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First-trimester contingent screening for trisomies 21, 18 and 13 by fetal nuchal translucency and ductus venosus flow and maternal blood cell-free DNA testing

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KEYWORDS: cell-free DNA; ductus venosus flow; first-trimester screening; nuchal translucency; trisomy

ABSTRACT

Objective *To examine performance of screening for major trisomies by a policy of first-line assessment of risk according to maternal age, fetal nuchal translucency thickness (NT) and ductus venosus pulsatility index for veins (DV-PIV) followed by cell-free DNA (cfDNA) testing in pregnancies with an intermediate risk.*

Methods *We estimated the distribution of risks based on maternal age, fetal NT and DV-PIV in a dataset of 86 917 unaffected and 491 trisomic pregnancies undergoing prospective screening for trisomies. Performance of screening for trisomies by cfDNA testing was derived from a meta-analysis of clinical validation studies. We estimated performance and cost of screening for trisomies using different combinations of ultrasound screening and cfDNA testing.*

Results *Screening for trisomies 21, 18 and 13 according to a combination of maternal age, fetal NT and DV-PIV in all pregnancies, followed by invasive testing in the high-risk group (≥ 1:10) and cfDNA testing in the intermediate-risk group (1:11–1:3000) can potentially detect about 96%, 95% and 91% of cases, respectively, with a false-positive rate (FPR) of 0.8%. On the assumption that the costs for ultrasound screening, cfDNA testing and invasive testing are €150, €500 and €1000, respectively, the overall cost of such a policy would be about €250 per patient. The alternative policy, of universal screening by cfDNA testing, can potentially detect about 99%, 97% and 92% of cases of trisomies 21, 18 and 13, but at an overall cost of more than €500 per patient.*

Conclusion *Incorporation of cfDNA testing into a contingent policy of early screening for the major trisomies, based on the risk derived from first-line screening by a combination of maternal age, fetal NT and DV-PIV, can detect a high proportion of affected cases with a low FPR.* *Copyright © 2014 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

First-trimester combined screening based on maternal age, fetal nuchal translucency thickness (NT) and serum markers, free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A), detects about 90% of fetuses with trisomy 21 with a false-positive rate (FPR) of about 5%[1]. Screening for trisomy 21 can be further improved by assessing additional ultrasound markers such as the nose bone or Doppler flow in the ductus venosus and across the tricuspid valve[2–5]. In a study of 44 756 euploid pregnancies and 202 pregnancies with trisomy 21, the addition of ductus venosus pulsatility index for veins (DV-PIV) with the combined test resulted in a detection rate (DR) of 96% and a FPR of 2.6%[5]. The performance of screening by incorporating assessment of the additional ultrasound markers in all pregnancies is similar to that achieved by limiting these markers to pregnancies with an intermediate risk (1:50–1:1000), determined by the first-trimester combined test, which constitute only 15% of the total[2–4,6,7]. An alternative strategy is to carry out first-line screening according to maternal age, fetal NT and one of the additional ultrasound markers and to reserve assessment of serum-free β-hCG and PAPP-A for the intermediate-risk group; this approach provides a DR of 95% and a FPR of 2.5%[8].

Recently, analysis of cell-free DNA (cfDNA) in maternal blood has been introduced as a method of screening for fetal aneuploidies. The performance of cfDNA testing in screening for trisomies 21 and 18 is

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superior to that of all other methods of screening and it is therefore likely that the test will become widely available[9]. However, at present, the cost of cfDNA testing is too high for this to be adopted as the primary method of screening. In order to keep the costs of screening low, the first-trimester combined test could serve as a triage examination for the cfDNA assessment[10,11]. Such a policy is generally based on first-trimester combined testing for all patients and assessment of cfDNA as a secondary test in a smaller proportion of pregnancies. For example, if the cfDNA test is reserved for the 25% of pregnancies with a risk from the combined test of $\geq$ 1:2500, the overall performance of screening would be almost as good as if cfDNA testing was carried out in all pregnancies[10].

