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A Mixture Model of Ductus Venosus Pulsatility Index in Screening for Aneuploidies at 11–13 Weeks' Gestation

Nerea Maiz[a, c] David Wright[b] Ana Fatima A. Ferreira[c] Argyro Syngelaki[c]
Kypros H. Nicolaides[c, d]

[a]Fetal Medicine Unit, Obstetrics and Gynecology Department, Cruces University Hospital, Biscay, Spain; [b]School of Computing and Mathematics, University of Plymouth, Plymouth, [c]Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, and [d]Department of Fetal Medicine, University College Hospital, London, UK

Key Words
Trisomy 21 screening · Ductus venosus flow · Nuchal translucency · Chromosomal defects · First trimester screening

Abstract
Objective: To assess the value of ductus venosus pulsatility index for veins (DV PIV) in screening for aneuploidies at 11–13 weeks' gestation. ***Methods:*** Fetal DV PIV was measured in singleton pregnancies undergoing first-trimester screening for aneuploidies. In euploid (n = 44,756) and aneuploid (202 cases of trisomy 21, 72 cases of trisomy 18 and 30 cases of trisomy 13) fetuses, DV PIV was best described by a mixture model of distributions. Performance of screening for aneuploidies by DV PIV alone and in combination with fetal nuchal translucency (NT) thickness and serum free β-hCG and PAPP-A was estimated. ***Results:*** In euploid pregnancies there was a bimodal distribution of DV PIV with a dominant crown-rump length (CRL)-dependent part, accounting for around 97% of cases in Caucasians and around 93% in Afro-Caribbeans, and a smaller CRL-independent distribution. In aneuploidies the dominant part was the CRL-independent distribution, which accounted for around 85% cases of trisomies 21 and 18 and 70% of cases of trisomy 13. In screening for trisomy 21 by maternal age, NT and biochemistry at a risk cutoff of 1 in 100, the detection rate was 89.7% and false positive rate was 2.74%; with addition of DV PIV, the values were 93.5 and 1.63%, respectively. ***Conclusions:*** Measurement of DV PIV improves the performance of first-trimester combined test for aneuploidies.

Introduction

Increased impedance to flow in the fetal ductus venosus (DV) at 11–13 weeks' gestation is associated with fetal aneuploidies, cardiac defects and other adverse pregnancy outcomes [1]. Most studies examining DV flow have classified the waveforms as normal when the a-wave observed during atrial contraction is positive or abnormal when the a-wave is absent or reversed [2–5]. The preferred alternative in the estimation of patient-specific risks for pregnancy complications is measurement of the pulsatility index for veins (PIV) as a continuous variable. However, this measurement has been limited because its distribution is not Gaussian. A study examining the poten-

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Prof. K.H. Nicolaides
Harris Birthright Research Centre for Fetal Medicine
King's College Hospital Medical School
Denmark Hill, London SE5 8RX (UK)
Tel. +44 20 7346 3040, E-Mail kypros@fetalmedicine.com

tial value of DV PIV in first-trimester screening for trisomy 21 demonstrated that in order to apply a Gaussian risk algorithm to log-transformed DV PIV multiple of the median (MoM) values for trisomy 21 and unaffected pregnancies, it was necessary to apply truncation limits which resulted in the exclusion of more than half of the data [6].

We have observed that in both euploid and aneuploid pregnancies there is a bimodal distribution of DV PIV, which is analogous to the mixture model of distributions of nuchal translucency (NT) thickness [7]. Fetal NT follows two distributions, one in which NT increases with crown-rump length (CRL) and another which is CRL-independent. The distribution in which NT increases with CRL is observed in about 95% of euploid and in 5, 30 and 15% of trisomies 21, 18 and 13, respectively. In contrast, the proportion of cases in which NT does not change with CRL is, firstly, small for euploid and large for aneuploid pregnancies and, secondly, the median NT is different, being 2.0 mm for the euploid group and 3.4, 5.5 and 4.0 mm for trisomies 21, 18 and 13, respectively [7]. This mixture model of NT distributions, which is compatible with our understanding of the pathophysiology of increased NT in both euploid and aneuploid fetuses has provided accurate patient-specific risks and a high performance in screening for aneuploidies [7].

In this paper we present a mixture model for DV PIV and screening performance for trisomy 21 achieved from this model in combination with fetal NT and serum free β-hCG and PAPP-A in a large database derived from screening for aneuploidies at 11–13 weeks' gestation.

Methods

Study Population

In this study we present the results of analysis of prospectively collected data on DV PIV at 11–13^{+6} weeks' gestation from singleton pregnancies undergoing screening for aneuploidies at University College London Hospital and King's College Hospital, UK, between March 2006 and November 2010.

