METHODS TO DIAGNOSE CONGENITAL MALFORMATIONS IN NEWBORNS

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Abstract

Congenital malformations, congenital anomalies and innate defects (present at birth) are synonymous terms used to describe structural, functional or metabolic disorders present at birth. The science that studies the causes of these disorders is called teratology (Greek *teratos* = monster).

As genetic and malformative disorders are very diverse, appear at different ages and affect any system or organ, the patients who suffer of these diseases can be examined by a specialist doctor, all the medical practitioners facing genetic pathology should know some principles of genetic medicine. They also should know and apply the general methodology of genetic examination, should be able to indicate the necessary genetic explorations, as well as to correctly understand and interpret their results and should be able to advice genetically in a correct manner - within his/her area of competence – the patient and/or the family facing a genetic risk.

Major structural anomalies appear in 2-3% in live newborns and other 2-3% are discovered in children up to 5 years old, summarizing 4-6%. Birth defects are the first causes of infantile mortality, accounting for approximately 25% of all neonatal deaths.

Minor anomalies appear in approximately 15% out of the total of newborns. These anomalies do not alter the individual's health status, but they are associated with major defects in some cases, therefore they can serve as key elements for the diagnosis of more serious, hidden defects.

Key words: congenital malformations, genetic examination.

Classifying and defining congenital defects[1]

1. Malformation – anomaly of morphogenesis produced through a primary, intrinsic and precocious process of abnormal development (morphogenesis). The organ does not develop normally (although the tissues are normal) or the differentiation is incomplete (the morphogenesis process doesn't end).

Examples: *spina bifida*, labial/palatine rupture, congenital heart defects, sindactilia.

2. Deformation – shape or position anomaly of a body part produced through the compression and deformation of a region otherwise normally developed both morphologically and structurally (fetopatic). Deformations affect the muscular-skeletal system and lead to the loss of symmetry, altering the alignment, distorting the configuration and the abnormal position of some structures; they are determined by multiplication factors that produce the limitation of the uterine space and/or the inability to move (small /malformed uterus, big fetus, twin pregnancy, oligohydramnios). Unlike malformations and disruptions, deformations are reversible (if compression is seized) spontaneously or after orthopedic maneuvers.

Examples: some skull and face asymmetries, anomalies of the ear, crooked leg, deformations of the members.

3. Disruption – morphogenesis anomaly produced by altering or secondary destruction, extrinsic and late (fetopathy) of a fetal structure otherwise normally developed. The destructive processes produce shape and configuration modifications, unusual diffusions or fusions, loss of component parts. Disruptions are determined by extrinsic agents / ischemia, infections, embrionary forces (amniotic brides) / that destroy normal embrionary structures through compression and/or necrosis (consequence of thrombosis, embolies, compressions). By definition disruption is not genetic, although genetic factors may predispose to disruptive events (for example collagen defects that reduce the resistance of the amnion and make it more vulnerable to spontaneous ruptures). Disruptions usually appear in the form of multiple congenital anomalies.

Example: the absence of fingers or members.

4. Dysplasia – morphogenesis anomaly determined by the abnormal organization and functioning of the cells in a specific tissue. The effects of dysplasia are usually seen in all the body structures in which the tissue is placed. Therefore monogenic mutations appear and they are highly recurrent.

Examples: skeletal disorders, ectodermal dysplasia, connective tissue anomalies.

The diagnosis of congenital defects[2,3]

For a correct and complete diagnosis of the different congenital anomalies, the following steps must be followed:

1. Family background (personal and family's antecedents).
2. Genetic examination and advice.
3. Clinical examination.
4. Paraclinical investigations.
5. Analysis and interpretation of data.

1. Family background

a) Personal antecedents refer to every stage in the individual's life and the history of the disease.

Schematically, we can distinguish:

1. Gestational anamnesis – that will refer to conception, the evolution of the pregnancy and birth.

Conception: reproductive antecedents and the age of the parents, their eventual chronic diseases, blood types, the date of the pregnancy's debut.

