Review article

PRENATAL DIAGNOSTICS

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Pregnancy is an exquisite period of life rich in physical and emotional changes. The beginning of new life is exciting not only for future parents but also for the doctor following and supervising the development and growth of a new human being up to its birth after forty weeks of pregnancy. There are many questions, fears and concerns which rise over and over again during this long but also short period of time. However, the consoling truth is that pregnancy has never been as safe as nowadays. Never before in the history of obstetrics have the babies had so many chances to be born alive and healthy. Unnecessary fears can make pregnancy an upsetting event. To prevent it, pregnant woman should be educated and advised on the possibilities of modern prenatal medicine and directed to choose the best ways of prenatal medicine to solve their dilemmas. The aim of this paper was to help pregnant woman and her doctor to find the appropriate treatment in every single case. *Acta Medica Medianae 2008;47(2):58-66.*

***Key words**: prenatal medicine, amniocentesis, ultrasonography*

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Introduction

Prenatal diagnostics includes the diagnostic methods which prove or exclude chromosomal anomalies, a great number of congenital metabolic defects and X-bound hereditary diseases, spine fissures (spina bifida) as well as other morphological abnormalities of a fetus. Congenital defects appear in 3–5% of all newborn infants. In addition, there can be a defect in a single gene or more genes and/or environmental factors (multifactorial occurrence). The greatest number of congenital defects arises regardless of the parents' age. The risk of disease increases with mother's age only when the chromosomal anomalies are found (trisomy 21 – Down Syndrome). The total risk of spontaneous appearance of trisomy is estimated to be between 1:650 and 1:700 births. Besides, this frequency varies depending on mother's age and ranges between 1:40 and 1:2000. Thus, a pregnant woman at the age of 31 carries the risk of getting Down syndrome which is estimated at 1:800, whereas this risk is 1:400 at the age of 35. From this very reason, amniocentesis and chromosomal analysis are advised to pregnant women already after the age of 35. Very rarely, father's age (after the age of 45) influences the appearance of dotted mutations (changes of gene units). (1) The most significant, noninvasive test which is used in prenatal diagnosis is the combined screening ("combined test"), presented by "Fetal Medicine Foundation" (prof. Kypros Nicolaides), London, which is obligatory in all countries of the European Union (with the consent of a pregnant woman). This test is based on the combination of the ultrasonic screening – detecting the "ultrasonic markers', and above all the thickness of the nuchal fold as well as detecting the blood parameters – PAPP-A and "free" ßhCG, after what the level of the risk of Down syndrome appearance can be determined in the current pregnancy. According to the result of combined screening pregnant women are classified in the groups with low, middle and high risk. The classification of the risk means the following: from the group with the high risk there will be 82% of children born with trisomy 21 (T21), from the group with the middle risk there will be 16% of children born with T21 and from the group with the low risk there will be 2% of children born with T21. Therefore, those pregnant women who are classified in the group with the high risk are advised on karyotypisation – the chorionic villus sampling (CVS) and the puncture of the amniotic fluid as well as detecting the fetal karyotype, which stands for the number and appearance of baby's chromosomes. All pregnant women from the latter two groups, with the risk>1:300 (1:250 in CGO CC Kragujevac) in the combined test or if any of the ultrasonic markers are positive, are advised on karyotypisation. Karyotypisation – the chromosomal analysis of an embryo or a fetus is possible due to

the procedures such as the chorionic villus sampling (11^{th} – 14^{th} week of pregnancy) and amniocentesis (16^{th} – 18^{th} week of pregnancy). The most significant risks of these procedures are spontaneous miscarriage during the procedure – the risk is estimated 1–2% during the chorionic villus sampling and 0,3–1% during amniocentesis (2). Injuries of embryos or fetuses practically cannot be seen in these procedures. In amniocentesis, from the amniotic fluid, the levels of AFP and acetylcholinesterase (an enzyme of the nervous system) can be determined additionally and they can indicate the defects of the neural tube (spina bifida).

Indications of the invasive prenatal diagnostics.

- Mother's age > 35 (37)
- father's age > 42 (45),
- chromosomopathies in the family,
- abnormalities of chromosomes in a previously born child or in the parents,
- previously born children with multiple malformations,
- abnormalities found by ultrasound,
- positive combined test and,
- other indications.

