Prenatal Risk Calculation (PRC) 3.0: An Extended DoE-Based First-Trimester Screening Algorithm Allowing For Early Blood Sampling

Authors E. Merz[1], C. Thode[2], B. Eiben[3], S. Wellek[4,5]

Affiliations Affiliation addresses are listed at the end of the article

Key words
- prenatal diagnosis
- first trimester screening
- risk calculation

received 05.10.2015
accepted 25.11.2015

Bibliography
DOI http://dx.doi.org/10.1055/s-0035-1569403
Published online: February 23, 2016
Ultrasound International Open 2016; 2: E19–E26

ISSN 2199-7152

Correspondence
Prof. Dr. E. Merz
Center for Ultrasound and Prenatal Medicine
Center for Ultrasound and Prenatal Medicine
Steinbacher Hohl 2–26
60488 Frankfurt
Germany
Tel.: +49/69/7601 3579
Fax: +49/69/7601 3613
merz.eberhard@web.de

Abstract

Aim: Both previous versions of the German PRC algorithm developed by our group for routine first-trimester screening relied on the assumption that maternal blood sampling and fetal ultrasonography are performed at the same visit of a pregnant women. In this paper we present an extension of our method allowing also for constellations where this synchronization is abandoned through preponing blood sampling to dates before 11 weeks of gestation.

Methods: In contrast to the directly measured concentrations of the serum parameters PAPP-A and free ß-hCG, the logarithmically transformed values could be shown to admit the construction of reference bands covering the whole range from 16 to 84 mm CRL [corresponding to 63 to 98 days of gestation]. Prior to determining reference limits from which the DoEs for each individual patient had to be calculated, the log concentrations of all PAPP-A and free ß-hCG values were transformed once more using the calibration approach established in [1] for the elimination of the influence of maternal weight.

Results: Although that part of the database which was available for estimating the reference bands for blood sampling times prior to 11 weeks of gestation was comparatively sparse (898 out of 186215 pregnancies with euploid outcome), the key statistical characteristics of the extended risk-calculation procedure turned out to be very satisfactory. Using the same cutoff value of 1:150 for the posterior risks of trisomy 21 and 13/18, the overall FPR (false positive rate) for diagnosing a T21 was found to be 3.42%. The corresponding DTR (detection rate) was obtained to be 86.8% and thus exceeded the DTR attained by PRC 2.0 for trisomy 21. For trisomies 13 and 18, the proportions of patients with calculated posterior risks exceeding the cutoff value of 1:150 were obtained to be 1.60% (=FPR) and 86.4% (=DTR).

Conclusion: Transforming the measured concentrations of PAPP-A and free ß-hCG to the logarithmic scale allows one to extend the DoE-based algorithm developed by the FMF Germany for diagnosing trisomies 21 and 13/18 in such a way that it can be applied to constellations where blood sampling is done before 11 weeks of gestation.

Introduction

During the last few years an increasing tendency to favor a modified schedule for performing the tests carried out in routine first-trimester screening could be observed. According to this modified schedule, each patient should be seen at 2 different visits. The first one should take place between 9+0 and 11+0 weeks of gestation and be used for drawing the blood to determine the biochemical marker concentrations. The sonographical examinations should be carried out at a second visit with the usual timing, i.e., between 11+1 and 14+0 weeks. Several authors have argued that preponing the date of blood sampling leads to better diagnostic accuracy of the screening procedure [2–4].

The major challenge which had to be addressed in adapting the statistical approach behind the previous versions of the first-trimester screening algorithm of the FMF-Germany to this modified time schedule was to recruit sufficiently large samples of patients with blood sampling performed before 11+1 weeks of gestation and extend the reference bands to be used for assessing the degree of outlyingness of test results.

Materials and Methods

Patients

In developing a new algorithm for the assessment of findings from first-trimester screening allowing for early blood sampling a database comprising n = 186 215 euploid pregnancies and n = 925 pregnancies with chromosomal defects (trisomies 21, 18 and 13) was established. In the reference group with negative outcome n = 898 patients provided blood samples drawn before 11 + 1 weeks of gestation. In the group with a pathological outcome n = 726 fetuses had a trisomy 21, and in the remaining 199 fetuses either a trisomy 18 or 13 was found. All these samples consisted of data taken in singleton pregnancies with well-documented outcome.

Ultrasound and biochemical procedures

The basic parameters analyzed for the purpose of first-trimester screening were maternal age and weight, the sonographic parameters crown-rump length (CRL) and nuchal translucency (NT) of the fetus, and the 2 biochemical markers PAPP-A (Pregnancy associated plasma protein A) and free ß-hCG (free beta-human Chorionic Gonadotropin) whose concentrations were measured in maternal serum.

For the sake of quality assurance of the sonographical methods employed, only data provided by level-II or level-III certified ultrasound investigators were included. The biochemical investigations were carried out in certified laboratories using the Brahms Kryptor® system.