In this study, we examined the potential performance of a policy of prenatal screening for trisomies 21, 18 and 13, in which first-line assessment of risk is based on maternal age, fetal NT and DV-PIV, and cfDNA testing is reserved for pregnancies with an intermediate risk. Such a policy would have two advantages: the costs for the measurement of free β-hCG and PAPP-A could be avoided; and, in a prenatal screening service in which biochemical markers are measured after the ultrasound examination, a direct decision about the necessity of further cfDNA analysis would be more time-effective.

METHODS

We present the results of analysis of prospectively collected data on fetal NT and DV-PIV at 11 + 0 to 13 + 6 weeks' gestation, from singleton pregnancies undergoing screening for aneuploidies at King's College Hospital, London, University College London Hospital, London and Medway Maritime Hospital, Gillingham, UK, between March 2006 and May 2012. Gestational age was determined from measurement of fetal crown–rump length. The patient-specific risks for trisomies 21, 18 and 13 were estimated from a combination of maternal age, fetal NT, fetal heart rate and serum free β-hCG and PAPP-A[12]. Women with risks considered to be high were offered chorionic villus sampling (CVS) or amniocentesis for fetal karyotyping.The overall results of this study have been previously published[13]. In this subanalysis, we used the distribution of risks based on maternal age, fetal NT and DV-PIV in pregnancies with trisomies 21, 18 and 13 and in those unaffected by these aneuploidies. The unaffected group included pregnancies that were found to be euploid by prenatal karyotyping or resulted in the birth of phenotypically normal neonates.

To estimate the effect of cfDNA testing in screening for trisomy, the following assumptions were made: first, the DRs for trisomies 21, 18 and 13 are 99.0%, 96.8% and 92.1%, respectively, and the respective FPRs are 0.08%, 0.15% and 0.20%, resulting in a total of 0.43%[9]; and, second, the failure rate of cfDNA testing in providing results is 3% and, in these cases, invasive tests are performed. We estimated the effectiveness and the costs of screening for trisomies based on different combinations of first-trimester ultrasound screening and cfDNA testing in 100 000 pregnancies, including 98 975 with euploid fetuses, 701 with trisomy 21, 216 with trisomy 18 and 108 with trisomy 13[12]. The costs for first-trimester ultrasound screening, cfDNA testing and invasive testing were set at €150, €500 and €1000, respectively. For selected screening policies, we additionally estimated the costs for screening based on a price for cfDNA testing of €250.

The following screening policies were examined: first, screening according to maternal age, fetal NT and DV-PIV only; second, screening according to cfDNA testing only; and, third, screening according to a combination of first-trimester ultrasound assessment and cfDNA testing. In the third strategy, all women underwent screening according to a combination of maternal age, fetal NT and DV-PIV. Those with a risk above a high cut-off were classified as screen positive and those with a risk below a low cut-off were classified as screen negative. Patients with an intermediate risk (between the upper- and lower-risk cut-offs) underwent cfDNA testing and were classified as screen positive if the result was abnormal or uninformative and screen negative if the result was normal.

Statistical analysis

For the calculation of risks based on the ultrasound examination, Bayes theorem was used by combining the likelihoods of trisomy with the maternal age-specific prior risk of trisomy 21, trisomy 18 and trisomy 13 at 12.5 weeks' gestation[14]. The resultant risks were compared with the risk cut-off to obtain an age-specific DR for each year of maternal age, from 12 to 50 years. All likelihoods were used for each maternal age. The weighted average of these age-specific DRs was then computed to produce a standardized DR. The weights used were obtained from the maternal age distribution of pregnancies in England and Wales in 2011 at 12.5 weeks' gestation, and the gestational- and maternal-age specific risk of each trisomy[14]. Similarly, standardized FPRs were computed by obtaining the likelihoods in unaffected pregnancies and then applying these to each year of maternal age, from 12 to 50 years, to estimate the age-specific FPRs. These were then weighted according to the maternal age distribution of unaffected pregnancies in England and Wales in 2011[14]. Empirical estimates of performance were obtained using likelihoods for the sample data. Modeled performance was obtained using likelihoods from simulated data from the fitted model. Samples of 100 000 unaffected, trisomy 21 and trisomy 18 or 13 pregnancies were used in these simulations.

