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Fetal Nasal Bone in Screening for Down's Syndrome

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INTRODUCTION

The major cause of perinatal death and childhood disabilities are chromosomal abnormalities. Prenatal diagnosis of chromosomal defects necessitates invasive testing, by chorionic villous sampling, amniocentesis or cordocentesis, which is associated with a risk of miscarriage of about 1%. For this reason, these techniques are reserved for pregnancies considered to be at high risk for chromosomal defects. The methods of screening to identify the high-risk group are described in Table 80.1. Screening using ultrasound is based on the fact that most fetuses with chromosomal abnormalities have structural defects that can be detected by sonographic examination, both in the first and the second trimesters of pregnancy. In order to calculate the individual patient-specific risk of chromosomal defects, it is necessary to take into account the *background risk* (which depends on maternal age and gestational age) and multiply this by a series of *factors*, which depend on the results of a series of screening tests carried out during the course of the pregnancy. Every time a test is carried out the *background risk is multiplied by the test factor* to calculate a new risk, which then becomes the background risk for the next test. This process is called *sequential screening*[1].

Table 80.1: The screening performance of the various tests is compared by examining the detection rate (DR) for a fixed screen positive rate of 5%

Screening test	*DR*
MA (≥ 37 years)	30%
Maternal serum biochemistry at 16 weeks (AFP and β-hCG and uE3)	65%
NT at 12 weeks	80%
NT and β-hCG and P-APPA at 12 weeks	90%
NT and NB and β-hCG and P-APPA at 12 weeks	96%

MA: maternal age; AFP: α-fetoprotein; β-hCG: free β-human chorionic gonadotropin; uE3: unconjugated estriol; NT: nuchal translucency; PAPP-A: pregnancy-associated plasma protein-A; NB: nasal bone.

BACKGROUND

The physical characteristic of the individuals affected by trisomy 21, were described for the first time in 1866 by the physician Langdon Down[2]. He reported that the skin is too large for their body, their face is flat and the nose is small[2]. In recent years it has become possible to observe these features by ultrasound examination during the third month of intrauterine life. Extensive studies over the last decade have demonstrated that the most effective sonographic marker of trisomy 21, and other chromosomal abnormalities, is increased nuchal translucency (NT) at 11-13^{+6} weeks' gestation. Another promising marker for trisomy 21, which it has been extensively studied over the past four years, is the absence of the fetal nasal bones (NB), both in the first and in the second trimesters of pregnancy.

This chapter reviews the association between absence or hypoplasia of the fetal NB and Down's

syndrome in the first and second trimester of pregnancy, and examine the value of incorporating this novel marker in screening policies.

DEVELOPMENT OF THE NASAL BONES

During the fourth week of gestation, collections of neural crest cells undergo proliferation, forming the nasal placodes. In the sixth week, the nasal placodes of the frontonasal prominence invaginate to form the nasal pits and the lateral and medial processes. The medial nasal processes will fuse with the nasofrontal process to form the nasal septum, which in turn will grow toward the forming palate, defining the left and right nasal cavities. While this is occurring, the cartilaginous frame of the nose is developing. During the eighth week, initial centres of ossification of the NB will appear in the membrane covering the cartilaginous nasal capsule.[3-7]

In 1994 Sandikcioglu et al[8], established normal prenatal development standards for the NB. This study showed that the two bilateral NB appear as a thin bony contour ventral to the cartilaginous nasal septum in the sagittal plane, and changes gradually during growth to a wedge-shaped bone. The initial appearance of the NB occurred at different developmental stages in normal fetuses. The smallest crown-rump length at which NB was observed histologically was 42 mm corresponding to approximately 10.9 weeks' gestation. Furthermore, the NB increased in length with advancing gestation and their appearance changed morphologically, being wider and more pointed at the anterior tip in the most mature specimens.[8]

ANTHROPOMETRICS AND RADIOLOGICAL STUDIES

An anthropometric study in 105 patients with Down's syndrome at 7 months to 36 years of age reported that the nasal root depth was abnormally short in about 50% of cases.[9]

Sandikciolglu et al[8] demonstrated the feasibility of post-mortem radiological evaluation the NB in 62 chromosomally normal aborted fetuses at 9-24 weeks' gestation age, and a crown-rump length (CRL) ranging from 33 to 225 mm. Radiographically, the first appearance of the NB was documented in a specimen with a CRL of 50 mm.[8]