Statistical Methods

Adjusting the measured concentrations of PAPP-A and free ß-hCG for maternal weight

In a previous paper [1], the problem of eliminating a systematic influence of maternal weight on the false-positive rates of the diagnostic procedures to be based on the posterior risks calculated for the respective type of trisomy was successfully addressed by fitting the following exponential regression model to the measured concentrations of *PAPP-A and free ß-hCG*: the following exponential regression model:

$$Y = \beta_0 \cdot \exp\{-\beta_1 * W\} + \varepsilon.$$

In this equation, Y represents the concentration of PAPP-A or free ß-hCG measured in a randomly selected patient, and W denotes maternal weight in kg. Furthermore, β_0 and β_1 are unknown constants which have to be estimated from the data, and ε is an error term assumed to be stochastically independent of Y. For our present purposes, it will be more convenient to replace the above model with the log-linear regression model

$$\log Y = \tilde{\beta}_0 - \tilde{\beta}_1 * W + \tilde{\varepsilon}.$$

As soon as the parameters $\tilde{\beta}_0$ and $\tilde{\beta}_1$ have been estimated by means of standard least squares fit, weight-corrected log-concentrations $\tilde{Y}_{corr}$ are obtained by means of the formula

$$\tilde{Y}_{corr} = \tilde{Y} + \tilde{\beta}_1 * (W - \bar{W}).$$

Performing this correction in any individual case simply requires to add the difference between individually observed and average maternal body weight multiplied by the regression coefficient $\tilde{\beta}_1$ to the natural logarithm $\tilde{Y}$ of the measured concentration Y. As a fraction of the deviation of a pregnant woman's actual weight from the average, the amount of this correction equals the slope of the regression line describing the relationship between log-concentration and maternal weight.

Modelling the regression of NT and the weight-corrected concentrations of the biochemical parameters on gestational age

As previous experience has clearly shown, the form of the regression of the quantities measured in the context of first-trimester screening on gestational age cannot simply be inferred from a well-established biological theory and varies considerably between the 3 parameters under assessment. Therefore, appropriate modelling of this relationship requires to refer to a class of regression functions which is sufficiently rich in its coverage of different shapes and can be specified without relying on theoretical assumptions. A good choice that takes both aspects into account, and which was introduced from the beginning of the FMF-Germany project, is the class of all polynomials of degree up to 10. What degree is really needed was determined automatically in each of the 3 cases (i. e., for NT, PAPP-A and free ß-hCG) by means of stepwise multivariable regression treating the first 10 powers of CRL as potentially relevant regressors.

For fitting a regression model of this form to NT, the directly measured quantity was taken as the dependent variable. In contrast, in the regression analysis of the biochemical parameters, the originally measured concentrations were replaced by their weight-corrected log-transforms. Furthermore, in analyzing NT, gestational age as measured in terms of CRL was allowed to vary over the traditional range of 45–84 mm. Extension of this range to a range of 16–84 mm in constructing reference bands for the biochemical parameters was the crucial step in re-designing the screening algorithms in a way making them suitable for evaluating constellations with early blood sampling. Typically, preponing the date of blood sampling will entail temporal dissociation between the sonographical and the biochemical part of the underlying clinical investigation. In accordance with this, all analyses have to deal with 2 different values of CRL for each individual patient, denoted by CRL and CRLBE in the sequel.

Construction of reference bands and computation of DoE's

In constructing reference bands for NT and the weight-corrected, logarithmically transformed concentrations of PAPP-A and free ß-hCG, we adhered to the same general principles as have made up the statistical basis behind PRC from the beginning (for details see Merz et al., 2008 [5]). Again, all 3 reference bands were to provide an overall coverage proportion of 90%, and through allowing for asymmetry of the conditional distributions of the measurements it was ensured that the proportions of euploid patients with values below the lower and above the upper limit both equal 5%. Reference bands exhibiting these coverage properties were generated by means of the method originally introduced by Wellek and Merz, 1995 [6] which essentially consists of computing smallest admissible vertical distances between the bounds of the band and the regression line around which it is spanned.

Upon determing CRL-dependent reference limits for the quantity under consideration, each individual data point was evaluated in terms of its degree of outlyingness through computing the corre-

sponding DoE. This measure is defined as the signed vertical distance of a point in the respective scatterplot from the regression line as expressed as a multiple of the width of the upper or lower part of the reference band at the actual value of CRL as abscissa (for a complete formal definition see Merz et al., 2008 [5]).

Establishing the diagnostic decision rule

Like all widely-used procedures for evaluating standard first-trimester screening data [5, 7–12], the newly developed version of PRC uses the Bayesian posterior risk of trisomy as the basis for deciding between euploidy and (potential) presence of the chromosomal anomaly under investigation. In order to calculate the posterior probability of a pregnant woman to carry a fetus exhibiting the currently considered form of trisomy, we apply Bayes' theorem with the following specifications:

(i) Parametric estimate of the joint likelihood of the DoE's for the 3 markers (i. e., NT, weight-corrected logarithms of PAPP-A and free ß-hCG) among euploids and pregnancies with positive outcome, respectively.

(ii) Prior probability of carrying a trisomy-fetus among pregnants of specified age in the given week of gestation.

The components of the likelihood are obtained as values of densities of Gaussian normal distributions with population parameters substituted by sample means and variances. The DoE's of the 3 markers are assumed to be mutually independent so that the joint likelihood can be computed as the product of the likelihood of the individual DoE's. The prior probabilities were determined through interpolating the values tabulated by Snijders et al., 1999 [13] and Snijders et al., 1995 [14] for trisomy 21 and trisomy 13/18 respectively.