The evolution of the pregnancy: diseases and teratogenous exposures in the 1st trimester, the beginning and evolution of fetal movements, the quantity of amniotic liquid and echographical data.

In the anamnesis of the evolution of the pregnancy the emphasis will be on the mother's health or sickness, especially in the first 12 weeks of gestation, on the medication received and the eventual X/ray irradiation. The diseases (anomalies) induced in the next 6 months are usually the result of the action of mechanical factors (ambiguous), of infections or other factors that interfere with the growth of fully structured organs (hydrocephaly, microphthalmia, corioretinitis, malposition of the extremities). From the anamnesis of the anterior pregnancies, the eventual abortions or abortion risks will be considered.

Birth: gestational birth, the duration of labor, presentation, anomalies of the amniotic liquid, umbilical cord and placenta, APGAR score / resuscitation of the newborn, its morphological coordinates (weight, length, skull perimeter).

2. Neonatal anamnesis – it is equally important, as the difficulties in "the adaptation of the child to the extra uterine life" or a series of abnormal manifestations (apnea crises, sucking difficulties, eructation, hypotony, convulsions, cyanosis, and prolonged icter) can signal congenital malformations.

3. Postnatal anamnesis – refers to the evaluation of growth, social and psychomotoric development.

4. The history of the patient's disease: the moment of the debut, manifestations, clinical and paraclinical evaluations, medical care etc.

b) Family antecedents

The family's anamnesis offers a lot of information about the biological and social/legal relations, about the physical and mental state of the individuals in the family, about their reproductive function, which are all important information for establishing: the medical diagnosis, testing strategies, the way the malformation is transmitted, determining the recurrence risk, the identification of the persons with a high genetic risk etc. A history of the family is obtained in a "face to face" interview (with the patient and his/her parents) in a comfortable setting, without disturbing elements, in order to assure the confidentiality.

The family anamnesis will include the mother's and father's age, the eventual consanguinity, the genealogical tree (as detailed as possible) which records al the brothers and sisters, parents and all the ascending and collateral relatives, the sick and healthy ones, too.

2. Genetic consultation and advice

Most frequently, people ask for genetic advice in two situations:

a) if a person or its relatives have a congenital defect, in order to assess the risk of giving birth to sick children:

b) if in a family with healthy parents and one or more sick children there is a recurrence risk and the parents want to know the risk recurrence ratio.

The genetic examination and advice is performed by the genetician who is required to know and discover if the disease (defect) is hereditary or determined after conception (the transmission risk and even the prognosis differ a lot).

This is often difficult because the congenital element (present at birth) is not conclusive, since not all hereditary anomalies are congenital (present at birth) and not all congenital anomalies (present at birth) are hereditary. The familial character of the disease does not show beyond any doubt the genetic cause of the disease. The recurrence of the same disease in a family can be the consequence of the family's exposure to the same environmental factors. A genetic defect may also appear sporadically if it is a new mutation.

Differentiating a hereditary disease from a non-hereditary one can be complicated, as the clinical signs of a non-hereditary disease may be similar with those of a disease resulting from a genetic mutation.

If the disease is hereditary, knowing the dominant or recessive way of disease transmission is of great interest.

If a male individual who showed a pathological recessive feature linked to X marries a normal woman, none of his boys will carry and transmit the feature, but all the girls will carry and transmit it, although the disease will have no manifestations. The sons of such a carrier mother will have the risk of 1:1 to carry and express the abnormal feature (if they marry a normal woman, they will not transmit the disease). All the daughters have the risk of 1:1 of being carriers, like their mothers, but they are apparently normal.

If a congenital anomaly has been caused by environmental factors, the prognosis over the next pregnancy depends upon the possibility of the etiological agent (for example congenital rubeola, toxoplasmosis, X-rays administered therapeutically or teratogenous medicine) to interfere (reject, neutralize).

