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ABSTRACT

Background: There is limited data regarding non-invasive prenatal testing (NIPT) in low risk populations and the ideal aneuploidy screening model for a pregnant population has yet to be established.

Aims: To assess the implementation of NIPT into clinical practice utilising both first and second line screening models.

Materials and Methods: Three private practices specializing in obstetric ultrasound and prenatal diagnosis in Australia offered NIPT as a first line test, ideally followed by combined first trimester screening (cFTS), or as a second line test following cFTS, particularly in those with a calculated risk between 1:50 and 1:1000.

Results: NIPT screening was performed in 5267 women, and as a first-line screening method in 3359 (63.8%). Trisomies 21 and 13 detection was 100% and 88% for trisomy 18. Of cases with known karyotypes, the positive predictive value (PPV) of the test was highest for trisomy 21 (97.7%) and lowest for monosomy X (25%). Ultrasound detection of fetal structural abnormality resulted in the detection of 5 additional chromosome abnormalities, two of which had high risk cFTS results. For all chromosomal abnormalities, NIPT alone detected 93.4%, a contingent model detected 81.8% ($p=0.097$) and cFTS alone detected 65.9% ($p<0.005$).

Conclusions: NIPT achieved 100% T21 detection and had a higher DR of all aneuploidy when used as a first line test. Given the false positive rate for all aneuploidies, NIPT is an advanced screening test, rather than a diagnostic test. The benefit of additional cFTS was the detection of fetal structural abnormalities and some unusual chromosomal abnormalities.

INTRODUCTION

Prenatal aneuploidy screening is an integral part of obstetric management and all women should "receive the best possible estimate of her personal risk of fetal aneuploidy".[1] Until recently this was best achieved using combined nuchal translucency (NT) and biochemical first trimester screening (cFTS), with trisomy detection rates (DR) of 85% for a 4.8% false positive rate (FPR).[2] The recent introduction of non-invasive prenatal testing (NIPT) analysing DNA released from cytotrophoblastic cells into maternal plasma (cell-free fetal DNA), has improved detection of the main trisomies (13, 18 and 21) at a very low FPR .[3] The disorders detected has increased to include sex chromosomal abnormalities, while the cost has continued to fall.

The ideal method for aneuploidy screening in the pregnant population is yet to be established, and the mechanism for integration of NIPT into current screening is unclear. The International Society for Prenatal Diagnosis and the International Society for Ultrasound in Obstetrics and Gynaecology both recommended NIPT use only for high risk patients during the time of this study.[1,4] Their concerns relate to limited data regarding NIPT in low risk populations (as this cohort is likely to have a lower aneuploidy DR, the test will have a lower positive predictive value and higher FPR), the availability of appropriate pre and post-test counselling, the cost of NIPT and the continued importance of first trimester ultrasound to assess fetal morphology and NT.[1,4] The performance of NIPT as a second-line screening test will be limited by the DR and screen positive rate of the original test and the result delayed by an additional 7 - 14 days.[5,6]

The aim of this study is to assess the implementation of NIPT in singleton pregnancies, either as first-line screening (Model 1) or as second-line screening (Model 2) following cFTS or the detection of a structural abnormality, in conjunction with specialized ultrasound and counselling services in a mixed-risk Australian obstetric population.

MATERIALS AND METHODS

Three metropolitan private practices specializing in obstetric ultrasound and prenatal diagnosis in Australia combined data regarding NIPT. Each practice has a well-established cFTS program incorporating detailed fetal structural assessment, NT measurement, nasal bone assessment and placental serology (free beta human chorionic gonadotrophin and pregnancy-associated plasma protein A, with placental growth factor additionally incorporated in one of the practices).

There was no patient selection process specified by the tertiary referral practices. Patients were referred for NIPT either as a first-line screening test (Model 1) or, where the primary referral was for cFTS, NIPT was discussed during post-test counselling, particularly when the 'intermediate' a posteriori risk was between 1 in 51 and 1 in 1000, or in those where a fetal structural anomaly or aneuploidy marker was identified (Model 2). Approximately 350 of these patients have been included in a prior publication by Hui et al.[6]

For the Model 1 patients, preliminary dating ultrasound was performed and pre-test counselling was provided by a prenatal diagnosis specialist or genetic counsellor. The benefits of additional cFTS at approximately 13 weeks gestation were also discussed, including structural assessment

along with screening for early-onset pre-eclampsia and other adverse pregnancy outcomes. A high or low risk status from an NIPT result was not altered by a differing cFTS result.