Keeling et al[10], investigated the abnormal development of the NB in aborted Down syndrome fetuses. They examined the development of the axial skeleton in 31 human trisomy 21 aborted fetuses at 12-24 weeks' gestation and found a 60% incidence of either NB absence (26%) or NB hypoplasia (34%).[10] Stempfle et al[11], conducted a radiological study and found that the NB was absent in 23% of the 60 Down's syndrome aborted fetuses between 15 and 40 weeks' gestation. The authors also observed that, when present, the NB in the Down syndrome fetuses were short.[11]

SONOGRAPHIC, RADIOLOGICAL AND HISTOLOGICAL CORRELATION

Tuxen et al[12] conducted a post-mortem radiological study to investigate the presence of the NB in 33 aborted Down's syndrome fetuses at 14-25 weeks' gestation. They found that 30% (10 of 33) of fetuses had either bilateral or unilateral absence of NB. They also performed a histological evaluation of the specimens, which confirmed a complete NB absence in 7 of 10 fetuses. Histological evaluation was not performed in the remaining 3, including the 2 that had unilateral absence of the NB. Presence of a NB was confirmed in all 23 fetuses that had a radiological evidence of NB formation.[12]

Minderer and his colleagues[13] compared prenatal sonographic findings on the NB in 17 Down's syndrome fetuses between 11 and 14 weeks' gestation, to those obtained by histological study performed after termination of pregnancy. In this report, the NB could not be examined sonographically in 1 of the 17 cases, due to fetal position, and in the remaining 16 cases the NB was either absent or hypoplastic. By contrast, the histological evaluation of the NB area showed evidence of NB formation in 16 of the 17 cases. Armed with the knowledge of the results of the histological evaluation, the investigators reviewed the ultrasound

images of the fetuses originally classified as having an absent NB. They claimed that they were able to now detect evidence of a NB albeit "smaller, less distinct, and less echogenic".[13]

A study by Larose et al[14] compared sonographic and radiological findings on the NB in 21 aborted fetuses with trisomy 21. The ultrasonographic evaluations were done between 11^{+3} and 13^{+5} weeks' gestation. The subsequent radiological studies on the aborted fetuses were performed between 13 and 25^{+5} weeks' gestation. Interestingly, the incidence of NB absence on ultrasound was very similar to the one noted on X-ray. However, the ultrasound and X-ray findings were discordant in 9 of the 21 cases (43%). Four of the cases where the NB was present on ultrasound showed no evidence of a NB on X-ray, indicating a possible wrong assessment of the fetal profile by ultrasound. In the other 5 cases where the NB was noted to be absent on ultrasound, this resulted to be present on X-ray. However, radiological examinations were performed at a later gestational age than that of the ultrasound examination, suggesting that the NB could have developed in the period of time occurred between the two examinations[14].

The apparent discrepancies in the NB identification among the three modalities (ultrasound, radiology and histology) have a number of possible explanations. It is likely that small areas of calcification can be seen on histological evaluation even if those cannot be detected on either ultrasonography or X-ray. The likelihood of picking these up will depend on the number of sections done. Different types of staining were used in the two studies mentioned above possibly contributing to the contradictory results. In the study by Larose et al[14], the ultrasounds and the X-rays were done at very different gestational ages (11^{+3}-13^{+5} weeks for ultrasound and 13-25^{+5} weeks for X-ray). Since the prevalence of NB absence changes with gestational age, they are not truly comparable. Just as is the case with the ultrasound examination, controlling for the gestational age and standardization of the technique for both the radiological and the histological examinations is crucial.

SONOGRAPHIC STUDIES

The observation that in fetuses affected by trisomy 21 sonographic examination may reveal absence of the NB was only made at the beginning of 2001. In the initial description[15], three Down's syndrome fetuses were evaluated in the second trimester. Two of them had no identifiable NB and one had a hypoplastic NB. A review of the videotaped exam from the first trimester examination of one of the fetuses at the time of the NT evaluation revealed that the NB could not be identified at that point in pregnancy[15]. Armed with this information, observational studies were undertaken to investigate the role of NB examination in screening for Down syndrome.

