Variability of "Reported Fetal Fraction" in Noninvasive Prenatal Screening (NIPS)

Fredrik Persson[a,*] and Lawrence Prensky[b]

BACKGROUND: Fetal fraction is often used to designate no-calls in noninvasive prenatal screening (NIPS). We wished to compare the variability in determining fetal fraction to gold standard methods.

METHODS: We identified 6 publications with datasets consisting of methods capable of measuring fetal fraction for all samples that also had comparison data from gold standard methods. Examples of gold standard methods included relative Y-chromosome quantification in cases of male fetus pregnancies or relative quantification of the relevant chromosome for pregnancies affected by one of the 3 major trisomies.

RESULTS: The studies showed that the differences of the various fetal fraction measurement assays as compared to a gold standard measurement displayed a standard deviation (SD) in the range of 1.3–3.4% fetal fraction (FF). The 4 studies that measured FF from fragment size and genomic coordinates or single nucleotide polymorphisms had a lower variability, with a median SD of about 1.6%, whereas 2 other studies using different methods displayed significantly higher variability.

CONCLUSION: When deciding whether to use the reported FF as a reason to discard samples as no-calls or not, we recommend taking the variability of the FF measurement into consideration.

Introduction

In clinical practice, prenatal aneuploidy screening is transitioning from traditional biochemistry-based assays toward noninvasive prenatal screening (NIPS) based on cell-free DNA (cfDNA). In Europe, both the Netherlands and Belgium are offering NIPS as a first line screening option (1), and recently the American College of Obstetrics and Gynecology has recommended that it should be offered to all woman regardless of a priori risk (2).

Although there are many different NIPS molecular approaches [sequencing reads (3), rolling-circle replication products (RCPs) (4), single nucleotide polymorphisms (SNPs) (5), or microarray intensities (6)], all use the same principle of measuring a chromosomal ratio (CR) between a chromosome (such as chromosome 21) and one or more reference chromosomes. This can be normalized using a multiple of median (MoM) approach ensuring that unaffected samples obtain values centered around 1 (7). The increase in normalized CR for a trisomic pregnancy should be half of the proportion of cfDNA that has a fetal (placental) origin [commonly referred to as the fetal fraction (FF)] and leads to the following relation for the normalized CR (normCR):

$$\text{Average normCR} = \begin{cases} 1 & \text{For unaffected pregnancies} \\ 1+FF/2 & \text{For affected pregnancies} \end{cases}$$

The ability to differentiate an affected sample from an unaffected sample is determined by how far away the affected sample's normalized CR is in relation to how much the unaffected samples vary around its average value of 1.

Given the same NIPS test, the further the sample is above a normalized CR of 1, the greater the likelihood that it originates from an affected pregnancy. Since the FF essentially determines the CR for an affected sample, it is often viewed as the key factor in the determination of the affected status of a sample.

Many laboratories try to estimate the "true FF" in each sample, resulting in a "reported FF" that is compared to a set FF cutoff, often at 4% (8), to decide when to report a result or not, i.e., classify it as a no-call.

[a]Vanadis Diagnostics, PerkinElmer Inc., Sollentuna, Sweden; [b]PerkinElmer Inc., Waltham, MA.
*Address correspondence to this author at: Vetenskapsv. 10, SE-191 38 Sollentuna, Sweden. E-mail fredrik.persson@perkinelmer.com.
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In 2015, Wright et al. showed the large impact the imprecision of FF measurement can have, especially with low FFs (9). They found that 2.99% of all samples have a reported FF below 4%, while only 0.37% of all samples would actually have a true FF below 4%. This means that at least 88% of the samples with a reported FF below 4% are erroneously classified as having a low FF. Due to the substantial implications of this information, there is a need to understand the variability in the different methodologies that have been implemented and presented over the years to try to determine the FF of all individual samples.

Methods

To assess the variability of different methods of measuring FF for all samples in the context of NIPS, a set of 6 publications were identified that contained either stated values of the variability (10–12), or presented data in comparison to a gold standard, enabling a secondary analysis to determine the variability (7, 13, 14). Gold standard measurements here include results based on the relative quantification of the Y-chromosome in male fetus singleton pregnancies or the relative quantification of the trisomic chromosome in a singleton pregnancy affected by Down, Edwards, or Patau Syndrome.

For the secondary analysis, the variability between the gold standard value and the measured value was determined and quantified as the standard deviation (SD) of this difference. It is important that it is the SD between unique samples, not replicates of the same sample, to also take into account biological variabilities in measures such as fragment size distributions or SNPs.

