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UOG Journal Club: July 2013

Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies

M. M. Gil, M. S. Quezada, B. Bregant, M. Ferraro, K. H. Nicolaides

Volume 42, Issue 1, Date: July 2013, pages 34–40

First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing

K. H. Nicolaides, D. Wright, L. C. Poon, A. Syngelaki, M. M. Gil.

Volume 42, Issue 1, Date: July 2013, pages 41–50

Journal Club slides prepared by Dr Leona Poon
(UOG Editor for Trainees)

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Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies

Gil et al., UOG 2013

Objective

To explore the feasibility of routine maternal blood cfDNA testing in screening for trisomies 21, 18 and 13 at 10 weeks' gestation.

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Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies

Gil et al., UOG 2013

Patients and Methods

- Prospective observational study
- Singleton pregnancy and live fetus with CRL 32–45 mm
- Women attending The Fetal Medicine Centre, London, between October 2012 and April 2013, were screened for trisomies 21, 18 and 13 by cfDNA testing at 10 wks and the combined test at 12 wks

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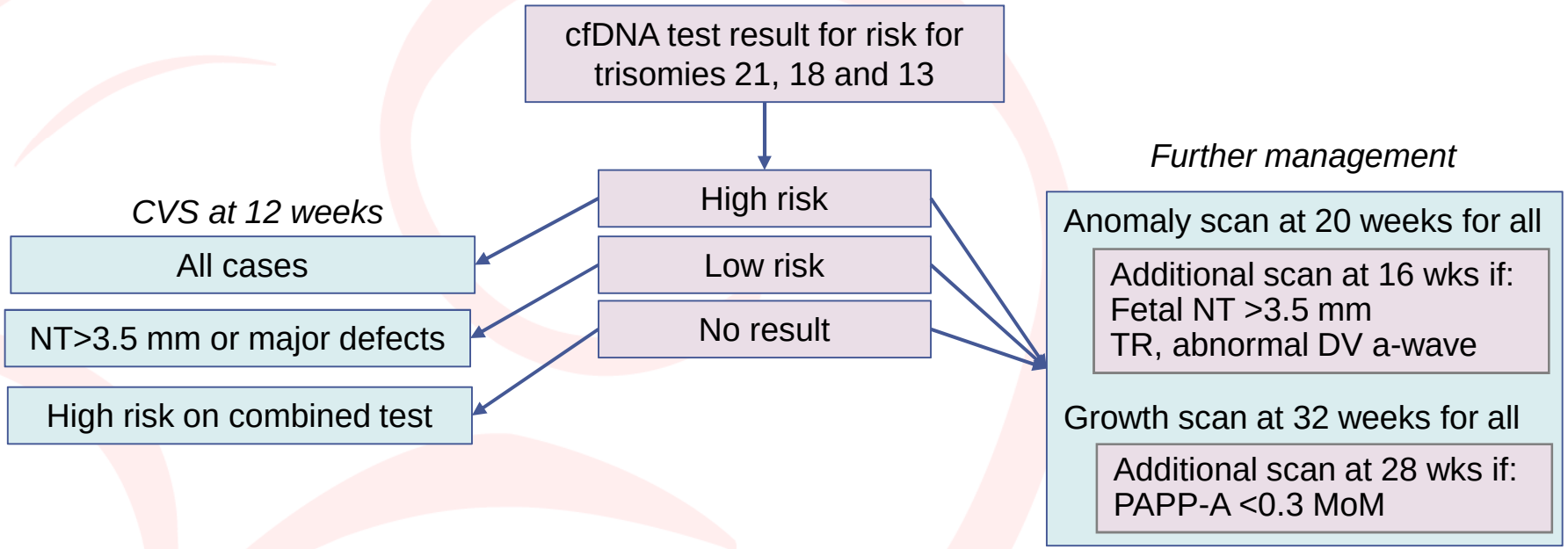
Patients and Methods



Protocol for pregnancy management according to results of maternal blood cfDNA testing and the combined test