Amniocentesis is a procedure in which one, under the control of the ultrasonic probe, enters through the mother's abdomen with a needle and aspires the amniotic fluid for further analysis. Before the puncture, one must perform a detailed ultrasonic survey in which one must determine the position of the baby, as well as of the placenta so that one could avoid impreciseness of the action as well as injuries of the fetus. The amniotic fluid is a liquid in which the fetus is located, so according to that it contains peeled cells of the skin and the fetus's organs. For the analysis of the fetal cells one needs 15 – 20 ml of the amniotic fluid, and the cultivation of fetal cells and karyotypisation (the survey of fetal chromosomes) last three weeks. Amniocentesis provides not only the detecting of the number and appearance of chromosomes and defining the sex of the fetus but it is also the analysis which is used for some other special questions during pregnancy, especially when it is necessary to define maturity of a child or the quantity of bilirubin in the amniotic fluid for Rh differences (3). Similar to amniocentesis, one can perform cordocentesis – the puncture of umbilical vein and cardiocentesis – taking blood from the left ventricle of the fetal heart. During cardiocentesis one must tap at least 1 cm from the place where the umbilical cord is set apart from the placenta and aspire a little quantity of blood from the umbilical vein. These extremely demanding procedures are practiced only for special questions during pregnancy like hereditary hemoglobinopathies, fetal infections, intrauterine transfusion of blood for fetal anemia, immunologic diseases, and for hereditary diseases only when the culture of the amniotic fluid is not successful, that is for mosaicism. The risks during these procedures include spontaneous miscarriage, premature birth, bleeding and infections, and they occur in 1–2% of all punctures.

It is recommended to perform all invasive methods of prenatal diagnostics after the genetic consultation when a pregnant woman must be warned of the possible risks of these procedures: bleeding, infection and the possibility of the spontaneous miscarriage which is 0,5–1%. Invasive prenatal diagnostics has its place in the antenatal protection of a pregnant woman only if the risk of chromosomopathy exceeds the risk of the complications of the very procedure. The indications for the prenatal diagnostics: the analysis of chorionic villi (biopsy of chorionic villi or aspiration of chorionic villi – chorionic villus sampling (CVS) is the oldest invasive method of prenatal diagnostics which can be used between 11^{th} and 12^{th} weeks of pregnancy. This procedure includes the aspiration of the part of placenta tissue (chorion) for the analysis with a thin catheter which is taken into the uterus through the vagina (transcervical approach) or through the abdomen (transabdominal approach) with ultrasonic leading. Chorion villi are fingerlike sprouts of the placenta tissue which are genetically identical to the fetus, and they develop early in pregnancy so that their analysis is possible before the analysis of the amniotic fluid. The cultivation of the chorionic cells (from the aspired tissue) with the method of direct karyotypisaton gives the findings in only three days. With this method, the hereditary metabolic errors can be successfully diagnosed as well as the lack or insufficient function of an enzyme. The typical disorders that can be diagnosed with this method are: cystic fibrosis, hemophilia, thalassemia and other hemoglobinopathies, Huntington's chorea and muscular dystrophies.

The key question which can be asked by a physician who follows the pregnancy, as well as the pregnant woman herself, is to what level is the current pregnancy burdened with the risk of chromosomopathy – disorders of number and structure of fetal chromosomes, above all trisomy (21,18,13), triploidy, Turner's and Klinefelter's syndrome. If the risk of trisomy is estimated only by the age of a pregnant woman (>35) and if it is recommended to tap the amniotic fluid with the chromosomal analysis, one can diagnose only about 30% of all possible trisomies. By introducing 3D mini anomaly scan in the 12^{th} week of pregnancy and by estimating the ultrasonic markers of chromosomopaties: the nuchal fold, nasal bone, maxillary nucleus, the flow through the ductus venosus and the presence of the tricuspidal regurgitation, it is possible to classify the pregnancy into the group with the low, middle or high risk with a high level of precision of 90% and a part of 5% of false positive results. The division of the risk means the following: from the group with the high risk there will be 82% of children born with trisomy 21 (T21), from the group with the middle risk there will be 16% children born with T21 and from the group with the low risk there will be 2% of children born with T21. Therefore, all those pregnant women who are classified in the group with the high risk are advised on karyotypisation