RESULTS

The study population for assessing the effectiveness of first-trimester ultrasound screening consisted of 86 917 unaffected pregnancies, 324 with trisomy 21, 125 with

Table 1 Maternal characteristics of the study population undergoing screening for aneuploidies, according to karyotype

Characteristic	*Euploid (n = 86 917)*	*Trisomy 21 (n = 324)*	*Trisomy 18 (n = 125)*	*Trisomy 13 (n = 42)*
Maternal age (years)	31.2 (26.7–35.1)	37.9 (34.6–40.2)	37.5 (32.8–41.1)	34.5 (28.8–37.8)
Maternal weight (kg)	65.5 (58.9–75.5)	65.0 (60.0–74.0)	66.8 (59.5–76.4)	68.5 (60.0–77.2)
Crown–rump length (mm)	63.1 (58.1–68.7)	63.8 (58.5–70.0)	55.0 (51.0–60.1)	57.6 (53.5–61.5)
Nuchal translucency (mm)	1.8 (1.5–2.1)	3.5 (2.4–5.0)	5.1 (2.2–7.3)	3.9 (2.1–6.4)
Ductus venosus PIV	1.059 (0.950–1.160)	1.561 (1.210–1.995)	1.730 (1.320–2.290)	1.500 (1.170–1.950)

Data are given as median (interquartile range). PIV, pulsatility index for veins.

Table 2 Effectiveness and cost of screening women with singleton pregnancy for trisomies (T) 21, 18 and 13 according to a combination of maternal age, fetal nuchal translucency thickness and ductus venosus pulsatility index for veins

Risk cut-off	*Screen-positive rate (%)*				*Invasive testing rate (%)*	*Cost (€)*
	Euploid	*T21*	*T18*	*T13*		
1 in 10	0.2	64.9	69.0	58.2	0.9	15 857 000
1 in 20	0.4	71.9	75.1	64.6	1.1	16 147 000
1 in 30	0.6	75.7	78.2	68.1	1.4	16 399 000
1 in 40	0.8	78.1	80.2	70.6	1.6	16 617 000
1 in 50	1.0	79.9	81.7	72.4	1.8	16 811 000
1 in 100	1.8	84.7	85.7	77.7	2.6	17 645 000
1 in 500	5.4	91.9	91.6	87.1	6.3	21 323 000
1 in 1000	8.7	93.9	93.2	89.9	9.5	24 519 000
1 in 1500	11.5	94.9	94.1	91.4	12.4	27 379 000
1 in 2000	14.2	95.6	94.7	92.4	15.1	30 071 000
1 in 2500	16.8	96.1	95.2	93.2	17.6	32 600 000
1 in 3000	19.2	96.5	95.7	93.8	20.0	35 008 000

Patients with a risk above the upper risk cut-off were classified as screen positive. Costs refer to a population of 100 000 pregnancies including 701 with trisomy 21, 216 with trisomy 18 and 108 with trisomy 13.

trisomy 18 and 42 with trisomy 13. The characteristics of the study population are summarized in Table 1.

The performance of screening for trisomies 21, 18 and 13 according to the combination of maternal age, fetal NT and DV-PIV is summarized in Table 2. For a risk cut-off of 1:100, the respective DRs were 84.7%, 85.7% and 77.7%, with a total FPR of 1.8%. Such a policy applied to 100 000 pregnancies would result in 2645 (2.6%) invasive tests. The costs would be 100 000 × €150 for screening, plus 2645 × €1000 for invasive testing, resulting in a total cost of €17 645 000.

