

Measurement of nasal bone length at 11–14 weeks of pregnancy and its potential role in Down syndrome risk assessment

F. ORLANDI*, C. M. BILARDO†, M. CAMPOGRANDE‡, D. KRANTZ§, T. HALLAHAN§, C. ROSSI* and E. VIORA‡

*Centro Diagnosi Prenatale, Palermo and ‡Div. Ost. Gin. Ospedale S. Anna, Torino, Italy, †Academic Medical Centre, Amsterdam, Netherlands and §NTD Laboratories, Huntington Station, New York, NY, USA

KEYWORDS: Down syndrome; free beta-hCG; nasal bone; PAPP-A; pregnancy; screening

ABSTRACT

Objectives To assess the feasibility of measuring nasal bone length in first-trimester pregnancy and to confirm if the absence of a fetal nasal bone is a marker for Down syndrome.

Methods Fetal nasal bone assessment was attempted in 1089 consecutive singleton pregnancies between 11 and 14 weeks' gestation. All ultrasound examinations were performed transabdominally in three separate centers. If the nasal bone was present, nasal bone length was measured.

Results Nasal bone assessment was successfully achieved in 1027 of 1089 (94.3%) ultrasound examinations. Within this group nasal bone was absent in 10 of 1000 (1.0%) unaffected cases, 10 of 15 (66.7%) Down syndrome cases and 5 of 12 (41.7%) cases with other pathological conditions. Regression analysis showed a significant increase ($P < 0.0001$) in nasal bone length from 2.48 mm at a crown–rump length of 45 mm to 3.12 mm at a crown–rump length of 84 mm. The nasal bone length in the five cases of Down syndrome in which the nasal bone was present was less than the median measurement of unaffected cases. Using modeling, the combination of nasal bone with maternal age, nuchal translucency, free beta-human chorionic gonadotropin (hCG) and pregnancy associated plasma protein-A (PAPP-A) achieved a detection rate of 95% with a false-positive rate of 2.9%. At a fixed 1% false-positive rate, the detection rate was 91%.

Conclusions Absence of the nasal bone can be used as a marker for Down syndrome in the first trimester of pregnancy. Inclusion of the nasal bone in the current

first-trimester screening protocol along with nuchal translucency, free beta-hCG and PAPP-A can achieve high detection at a very low false-positive rate. Large datasets are needed to confirm whether the measurement of nasal bone length provides additional benefits beyond the assessment of the presence or absence of the nasal bone. Copyright © 2003 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

First-trimester Down syndrome screening using a combination of nuchal translucency, free beta-human chorionic gonadotropin (hCG) and (pregnancy-associated plasma protein-A) PAPP-A has become common practice in many centers throughout the world. Such screening can detect approximately 90% of Down syndrome cases with a false-positive rate of 5%^{1–11}. The recent report by Cicero *et al.*¹² in which absence of the fetal nasal bone in the first trimester of pregnancy was observed in 73% of Down syndrome cases and in only 0.5% of unaffected cases is suggestive of the potential to further improve the performance of first-trimester screening. Projections based on the data of Cicero *et al.*¹² indicate that the inclusion of the nasal bone in first-trimester Down syndrome screening could result in a detection efficiency as high as 98%¹³.

The nasal bone may be absent in first-trimester cases of Down syndrome due to delayed ossification. We hypothesized that in Down syndrome cases in which the nasal bone is present in the first trimester, it may be hypoplastic. Therefore, we conducted a multicenter trial to determine if the measurement of nasal bone length

Correspondence to: Dr F. Orlandi, Centro di Diagnosi Prenatale, Via Villareale 35, 90141 Palermo, Italy (e-mail: orlandi@tin.it)

Accepted: 22 February 2003

could improve the effectiveness of this marker and to confirm the findings of Cicero *et al.*¹².