The underlying data for the FF variability from Kim et al. (13) and Wald et al. (7) was digitized from the relevant figures in those publications using the freeware ScanIt (AmsterCHEM). As the gold standard, Wald et al. presented the normalized relative amount of the trisomic chromosome in singleton pregnancies affected by Down, Edwards, or Patau Syndrome as MoM values. These were converted to a fetal fraction using the equation $MoM = 1 + FF/2$, as stated in the publication. All digitized data can be found in the online Data Supplement. In the case of Schmid et al. (14), the relevant figure could not be digitized in the same way owing to the vast number of datapoints (47 512). Instead, data were digitized using image analysis (see Supplemental Information).

Results

The 4 studies that measured FF from fragment size and genomic coordinates or SNPs had a lower variability, with a median SD of about 1.6%, while the other 2 displayed significantly higher variability.

The published data revealed that these methods of measuring the FF have a variability in reported FF, as compared to a gold standard, that can be represented by an SD that is between 1.3 and 3.4% FF as shown in Table 1.

It can be noted that Schmid et al. indicated a variability of the FF measurement equivalent to an SD of 0.39% FF (14), seemingly 3–9 times more precise than the other methods. However, those numbers relate to the reproducibility (the variability between multiple aliquots of the same sample) and not true measurement error between unique samples. By estimating the measurement error from the relevant figure in the publication, the variability between samples in this study would correspond to an SD of 1.6% FF, on par with the other studies.

Discussion

All the studies examined showed that the current methodologies used in the measurement of FF have considerable variability, with a median SD of at least 1.6%.

Assuming FF measurements follow a Gaussian distribution and the SD is 1.6% FF, then about 95% of results (corresponding to ±2SD) would fall within ±3.2% FF of the true FF. Therefore, when a reported FF of 4% is stated for a sample, there is a 95% chance that it originated from a sample with an FF in the range of 0.8–7.2%. This shows the inherent uncertainty with reported FF. However, a more direct consequence, also demonstrated by Wright et al. (9), is that most samples discarded due to low reported FF are done so unnecessarily.

Furthermore, using a reported FF cutoff of 4% means that not all samples with a true FF below 4% will be discarded. If a laboratory wished to ensure that most (97.5%) samples with a true FF of 4% would be discarded, the cutoff in reported FF would have to be set at approximately 7%. Using data from a previously published prospective series of 10 472 unaffected singleton pregnancies tested at 10–14 weeks gestation (15), this would mean that every sixth woman would not get a NIPS result.

Using a reported FF cutoff will cause an increase in the no-call rate. Although this can provide the test with an improved apparent performance (the performance of the test for patients who were provided a result), it does so at the cost of the effective performance (the performance of the test for all patients who took the test) (16).

As a real-world example, the NEXT study reported a 100% apparent detection rate for T21 (38 of 38) from a cohort of 15 841 women with a NIPS result (17). However, there were 3 additional T21 cases among the 3% (488) of women who did not receive a NIPS result (most due to low FF). Therefore, the effective detection rate of the NIPS screening would be decreased from

Table 1. The variability of different methods of measuring fetal fraction taken from the literature or secondary analysis of data presented in the literature

Method	FF variability (SD)	Source	Comment	Reference
Fragment size and genomic coordinates	1.3%	Stated	No data nor experimental details presented	Illumina (10)
Nucleosome pattern (SANEFALCON)	2.5% (<15% FF) 3.3% (All FF)	Stated		Straver et al. (11)
Fragment size	3.4%	Stated		Yu et al. (12)
Fragment size and genomic coordinates (seqFF)	1.5%	Secondary analysis	Data from fig. 2a	Kim et al. (13)
SNP	1.6%	Secondary analysis	Data from fig. 2b	Kim et al. (13)
Fragment size and genomic coordinates (seqFF)	2.1%	Secondary analysis	Data from fig. 3	Wald et al. (7)
SNP (DANSR)	1.6 % (FF > 4%)	Secondary analysis	Data from fig. 2	Schmid et al. (14)

100 to 93% when considering the no-calls as well. More recently, Hancock et al. found in a retrospective study of 58 105 samples that the apparent performance stayed the same when including low reported FF samples (FF < 4%) while the no-call rate decreased, creating a significantly better effective performance (18).

In conclusion, although determining the FF of a sample would be very useful, there does not currently seem to exist any way to do this with a sufficiently low variability. Since a reported FF for a given sample can be incorrect by approximately ±3.2% FF or more, it is undesirable to discard samples from a screening test to increase the apparent performance, especially with NIPS becoming more widely used for general population screening. Doing so would result mainly in unnecessarily denying women a reliable test result, along with increasing the associated anxiety and issues surrounding follow-up tests including pregnancy loss, parental stress, financial costs, and clinical burden.

Nonstandard Abbreviations: NIPS, noninvasive prenatal screening; SD, standard deviation; cfDNA, cell-free DNA; RCPs, rolling-circle replication products; SNPs, single nucleotide polymorphisms; CR, chromosomal ratio; MoM, multiple of median; FF, fetal fraction; normCR, normalized chromosomal ratio; T21, trisomy 21.

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