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Review article

Prenatal diagnosis - principles of diagnostic procedures and genetic counseling

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Abstract: The frequency of inherited malformations as well as genetic disorders in newborns account for around 3-5%. These frequency is much higher in early stages of pregnancy, because serious malformations and genetic disorders usually lead to spontaneous abortion. Prenatal diagnosis allowed identification of malformations and/or some genetic syndromes in fetuses during the first trimester of pregnancy. Thereafter, taking into account the severity of the disorders the decision should be taken in regard of subsequent course of the pregnancy taking into account a possibilities of treatment, parent's acceptation of a handicapped child but also, in some cases the possibility of termination of the pregnancy. In prenatal testing, both screening and diagnostic procedures are included. Screening procedures such as first and second trimester biochemical and/or ultrasound screening, first trimester combined ultrasound/biochemical screening and integrated screening should be widely offered to pregnant women. However, interpretation of screening results requires awareness of both sensitivity and predictive value of these procedures. In prenatal diagnosis ultrasound/MRI searching as well as genetic procedures are offered to pregnant women. A variety of approaches for genetic prenatal analyses are now available, including preimplantation diagnosis, chorion villi sampling, amniocentesis, fetal blood sampling as well as promising experimental procedures (e.g. fetal cell and DNA isolation from maternal blood). An incredible progress in genetic methods opened new possibilities for valuable genetic diagnosis. Although karyotyping is widely accepted as golden standard, the discussion is ongoing throughout Europe concerning shifting to new genetic techniques which allow obtaining rapid results in prenatal diagnosis of aneuploidy (*e.g.* RAPID-FISH, MLPA, quantitative PCR).

Key words: Prenatal diagnosis - Prenatal screening - Prenatal rapid testing - Prenatal genetic counseling

Introduction

Prenatal diagnosis enables early diagnosis of congenital anomalies and genetic disorders in utero. The population risk of having a child with some congenital abnormality, whether genetically and/or environmentally determined, varies between 3 and 5%. In families at risk of a genetic disorder the probability of having an affected child can exceed several fold the population risk, therefore in these families prenatal diagnostic procedures should be strictly applied [1].

Advanced imagining techniques as well as cytogenetic and molecular biology methods provide the means to diagnose prenatally numerous congenital structural malformations and genetic disorders in high-risk families. Early diagnosis in utero can prove essential to management of the pregnancy, prenatal and postnatal medical care, and treatment. It is also crucial to making informed decisions about continuing or terminating the pregnancy.

Genetic counseling in association with modern prenatal diagnostic procedures constitutes a basic element of prevention of congenital anomalies and genetic disorders. The process of prenatal counseling and diagnosis is committed mainly to transferring information which aims to help the parents to:

1. understand and acknowledge the indications for prenatal diagnosis,
2. understand the medical aspects of making the diagnosis of a genetic disease or a congenital abnormality (by characterizing the disorder, pattern of inheritance, the risk of having an affected child in successive generations),
3. make informed choices about the adequate for a given pathology and acceptable diagnostic scheme (by describing the potential diagnostic methods and procedures, their benefits, limitations and risks).

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The decision about prenatal diagnosis should be made solely by the woman/couple concerned (the principle of informed consent). The genetic counselor serves only as an advisory body (non-directional counseling) for the patient and enables her to consider and assess the advantages and disadvantages of suggested prenatal diagnosis.

However, it is receiving the genetic and clinical diagnosis (the interpretation of the test result) and the consequences of diagnosing a syndrome or fetal anomalies (informed decision about continuing or terminating the pregnancy) that are crucial points in the counseling process.

According to WHO and European Commission's recommendations, prenatal diagnosis should be voluntary and performed only in order to gain knowledge about fetal health status (as described by medical indications). Feasibility of prenatal diagnosis should be equal, fair, and available to anyone, irrespective of the couple's or medical practitioner's attitude towards termination of pregnancy. In case of receiving an abnormal result the decision about termination of the pregnancy should be made independently by the woman or the couple. People making such decisions should not be discriminated against, whatever decision they have made: either terminating the pregnancy or giving birth to a handicapped child [1,2].