In universal screening according to cfDNA testing, the respective DRs for trisomies 21, 18 and 13 were 99.0%, 96.8% and 92.1%, with a total FPR of 0.43%. Such a policy applied to 100 000 pregnancies would result in 1428 (1.4%) invasive tests. The costs would be 100 000 × €500 for screening, plus 1428 × €1000 for invasive testing, resulting in a total cost of €51 428 000. If the price for cfDNA testing is halved to €250, the total cost would be €26 428 000. However, these estimates are based on the assumption that all results from cfDNA testing were informative, but in reality there is test failure in about 3% of cases and if all such cases undergo invasive testing there would be an additional cost of €3 000 000.

Table 3 Effectiveness of contingent screening for trisomies (T) 21, 18 and 13, in which all women underwent screening according to a combination of maternal age, fetal nuchal translucency thickness and ductus venosus pulsatility index for veins

Lower risk cut-off	*Upper risk cut-off*					
	1 in 10	*1 in 20*	*1 in 30*	*1 in 40*	*1 in 50*	*1 in 100*
1 in 500						
FPR	0.37	0.59	0.80	0.99	1.16	1.92
DR for T21	91.6	91.7	91.7	91.7	91.8	91.8
DR for T18	90.9	91.1	91.2	91.2	91.3	91.4
DR for T13	84.9	85.4	85.6	85.8	86.0	86.4
1 in 1000						
FPR	0.48	0.70	0.91	1.10	1.27	2.03
DR for T21	93.6	93.7	93.7	93.7	93.7	93.8
DR for T18	92.4	92.6	92.7	92.8	92.8	93.0
DR for T13	87.4	87.9	88.2	88.4	88.5	88.9
1 in 1500						
FPR	0.58	0.80	1.00	1.19	1.37	2.13
DR for T21	94.6	94.7	94.7	94.7	94.7	94.8
DR for T18	93.3	93.5	93.6	93.6	93.7	93.8
DR for T13	88.8	89.3	89.6	89.8	89.9	90.3
1 in 2000						
FPR	0.67	0.89	1.10	1.29	1.46	2.22
DR for T21	95.3	95.3	95.4	95.4	95.4	95.5
DR for T18	94.0	94.1	94.2	94.3	94.3	94.5
DR for T13	89.8	90.3	90.6	90.7	90.9	91.3
1 in 2500						
FPR	0.76	0.98	1.18	1.37	1.55	2.31
DR for T21	95.8	95.8	95.9	95.9	95.9	96.0
DR for T18	94.4	94.6	94.7	94.8	94.8	95.0
DR for T13	90.5	91.0	91.3	91.5	91.6	92.0
1 in 3000						
FPR	0.85	1.06	1.27	1.46	1.63	2.39
DR for T21	96.3	96.2	96.3	96.3	96.3	96.3
DR for T18	94.9	95.0	95.1	95.2	95.2	95.3
DR for T13	90.7	91.6	91.9	92.1	92.2	92.6

Values are given as %. Patients with a risk above the upper cut-off were classified as screen positive and those with a risk below the lower risk cut-off were screen negative. DR, detection rate; FPR, false-positive rate.

The effectiveness of contingent screening is shown in Table 3. After first-line screening according to a combination of maternal age, fetal NT and DV-PIV, the patients were divided into a high-risk group in need of invasive testing, an intermediate-risk group that underwent cfDNA testing and a low-risk group that had no further testing. At the upper- and lower-risk cut-offs of 1:10 and 1:3000, respectively, the high-risk group (≥ 1:10) consisted of 0.2%, 64.9%, 69.0% and 58.2% euploid, trisomy 21, trisomy 18 and trisomy

