

One-stop clinic for assessment of risk for trisomy 21 at 11–14 weeks: a prospective study of 15 030 pregnancies

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ABSTRACT

Objective To evaluate the performance of a one-stop clinic for assessment of risk (OSCAR) for trisomy 21 by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β -human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11–14 weeks of gestation.

Method Screening for trisomy 21 was carried out by OSCAR in 15 030 singleton pregnancies with live fetuses at 11–14 weeks. The estimated risk for trisomy 21 was calculated, and the women were counseled regarding this risk and the option of invasive testing or expectant management. Follow-up of the outcome of all pregnancies was carried out. The detection and false-positive rates for different risk cut-offs were calculated.

Results Fetal NT and maternal serum free β -hCG and PAPP-A were successfully measured in all cases. Pregnancy outcome, including karyotype results or the birth of a phenotypically normal baby, was obtained from 14 383 cases. The median maternal age of these cases was 34 (range 15–49) years and in 6768 (47.1%) the age was 35 years or greater. The median gestation at screening was 12 (range 11–14) weeks and the median fetal crown–rump length was 64 (range 45–84) mm. The estimated risk for trisomy 21 based on maternal age, fetal NT and maternal serum free β -hCG and PAPP-A was 1 in 300 or greater in 6.8% (967 of 14 240) normal pregnancies, in 91.5% (75 of 82) of those with trisomy 21 and in 88.5% (54 of 61) of those with other chromosomal defects. For a fixed false-positive rate of 5% the respective detection rates of screening for trisomy 21 by maternal age alone, maternal age and serum free β -hCG and PAPP-A, maternal age and fetal NT, and by maternal age, fetal NT and maternal serum biochemistry were 30.5%, 59.8%, 79.3% and 90.2%, respectively.

Conclusion Screening for trisomy 21 by a combination of maternal age, fetal NT and maternal serum biochemistry at 11–14 weeks can be provided in an OSCAR setting and is associated with a detection rate of about 90% for a false-positive rate of 5%.

INTRODUCTION

Trisomy 21 is associated with increased maternal age, increased fetal nuchal translucency (NT) thickness, increased maternal serum free β -human chorionic gonadotropin (hCG) and decreased serum pregnancy-associated plasma protein-A (PAPP-A) concentration. We have previously estimated that the most effective method of screening for trisomy 21 would be by a combination of maternal age, fetal NT and serum biochemistry at 11–14 weeks of gestation¹. It was predicted that for a false-positive rate of 5% the detection rate of trisomy 21 by this method would be about 90%, which is superior to the 30% achieved by maternal age alone, the 65% by maternal age and second-trimester serum biochemistry, and the 75% by maternal age and first-trimester fetal NT^{1–3}. With the advent of rapid immunoassays, it has become possible to provide pretest counseling, biochemical testing of the mother, ultrasound examination of the fetus and post-test counseling of a combined risk estimate, all within a 1-h visit to a multidisciplinary one-stop clinic for assessment of risk (OSCAR) for fetal anomalies⁴.

This prospective study evaluates the effectiveness of early screening for trisomy 21 in an OSCAR setting.

METHODS

All women booked for maternity care at King's College Hospital, London (between January 1999 and February 2000) and those attending The Fetal Medicine Centre, London (between July 1999 and October 2001) were offered screening for trisomy 21 by a combination of fetal NT and maternal serum free β -hCG and PAPP-A at 11–14 weeks. Women received an information leaflet about the OSCAR service and gave details about their demographic characteristics and medical history, which were entered into a computer database. The maternal serum free β -hCG and PAPP-A were measured using the Kryptor analyser (Brahms Diagnostica GmbH, Berlin—formerly CIS), and an ultrasound examination was carried out to measure the fetal NT and crown–rump length (CRL), and to diagnose any major defects. All scans

were carried out by sonographers who had obtained The Fetal Medicine Foundation Certificate of Competence in the 11–14-Week Scan (<http://www.fetalmedicine.com>). The blood results were available within 20 min, thus allowing for calculation of a composite risk based on the biochemical and sonographic findings in the same visit.

