Impact of Ductus Venosus Assessment in Screening Down Syndrome Protocols: An Improved Strategy in a Fetal Medicine Unit

[1]Carmen Comas, [1]M Angeles Rodríguez, [1]Mónica Echevarría, [1]Joan Nicolau, [1]Bernat Serra [2]Concha Bach [2]Josep Sabrià

[1]Fetal Medicine Unit, Department of Obstetrics and Gynecology, Institute Dexeus, Barcelona, Spain
[2]SBP Soft 2007 SL Girona, Spain

Correspondence: Carmen Comas, Fetal Medicine Unit, Department of Obstetrics and Gynecology, Institute Dexeus, Gran Via Carles III 71-75 08028 Barcelona, Spain, Phone: 0034-932274706, Fax: 0034-934187832, e-mail: carcom@dexeus.com

ABSTRACT

Objective: To estimate the improvement in screening efficiency when ductus venosus (DV) Doppler studies are added to existing Down syndrome (DS) screening protocols.

Methods: First-trimester combined screening for trisomy 21 was prospectively carried out, from October 2003 to March 2008, in 8842 consecutive singleton pregnancies attended in our tertiary reference center. The nuchal translucency (NT) and the pulsatility index for veins for DV were calculated. The maternal serum biochemistry was measured using the Kryptor analyzer, at the same time of the scan (one step strategy) or before it (two step strategy). The detection rate (DR) and false-positive rates for standard screening strategy (maternal age, NT and biochemistry) and the same strategy but including DV assessment were calculated.

Results: Successful DV assessment was possible in the 95.3% of cases, representing a total of 8426 cases. Down syndrome was identified in 34 pregnancies (prevalence of DS 1:250). For a fixed screen positive rate of 5%, the addition of the DV assessment improves the DR from 85 to 94% and, for a fixed DR of 85%, it reduces the number of unnecessary invasive tests from 3.7 to 3.2%.

Conclusion: Early evaluation of DV can be introduced to standard DS screening strategies in experienced centers as a first level test to reduce invasive test rate derived from the existing protocols.

Keywords: Ductus venosus, trisomy 21, first-trimester screening, Doppler ultrasound.

INTRODUCTION

There is extensive evidence that screening by combination of nuchal translucency (NT) and maternal serum free beta-human chorionic gonadotropin (free β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in the first trimester is the most effective current strategy to detect Down syndrome (DS).[1-4] Recently, several studies have demonstrated an increased impedance to flow in the ductus venosus at the 11 to 13^{+6} weeks' scan in DS fetuses.[5-12] Therefore, ductus venosus (DV) Doppler studies could be used to modify the estimated DS risk either selectively in women referred because high-risk for this condition as a secondary screening method,[5-7,11,13-15] or incorporated routinely into existing screening protocols as a primary screening method.[6,9,12] Technical difficulties in DV assessment have been postulated as the main limitation to incorporate this marker as a first line screening test to evaluate individual DS risk in combination with NT, although competence in Doppler assessment of the DV can be achieved after extensive supervised training. The aim of this prospective non-interventional study was to estimate the improvement in screening efficiency in trained operators when ductus venosus Doppler studies are routinely added to existing Down syndrome screening protocols.

METHODS

First-trimester combined screening for trisomy 21 (including maternal age, biochemistry and nuchal translucency) was prospectively carried out to singleton pregnancies which were attended in our tertiary referral fetal medicine unit from October 2003 to March 2008. From October 2003 to January 2007, Down syndrome was screened by a one-step protocol, with blood taken for maternal serum pregnancy associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotrophin (free β-hCG) the same day the ultrasound was performed. From February 2007 we decided to move to two step strategy, measuring biochemical markers between two to four weeks before the NT was assessed. Scans were performed trans-vaginally, or combined with transabdominal route depending on patients and fetal conditions, by eight experienced operators. NT was measured according to Fetal Medicine Foundation criteria, considering CRL from 40 to 85 mm. Although the usual

rank for the NT measurement corresponds to CRL levels of 45-84 mm, our program for risk calculation permits the risk estimation in a wider CRL spectrum. This is the reason why we consider the rank mentioned above. The equation that relates the NT with the CRL is, by default, a polynomial function with exponential transformation 10 (10^), applied to the formula: NT = 10 ^ (– 0.3599 + 0.0127 CRL – 0.000058 CRL^2).[16] For Doppler studies, a fetal mid-sagital view was obtained during fetal quiescence, and ductus venosus pulsatility index for veins (DVPIV) was calculated according to previous published methodology.[17] The maximum time for DV assessment was predefined as up to 5 minutes. The maternal serum biochemistry was measured using the Kryptor analyzer (Brahms AG, Berlin, Germany). According to our protocol, fetal karyotyping was performed as a result of advanced maternal age (over 38), family history of aneuploidy, parental anxiety, presence of ultrasound anomalies and increased combined risk for DS (>1/270). Postnatal follow-up confirming normal outcome was obtained after delivery in our center or by a telephone questionnaire.

