong Kong, Hungary, Israel, Poland si Uruguay, all of them bein intrested in the colaboration with laboratories

world wide. Some of the reports written by these groups were published in well known magazines, like Blood, Ann Hematol, Lancet, Leukemia, Br J Haematol, Best Pract Res Clin Haematol, J Clin Oncol, Klin Padiatr, Nature, Science, New England J Med, Hematology.

**In conclusion, the complex diagnostic – morfological, imunophenotipical, citogenetic and molecular – is an obligation in all the international protocols refering to the treatment of ALL sufering patients.**

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## II. NEONATOLOGY

# THE PREVALENCE OF THE HEART CONGENITAL MALFORMATIONS TO THE PREMATURE NEW BORN

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### Abstract

Congenital heart diseases occur in approximately 1% of live-born infants and represent an important problem in pediatry. The objectives of this study are to establish the incidence of heart congenital malformations when compared to the other congenital malformations and their frequency according to some factors: risk, social background, sex and prematurity.

**Key words:** heart congenital malformations, prematurity.

### Introduction

Heart congenital malformations represent an important problem in pediatry because of the growth incidence and of the medical and social implications.

The diagnostic dilemma of the newborn with congenital heart disease must be resolved quickly since therapy may prove lifesaving for some of these infants. Congenital heart disease occurs in approximately 1% of live-born infants. Nearly half of all cases of congenital heart disease are diagnosed during the first week of life. The most frequently occurring anomalies seen during this first week are patent ductus arteriosus, transposition of the great arteries, hypoplastic left heart syndrome, tetralogy of Fallot, and pulmonary atresia.

Symptoms and signs in newborns with heart disease permit grouping according to levels of arterial oxygen saturation (cyanotic heart disease and acyanotic heart disease). Further classification (based on other physical findings and laboratory tests) facilitates delineation of the exact cardiac lesion present.

### *Cyanotic heart disease*

Infants with cyanotic heart disease are usually unable to achieve a PaO<sub>2</sub> above 100 mm Hg after breathing 100% inspired oxygen for 10-20 minutes. Because of intracardiac right-to-left shunting, the newborn with cyanotic congenital heart disease (in contrast to the infant with pulmonary disease) is unable to raise the arterial saturation, even in the presence of increased ambient oxygen.

Care must be taken in evaluating cyanosis by skin color, since polycythemia, jaundice, racial pigmentation, or anemia may make clinical recognition of cyanosis difficult.

The infant with cyanotic congenital heart disease often does not have a distinctive murmur. In fact, the most serious of these anomalies may not be associated with a murmur at all.

Cyanotic infants may be further classified on the basis of pulmonary circulation on chest x-ray and electrocardiographic findings.

The most frequent cyanotic heart disease abnormalities are:

### 1. Transposition of the great arteries

Transposition of the great arteries is the most common cardiac cause of cyanosis in the first year of life, with a male/female ratio of 2:1. The aorta comes from the right ventricle and the pulmonary artery from the left ventricle. With modern newborn care, 1-year survival approaches 80%.

Typical presentation is a large, vigorous infant with cyanosis but little or no respiratory distress. There may be no murmur or a soft, systolic ejection murmur. Chest x-ray study may be normal, but typically it reveals a very narrow upper mediastinal shadow ("egg on a stick" appearance).

There are no characteristic ECG findings but echocardiography is diagnostic and typical findings are branching of the anterior great vessel into the innominate, subclavian, and carotid vessels and branching of the posterior great vessel into the right and left pulmonary arteries.

Like echocardiography, cardiac catheterization is diagnostic and often therapeutic. Urgent cardiac catheterization with balloon septostomy and later Mustard or Senning procedures (transposing venous return via an intra-arterial baffle) or early arterial switch operation are the methods of treatment.

### 2. Tetralogy of Fallot (TOF)

TOF is characterized by 4 anomalies: pulmonary stenosis, ventricular septal defect, overriding aorta, and right ventricular hypertrophy (RVH). There is a slight male predominance. Cyanosis usually signifies complete or partial atresia of the right ventricular outflow tract or extremely severe pulmonary stenosis with hypoplastic pulmonary arteries. The degree of right ventricular outflow obstruction is inversely proportional to pulmonary blood

flow and directly proportional to the degree of cyanosis. TOF with absent pulmonary valve may present later in infancy due to poor feeding (due to very large pulmonary arteries causing esophageal compression).

At physical examination the patient is cyanotic with a systolic ejection murmur along the left sternal border. Loud murmurs are associated with more flow across the right ventricular outflow tract, and softer murmurs, with less flow.

Chest x-ray study shows a small, often "boot-shaped" heart, with decreased pulmonary vascular markings. A right aortic arch is seen in about 20% of these infants.

The echocardiogram may be normal or demonstrate right ventricular hypertrophy (RVH). The only sign of RVH may be an upright T wave in V4R or V1 after 72 hours of age.

Echocardiography is usually diagnostic, with the demonstration of an overriding aorta, ventricular septal defect (VSD), and small right ventricular outflow tract.

Pulmonary blood flow may be ductal-dependent with severe cyanosis and may respond to ductal dilation using prostaglandin E. This measure allows more flexibility for planning cardiac catheterization and surgical correction. Surgery (shunting or total correction) may be palliative.

#### *Acyanotic heart disease*

Infants with acyanotic heart disease will achieve PaO<sub>2</sub> levels of over 100 mm Hg after breathing 100% inspired oxygen for 10-20 minutes.

The infant who is not cyanotic will present with either a heart murmur or symptoms of congestive heart failure.

Specific acyanotic heart disease abnormalities:

1. Ventricular septal defect (VSD) is the most common congenital heart abnormality, with equal sex distribution. Murmurs are not heard at birth but typically appear between 3 days and 3 weeks of age. Congestive heart failure is unusual before 4 weeks of age but may develop earlier in premature infants. Symptoms and physical findings vary with the age of the patient and the size of the defect.

Spontaneous closure occurs in half. Surgical correction is reserved for large, symptomatic VSD only.

2. Atrial septal defect (ASD) is not an important cause of morbidity or mortality in infancy. Occasionally, congestive heart failure can occur in infancy but not usually in the neonatal period.

3. Endocardial cushion defects include ostium primum-type ASD with or without cleft mitral valve and atrioventricular (AV) canal. These defects are commonly associated with multiple congenital anomalies, especially Down's syndrome. If marked AV-valve insufficiency is present, the patient may present with congestive heart failure at birth or in the neonatal period.

#### **Objectives**

This study wants to establish the incidence of heart congenital malformations when compared to the other congenital malformations and their frequency according to some factors: risk, social background, sex and prematurity.

#### **Material and method**

The study is based on clinical, paraclinical examinations and imagistic explorations which were performed on premature new born hospitalized in the Neonatology and Health Care Clinic Timisoara between 2003 and 2005.

#### **Results and discussions**

Of the 72 studied with congenital malformations, 33 (45,8%) had heart congenital malformations. Of these 3 (9%) were cyanotic lesions (transpositions of the great arteries) and 30 (91%) – acyanotic lesions.

Regarding the social background 18 (54,5%) were from urban areas and 15 (45,5%) from the rural areas (fig 1).

The repartition of the cases according to sex showed that 16 (48,4%) were female and 17 (51,6%) were male (fig. 2).

According to the prematurity: 12 (36,3%) were 1<sup>st</sup> grade prematures, 12 (42,4%) – second grade prematures, 3 (9%) – 3<sup>rd</sup> grade prematures and 4 (12%) – 4<sup>th</sup> grade prematures (fig. 3).

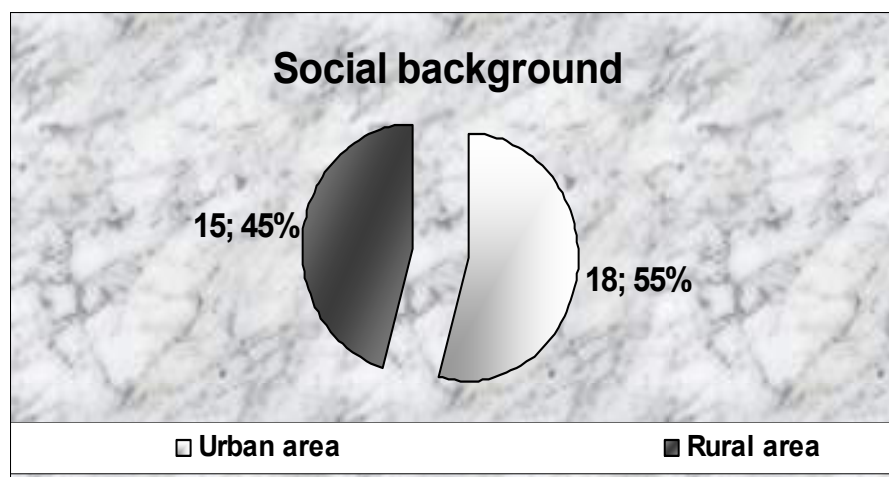


Fig. 1. Repartition of cases according to social background.

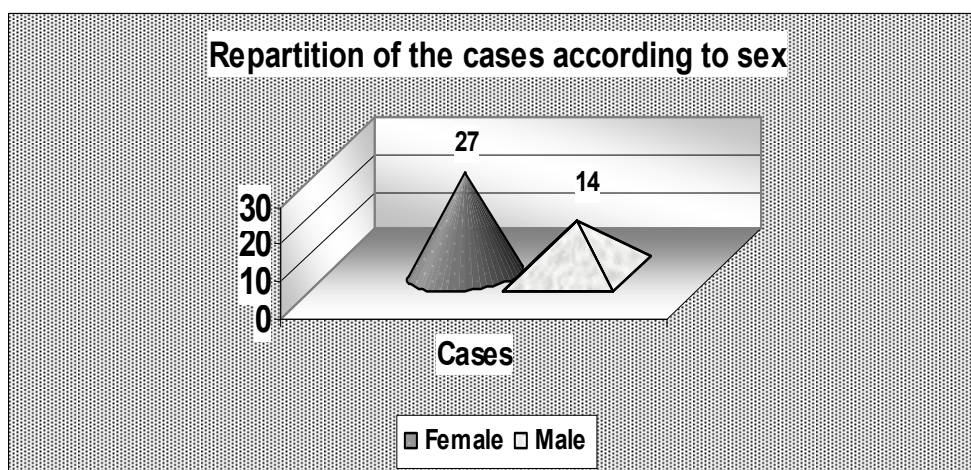


Fig. 2. Distribution of cases according to sex.

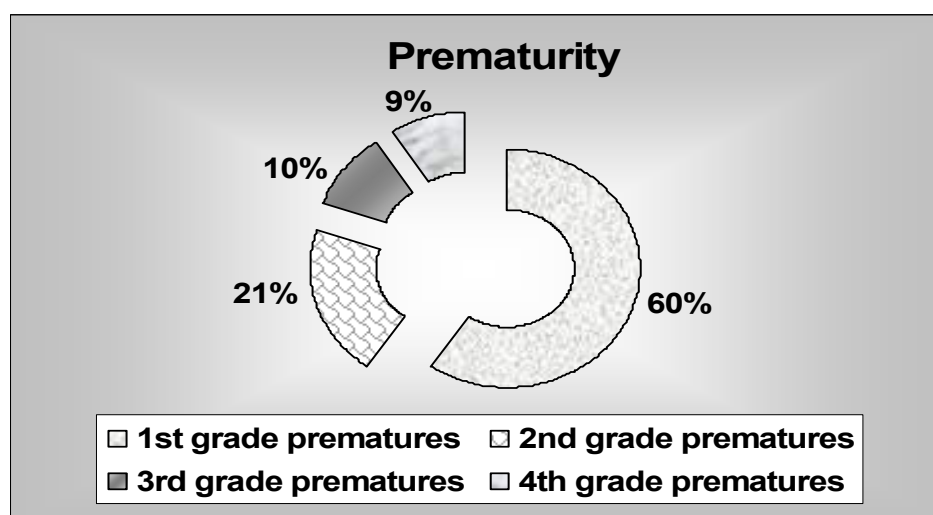


Fig. 3. Repartition of cases related to grade of prematurity.

### Conclusions

1. The incidence of heart congenital malformations is high, representing 45,8% of the total congenital malformations.
2. The frequency of the acyanotic lesions is superior to the cyanotic lesions.
3. There is a slight predominance of the cases which come from the urban areas comparatively to those from the rural areas, possibly because of higher pollution.
4. Distribution according to sex is approximately equal.

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### III. PEDIATRICS

## THE ROLE OF DENDRITIC CELLS IN ATOPIC DISEASE

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#### Abstract

Large populations of dendritic cells (DCs) are found throughout the respiratory tract, the most prominent comprising a contiguous network dispersed throughout the epithelium and underlying mucosa of the conducting airways. These populations of DCs in the lung and airway wall are now known to play a central role in the maintenance of immunological homeostasis in the respiratory tract. Dendritic cells play a critical role in the initiation of allergic pulmonary inflammation. Pulmonary DCs total number is increased in the asthmatic pulmonary tissue, even though it is not known if these pulmonary DCs are phenotypically or functionally different from those present in the bronchia and bronchioles of non-asthmatic individuals.

**Key words:** dendritic cell, allergy, Th<sub>2</sub>, asthma

Atopic diathesis is characterized by three main diseases: allergic rhino-conjunctivitis, allergic asthma and atopic dermatitis, usually associated with increased levels of IgE. Approximately 3 millions Romanians are suffering of allergic diseases. The spring is accompanied for them with an exacerbation of symptoms, determined by an increase in aeroallergens. In many cases, the symptoms are only annoying (rhinorea, sneezing etc.); however, more severe consequences, as exacerbation of bronchic asthma, are possible.

Research regarding asthma revealed the essential role of airway dendritic cells in inducing allergen sensitivity. This paper describes the physiology of DCs, as well as the mechanisms of allergic sensitization through dendritic cells and provides a summary of a recent proved theory that DCs function regardless of sensitization degree.

Many factors may influence the establishment of allergic disease, including genetic susceptibility, environmental factors as microbial exposure and allergen dose, the time of allergen exposure, and the subtype/function of dendritic cells that initiate Th<sub>2</sub> polarization. Although genetic component is important, it seems that environmental interactions during the first years of life, a concept called hygiene hypothesis, are crucial in rhinitis development. Childhood exposure to microbial endotoxines with Th<sub>1</sub> immunologic programming may have a protective effect. For example, animal contacts during the

childhood (children that grow in farms or that have pets), provide protection against atopic sensitization.

Allergy thus may originate in a fail to change allergen-specific Th<sub>2</sub> response in protective Th<sub>1</sub> response. It has been also suggested that impaired regulatory T cells activity in atopic individuals would be a cause of disease development.

Asthma is a common, hardly treatable disease, with an incidence that doubled in the last two decades, its global costs exceeding those for tuberculosis and HIV/AIDS together. Asthma is a Th<sub>2</sub>-type inflammatory disease of the airways characterized by airway eosinophilia, increase of mucus production and structural remodeling or airway walls. All these features lead to airways obstruction and bronchial hyper-reactivity (BHR) to non specific stimuli.

The presence of high levels of allergen-specific IgE in allergic asthma is the reflection of a Th<sub>2</sub> immune reaction to common environment allergen as HDM or aeroallergens. This Th<sub>2</sub> sensitization process to inhaled allergens occurs in the childhood and is influenced by genetic factors such as infections and exposure to microbial compounds.

Naive T cells require antigen-presenting cells (APC), such as dendritic cells, to clonally proliferate and to acquire Th<sub>2</sub> effector function so that to react at the time of antigen contact. The studies conducted in the late 90's clearly showed that DCs play a vital role in deciding the result of antigen integration in the immune system and in the integration of antigen-derived signals, inflammation context and host environment into a signal that can be read by naïve T cells in lymphoid tissues.

DCs play a unique role in the initiation of specific immune responses, beside their role in the process of differentiation and polarization of antigen-specific T cell responses. Even though it has been suggested that other cells, such as B cells, macrophages, epithelial cells and even eosinophils, take part in antigen presentation, it became more and more clearly that DCs are the most important APC. For instance, ovalbumin (OVA)-sensitized B cell-deficient mice further develop airway inflammation. Similarly, the eosinophils seem to amplify Th<sub>2</sub> responses, but they lack the capacity to present the antigen to naïve T cells. In a murine model of human severe combined immunodeficiency, reconstituted with peripheral blood mononuclear cells from *Dermatophagoides pteronyssinus*

(Der p1)-sensitive patients, it has been shown that human DCs are mainly localized in the alveolar spaces of the lungs of mice, which developed a pulmonary inflammatory infiltrate. After exposure to Der p1, the number of DCs in the airways decreased and subsequently it was detected an increased production of IgE as compared to mice in the control group. It has been recently shown that adoptive transfer of DCs from mice with cow's milk food allergy also induces allergen-specific IgE in naïve syngenic mice in absence of antigen challenge. Interestingly, allergen-specific IgE response was induced without altering Th<sub>1</sub>/Th<sub>2</sub> balance, indicating that Th<sub>2</sub>-skewed responses were not involved in early phases of allergic responses. Moreover, OVA-sensitized transgenic mice, with selectively depleted airway DCs, but not macrophages and B cells, displayed the suppression of eosinophilic airway inflammation after OVA exposure, compared to control mice, thus proving DCs contribution to the pathogenesis of this disease.

After neglecting DCs research for some years, investigators are now showing a great interest on the subject, because of the central role these cells in the complex processes of adaptive immune reaction. Furthermore, understanding the role of DCs in physiopathologic conditions might be an important step in developing a therapy for many diseases. As several types of DCs, including follicular and thymic DCs, were identified in the last years, this paper will focus on classic DC.

Over the last decade, airways DCs were shown to be vital in Th<sub>2</sub> allergic sensitization process, especially in asthmatic gerbils. The gerbils were induced with transgenic disease to study DCs role in pulmonary allergic reactions. These experiments lead to the conclusion that airways DCs are vital not only in the regulation of inhaled allergen sensitization process, but also in controlling established allergic inflammation. Change of DCs function is a therapeutic concept that will be able either to prevent establishment of sensitization, or to treat the already established disease.

DCs were found in all epithelial types (subcutaneous tissue, mucosa, lungs), as well as in heart, kidneys and other organs. In addition, various DCs subtypes were found in the blood and lymphatic system. These represent different maturation stages (depending on antigenic load) and are connected through circulatory pathways. Beside the typical dendritic structure in tissue and suspension, DCs are initially characterized by the expression of major histocompatibility complex (MHC), class II HLA-DR and by high stimulatory activity towards allogeneic T cells. Immature DCs are distributed all over the lungs area, playing a pivotal role in the control of immune reactions of inhaled auto-antigens. A network of airway DCs is situated immediately above or beneath the basement membrane of respiratory epithelium in all studied species.

Even though they act as specialized antigen-presenting cells (APC), DCs must undergo 4 main differentiation and maturation stages before effectuating their main function in lymphoid organs.

Once situated in the peripheral blood, DCs are considered functionally immature. This means that tissue DCs are specialized in capturing and processing both self

and non-self antigens. Another DCs feature is the stability of MHC class I or class II molecules on the cell surface, enabling them to remain loaded with defined antigens for a long time. In this maturation state, DCs are able to stimulate memory T cells by migrating throughout the tissue, and initiating a secondary immune response in this site of captured antigen contact. However, as the macrophages and other cells are equally effective in this type of stimulatory activity, it is conceivable that activation of secondary immune response is the main role of DCs under normal conditions.

Over the last years DCs migration has been shown to be up-regulated by chemokines. Expression of chemokines in various anatomic sites and under different pathological conditions, combined with expression of chemokine receptors on the cells during different maturation stages represent the basic events for the initiation of a complex signaling network that directs their migration and interaction in the process of immune response. It has been shown (characteristic for DCs) that chemokine receptor profile expressed on immature DCs (CCR1, CCR2, CCR5 and CCR6) recognizes mainly the chemokines released during the inflammatory process. This allows for DCs to accumulate for the antigen uptake at sites of inflammation. When chemokines like IL-1 and TNF- $\alpha$  occur, this process continues by induction of immature DCs that will release more chemokines. Mature DCs regulate their inflammatory chemokines receptors and also express different chemokine receptors (CCR4, CCR7, CXCR4, SLC and ELC).

After antigen uptake, DCs migrate from the tissue to the regional lymph nodes. For instance, LC appear to migrate quite fast, several millimeters in 30 minutes. On their way to the lymph nodes, DCs start a profound metamorphosis leading to significant changes in structure and phenotype. DCs in afferent lymphatic vessels were described as veil (circulating) cells, while DCs in the T cell-rich paracortical areas of secondary lymph tissues as interdigital cells. Mature DCs loose their antigen uptake capacity in the process and acquire their antigen-presentation function. One of the major steps of this development is the regulation of costimulatory and peptide-loaded MHC class II molecules (CD80, CD86) on these cells surface. Meanwhile, DCs rapidly regulate Fc receptors expression. DCs migration and maturation seem to be interconnected *in vitro*, as factors like lipopolysaccharide (LPS), TNF- $\alpha$  and IL-1 induce the both processes. *In vitro* TNF- $\alpha$  induces the maturation of monocytes-derived DCs, leading to regulation of CD80, CD86, CD83 and MHC class II molecules, which are all crucial in effective antigen presentation.

Naïve T cells activation is a crucial role of DCs. For this, DCs and naïve T cells must meet in the paracortical area of lymph nodes. An interesting finding is that naïve T cells express chemokine receptors (e.g. CCR7) which enable them to receive signals from mature DCs releasing ELC and specific chemokine receptors. After reaching the T cell zone, a single DC can activate hundreds of naïve T cells. In this process, peptides bound to MHC class II or MHC class I by DCs are presented to T cells *versus* T cell receptor complex (TCR). Recently, in addition to the signals received *versus*

TCR, co-stimulatory were shown to be of key importance in initiating and directing the T cell response. The interaction between co-stimulatory molecules CD80/CD86 and CD28 or CTLA-4 T cells determines whether the stimulation process will result in a T cell antigen- or tolerance-specific proliferation. Indeed, additional factors, as IL-10, present in the site of T cell – DCs interaction can change CD80/CD28 signaling by blocking the events of signal delivery, thus leading to an antigen-specific tolerance.

An important observation is that DCs releases IL-12. This cytokine is involved in the induction of a Th<sub>1</sub> response of T cell. In the same way, other cytokines, like IFN- $\gamma$ , may induce a Th<sub>1</sub> response, even though IL-4 directs T cell response towards Th<sub>2</sub>. This ability to influence the type of T cell response may explain the reason why some antigens induce an allergic reaction, while the others don't. It is interesting that cytokines and factors released during T cell activation induce a different chemokine repertoire on stimulated T cells. Although Th<sub>1</sub> cells express CCR1, CCR2, CCR5, CXCR3 and CXCR5, Th<sub>2</sub> cells are characterized by the expression of CCR2, CCR3, CCR4 and CXCR5.

Differential expression pattern could recruit these cells to specific types of inflammation. As the allergic reactions concern, Th<sub>2</sub>, eosinophil and basophil cells are known to share the expression of chemokine receptors CCR3, even if Th<sub>1</sub> cells and monocytes, that are able to differentiate into DCs, share CCR1 and CCR5.

Once antigen presentation accomplished, DCs cannot recirculate in the peripheral blood or lymph vessels; it is assumed DCs will be killed by T cells and will die by apoptosis.

DCs are thought to be the best candidates for T cell activation against environmental allergens. In the context of Th<sub>1</sub>/Th<sub>2</sub> dichotomy that dominated immunologic research over the last years, it has been intensively discussed the way T cells are directed towards Th<sub>1</sub> or Th<sub>2</sub> during antigen presentation. Although it became clear that IL-12 secreted by DCs is responsible for the skewing towards Th<sub>1</sub>, which cells are the source of IL-4 – that skews the T cell response towards Th<sub>2</sub> remains to be further discussed. Kalinski *et al.* showed that prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) may be the signal that directs Th<sub>0</sub> cells towards Th<sub>2</sub>. Recently Rissouan *et al.* showed that myeloid DCs are responsible for the skewing of T cells towards Th<sub>1</sub> (DC<sub>1</sub>), while lymphoid DCs are skewed towards Th<sub>2</sub> independently of IL-4 (DC<sub>2</sub>). Moreover, there are feedback mechanisms acting between DCs and T cells.

LC, monocytes and myeloid DCs were reported to express the IgI and Fc $\epsilon$ RI high affinity receptor. Fc $\epsilon$ RI on LC and DC<sub>1</sub> shows important differences from this receptor on effector cells of anaphylaxis.

It is indeed not expressed on these cells, but seems to be regulated by the signals of inflammatory micromilieu that surrounds the cells. Therefore, the highest Fc $\epsilon$ RI expression is displayed on LC and a recently described inflammatory dendritic epidermal cell (IDEC, presumably DC<sub>1</sub>) from lesional skin of atopic dermatitis.

The lack of receptor complex is due to the low expression of signal transmission chain that is important for the expression of heterotrimeric structure surface, whereas

the chain IgE-bound is present inside the cells. Furthermore, Fc $\epsilon$ RI on LC and DC<sub>1</sub>, as well as on monocytes, lacks 4 transmembrane chains. As a consequence, in contrast with LC and DC<sub>1</sub> from atopic individuals, normal LCs (with low expression receptors) are not activated. There are proofs of Fc $\epsilon$ RI role in antigen presentation that emphasizes blood monocytes, LC and DC. Multimeric ligations taken over by the endocytosis-mediated Fc $\epsilon$ RI receptors are effectively directed in MHC class II compartments, like the organs in which processing-dependent cathepsin S and MHC class II-loading peptides occur. This results in the optimal antigen presentation by CD4<sup>+</sup>, similar to a mechanism in the first line of antigen recognition. In this context, a carrier role for DCs expressing Fc $\epsilon$ RI in the regulation of IgE synthesis is conceivable. It is generally accepted that IgE molecules and effector cells are the result of the efficient anti-parasitic defending mechanism. This system was proposed to be redirected towards a harmless allergen environment because of the lack of physiologic/pathologic partners.

As mentioned before, allergen uptake and presentation are the primary functions of DCs. Among the methods of allergen capture, classically including nonspecific absorption, fluid phase pinocytosis and cell surface receptor endocytosis, the last is the most effective.

Expression of high Fc $\epsilon$ RI density in atopic DCs patients involves several important features. Firstly, DCs extend their ability to react to allergens by binding large amounts of IgE molecules with specific variants. This significantly increases the probability of Fc $\epsilon$ RI cross-linking to a different allergen on the cell surface.

Secondly, IgE/Fc $\epsilon$ RI complexes allow the allergen capture; under normal circumstances, they don't undergo phagocytosis via normal route (e.g. by pinocytosis). Thirdly, Fc $\epsilon$ RI aggregation on DCs is followed by their internalization through receptor-mediated endocytosis. However, similar to B cell receptor, where Ig $\alpha$  and Ig $\beta$  specialize on various endosomal behaviors, this route used for the antigen uptake by DCs, especially via IgE and Fc $\epsilon$ RI, can determine if the foreign structures will be effectively processed and orientated to MHC class II-rich compartments, finally leading to a higher density of specific peptides on MHC class II molecules surface; lastly, DCs that express high receptor densities show the total activation of the cell after Fc $\epsilon$ RI ligation, most probably including synthesis and release of mediators to be finalized. Such mediators are able to influence antigen presentation.