Model 2 patients either had cFTS as a direct referral to the practice or were referred for NIPT having had cFTS performed elsewhere. Some patients with no prior screening or low risk cFTS had sonographic aneuploidy markers or a fetal structural abnormality identified at the routine morphology ultrasound and were offered the option of NIPT. A geneticist was involved as necessary. Blood was transported to the USA (Harmony™, Ariosa, California) and the cost fell from $1500 to $495AUD during the study.

Invasive testing was discussed with all patients who had: 1) a high risk result from primary cFTS utilizing the Fetal Medicine Foundation algorithm[7], 2) a high risk NIPT result, utilizing the FORTE™ algorithm[8] or 3) in those where a fetal structural anomaly was detected, despite a low risk on prior screening. Invasive testing utilised quantitative fluorescent polymerase chain reaction or fluorescence in-situ hybridization for rapid analysis, and karyotyping (in one practice) or microarray comparative genomic hybridization (aCGH) in the other 2 practices.

Pregnancy outcome information was gathered from referring doctors and both antenatal and postnatal laboratory results. The outcome of the high risk cases were specifically requested, however failure to karyotype miscarriages and some of those with high risk results for sex chromosomal abnormalities affected the outcome ascertainment. Low risk results were not individually followed up, expecting adverse outcomes to be notified to the practice by the referring doctors.

Descriptive statistics were applied, using chi-square analysis when comparing discrete categorical variables and Students t-test when comparing means, with significance determined by a p-value < 0.05.

Ethical approval was provided by the Royal Women's Hospital (Victoria) Research Committee and Human Research Ethics Committee (29.7.2015) who decreed that this study meets the National Health and Medical Research Council requirements for quality assurance / audit projects.

RESULTS

Between March 2013 and August 2014, 5267 women undertook NIPT, with the 3 practices contributing 2490, 1460 and 1317 patients respectively. The median maternal age was 36.0 years and the median body mass index (BMI) was 23.3 kg/m^2, with no significant differences between those undertaking NIPT as a first or second-line test, or between the practices.

The mean gestation at time of blood collection for NIPT was 12.2 weeks (range 10.0 - 35.7) with 42.8% of tests performed under 11 weeks gestation (Figure 1).

NIPT was used as a first line method of screening in 3359 cases (63.8%) and second line in 1908 cases (36.2%), including 1872 cases following cFTS and 36 cases following the finding of a structural abnormality with no prior cFTS. The pathways and outcomes of screening and prenatal diagnosis for first line NIPT and NIPT contingent on the cFTS result are depicted in Figure 2. Of those with known karyotypes, there was no difference in numbers of trisomy 21 fetuses between those undertaking Model 1 [n=28 (1 in 120)] and those undertaking Model 2 [n=15 (1 in 127)], p=0.97.

The primary test failure rate was 2.4% (129) and 49 (38.0%) chose not to repeat the test as they had a low risk result from cFTS. Of those opting for repeat sampling, 47 (58.8%) successfully obtained a result. The most common association for repeated failure was BMI exceeding 30kg/m^2. None of the original test failure cases had an aneuploid pregnancy.

Of those participants with a recorded result following NIPT, the aneuploidy risks were less than 1/10000 in 5196 (98.7%) patients, 3 patients had an 'intermediate' risk result (between 1/100 and 1/1000) and 116 recorded a 'high' risk result (1/99 to >99%). From the 119 participants with high or intermediate NIPT risk results, there were 14 with unknown outcomes (due to miscarriage without karyotyping [5, including 3 with > 99% risk for trisomy 21], continuing pregnancies with no available antenatal or postnatal testing [7; the majority with high risk results for sex chromosome abnormalities including 4 with normal newborn examinations, plus a 27 week stillbirth] or lost to follow-up [2]). Of those with known chromosome results, the positive predictive value of the test was highest for trisomy 21 (43 true positive cases from 44 high risk tests; 97.7%), followed by trisomy 18 (7/8; 87.5%) and XYY (3/4; 75%) and less than 55% for all other trisomies, the poorest performance being for monosomy X (6/24; 25.0%) (Table 1).