All four markers, DVPIV, NT, PAPP-A and free β-hCG were expressed as multiples of the gestation-specific normal median (MoM) based on regression. The serum markers were also adjusted for maternal weight, ethnicity, self-recorded smoking status and previous diagnosis of maternal type 1 diabetes mellitus. Distribution curves of DVPIV and biochemical parameters were estimated from the current study. Mean values and standard deviation values were calculated, excluding chromosomal abnormalities. Pearson's correlation was used to determine linear relationships between variables. For combined risk calculation the commercial available SsdwLab5-software (SBP-Software) was used.[18] As these variables present a Gaussian distribution, it is possible to estimate the likelihood ratio (LR) for each variable or the combination of all of them, considering the correlation between them, because the "Likelihood method" is based on the normal multivariate distribution.[19] The software, which uses the multivariate normal distribution function, combines the MoM of the markers to obtain the LR for a combined screening test. Two screening policies were considered, the standard screening strategy (maternal age, NT and biochemistry) and the same strategy but including DVPIV assessment. Performance was expressed in three ways: the detection rate (DR) and false positive rate (FPR) for a fixed cut-off risk at screening and term, the DR for a fixed FPR and the FPR for a fixed DR. Receiver-operating characteristic (ROC) curve analysis were used to analyze the accuracy of the different strategies. The area under curve is displayed; the coordinates of the curve provided the guidance for determining what should serve as the cut-off for determining positive and negative screening results. The Chi-Square test was used to compare DR and FPR between strategies.

RESULTS

First-trimester combined screening for trisomy 21 was prospectively carried out, from October 2003 to March 2008, in 8842 consecutive singleton pregnancies at 10^{+0} to 13^{+6} gestational weeks, including maternal age, biochemistry and nuchal translucency. Successful DV assessment was possible in the 95.3% of cases, representing a total of 8426 cases included in the study. The mean maternal age was 33 (range 17-45) years and the mean gestational age at scan was 11 (range 10-13.6) weeks. The population included 31.6% over 35 years. An invasive procedure was done in 28.8% of our population. Down syndrome was identified in 34 pregnancies: 29 Down syndrome cases prenatally detected by the current combined screening strategy, 3 cases prenatally diagnosed later on (by detection of structural abnormalities at the morphological scan at 20 weeks) and 2 postnatal diagnosed cases. Follow-up was achieved in 87.1% of pregnancies in which an invasive procedure was not performed.

1. *Distribution values of biochemical markers:* PAPP-A and free β-hCG distribution parameters were estimated from the current study, excluding chromosomal abnormalities (Table 1 and Fig. 1). Our study allows us to obtain the distribution of biochemical values at early stages of pregnancy.
2. *Comparison between one-step and two-step strategies:* During the first period of the study, Down syndrome was screened by a one-step protocol, with blood taken for maternal biochemistry the same day the ultrasound was performed. A total of 6120 patients were included in the one-step strategy, and further 2306 patients were included in the two-step strategy. There was a statistically significant difference in the gestational age at which the NT measurements were done in the one-step and two-step populations (one step: CRL 61.77 mm +/–9.3; two-steps CRL 54.29 mm +/–7.72; $p < 0.05$). Our experience has demonstrated a higher efficiency when biochemistry is performed earlier (FPR of 8.6% and 2.4%, respectively, in one-step and two-step strategy) ($p < 0.001$). Therefore, from February 2007 onwards we decided to move to the two-step strategy, measuring biochemical markers from two to four weeks before the ultrasound is done.
3. *Distribution values of DVPIV:* Ductus venosus pulsatility indices are not normally distributed, while Log 10 MoM (DVPIV) is normally distributed (Q-Q Normal Plot). DVPIV distribution parameters were estimated from the current study, according to the following quadratic model: DVPIV median = EXP (–0.2277417054 + 0.0119299157 × CRL– 0.0001180264 × CRL^2). Table 2 shows the DPIV values across

