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Population screening for fetal trisomy 21: easy access to screening should be balanced against a uniform ultrasound protocol

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Objectives To evaluate the performance of a first-trimester fetal aneuploidy screening program, with a documented underestimation of nuchal translucency thickness measurements (NT) compared to the Fetal Medicine Foundation (FMF) reference range.

Methods We analysed the data of Algemeen Medisch Laboratorium (AML) in Antwerp, Belgium, on combined screening with pregnancy-associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (FB-hCG) and NT. NT-multiples of the median (MoM), relative to the FMF reference range, were used for risk calculations.

Results The proportion of first-trimester screening tests in the total of serum screening tests increased from 1.3% (125/9424) in 2000 to 53.1% (6577/12 377) in 2003. Only 11.4% (1514/13 267) of NT measurements were performed according to FMF criteria. The 80.8% (21/26) trisomy 21 (T21) detection rate (DR) at cut off 1 : 300 resulted from maternal serum screening. NT measurements did not add to this DR, but reduced the false-positive rate from 16.8% (2212/13181) to 8.6% (1130/13181). Only 23.8% (5/21) of T21 detections were by FMF trainees.

Conclusion Easy access to screening and maternal serum parameters accounted for the majority of T21 detections in our first-trimester combined screening program.

KEY WORDS: population screening; trisomy 21; nuchal translucency; combined screening; first-trimester screening

INTRODUCTION

In Flanders, North Belgium, over 95% of prenatal care is provided by private obstetricians, who deal with fetal aneuploidy screening and most of the obstetric ultrasound scanning (http://www.kindengezin.be). First-trimester nuchal translucency (NT) thickness measurement for fetal aneuploidy screening was introduced in 1996. In combined screening algorithms, NT was first combined with second-trimester maternal serum parameters, and a few years later also with first-trimester maternal serum parameters (Gyselaers *et al*., 2004b). We recently reported our audit on NT measurements from a group of 264 Flemish obstetricians: compared to the Fetal Medicine Foundation (FMF) reference range, a systematic underestimation of NT values was found (Gyselaers *et al*., 2004a). Failure to comply with the guidelines for NT measurement, as recommended by FMF (Nicolaides *et al*., 2002), was considered to be the most likely explanation for this observation. In this article, we discuss the introduction of our first-trimester fetal aneuploidy screening program, which combines NT with maternal serum concentrations of pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotrophin (FB-hCG), and we evaluate the influence of these underestimated NT values on the screening results. The performance of our screening program is compared to published population studies on first-trimester combined screening.

METHODS

Between 1 January 2004 and 30 April 2004, maternal serum samples for first- and second-trimester fetal aneuploidy screening were analysed by the Algemeen Medisch Laboratorium (AML) in Antwerp, Belgium. These samples were obtained from 264 obstetricians, active in 35 centres located in all geographic regions of Flanders. All serum samples, sent for combined screening, were accompanied with data on first-trimester NT and crown–rump length (CRL) in mm. The obstetricians, referring the blood samples for analysis, performed the first-trimester ultrasound scans. The FMF-accredited ultrasonographers were identified at the official FMF-website (http://www.fetalmedicine.com/f-competence.htm). From our audit, it was concluded that the NT measurements from the FMF trainees were according to the FMF criteria. As no information was available on the ultrasound qualifications of the other

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ultrasonographers, their NT measurements were defined 'unspecified'.

We calculated the yearly number of all maternal serum tests performed by AML during the period 2000–2003, to be the sum of all second-trimester maternal serum screening tests, with and without NT, and the first-trimester combined screening tests.

A total of 2700 NT/CRL measurements were mailed to FMF. From these data, CRL-related multiples of the median (NT-MoM), relative to the FMF reference range, were mailed back to us. From this, FMF-specific NT-MoM values could be defined for every NT measurement in our database, which were used to calculate risks for fetal aneuploidy. Biochemical analysis for PAPP-A was performed using an enzyme-linked immunosorbent assay (ELISA 2397, DRG International Inc, Mountainside New Jersey, USA). FB-hCG was measured by radioimmunoassay (free β-hCG IRMA K1P1001, BioSource Europe SA, 1440 Nivelles, Belgium). For PAPP-A, the intra- and inter- assay coefficients of variation were less than 10%, and for FB-hCG they were less than 5% and 10% respectively. Each result was adjusted for maternal weight (de Graaf *et al*., 1999), multiple gestation (Spencer, 2000), smoking (de Graaf *et al*., 2000) and ethnicity (Hallahan *et al*., 2004) and was expressed as a MoM. Medians and MoM values were controlled and improved each month with an exponential regression analysis using NCSS Statistical Software (NCSS, 329 North 1000 East-Kaysville, Utah 84037, USA).