– the chorionic villus sampling (CVS) and the puncture of the amniotic fluid as well as the detecting of fetal karyotype, which stands for the number and appearance of baby's chromosomes. If a pregnant woman is classified into the group with the low or middle risk, it is recommended to perform the combined screening in order to improve secureness in the diagnostics of chromosomopathies. This test is based on the combination of the ultrasonic screening – detecting the "ultrasonic markers", and above all the thickness of the nuchal fold as well as detecting the blood parameters – PAPP-A and "free" ßhCG, after what the level of the risk of Down syndrome appearance is determined in the current pregnancy. All pregnant women from these two groups, with the risk >1:250 in the combined screening or if any of the ultrasonic markers are positive, are advised on karyotypisation. The precision of the combined screening (PAPP-A, free ßhCG, nuchal fold, nasal bone) is estimated 95% with 5% of the false positive results. The triple test appeared much earlier than the combined test, and it allows the comparison of the definite parameters in the blood of a pregnant woman (alpha-fetoprotein, ß-hCG and estriol) and her age with the standard curves as well as the estimation of the individual risk of Down syndrome (trisomy 21 or mongolism). The precision of the test itself is estimated to be 65%. The test is performed a bit latter, usually between the 15^{th} and 18^{th} weeks of pregnancy. If this test is positive, or in the case of those pregnant women from the group with the high risk, the puncture of the amniotic fluid is planned (amniocentesis) and the detecting the fetal karyotype, that is the number and appearance of baby's chromosomes. The sensitivity of the Triple test for other disorders in the number of chromosomes is a bit lower and is about 50%. The Double test (alpha-fetoprotein, free ß-hCG) is a bit more sensitive than the Triple test: with this test one can succeed to find 70 % of the children with the hereditary diseases, with 5 % of the false positive results. Here it must be emphasized that the estimation of the trisomy risk depending on the age or the estimation of the Triple / Double tests results only gives the estimation of the individual risk. The negative Triple or Double test, unfortunately, does not give the complete warranty that the child is healthy (there is still one case of trisomy undiscovered in 1.000 accomplished tests). When the results of the test – if the individual risk of trisomy appearance is 1:200 (in some countries 1:300), in our country 1:250, genetic consultation is recommended and the methods of the prenatal diagnostics must be undertaken. Only the serum markers (Triple/Double test) are not highly sensitive during the discovery of the hereditary diseases and are always used together with the ultrasonic diagnostics. The greatest mistakes of the test appear among the pregnant women older than 35. At this age the level of AFP is naturally reduced and the level of free ß-hCG is raised, that is why the test is very often positive. For the same reason, although these tests are useful for all pregnant women, for the group of pregnant women over 35 with the high risk, they are no longer reliable. The most famous and the oldest method which is used in prenatal diagnostics is the ultrasonic screening. With the ultrasonic survey the fetal length, the diameter of the head, abdomen, femur can usually be measured and thus the growth and development of the fetus can be followed. Within the frame of the antenatal protection – for the sake of following the growth and development of the still unborn child in most European countries, it is commonly recommended to perform three ultrasonic surveys: between the 9^{th} and 12^{th} weeks, then between the 19^{th} and 22^{nd} weeks and between 29^{th} and 32^{nd} weeks (4). If there are any irregularities or complications during pregnancy, an additional ultrasonic survey gives an additional secureness to a pregnant woman as well as to the gynecologist who follows the pregnancy. In the search for the early ultrasonic signs – markers which could point to the increased risk of hereditary diseases or acquired disorders – chromosomopathy of the fetus, the scientific researches confirm the exceptional validity of the ultrasonic finding of the nuchal fold (nuchal transparency). The nuchal fold marks the ultrasonic finding of liquids (lymph) gathered between the skin and subcutaneous fascia in the neck area or between the neck and the back of the embryo, which can be discovered with the ultrasonic survey between the 11^{th} and 14^{th} weeks of pregnancy, that is when the crown – rump length (CRL) is between 45 – 84 mm. It can be usually tolerated when the thickness of the fold is less than 99 percentile for CRL. Many studies show the connection between the findings of this ultrasonic marker (nuchal fold > 3 mm) with the exactly defined chromosomal aberration, and above all with the aneuploidy and Down syndrome. The connection of these findings with Down syndrome is so important that most authors who study this phenomenon classify the ultrasonic survey of the neck fold into the screening procedures for Down syndrome. In the greatest of these studies (King's group) in more than 96.000 pregnancies (22 perinatal centers, 306 gynecologists) this ultrasonic finding is discovered among 82% of fetuses with Down syndrome (the frequency of the false positive results: 8.3%). Besides the connection with the chromosomal aberrations, the findings for the neck fold are also markers for the other genetic syndromes, mostly the heart anomalies. If the measurement of the nuchal fold is simultaneously done with the Double test, in this early stage of pregnancy it is possible to discover almost 90 % of all hereditary diseases. Since 1997 the technology of measurement of the nuchal fold in the 12th week of pregnancy has been performed on all the pregnant women in The Great Britain, and since 1998 this procedure is compulsory in Austria and Slovenia. The ultrasonic survey at the