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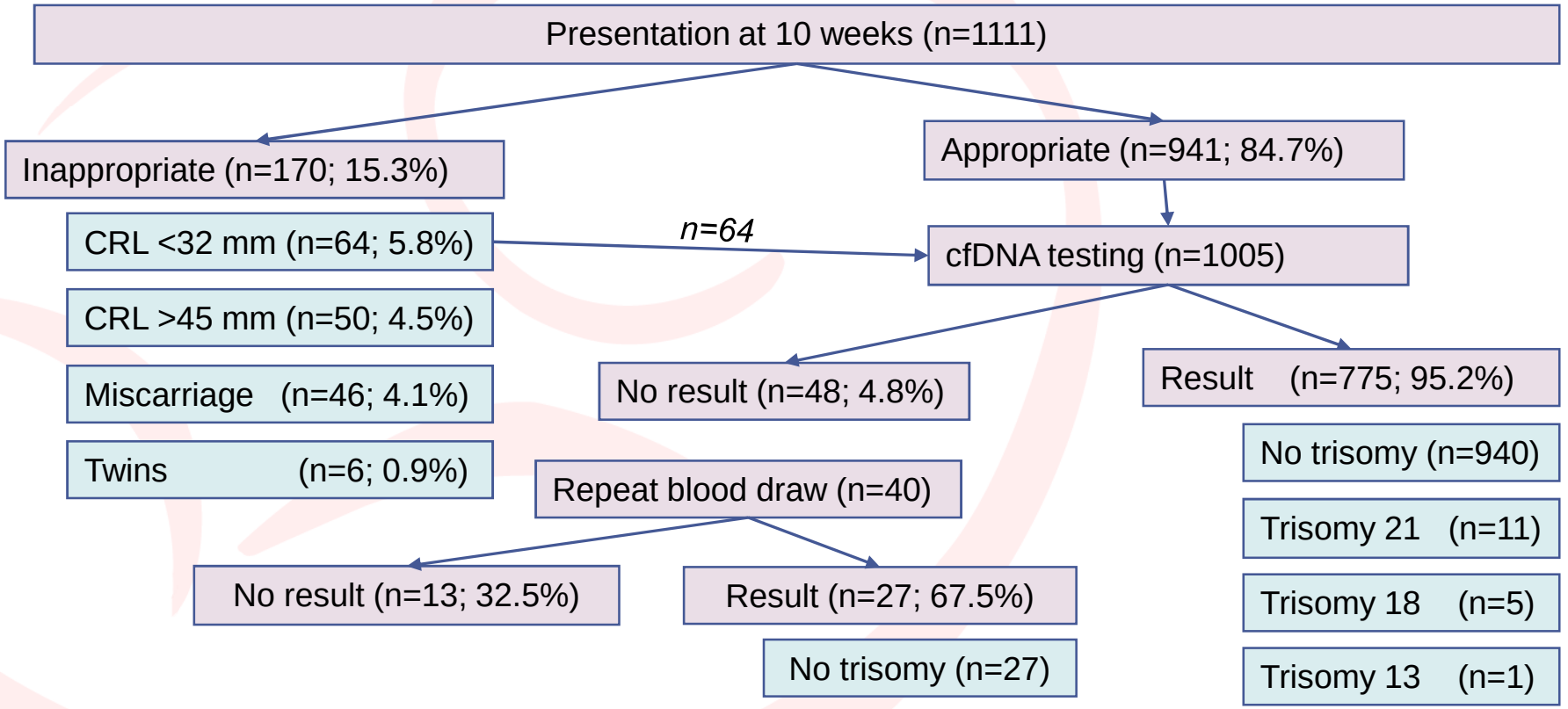
Results