The conclusion is that DCs expressing Fc $\epsilon$ RI coupled with IgE can activate the second immune response and IgE synthesis by recruiting and activating antigen-specific Th<sub>2</sub> cells. In the case of Fc $\epsilon$ RI mediated antigen, the uptake and the presentation seem not to occur in the primary response, as specific IgE should be present from the beginning. However, the hypothesis that allergen structures captured via Fc $\epsilon$ RI on DCs are processed by these cells in a manner that leads to peptide presentation to T cell should not be excluded. This initiates a response to the antigens, resulting in the increase of specific IgE diversity. This concept of DCs expressing Fc $\epsilon$ RI study remains to be further explored, considering particularly the recent research

suggesting an important role of DC-derived IL-12 from PGE<sub>2</sub> in skewing T cells towards Th<sub>2</sub> or Th<sub>1</sub>, respectively.

Although most asthmatic patients are atopic, only some of them develop the disease. Asthma is a complex clinic entity, characterized by chronic and acute stages. Characteristic for the acute stage is histamine release from respiratory airways cells, while the chronic stage is induced by an inflammatory intruder in the airways mucosa. In the end, chronic inflammation leads to a permanent lesion of respiratory airways. Asthma is a proto-typical allergic disease associated with Th<sub>2</sub> response and with an increased level of IgE serum.

Recently it has been speculated that increasing incidence of asthma or any other allergic disease could be caused by the high hygiene standards. However, the newborns encounter less pathogens that activate the Th<sub>1</sub> immune response. In addition, postnatal stimulation of a recently allergen-activated immune system predisposes to the positive Th<sub>2</sub> selection, thus favoring the atopic diseases-associated type of immune response. Maturation of airways DCs function after birth represents an important factor for the result of Th<sub>1</sub>/Th<sub>2</sub> memory cell selection. Variations in the effectiveness of maturation process may play a key role in determining the genetic risk of asthma.

The first requirement for the induction of an immune response to allergens is for these molecules to gain access to the immunocompetent cells. Even if the airway epithelium is a regulated barrier, transepithelial permeability is increased in asthma. Even the bronchic epithelium becomes permeable to macromolecules after allergen deposition. Moreover, allergen exposure induces the

expression of GM-CSF by asthmatic epithelium, which attracts DCs to the antigen contact site.

As for the antigen uptake by DCs, the most rapid cell reaction detectable in the tracheal tissue is the recruitment of complex MHC class II-carrying DCs. The cells remain in the epithelium, reaching their maximum within an hour since the antigen exposure. After that, DCs morphologically convert from the round form to the veil cells form. Surveillance of active DCs in the epithelium is amplified and an increase of their traffic from epithelium to the lymph nodes results. Another mechanism that may contribute to an increased reaction of asthmatic patient to inhaled allergens can be the same as in the inflammatory process, i.e. recruitment of “new” DCs from monocytes. Monocyte-derived DCs in patients with allergic asthma are known to show phenotypic variations in HLA-DR, CD11b and IgE high affinity receptor expression and even a B7-2 (CD86) regulation, and develop in stronger accessory cells than in normal patients.

Data from mice and humans show that DCs play a crucial role in the pathogeny of pulmonary allergic reaction, both during the sensitization and the disease. Therefore, the dendritic cells are leading a complex multi-cellular process, in which T cell aberrant responses, genetic influence in the process of allergen reconstitution, structural changes of respiratory airways walls and inherent epithelial defects play an important role.

Airways DCs are critical for the activation of the immune system to inhaled allergens, their interaction with APC, as well as with other effector cells remaining an active research field.

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# ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS – A PREDICTOR FOR WORSE OUTCOME IN CYSTIC FIBROSIS PATIENTS (CASE REPORT)

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## Summary

Allergic Bronchopulmonary Aspergillosis (ABPA) is a disease primarily occurring in patients with asthma or Cystic Fibrosis (CF) and develops from sensitization with allergens from *Aspergillus fumigatus* (Af) present in the environment. It is manifested by wheezing, pulmonary infiltrates and bronchiectasis and fibrosis. Immunological manifestations are peripheral blood eosinophilia, immediate cutaneous reactivity to Af antigen, elevated total serum Ig E, precipitating antibody to Af, and increased serum IL2 receptor concentrations.

The diagnosis of ABPA in CF may be difficult and often delayed because of overlapping of diagnostic criteria with common manifestations in CF. The typical presentation of ABPA in CF is with wheezing, new pulmonary infiltrates, a rise in total serum IgE and specific Ig E to *A. fumigatus*, with a fall in pulmonary function. Early diagnosis and treatment aiming to suppress the inflammation is however important to prevent irreversible lung damage.

This is a case report of 11 years and 7 months old boy diagnosed with CF at the age of 4 months but started to be followed up on a regular basis at the age of 9 years.

By the time of first presentation in our clinic he had a poor nutritional status and a poor lung function. Partial recovery followed after including him in standardized treatment regimens. He presented in January 2006 with a severe pulmonary exacerbation diagnosed as ABPA. Decision making and treatment options are discussed.

**Key words:** Allergic bronchopulmonary aspergillosis, cystic fibrosis, lung function

## Introduction

Allergic Bronchopulmonary Aspergillosis (ABPA) develops from sensitization with allergens from *Aspergillus fumigatus* present in the environment. ABPA is a disease primarily occurring in patients with asthma (1-2%) or Cystic Fibrosis (CF) (2-15%).

It is manifested by wheezing, pulmonary infiltrates and bronchiectasis and fibrosis. Some immunological manifestations are peripheral blood eosinophilia, immediate cutaneous reactivity to Af antigen, elevated total serum Ig E, precipitating antibody to Af, and increased serum IL2 receptor concentrations.

The diagnosis of ABPA in CF is usually difficult and may be often delayed because many of the diagnostic

criteria overlap with common manifestations in CF. The typical presentation of ABPA in CF is with wheezing, new pulmonary infiltrates, a rise in total serum IgE and specific Ig E to *A. fumigatus*, with a fall in pulmonary function.

The hyphae of Af that grow saprophytically in the bronchial lumen result in persistent bronchial inflammation leading to proximal bronchiectasis.

Early diagnosis and treatment aiming to suppress the inflammation is however important to prevent irreversible lung damage.

A consensus guideline on management of ABPA in CF has been published recently (1). The mainstay of treatment is oral corticosteroid therapy, but this need to be continued for several months and may be associated with significant adverse effects. It seems reasonable as well to attempt to reduce the burden of *A. fumigatus* in the respiratory tract (2). Studies of Itrakonazole in CF uncontrolled setting (3) and in randomized trials in adults with asthma and ABPA (4) have shown evidence of benefit, including the ability to reduce steroid dosage.

## Case presentation

We describe one case with CF and ABPA with difficult decision making regarding treatment options.

A 11 years and 8 months old boy was admitted on the 15th of January 2006 in the Children's Hospital of Brasov for frequent coughing, brown sputum plugs, exertional dyspnoea, left-sided chest pain, fatigue, respiratory distress. The patient was known as having CF, homozygote for del F508 mutation, diagnosed at the age of 3 months. He was unfortunately followed up and treated on a regular basis only starting with the age of 9 years.

His actual status was of severe pulmonary involvement with extensive bronchiectasis, low lung function (FEV1 50-60%pred), satisfactory nutritional status (BMI 16). During the 3 years prior to his current consultation, the patient has been admitted to the Children's Hospital of Brasov on several occasions for pulmonary exacerbations. He is chronically infected with *Staphylococcus aureus*, intermittently colonized with *Pseudomonas aeruginosa*.

The onset of symptoms goes back to December 2005 when he experienced a pulmonary exacerbation with intense coughing and brown sputum. He had a short course of iv Cefuroxime followed by oral Ciprofloxacin with attenuation of symptoms.

The actual episode started 4 days before the hospital admission with frequent coughing, minor hemoptizia and brown sputum plugs, exertional dyspnoea, no appetite.

On clinical examination, he was found to have a weight of 31 kg, height 138 cm, afebrile, dyspnoeic, with frequent cough and perioral cyanosis, wheezing, small

amounts of brown sputum, some crackles over the left hemithorax. Oxygen saturation was 90%. The chest X-ray showed bronchiectasis in the upper and lower lobes, new patchy infiltrates in the left lower lobe, right mucus hilar impaction (fig.1), compared to his last examination (fig.2).



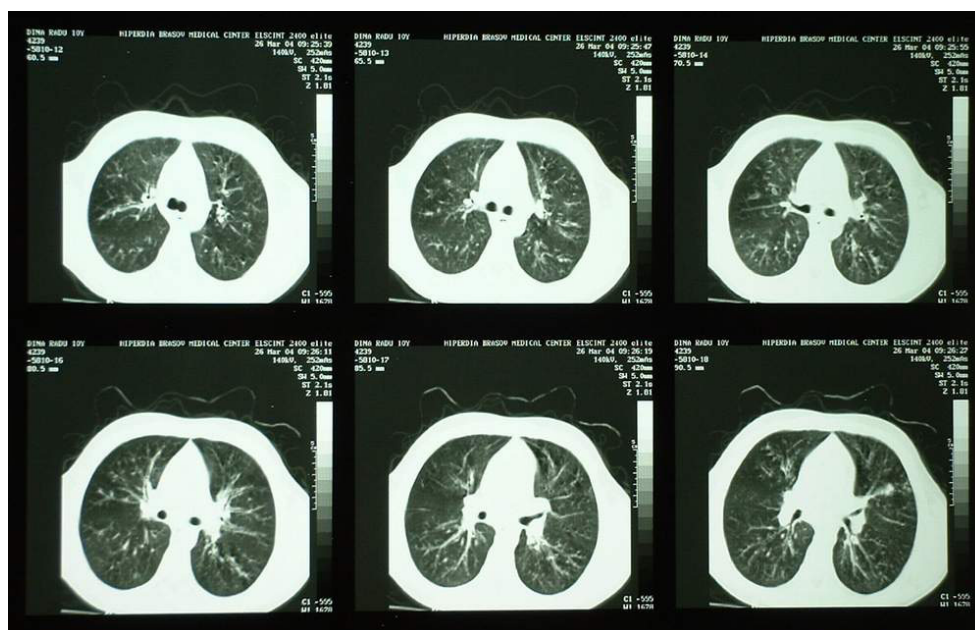
Chest x-ray January 2006 (fig.1).



Chest x-ray November 2005 (fig.2).

The CT scan confirmed the extensive bronchiectasis, bronchial wall thickening and mucus plugging, air trapping and new infiltrates in the left lower lobe.

There was a significant worsening of CT scores compared with last CT performed in 2003 (see fig.3).



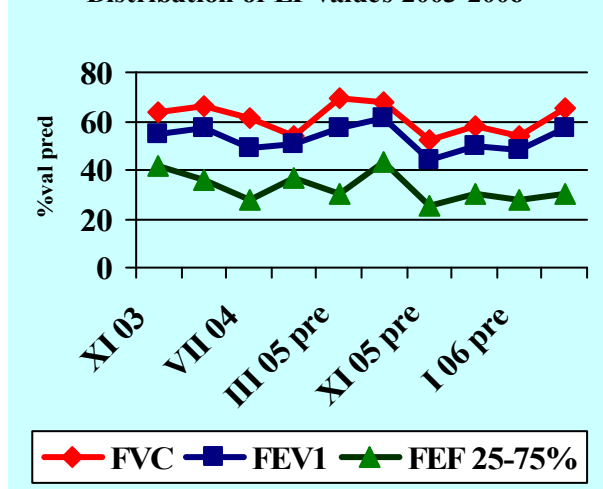
CT aspect in 2003 (fig.3).

The laboratory data were: WBC 14 600/mm<sup>3</sup>, 61%granulocytes, Hb 11.9 g/dl, ESR 68 mm/h, CRP 24mg/l, IgG 368 IU/ml; IgM 600 IU/ml. The sputum culture showed *Aspergillus fumigatus*, there was no growth of other bacteria.

Spirometry: FVC 1.26 (54%pred), FEV1 0.98(48% pred); FEF 25-75% 0.80 (28% pred).

In order to show variation of lung function over time, we attach the following graph:

Distribution of LF values 2003-2006



Other laboratory findings regarding glycaemic and hepatic status were normal (regular annual OGTT normal)

Considering the clinical symptoms, the new infiltrates on the chest x-ray, sudden decline of lung function and positive sputum culture for *A. fumigatus*, there was a high suspicion for ABPA. Skin prick test for *Af* was positive, total serum IgE were 13 579 IU/ml and IgE to *Af* >100 chiroU/l (ref values <0.35 chiroU/l).

At this stage, the patient encountered sufficient diagnostic criteria for ABPA. This determined us to start treatment with Prednisolone 2 mg/kg/day for the first 2 weeks followed by 1 mg/kg/day 1 week and 1 week of alternate day therapy. Dose was tapered to 15 mg/day alternate day till present. Itrakonazole capsules 200mg/day was associated for 6 weeks along Pulmozyme, physiotherapy and hypercaloric diet.

After the first 5 weeks of treatment the total serum IgE dropped to 8 300 IU/ml, the clinical status significantly improved, there was occasional coughing with clear sputum, diminished infiltrates on the chest x-ray, lung function increased with ~7%. There were no adverse reactions from corticosteroid and antifungal therapy.

## Discussions

Out of 25 CF patients followed up during a 7 years period in the Children's Hospital of Brasov, only 3 were colonised with *Af* and one had ABPA. All 3 patients had lower levels of lung function and didn't benefit from early treatment and follow up.

ABPA occurred in a patient with severe lung disease, with already important lung tissue scarring and who unfortunately didn't benefit from regularly follow up and treatment from diagnosis till the age of 9 years.

The patient also had a former exacerbation during November 2005 (2 months before the actual episode), with particular infiltrates on his x-ray, but no evidence of *Af* in

the sputum, positive response to antistaphylococcal therapy. There were several exacerbations when he experienced wheezing, probably showing an asthmatic pattern of response to different viral triggers.

In this particular case, the short term outcome seems to be somehow favorable, but is well known that ABPA negatively influences the pulmonary status in CF, which will be, at a certain moment a marker for a worse outlook.

Chronic infection with *Staph. aureus* seemed to be a risk factor for the developing of ABPA in this patient, more so being a determinant of extensive bronchiectasis. This could allow (as mentioned in some reports) colonisation by fungi, particularly the thermotolerant *A. fumigatus*.

Interestingly, this patient probably was colonised with *Af* following heavy rains and floods that were encountered in the county region where he lives, during the rainy season of last summer.

It has been shown that there are much higher air counts of moulds during summer-autumn season; the hypothesis that increased humidity, coupled with higher winds may trigger increased spore production and dissemination.

It is mentioned that the proof of efficiency of corticosteroid therapy in ABPA is demonstrated when total serum IgE decrease more than 50% of the initial value, which is still not our case. It seems that there is still a long way to go regarding corticosteroid therapy (probably several months) in terms of reducing the level of allergic response to *Af*.

Knowing that the bioavailability of Itrakonazole capsules is low in CF and being in the situation of very high values of total serum IgE even after 2 months of corticosteroid therapy, it seems reasonable to try to attempt reducing the burden of *Af* antigen in this patients' airways; meantime hoping to be able to reduce the doses of Prednisolone.

Vorikonazole (Vfend – Pfizer), as mentioned in the literature, even an expensive alternative, could offer a better treatment option for a patient with altered lung function, extensive bronchiectasis and very important allergic response to *Af*.

Decision making on using this antifungal agent along prolonged corticosteroid therapy, will depend on hospital policy and judging of benefits for the patient.

The question that remains is which treatment would be the best choice for this child who has already a severe impairment of lung function. It has to be considered the effect of prolonged treatment with prednisolone that apparently doesn't work as expected (only a small reduction in total serum IgE) and its adverse effects on glucose tolerance and osteopenia. In this respect, it would seem reasonable to try to attempt treatment with Vorikonazole in a supervised hospital setting, at least in order to lowering the burden of *Af* antigen response and try to restore residual lung function.

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# IMPACT OF OTHER RISK FACTORS THAN AGE AT DIAGNOSIS ON OUTCOME IN CYSTIC FIBROSIS PATIENTS

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## Abstract

Cystic Fibrosis (CF) is a complex disease requiring early diagnosis and treatment in order to improve survival. Objective: to determine the impact of risk factors (RF) other than age at diagnosis on outcome in CF patients.

Methods: Retrospective study of 24 clinical files of CF patients (age: birth-22 years) followed up during a 7 year period (1999-2006) correlated with age at diagnosis and associated RF: early *P aeruginosa* acquisition; frequent pulmonary exacerbations; del F508 homozygous; poor socioeconomic status (PSES); severe malnutrition at diagnosis (SMD); associated conditions; age when started follow up. Study population was divided in group A (early diagnosis <1 year of age) and group B (late diagnosis).

Results: Mean age at diagnosis was 4.2 months in 20/24 patients (group A) vs. 4.3 years in 4/24 patients (B). 9 patients (A) died, the majority under the age of 1 year. Major RF for deceased patients was PSES and SMD. Age for first acquisition of *P aer* (A) was 4.2 years compared to 5 years (B). There was no correlation between genotype and outcome, 75% patients had severe mutations. 50% patients (A) had frequent exacerbations compared to 25% (B). PSES was an independent RF for not deceased patients. 66% patients (A) had SMD, percentage diminished after inclusion in standard care program. LF was performed in 5/14 patients with mean decline of 4%/year, variation depending on complications of disease. Patients with early diagnosis and follow up had significantly better outcomes.

Conclusions: early *P aeruginosa* acquisition, PSES and associated conditions adversely affected outcomes. Late age when starting follow up independently of age at diagnosis predicted worse outcome.

**Key words:** cystic fibrosis, risk factors, outcome

## Introduction

Cystic Fibrosis (CF) is the most common autosomal recessive inherited disease in the caucasian population, with a frequency of one in 2500-3000 live births and a heterozygote carrier rate of approximately one in 25. The mutation is found in the CF transmembrane conductance regulator (CFTR) gene on chromosome 7. Over 1300 different mutations have been identified.

Without normal CFTR protein there is excess sodium and defective chloride transport across the apical membrane of secretory epithelial cells with dehydration of the surface epithelium and abnormal ion concentrations in

the surface liquid. Diagnostic tests for CF exploit this ionic imbalance. The clinical consequence is a multisystem disease involving predominantly the respiratory, gastroenterology, hepatobiliary and male reproductive systems. Patients are susceptible to recurrent respiratory infections with a variety of microorganisms but especially *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, *Staphylococcus aureus*, *Haemophilus influenzae* and *Aspergillus* species. Respiratory failure is the cause of death in approximately 80% of patients (1). Lack of pancreatic enzymes and bicarbonate results in steatorrhea and malnutrition. Abnormal gut motility results in acute obstruction. Liver disease may progress from focal biliary fibrosis to cirrhosis with splenomegaly, varices and cirrhosis. Men with CF are universally infertile due to bilateral absence of vas deferens.

Treatment is mostly directed at the life-threatening aspects of this multisystem disease: preventing respiratory infections as much as possible, minimizing lung damage by prompt treatment of acute infective exacerbations and use of antiinflammatory therapies, maintaining normal growth and nutrition and potentially reducing the risk of liver disease.

Patients with CF should be monitored at regular and frequent intervals, at least every 3 months, for early detection of deterioration (2).

Early diagnosis, regular monitoring and aggressive treatment protocols in CF have shown their benefits in terms of better lung function, higher life span and improved quality of life.

## Objective

The objective of the study was to determine the impact of other risk factors (RF) than age at diagnosis on the outcomes of CF patients.

## Methods

The study was represented by the retrospective evaluation of 23 clinical files of CF patients.

Age range of patients was from birth to 22 years.

The study was carried out in the Children's Hospital of Brasov during a seven years period: 1999-2006.

The evaluation of clinical parameters and outcomes were correlated with several risk factors: age at diagnosis and associated RF: early *P aeruginosa* acquisition; frequent pulmonary exacerbations; del F508

homozygous; poor socioeconomic status (PSES); severe malnutrition at diagnosis (SMD); associated conditions; age when started follow up.

Study population was divided in two groups: group A (early diagnosis <1 year of age) and group B (late diagnosis).

#### Results

Table 1, represents the patient demographics and baseline characteristics of the study population.

Table 1. Patient demographics and baseline characteristics:

	Group A (dg<1y) (n=20)	Group B (dg>1y) (n=4)
Male	13	1
Female	6	3
Del F 508 homozygous	10 (41.6%)	1 (4.16%)
Del F508 heterozygous (severe)	4 (16.6%)	2 (8.33%)
Other mutation (severe/severe)	1 (4.16%)	0
Undetermined mutations	5	1
Mean age at diagnosis (months/years)	4.2 months	4.3 years
Mean age 1-st CF symptoms	2 months	3 months
Mean age when started follow up	3 months	3.5 years
Mean age 1-st <i>Paer</i> acquisition	4.2 years	5 years
Deceased<1 year of age	6	0
Deceased >1 year of age	3	0
Mean Schwachman score*	71.16	74.6
Mean Chrispin-Norman score*	4.25	5
Associated conditions **	10	0

\*Evaluation done at the end of the study period

\*\* MI, ABPA, CFRLD, other

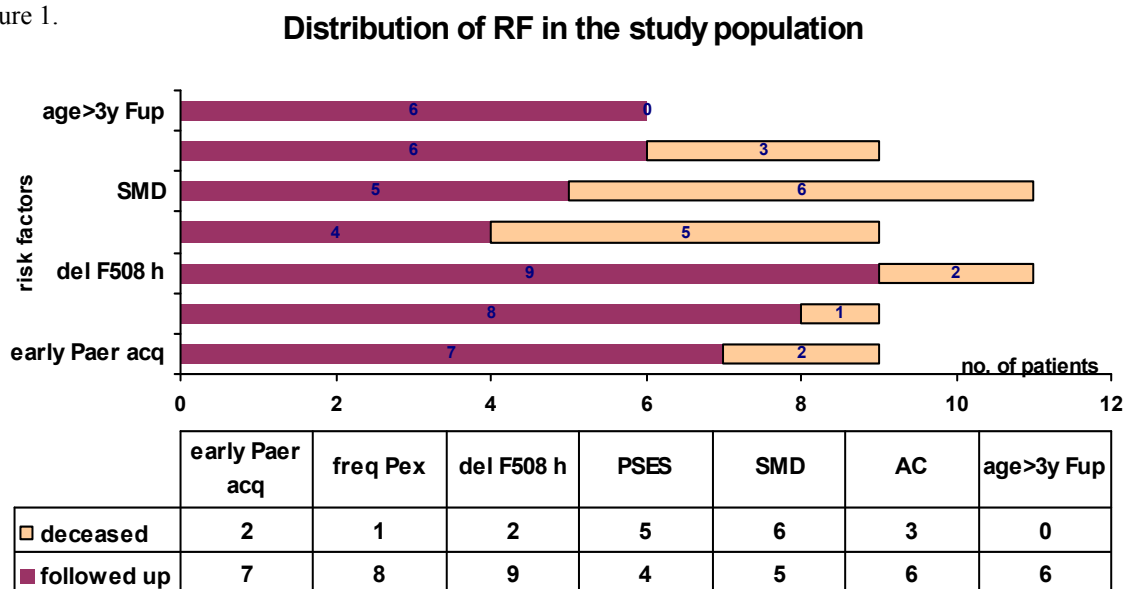
At that moment of the study, the following observations were done:

- There was no correlation between genotype and clinical outcomes (as seen in Schwachman scores and incidence of severe mutations)
- 75% of patients had severe mutations (42% del F508 homozygous)

- There was no significant difference between Schwachman scores and Chrispin-Norman scores ( $p=0.20$ )

The distribution of evaluated risk factors is shown in figure 1.

Figure 1.



Among the evaluated risk factors, being a del F508 homozygous seemed to have a major impact in the study population, as well as early colonisation with *P aeruginosa* (as mentioned in the literature).

Deceased patients (37.5% of the study population) had a greater impact on worse outcome by poor socio-economic status (the majority being from rural areas with low incomes of the families and poorer understanding of the chronic condition of disease. Severe malnutrition at diagnosis (SMD) was also predominant in this group of

patients, probably correlated as well with the socio-economic status.

There was a significant difference between ages when 1-st symptoms of CF occurred and age when diagnosis was done (age when diagnosis was done usually corresponded with age when follow up started) as it is illustrated in figure 2.

Late age by starting follow up and associated conditions have had influenced outcomes as observed in figure 3.

Figure 2.

### Distribution of ages at 1-st symptoms and at diagnosis among followed up patients

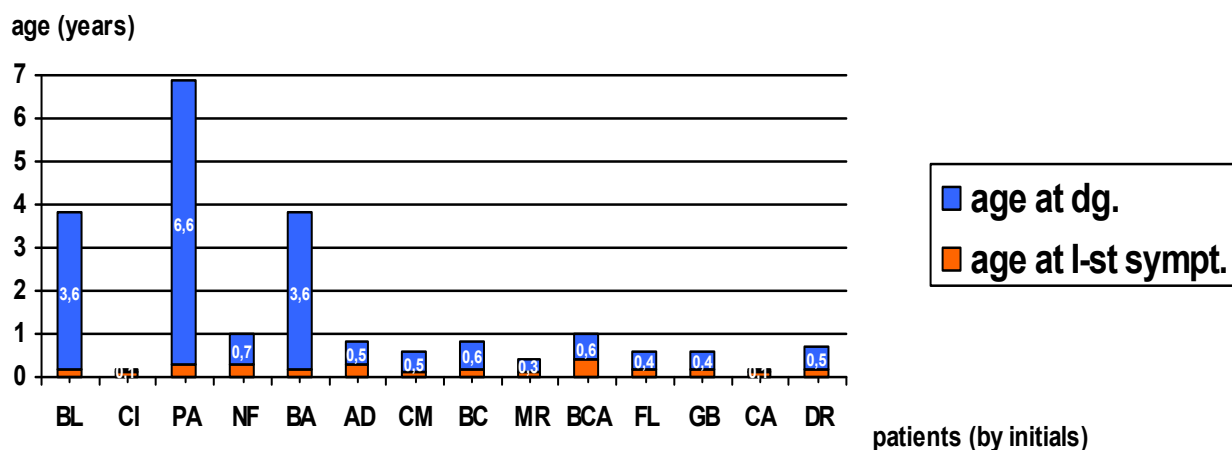
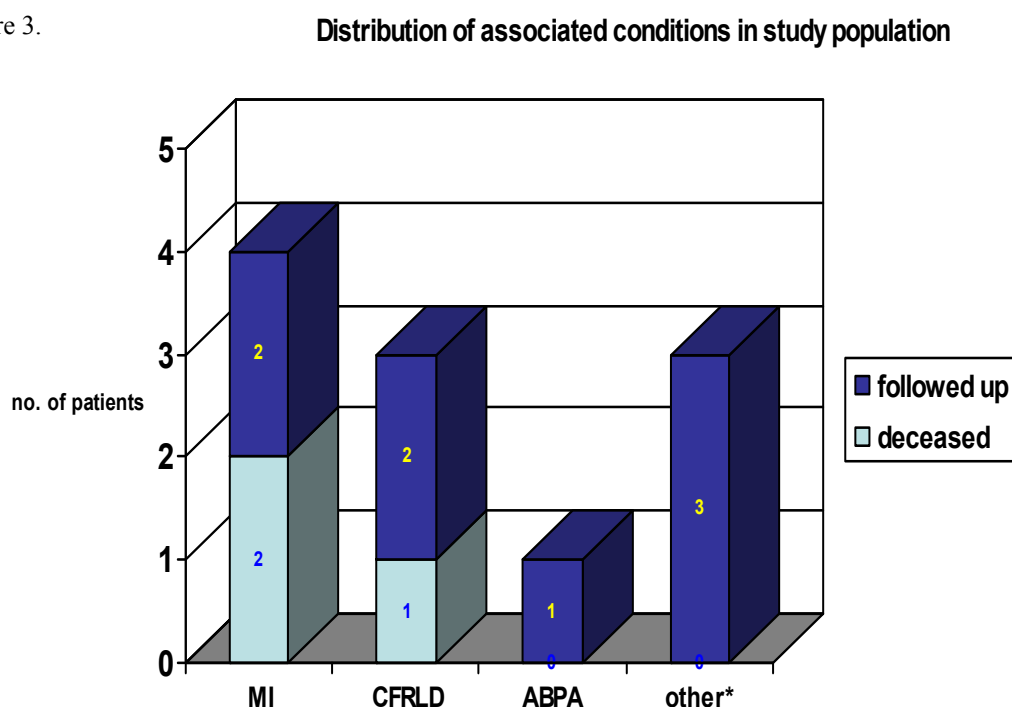


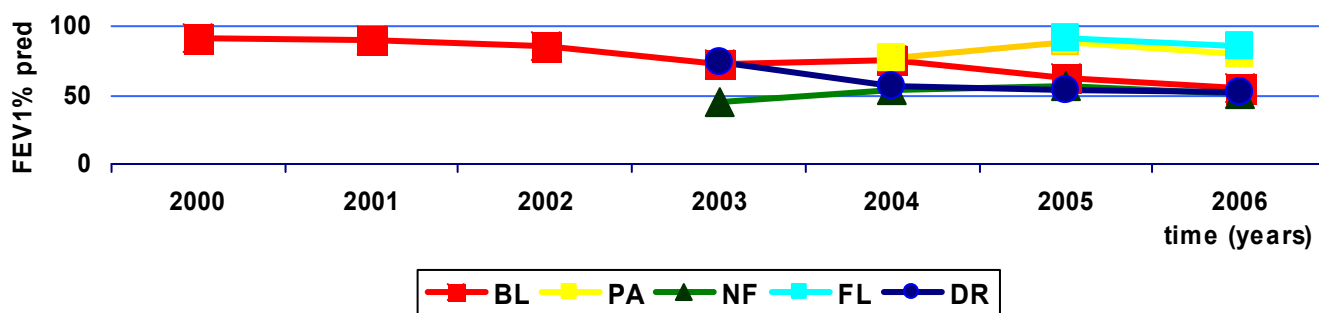
Figure 3.