Comparison was made of aneuploidy detection rates between three possible screening models: 1) NIPT alone, 2) a contingent model using cFTS as a first line screening test (CVS offered if > 1 in 50, NIPT offered if 1/50-1000 and no further testing if < 1/1000) or, 3) cFTS alone using a risk cut-off of 1/300 (Table 2). NIPT alone identified 93.4% of all chromosome abnormalities compared with 81.8% for the contingent model and 65.9% for cFTS alone (Model 1 vs 3, p<0.005). There was no significant difference in the detection of trisomies 21, 18 or 13 between the models. NIPT performed superiorly to the other models in sex chromosome abnormality detection (100%, 58.3% and 8.3% respectively: 1 vs 2 p=0.0135; 1 vs 3 p<0.0005; 2 vs 3 p=0.0321).

Invasive prenatal tests (CVS = 64, amniocentesis = 77) were performed in 139 participants (2.6% of the cohort), with two additionally undertaking amniocentesis following a mosaic CVS result. The most common indication was a high risk NIPT result (n=88) or a fetal structural abnormality (n=29). The indication for the remaining 24 cases comprised maternal concern [12], failed NIPT [7], parental chromosomal rearrangement [3], early fetal growth restriction [1] and fragile X testing [1]. If the current study cohort only underwent cFTS, the age-matched screen positive rate would have resulted in a significant increase in invasive tests (n=293, 5.5%; p<0.0001). Invasive prenatal testing was more likely in the event of a high NIPT risk for trisomies 21, 18, 13 and monosomy X (85.7%) compared to those with a high risk for XXX, XXY or XYY (46.7%: p < 0.0005).

Five additional chromosome abnormalities were detected by invasive testing in fetuses with low NIPT aneuploidy risks where structural abnormalities were identified in the second (n=143) or third trimesters (n=3). These comprised a deletion of 5p [1], mosaic trisomy 22 [1] and pathogenic copy number variants only detectable by microarray CGH [3]. No additional rare chromosome abnormalities were found in the 26 fetuses with structural abnormalities identified in the first trimester. Of the 29 invasive tests for structural issues, 23 had a micro-array and 6 had karyotyping. Three of the karyotypes were aneuploid, leaving only 3 cases where micro-array testing may have been of benefit.

DISCUSSION

This is the first Australian study reporting on the prospective use of NIPT within an existing first trimester screening program in an unselected population. The principal finding was a 100% detection of trisomies 21 and 13 and 88% for trisomy 18, a screen positive rate of 2.2% and a FPR of only 0.7%, although the certainty of these rates may be influenced by incomplete outcome ascertainment. The PPV of screening was high for trisomy 21, as noted in the NEXT study, but reduced for the other trisomies, notably for monosomy X.[9] Although NIPT detects the majority of major trisomies, there are false positive and false negative results which confirms NIPT as an advanced screening test rather than a diagnostic prenatal test. Despite NIPT being neither locally available nor publicly funded during the study period, there was increasing demand for NIPT testing which corresponded to greater public knowledge of NIPT and reducing cost. Despite a low risk cFTS (<1:1000) and counselling, 908 women still had NIPT. These facts suggest that the patients in this cohort favoured a prenatal screening model with high detection rate and safety, with cost being a secondary concern.

Nearly two-thirds of the patients had NIPT as a first-line screen, usually performed under 11 weeks gestation. This model has the advantage of the highest aneuploidy detection of all current screening modalities with the lowest FPR and results are available before the detailed first trimester ultrasound. Thus all relevant information is available in a timely fashion should invasive testing by CVS be necessary. CVS tests placental mesenchymal DNA which is derived from the inner cell mass like the fetus, so is more likely to be representative of the fetal DNA, while NIPT tests trophoblastic DNA [10].