Median maternal age of 37 (range 20-49) years; 98% had results within 14 days



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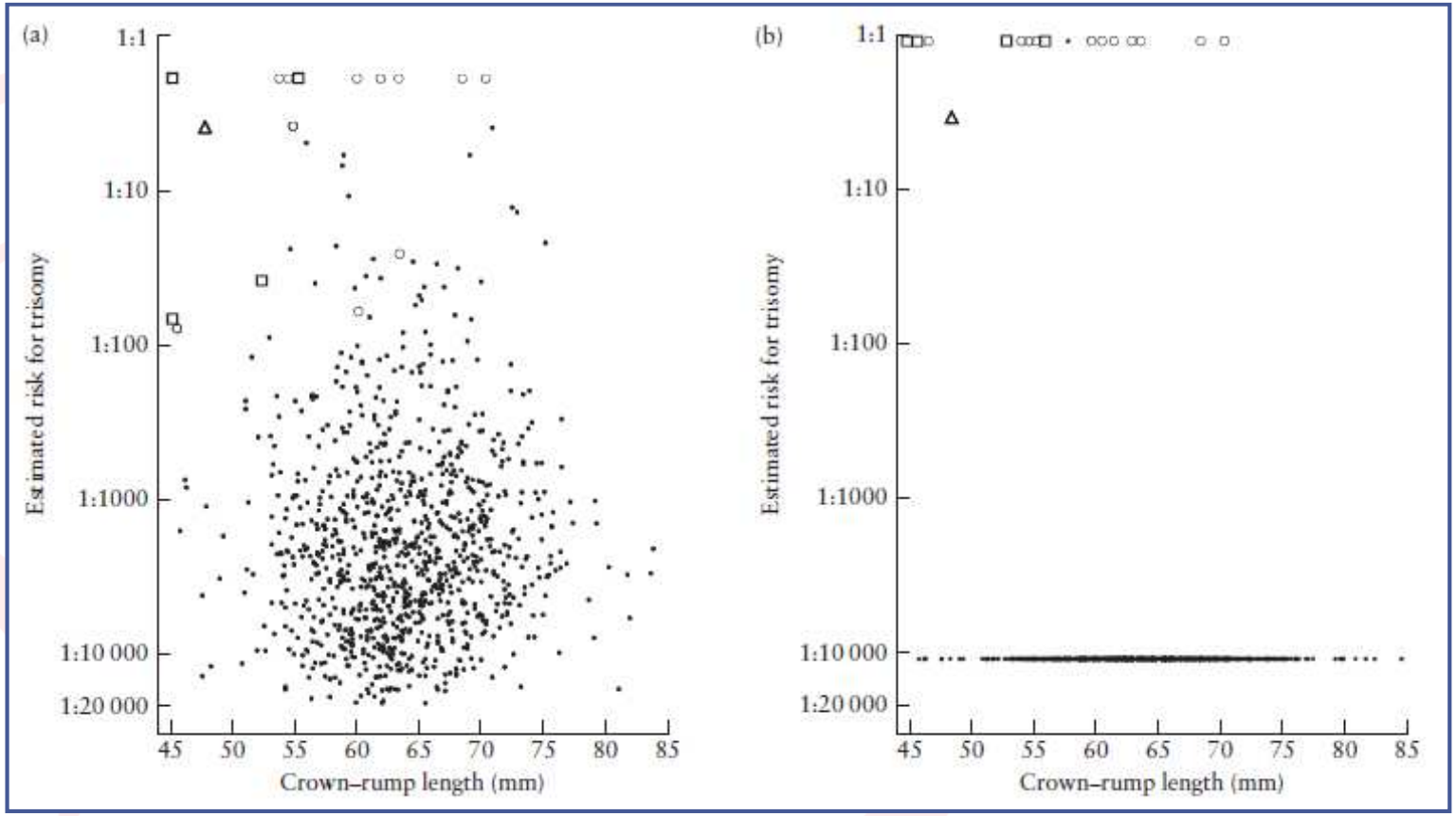

Estimated risk for trisomy in the pregnancies with trisomy 21 (○), trisomy 18 (□) or trisomy 13 (Δ) and assumed euploid fetuses (●), by combined test (a) and cfDNA test (b)

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Results

Findings/outcomes in the 17 pregnancies classified by cfDNA testing as being at high risk for aneuploidy

cfDNA risk	*Combined test risk*	*Karyotype*	*Pregnancy outcome*	*No. of cases*
T21 risk >99%	1:2	T21	TOP	7
T21 risk >99%	1:4	T21	TOP	1
T21 risk >99%	1:27	T21	TOP	1
T21 risk >99%	1:81	Not done	Miscarriage	1
T21 risk >99%	1:65	T21	TOP	1
T18 risk >99%	1:2	T18	TOP	2
T18 risk >99%	1:39	T18	TOP	1
T18 risk >99%	1:71	T18	TOP	1
T18 risk >99%	1:5861	Normal	Continue	1
T13 risk 34%	1:4	T13	TOP	1