All patients who could perform lung function testing\* were followed up starting over 3 years of age (figure 4). Usually the lung function trendline had a

descendent direction. The rate of FEV1 decline was higher than 4%/year.

**Distribution of mean FEV1/year along the study period  
in 5 patients\***



### Discussions

Even the study groups were not homogenous as number, there is a paradoxal difference between ages at first suggestive symptoms for CF (around 4 months of age) and the age when diagnosis was done (4.2 months vs. 4.3 years). This had a direct impact on the age when follow up on a regular basis was started. Clinical outcomes as reflected by Schwachman and Chrispin Norman scores did not vary significantly between the two groups, which was not the same for the percentage of deceased patients, the majority being from the group with early diagnosis. For that reason, it seems normal to presume that the high incidence of deaths in the early diagnosis group is

correlated to some risk factors as it was shown (poor socio-economic status, severe malnutrition at the time of diagnosis and probably associated conditions as meconium ileus)

To mention the associated conditions, only one patient that had a relative favorable outcome till the moment of death (mostly in terms of lung disease and *P aer* colonisation), and died from early liver disease (CFRLD) at the age of 3years 6 months. The patient had meconium ileus and several surgical interventions, as well as recurrent episodes of distal intestinal obstruction syndrome (DIOS).

The other deceased patients died under the age of one year and had worse nutritional status and poor socio-

economic status which probably interfered with the access to specialized care.

One patient had allergic bronchopulmonary aspergillosis (ABPA), even being from the early diagnosed ones, but started follow up on a regular basis at the age of 9 years when lung function was severely affected. ABPA worsened the outcome of lung disease with a consecutive fall in FEV<sub>1</sub> despite intensive treatment.

All five patients who could perform lung function testing over the time of the study period had a fall in FEV<sub>1</sub> higher than 4%/year belonging to the category of late start of follow up (despite early diagnosis in some of them).

### Conclusions

Late age when started follow up independently of age at diagnosis predicted worse outcomes.

There was a high rate of decline of lung function (FEV<sub>1</sub>) in older patients and also lower Schwachman scores and BMI in late followed up patients.

There was a high mortality rate, mainly influenced by associated risk factors as poor socio-economic status and severe malnutrition at the time of diagnosis which could have had influenced the outcome of lung disease.

Early diagnosis followed by regular monitoring, early and aggressive intervention regarding pulmonary infection and nutritional support could influence the outcome of CF patients.

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# BIOLOGICAL THERAPY WITH ANTI-TNF AGENTS IN JUVENILE ARTHRITIS CONSIDERATIONS ON A CASE REPORT

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## Abstract

The management of juvenile rheumatoid arthritis has advanced dramatically in the last years based on a more effective use of available drugs and on the application of newly discovered ones. More judicious use of corticosteroids and techniques such as intravenous pulse therapy rather than long-term high-dose use of oral corticosteroids, besides the therapy with methotrexate in moderate to severe JRA still represent the gold standard for polyarthritis management. However, the introduction of anti-TNF agents, such as Etanercept or Infliximab could represent a major shift to the use of biological therapy in patients intolerant to or unresponsive to standard disease modifying antirheumatic drugs (DMADs). We present a case of therapy with Etanercept in a boy with refractory JRA and discuss on the perspective of use of the biological agents.

**Keywords:** etanercept, JRA, children

## Background

Juvenile rheumatoid arthritis (JRA) is the most common rheumatic disease in childhood. Unfortunately, less than one third of patients can have their disease controlled by nonsteroidal or steroidal anti-inflammatory drugs, the remainders being candidates for a more aggressive therapy.

Methotrexate was shown to have a therapeutic advantage over placebo, with an acceptable safety profile in randomized controlled trials in children with JRA. However, many patients do not have an adequate response to methotrexate, even at doses of up to 1mg/kg/week. Moreover, the severity and frequency of side effects increase with higher doses of methotrexate and the consequences of long-term use are not known.

Other disease-modifying antirheumatic drugs (DMARDs) such as sulphasalazine, cyclosporine or cyclophosphamide are finding a specific role for resistant disease where they may be used in combination with methotrexate for example.

In some controlled studies the use of intravenous immunoglobulin (IVIG) in patients with systemic-onset JRA suggested that it was of limited benefit, whereas in a controlled phase I/II trial by Giannini et al substantial short-term benefit was demonstrated in about 75% of individuals, particularly if it was used early in the course of disease. Another retrospective study noted benefit for the systemic features and steroid dependency but limited effect on the polyarthritis. Thus, based on their still incompletely understood pathogenic action in blocking the autoimmune

phenomena, IVIG may have a role in selected patients with severe disease unresponsive to other approaches.

The introduction of antitumor necrosis factor (anti-TNF) agents represents a real revolution in the treatment of rheumatic disorders. TNF is a proinflammatory cytokine that has a complex role in the pathogenesis of JRA. TNF was found elevated in both the serum and the synovial fluid of children with rheumatoid arthritis. Serum levels of soluble TNF receptor are elevated in patients with all subtypes of JRA and the level is correlated with the activity of the disease. Thus, the rationale for the introduction of anti TNF therapy is based on the understanding that cytokines such as TNF are critical molecules lying at the heart of the chronic autoimmune/inflammatory disease process. This has resulted in the introduction into the clinic of 2 inhibitors of TNF, the soluble TNF receptor (etanercept) and the anti-TNF monoclonal antibody (infliximab).

Etanercept (Enbrel, Immunex, Seattle) is a genetically engineered fusion protein consisting of two identical chains of the recombinant extracellular human P75 TNF-receptor molecules fused with the Fc domain of human IgG1, which effectively binds TNF and lymphotoxin- $\alpha$  and inhibits their activity. It is given 0.4mg/kg twice weekly by subcutaneous injection with onset of effect anticipated within 3-4 weeks. Randomized multicentre double-blind placebo-controlled studies of etanercept for the treatment of active polyarticular JRA in children showed its effectiveness in 74% of pediatric patients with severe polyarticular JRA (regardless of the type of onset) who did not tolerate well or had an inadequate response to methotrexate.

We present a case of polyarticular JRA in a 15y-old boy and his various therapeutic regimes over the time.

## Case presentation

The patient was admitted at the hospital for the first time at the age of 10 for swelling, tenderness, pain and limitation of range of motion in small joints (wrists, interphalangeal and metacarpophalangeal joints), but also with knees, ankles and temporomandibular involvement. The onset was approximately 2 months before admittance and the physical examination confirmed the arthritis of the above mentioned joints. Biological findings revealed signs of articular inflammation: elevated ESR (58/95cm) and CRP values, positive rheumatoid factor (RF), whereas antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-dsDNA) were negative. The radiographic changes of hands and knees consisted of soft tissues swelling and discreet periosteal new bone formation. The diagnosis of

polyarticular JRA was established based on the American College of Rheumatology classification criteria.

The initial treatment consisting of nonsteroidal anti-inflammatory therapy was started but within one month no clinical improvement was noted, so the steroid regime was initiated with Prednison 1mg/kg/day. The clinical signs improved, but after 6 months of therapy, MRI examination revealed signs of osteoporosis, so the corticoid therapy was stopped. Further on, the treatment was continued with Na-uro-thiomalate (Tauredon) for a period of 20 months until a total dose of 700mg was reached. The clinical signs did not improve much, moreover, a thoracic cifosis occurred and a certain grade of ankylosis of hands and elbows joints was noticed with limitation of motion. The next approach chosen was methotrexate, hoping it could bring about some improvement, but unfortunately in less than one month a severe cytopenia (leucopenia and thrombocytopenia) was observed which constrained us to stop this last treatment.



The figure presents the limitation of range of motion of the hands and elbows joints after 3 years from the onset.

Having said the above course of the disease, the next recently available choice was to start therapy with anti-TNF agents. The patient received 25mg of Etanercept subcutaneously twice weekly for a period of 24 months. A complete check for infections was performed before the treatment was started. Physical examination, laboratory tests (ESR, CRP, RF, ANA, anti-dsDNA, liver enzymes, renal function, cultures) and evaluation of disease activity measures and response were performed monthly.

The outcome measures used to assess disease response consisted of the following set of 6 response variable (the JRA core criteria): 1) global assessment of disease severity by the physician, 2) global assessment of overall well-being by the patient or the patient's parent or guardian, 3) number of joints with active disease (joints with swelling not due to deformity or joints with limitation of motion and with pain, tenderness or both), 4) number of joints with limitation of motion, 5) functional ability, assessed by Childhood Assessment questionnaire (C-HAQ) and 6) a laboratory marker of inflammation, defined as ESR and CRP.

Response was evaluated according to the JRA definition of improvement (DOI) criteria (the patient had to have 30% of improvement from baseline in at least 3 of the 6 response variable, with no more than 1 variable worsening by more than 30%).

The table below presents JRA core criteria at start and after 24 months of therapy showing more than 30% JRA definition of improvement:

Parameter	Month 1	Month 24	% Improvement
<b>JRA core criteria</b>			
Total no. of active joints	26	18	31%
No. of joints with limitation of motion	18	15	17%
Physician's global assessment	2.0	2.0	0
Patient's/parent's global assessment	2.0	1.0	50%
C-HAQ score	0.8	0.5	38%
ESR	66	58	15%

The patient was followed up for adverse effects such as infections, occurrence of other autoimmune disorders, tuberculosis or malignancies. The main incident that occurred was a severe bronchopneumonia after 18 months of therapy which hardly responded to antibiotic treatment.

### Discussions

The treatment of JRA continues to evolve, with the introduction of many new pharmacologic interventions. Although methotrexate continues to be the DMARD used most frequently for the treatment of refractory JRA, it is very often not effective or not well-tolerated by some patients. So, the therapy with etanercept (a new anti-TNF agent) becomes the issue of choice in refractory cases. Even if in some adults etanercept is associated with methotrexate, we decided not to add it in this case because the side effects

to methotrexate already occurred in our patient. He tolerated well the therapy with etanercept with except an infectious incident (bronchopneumonia) which happened to occur at some point and which was difficult to treat. There was no notice of other adverse events such as tuberculosis, varicella infection, pancitopenia, or new auto antibodies. Unfortunately, when the therapy with etanercept was started a certain grade of ankylosis was already present, so the therapy was effective only on active joint, with an improvement of more than 30% in 3 parameters of core criteria for definition of improvement. Studies coordinated by Daniel Lovell and coworkers have shown that long-term treatment with etanercept can provide significant clinical benefit to pediatric patients with severe polyarticular-course JRA, regardless of disease type of onset and that prolonged use has not been associated with increases in the rates of adverse events or infections. However, the question in

matter is now, for how long can we continue the treatment in our patient or what other alternatives are available (autologous stem cell transplantation, anti IL-1 - Anakira, or B-cell-targeted therapy with anti-CD20 monoclonal antibody- Rituximab).

### Conclusions

Early consideration of more aggressive therapy at start should be considered in children with severe arthritis as irreversible joint damage can occur within 1 to 2 years and

better predictors of prognosis and responses to therapy are required to identify patients requiring early aggressive treatment. Anti-TNF agents represent a real alternative in children with refractory clinical signs or with severe side effects to methotrexate. National registries of patients treated with anti-TNF agents would be extremely important to support long-term safety profiles, effectiveness and the true risk of potential complications such as severe infections, malignancies or autoimmune disorders.

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## GOLDENHAR-GORLIN SYNDROME – CASE PRESENTATION

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### Abstract

The paper presents the case of a 6-week-old female infant, diagnosed with Goldenhar-Gorlin syndrome according to the clinical picture (facial dysmorphism associated with left microsomia, left microtia, preauricular appendage, and unilateral macrostomia with malocclusion) and non-cyanotic heart malformation – DSA of an ostium secundum type, poorly tolerated.

**Key words:** Goldenhar-Gorlin Syndrome, infant, diagnosis.

### Introduction

The Goldenhar-Gorlin syndrome represents a complex of anomalies, especially ocular, aural, and mandibular, unilateral; it is frequently associated with vertebral anomalies or/and malformations of internal organs. The affection is a very rare one (1/25.000 live births) with a M:F report of 3/2.

### Clinical case

We present the case of a 6-week-old female infant, who was admitted in 2<sup>nd</sup> Clinic Pediatric (FO 46743/2005) after transferring her from a Pediatric section of a county hospital, in order to be diagnosed with a precordial systolic breath, level III and a persistent respiratory functional syndrome.

The anamnesis reveals that she is the first child of a young, healthy couple. She is the result of a pathologic pregnancy (which required repeated hospitalizations of the pregnant woman, without her presenting their nature), born dismature. She delivered spontaneously, on term, with a pelvic presentation, W = 1,900 g; the Apgar score could not be mentioned by the mother, nor the immediate postnatal evolution. The new born was breast fed for two weeks, then with Lactovit. From the age of three weeks, the infant has presented generalized cyanotic stirring episodes, which required hospitalization.

Objective exam at admission: altered general state, without fever, deficitary nutrition state (G=3,000g), pale teguments and mucosa, perioronasal and extremities cyanosis, dysmorphic, asymmetric with left microsomia, left microtia, preauricular appendages, cheek clefting unilateral macrostomia with homolateral occlusion. Absent subcutaneous cellular tissue at all levels, moderate polypnea with subcostal draught, spastic cough, AV=148/min, systolic breath level III precordial, liver at 1 cm under the right lower rib, low appetite, present archaic reflexes.

The clinical aspect of the infant pleads for a syndrome with facial asymmetric, unilateral affection, the Goldenhar-Gorlin Syndrome [fig.1].



Fig.1.

In order to specify the infant's state and the evaluation of the associated anomalies, we have performed paraclinic investigations and laboratory exams. The

biological tests indicate a moderate anemia (Hb=11.20g/dl, Ht=34%, anisocytosis), hypocalcaemia (Ca=1.86mmol/l), the other laboratory findings being within normal limits (acute



Sometimes, the Goldenhar syndrome can be associated with a dysplasia of the axial skeleton (especially of the cervical region): hypoplasia, the fusion or/and absence of certain vertebrae. (4,5).

The associated anomalies of the internal organs are:

- Congenital heart defects in 5-58% of cases: DSV, persistence of arterial conduct, tetralogy of Fallot, big vessel transposition (1,4)
- Esotracheal fistula (7)
- Pulmonary agenesis (1,7)
- Renal affections: renal agenesis, ectopia or renal fusion, multicystic kidney, double ureter, vesico-ureteral reflux.
- Neurological affections: microcephaly or hydrocephaly, occipital encephalocele etc.

Moderate mental retardation may be present in 10% of the cases (2).

The diagnosis can be established during intrauterine life by means of fetal echography which shows different levels of underdevelopment (more frequent – unilateral) of the craniofacial structures. Hemifacial microsomia, unilateral maxillary hypoplasia, microphthalmia can be detected.

After birth it is necessary to perform a skull CT scan and a NMR, which allow us to notice NCS malformations: intracranial dermoid cysts, brain scythe calcification (4).

The differential diagnosis is performed by hemifacial microsomia (the anomalies are confined to the viscerocranium), other syndromes which can associate the Goldenhar syndrome and which could have common pathogenic mechanisms (the abnormal development of the

neural crest): Charge syndrome, Townes-Brocks syndrome. (4).

The prognostic quo ad vitam is considered favorable when there are no complications. The eye and ear anomalies can be corrected by plastic surgery (indicated in early years). Thus, the epibulbar dermoids can be removed, the palate or the lip reconstruction can be performed, and also the resection of the preauricular appendage, the reconstruction of the outer ear (after the age of 4) and even palate and lip shortening and extension, orthodontic treatment (10). The treatment is a supporting one and it supposes regular audiometric controls.

The genetic advice is useful; cases with familial affection based on familial studies are cited (6-8%) (9). Since most forms are sporadic, the risk that an individual with Goldenhar syndrome have a child bearing this affection is unlikely to happen.

### Conclusions

The above presented case was diagnosed with Goldenhar-Gorlin syndrome by the clinical aspect (facial dysmorphism marked with facial asymmetry, left preauricular appendage, unilateral macrostomia by cheek clefting with homolateral malocclusion). The ENT exam confirms the presence of the outer ear canal and it is not associated with vertebral anomalies. The infant also presents heart congenital malformation (more rarely met) – DSA ostium secundum badly tolerated, appearance of heart failure until the age of 4 months with consequences upon the weight gain, thus hiding the prognostic.

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## THE ROLE OF PARACLINICAL INVESTIGATIONS IN DIAGNOSING ADENOPATHIES IN CHILDREN

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### Abstract

No laboratory investigations are necessary for most of the children with peripheral adenopathy, as the etiological diagnosis can be performed only based on the anamnesis and clinical examination. Paraclinical investigations and ganglionic biopsy may be necessary in order to make a diagnostic if there are signs of malignity or if the adenopathy persists even under proper antibiotics therapy. This study refers primarily to cases of adenopathy requiring ganglionic biopsy in order to identify the etiology.

**Key words:** adenopathy, biopsy, child.

### Introduction

In most cases, based on the anamnesis and the clinical exam, the cause of adenopathy can be diagnosed. However, a part of the patients still remain with an unknown etiology adenopathy. In these cases, if the adenopathy is localized, without other associated clinical signs, a period of monitoring is required for 3 to 4 weeks, before the ganglionic biopsy. More complete investigations, including ganglionic biopsy, are to be performed on patients with localized adenopathy with systemic signs and those with generalized adenopathy of unknown cause.

### Patients and methods

The study was conducted on a group of 1,112 children with various diseases, who had peripheral adenopathy and needed hospitalization to identify the etiology. The cases were between 1 and 16 years of age and were hospitalized in the Clinical Emergency Hospital and the Infectious Diseases Hospital of Craiova, for 11 years (01.01.1994 – 31.12.2004).

In all the cases, the common anamnesis, clinical exam and biological investigations were performed. More complex investigations, including ganglionic adenopathy, were performed on patients with localized adenopathy and systemic signs.

### Outcome and discussions

In most cases, the cause of the adenopathy can be identified based on the anamnesis and clinical exam. In these situations, no additional investigations are required. In other cases, the etiological diagnostic cannot be established only based on the anamnesis and the clinical exam, although the latter may suggest a certain etiology; the paraclinical

investigations are not needed to identify the diagnostic. The paraclinical investigations aim to find the local or general cause of the ganglionic hypertrophy. These investigations must develop from simple to complex ones.

Although it is difficult to say which dimensions of the ganglions are pathological, most researchers believe that a ganglion exceeding 2 cm in diameter is surely pathological; those with 1 – 2 cm diameter need additional investigations if they are not solved after 2 weeks of treatment.

In the studied group, made up of children selected by the family doctory, who raised problems of differential diagnosis, the following paraclinical investigations were performed:

- ♦ A complete hemoleukogram, with a careful examination of the peripheral blood sample was performed in all the cases. The aim was to notice: the leukocytosis in the piogenic infections, the non-typical lymphocytes in the infectious mononucleosis, the infection with the cytomegalic virus, other viral infections, the eosinophilia in the hypersensitization reactions, the pancytopenia or thrombocytopenia in leukemia, HIV infection;

- ♦ the speed of hematias (VSH) is a non-specific, yet helpful test that was also performed in all the cases, as it was significantly high in bacterial infections, inflammatory and neoplastic diseases;

- ♦ the cultures in the ganglionic product were positive in 58 cases (18.5%), with pyogenic adenitis;

- ♦ the cultures in the pharyngeal exudate were positive in 40 cases (12.7%), and the skin lesion in 18 cases (5.7%) with pyogenic adenitis;

- ♦ the skin test in tuberculine, for the diagnosis of tubercular adenite was positive in 76 cases (87.3%); in the adenites with non-typical mycobacteria it was positive in all the 12 cases (100%).

- ♦ the serum tests: determining the IgG and IgM titre was performed in the 16 cases (100%) with toxoplasmosis, in the infection with citomegalic viruse in the 3 cases; determining the heterophilic antibodies was performed in the 36 cases with infectious mononucleosis, with 28 cases identified as positive (77.7%).

- ♦ the ELISA test to determine the HIV infection, positive in 184 cases (100%);

- ♦ antinuclear antibodies were present in 13 cases (37,1%) with ARJ and in the 4 cases with LES, while the

rheumatism factor was positive in 10 cases (28,5%) with ARJ;

The following *image explorations* were conducted:

- ♦ lung radiography that revealed mediastinal adenopathy in 12 children with tuberculosis adenitis, 6 cases of leukemia, 1 case of Hodgkin disease and 3 cases with non-Hodgkin lymphomas.

- ♦ abdominal ecography, computer tomography or nuclear magnetic resonance were sometimes needed to highlight associated abdominal adenopathy in lymphomas, abdominal tumors.

In patients with localized adenopathy, without other symptoms and specific anamnesis data, with normal clinical exam, the etiology is most likely benign, unspecific. In general, these patients were kept under observation, with the adenopathy being solved spontaneously.

The patients were informed to come back to the doctor for reassessment after 2 to 4 weeks or immediately, if the ganglions grow larger or other signs and symptoms appear. At the following examination, if the adenopathy was not in regression and no improvement was noticed, complementary investigations were conducted to set the diagnostic.

Some guideline criteria for ganglionic biopsy were the followings:

- abscesses;
- a significant increase in the ganglions after 2 weeks of proper antibiotic treatment;
- a significant adenopathy that failed to respond to the antibiotics therapy after 4 – 6 weeks or did not solve in 8 weeks;
- ganglions that grew rapidly, were conglomerated or fixed on the structures around them;
- ganglions localized in the rear triangle of the neck or in the over-clavicle region;
- other new signs and symptoms: astenia, weight loss, perspiration.

Ganglionic biopsy was conducted, during the 11 years of study of the 1,112 cases hospitalized with significant peripheral adenopathy, on 225 cases (21.2%).

Judging by the age of the children that needed ganglionic biopsy, the frequency of the cases needing biopsy increased with the age, reaching a maximum at the 10 – 16 years of age (42.6%). A slight predominance of the male cases was noticed (56.8%), as compared to the female cases (43.1%). Judging by the location of the adenopathy, most of the ganglionic biopsies were conducted on ganglions in the lateral-cervical region (45.3%).

Table no. 1 – Characteristics of the group of children requiring ganglionic biopsy (N=225)

Characteristics		No.	%
<i>Gender</i>	Male	128	56,8
	Female	97	43,1
<i>Age groups</i>	0 – 1 year	3	1,3
	1 – 3 years	27	12
	3 – 6 years	41	18,2
	6 – 10 years	58	25,7
	10 – 16 years	96	42,6
<i>Location of adenopathy</i>	Lateral-cervical	102	45,3
	Axillary	43	18,1
	Submandibular	41	18,2
	Inguinal	23	1,2
	Over-clavicular	11	4,8
	Lateral-thoracic	2	0,8
	Preauricular	3	1,3

The cases in which ganglionic biopsy was conducted were divided in two main categories, based on the hysto-pathological exam: reactive adenopathies in 200 cases (88.9%) and malignant adenopathies in 25 cases (11.1%).

The reactive adenopathies were classified in 5 characteristic patterns: inter-follicular pattern (50.2%),

follicular pattern (25.8%), mixed pattern (8%), sinus pattern (2.7%), diffuse pattern (2.2%).

We noticed changes characteristic to the *follicular pattern* in 58 cases (25.8%), out of which: 46 cases (20.4%) with unspecific follicular hyperplasia, in response to some infectious diseases, 3 cases (1.3%) with Castleman disease and 9 cases (4%) with HIV infection.

Table no.2 – Hystopathological Types of Adenopathies (N=225)

Types of adenopathies	No.	%
<i>Reactive Adenopathies</i>	200	88,9
▪ Follicular pattern	58	25,8
<b>Non-specific follicular hyperplasia</b>	46	20,4
Rheumatoid arthritis / Sjögren syndrome	0	0
Syphilis	0	0
Angiofollicular hyperplasia (Castleman disease)	3	1,3
AIDS	9	4
Progressive change of germinating centers	0	0
▪ Inter-follicular pattern	113	50,2
Non-specific inter-follicular hyperplasia	36	15,5
Dermatopathic lymphadenitis	1	0,4
Histiocitary necrosing lymphadenitis (Kikuchi disease)	1	0,4
Granulomatous lymphadenitis	75	33,3
▪ Mixed follicular and interfollicular pattern	18	8,0
Toxoplasmosis	7	3,1
Cat claw disease	11	4,8
Venereum lymphogranulomatosis	0	0
Mesenteric lymphadenitis	0	0
Kimura disease	0	0
▪ Diffuse pattern	5	2,2
Infectious mononucleosis and other viral adenites	4	1,7
Angioimmunoblastic adenopathy	0	0
Drug-induced adenopathy	1	0,4
Systemic Lupus erythematosus	0	0
Kawasaki disease	0	0
▪ Sinus pattern	6	2,7
Sinus histiocytosis	3	1,3
Hemophagocytic syndrome	0	0
Sinus histiocytosis with massive adenopathy	3	1,3
Whipple disease	0	0
<i>Malignant Adenopathies</i>	25	11,1
Non-Hodgkin lymphomas	14	5,7
Hodgkin disease	7	3,1
Ganglionic metastases	4	1,7

There were 113 cases (50.2%) showing the *inter-follicular pattern* in which the germinating centers are small, with the change of the para-cortex by the proliferation of the T lymphocytes. The followings are included in this pattern:

- ♦ unspecific inter-follicular hyperplasia was noticed in 36 cases (15.5%);
- ♦ dermatopathic lymphadenitis associated to chronic dermatitis in 1 case (0.4%);
- ♦ histiocitary necrosing lymphadenitis (the Kikuchi disease) in 1 case (0.4%);
- ♦ specific granulomatous adenitis (tuberculosis, non-typical mycobacteria) were noticed in 68 cases (30.2%), while non-specific granulomatous adenitis were noticed in 7 cases (3.1%).