The patients were counseled with regards to their combined estimated risk and the available options for the subsequent management of the pregnancy, including chorionic villus sampling. They were informed that a risk of 1 in 300 or more was generally considered to be high, but were allowed to decide for themselves for or against invasive testing, irrespective of the risk estimate. Those choosing to have an invasive test had the option of having chorionic villus sampling at the time of the OSCAR. Provisional results from quantitative polymerase chain reaction (PCR) analysis were available within 48 h⁵ and a confirmed diagnosis by conventional karyotyping was available within 7 days. Data on pregnancy outcome were obtained from the cytogenetics laboratory, the patients themselves, their general practitioners or the maternity units in which they delivered. Cases were also linked to those recorded in the National Down Syndrome Register.

Patient-specific risks were calculated by a multivariate approach using population parameters established in our retrospective study and the maternal age and gestation-related risk of trisomy 21 at the time of OSCAR^{1,6}. Essentially, the maternal age-related risk was multiplied with each likelihood ratio (LR) derived from the fetal NT and maternal weight-adjusted serum free β -hCG and PAPP-A. The maximum and minimum LR allowed were 0.12 and 55 for NT, 0.018 and 7.138 for each metabolite, and 0.1 and 80 for the combined sonographic and biochemical markers.

The detection rate of trisomy 21 and false-positive rate of the screening test for a risk cut-off of 1 in 300 was calculated. To allow comparison with other screening tests, we also calculated the detection rates for fixed false-positive rates between 1% and 5% and the false-positive rates for fixed detection rates between 60% and 90% by maternal age alone, maternal age and fetal NT, maternal age and serum free β -hCG and PAPP-A and by a combination of maternal age, fetal NT and maternal serum biochemistry.

RESULTS

Screening for trisomy 21 by OSCAR was carried out in 15 030 singleton pregnancies with live fetuses at 11–14 (median 12) weeks. Fetal NT and maternal serum free β -hCG and PAPP-A were successfully measured in all cases. Pregnancy outcome, including karyotype results or the birth of a phenotypically normal baby, was obtained from 14 383 cases. Excluded from further analysis were 647 cases, because the fetal karyotype was not known and they resulted in spontaneous fetal loss ($n = 41$), termination of pregnancy ($n = 32$) or were lost to follow-up ($n = 574$). All cases in the latter group were cross referenced against the cases of trisomy 21 recorded in the National Down Syndrome Register but none of these pregnancies were found to be affected.

The median maternal age of the 14 383 cases was 34 (range 15–49) years and in 6768 (47.1%) the age was 35 years or

greater. The median fetal crown–rump length was 64 (range 45–84) mm. Chromosomal abnormalities were identified in 143 pregnancies, including 82 cases of trisomy 21 (Table 1).

The distribution of NT for CRL in normal and trisomy 21 pregnancies is shown in Figures 1 and 2, respectively. In 74.4% (61 of 82) of the trisomy 21 pregnancies, the fetal NT was above the 95th centile of the normal range. In the trisomy 21 pregnancies, the median distance in NT from the normal median for CRL (delta value) did not change significantly with gestation (median = 1.96 mm, $r = 0.08$, $P = 0.48$; Figure 3). Similarly, in the trisomy 21 pregnancies the median free β -hCG (in MoM corrected for fetal CRL and maternal weight) did not change significantly with CRL (median =

Table 1 Estimated risk for trisomy 21 of 1 in 300 or greater, based on the combination of maternal age, fetal nuchal translucency, and maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A in chromosomally normal and abnormal pregnancies

Fetal karyotype	n	Estimated number at risk of ≥ 1 in 300
Normal	14 240	967 (6.8%)
Trisomy 21	82	75 (91.5%)
Trisomy 18	21	21 (100%)
Trisomy 13	11	10 (90.9%)
Turner syndrome	11	10 (90.9%)
Triploidy	8	7 (87.5%)
Other*	10	6 (60.0%)
Total	14 383	1096 (7.6%)

*Deletions, partial trisomies, unbalanced translocations or sex chromosome aneuploidies.

