The Accuracy of Fetal Cell Free DNA in the Assessment of Fetal Karyotype: A Systematic Review of Literature

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Abstract: *Objective*: To review literature about the accuracy of non invasive prenatal testing by cell free fetal DNA (NIPT) to detect fetal trisomies (T).

Methods: A search in PubMed, EMBASE, Medline, reference lists was performed (January 2009 – December 2013). Inclusion criteria for study selection: fetal karyotype assessed at birth or by invasive procedures, report of true positive, false positive, true negative, and false negative rates of trisomy 21, 18, 13, and other aneuploidies, data reported as proportional rates. Exclusion criteria: articles aimed to describe genetic procedures, data reported in graphs or percentage. Data abstracted from each article were: sensitivity, specificity, failure rate, demographic characteristics. Pooled sensitivity and specificity and 95% Confidence Interval (95% CI) were calculated by using Der Simonian Laird methods.

Results: From 15 articles, 11,512 women were screened with cfDNA. Sensitivity was 99% (98-100%) for T21, 96% (92-98%) for T18, 86% (71-95%) for T13, and 90% (81-96%) for other aneuploidies. Failure rate of blood analysis was 12% with DANSR and 3% with MPSS technique. Sensitivity for T21 was 99% (98-100%) and 85% (69-94%) following MPSS and DANSR, respectively.

Conclusion: Paucity of data about gestational age at time of NIPT, placental mosaicism, direct comparison between techniques. Fetal cfDNA is an efficacious method to detect fetal T. Further studies are needed to standardize criteria for cfDNA isolation, select population and define the optimal time for NIPT assessment.

Keywords: Aneuploidy, non invasive prenatal testing, karyotype, prenatal diagnosis, prenatal screening, cell free DNA.

INTRODUCTION

Prenatal screening of fetal aneuploidy is generally performed with serial sonographic examinations and maternal serum biochemistry in the first or second trimester of pregnancy. A relatively new approach for screening of fetal abnormal karyotype consists in the assessment of fetal cell free DNA (cfDNA) in maternal blood. Although this approach is usually indicated as non invasive prenatal testing (NIPT), we believe that this term might be improper, since the other screening tests, such as combined and integrated tests, are also non invasive methods. Nonetheless, in this review the term NIPT will be used to refer to cfDNA testing in maternal blood sample.

NIPT is based on the rationale that a fetus affected with a trisomy releases an increased amount of that chromosome in maternal blood [1]. Two genetic techniques have been employed to assess cfDNA: massive parallel DNA shotgun sequencing (MPSS) and digital analysis of selected regions (DANSR). The former randomly analyses DNA from the whole genome, whereas the latter select specific genomic fragments of DNA.

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The advantage of NIPT is the very high sensitivity for fetal trisomies, which is achieved by a single maternal blood sample. The disadvantages are the high cost and complexity of the required technology, the low concentration of cfDNA and variation according to maternal characteristics. The demonstration that the majority of circulating cfDNA is maternal in origin further made the efficacy of NIPT questionable. In addition, studies are limited by the small sample size [2-4] and encourage large clinical trials, mainly for trisomy 13 and 18, which are uncommon disorders. We therefore performed a review of literature in order to detect sensitivity and specificity of NIPT in identifying fetal trisomies.

MATERIALS AND METHODS

A search in PubMed, EMBASE, Medline, Clinicaltrial.org, reference list was performed from January 2008 to December 2013 to find articles that described the efficacy of NIPT in identifying fetal aneuploidies. Key words were: first trimester, antenatal screening, abnormal karyotype, fetal aneuploidies, non invasive prenatal testing, cell free DNA, trisomy, massively parallel sequencing, target / selective sequencing. Inclusion criteria were: fetal karyotype assessed by invasive procedure or at birth, detection of:

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- True positive rate: number of fetuses with positive NIPT and abnormal karyotype;
- False positive rates: number of fetuses with positive NIPT and normal karyotype;
- False negative cases: number of fetuses with negative NIPT and abnormal karyotype;
- True negative cases: number of fetuses with negative NIPT and normal karyotype.