The *mixed pattern*, characterized from the hystologic viewpoint by areas of the follicular pattern type and areas

similar to the diffuse pattern was noticed in 18 cases (8%): 7 cases (3.1%) of toxoplasmosis and 11 cases (4.8%) with the cat claw disease. No cases of venereum lympho-granulomatosis and the Kimura disease were noticed. This study does not focus on mesenteric lymphadenitis, but on peripheral adenopathies.

The *diffuse pattern* was noticed only in 5 cases (2.2%): 2 cases (0.8%) of infectious mono-nucleosis, 2 cases (0.8%) of post-vaccine adenitis, and one case (0.4%) with adenopathy induced by chronic intake of anti-convulsives.

In this pattern, the ganglionic architecture is generally diffuse, and the modifications may often be mistaken for lymphomas.

No cases of either angioimmunoblastic adenopathy or the Kawasaki disease were noticed in this study.

The LES was noticed in 4 cases, but no ganglionic biopsy was conducted on them.

In the *sinus pattern*, the ganglions kept their architecture, but the sinuses were widened and infiltrated with histiocytes.

3 cases (1.3%) of histiocytosis with Langerhans cells and 3 cases (1.3%) of sinus histiocytosis with massive adenopathy (the Rosai – Dorfman disease) were noticed in this group of reactive adenopathies. No case with either hemophagocytic syndrome or the Whipple disease was found.

*Malignant adenopathies* were found in 25 cases (11.1%) of the total ganglionic biopsies, out of which: 14 cases (5.7%) with non-Hodgkin malignant lymphomas, 7 cases (3.1%) with the Hodgkin disease, and 4 cases (1.7%) with ganglionic metastases.

In a study conducted by Moore and fellows (1999) on 1,332 cases under 16 years of age, for a span of time of 23 years (1976 – 1999), in which the cervical lymphatic ganglions were excised, the results were the followings: normal ganglions in 1.5% cases, non-specific reactive hyperplasia in 637 cases (47.8%), chronic granulomatous modifications in 484 cases (36.3%). In cases of granulomatous diseases, the tuberculosis adenitis was confirmed in 332 cases (25%), the Rosai Dorfman disease in 3 cases, syphilis in 4 cases, toxoplasmosis in 1 case. The neoplasia was confirmed in 154 cases, out of which lymphomas in 108 cases, acute leukemia in 10 cases.

Bases on two retrospective studies, Pangalis and fellows developed (1993) a useful algorithm to select the patients with adenopathies of nonspecified etiology requiring ganglionic biopsy. Three variables with predictable value were identified in the first study, for patients aged between 9 and 25 that needed to undergo biopsy: the size of the lymphatic ganglions of over 2 cm and the modifications of the thoracic radiography had a positive prediction value; the signs of recent infection in the ORL area had a negative prediction value. In the second study conducted on adults, the followings had positive prediction value: age over 40, the size of the ganglions of over 1.5 x 1.5 cm, the over-clavicle location, the hard texture of the ganglions and the lack of sensitivity or ganglionic pain. The followings had a negative prediction value: ages under 40, the size of the ganglions under 1 x 1 cm, the elastic ganglionic texture and sensitive ganglions presenting pain when touched.

If a ganglionic biopsy is decided, the largest ganglion or the ganglion that has recently increased and shows abnormal clinical characteristic will be excised. If a lymphoma is suspected, it is useful to conduct biopsy on more than one ganglion, because the specific modifications may not be present in all the ganglions. One should avoid conducting biopsy on the axillary or inguinal ganglions if the adenopathy is generalized, as the ganglions in these regions often show only reactive hyperplasia. The ganglion on which the biopsy is to be performed will be completely excised, with intact capsule (Twist and fellows, 2000).

In his studies, Ferrer (2003) underscores that the definitive diagnostic can be established only in 40 – 60 %

cases, for the following reasons: improper size of the sample, unsuitable restoration, and an unsuitable choice of the ganglions for the biopsy. If, after the biopsy, one cannot set a diagnostic, it is recommended to repeat the biopsy when the problems persist or grow.

The histological examination of the ganglionic product is often not enough. The flowcytometry and the imunohistochemical genetic exams can bring additional information.

In case an infectious mononucleosis or a hypersensitivity to drugs is suspected, one should avoid performing a biopsy, because the histological changes are very similar to those in lymphomas and can be misinterpreted (Pangalis, 1993).

The FNA is primarily used for adults, because the tissue obtained is not enough for complete investigations and does not enable the examination of the ganglionic architecture. The main difficulty is to establish a differential diagnosis between the lymphomas having a low degree of malignancy and the reactive hyperplasia (Lioe and fellows, 1999). So, at an early stage, the diagnostic of lymphoma may be omitted (Moore, 2001). In a study conducted on 157 patients, dummy-negative results at the FNA were obtained in 3.5% patients, when the cases of lymphoma were excluded; when included, the percentage of fake-negative was 12.5%. So, the overall precision of the diagnostic was 24.4%, the sensitivity was 85.4% and the specificity 100%.

The FNA is recommended in infections located at the level of the ganglions, in ganglionic metastases, in order to assess the thyroid nodule or to investigate some ganglions that were not accessible to the biopsy, such as the retro-peritoneal ones.

In association with the flowcytometry, the FNA allows a better assessment of the development of lymphomas under treatment.

The material vacuumed out through the FNA will be sent to the lab to perform the Gram and Ziehl-Nielsen colorations and for cultures to highlight the aerobe and anaerobe bacteria, the mycobacteria and the fungi (Umpathy and fellows, 2003).

## Conclusions

1. Ganglionic biopsy was conducted in 225 cases (21.2%), particularly at the children aged 10 – 16 (42.6%) that run a higher risk of having serious diseases.

2. The histopathological exam showed that most of them were reactive adenopathies (88.9%), with the malignant adenopathies being present in 11.1% cases.

3. Within the reactive adenopathies, the most frequently noticed were the granulomatous adenitis (33.3%), followed by nonspecific follicular hyperplasia (20.4% and nonspecific inter-follicular hyperplasia (15.5%).

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## EMOTIONAL AND BEHAVIOR DISORDERS IN CHILDREN WITH CHRONIC DISEASES

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### Summary

The children with chronic diseases are subjected to a psychic stress that generates unexpected emotional and behavior reactions, which surprise both physicians and parents. The study performed at the 2<sup>nd</sup> Pediatrics Clinic Timisoara has as a goal the setting of a diagnosis of the emotional and behavior disorders of the children with chronic diseases to ease up the interaction of the physicians with these patients and also to come to the aid of parents with new information in the domain.

**Key words:** emotional and behavior disorders, children, chronic diseases.

### Introduction

Since 1946, W.H.O. has defined health as being “a complete physical, mental and social well-being which consists in the lack of disease and infirmities”(4).

This definition “underlines the dynamic interaction and interdependence between the three components of health: the physic condition, the psychic equilibrium and the social environment”(4).

“A child with chronic diseases or infirmity is two times sick: through the basic disease or infirmity and through it’s perturbation of his morph functional, physical, intellectual, behavioral and social-emotional development”(2). Once with the detection of a chronic

disease the children go over an important period of their life (childhood and/or adolescence), bearing on their shoulders a much to heavy burden, which is the chronic disease with all it’s implications that unfold in time(1).

### The goal of the study

We set ourselves the goal to trace out the emotional and behavior disorders in the investigated children with three types of chronic diseases: mucoviscidosis, diabetes mellitus, bronchial asthma and also the find out, if possible, some correlations between the disease and the emotional and behavior disorders of these children.

### Working method

The studied group consisted of 33 subjects hospitalized in the 2<sup>nd</sup> Pediatrics Clinic Timisoara in the period 2005 – 2006, having an average age of 10 years, of which:

- 18 cases of mucoviscidosis
- 10 with insulin dependent diabetes mellitus
- 5 with persistent bronchial asthma.

All the cases were evaluated at two consecutive hospitalizations and a third examination was made at a distance of one year (Fig.1).

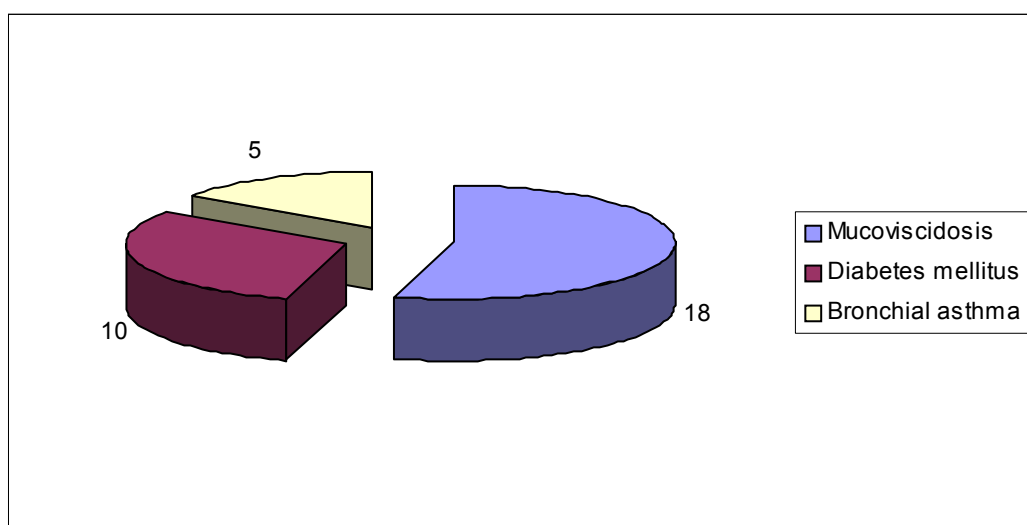


Fig.1.The cases were evaluated.

### The used methods and tests

- a. The direct observation of the subject's behavior, was done in the Psychological consulting room of the Mucoviscidosis Center Timisoara regardless of the type of chronic disease.
- b. The clinical interview – was aimed at obtaining information and the understanding of the psychological functioning of the patient through focusing on his life experience and with emphasis on the relationship established in the interview situation (3).
- c. The anamnesis – has as a goal the collecting of data referring to the important events in the life of the subject, and also to the eventual clinical records.
- d. The specific tests used in the psychological examination of children:
  - The “draw a person” test (Machover test) – is a projective test, the drawing of the person representing a real personal stamp. The analysis of the drawing gives information about the characteristic traits of the child and about the existence of eventual psychic, intellectual or emotional disorders.

- The tree test – is also a projective test. The study of the drawings gives information about the social attitude of the subject, about his intimate self (EGO), about his endeavors, wishes and needs.
- The family test. Projective test that highlights the relationship of the subject with his family, attracting attention on eventual conflicts inside the family which could have negative responses in the psychic life of the child.
- The Raven test. It is a perceptive, non-verbal test for the assessment of general intelligence.

### Results (Fig.5).

57,14% of the children with mucoviscidosis have attachment disorders and 42,85% from them are affectively immature (Fig.2). In the group of children with bronchial asthma 66,66% of them suffer from anxiety, emotional lability and the rest of 33,33% of them are affectively immature (Fig.3).

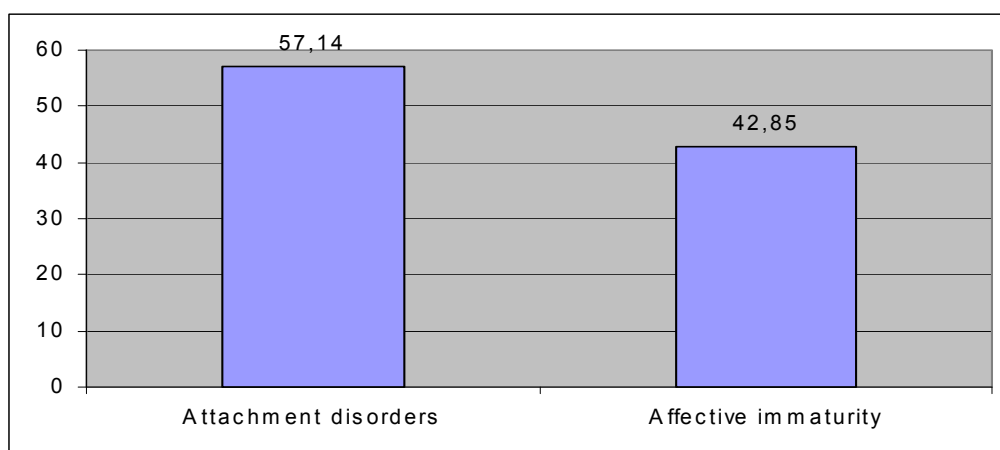


Fig.2. Emotional and behavior disorders in children with mucoviscidosis.

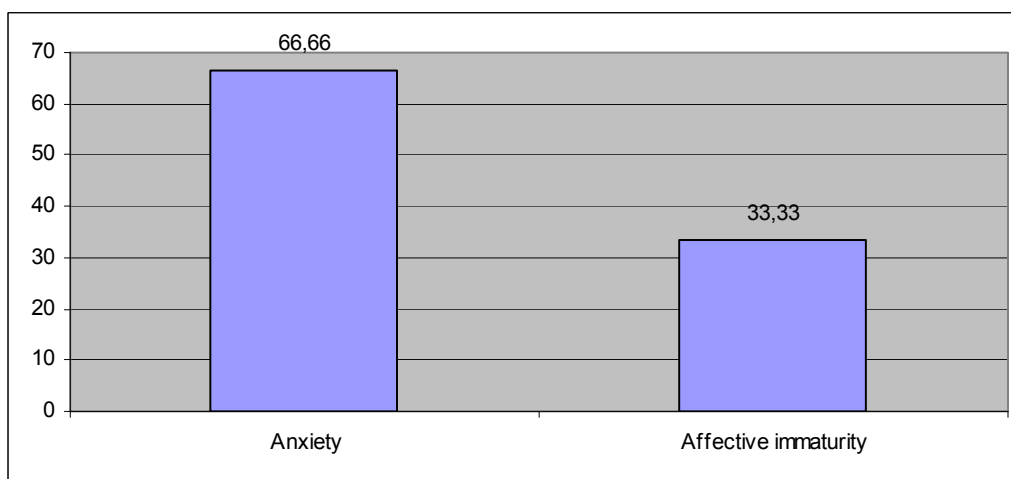


Fig.3. Emotional and behavior disorders in children with bronchial asthma.

From the analyzed children with insulin dependent diabetes mellitus 50% were affectively immature and 25% of them showed irritability and even hostility to the persons around them.(Fig.4).

The comparative analysis of the three groups of patients shows that from the point of view of the affective immaturity the first place is taken by the children with insulin dependent diabetes mellitus (Fig.6).

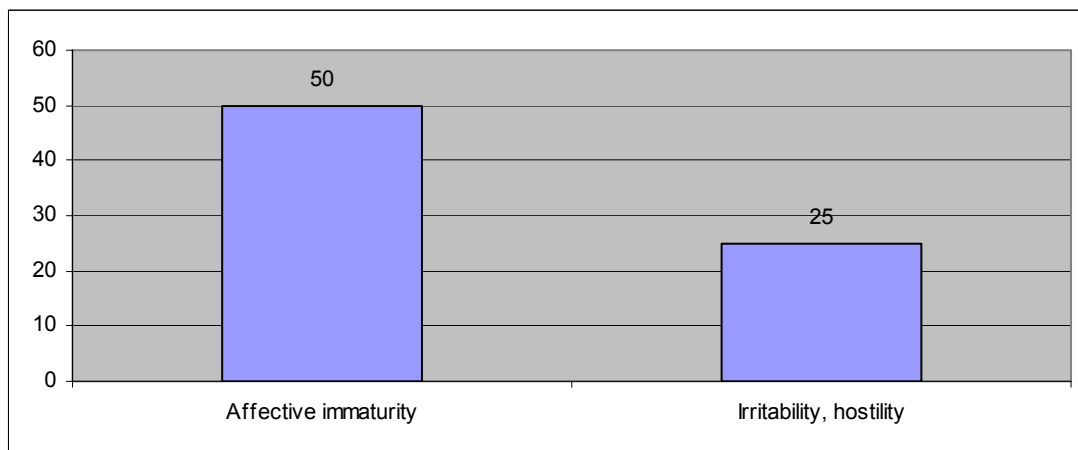


Fig. 4. Emotional and behavior disorders in children with insulin dependent diabetes mellitus.

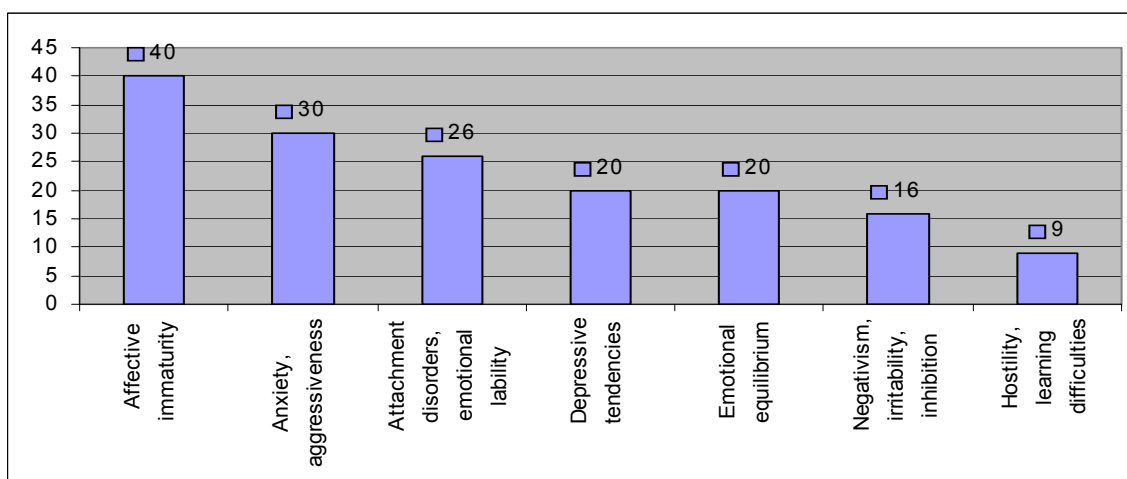


Fig.5 Comparative chart of the general frequency with which emotional and behavior disorders appear in children with chronic diseases.

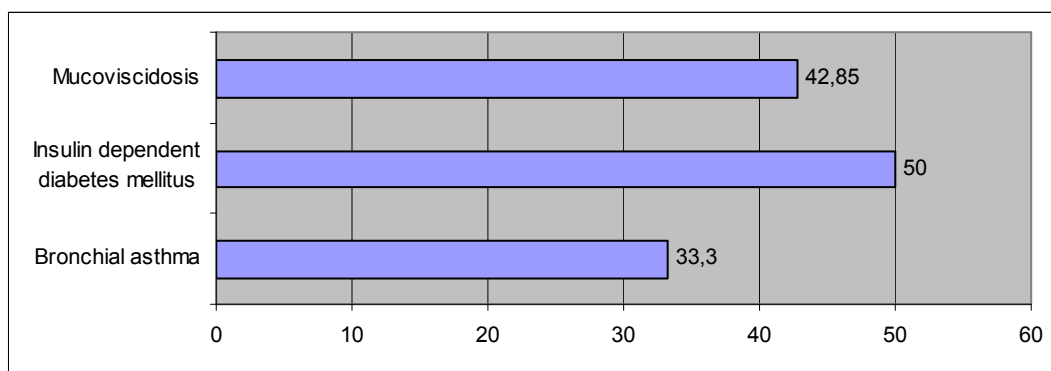


Fig. 6 Comparative chart, of the affective immaturity percentage, on groups of diseases, in the established batch.

**Discussions**

The parents-child-disease relationship in mucoviscidosis. The family is a complex system (in this environment every interaction between it's components has an echo at the level of the whole system). More than that, the intervention of a powerful and unexpected vector can break the fragile equilibrium of the interaction field, which characterizes the family life.

The family dysfunctions have negative effects on the behavioral and health status of the child with cystic fibrosis. Based on the experience of the Mucoviscidosis Center it was established that:

- Most of the parents react with a psycho-emotional block after hearing the diagnosis.
- In the next phase appears confusion (they are not sure that they have understood well).
- Some of them deny the possibility that this disease affects their child and even want to repeat the tests.

Parents react in accordance with their personality:

- some become excessively anxious,
- others become depressive,
- they try to maintain the equilibrium and not induce fear in the sick child,
- the partner is blamed for the child's disease (sometimes). We have not encountered cases

of hostility towards the physicians or the medical profession.

In most of the cases parents offer their full support to the child. There are also dysfunctional families, in which the relationship disorders have a negative impact on the sick child. The psychological implications of bronchial asthma on different age groups. The small child is more vulnerable emotionally to the asthma crises, because he does not understand them. When the family climate is very stern, the parents not showing clearly their affection to the child and often applying punishments, the small patient can perceive wrongly the disease. The psychological implications that appear in time in the child with diabetes mellitus. The child has to gain confidence in him, in the family and in the medical team, which is why his education raises the problem of finding a direct way to transmit information, having in mind the limits of his understanding.

**Conclusions**

The work hypothesis was confirmed, namely, in children with chronic diseases the presence of emotional and behavior disorders was established as statistically significant.

The obtained results are valid only for the investigated subjects, being impossible to extrapolate them to all the children suffering from the chronic diseases mentioned in the paper.

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## IV. PEDIATRIC SURGERY

### MODIFICATIONS OF TESTICULAR OXYGEN SATURATION (SpO<sub>2</sub>) IN EXPERIMENTAL ORCHIOPEXY

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#### Abstract

When performing orchiopexy in undescended testicle, an essential demand for the gonad functionality and viability is the absence of tension in the spermatic cord. Currently it is not fully known the relation between spermatic cord elongation and testicular perfusion; this paper aims to study the above mentioned aspects by determining oxygen saturation (SpO<sub>2</sub>) in the testicles as compared with the spermatic cord elongation.

**Key words:** orchiopexy, spermatic cord elongation, testicular oxygenation.

#### Introduction

Testicular migration anomalies are mentioned in the literature since Galen and Vesalius, the empty scrotum representing an important source of anxiety accompanied by disorders of behavioral development and body scheme. On birth, both testicles must be placed in the bursae (in 3-4% of cases, this is not occurring). If this is not the situation, one may wait at most a year (the incidence drops to 1%), and after that, during the second year of life, surgical descent of the testes should be performed (electronic microscopy studies reveal anatomopathologic modifications in the undescended testicle as early as from 1 year of age). Surgical orchiopexy must be performed by a surgeon trained in pediatric surgery, familiarized with child's delicate anatomic structures. There is no use to wait if testicular ectopia is accompanied by inguinoscrotal hernia, and the surgery must be performed immediately. Any testis that remains outside the bursa has a high risk of malignant transformation, the higher retention in the abdomen, the more frequent occurrence.

Regarding strictly aspects of therapeutic conduct in undescended testis, we mention that palpable testis in the inguinal canal or in the pubis area can be brought in the bursa by simple surgical procedures.

In infant, all the testes disposed distally of the internal orifice of the inguinal canal and half of the abdominal testes can be descended by simple orchiopexy, when using proper technique. Because of the reduced size of inguinal region and scrotum, testis descent is performed without great difficulties, retroperitoneal mobilization of spermatic vessels being rarely necessary. Even though at

this age the vaginal process is an extremely delicate membrane and its mobilization from the elements of funiculum difficult, the orchiopexy is possible and safe for experienced surgeons. Although surgical risk is higher for the small child, as far as pathological changes in the undescended testis concerns, the orchiopexy should be performed before the age of two years.

In newborn and suckling babies, even high abdominal testes can be brought in scrotum, while post puberty, due to the increase of inguinal, pubian and scrotal regions size, the simple orchiopexy sometimes fails even under the circumstances in which the testicles are disposed in the inguinal canal.

In the case of testis retained in a high position in the abdomen, performing classic orchiopexy is limited by the length of spermatic vessels so that the testis can be descended only by vascular microsurgery techniques. Orchiopexy techniques in two operating sequences or with the previous section of spermatic vessels - Fowler-Stepens technique – can be successful in certain situations, but carry the risk of testicular atrophy. In 5% of the cases, the very short vascular pedicle does not allow the performing of conventional orchiopexy techniques. Under these circumstances, the microsurgery techniques are used.

The term “orchiopexy” is derived from the Greek word pexis - fixation, even though the essential sequences of operation are the hernial sac resection, spermatic vessels dissection and supra-pubian transposition of cord elements, procedures by which the testis is brought in the scrotum. The orchiopexy it is not a surgical intervention without difficulties, requiring a proper technique, experience and a lot of patience. Postoperative testicular atrophy, in some older statistics, reaches up to 35%, while the most optimistic statistics mention postoperative testicular atrophy in 1-2% of the cases. Only after hernioraphy performed before the age of one year, the testicular atrophy is mentioned in 2% of the cases.

Correctly performed orchiopexy means to respect the following principles:

- Taking into account that most of the undescended testes are placed in the inguinal canal, the incision must offer a good approach of it;

- The spermatic cord will be dissected cleanly, as higher as possible, without harming its elements;
- To obtain an adequate length of spermatic cord, the resection of the rest of the peritoneovaginal canal is absolutely necessary;
- If the testis presents modifications in consistency, sizes and aspect, testicular biopsy will be performed;
- Tunnelization of the scrotum descent route must be the shortest possible;
- The testis will be descended in the scrotum as lower as possible, on the same side (homolateral orchiopexy), in a pocket created between skin and dartos;
- When performing the testicular descent it is vital to avoid the torsion of spermatic cord;
- *An essential demand for the gonad functionality and viability is the absence of tension in the spermatic cord;*
- To avoid the subsequent ascend or torsion, the descended testis will be fixed to dartos with unresorbable suture material passed through albuginea;
- Rebuilding of the inguinal canal will be performed so that to not weaken the abdominal wall architecture, but

also to not hamper the blood circulation in the descended testicle.

### Objectives

Although it is known that the tension in the spermatic cord should be avoided when performing orchiopexy (risk of subsequent gland atrophy), the relation between spermatic cord elongation and testicular perfusion is not fully known, the present paper aiming to study these aspects.

### Material and method

To perform the experiment, we studied a lot of 10 adult male dogs of different sizes, in which, by the means of a pulsoximeter, we have measured the variations in oxygen saturation at the site of penetration of vessels in the testicular parenchyma, depending on the elongation degree of spermatic cord.