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Discussion

- Study demonstrates the feasibility of implementing cfDNA testing in early pregnancy screening for trisomies in singleton pregnancies
- Blood sampling at 10 weeks and USS at both 10 and 12 weeks retains the advantage of 1st trimester diagnosis as for the combined test
- It is necessary to confirm +ve results of cfDNA testing by fetal karyotyping, as one of the cases of suspected trisomy 18 had a normal karyotype
- In this population (median maternal age, 36.7 yrs), the screen-positive rate on cfDNA testing was 1.7% *vs* 5.0% for the combined test (risk cut-off 1:100)

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Discussion

- The main advantages of cfDNA testing, compared with the combined test, are:
  - Substantial reduction in FPR
  - Reporting of results as very high or very low risk, which makes it easier for parents to decide in favour of or against invasive testing
- cfDNA testing has substantially reduced the rate of invasive testing but some women still desire a diagnostic test to provide certainty of exclusion of not only the common trisomies but all other aneuploidies

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Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies

Gil et al., UOG 2013

Limitations

- Since most pregnancies are continuing at the time of writing, it is not possible at present to assess the sensitivity of the screening tests in identifying trisomic pregnancies
- The study did not diagnose aneuploidies other than trisomies 21, 18 and 13, however, invasive testing should be recommended in cases with high fetal NT even if cfDNA test gives low risk results, because in such cases these trisomies account for only 75–80% of clinically significant aneuploidies

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Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies

Gil et al., UOG 2013

Conclusions

- This study has shown that routine screening for trisomies by cfDNA testing at 10 weeks is feasible, allowing diagnosis of aneuploidies and the option of pregnancy termination within the first trimester

- Advantages of cfDNA testing:
  - Substantial reduction in FPR
  - Clear separation of high and low risk results

- Disadvantages of cfDNA testing:
  - Failure of cfDNA testing in providing results
  - Need to investigate abnormal results by invasive testing

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First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing

Nicolaides et al., UOG 2013

Objective

To define risk cut-offs with corresponding detection rates and false-positive rates in screening for trisomy 21 using maternal age and combinations of first-trimester biomarkers in order to determine which women should undergo contingent maternal blood cfDNA testing

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First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing

Nicolaides et al., UOG 2013

Patients and Methods

- Analysis of prospectively collected data between March 2006 and May 2012 on the following biomarkers: fetal NT, DV PIV at 11-13 wks gestation and serum free β-hCG, PAPP-A, PlGF and AFP at 8-13 wks gestation

- Patient-specific risks for trisomies 21, 18 and 13 were estimated from a combination of maternal age, fetal NT, serum free β-hCG and PAPP-A and women considering their risks to be high were offered CVS or amniocentesis

- Estimates of risk cut-offs, DRs and FPRs were derived for combinations of biomarkers and these were used to define the best strategy for contingent cfDNA testing.

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First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing

Nicolaides et al., UOG 2013

Results

- Study population of 87,241 cases, including 324 cases of trisomy 21 and 86,917 unaffected pregnancies with normal fetal karyotype or the birth of a phenotypically normal neonate

- The observed number of cases of trisomy 21 is consistent with that expected 333.1 (P=0.61) given the maternal and gestational age distribution of the cohort

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Results

	Nuchal translucency with:					
	PAPP-A and β-hCG		PAPP-A, β-hCG, AFP and PlGF		PAPP-A, β-hCG, AFP, PlGF and DV-PIV	
Risk cut-off (1:x)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)
100	2.1	85.2	1.8	87.9	1.3	92.4
500	7.2	92.9	6.1	94.4	3.9	96.3
1000	11.9	95.3	9.9	96.3	6.1	97.5
1500	15.7	96.4	12.9	97.2	8.0	98.0
2000	19.0	97.1	15.4	97.7	9.5	98.3
2500	21.8	97.5	17.5	98.1	10.9	98.6
3000	24.3	97.9	19.5	98.3	12.2	98.7
3500	26.6	98.1	21.2	98.5	13.3	98.9
4000	28.7	98.3	22.7	98.7	14.3	99.0
5000	32.4	98.6	25.6	98.9	16.2	99.1
6000	35.6	98.9	28.0	99.1	17.9	99.2
7000	38.4	99.0	30.1	99.2	19.5	99.3
8000	41.0	99.1	32.1	99.3	20.9	99.4

Modeled DR and FPR in first-trimester screening for trisomy 21 by various combinations of biomarkers at fixed risk cut-offs. Rates were standardised so that they relate to the pregnant population of England and Wales in 2011.

