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First trimester contingent testing with either nuchal translucency or cell-free DNA. Cost efficiency and the role of ultrasound dating

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Conflict of interest
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Abstract

Objective. To evaluate the performance and cost efficacy of different first-trimester contingent screening strategies based on an initial analysis of biochemical markers. *Design.* Retrospective study. *Setting.* Swedish National Quality Register for prenatal diagnosis. *Population.* 35 780 women with singleton pregnancies. *Methods.* Serum values from first trimester biochemistry were re-analyzed in a contingent approach. For risks between 1:40 and 1:1000, risk estimates from nuchal translucency measurements were added and outcomes were compared using either a final cut-off risk of 1:200 to proceed with invasive testing or offering non-invasive prenatal testing. In a subgroup of 12 836 women with regular menstrual cycles the same analyses were performed using data on the last menstrual period for determining gestational age. The costs of detecting one case of aneuploidy were compared. *Main outcome measures.* Comparison of screening strategies. *Results.* The detection rate was the same (87%) in the contingent group as in complete combined screening, with only 41% requiring a nuchal translucency scan. As an alternative, offering non-invasive prenatal testing to the intermediate risk group would result in a detection rate of 98%, but the cost to detect one case of trisomy 21 would be 83% higher than the cost associated with traditional combined screening. *Conclusions.* First trimester examination using a contingent approach will achieve similar results compared with full combined screening. Non-invasive prenatal testing will not be cost-effective when a high proportion of pregnancies need further testing.

Abbreviations: β-hCG, serum free β-human chorionic gonadotropin; CRL, crown-rump length; CUB, combined ultrasound and biochemical; ET, embryo transfer; LMP, last menstrual period; NIPT, non-invasive prenatal testing; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A; T13/18, trisomy 13/18; T21, trisomy 21.

Introduction

First trimester screening based on nuchal translucency (NT) and serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) is the most common method used for prenatal detection of trisomies 21 (T21) and 13/18 (T13/18) (1,2). Using combined ultrasound and biochemical (CUB)

Key Message

Contingent first trimester screening utilizing non-invasive prenatal testing (NIPT) increases sensitivity for trisomy 21 but will be cost-effective only when a small proportion of women need additional testing.

screening, detection and false-positive rates of 90%/3–5% are observed (3–6). However, to maintain these levels of performance requires specifically trained ultrasound operators and quality assurance programs with continuing monitoring and audits (7). These prerequisites for establishing an efficient screening program have probably hampered the implementation of the method. An alternative method would be to offer the CUB test in a contingent approach (8,9). Women would then be stratified into three groups depending on their risk estimates following biochemical testing. Women with a high risk following serum testing would be offered an invasive test immediately, whereas women in the low risk group would not be offered any further tests. In the intermediate risk range, full CUB screening including NT scans would be offered. It has been demonstrated that the number of women requiring NT scans using this contingent approach can be substantially reduced with only a minor decrease in sensitivity (8–10). Non-invasive prenatal testing (NIPT) may here be a more discriminatory alternative. Analysis of cell-free DNA in maternal blood is a method for determining the fetal karyotype non-invasively with a high sensitivity and specificity (99%/0.1%) (11–13). As both false-negative and false-positive cases have been reported, it is still regarded as a screening test rather than a diagnostic method and screen-positive cases should be confirmed by an invasive test (14–18). An important limitation to contingent screening strategies has been the requirement of accurate pregnancy dating, as both ultrasound and biochemical markers vary with gestational age. Previous studies have indicated a need for ultrasound dating measuring the crown–rump length (CRL) rather than information based on the last menstrual period (LMP) (19). The aim of this study was to investigate the performance of a contingent model and to compare the results with regard to different methods for estimating gestational age. The costs of detecting one case of aneuploidy with different strategies were compared.