Under conditions of general anesthesia isolation and disinfection of scrotal teguments was performed. By a longitudinal incision that included the vaginal tunica (fig. 1), we opened the left scrotal bursa and exteriorized the testis suspended by the spermatic cord (fig. 2).



Fig. 1. The longitudinal incision of the scrotum.



Fig. 2. The testis suspended by the spermatic cord.

We passed a thread through albuginea on the free testicular margin for anchorage, with a marker, we established two marks on the spermatic cord at 2 cm distance and we placed the pulsoximeter sensor at the terminal side of the spermatic cord (fig. 3). We recorded the oxygen saturation, then we performed an elongation of the cord, so that the distance between the marks to increase by 1

mm (a 5% elongation), and after a minute, we measured the oxygen saturation; we repeated the process after each 1 mm elongation until the oxygen saturation could not be recorded (fig. 4). The same measurements were performed in the right testis, repeating the process in each specimen (a total of 20 testicles).



Fig. 3. Establishing two marks on the spermatic cord at 2 cm distance.



Fig. 4. SpO<sub>2</sub> determination.

## Results and discussions

Values of oxygen saturation, cardiac frequency and elongation are detailed in the following table:

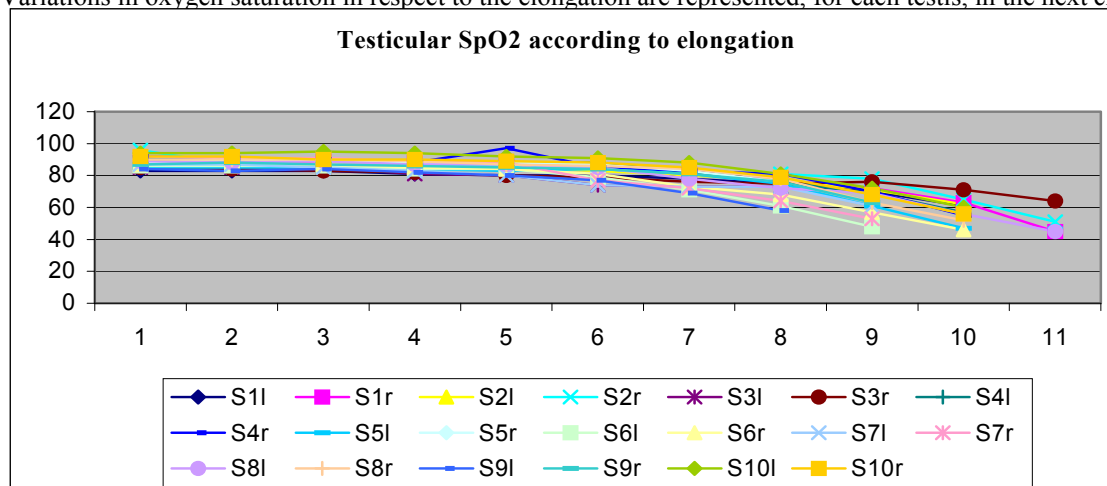
Elong.	S1l	S1r	S2l	S2r	S3l	S3r	S4l	S4r	S5l	S5r	S6l	S6r	S7l	S7r	S8l	S8r	S9l	S9r	S10l	S10r
0%	83	88	87	96	85	85	90	92	85	88	86	87	87	92	89	90	84	87	94	92
5%	83	88	87	87	85	84	90	91	85	90	86	88	87	91	88	90	83	88	94	92
10%	83	89	85	86	85	83	90	90	85	89	86	87	85	91	89	90	84	87	95	90
15%	81	88	85	86	81	82	89	88	84	88	84	85	83	89	87	89	82	86	94	90
20%	80	86	85	86	80	80	88	97	83	88	84	84	80	88	87	87	80	85	92	89
25%	80	84	83	84	74	79	88	85	84	87	79	81	74	77	85	86	77	84	91	88
30%	78	81	80	82	73	76	82	83	81	83	71	73	73	72	78	84	69	81	88	85
35%	75	77	77	81	73	75	76	81	75	76	61	68	73	64	72	78	58	76	81	79
40%	70	72	71	78		76	69	70	61		48	57	61	53	69	63		63	72	68
45%	61	63	62	65		71	57	55	47			46			56	52			60	56
50%		45		51		64									45					

S1.....S10 = number of subject.

r = right testicle.

l = left testicle.

Variations in oxygen saturation in respect to the elongation are represented, for each testis, in the next chart:



It can be seen that there is not a linear variation of oxygenation in respect to the elongation of the spermatic cord. Up to 30-35% elongation, the oxygenation is relatively constant, followed by an interval in which oxygenation drops down dramatically (up to 65-70% elongation), after that becoming undeterminable, indicating the interruption of testicular perfusion. By inducing tachycardia (due to the pain caused by elongation in the case of an insufficient anesthesia) maintenance of the same oxygenation is possible even in greater elongation.

To explain this behavior of oxygenation level in respect to the spermatic cord elongation, we studied the disposal of spermatic artery in this site. We discovered the spermatic cord following the above mentioned procedure, and after that, at the profound orifice of inguinal canal, we isolated the spermatic artery from the rest of cord elements and catheterized it (fig. 5), then we performed an arteriography using iopamiro 370 (fig. 6).

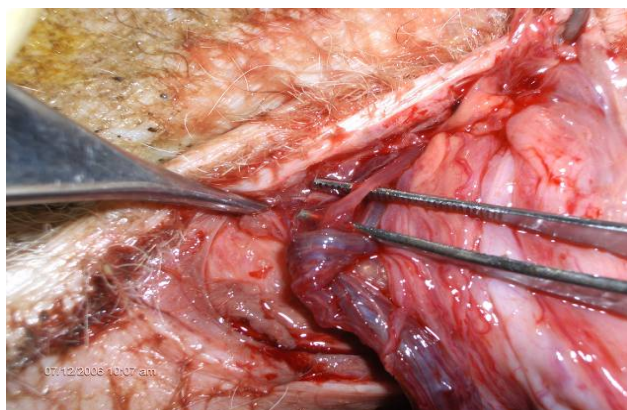


Fig. 5. The spermatic artery.

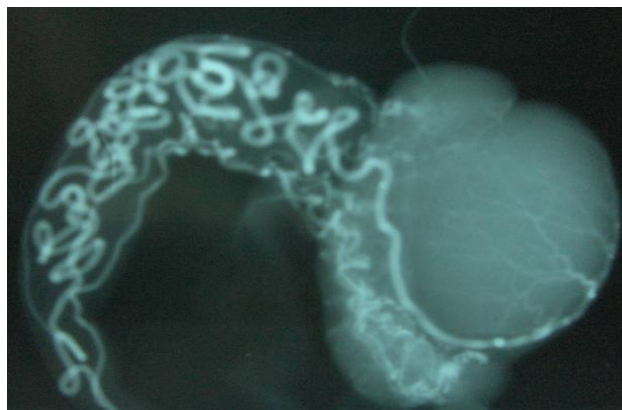


Fig. 6. The arteriography of the spermatic artery.

Sinuuous disposal of artery in the spermatic cord is observed, so that by its elongation it is possible in a first phase to maintain the oxygenation close to the normal level by unfolding the artery without affecting its caliber.

#### Conclusions

1. Testicular oxygenation does not vary linearly in relation with the spermatic cord elongation:

- recording a plateau phase;

- followed by an unlinear descending slope.

2. Elongations of 30-35% of the spermatic cord have no significant impact on the tissue perfusion.

3. A good oxygenation can be achieved even in greater elongation by increasing the cardiac frequency.

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# CONGENITAL DIAPHRAGMATIC HERNIA

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## Abstract

Five defects might develop in the diaphragm to create intraabdominal viscera herniation:

1. The esophageal hiatus is the most frequent area, wherein the stomach prolapses into the mediastinum.
2. A congenital posterolateral defect occurs from maldevelopment of the diaphragm.
3. Anomalous attachment of the diaphragm to the sternum and adjacent ribs results in a foramen, which allows the bowel to extend into the anterior mediastinum.
4. The association of an epigastric omphalocele and a retrosternal defect in the diaphragm and pericardium (pentalogy of Cantrell) results in herniation within the pericardium.
5. Attenuation of the tendinous or muscular portion of the diaphragm produces eventration.

Paralysis of the muscles of the diaphragm either from trauma to the phrenic nerve or a congenital defect in the anterior horn cells of cervical spinal cord (C3,4) -Werdnig-Hoffmann disease, results in herniation of the intraabdominal contents into the thoracic cavity.

## DEFINITIONS

Congenital diaphragmatic hernia is characterized by a defect in the posterolateral diaphragm (foramen of Bochdalek) through which the abdominal viscera migrate into the chest during fetal life.

**Key words:** congenital diaphragmatic hernia (CDH), diaphragm.

## ANATOMY OF THE DIAPHRAGM

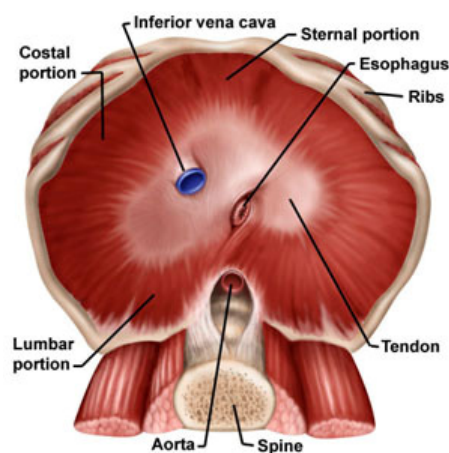
The diaphragm is a fibromuscular sheet that separates the thoracic and abdominal cavities. It is the principal muscle of inspiration.

1. The fibrous portion of the diaphragm - the central tendon, is more anteriorly than centrally oriented and it accounts for about 35% of the total surface of the diaphragm.
2. The muscular portion consists of:
  - the short muscle slips arising from the xiphoid process
  - the origin from the lowest six ribs of the costal arch
  - the muscle arising from the medial and lateral arcuate ligaments, overlying the psoas and quadratus lumborum muscles respectively
  - the vertebral part, the crura, arising from the first three lumbar vertebrae

In 80% of bodies, there is a gap between the muscles arising the lateral arcuate ligament and those of costal origin as they traverse to the central tendon, which is called the vertebral-costal or lumbo-costal triangle. This muscular gap is covered by a fibrous membrane - foramen Bochdalek.

In the diaphragm are three significant openings:

- the vena cava traverses the central tendon to the right of the midline;
- the esophageal hiatus is just to the left of the midline and slightly posterior to the plane of vena cava;
- the aorta lies on the vertebral bodies



## EMBRYOLOGY OF THE DIAPHRAGM

The normal diaphragm is derived from several components. The central tendon is formed from the septum transversum; small dorsolateral portions are from pleuroperi-toneal membranes; dorsal crura are from the esophageal mesentery; the posterior lateral muscle is derived from the inter-costal muscle groups.

Development of the diaphragm begins at week four in human gestation with formation of the septum transversum, which separates the thoracic and abdominal cavities of the embryonic coelom leaving two pleuroperitoneal canals dorsolaterally. The pleuroperitoneal folds extend from the late-lateral body wall and grow medial and ventral until week seven - when they fuse with septum transversum and the mesentery of the esophagus.

The last area for fusion of the septum transversum and the pleuroperitoneal membranes is a foramen called pleuro-peritoneal canal. CDH is a consequence of a persistent pleuroperitoneal canal that results in a defect in the posterolateral portion of the diaphragm. This defect occurs five times more commonly on the left side, because the liver facilitates closure of the right pleuroperitoneal canal.

During the early development of the diaphragm, the midgut is herniated into the yolk sac. If closure of the pleuroperitoneal canal has not occurred by the time the midgut returns to the abdomen (weeks 9 through 10), the abdominal viscera herniate through the lumbocostal trigone into the

ipsi-lateral thorax. This prevents the normal counterclockwise rotation and fixation of the midgut. No hernia sac is present if the herniation occurs before complete closure of the pleuro-peritoneal canal.

### LUNG DEVELOPMENT

At 26 to 33 days gestation, the tracheal diverticulum from the foregut is identified and this splits into two lung buds. The subsequent development of the airways is divided into:

- The glandular phase (day 52 to the end of the 16<sup>th</sup> week of gestation), when bronchopulmonary segments are forming by dichotomous branching of the terminal buds;
- The canalicular phase (17 to 26 weeks of gestation) is characterized by continued budding of air spaces, which form the respiratory bronchi, atria and alveoli of the lung. During this period are forming the type I pneumocytes, the type II pneumocytes. This last type produces surfactant beginning at the 24<sup>th</sup> week of gestation.
- The alveolar phase extends from 26 weeks to full term. At this stage, progressive elongation and budding of the thin-walled air spaces occur beyond the transitional ducts, respiratory bronchioles and saccules.

At birth there are about 24 million saccules and these saccules develop septa to form distinct alveoli. The alveoli increase rapidly in number but not in size for 1<sup>st</sup> 3 years. Between 3 and 8 years, the alveoli continue to increase in number and size. At 8 years and older, they enlarge as the chest cavity increases in volume, but no new alveoli form.

### EPIDEMIOLOGY

CDH probably occurs once in 1/2200 births, when stillbirths are included. The natural history of CDH has become clarified with prenatal ultrasound imaging of the fetus.

The cause of CDH is unknown, but there may be a genetic factor; it has been reported in identical twins, sibling and uncles and cousins. Females are affected almost twice as often as males.

### PATHOPHYSIOLOGY

CDH is characterized by a defect in the posterolateral diaphragm (foramen of Bochdalek), through which the abdominal viscera migrate into the chest during fetal life.

The resultant compression of the lungs during their growth and development causes pulmonary hypoplasia to a greater or lesser degree. It is this pulmonary hypoplasia which is responsible for the high rate of mortality associated with this lesion.

Pulmonary hypoplasia associated with CDH is characterized by a diminished number of bronchial branches and total alveoli for gaseous exchange. The pulmonary vasculature is also affected by pulmonary hypoplasia. The arteries are smaller in diameter and have a thicker muscular wall.

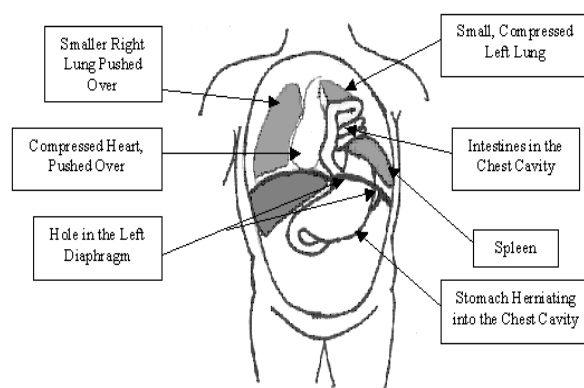
Pulmonary hypertension frequently occurs in infants with pulmonary hypoplasia. The hypertension is the result of a diminished number of pulmonary vessels and

capillary bed, an increased resistance from the abnormally thick muscular arterial walls and an abnormally increased reactivity of arterial musculature to hypoxia, hypercarbia, acidosis and other mediators of vascular tone.

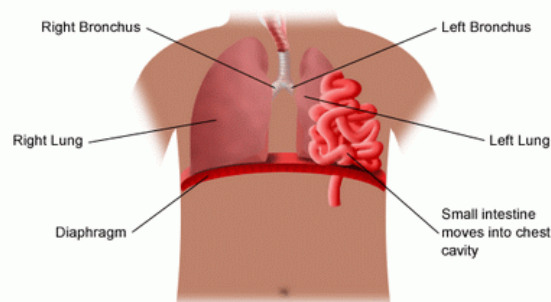
At birth, the infant has difficulty attaining air-entry into the lungs, because the most important mechanism of inspiration is contraction of the diaphragm and because the mediastinum is pushed to the opposite chest and both lungs are compressed. The negative pressure generated during inspiration produces further herniation of bowel within the thorax. During respiratory distress, the infant swallows large volumes of air, which distend the bowel and further compress the lung.

The number of pulmonary artery branches is diminished, so that the right ventricular workload requires pushing blood through a small vascular bed, which results in increased pulmonary artery and right heart pressure.

Shunting of blood from right to left occurs through the ductus arteriosus and foramen ovale, exacerbating systemic hypoxia, hypercarbia and acidosis.



**Congenital Diaphragmatic Hernia**



### DIAGNOSIS

#### PRENATAL DIAGNOSIS

Most series of CDH presenting after birth have quoted a mortality of approximately 50%. However, the advent of widespread maternal ultrasonography has resulted in the identification of a large group of fetuses with CDH

who die in utero, or soon after birth and never present to the paediatric surgeon. The impact of this “hidden mortality” on the management of a fetus with CDH is considerable, as the outlook is much more grave than previously appreciated, with a mortality between 60% and 80%.

There are a number of features which have prognostic value in prenatally diagnosed CDH. Prenatal diagnosis itself may indicate a worse prognosis. Other poor<sup>2,6,11</sup> prognostic indicators include:

- polyhydramnios
- small left ventricular size
- presence of the stomach above the diaphragm
- the liver within the thorax
- lung area-to-head circumference ratio <1
- early gestation diagnosis (less than 24 weeks)

Critical ultrasound findings include the presence of viscera in the right or the left hemithorax above the level of the inferior margin of the scapula or at the level of the four-chamber view of the heart. The hypoechoic signal of the fluid-filled stomach, gallbladder or bowel can be distinguished from the hyperechoic signal of the fetal lung.

Other common findings include:

- A small ipsilateral lung
- A defect in the ipsilateral diaphragm
- A shift of the mediastinum away from the affected side.

Identification of abnormal upper abdominal and presence of peristalsis in herniated bowel loops helps distinguish congenital diaphragmatic hernia from other diagnoses.

After CDH is identified, the fetus is studied for additional anomalies by thorough scanning of the head, spine, heart and kidneys. Amniocentesis or villus biopsy is performed to screen for chromosomal and metabolic anomalies. Associated anomalies are seen in 25% to 57% of all cases of CDH and 95% of stillborns with CDH and include:

- congenital heart defects
- hydronephrosis
- renal agenesis
- intestinal atresia
- extralobar sequestrations
- neurologic defects, including:
  - hydrocephalus,
  - encephalocele,
  - anencephaly,
  - spina bifida.

Chromosomal anomalies, including trisomy 21, 18 and 13, occur in association with CDH in 10% to 20% of cases diagnosed prenatally.

#### POSTNATAL DIAGNOSIS

The typical neonate with CDH presents within hours of birth with severe respiratory distress. The following are the most common symptoms of a CDH:

- difficulty breathing
- fast breathing
- fast heart rate
- cyanosis
- scaphoid abdomen
- shifting of the heart sounds to the right and bowel sounds in the chest.

Gastrointestinal symptoms of abdominal pain, nausea and vomiting may be from compression of the bowel through the diaphragm, producing obstruction, or from gangrene of the bowel, with or without volvulus.

The definitive diagnosis is usually made by chest X-ray, which shows bowel loops in the chest and a significant mediastinal shift. A chest radiograph should be obtained, preferably after an orogastric tube has been passed into the stomach.

If the radiograph is taken before air enters the bowel, the affected chest is radiopaque, but the trachea and heart are shifted to the contralateral side and the aerated lung is diminished.



Although most infants with CDH present in the first 24 hours of life, some can appear later. These children have a variety of presentation including mild respiratory distress, an incidental finding in chest radiograph, chronic pulmonary disease, pneumonia, pleural effusion, empyema or gastric volvulus. Presentation with gastric volvulus is worthy of emphasis because it is an indication for emergent surgical intervention.

#### DIFFERENTIAL DIAGNOSIS

The prenatal and neonatal diagnosis of CDH can be confused with a variety of other lesions including:

- the eventration of the diaphragm
- pentology of Cantrell
- Morgagni hernia
- congenital cystic disease of the lung
- primary agenesis of the lung
- mucoviscidosis
- esophageal atresia
- pneumothorax
- tracheomalacia

Diaphragmatic eventration has many causes, but in new born, it commonly results from birth trauma or Wernig-Hoffman anterior horn-cell disease. The physiologic consequences are quite variable, ranging from asymptomatic infants to acute respiratory distress.

A Morgagni hernia occurs in the anterior muscular diaphragm at the hiatus for the internal mammary artery and is rarer than the Bochdalek hernias. Affected infants can

present with gastrointestinal crisis because of incarceration and need emergent surgery; most are asymptomatic.

## TREATMENT

### PRENATAL TREATMENT

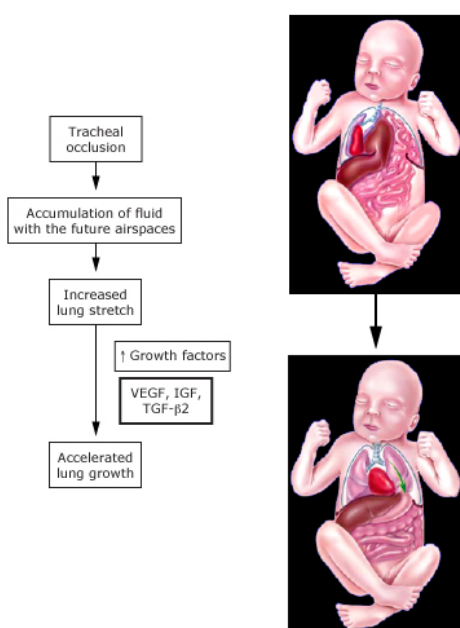
The advantage of prenatal diagnosis is not so much for surgical preparation as it is for educating parents about possible treatments and outcomes. It also allows the fetus and mother to be referred safely to an appropriate level III tertiary perinatal center where the full array of respiratory care expertise and strategies are immediately available.

<sup>3</sup>As a matter of principle, a spontaneous vaginal delivery is preferred unless obstetric issues supervene. The mere diagnosis of CDH is not an indication for elective cesarean section.

Correction of the lung compression using open fetal surgery permits sufficient lung growth to support respiratory function at birth.<sup>2</sup> Morphometric analysis of the lung in this model has confirmed that *in utero* repair results in improved lung growth and reversal of the characteristic pulmonary vascular changes.

In the animal models of CDH, <sup>8</sup>tracheal occlusion induces lung growth, increases alveolar surface area and alveolar number, as well as visceral reduction from the chest. The results of these experiments were so compelling that the fetal occlusion was applied in human fetuses with severe CDH.

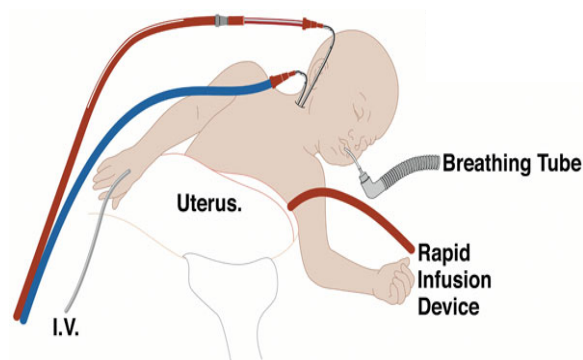
The fetal lung secretes fluid by active ion transport through gestation, and this lung fluid provides a template for lung growth. Occlusion of the fetal trachea traps this fluid and stimulates lung growth, either by retention of growth factors within the lung or stimulation of local growth factors by the gentle distension provided by the fluid. A randomized trial in humans found that tracheal occlusion did not improve outcome compared with standard treatment.



As a result of the poor outcomes with the procedure, a procedure was described using transuterine endoscopy or FETENDO. The results from the FETENDO<sup>10</sup> approach in high risk CDH were promising. Shortly after initiation of the FETENDO trial for CDH it is developed a less invasive endoluminal balloon tracheal occlusion technique<sup>9</sup>.

Depending on the nature of CDH, the fetus may be a candidate for reversible balloon tracheal occlusion or EXIT-to-ECMO (ex utero intrapartum treatment to extracorporeal membrane oxygenation) procedures, both of which are types of fetal surgical intervention.

Some prognostic information may be gained by noting the gestational age at diagnosis, the presence of polyhydramnios and the size of the defect.



### PREOPERATIVE TREATMENT

<sup>1</sup>Newborn infant who present with respiratory insufficiency require endotracheal ventilatory support. Initial mask ventilation is avoided, because insufflation of air is produced into the stomach and bowel.

As soon as endotracheal ventilation is achieved, the baby is paralyzed to facilitate ease of ventilation without the baby struggling and displacing bowel into the chest. The stomach is decompressed with a nasogastric tube which is connected to a vacuum. The ventilatory pressures must be carefully restricted to less than 45 mmHg.

Appropriate fluid management and monitoring with central venous and urinary catheters is important. Umbilical artery and right atrial catheters are inserted. Cannulation of the right radial artery is desirable to detect any difference in the PaO<sub>2</sub> in the aorta, proximal and distal to the ductus arteriosus. When the PaO<sub>2</sub> in the distal aorta is lower than that in the right radial artery, pulmonary hypertension and/or hypoplasia is present.

The objective of ventilation is to maintain the postductal PaO<sub>2</sub> above 40mmHg, the PaCO<sub>2</sub> less than 30mmHg and the pH greater than 7.5 to achieve maximum pulmonary vasodilatation.

A small number of babies have minimal to moderate respiratory distress, which responds well to ventilation. A larger number are born in extremis, are never resuscitable and die within the first few hours or days of life. There is a middle group, who have a brief "honeymoon period" after which rapid deterioration takes place. This is

probably due to vasoconstriction of the abnormally reactive pulmonary vascular bed.

A battery of pharmacological agents was used in an attempt to decrease pulmonary vascular resistance. These drugs contribute to capillary leak, hypovolemia and systemic hypotension.

More recently, nitric oxide has been used as a selective pulmonary vasodilator when added to ventilatory gases. This agent is rapidly degraded so that systemic hypotension does not occur.

Some authors have suggested surfactant replacement, because there is some evidence that infants with CDH may be surfactant deficient. None of these approaches, appears to have had a major impact.

<sup>2</sup>There are three new techniques which have some promise to improve survival in CDH:

1. **ECMO** - the patient's blood is perfused through a membrane oxygenator for gaseous exchange and then returned to the patient. The blood may be withdrawn from the right atrium and returned to the venous circuit, but the most frequent technique is to return it to the arterial circuit. An incision in the neck gives access to the deep jugular vein, where a large-bore catheter with multiple holes can be passed into the right atrium. A common carotid arteriotomy allows passage of a cannula into the arch of the aorta. The venous blood is drawn by gravity into a reservoir. The blood is pumped around a silicone rubber membrane, which is permeable to oxygen and carbon dioxide. While the patient is on by-pass, the lungs are ventilated at a rate of 10 times per minute.

A peak inspiratory pressure (PIP) is used at 20 cm H<sub>2</sub>O and the positive end-expiratory pressure (PEEP) is used at 5 cm H<sub>2</sub>O. This pressure places the lungs at relative rest while they are expanded for expected ideal blood flow and ventilation.

The most serious complication is intracranial bleeding, which is much more common in premature infants.

Indications for neonatal ECMO<sup>2</sup>:

- Oxygenation index > 25
- No congenital anomaly incompatible with normal life
- Gestational age > 35 weeks
- Mechanical ventilation less than 7-10 days
- No evidence of intracranial haemorrhage
- In CDH evidence of a 'honeymoon period'

Several investigations have reported a survival of greater than 70% using ECMO in selected high-risk newborns with CDH.