period between the 11th and 14th weeks of pregnancy recently includes the review, as well as the measuring the nasal bone and maxillary nucleus, and the Doppler reviews of the flow through the ductus venosus as well as the presence of the tricuspidal regurgitation. At this period, the nasal bone cannot be shown for 60 – 70% of fetuses with trisomy 21, and for 2% of chromosomally normal fetuses. It is the similar case with the maxillary nucleus, whose hypoplasia ("short maxilla") can be found in 25% of the fetuses with trisomy 21.

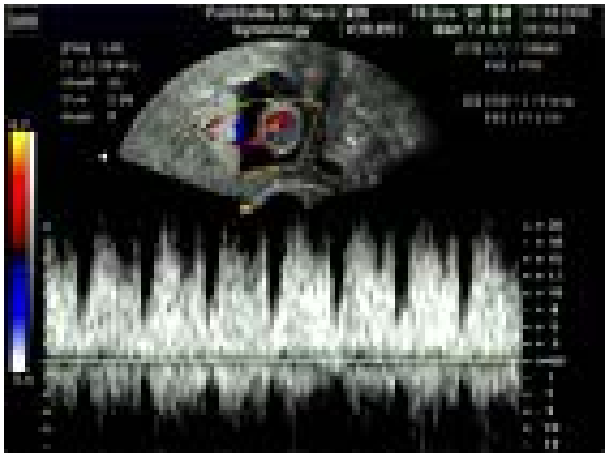

Figure 1. Color Doppler

During the Color Doppler survey between the 11th and 14th weeks of pregnancy, one can discover in 80 % of fetuses with trisomy 21 "zero flow' or "reverse flow" during the atrium contraction in the ductus venosus. The ductus venosus is a blood vessel which connects umbilical vein and the right auricle of the fetal heart, and in the case of trisomy, most probably because of the accompanying heart defects during the contraction of the auricle the flow through this blood vessel cannot be registered or it is negative. Because of that, the survey of the flow through the ductus venosus by using the color Doppler technique, with all described "soft markers" is an integral part of every serious screening of ultrasonic markers of chromosomopathies. During the discovery of the fetal structural malformations, one must carefully examine and follow the morphology – the appearance of the fetus and some fetal organs, and the most perfect time for the morphological examination of the fetus is around the 22nd week of pregnancy (20th – 24th weeks in CGO CC Kragujevac). At this period all vital organs can be seen, and their size allows the precise estimation of their appearance and the discovery of the possible deviations. Doppler sonography (popular term color-Doppler) is an ultrasonic method which can provide the review of changes in the speed of the blood flow through the blood vessels of an organ by using the Doppler effect. The Doppler Effect is considered to be the change in frequency and wavelength of a wave for an observer moving relative to the source of the waves. Practically, we use the change in frequency of the ultrasonic waves, which occurs when they are reflected from the moving blood cells. While the scientists around the world still hesitate about the significance of this method in the sense of screening procedures in gynecology (for example the early discovery of the ovarian cancer) – in obstetrics there are completely clear indications for the necessity of this survey during the pregnancy.

Indications of the color Doppler in pregnancy:

- hypertension (preeclampsia / eclampsia) in the current or previous pregnancies,
- suspicion of the growth stoppage of a child in the current pregnancy,
- low weight of the child born in previous pregnancies,
- intrauterine death of a child in the previous pregnancies,
- abnormalities in the heart frequency of a child
- multiple-prolific pregnancies with discordant growth,
- suspicion of the heart anomalies or cardiovascular system of a child.

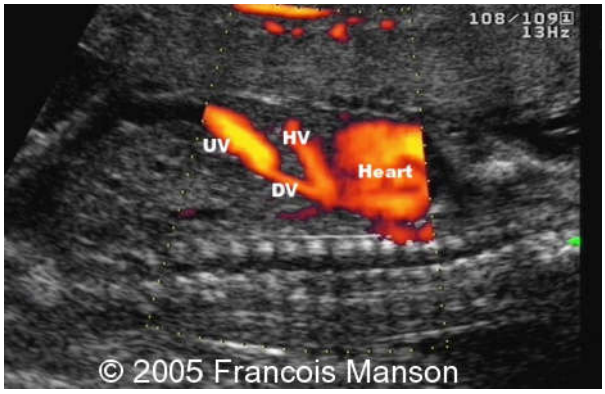

Figure 2.