Table 1: Population parameters for PAPP-A and free β-hCG using Kryptor analyzer

Gestational age (completed weeks)	*Median(days of gestation)*	*n*	*PAPP-A mIU/l Median*	*PAPP-A mIU/l Regressed*[1]	*β-hCG ng/ml Median*	*β-hCG ng/ml Regressed*[2]
8	60	589	317.17	295.58	73.72	74.81
9	66	896	630.27	627.67	74.36	72.10
10	72	524	1079.39	1193.71	62.19	64.79
11	80	244	1991.17	2033.88	53.83	54.14
12	87	293	3069.07	3104.63	40.93	41.95
13	94	133	3639.70	4245.74	34.23	30.06

Polynomial exponential equation with 3 coefficients for PAPP-A[1]
a = –5.0233364486
b = 0.24775223
c = –0.0011217409

Polynomial exponential equation with 4 coefficients for free β-hCG[2]
a = 2.2344743609
b = 0.0661916474
c = –0.0004438673
d = –0.0000013621

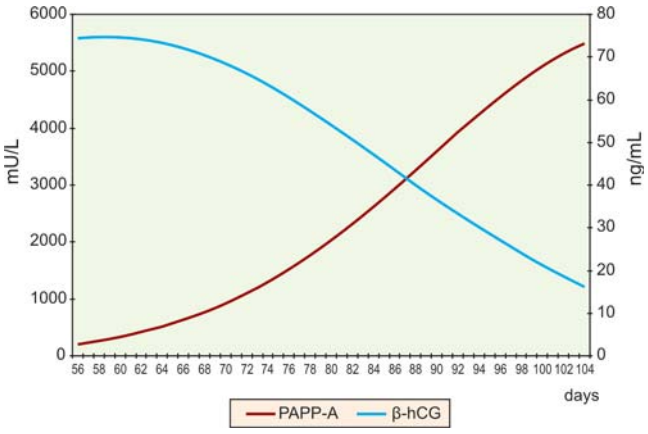
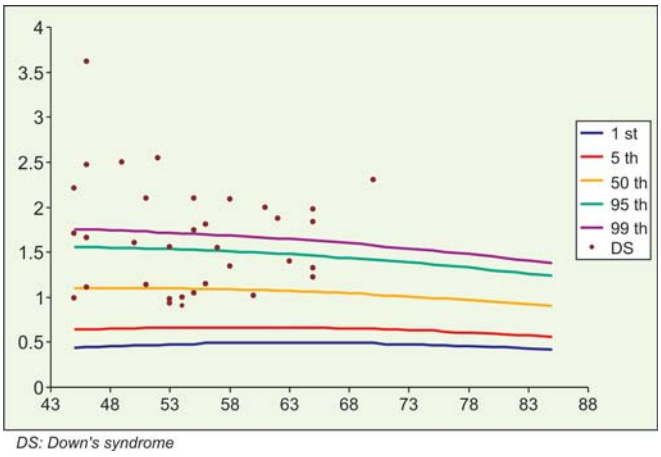


Fig. 1: PAPP-A and free β-hCG distribution



DS: Down's syndrome

Fig. 2: DVPIV values at 11-14 weeks' scan. DVPIV median = EXP (–0.2277417054 + 0.0119299157 × CRL–0.0001180264 × CRL^2

gestational ages from a CRL of 45 mm to 85 mm, excluding the cases between 40 and 45 mm due to the low casuistic in such pregnancy period. Figure 2 shows the DVPIV values in chromosomically normal fetuses, expressed as centiles of population, plotted against the individual values in DS cases, expressed as dots.

4. *Correlation between markers:* In those fetuses with a normal karyotype, DVPIV correlated negatively with gestational age (r = –0.067), PAPP-A (r = –0.0274) and free β-hCG (r = –0.0227) and weak positively with NT (r = 0.1488). In Down syndrome pregnancies, a statistically significant positive correlation between DVPIV and NT was found (r = 0.284) (Table 3).

