

Maternal serum biochemistry at 11–13⁺⁶ weeks in relation to the presence or absence of the fetal nasal bone on ultrasonography in chromosomally abnormal fetuses: an updated analysis of integrated ultrasound and biochemical screening

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Background Screening for trisomy 21 by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β -hCG and pregnancy associated plasma protein-A (PAPP-A) at 11–13⁺⁶ weeks of gestation is associated with a detection rate of 90%, for a false-positive rate of 5%. Recent evidence suggests that in about 70% of fetuses with trisomy 21 the nasal bone is not visible at the 11–13⁺⁶ week scan and that the frequency of absence of nasal bone differs in different ethnic groups. In addition, there is a relationship between absent nasal bone and nuchal translucency thickness. In a preliminary study we showed that while PAPP-A levels were lower and free β -hCG levels were higher in trisomy 21 fetuses with an absent nasal bone, this difference was not statistically different. In fetuses with trisomy 13 and trisomy 18, there is also a high (57 and 67%) incidence of an absent nasal bone. The aim of this present study was to extend our examination of whether the level of maternal serum biochemical markers is independent of the presence or absence of the nasal bone in cases with trisomy 21 and to ascertain if any differences exist in cases with trisomies 13 and 18.

Methods This study data comprised 100 trisomy 21 singleton pregnancies at 11–13⁺⁶ weeks of gestation from our previous study and an additional 42 cases analysed as part of routine OSCAR screening. A total of 34 cases with trisomy 18 and 12 cases with trisomy 13 were also available. Ultrasound examination was carried out for measurement of fetal NT and assessment of the presence or absence of the fetal nasal bone. Maternal serum free β -hCG and PAPP-A were measured using the Kryptor rapid random access immunoassay analyser (Brahms Diagnostica AG, Berlin). The distribution of maternal serum free β -hCG and PAPP-A in chromosomally abnormal fetuses with absent and present nasal bone was examined.

Results The nasal bone was absent in 29 and present in 13 of the new trisomy 21 cases and in 98 (69%) and 44 respectively in the combined series. For the trisomy 18 cases, the nasal bone was absent in 19 (55.9%) cases and in 3 (25%) of cases of trisomy 13. There were no significant differences in median maternal age, median gestational age, NT delta, free β -hCG MoM and PAPP-A MoM in trisomy 21 fetuses with and without a visible nasal bone, and similarly for those with trisomies 13 or 18. For a false-positive rate of 5%, it was estimated that screening with the four markers in combination with maternal age would be associated with a detection rate of 96% of cases with trisomy 21. For a false-positive rate of 0.5%, the detection rate was 88%.

Conclusions There is no relationship between an absent fetal nasal bone and the levels of maternal serum PAPP-A or free β -hCG in cases with trisomies 13, 18 or 21. An integrated sonographic and biochemical test at 11–13⁺⁶ weeks can potentially identify about 88% of trisomy 21 fetuses for a false-positive rate of 0.5%. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: prenatal screening; chromosomal anomalies; Down syndrome; OSCAR; nuchal translucency; nasal bone; free β -hCG; PAPP-A

INTRODUCTION

In an initial study examining the fetal profile in 701 fetuses prior to chorionic villus sampling (CVS) at 11–13⁺⁶ weeks, an absent nasal bone was found in

72.9% of cases with trisomy 21 and in 0.5% of chromosomally normal fetuses (Cicero *et al.*, 2001). In an extension to this series, Cicero *et al.* (2002) in 1046 singleton pregnancies observed an absent nasal bone in 68.4% of cases with trisomy 21, 50% of cases with trisomy 18 and 80% of cases with trisomy 13, while in chromosomally normal fetuses this was absent in 0.5% of cases. Additionally, it was observed that in the chromosomally normal group the nasal bone length increased significantly with crown-rump length (CRL) (Cicero *et al.*, 2002). The high association between trisomy 21

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and absent nasal bone has been confirmed in at least four other studies. Otano *et al.* (2002) in a study of 194 pregnancies reported an absent nasal bone in 60% (3/5) of cases with trisomy 21 but only 0.6% (1/175) normal fetuses. Zoppi *et al.* (2003) reported an absent nasal bone in 70% (19/27) of cases with trisomy 21 and in 0.2% (8/5485) normal fetuses. Orlandi *et al.* (2003) reported an absent nasal bone in 67% (10/15) of cases with trisomy 21 and in 1.0% (10/1000) normal fetuses. Viora *et al.* (2003) reported an absent nasal bone in 80% (8/10) of cases with trisomy 21 and in 1.4% (24/1733) normal fetuses. Absent or hypoplastic development of the nasal bone in cases with trisomy 21 have also been confirmed both radiologically and histologically (Larose *et al.*, 2003; Minderer *et al.*, 2003; Tuxen *et al.*, 2003; Wong *et al.*, 2003).