For all parameters, we used the likelihood ratios and correlation coefficients as reported by de Graaf *et al*. (1999). Risk calculations were performed using the algorithm reported by Wald *et al*. (1988) and Reynolds *et al*. (1989). We validated our risk calculation algorithm by logarithmic correlation of the calculated risks with the observed risks in our population (Wald *et al*., 1997; Onda *et al*., 1998).

At least once a year, obstetricians reported the outcome of the screened pregnancies by mail. Non-responding obstetricians were contacted personally to collect missing data on chromosomal anomalies. The data were grouped in trisomy 21 (T21)-affected pregnancies, pregnancies affected by fetal chromosomal anomalies different from T21, pregnancies with spontaneous fetal loss and unaffected pregnancies. For this study, only the T21-affected and unaffected pregnancies were considered.

The validity of our registration was evaluated by comparing the prevalence of T21 at term, as expected from our observations, with the prevalence of T21, as expected from the maternal age distribution in our population and the data on maternal age and gestation related prevalence as registered in the British National Down Syndrome Cytogenetic Register (Morris *et al*., 1999, 2002a, b).

In the group of affected pregnancies, we defined the T21 detection rate (DR) at 12-week risk cut off values 1 : 200 and 1 : 300 for 3 different screening methods: screening by maternal age and first-trimester NT (AN), by maternal age and first-trimester maternal serum parameters (AMS) and by maternal age, NT and serum (ANMS). In the group of unaffected pregnancies, we defined for each screening method the false-positive rate (FPR) at cut off 1 : 200 and 1 : 300 and the cut off value at 5% false-positive screening results.

We searched the electronic database of Medline and PubMed for publications on population screening for fetal aneuploidy by first-trimester combined algorithms, using the keywords Down's Syndrome, fetal aneuploidy, population screening, first-trimester screening, combined screening, NT, ultrasound screening, PAPP-A, hCG, maternal serum screening. According to the methodology reported in the Materials and Methods section, we grouped these studies in single ($\leq$2 centres) studies using FMF criteria for NT measurement, multicentre ($\geq$3 centres) studies using FMF criteria for NT measurement and studies using population-, group- or performer-specific NT medians.

RESULTS

The number of maternal serum screening tests in the AML-database increased from 9424 in 2000 to 12 377 in 2003. The 3-year evolution of the numbers and proportions of different tests is shown in Table 1. All samples were obtained from a total of 264 obstetricians, of whom only 6 were enlisted as FMF-accredited ultrasonographers at the FMF-website. The FMF trainees measured 11.4% (1514/13 267) of all NT values and all of them screened a minimum of 100 pregnancies. Of the other ultrasonographers, 31 screened more than 100 pregnancies, 19 screened between 50 and 100 and 209 screened less than 50. Blood was sampled more than 5 days before the NT scan in 36.3% (4816/13 267) of pregnancies.

We evaluated a total of 13 267 first-trimester screening tests. In this total population, 26 T21 affected pregnancies and 23 pregnancies with other fetal chromosomal anomalies were present. Of the other pregnancies, a total of 49 were reported as intrauterine fetal deaths, 12 before the 14th week of gestation, 23 between 14 and 24 weeks and 14 after 24 weeks. In these pregnancies, 2 fetal chromosomal anomalies different from T21 and 11 normal fetal karyotypes were reported. Five cytogenetic cell cultures failed. Four of the 12 first-trimester miscarriages did not have any result on NT measurement, as the fetus died between the moment of blood sampling and the first-trimester ultrasound. After exclusion

Table 1—Evolution between 2000 and 2003 of the different screening tests performed by AML in Antwerp, Belgium

Year	Total *n*	2 AMS *n* (%)	2 ANMS *n* (%)	1 ANMS *n* (%)
2000	9424	7502 (79.6)	1797 (19.1)	125 (1.3)
2001	10 534	6895 (65.4)	1999 (19.0)	1640 (15.6)
2002	12 107	7148 (59.0)	1873 (15.5)	3086 (25.5)
2003	12 377	4053 (32.7)	1747 (14.2)	6577 (53.1)

(2 AMS = maternal age + second trimester maternal serum parameters, 2 ANMS = maternal age + nuchal translucency + second trimester maternal serum parameters, 1 ANMS = maternal age + nuchal translucency + first-trimester maternal serum parameters).

of the 23 fetal chromosomal anomalies, different from T21, and the 37 intrauterine fetal deaths with unknown genetic constitution, a total of 13 181 unaffected and 26 T21-affected pregnancies were left for further evaluation.

As is shown in Figure 1, the correlation between the logarithmic values of our algorithm's risk calculations and the observed prevalence of T21 in our population was 0.93.