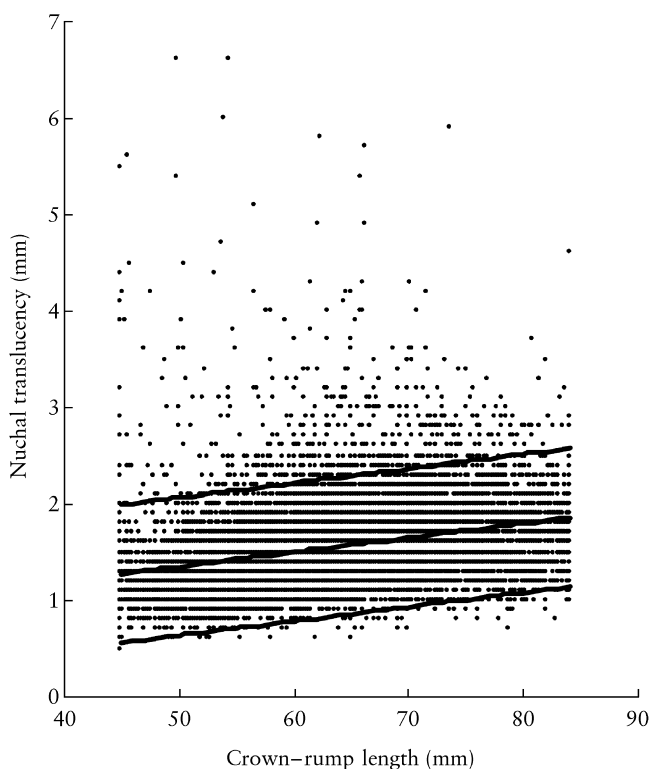


Figure 1 Distribution of fetal nuchal translucency for crown–rump length in the 14 240 normal pregnancies (median, 95th and 5th centiles).

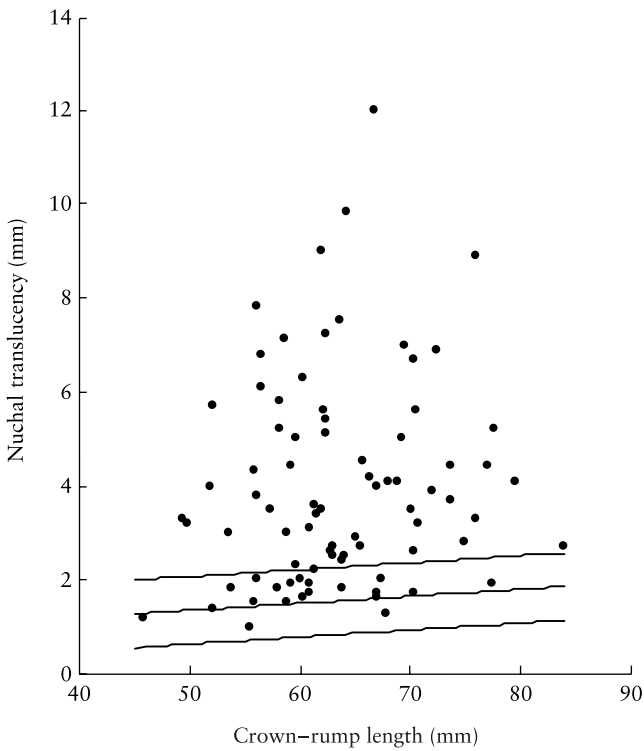


Figure 2 Distribution of fetal nuchal translucency for crown-rump length in the 82 pregnancies with trisomy 21 plotted on the normal range (median, 95th and 5th centiles).