In a further extension to the original study, Cicero *et al.* (2004) examined 5918 fetuses and in the chromosomally normal group found that the incidence of absent nasal bone was related to the ethnic origin of the mother (with a higher incidence in Afro-Caribbeans); to fetal CRL (with a higher incidence in earlier gestation) and to the nuchal translucency (NT) thickness (with a higher incidence with greater NT thickness). In the chromosomally abnormal group, absent nasal bone was found in 68.8% (229/333) of cases with trisomy 21, 54.8% (68/124) of cases with trisomy 18 and 34.2% (13/38) of cases with trisomy 13. The influence of Afro-Caribbean ethnicity on the incidence of absent nasal bone was also confirmed by Prefumo *et al.* (2004).

Our previous analysis (Cicero *et al.*, 2003a) of the relationship between absent nasal bone in cases with trisomy 21 and the first-trimester maternal serum biochemical markers showed lower levels of PAPP-A and higher levels of free β -hCG in cases in which the fetal nasal bone was absent, although this was not statistically significant. Our initial estimates of combining absent fetal nasal bone, fetal NT thickness and the maternal serum biochemical markers showed that this integration of ultrasonographic and biochemical markers would allow a detection rate of 90% of cases of trisomy 21 for a 0.5% false-positive rate.

In this present study, we wish to firstly evaluate in an extended series of cases of trisomy 21 if the trend to lower PAPP-A and higher free β -hCG becomes statistically significant and hence requires correction for. Secondly, to examine the screening performance of an integrated first-trimester test that takes into account the

relationship between absent nasal bone and ethnic origin of the mother, fetal CRL and NT thickness (Cicero *et al.*, 2004). Thirdly, to evaluate if there are differences also for these biochemical markers with absent nasal bone in cases with trisomies 13 and 18.

METHODS

The study population was composed of data from the 100 trisomy 21 cases of singleton pregnancies at 11–13⁺⁶ weeks of gestation from our previous study, supplemented with an additional 42 cases analysed as part of routine OSCAR screening (Spencer *et al.*, 2000c, 2003a; Bindra *et al.*, 2002). Additionally, a total of 34 cases with trisomy 18 and 12 cases with trisomy 13 were also available. All fetuses had been found to have possible chromosomal defects after screening with a combination of maternal age and fetal NT or additionally maternal serum free β -hCG and PAPP-A. In all cases, CVS for fetal karyotyping was carried out at the request of the parents after first-trimester screening. Ultrasound examination was carried out for measurement of fetal CRL and NT and prospective assessment of the presence or absence of the fetal nasal bone as previously described (Snijders *et al.*, 1998; Cicero *et al.*, 2001). Immediately before CVS, maternal blood samples were collected and the serum was aliquoted and stored at -20°C prior to analysis in those women not having already had biochemical marker assessment as part of routine first-trimester screening. Serum free β -hCG and PAPP-A were measured using the Brahms Kryptor rapid random access immunoassay analyser (Brahms AG, Henningsdorf, Berlin) using time resolved amplified cryptate emission technology (TRACE). The analytical performance of this system has been described before (Spencer *et al.*, 1999). To evaluate if there were any difference between biochemical marker levels in normal pregnancies in which the nasal bone was absent or present, we analysed a set of 7638 normal pregnancies screened at the Fetal Medicine Centre (London). Table 1 summarises the demographic characteristics of the three aneuploidy groups and the normal group.