As usual, the final step of the diagnostic screening procedure consists of comparing the posterior risk calculated for the given patient to the predefined cutoff, i. e., to the number $1/150 \approx 0.67\,\%$. If the cutoff is exceeded, the decision is to recommend amniocentesis or performance of some other procedure providing very high diagnostic accuracy. Otherwise, the findings from first-trimester screening are classed as free of evidence of trisomy.

Investigating the robustness of the false positive rates against changes in maternal weight

In order to ascertain sufficient robustness of the algorithm against changes in maternal weight, the interval over which maternal weight ranged within the reference sample of euploids was partitioned into 18 adjacent classes. The lengths of these sub-intervals were chosen depending on the density of the weight-distribution in the respective part of the total range. In the upper tail of the distribution, groups of length 10 or even 15 kg were formed. All other classes were defined to have length 2.5 kg. For each of the 18 strata, the group-wise false positive rate was determined and the variability of the FPR over the strata measured in terms of an ordinary coefficient of variation. In addition to quantifying the lack of robustness in that way, a graphical check was performed by means of bar graphs providing direct visualization of the amount of fluctuation between the weight groups.

Incorporating add-in markers

In addition to the parameters maternal weight, ethnicity and smoking status which we already included in our risk calculation program PRC 2.0 [1], we have now included as further parameters Ductus venosus flow and Tricuspid regurgitation [15, 16] for a multi-variate analysis.

Results

Weight correction for the log-concentrations of the biochemical parameters

Fig. 1 shows the measurements taken on PAPP-A in the whole sample of patients with negative outcomes as points in the plane, with maternal weight as abscissa and log-concentration level as ordinate, together with the fitted regression line. The coefficients were estimated by means of ordinary least squares fit to be $\hat{\hat{\beta}}_0 = 2.36735$ and $\hat{\hat{\beta}}_1 = 0.01654$.

Analogously, linear regression analysis with the values behind the log ß-hCG * WEIGHT scattergram shown in **Fig. 2** gave the numbers $\hat{\hat{\beta}}_0 = 4.27126$, $\hat{\hat{\beta}}_1 = 0.00972$ as parameter estimates.

Since in the reference sample of all euploid pregnancies, mean maternal weight was $\overline{W} = 68.341242$, we obtained the following formulae for computing weight-corrected log-concentrations of PAPP-A and free ß-hCG:

$$Log\ PAPP\text{-}A_{corr} = log\ PAPP\text{-}A + 0.01654 * (W - 68.341242),$$
$$Log\ \beta\text{-}hCG_{corr} = log\ \beta\text{-}hCG + 0.00972 * (W - 68.341242).$$

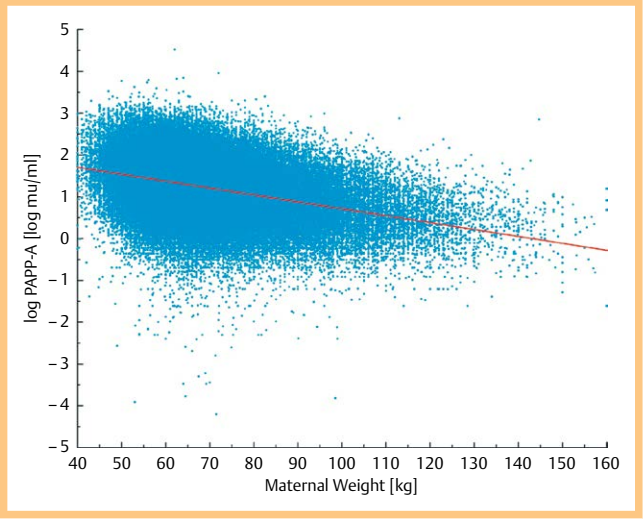

Fig. 1 Scatterplot with linear regression relating log-concentrations of PAPP-A to maternal weight.

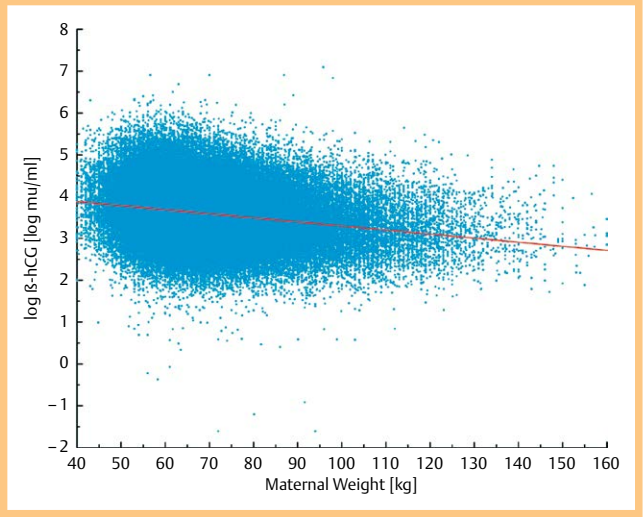

Fig. 2 Scatterplot with linear regression relating log-concentrations of free β-hCG to maternal weight.