Comparing three possible screening models in the current cohort showed NIPT alone had the highest DR for all chromosome abnormalities, followed by the contingent model, then cFTS alone, although the numbers were insufficient to reach statistical significance in all but the comparison between NIPT alone and cFTS alone. This infers that first line NIPT achieves a higher DR as it is not restricted by the performance of the primary screening test. The difference was most marked for sex chromosome aneuploidy and least for trisomies 21, 18 or 13. Combined FTS assisted in the detection of other chromosomal abnormalities not detectable by NIPT, even though it was not designed for that purpose. First trimester anatomical assessment detected two cases with a severe structural anomaly but normal karyotype, which resulted in pregnancy termination.

NIPT was typically used in addition to cFTS or early anatomical assessment in this study cohort, rather than as a replacement for it, which was similar to the findings of an early survey of NIPT use by Australian sonologists.[11] Three of the false positive cases and the one false negative trisomy 18 case from NIPT had correct assignment of risk by cFTS, hence the two in tandem may be of assistance in determining appropriate management.[12] The single false positive trisomy 21 result on NIPT was also high risk on cFTS.

Additional benefits of cFTS include fetal structural assessment, NT measurement, and risk assessment for adverse pregnancy outcomes such as early onset pre-eclampsia.[13,14,15] Fetal structural anomalies and enlarged NT measurements are found in a number of chromosome abnormalities and genetic syndromes that are not detected on current NIPT.[16, 17,18] The current cohort included 5 additional chromosome abnormalities found as a result of invasive testing in fetuses with structural abnormalities and low NIPT aneuploidy risks Four of these 5 cases were identified after the first trimester. Combined FTS was performed in 3 of these cases, two of which were at high risk. In a large Danish cohort undertaking cFTS, 23% of phenotypically important atypical chromosome abnormalities found would not have been detected using current NIPT.[16] Similarly Susman et al. found that NIPT would not have detected 11% of the chromosomal abnormalities which were detected due to cFTS in Victoria.[19]

Some patients used NIPT as added reassurance after low risk assessment with cFTS. The risk used as the cut-off for each patient was self-determined, although guidance was given based on published contingent models.[11,20]

Sex chromosome abnormalities are more common at birth than the major trisomies and are often phenotypically normal.[26,27] Conventional prenatal screening did not directly seek to identify sex chromosomal abnormalities and whether they should be detected antenatally has been questioned.[22,26] Earlier detection enables early intervention, particularly with hormonal therapy for Turner's and Kleinfelter's syndromes, as these syndromes are often not diagnosed until puberty.[28] Skilled post-diagnosis counselling has been shown to assist understanding of the condition and reduce pregnancy termination rates.[28] NIPT has poorer detection and a higher FPR for sex chromosome abnormalities than for the other trisomies, particularly for monosomy X, which was confirmed in this study.[26] Causes for this include sequencing bias of guanine and cytosine, maternal mosaicism, maternal XXX and placental mosaicism.[26,27] NIPT may lead to

maternal testing and the discovery of a maternal sex chromosome aneuploidy or mosaicism, complicating the counseling.[26]

NIPT caused a significant reduction in invasive testing compared with the previous cFTS program. Some women will undertake NIPT as it is perceived as being without risk, even though they would not consider invasive testing or pregnancy termination. Others with a low risk on both NIPT and cFTS are still not sufficiently reassured and will have definitive testing. Structural abnormalities found at a detailed first trimester scan or second trimester morphology assessment added 29 invasive tests in this cohort but identified 5 atypical chromosomal abnormalities (17.2%).

The strengths of this study include the large cohort, appropriate counselling and pre-test ultrasound. Many patients also had cFTS and the option of invasive testing with microarray analysis. Potential weaknesses of the study include: incomplete pregnancy outcome ascertainment, a private practice population that may not be representative of the Australian obstetric community, being older, predominantly Caucasian, from a higher socioeconomic group and some patients referred after a high risk screening result elsewhere. The trisomy 21 prevalence was similar for both first and second line NIPT screening groups (1 in 120 and 1 in 127 respectively) but higher than expected from a cohort with a median age of 36 years (1 in 196 at 12 weeks gestation), suggesting the potential of referral and self-selection bias.[29]

Conclusion

This large study confirms that NIPT has a very high aneuploidy detection rate with a very low FPR. The DR is limited by the first screening test, so it would not have been the same had NIPT only been used contingent on the cFTS result. Sex chromosome abnormalities accounted for half of all high risk NIPT results, but have a high FPR and create challenging counselling. The study also demonstrated the benefits of additional cFTS in detection of structural anomalies and atypical chromosomal abnormalities. The use of NIPT will increase as the number of screened conditions increases and the cost reduces, but the introduction is best handled within the context of an experienced first trimester screening program with access to appropriate counselling and prenatal diagnosis.