We calculated the expected number of T21 at term from the maternal age distribution in our population and the maternal age related prevalence of T21 as registered in the British Down syndrome cytogenetic register (Morris *et al*., 2002b): the expected number of T21 at birth was 19.4. Considering a 43% spontaneous abortion rate, as reported by Morris *et al*. (Morris *et al*., 1999, 2002a) and assuming no intervention was performed for the 17 terminated T21-affected pregnancies in our population (Table 2), we calculated from our observations a total of 18.7 expected cases of T21 at term (9 live births + 17 terminations × 0.57). The correlation was 0.96 (18.7/19.4) between the expected number of T21 from our observations and the expected number

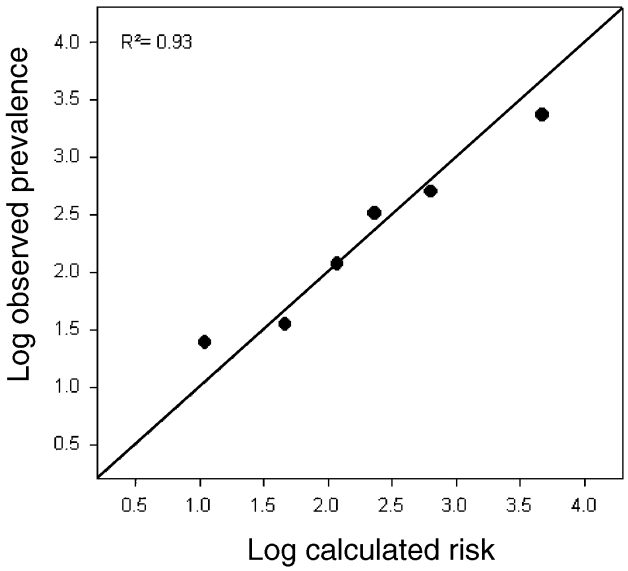

Figure 1—The correlation and correlation coefficient between the logarithmic values of our algorithm's risk calculations and the observed prevalence in our population

Table 2—Details on the 26 T21 affected pregnancies in our population: maternal age, gestation at blood sampling (Gest) in weeks and days (w,d), PAPP-A and free β-hCG (FB-hCG) in mIU/ml and in corrected MoM values, fetal crown rump length (CRL) and nuchal translucency (NT) in mm and subsequent NT-MoM values, and the calculated risk by maternal age + NT (AN), by maternal age and serum (AMS) and by maternal age, NT and maternal serum (ANMS). The five pregnancies screened by the FMF-accredited ultrasonographers are in bold

Age years	Gest w,d	PAPP-A mIU/ml	PAPP-A corr MoM	FB-hCG mIU/ml	FB-hCG corr MoM	CRL mm	NT -mm	NT -MoM	Risk AN	Risk AMS	Risk ANMS	Outcome
37.07	10.5	1.53	0.672	124.3	3.159	38.5	1.2	1.434	182	24	29	TOP
39.05	10.3	0.38	0.261	14.6	0.311	37.4	0.5	0.627	423	23	129	TOP
28.02	11.6	0.55	0.202	91.9	2.478	50.0	1.2	0.967	2921	28	67	TOP
29.06	12.4	0.67	0.214	33.5	0.985	63.0	2.0	1.274	1292	80	127	TOP
30.08	13.6	5.60	0.950	40.9	1.402	75.7	1.1	0.635	2820	1143	8386	LB
36.03	12.5	3.05	0.610	266.8	7.059	63.2	1.9	1.207	457	12	23	TOP
30.01	**10.1**	**0.10**	**0.081**	**34.3**	**0.692**	**72.0**	**2.1**	**1.237**	**1317**	**11**	**15**	**LB**
30.02	**13.2**	**0.95**	**0.257**	**34.2**	**1.180**	**72.0**	**1.7**	**1.001**	**2232**	**99**	**299**	**LB**
30.00	12.4	0.53	0.174	12.4	0.336	56.0	2.2	1.555	586	55	39	TOP
41.08	12.3	1.38	0.378	138.0	3.448	69.0	2.9	1.744	26	3	1	LB
38.00	11.3	0.85	0.418	96.2	2.221	51.0	1.7	1.336	213	22	32	TOP
31.08	11.2	0.74	0.399	55.5	1.410	54.0	2.7	1.984	140	170	41	TOP
31.07	11.2	3.82	1.606	54.7	1.209	46.0	1.7	1.537	485	3219	4155	LB
40.06	**11.0**	**2.62**	**1.126**	**67.2**	**1.372**	**56.0**	**3.5**	**2.473**	**5**	**189**	**13**	**LB**
27.02	11.1	0.59	0.369	40.2	0.947	41.6	0.7	0.738	4016	404	2287	LB
33.00	12.0	0.62	0.224	69.4	1.759	52.5	1.0	0.759	1914	28	96	TOP
22.03	10.6	1.07	0.730	220.9	4.405	40.0	4.0	4.490	1	82	1	TOP
26.10	12.0	1.54	0.710	17.8	0.484	60.6	1.0	0.657	4132	3028	28 436	LB
24.02	9.0	0.13	0.144	30.3	0.428	47.0	0.8	0.701	4718	48	200	TOP
37.06	10.5	0.57	0.301	11.5	0.230	36.2	3.7	4.903	1	57	1	TOP
28.06	12.3	1.87	0.505	84.0	2.118	52.0	1.0	0.768	3598	196	865	LB
32.08	**12.4**	**1.70**	**0.494**	**87.8**	**2.349**	**65.0**	**2.9**	**1.807**	**190**	**86**	**36**	**TOP**
35.00	8.5	0.09	0.124	39.2	0.473	61.0	3.5	2.286	31	8	1	TOP
32.08	11.1	0.71	0.320	170.2	3.290	48.0	2.3	1.958	125	21	6	TOP
39.03	**10.3**	**0.15**	**0.107**	**28.1**	**0.499**	**78.6**	**1.8**	**1.026**	**323**	**2**	**5**	**TOP**
27.04	11.6	1.80	0.659	34.6	0.820	48.9	4.4	3.652	3	1945	4	TOP
Median MoMs												
All			0.37		1.29			1.31				
FMF trainees			0.26		1.18			1.24				
Non-FMF trainees			0.38		1.40			1.34				