In the above equations, *W* stands for the weight measured [in kg] in the constellation to be evaluated in terms of trisomy risk. Back-transforming them to the original scale yields the correction formulae

$$PAPP\text{-}A_{corr} = PAPP\text{-}A * \exp\{0.01654 * (W - 68.341242)\}$$

and

$$\beta\text{-}hCG_{corr} = \beta\text{-}hCG * \exp\{0.00972 * (W - 68.341242)\}.$$

The values of the correction factors to be used according to these formulae are tabulated in **Table 1** for maternal weights *W* ranging between 60 and 120 in steps of 5 [kg].

Reference Bands and Transformation of Individual Measurements Into DOE's

Nuchal translucency (NT)

Stepwise regression analysis with the originally measured values of *NT* lead to selecting the following polynomial (with coefficients rounded to 5 significant digits) for modeling the dependence of the mean of that variable on gestational age as expressed in terms of CRL:

$$Y = 152.16888 - 12.35168 \times x + 0.39173 \times x^2 - 0.00581 \times x^3 + 0.00003 \times x^5 - 7.23565 \times 10^{-12} \times x^7 + 2.08419 \times 10^{-18} \times x^{10}.$$

In **Fig. 3**, the graph of the curve given by this equation is plotted in cyan. The conditional variance of *NT* turned out to be sufficiently stable for justifying the assumption of approximate CRL-independence of the variability. Accordingly, the width of the reference band constructed with the data represented in the diagram in the usual form of a scatterplot is constant over the whole range of CRL values at which the measurements of *NT* were taken in the total sample of euploid pregnancies. More precisely speaking, the vertical distance of the estimated upper and lower 5% reference limits (plotted in red) from the regression curve was computed to be 0.65869 and 0.57159 mm, respectively.

Weight-corrected logarithmic concentration of PAPP-A

Plotting $\log PAPP - A_{corr}$ against CRL for all n = 186 215 euploidies gave the blue cloud of points shown in **Fig. 4**. In order to indicate that the gestational age at which the blood samples for determining the biochemical concentration levels were taken were allowed to differ from that at which sonography was performed, the horizontal axis is labeled CRLBE instead of CRL. Furthermore, the range of CRLBE extends to the left until 16 mm, and the density of the cloud is much lower below the traditional limit of 45 mm. With these data, stepwise least squares fit of polynomials of degree up to 10 resulted in a distinctly simpler regression equation as compared with that obtained for *NT*, namely

$$y = -1.95000 + 0.07313 \times x - 3.63693 \times 10^{-4} \times x^2 + 1.39983 \times 10^{-20} \times x^{10}.$$

Except for the changes concerning the labeling and spacing of both coordinate axes, all other components of the graph have the same meaning as in **Fig. 3**. The points on the curves plotted in red have as ordinates the estimated age-specific 5th and 95th percentiles, respectively, and the regression curve is represented by the cyan line.

Table 1 Multiplication factors for calculating weight-corrected concentrations of the biochemical markers [mU/ml] for maternal weights *W* ranging between 60 and 120 in steps of 5 [kg].

W	PAPP-A	ß-hCG
60	0.87113	0.92212
65	0.94624	0.96804
70	1.02782	1.01625
75	1.11643	1.06686
80	1.21268	1.11999
85	1.31724	1.17577
90	1.43080	1.23432
95	1.55416	1.29579
100	1.68815	1.36032
105	1.83370	1.42807
110	1.99179	1.49919
115	2.16352	1.57385
120	2.35005	1.65223

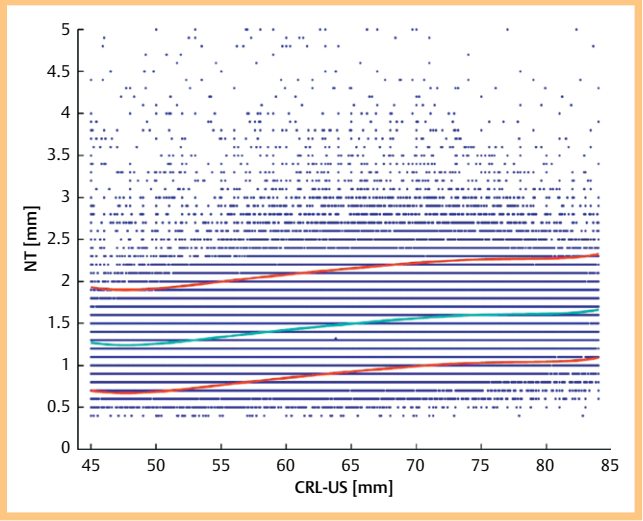

Fig. 3 90% reference band for NT computed from the data of n = 186 215 euploid pregnancies.