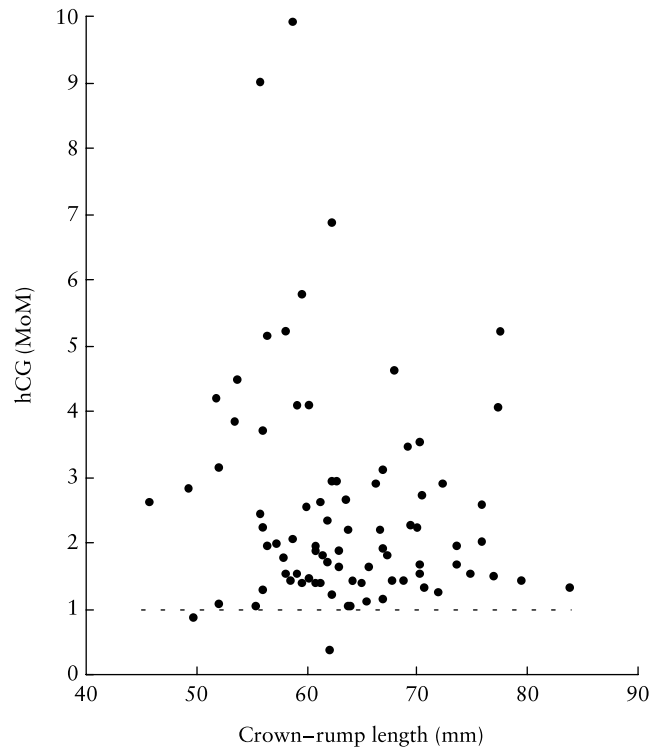


Figure 4 Maternal serum free β -human chorionic gonadotropin (hCG), in MoM for crown-rump length and maternal weight, in the 82 pregnancies with trisomy 21. The normal median is 1 MoM (horizontal dotted line).

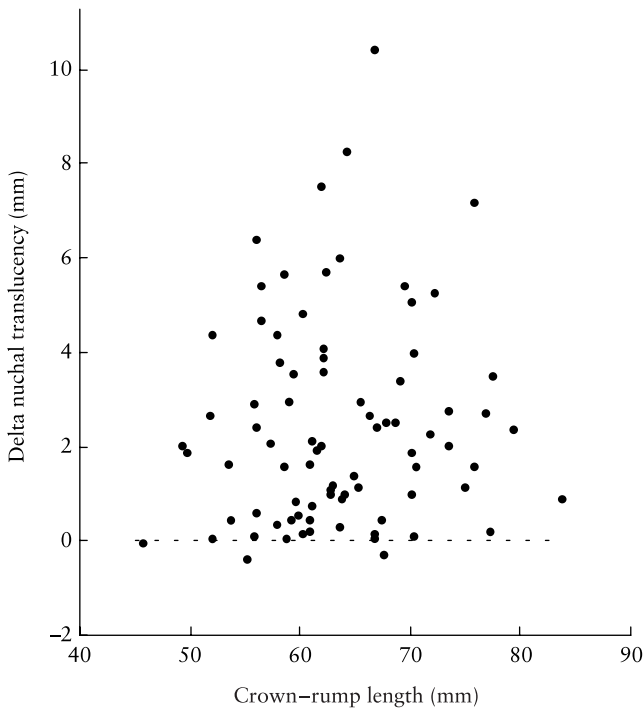


Figure 3 Delta fetal nuchal translucency (NT) for crown-rump length in the 82 pregnancies with trisomy 21. The delta value is the difference in observed NT from the median for crown-rump length of the normal pregnancies. The normal median value of delta is 0 mm (horizontal dotted line).

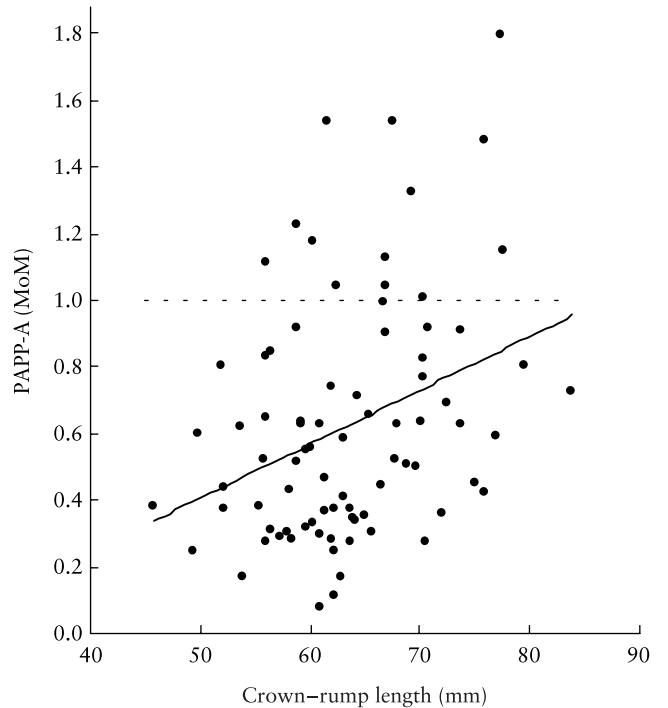


Figure 5 Distribution of maternal serum pregnancy-associated plasma protein-A (PAPP-A), in MoM for crown-rump length (CRL) and maternal weight, in the 82 pregnancies with trisomy 21 demonstrating a significant increase with CRL (regression line). The normal median is 1 MoM (horizontal dotted line).