Exclusion criteria were: omitting at least one inclusion criterion, data reported in graphs or percentage, personal communication and non-English language publication.

From each article the following data were abstracted: maternal demographic characteristics, study sample (unselected or high risk population), sensitivity and specificity of NIPT, type of genetic technique to isolate cfDNA (MPS, MPS-GC correction, DANSR), failure rates, type of algorithm to assess the risk score (z-score, FORTE), and rates of trisomy 21, 13, 18, and any other aneuploidies. These consisted in sex chromosome aneuploidies, such as Klinne felter and Turner syndromes.

High risk for aneuploidies was defined as the presence of at least 1 of the following: maternal age >35 years, positive combined test, positive integrated test, positive family history for fetal aneuploidy, abnormal ultrasound, i.e. presence of structural anomalies that are highly correlated to abnormal karyotype. Failure rate was defined as inadequate blood sample, assay failure, insufficient DNA.

An effort to contact the corresponding Author was performed in order to obtain unpublished data. The two Authors independently selected articles and discordance was resolved with consensus.

Pooled sensitivity and specificity with 95% Confidence Interval (95% CI) were calculated with DerSimonian Laird method. Sensitivity and specificity

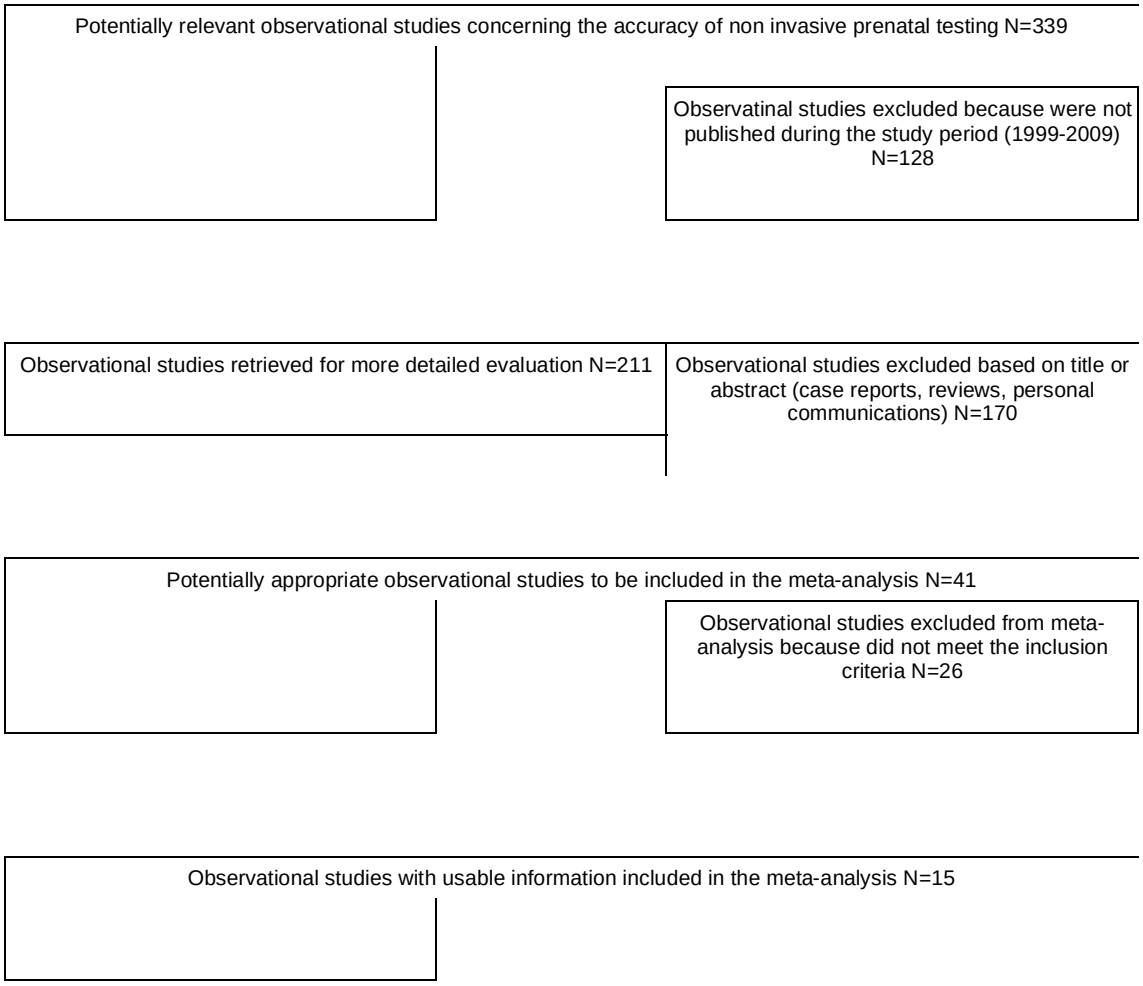

Figure 1: Steps for study selection.

Table 1: Characteristics of the Included Studies

Author	Year	Sample size	GA week	Technique	Risk calculator
Stumm	2014	472	11-32	random massively parallel sequencing	z
Verweij	2013	502	10-28	selective sequencing	forte
Song	2013	202	11-21	random massively parallel sequencing	z
Nicolaides	2013	229	11-13	snp	natus
Liang	2013	412	na	random massively parallel sequencing	z
Palomaki	2011	1471	11-20	random massively parallel sequencing	z
Ashoor	2013	1949	11-13	selective sequencing	forte
Hooks	2014	414	10-34	selective sequencing	forte
Norton	2012	3228	10-38	selective sequencing	forte
Lau	2012	108	11-28	random massively parallel sequencing	z
Ehrich	2011	480	8-36	multiplex sequencing	z
Ashoor	2012	300	11-13	selective sequencing	forte
Sparks	2012	171	11-36	selective sequencing	forte