Material and methods

This retrospective study of pregnancies included in the Swedish National Quality Registry for prenatal diagnosis (from 2013 The Swedish Pregnancy Registry) was carried out over a 5-year period from 2006 to 2011. The cohort in the study group consisted of 35 780 women with singleton pregnancies including in vitro fertilization where CUB screening had been performed in the Stockholm area at five different ultrasound units. All outcomes of the pregnancies including any chromosomal aberrations were known. The screening protocol has been described more extensively in a previous report (20). All data were de-identified following reports of pregnancy outcomes and before comparisons were made between the different groups. Ethical approval for the study was obtained from the local Research Ethics Committee.

There were 176 cases of T21, 65 cases of T13/18 and 35 539 unaffected pregnancies within the cohort. The median maternal age at the time of NT scan was 35 years. In 27 821/35 780 women (78%), serum samples were taken at 9–11 gestational weeks, a median of 12 days before the NT scan, which was booked at 12 gestational weeks. The maternal serum samples were collected at outlying antenatal clinics and analyzed using the AutoDELPHIA analyzer (PerkinElmer Life Science, Waltham, MA, USA) at the laboratory of the Karolinska University Hospital with standard kits. NT measurements were carried out by midwives and doctors certified by and in accordance with the guidelines of the Fetal Medicine Foundation in London (www.fetalmedicine.com). All biochemical marker and NT measurements were converted to multiples of the gestational medians (MoM) based on CRL measurements at the time of the NT scan. Biochemical markers were adjusted for maternal weight, smoking, ethnicity, in vitro fertilization pregnancy or a previous pregnancy affected with a trisomy. Individual risk estimates were calculated using an algorithm developed by the Swedish National Quality Register for prenatal diagnosis and previously reported by our group based on likelihood ratios of the serum markers (free β-hCG and PAPP-A) and NT from Gaussian distributions in normal and affected pregnancies in our local population (21). Women with a risk ≥1:200 at the time of the scan were considered screen-positive and offered further invasive diagnostic testing. The detection rate for T21 in this cohort with complete CUB screening was 87% at a false-positive rate of 5.1%.

To compare traditional complete CUB screening with that of a contingent approach described by previous investigators (8,9), the same final risk cut-off of 1:200 as in the complete CUB screening program was used. We applied a two-stage protocol where women with a high-risk cut-off of 1:40 and a low-risk cut-off of 1:1000 following assessment of the double test were identified using a statistical approach described by Christiansen and Larsen (8).The high-risk cut-off was chosen at this level as an additional NT scan would presumably not be able to shift the woman to the low risk group following complete screening (8). The study group was divided into three groups according to the women's initial risk from the double test. Women with a high risk (≥1:40) would be offered an invasive diagnostic test immediately, women with a low-risk (≤1:1000) would not be offered any further testing and the remaining women with an intermediate risk between 1:40 and 1:1000 would be offered an additional NT scan. In the latter group, a combined risk calculation would be made and women with a final risk

greater than 1:200 were defined as screen-positive and added to the initial high-risk group. Pregnancies with a final combined risk of <1:200 were defined as screen-negative and added to the initial low risk group.

Calculations concerning the sensitivity and specificity of the contingent test at various cut-off limits for high and low risk, respectively, using a final cut-off risk of 1:200 for T21 alone or for T21 as well as T13/18, were computed. The outcomes of the contingent approach were also investigated if NIPT was undertaken in the intermediate risk group as an alternative to offering a NT scan. Information on the dates of the LMP or embryo transfer (ET) were recorded in the register at the time of the examination from 2009 onwards. A total of 12 836 women from this period (79%) had a history of either regular menstrual cycles or an ET date that could be used for dating the pregnancies instead of using ultrasound and the performance of the contingent model was also examined in this group. Only women where the difference in estimation of gestational age according to the LMP compared with CRL dating through ultrasound was less than 14 days were included. In pregnancies with aneuploidies, 62/65 pregnancies with T13/18 and 44/176 cases of T21 matched these criteria and could be dated through either LMP or ET and assessed in a contingent model. In our economic analysis, we applied local costs in euros (€) for NT (90€), the double test (36€), invasive testing through chorion villus sampling (755€) and for cell-free DNA (744€). The cost of detecting one case of either T21 or T13/18 including costs for invasive testing using the contingent approach as compared with complete CUB screening was calculated with the assumption that all test-positive cases underwent invasive testing.