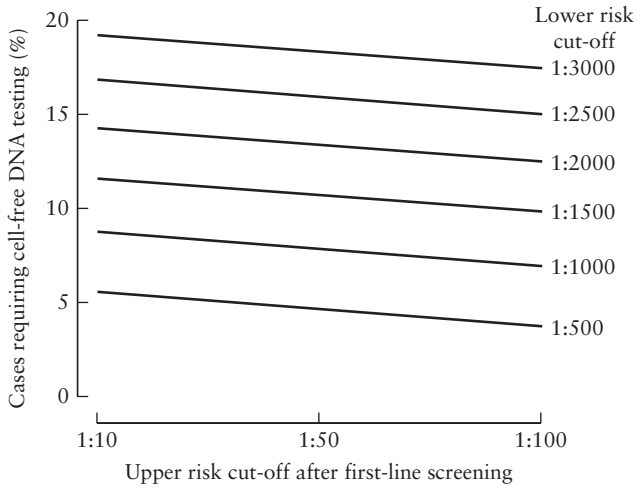

Figure 1 Proportion of women undergoing screening for aneuploidies requiring cell-free DNA testing in contingent screening, according to the risk cut-off used to define the high- and low-risk groups.

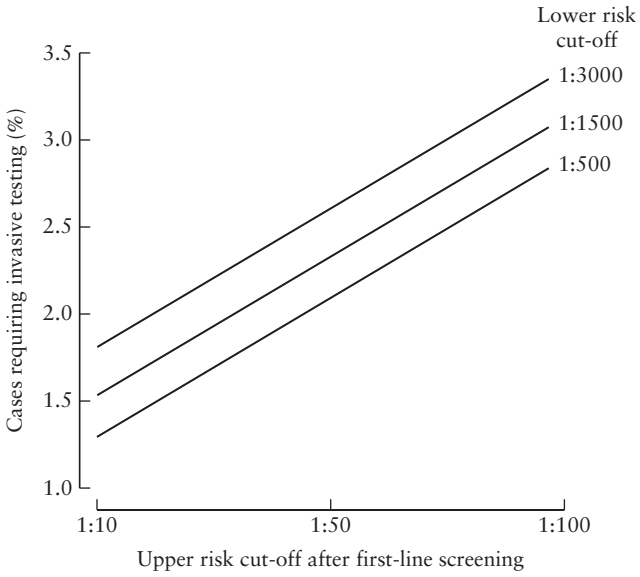

Figure 2 Proportion of women undergoing screening for aneuploidies requiring invasive testing in contingent screening, according to the risk cut-off used to define the high- and low-risk groups.

13 fetuses, respectively. The respective values for the intermediate-risk group (1:11–1:3000) were 19.0%, 31.6%, 26.7% and 35.6% and the total proportion of pregnancies belonging to this group was 19.1%. Such a policy would result in an overall FPR of 0.85%, DRs for trisomies 21, 18 and 13 of 96.3%, 94.9% and 90.7%, respectively, and a total invasive-testing rate of 1.82%.

The cost of the contingent policy applied to 100 000 pregnancies would be 100 000 × €150 for ultrasound screening plus 19 123 × €500 for cfDNA testing in the intermediate-risk group, plus 392 × €1000 for invasive testing as a result of positive cfDNA results, plus 865 × €1000 for invasive testing in the high-risk group after first-line screening, resulting in a total cost of €25 818 500. If the price for cfDNA testing is halved to €250, the total cost would be €21 037 750. These cost estimates are based on the assumption that all results from cfDNA testing were informative, but if the test failure is 3% and these cases undergo invasive testing, there would be an additional cost of €574 000.

Figures 1 and 2 demonstrate the proportion of cases requiring cfDNA analysis and invasive testing in contingent screening, according to the risk cut-off used to define the high- and low-risk groups.

DISCUSSION

Main findings of the study

Screening for trisomies 21, 18 and 13 according to a combination of maternal age, fetal NT and DV-PIV in all pregnancies, followed by invasive testing in the high-risk group (≥ 1:10) and cfDNA testing in the intermediate-risk group (1:11–1:3000) can potentially detect about 96%, 95% and 91% of cases, respectively, with a total FPR of 0.8%. On the assumption that the costs for first-trimester ultrasound screening, cfDNA testing and invasive testing are €150, €500 and €1000, respectively, the overall cost of such a policy would be approximately €250 per patient. If the price for cfDNA testing is halved to €250, the total cost would be about €210 per patient.