First Trimester: the 11-13^{+6} Week Scan

Several studies have demonstrated that the fetal NB can be visualized by sonography in the first trimester of pregnancy, and that there is a high association between absent NB at 11-13^{+6} weeks and trisomy 21, as well as other chromosomal abnormalities.[16-25]

Cicero et al[16] reported for the first time on the absence of the NB in trisomy 21 fetuses in the first trimester of pregnancy. In this observational study ultrasound examination of the fetal profile, for evaluation of absence or presence of the NB, was performed in 701 fetuses at 11-13^{+6} weeks' gestation following screening test by maternal age and NT, and immediately before chorionic villous sampling. In this series, the NB was absent in 73% (43 of 59) of Down's syndrome fetuses and in only 0.5% (3 of 603) of chromosomally normal fetuses. In this initial study, presence or absence of the NB was found to be independent of other fetal and maternal variables. It became clear that incorporation of the examination of the NB into the screening for trisomy 21 by maternal age and NT could increase the sensitivity of the test and reduce, at the same time, the false positive rate.[16]

In an extended series[18] of 5,818 fetuses undergoing prenatal diagnosis by chorionic villous sampling at 11-13^{+6} weeks, the fetal profile was

successfully examined in 5,851 (98.9%) cases. Furthermore, the NB was absent in 129 of 5,223 (2.5%) chromosomally normal fetuses, in 229 of 333 (68.8%) fetuses with trisomy 21 and in 95 of the 295 (32%) with other chromosomal defects. An important finding of this study was that the incidence of absent NB is higher in fetuses of Afro-Caribbean origin than in Caucasians, it decreases with fetal CRL and increases with fetal NT. Consequently, in the calculation of an individual patient-specific risk for trisomy 21 it is necessary to take into account these demographic and ultrasound findings.[18]

The fact that ethnic origin of the mother may play a role on the evaluation of the fetal NB, was also suggested by Prefumo et al.[26] The authors conducted a prospective study in 4492 fetuses. Due to chromosomal abnormalities or an unsatisfactory examination 500 cases were excluded from the analysis. In the remaining 3992 fetuses, the failure to visualise the fetal NB was significantly higher in women of African but not Asian origin, compared to the Caucasian origin. In this study, it was demonstrated that having a mother of African origin is significantly associated with an increased likelihood of absent fetal NB compared with Caucasians, even after correcting for maternal age, parity and crown-rump length. The authors suggested that corrections for maternal ethnicity might be required to ensure equity of fetal NB screening in multiracial populations.[26]

There are seven additional studies[19-25] that support the high association between trisomy 21 and absent NB at 11-13^{+6} weeks. In their combined data on 12,315 fetuses the fetal profile was successfully examined in 11,973 (97.2%) cases. The NB was absent in 56 of 9,825 (0.6%) chromosomally normal fetuses and in 53 of 79 (67.1%) fetuses with trisomy 21 (Table 80.2).

Malone et al[27], reported that they were able to examine the fetal nose in only 4,796 of 6,316 (75.9%) fetuses scanned at 10-14 weeks and that the NB was apparently present in all nine of their trisomy 21 fetuses. Their results contrast significantly with the above published studies in a number of ways. In this study, the fetal nose could be examined in only 75.9% of the cases and the NB was reported as being present in all nine of the trisomy 21 fetuses. Issues regarding adequacy of training in this study remain to be elucidated. Furthermore, images published by the lead authors of this study suggest that their technique may not be consistent with that used by others.[28] Similarly, De Biasio and Venturini[29], who examined retrospectively the photographs obtained for measurement of NT, reported that the NB was present in all five fetuses with trisomy 21. However, the five images that they published were inappropriate both for the measurement of NT and for examination of the NB, because they were either too small or the fetus was too vertical or too oblique.

Preliminary data of a prospective study conducted by the Fetal Medicine Foundation on a series of 18,636 fetuses[30], who underwent screening by maternal age, NT, maternal serum free b-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11-13^{+6} weeks, the fetal profile was successfully examined in 18,405 (98.8%) cases. The NB was absent in 101 of 18,388 (0.5%) chromosomally normal fetuses, in 85 of 138 (61.6%) fetuses with trisomy 21 and in 34 of the 103 (33%) with other chromosomal defects.[30]

Based on the available data (Table 80.2), it can be concluded that at 11-13^{+6} weeks the fetal profile can be successfully examined in about 95% of cases, and that the NB is absent in about 65% of trisomy 21 fetuses and in about 1% of chromosomally normal fetuses. Consequently, absence of the NB is an important marker of trisomy 21. However, appropriate adjustments need to be made on the basis of maternal ethnic origin, fetal crown-rump length and NT when calculation of individual patient-specific risk for trisomy 21 is performed.

Second Trimester: 15-24 Week Scan

Absence or hypoplasia of the NB represents a new ultrasound marker also in the second trimester of pregnancy, and it is likely to have a major impact on screening for trisomy 21 during the 16-24 week scan. Its role has now been confirmed in large trials.