METHODS

From February 2002 to April 2002 nasal bone assessment was evaluated in 1089 fetuses at 11–14 weeks of pregnancy (crown–rump length (CRL), 45–84 mm) in three separate centers (Turin, Palermo and Amsterdam). The ultrasound examinations were conducted by five sonographers in Turin, by two sonographers in Palermo and by one sonographer in Amsterdam. In Palermo and Turin, the nasal bone measurement was conducted as part of routine first-trimester Down syndrome screening. In Amsterdam, the evaluation was performed mainly in patients referred for prenatal diagnosis. The result of the nasal bone measurement was not included in the clinical assessment of Down syndrome risk but was recorded for retrospective analysis. Patients were not counseled about the nasal bone results.

Karyotyping was performed by chorionic villus sampling (CVS) or amniocentesis or was obtained postnatally. Prenatal diagnostic testing was performed if the patient was at increased risk for Down syndrome (nuchal translucency, free beta-hCG and PAPP-A in Palermo or nuchal translucency only in Turin and Amsterdam). In addition, some patients had requested a diagnostic procedure based on maternal age or anxiety.

As part of the ultrasound examination for nuchal translucency, an additional 3–4 min was allowed for nasal bone assessment. For the nasal bone measurement a mid-sagittal view of the fetal face profile was obtained transabdominally with the beam of the ultrasound transducer perpendicular to the nasal bone. A precise midline view was obtained so that the nasal bone appeared as a thin echogenic line. In addition, the ultrasound transducer was tilted from side to side to ensure that the skin was seen separate from the nasal bone. An insonation angle of approximately 45° to the fetal face was used to ensure that the nasal bone would not falsely appear to be absent. The nasal bone measurement was made by placing the calipers in the out-to-out position (Figure 1).

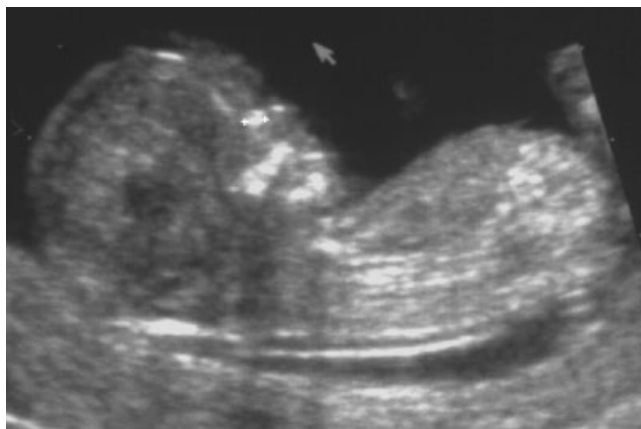


Figure 1 Ultrasound image showing measurement of nasal bone length with calipers in the out-to-out position.

A regression formula for measurable nasal bones was calculated to determine the growth rate of nasal bone length vs. CRL. The regression formula was then used to convert each patient's nasal bone length to a multiple of the median (MoM) value. To determine likelihood ratios for nasal bone MoMs, results were grouped into three separate categories: 'absent', 'small' (present but $\leq 10^{\text{th}}$ percentile of the unaffected cases) and 'within range' (those $> 10^{\text{th}}$ percentile). The likelihood ratios were then calculated by dividing the percentage of Down syndrome cases in each category by the percentage of unaffected cases in that category. Down syndrome risks were then calculated by multiplying these likelihood ratios by the patient's *a priori* risk based on gestational and maternal ages.

To assess the usefulness of the nasal bone in a multiple-marker screening protocol we used a random number generator to assign to each patient one of the three nasal bone length categories described above. The assignment was based on the observed percentage of Down syndrome and unaffected pregnancies within each nasal bone length category. A new likelihood ratio based on the combination of nasal bone, delta-nuchal translucency, free beta-hCG and PAPP-A was then calculated by multiplying the randomly generated nasal bone likelihood ratio by the observed likelihood ratio from 4953 unaffected and 29 Down syndrome cases between 11 + 1 weeks and 13 + 6 weeks' gestation from our previous prospective study on nuchal translucency, free beta-hCG and PAPP-A⁷. The modified likelihood ratios were then modeled using the maternal age distribution of live births in the United States to determine the false-positive rate and the detection rate. The model procedure was repeated 100 times and the median false-positive and detection rates determined.