3. Clinical consultation

The physical examination is extremely important, because it represents, along with the medical history, the basis for a correct and complete clinical diagnosis. It is the first test of a clinician's qualification and it requires:

- optimal conditions (cooperative and relaxed patient, optimal light and temperature and, for a child, the presence of the mother):
- excellent technique, which includes a complete and methodical evaluation, attentive and comprehensive;
- a background of basic knowledge, represented by the knowledge of morphology and the understanding of the normal and abnormal morphogenesis.

a) General examination

The basic elements of the physical examination of a patient are:

- evaluation of vital signs (cardiac frequency, respiratory frequency, blood pressure);

- assessment of the growth parameters (weight, height, skull perimeter), the general habitus of the body and its proportions;

- evaluation of the general health and nutritional status;

- methodic evaluation of the systems and anatomical structures; the order of examination can vary, according to the patient's age and the main problems, but it is essential to perform a complete examination.

b) Dysmorphic examination

The objective is the recognition of the abnormal forms (dysmorphism), and so the identification of minor anomalies which can make a characteristic model.

Sometimes the diagnosis is instantaneous, because many syndromes have a facial stereotype appearance that allows for a rapid recognition, an immediate visual and mental diagnosis (for example Down syndrome, achondroplasia etc.).

The instantaneous recognition requires experience, which is acquired in time and may frequently lead to errors either because of the variable manifestations of the syndromes, or because of the temptation of making a quick diagnosis (before finalizing the evaluation of the patient or ignoring significant clinical data).

The diagnosis is most of the times analytical, based on a complete and detailed clinical evaluation. The shape, size, proportions, position, contour, folds, spacing, and symmetry of all the anatomical elements (whose normal morphology with the described variants must be well known) will be closely observed and described correctly (using the malformative semiology terminology). Special attention will be given to minor anomalies / subtle modifications of structures. The regional inspection of the surface structures will be completed by palpating them and through a series of active maneuvers.

Beside the initial examination (anamnesis, physical examination), psychological support and medical advice will be offered to the couple /the parents. At the end of the evaluation, the doctor has to present in a simple and accessible way a map of the identified problems (anomalies, signs, and symptoms) and a medical evaluation and health care plan (management).

4. Paraclinical investigations

These are focusing on the prenatal and postnatal period (in the last case some are specific to the investigation of certain apparatus and systems).

Prenatal diagnosis

Prenatal diagnosis is a complex, highly informative medical act, which allows for the diagnosis of numerous congenital anomalies and genetic diseases in the fetal life. This is correctly done only through a strong multidisciplinary collaboration, in which the genetician has an essential role in evaluating, diagnosing and eventually giving the genetic advice.

Establishing a prenatal diagnosis has been until recently done at the beginning of the second trimester of pregnancy. Prenatal diagnosis in the first trimester represents a great progress and not only the "last fashion", allowing for and facilitating the diagnosis of chromosomal aberrations, hereditary disease in general, and especially of the hemoglobinopathies. Traditional or modified (modernized) techniques are being used. Direct visualization (fetoscopy), followed by chorion biopsy can be done, nowadays, between the 6^{th} and the 13^{th} gestational week. The chorionic villi suction biopsy can be guided through ultrasonography. The trophoblastic tissue obtained produces analyzable metaphases to determine the karyotype, culture tissue, good material for cytogenetic and biochemical analysis or for DNA extraction. Through specific Y-chromosomal probes the fetal sex can be established. But there are major risks for the fetus: injury or even death through bleeding, infection or tissue damage. The maternal risk appears to be insignificant. If the attempt to obtain tissue samples fails, the second option will be the amniocentesis, but this is more effective in order to assess the neural tube defects. The cytogenetic studies performed in the first trimester of pregnancy allow for the more precise and frequent detection of the chromosomal anomalies that the one made through the analyses of amniotic cells in culture, obtained in the second trimester of pregnancy (this is because many conception products are lost through spontaneous abortion in the first trimester). The higher frequency of miscarriages requires comprehensive studies to differentiate between spontaneous and induced miscarriages. There are many advantages of the prenatal diagnosis in the first trimester of pregnancy, including the optimization of therapeutic effects, the simpler and safer indication of therapeutic abortion, decrease of mother's anxiety etc[4].

There is – in the future – the possibility that more pregnant women can be prenatally investigated through simpler and less harmful procedures.