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REFERENCES

1 Benn P, Borell A, Chiu R, et al. Position statement from the Aneuploidy Screening Committee on Behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn 2013; 33:622-629.

2 Russo ML, Blakemore KJ. A historical and practical review of first trimester aneuploidy screening. Semin Fetal & Neonatal Med 2014: 19(3):183-187.

3 Benn P, Cuckle H and Pergament E. Non-invasive prenatal testing for aneuploidy: current status and future prospects. Ultrasound Obstet Gynecol 2013; 42: 15-33.

4 ISUOG consensus statement on the impact of non-invasive prenatal testing (NIPT) on prenatal ultrasound practice. Ultrasound Obstet Gynecol 2014; 44: 122-123.

5 Royal College of Obstetricians and Gynaecologists, Non-invasive prenatal testing for chromosomal abnormality using maternal plasma DNA, March 2014, Scientific Impact Paper No. 15, www.rcog.co.uk (accessed March 2015)

6 Hui L, Teoh M, da Silva Costa F, et al. Clinical implementation of cell-free DNA-based aneuploidy screening: perspectives from a national audit. Ultrasound Obstet Gynecol 2015; 45: 10-15.

7 Wright D, Spencer K, Kagan O, et al. First trimester combined screening for trisomy 21 from 7-14 weeks gestation. Ultrasound in Obstet Gynecol; 36(4): 404-411.

8 Sparks AB, Struble CA, Wang ET, et al. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012; 206: 319.e1-9.

9 Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for Non-invasive Examination of Trisomy. April 1, 2015 DOI: 10.1056 New Engl J Med oa1407349

10 Grati F, Malvestiti F, Ferreira J, et al. Fetoplacental mosaicism: potential implications for false-positive and false-negative noninvasive prenatal screening results. Gen Med 2014; 16: 620-624.

11 Hui L, Teoh M, Piessens S, Walker SP. Early clinical experience of cell-free DNA-based aneuploidy screening: a survey of obstetric sinologists in Australia and New Zealand. Aust NZ J Obstet Gynaecol 2015; 55(2): 138-143.

12 Quezada MS, Gil MM, Francisco C, et al. 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.

13 Poon LC, Syngelaki A, Akolekar R, Lai J, Nicolaides KH: Combined screening for preeclampsia and small for gestational age at 11–13 weeks. Fetal Diagn Ther 2013; 33: 16–27.

14 Park FJ, Leung CHY, Poon LCY, Williams PF, Rothwell SJ, Hyett JA. Clinical evaluation of a first trimester algorithm predicting the risk of hypertensive disease of pregnancy. Aust NZ J Obstet Gynaecol 2013; 53: 532-539.

15 Zhong Y, Zhu F, Ding Y. Serum screening in first trimester to predict pre-eclampsia, small for gestational age and preterm delivery: systematic review and meta-analysis. BMC Pregnancy and Childbirth 2015; 15:191

16 Petersen OB, Vogel I, Ekelund C, et al. Potential diagnostic consequences of applying non-invasive prenatal testing: population-based study from a country with existing first-trimester screening. Ultrasound Obstet Gynecol 2014; 43: 265-271.

17 Syngelaki A, Pergament E, Homfray T, et al. 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(3): 174-184.

18 Chitty LS, Hill M, White H, et al. Noninvasive prenatal testing for aneuploidy – ready for prime time? Am J Obstet Gynecol 2012; 206(4): 269-275.

19 Susman MR, Amor DJ, Muggli E, Jaques AM, Halliday J. Using population-based data to predict the impact of introducing noninvasive prenatal diagnosis for Down syndrome. Genet Med 2010; 12(5): 298-303.