2. **HFO (HIGH FREQUENCY OSCILLATION)** is being used experimentally in respiratory distress syndrome and meconium aspiration, with encouraging preliminary results. There is good evidence that HFO permits ventilation with a lower mean airway pressure.

3. **DELAYED SURGICAL REPAIR OF CDH** has many advantages. Although it had been assumed for many years that immediate operation to decompress the chest was necessary, there are a number of problems with this approach. Ventilatory management is not as good during transport to and from the operating room, infants often

deteriorate after surgery. After paralysis and positive pressure ventilation, it is common on chest X-ray to see the lungs expand, the mediastinal shift resolve and the bowel move down into the abdomen.

Sakai and colleagues have shown that surgical repair of the defect actually worsens thoracic compliance and PCO<sub>2</sub>, suggesting that emergency repair may be harmful to the already borderline infant.

Repair of the hernia does not increase the surface area available for gas exchange in hypoplastic lungs. The alveoli are not atelectatic and do not expand upon decompression of the chest.

A consensus has developed recently that surgery be performed when pulmonary vascular tone is maximally stabilized. This is followed by preductal-postductal oximetry. Recent reports on infant with CDH studied by whole body plethysmography during prolonged preoperative resuscitation demonstrate that infant minute ventilation improves with decreased mechanical support over the first several days of life with concomitant improvement in blood gas parameters.

A timely operation can often be performed after 100 hours with minimal supplemental oxygen and airway pressure requirements, particularly if therapy is guided primarily by preductal oxygen saturation.

### **SURGICAL REPAIR**

*It is essential to consider that CDH is a physiologic, not a surgical emergency!*

Repair of the defect is usually the most straightforward part of the management of CDH. Preoperative antibiotics are usually used. Blood should be available.

<sup>1</sup>Once the infant has stabilized, a general anesthetic using halothane and pancuronium bromide (Pavulon) is preferred to minimize vasoreactivity.

Through a left subcostal abdominal incision, the defect is exposed and the viscera are pulled down out of the chest. The stomach, the spleen, part of the pancreas, the small bowel and the proximal colon are often in the chest.

The spleen on the left and the liver on either side can be difficult to reduce, but this must be done without injury. On the right side, the kidney and adrenal gland may be found in the chest.

When there is a true hernia sac with a membrane of parietal pleura and peritoneum, this should be resected to achieve adequate healing.

Usually the anterior rim of the diaphragm is better developed than the posterior component. The posterior aspect of the defect is covered by peritoneum. An incision is made in the peritoneum posteriorly and the posterior muscular edge freed to develop as much posterior diaphragm as possible.

Much of the time it is possible to carry out direct closure using interrupted non-absorbable sutures. The one found to be most secured is a vest-over-pants closure using horizontal mattress sutures as the first row and then a simple suture between each of these to reinforce the first suture line.

In some cases, the defect is too large for primary closure and prosthetic material (Gortex or Marlex) is used.

An alternative to this approach is a muscle flap taken from the transversus abdominus, leaving the outer abdominal muscle layers intact.

The lateral rim may be absent, making it necessary to put the lateral sutures either around the rib or into the intercostal muscle.

Procedure for nonrotation of the midgut as well as appendectomy is not needed and potentially dangerous because of the risk for hemorrhage.

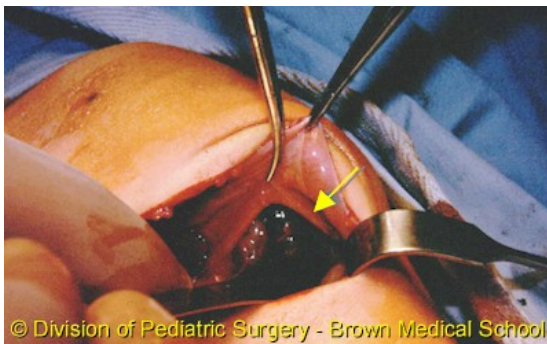
Prior to closure, the abdomen is manually stretched to make room for the herniated viscera. Wound closure may be difficult, because the peritoneal cavity is small; on occasion, a Silastic gusset is required for abdominal wound closure. Primary closure in layers is usually possible.

A tube thoracostomy is generally not needed in either hemithorax for the CDH infant, unless there is a pneumo-thorax, a bronchopleural fistula, bleeding or some other specific indication. The ipsilateral lung is hypoplastic and hence smaller than that hemithorax. Because the pleural space is driven to obliterate itself, and because the lung is not yet capable of filling it, the remaining space will be filled with pleural fluid.

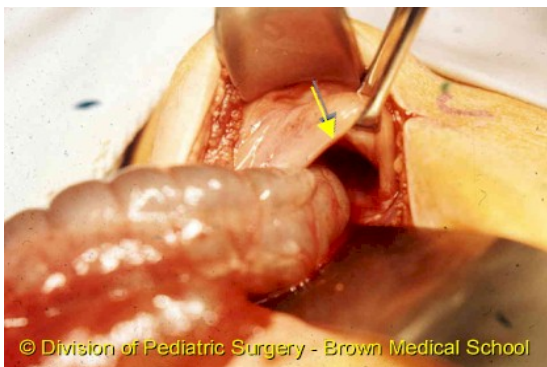
If a chest tube is used, it is important to avoid applying suction, as it increases the transpulmonary pressure gradient and predispose to pneumothorax.

<sup>3</sup>**LUNG TRANSPLANTATION** - may be an option for the infant with severe pulmonary hypoplasia, who is truly refractory to all forms of therapy.

Transplantation of a single lung has been reported in one case. Lung transplantation may allow the remaining hypoplastic lung to grow and to recover from injury while still allowing adequate oxygenation and ventilation. This approach has not been widely used because of the substantial problems associated with donor lung availability and immunosuppression.



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## POSTOPERATIVE CARE

<sup>1</sup>It is primarily based on close fluid management, ventilatory support, which often requires paralysis and haemodynamic monitoring:

- The chest tube is connected to a water seal
- The ventilatory care continues postoperatively with the goal of maintaining hypocarbia and a pH greater than 7.5
- In the first 24 to 48 hours postoperatively, intravenous fluid infusions consists of
- Ringer lactate solution

Depending on the previous intensity of hypoxia, cardiomyopathy and renal failure may occur.

## FOLLOW-UP

### Pulmonary care

Severely affected infants have chronic lung disease.

These infants may require prolonged therapy with supplemental oxygen and diuretics, an approach similar to that for bronchopulmonary dysplasia.

Late pulmonary hypertension has been successfully treated with low-dose inhaled nitric oxide. This therapy can be delivered via nasal cannula following extubation. In a recent report, the median duration of treatment using inhaled NO delivered via nasal cannula was 17 days.<sup>4</sup>

### Neurologic evaluation

The incidence of hearing loss appears to be particularly high in patients with CDH (approximately 40% of infants). An automated hearing test should be performed prior to discharge.

### Gastroesophageal reflux

The incidence of significant gastroesophageal reflux is very high in patients who survive CDH (45-85%). The need for a diaphragmatic patch may be a significant predictor of gastroesophageal reflux.

### Growth assessment

Failure to thrive is common. In one study, one third of infants required gastrostomy tube placement to improve caloric intake. The need for supplemental oxygen at the time of discharge is a significant predictor for subsequent growth failure. Possible causes include increased caloric requirements due to chronic lung disease, oral aversion after prolonged intubation, poor oral feeding due to neurologic delays and gastroesophageal reflux.

## PROGNOSIS

Of those pediatric patients who are born alive at a medical center where the operative treatment is undertaken, the mortality exceeds 60 to 65%.

Those infants who are symptomatic at birth and who survive transport to a pediatric surgical center have a mortality of 35 to 50%.

The mortality is much greater in babies who require urgent treatment within the first 6 to 24 hours of life compared with older babies.

<sup>4</sup>Survivors are at risk for significant longterm morbidity, including chronic lung disease, growth failure, gastroesophageal reflux, hearing loss and neurodevelopmental delay.

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## BILIARY ATRESIA

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### Abstract

Extrahepatic biliary atresia (EHBA) is an inflammatory fibrosing process affecting the extrahepatic and intrahepatic biliary tree resulting in fibrous obliteration of the extrahepatic biliary tract, ductopenia of intrahepatic bile ducts, and biliary cirrhosis. EHBA is divided in a fetal, prenatal or embryonic, and a more common, perinatal form. The symptoms of the fetal form start shortly after birth and there is frequently an association with a variety of congenital anomalies. Children with the perinatal form become jaundiced several weeks after birth; no associated congenital anomalies are present. Morphologically, an inflammatory and fibrosing process of the extrahepatic biliary tree leads to complete luminal obliteration. The liver is characterized by a nonspecific giant cell transformation, and portal expansion by fibrous connective tissue with marked ductular proliferation. The differential diagnosis with other conditions with similar microscopic patterns such as alpha-1 antitrypsin deficiency, total parental nutrition, obstruction by a choledochal cyst, arteriohepatic dysplasia, familial progressive intrahepatic cholestasis a. s. o. is discussed. Different etiologies have been postulated in the perinatal form of EHBA: genetic susceptibility, vascular factors, toxins, and infections. EHBA is a heterogenous disease, resulting from a combination of genetic factors, insults, and immunologic pathways. The treatment of EHBA is surgical, whit anastomosis between the biliary tree and the intestine in the “correctable” type and a hepatic portoenterostomy (HPE) for “noncorrectable” group. HPE is a temporizing treatment allowing the infant to develop and grow, followed in the majority of the patients by liver transplantation.

**Key words:** cholestasis, conjugated hyperbilirubinemia, extrahepatic biliary atresia, and pediatric diseases of the liver.

### INTRODUCTION

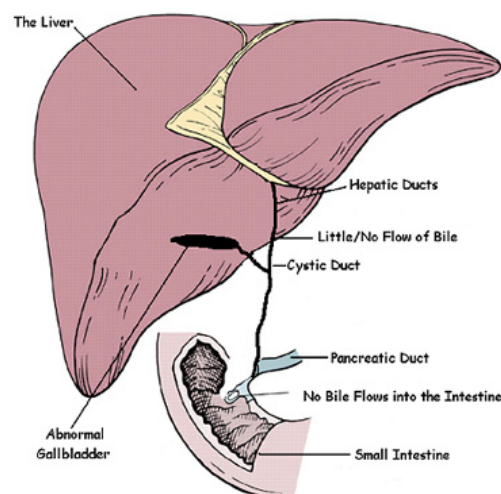
EHBA is defined as a complete fibrous obliteration of a portion or of entire extrahepatic biliary tree, not associated with calculi, neoplasm or rupture (fig. 1). This process is dynamic, inflammatory and fibrosing and involves the extra- and intrahepatic biliary tree and leads to obliteration of the extrahepatic biliary tree, biliary cirrhosis and ductopenia.

### HISTORY

J. Burke made the first description of the disease in 1817. In 1916 Holmes introduced in a review the concept of correctable and noncorrectable types of disease. Ladd reported the first successful surgery for the correctable type

in 1928. In 1953, Gross documented that extrahepatic biliary atresia was the most common condition causing obstructive jaundice in the first month of life, and that most patients had he noncorrectable type of disease. In 1957, Kasai et al introduced HPE for surgical treatment of biliary obstruction in infants considered to have noncorrectable biliary atresia. (2) Increased experience with hepatic portoenterostomy has revealed that the intrahepatic component of ductal pathology is of great importance in determining prognosis. Liver transplantation was presented by Starzl et al in 1963 as optional therapy for patients in whom hepatic portoenterostomy was unsuccessful. (3)

Fig 1: the disease (1)



### EMBRYOLOGY

The liver and biliary tree develops inseparably. The hepatic bud is an endodermal diverticulum that arises late during the third embryonic week from the ventral portion of the foregut, in the section corresponding to the duodenum. This diverticulum has a caudal and a cranial portion. The cranial portion develops into liver, with intrahepatic bile ducts and a portion of the hepatic ducts, including these ducts cholangiocytes. The caudal portion becomes bile duct, cystic duct, and gallbladder.

The epithelium of the cranial portion of the hepatic bud grows into the mesenchyme of the septum transversum. This mesenchyme appears necessary for the induction of hepatocyte differentiation. The liver-committed epithelium cloaks vessels of the vitelline venous plexus; blood leaving the developing liver drains into the sinus venosus. As the embryo lengthens, the duodenum no longer is apposed to the septum transversum, so that the ventral mesentery (the lesser omentum) comes to contain the extended stalk derived from

the caudal hepatic bud. (4,5) This is the anlage of extrahepatic bile ducts.

Within the embryonic liver, portal tracts are defined by condensations of mesenchyme around vessels. Hepatoblasts are a cell type capable of differentiation into either hepatocyte or cholangiocyte, at the margin of these tracts, constitute the ductal plate. This becomes apparent between the 9<sup>th</sup> and 10<sup>th</sup> embryonic week as a layer of cells; this layer is then duplicated focally and by the 12<sup>th</sup> embryonic week begins to develop lumina. Regression of portions without lumina and outward growth of mesenchyme bring the persistent portions within the portal tract. This process moves outward along the portal tracts as the liver grows, establishing an anastomosing network through which bile can drain toward the hilum. (6,7)

### BILIRUBIN PHYSIOLOGY

Newborns are at increased propensity for developing hyperbilirubinemia due to the following

physiological handicaps leading to either increased bilirubin production or its decreased excretion.

#### Increased production

- Newborns have a greater red cell (RBC) mass per kg as compared to that in adults; so they produce 6-10 mg of bilirubin/kg/day as opposed to the production of 3-4 mg/kg/day in adults.
- Newborns have a shorter life span of RBC (80-90 day as compared to 120 day in adults).

#### Decreased excretion

- Defective uptake of bilirubin due to hepatic immaturity and decreased ligandin.
- Defective conjugation due to decreased UDPG-T activity (Uridine diphospho glucuronyl transferase activity).
- Decreased hepatic excretion of bilirubin.
- Increased entero-hepatic circulation due to higher levels of beta-glucuronidase enzymes in neonatal gut and decreased intestinal bacteria.

### METABOLISM OF BILIRUBIN – see table 1 and table 2

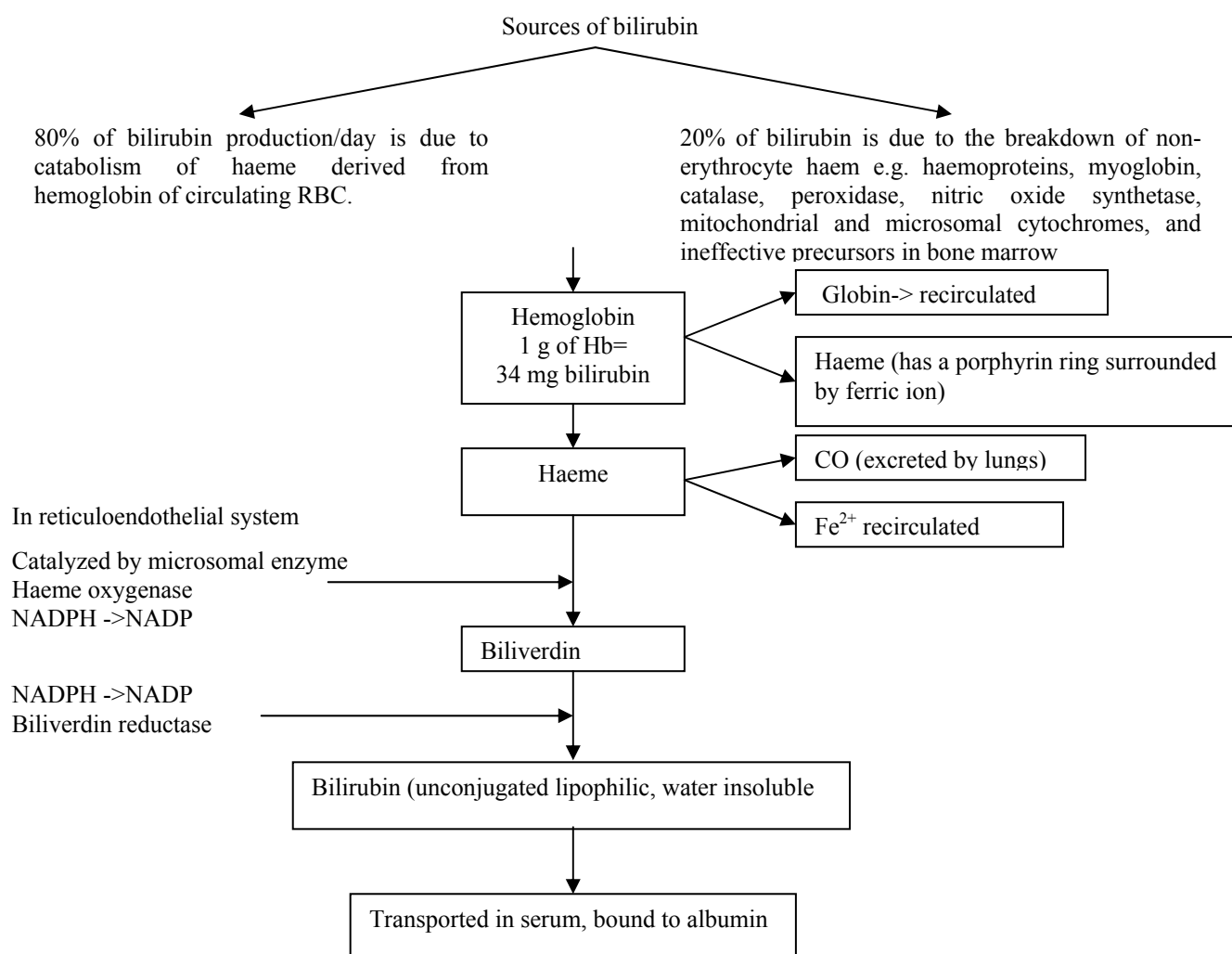


Table 1

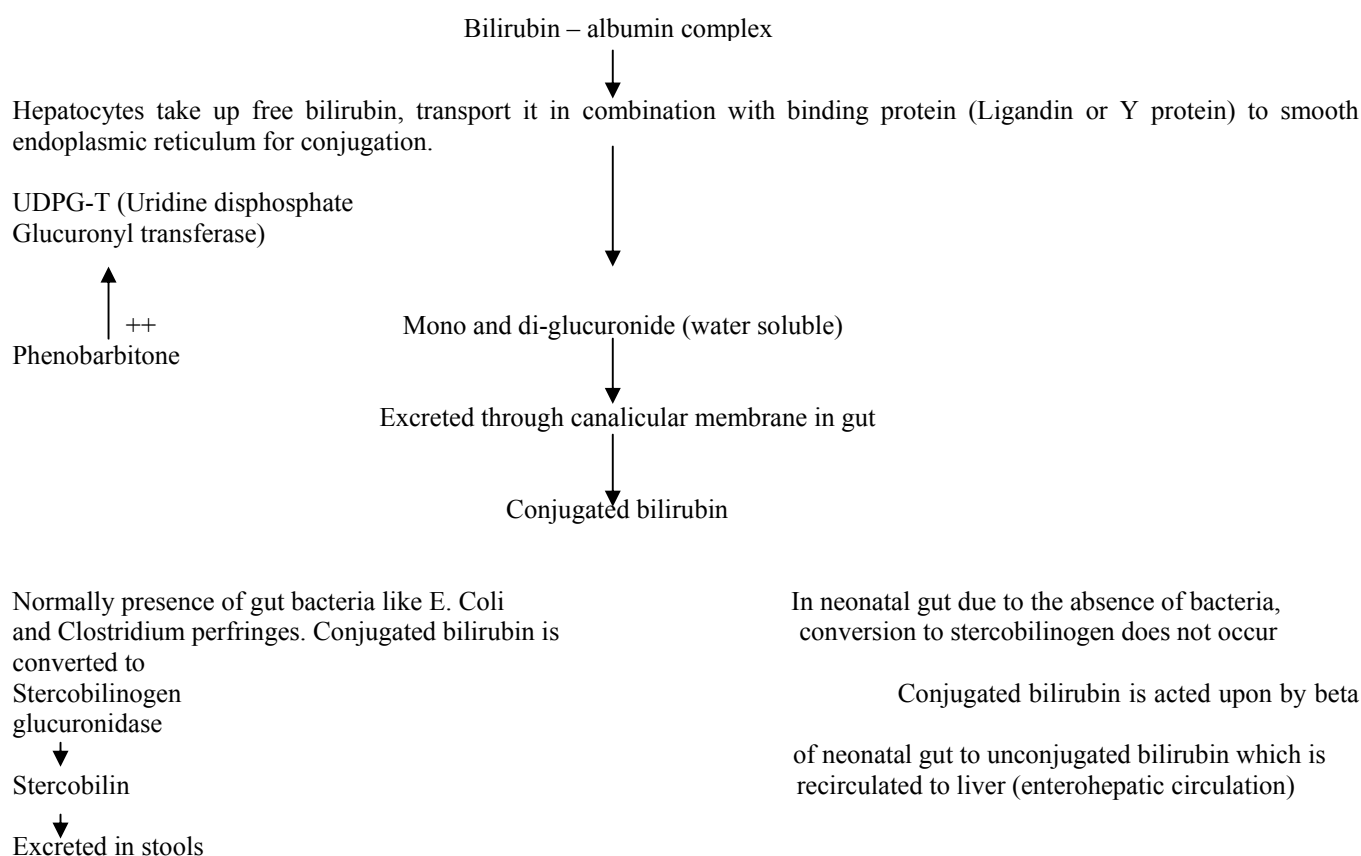


Table 2

### FETAL BILIRUBIN METABOLISM

Most of unconjugated bilirubin formed by fetus is cleared by placenta into maternal circulation. Conjugation of bilirubin is limited in fetus due to the decreased hepatic blood flow and the decreased uridine diphosphoglucuronyl transferase (UDPGT) activity. Bilirubin is normally present in amniotic fluid by 12 weeks. Increased amniotic fluid bilirubin level is found in hemolytic disease of the newborn and the fetal intestinal obstruction below the bile ducts. (8)

### GENERAL CONSIDERATIONS

- A whole or a part of the extrahepatic bile ducts is absolutely atretic in biliary atresia and completely obstructs bile flow.
- The incidence of biliary atresia is about 1 in 8,000 to 12,000 live births.

- Is a moderate predominance of the disease in females (1: 0, 64).
- Mortality/morbidity: the long-term survival rate for infants' with biliary atresia following portoenterostomy was 45-50% at 5 years and 25-35% at 10 years.
- Liver transplantation may be the only option for long-term survival in the majority of patients
- Patients with biliary atresia may be subdivided into 2 distinct clinical forms: (1) those with isolated EHBA (postnatal form), accounting for 65-90% of cases and (2) patients with associated situs inversus or polysplenia/asplenia with or without other congenital anomalies (fetal/embryonic form), comprising 10-35% of cases.

Table 3: Comparison between fetal and perinatal biliary atresia: incidence, predisposing factors, associations and time of presentation

EHBA type	Incidence	Insult	Congenital anomalies	Onset
Fetal	10-25%	Antenatal	Present	Early
Perinatal	Common	Perinatal	Absent	After 2 weeks

## Associated anomalies:

- Cardiovascular
- Preduodenal portal vein
- Meckel's Diverticulum
- Malrotation
- Polysplenia
- Asplenia
- Situs Inversus
- Urological
- Pulmonary Hypogenesis
- Umbilical Hernia
- Inguinal hernia
- Others

**MORPHOLOGY**

The morphologic changes of the extrahepatic tree consist of a spectrum of alteration from epithelial damage of a patent bile duct, to inflammation and fibrosis with obliteration of the lumen, a progressive and dynamic process. The gallbladder is frequently shrunken or atretic. The microscopic changes of the gallbladder and cystic duct are less severe than those of the extrahepatic biliary tree. Similar changes affect the bile ducts of the porta hepatis resulting in several subtypes such as complete fibrous obliteration of the bile ducts, presence of small duct or gland-like structures, and presence of large ducts.

Initial studies of atretic ducts in infant with EHBA grouped the findings into three categories based on the size of the lumen of the bile ducts: type 1 – lumen 150 micrometer or greater; type 2 – ductal structures less than 150 micrometer; type 3 – without epithelium-lined structures, and concluded that presence of bile ducts with a diameter of 150 micrometer or more are correlated with adequate bile flow after surgical intervention and therefore predicts a better outcome. (11) Now, is generally accepted that there is no valid correlation between duct size at time of surgery and prognosis. Absence of bile flow in fibrous remnants has been reported a poor prognostic sign in terms of postoperative bile drainage.

The intrahepatic changes of EHBA at an early stage of disease, before 3 months of age are characterized by nonspecific lobular giant cell transformation with cholestasis, extramedullary hematopoiesis, hemosiderin in Kupffer cells), and diagnostic portal changes. All portal spaces are expanded by fibrosis, contain an apparently increased number of interlobular bile ducts, and show marked ductular proliferation. A ductal plate malformation pattern of the ductular proliferation has been observed in patients with a severe form of EHBA. Ductal plate refers to phenotypic changes of periportal hepatocytes characterized by the expression of a biliary phenotype in the cytokeratin stain (CK19 positivity in addition to CK18 expression) around 8-week gestation. These transformed hepatocytes become flattened, form first uni-cell and subsequently double-cell layer that acquires a lumen and extends into the portal space, followed by disruption of the connection to the ductal plate. (11) The cell of the ductal plate will disappear subsequently. This phenomenon of bile duct formation with regression of the ductal plate is called ductal plate

remodeling. Bile duct malformation refers to the disturbance in this process with persistence of bile ducts in a ductal plate configuration, numerous dilated bile ducts surrounding a portal space as well as an increase in the number of interlobular bile ducts in the portal space. The ductular proliferation is the typical morphologic presentation of EHBA (a diagnostic biopsy must contain at least five complete portal spaces). (13,14)

After 3 months of age in nonoperated and in most of the operated patients, the portal fibrosis progresses surrounding and subdividing the hepatic lobule, thus resulting in biliary fibrosis and cirrhosis. The number of interlobular bile ducts is considerably reduced by 5 months of age.

Morphologic classification of types of biliary atresia (proposed by the Japanese Society of Pediatric Surgeons)

- Principal types:
  - type I: atresia of common bile duct
  - type II: atresia of hepatic duct
  - type III: atresia of bile duct at the porta hepatis
- Subtypes according to the patterns of distal bile ducts:
  - a: patent common bile duct
  - b: fibrous common bile duct
  - c: aplasia of common bile duct
  - d: miscellaneous
- Subtypes according to patterns of hepatic radicles at the porta hepatic:
  - dilated hepatic radicles
  - hypoplastic hepatic radicles
  - bile lake
  - fibrous hepatic radicles
  - fibrous mass
  - aplasia of hepatic radicles

**ETIOLOGY**

The etiologic factors are different in the fetal and perinatal group.

## ➤ The fetal group

A defect in a yet undefined gene has been postulated for patients with EHBA with extrahepatic malformation; for those without extrahepatic malformation, an abnormal morphogenesis of the bile ducts seems more likely. Several mutated genes have been considered: inversin gene, Kartagener gene, CFC1, JAG1, and HLA class II gene. (15)

## ➤ The perinatal form

- a genetic susceptibility of patients with EHBA expressed by a high frequency of HLA-B12, with haplotypes A9-B5, A28-B25, B8, DR3.
- Toxin: bile acids, alcohol, environmental factors
- Vascular: patent ductus arteriosus, hepatic arteriopathy
- Infectious: hepatitis C virus, rubella, cytomegalovirus, Epstein-Barr virus, human papilloma virus, reovirus 3, rotavirus. (16,17)

**PATHOGENESIS**

The pathogenesis of EHBA is controversial. It is a heterogeneous disease, the end result of different causal and

pathogenetic mechanisms operating at different periods of gestational and postnatal development.

