Chapter 3

Prenatal Screening and Diagnosis

Jaana Marttala

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52861

1. Introduction

1.1. Maternal age

In recent years the prevalence of Down syndrome has been increasing. The increase in the prevalence might be partly explained by better compilation of statistics on Down syndrome today. Also, the mean maternal age at first delivery as well as the proportion of older mothers is increasing in all western countries and the risk of Down syndrome increases with advancing maternal age [1]. The proportion of mothers aged 35 years or older in France, Finland, Germany, Greece and United Kingdom were 15.8 %, 19.0 %, 17.0 %, 14.2 % and 17.2 % in 2001, respectively, in 2008 the proportions were 18.9 %, 18.2 %, 21.8 %, 20.9 % and 20.1 %, respectively (Eurostat). Screening for Down syndrome was first performed in 1970's using advanced maternal age or previous history of chromosomal abnormality. The prevalence of Down syndrome at term rises from 1/1527 at the maternal age of 20 years to 1/895 at age 30 and to 1/97 at age 40 [11]. Also the gestational age affects the prevalence of Down syndrome. The estimated rate of fetal loss in Down syndrome pregnancies is 43 % between gestational week 10 and term, 23 % between gestational week 15 and term and 12 % of births are stillbirths or result in a neonatal death [12]. Therefore, the risk of Down syndrome decreases as the pregnancy progresses. Table 1 presents the prevalence of Down syndrome pregnancies in different maternal age groups according to the gestational age.

Maternal age of 35 years or more used as a screening method can detect approximately 43-61 % of Down syndrome cases [13, 14]. However, the false positive rate (FPR) is high since the proportion of women aged 35 years or older is approximately 20 % in western countries. Chorionic villus sampling (CVS) and amniocentesis (AC) carry a 0.5-1.0 % risk of fetal loss [15]. Maternal age of 35 is an arbitrary threshold and there are better screening methods available today. The invasive test should not be offered only because of increased maternal age.

Maternal age (years)	Gestational age (weeks)					
10	12	14	16	20	40	
20	1/983	1/1068	1/1140	1/1200	1/1295	1/1527
25	1/870	1/946	1/1009	1/1062	1/1147	1/1352
30	1/576	1/626	1/668	1/703	1/759	1/895
35	1/229	1/249	1/266	1/280	1/302	1/356
40	1/62	1/68	1/72	1/76	1/82	1/97
45	1/15	1/16	1/17	1/18	1/19	1/23

Table 1. The prevalence of Down syndrome according to maternal age and gestational age. (Modified from Snijders *et al.* 1999).

1.2. Second trimester screening

Abnormal levels of specific maternal serum markers were associated with Down syndrome in 1980's. Second trimester screening with maternal age and maternal serum markers was developed consisting of either double, triple or quadruple serum screening. Optimal window for second trimester serum screening is between 15 and 22 weeks of gestation. Double test includes maternal age, maternal serum free beta human chorionic gonadotropin (fβ-hCG) and alfafetoprotein (AFP). The additional serum markers are unconjugated oestriol (uE3) in triple screening and uE3 and inhibin-A in quadruple screening. The estimated FPRs for an 85 % detection rate (DR) for double, triple and quadruple screening are 13.1 %, 9.3-14 % and 6.2-7.3 %, respectively [9, 16]. For a 5 % FPR the DRs for double, triple and quadruple screening are approximately 59 %, 63 % and 72 %, respectively.

2. Screening for Down syndrome today

Screening for Down syndrome has moved from second trimester to first trimester during the last two decades. The most popular screening method today is combined first trimester screening where maternal serum biomarkers fβ-hCG and pregnancy associated plasma protein-A (PAPP-A) are used in combination with fetal nuchal translucency (NT) measurement, ultrasound dated gestational age and maternal age to calculate a woman's risk for Down syndrome using a computer based program. The serum markers and NT do not correlate with each other in chromosomally normal or abnormal fetuses [17]. Each screened woman has a priori risk which is based on her age and the gestational age. The risk calculation software program uses the Gaussian distributions of NT and serum values of normal and affected cases to calculate the LRs. These are described by their means of $\log_{10}$ MoMs, standard deviations and correlation coefficients between the markers. The screening test performs well if the Gaussian distributions of the markers in the normal and affected populations are separated. Alternatively, the screening test is impractical if the distributions overlap widely.

http://dx.doi.org/10.5772/52861

The median MoMs and standard deviations in the populations influence the degree of the overlap. A patient-specific risk for each screened woman is calculated by multiplying the a priori risk based on maternal age with the LR [18-21].

Maternal serum biochemistry reflects the degree of maturity of the placenta rather than directly measuring the presence or absence of Down syndrome. These markers have also limitations, such as the relatively narrow gestational window in which they can be used. In pregnancies that are affected by fetal chromosomal abnormalities the placental function is impaired and the levels of fβ-hCG and PAPP-A differ from normal pregnancies. The results of the maternal serum biochemistry are reported as multiples of the median (MoM) specific to the gestational week. MoM values are calculated by dividing a woman's marker level by the median level of that marker for the entire population at that gestational age in each laboratory. The use of MoM values therefore also allows the interpretation between the results from different laboratories in different countries. The expected levels of maternal serum markers are not only affected by maternal age and gestational age but also other factors like maternal weight, ethnic origin, the presence of insulin dependent diabetes mellitus, multiple pregnancy, smoking and vaginal bleeding. Screening program takes into account certain variables.

2.1. Screening markers

Human chorionic gonadotropin (hCG) was first purified from the pregnant women's urine. hCG is produced by the trophoblastic cells of the placenta from the 10^{th} to 12^{th} day after conception and it reaches its peak value in maternal circulation at 8 to 10 weeks of gestation. Then, a rapid decrease is seen and a plateau is reached at 20^{th} week of gestation [22]. hCG was first used as a second trimester screening marker for Down syndrome. Later, it was shown that the free beta subunit of hCG (fβ-hCG) is an effective screening marker for Down syndrome in the first trimester of the pregnancy. In Down syndrome pregnancies maternal serum fβ-hCG levels are higher than in normal pregnancies during the first trimester of the pregnancy. The reported DRs for fβ-hCG alone are around 19-42 % for a 5 % FPR [9, 16]. The DR of fβ-hCG for Down syndrome is better at gestational week 13 than at gestational week 10 [9].

The association between abnormal levels of maternal serum pregnancy associated plasma protein-A (PAPP-A) and fetal aneuploidy was made in late 1980's and early 1990's [22, 23]. PAPP-A levels normally rise during pregnancy all the way to the delivery. PAPP-A is a metalloproteinase that cleaves insulin-growth factor binding protein-4 (IGFBP-4) which binds IGFs with high affinity thus preventing their interaction with the IGF-receptors that mediate cell growth and survival signals [24, 25]. IGFs are important in implantation, placental physiology and fetal growth [25]. Therefore, PAPP-A is believed to function as a growth factor of both fetus and placenta during the pregnancy. PAPP-A levels are lower in Down syndrome pregnancies during the first trimester of the pregnancy but the deviation from normal decreases with gestational age [20]. The DR for PAPP-A alone is approximately 52 % for a 5 % FPR [16].

Fetal NT in the first trimester of the pregnancy was described as the fluid-filled space under the skin behind the fetal neck in 1992 [7, 8]. NT is measured during first trimester ultrasound scan at gestational weeks 10-13. Ultrasound scan also offers accurate dating of the

pregnancy, ascertainment of viable fetus or missed abortion, detection of multiple pregnancies, accurate dating of the pregnancy, identification of chorionicity and detection of some major fetal anomalies. NT measurement is not altered in multiple pregnancies or by assisted reproduction techniques. Large studies in low risk populations have shown the association between increased NT and chromosomal defects. The combination of maternal age and NT was reported to have a DR of 63.0-90.0 % for a FPR of 5.0-13.0 % [16, 26]. Therefore, NT measurement is the best single marker in screening for Down syndrome [16, 27].

The incidence of chromosomal defects is related to the thickness rather than the appearance of NT [28]. In initial studies, single millimeter cut-offs like 2.5 mm or 3.0 mm were used to define screen positivity but as it was learned that NT increases with CRL it was realized that it is important to take gestational age into account [29]. Later certain percentile cut-offs, like the fetal NT measurement equal to or above the 95th or 99th centile for CRL, were used. Today, most current screening programs advocate the use of gestational age based cut-offs for risk assessment of MoMs. However, some recent studies like a study of 36120 singleton pregnancies with complete first trimester NT and serum marker data have concluded that immediate invasive testing should be offered to all patients with NT measurement of 3 mm or greater since the addition of the first trimester serum markers do not seem to significantly reduce the final risk of fetal aneuploidy [30].