Bianchi	2012	534	8-22	random massively parallel sequencing	na
Dan	2012	3078	9-28	random massively parallel sequencing	Na

were stratified for type of laboratory technique used to isolate cfDNA and algorithm for risk score calculation.

RESULTS

Because only 1 article was performed in unselected population, we limited the review to studies enrolling women at high risk for fetal aneuploidy.

Fifteen articles met the inclusion criteria [2-16]. Figure **1** shows the steps for study selection. Characteristics of each study are summarized in Table **1**. Overall, 11,512 pregnancies were screened by cfDNA. Of these, 311 (2.7%) were excluded due to failed technique, leaving 11,201 pregnancies available for review. Median maternal age at time of screening ranged from 29 [14] to 37 [2,9,13] years. Ethnicity was reported in 6877 women (59.7%). The most prevalent ethnicity was Caucasian (61%) and the less prevalent ethnicity was the Jewish (0.6%). The most common indications for studying fetal karyotype were positive first trimester screening (56.6%), advanced maternal age (22.6%), and ultrasound structural anomalies (9.9%). Fetal karyotype was assessed by amniocentesis (46.9%), CVS (51.9%), umbilical cord sampling (0.9%) and product of conception (0.06%). The lowest gestational age of cfDNA sampling was 8 weeks [7,9], whereas the highest gestational age was 38 weeks. Fetal fraction ranged from 10% to 14%. Table **2** resumes maternal demographic characteristics.

Table 2: Demographic Characteristics; CVS: Chorionic Villous Sample; UCS: Umbilical Cord Sample

		Total	%
Ethnicity	Caucasian	4247	61.7
	African	919	13.4
	Asian	945	13.7
	Hispanic	579	8.4
	Jewish	45	0.6
	Other	142	2
	Unreported	0	
Indication for enrollement	Positive first trimester screening	3725	56.6
	Positive second trimester screening	203	3.1
	Positive integrated screening	244	3.7
	Abnormal ultrasound	652	10
	Advanced maternal age	1492	22.6
	Family history for abnormal karyotype	89	1.3
	Unreported	180	2.7
Invasive diagnostic procedure	Amniocentesis	4730	47
	CVS	5233	52
	UCS	100	10
	Post-Natal	6	<1

The risk of chromosomal aneuploidies was assessed by z-score in 6 articles, by the analytical performance of DANSR in combination with FORTE algorithm in 6 articles, and other algorithms in the remaining 2 articles.