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Results

	NT, PAPP-A, β-hCG			NT, PAPP-A, β-hCG, PlGF, AFP			NT, DV-PIV, PAPP-A, β-hCG, PlGF, AFP		
Risk cut-off	*cfDNA (%)*	*DR (%)*	*IR (%)*	*cfDNA (%)*	*DR (%)*	*IR (%)*	*cfDNA (%)*	*DR (%)*	*IR (%)*
100	2.3	84.7	0.34	2.1	87.4	0.33	1.6	92.2	0.32
500	7.5	92.2	0.36	6.4	93.9	0.35	4.2	95.9	0.34
1000	12.1	94.6	0.37	10.2	95.6	0.36	6.4	96.9	0.34
1500	15.9	95.6	0.38	13.1	96.6	0.37	8.3	97.3	0.35
2000	19.2	96.3	0.39	15.6	96.9	0.37	9.8	97.6	0.35
2500	22.0	96.9	0.39	17.7	97.3	0.38	11.2	98.0	0.35
3000	24.5	96.9	0.39	19.7	97.6	0.38	12.5	98.0	0.35
3500	26.8	96.9	0.39	21.4	98.0	0.38	13.6	98.3	0.35
4000	28.9	97.3	0.40	22.9	98.0	0.38	14.5	98.3	0.36
5000	32.6	97.6	0.40	25.8	98.3	0.39	16.4	98.3	0.36
6000	35.8	98.0	0.41	28.2	98.3	0.39	18.1	98.6	0.36
7000	38.6	98.0	0.41	30.3	98.6	0.39	19.7	98.6	0.36
8000	41.2	98.0	0.41	32.3	98.6	0.39	21.1	98.6	0.36

Overall cfDNA testing rate, DR and rate of invasive testing (IR) by CVS in contingent first-trimester screening for trisomy 21. In these calculations it was assumed that cfDNA testing failed to provide a result in 4% of cases, and that in those with a result, DR was 99.5% and FPR was 0.1%.

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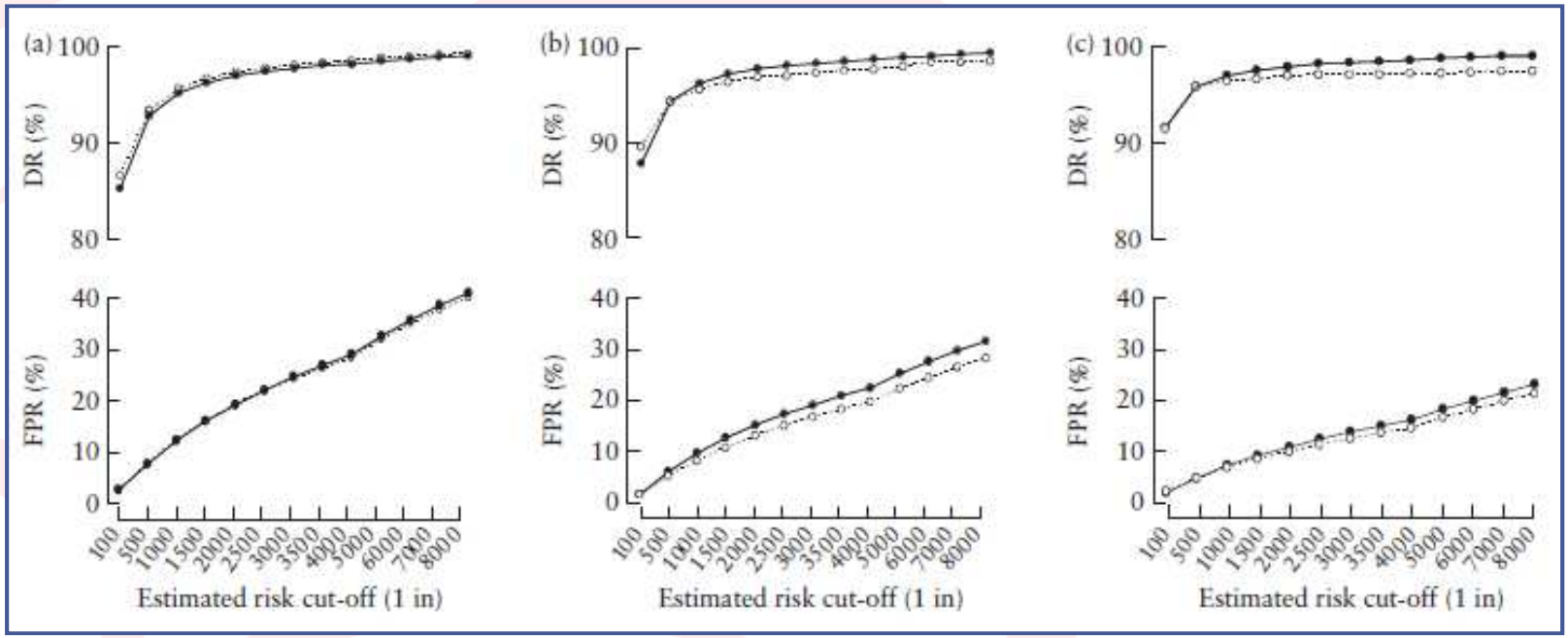