Ductus venozus – Nuchal fold
UV= umbilical vein, DV= ductus venosus
i HV= hepatic vein Normal finding

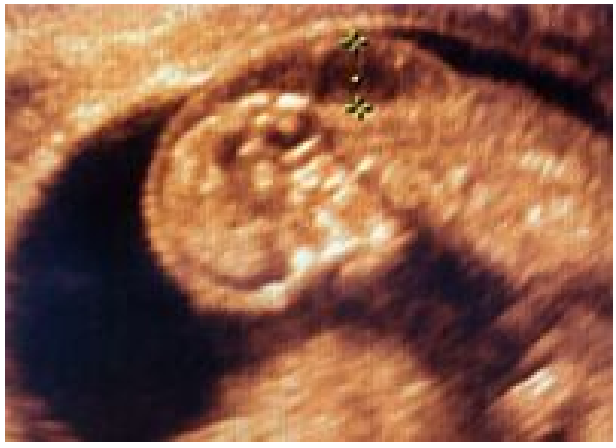

Figure 3.

The correct estimation of the gestational age is one of the most important contributions of the ultrasonic technique in the obstetrics (5). The most significant measures involve the defining of the embryo's crown – rump length – CRL, (Table 1), defining biparietal diameter – BPD, (Table 2), as well as measuring the femur length, (Table 4).

Of these three parameters, defining the embryo's length is the measure which can most precisely assume the birth term.

The values given in this table are based on the following mathematical estimate: GD = [8.052 (CRL) ½ + 23.73] / 7 corrected by adding 1 mm and 3.7 % according to the Robinson and Fleming's recommendation (1975), where GD (gestational age) is given in weeks, and CRL (crown – rump length) is given in millimeters. (Table 1).

Table 1. CRL: crown-rump length (Robinson and Fleming, 1975)

CRL (mm)	GD: gestational age			error (±2 sigma)
	weeks	days	total	
4	6	0	6.0	
6	6	3	6.4	
8	6	6	6.8	
10	7	1	7.2	
12	7	4	7.5	
14	8	0	8.0	4.7 days
16	8	2	8.3	
18	8	4	8.5	
20	8	5	8.8	
22	9	0	9.0	
24	9	2	9.3	
26	9	3	9.5	
28	9	5	9.7	
30	9	6	9.9	
32	10	1	10.1	
34	10	2	10.3	4.7 days
36	10	3	10.5	
38	10	5	10.7	
40	11	0	10.9	
42	11	1	11.1	
44	11	2	11.3	
46	11	3	11.4	
48	11	4	11.6	
50	11	5	11.8	
52	11	6	11.9	4.7 days
54	12	1	12.1	
56	12	2	12.2	
58	12	3	12.4	
60	12	4	12.5	
62	12	5	12.7	
64	12	6	12.8	
66	13	0	12.9	4.7 days
68	13	1	13.3	
70	13	2	13.4	
72	13	3	13.4	
74	13	4	13.5	
76	13	5	13.7	
78	13	6	13.8	
80	14	0	13.9	

The sign BPD is used in the ultrasonic measures as an abbreviation for biparietal diameter and marks the distance between the two parietal bones. The British Society of radiologists recommends Hadlock's table for the estimation of the gestational age according to this parameter. (Table 2).

Table 2. BPD: biparietal diameter (r Hadlock et al, 1982a)*

BPD (mm)	GD: gestational age			error (± 2 sigma)
	weeks	days	weeks	
20	12	2	12.2	
22	12	5	12.8	
24	13	2	13.3	
26	13	6	13.9	
28	14	3	14.5	
30	15	0	15.0	0.8 weeks
32	15	4	15.6	
34	16	1	16.2	
36	16	6	16.8	
38	17	3	17.4	
40	18	0	18.0	
42	18	4	18.6	
44	19	2	19.2	
46	19	6	19.9	
48	20	4	20.5	
50	21	1	21.2	1.4 weeks
52	21	6	21.8	
54	22	4	22.5	
56	23	1	23.2	
58	23	6	23.9	
60	24	4	24.6	
62	25	2	25.3	
64	26	0	26.1	
66	26	6	26.8	
68	27	4	27.6	1.3 weeks
70	28	2	28.3	
72	29	1	29.1	
74	30	0	30.0	
76	30	5	30.8	
78	31	4	31.6	
80	32	3	32.5	2.0 weeks
82	33	2	33.3	
84	34	2	34.2	
86	35	1	35.1	
88	36	0	36.1	
90	37	0	37.0	
92	38	0	38.0	3.6 weeks
94	39	0	39.0	
96	40	0	40.0	
98	41	0	41.0	
100	42	0	42.0	