Trisomy 21, trisomy 18, trisomy 13 and other aneuploidies were detected by fetal karyotype examination in 764 (6.8%), 208 (1.8%), 38 (0.3%), and 73 (0.6%) pregnancies, respectively.

Sensitivity of cfDNA test was 99% (95% CI: 98 – 100%) for trisomy 21 (Figure **2**), 96% (95% CI: 92 – 98%) for trisomy 18 (Figure **3**), 86% (95% CI: 71 – 95%) for trisomy 13 (Figure **4**), and 90% (95% CI: 81 – 96%) for any other aneuploidies (Figure **5**). Specificity was 100% (95% CI: 100 – 100%) in all types of trisomies.

With regard to the risk assessment, sensitivity of trisomy 21 was 99% with both FORTE and z-score,

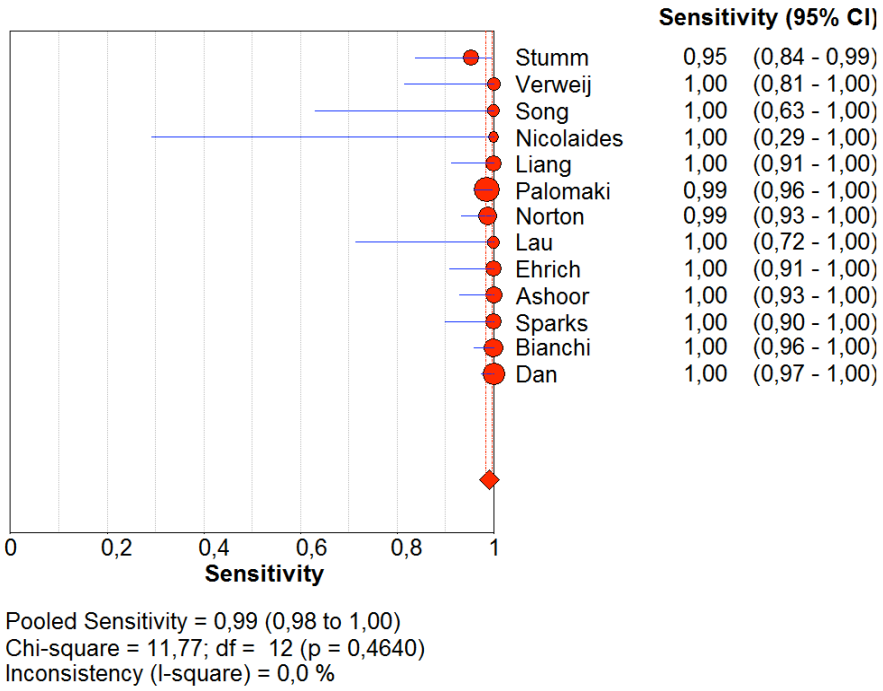

Figure 2: Sensitivity for trisomy 21.