Statistical analysis

Delta NT (Spencer *et al.*, 2003b) was calculated for each case using median values established from a previous

Table 1—Demographic characteristics of trisomy 21, trisomy 18, trisomy 13 and unaffected pregnancies

	Trisomy 21	Trisomy 18	Trisomy 13	Unaffected
Number	142	34	12	7638
Absent nasal bone	98 (69%)	19 (55.9%)	3 (25%)	49 (0.6%)
Median (range) maternal age (years)	38.6 (24.4–45.4)	39.1 (25.1–43.2)	36.9 (31.5–44.9)	35.0 (16.2–48.3)
Median (range) fetal CRL (mm)	63.35 (47–84)	49.8 (45–72)	63.9 (48–74)	61.8 (45–84)
Median (range) maternal weight (kg)	63.6 (42–102)	63.6 (47–110)	63.0 (50–80)	63.6 (33.6–186.0)
Caucasian (%)	80%	91%	100%	93%
Asian (%)	16.9%	2.9%	0%	3.0%
Afro-Caribbean (%)	2.1%	2.9%	0%	0.7%
Non-cigarette smoker (%)	97%	96%	100%	96%
Male fetus (%)	50.4%	46.4%	81.8%	48.3%

large study (Snijders *et al.*, 1998). All biochemical marker measurements were converted to multiple of the median values (MoM) using median values derived from previous studies of unaffected pregnancies with gestational age calculated in weeks and days from the fetal CRL (Ong *et al.*, 2000). MoM values for biochemical markers were calculated with correction for maternal weight using the linear weight correction procedure (Spencer *et al.*, 2000a, 2003c). Statistical analysis of data was performed with Analyse-It (Smart Software, Leeds, UK), a statistical software add-in for Microsoft Excel.

In order to simulate the performance of population screening using the four markers (fetal NT, maternal serum free β -hCG and PAPP-A, absent fetal nasal bone) in combination with maternal age, we used the maternity age distribution of England and Wales (ONS, 2000) and standard statistical modelling techniques (Royston and Thompson, 1992). We used the distribution parameters and inter-relationships for the first three markers from a well-described large population of trisomy 21 cases and from a large unaffected population (Spencer *et al.*, 1999; Spencer *et al.*, 2003a) and the frequency of absent nasal bone in the affected and unaffected populations from the cases included in the study by Cicero *et al.* (2004). We assumed that the lack of a significant association between maternal serum metabolites and absence of the nasal bone in the trisomic fetuses we identified by the OSCAR approach (see Results) would apply equally to those trisomic fetuses that were in the screen negative group. A series of 15 000 delta NT values, and MoM's for free β -hCG and PAPP-A for the unaffected pregnancy group and for the trisomy 21 group were selected at random from within the Gaussian distribution of each marker in each pregnancy group. To take into account the recently described relationship between CRL, NT, ethnicity and absence of nasal bone (Cicero *et al.*, 2004) we randomly assigned to the 15 000 data sets a CRL value with 25% of cases in the range 44–54 mm, 50% in the range 55–69 mm and 25% in the range 70–84 mm, this being the distribution seen in our centre over the past 5 years. From the CRL and delta NT, we then calculated a measured NT. We then randomly assigned to the data set ethnic origin with 90% of the data being Caucasian, 5% being Afro-Caribbean and 5% being Asian, as per our routine screened population distribution. We then randomly assigned the presence or absence of nasal bone based on the individual ethnic group frequencies described by Cicero *et al.* (2004). From these data sets, we firstly calculated a likelihood ratio for trisomy 21 based on the described relationship between absent nasal bone, NT, CRL and ethnicity (Cicero *et al.*, 2004). We then calculated a likelihood ratio for trisomy 21 on the basis of the described relationship of delta NT (Spencer *et al.*, 2003b) and then a likelihood ratio for trisomy 21 on the basis of the Gaussian distributions of the two biochemical markers (Spencer *et al.*, 1999). The nasal bone likelihood ratio was then multiplied by the delta NT and biochemistry likelihood ratio to provide an integrated likelihood ratio for all four markers. The integrated likelihood ratios for the two

pregnancy populations were then used together with the age-related risk of trisomy 21 at 12 weeks (Snijders *et al.*, 1999) to calculate the expected detection rate of affected pregnancies at various false-positive rates in a population with the maternal age distribution of pregnancies in England and Wales (ONS, 2000).