20 Nicolaides KH, Syngelaki A, Poon LC, et al. 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

21 Faas BH, Ligt J, Janssen I, et al. Non-invasive prenatal diagnosis of fetal aneuploidies using massively parallel sequencing-by-ligation and evidence that cell-free fetal DNA in the maternal plasma originates from cytotrophoblastic cells. Expert Opin Biol Ther 2012; 12(1): S19-S26.

22 Samango-Sprouse C, Banjevic M, Ryan A, et al. SNP-based non-invasive prenatal testing detects sex chromosome aneuploidy with high accuracy. Prenat Diagn 2013; 33: 643-649.

23 Struble CA, Syngelaki A, Oliphant A, et al. Fetal fraction estimate in twin pregnancies using directed cell-free DNA analysis. Fetal Diagn Ther 2014; 35(3): 199-203.

24 Bevilacqua A, Gil MM, Nicolaides KH, et al. Performance of screening for aneuploidies by cell-free DNA analysis of maternal blood in twin pregnancies. Ultrasound Obstet Gynecol 2015; 45: 61-66.

25 Wright D, Wright A and Nicolaides KH. A unified approach to risk assessment for fetal aneuploidies. Ultrasound Obstet Gynecol 2015; 45: 48-54.

26 Nicolaides KH, Musci TJ, Struble CA, et al. Assessment of fetal sex chromosomes aneuploidy using directed cell-free DNA analysis. Fetal Diagn Ther 2014; 35(1): 1-6.

27 Mazloom AR, Dzakula Z, Oeth P, et al. Noninvasive prenatal detection of sex chromosomal aneuploidies by sequencing circulating cell-free DNA from maternal plasma. Prenat Diagn 2013; 33: 591-597.

28 Bourke E, Snow P, Herlihy A, et al. A qualitative exploration of mothers' and fathers' experiences of having a child with Kleinfelter syndrome and the process of reaching this diagnosis. Euro J Hum Genet 2014; 22(1): 18-24.

29 Nicolaides, Fetal Medicine Foundation, 2004, accessed 15 June 2015, <www.fetalmedicine.com/synced/fmf/FMF-English.pdf>

TABLES & FIGURES

Table 1. NIPT performance by aneuploidy type

	Total high risk results	True positive results	False positive results	Positive predictive value (%)*	Unknown
T21	47	43	1	91.4	3 (miscarriage=3)
T18	12	7	4	58.3	1 (miscarriage=1)
T13	2	1	1	50.0	0
XO	27	6 (mosaic=2)	18	25.0	3 (miscarriage=1)
XXX	14	3	6	33.3	5
XXY	13	6	5	54.5	2
XYY	4	3	1	75.0	0
	119	**69**	**36**	**57.9**	**14**

* Where outcome is known

Table 2. Detection of pregnancies with known abnormal chromosomes using 1)NIPT only; 2) A contingent model, using cFTS as a first line test with invasive testing for those with risk above 1:50 and NIPT also for those at intermediate risk (1:51 – 1:1000) and 3) cFTS only with a 1:300 cut-off. Percentages show detection rates.

	All chromosome abnormality (n = 5267) *	Patients undertaking NIPT only		Chromosome abnormalities in those undertaking cFTS (n = 3311) **	Contingent	cFTS only
T21	43	43 (100%)		22	21 (95.5%)	20 (90.9%)
T18	8	7		7	7	6
T13	2	2		0	0	0
45X	6	6		3	3	1

XXX	3	3		3	1	0
XXY	6	6		5	2	0
XYY	3	3		1	1	0
Other	5	0		3	1	2
TOTAL	76	71/76 (93.4%)		44	36/44 (81.8%)	29/44 (65.9%)