Table 3. AC: abdominal circumference (Deter et al, 1982)

	GD: gestational age	
AC (mm)	weeks	error in mm (± 2 sigma)
35	10	
46	11	
57	12	
69	13	
80	14	14
92	15	
103	16	
114	17	
126	18	
137	19	15
149	20	
160	21	16
171	22	17
183	23	18
194	24	19
206	25	20
217	26	20
228	27	21
240	28	22
251	29	23
263	30	24
274	31	25
285	32	27
297	33	28
308	34	29
320	35	30
331	36	32
342	37	33
354	38	34
365	39	35
377	40	36

The values given in this table are based on the following mathematical estimate: GD = 6.8954 + 0.26345 (BPD) + 0.000008771 (BPD)3 where GD (gestational age) is given in weeks, and CRL (crown – rump length) is given in millimeters. The sign AC is used in the ultrasonic measures as an abbreviation for abdominal circumference and it marks the circumference of the abdomen (Table 3). The British Society of Radiologists recommends Deter and collaborators' table for the estimation of the gestational age according to this parameter.

The values given in this table are based on the following mathematical estimate: AC = -56.582 + 11.402 (GA - 2), where AC (abdominal circumference) is given in millimeters, and GD (gestational age) is given in weeks of pregnancy. The sign FL is used in the ultrasonic measures as an abbreviation for femur length and it marks the length of the mentioned bone (6.7).

The values given in this table are based on the following mathematical estimate: logeGD = 2.35301 + 0.023185 (FL) - 0.00007804 (FL)2, where GD (gestational age) is given in weeks, and FL (femur length) is given in millimeters.

Table 4. FL: femur length *(r Warda et al, 1985)

FL (mm)	GD: gestational age			error (± 2 sigma)
	weeks	days	total	
10	13	1	13.2	
12	13	5	13.7	
14	14	2	14.3	
16	15	0	14.9	
18	15	4	15.6	
20	16	1	16.2	0.8 weeks
22	16	6	16.9	
24	17	4	17.5	
26	18	2	18.2	
28	19	0	18.9	
30	19	5	19.7	
32	20	3	20.4	
34	21	1	21.1	
36	21	6	21.9	
38	22	5	22.7	
40	23	3	23.5	1.4 weeks
42	24	2	24.3	
44	25	1	25.1	
46	25	6	25.9	
48	26	5	26.7	
50	27	4	27.6	
52	28	3	28.4	
54	29	2	29.3	
56	30	1	30.2	
58	31	0	31.0	1.3 weeks
60	31	6	31.9	
62	32	6	32.8	
64	33	5	33.7	
66	34	4	34.6	
68	35	3	35.5	
70	36	3	36.4	
72	37	2	37.3	2.0 weeks
74	38	1	38.1	
76	39	0	39.0	
78	39	6	39.9	
80	40	6	40.8	

The American Association of Gynecologists - The American College of Obstetrics and Gynecology (ACOG) recommends the prenatal screening for

Down syndrome for all pregnant women, regardless of their age, as it was declared in their directives in the January issue of the scientific magazine Obstetrics and Gynecology.