The alternative policy of universal screening by cfDNA testing can potentially detect about 99%, 97% and 92% of cases of trisomies 21, 18 and 13, respectively, with a total FPR of 0.4%, but at the overall cost of more than €500 per patient.

In this study, we defined the intermediate-risk group according to the cut-offs of 1:11 and 1:3000. However, we provide data that allow modifications of these cut-offs and the consequences of the proportion of the population requiring cfDNA testing and their effects on DR, FPR and cost.

Limitations of the study

This was not a prospective study, but a modeled analysis based on a series of assumptions. However, the model is based on reliable extensive data allowing general conclusions for population-based screening. The ultrasound examination data were obtained from a large prospective study including more than 85 000 normal and 490 trisomic pregnancies[13] and the performance of screening by cfDNA testing was summarized in a recent meta-analysis, which included 809 cases of trisomy 21, 301 of trisomy 18 and 85 of trisomy 13[9].

The overall screening performance of the contingent model relies on the quality of fetal NT and DV-PIV measurements. Assessment of DV flow was previously based on the classification of the shape of the a-wave into normal or abnormal[2], with very high positive- or negative-likelihood ratios, and therefore susceptible to operator bias. This problem has been overcome with the use of DV-PIV[5], which reduces the risk of bias and,

more importantly, facilitates ongoing quality assurance comparable with that for NT.

Comparison with previous studies

There is increasing evidence, from studies in both high-risk pregnancies and the general population, that the performance of screening for the major trisomies by cfDNA testing is superior to that achieved by previous methods of screening[9,15–18]. However, the test is expensive and therefore unlikely to be implemented as a first-line method of screening for the whole population. Consequently, it was proposed that cfDNA testing should be reserved for high- or intermediate-risk groups, as an effective, but cheaper, primary method of screening[9–11]. Previous health economic assessment studies have also concluded that cfDNA testing is cost effective only if it is embedded into a contingent screening policy[19,20].

In contingent screening, the group that would benefit most from cfDNA testing are patients with an intermediate risk. This group constitutes about 20% of the population and contains > 30% with trisomy 21 and > 25% with trisomy 18 or trisomy 13. The high-risk group (≥ 1:10) is very small (< 0.5% of the population) and yet contains a high proportion of trisomies 21, 18 and 13, as well as many other chromosomal abnormalities, and would therefore benefit from invasive testing[21]. The low-risk group (< 1:3000) is very large (>80% of the population) and contains less than 5% of the trisomies; it could therefore be argued that the cost and anxiety generated from cfDNA testing in this group are too high to justify such a policy.

In a recent screening study using the first-trimester combined test in about 21 000 pregnancies, the karyotype was abnormal in 212 cases, including 23 (11%) with an atypical result (aneuploidy other than trisomy 21, 18, 13 and sex chromosomal abnormality)[22]. It was demonstrated that a contingent policy with combined screening for all pregnancies and subsequent cfDNA testing only in cases with a risk between 1:50 and 1:1000 would detect 94% of all chromosomal abnormalities and about 70% of the atypical cases.

Implications for practice

The high cost of cfDNA testing at present necessitates that this high-performance test is offered to some, but not all, pregnancies. As demonstrated in this and previous studies, the best policy would be to offer cfDNA testing contingent on the results of a first-trimester method of screening and that the group which would benefit most from such a policy is the one with intermediate risks.

In previous studies, first-line screening was provided using the combined test[10,11], and in this study we demonstrated that results of a similar standard can be achieved by the use of ultrasound markers alone. Ultimately, the best option for a first-line screening test will depend on the expertise of sonographers at the screening center in measuring DV-PIV, in addition to NT, and whether the results from serum biochemical testing can be readily available at the time of the ultrasound examination. Ideally, patients should receive the best estimate of their risk in the same hospital visit as for the scan so that they can decide whether to undergo invasive testing, cfDNA testing or no further testing[23].