* Data from the entire cohort

** Data only from cases with cFTS data, either before or after NIPT

Figure 1. Distribution of gestation at non-invasive prenatal testing

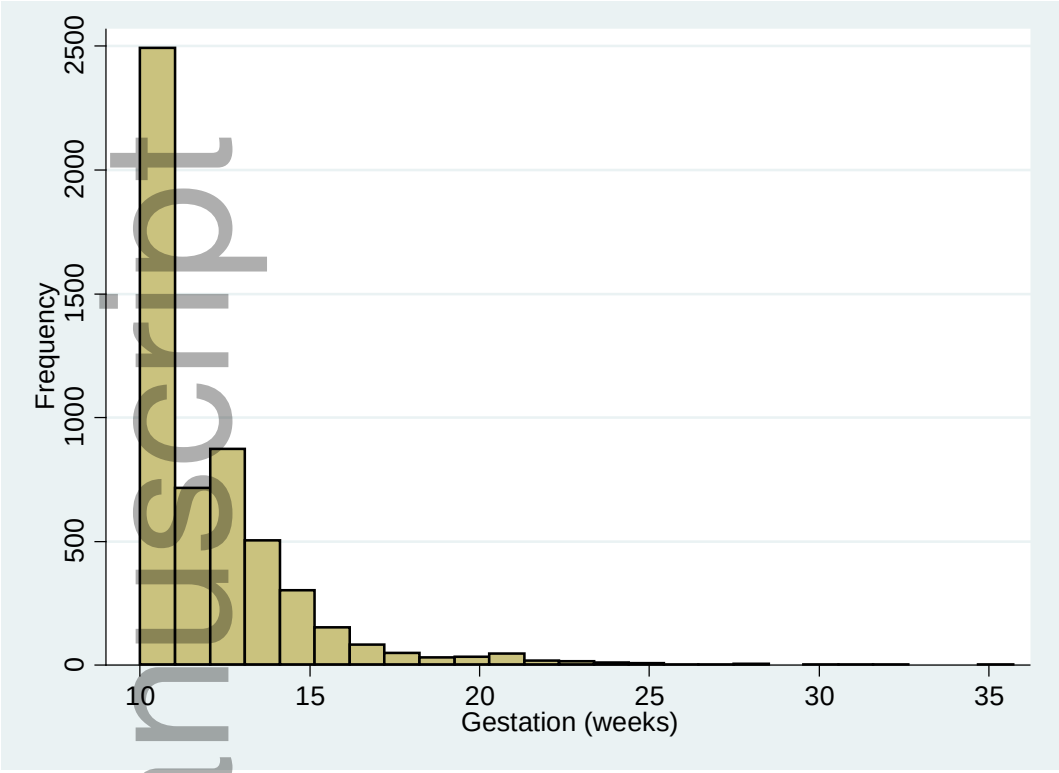
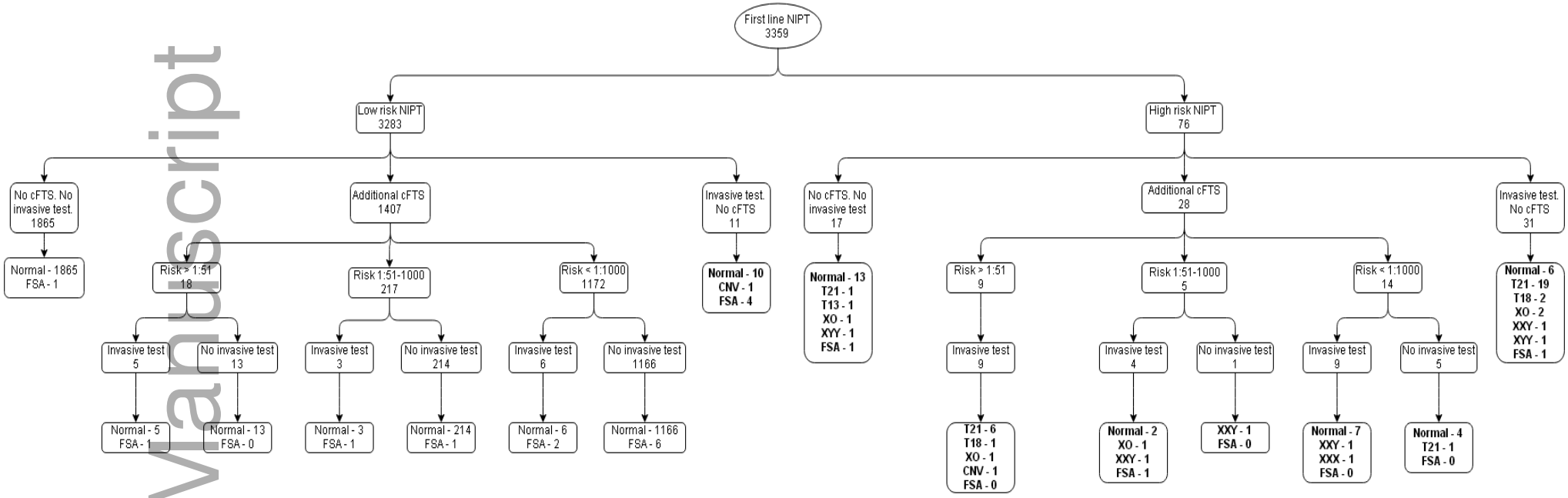