Also the risk of other adverse pregnancy outcomes increases with enlarging NT measurement. Between NT values of the 95th and 99th percentiles, the prevalence of major anomalies is 2.5 %. With NT measurement of 6.5 mm or larger, the risk is approximately 45 % [31]. The causes behind increased NT measurement are heterogenic which is in relation to the variety of adverse pregnancy outcomes that increased fetal NT has been associated with [32]. Congenital heart defect is the most common adverse pregnancy outcome that has been associated with increased NT [31]. The prevalence of congenital heart defects in children with Down syndrome is approximately 43 % [33]. Other suggested mechanisms include impaired or delayed development of lymphatic drainage [34], mediastinal compression and impedence to venous return caused by for example diaphragmatic hernia or skeletal dysplasias [35, 36], over-expression of certain collagen genes in trisomic fetuses [37], exomphalos, body stalk anomaly, fetal akinesia deformation sequence and genetic syndromes [38, 39].

2.2. Performance of the combined first trimester screening

Screening works better among a population where the incidence of the screened condition is high. Therefore, since the risk of Down syndrome increases with advancing maternal age, screening works better among the older women. Overall, more than half of the Down syndrome cases occur among the women aged 35 years or older [13, 14]. Reported screening perfomances are better in studies that have been conducted in high risk populations where the median maternal age is high and thereby the incidence of Down syndrome is also high. When the screened population reflects well the general low risk population and united screening strategy and high quality ultrasound machines are used, reliable screening results are drawn. Table 2 summarizes the performance of combined first trimester screening for trisomy 21 in large studies reported in the literature.

Study	Gestation	Sample size	Trisomy 21 (N)	Incidence of trisomy 21	Median age	Women at age of ≥ 35 (%)	Cut-off level	DR %	FPR %
Bindra *et al.* 2002 [40]	11 – 14	15030	82	1:175	34.0	47.1	1:300	90.2	5.0
Crossley *et al.* 2002 [41]	10 – 14	17229	45	1:383	29.9	15.4	1:250	82	5
Wald *et al.* 2003 [16]	10 – 13	39983	85	1:470	-	-	1:310	83	5
Wapner *et al.* 2003 [42]	10 – 14	8514	61	1:135	34.5	50.0	1:270	85.2	9.4
Malone *et al.* 2005 [9]	11 12 13	38167	117	1:326	30.1	21.6	1:300	87 85 82	5.0
Rozenberg *et al.* 2006 [43]	11 – 13	14934	51	1:293	30.9	-	1:250	79.6	2.7
Kagan *et al.* 2008 [44]	11 – 13	56771	395	1:143	35.4	-	1:200	89	4.6
Okun *et al.* 2008 [45]	11 – 13	14487	62	1:234	34.0	-	1:200	83.9	4.0
Borrell *et al.* 2009 [46]	11 12 13	7250	66	1:110	32.0	-	1:250	86 84 83	4.9 5.4 6.1
Kagan *et al.* 2009 [47]	11-13	19736	122	1:162	34.5	-	1:150	91.0	3.1
Leung *et al.* 2009 [48]	11 – 13	10363	38	1:272	32.0	27.4	1:300	91.2	5.4
Schaelike *et al.* 2009 [49]	11-13	10668	59	1:181	-	31.0	1:300	88.1	4.9
Wortelboer *et al.* 2009 [50]	10 – 14	20293	87	1:233	34.3	"/>36 yr 38.7	1:250	75.9	3.3
Salomon *et al.* 2010 [51]	11-13	21492	80	1:269	30.7	-	1:250	80.0	8.8
Wright *et al.* 2010 [52]	7-14	223361	886	1:252	31.9	-	1:100	90.0	3.0
Engels *et al.* 2011 [16]	9-14	26274 <36 17970 ≥36 8304	121 <36 52 ≥36 69	1:217 <36 1:346 ≥36 1:120	34.1	≥36 yr 31.6	1:200	95.2 <36 94.5 ≥36 95.8	6.6 <36 4.1 ≥36 13.0
Marttala *et al.* 2011 [53]	9 – 13	76949	188	1:409	29.3	19.3	1:250	81.9	4.3
Yeo *et al.* 2012 [54]	10-13	12585	31	1:406	-	-	1:300	87.1	5.1
Peuhkurinen *et al.* 2012 [55]	9-13	63945 <35 50941 ≥35 13004	<35 73 ≥35 115	<35 1:876 ≥35 1:113	<35 27.9 ≥35 37.8	16.9	1:250	<35 74.0 ≥35 87.0	<35 2.8 ≥35 11.9

Table 2. Performance of first trimester combined screening of Down syndrome in different studies.

Improving the screening means increase in the DR and decrease in the FPR and thus decrease in the number of invasive procedures needed to detect one case of Down syndrome and number of procedure related miscarriages. However, with current screening strategies, increase in DR means an increase also in the FPR. A decrease in invasive procedures is an important goal and therefore special attention should be given to decreasing the FPR.

As screening performance depends on maternal age the screening program takes into account maternal age [55]. DR and FPR increase with advancing maternal age. Worst screening performance is among the women aged 25-29 years [14, 43]. The overall screening performance may be an underestimation or overestimation on individual level depending on the screened woman's age. More focus on individual risk in counseling is needed. Among younger women, the possibility of a false negative screening result is higher and among older women the possibility of false positive screening result is higher. Possibly, lowering the screening cut-off level among women aged 35 or more could improve the balance between DR and FPR [14]. For example, in USA, improved prenatal screening tests and increased availability of screening for also the older women has declined the uptake of invasive testing over the past decade. Also the risk of procedure related miscarriage affects women's decision. The possibility for earlier screening during the first trimester has decreased the number of invasive tests more than the second trimester screening. Also a screening strategy that excludes maternal age, called advanced first trimester screening, might be an option among older women.

Most common factor for a false negative screening result is NT. Therefore, appropriate training and constant audit as well as possibly the certification of the competence should be required from the examiners performing ultrasound scans and NT measurements. Even more competence will be required if additional ultrasound markers like nasal bone will be included into the screening program. The quality of ultrasound machines is also important.

It is possible to provide pretest counseling, biochemical testing of the mother, and NT measurement at the same visit and post-test counseling on a combined risk estimate within a one-hour visit to a one-stop clinic [40]. However, screening performance differs according to the gestational age. The difference between fβ-hCG MoM values increases between unaffected and affected pregnancies as the pregnancy progresses. On the contrary, the difference in PAPP-A values decreases and PAPP-A is more effective screening marker than fβ-hCG. The maximum separation in PAPP-A levels is seen at 9-10 gestational weeks. Therefore, screening works better when PAPP-A is measured during 9-10 weeks of gestation rather than during gestational weeks 7-8 or 11-14. First trimester ultrasound scan is more accurate during the late first trimester. Therefore it would be rational to draw blood samples for the measurements of PAPP-A and fβ-hCG at gestational weeks 9-10 and have another visit at 12th gestational week for the ultrasound scan. Another option could be to measure PAPP-A at gestational weeks 9-10 and NT and fβ-hCG at 12th gestational week. This could improve DRs from 90 % to 92 % for a FPR of 3 % and from 93 % to 95 % for a FPR of 5 % [20, 47, 48, 50].

Also, fetal gender has been shown to affect the levels of maternal serum PAPP-A and fβ-hCG in Down syndrome pregnancies. The levels of fβ-hCG and PAPP-A were shown to be

significantly increased and NT measurements significantly reduced in women carrying female fetuses compared to women carrying male fetuses [51]. In future, NIPD may replace contemporary prenatal diagnosis in those women who are at risk of fetal chromosomal abnormality after Down syndrome screening. However, at the moment, research should also focus on improving the sensitivity and specificity of the combined screening. This might happen by adding new biochemical and sonographic markers into screening.

2.3. Invasive testing

After a positive screening result, a diagnostic test is offered. Also women who are in increased risk for Down syndrome due to increased maternal age or have a family history of Down syndrome are offered invasive testing. CVS can be performed at 11-14 weeks of gestation and AC from 15 weeks of gestation. CVS and AC carry an approximately 0.5-1 % risk of miscarriage [15].

2.4. Other investigated screening markers

2.4.1. Additional ultrasound markers

Ductus venosus (DV) shunts approximately half of the well-oxygenated blood from the umbilical vein directly into the inferior vena cava thus bypassing the liver. The blood flow in the DV is normally forward and triphasic. The waveform of the blood flow has a peak during ventricular systole (S-wave) and diastole (D-wave), during the atrial contraction in late diastole there is a nadir (A-wave). Abnormal flow in the DV in the first trimester of the pregnancy has been associated with chromosomal abnormalities. The abnormal DV flow has been reported to detect approximately 65-75 % of the Down syndrome cases for a FPR of 5.0-21 % [56, 57]. Addition of DV assessment to combined screening can improve the DR from 89 % to 96 % with an increase in FPR from 2.3 % to 2.5 % [58].

