Posttest risk calculation following positive noninvasive prenatal screening using cell-free DNA in maternal plasma

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Noninvasive prenatal screening (NIPS) for fetal chromosome defects has high sensitivity and specificity but is not fully diagnostic. In response to a desire to provide more information to individual women with positive NIPS results, 2 online calculators have been developed to calculate posttest risk (PTR). Use of these calculators is critically reviewed. There is a mathematically dictated requirement for a precise estimate for the specificity to provide an accurate PTR. This is illustrated by showing that a 0.1% decrease in the value for specificities for trisomies 21, 18, and 13 can reduce the PTR from 79-64% for trisomy 21, 39-27% for trisomy 18, and 21-13% for trisomy 13, respectively. Use of the calculators assumes that sensitivity and specificity are constant for all women receiving the test but there is evidence that discordancy between screening results and true fetal karyotype is more common for older women. Use of an appropriate value for the prior risk is also important and for rare disorders there is considerable uncertainty regarding prevalence. For example, commonly used rates for trisomy 13, monosomy-X, triploidy, and 22q11.2 deletion syndrome can vary by >4-fold and this can translate into large differences in PTR. When screening for rare disorders, it may not be possible to provide a reliable PTR if there is uncertainty over the false-positive rate and/or prevalence. These limitations, per se, do not negate the value of screening for rare conditions. However, counselors need to carefully weigh the validity of PTR before presenting them to patients. Additional epidemiologic and NIPS outcome data are needed.

Key words: cytogenetic abnormalities, noninvasive prenatal screening, positive predictive value, prenatal screening, risk calculation, sensitivity, specificity

Introduction

When prenatal screening is provided using conventional screening tests (maternal serum and ultrasound markers), it has been common practice to report a patient-specific risk for each woman tested.[1] Numerical estimates of risk have facilitated the combination of multiple risk factors such as maternal age and family history, as well the individual test components. Patients have been counseled according to risk and have made their decisions about whether or not to accept invasive testing on the basis of these risks.

The introduction of noninvasive prenatal screening (NIPS) using cell-free DNA (cfDNA) in maternal plasma changed the paradigm.[2] These results are generally reported as test positive (increased, or high risk) or test negative (low risk). Some approaches present a risk score on the report[3,4] but this only provides a measure of the likelihood that DNA from aneuploid cells is present in the maternal circulation; this is not a measure of the probability of true fetal chromosome imbalance. NIPS for various fetal chromosome abnormalities is associated with sensitivities and specificities that are much higher than conventional screening but the testing is not diagnostic. Moreover, it has been recognized that when the test is provided to women with low prior risks, the positive predictive value (PPV) of the testing is expected to be lower.[2] Uncertainty about the true risk for fetal imbalance and the desire to counsel women more precisely has prompted the development of online calculators to assess individual women's post-NIPS risk.[5-7]

In this article I review these calculators. I explain why considerable caution is needed when using these calculators to compute a risk for individual patients.

General considerations

The online calculators use the formula:

$$q_i = p_i \times D/[(p_i \times D) + (1 - p_i) \times (1 - C)]$$

(supplemental material, formula 5) where:

q_i is the posttest risk (PTR) (usually expressed as percentage) carried out on the i^{th} patient; p_i is the pretest risk; D is the detection rate (sensitivity); and C is the specificity.

The PTR (q_i) for each patient has been referred to as a PPV but this can be a source of confusion because there will be a PPV that reflects the overall test performance for the entire population of women screened. For the purposes of evaluating risk for individual women it would therefore be more accurate to refer to the number generated by the calculators as a personalized PPV (which would be equivalent to testing a population with the same prior risk, using a test with the same sensitivity and specificity) or, more simply, PTR. The distinction between PTR and PPV is discussed in more detail in the online supplementary material accompanying this article.