Figure 2: Flow diagram of NIPT testing performed as a first line test



cFTS = Combined first trimester screening

FAS = Fetal structural abnormality

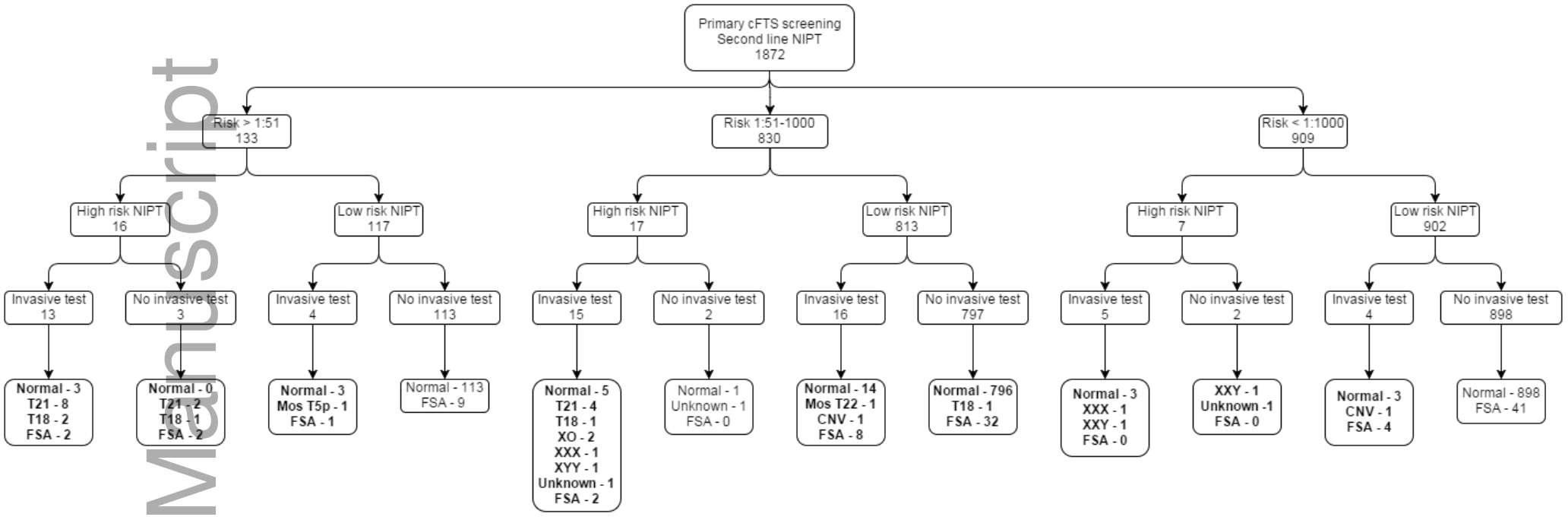
T21 = Trisomy 21

T18 = Trisomy 18

T13 = Trisomy 13

CNV = Copy number variant

Figure 3: Flow diagram of NIPT testing performed as a second line test. 36 cases having NIPT entirely due to a structural abnormality are not included below.



cFTS = Combined first trimester screening

FAS = Fetal structural abnormality

T21 = Trisomy 21

T18 = Trisomy 18

CNV = Copy number variant

Mos T5p = Mosaic trisomy 5p

Mos T22 = Mosaic trisomy 22

Unknown = Karyotyping was not performed, often due to miscarriage or unconfirmed sex chromosomal abnormality