RESULTS

The median delta NT, and free β -hCG and PAPP-A MoM in all cases with a trisomy 21 fetus was 2.17 mm and 1.96 and 0.43 MoM respectively and these levels are similar to previously published series (Spencer *et al.*, 1999; Cicero *et al.*, 2003a). In the cases with trisomy 18, the median delta NT was 2.81 mm, free β -hCG MoM was 0.193 and that for PAPP-A was 0.181 MoM, being similar to our previously published larger series (Tul *et al.*, 1999). In cases with trisomy 13, the median delta NT was 1.13 mm, free β -hCG MoM was 1.118 and that for PAPP-A was 0.453 MoM. These results are somewhat atypical for trisomy 13 in that the free β -hCG is much higher than the 0.581 in our combined series data (Spencer *et al.*, 2005) and the delta NT is considerably lower than the 2.864 found in our previous series (Spencer *et al.*, 2000b (data originally published as MoM)). Table 2 shows the data for each trisomy and each marker in the presence of absence of nasal bone, indicating that in only one case (lowered PAPP-A in trisomy 13 cases with an absent nasal bone) did this difference possibly reach significance, although this is questionable because of the small number of cases ($n = 3$) in this group. Figures 1 and 2 show the distribution of the biochemical markers for trisomy 21 in the groups with absent and present nasal bone along with the median and 95% confidence intervals. Since the markers' free β -hCG and PAPP-A are known to fit a Gaussian distribution after log transformation in both unaffected and trisomy 21 pregnancies (Spencer *et al.*, 1999) and trisomy 18 pregnancies (Tul *et al.*, 1999), further tests of significance between those with and without nasal bone were carried out using t-tests of unequal variance on the \log_{10} transformed MoMs. Each marker showed no significant difference ($p > 0.05$) in MoM between the two groups for either trisomy 21 or 18. Figures 3 and 4 show the distribution of the biochemical markers for trisomy 18 in the groups with absent and present nasal bone. In all cases, the medians and 95% confidence intervals overlapped.

In the normal pregnancy population the median MoM free β -hCG was 1.195 (95% confidence interval 1.020 to 1.718) in the group with an absent nasal bone and 1.034 (95% confidence interval 1.018 to 1.051) in the group with a present nasal bone. For PAPP-A, the median was 0.990 (95% confidence interval 0.746 to 1.226) in the absent nasal bone group and 1.063 (95% confidence interval 1.048–1.077) in the group with a present nasal bone. When the \log_{10} MoMs were compared by t-test of unequal variance there was no significant difference between the groups ($p > 0.05$). Figure 5 demonstrates the distribution of MoMs in the two groups for each

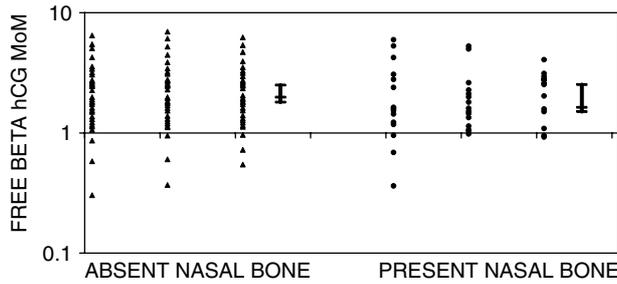


Figure 1—Free β -hCG MoM in cases of trisomy 21 with an absent and present nasal bone. The median and 95% confidence intervals for both groups are also shown

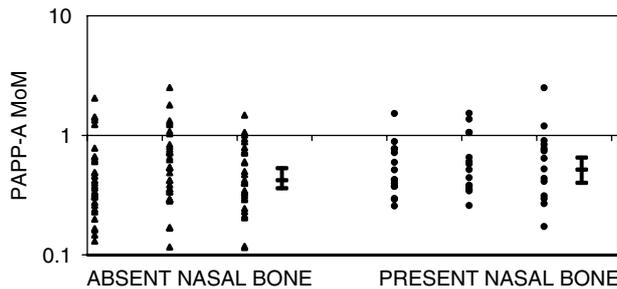


Figure 2—PAPP-A MoM in cases of trisomy 21 with an absent and present nasal bone. The median and 95% confidence intervals for both groups are also shown

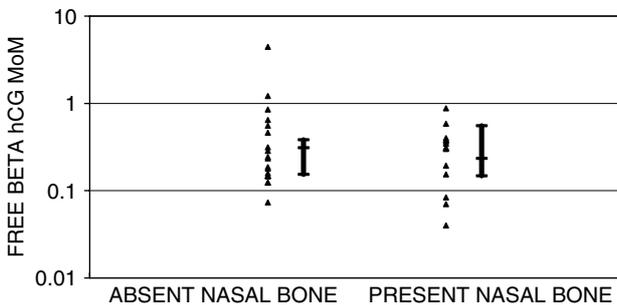


Figure 3—Free β -hCG MoM in cases of trisomy 18 with an absent and present nasal bone. The median and 95% confidence intervals for both groups are also shown

marker. It can safely be assumed that in both the affected and unaffected populations that there is no relationship between biochemical marker MoMs to the observation of an absent nasal bone.