(TOP = termination of pregnancy, LB = live birth).

Table 3—Performance of three different first-trimester fetal aneuploidy screening methods: screening by maternal age + nuchal translucency (AN), by maternal age + serum (AMS) and by maternal age, nuchal translucency and serum (ANMS). The detection rate of trisomy 21 (DR T21) and the false-positive rate (FPR) at cut off 1 : 200 and 1 : 300 are enlisted, as well as the detection rate of T21 at a fixed 5% false-positive rate and the cut off values at 5% FPR

	DR T21		FPR		DR T21 at 5% FPR	
	1 : 200 *n*/26 (%)	1 : 300 *n*/26 (%)	1 : 200 *n*/13 181 (%)	1 : 300 *n*/13 181 (%)	Cut-off	DR T21 *n*/26 (%)
AN	10 (38.5)	11 (42.3)	419 (3.2)	635 (4.8)	1 : 310	11 (42.3)
AMS	21 (80.8)	21 (80.8)	1529 (11.6)	2212 (16.8)	1 : 85	16 (61.5)
ANMS	20 (76.9)	21 (80.8)	724 (5.5)	1130 (8.6)	1 : 180	19 (73.6)

from the maternal age distribution in our population.

In Table 2, details are enlisted on the different parameters and the screening results in the 26 T21-affected pregnancies. Of these pregnancies, a total of 17 were terminated and 5 T21-live births were reported. Four pregnancies with positive screening results were not terminated: after counselling, two women refused further invasive testing by amniocentesis or chorionic villus sampling and two other women accepted the birth of an affected child. Of all prenatally detected T21-affected pregnancies, 23.8% (5/21) were in the population screened by the FMF trainees. The median MoM values for all parameters were similar in the affected pregnancies screened by the FMF trainees and the pregnancies screened by the other ultrasonographers.

From Table 2, the T21 DR at cut off 1 : 200 and 1 : 300 was defined for three different screening methods: screening by maternal age and NT (AN), by maternal age and maternal serum parameters (AMS) and by maternal age, NT and maternal serum (ANMS). The results are shown in Table 3, which also shows the numbers of positive screening results and the FPR in the unaffected pregnancies. The DR at a fixed 5% FPR was 42.3% (11/26) for AN, 61.5% (16/26) for AMS and 73.6% (19/26) for ANMS at cut off values of respectively 1 : 310, 1 : 85 and 1 : 180.

DISCUSSION

In this report, we describe the introduction and the performance of our first-trimester fetal aneuploidy screening program in Flanders (North Belgium) and we compare our results with other publications.

As is shown in Table 1, the introduction of first-trimester combined screening in our population was very efficient: within 3 years from introduction, first-trimester combined screening became the most important serum-related screening method (Table 1).

We validated our algorithm for the method of risk calculations and the method of registering the T21-affected pregnancies. A good correlation ($R^2 = 0.93$) was found between the logarithmic values of the algorithm's calculated risks and the observed prevalence of T21 in our population (Figure 1). We also found a good correlation ($R^2 = 0.96$) between the expected number of T21-affected pregnancies from our observations and the expected number from the maternal age distribution in our population. We are aware that our analysis does not include the T21-affected pregnancies, missed by screening in the first trimester and resulting in a spontaneous fetal loss before term. It is likely that some chromosomal defects were present in our group of 37 pregnancies with fetal loss of unknown genetic constitution. Therefore, our calculations on the algorithm's performance may be overestimating the detection of T21. This problem of inaccuracy in reporting screening performance of first-trimester screening methods has already been highlighted before (Haddow, 1998). Our approach, however, is similar to the methods used in most reports enlisted in Table 4, which allows for comparison of our data with the results from these studies.