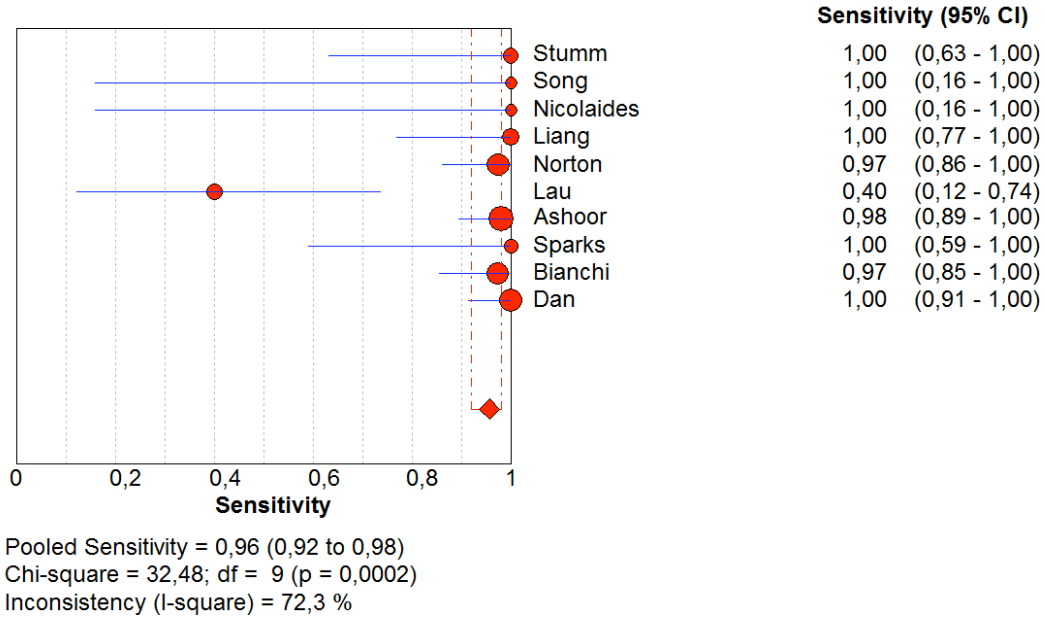

Figure 3: Sensitivity for trisomy 18.

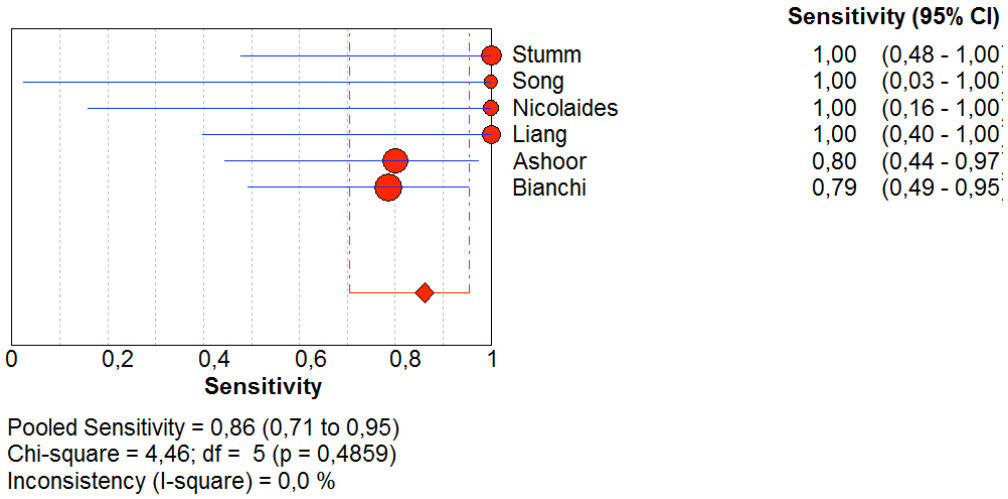

Figure 4: Sensitivity for trisomy 13.

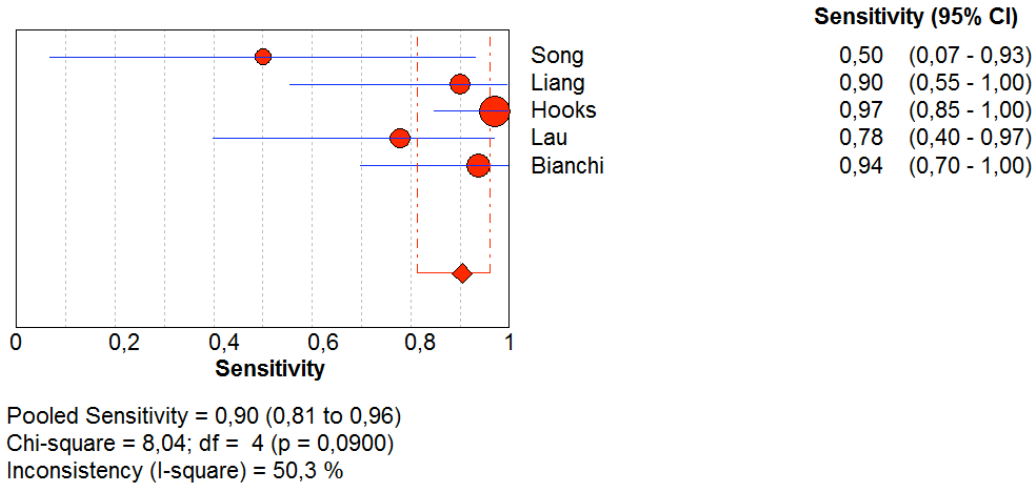

Figure 5: Sensitivity for other aneuploidies.