With the new information on absent nasal bone and its relationship to CRL, delta NT and ethnicity and our reconfirmation in this extended series of no relationship between absent nasal bone and maternal serum biochemical markers, we evaluated the expected screening performance when using this integrated ultrasound and maternal serum biochemical screening approach. Table 3 shows the detection rate at a variety of different false-positive rates and confirms that this approach, which can be delivered in a one hour visit to an OSCAR clinic, can achieve detection rates of 96% for a 5% false-positive rate or 90% at a 1% false-positive rate or 88% at a false-positive rate of 0.5%.

Table 2—Median marker MoMs and delta NT in trisomic pregnancies in the presence or absence of fetal nasal bone

	Absent nasal bone (n = 98)	Present nasal bone (n = 44)	Probability of difference
Trisomy 21			
Median Delta NT mm	2.24	1.70	0.1529
Median free β -hCG MoM	1.98	1.63	0.3729
Median PAPP-A MoM	0.42	0.52	0.1517
Trisomy 18			
Median Delta NT mm	3.75	1.17	0.1599
Median free β -hCG MoM	0.12	0.27	0.9424
Median PAPP-A MoM	0.06	0.18	0.3661
Trisomy 13			
Median Delta NT mm	0.95	1.30	0.4054
Median free β -hCG MoM	0.34	0.82	0.4046
Median PAPP-A MoM	0.13	0.34	0.0124

^a Accuracy of *p*-value questionable because of small number of cases.

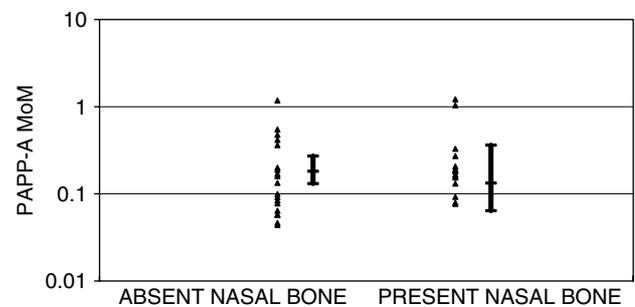


Figure 4—PAPP-A MoM in cases of trisomy 18 with an absent and present nasal bone. The median and 95% confidence intervals for both groups are also shown

DISCUSSION

The results of this study have confirmed our previous observation (Cicero *et al.*, 2003a) that there is no relationship between the levels of maternal serum biochemical markers of trisomy 21 in the first-trimester and an absent nasal bone on ultrasound examination at the same time. Thus, examination of the nasal bone can effectively be combined with maternal serum biochemistry and fetal NT thickness to achieve detection rates of 96% at a 5% false-positive rate. Additionally, since there appears to be no relationship between absent nasal bone and the same biochemical markers in cases with trisomies 13 or

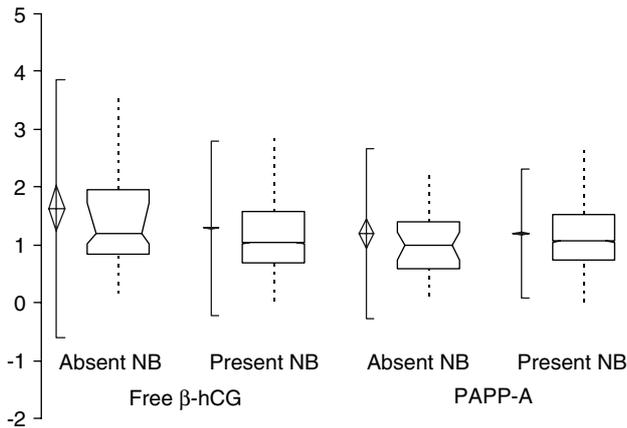


Figure 5—Box-whisker plots of free β -hCG MoM and PAPP-A MoM in the groups with and without a nasal bone in normal pregnancies. The diamond shows the mean and the 95% confidence interval and the notched lines the parametric percentile range. The notched box shows the median, lower and upper quartiles and confidence interval around the median

18, the nasal bone can also be incorporated into existing algorithms (Spencer and Nicolaides, 2002) for these aneuploidies when it could be expected to increase the detection rate by a further 2–3% over and above the 95% achieved at a false-positive rate of 0.3%. Using this combined approach, it would also be possible to maintain the same detection rate as achieved with NT and maternal serum biochemistry, but to have a consequent tenfold reduction in the false-positive rate.