Maternal weight and height were measured. Maternal characteristics and medical history were recorded in computer databases. Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or in vitro fertilization), cigarette smoking during pregnancy (yes or no), pre-existing diabetes mellitus (type 1, type 2 or no) and obstetric history including parity. The questionnaire was then reviewed by a doctor together with the patient.

A maternal blood sample was analyzed within 10 min of collection for measurement of serum PAPP-A and free β-hCG using automated machines that provide reproducible results (DELFIA Xpress system; PerkinElmer Life and Analytical Sciences, Waltham, Mass., USA). An ultrasound scan was carried out transabdominally to firstly determine gestational age from the measurement of the fetal CRL, secondly to measure fetal NT thickness, thirdly to examine the fetal anatomy for the diagnosis of major fetal defects and fourthly to measure DV PIV [5, 8–10].

The patient-specific risks for trisomies 21, 18 and 13 were estimated from a combination of maternal age, serum free β-hCG and PAPP-A and fetal NT [11]. Women considering their risks to be high were offered chorionic villus sampling or amniocentesis for fetal karyotyping. Karyotype results and details on pregnancy outcomes were added into the database as soon as they became available.

In this study we examine the distribution of DV PIV in fetuses with aneuploidies and normal pregnancies. In the normal group we include pregnancies that were euploid or resulted in the birth of phenotypically normal neonates. We excluded pregnancies that developed preeclampsia and those resulting in stillbirth or delivery of small for gestational age neonates. The definition of preeclampsia was that of the International Society for the Study of Hypertension in Pregnancy [12]. The birth weight percentile for gestation at delivery was calculated using a reference range derived from our population and the neonate was considered to be small for gestational age if the birth weight was below the 5th percentile [13].

Measurement of DV PIV

In the DV studies the following criteria were fulfilled [14]: (1) the examinations were undertaken during fetal quiescence; (2) the magnification of the image was such that the fetal thorax and abdomen occupied the whole screen; (3) a right ventral mid-sagittal view of the fetal trunk was obtained and color flow mapping was used to demonstrate the umbilical vein, DV and fetal heart; (4) the pulsed Doppler sample was small (0.5–1.0 mm) to avoid contamination from the adjacent veins and it was placed in the yellowish aliasing area which is the portion immediately above the umbilical sinus; (5) the insonation angle was less than 30°; (6) the filter was set at a low frequency (50–70 Hz) to allow visualization of the whole waveform, and (7) the sweep speed was high (2–3 cm/s) so that the waveforms were widely spread. The DV PIV was measured by the machine after manual tracing of the outline of the waveform.

Statistical Analysis

Comparison of Groups in the Study Population

Comparison between outcome groups was done by a Kruskal-Wallis test with post hoc Bonferroni correction for continuous variables and χ^2 test or Fisher's exact test with post hoc Bonferroni correction for categorical variables.

Two-Component Mixture Model

In normal pregnancies it is assumed that DV PIV arises from a mixture of two Gaussian distributions: firstly, a proportion (1 – p) in which there is a quadratic relation between mean (μ_o) and CRL [$\mu_0 = \beta_0 + \beta_1 CRL + \beta_2 CRL^2 + \beta_3$ (racial group = Afro-Caribbean)] with a constant standard deviation (σ_0), and secondly, a proportion (p) in which DV PIV arises from a distribution with a substantially higher mean μ_1, independent of CRL, and SD of σ_1.

Table 1. Characteristics of the study population

Characteristics	Normal (n = 44,756)	Trisomy 21 (n = 202)	Trisomy 18 (n = 72)	Trisomy 13 (n = 30)
Maternal age in years, median (IQR)	32.1 (27.9–35.6)	38.1 (35.0–40.2)**	37.8 (33.8–41.1)**	34.7 (29.1–38.2)**
Maternal weight in kg, median (IQR)	65.0 (59.0–75.0)	65.0 (60.0–72.3)	66.7 (59.5–77.6)	70.0 (63.5–82.8)
Spontaneous conception, n (%)	43,185 (96.5)	179 (88.6)*	61 (84.7)*	27 (90.0)
Smoker, n (%)	3,248 (7.3)	14 (6.9)	5 (6.9)	1 (3.3)
Racial origin, n (%)				
Caucasian	32,068 (71.6)	172 (85.1)	52 (72.5)	25 (83.3)
Afro-Caribbean	8,062 (18.0)	21 (10.4)*	15 (20.8)	4 (13.3)
South Asian	2,190 (4.9)	2 (1.0)	4 (5.6)	1 (3.3)
East Asian	1,186 (2.6)	4 (2.0)	0	0
Mixed	1,251 (2.8)	3 (1.5)	1 (1.4)	0
CRL in mm, median (IQR)	63.4 (58.4–68.7)	64.0 (58.6–70.0)	55.4 (50.0–60.6)**	58.0 (53.2–61.9)**
Delta NT in mm, median (IQR)	0.20 (0.00–0.42)	2.16 (1.15–3.71)**	3.84 (0.82–6.13)**	1.23 (0.55–4.96)**
Serum PAPP-A in MoM, median (IQR)	1.04 (0.71–1.47)	0.56 (0.35–0.83)**	0.21 (0.13–0.37)**	0.25 (0.17–0.41)**
Serum free β-hCG in MoM, median (IQR)	0.98 (0.66–1.47)	2.06 (1.37–3.00)**	0.19 (0.12–0.30)**	0.49 (0.39–0.77)**