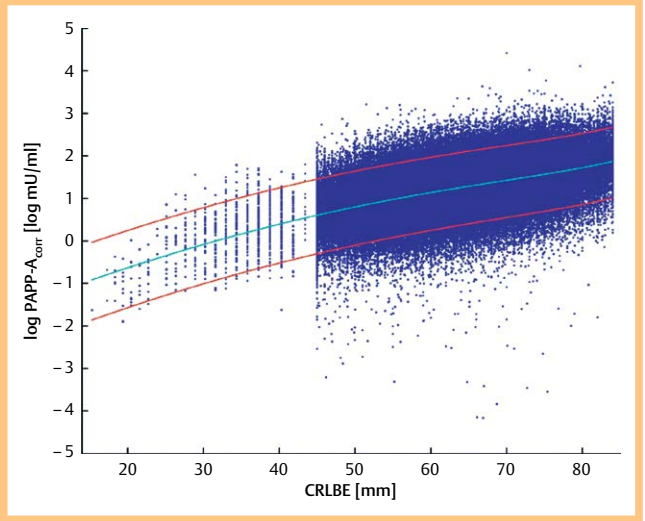

Fig. 4 90% reference band for weight-corrected log-concentrations of PAPP-A computed in a sample of n = 186 215 euploid pregnancies.

Weight-corrected logarithmic concentration of free ß-hCG

Fig. 5 is the analogue of **Fig. 4** for the second biochemical marker, i.e., the log-serum concentration of free ß-hCG. In contrast to PAPP-A, the mean levels of free ß-hCG decrease with gestational age. Precisely, stepwise polynomial regression of the logarithmic free ß-hCG level on CRL yielded the model equation

$$y = 5.05336 - 0.03595 \times x + 2.18174 \times 10^{-4} \times x^2 - 8.68749 \times 10^{-15} \times x^7 - 2.36687 \times 10^{-21} \times x^{10}.$$

Again, no marked asymmetry of the distribution of the residuals around this curve was found, and the bandwidth could be chosen nearly constant in time, due to the fact that the variance around the regression curve changes only slightly from left to right.

Example illustrating the use of the reference bands for transforming individual measurements to degrees of outlyingness (DoE's)

Table 2 shows the data obtained from a patient randomly selected from the reference sample of pregnancies with an euploid fetus. The entries in the right-most column are obtained through calculating the difference between the measured value and the ordinate of the corresponding point on the regression curve making up the central line around which the reference band is spanned, and dividing the result by the width of the upper and lower part of the band, respectively. If the data point corresponding to the measured value falls below the regression line, a negative sign has to be added in front of the result. For a non-verbal, mathematically precise general formulation of the rule for computing DoEs for arbitrary values of NT and the 2 biochemical markers, the reader is referred to Merz et al., 2008 [5].

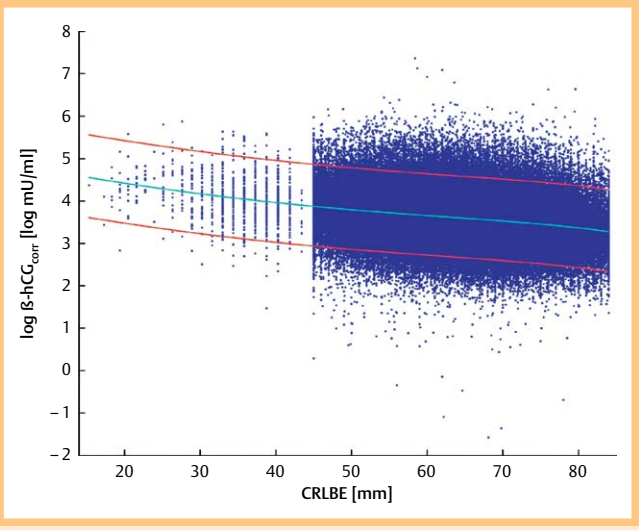

Fig. 5 Analogue of **Fig. 4** for free β-hCG.

Likelihood ratios to be entered in Bayes' formula for calculating posterior risks of trisomy

In determining the likelihood ratios required for calculating the posterior risks, we relied on the following assumptions:

(i) Except for some normalizing transformation, the distributions of the DoE's have (approximately) Gaussian form with both parameters potentially depending on a patient's ploidy status.

(ii) Both for normal and aneuploid pregnancies, the DoE's associated with the 3 markers are mutually independent.

From (i), it follows that any observed value x of the DoE of one of the 3 markers under consideration has the likelihood ratio

$$LR = \frac{\sigma_{eu}}{\sigma_{aneu}} \times exp\{-0.5 \times ((x - \mu_{aneu})/\sigma_{aneu})^2 + 0.5 \times ((x - \mu_{eu})/\sigma_{eu})^2\}, \quad \otimes$$

where μ_{eu} and σ_{eu} denotes, respectively, the population mean and standard deviation for euploid pregnancies, and the symbols μ_{aneu} and σ_{aneu} have the analogous meaning for the aneuploid population. Since the populations involved are not known as a whole, the μ's and σ's must be replaced by the corresponding sample means and standard deviations, respectively. The values which were obtained for the latter are displayed in **Table 3**.

In the example of **Table 2**, calculating the likelihood ratios for the DoEs of the individual markers gave the results shown in **Table 4**.

Eventually needed is the joint likelihood ratio for the DoE's of all 3 markers. Under the independence assumption (ii), this is simply the product of the likelihood ratios calculated for the individual markers. Combining the entries in **Table 4** in this way gave $LR_{joint} = 0.434902 \times 2.855837 \times 4.727462 = 5.871551$ and $LR_{joint} = 0.384972 \times 1.663977 \times 0.130494 = 0.083592$ for trisomy 21 and trisomy 13/18, respectively.