The overall impact of screening a population of 100 000 pregnancies with the integrated first-trimester

Table 3—Modelled detection rates for different fixed false-positive rates using various marker combinations with maternal age

False-positive rate (%)	NT and free β -hCG and PAPP-A Detection rate (%)	NT and free β -hCG and PAPP-A and nasal bone Detection rate (%)
0.5	69.5	88.0
1.0	75.2	90.2
2.0	80.4	93.0
3.0	83.8	94.3
4.0	86.2	95.2
5.0	88.9	96.1

approach, compared with alternative strategies, is depicted in Figure 6. Assuming a 5% invasive testing rate and a procedure-related fetal loss rate of 1%, using conventional combined first-trimester screening would result in the loss of 50 normal fetuses to detect 184 cases with trisomy 21—a detected to fetal loss ratio of 3.7 to 1. With the addition of nasal bone, this ratio improves only slightly to a ratio of 3.8. However, lowering the false-positive rate to 0.5%, with the detection rate still being close to 90%, results in a detected loss ratio of 35 to 1.

The first-trimester integrated screening program, which can be accomplished during one visit in the first trimester to an OSCAR centre, is much more likely to be favoured by women, rather than a protracted first- and second-trimester screening program (Spencer and Aitken, 2004), which in mathematical modelling exercises is expected to detect 83% of cases at a 1% false-positive rate or 78% at a 0.5% false-positive rate

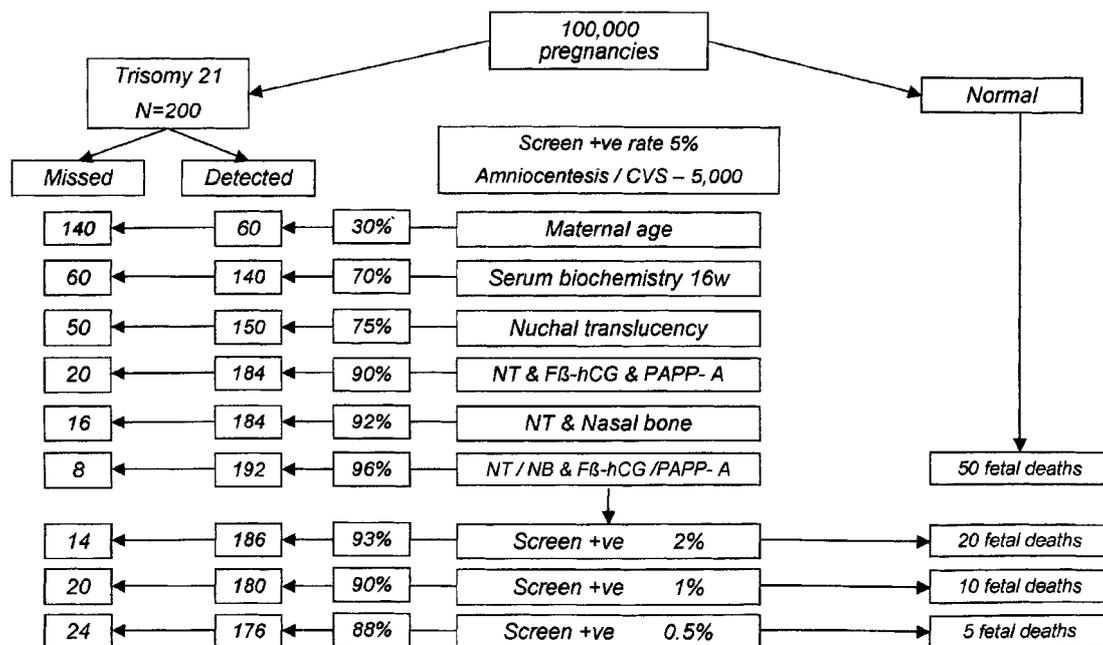


Figure 6—Results of screening a hypothetical pregnant population of 100 000 women with a trisomy 21 incidence of 1 in 500. A variety of screening modalities are present showing cases detected and missed for an invasive testing rate of 5% with a 1% procedural related fetal loss or at lower invasive testing rates for the combination with nasal bone

(Wald *et al.*, 1999; Wald *et al.*, 2003). Such a theoretical program—referred to as the Integrated first- and second-trimester test has a detected fetal loss ratio of 31 to 1.