Prenatal diagnosis techniques

Methods of prenatal diagnosis can be divided into non-invasive and invasive techniques.

Non-invasive procedures

Used for diagnosing congenital anomalies and risk assessment of given genetic disorders (screening) [3]

- ultrasound:
  - routine obstetric ultrasound scan
  - high-resolution ultrasound scan and Doppler studies
  - fetal heart echocardiography
- magnetic resonance imaging (MRI)
- maternal serum biochemistry testing (measurement of indicative enzymes in maternal blood serum)

Routine obstetric ultrasound scanning. Performed by the obstetrician managing the pregnancy. Standards for normal pregnancies provide for four scans carried out at: 11-14 weeks, 21-26 weeks, 27-32 weeks, and 40 week of gestation (as recommended by the Ministry of Health, 10 July 2003).

High resolution ultrasound scanning. Performed in any pregnancy with an increased risk of fetal structural abnormalities, isolated or part of a genetic syndrome (Table 1). Women are referred for high-resolution ultrasound to specialist centers managing high-risk pregnancies. According to the Ultrasound Section of Polish Gynecological Society's recommendations the first scan should take place at 11-13 (+6 days) weeks of gestation (crown rump length 45-84 mm), followed by another scan at 18-23 weeks of gestation.

In recent years three-dimensional ultrasound (3D) and four-dimensional ultrasound (4D) have started to play an increasing role in prenatal diagnosis. They can be applied in assessing facial features, central nervous system abnormalities and skeletal defects [4].

Doppler studies. Detect abnormal blood flow in umbilical, placental, and fetal vessels that may be suggestive of a genetic syndrome (Table 1).

Fetal heart echocardiography. Performed at 18-23 weeks of gestation in the presence of an increased risk of heart defect (for example: heart defect in a parent or sibling, abnormal routine ultrasound) [5].

Magnetic Resonance Imaging. MRI is used in combination with ultrasound, usually at or after 18 weeks' gestation. MRI provides a tool for examination of fetuses with large or complex anomalies, and visualization of the abnormality in relation to the entire body of the fetus. Apparently MRI is a risk-free method [6].

Biochemistry testing (maternal serum markers) can be applied as a screening technique for every pregnant woman.

Screening in the first trimester involves the measurement of PAPP-A (pregnancy associated plasma protein A) and free -HCG (-human chorionic gonadotropin) levels in maternal serum. These measurements are used in conjunction with ultrasound scanning that includes assessment of ultrasound markers such as nuchal translucency (NT) thickness and absence/presence of the nasal bone (NB). The detection rate (DR) of these combined methods is about 85-90% in regard to trisomy 21 and 18, for a false positive rate of 5%. DR for nuchal translucency alone is 75% for a false positive rate of 5%. Abnormal nuchal translucency thickness measurements can be associated with other disorders such as: heart defects, Beckwith-Wiedemann syndrome, achondroplasia, Smith-Lemli-Opitz syndrome, osteogenesis imperfecta, Noonan syndrome, and with pregnancies complicated by arterial hypertension or gestosis [7].

Recent studies have indicated the possibility of introducing a new biochemical marker for Down and Edwards syndrome: ADAM 12 (A Disintegrin And Metalloprotease 12) that can be used in the first trimester screening. Combining ADAM 12, PAPP-A,

Table 1. Ultrasound markers of fetal congenital abnormalities or genetic syndromes found in: (**A**) first trimester scanning [at 11-13 (+6 days) weeks' gestation], (**B**) second trimester scanning [at 18-24 (+6 days) weeks' gestation].