5. *Comparison between screening policies:* The addition of DVPIV to NT, PAPP-A and free β-hCG, the addition of DVPIV (at screening time and using the cut-off value of 1/270) increases the DR from 85.3 to 88.2% and significantly reduces the FPR from 6.5 to 4.4% ($p < 0.001$) (Table 4). Figure 3 and Table 5 show the receiver-operating curves for the performance of the different strategies. Table 6 displays the DR for a fixed 5% FPR. Table 7 demonstrates the FPR for a fixed 85% DR. Although the AUC were not significantly different, the addition of the DV assessment increases the DR (from 85.3 to 94.1% at a fixed 5% FPR), and decreases the FPR (from 3.7 to 3.2% at a fixed 85% DR). Looking at the original policy in our unit (one-step standard strategy) and the current one (two-steps strategy including the DV

Table 2: DVPIV values at 11-14 weeks' scan

CRL	*1 st*	*5 th*	*50 th*	*95 th*	*99 th*
45	0.44	0.64	1.1	1.56	1.75
46	0.45	0.64	1.1	1.56	1.75
47	0.45	0.64	1.1	1.56	1.75
48	0.46	0.65	1.1	1.55	1.74
49	0.46	0.65	1.1	1.55	1.74
50	0.47	0.65	1.1	1.55	1.73
51	0.47	0.66	1.1	1.54	1.73
52	0.47	0.66	1.1	1.54	1.72
53	0.48	0.66	1.1	1.54	1.72
54	0.48	0.66	1.1	1.53	1.71
55	0.48	0.66	1.09	1.53	1.71
56	0.49	0.66	1.09	1.52	1.7
57	0.49	0.66	1.09	1.52	1.69
58	0.49	0.66	1.09	1.51	1.69
59	0.49	0.66	1.08	1.5	1.68
60	0.49	0.66	1.08	1.5	1.67
61	0.49	0.66	1.08	1.49	1.66
62	0.49	0.66	1.07	1.48	1.65
63	0.49	0.66	1.07	1.48	1.65
64	0.49	0.66	1.06	1.47	1.64
65	0.49	0.66	1.06	1.46	1.63
66	0.49	0.66	1.05	1.45	1.62
67	0.49	0.65	1.05	1.44	1.61
68	0.49	0.65	1.04	1.44	1.6
69	0.49	0.65	1.04	1.43	1.59
70	0.49	0.65	1.03	1.42	1.58
71	0.48	0.64	1.02	1.41	1.56
72	0.48	0.64	1.02	1.4	1.55
73	0.48	0.63	1.01	1.39	1.54
74	0.48	0.63	1	1.38	1.53
75	0.47	0.63	0.99	1.36	1.52
76	0.47	0.62	0.99	1.35	1.5
77	0.46	0.61	0.98	1.34	1.49
78	0.46	0.61	0.97	1.33	1.48
80	0.45	0.6	0.95	1.3	1.45
81	0.45	0.59	0.94	1.29	1.44
82	0.44	0.58	0.93	1.28	1.42
83	0.43	0.58	0.92	1.26	1.41
85	0.42	0.56	0.9	1.24	1.38

Table 3: Population parameters for ductus venosus

	Unaffected pregnancies (n = 8375)	*Down syndrome (n = 34)*
Mean Log 10 MoM	–0.0017	0.1681
Standard deviation log 10 MoM	0.0933	0.1539
Correlation coefficient between log 10 MoM values		
DVPIV - NT	0.1488	0.2841
DVPIV - PAPP-A	–0.0274	–0.1352
DVPIV - free β-hCG	–0.0227	–0.3351
MoM truncation limits		
Superior	1.5	
Inferior	0.67	

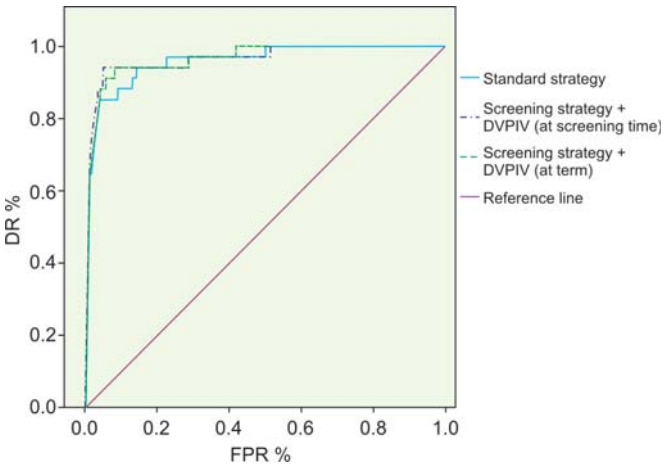


Fig. 3: Receiver-operating (ROC) curves for the performance of screening for DS considering standard strategy (maternal age, NT and biochemistry) and the same strategy including DVPIV, at screening time and at term. DVPIV: Ductus venosus pulsatility index for veins, DR: Detection rate, *FPR: False positive rate*