Results

The analysis included 35 780 singleton pregnancies and there were 176 cases of T21, 65 cases of T13/18 and 35 539 unaffected pregnancies within the cohort. The detection rates and false-positive rates for T21 and T13/18 in this cohort using complete CUB screening were 87 and 85% vs. 5.1 and 1.9%, respectively. The results of the contingent screening approach concerning the detection of T21 (Table 1) as well as both T21 and T13/18 (Table 2) are demonstrated in the cohort at various cut-off levels. Using initial high and low risk cut-offs of 1:40 and 1:1000, respectively, and employing a final cut-off of 1:200 to have an invasive procedure for those women identified in the intermediate risk category, would achieve a detection rate of 86.9% at a false-positive rate of 5.3%, with only 41% of women requiring a NT scan (Figure 1). Offering the contingent approach for detection of T13/18 as well as T21 with the same cut-offs as described above would result in a detection rate of 88% of all trisomies at a false-positive rate of 6.7%, with 46% of patients requiring a NT scan (Figure 2). In the subgroup of women with gestational age determined using either the date of their LMP or ET and employing contingent testing with the same cut-offs as described above would result in a 79% detection rate of all trisomies at a false-positive rate of 3%, with 61% requiring a NT scan (Table 3). The costs to detect one case of a chromosomal anomaly with

Table 1. Screening performance and need for an additional nuchal translucency scan for the intermediate risk group following contingent testing for trisomy 21 (n = 176) with various risk cut-off values in 35 715 women with gestational age determined by ultrasound.

Biochemistry High-risk cut-off ≥	Biochemistry Low-risk cut-off ≤	Nuchal translucency frequency, %	Detection rate, %	False-positive rate, %
1:20	1000	43.4	86.9	4.7
1:40	1000	41.4	86.9	5.3
1:100	1000	36.2	90.3	9.6

Table 2. Screening performance and need for an additional nuchal translucency scan for the intermediate risk group following contingent testing for trisomies 21 and 13/18 (n = 241) with various risk cut-off values in 35 780 women with gestational age determined on the basis of ultrasound.

Biochemistry High-risk cut-off ≥	Biochemistry Low-risk cut-off ≤	Nuchal translucency frequency, %	Detection rate, %	False-positive rate, %
1:20	1000	48.4	86.7	5.7
1:40	1000	45.8	87.6	6.7
1:100	1000	39.6	91.3	12.0