Conclusion

Incorporation of cfDNA testing into a contingent policy of early screening for the major trisomies, based on the risk derived from first-line screening according to a combination of maternal age, fetal NT and DV-PIV, can potentially detect a high proportion of affected cases with a very low FPR. Such a policy would be particularly attractive for prenatal screening centers, in which the results of serum biochemistry are not available in the same visit as for the ultrasound examination.

REFERENCES

1. 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:** 618–624.
2. 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:** 512–517.
3. Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11 + 0 to 13 + 6 weeks of gestation. *Ultrasound Obstet Gynecol* 2009; **33:** 18–22.
4. Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. *Ultrasound Obstet Gynecol* 2009; **33:** 259–264.
5. Maiz N, Wright D, Ferreira AFA, Syngelaki A, Nicolaides KH. A mixture model of ductus venosus pulsatility index in screening for aneuploidies at 11–13 weeks' gestation. *Fetal Diagn Ther* 2012; **31:** 221–229.
6. Wright D, Bradbury I, Benn P, Cuckle H, Ritchie K. Contingent screening for Down syndrome is an efficient alternative to non-disclosure sequential screening. *Prenat Diagn* 2004; **24:** 762–766.
7. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005; **25:** 221–226.
8. 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:** 542–547.
9. Gil MM, Akolekar R, Quezada MS, Bregant B, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal Diagn Ther* 2014; **35:** 156–173.
10. Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM. First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing. *Ultrasound Obstet Gynecol* 2013; **42:** 41–50.
11. Nicolaides KH, Syngelaki A, Poon LC, Gil MM, Wright D. First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing. *Fetal Diagn Ther* 2014; **35:** 185–192.

12. Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free b-hCG and pregnancy-associated plasma protein-A. *Hum Reprod* 2008; **23:** 1968–1975.
13. Wright D, Syngelaki A, Bradbury I, Akolekar R, Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. *Fetal Diagn Ther* 2014; **35:** 118–126.
14. Wright DE, Bray I. Estimating birth prevalence of Down's syndrome. *J Epidemiol Biostat* 2000; **5:** 89–97.
15. Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first trimester population. *Am J Obstet Gynecol* 2012; **207:** 374.e1–e6.
16. Song Y, Liu C, Qi H, Zhang Y, Bian X, Liu J. Noninvasive prenatal testing of fetal aneuploidies by massively parallel sequencing in a prospective Chinese population. *Prenat Diagn* 2013; **33:** 700–706.
17. Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ; CARE Study Group. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med* 2014; **370:** 799–808.
18. Quezada MS, Gil MM, Francisco C, Oròsz G, Nicolaides KH. Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10–11 weeks' gestation and the combined test at 11–13 weeks. *Ultrasound Obstet Gynecol* 2015; **45**: 36–41.
19. Cuckle H, Benn P, Pergament E. Maternal cfDNA screening for Down syndrome – a cost sensitivity analysis. *Prenat Diagn* 2013; **33:** 636–642.
20. Morris S, Karlsen S, Chung N, Hill M, Chitty LS. Model-based analysis of costs and outcomes of non-invasive prenatal testing for Down's syndrome using cell free fetal DNA in the UK National Health Service. *PLoS One* 2014; **4:** e93559.
21. Syngelaki A, Pergament E, Homfray T, Akolekar R, Nicolaides KH. Replacing the combined test by cell-free DNA testing in screening for trisomies 21, 18 and 13: impact on the diagnosis of other chromosomal abnormalities. *Fetal Diagn Ther* 2014; **35:** 174–184.
22. Kagan KO, Hoopmann M, Hammer R, Stressig R, Kozlowski P. Screening for chromosomal abnormalities by first trimester combined screening and noninvasive prenatal testing. *Ultraschall Med* 2014 Sep 25. [Epub ahead of print]
23. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999; **13:** 231–237.