Table 80.2: Summary of studies reporting on the incidence of absent nasal bone in first-trimester trisomy 21 fetuses

Author	*Study*	*Successful examination (n (%))*	*Absent nasal bone*	
			Normal (n (%))	*Trisomy 21 (n (%))*
Cicero et al 2001[16]*	Pre-CVS	701/701 (100%)	3/603 (0.5%)	43/59 (72.9%)
Otano et al 2002[19]	Pre-CVS	183/194 (94.3%)	1/175 (0.6%)	3/5 (60.0%)
Zoppi et al 2003[20]	Screening	5,525/ 5,532 (99.8%)	7/3,463 (0.2%)	19/27 (70.0%)
Orlandi et al 2003[21]	Screening	1,027/1,089 (94.3%)	10/1,000 (1.0%)	10/15 (66.7%)
Viora et al 2003[22]	Screening	1,752/1,906 (91.9%)	24/1,733 (1.4%)	8/10 (80.0%)
Senat et al 2003[23]	Retrospective	956/1,040 (91.9%)	4/944 (0.4%)	3/4 (75%)
Wong et al 2003[24]	Pre-CVS	119/143 (83.2%)	1/114 (0.9%)	2/3 (66.7%)
Cicero et al 2003[17]*	Pre-CVS	3,788/3,829 (98.9%)	93/3,358 (2.8%)	162/242 (67%)
Cicero et al 2004[18]	Pre-CVS	5,851/5,818 (98.9%)	129/5,223 (2.5%)	229/333 (68.8%)
Orlandi et al 2005[25]	Screening	2,411/2,411 (100%)	9/2,396 (0.4%)	8/15 (53%)
FMF[30]	Screening	18,405/18,636 (98.8%)	101/18,388 (0.5%)	85/138 (61.6%)
Total		36,229/36,769 (95.5%)	286/33,436 (0.9%)	367/550 (66.7%)

* included in Cicero et al 2004[18]

Bromley et al[31], assessed the NB using ultrasound in 239 fetuses between 15 and 20 weeks' gestation. Six (37%) of the 16 Down's syndrome fetuses had no detectable NB. Among the fetuses with a normal karyotype, absence of the NB had a prevalence of 0.5%. In this study, absence of the NB was associated with a likelihood ratio for Down's syndrome of 83. Interestingly, in 2 of the 16 (13%) Down's syndrome fetuses, NB absence was the only abnormal finding. In addition, the NB length was found to play an important role. Biparietal diameter (BPD) to NB length (NBL) ratio was generated to control for the gestational age and the size of the fetus. This ratio increases as the NB becomes shorter. Using the BPD/NBL ratio of 10 or grater as a cut off in screening for Down syndrome gives this test sensitivity of 81% with a false positive rate of 11%. This study confirmed that NB length increases linearly with gestation in chromosomally normal fetuses. However, the NB length in Down syndrome fetuses was found to be remarkably uniform over the gestational age investigated (3.5mm +/-.47) suggesting the possibility that a single cut-off value for NB length may be appropriate in Down syndrome screening in the first half of the second trimester.[31]

Such approach was used in another study[32] looking at the utility of NB evaluation in the second trimester (15-22). The authors examined the fetal profile in 1046 singleton pregnancies undergoing amniocentesis for fetal karyotyping at 15 to 22 weeks. The NB was absent or hypoplastic (<2.5mm) in 21 of the 34 (61.8%) fetuses with Down syndrome, in 12 of 982 (1.2%) chromosomally normal fetuses, and in 1 of the 30 (3.3%) with other chromosomal defects. It was noted that the prevalence of NB hypoplasia was higher in the euploid Afro-Caribean population (8.8%) in comparison to the Caucasian population (0.5%), suggesting for the first time that adjustments based on ethnicity may need to be made when using the NB for screening. Furthermore, the overall likelihood ratio for trisomy 21 for hypoplastic NB was 50.5 (95% CI 27.1–92.7) and for present NB it was 0.38 (95% CI 0.24–0.56).[32]

Bunduki et al[33] looked at the utility of NB measurement in the second trimester (16-24 weeks' gestation) in 1631 patients. The association between hypoplasia of the NB and Down's syndrome was also demonstrated. Using the 5th percentile of the normal curves generated in the same study as a cut off for screening for trisomy 21, a sensitivity of 59% was achieved.[33]