Fetal tachycardia has been associated with Down syndrome. However, the results have been controversial and even when the association has been made the authors have not always suggested the use of fetal heart rate (FHR) in the screening program. Addition of FHR to combined screening improves the DR only marginally, from 89 % to 90 % for a FPR of 3.0 % [58].

Frontomaxillary facial (FMF) angle decreases normally with CRL from 85° at 45 mm to 75° at 84 mm [59]. The FMF angle measurements are above the 95th centile in approximately 69 % of Down syndrome fetuses and 5 % of euploid fetuses. Addition of FMF angle to combined screening can improve the DR from 90 % to 95 % for a FPR of 5.0 % [60].

Nasal bone (NB) has been found to be absent or hypoplastic in fetuses with Down syndrome. NB is classified as being absent in cases where NB appears as a thin line, or less echogenic than the overlying skin suggesting that the NB is not yet ossified. The DR for NB alone is approximately 73 % for a FPR of 0.5 % [83]. Addition of NB to combined screening can improve the DR from 89 % to 91 % for a FPR of 2.5 % [58].

Tricuspid regurgitation (TR) is defined by the Fetal Medicine Foundation as when the velocity of the flow exceeds 60 cm/s and occurs during at least half of the systole. In some studies,

however, TR has been defined as when the flow exceeds 80 cm/s [62]. The DR for TR alone is approximately 59.4 % for a FPR of 8.8 % [63]. Addition of TR to combined screening can improve the DR from 75 % to 87 % for a FPR of 1.0 % [62]. Table 3 presents the reported DRs and FPRs for additional ultrasound markers alone and in combination with first trimester combined screening.

Ultrasound marker	Ultrasound marker + maternal age		Combined screening + ultrasound marker	
	Detection rate (%)	False positive rate (%)	Detection rate (%)	False positive rate (%)
Ductus venosus flow	65-75	5-21	96	2.5
Fetal heart rate	-	-	90	3
Frontomaxillary facial angle	69	5	95	5
Nasal bone	59.8-73	0.5-2.6	91	2.5
			97	5
Tricuspid regurgitation	59.4	8.8	87	1
			96	2.6

Table 3. Screening performance of the additional ultrasound markers used alone and in combination with combined first trimester screening markers.

There is no significant association between DV flow, FMF angle, NB or TR and the combined screening markers PAPP-A, fβ-hCG and NT [59, 64]. New sonographic markers may also be used in combination. Inclusion of the new sonographic markers in to screening requires appropriate training of the examiners and the imagining protocols need to be standardized.

2.4.2. Genetic sonogram

Genetic sonogram is an ultrasound examination performed during the second trimester of the pregnancy. During the genetic sonogram fetuses are evaluated for structural malformations and also searched for the sonographic markers of Down syndrome. Main markers include nuchal fold, short femur and humerus, pyelectasis, echogenic intracardiac focus, hyperechoic bowel and any major anomaly. Major abnormalities can be recognized approximately in 25 % of the Down syndrome pregnancies [65]. If there are one or more sonographic markers present, the baseline risk of Down syndrome increases. Similarly, the absence of markers conveys a reduction in the risk based on for example combined first trimester screening, previous chromosomal abnormality or advanced maternal age [66].

Besides major markers there are also minor, "soft", markers that can be evaluated during the scan. These include nuchal skinfold of 6 mm or more, choroid plexus cyst, enlarged cisterna magna over 10 mm, ventriculomegaly 10 mm or more, echogenic intracardiac focus, pericardial effusion, hydrops, two-vessel umbilical cord, polydactyly, clinodactyly, sandal gap, and

club foot. The genetic sonogram has been reported to have a DR of 66.6 – 83.0 % for a 6.7 – 19.3 % FPR depending on the population. The screening performance is naturally lower in a low risk population [67, 68]. Combining the genetic sonogram into combined first trimester screening can improve the DR from 81 % to 90 % for a 5 % FPR [69].

If major defects are detected during the scan, fetal karyotyping is offered to determine the underlying cause and the risk of recurrence. Even if the condition, like diaphragmatic hernia, is treatable by a surgery, there might be a chromosomal abnormality behind it. Unlike major defects, minor defects are common and rarely associated with any other handicap than chromosomal abnormality. Therefore, detection of a minor defect should lead to a thorough search for other defects. The risk of a fetal anomaly should be individually evaluated since it increases with the number of minor defects detected. Second trimester ultrasound scan will likely have an important role also in the future in the detection of fetal Down syndrome and other chromosomal abnormalities.

2.4.3. Other biochemical screening markers

New biochemical screening markers are under investigation. A disintegrin and metalloprotease 12 (ADAM12) is a glycoprotein that is synthesized by placenta. Lowered levels of ADAM12 in maternal serum have been associated with Down syndrome and other chromosomal abnormalities such as trisomies 18 and 13 during the early first trimester of the pregnancy but its deviation from normality decreases as the pregnancy progresses [69-71]. ADAM12 is not a good screening marker for Down syndrome during gestational weeks 11-13 since its levels are not significantly different from normal. Although in other chromosomal abnormalities the levels differ significantly from normal, there is a significant association between ADAM12 and fβ-hCG and PAPP-A [95, 96]. Modeled DRs for ADAM12 in combination with first trimester combined screening markers are 97 % and 89 % for FPRs of 5 % and 1 % at gestational week 12 [70]. However, it seems that no additional benefit could be obtained be the inclusion of ADAM12 into the first trimester combined screening.

Inhibin A has been long used as a part of second trimester quadruple screening. High levels of inhibin A in Down syndrome pregnancies have also been found during the first trimester of the pregnancy. Using inhibin A with combined screening markers during the gestational weeks 9-11 can achieve an approximately 82.6 % DR for a 1.0 % FPR which is close to the performance of the integrated test [5].

Placental protein 13 (PP13) levels are not altered significantly in Down syndrome pregnancies but its levels are significantly decreased in trisomy 18 and 13, Turner syndrome and triploidy pregnancies [72, 73]. Placental growth factor (PlGF) levels in maternal serum have been reported to be decreased, increased or the same in Down syndrome pregnancies compared to unaffected pregnancies during the first and second trimester of the pregnancy. According to the literature, maternal serum PlGF is potentially useful in first trimester screening for fetal chromosomal abnormalities.

Using second trimester serum markers AFP, inhibin A and uE3 during the first trimester has also been studied. For the combination of PAPP-A, fβ-hCG, AFP and NT the estimated DR

is 87.2 %, when AFP is replaced with uE3 the estimated DR is 87.9 % and for all the markers, 88.3 % for a 5 % FPR [20]. Inhibin A with combination of first trimester combined screening markers has been shown to achieve DRs of 81.4 % and 82.6 % at gestational weeks 7-8 and 9-11, respectively, for FPRs of 0.9 % and 1 % [5]. The studies on inhibin A have been controversial and some have found that inhibin A does not increase the screening performance in the first trimester [74].

Besides the biomarkers mentioned above, also other maternal serum proteins have been shown to be more abundant in control versus Down syndrome pregnancies in both first and second trimester of the pregnancy [75]. Large scale prospective studies in low risk populations evaluating the new maternal serum biomarkers need to be conducted before these markers could be implemented into the routine first trimester screening.

2.5. Integrated screening and contingent screening

In 1999 first trimester and second trimester screening were combined to create an integrated screening method which has been shown to achieve DRs around 85 %, 90 % and 94 % for FPRs of 1 %, 2 % and 5 %, respectively [76]. After first trimester combined screening is performed, no risk assessment is provided, instead, women return between gestational weeks 15 and 20 for measurements of serum quadruple markers. These screening methods are then combined with maternal age and an individual risk for Down syndrome is calculated. The advantage of integrated screening is its high sensitivity and specificity. However, first trimester screening results are withheld and the screening results are not available until the second trimester of the pregnancy. In the FaSTER trial, with a 5 % FPR, modeled DRs for integrated screening method were 96 %, 95 % and 94 % when the PAPP-A was measured during the gestational weeks 11, 12 or 13, respectively [9]. In the SURUSS study, integrated screening achieved a 93 % DR for a 5 % FPR. At an 85 % DR the FPR was 1.2 % [16].