Table 4: Efficiency according to the screening strategy

Standard screening strategy					*Screening strategy including DVPIV*				
Cut-off	*Risk at screening*		*Term risk*		*Cut-off*	*Risk at screening*		*Term risk*	
	DR%	*FPR%*	*DR%*	*FPR%*		*DR%*	*FPR%*	*DR%*	*FPR%*
1/250	85.3	6.1	85.3	3.9	1/250	88.2	4.2	85.3	3.1
1/270	85.3	6.45*	85.3	4.1	1/270	88.2	4.4*	88.2	3.3
1/300	85.3	7	85.3	4.45	1/300	94.1	4.8	88.2	3.6
1/350	85.3	7.8	85.3	5	1/350	94.1	5.3	88.2	3.9
1/400	85.3	8.7	85.3	5.5	1/400	91.2	5.9	88.2	4.4

DR: Detection rate
FPR: False positive rate
*p < 0.001

Table 5: Strategy comparison by mean of ROC curves

Strategies	AUC	signi-ficance	CI 95%	
Standard strategy	0.959	< 0.001	0.932	0.986
Screening strategy + DVPIV (at screening time)	0.963	< 0.001	0.932	0.995
Screening strategy + DVPIV (at term)	0.962	< 0.001	0.931	0.994

ROC: Receiver-operating curves
AUC: Area under curve
CI: Confidence interval

Table 6: Performance of DS screening expressed as the DR for a fixed 5% FPR

First trimester Down's syndrome screening	*DR for 5% FPR*	*Detected/Total*	*n*
One-step standard strategy	83.3	25/30	6120 (30 DS)
One-step standard strategy + DVPIV	93.3	28/30	
Two-step standard strategy	100	4/4	2306 (4 DS)
Two-step standard strategy + DVPIV	100	4/4	
Standard strategy (one or two-steps)	85.3	29/34	8426 (34 DS)
Standard strategy (one or two-steps) + DVPIV	94.1	32/34	

DVPIV: Ductus venosus pulsatility index for veins
DR: Detection rate
FPR: False positive rate
DS: Down's syndrome

Table 7: Performance of DS screening expressed as the FPR for a fixed 85% DR

First trimester Down's syndrome screening	*FPR at 85% DR*	*nº invasive test*	*n*
One-step standard strategy	8.6	544	6120 (30SD)
One-step standard strategy + DVPIV	3.5	237	
Two-step standard strategy	2.4	58	2306 (4SD)
Two-step standard strategy + DVPIV	3.0	71	
Standard strategy (one or two-steps)	3.7	338	8426 (34 SD)
Standard strategy (one or two-steps) + DVPIV	3.2	293	

DVPIV: Ductus venosus pulsatility index for veins
DR: Detection rate
FPR: False positive rate
DS: Down's syndrome

assessment), we could reduce the FPR from 8.6 to 3.0% (avoiding 472 invasive tests in our group population).

DISCUSSION

Combined standard screening strategies at 11-14 weeks gestation are able to prenatally detect up to 90% of the trisomy 21 fetuses, selecting 5% of the chromosomally normal population for invasive testing. However, new strategies are desirable to raise the detection rate and/or decrease the false-positive rate (viewed as unnecessary invasive testing). With this purpose, ductus venosus Doppler studies could be used to modify the estimated DS risk either selectively in women referred because of high-risk for this condition as a second line screening test strategy[5-7,11,13-15] or incorporated routinely into existing screening protocols.[6,9,12] Technical difficulties in DV assessment have been postulated as the main limitation for its incorporation as a first line screening test to evaluate individual DS risk in combination with NT, although competence in Doppler assessment of the DV can be achieved after extensive supervised training. Maiz et al proved that sonographers with a prior experience in the 11 to 13^{+6} weeks' scan need a learning curve of 80 examinations to achieve an adequate skill in DV measurements.[20] This finding is compatible with the results of two previous studies reporting that also a minimum of 80 sans are needed to achieve and adequate experience in the measurement of the NT thickness and the assessment of the