* $p < 0.05$; ** $p < 0.01$.

In each aneuploidy (trisomy 21, 18, and 13) it is assumed that DV PIV also arises from a mixture of two Gaussian distributions: firstly, a proportion $(1 - p_{aneuploidy})$ with the same CRL-dependent distribution as in euploid pregnancies, and secondly, an aneuploidy-specific proportion ($p_{aneuploidy}$) in which DV PIV arises from a distribution with a substantially higher mean $\mu_{aneuploidy}$ than μ_o and standard deviation of $\sigma_{aneuploidy}$. The proportions $p_{aneuploidy}$ defining the mixture, and the mean and standard deviation of the CRL-independent component differ according to aneuploidy (trisomy 21, 18 and 13).

For euploid pregnancies the CRL-dependent component dominates and p is close to 0 whilst for the aneuploidies the CRL-dependent component is uncommon and p is close to 1. In euploid pregnancies p is dependent on CRL and racial origin according to a logistic regression model. In this model CRL was centered by subtraction of 65.

We assume that variation between operators is represented by random effects acting additively on DV PIV with mean 0 and standard deviation σ_{op}. The degree of heterogeneity between operators is reflected in the magnitude of σ_{op}.

The mixture model was fitted within a Bayesian framework using Markov chain Monte Carl implemented in WInBUGS [15] with vague priors. Details are available on request from the authors.

Assessment of Screening Performance

Standardized detection rates and false positive rates of screening by serum free β-hCG and PAPP-A, fetal NT and DV PIV, separately and in combination, at different risk cutoffs were estimated. Standardized rates were produced by first calculating age-specific detection rates and false positive rates and then weighting them according to the maternal age distributions of affected and unaffected pregnancies in England and Wales in 2002 [16]. Confidence intervals for detection rates and false positive rates were obtained by bootstrapping.

In these calculations the following steps were taken to produce patient-specific risk of trisomy 21. First, the maternal age-related risk at term was calculated and adjusted according to the gestational age at the time of screening. Second, the likelihoods of normal and trisomy 21 pregnancies for given NT were obtained from the mixture model for NT [7]. Third, the likelihoods for given DV PIV were obtained from the model presented in this paper. Fourth, the measured free β-hCG and PAPP-A were converted into MoM for gestational age adjusted for maternal weight, racial origin, smoking status, method of conception, parity and machine for the assays [17]. Fifth, bivariate Gaussian distributions were used for the distribution of log MoM free β-hCG and log MoM PAPP-A in normal and trisomy 21 pregnancies. Sixth, the likelihoods for normal and trisomy 21 pregnancies for given NT, DV PIV and biochemical markers were assumed to be independent conditionally on outcome. Their product was then combined with the prior distribution over the possible outcomes using Bayes theorem to form a posterior risk.

Results

Characteristics of the Study Population

During the study period, we examined 50,804 singleton pregnancies. We excluded 2,586 (5.1%) cases because they had missing outcome data (n = 1,671) or the fetal karyotype was not known and the pregnancies resulted in termination, miscarriage or stillbirth (n = 915).

In the study population of 48,218 cases there were 47,830 (99.2%) with normal fetal karyotype or the birth of a phenotypically normal neonate (euploid group) and 388 (0.8%) cases with prenatal diagnosis of fetal trisomy