Successful implementation of an integrated first-trimester ultrasound and maternal serum biochemistry screening program, including examination of the nasal bone, will be dependant upon sonographers and obstetricians being adequately trained and achieving satisfactory audit performance, as is the case now with NT measurement. It has been shown that the skill in clearly and accurately identifying an absent nasal bone takes considerable time to acquire (Cicero *et al.*, 2003b).

One possible method of implementation of the integrated first-trimester test, which would enable the benefit of increased detection and low false-positive rate and hence lessen the demand for CVS, is by concentrating the expertise for CVS and nasal bone scanning in a tertiary referral centre. It is possible that by selecting an appropriate risk cut-off of 1 in 500 for the conventional first-trimester combined test to classify as screen positive 10% of women, including 95% of those with trisomy 21, and to refer this group to a tertiary centre for an ultrasound scan for the presence or absence of nasal bone and only then decide on the need for invasive testing. This approach would be associated with a detection rate of about 90% for a 0.5% false-positive rate. Further improvement may then be possible, by including other specialist techniques such as ductus venosus flow (Matias *et al.*, 1998; Borrell, 2004).

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REFERENCES

- 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. 2004. The ductus venosus in early pregnancy and congenital anomalies. *Prenat Diagn* **24**: 688–692.
- Cicero S, Curcio P, Papageorgiou A, Sonek J, Nicolaides K. 2001. Absence of nasal bone in fetuses with trisomy 21 at 11–14 weeks of gestation: an observational study. *Lancet* **358**: 1665–1667.
- Cicero S, Bindra R, Rembouskos G, Tripsanas C, Nicolaides KH. 2002. Fetal nasal bone length in chromosomally normal and abnormal fetuses at 11–14 weeks of gestation. *J Matern Fetal Neonatal Med* **11**: 400–402.
- Cicero S, Bindra R, Rembouskos G, Spencer K, Nicolaides KH. 2003a. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free β -hCG and PAPP-A at 11 to 14 weeks. *Prenat Diagn* **23**: 306–310.
- Cicero S, Dezerega V, Andrade E, Scheier M, Nicolaides KH. 2003b. Learning curve for sonographic examination of the fetal nasal bone at 11–14 weeks. *Ultrasound Obstet Gynecol* **22**: 135–137.
- Cicero S, Rembouskos G, Vandecruys H, Hogg M, Nicolaides KH. 2004. Likelihood ratio for trisomy 21 in fetuses with absent nasal bone at the 11–14 week scan. *Ultrasound Obstet Gynecol* **23**: 218–223.
- Larose C, Massoc P, Hillion Y, Bernard JP, Ville Y. 2003. Comparison of fetal nasal bone assessment by ultrasound at 11–14 weeks and by post-mortem X-ray in trisomy 21: a prospective observational study. *Ultrasound Obstet Gynecol* **22**: 27–30.
- Matias A, Gomes C, Flack N, Montenegro N, Nicolaides KH. 1998. Screening for chromosomal abnormalities at 10–14 weeks: the role of ductus venosus blood flow. *Ultrasound Obstet Gynecol* **12**: 380–384.
- Minderer S, Gloning KP, Henrich W, Stoger H. 2003. The nasal bone in fetuses with trisomy 21: sonographic versus pathomorphological findings. *Ultrasound Obstet Gynecol* **22**: 16–21.
- Office for National Statistics. 2000. *Birth Statistics, Series FMI, No. 28*. London: Stationery Office.
- Ong CYT, Liao AW, Spencer K, Munim S, Nicolaides KH. 2000. First trimester maternal serum free β human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *Br J Obstet Gynaecol* **107**: 1265–1270.
- Orlandi F, Bilardo CM, Campogrande M. *et al.* 2003. Measurement of nasal bone length at 11–14 weeks of pregnancy and its potential role in Down syndrome risk assessment. *Ultrasound Obstet Gynecol* **22**: 36–39.
- Otano L, Aiello H, Igarzabal L, Matayoshi T, Gadow EC. 2002. Association between first trimester absence of fetal nasal bone on ultrasound and Down syndrome. *Prenat Diagn* **22**: 930–932.