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The National Society of Genetic Counselors/Perinatal Quality Foundation calculator

This calculator was developed by members of the National Society of Genetic Counselors (NSGC) and the Perinatal Quality Foundation (PQF) and is designed for health care professionals with an understanding of predictive values; it is "intended to aid such health care professionals in counseling their patients."[7] PTR can be calculated for a variety of specific chromosome abnormalities. Users can enter sensitivity and specificity for NIPS or use default rates based on the metaanalyses of Gil et al[8]. For some chromosome abnormalities default rates are unavailable. Prior risk (or prevalence) can be entered or, for some disorders, the prior risk can be based on maternal age at delivery. Prior risk is based on a fixed time point in pregnancy (gestational age 16 weeks). As well as PTR, the posttest level of reassurance provided by a negative result for an individual patient (referred to in the calculator as the negative predictive value) can be calculated.

TABLE 1
Examples of change in posterior risks when false-positive rates or detection rates are altered

Disorder	Prior risk[a]	Sensitivity	Specificity	Posterior risk	Comment[b]
t21	1/296	99.2%	99.91%	78.8%	Default rates
		99.2%	99.81%	63.7%	0.1% Higher FPR
		99.2%	99.71%	53.5%	0.2% Higher FPR
		89.2%	99.91%	76.9%	10% Lower DR
		79.2%	99.91%	74.8%	20% Lower DR
t18	1/1152	96.3%	99.87%	39.1%	Default rates
		96.3%	99.77%	26.6%	0.1% Higher FPR
		96.3%	99.67%	20.2%	0.2% Higher FPR
		86.3%	99.87%	36.5%	10% Lower DR
		76.3%	99.87%	33.7%	20% Lower DR
t13	1/2576	91.0%	99.87%	21.4%	Default rates
		91.0%	99.77%	13.3%	0.1% Higher FPR
		91.0%	99.67%	9.7%	0.2% Higher FPR
		81.0%	99.87%	19.5%	10% Lower DR
		71.0%	99.87%	17.5%	20% Lower DR

t13, trisomy 13; *t18*, trisomy 18; *t21*, trisomy 21; *DR*, detection rate; *FPR*, false-positive rate.

[a] Based on woman age 35 y (at delivery) and 16 wk' gestation; [b] Default rates are sensitivity and specificity used in National Society of Genetic Counselors/Perinatal Quality Foundation calculator.[7]

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The University of North Carolina calculator

The calculator developed by Grace et al[5] from the University of North Carolina is designed "as a teaching tool to demonstrate the relationship between a priori risk, sensitivity, and specificity and to underline that cfDNA screening is not a diagnostic test."[6] The calculator is limited to trisomy 21, 18, and 13. Recognizing that the different NIPS tests available in the United States can be expected to have different performance characteristics, users can select the test (Verifi Illumina Inc, Redwood City, CA; Harmony Ariosa Diagnostics Inc, San Jose, CA; Materniti21 Sequenom Inc, San Diego, CA; or Panorama Natera Inc, San Carlos, CA). The option to use custom sensitivity and specificity rates is not available. Prior risk can be a user-specified value (gestational age for this is not needed), or it can be based on maternal age (range 20-44 years). When maternal age is used, the gestational age (between 10-20 weeks) also needs to be specified.

Limitations and implicit assumptions

Positive, negative, and intermediate results

Both calculators assume that the initial determination that a case is positive or negative will be independent of the prevalence. In fact, 2 of the commonly used NIPS methods compute a risk score that already incorporates maternal age in the algorithm and cases are only considered positive if this risk score is >1%.[3,4] Use of the calculators is problematic in this situation because a case might have been classified differently if age had not been used. In effect, age is being used twice to compute the PTR in some cases.

Although age is a relatively weak contributor to risk (most cases have risk scores >99/100 and would likely test positive even maternal age were not included), there are some positive cases that fall close to the 1% cutoff. One of the test methodologies also presents some findings as having intermediate risk or "aneuploidy suspected"[9] and for these cases there are insufficient data to assess a PTR.

Independence of sensitivity, specificity, and prevalence

A second assumption implicit in the calculation of patient-specific posterior risk is that sensitivity and specificity are independent of prior risk (prevalence); ie, the composite values of sensitivity and specificity that were derived from trials can be used for any woman. This assumption is widely accepted as valid for conventional screening and is the basis for computing patient-specific risks using single values for specificity and sensitivity. However, there are grounds to question the validity of the assumption for NIPS.

Reasons for a discordance between the NIPS result and the true fetal karyotype are becoming increasingly well understood and may be due to true fetal mosaicism, confined placental