The selection of pregnant women for establishing the prenatal diagnosis is based on the principle that the risk of a fetal anomaly can be at least equal to the risk of abortion induction by using the procedure of prenatal diagnosis. Over 200 genetic diseases can be diagnosed prenatally today.

Unlike non-invasive, without fetal risk screening methods, applied in a large number of pregnant women to identify pregnancies with high risk of abnormalities, prenatal diagnosing techniques are usually invasive (chorionic villi biopsy, amniocentesis, cordocentesis). This involves the harvesting and analysis of fetal cells to establish – for pregnant women selected on the basis of their high genetic and/or malformative risk – if the fetus is normal or not[5].

Regardless of what procedure is used, prenatal diagnosis techniques must fulfill the following applicability conditions:

- security – depends on the experience of the person applying the procedure and is expressed through the ratio of immediate or late abortions following the procedure;

- accuracy – expressed through the quality of the results;

- quality control – which is done through the use of standardized procedures that refer to the qualification of the personnel, the functioning of the equipments and the accuracy of the results.

The tests have to be performed as soon as possible and the results must be obtained rapidly, so that if the pregnant woman carries an abnormal fetus, she can benefit of selective abortion, possible in the terms established by law[6].

Prenatal diagnosis techniques are:

1. Fetal echography

The echography is the most frequently used method of visualizing the fetus (exceeding the radiography or RMN) as it has no risks for the mother and the fetus. It has been used as a prenatal diagnosis method since 1972, the initial purpose being that of detecting anencephaly. The informative capacity of the echographical investigations grew over the last years, as the apparatus have been improved and also fetal medicine experts have appeared.

The obstetrical information offered by the echography depends on the trimester in which the examination is done.

- in the 1st trimester (usually 10±2 weeks of amenorrhea) the echographical examination establishes the age of the pregnancy and determines the viability.

- in the 2nd trimester (usually 18±2 weeks) the echography allows for the diagnosis of twin pregnancy, assessment of the position of the placenta, gestational age – by measuring the biparietal diameter and the length of the femur, the screening of the anomalies of the fetal structures.

-in the 3rd trimester (usually at 32±34 weeks), the echographical investigations allow for the physician to establish the size and position of the fetus, the growth ratio, the intensity of the fetal movements, the gender, the anomalies of the amniotic liquid, the screening of the anomalies.

At present, in many countries the echography is a routine procedure for every woman in order to assess the fetus morphology and growth.

The sensitivity of the method in establishing the major congenital malformations is of 40-60%, and even higher for some types of anomalies (almost 100% for anencephaly, 85-90% for spina bifida).

The fetal defects that can be detected in the 2nd trimester of pregnancy are:

- anomalies of the nervous system: anencephaly, posterior fossa cyst, encephalocele, facial dysplasia, holoprosencephaly (anomalies of the cerebral ventricles and of the face), hydrocephaly, microcephaly, myelomeningocele, porencephaly (cystic lesions of the brain), rachischisis, *spina bifida*;

- cardiovascular defects: pericardial liquid collections, septal defects, *situs inversus*, valve defects, vascular anomalies, ventricular hyperplasia or hypoplasia;

- thoracic anomalies: esophagus atresia, diaphragmatic hernia, pleural effusions, intrathoracic cysts;

- gastrointestinal malformations: absence of abdominal muscles, ascites, cystic lymphangioma, intestinal atresia, laparoschisis (paraumbilical extrusion of abdominal viscera), mesenteric cysts, omphalocele (umbilical hernia of abdominal viscera);

- urogenital malformations: hydronephrosis, hydroureter, polycystic kidneys, renal atresia, teratomas, urethral valve;

- muscular-skeletal malformations: arthrogryposis, bone dysplasia, crooked leg, fractures, limbs paralysis, limbs reduction, mineralization defects;

- other anomalies: amniotic bride, Siamese twins, teratomas, tumors.