1.94 MoM, $r = 0.16$, $P = 0.14$; Figure 4), but the median PAPP-A (in MoM corrected for fetal CRL and maternal weight) increased with fetal CRL (median = 0.56 MoM, $r = 0.33$, $P = 0.002$; Figure 5).

The estimated risk for trisomy 21 based on maternal age, fetal NT and maternal serum free β -hCG and PAPP-A was 1 in 300 or greater in 6.8% (967 of 14 240) normal pregnancies, in 91.5% (75 of 82) of those with trisomy 21 and in

Table 2 Detection rates for different fixed false-positive rates in screening for trisomy 21, based on the combination of maternal age, fetal NT, and maternal serum free β -hCG and PAPP-A

Method of screening	Detection rate with false-positive rate fixed at				
	1%	2%	3%	4%	5%
Maternal age	9 (11.0%)	14 (17.1%)	19 (23.2%)	23 (28.0%)	25 (30.5%)
β -hCG and PAPP-A	22 (26.8%)	33 (40.2%)	39 (47.6%)	42 (51.2%)	49 (59.8%)
NT	53 (64.6%)	60 (73.2%)	62 (75.6%)	64 (78.0%)	65 (79.3%)
NT and β -hCG and PAPP-A	63 (76.8%)	65 (79.3%)	69 (84.1%)	72 (87.8%)	74 (90.2%)

In this population there were 82 cases of trisomy 21. hCG, human chorionic gonadotropin; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

Table 3 False-positive rates for different fixed detection rates in screening for trisomy 21 by the combination of maternal age, fetal NT and maternal serum free β -hCG and PAPP-A

Method of screening	False-positive rate with sensitivity fixed at						
	60%	65%	70%	75%	80%	85%	90%
Maternal age	1993 (14.0%)	2724 (19.1%)	3577 (25.1%)	3939 (27.7%)	4782 (33.6%)	6603 (46.4%)	7537 (52.9%)
β -hCG and PAPP-A	723 (5.1%)	815 (5.7%)	1002 (7.0%)	1433 (10.1%)	1866 (13.1%)	2167 (15.2%)	2594 (18.2%)
NT	80 (0.6%)	140 (1.0%)	193 (1.4%)	367 (2.6%)	874 (6.1%)	1299 (9.1%)	2276 (16.0%)
NT and β -hCG and PAPP-A	37 (0.3%)	68 (0.5%)	91 (0.6%)	128 (0.9%)	305 (2.1%)	432 (3.0%)	718 (5.0%)

In our population there were 14 240 normal and 82 trisomy 21 pregnancies. hCG, human chorionic gonadotropin; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

88.5% (54 of 61) of those with other chromosomal defects (Table 1). In the trisomy 21 group, there were 76 cases where the diagnosis was made prenatally (73 in the screen-positive and three in the screen-negative pregnancies, in which the parents chose to have invasive testing) and six where the diagnosis was made postnatally in live births (two in the screen-positive pregnancies, in which the parents chose to avoid invasive testing, and four in the screen-negative pregnancies).