sensitivity of trisomy 18 was 98% (95% CI: 93 – 100%) with FORTE and 82% (95% CI: 65 – 93%) with z-score, sensitivity of trisomy 13 was 80% (95% CI: 44 – 96%) with FORTE and 100% (95% CI: 69 – 100%) with z-score. Other aneuploidies were detected with sensitivity of 97% (95% CI: 82 – 99%) and 100 (95%CI: 99 – 100%) with FORTE and z-score, respectively.

Comparison between MPSS and DANSR showed a slightly lower sensitivity of DANSR than MPSS (Table **3**). Failure rate was 205/6564 (3.1%) following MPSS and 205/6343 (3.2%) following DANSR. Reasons for failure are reported in Table **4**.

CONCLUSION

This review shows that NIPT is a very efficacious screening test for fetal aneuploidies, mainly for trisomy 21. With regard to the technique, MPSS is not selective in the chromosomal origin of the sequenced DNA fragments. Because chromosome 21 represents only 1.5% of the human genome, a high quantity of fragments are required to ensure sufficient chromosome 21 counts to achieve accurate results [11]. In contrast, DANSR is a selective technique of loci from chromosomes 21, 18 and 13 with tenfold decrease in the required DNA sequenced compared with MPSS technique [11]. This leads to more efficiency and less expenses of DANSR in comparison with MPSS [11]. However, we did not find clinically relevant differences between DANSR and MPSS. Noteworthy, we did not find articles that compared the two genetic techniques directly, hence we could not perform a comparative meta-analysis. Further studies are needed to investigate which of the two techniques is more sensitive for the detection of fetal aneuploidies.

Table 3: Comparison between DANSR and MPSS

	DANSR N (%)	MPSS N (%)	MPSS-GC Correction N (%)
FAILURE RATE	476/3949 (12.0)	185/5515 (3,3)	137/504 (27,2)
inadequate blood sample	280 (58,8)	116/185 (62,7)	0
assay failure	109/476 (22,8)	37/185 (20)	75/137 (54,7)
insuff. DNA	87/476 (18,3)	32/185 (17,3)	62/137 (45,2)
SENSITIVITY	% (95% CI)	% (95% CI)	% (95% CI)
trisomy 21	85 (69-94)	99 (98-100)	98 (96-99)
trisomy 18	98 (93-100)	99 (93-100)	81 (64-93)
trisomy 13	80 (44-96)	78 (48-94)	99 (66-100)
other trisomies	98 (97-99)	93 (67-99)	84 (60-97)

Table 4: Reason for Failure

	DANSR	MPS	MPS GC Correction
Inadequate blood sample	280 (58.8%)	116 (62.7%)	0
Technical reason	109 (22.8%)	37 (20.0%)	75 (54.7%)
Insuff. DNA	87 (18.3%)	32 (17.3%)	62 (45.3%)

The reason for assay failure is unknown, but seems to equally affect trisomic and euploid fetuses [12]. When fetal fraction is <4%, differences in circulating cfDNA between disomic and trisomic karyotypes might be undetermined [3,9]. However, in case of failure secondary to low fetal fraction or assay failure, repeat sampling is successful in 60% of cases [17]. In our review, failure rate was similar between MPSS (3.5%) and DANSR (3.2%). Noteworthy, the most frequent reason for failure of both the two methods was inadequate blood sample, whereas insufficient DNA fraction occurred in approximately 17% of cases.

Following the quantitative analysis of cfDNA, the risk of abnormal karyotype is calculated. Two statistical calculations have been proposed: FORTE and z-score. FORTE analysis does not require external reference samples or normalizing adjustments based on historical information. In addition, FORTE discerns trisomic from disomic karyotype, since it is responsive of fetal fraction in both trisomic and disomic karyotypes, whereas z-score is only responsive to fetal fraction in the trisomic samples. Another advantage of FORTE is the possibility to incorporate maternal age in the calculation of the risk of fetal aneuploidy. We observed that FORTE was similar to z-score in detecting trisomy 21 but more robust in identifying trisomy 18 and less efficacious in finding trisomy 13 compared with z-score.