Parameter	Days of Gestation	Value		Reference Limit		DoE
		Measured	Predicted†	Lower	Upper	
NT	81	1.5	1.24928	0.67768	1.90797	0.38064
Log PAPP-A$_{corr}$	65	−1.15671	−0.27541	−1.20614	0.59482	−0.94689
Log β-hCG$_{corr}$	"	5.38482	4.25754	3.31495	5.25608	1.12893

†by means of the polynomial regression model obtained in §5.2

Table 2 Example illustrating the computation of DoE's for given values of NT and corrected log-concentrations of the 2 biochemical markers.

Table 3 Constants for be used in calculating the likelihood ratios according to formula ⊗ for both types of trisomy.

Parameter	Transformation of DoE	$\bar{x}_{eu}$	S_{eu}	$\bar{x}_{aneu}$		S_{aneu}	
				T21	T13/18	T21	T13/18
NT	$\sqrt[7]{DoE + 2.5}$	1.13263	0.04320	1.23730	1.24310	0.09251	0.11135
Log PAPP-A$_{corr}$	None	0.01629	0.61358	−0.85335	−1.70421	0.73057	0.85010
Log β-hCG$_{corr}$	" "	−0.01405	0.61453	0.78042	−1.30688	0.62854	0.94820

The final decision rule and its properties

As soon as the formula for computing the joint likelihood ratios has been established, completing the construction of the desired diagnostic decision rule requires only to link the quantity LR_{joint} with the prior probability of finding an aneuploidy of the type under consideration. Denoting the latter by π, Bayes' rule states how this has to be done in the appropriate way, namely by evaluating the expression

$$PPOST = \frac{1}{1 + LR_{joint}^{-1} \times (1-\pi)/\pi}.$$

Given the type of trisomy, the prior probability π (also called "background risk" in the usage of first-trimester screening) depends on both maternal age and gestational week. There is no mathematical model which describes the relationship between π and these baseline variables. Instead, one can recourse to tabulations of proportions of aneuploidies observed for specific combinations of age and week of gestation in large samples. The results shown in **Table 5** for a selection of exemplary findings from standard first trimester screening were obtained with the background risks tabulated by Snijders et al. for trisomy 21 [13] and for trisomy 13/18 [14], and the same data are used throughout the implementation of the algorithm distributed by the FMF-Germany.

Which diagnostic decision has to be taken in an individual case depends on the cutoff value *C* to which the calculated value of *PPOST* is compared. Speaking in general terms, the proportion of euploid pregnancies for which *PPOST* turns out to exceed the prespecified value of *C* is the false positive rate (*FPR*) of the corresponding diagnostic procedure, and analogously, the proportion of trisomies 21 or 13/18 for which one finds that *PPOST* > *C* is its detection rate (*DTR*).

In order to keep consistent with the previous version of PRC, we determined both rates using the same cutoff value for both types of trisomy. **Table 6** shows the results for 2 different choices of *C*, namely *C* = 1/150 = 0.0067 and *C* = 1/500 = 0.002. With the stricter specification of *C* to be considered as "red line" beyond which a patient should be advised to undergo invasive diagnostics, the FPR turned out to be as small as 3.42 and 1.60 % for trisomies 21 and 13/18, respectively. Both of the corresponding detection rates exceeded 86 %, and Youden's index, a customary overall measure of diagnostic accuracy (defined as the difference between DTR and FPR), came out greater than 83 % for both types of trisomy.

Table 4 Likelihood ratios for the DoE's of the individual markers obtained in the example of **Table 2**.

Parameter	Type of trisomy to be diagnosed	
	T21	T13/18
NT	0.434902	0.384972
Log PAPP-A$_{corr}$	2.855852	1.663988
Log β-hCG$_{corr}$	4.727560	0.130494

Stability of the false positive rate under stratification with respect to maternal weight

Figs. 6,7 show the results of a stratified analysis of the false positive rates using maternal weight as grouping variable. For trisomy 21, the weight-group-wise FPRs ranged from 2.74 to 4.50 % with a coefficient of variation of 14.4 %. The maximum was reached in the class 87.5–95.0 kg whereas in terms of FPR, the heaviest patients (weighing more than 120 kg) ranked only 7th from the bottom. Altogether, these results admit the conclusion that the FPR entailed in diagnosing trisomy 21 by means of the new algorithm remains at least as stable over the weight groups as held true for the previous version of PRC. The analysis of the weight-group-wise FPR's for trisomy 13/18 gave similar results: The range was still narrower (0.76–1.89 %), and for the trisomy 13/18 related part of the algorithm, the lowest FPR was even found in the highest weight group. The coefficient of variation over all 18 groups was again reasonably low (19.4 %).

Discussion

The objective of the project presented in this paper was to extend our approach to developing a statistical algorithm for first-trimester screening in a way which makes it suitable also for the evaluation of findings relating to blood samples taken before the left-hand endpoint of the usual time window for performing sonography. The key idea which lead to a satisfactory solution to this problem was to use the logarithmic transformation not only for the purpose of correcting the biochemical marker concentrations for maternal weight but at the same time for establishing a modified database for centile estimation for PAPP-A and free ß-hCG. Working on the logarithmic scale the reference bands for these parameters could be extended without affecting the smoothness of the boundaries to a time window

Table 5 Results of trisomy risk calculations in a group of patients with euploid pregnancies randomly selected from the database of FMF Germany. [Entries in columns 8–11: reciprocal risks rounded to the nearest integer.]