- ❖ For the fetal type, genetic mutations and ductal plate malformation seem to play a major role. Mutation of the JAG1 gene may lead to malformation or dysfunction of the intrahepatic bile ducts. The defect in remodeling of the ductal plate is a result in an inadequate mesenchymal cuff around the hilar bile ducts. These deficient bile ducts rupture resulting in inflammation and fibrosis and bile duct obliteration at the porta hepatis.

- ❖ For the perinatal type, the infection, apoptosis and cell necrosis, inflammation, fibrosis – play a major role.

-infection: no single agent has been identified as causative for biliary atresia, though the role of infecting organisms has been the most extensively studied. CMV, rotavirus, reovirus, common hepatitis A, B, C was studied, but no clear associations have been found. (steroid therapy may modulate the inflammation)

-apoptosis and cell injury. Apoptosis is increased in EHBA. Apoptosis results in cell death activation of caspase

protease, and upregulation of Fas ligand (FasL). Injured hepatocytes lead to induction of cytokine expression and subsequent activation of hepatic stellate cells, thus perpetuating inflammation and fibrosis.

Caspase inhibitor constitutes a potential therapeutic modality to interrupt the ongoing bile duct destruction.

-inflammation (high level of serum interferon-inducible protein-10, TNF-alpha correlated with abnormal liver function test results). (18,19)

-fibrosis – occurs through activation of macrophages. It was observed elevation of interleukin-18 (IL-18) in the serum and activation of Kupffer cells.

The antibody therapy against IL-18 may arrest progression of the disease. (19)

Other causes

Disorders of bile acid synthesis are part of the differential diagnosis of EHBA. In fact, bile acids almost certainly contribute to ongoing hepatocellular and bile ductular damage in infant with the disorder. Although associated defects in bile acid metabolism may hasten progression of liver disease, no primary role for bile acids in the development of biliary atresia has been identified.

Table 4: Pathogenesis: factors implicated in the different types of EHBA.

Fetal type	Perinatal type
Genetic alteration	Infection
Ductal plate malformation	Apoptosis and cell necrosis
	Inflammation
	Fibrosis

## CLINICAL PRESENTATION

- Typical symptoms include variable degrees of jaundice, dark urine, and acholic stools, hepatomegaly.
- Most of biliary atresia, most infants are full-term, though a higher incidence of low birth weight may exist; they manifest normal growth and weight gain during the few weeks of life.
- In most cases, acholic stools are not noted at birth but develop over the first few weeks of life. Appetite, growth, and weight may be normal.

Physical findings do not identify biliary atresia. No findings are pathognomonic for the disorder.

*Jaundice* follows neonatal jaundice in perinatal forms, but appears shortly after birth in others. Pathological jaundice should be considered in a patient with hyperbilirubinemia when the conjugated fraction comprised more than about 20% of the total, or greater than 2mg/dl.

Infants with neonatal cholestasis, pose a unique diagnostic challenge of distinguishing between two broad etiologic categories of neonatal hepatitis and extra hepatic biliary obstruction. Early distinction between the two diagnostic categories has important prognostic implications because an appropriate surgical intervention done before 8-10 weeks, significantly improves the outcome of obstructive lesions.

Neonatal cholestasis should be suspected in a child presenting with a history of jaundice at an age of more than 2 weeks with mustard or turmeric colored urine, with or

without other associated histories of pale colored stools, maternal or neonatal morbidity.

Meconium color is normal in most patients. The feces in neonatal period usually are yellowish or light yellowish in most of patients.

*The liver* gradually increases in size and consistency with aging, a characteristic that reflects the duration of bile stasis.

Splenomegaly follows hepatomegaly.

Patients are active; their growth appears normal during the first few months after birth.

Later, anemia, malnutrition, and maldevelopment result because of malabsorption of fat-soluble vitamins; the liver cirrhosis are develops.

Intracranial bleeding caused by vitamin K deficiency is occasionally encountered.

Most patients, who do not undergo surgery, die of hepatic decompensation, esophageal bleeding, or infection.

The 3-year survival rate for children who did not have any drainage procedure was less than 10%. (20)

## DIAGNOSIS

*Antenatal diagnosis* of EHBA is exceptional. EHBA types 1 and 2, which is rare, can be suspected on antenatal ultrasonography scans when a cystic structure is detected in the liver hilum. Postnatal examination has to distinguish the cystic form of EHBA, which requires urgent surgery, from a choledochal cyst for which surgery may be delayed.

Non-visualization of the foetal gallbladder in early pregnancy (14-16 weeks gestation) may be associated with severe foetal anomalies, including polymalformation syndromes, chromosomal aberrations, cystic fibrosis: amniocentesis is recommended for cystic fibrosis screening, hepatic enzymes tests and chromosomal analysis. Gallbladder may be visualized later in pregnancy, suggesting a delay in its recanalization process. When the gallbladder remains undetectable after birth, the possibility that the patient has EHBA has to be carefully investigated. The incidence of agenesis of the gallbladder (without EHBA) is estimated at approximately 1/6000 pregnancy.

Features of polysplenia syndrome may be detected by antenatal ultrasonography. They may be part of a cardiosplenic syndrome whose prognosis depends mainly on the underlying cardiopathy. Interrupted inferior vena cava may be isolated and benign. Neonates with features of polysplenia syndrome should be carefully followed in order to rule out EHBA.

#### Postnatal diagnosis

Pathologic jaundice should be considered in a patient with hyperbilirubinemia when the conjugated fraction makes up more than approximately 20% of the total.

Many factors may cause pathologic jaundice in the neonatal period. A series of blood studies can indicate a diagnosis of infection, hematologic problems, metabolic diseases, or genetic disorders (e.g. alpha-1 antitrypsin deficiency).

Neonatal hepatitis and interlobular biliary hypoplasia (paucity of interlobular bile ducts) are most likely to be confused with biliary atresia.

According to Alagille, clinical features and laboratory data allow differentiation between biliary atresia and neonatal hepatitis in 83% of cases before the age of 3 months. (21)

### CAUSES OF NEONATAL HYPERBILIRUBINEMIA

#### Unconjugated hyperbilirubinemia (Table 5)

##### Hemolytic causes

- *Overproduction* – blood group incompatibility: ABO, Rh
- *RBC membrane defects*
  - Hereditary eliptocytosis
  - Hereditary spherocytosis
  - Hereditary spherocytosis
  - Hereditary poikilocytosis
- *RBC enzyme defects*
  - Glucose 6 phosphate deficiency
  - Pyruvate kinase deficiency
- *Haemoglobinopathies*
  - Alpha thalasemia
  - Delta-beta thalasemia
- *Acquired haemolysis*
  - Vit K3, nitrofurantoin, sulphon- amides, antimalarials, sepsis.

##### Non-hemolytic causes

- *Overproduction*
  - Extravasated blood
  - Cephalhematoma
  - Polycythemia
- *Impaired conjugation*
  - Hypotiroidism
  - Gilbert syndrome
  - Crigler-Najjar syndrome
  - Breast milk jaundice
- *Increased enterohepatic circulation*
  - Pyloric stenosis
  - Intestinal obstruction, ileus

#### Conjugated hyperbilirubinemia (Table 6)

##### Obstructive disorders:

1. Tumor or band
2. Rotor and Dubin Johnson syndrome
3. Paucity of intralobular bile ducts (syndromic = Alagille's syndrome or non-syndromic)
4. Choledochal cyst and pseudocholedochal cyst
5. Inspissated bile syndrome
6. Cystic fibrosis
7. Biliary atresia

##### Non-obstructive disorders:

1. Infective causes: bacterial (syphilis), viral (rubella, CMV, hepatitis, coxsackie, enterovirus), protozoal (toxoplasmosis), idiopathic: Neonatal hepatitis
2. Toxic: Novobiocin; IV hyperalimentation
3. Metabolic defects: galactosemia, alpha 1 antitrypsin deficiency, Tyrosinemia, hereditary fructosemia, hypothyroidism

**PHYSIOLOGICAL JAUNDICE***In term babies:*

- Appears between 30-72 hours of age
- Maximum intensity by 4<sup>th</sup>/ 5<sup>th</sup> day
- Serum bilirubin does not exceed 12 mg%
- Disappears by 7-10 days of age

*In preterm babies:*

- It appears slightly earlier but not before 24 hours of age
- Maximum intensity is by 5<sup>th</sup> or 6<sup>th</sup> day
- Serum bilirubin may go up to 15mg%
- Disappears by 8<sup>th</sup> – 14<sup>th</sup> day

*Factors who accentuated and prolonged physiological jaundice:*

- Immaturity
- Birth asphyxia, acidosis, hypothermia, hypoglycemia
- Drugs like vitamin K, salicylates, sulpha drugs, gentamycin, fructosemide, novobiocin.
- Cephalhematoma and concealed hemorrhage.
- Polycythemia and hypothyroidism
- Intrauterine infections.
- Breast milk jaundice.

*Criteria to exclude the diagnosis of physiological jaundice, proposed by Maisels (1981):*

- Clinical jaundice in first 24 hours of age.
- Total serum bilirubin concentration increasing by more 5 mg/day.
- Total serum bilirubin exceeds 13 mg% in full term and 15 mg% in preterm.
- Direct serum bilirubin more than 1,5-2 mg%.
- Clinical jaundice persisting more than one week in full term and more than two weeks in preterm.

**EVALUATION OF JAUNDICED INFANT****I. History:**

1. Time of detailed history is suggestive of probable etiology. The age of onset of jaundice gives an important clue to diagnosis. The appearance of jaundice on first day of life is suggestive for a serious disease process like hemolytic disease of newborn, intrauterine infections (TORCH), Crigler-Najjar syndrome. Between 24-72 hours of age, the jaundice is usually physiological. If the jaundice appears after 72 hours then one should think of causes like sepsis, neonatal hepatitis, extrahepatic biliary atresia, breast milk jaundice, metabolic causes, hypertrophic pyloric stenosis or

other causes of intestinal obstruction leading to increased enterohepatic circulation.

2. History of clay coloured stools with dark urine is suggestive of cholestatic jaundice.

3. History of delayed passage meconium, with or without vomiting is suggestive of intestinal obstruction.

4. Family history of jaundice in previous sibling in neonatal period is suggestive of ABO, Rh incompatibility.

5. Presence of vomiting with temperature instability and lethargy is suggestive of sepsis.

6. A family history of jaundice, anemia or early gall bladder disease is suggestive of hereditary hemolytic anemia.

**II. Antenatal and obstetric history:**

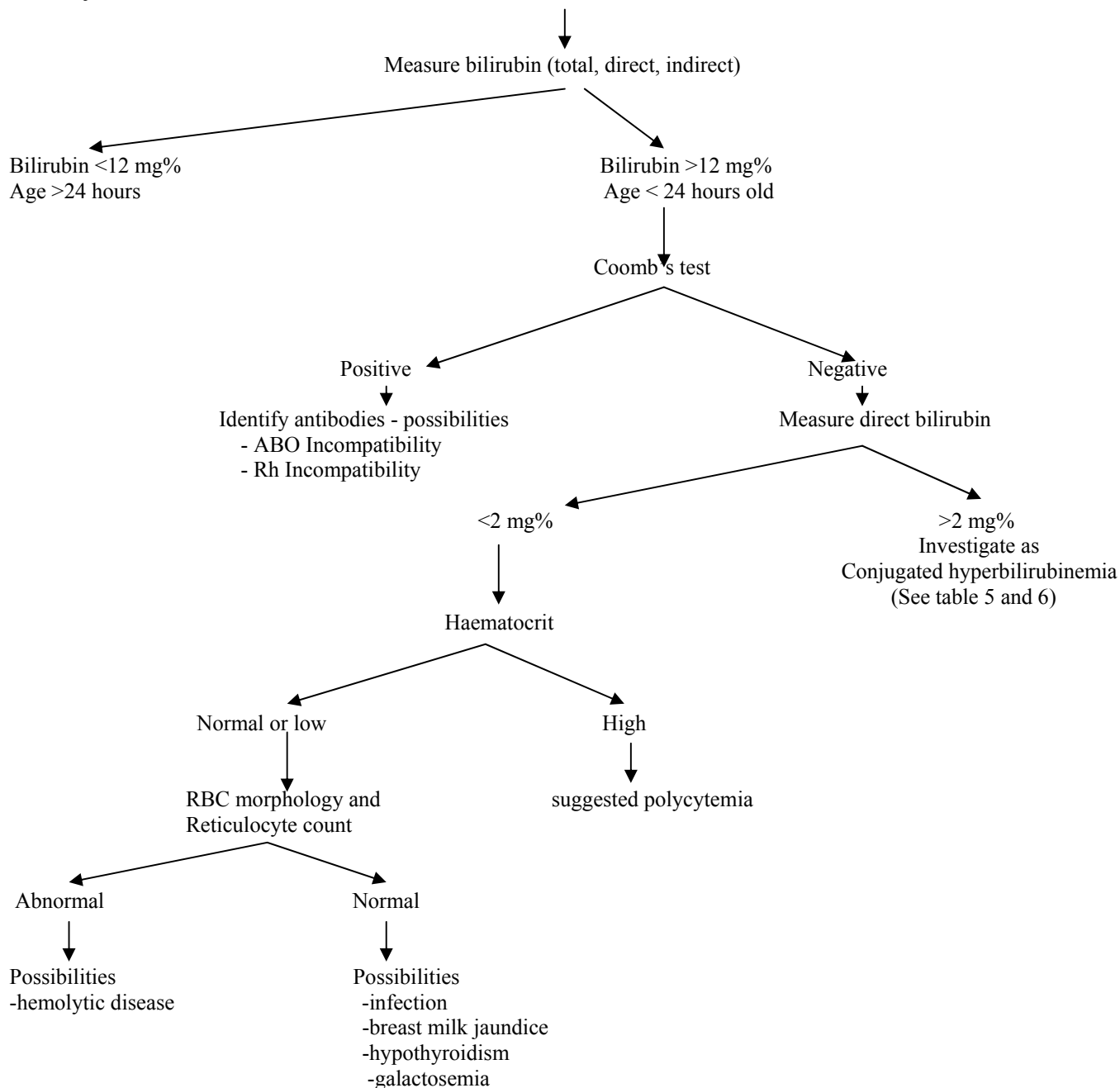
1. antenatal maternal illness may suggested congenital infection (TORCH)
2. maternal diabetes mellitus increased risk of jaundice in infancy
3. maternal drug intake during pregnancy like nitrofurantoin or antimalarials, can lead to hemolysis in G-6 PO4 deficient babies.
4. history of vacuum extraction leads to increased incidence of cephalhaematoma.
5. APGAR score at birth is significant as there are increased chances of neurotoxicity in asphyxiated babies.
6. history of breast feeding might be significant. A distinction should be made between breast milk jaundice in which jaundice is thought to be due to the breast milk itself and breast-feeding jaundice in which low caloric intake may be responsible.

**III. Physical examination:**

1. jaundice is detected by blanching the skin with finger pressure to observe the color of the skin and subcutaneous tissue in day light. Jaundice progresses in cephalocaudal direction.
2. gestational age and birth weight.
3. microcephaly if is present – may suggested intrauterine infections
4. cephalhaematoma – leads to increased bilirubin load
5. pallor is suggestive for anemia
6. plethora is suggestive for polycythemia
7. petechiae and scratch marks may suggest congenital infections, sepsis, severe hemolytic disease.
8. hepatosplenomegaly – congenital infections, hemolytic anemia
9. umbilical hernia with hoarse cry, large tongue and hypotonia – cretinism.
10. optic fundi if shows chorioretinitis is suggestive for congenital infections.

Table7: Protocol for work up

## Clinical jaundice

*Laboratory studies for conjugated hyperbilirubinemia:*

1. serum bilirubin
2. SGOT, SGPT, alkaline phosphatase, GPT, total protein, albumin
3. IgM antibody for TORCH
4. alpha-1 antitrypsin level
5. urine, including determination for reducing substances (for galactosemia)
6. abdominal ultrasound
7. hepatobiliary scan
8. percutaneous needle liver biopsy
9. exploratory laparotomy.

*Clinical findings and examination for diagnosis of biliary atresia:**\* Routine examination:*

Color of stool

Consistency of liver

Conventional liver function tests, including test for gamma-glutamyl transpeptidase

*\* Special examination:*

Special biochemical studies:

-serum lipoprotein-X

-serum bile acid

Confirmation of patency of extrahepatic bile ducts:

- duodenal fluid aspiration
- ultrasonography
- hepatobiliary scintigraphy
- endoscopic retrograde colangiopancreatography
- laparoscopy
- surgical colangiography
- near-infrared spectroscopy

Needle biopsy of the liver for histopathological studies.

## I. Special biochemical studies

### o serum lipoprotein-X:

1. -results of tests for Lp-X are positive in all patients with biliary atresia
2. -results of tests for Lp-X are positive in 20-40% of patients with neonatal hepatitis
3. -absence of Lp-X in the serum excludes biliary atresia.

### o serum bile acids:

- the serum bile acids level increase in infants with cholestatic disease
- is not useful for differentiating biliary atresia from other cholestatic disease
- serum levels of Lp-X greater than 300mg/dl strongly suggest BA.

## II. Confirmation of patency of extrahepatic bile ducts

### a. Duodenal fluid aspiration

#### Protocol:

-intravenous support with 5% dextrose-saline solution (120 ml/kg/day)

-an 8 Fr polyethylene tube (90 cm long) was placed via the nostril in the third portion of the duodenum; its position was verified by abdominal radiography after instillation of 3-ml solution of soluble radiopaque material through the tube;

-the duodenal fluid was collected by gravity (without suctioning) every 2 hours in assay tube;

-to stimulate biliary flow, 5 ml of a 20% magnesium sulfate solution was introduced into the tube every 4 hours, and the proximal end was closed 15 minutes;

-every 2 hours, the pH of the fluid collected was assessed by a semiquantitative test. If the pH was less than 6.5, an abdominal radiograph was taken to verify the correct distal position of the duodenal tube;

-permeability of the collecting tube was maintained by instilling 5 ml sterile water every 4 hours, alternated with magnesium sulfate.

#### Results:

-the test was considered bile positive when a yellow biliary fluid was observed, and the test was concluded at this time.

-when no yellow biliary duodenal fluid was observed, the fluid was collected for another 24 hours and, if negative, was reported as such.

-in the majority of the series reported, the sensitivity (the proportion of patients with BA who were identified by bile negative in the fluid aspiration), was around 97%, the positive predictive value was around 92% and the specificity was higher than 90%

#### Conclusions:

-this test is inexpensive, not highly invasive; trained personnel with few specialized resources may perform it. (22)

### b. Ultrasonography

-is a rapid, non-invasive, relatively inexpensive investigative method and produces images in real time

-if is performed by a well-trained professional, provides excellent results;

-the characteristic sign in EHBA is “triangular cord” which represents a cone-shaped fibrotic mass cranial to the bifurcation of the portal vein. This sign is a simple, time- saving, highly reliable, and definite tool in diagnosis of EHBA, representing a high positive predictive value.

-in 2003, Hee-Jung Lee et al, from the Departments of Diagnostic Radiology and Pediatric Surgery in Donsang Medical Center in Korea was studied and measured the thickness of the echogenic anterior wall of the right portal vein (EARPV), and said that the sole criterion for the TC sign was an EARPV thickness of more than 4 mm on a longitudinal scan. (a thickness of 4 mm was chosen as the upper limit for all normal possible structures that could be possible along the anterior aspect of the right portal vein, including the anterior wall of the right portal vein (1mm), anterior wall of the right hepatic artery (1mm), and the common hepatic duct (1-2mm). (23)

-other observation on the ultrasound images (fig. 2) in EHBA was the gallbladder ghost triad: gallbladder length < 1.9 cm, lack of smooth/complete echogenic mucosal lining with indistinct wall and irregular/lobular contour.



Fig 2: Sonogram illustrates method of measuring gallbladder – images 1, and sonogram reveals tubular echogenic cord – images 2. (24)

-the most frequently used radioisotope is DISIDA Tc99m, which has a very short half-life, low gamma ray emission, very good concentration in the liver, non-conjugated excretion in the bile and a low renal excretion level.

-literature refers to false positive/negative levels of 10%.

-the BRIDA Tc99m isotope is also recommended.

-the DISIDA Tc99m test is not recommended when conjugated bilirubin levels are over 20mg/dl; in such case BRIDA Tc99m should be employed.

-in the normal individuals, the radioisotope is captured by the liver and excreted by the bile ducts to the duodenum.

-the iv administration of the DISIDA Tc99m, should be preceded by the use of Phenobarbital (5mg/kg/day, orally), during 3 to 5 days, aiming at reducing the number of false positive results.

-the immense majority of this test showed the absence of the radioisotope flow to the duodenum, independently on the cause of the cholestasis; the absence of the isotope in the intestine does not indicate with certainty the existence of an extrahepatic biliary obstruction; the presence of the radiotracer in the intestine rule out the hypothesis of EHBA. (25)

#### c. Colangiography

-preoperative colangiography through retrograde laparoscopic or endoscopic via, which are unavailable in most urban centers, have their use quite limited. After the

application of the radiological contrast into the papilla of Vater, we may observe three types of image: (1) bile duct not seen; (2) distal common bile duct and gallbladder seen, no sight of the principal hepatic duct; (3) opacity of the distal common bile duct, gallbladder and principal segment of the hepatic duct, with bile lakes at the porta hepatis.

-colangiography by magnetic resonance imaging.

-operative colangiography, performed after a minilaparotomy, constitutes the last diagnostic method in cases of probable extrahepatic biliary atresia or in those impossible to discard an extrahepatic biliary obstruction.

-in infants with extrahepatic biliary atresia, surgical colangiography is possible to be performed in only 17 to 25% of the cases, due to impossibility of injecting contrast agents through the gallbladder in atresia.

#### d. Hepatic biopsy

-although there are no pathognomonic aspects in the histological analysis of the hepatic material collected by percutaneous or wedge biopsy, the sum of findings provides an important supplement for the formulation of a definitive diagnosis.

-the following were described as findings suggestive of extrahepatic cholestasis: portal duct proliferation, cholestasis in newly formed ducts, pronounced canalicular cholestasis, biliary thrombi in the portal area and accentuated portal and peritubal fibrosis.

-discrete or absent ductile proliferation and the non-existence of portal fibrosis would rule out the possibility of extrahepatic cholestasis

Table 8: Histopathologic differences between idiopathic neonatal hepatitis and extrahepatic biliary obstructions.

Idiopathic neonatal hepatitis	Extrahepatic biliary obstruction
Intracytoplasmic and canalicular cholestasis	Intracytoplasmic and canalicular cholestasis
Disarranged architecture	Preserved architecture
<b>Gigantocellularis transformation</b>	Rare giant cells
<b>Inflammatory infiltrate in the portal space</b>	Discrete inflammatory infiltrate
Little fibrosis	Portal and periportal fibrosis
Extramedullary hematopoiesis	<b>Ductal proliferation</b>
	<b>Biliary thrombus in the interlobular ducts</b>

#### DIAGNOSIS CONFIRMED

Once a EHBA diagnosis has been confirmed, the following conduct is indicated: (1) the Kasai portoenterostomy is the first surgical treatment indicated; (2) liver transplantation is indicated in cases of Kasai portoenterostomy failure; (3) the liver transplant should be delayed by as long as possible, with the intention of allowing for maximum patient growth; (4) the liver transplant should not be performed until the occurrence of severe aggravation of the cholestasis, hepatocellular decompensation or severe portal hypertension; (5) multiple attempts to correct an unsuccessful portoenterostomy are not recommended, since the performance of the transplant becomes more difficult and dangerous.

#### PREOPERATIVE MANAGEMENT

-daily doses of vitamin K (1-2 mg/kg) usually given for several days before surgery;

-prepared the bowel with oral kanamycin in a dose of 50 mg every 8 hours, starting 36 hours before surgery (other authors doesn't recommend the bowel preparation).

-oral feedings are discontinued and saline enemas are given 24 hours before surgery.

-blood is cross-matched and preoperative broad spectrum antibiotics are administered.

#### SURGICAL MANAGEMENT

Many technical variants are possible, according to the anatomical pattern of the biliary remnant.

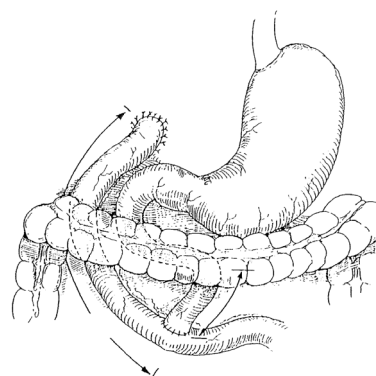
Table 9: Anatomical types of biliary atresia (Bicetre) (1)

French classification	Frequency	Description	Upper level of obstruction of the extrahepatic bile ducts	US/UK/Japan classification
Type 1	3%	Atresia limited to common bile duct	Common bile duct	Type 1
Type 2	6%	Cyst in the liver hilum communicating with dystrophic intrahepatic bile ducts	Hepatic duct	Type 2
Type 3	19%	Gallbladder, cystic duct and common bile duct patent	Porta hepatis	Type 2
Type 4	72%	Complete extrahepatic biliary atresia	Porta hepatis	Type 3

- Type 1 EHBA: *cholecysto-enterostomy*, or *hepatico-enterostomy*.
- Type 2 EHBA: *cysto-enterostomy*. This operation can be performed only if the hilar cyst communicates with the dystrophic intra-hepatic bile ducts (colangiography).
- Type 3 EHBA: *hepato-cholecystostomy*. The patent gallbladder, cystic duct and common bile duct are preserved. The gallbladder is mobilized with preservation of this pedicle. An anastomosis is

performed between the gallbladder and the transected tissue in the porta hepatis. Since there is no direct contact between the porta hepatis and the intestine, this operation reduces the risk of postoperative cholangitis. Its specific complications are however, bile leaks and post-operative biliary ascites due to kinking and obstruction of cystic duct and common bile duct.

- Type 4: *hepatic portoenterostomy* (Kasai's procedure)

Type 4: *hepatic portoenterostomy* (Kasai's procedure)

**Hepatic portojejunostomy (HPE)** is the standard procedure for treatment of noncorrectable type of biliary atresia. In this procedure, the extrahepatic bile ducts are totally removed en bloc and the exposed area of the crude transected surface at the liver hilum is anastomosed to the intestine. If is present, the microscopic biliary structures drain bile into the intestine.

The common bile duct remnant is carefully dissected, because it often adheres to the surrounding tissues. After the common bile duct remnant is severed at the duodenal portion, it is pulled up and the hepatic duct is freed from underlying hepatic arteries and the portal vein, which should be clearly identified and exposed. The hepatic ducts usually transform into a cone-shaped fibrous bile duct remnant that is located cranially to the bifurcation of the portal vein. The separation of the portal bile duct remnants from the right and left portal veins is carefully dissected posteriorly. Some surgeons recommend retraction of the main branches of the portal vein or of the branches of the portal bile duct remnants during portal dissection. The portal bile duct remnants are transected at the level of the posterior

surface of the portal vein, bile sometimes comes out of the transected surface of the bile duct remnants.