On the basis of the maternal age and gestational age at the time of screening it was estimated that in the 14 383 pregnancies examined there would have been 79 cases of trisomy 21, which is similar to the observed 82 cases⁶. We also estimated, on the basis of the maternal age-related prevalence of trisomy 21 in live births⁷, that 54 babies with trisomy 21 would have been live-born had there not been any antenatal testing and selective termination of affected pregnancies. In our screen-negative group there were four live births and three cases that were diagnosed prenatally, and these pregnancies were terminated at the request of the parents. The rate of intrauterine lethality of trisomy 21 pregnancies between 12 weeks and term is about 30%^{6,7}, and it is therefore not certain how many of the trisomy 21 pregnancies that were terminated would have resulted in live births. On the extreme assumptions that first, all intrauterine deaths are from the screen-positive group and secondly, invasive testing is confined to the screen-positive group, then screening by a combination of maternal age, fetal NT and serum free β -hCG and PAPP-A, followed by invasive diagnostic testing for those with a risk of 1 in 300 or greater, and selective termination of affected fetuses would have reduced the potential live-birth prevalence of trisomy 21 by 87% (47 of 54).

The detection rates for fixed false-positive rates between 1% and 5%, and the false-positive rates for fixed detection rates between 60% and 90% of screening for trisomy 21 by

maternal age alone, maternal age and fetal NT, maternal age and serum free β -hCG and PAPP-A, and by maternal age, fetal NT and maternal serum biochemistry are shown in Tables 2 and 3, and Figure 6. The best method of screening was the combination of ultrasound and biochemistry, with a sensitivity of 90.2% for a false-positive rate of 5%. The risk cut-off to achieve these results was 1 in 215. In our population of 14 383 pregnancies, 839 women had a risk of 1 in 215 or more, including 712 normal pregnancies, 74 with trisomy 21 and 53 with other chromosomal defects. Therefore, if all

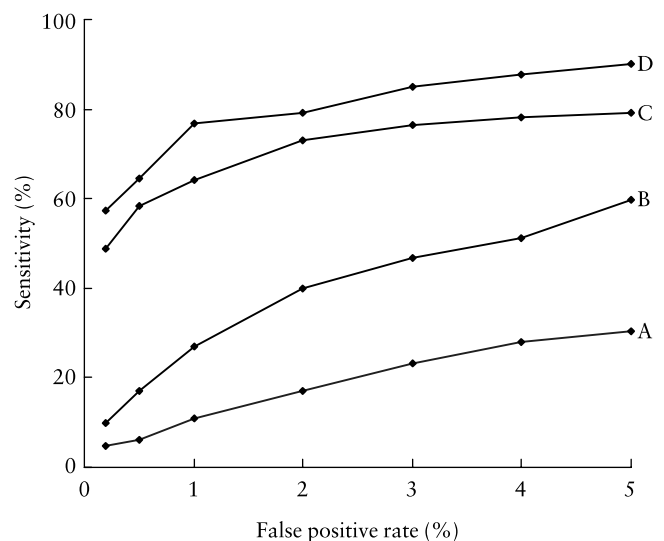


Figure 6 Relationship between detection rates and false-positive rates in screening for trisomy 21 by maternal age alone (A), maternal age and serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A (B), maternal age and fetal nuchal translucency (NT) (C), and by the combination of maternal age, fetal NT and maternal serum biochemistry (D).

Table 4 Risk cut-off and screen-positive rate (false- and true-positive rates) to achieve detection rates between 60% and 90% by a combination of maternal age, fetal nuchal translucency and maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A

Detection rate (%)	Risk cut-off	Type of pregnancy				Screen-positive rate (%)	Abnormal fetuses per 1000 invasive tests*
		Normal n = 14 240	Abnormal				
			Total n = 143	Trisomy 21 n = 82	Other n = 61		
60	1 in 9	37	81	50	31	0.8	680
65	1 in 17	68	88	53	35	1.1	564
70	1 in 22	91	94	57	37	1.3	508
75	1 in 30	128	104	62	42	1.6	457
80	1 in 86	305	116	66	50	2.9	276
85	1 in 132	432	123	70	53	3.9	222
90	1 in 215	718	127	74	53	5.8	151

*The last column shows the number of abnormal fetuses (trisomy 21 and other chromosomal defects) detected per 1000 invasive tests in all screen-positive pregnancies. When the detection rate is fixed at 60% for trisomy 21 the risk cut-off is 1 in 9 and the screen-positive rate is only 0.8% but the yield of abnormal results for every 1000 invasive tests is 680. In contrast, to achieve a detection rate of 90% the risk cut-off is 1 in 215 and the yield of abnormal results for every 1000 invasive tests is 151.

women with a risk estimate of 1 in 215 or more had an invasive test, 15.1% would have an abnormal result. The rates of abnormal results per 1000 invasive tests for different detection rates are shown in Table 4.