As shown in Table 3, the performance of screening by AN shows a 42.3% T21 DR at 5% FPR. This is considerably less than the 75–80% DR deducted from a series of publications on NT screening in unselected populations (Brigatti and Malone, 2004). In our algorithm, we did not use population-, centre- or performer-specific reference values for NT measurements, as proposed by others (Logghe *et al*., 2003; Wald *et al*., 2003). We implemented NT values, expressed as a MoM relative to the FMF-reference range. We recently reported a systematic underestimation of the NT-MoM values in our database, in comparison to this FMF-reference range (Gyselaers *et al*., 2004a). This underestimation may explain the poor performance of AN screening in our population. FMF-trained ultrasonographers performed less than 12% of all NT measurements in our population. Of the other ultrasonographers, no information was available on their ultrasound qualifications and the majority of them screened less than 50 pregnancies in the 3-year study period. The implementation of NT measurements, without active training and quality control, was reported to be unreliable in combination with first-trimester maternal serum screening (Haddow *et al*., 1998). In a careful review of NT screening studies by Malone and D'Alton *et al*. (2003), there was a broad range of DRs and FPRs in 30 studies of unselected patient populations. The performance of the combined algorithm ANMS in our study showed an acceptable T21 DR of 80.8% at FPR of 8.6%, which was similar to other multicentre studies (Muller *et al*., 2003; Niemimaa, 2003; Wald *et al*., 2003; Wapner *et al*., 2003). As is shown in Table 3, in our algorithm the T21 DR was mainly achieved by AMS only. NT did not add to this DR, but was an important parameter to reduce FPR from 11.6% to 5.5% at cut off 1 : 200 and from 16.8% to 8.6%

Table 4—List of publications of prospective population studies on first-trimester combined fetal aneuploidy screening (Orlandi *et al*., 1997; De Biasio *et al*., 1999; Krantz *et al*., 2000; Bindra *et al*., 2002; Crossley *et al*., 2002; Schuchter *et al*., 2002; von Kaisenberg *et al*., 2002; Muller *et al*., 2003; Niemimaa, 2003; Spencer *et al*., 2003; Wald *et al*., 2003; Wapner *et al*., 2003; Borrell *et al*., 2004; Scott *et al*., 2004; Stenhouse *et al*., 2004). The studies are grouped in (1) single centre (≤2 centres) studies with FMF scanning criteria, (2) multicentre (≥3 centres) studies with FMF scanning criteria and (3) studies with population-specific NT Medians

				Detection rate		False-positive rate		DR at 5% FPR
Author	Centres	*n*	Cut-off	%	(*n*/*n*)	%	(*n*/*n*)	%
Single centre, FMF criteria								
Stenhouse		5084	1/250	93	14/15	5.5	274/4974	
Scott		2121	1/300	100	5/5	7.1	148/2104	
Spencer		12 339	1/300	92	23/25	5.2	577/11 105	89
Krantz		5809	1/270	91	30/33	8.6	451/5223	91
Bindra		14 383	1/300	91	75/82	6.8	967/14 240	90
Schuchter		4939	1/250	86	12/14	5	245/4925	86
Orlandi		2010	1/380	100	11/11	9	165/1833	87
DeBiasio		1467	1/350	85	11/13	3.3	48/1454	
		48 152		91.4	181/198	6.3	2875/45 858	89.5
Multicentre, FMF criteria								
Wapner	12	8216	1/270	85.2	52/61	9.4	766/8144	79
von Kaisenberg	9	3864	1/300	84	16/19	6.6	233/3505	74–84
Crossley	25	17 229	1/250	62–82	28/34–45	5	(649/12 983)[a]	82
	46	29 309		82.5	94/114	6.7	1648/24 632	79.9
Population-specific reference								
Borrell	1	2780	1/250	88	7/8	3.3	92/2765	88
Niemimaa	2	3178	1/250	77	10/13	5.8	184/3165	77
Muller	9	5694	1/250	74	19/26	4.7	265/5644	77
SURUSS	25	39 983	1/250	80	65/85	4.7	1875/39 898	83
	37	51 635		76.5	101/132	4.7	2416/51 472	81.5

[a] Numbers deducted from the original data.