(A)

- nuchal translucency > 3 mm: trisomy 21, Turner and other syndromes; heart and great vessels defects
- absence of the fetal nasal bone: trisomy 21
- hypoplastic maxilla: about 50% of fetuses with trisomy 21
- abnormal blood flow velocity in the fetal ductus venosus: in 80% of fetuses with trisomy 21
- omphalocoele: trisomy 18
- hypoplastic bladder: trisomies 18 and 13
- single umbilical artery: trisomy 18
- intra-uterine growth trisomy 18 and triploidy, rare in trisomy 13 and Turner syndrome

(B)

- congenital heart defects, ventriculomegaly - trisomies 13, 18, 21; triploidy, Turner syndrome
- echogenic intracardiac focus (EICF) found at 16-20 weeks of gestation; right-handed, bilateral, large, isolated foci in women aged above 35 or in women with abnormal biochemistry tests results – trisomies 13, 18, 21
- holoprosencephaly - trisomies 13 and 18
- choroid plexus cysts > 3mm (found at 14-24 weeks of gestation) in women > 35 years of age, in women with abnormal biochemistry tests results – trisomies 13, 18, 21; in fetuses with other malformations – trisomies 18, 21
- agenesis/hypoplasia of corpus callosum - trisomy 18
- enlarged cisterna magna >10 mm with additional abnormalities found in ultrasound scan – trisomy 18, di George syndrome, Merkel-Gruber syndrome
- Dandy-Walker complex – trisomies 13,18, triploidy
- cleft lip - trisomies 13, 18
- micrognathia - trisomy 18, triploidy
- diaphragmatic hernia – trisomies 13, 18
- encephalocele - trisomy 18, triploidy
- omphalocoele - trisomy 18
- polydactyly/syndactyly - trisomy 13, triploidy
- thickened nuchal fold or nuchal oedema (>5mm at 16-18 weeks of gestation, >6mm at 18-24 weeks of gestation), cystic hygroma – Turner syndrome, trisomies 13, 18, 21, Noonan syndrome, neonatal bone dysplasia, heart defects
- duodenal atresia & echogenic bowel - trisomies 13, 18 , 21, cystic fibrosis, congenital infections, bowel malformations)
- kidney defects - trisomies 13, 18, 21, Turner syndrome
- mild ventriculomegaly (10-15 mm) found at 16-20 weeks of gestation – trisomy 21, central nervous system abnormalities, congenital infections
- short femur/humerus length - trisomy 21, neonatal bone dysplasia
- fifth finger clinodactyly (absence or shortening of the mid-phalanx of the fifth finger) - trisomy 21
- intra-uterine growth retardation - trisomy 18, Turner syndrome, triploidy

β-HCG and NT measurements at 8-9 and 12-13 weeks of gestation increases the DR to 97%, for a false positive rate of 1% [8-10].

Second trimester maternal serum biochemistry (at 14-18 weeks of gestation) involves the "triple", "quadruple" (triple screen and inhibin A) and "integrated" screen.

The "triple" screen is the measurement of alpha-fetoprotein (AFP), free beta human chorionic gonadotropin (β-HCG), free estriol (uE3) levels in maternal serum. The values of these parameters can be influenced by the presence of maternal diabetes type 1, smoking and pregnancy-related weight gain [11]. The detection rate for this test is increased by determining the Ulm index (which eliminates the influence of maternal age on test results). Second trimester maternal serum biochemical testing is carried out as a screening method for Down and Edwards syndrome, open neural tube defects (anencephaly, myomeningocele, omphalocele and gastroschisis). DR for trisomy 21 and 18 is 60-70%, for a false positive rate of 6%. Abnormal second trimester maternal serum biochemical test results are an indication for high-resolution ultrasound in the second and third trimester and/or invasive prenatal diagnosis. Evaluation of the severity and the etiology of the anomaly is an important prognostic factor [12].

Invasive procedures

Invasive procedures involve direct examination of fetal cells or tissues. Classical cytogenetic, molecular and biochemical methods (performed on uncultured or cultured cells) are the most frequently used in prenatal invasive diagnosis. The procedures should take place in specialist centers that manage high-risk pregnancies. When considering invasive methods all indications and criteria need to be carefully evaluated as there is a considerable risk to the pregnancy [13].