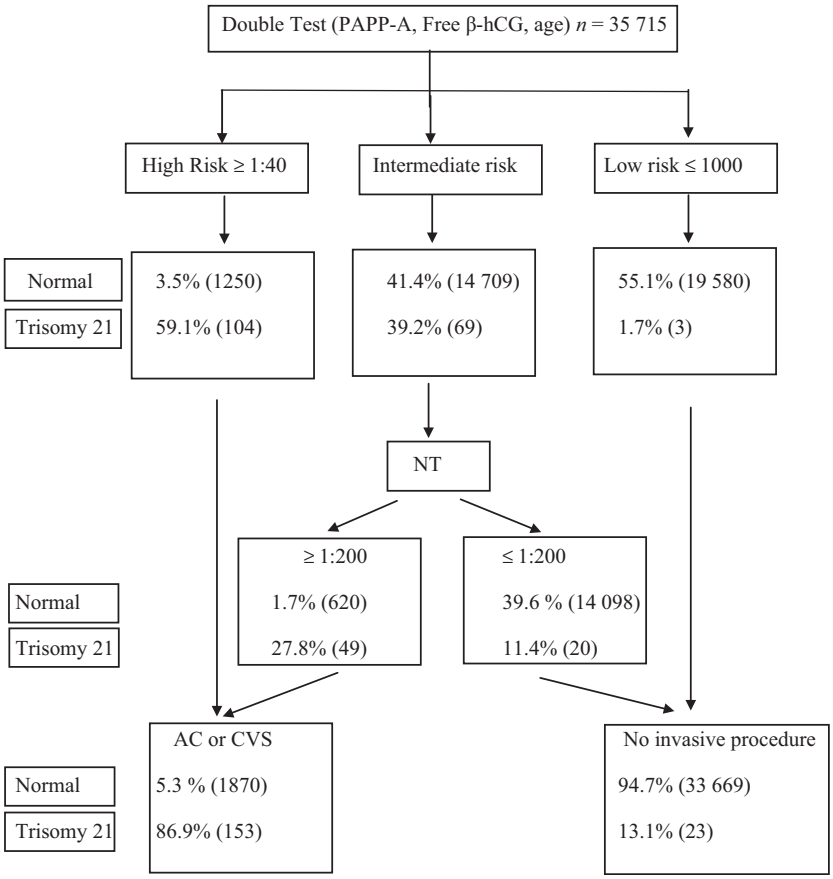

Figure 1. Contingent screening model for trisomy 21. Risk estimates following an initial screening with maternal serum biochemistry and age would be stratified into three groups, high, intermediate and low risk. Pregnancies with a risk greater than 1:40 would be offered invasive testing immediately, low risk women with a risk lower than 1:1000 would not be offered any further testing. Pregnancies with an intermediate risk between 1:40 and 1:1000 would be offered a nuchal translucency scan. Women with a final risk greater than 1:200 would be offered invasive testing. AC, amniocentesis; CVS, chorionic villus sampling; NT, nuchal translucency.

the various screening and dating strategies are shown in Table 4. Using the contingent CRL-dependent approach for all trisomies, the cost per detected case was 25% lower than with complete CUB screening. In a similar comparison with full CUB screening, the cost per detected case of any trisomy could be reduced by 38% using the LMP-dependent contingent test but with 61% of women requiring an NT scan. If instead NIPT was used as a second line test for detection of only T21 and offering this test to the 41% of pregnancies in the intermediate risk group, the detection rate would increase to 98%, reducing the false-positive rate by a third but the cost per detected case would almost triple compared with using NT in a contingent approach.

Discussion

In this study, the results from our cohort demonstrate that using the contingent approach based on initial serum biochemistry, the same performance may be achieved as with traditional complete CUB screening but at a substantially lower cost per detected case. We chose to include cases of T13/18 in our comparison as these aneuploidies are also part of the first trimester screening test. Using the contingent approach, the same detection rate of 88% could be achieved with less than half (46%) of the pregnant women requiring an NT scan and with a 25% reduction of costs per detected case of aneuploidy. These results are calculated under the assumption that the gestational age could be assessed with ultrasound through measurement of CRL by a midwife or gynecologist at the woman's referring unit during her first visit, which is already common practice in many settings today. A dating scan assessing CRL with ultrasound does not require high-resolution machines, advanced training and certification of competence of the operators or external quality control, as opposed to NT screening programs (7). The alternative of only using data from the LMP for