Modeled (●) and empirical (○) DR (upper lines) and FPR (lower lines) in screening for trisomy 21 by combined testing (maternal age, NT, free β-hCG and PAPP-A) (a), combined test with addition of PlGF and AFP (b) and combined test with addition of DV PIV, PlGF and AFP (c)

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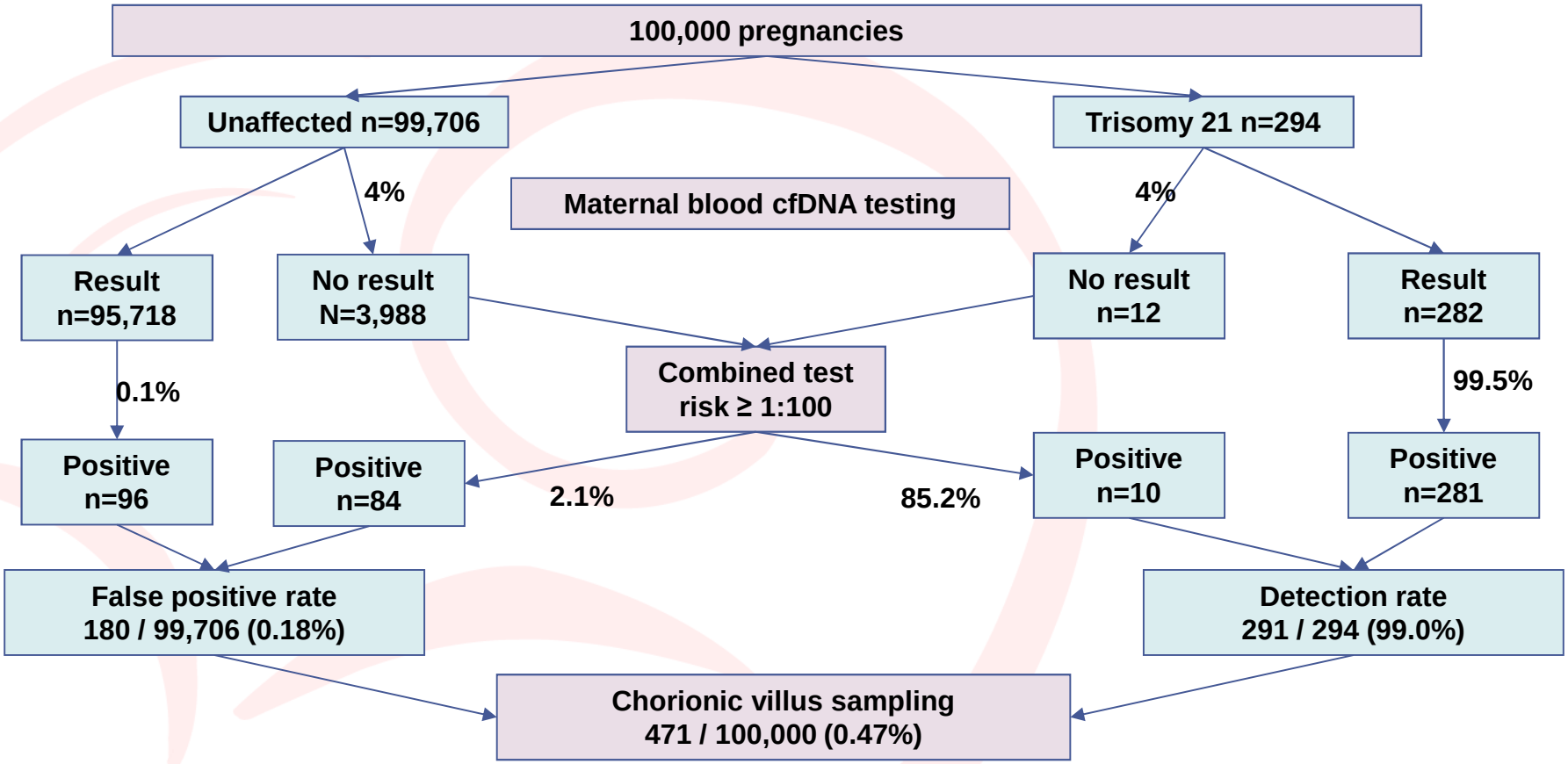