DISCUSSION

This prospective study of screening for trisomy 21 by a combination of maternal age, fetal NT and maternal serum free β -hCG and PAPP-A at 11–14 weeks has demonstrated that screening can be provided in an OSCAR setting and is associated with a detection rate of 90% for a false-positive rate of 5%. For the same false-positive rate the detection rate of screening by maternal age alone is 30%, and by maternal age and serum biochemistry it is about 60%. This is identical to the prediction provided from a study of 210 singleton pregnancies with trisomy 21 and 946 chromosomally normal controls, matched for maternal age, gestation and sample storage time¹. This method has also identified 94% of all major chromosomal defects, such as trisomies 18 and 13, triploidy and Turner syndrome, and 60% of other chromosomal defects, such as deletions, partial trisomies, unbalanced translocations, and sex chromosome aneuploidies other than Turner's (Table 1).

Screening for chromosomal defects in the first rather than the second trimester provides earlier reassurance for those with a normal result and less traumatic termination for those choosing this option. A potential disadvantage is that earlier assessment of risk and prenatal diagnosis preferentially identifies those chromosomally abnormal pregnancies that are destined to miscarry. Approximately 30% of trisomy 21 fetuses die between 12 weeks and term⁶. This issue of intrauterine lethality of chromosomal defects is of course a potential criticism of all methods of antenatal screening, including second-trimester maternal serum biochemistry; the estimated rate of intrauterine lethality between 16 weeks and term is about 20%⁶. In any case, as demonstrated in this study, assessment of risk by a combination of maternal age, fetal NT and maternal serum free β -hCG and PAPP-A, followed by

selective termination of affected fetuses, reduces the potential live-birth prevalence of trisomy 21 by at least 87%.

In this study fetal NT was successfully measured in all cases. This is because the ultrasound scans were carried out by appropriately trained sonographers, who adhered to the criteria established by The Fetal Medicine Foundation. Thus, the ultrasound equipment was of good quality, the gestational age was 11–13+6 weeks and the fetal crown–rump length was 45–84 mm, a good sagittal section of the fetus away from the amnion was obtained and the NT was measured with the fetus in the neutral position, the maximum thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine was measured, and the magnification was such that each increment in the distance between callipers was only 0.1 mm. During the scan, three measurements were taken and the maximum one was recorded. The ability to achieve a reliable measurement of NT is dependent on adherence to the criteria outlined above and on the motivation of sonographers. For example, in a screening study in which the time spent in examining patients was less than 3 min and in which 54% of cases were examined before 10 weeks, the sonographers were unable to measure NT in 42% of the cases⁸. A study comparing the results obtained from hospitals where NT was used in clinical practice (interventional) compared to those from hospitals where they merely recorded the measurements but did not act on the results (observational), reported that, in the interventional group, successful measurement of NT was achieved in 100% of cases and the measurement was > 2.5 mm in 2.3% of cases; the respective percentages in the observational group were 85% and 12%^{9,10}. Appropriate training, high motivation and adherence to a standard technique for the measurement of NT are essential prerequisites for good clinical practice. Monni *et al.* reported that, after modifying their technique of measuring NT, by following the guidelines established by The Fetal Medicine Foundation, their detection rate of trisomy 21 improved from 30% to 84%¹¹.

The fetal NT was above the 95th centile of the normal range in 74% of the trisomy 21 pregnancies (Figure 2). This

is similar to the results of the previous 14 prospective studies examining the implementation of fetal NT measurement in screening for chromosomal defects^{2,12-24}. Thus, the combined results on a total of 174 473 pregnancies, including 728 with trisomy 21, demonstrated a detection rate of 77% for a false-positive rate of 4.7%. Our study has also demonstrated that within the gestational range of 11–14 weeks the increased NT of trisomy 21 fetuses did not change with fetal CRL (Figure 3). The implication of this finding is that NT screening is as effective at 11 weeks as it is at 12 and 13 weeks.