Contingent screening policy was developed to reduce the number of NT measurements needed. This can be beneficial in the areas where there are no qualified personnel or high-quality ultrasound machines available or where distances are long. Firstly, first trimester serum sample is analyzed for the levels of PAPP-A and fβ-hCG. Secondly, women are divided into three groups, women in low, intermediate and high risk for chromosomal abnormalities according to the serum markers. Women in low risk are offered no further screening. Women in high risk are offered immediate invasive testing. NT screening is offered for those in intermediate risk and new risk calculation using first trimester serum markers and NT measurement is performed and invasive testing is offered for those in high risk. This method has been estimated to achieve DRs of 67.6 % and 88.6 % for FPRs of 2.3 % and 6.4 %, respectively [77, 78]. Contingent screening might put women in unequal positions as first trimester combined screening is known to achieve higher screening performances. Moreover, major structural abnormalities can be detected during the first trimester ultrasound scan [79, 80]. Also other variations of contingent screening including for example new sonographic markers have been developed.

3. Screening for Down syndrome in the future

3.1. Non-Invasive Prenatal Diagnosis (NIPD)

One of the hottest topics in prenatal medicine today is the noninvasive prenatal diagnosis (NIPD). Since 1997 many approaches have been made in the field of NIPD and today it is possible to determine fetal sex, fetal Rhesus D status and diagnose genetic disorders or carrier status for paternally inherited mutations [81]. Women in high risk of X-linked disorders like hemophilia can be offered noninvasive fetal sex determination. Y chromosome derived sequences can be found in maternal blood as early as eight weeks of gestation [82]. The detection of Y chromosome material indicates further investigations but if no evidence of detectable Y chromosome is found, unnecessary invasive testing with the risk of pregnancy loss, can be avoided. The costs of NIPD of fetal gender and invasive testing are similar [83, 84]. Y chromosome sequences can be detected with approximately 95.4 % sensitivity and 98.6 % specificity. Best test performance reported is for the real-time quantitative polymerase chain reaction (RTQ-PCR) after 20 weeks of gestation. Tests performed before seventh gestational week or using urine sample have been reported to be unreliable [85].

Detection of fetal rhesus D status can reduce the use of D immunoglobulin to prevent immune hemolytic disease of the newborn. The reported sensitivities and specificities for fetal Rhesus D sequence are greater than 95 % [86]. Reported false negative results are mainly due to a lack of fetal DNA in maternal blood sample due to too early gestation or insensitive methods. The presence of pseudogenes, mainly in African women, can lead to false positive results. However, current genotyping protocols in molecular diagnostic laboratories acknowledge the possibility of the pseudogene and do not amplify this region of the genome [87]. The first study evaluating the national clinical application of NIPD of fetal Rhesus D status conducted in Denmark, reported a sensitivity of 99.9 % and specificity of 96.5 % [88].

Fetal hemoglobin in maternal circulation was detected in 1956 indicating transplacental transmission of fetal erythrocytes [89]. Fetal cells were found in maternal blood during pregnancy in 1958 [90]. Nucleated red blood cells have a relatively short lifespan in maternal blood but other cells can reside in maternal blood for decades after delivery and therefore cause false positive or negative test results in subsequent pregnancies [87, 91]. Other problems besides the possibility of the presence of previous pregnancy include the rare number of fetal cells in maternal plasma, one cell per ml, and low efficiency of enrichment methods.

CffDNA, originating from the apoptotic trophoblasts derived from the embryo, was first detected in maternal circulation in 1997 [92, 93]. It has been shown that cffDNA is present in maternal circulation even before placental circulation has been established. It is present also in anembryonic gestations. Detected cffDNA sequences in maternal blood have been shown to reflect the placental genotype in cases of confined placental mosaicism [87]. Compared to intact fetal cells cffDNA has many advantages; it is almost a thousand times more present in maternal circulation than fetal cells, its mean half-life in maternal blood is approximately 16-30 minutes making it a marker of the current pregnancy [94, 95]. Even though the concentration of cffDNA in maternal blood is higher than that of the intact fetal cells, it is still low

and it only comprises 3-6 % of the total cell-free DNA in maternal blood since the majority of cell-free DNA is of maternal origin. Also, half of the fetal genome is inherited from the mother and there are individual differences in the concentration of the total cffDNA [94, 96].

The newest strategy for noninvasive prenatal gene profiling is the maternal blood analysis of fetal mRNA. Discovery of fetal placenta-specific expressed mRNAs in the maternal serum and plasma was made in 2000 [97]. Fetal mRNA molecules have been shown to be easily detectable since they are very stable in maternal blood probably due to the association with particulate matters [99]. Numerous pregnancy-specific, fetal-specific mRNA transcripts that are independent from fetal gender and fetal genetic polymorphisms have been identified in maternal circulation [99, 100]. Studied noninvasive prenatal screening mRNA markers include for example placenta-specific 4 (PLAC4) which is cleared rapidly after delivery and has been reported to have a 90 % DR for a 3.5 % FPR for Down syndrome [100].

3.1.1. Current state of art in NIPD

Various methods for NIPD using cffNA in maternal circulation have been introduced. Massively parallel sequencing (MPS) of fetal DNA has high sensitivity and specificity for the detection of trisomy 21. The reported sensitivities range between 79.1 % and 100 % and specificities between 97.9 % and 100 %, respectively [101-104]. Similar sensitivities and specificities for trisomies 21 and 18 have been reported for targeted MPS method and for trisomy 21 with differential methylation and real-time multiplex ligation-dependent probe amplification (RT-MLPA). One study achieved a 100 % sensitivity and specificity for trisomy 21 by a targeted approach that was based on calculation of haplotype ratios from tandem single nucleotide polymorphisms (SNP) sequences on chromosome 21 combined with a quantitative DNA measurement technology [105].

The use of MPS as the screening strategy has been reported to achieve sensitivities of 91.9-100 % and 100 % with specificities of 98.9-100 % and 98.4-100 % for trisomy 18 and trisomy 13, respectively [106-108]. MPS combined with improved z-score test methodology, was reported to achieve 100 % DR with a 0 % FPR for Down syndrome, trisomy 18, trisomy 13, Turner syndrome and Klinefelter syndrome [109]. High troughput DNA sequencing has many advantages as the entire process can be automated and multiple samples be analyzed simultaneously so that thousands of sequencing reactions can occur in parallel as the test DNA is bound to a solid support such as an array.

One method called the RNA-SNP approach measures the ratio of alleles for a SNP in placenta-derived mRNA molecules in maternal plasma [100]. PLAC4 mRNA has been used for this method [110]. The RNA-SNP method detects the deviated RNA-SNP allelic ratio on PLAC4 mRNA which is caused by the imbalance in chromosome 21 dosage. The RNA-SNP strategy is only suitable to women with a fetus heterozygous for the studied SNP in the PLAC4 gene. Method can be based on a mass spectrometry (MS) method or digital-PCR which enhances the precision [100, 111]. Digital-PCR method is more costly but it can be used in analysis of plasma samples with low concentration of PLAC4 mRNA such in early pregnancy samples.

Another method used is the measurement of the total concentration of PLAC4 mRNA in maternal plasma is increased in Down syndrome pregnancies because of the extra gene copy in the placenta [112]. The mRNA quantification method can be used for pregnancies with homozygous fetuses. However, it is not yet known if there are other factors such as increased apoptosis in aneuploid placentas that might contribute to the increase of circulating PLAC4 mRNA in maternal plasma. The diagnostic accuracies of RNA-SNP approach, using blood samples from women carrying heterozygous fetuses for the PLAC4 mRNA, on the MS and digital-PCR platforms have identical sensitivities and specificities of 90-100 % and 89.7-96.5 %, respectively [100, 112].

Also gene sequences present in neonatal and maternal whole blood have been studied [81, 87]. In amniotic fluid, abundant amounts of both cffRNA and cffDNA can be found and the present cell-free nucleic acids (cffNA) are nearly exclusively of fetal origin. Also, the cffNA appears to originate from fetal tissues that are either in direct contact with the amniotic fluid or drain into the amniotic fluid and there seems to be no NA derived from the placenta. Intial studies on the molecular pathophysiology in the living fetus suggest that the majority of dysregulated gene espression in aneuploid fetuses occurs in genes present in other chromosomes than the one involved in the chromosomal abnormality. Another finding is the oxidative stress in fetuses affected by Down syndrome which may result in the mental retardation and Alzheimer's disease [87]. After birth, analysis of cffNA from neonatal saliva can be used to monitor neonatal health and development. This offers comprehensive, real-time information regarding many organs and tissues which could allow the monitoring of premature neonates in terms of health, disease and development [113].