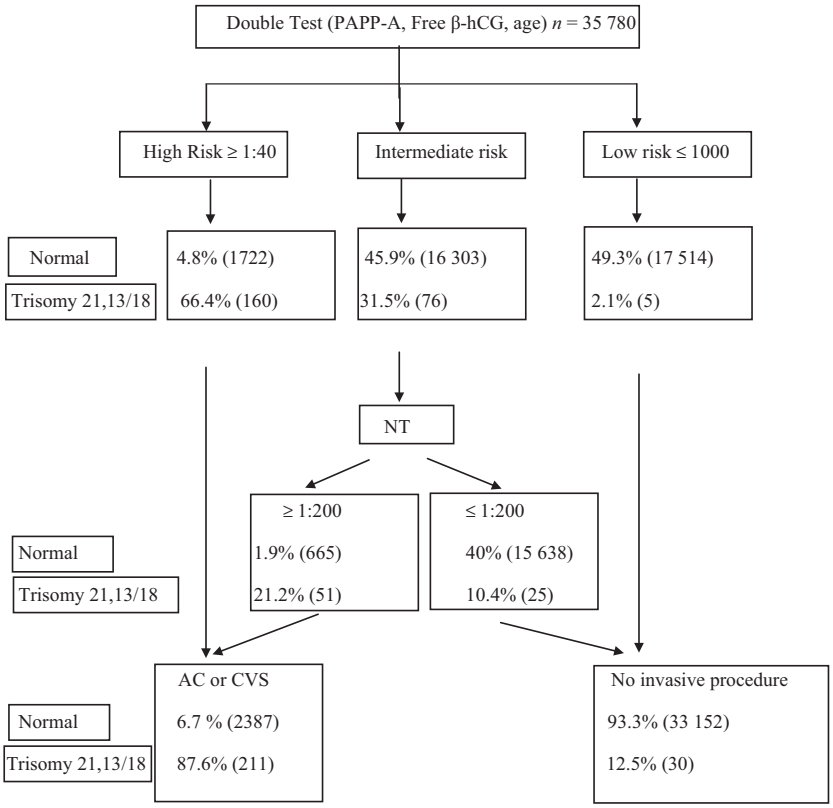

Figure 2. Contingent screening model for trisomy 21, 13/18. Risk estimates following an initial screening with maternal serum biochemistry and age would be stratified into three groups, high, intermediate and low risk. Pregnancies with a risk greater than 1:40 would be offered invasive testing immediately, low risk women with a risk lower than 1:1000 would not be offered any further testing. Pregnancies with an intermediate risk between 1:40 and 1:1000 would be offered a nuchal translucency scan. Women with a final risk greater than 1:200 would be offered invasive testing. AC, amniocentesis; CVS, chorionic villus sampling; NT, nuchal translucency.

assessing risk with the double test has a higher frequency of false-positive cases according to a previous report from van Heesch et al. in a smaller group of a 100 women (19). To our knowledge, no previous studies have reported on the outcomes of a contingent approach for first trimester screening when determining gestational age with data based on LMP/ET. In our cohort, 80% of women (12 836) had either an optimal menstrual history with a record of their LMP or ET date that could be used for analysis, which is consistent with previous studies from our region but higher than in the study by van Heesch et al. (19,22). However, the results utilizing this approach demonstrate that using the same cut-offs as in the CRL-dependent analysis, the detection rate would be almost 10% lower (79% vs. 88%). The majority of women (61%) would also be required to have an NT scan in the end even though the overall cost per detected case could be reduced. Therefore, this method for carrying out contingent screening would probably not be used other than in remote areas or in settings offering only primary care.

A contingent protocol based on the results of maternal serum biochemistry could be a way to introduce first trimester risk assessment in regions that today cannot provide screening options for pregnant women due to lack of specifically trained ultrasound operators. Here, the offer of an NT scan could focus on the group of women who have most need of it. Another option would be to offer contingent screening and utilize cell-free DNA analysis in maternal blood in the intermediate risk group. Cell-free DNA testing can detect at least 99.5% of cases of T21 with an FPR of 0.1% (11,13,23,24). In our study group, there would be a benefit of a greater than 10% increase in the detection rate from 87 to 98% and a substantial lowering of the false-positive rate by a third. However, the cost per detected case of T21 would almost double compared with traditional CUB screening (77 000 vs. 42 000 €). The results in our study can be added to the conclusions of Johnson et al. (25) who found that offering NIPT in a contingent model to the 10–20% of pregnancies with the highest risk cut-offs following a first trimester quad test (PAPP-A, β-hCG, AFP, placental

Table 3. Screening performance and need for an additional nuchal translucency scan for the intermediate risk group following contingent testing for trisomies 21 and 13/18 (n = 106) with various risk cut-off values in 12 836 women with gestational age determined on the basis of the last menstrual period or embryo transfer date.