First-line screening by *cfDNA testing alone* in a population of 100000 pregnancies, including 294 with trisomy 21

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First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing

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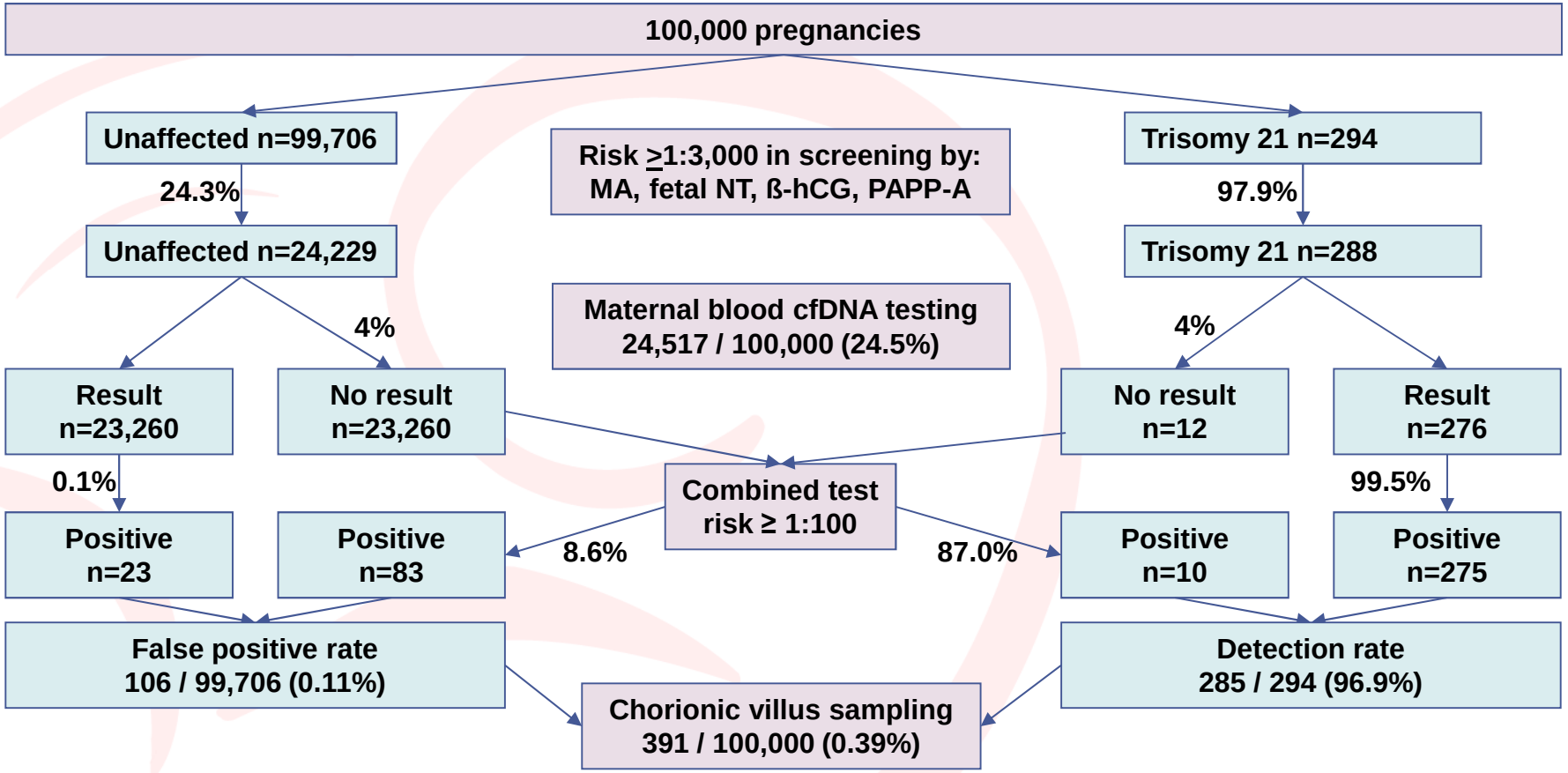