"Historically observed, the maternal age of 35 or older was used for the recognition of those women with the greatest risk of having the baby with Down syndrome, so these women were automatically offered genetic counseling and amniocentesis or chorionic villus sampling," as it is quoted in the recommendations of ACOG Committee on Genetics and The Society for Maternal-Fetal Medicine Publications Committee (8). "Biochemical tests for Down syndrome for the women younger than 35 were put into practice in 1984, when the connection between the low level of alpha-fetoprotein (AFT) in the mother's serum and the appearance of Down syndrome was noticed. Only then was the former practice of the use of the age for determining pregnant women who will be offered screening or the invasive diagnostic test changed." The current directives have been developed in order to show and estimate the justification of the use of ultrasonic and serum markers in the selection of the tests for aneuploidy in pregnancy, and to provide the practical recommendations for screnning Down syndrome in the clinical practice. During the recent years numerous markers and strategies of searching for Down syndrome in pregnancy, as well as the algorithms of the combined use of ultrasound and serum markers in the first and second trimester of pregnancy, have been described. Different serum markers have included the human chorionic gonadotrophin (hCG) and unconjugated estriol in the combination with defining the height of the alpha-fetoprotein in mother's serum. The use of all three markers ("triple test") shows the rate of discovering Down syndrome of approximately 70 %, where the frequency of the positive results is approximately 5% in all pregnancies. Simultaneous measuring inhibin A with the triple test ("quadruple test") improves the level of detection of Down syndrome in approximately 80 %. "Biochemical tests, ultrasonic tests or both, are gradually widened to the whole population of pregnant women in order to provide as more precise estimation of the individual risk for Down syndrome" say the authors. "Greater sensitivity, and according to that greater level of the correctness of the diagnostics (what is defined by the percent of the children with Down syndrome out of the total number of the positive results of the test) with a small number of false positive results, has caused the increase of the use of the non-invasive screening tests and the reduction of the number of the accomplished amniocenteses." "The other way of survey is measuring the nuchal fold – the early joined finding in the case of the wide spectrum of the fetal chromosomal, genetic and structural abnormalities. The directives for the systematic measuring of the nuchal fold are standardized, and they improve the level of the precision in discovering Down syndrome. For the use of the screening programme which includes the measuring the nuchal fold, it is recommended to perform a specialized training, standard methods of measuring and regular control of the technique. The important shift in the screening tests of Down syndrome in the first trimester is achieved by the combined test – showing the measures of the nuchal fold in the MoM values ("multiple of the median") and in combination with the detecting the free subunit of the ß-hCG hormone ("free ß-hCG") and PAPP-A protein ("pregnancy-associated plasma protein A"). The detecting of PAPP-A and hCG or free ß-hCG in mother's serum is a significant test in the first trimester, while it is more effective to detect the level of alpha-fetoprotein, unconjugated estriol and inhibin A in the mid-trimester using the biochemical test. Given results are considered to be good scientific proofs ("evidence-based medicine": level A), and according to them we can give the following recommendations:

- The use of the combined test – measuring the nuchal fold as well as detecting the biochemical markers represents the reliable test for the discovery of Down syndrome in the whole population. This strategy shows a greater level for the discovery of Down syndrome than it can be provided by the triple and quadruple tests in the mid-trimester of pregnancy, whereas the frequency of the false positive results of the test is equal.
- Searching for the Down syndrome in the first trimester which includes only the ultrasonic measuring the nuchal fold is less reliable than the combined test: measuring the nuchal fold and detecting the biochemical markers.
- If the risk for the appearance of the aneuoploidy is higher according to the tests in the first trimester, the pregnant woman should be offered genetic counseling and the option of chorionic villus sampling or mid-trimester amniocentesis.
- Measuring the nuchal fold during the survey of Down syndrome should be limited to the institutions and physicians who fulfill the following criteria: specific training, use of standardized technique for the measuring the nuchal fold and appropriate ultrasound equipment and ongoing quality assessment.
- Those pregnant women who elect only first-trimester screening should be offered the ultrasonic screening for the defects of the neural tube in the mid-trimester.

Table 5. Ultrasonic markers: Trisomy 21

Structural anomalies - "Hard markers"	**"Soft markers"**
• Hypoplasia / aplasia of the nasal bone (60-70%) • Ventriculomegalia (6-8%) • Cystic hygroma • Hypoplasia of maxillae (25%) • Esophageal atresia • Duodenal atresia ("Double bubble") • Heart defects: AVSD, Tetralogia Fallot (40-50% CHD) • Abnormal Doppler flow in Ductus venosus (80%) • Clinodactilia and/or hypoplasia of the middle phalanx of the fifth digit • "Sandal gap"	• Increased nuchal fold - 2.5 mm above the median for CRL (42-83%) • Hyperechogene intestine (T18) • Pyelectasia (T18, T13) • "Short limbs": length of femur and humerus < 2 SD for the gestational age • "Cardiac soft marker": o echogenic focus - "white spot" (16-18%) (T13) o perikardial effusion o tricuspidal regurgitation o linear insertion AV valvula o narrow "LV/RV width" ("narrow left ventricle") o "ARSA": aberrant right subclavian