Limitations of literature should be pointed out. Firstly, the primary source of cfDNA is the placenta, in fact circulating cfDNA is positively correlated with placental mass [18]. However, we found paucity of data about placental mosaicism, which should be considered in cases of false positive detection.

Secondly, fetal DNA can be detected from the 4th weeks [19], increases with gestational age, reaching a sharp peak in the last 8 weeks of pregnancy [20], and disappears within 2 hours after delivery. Using digital PCR, cfDNA in maternal plasma increases from approximately 10% in the first trimester to 20% in the third trimester [21]. Nonetheless, the relationship between gestational age and fetal DNA amounts remains controversial. Earlier studies have shown that the increase of fetal DNA occurs in the third trimester, whereas subsequent studies did not demonstrate any correlation between gestational age and fetal DNA amounts. cfDNA has been successfully tested as early as 10 weeks [17]. This would be the ideal time for applying NIPT, because in case of failure or detection of high risk for fetal aneuploidy, parents would still have the possibility to be screened by combined test at 11-14 weeks and, eventually, undergo CVS. However, literature is limited with regard to the optimal gestational age associated with the highest accuracy of NIPT. We found only 3 articles that were performed in the first trimester, whereas the other articles sampled cfDNA in all the gestational weeks without stratifying outcomes for gestational age.

In a sub-population, cfDNA sampling was performed in the late second or early third trimester, probably after the invasive procedure that was used to assess fetal karyotype. Consequently, additional fetal DNA might have been released into the maternal circulation, leading to an increased fetal fraction of DNA. However, the procedure-related increase of fetal DNA is still theoretical, since, to our knowledge, no studies have actually demonstrated an association between invasive prenatal testing and fetal fraction.

Although fetal fraction is associated with maternal characteristics, such as weight and ethnicity [6], we could not control our results according to these

variables. This is because the reviewed articles did not stratify outcomes of NIPT with regard to maternal factors.

Cost is an important factor to be considered before the introduction of NIPT in clinical practice. In most countries the cost of this technology is higher than the current screening methods [22]. However, because this review included studies performed in Europe, USA, and Asia, where costs of healthcare are very different and change according to local policy, it was not possible to pool this very heterogeneous variable in a meta-analysis.

Finally, the reviewed articles were based on high risk pregnancies for fetal aneuploidy. Because the efficacy of NIPT is based on assay precision and fetal fraction in the sample rather than the prevalence of the aneuploidy [6], it is reasonable to assume that NIPT is applicable to the general population. Further studies are needed to assess the efficacy of NIPT in the general, unselected population.

In conclusion, this review of current literature supports the guidelines established by the American College of Obstetrics and Gynecology and the International Society of Prenatal Diagnosis, which advocate the implementation of cfDNA screening in high risk pregnancy. In addition, NIPT should not replace current first trimester screening of aneuploidies and should be confirmed by invasive testing when positive results are provided by NIPT. In spite of the high sensitivity of NIPT, first trimester ultrasound should not be replaced with NIPT, because about 50% of fetal malformations can be detected as early as 11-14 weeks. In addition, abnormal nuchal translucency is predictive of cardiac defects and other adverse outcomes, as well as biochemical analysis of PAPPA-A may identify adverse obstetric outcomes, including pre-eclampsia and intrauterine growth restriction [23,24]. On the other hands, NIPT is efficacious not only for detection of trisomies, but also to identify or exclude gene disorders, such as beta-thalassemia, haemophilia, and X-linked conditions as congenital adrenal hyperplasia and Duchenne muscular dystrophy [25]. Therefore, first trimester screening should be personalized according to parental genetic factors, whereas further studies are needed to establish the optimal screening in the low risk population.

DISCLOSURE

This paper was presented at the 13th World Congress of Fetal Medicine (Fetal Medicine Foundation, 28 June – 4 July 2014, Nice, France) as poster presentation.

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