- Prefumo F, Sairam S, Bhide A, Penna L, Hollis B, Thiliganathan B. 2004. Maternal ethnic origin and fetal nasal bone at 11–14 weeks of gestation. *BJOG* **111**: 109–112.
- Royston P, Thompson SG. 1992. Model based screening for risk with application to Down's syndrome. *Stat Med* **11**: 257–268.
- Snijders RMJ, Noble P, Sebire N, Souka A, Nicolaides KH. 1998. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. *Lancet* **351**: 343–346.
- Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. 1999. Maternal age- and gestation-specific risk for trisomy 21. *Ultrasound Obstet Gynecol* **13**: 167–170.
- Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. 1999. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β -human chorionic gonadotrophin and pregnancy associated plasma protein-A. *Ultrasound Obstet Gynecol* **13**: 231–237.
- Spencer K, Ong CYT, Liao AWJ, Nicolaides KH. 2000a. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn* **20**: 491–494.
- Spencer K, Ong C, Skentou H, Liao AW, Nicolaides KH. 2000b. Screening for trisomy 13 by fetal nuchal translucency and maternal serum free β -hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* **20**: 411–416.
- Spencer K, Spencer CE, Power M, Moakes A, Nicolaides KH. 2000c. One stop clinic for assessment of risk for fetal anomalies; a report of the first year of prospective screening for chromosomal anomalies in the first trimester. *Br J Obstet Gynaecol* **107**: 1271–1275.
- Spencer K, Nicolaides KH. 2002. A first trimester trisomy 13/trisomy 18 risk algorithm combining fetal nuchal translucency thickness, maternal serum free β -hCG and PAPP-A. *Prenat Diagn* **22**: 877–879.
- Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. 2003a. 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. *Br J Obstet Gynaecol* **110**: 281–286.
- Spencer K, Bindra R, Nix ABJ, Heath V, Nicolaides KH. 2003b. Delta NT or NT MoM: which is the most appropriate method for calculating accurate patient specific risks for trisomy 21 in the first trimester? *Ultrasound Obstet Gynecol* **22**: 142–148.
- Spencer K, Bindra R, Nicolaides KH. 2003c. Maternal weight correction of maternal serum PAPP-A and free β -hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. *Prenat Diagn* **23**: 851–855.
- Spencer K, Aitken D. 2004. Factors affecting women's preference type of prenatal screening test for chromosomal anomalies. *Ultrasound Obstet Gynecol* **24**: 735–739.
- Spencer K, Crossley JA, Aitken DA, Nicolaides KH. 2005. Second trimester levels of pregnancy associated plasma protein A and free β -hCG in pregnancies with trisomy 13. *Prenat Diagn* **25**: 358–361.

- Tul N, Spencer K, Noble P, Chan C, Nicolaides K. 1999. Screening for trisomy 18 by fetal nuchal translucency and maternal serum free β -hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* **19**: 1035–1042.
- Tuxen A, Keeling JW, Reintoft I, Hansen BF, Nolting D, Kjaer I. 2003. A histological and radiological investigation of the nasal bone in fetuses with Down syndrome. *Ultrasound Obstet Gynecol* **22**: 22–26.
- Viora E, Masturzo B, Errante G, Sciarrone A, Bastonero S, Campogrande M. 2003. Ultrasound evaluation of fetal nasal bone at 11 to 14 weeks in a consecutive series of 1906 fetuses. *Prenat Diagn* **23**: 784–787.
- Wald NJ, Watt HC, Hackshaw AK. 1999. Integrated screening for Down's syndrome based on tests performed during the first and second trimesters. *N Engl J Med* **341**: 461–467.
- 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). *Health Technol Assess* **7**: 1–88.
- Wong SF, Ng WF, Ho LC. 2003. Histopathological findings of the nose of Down syndrome abortuses. *Prenat Diagn* **23**: 561–563.
- Zoppi MA, Ibba RM, Axiana C, Floris M, Manca F, Monni G. 2003. Absence of fetal nasal bone and aneuploidies at first trimester nuchal translucency screening in unselected pregnancies. *Prenat Diagn* **23**: 496–500.