After hemostasis is ensured by irrigation with warm saline, the end of the intestine is anastomosed around the transected area at the porta hepatis with full-thickness, interrupted sutures of Maxon 5-0.

A drain is placed in the foramen of Winslow, and the abdominal wound is closed in layers. (27)

There are minimum requirements to achieve *successful results of hepatic portoenterostomy*: early operation within 60 days after birth; adequate operative technique, sufficient wide proper portal dissection with full recognition to the normal anatomy of porta hepatis; precise postoperative management to increase bile flow, to prevent cholangitis, and to maintain good nutritional states; and re-do of hepatic portoenterostomy in indicated cases.

With regard to the *re-do of hepatic portoenterostomy*, favorable results were expected only in cases with active bile excretion after the initial operation. In the era of liver transplantation, the re-do portoenterostomy should be performed only in patients with sudden cessation

of good bile flow after the first operation to avoid excessive adhesion in the abdominal cavity, in patients with favorable hepatic and biliary duct remnant histology at initial operation, who do not successfully drain bile, in patients who may have had an inadequate initial surgery.

## POSTOPERATIVE MANAGEMENT

-patients are placed in an oxygen tent with nasogastric drainage and given intravenous fluids.

-oral feedings is usually possible on the fifth to sixth postoperative day when bowel activity resumes.

-is used medication to avoid postoperative cholangitis:

### ○ Choleraics

-Dehydrocholic acid, 100 mg twice daily i.v. a few months

-Ursodeoxycholic acid, 20-40 mg/kg of body weight per day, orally

-Prednisolone, 10 mg twice daily, i.v., start on the seven postoperative day for 4 days, then switch to oral administration and continue every other day.

### ○ Antibiotics

-intravenous: Cephalosporine, 50-80 mg/kg/day for 1-2 months; Aminoglycoside, 4-8 mg/kg/day for 1-2 weeks.

-oral prophylaxis: Aminobenzyl penicillin, 200-400mg/day for 2-3 months; Cotrimoxazole, half tablet per day.

### ○ Metabolic and nutritional care

-vitamin D supplement

-vitamin E supplement

-essential fatty acid supplement.

## COMPLICATIONS

### I. Cholangitis

-is the most frequent and serious complication after hepatic HPE.

-the incidences are 40% to 60% of cases.

-the cause and pathogenesis of postoperative cholangitis is not entirely clear; it is possible to be do to the reflux of intestinal contents from the draining intestinal loop toward the porta hepatis. Other explanations: portal venous infections, destruction of lymph drainage at the porta hepatis, bacterial translocation.

-clinical manifestation: fever, decreased quantity and quality of bile, and a progressive increase in serum bilirubin levels; laboratory: leukocytosis, elevation of C-reactive protein levels.

-early postoperative cholangitis (within 3 months) is frequently followed by cessation of bile flow, and repeated attacks cause a progressive deterioration of hepatic function.

-in this situation, the patient should be maintained on fluid therapy and should receive heavy coverage with antibiotics and choleraics.

-6 to 9 month after surgery, the incidence of cholangitis decreases.

Modifications for prevention of cholangitis:

-Roux-en-Y biliary construction has been modified by various maneuvers: a long Roux-en-Y limb that is to 50 to 70 cm long, total diversion of biliary conduit, a partially

diverted stoma for decompression of the biliary conduit, intestinal valve formation, and use a physiologic intestinal valve.

-many studies confirm that the Roux-en-Y with a 50-70-cm limb is the better procedure to avoid cholangitis.(28)

### II. Portal hypertension

-is the most serious complication, even in jaundice free survivors.

-the incidence varies from 30% to 75%.

-cholangitis is reported to be a risk factor associated with portal hypertension.

-the presence of esophageal varices should be checked even in patients with good bile drainage.

-variceal bleeding occurs in 20-60% of patients with esophageal varices.

-the initial treatment of severe esophageal varices is endoscopic injection sclerotherapy.

-hypersplenism is another complication in long-term survivors.

-severe thrombocytopenia is found sometimes leads to alimentary tract bleeding.

### III. Other complications

-hepatopulmonary syndrome: characterized by diffuse formation of intrapulmonary arteriovenous shunts, and hepatopulmonary hypertension; occasionally encountered in long-time survivors.

-intrahepatic biliary dilatation, associated with or without stones.

-malabsorption of fatty acids, fat-soluble vitamins and trace minerals.

-pruritus.

-retarded growth/development and delayed puberty.

-malignancies: hepatocarcinoma, hepatoblastoma, cholangiocarcinoma have been described in the cirrhotic livers of patients with EHBA in childhood or adulthood.

## PROGNOSIS

### • Postsurgical prognosis

-the result are different from different centers with initial success of the Kasai portoenterostomy ( for achieving bile flow) ranging from 60-80%.

-one third of patients require early (<2 years) liver transplantation.

-factors that predict improved long term outcome after Kasai portoenterostomy: younger than 10 weeks at operation; preoperative histology and ductal remnant size; absence of portal hypertension, cirrhosis, and associated anomalies; experience of the surgical team; postoperative clearing of jaundice.

### • Liver transplantation

-EHBA is the most common primary diagnosis in children requiring orthotopic liver transplantation.

-65% of infants undergoing the Kasai procedure ultimately required liver transplantation, including more than 50% of patients who initially achieved bile drainage.

-the primary indications for liver transplantation are the symptoms of end-stage liver disease and/or hepatic failure, including progressive cholestasis, recurrent cholangitis, poorly controlled portal hypertension,

intractable ascites, decreased hepatic synthetic function (e.g. hypoalbuminemia, coagulopathy unresponsive to vitamin K), and growth failure.

-long-term outcomes following liver transplantation in children continue to improve.

-with increased living donor availability, using split-liver grafts, application of this surgical modality for early treatment of biliary atresia (certainly, in the face of inadequate bile flow following HPE) will increase.

-some controversy exists regarding whether HPE or liver transplantation is the best initial therapy for extrahepatic biliary atresia. Transplantation certainly has been suggested as the initial procedure of choice, given its excellent long-term survival statistics and the fact that more than 60% of infants undergoing the Kasai procedure ultimately require liver transplantation. The available data indicates that overall survival statistics are not significantly altered by primary transplantation. (29)

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## POLYDACTYLY OF THE HAND AND FOOT CASE REPORT

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### Abstract

Congenital malformations of the limbs are among the most frequent congenital anomalies found in humans, and they preferentially affect the distal part, the hand or foot.

They exhibit a wide spectrum of phenotypic manifestations and may occur as an isolated malformation and as part of a syndrome.

We report a case of both hand and foot polydactyly in a patient from a family where for three generations all males have been affected by anomalies regarding the number of digits on hands or feet.

**Key words:** congenital deformities of the hands, polydactyly, limb malformations.

### Introduction

Polydactyly is one of the most common congenital deformities of the hands. It can occur as an isolated disorder, in association with other malformations of the hands or feet, or as part of a syndrome. It can occur sporadically but it can also be inherited with a mainly autosomal dominant inheritance. (Karaaslan 2003)

Genetic developmental malformations on the limbs, in man, in many instances do not interfere with reproductive fitness, yet they are likely to be recognized and reported by the physician.

A common and conspicuous congenital hand anomaly, polydactyly commonly involves only the hand or the foot. Polydactyly involving both hands and feet is rare. (Hosalkar 1999)

### Case report

Our case is a female, the first born child of healthy non consanguineous parents aged 25 and 28 years. There was no previous history of other pregnancies or abortions for the mother. The patient was born at 40 weeks of gestation with a normal body weight (3100g) and both head circumferences and body length within normal range.

Family history was positive for hand and foot malformations. For three generations the males of the family were affected by both hand and foot polydactyly or just by isolated hand or foot malformations. Both the father of the child and his brother have hand polydactyly but no other associated malformations. A familial pedigree analysis suggested that polydactyly was inherited as an autosomal dominant trait in the family.

Postnatal examination revealed a hand and foot polydactyly with 6 finger bilateral hands and 7 toes at both legs. Other than these findings there were no other malformations or conditions noted after intensive investigation. (Figure 1. A, B).

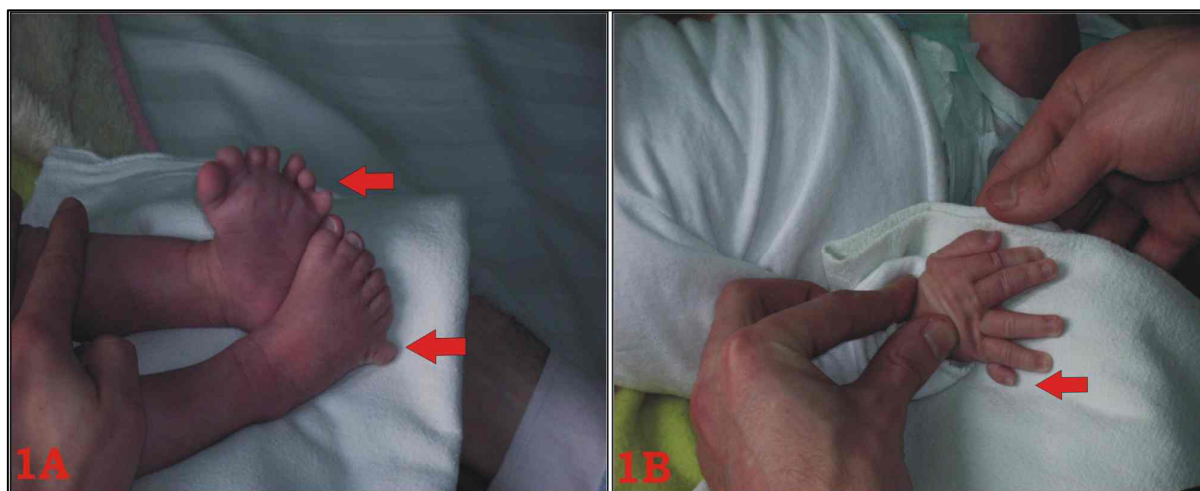


Figure 1. Hand (1B) and Foot polydactyly (1A).

Postnatal X-ray of the patient's hands and feet was performed in order to determine which surgical approach should be considered.

At the level of his hands, the 6<sup>th</sup> finger was composed of two phalange segments of cartilaginous origin without any modifications to be mentioned regarding carps and meta-carps. The postaxial digits in the hands were floating, with no palpable bones or active movements. Further clinico-radiological examination revealed no other congenital anomaly.

### Discussions

The patient described in this report is one of the 6 cases of both hand and feet polydactyly, that we encountered in a retrospective study from 1995 to 2005 conducted at the Children's Hospital "Louis Turcanu", Department of Pediatric Surgery in Timisoara. Out of 64 cases of polydactyly, in 41 cases the hands were affected (13 left, 19 right, 9 both hands), in 17 cases the feet were malformed (4 left, 7 right, 6 both feet) and only in 6 cases both hands and feet were affected.

With regards to the surgical correction of the polydactyly, is almost always indicated, not only for cosmetic improvement but also for better function. Surgical reconstruction generally is performed between 18 months to 5 years.

The presence of extra digits is the most common limb deformity of the human hand and is the consequence of disturbances in the normal program of limb development. However, despite the extensive use of the developing limb as a classical developmental model, the cellular and genetic mechanisms that control the number and identity of the digits are not completely understood. (Talamillo and Bastida 2005).

There are several growth factors that can modify in certain conditions the outcome of the limb development. Fibroblastic growth factors like FGF-2, FGF-4 and FGF-8 are just some of the elements that cause the mesodermal cells to proliferate and expand outward in different directions. FGF8 is unique not only in its expression pattern, but also because it is the only such FGF gene that causes limb skeletal abnormalities, like polydactyly, when individually inactivated. (Lu 2006)

Lu et al. (2006), reported that the increase in FGF signaling that occurs when the FGF4 gain-of-function allele is activated in a wild-type limb bud causes formation of a supernumerary posterior digit, postaxial polydactyly, as well as cutaneous syndactyly between all the digits.

In recent years, increasing knowledge of the molecular basis of embryonic development has significantly enhanced our understanding of congenital limb malformations.

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## V. DENTISTRY

# COMPARATIVE STUDY BETWEEN CONVENTIONAL AND MODERN METHOD OF PROFESSIONAL DENTAL HYGIENE

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### Abstract

**Objective.** The aim of this study is to demonstrate the superiority of one modern dental hygiene technique comparative with conventional methods including manual scaling, ultrasonic scaling and professional toothbrushing.

**Materials and methods.** 94 patients, divided in 3 groups were included in a preventive program for a 2 years period, applying to each group one professional hygiene method every 3 months. Using the following indices: Oral Calculus Index (OCI), plaque index Quigley-Hein modified by Turesky (QH) and the gingival index Loe and Silness (GI) it was evaluated the efficiency of each method.

**Conclusion.** The results indicate that Prophyflex is a good alternative to conventional methods of professional dental hygiene.

**Key words:** plaque control, scaling, modern prophylaxis, Prophyflex

### Introduction

There is no universal or simple way to define what is "health" and what is "disease". Indeed, these very notions may differ according to regions, populations or age categories in the world. Furthermore, our perception of health and disease has changed over the years. Health was previously defined as a "state of physical, mental and social wellbeing and ability to function and not merely the absence of illness or infirmity". Today, health is a relative entity and a healthy person is someone who is able to lead an economically and socially productive life (WHO 1995). Disease is actually defined as a definite deviation from the normal state (American Academy of Periodontology 1992).

Oral health certainly fits within the broader domain of health related quality of life. Quality of life has been profoundly influenced by modern economic development (Feinstein 1993, Govaerts 1995). This applies particularly to industrialized countries where the quality of life of populations is part of economic values and has reached high standards (Diderichsen 1990, Scheuch, 1995). The oral health of a population is influenced by many parameters, including exposure to risk factors, susceptibility to disease and psychosocial and behavioral factors.

Throughout life, all external surfaces of the body are exposed to colonization by a wide range of microorganisms. In general, the establishing microbial flora lives in harmony with the host. Constant renewal of the surfaces by shedding prevents the accumulation of large masses of microorganisms. In the mouth, however, teeth, implants and prosthodontic devices provide hard, non-shedding surfaces for the development of extensive bacterial deposits.

It is well documented that toothbrushing and other oral hygiene procedure can prevent or control gingivitis and periodontal diseases. The caries-preventive effect of daily toothbrushing has been labeled as controversial in earlier reviews (Hine 1948, Andlaw 1978, Bellini et al. 1981). However, several clinical trials in the last two decades have demonstrated that more frequent professional tooth cleaning can produce a dramatic reduction in dental disease, including dental caries. These observations have created new interest in the association between mechanical oral hygiene and caries.

The control of dental plaque is the key factor in preventing gingivitis, periodontitis and dental decay. It is linked directly to the only etiological factor of these diseases: oral pathogenic bacteria which colonize the dental surfaces and forms the dental plaque. Mechanical plaque control by professional tooth cleaning involves removal of supragingival plaque and 1-3 mm of subgingival plaque with the use of a mechanically driven instrument and prophylaxis paste. It may also include the removal of calculus and deep subgingival plaque.

At the workshop in Bethesda in 1986 it was recognized that the need for professional care differs for different individuals and is related to patient attitudes, skills and individual susceptibility to disease (Frandsen, 1986). Hygiene in dentistry has reached today new shapes and dimensions. A delicate issue like the removing of dental plaque, of soft deposits and smoke and coffee discolorations it could be solved with the help of modern methods of professional hygiene. Modern prophylaxis admits the importance of clean dental surfaces and it was born from the necessity of obtaining good results in removing dental plaque and discoloration.

### Material and method

In this study were included 135 patients with age between 6-18, from which were selected 94 patients with calculus deposits and pigmentation of the dental surfaces. The patients were divided in 3 groups and they were included in a preventive program. Prophylaxis included demonstration of oral hygiene technique, professional cleaning of all tooth surfaces and topical application of fluoride. The study was developed over a two years period and the patients were recalled every 3 months.

Prophylaxis also included oral hygiene instruction with emphasis on interproximal cleaning and professional mechanical plaque control including scaling and polishing with abrasive pastes as classic preventive measures and the use of Prophyflex as a modern alternative in prophylaxis. Prophyflex releases a homogeneous water-air-bicarbonate of potassium mixture at the tip of the hand piece just before the emission of the stream. This thing provides the rounding of the crystals of bicarbonate, the mixture being abrasive and also having a gentle action on the dental surfaces and implants of titan on witch they didn't determinate rugs surfaces. (Barnes et.al.1994).

The selection of the patients was made randomly having in mind only the quantity of the calculus deposits evaluated to a clinical examination in the first appointment by using oral calculus index (OCI). Many of the indices used to evaluate the presence of calcified deposits are components of other indices evaluating the oral hygiene status.

The Oral Calculus Index (OCI, Greene & Vermillion 1960, 1964) scores are assigned according to the following criteria:

0- no calculus present

1- supragingival calculus covering not more than one-third of the exposed tooth surface being examined

2- supragingival calculus covering more than one-third but not more than two-thirds of the exposed tooth surface, or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth

3- supragingival calculus covering more than two-third of the exposed tooth surface, or a continuous heavy bank of subgingival calculus around the cervical portion of the tooth

Patients were divided into 3 groups, according to the OCI index, as follows:

Group-patients	OCI
1- 25	2,6-2,9
2- 37	2,1-2,5
3- 32	1,3-1,9

Group 1- 25 patients with massive calculus deposits, present on lingual, facial surfaces-OCI 2,6-2,9

Group 2- 37 patients with calculus deposits present only on lingual surfaces- OCI 2,1-2,5

Group 3 – 32 patients with moderate calculus deposits present on lingual surfaces especially on the interproximal surfaces OCI 1,3-1,9.

In the first appointment without taking in consideration the professional hygiene method applied, it were filled in the dental files to all of the patients registering the dental plaque and gingival indices.

It was used as dental plaque index the Quigley-Hein modified by Turesky plaque index (QH).

This index represents another system for evaluating the occlusal extension of plaque. The labial surfaces of the anterior teeth are divided into four segments.

The amount of plaque is determined with disclosing solution and scores ranging from 0 to 5 are assigned.

The average amount of plaque per tooth surface and per person was then computed. A modification of this Quigley-Hein plaque index was used by Turesky et al. (1970) and included both the facial and lingual surfaces of all teeth.

The score per person is derived by a sum of the plaque scores divided by the number of surfaces examined.

The reason for that we used this index is the fact that it could register the dental plaque to all of the dental surfaces.

For evaluating the status of the gingival tissue we used the gingival index Loe and Silness (GI).

Scaling is the basic procedure by which calculus is removed from the surface of the teeth. Scaling is divided into supragingival and subgingival scaling, depending on the location of the calculus in relation to the gingival margin.

For the supragingival scaling it was been used scalers: sickle, hoe and chisel.

To the first group formed by 25 patients with massive tartaric deposits we calculated the dental plaque indices, witch had the following values:

- 11 patients had score 3,3-3,7 which showed that the facial surface was covered with dental plaque more than 1/3 but less than 2/3 and
- 14 patients had score 4,2-4,8 which meant that more than 2/3 of the facial surface was covered with dental plaque. In order to reveal the dental plaque we used as disclosing agent the Plak –Lite system a more efficient method for observing the dental plaque deposits with the help of a light source which reveals the dental plaque in yellow.

It was proceeded manual scaling and professional tooth brushing with abrasive pastes and after that it was used again a disclosing agent and it was recorded the new value of the dental plaque index.

Table with Quigley-Hein( OH indices modified by Turesky before and after manual scaling

Group 1	patients	OH indices
BEFORE	14	4,2-4,8
	11	3,3-3,7
AFTER	19	2,4-2,9
	6	1,7-1,9

The values of the gingival indices were:

- 17 patients had score 1,8-2,3 moderate inflammation-with erythema, edema, hypertrophy and bleeding on probing,

- 8 patients had score 0,7-1,5 mild inflammation with a slight change in color and structure of the gingival tissue

Group 1	patients	GI indices
BEFORE	17	1,8-2,3
	8	0,7-1,5
AFTER	14	1,3-1,8
	11	0,6-0,9

Group 2	patients	OH indices
BEFORE	29	3,8-4,4
	11	2,8-3,5
AFTER	31	2,1-2,3
	6	1,2-1,6

Group 2	patients	GI indices
BEFORE	20	1,4-1,7
	17	0,9-1,2
AFTER	12	1,1-1,3
	25	0,4-0,6

To the second group formed by 37 patients with calculus deposits we calculated the initial value of the dental plaque index: 29 patients had score 3,8-4,4, 11 had score 2,8-3,5. At this group we carried out professional cleaning by using ultrasonic scaling, followed up by professional tooth brushing and the determination of the new dental plaque index. It was determined for the second group the GI indices before and after the professional cleaning. The values of the gingival index (GI) were the following: before the procedure for 20 patients score 1,4-1,7 and for 17 patients score 0,9-1,2 and after the procedure for 12 patients score 1,1-1,3 and for 25 score 0,4-0,6.

Group 3	patients	OH indices
BEFORE	18	2,5-2,8
	14	1,7-2,1
AFTER	12	0,8-1,2
	20	0,4-0,6

The third group was formed by 32 patients and they were cleaned with the help of Prophylflex. The initially scores of the dental plaque indices were: for 18 patients score 2,5-2,8, for 14 patients score 1,7- 2,1 and after cleaning 12 patients had score 0,8-1,2 and 20 patients had score 0,4-0,6.

The gingival index (GI) had the score 1,1-1,3 for 7 patients and score 0 for 25 patients.

The patients were recalled every 3 months for a period of two years. To every 3 months we proceeded to the evaluation of dental plaque indices and gingival indices and it was initiated a cleaning program; it was individualized to each patient his personal needs for treatment. For that, there were combined the methods that it was used in this study: conventional methods consisting of manual scaling, ultrasonic scaling associated with professional tooth cleaning with modern cleaning using Prophylflex. So, in this second part of the study it was also applied to the first and to

the second group professional cleaning with Prophylflex. The results obtained were similar to the results that were obtained to the third group: score 0 at the dental plaque index. Obviously the maintenance of these results is related to each individual personnel skills and ability to practice every day proper dental care.

### Results and discussion

*The first objective of treatment is to create an environment in which the tissues can return to health. In the sequence of patient treatment, introduction to preventive measures is first, before professional instrumentation. After health has been attained, the patient's self-care on a daily basis is essential to keep the teeth and gingival tissues free from disease caused by the microorganism of bacterial plaque. Professional instrumentation makes a limited contribution to arresting the progression of disease without daily plaque control measures by the patient.*

*General objectives are that dental hygiene instrumentation will:*

- *create an environment in which the tissues can return to health and then be maintained in health*
- *aid in the prevention and control of gingival and periodontal diseases by removal of factors that predispose to the retention of bacterial plaque(dental calculus, irregular and overhanging restorations)*
- *provide the patient with smooth surfaces which are easier to clean and to keep plaque free*

After it was finished the professional cleaning to each patient it was preceded to the registration of the dental plaque indices in order to evaluate the efficiency of the prophylactic method that it was chosen.

To the first group of patients to which we carried out manual scaling, after we used a disclosing substance the value of the dental plaque index was in the most of the cases 2,1-2,4 (for 17 patients) and 1,3-1,5 (for 8 patients). It was observed fine dental plaque and soft deposits to the gingival border. At the next appointment it was calculated the gingival index Silness and Loe (GI) which had a decrease from 1,5- 1,3 to 1,1-0,9 for 12 patients and a decrease from 0,6-0,3 to 0,2-0,1 to 7 patients. 6 patients from this group showed the same values of the gingival index: 2 patients had score 2, 2 patients had score 1 and 2 patients had score 0. These results indicated us that were necessary an improving on the cleaning method that we had chosen and that only the manual scaling was not enough to obtain healthy gingival tissue.

The second group benefited of ultrasonic scaling and professional tooth brushing. It was obtained were the following data: dental plaque indices (OH) decreased from values 1,6-1,8 to values 1,3-1,5. The gingival index of Silness and Loe (GI) also showed a decrease from values 0,4-0,3 to 0,2-0,3. It could be discussed the results to this group in the way that for obtaining best therapeutically improvement and esthetic aspects an additional cleaning method was imperative.

In clinical practice there are frequently situations in which after classic therapeutic measures including manual

scaling, ultrasonic scaling, dental surfaces or restorations showed fine dental plaque tracks or calculus deposits. These remarkable results are due to using Prophyflex which incorporate a unique system of cleaning and we don't have to make confusions with other system which are pulverizing separately the water and the air / abrasive particles.

The third group was cleaned using Prophyflex. The plaque indices decreased obviously (value 0). There were obtained the best results in the case of using Prophyflex fact that it was noticed by using a disclosing agent (Plak-Lite system). When it was used the dental probe to the first two groups it was detected roguishly surfaces (it was a sign that the dental surface was not proper cleaned existing the risk of the reconstruction of the calculus deposits and dental plaque deposits). In the case of using Prophyflex all this inconvenience was non-existent.

### Conclusions

Comparative with the dental hygiene units that are existing on the market in this moment, Prophyflex utilizes this homogeneous mixture which can get access in places witch are not accessible to usual cleaning systems like the interproximal areas, the spaces between brackets and orthodontic ring- without showing loses of composite or cement because of the diffusion of the stream around of this elements.

The efficiency of the Prophyflex system derives from the numerous advantages witch it have by comparing with the conventional methods: it is pain-free, it does not have negative effects on the human body, it does not need special installation (it uses a low pressure 35psi-it permits the adaptation to the dental unit in the place of the turbine), it is small, portable, easy to use, the hand piece is removable, easy to clean, it could be used for bleaching of tooth, it is an alternative to the scaling of the teeth with high sensitive and the time for work is reduce to half.

There were obtained better results concerning dental surfaces which were clean, smooth, bright to the patients that had professional tooth cleaning with the help of

Prophyflex in stead of the patients witch were cleaned by using conventional methods. The use of Prophyflex increased effectiveness of plaque control measures. A smooth surface is easier for the patient to keep clean.

Removal of subsequent calculus deposits is easier because calculus will be less firmly attached to a smooth surface than to a rough one in which the calculus could become embedded in undercuts and other tooth surface irregularities.

The quantification of the results it was made by following the values of the dental plaque and gingival indices before and after we initiated the dental hygiene measures. With regard to the effectiveness of instrumentation, it is apparent that there is little difference in clinical response between manual, sonic and ultrasonic instrumentation. It may be that a technique for scaling and root planing that is instrument-driven, requiring less time and endurance (for both clinician and patient) is more cost-effective.

As a conclusion it is indicate the use of the Prohyflex in the situation in which it must be obtained aesthetic results, but in the case of massive calculus deposits the therapeutically measure is oriented on using manual scaling, ultrasonic scaling and professional cleaning.

Future research into the most cost-effective methods of education and instruction in order to achieve adequate self-care for sustained periods of time would be of value. The development and adequate evaluation of new mechanical plaque control aids which may increase the efficacy of plaque control and in particular enhance interproximal plaque control is warranted. In a report by Fejerskov (1995) dealing with the design of preventive programs, it was concluded that until tests with sufficient predictive power are developed for the identification of groups or individual at higher risk, a population strategy for prevention should be maintained and developed further, with emphasis on oral hygiene as it influences norms and behavior.

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