at cut off 1 : 300 (Table 3). The median MoM values of PAPP-A in our group of affected pregnancies were appropriately low (Table 2). The median MoM values of FB-hCG and NT were not as elevated as expected: it is likely that higher values of one or both would improve our screening results. NT-MoM medians were not different between the pregnancies screened by the FMF trainees and those screened by the other ultrasonographers: we think this is coincidental. From these data, it is concluded that PAPP-A is by far the most important screening parameter in our algorithm. Muller *et al*. reported different screening results from second-trimester maternal serum screening algorithms, depending on the relative weight of the parameters (Muller *et al*., 1999). In our algorithm, the relative weight of NT is low, and this may explain the minor impact of underestimated NT values on the final screening results of our combined algorithm. A theoretical calculation in the SURUSS report also showed a minor impact of increasing fractions of sub-optimal NT measurements on the final results of combined or integrated population screening (Wald *et al*., 2003). The results from our study add to the growing evidence that NT measurements, performed under different conditions as recommended by FMF, may still be valuable in fetal aneuploidy screening, when used in combination with other parameters.

Table 4 enlists publications of population studies on combined first-trimester screening by maternal age, NT, PAPP-A and FB-hCG, grouped by the number of centres involved in each study and the methodology used for implementation of NT measurements. The overall prevalence of T21 in the single centre studies (≤2 centres) is 1/243 (198/48 152), in the multicentre studies (>2 centres) using FMF criteria for NT measurement is 1/257 (114/29 309) and in the studies using population-specific NT medians is 1/411 (158/64 904). As is shown, the overall performance of the algorithms using population-specific medians is lower than the overall performance of the algorithms from the single centre studies using FMF criteria. However, when the FMF criteria are used in multicentre studies, the overall screening performance is also lower than in the single centre studies and equal to the overall performance of algorithms with population-specific NT medians: for both methods the T21 DR at 5% FPR varies around 80% compared to 89.5% for the single centre studies (Table 4). The 3 multicentre studies with FMF criteria reported difficulties with compliance to the protocol for ultrasonic NT measurement. This clearly illustrates the difficulty of introducing stringent US methods in population screening for fetal aneuploidy and of moving expertise from the specialist centres to prenatal screening clinics. However, the Scottish group recently reported that an optimal performance of first-trimester combined screening was achievable in a routine prenatal clinic after motivation for adherence to an ultrasound screening protocol and reorganisation of busy prenatal clinic activities (Stenhouse *et al*., 2004).

At present, the bottleneck in the organisation of first-trimester population screening for fetal aneuploidy is

the availability of appropriately trained and equipped ultrasonographers (Benn, 2002). Both in the United Kingdom and in the United States, it was reported that only a minority of pregnant women had access to high quality NT-related screening (Whittle, 2001; Egan *et al*., 2002; Welch and Malone, 2003; Brigatti and Malone, 2004). In our population, only 11.4% of the pregnant women had access to NT measurements according to FMF criteria and this allowed a detection of less than 20% of all T21-affected pregnancies in the population. Programs have been developed towards training and audit of an increasing number of obstetric ultrasonographers, in order to offer high quality ultrasound examinations to as many pregnant women as possible (Braithwaite *et al*., 1996; Wojdemann *et al*., 2001; Snijders *et al*., 2002). The medical and ethical implications of the general introduction of these Programs for Certification of Competence have been highlighted (Wald, 2003). Our data show that unspecified NT measurements for fetal aneuploidy screening are easier to introduce in population screening than those according to FMF criteria. Our data also show that this practice does not necessarily imply poor medical practice, as the screening results from our algorithm are acceptable and comparable to other population screening studies, using FMF criteria (Wapner *et al*., 2003) or population-specific medians (Muller *et al*., 2003; Niemimaa, 2003; Wald *et al*., 2003). Finally, our data show that the large number of women screened by the non-FMF-trained ultrasonographers allowed to detect three times the number of T21-affected pregnancies detected by the FMF trainees.

We acknowledge, however, the importance of further improving the screening performance of our screening program. We are currently investigating four measures: (1) an increase of the number of parameters, such as the ultrasonic evaluation of the fetal nasal bone (Cicero *et al*., 2003) and/or the integration of first and second-trimester parameters (Wald *et al*., 2003), (2) the application of population-, centre- or performer-specific reference values and correlations for all screening parameters (Logghe *et al*., 2003; Wald *et al*., 2003), (3) further training and audit in ultrasound methodology for obstetricians involved in first-trimester ultrasound screening (Braithwaite *et al*., 1996; Snijders *et al*., 2002), which has already started following a survey on the future organisation of ultrasound screening in Flanders (Gyselaers *et al*., 2005) and (4) the introduction of contingent testing, which offers maternal serum screening to all pregnant women in the program, and subsequent high quality ultrasound screening to only those who are considered at higher risk (Christiansen and Olesen Larsen, 2002).