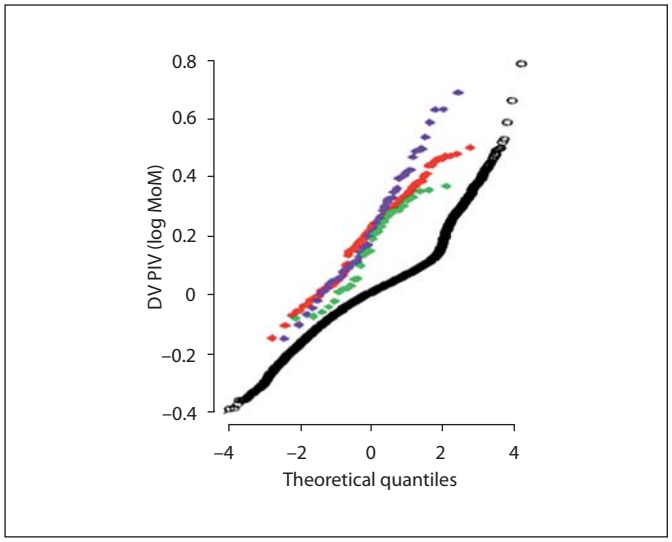

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Fig. 1. Gaussian probability plots of log MoM values for DV PIV in normal (black), trisomy 21 (red), trisomy 18 (purple) and trisomy 13 (green) pregnancies (colors refer to the online version only).

Table 2. Fitted mixture model for DV PIV

Parameter	Estimate	95% CI
CRL-dependent		
Intercept (β_0)	0.6977	0.6238, 0.7708
Coefficient of CRL (β_1)	0.01189	0.00960, 0.01418
Coefficient of CRL^2 (β_2)	–0.0001036	–0.0001212, –0.0000859
Afro-Caribbean race (β_3)	0.01768	0.01404, 0.02139
Standard deviation (σ_0)	0.1475	0.1464, 0.1487
CRL-independent		
Logistic model intercept for mixture proportion		
Intercept (α_0)	–3.3670	–3.4620, –3.2730
Coefficient of CRL-65 (α_1)	–0.02474	–0.03260, –0.01701
Afro-Caribbean race (α_2)	0.8684	0.7494, 0.9890
Mean (μ_1)	1.5610	1.5210, 1.6030
Standard deviation (σ_1)	0.5009	0.4824, 0.5192
Trisomy 21		
Proportion (p_{T21})	0.8682	0.7915, 0.9411
Mean (μ_{T21})	1.8340	1.7420, 1.9240
Standard deviation (σ_{T21})	0.5009	0.4824, 0.5192
Trisomy 18		
Proportion (p_{T18})	0.8327	0.6791, 0.9998
Mean (μ_{T18})	2.0780	1.8010, 2.3590
Standard deviation (σ_{T18})	0.9004	0.7489, 1.0870
Trisomy 13		
Proportion (p_{T13})	0.7819	0.5150, 1.0000
Mean (μ_{T13})	1.7280	1.4510, 2.0140
Standard deviation (σ_{T13})	0.5009	0.4824, 0.5192
Operator standard deviation (σ_{op})	0.0354	0.0317, 0.0395

21 (n = 202), trisomy 18 (n = 72), trisomy 13 (n = 30) or other aneuploidy (Turner syndrome, n = 25; triploidy, n = 20; sex chromosome aneuploidies, mosaicisms, deletions or translocations, n = 39). The observed number of trisomies 21, 18 and 13 was not significantly different from the expected [18] on the basis of maternal age and gestational age at the time of screening (trisomy 21 expected 199.8, p = 0.88; trisomy 18 expected 81.8, p = 0.28; trisomy 13 expected 26.1, p = 0.44).

In the euploid group there were 44,756 (93.6%) cases with normal pregnancy outcome (normal group) and 3,074 (6.4%) that developed preeclampsia and/or delivered small for gestational age neonates. The characteristics of the normal group and the cases of trisomies 21, 18 and 13 are presented in table 1.

Mixture Model

Probability plots of log MoM DV PIV in normal, trisomy 21, trisomy 18 and trisomy 13 pregnancies showed substantial departures from Gaussian distributions (fig. 1).

The fitted mixture model is described in table 2 and figure 2. In the normal pregnancies, the dominant part of the mixture was the CRL-dependent Gaussian distribution which accounted for about 97% of cases in Caucasians and 93% in Afro-Caribbeans. In contrast, in all aneuploid pregnancies the dominant part of the mixture was the CRL-independent Gaussian distribution, which accounted for about 85% of cases with trisomies 21 and 18 and 70% of trisomy 13. However, because of relatively small numbers there is considerable uncertainty about the mixing proportions for the cases of trisomies 18 and 13. There was little evidence of any difference in the standard deviations of the CRL-independent distributions for normal, trisomy 21 or trisomy 13 outcomes. These were constrained to be the same in the final model presented in table 2.