The reported data indicates that highly accurate NIPD of chromosomal abnormalities by maternal blood sample is achievable during the first trimester of the pregnancy. However, the gestational window of NIPD is still to be researched. Although studies have reported high sensitivities and specificities, approximately 1 % FPRs have been reported. Therefore, at the moment, invasive testing is still required after positive test result and the method might be more incisively regarded as an "advanced screening test" rather than a diagnostic test and pregnancy termination should not be offered only based on a positive NIPD test. However, it has been estimated that 98 % of the invasive procedures could be avoided if AC or CVS were based on the MPS test results [101]. Most studies to date have been small and conducted in high risk women. Large-scale objective clinical trials are needed to evaluate the sensitivity and specificity of NIPD in low risk general populations. The future costs of NIPD can be only estimated and are dependent on the relative costs of NIPD, Down syndrome screening and number of invasive tests that are performed.

NIPD of fetal Rhesus D genotype has been widely validated in Europe but it is slower been undertaken in United States of America. It is anticipated that besides fetal sex determination and Rhesus D detection, over the next few years also the NIPD of fetal aneuploidy will be possible and NIPD will be refined to include also other trisomies than trisomy 21. However, it may take longer to develop proper techniques to detect other pathogenic rearrangements. Ultrasound scan during the early pregnancy will be necessary even if NIPD would become a routine screening method. Increased levels of cffNA in maternal blood have been associated,

besides chromosomal abnormalities, with various pathological conditions like pre-eclampsia, hemolytic anemia, elevated liver enzymes, low platelets syndrome and placental abnormalities like placenta accrete [87, 114].

3.1.2. Ethics in NIPD

NIPD has many benefits as definitive diagnoses can be made earlier in the pregnancy when termination of an affected pregnancy is safer, parental anxiety is reduced and costs are decreased. As testing becomes safer the uptake will probably increase and thus additional health and economic benefits can be reached. However, NIPD also raises many ethical issues. Counseling needs to be informative so that women could make the decision fully aware of the consequences of possible findings. At the moment, counseling is offered for every woman but only those who have received a positive screening result are offered more detailed information about Down syndrome as they are offered invasive testing. The nature of NIPD, however, is closer to invasive diagnosis than screening. Therefore, all women should be comprehensively counseled before the testing. This probably requires much more genetic counselors than are currently available.

In recent years, private sector has been funding research around NIPD. This might lead to expensive testing. Until now, Down syndrome screening has had a minimal effect on birth incidence of genetic disorders. As testing becomes safer and more accurate than before more affected pregnancies may be found and possibly terminated. This might affect the public attitudes towards affected individuals and their families. Women might feel more pressured by the society to test and terminate affected pregnancies. Also commercial and insurance sectors might perceive economic benefits in decreasing the prevalence of disorders. As the technology develops, also less severe disorders, late-onset disorders, nonmedical traits and predispositions can be detected prenatally. Codes of practice should be developed as well as regulatory recommendations made [158]. In United States of America, several professional organizations have stated that noninvasive fetal gender determination should only be offered for medical indications. However, via the internet the test is available directly to the consumers and the technology might also be used for fetal sex selection.

Women seem to feel positive about the new improvements in the screening field. However, they find it hard to fully realize the new choices and consequences that will follow with NIPD [115]. Among the healthcare providers there seems to be a lack of knowledge or conviction about using NIPD. Healthcare providers hold genetic counseling and professional society approval important and they are more willing to offer cffDNA testing for chromosomal abnormalities and single-gene disorders than determination of sex and behavioral or late-onset conditions. Standards of care and professional guidelines are necessary.

4. Other implications for combined Down syndrome screening method

Using the algorithm for Down syndrome, combined screening detects approximately 55.6 % of trisomy 18 cases, 36.4 % of trisomy 13 cases and 60 % of other aneuploidies for a 4.3 %

FPR. When specific algorithm for trisomy 18 is used, the DR for trisomy 18 is reported to be 74.0 - 88 % with a slight increase of 0.1 % in the FPR. Using the specific algorithm for trisomy 13 improves the DR for trisomy 13 to approximately 54.5 - 73 % for an additional 0.1 % increase in the FPR [116, 117].

Adverse pregnancy outcomes like pregnancy loss, hypertension, preeclampsia, eclampsia, preterm delivery, small for gestational age newborns and fetal death cannot yet be predicted in the early pregnancy. Closer surveillance and possible new treatments could be studied on women in high risk to avoid the adverse pregnancy outcomes in the future. As well as increased NT measurements, also abnormal levels of maternal serum biochemical markers have been associated with pregnancy complications.

5. Ethical aspects of the first trimester screening

Participating in the screening for chromosomal abnormalities and the diagnostic testing is voluntary. Women have an opportunity to retrieve screening at any point. It is essential that women make an informed decision when they decide to participate in the screening. When a positive screening result is received, detailed and objective counseling should be offered about the condition at issue and about the procedure and its risks. Health professionals' personal opinions should not affect the woman's decision. However, it is known that the many issues like the age, level of medical knowledge, opinion about the screening test, specialty and attitudes towards the patients affect the counseling. Due to the complexity of the screening, women need to assimilate a lot of information which might not always be successful. If the possibility of a chromosomal abnormality is introduced for the first time when the screening is offered, worry can be caused. The possibility to terminate the pregnancy after a chromosomal abnormality is detected raises many ethical issues about the right of the disabled to be born regardless of their disability. Screening is also thought to be insulting towards people with a chromosomal or a structural anomaly. Screening does not produce diagnoses, only risks for chromosomal abnormalities. The limitations of the screening should be told for the women participating in the screening. One redeeming feature of the screening is that it provides a great deal of knowledge about chromosomal and structural abnormalities equally for every screened woman.

6. Screening in multiple pregnancies and in ART pregnancies

Screening in multiple pregnancies is more difficult than in singleton pregnancies. Firstly, maternal serum biochemistry is less effective in multiple pregnancies since placental analytes from normal fetus/fetuses can mask abnormal levels in the affected fetus. Moreover, abnormal levels of maternal serum biochemical markers cannot distinguish which fetus is the affected one [118]. Secondly, second trimester ultrasound examination is more challenging because of the limitations due to the positions of the fetuses and interposition of fetal parts.

Nuchal translucency measurement together with maternal age, however, has been shown to be an effective screening method in multiple pregnancies. The DR is comparable to that in singleton pregnancies for a slightly higher FPR. Also, determination of fetus-specific risk is possible with this technique. The limitation of ultrasound in twins is that they can be influenced by hemodynamic imbalance between the twins' circulation. Other possible screening markers in multiple pregnancies are DV flow and NB [119-121].

Screening in pregnancies conceived using assisted reproductive technologies (ART) has been studied by different research groups and contradictory results have been reported. In some studies fβ-hCG and NT have been enlarged in ART pregnancies and PAPP-A levels decreased, while others have reported no significant differences in these markers. It seems that decreased PAPP-A levels in ART pregnancies is the most discriminating factor leading to increased FPR in these pregnancies. However, some have reported no significant difference in FPR in ART pregnancies compared with spontaneous pregnancies [191].

7. Cost-effectiveness of the screening and international differences in screening strategies

The demands for the prenatal screening performance are high. Also, the cost-effectiveness of the screening should be good. There are some estimations about the screening costs in different countries but overall, the cost and patient acceptability of the alternative policies of screening tests depend on the existing infrastructure of antenatal care, which varies between different countries and centers. Screening and diagnostic tests for chromosomal abnormalities have been developed and been available for several decades and the research for new strategies is ongoing. National committees review available evidence and national screening statistics and each country adopts testing modalities in its own way. In dissimilar healthcare systems guidelines for best practice evolve different ways. There are differences in what tests are offered, insurance coverage, counseling and the national legal situation for terminating an affected pregnancy. Global knowledge about testing practices gets more and more important for the counselors as people immigrate between the countries and into different cultures. In Europe, almost 90 % of couples who receive a prenatal diagnosis of Down syndrome decide to terminate the pregnancy. However, the legal situation concerning pregnancy termination differs between countries [123]. Most couples that feel that they would continue the pregnancy even though the fetus would be diagnosed with a chromosomal abnormality do not participate in the screening program [124]. There are significant differences in screening modalities between for example United Kingdom and the United States of America despite many similarities between the countries [125]. The introduction of prenatal screening has, however, led to a reduction in live-births of Down syndrome cases internationally.

Author details

Jaana Marttala

Department of Obstetrics and Gynecology, Oulu, Finland

References

[1] Hook EB (1981) Rates of chromosome abnormalities at different maternal ages. Obstet Gynecol 58(3): 282-285.

[2] Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol. 1984; 148: 886-94.

[3] Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn. 1987; 7: 623-30.

[4] Canick JA, Knight GJ, Palomaki GE, Haddow JE, Cuckle HS, Wald NJ. Low second trimester maternal serum unconjugated oestriol in pregnancies with down's syndrome. Br J Obstet Gynaecol. 1988; 95: 330-3.

[5] Christiansen M, Norgaard-Pedersen B. Inhibin A is a maternal serum marker for down's syndrome early in the first trimester. Clin Genet. 2005; 68: 35-9.