Vintzileos et al[34] looked retrospectively at profiles of 29 Down's syndrome fetuses between 17.7 and 20.7 weeks' gestation. The nasal bone was absent in 12 of the 29 (41%) fetuses with trisomy 21 and in none of the 102 chromosomally normal fetuses.[34]

A prospective study involving ultrasound evaluation of the fetal NB between 19 and 22 weeks' gestation[35] also looked at the utility of NB length and its presence or absence in screening for Down's syndrome. The normal NB ranges were based on examinations of 1913 fetuses. All of the five Down's syndrome fetuses in the study had either an absent NB or a NB length below the 2.5th percentile. None of the fetuses with other chromosomal abnormalities had a short or absent NB.[35]

It is premature to speculate on the precise detection rates that could be achieved in the second trimester by a combination of maternal age, serum biochemistry and ultrasound examination for the fetal NB and other sonographic markers. Nevertheless, on the basis of currently available data, nasal hypoplasia is likely to be the single most sensitive and specific second trimester marker of trisomy 21.

ULTRASOUND EXAMINATION OF THE NASAL BONE: TECHNIQUE

Ultrasound evaluation of the NB requires strictly adherence to standard criteria and it is essential that the operators performing this examination undergo adequate training and gain extensive experience.[36,37]

The need for adequate training is highlighted by a study published in 2003[37], which looked at the extent of training needed for 15 sonographers experienced in measuring fetal NT, to become competent in examining the fetal NB at 11^{+0}-13^{+6} weeks' gestation. The study demonstrated that the number of supervised scans required to achieve proficiency is on average 80 with a range of 40 to 120. However, evaluation of the NB does not appear to significantly impact the length of the ultrasound examination.[38]

This was confirmed in a study of 501 consecutively scanned fetuses by experienced sonographers. The authors reported that the fetal NB could be successfully examined and measured in all cases without extending the length of time required for scanning.[39]

With a few minor exceptions, the method of NB evaluation is very similar in both the first and second trimesters of pregnancy. The NB should be seen as an echogenic line within the nasal bridge, i.e. underneath the nasal skin. This is usually not a difficult task in the second trimester.

1. The gestation should be 11-13^{+6} weeks and the fetal crown–rump length should be 45-84 mm. There is no value on examining the fetal NB before this gestational age, as the NB first appear at a crown-rump length of 42 mm and increase linearly with gestation.[8]
2. The image should be magnified so that the head and the upper thorax only are included in the screen (Figs 80.1 and 80.2).
3. A mid-sagittal view of the fetal profile should be obtained with the ultrasound transducer held in parallel to the direction of the nose (i.e. ultrasound beam perpendicular to longitudinal axis of the NB). The ultrasound transducer should then be gently tilted from side to side to ensure that the NB is seen separate from the nasal skin. The fetus should be facing the transducer.
4. When the correct view is obtained, three distinct lines will be visualized: the first two lines, which are proximal to the forehead, are horizontal and parallel to each other and resemble an "equal sign" (Fig. 80.1). The top line represents the skin and bottom one, which is usually thicker and more echogenic than the overlying skin, represents the NB. A third line, distal to the forehead, and almost in continuity with the skin, but at a higher level, represents the tip of the nose. Therefore, is the absence of the bottom line of the equals sign that represents the absence of the fetal NB (Fig. 80.2).
5. If the bottom line of the "equal sign" is absent, the diagnosis of nasal bone absence is fairly straightforward. Occasionally, a line that is thinner and less echogenic than the skin line is noted within the nasal bridge. This may either represent a nasal bone that is not yet ossified or an unusually prominent cartilage. Either way, this finding should also be classified as nasal bone

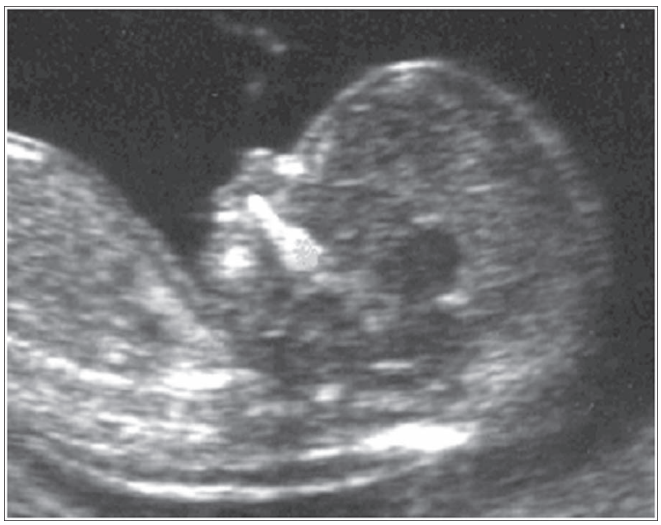


Fig. 80.1: Fetal profile at 12 weeks of gestation in a normal fetus showing the nasal bone