Biochemistry High-risk cut-off ≥	Biochemistry Low-risk cut-off ≤	Nuchal translucency frequency, %	Detection rate, %	False-positive rate, %
1:20	1000	62.0	79.3	2.7
1:40	1000	61.2	80.2	3.0
1:100	1000	57.0	90.6	6.3

Table 4. Screening performance, need for either an additional nuchal translucency (NT) scan or non-invasive prenatal testing (NIPT) and the cost per detected case of chromosomal anomaly following different strategies: traditional combined ultrasound and biochemical (CUB) screening, contingent testing for trisomies 21 (T21) and 13/18 (T13/18) in either crown–rump length (CRL) or last menstrual period (LMP) dated pregnancies with high and low risk cut-offs (40/1000) and a final risk after NT examination greater than 1:200 or NIPT in the intermediate group.

Method	*n*	NT/NIPT frequency, %	Detection rate n	%	False-positive rate, %	Cost per detected case, €
CUB T21	35 715	100	153/176	87	5.1	42 000
CUB T21 + 13/18	35 780	100	211/241	88	5.8	31 000
Contingent test T21 NT: 40/1000 CRL dated	35 715	41	153/176	87	5.3	28 000
Contingent test T21 + 13/18 NT: 40/1000 CRL dated	35 780	46	211/241	88	6.7	23 000
Contingent test T21 NIPT: 40/1000 CRL dated	35 715	41	173/176	98	3.5	77 111
Contingent test T21 NT: 40/1000 LMP dated	12 774	58	36/44	82	2.7	41 000
Contingent test T21 + 13/18 NT: 40/1000 LMP dated	12 836	61	84/106	79	3.0	19 000

growth factor (PlGF)) would be cost-neutral compared with performing NT scans in the same group (25). Clearly, as demonstrated in this study, employing a contingent model with current pricing, there is an upper limit to how many pregnancies can be examined with NIPT before losing cost efficacy of the program. Selecting optimal cut-offs will always be a trade-off between detection rates and false-positive rates. As demonstrated in Tables 1 and 2, altering the high-risk cut-off from 1:40 to 1:100 would result in a 3% increase in detection rate but at the same time double the false-positive rate to more than 10%, which most clinicians would consider unacceptably high. There are several limitations to our study. Both the detection and false-positive rates are influenced by the rather high median maternal age (35 years) in our cohort and these may have been different if the age distribution had been closer to that of pregnant women in general. The cost estimates for CRL-dependent contingent testing were analyzed with the assumption that gestational age could be determined by ultrasound at the antenatal clinics without additional costs. The actual costs of these examinations are hard to assess, as both ultrasound machines and staff are present and have many different clinical commitments. Also the economic costs and social as well as psychological implications of non-detected cases and false-positive test results were not addressed. The costs of lost lives following invasive procedures were not estimated. This question has become even more complex as a recent meta-analysis of miscarriages following invasive procedures indicated a non-significant increase of only 0.1–0.2% (26). As only a smaller proportion of women with T21 pregnancies had an optimal menstrual history or ET data to rely on for analysis, the costs for detecting a case of T21 using the LMP-dependent contingent test were overestimated compared with the costs for detecting all trisomies using the same method. Miscarriages and twin pregnancies could be identified through a basic dating scan at antenatal clinics but a potential

negative effect of the contingent approach is that fetal malformations would fail to be detected at the time of the NT scan if not all women were examined. However, even though there have been several convincing reports on the efficacy of first trimester examination in diagnosing malformations (27–29), currently most anomalies are detected later at the 18–20-week scan and the main role for the first trimester scan is still the detection of aneuploidies (30).

In conclusion, a contingent approach based on initial maternal serum biochemistry will achieve similar results to full CUB screening. NT scans could be offered only to pregnancies with intermediate risk estimates assessed with the double test in regions where ultrasound units and trained staff are not already available. This would reduce the need for NT scans in the majority of women without compromising efficacy and offer an opportunity to allocate resources to other areas of women's health care. NIPT is not a cost-efficient alternative when used in a contingent model and a high proportion of pregnancies require further examination.

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