RESULTS

Nasal bone evaluation was successful in 1027 of 1089 (94.3%) ultrasound examinations. Among the group in which the nasal bone was successfully evaluated, there were 1000 unaffected fetuses, 15 Down syndrome cases and 12 with other pathological conditions (three trisomy 18, two trisomy 13, three Turner syndrome, one trisomy 18 mosaicism, one duplication of chromosome 5, and two other fetuses with a very large nuchal translucency, normal karyotype and intrauterine fetal death within a few days from the first ultrasound examination). Table 1 summarizes the unaffected and Down syndrome cases. There was a statistically significant difference in maternal age ($P < 0.0001$) and delta-nuchal translucency ($P < 0.0001$) but not in CRL ($P = 0.8865$) between the unaffected and Down syndrome groups.

The nasal bone was absent in 10/1000 (1.0%; 95% CI, 0.5–1.8%) unaffected cases, 10/15 (66.7%; 95% CI, 38.4–88.2%) Down syndrome cases, 2/2 (100%) cases of intrauterine fetal death, 2/3 (66.7%) cases of trisomy 18, 1/2 (50.0%) cases of trisomy 13, 0/3 (0.0%) cases of Turner syndrome, and 0/2 (0.0%) cases with other

Table 1 Mean (SD) of other screening variables in unaffected and Down syndrome cases

| Variable | Unaffected cases | Down syndrome cases | P* |
|----------------------|------------------|---------------------|----------|
| Maternal age (years) | 31.7 (4.00) | 36.5 (4.09) | < 0.0001 |
| CRL (mm) | 62.17 (8.089) | 62.45 (9.775) | 0.8865 |
| Delta-NT (mm)† | 0.01 (0.498) | 3.04 (2.241) | < 0.0001 |

*Mann–Whitney *U*-test. CRL, crown–rump length; NT, nuchal translucency. †Delta-NT is defined as the difference between the measured NT and the median NT for the same gestational age.

chromosomal abnormalities. Amongst both the affected and unaffected cases with present nasal bone there was only one case with a nasal bone length < 1.5 mm.

Using the unaffected cases with measurable nasal bone, a regression line was calculated (Figure 2). The regression line (nasal bone = $0.5998 \times \text{CRL}^{0.3721}$) showed a significant positive slope ($P < 0.0001$) with increasing CRL. As the CRL increased from 45 to 84 mm, the nasal bone showed an increase from 2.48 mm to 3.12 mm, a 25.8% increase. The 10th, 50th and 90th percentiles of nasal bone length MoMs were 0.80, 1.01 and 1.24, respectively. The median MoM was 0.94 in Palermo, 1.06 in Turin and 0.84 in Amsterdam. The difference in medians was significant ($P < 0.001$, Kruskal–Wallis test). Spearman's rank correlation coefficient in unaffected cases was 0.08 ($P = 0.009$) between nasal bone MoM and delta-nuchal translucency and it was 0.05 ($P = 0.13$) between nasal bone MoM and maternal age. All five Down syndrome cases with present nasal bone had a nasal bone length < 50th percentile.

The likelihood ratios for absent, small and within-range nasal bone results were 66.667, 1.347 and 0.224, respectively. Using these likelihood ratios, a 4.8% (95% CI, 3.6–6.3%) false-positive rate and an 80% (95% CI, 51.9–95.7%) detection rate was observed at a cut-off risk of 1/250 for the nasal bone plus maternal age cut-off.