Invasive techniques include:

- chorionic villus sampling (trophoblast cells analysis)
- amniocentesis (amniotic fluid cells analysis)
- cordocentesis (Percutaneous Umbilical Blood Sampling)

Chorion villi sampling (CVS) - a sample of the developing placenta is obtained transcervically or transabdominally at 8-11 weeks of gestation under ultrasound guidance. A variety of diagnostic techniques can be employed on CVS cells:

- karyotype analysis (classical and molecular cytogenetic methods) - detects all numerical and many structural chromosome aberrations, including microdeletions that cause syndromes such as Prader-Willi or William's syndrome,
- enzyme studies, for example when there is a risk of inborn errors of metabolism (phenylketonuria, Gaucher disease, mucopolysaccharidosis, haemoglobinopathies such as thalassaemia),
- DNA analyses in monogenic disease (a preferred method for molecular studies).

The risk to the pregnancy is about 2% (most frequently: miscarriage, infection, bleeding, limb defects). The benefit of CVS is an early diagnosis and the chance to verify the results by other invasive methods.

The following problems may arise in CVS:

- placental mosaicism (confined to trophoblast tissue not fetal tissue),
- contamination by maternal tissue [13].

Amniocentesis - can be performed at 13-15 weeks of gestation (early amniocentesis) but usually done at 16-18 weeks of gestation. A sample of about 15 ml of amniotic fluid is obtained transabdominally under ultrasound guidance.

Employed methods of analysis include:

- karyotyping (cytogenetic as well as molecular cytogentic methods)
- DNA analysis (monogenic disease diagnosis, such as congenital adrenal hyperplasia, cystic fibrosis)
- biochemical studies:
  - measurement of AchE and AFP levels when considering neural tube defects
  - measurement of 17α-hydroprogesterone when a risk of congenital adrenal hyperplasia
  - inborn errors of metabolism diagnosis (mucopolysaccharidosis, familial hypercholesterolaemia, adrenoleucodystrophy, homocysteinuria, maple syrup urine disease)

The risk of amniocentesis is around 0.5-1% and it includes miscarriage, transient amniotic fluid leakage and intrauterine infection [13].

Cordocentesis (Percutaneous Umbilical Blood Sampling) - a sample of 0.5-1 ml fetal blood is obtained from the umbilical vein (close to the placenta) usually at 18-23 weeks of gestation under ultrasound guidance. The blood sample can be used for genetic and biochemical studies, including chromosomal analysis and monogenic disease diagnosis (phenylketonuria, cystic fibrosis, Duchenne muscular dystrophy). Furthermore it is also possible to detect haemoglobinopathies, immunological deficiency syndromes (ataxia-teleangiectasia) and intrauterine infections (toxoplasmosis, rubella, cytomegaly).

The risk is estimated to be around 2% with fetal death, premature birth, bleeding (usually transient) and fetal bradycardia (usually short lasting) being the most frequent complications [14].

Optimal diagnostic algorithm

The criteria for prenatal diagnosis classification are presented in Table 2 (low-risk and high-risk pregnancies). The approach to prenatal diagnosis in these groups is aimed at 1) estimating early and accurately the risk of genetic disorder or congenital abnormality in the fetus (genetic counseling), 2) choosing the appropriate diagnostic methods (informed decision).

The optimal time for genetic counseling referral is 10th week of gestation [15]. This is early enough to implement the correct diagnostic scheme: risk evaluation, effective non-invasive procedures, and invasive diagnosis if warranted.

The best approach to prenatal diagnosis in a lower-risk pregnancy (example: high level of anxiety) involves referring the woman for genetic counseling (family history, pedigree analysis), risk evaluation and implementing non-invasive methods such as high-resolution ultrasound scan with nuchal translucency thickness assessment and screening tests: PAPP-A at 11-13(+6) weeks' gestation and/or "triple" test at 14-16 weeks' gestation. Invasive procedures may be implemented in case of abnormal screening results. An individual approach to non-invasive methods can depend on some other factors, for example the kind of congenital abnormality found in the previous child. In case of neural tube defects the algorithm includes:

1. folic acid supplementation (4 mg) preconceptually (at least 3 months) continuing throughout 12 weeks of gestation
2. AFP/AchE measurements in amniotic fluid
   or:
3. "triple" screen of maternal serum at 14-16 weeks of gestation
4. high-resolution ultrasound or MRI.