							Trisomy 21		Trisomy 13/18	
Age	Weight	CRLBE	CRL	NT	PAPP_A	β-hCG	Backgr	Post	Backgr	Post
37	74.5	58	86	2.3	0.330	52.5	145	486	256	1083
33	65.1	58	86	2.0	0.526	124.7	349	1022	617	49471
27	48.6	59	88	1.2	0.210	71.6	876	2901	1651	2492
23	65.4	58	88	1.9	0.725	96.0	1021	13238	1928	378897
42	72.8	59	89	2.0	0.359	77.0	43	176	82	2027
37	69.0	58	89	1.8	0.241	47.2	147	1355	278	974
36	84.4	58	90	2.0	0.290	51.9	225	2198	425	7541
41	61.6	55	90	1.7	0.219	84.6	55	264	104	1087
30	57.6	59	91	1.6	0.349	75.3	677	6329	1278	25491
26	64.7	59	92	1.6	0.230	73.9	945	3983	1782	13570
35	73.3	59	92	1.5	0.399	69.6	233	5072	440	39012
34	51.6	58	93	2.1	0.563	142.4	338	902	638	40802

corresponding in terms of CRL to 16–84 mm rather than 45–84 mm. Upon completion of the construction of these bands the same concepts and computational procedures as for PRC 2.0 could be used for building the diagnostic algorithms eventually needed. A crucial role was again assigned to measuring the degree of outlyingness of an arbitrary data point by means of an index (called DoE) which compares the vertical distance of the point from the center of the band to the width of the latter. The criterion to be used for diagnostic decision making was derived from the Bayesian posterior distribution obtained from modeling the joint distributions of all 3 markers involved (i. e., NT and the concentrations of the 2 biochemical markers) among euploid pregnancies on the one hand and pregnancies showing a trisomy of the kind under consideration on the other.

Although the number of data points available in the left-most part of the extended time window was rather small as compared with the traditional range 45–84 mm in which the vast majority of the investigations were made, the overall diagnostic accuracy eventually attained was even better than that provided by PRC 2.0 [1]. Furthermore, we were able to show that approximate independence of the false positive rates of maternal weight can still be taken for granted.

In principle, the approach described in the previous sections can easily accommodate additional markers like (i) smoking status, (ii) ethnicity, (iii) fetal nasal bone, (iv) tricuspid regurgitation, and (v) ductus venosus Doppler. Likelihood ratios obtained for these markers with the database of the FMF-Germany will be reported in a separate paper.

It is important to note that the major results presented in this paper are specific for the platform used for ascertaining the biochemical marker levels. Adapting the algorithms for a different platform requires to repeat all steps of the construction with data obtained in environments where this platform is in large-scale use. Recently, we re-designed the complete diagnostic procedure for use in connection with the Perkin-Elmer device and the Roche system. Thus PRC 3.0 can be used with results of the biochemical markers measured with Brahms Kryptor®, Roche Elecsys®, or PerkinElmer DELFIA®, Xpress.

Table 6 False positive and detections rates and Youden's index for 2 different cutoff specifications.

	Trisomy 21			Trisomy 13/18		
Cutoff Value	***FPR***	***DTR***	***YI***	***FPR***	***DTR***	***YI***
1:150	3.42 %	86.8 %	83.4 %	1.60 %	86.4 %	84.8 %
1:500	9.25 %	94.5 %	85.3 %	3.27 %	89.5 %	86.2 %

Conclusion

A new version of the risk calculation procedure of FMF-Germany (PRC) has been developed and made available as a CE-certified computer program. It is based on an algorithm which provides control over the rate of false positive decisions not only for the total sample of all patients with negative outcome but over the

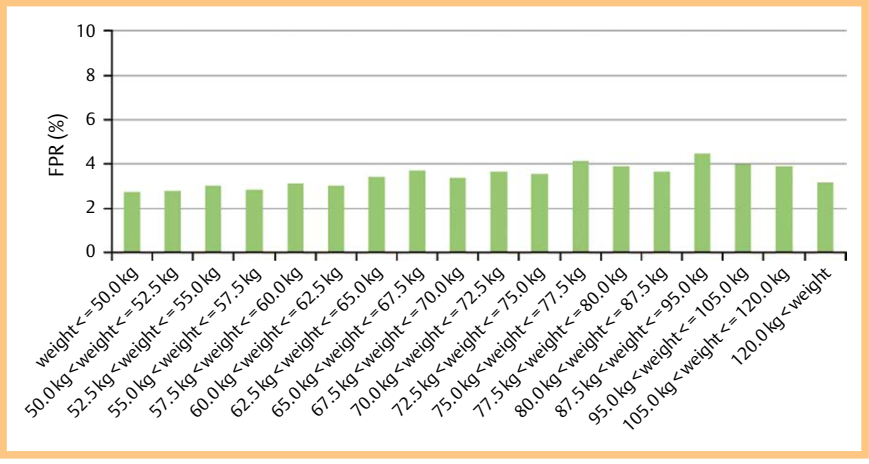

Fig. 6 False positive rate of the decision rule for the diagnosis of trisomy 21, by maternal weight.