nasal bone in the first-trimester scan.[21,22] On the other hand, Borrell et al. showed that the quantitative assessment of the ductus venosus using the DVPIV is superior to the qualitative analysis.[9] A recent study from the same group demonstrates the substantial interobserver reliability of DVPIV measurements if compared with the end-diastolic velocity, allowing its use for clinical purposes.[23] Considering the demanding expertise of trained operators in a fetal medicine unit, the current sophisticated ultrasound machines equipped with color and pulsed Doppler technology and the reproducibility of objective DV parameters, some of the drawbacks claimed for applying DV Doppler studies should be reconsidered. The fetal medicine foundation has established a process of training and quality assurance for the appropriate introduction of NT screening into clinical practice,[1,2] and several studies have demonstrated that an ongoing regular audit of images and the distribution of measurements of NT are essential for the assessment of the quality of a center.[24-27] A similar process of training, certification and quality assurance oriented to fetal medicine units in DV flow can be suggested after our experience.

In terms of the performance of screening for DS in a set-up in which biochemical testing and the ultrasound scan are carried out in the same visit, the ideal gestational age would be around 12 weeks of gestation, as at this pregnancy week the suitable biochemical (PAPP-A and free β-hCG) and ultrasound markers (NT) are given (OSCAR-one stop clinic assessment of risks). An alternative strategy for first-trimester combined screening would be to carry out the biochemical testing and ultrasound scanning in two separated visits, with the first done at 8-10 weeks and the second at 11-13 weeks. This two-stop approach has been suggested on the basis of the best performance of PAPP-A from 8 to 11 weeks and NT from 11 to 14 weeks. The cost and acceptability of these policies will depend on the existing infrastructure of antenatal care. From a methodological point of view, during the first period of the study Down syndrome was screened by a one-step protocol, with blood taken for maternal biochemistry the same day the ultrasound was performed. Published experiences[4,28-30] and our own results have demonstrate a higher efficiency when biochemistry and ultrasound are assessed at individually established optimal gestational ages. As suggested by Spencer et al, screening algorithms modified to take account of the significant temporal variation in the screening markers should allow more accurate gestation-specifics risks.[29] According to these observations, from February 2007 we decided to move from the one to the two-steps strategy, measuring biochemical markers from two to four weeks before the ultrasound measurement of NT was done. Contrary to the first observations, there is no doubt that the most efficient and simple strategy to improve the effectiveness of the current screening programmes is the early determination of the biochemical markers in a two-step strategy provided that the health system infrastructure enables this logistics. Our study allows us to obtain the distribution of biochemical values at early stages of pregnancy. As far as we know, these data have not been previously published in the literature.

Several studies have described an association between DV and NT or biochemical markers, reporting confusing results mostly related to methodological aspects (qualitative and subjective assessment of the A-wave, population selected, markers not adjusted for gestational age).[9,12] When considering a nonselected population and adjusting parameters for gestational age, our results do not agree with previous studies.[9,12] Unlike Borrell's studies, our study demonstrates a week positive significant correlation between DVPIV and NT thickness among unaffected pregnancies. Due to the fact that both parameters show to be dependent variables in the normal chromosomal population, these could be used together for the combined risk calculation by using the likelihood ratio method.[20]

This study demonstrates the feasibility of assessing DV blood flow at the 10-14 weeks' scan, either transvaginal or transabdominally, by trained operators. Our study demonstrates an improvement in screening efficiency in experienced operators in fetal medicine units when DV Doppler studies are routinely added to the existing Down syndrome screening protocols, improving the detection rate and particularly the specificity. This improvement does not justify the introduction of DV as a primary test, which requires considerable skills, but can be applicable in specialized centers. Considering these conditions, DVPIV could be incorporated to the routine 11 to 13^{+6} scan in experienced centers. Such a policy could significantly reduce the need for unnecessary invasive testing, maintaining the same detection rate.

Moreover, DV assessment in fetuses with enlarged NT and normal karyotype was found to be useful in the prediction of congenital heart diseases an adverse perinatal outcome.[3,5,10,14,31,32] This additional utility increases the clinical interest of the DV assessment. More research is needed to quantify the benefit of measuring DV flow in routine obstetric practice.

In conclusion, we suggest that evaluation of ductal flow at 11-14 weeks gestation can be introduced to standard DS screening strategies in experienced centers as a first level screening test to reduce invasive test rate derived from the extended existing DS screening protocols.

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CONFLICT OF INTEREST STATEMENT

J Sabria and C Bach are, respectively, director and research manager of SBP Soft 2007 SL, which produces the commercial software SsdwLab version 5, for antenatal screening for Down's syndrome and neural tube defects.

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