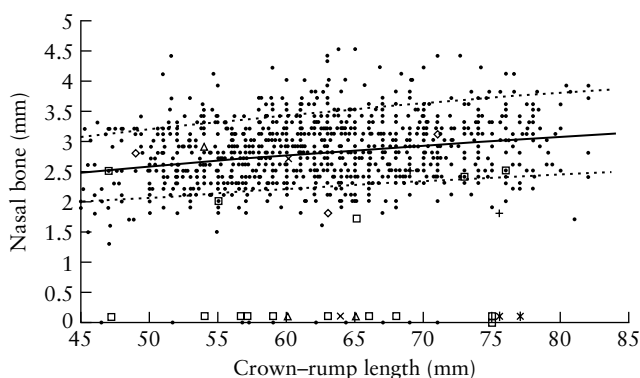


Figure 2 Nasal bone length in 1027 first-trimester fetuses. Fetuses with absent nasal bone are shown at 0.1 mm and below. The solid line indicates the median and the dotted lines are the 10th and 90th percentiles of nasal bone length. ●, unaffected; □, Down syndrome; △, trisomy 18; ×, trisomy 13; ◇, Turner syndrome; +, other chromosomal abnormality; *, intrauterine fetal death.

Table 2 Projected screening results using maternal age plus nasal bone, nuchal translucency, free beta-human chorionic gonadotropin (beta-hCG) and pregnancy-associated plasma protein (PAPP-A)

| Risk cut-off | False-positive rate (%) | Detection rate (%) |
|------------------------|-------------------------|--------------------|
| 1% false-positive rate | 1.0 | 91 |
| 35-year-old risk* | 2.9 | 95 |
| 5% false-positive rate | 5.0 | 98 |

*Risk for a 35-year-old woman at 12 weeks' gestation is 1/250.

Table 2 shows the false-positive rate and detection efficiency for the first-trimester Down syndrome screening protocol using maternal age plus the combination of nasal bone, nuchal translucency, free beta-hCG and PAPP-A. At a 1/250 cut-off, the model indicated a false-positive rate of 2.9% and a detection rate of 95%. Using a fixed 1% false-positive rate, the detection rate was 91% while at a 5% cut-off the detection was 98%.

DISCUSSION

Our results demonstrate that it is possible to visualize and measure by ultrasound the fetal nasal bone between 11 and 14 weeks of pregnancy. In 66.7% (10/15) of trisomy 21 fetuses and in 1% (10/1000) of those unaffected the nasal bone was not visible at the 11–14-week scan. The nasal bone was also absent in 5/12 fetuses with other pathological conditions. These data confirm the finding of Cicero *et al.*¹² that absence of the nasal bone at 11–14 weeks is a marker for Down syndrome.

There are several technical issues regarding the assessment of the nasal bone that must be addressed prior to implementation of this marker in a Down syndrome screening program. First, the ultrasound machine must be of sufficient quality to produce a clear image of the nasal bone and care must be taken to evaluate the nasal bone in cases in which the fetal position or the patient's body mass causes a loss of ultrasound image quality. Second, the angle of insonation between the ultrasound beam and the longitudinal axis of the nasal bone should be 45° since even small changes in this angle can cause misinterpretation of the presence of the nasal bone. Third, care must be taken to distinguish between the echo produced by nasal skin and that of nasal bone. Fourth, since there was a significant difference in nasal bone echogenicity among different patients, care must be taken to visualize the nasal bone in cases in which its appearance is only that of a very thin line. Fifth, several attempts to visualize the nasal bone must be undertaken to ensure that the nasal bone really is absent. Finally, the data suggest that there may be differences in measuring technique. The data showed that there was a significant difference in the median nasal bone length between the three centers. The data were also significantly different from the data of Cicero *et al.*¹⁴ which showed a median nasal bone length of 1.3–2.1 mm for a CRL between 45 and 84 mm, but they were similar to the data of Sonek *et al.*¹⁵ showing

a median nasal bone length of 2.3–3.1 mm prior to 14 weeks. Therefore, it is imperative that sonographers undergo a standardized training regimen and participate in quality assessment programs prior to implementing this marker into clinical practice.