In the OSCAR setting, measurements of free β -hCG and PAPP-A were carried out using a random access immunoassay analyser and automated immunofluorescent assays, which are highly accurate and reproducible^{1,25,26}. External quality assessment of centers using this system has demonstrated that on average the between-laboratories coefficient of variation for both free β -hCG and PAPP is 4%, whereas with the Perkin Elmer Delfia system the variation is 10% for PAPP-A and 8% for free β -hCG²⁶.

In the trisomy 21 pregnancies, maternal serum free β -hCG was on average twice as high as in normal pregnancies. As with fetal NT, within the gestational range of 11–14 weeks the increased free β -hCG did not change with fetal CRL (Figure 4) and, therefore, screening with this marker is as effective at 11 weeks as it is at 13 weeks. In contrast, maternal serum PAPP-A, which is the most effective biochemical marker in the first trimester, is a better discriminator between affected and normal pregnancies at 11 weeks than at 13 weeks (Figure 5). It could therefore be argued that the combined test is best carried out at 11 or indeed at 10 weeks; we have previously shown that effective NT screening can be carried out from 10 weeks². However, in the last decade it has become increasingly apparent that the first-trimester scan should not be restricted to the measurement of fetal NT and CRL but is valuable in the early detection of a wide range of fetal defects^{27,28}. As at 10 weeks even major defects such as anencephaly can be missed, it is better to delay the scan until 12–13 weeks.

One option would be to perform serum testing at 10–11 weeks and the scan at 12–13 weeks, because such an approach might even improve the 90% detection rate observed in this study. However, the advantage of the OSCAR approach is that biochemical testing, ultrasound scanning and counseling can be integrated into one visit and provided efficiently by a multidisciplinary team of experts.

The data on detection and false-positive rates (Tables 2–4) are useful for healthcare planners because in nationalized health care systems, such as in the UK (<http://www.doh.gov.uk/nsc/>), a governmental decision may be taken to fix these rates but allow freedom to health care providers to select the method of screening. If the false-positive rate is fixed at 5%, the choice of screening methods would be maternal age, first- or second-trimester maternal serum biochemistry, fetal NT, or the combination of fetal NT and serum biochemistry at 11–14 weeks, with respective detection rates of about 30%, 60%, 65%, 80% and 90%. If the choice is made in favor of fetal NT and serum biochemistry at 11–14 weeks, the risk cut-off for invasive testing would be 1 in 215.

As shown in Table 4, invasive testing in all pregnancies with a minimum risk of 1 in 215 would not only lead to the detection of 90% of trisomy 21 pregnancies but also 86% of those with other major chromosomal defects. Therefore, if all women with a minimum risk of 1 in 215 choose to have invasive testing, for every 1000 invasive tests carried out there would be 151 abnormal results (rate of 1 in 6.6) and 849 normal fetuses would be subjected to the 1% procedure-related risk of death, resulting in 8.5 miscarriages. Consequently, with this method of screening and invasive testing for all screen-positive pregnancies, one chromosomally normal fetus will die for every 18 abnormal fetuses that are detected. In these calculations it is assumed that the doctors performing chorionic villus sampling are appropriately trained, in which case the procedure-related risk of miscarriage would be 1%, which is the same as for second-trimester amniocentesis.

Alternatively, healthcare planners may recommend that the minimum detection rate should be 60%, which can be achieved with screening by fetal NT and serum biochemistry at 11–14 weeks, at a false-positive rate of less than 1% and a risk cut-off for invasive testing of 1 in 9. In this case, one chromosomally normal fetus will die for every 213 abnormal fetuses that are detected.

In counseling women, an alternative approach is to accept that decisions taken by healthcare planners based on arbitrary comparisons of the burdens of miscarriage to those of the birth of a chromosomally abnormal baby are contrary to the basic principle of informed consent²⁹. Our responsibility is to assess the risk of a pregnancy being affected using the most accurate method and to allow the parents decide for themselves in favor or against invasive testing.

The data of this study provide further evidence that, currently, the most effective method of screening for chromosomal defects is that provided by a combination of maternal age, fetal NT and maternal serum free β -hCG and PAPP-A at 11–14 weeks, and they support the view that the time has come for a total shift to first-trimester screening³⁰.

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