In the implementation of the mixture model it is necessary to incorporate truncation limits to prevent misleading results at extreme values and ensure that the likelihood ratios are a monotonic function of DV PIV. The lower truncation limits for DV PIV, which depend on CRL and racial origin, were set at the point where the likelihood ratios (unaffected/affected) reach their maxima. We truncated the upper limits on the basis of the 90th percentile of the CRL-independent process in trisomy 21, which occurs at a DV PIV of 2.45. Figure 3 shows the likelihood ratios (unaffected/affected) for Afro-Caribbean and other racial origins at a CRL of 65 mm. It is notable that larger DV PIV are associated with lower risks in Af-

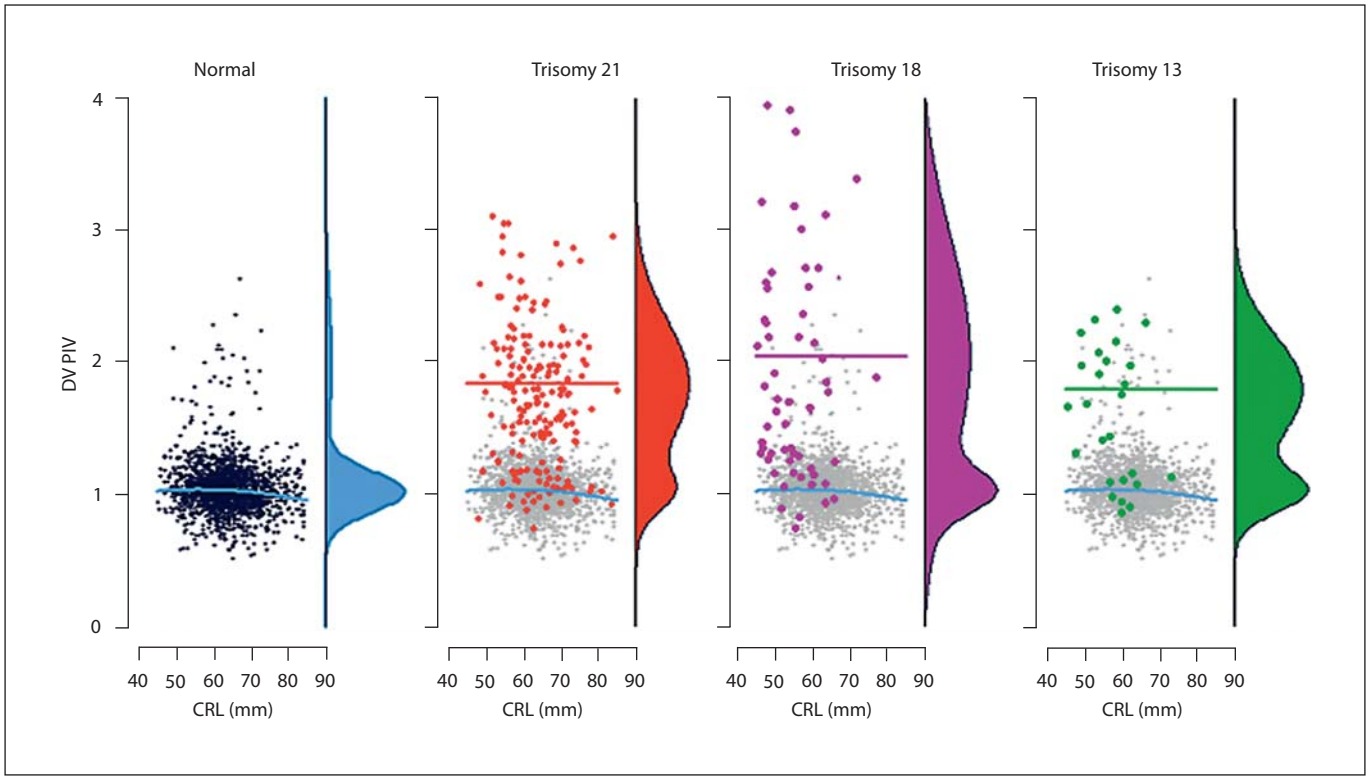

Fig. 2. Mixture model with CRL-independent and CRL-dependent distribution of DV PIV in euploid, trisomy 21, trisomy 18 and trisomy 13 pregnancies.