If multiple fetal anomalies are seen, amniocentesis and cytogenetic analysis should be performed. Between 15% and 30% of the fetuses that show echographically morphologic anomalies have chromosomal anomalies. A series of alarming echographic signs are associated with a high risk of chromosomal anomalies:

- the thickening of the nucal fold identified in the 1st trimester of pregnancy is suggestive for Down syndrome;

- the excess of skin on the nape is suggestive for Turner or Down syndrome;

- the big placenta suggests the presence of triploids, fetal hydrops or thalassemia;

- precocious delay of growth appears in the case of trisomy and triploids;

- labial-palatine crests are visible in fetuses with trisomy or triploidy;

Echographical markers suggestive for the presence of fetal anomalies are:

- abdominal calcifications (meconium peritonitis)

- permanently flexed fingers (trisomia 18, arthrogryposis)

- defect of the common trunk, displayed as Fallot teratology or vascular defect (velo-cardio-facial syndrome)

- defects of ossification of the skull (anencephaly)

- thoracic deformations (skeletal dysplasia)

- urinary bladder hypertrophy (urethral valve)

- bone hypodensity (hypophosphatasia)

- cystic hygroma (Turner syndrome)

- facial hypoplasia and palatine rupture (trisomy 13 – holoprosencephaly)

- fractures (osteogenesis imperfecta)

- high number of choroidal cysts (trisomies)

- polydactylia (trisomy 13, Elis Van-Creveld syndrome)

- pterygium colli (Turner syndrome)

- lemon sign – skull in the form of a lemon (spina bifida)

- shortening of long bones (bone dysplasia)

- high volume of cerebral ventricles (hydrocephaly)

2. Amniocentesis

Amniocentesis is the procedure through which a sample of amniotic liquid (AL) is harvested through echographically guided transabdominal punction. AL contains fetal cells that can be either DNA tested to detect the mutations, or cultivated for chromosomal analysis.

Over the last decade the health programmes in some countries confirmed the safety (lack of risks) and accuracy of prenatal diagnosis through amniocentesis. Abdominal

amniocentesis can be routinely practiced in the 16th week of gestation.

Amniocentesis is preceded by a thorough echographical examination to establish the number of fetuses, the conformation and viability of the fetus, the gestational age, the position of the placenta, the approximate volume.

Indications for amniocentesis:

- old age of the mother;
- the existence of a parental chromosomal translocation;
- older child that presented or still presents a chromosomal aberration;
- defects of neural tube to another descendant;
- congenital nephrosis in family antecedents;
- the presence in the family of a hereditary X-linked disease;
- the presence in the family of a metabolic hereditary disease.

The major complication of amniocentesis is the risk of 0.1-1% of inducing abortion, over the main risk of 2-3% for every pregnancy in the 2nd trimester.

Other rare complications are: small leaks of AL, infections, injury to the fetus. Further risks include the respiratory distress of the newborn and other benign orthopedic deformations.

3. Chorionic villi biopsy

It is the method of obtaining fragments of chorionic villi, which are embryonic structures that derive from the trophoblast, the external part of the blastocyst. The harvested samples contain both fetal cells and cells from the maternal decidua, which have to be carefully taken away before the analysis.

4. Cordocentesis

This is the procedure through which a sample of 2-3 ml of fetal blood is obtained, under echographical guidance, from the root of the umbilical cord. This is usually performed at 18-21 weeks of pregnancy in patients that come late for a prenatal diagnosis, when the cultures of amniotic cells give ambiguous results (mosaicism) or when the DNA diagnosis is not possible for a disease that can be identified through biochemical tests of the plasma or of the sanguine cells (some hematological diseases or hereditary immunological ones, bacterial infections, viral or parasite ones). The fetal blood needs only a few culture days for obtaining good quality chromosomal preparations. The abortion risk due to the procedure is of 2-3%.

5. Fetoscopy

The fetoscopy consists of introducing in the uterus, on a transabdominal way, a small and flexible instrument, called fetoscope that allows the visualization of the embryo and of the fetus and the collection of tissue from the conception product (skin, muscle, hepatic tissue). Fetoscopy detects defects and diseases that can not be detected through other invasive techniques (genodermatosis, muscular dystrophies, hepatic diseases).

It has to be practiced in aseptic conditions, with local anesthesia.