[6] Haddow JE, Palomaki GE, Knight GJ, Williams J, Pulkkinen A, Canick JA, et al. Prenatal screening for down's syndrome with use of maternal serum markers. N Engl J Med. 1992; 327: 588-93.

[7] Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: Ultrasound screening for chromosomal defects in first trimester of pregnancy. BMJ. 1992; 304: 867-9.

[8] Ville Y, Lalondrelle C, Doumerc S, Daffos F, Frydman R, Oury JF, et al. First-trimester diagnosis of nuchal anomalies: Significance and fetal outcome. Ultrasound Obstet Gynecol. 1992; 2: 314-6.

[9] Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First- and Second-Trimester Evaluation of Risk (FASTER) Research Consortium. First-trimester or second-trimester screening, or both, for down's syndrome. N Engl J Med. 2005; 353: 2001-11.

[10] de Graaf IM, Tijmstra T, Bleker OP, van Lith JM. Womens' preference in down syndrome screening. Prenat Diagn. 2002; 22: 624-9.

[11] Snijders RJ, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age- and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol. 1999; 13: 167-70.

[12] Morris JK, Wald NJ, Watt HC. Fetal loss in down syndrome pregnancies. Prenat Diagn. 1999; 19: 142-5.

[13] Marttala J, Yliniemi O, Gissler M, Nieminen P, Ryynanen M. Prevalence of down's syndrome in a pregnant population in finland. Acta Obstet Gynecol Scand. 2010; 89: 715-7.

[14] Engels MA, Heijboer AC, Blankenstein MA, van Vugt JM. Performance of first-trimester combined test for down syndrome in different maternal age groups: Reason for adjustments in screening policy? Prenat Diagn. 2011; 31: 1241-5.

[15] Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther. 2010; 27: 1-7.

[16] Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for down's syndrome: The results of the serum, urine and ultrasound screening study (SURUSS). J Med Screen. 2003; 10: 56-104.

[17] Brizot ML, Snijders RJ, Butler J, Bersinger NA, Nicolaides KH. Maternal serum hCG and fetal nuchal translucency thickness for the prediction of fetal trisomies in the first trimester of pregnancy. Br J Obstet Gynaecol. 1995; 102: 127-32.

[18] Reynolds TM, Penney MD. The mathematical basis of multivariate risk screening: With special reference to screening for down's syndrome associated pregnancy. Ann Clin Biochem. 1990; 27: 452-8.

[19] Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for down's syndrome. Prenat Diagn. 1997; 17: 821-9.

[20] Cuckle HS, van Lith JM. Appropriate biochemical parameters in first-trimester screening for down syndrome. Prenat Diagn. 1999; 19: 505-12.

[21] Tsukerman GL, Gusina NB, Cuckle HS. Maternal serum screening for down syndrome in the first trimester: Experience from belarus. Prenat Diagn. 1999; 19: 499-504.

[22] Talmadge K, Boorstein WR, Fiddes JC. The human genome contains seven genes for the beta-subunit of chorionic gonadotropin but only one gene for the beta-subunit of luteinizing hormone. DNA. 1983; 2: 281-9.

[23] Brambati B, Macintosh MC, Teisner B, Maguiness S, Shrimanker K, Lanzani A, et al. Low maternal serum levels of pregnancy associated plasma protein A (PAPP-A) in the first trimester in association with abnormal fetal karyotype. Br J Obstet Gynaecol. 1993; 100: 324-6.

[24] Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease se-

creted by human fibroblasts is pregnancy-associated plasma protein-A. Proc Natl Acad Sci U S A. 1999; 96: 3149-53.

[25] Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. Cell. 1993; 75: 73-82.

[26] Malone FD, D'Alton ME, Society for Maternal-Fetal M. First-trimester sonographic screening for down syndrome. Obstet Gynecol. 2003; 102: 1066-79.

[27] Marttala J, Kaijomaa M, Ranta J, Dahlbacka A, Nieminen P, Tekay A, et al. False-negative results in routine combined first-trimester screening for down syndrome in finland. Am J Perinatol. 2012; 29: 211-6.

[28] Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. fetal medicine foundation first trimester screening group. Lancet. 1998; 352: 343-6.

[29] Braithwaite JM, Morris RW, Economides DL. Nuchal translucency measurements: Frequency distribution and changes with gestation in a general population. Br J Obstet Gynaecol. 1996; 103: 1201-4.

[30] Comstock CH, Malone FD, Ball RH, Nyberg DA, Saade GR, Berkowitz RL, et al. Is there a nuchal translucency millimeter measurement above which there is no added benefit from first trimester serum screening? Am J Obstet Gynecol. 2006; 195: 843-7.

[31] Bilardo CM, Muller MA, Pajkrt E, Clur SA, van Zalen MM, Bijlsma EK. Increased nuchal translucency thickness and normal karyotype: Time for parental reassurance. Ultrasound Obstet Gynecol. 2007; 30: 11-8.

[32] Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. Am J Obstet Gynecol. 2005; 192: 1005-21.

[33] Weijerman ME, van Furth AM, van der Mooren MD, van Weissenbruch MM, Rammeloo L, Broers CJ, et al. Prevalence of congenital heart defects and persistent pulmonary hypertension of the neonate with down syndrome. Eur J Pediatr. 2010; 169: 1195-9.

[34] Haak MC, Bartelings MM, Jackson DG, Webb S, van Vugt JM, Gittenberger-de Groot AC. Increased nuchal translucency is associated with jugular lymphatic distension. Hum Reprod. 2002; 17: 1086-92.

[35] Sepulveda W, Wong AE, Casasbuenas A, Solari A, Alcalde JL. Congenital diaphragmatic hernia in a first-trimester ultrasound aneuploidy screening program. Prenat Diagn. 2008; 28: 531-4.

[36] Ngo C, Viot G, Aubry MC, Tsatsaris V, Grange G, Cabrol D, et al. First-trimester ultrasound diagnosis of skeletal dysplasia associated with increased nuchal translucency thickness. Ultrasound Obstet Gynecol. 2007; 30: 221-6.

[37] von Kaisenberg CS, Krenn V, Ludwig M, Nicolaides KH, Brand-Saberi B. Morphological classification of nuchal skin in human fetuses with trisomy 21, 18, and 13 at 12-18 weeks and in a trisomy 16 mouse. Anat Embryol (Berl). 1998; 197: 105-24.

[38] Daskalakis G, Sebire NJ, Jurkovic D, Snijders RJ, Nicolaides KH. Body stalk anomaly at 10-14 weeks of gestation. Ultrasound Obstet Gynecol. 1997; 10: 416-8.

[39] Souka AP, Snijders RJ, Novakov A, Soares W, Nicolaides KH. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10-14 weeks of gestation. Ultrasound Obstet Gynecol. 1998; 11: 391-400.

[40] Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. One-stop clinic for assessment of risk for trisomy 21 at 11-14 weeks: A prospective study of 15 030 pregnancies. Ultrasound Obstet Gynecol. 2002; 20: 219-25.

[41] Crossley JA, Aitken DA, Cameron AD, McBride E, Connor JM. Combined ultrasound and biochemical screening for down's syndrome in the first trimester: A scottish multicentre study. BJOG. 2002; 109: 667-76.

[42] Wapner R. Thom E. Simpson JL. Pergament E. Silver R. Filkins K. Platt L. Mahoney M. Johnson A. Hogge WA. Wilson RD. Mohide P. Hershey D. Krantz D. Zachary J. Snijders R. Greene N. Sabbagha R. MacGregor S. Hill L. Gagnon A. Hallahan T. Jackson L. First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group. First-trimester screening for trisomies 21 and 18. N Engl J Med. 2003; 349: 1405-13.

[43] Rozenberg P, Bussieres L, Chevret S, Bernard JP, Malagrida L, Cuckle H, et al. Screening for down syndrome using first-trimester combined screening followed by second-trimester ultrasound examination in an unselected population. Am J Obstet Gynecol. 2006; 195: 1379-87.

[44] Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol. 2008; 31: 618-24.

[45] Okun N, Summers AM, Hoffman B, Huang T, Winsor E, Chitayat D, et al. Prospective experience with integrated prenatal screening and first trimester combined screening for trisomy 21 in a large canadian urban center. Prenat Diagn. 2008; 28: 987-92.

[46] Borrell A, Borobio V, Bestwick JP, Wald NJ. Ductus venosus pulsatility index as an antenatal screening marker for down's syndrome: Use with the combined and integrated tests. J Med Screen. 2009; 16: 112-8.

[47] Kagan KO, Etchegaray A, Zhou Y, Wright D, Nicolaides KH. Prospective validation of first-trimester combined screening for trisomy 21. Ultrasound Obstet Gynecol. 2009; 34: 14-8.