We conclude that the introduction of first-trimester combined screening with unspecified ultrasound methodology in a Belgian population was very easy. The performance of this screening method was less than in reported single centre studies using FMF scanning criteria, but the easy access to screening and the contribution from maternal serum parameters were responsible for the majority of T21 detections in our population. Our data illustrate that easy access to screening should be balanced against the uniform application of a single ultrasound protocol, in order to identify the highest possible number of fetal aneuploidy affected pregnancies in the population.

REFERENCES

Benn PA. 2002. Advances in prenatal screening for Down syndrome: II first trimester testing, integrated testing, and future directions. *Clin Chim Acta* **324**: 1–11.

Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. 2002. One-stop clinic for assessment of risk for trisomy 21 at 11–14 weeks: a prospective study of 15 030 pregnancies. *Ultrasound Obstet Gynecol* **20**: 219–225.

Borrell A, Casals E, Fortuny A. *et al*. 2004. First-trimester screening for trisomy 21 combining biochemistry and ultrasound at individually optimal gestational ages. An interventional study. *Prenat Diagn* **24**: 541–545.

Braithwaite JM, Kadir RA, Pepera TA, Morris RW, Thompson PJ, Economides DL. 1996. Nuchal translucency measurement: training of potential examiners. *Ultrasound Obstet Gynecol* **8**: 192–195.

Brigatti KW, Malone FD. 2004. First-trimester screening for aneuploidy. *Obstet Gynecol Clin North Am* **31**: 1–20.

Christiansen M, Olesen Larsen S. 2002. An increase in cost-effectiveness of first trimester maternal screening programmes for fetal chromosome anomalies is obtained by contingent testing. *Prenat Diagn* **22**: 482–486.

Cicero S, Bindra R, Rembouskos G, Spencer K, Nicolaides KH. 2003. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta-hCG and PAPP-A at 11 to 14 weeks. *Prenat Diagn* **23**: 306–310.

Crossley JA, Aitken DA, Cameron AD, McBride E, Connor JM. 2002. Combined ultrasound and biochemical screening for Down's syndrome in the first trimester: a Scottish multicentre study. *BJOG* **109**: 667–676.

De Biasio P, Siccardi M, Volpe G, Famularo L, Santi F, Canini S. 1999. First-trimester screening for Down syndrome using nuchal translucency measurement with free beta-hCG and PAPP-A between 10 and 13 weeks of pregnancy–the combined test. *Prenat Diagn* **19**: 360–363.

de Graaf IM, Pajkrt E, Bilardo CM, Leschot NJ, Cuckle HS, van Lith JM. 1999. Early pregnancy screening for fetal aneuploidy with serum markers and nuchal translucency. *Prenat Diagn* **19**: 458–462.

de Graaf IM, Cuckle HS, Pajkrt E, Leschot NJ, Bleker OP, van Lith JM. 2000. Co-variables in first trimester maternal serum screening. *Prenat Diagn* **20**: 186–189.

Egan JF, Kaminsky LM, DeRoche ME, Barsoom MJ, Borgida AF, Benn PA. 2002. Antenatal Down syndrome screening in the United States in 2001: a survey of maternal-fetal medicine specialists. *Am J Obstet Gynecol* **187**: 1230–1234.

Gyselaers W, De Catte L, Witters I, De Jonge E, Ombelet W, Nijhuis J. 2005. Survey on the future organisation of first trimester ultrasound screening in Flanders, Belgium. *Prenat Diagn* **25**: 518–519.

Gyselaers W, Vereecken A, Van Herck E, *et al*. 2004a. Audit on nuchal translucency thickness measurements in Flanders, Belgium: a plea for methodological standardization. *Ultrasound Obstet Gynecol* **24**: 511–515.

Gyselaers W, Vereecken A, Van Herck E. *et al*. 2004b. Screening for trisomy 21 in Flanders: a 10-years review of 40 490 pregnancies screened by maternal serum. *Eur J Obstet Gynecol Reprod Biol* **115**: 185–189.

Haddow JE. 1998. Antenatal screening for Down's syndrome: where are we and where next? *Lancet* **352**: 336–337.

Haddow JE, Palomaki GE, Knight GJ, Williams J, Miller WA, Johnson A. 1998. Screening of maternal serum for fetal Down's syndrome in the first trimester. *N Engl J Med* **338**: 955–961.

Hallahan T, Krantz D, Buchanan P, Larsen JW, Macri J. 2004. *Effect of Ethnicity of First-trimester Down Syndrome Screening*, http://genetics.faseb.org/genetics/ashg00/f2365.htm, (accessed 20-5/2004).

Krantz DA, Hallahan TW, Orlandi F, Buchanan P, Larsen JW Jr., Macri JN. 2000. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* **96**: 207–213.

Logghe H, Cuckle H, Sehmi I. 2003. Centre-specific ultrasound nuchal translucency medians needed for Down syndrome screening. *Prenat Diagn* **23**: 389–392.