The optimal period of harvesting blood is from the 18th to the 20th week of gestation. The prenatal diagnosis of hemoglobinopathies, hemophilia A, Christmas disease, and granulomatous disease is now possible by the analysis of blood samples or fetal serum. Skin biopsy is successfully practiced for the prenatal diagnosis of the harlequin ichthyosis and bullous epidermolysis.

The complete visualization of the fetus allows the prenatal diagnosis of some severe genetic diseases. The optimal period is 16-17 weeks of gestation. The echographic examination is required before fetoscopy.

Among the complications of fetoscopy, evaluated globally at 1-1.7%, the rise of premature birth ratio with 7.2% must be included. However, no orthopedic anomalies or neonatal respiratory distress (at term newborns) determined by the fetoscopy have been reported.

6. The analysis of the fetal cells in the maternal blood

Three types of fetal cells circulate in the maternal blood: lymphocytes, erythroblasts and cells of sincitiotrophoblast. They can be identified and isolated in the maternal blood due to the antigenic differences between the mother and the fetus. Erythroblasts seem to be the most adequate cells for neonatal diagnosis out of the three cell types present in the maternal blood. According to recent data, the number of fetal cells in the maternal blood is higher in the case of pregnancies with fetuses having aneuploidy, especially trisomy 21, which would facilitate the detection of such anomalies[7].

The lab analyses based on fetal cells are:

6.1. Chromosomes analysis

The diagnosis of chromosomal anomalies in the fetus represents the most frequent component of prenatal diagnosis, being indicated in the presence of a structural chromosomal anomaly in one of the parents or if in the family there is a child with a *de novo* chromosomal anomaly, such as pregnant women over 35 with a positive triple test or alarming echographical signs.

Sexual chromatin offers information regarding the number of sexual chromosomes and, through this, valuable data for establishing the genetic gender (XX or XY), and also the numeric gonosome anomalies.

- X chromatin results from the inactivation of an X chromosome at a XX woman who, through heterochromatization, will form the Barr corpuscle in the interphasic nucleus. The number of Barr corpuscles is equal to the sum of X chromosomes minus 1; therefore, the Bar test will be positive in a normal woman and negative for a normal man.
- Y chromatin represents the heterochromatin of the 2/3 distals of the long arm of the Y chromosome, visible in fluorescence under the microscope in the form of the F corpuscle; the number of F corpuscles is, of course, equal to the number of Y chromosomes.

The sexual chromatin test is simple, inexpensive and useful in practice, in well defined clinical situations. An

abnormal test represents a very serious option for a diagnosis, especially in a suggestive clinical context. However, we must add that the final diagnosis is made only through chromosomal analysis.

Determination of X chromatin is the most frequently used (Barr test) in practice, performed with a standardized method and a careful and correct interpretation. An abnormal result (negative Barr test in feminine patients, positive Barr test in masculine patients or the presence of two Barr corpuscles) will be confirmed by repeating the test and by an independent evaluation of another examiner. A delicate problem appears in the situation of certain positive results, but with a small percentage of positive X chromatin cells (under 10-12%); if a technical vice is excluded and the result is the same, a chromosomal analysis is required. Another delicate problem, rarely met, is the presence of a Barr corpuscle of abnormal dimensions, smaller or bigger than 1.5 microns, which can reflect a structural anomaly of Y chromosome: a deletion of X or an isochromosome X. The chromatin test is rarely used, as it is laborious and required UV microscopy.

6.2. Biochemical analyses for metabolic diseases

Over 100 metabolic diseases can be diagnosed through biochemical studies performed on fetal cells or amniotic liquid. Most of them (being recessive autosomally) have a high risk of recurrence, a fact that justifies prenatal diagnosis.

6.3. DNA analysis

Numerous monogenic problems can be diagnosed through DNA analysis, using methods of direct detection of mutations or indirect diagnosis by the study of markers linked to the mutant gene.

We shall further show the main possible ways of paraclinical investigation in the postnatal period, in order to find malformations of the cardio-vascular, digestive, renal-urinary and central nervous systems.

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