[48] Leung TY, Chan LW, Law LW, Sahota DS, Fung TY, Leung TN, et al. First trimester combined screening for trisomy 21 in hong kong: Outcome of the first 10,000 cases. J Matern Fetal Neonatal Med. 2009; 22: 300-4.

[49] Schaelike M, Kossakiewicz M, Kossakiewicz A, Schild RL. Examination of a first-trimester down syndrome screening concept on a mix of 11,107 high- and low-risk patients at a private center for prenatal medicine in germany. Eur J Obstet Gynecol Reprod Biol. 2009; 144: 140-5.

[50] Wortelboer EJ, Koster MP, Stoutenbeek P, Elvers LH, Loeber JG, Visser GH, et al. First-trimester down syndrome screening performance in the dutch population; how to achieve further improvement? Prenat Diagn. 2009; 29: 588-92.

[51] Salomon LJ, Chevret S, Bussieres L, Ville Y, Rozenberg P. Down syndrome screening using first-trimester combined tests and contingent use of femur length at routine anomaly scan. Prenat Diagn. 2010; 30: 783-9.

[52] Wright D, Spencer K, Kagan KK, Torring N, Petersen OB, Christou A, et al. First-trimester combined screening for trisomy 21 at 7-14 weeks' gestation. Ultrasound Obstet Gynecol. 2010; 36: 404-11.

[53] Marttala J, Ranta JK, Kaijomaa M, Nieminen P, Laitinen P, Kokkonen H, et al. More invasive procedures are done to detect each case of down's syndrome in younger women. Acta Obstet Gynecol Scand. 2011; 90: 642-7.

[54] Yeo GS, Lai FM, Wei X, Lata P, Tan DT, Yong MH, et al. Validation of first trimester screening for trisomy 21 in singapore with reference to performance of nasal bone. Fetal Diagn Ther. 2012.

[55] Peuhkurinen S, Laitinen P, Ryynanen M, Marttala J. First trimester down syndrome screening is less effective and the number of invasive procedures is increased in women younger than 35 years of age. J Eval Clin Pract. 2012.

[56] Maiz N, Staboulidou I, Leal AM, Minekawa R, Nicolaides KH. Ductus venosus doppler at 11 to 13 weeks of gestation in the prediction of outcome in twin pregnancies. Obstet Gynecol. 2009; 113: 860-5.

[57] Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH. Ductus venosus doppler in screening for trisomies 21, 18 and 13 and turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol. 2009; 33: 512-7.

[58] Kagan KO, Staboulidou I, Cruz J, Wright D, Nicolaides KH. Two-stage first-trimester screening for trisomy 21 by ultrasound assessment and biochemical testing. Ultrasound Obstet Gynecol. 2010; 36: 542-7.

[59] Borenstein M, Persico N, Kaihura C, Sonek J, Nicolaides KH. Frontomaxillary facial angle in chromosomally normal fetuses at 11 + 0 to 13 + 6 weeks. Ultrasound Obstet Gynecol. 2007; 30: 737-41.

[60] Borenstein M, Persico N, Kagan KO, Gazzoni A, Nicolaides KH. Frontomaxillary facial angle in screening for trisomy 21 at 11 + 0 to 13 + 6 weeks. Ultrasound Obstet Gynecol. 2008; 32: 5-11.

[61] Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: An observational study. Lancet. 2001; 358: 1665-7.

[62] Falcon O, Faiola S, Huggon I, Allan L, Nicolaides KH. Fetal tricuspid regurgitation at the 11 + 0 to 13 + 6-week scan: Association with chromosomal defects and reproducibility of the method. Ultrasound Obstet Gynecol. 2006; 27: 609-12.

[63] Huggon IC, DeFigueiredo DB, Allan LD. Tricuspid regurgitation in the diagnosis of chromosomal anomalies in the fetus at 11-14 weeks of gestation. Heart. 2003; 89: 1071-3.

[64] Cicero S, Bindra R, Rembouskos G, Spencer K, Nicolaides KH. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta-hCG and PAPP-A at 11 to 14 weeks. Prenat Diagn. 2003; 23: 306-10.

[65] Bromley B, Lieberman E, Shipp TD, Benacerraf BR. The genetic sonogram: A method of risk assessment for down syndrome in the second trimester. J Ultrasound Med. 2002; 21: 1087,96; quiz 1097-8.

[66] Benacerraf BR. The role of the second trimester genetic sonogram in screening for fetal down syndrome. Semin Perinatol. 2005; 29: 386-94.

[67] Benn PA, Kaminsky LM, Ying J, Borgida AF, Egan JF. Combined second-trimester biochemical and ultrasound screening for down syndrome. Obstet Gynecol. 2002; 100: 1168-76.

[68] Aagaard-Tillery KM, Malone FD, Nyberg DA, Porter TF, Cuckle HS, Fuchs K, et al. Role of second-trimester genetic sonography after down syndrome screening. Obstet Gynecol. 2009; 114: 1189-96.

[69] Laigaard J, Spencer K, Christiansen M, Cowans NJ, Larsen SO, Pedersen BN, et al. ADAM 12 as a first-trimester maternal serum marker in screening for down syndrome. Prenat Diagn. 2006; 26: 973-9.

[70] Laigaard J, Cuckle H, Wewer UM, Christiansen M. Maternal serum ADAM12 levels in down and edwards' syndrome pregnancies at 9-12 weeks' gestation. Prenat Diagn. 2006; 26: 689-91.

[71] Valinen Y, Peuhkurinen S, Jarvela IY, Laitinen P, Ryynanen M. Maternal serum ADAM12 levels correlate with PAPP-A levels during the first trimester. Gynecol Obstet Invest. 2010; 70: 60-3.

[72] Koster MP, Wortelboer EJ, Cuckle HS, Stoutenbeek P, Visser GH, Schielen PC. Placental protein 13 as a first trimester screening marker for aneuploidy. Prenat Diagn. 2009; 29: 1237-41.

[73] Akolekar R, Perez Penco JM, Skyfta E, Rodriguez Calvo J, Nicolaides KH. Maternal serum placental protein 13 at eleven to thirteen weeks in chromosomally abnormal pregnancies. Fetal Diagn Ther. 2010; 27: 72-7.

[74] Spencer K, Liao AW, Ong CY, Geerts L, Nicolaides KH. First trimester maternal serum placenta growth factor (PIGF)concentrations in pregnancies with fetal trisomy 21 or trisomy 18. Prenat Diagn. 2001; 21: 718-22.

[75] Nagalla SR, Canick JA, Jacob T, Schneider KA, Reddy AP, Thomas A, et al. Proteomic analysis of maternal serum in down syndrome: Identification of novel protein biomarkers. J Proteome Res. 2007; 6: 1245-57.

[76] Saller DN,Jr, Canick JA. Current methods of prenatal screening for down syndrome and other fetal abnormalities. Clin Obstet Gynecol. 2008; 51: 24-36.

[77] Christiansen M, Olesen Larsen S. An increase in cost-effectiveness of first trimester maternal screening programmes for fetal chromosome anomalies is obtained by contingent testing. Prenat Diagn. 2002; 22: 482-6.

[78] Vadiveloo T, Crossley JA, Aitken DA. First-trimester contingent screening for down syndrome can reduce the number of nuchal translucency measurements required. Prenat Diagn. 2009; 29: 79-82.

[79] Souka AP, Pilalis A, Kavalakis I, Antsaklis P, Papantoniou N, Mesogitis S, et al. Screening for major structural abnormalities at the 11- to 14-week ultrasound scan. Am J Obstet Gynecol. 2006; 194: 393-6.

[80] Oztekin O, Oztekin D, Tinar S, Adibelli Z. Ultrasonographic diagnosis of fetal structural abnormalities in prenatal screening at 11-14 weeks. Diagn Interv Radiol. 2009; 15: 221-5.

[81] Maron JL, Bianchi DW. Prenatal diagnosis using cell-free nucleic acids in maternal body fluids: A decade of progress. Am J Med Genet C Semin Med Genet. 2007; 145C: 5-17.

[82] Bustamante-Aragones A, Rodriguez de Alba M, Gonzalez-Gonzalez C, Trujillo-Tiebas MJ, Diego-Alvarez D, Vallespin E, et al. Foetal sex determination in maternal blood from the seventh week of gestation and its role in diagnosing haemophilia in the foetuses of female carriers. Haemophilia. 2008; 14: 593-8.

[83] Hill M, Finning K, Martin P, Hogg J, Meaney C, Norbury G, et al. Non-invasive prenatal determination of fetal sex: Translating research into clinical practice. Clin Genet. 2011; 80: 68-75.