Malone FD, D'Alton ME. 2003. First-trimester sonographic screening for Down syndrome. *Obstet Gynecol* **102**: 1066–1079.

Morris JK, Wald NJ, Watt HC. 1999. Fetal loss in Down syndrome pregnancies. *Prenat Diagn* **19**: 142–145.

Morris JK, Mutton D, Alberman E. 2002a. Potential biases in Down syndrome birth prevalence estimation (author's reply). *J Med Screen* **9**: 192.

Morris JK, Mutton DE, Alberman E. 2002b. Revised estimates of the maternal age specific live birth prevalence of Down's syndrome. *J Med Screen* **9**: 2–6.

Muller F, Aegerter P, Ngo S. *et al*. 1999. Software for prenatal down syndrome risk calculation: a comparative study of six software packages. *Clin Chem* **45**: 1278–1280.

Muller F, Benattar C, Audibert F, Roussel N, Dreux S, Cuckle H. 2003. First-trimester screening for Down syndrome in France combining fetal nuchal translucency measurement and biochemical markers. *Prenat Diagn* **23**: 833–836.

Nicolaides KH, Heath V, Cicero S. 2002. Increased fetal nuchal translucency at 11–14 weeks. *Prenat Diagn* **22**: 308–315.

Niemimaa M. 2003. *First Trimester Screening for Down Syndrome*. Thesis, University of Oulu, Finland; 1–62.

Onda T, Tanaka T, Takeda O. *et al*. 1998. Agreement between predicted risk and prevalence of Down syndrome in second-trimester triple-marker screening in Japan. *Prenat Diagn* **18**: 956–958.

Orlandi F, Damiani G, Hallahan TW, Krantz DA, Macri JN. 1997. First-trimester screening for fetal aneuploidy: biochemistry and nuchal translucency. *Ultrasound Obstet Gynecol* **10**: 381–386.

Reynolds T, Penney M. 1989. The mathematical basis of multivariate risk screening: with special reference to screening for Down's syndrome associated pregnancy. *Ann Clin Biochem* **27**: 452–458.

Schuchter K, Hafner E, Stangl G, Metzenbauer M, Hofinger D, Philipp K. 2002. The first trimester 'combined test' for the detection of Down syndrome pregnancies in 4939 unselected pregnancies. *Prenat Diagn* **22**: 211–215.

Scott F, Peters H, Bonifacio M. *et al*. 2004. Prospective evaluation of a first trimester screening program for Down syndrome and other chromosomal abnormalities using maternal age, nuchal translucency and biochemistry in an Australian population. *Aust N Z J Obstet Gynaecol* **44**: 205–209.

Snijders RJ, Thom EA, Zachary JM, *et al*. 2002. First-trimester trisomy screening: nuchal translucency measurement training and quality assurance to correct and unify technique. *Ultrasound Obstet Gynecol* **19**: 353–359.

Spencer K. 2000. Screening for trisomy 21 in twin pregnancies in the first trimester using free beta-hCG and PAPP-A, combined with fetal nuchal translucency thickness. *Prenat Diagn* **20**: 91–95.

Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. 2003. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years prospective experience. *BJOG* **110**: 281–286.

Stenhouse EJ, Crossley JA, Aitken DA, Brogan K, Cameron AD, Connor JM. 2004. First-trimester combined ultrasound and biochemical screening for Down syndrome in routine clinical practice. *Prenat Diagn* **24**: 774–780.

von Kaisenberg CS, Gasiorek-Wiens A, Bielicki M. *et al*. 2002. Screening for trisomy 21 by maternal age, fetal nuchal translucency and maternal serum biochemistry at 11–14 weeks: a German multicenter study. *J Matern Fetal Neonatal Med* **12**: 89–94.

Wald N. 2003. Certification of competence in performing specific procedures or tests in screening practice. *Prenat Diagn* **23**: 861.

Wald N, Cuckle H, Densem J, *et al*. 1988. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ* **297**: 883–887.

Wald NJ, Kennard A, Hackshaw A, McGuire A. 1997. Antenatal screening for Down's syndrome. *J Med Screen* **4**: 181–246.

Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. 2003. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *J Med Screen* **10**: 56–104.

Wapner R, Thom E, Simpson JL. *et al*. 2003. First-trimester screening for trisomies 21 and 18. *N Engl J Med* **349**: 1405–1413.

Welch KK, Malone FD. 2003. Nuchal translucency-based screening. *Clin Obstet Gynecol* **46**: 909–922.

Whittle M. 2001. Down's syndrome screening: where to now? *BJOG* **108**: 559–561.

Wojdemann KR, Christiansen M, Sundberg K, Larsen SO, Shalmi A, Tabor A. 2001. Quality assessment in prospective nuchal translucency screening for Down syndrome. *Ultrasound Obstet Gynecol* **18**: 641–644.