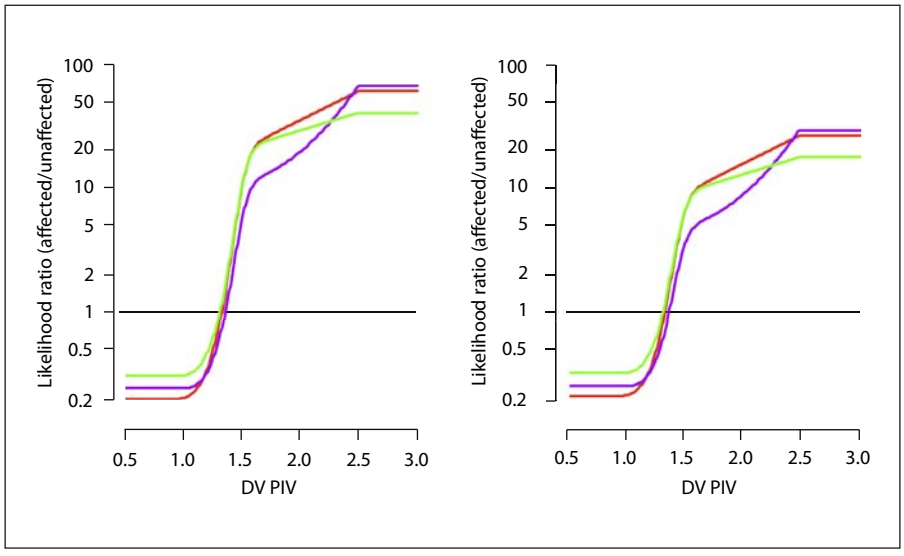

Fig. 3. Relationship between DV PIV and likelihood ratio for trisomy 21 (red), trisomy 18 (purple) and trisomy 13 (green) in Caucasian (left) and Afro-Caribbean (right) racial origin (colors refer to the online version only).

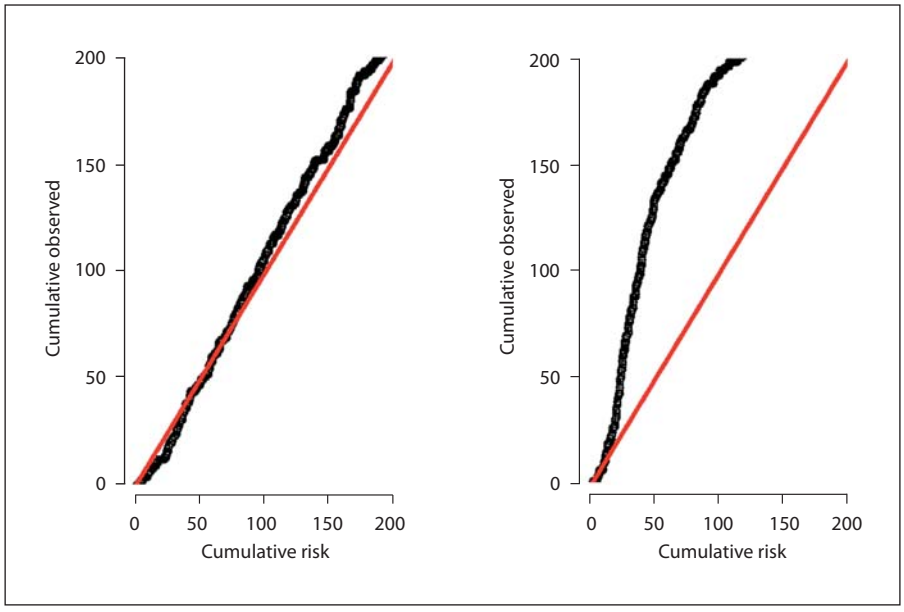

Fig. 4. Cumulative observed and expected numbers of trisomy 21 pregnancies obtained from the mixture model of DV PIV (left) and the previously reported MoM model (right). In the construction of these plots, risks were first ordered from highest to lowest. The expected and observed values should follow the red line for which the observed and expected values are equal.

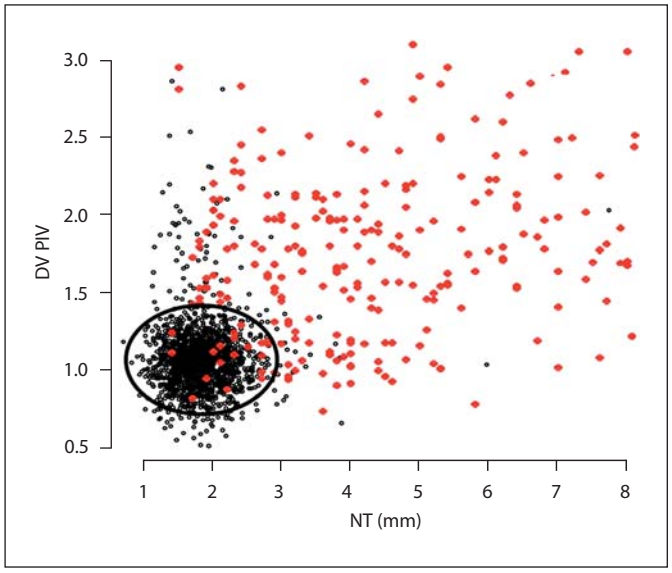

Fig. 5. Relationship between NT thickness and DV PIV in euploid (black) and trisomy 21 (red) pregnancies. The black ellipse contains 97% of the euploid pregnancies (color refers to the online version only).

ro-Caribbeans than in other races. This reflects the fact that in Afro-Caribbean women such values occur more frequently in unaffected pregnancies.