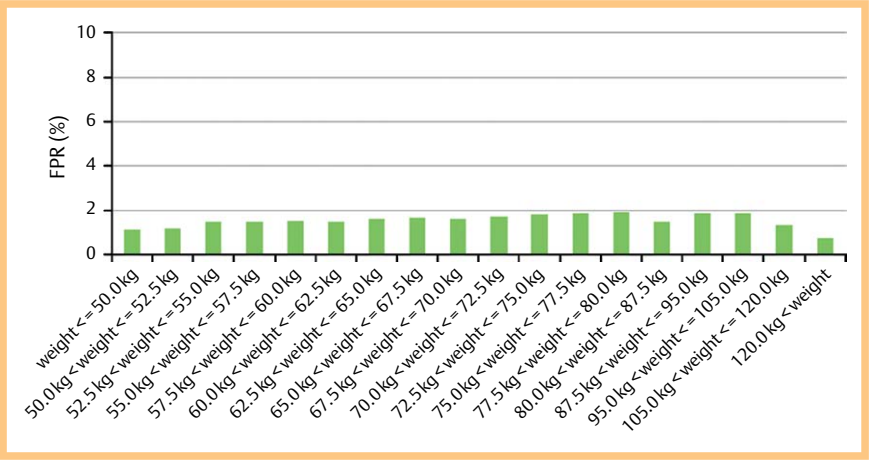

Fig. 7 Analogue of **Fig. 6** for trisomy 13/18.

whole range of maternal weight. This was made possible through deriving formulae allowing transformation of the originally measured values into weight-corrected concentrations of free ß-hCG and PAPP-A. In terms of diagnostic accuracy as assessed by means of Youden's index, an arithmetic combination of false positive and detection rates, the new version of the algorithm is even more effective than its predecessor.

Affiliations

[1] Center for Ultrasound and Prenatal Medicine, Frankfurt, Germany
[2] MVZ wagnerstibbe für Laboratoriumsmedizin und Pathologie GmbH, Laboratoriumsmedizin, Göttingen, Germany
[3] Labor Eiben Glaubitz, Institut für Klinische Genetik Nordrhein, Essen, Germany
[4] Department of Biostatistics, CIMH Mannheim, University of Heidelberg, Germany
[5] Department of Medical Biometry, Epidemiology and Informatics, Medical Center of the University of Mainz, Mainz, Germany

References

1 *Merz E*, *Ch Thode*, *Eiben B et al.* Individualized correction for maternal weight in calculating the risk of chromosomal abnormalities with first-trimester screening data. Ultraschall Med 2011; 32: 33–39
2 *Kirkegaard I*, *Petersen OB*, *Uldbjerg N et al.* Improved performance of first-trimester combined screening for trisomy 21 with the double test taken before a gestational age of 10 weeks. Prenat Diagn 2008; 28: 839–844
3 *Wright D*, *Spencer K*, *Kagan K et al.* First-trimester combined screening for trisomies 21 at 8–13 weeks. Ultrasound Obstet Gynecol 2010; 36: 404–411
4 *Tørring N*, *Petersen OB*, *Uldbjerg N*. Ten years of experience with first-trimester screening for fetal aneuploidy employing biochemistry from gestational weeks 6+0 to 13+6. Prenat Diagn 2015; 37: 51–57
5 *Merz E*, *Thode C*, *Alkier A et al.* A new approach to calculating the risk of chromosomal abnormalities with first-trimester screening data. Ultraschall in Med 2008; 29: 639–645
6 *Wellek S*, *Merz E*. Age-related reference ranges for growth parameters. Meth Inform Med 1995; 34: 523–528
7 *Nicolaides KH*, *Azar G*, *Byrne D et al.* Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. Br Med J 1992; 304: 867–889
8 *Snijders RJM*, *Noble P*, *Souka A et al.* UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. Lancet 1998; 351: 343–346
9 *Nicolaides KH*, *Spencer K*, *Avgidou K et al.* Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. Ultrasound Obstet Gynecol 2005; 25: 221–226
10 *Malone FD*, *Canick JA*, *Ball RH et al.* First- and Second-Trimester Evaluation of Risk (FASTER) Research Consortium. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med 2005; 353: 2001–2011
11 *Wald NJ*, *Rodeck C*, *Hackshaw AK et al.* First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). J Med Screen 2003; 10: 56–104 Erratum in: J Med Screen 2006; 13: 51–52
12 *Kagan KO*, *Wright D*, *Baker A et al.* Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 2008; 31: 618–624
13 *Snijders RJ*, *Sundberg K*, *Holzgreve W et al.* Maternal age- and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol 1999; 13: 167–170
14 *Snijders RJ*, *Sebire NJ*, *Nicolaides KH*. Maternal age and gestational age-specific risk for chromosomal defects. Fetal Diagn Ther 1995; 10: 356–367
15 *Kagan KO*, *Valencia C*, *Livanos P et al.* Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 18–22
16 *Maiz N*, *Valencia C*, *Kagan KO et al.* Nicolaides. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 512–517