Contingent screening by combined test and cfDNA testing in pregnancies with a risk of ≥ 1:3000 in a population of 100000, including 294 with trisomy 21

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Discussion

- This study has defined risk cut-offs with corresponding DRs and FPRs for first-line screening for trisomy 21, maternal age and combinations of fetal NT, DV PIV, free β-hCG, PAPP-A, PlGF and AFP
- Contingent screening could achieve a DR of 98% for trisomy 21, at an overall invasive testing rate <0.5%, with cfDNA test being offered to about 35%, 21% and 11% of cases identified by first-line screening using combined test alone, combined test with PlGF and AFP and combined test with PlGF, AFP and DV PIV, respectively
- An increase in DR from 98% to 99% would require a >10% increase in the number of cases requiring cfDNA testing

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Discussion

Content of first-line screening

- Measurement of serum PlGF and AFP can be performed in the same sample and by the same automated machines as for free β-hCG and PAPP-A at little extra cost. Inclusion of these metabolites would substantially increased the DR and reduce the FPR
- Fetal NT is an essential part of screening for aneuploidies with and without the use of cfDNA testing. It is also a marker for many other clinically significant aneuploidies, cardiac defects and several genetic syndromes
- 11-13 weeks scan is not only for the measurement of CRL and NT but also for detailed examination of the fetus and early diagnosis of major anomalies

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Discussion

Health economic evaluation

- First-trimester scan is essential for pregnancy care and it is therefore an integral part of any strategy of screening for aneuploidies
- The basis for any economic evaluation would be the relative cost of invasive testing and components of biochemical screening compared with cfDNA testing and also the DR for trisomy 21
- The cost of cfDNA testing is comparable to invasive testing and the substantial reduction of the latter by introduction of cfDNA testing would be cost neutral with the major advantage of avoidance of miscarriage

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Limitations

- This study was confined to the prediction of trisomy 21 because:
  - In the last 40 years this has been the main factor in defining strategies of screening for aneuploidies
  - To minimise the complexity of the model that would arise from inclusion of other aneuploidies
- Maternal serum PlGF and AFP were measured in a smaller population within the cohort, consequently, the modeled measures screening performance, especially at very high DRs, are somewhat speculative and may be biased optimistically

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Limitations

Assumptions:

- The only factor defining the decision for or against invasive testing was the estimated risk for trisomy 21, which is unlikely to be true
- The model assumes that cfDNA testing can detect 99.5% of cases of trisomy 21 with a FPR of 0.1%, based on published studies in high-risk pregnancies
- In 4% of cases the cfDNA test would fail to provide a result

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Conclusions

Screening for trisomy 21 by cfDNA testing contingent on the results of an expanded combined test would retain the advantages of the current method of screening, but with a simultaneous major increase in DR and decrease in the rate of invasive testing.

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Discussion points

- Should cfDNA testing be implemented as universal screening or contingent screening?
- How should we manage cases (4%) with failed cfDNA testing?
- In the case of high fetal NT or other major fetal anomalies, should we offer cfDNA testing?
- Can non-invasive prenatal testing replace the need of routine first-trimester scan?