There was good agreement between the observed and expected number of trisomy 21 pregnancies obtained by cumulative risk estimated from the mixture model of DV PIV (fig. 4). In contrast, the previously reported MoM model [6] was associated with a substantial underestimation of risk (fig. 4).

Performance of Screening

The relationship between NT thickness and DV PIV in euploid and trisomy 21 pregnancies is shown in figure 5. In 184 (91.1%) of the 202 cases of trisomy 21, the values were outside an ellipse that contained 97% of the euploid pregnancies. Although most fetuses with trisomy 21 had an increase in both NT thickness and DV PIV, there were some where only NT thickness or DV PIV was increased.

Standardized detection rates and false positive rates of screening for trisomy 21 by serum biochemistry, fetal NT and DV PIV, separately and in combination, at different risk cutoffs are shown in table 3. Addition of DV PIV to the combined test was associated with an increase in detection rate and decrease in false positive rate at all risk cutoffs.

Discussion

Effective first-trimester screening for trisomy 21 is provided by a combination of maternal age, fetal NT thickness and maternal serum free β-hCG and PAPP-A, with an estimated detection rate of about 90% at a false positive

Table 3. Estimates with 95% CI of standardized detection rates (DR %) and false positive rates (FPR %) of screening for trisomy 21 by serum biochemistry, fetal NT thickness and DV PIV, both separately and in combination at fixed cutoffs of risk at the time of screening

Method of screening	Risk cutoff 1 in 50		Risk cutoff 1 in 100		Risk cutoff 1 in 200		Risk cutoff 1 in 300	
	FPR	DR	FPR	DR	FPR	DR	FPR	DR
Free β-hCG, PAPP-A	2.85 (2.74–2.97)	54.3 (49.0–59.5)	5.26 (5.11–5.41)	65.0 (60.2–69.9)	9.19 (8.99–9.39)	75.0 (70.6–79.3)	12.48 (12.25–12.70)	80.5 (76.5–84.4)
NT	1.18 (1.10–1.25)	73.6 (67.6–79.6)	2.22 (2.12–2.32)	79.5 (73.9–85.1)	4.57 (4.44–4.70)	84.5 (79.6–89.4)	6.93 (6.79–7.07)	86.8 (82.4–91.2)
DV PIV	1.46 (1.39–1.54)	59.4 (54.5–64.4)	2.97 (2.84–3.10)	72.3 (66.7–77.9)	4.59 (4.44–4.73)	77.3 (72.1–82.5)	6.44 (6.29–6.59)	79.9 (75.2–84.7)
NT and DV PIV	0.91 (0.85–0.96)	80.9 (76.4–85.3)	1.71 (1.63–1.80)	86.7 (82.9–90.5)	3.17 (3.05–3.29)	90.7 (87.4–94.0)	4.39 (4.24–4.54)	92.7 (89.8–95.7)
Free β-hCG, PAPP-A and NT	1.53 (1.44–1.61)	85.8 (81.7–89.9)	2.74 (2.62–2.85)	89.7 (86.1–93.2)	4.75 (4.60–4.91)	92.9 (90.0–95.9)	6.47 (6.29–6.65)	94.4 (91.8–97.0)
Free β-hCG, PAPP-A and DV PIV	1.6 (1.51–1.69)	73.9 (68.8–78.9)	2.75 (2.63–2.86)	80.6 (76.1–85.2)	4.62 (4.46–4.77)	86.0 (82.1–89.8)	6.17 (6.0–6.35)	89.2 (85.8–92.5)
Free β-hCG, PAPP-A, NT and DV PIV	1.03 (0.96–1.11)	90.1 (86.8–93.4)	1.63 (1.54–1.72)	93.5 (90.8–96.2)	2.61 (2.49–2.73)	96.0 (93.8–98.1)	3.45 (3.32–3.58)	97.0 (95.1–98.9)

The reference population is the maternal age distribution of England and Wales 2000–2002.

rate of 3%. The findings of this study demonstrate that incorporating measurement of DV PIV in first-trimester combined screening can improve the detection rate to about 95% and reduce the false positive rate to about 2.5%. Alternatively, combining fetal NT with DV PIV has essentially the same high performance as screening by a combination of fetal NT with serum biochemistry.