Table 6. Ultrasonic markers: Trisomy 18

Structural anomalies - "Hard markers"	"Soft markers"
• "Strawberry shaped head" - dolichocephalia • Corpus callosum agenesia (T13) • Ventriculomegalia • Megacisterna magna, Dandy Walker complex • Hypoplasia / aplasia of the nasal bone (55%) • Micrognathia (T13, triploidia) • Cheiloshisis (T13) • Esophageal atresia • Diaphragm hernia (T13) • Exomphalos (T13) • Heart defects: AVSD, "overriding aorta"; DORV, LOTO (coarctatio aortae) • Kidney's malformations • Megacystis (7-15 mm) (T13) • "Overlapping fingers" - "clenched hand" • "Rocker bottom feet" • Pes equinovarus (T13, triploidia)	• Increased nuchal fold - 2.5 mm above the median for CRL (42-83%) • Cista plexusa chorioideus • Hyperechogene intestine (T21) • Pyelectasia (T21, T13) • Single umbilical artery (75%)
Other signs	
• hard IUGR "early onset" • Bradycardia	

Table 7. Ultrasonic markers: Trisomy 13

Structural markers	"Soft markers"
• Holoprosencephalia • Corpus callosum agenesia (T18) • Ventriculomegalia • Hypoplasia / aplasia of the nasal bone (30%) • Micrognathia (T18, triploidia) • Cheiloshisis (T18) • Diaphragm hernia (T18) • Exomphalos (T18) • Heart defects: LOTO (coarctatio aortae), HLHS (Turner) • Kidney's malformations • Megacystis (7-15 mm) (T18) • Polydactilia • Pes equinovarus (T18, triploidia)	• Increased nuchal fold - 2.5 mm above the median for CRL • Intracardial echogenic focus - "white spot" (T21) • Pyelectasia (T21, T18)
Other signs	
• mild IUGR "early onset" (Turner) • Tachycardia • Polyhydramnion	

Table 8. Ultrasonic markesr: Turner 45 XO

Structural anomalies	"Soft markers"
• Generalized hydrops • Cystic higrom • Mild pleural effusion and ascites • Heart defects: LOTO (coarctatio aortae); HLHS (T13) • Kidney's malformations - "horseshoe kidneys"	• the median for CRL • Pyelectasia • "Short limbs": length of femur and humeru < 2SD for gestational age
Other signs:	
• mild IUGR "early onset" (T13) • Tachycardia	

Table 9. Ultrasonic markers: Triploidia

Structural anomalies	"Soft markers"
• Ventriculomegalia • Micrognathia (T18, T13) • Heart defects • Kidney's malformation • Syndactilia • Pes equinovarus • "Hitch hiker toe"	• "Short limbs": length of femur and humerus < 2SD for gestational age
Other signs:	
• Hard asymmetric IUGR • Bradycardia • Oligohydramnion	

Conclusion

No matter what screening test you decide to advise to your patients, it is necessary to give all information about the precision, false positive results, advantages, disadvantages and limitations of the test, as well as about the risks and benefits of the diagnostic procedure. Pregnant women can refuse the survey of Down syndrome because they do not want to use the information for their decision or they want to avoid the possibility of having the false positive results. The result of the screening test depends on many factors, including the gestational age at the time of the first survey, number of fetuses, courses of the previous pregnancies, family anamnesis, the possibility of measuring the nuchal fold, sensitivity and limitations of the tests, risk of the invasive diagnostic procedures, need for the early results of the test and the opportunity to stop a pregnancy at an early stage.

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PRENATALNA DIJAGNOSTIKA

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Ni jedan drugi period života nije tako bogat, ne samo telesnim već i emocionalnim promenama kao što je to trudnoća. Sam nastanak novog života podjednako je uzbudljiv, ne samo za buduće roditelje već i za lekara koji će pratiti i nadgledati razvitak i rast novog bića sve do njegovog rođenja nakon 40 nedelja trudnoće. Mnogobrojna su pitanja, strepnje i strahovi koji se tokom ovog istovremeno dugog i kratkog perioda ponovo i ponovo stvaraju. Ipak je umirujuća istina da trudnoća nikada nije bila tako sigurna. Nikad ranije u istoriji akušerstva bebe nisu imale toliko izgleda da se rode žive i zdrave. Nepotrebni strahovi mogu učiniti trudnoću uznemirujućim događajem. Upravo zato, trudnicu treba savetovati i edukovati, predočiti joj mogućnosti savremene prenatalne medicine, usmeriti da pravilnim odabirom najcelishodnije reši sopstavne dileme. Cilj ovog rada bio je da svojim sadržajem pomogne kako trudnici tako i ordinirajućem lekaru u pronalaženju ispravnih postupaka u svakom pojedinačnom slučaju. *Acta Medica Medianae 2008; 47(2):58-66.*

Ključne reči : *prenatalna medicina, amniocenteza, ultrasonografija*