[84] Hill M, Taffinder S, Chitty LS, Morris S. Incremental cost of non-invasive prenatal diagnosis versus invasive prenatal diagnosis of fetal sex in england. Prenat Diagn. 2011; 31: 267-73.

[85] Devaney SA, Palomaki GE, Scott JA, Bianchi DW. Noninvasive fetal sex determination using cell-free fetal DNA: A systematic review and meta-analysis. JAMA. 2011; 306: 627-36.

[86] Macher HC, Noguerol P, Medrano-Campillo P, Garrido-Marquez MR, Rubio-Calvo A, Carmona-Gonzalez M, et al. Standardization non-invasive fetal RHD and SRY determination into clinical routine using a new multiplex RT-PCR assay for fetal cell-free DNA in pregnant women plasma: Results in clinical benefits and cost saving. Clin Chim Acta. 2012; 413: 490-4.

[87] Bianchi DW, Maron JL, Johnson KL. Insights into fetal and neonatal development through analysis of cell-free RNA in body fluids. Early Hum Dev. 2010; 86: 747-52.

[88] Clausen FB, Christiansen M, Steffensen R, Jorgensen S, Nielsen C, Jakobsen MA, et al. Report of the first nationally implemented clinical routine screening for fetal RHD in D- pregnant women to ascertain the requirement for antenatal RhD prophylaxis. Transfusion. 2012; 52: 752-8.

[89] Bromberg YM, Salzberger M, Abrahamov A. Transplacental transmission of fetal erythrocytes with demonstration of fetal hemoglobin in maternal circulation. Obstet Gynecol. 1956; 7: 672-4.

[90] Weiner W, Child RM, Garvie JM, Peek WH. Foetal cells in the maternal circulation during pregnancy. Br Med J. 1958; 2: 770-1.

[91] Lurie S, Mamet Y. Red blood cell survival and kinetics during pregnancy. Eur J Obstet Gynecol Reprod Biol. 2000; 93: 185-92.

[92] Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. The Lancet. 1997; 350: 485-7.

[93] Alberry M, Maddocks D, Jones M, Abdel Hadi M, Abdel-Fattah S, Avent N, et al. Free fetal DNA in maternal plasma in anembryonic pregnancies: Confirmation that the origin is the trophoblast. Prenat Diagn. 2007; 27: 415-8.

[94] Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: Implications for noninvasive prenatal diagnosis. Am J Hum Genet. 1998; 62: 768-75.

[95] Lo YM, Lau TK, Zhang J, Leung TN, Chang AM, Hjelm NM, et al. Increased fetal DNA concentrations in the plasma of pregnant women carrying fetuses with trisomy 21. Clin Chem. 1999; 45: 1747-51.

[96] Wright CF, Burton H. The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. Hum Reprod Update. 2009; 15: 139-51.

[97] Poon LL, Leung TN, Lau TK, Lo YM. Presence of fetal RNA in maternal plasma. Clin Chem. 2000; 46: 1832-4.

[98] Ng EK, Tsui NB, Lam NY, Chiu RW, Yu SC, Wong SC, et al. Presence of filterable and nonfilterable mRNA in the plasma of cancer patients and healthy individuals. Clin Chem. 2002; 48: 1212-7.

[99] Farina A, Chan CW, Chiu RW, Tsui NB, Carinci P, Concu M, et al. Circulating corticotropin-releasing hormone mRNA in maternal plasma: Relationship with gestational age and severity of preeclampsia. Clin Chem. 2004; 50: 1851-4.

[100] Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, Heung MM, et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. Nat Med. 2007; 13: 218-23.

[101] Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: Large scale validity study. BMJ. 2011; 342: c7401.

[102] Chiu RW, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci U S A. 2008; 105: 20458-63.

[103] Ehrich M, Deciu C, Zwiefelhofer T, Tynan JA, Cagasan L, Tim R, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: A study in a clinical setting. Am J Obstet Gynecol. 2011; 204: 205.e1,205.11.

[104] Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect down syndrome: An international clinical validation study. Genet Med. 2011; 13: 913-20.

[105] Ghanta S, Mitchell ME, Ames M, Hidestrand M, Simpson P, Goetsch M, et al. Noninvasive prenatal detection of trisomy 21 using tandem single nucleotide polymorphisms. PLoS One. 2010; 5: e13184.

[106] Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A. 2008; 105: 16266-71.

[107] Sehnert AJ, Rhees B, Comstock D, de Feo E, Heilek G, Burke J, et al. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem. 2011; 57: 1042-9.

[108] Chen EZ, Chiu RW, Sun H, Akolekar R, Chan KC, Leung TY, et al. Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. PLoS One. 2011; 6: e21791.

[109] Lau TK, Chen F, Pan X, Pooh RK, Jiang F, Li Y, et al. Noninvasive prenatal diagnosis of common fetal chromosomal aneuploidies by maternal plasma DNA sequencing. J Matern Fetal Neonatal Med. 2012.

[110] Kido S, Sakuragi N, Bronner MP, Sayegh R, Berger R, Patterson D, et al. D21S418E identifies a cAMP-regulated gene located on chromosome 21q22.3 that is expressed in placental syncytiotrophoblast and choriocarcinoma cells. Genomics. 1993; 17: 256-9.

[111] Lo YM, Lun FM, Chan KC, Tsui NB, Chong KC, Lau TK, et al. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. Proc Natl Acad Sci U S A. 2007; 104: 13116-21.

[112] Tsui NB, Akolekar R, Chiu RW, Chow KC, Leung TY, Lau TK, et al. Synergy of total PLAC4 RNA concentration and measurement of the RNA single-nucleotide polymorphism allelic ratio for the noninvasive prenatal detection of trisomy 21. Clin Chem. 2010; 56: 73-81.

[113] Maron JL, Johnson KL, Rocke DM, Cohen MG, Liley AJ, Bianchi DW. Neonatal salivary analysis reveals global developmental gene expression changes in the premature infant. Clin Chem. 2010; 56: 409-16.

[114] Benn PA, Chapman AR. Practical and ethical considerations of noninvasive prenatal diagnosis. JAMA. 2009; 301: 2154-6.

[115] Kooij L, Tymstra T, Berg P. The attitude of women toward current and future possibilities of diagnostic testing in maternal blood using fetal DNA. Prenat Diagn. 2009; 29: 164-8.

[116] Marttala J, Peuhkurinen S, Ranta JK, Laitinen P, Kokkonen HL, Honkasalo T, et al. Screening and outcome of chromosomal abnormalities other than trisomy 21 in northern finland. Acta Obstet Gynecol Scand. 2011; 90: 885-9.

[117] Ekelund CK, Petersen OB, Skibsted L, Kjaergaard S, Vogel I, Tabor A, et al. First trimester screening for trisomy 21 in denmark: Implications on detection and birth rates of trisomy 18 and trisomy 13. Ultrasound Obstet Gynecol. 2011; 38: 140-4.

[118] Matias A, Montenegro N, Blickstein I. Down syndrome screening in multiple pregnancies. Obstet Gynecol Clin North Am. 2005; 32: 81,96, ix.

[119] Sebire NJ, Snijders RJ, Hughes K, Sepulveda W, Nicolaides KH. Screening for trisomy 21 in twin pregnancies by maternal age and fetal nuchal translucency thickness at 10-14 weeks of gestation. Br J Obstet Gynaecol. 1996; 103: 999-1003.

[120] Matias A, Ramalho C, Montenegro N. Search for hemodynamic compromise at 11-14 weeks in monochorionic twin pregnancy: Is abnormal flow in the ductus venosus predictive of twin-twin transfusion syndrome? J Matern Fetal Neonatal Med. 2005; 18: 79-86.

[121] Sepulveda W, Wong AE, Dezerega V. First-trimester ultrasonographic screening for trisomy 21 using fetal nuchal translucency and nasal bone. Obstet Gynecol. 2007; 109: 1040-5.

[122] Kirkegaard I, Henriksen TB, Torring N, Uldbjerg N. PAPP-A and free beta-hCG measured prior to 10 weeks is associated with preterm delivery and small-for-gestational-age infants. Prenat Diagn. 2011; 31: 171-5.

[123] Boyd PA, Devigan C, Khoshnood B, Loane M, Garne E, Dolk H, et al. Survey of prenatal screening policies in europe for structural malformations and chromosome anomalies, and their impact on detection and termination rates for neural tube defects and down's syndrome. BJOG. 2008; 115: 689-96.

[124] Kobelka C, Mattman A, Langlois S. An evaluation of the decision-making process regarding amniocentesis following a screen-positive maternal serum screen result. Prenat Diagn. 2009; 29: 514-9.

[125] Tapon D. Prenatal testing for down syndrome: Comparison of screening practices in the UK and USA. J Genet Couns. 2010; 19: 112-30.

IntechOpen