The classical approach to prenatal diagnosis in high-risk pregnancies (example: high-risk of fetal aneuploidy because of advanced maternal age) includes:

1. ultrasound scan with nuchal translucency thickness evaluation and assessment of the presence/absence of nasal bone at 11-13(+6) weeks' gestation [2,16,17]
2. PAPP-A and β-HCG measurements in maternal serum at 11-13(+6) weeks' gestation [7]
3. amniocentesis at 13-17 weeks' gestation - fetal karyotype analysis [13,18]
4. high-resolution ultrasound scan at 20-24 weeks' gestation [19].

In most clinical situations an individual approach to prenatal diagnosis is necessary depending on indications for prenatal diagnosis, time of gestation at genetic counseling referral and maternal age. For example biochemistry testing is not recommended to women above 42 years of age, as there is a high risk of obtaining false results.

A woman who receives a prenatal diagnosis result that indicates serious fetus abnormality may consider continuing or terminating the pregnancy according to the current state of the law. If the pregnancy is to be

Table 2. The criteria for prenatal diagnosis classification.

Low-risk pregnancy	High-risk pregnancy
Risk of neural tube defects History of two or more miscarriages without the possibility of performing cytogenetic studies in both parents or non-invasive prenatal diagnosis Anxiety of the mother Maternal diabetes type 1 Maternal phenylketonuria	Advanced maternal age (>35) An affected child with chromosomal aberration Balanced chromosomal translocation in one of the parents Monogenic disease carrier status of one of the parents High-risk of X-linked disorders or inborn errors of metabolism Abnormal results of maternal serum biochemistry screening Structural malformations of the fetus found on ultrasound

continued it should be treated as high-risk and appropriate preparations should take place in order to implement treatment in utero if available and provide best possible medical care just after birth.

Genetic prenatal diagnosis

The development of genetic and molecular biology methods has opened up new opportunities in genetic prenatal diagnosis. Standard methods are based on culturing fetal cells and then implementing classical and molecular cytogenetic techniques or molecular methods. It takes on average 1 to 3 weeks to obtain a definitive result, the time depending on the method [20].

The increase in the number of invasive tests requires the introduction of reliable and rapid testing methods in regard to common chromosomal numerical aberrations (chromosome 13, 18, 21, X and Y aneuploidies) as well as rare genetic syndromes/disorders [21].

Currently in Europe there is an on-going discussion about allowing for changes to prenatal diagnosis algorithms because of the introduction of new rapid diagnostic techniques (Rapid Tests - RT), of chosen chromosomal defects. These recommended diagnostic techniques include:

1. Rapid-FISH (rapid fluorescence in situ hybridization),
2. MLPA (multiple ligation PCR amplification)
3. QF-PCR (Quantitative Fluorescent Polymerase Chain Reaction).

These methods of analysis do not require culturing, the amount of the sample material may be very small and the result is obtained in just few days. In comparison, classical cytogenetic analysis (karyotyping) after amniocentesis requires 15-20 ml of amniotic fluid, culturing of fetal cells (amniocytes) and takes around 10 to 21 days to produce the result [22-26].

New diagnostic standards assume performing classical cytogenetic analysis in cases of structural fetal malformations found on ultrasound scan, the presence of a balanced chromosomal translocation in one of the parents or chromosomal defects in previous child. Additionally the use of methods such as FISH, PCR, MLPA or array-CGH (micro array comparative genomic hybridization) is suggested, the latter being especially useful in detecting genomic imbalance in the fetus (duplications/deletions) [26-30].

The introduction of new diagnostic techniques requires changes in current standard procedures. The UK National Screening Committee recommends routine implementation of RT in pregnancies with high risk of aneuploidies (21, 13, 18, X and Y). This approach includes classical cytogenetic analysis only when there are premises to suspect other than mentioned chromosomal aberrations [31-33].

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