The mechanism that results in delayed ossification of the nasal bone is unclear. We hypothesized that in some cases of Down syndrome the nasal bone could be present but hypoplastic. Recent data show that this is the case in the second trimester¹⁶. Our data show that in the five cases of Down syndrome with present nasal bone, all measurements fell below the gestational-age-specific median supporting our hypothesis. Larger datasets are needed to confirm this result and to develop an algorithm to use nasal bone length as a quantitative marker. The addition of the quantitative measurement to the qualitative assessment of present/absent may improve the screening efficiency of this test.

Larger studies on low-risk populations are needed to confirm our data and to assess whether the measurement of nasal bone length provides additional benefits beyond the assessment of the presence or absence of the nasal bone.

REFERENCES

- Orlandi F, Damiani G, Hallahan TW, Krantz DA, Macri JN. First-trimester screening for fetal aneuploidy: Biochemistry and nuchal translucency. *Ultrasound Obstet Gynecol* 1997; **10**: 381–386.
- Biagiotti R, Brizzi L, Periti E, d'Agata A, Vanzi E, Cariati E. First trimester screening for Down's syndrome using maternal serum PAPP-A and free beta-hCG in combination with fetal nuchal translucency thickness. *Br J Obstet Gynaecol* 1998; **105**: 917–920.
- Benattar C, Audibert F, Taieb J, Ville Y, Roberto A, Lindenbaum A, Frydman R. Efficiency of ultrasound and biochemical markers for Down's syndrome risk screening. A prospective study. *Fetal Diagn Ther* 1999; **14**: 112–117.
- De Biasio P, Siccardi M, Volpe G, Famularo L, Santi F, Canini S. 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* 1999; **19**: 360–363.
- De Graaf IM, Pajkrt E, Bilardo CM, Leschot NJ, Cuckle HS, van Lith JM. Early pregnancy screening for fetal aneuploidy with serum markers and nuchal translucency. *Prenat Diagn* 1999; **19**: 458–462.
- Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Ultrasound Obstet Gynecol* 1999; **13**: 231–237.
- Krantz DA, Hallahan TW, Orlandi F, Buchanan P, Larsen JW, Macri JN. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* 2000; **96**: 207–211.
- Spencer K, Spencer CE, Power M, Moakes A, Nicolaides KH. OSCAR – 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* 2000; **107**: 1271–1275.
- Wapner, R for the Bun Study Group. First trimester aneuploid screening: results of the NICHD multicenter study. *Am J Obstet Gynecol* 2001; **185**(Suppl.): S70.
- Schuchter K, Hafner E, Stangl G, Metznerbauer M, Hofinger D, Philipp K. The first trimester 'combined test' for the detection of Down syndrome pregnancies in 4939 unselected pregnancies. *Prenat Diagn* 2002; **22**: 211–215.
- Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. One-stop clinic for assessment of risk for trisomy 21 at 11–14 weeks: a prospective study of 15 030 pregnancies. *Ultrasound Obstet Gynecol* 2002; **20**: 219–225.
- Cicero S, Curcio P, Papageorgiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11–14 weeks of gestation: an observational study. *Lancet* 2001; **358**: 1665–1667.
- Cuckle H. Time for total shift to first-trimester screening for Down's syndrome. *Lancet* 2001; **358**: 1658–1659.
- Cicero S, Bindra R, Rembouskos G, Tripsanas C, Nicolaides KH. Fetal nasal bone length in chromosomally normal and abnormal fetuses at 11–14 weeks of gestation. *J Matern Fetal Neonat Med* 2002; **11**: 400–402.
- Sonek JD, McKenna D, Webb D, Croom C, Nicolaides KH. Nasal bone length throughout gestation: normal ranges based on 3537 fetal ultrasound measurements. *Ultrasound Obstet Gynecol* 2003; **21**: 152–155.
- Cicero S, Sonek JD, McKenna DS, Croom CS, Johnson L, Nicolaides KH. Nasal bone hypoplasia in trisomy 21 at 15–22 weeks' gestation. *Ultrasound Obstet Gynecol* 2003; **21**: 15–18.