Sonographers undertaking risk assessment by Doppler examination of the DV should receive appropriate training and certification of their competence in performing such a scan and should adhere to a series of strict criteria for obtaining the appropriate waveform [14]. We have previously shown that sonographers with prior extensive experience in the 11–13 weeks scan require an average of 80 examinations before they can achieve this level of competence [14].

In our previous studies on DV flow, we have classified the waveforms as normal, when the a-wave observed during atrial contraction was positive, or abnormal, when the a-wave was absent or reversed [2–5]. Assessing the DV PIV and the associated likelihood ratio for aneuploidies as a continuous variable, rather than as a categorical variable, is particularly useful when the a-wave velocity is close to 0 and there is doubt as to whether to classify the waveform as normal or abnormal. In the categorical approach the likelihood ratio changes dramatically from very low, if the waveform is classified as normal, to very high, if classified as abnormal. When the a-wave velocity is high, the waveform is easily classified as normal, the DV PIV is invariably low and in both cases the likelihood ratio for aneuploidies is low. Similarly, when the a-wave has a high negative velocity, it is easily classified as reversed and the DV PIV is high. The overall performance of screening by the continuous and categorical approaches [5] is similar. However, the estimated patient-specific risk for aneuploidies in cases where the a-wave velocity is close to 0 is likely to be more accurate with the use of DV PIV rather than the classification of the waveform into normal or abnormal.

Our results indicate that the distribution of fetal DV PIV log MoM in euploid and aneuploid pregnancies is not Gaussian and demonstrate that DV PIV follows two distributions, one which is CRL-dependent and another which is CRL-independent. The CRL-dependent distribution is the same for normal and aneuploid pregnancies, but the proportion that follows this distribution is large in the normal group (about 97% for Caucasians and 93% for Afro-Caribbeans) and small in the abnormal group, being about 15% for trisomies 21 and 18 and 30% for trisomy 13. In contrast, the proportion of cases in which DV PIV does not change with gestation is, firstly, small for normal pregnancies and large for the aneuploidies and, secondly, the median and spread of DV PIV values are different for each aneuploidy.

A mixture model of the form we propose is useful in situations where a single Gaussian or other distribution fails to provide an adequate fit to the data. One of the earliest applications, published in 1894, was a mixture of two Gaussian distributions fitted by Karl Pearson on the ratio of forehead to body length in crabs sampled from the Bay of Naples. This led to the conjecture that the crabs came from different species [19]. In first-trimester screening for aneuploidies, we have successfully applied the mixture model to describe the distribution of fetal NT in euploid and aneuploid pregnancies [7]. This model provides a better fit to NT data than the Gaussian model for log-transformed MoM values.

The mixture model of both NT and DV PIV distributions is compatible with our understanding of the pathophysiology of increased NT and high DV PIV in both euploid and aneuploid fetuses. The CRL-dependent NT and DV PIV distributions in normal pregnancies presumably reflects a physiological development of the fetal nuchal region and cardiovascular dynamics during the gestational range of 11–13 weeks. The proportion of aneuploid fetuses following the CRL-dependent and CRL-independent distributions of NT and DV PIV is compatible with the wide range of phenotypic expression in these aneuploidies. The mixture models for NT and DV PIV recognize that a minority of aneuploid fetuses are similar in terms of their NT thickness and/or DV flow pattern to the majority of normal fetuses. This limits the extent to which a 'normal' NT or DV PIV reduces the risks for aneuploidies. Similarly, these models recognize that a minority of euploid fetuses have high NT and/or high DV PIV, like the majority of aneuploid fetuses. This limits the extent to which a high NT or DV PIV increases the risks for aneuploidies.

In normal pregnancies from women of Afro-Caribbean racial origin the proportion of fetuses in the CRL-independent distribution was twice as high as in Caucasians. There is no obvious explanation for this finding, but racial effects have also been observed for other sonographic and biochemical markers used in screening for aneuploidies and these should be taken into account in providing patient-specific risks. In women of Afro-Caribbean racial origin, compared to Caucasians, at 11–13 weeks' gestation the levels of PAPP-A, free β-hCG and placental growth factor are increased by about 60, 50 and 10%, respectively [17, 20], and there is a fivefold increase in absence of the fetal nasal bone [21].

In conclusion, Doppler assessment of flow in the fetal DV is useful in first-trimester screening for trisomy 21. However, in the estimation of patient-specific risk for aneuploidies it should not be assumed that the distribution of measurements of DV PIV or log MoM DV PIV is Gaussian. As in the case of fetal NT, the distribution of DV PIV is bimodal and is best described by the mixture model.

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