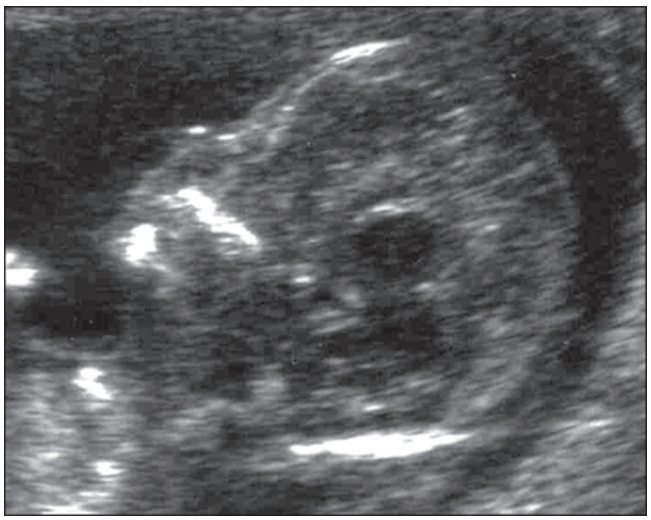
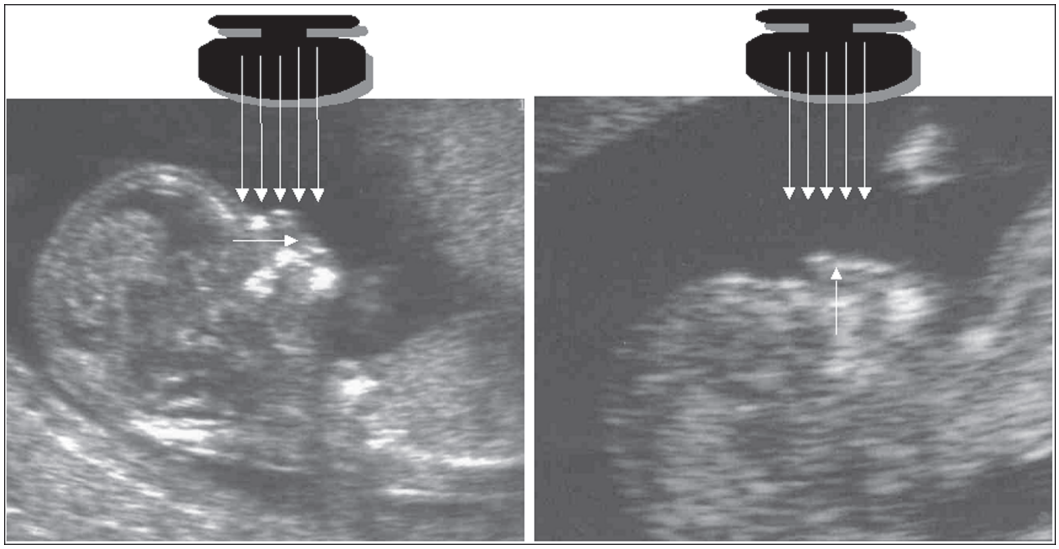


Fig. 80.2: Fetal profile at 12 weeks of gestation in a trisomy 21 fetus showing absence of the nasal bone

absence. Similarly, the presence of a tiny echogenic dot that can occasionally be seen in the area of the nasal bone should not be interpreted as nasal bone presence.

Two techniques for calliper placement have been employed to measure the NB in the first trimester of pregnancy: including the central hyperechogenic region only or including the entire length of the echogenic line.[21,40]

The angle of insonation used to evaluate the NB is extremely important. The best angle of insonation used to simply differentiate between the presence and the absence of the NB is 90 degrees to the longitudinal axis of the NB (i.e. the beam of the ultrasound perpendicular to the longitudinal axis of the NB) (Fig. 80.3). Evaluation of the NB should not be attempted with either a 0 or 180-degree angle of insonation (i.e. with the ultrasound beam parallel to the longitudinal axis of the NB) (Fig. 80.3). At this angle, the NB is insonated at its thinnest dimension



Figs 80.3: The best angle of insonation to assess the nasal bone in the first trimester is 90 degrees to the longitudinal axis of the nasal bone (i.e. the beam of the ultrasound perpendicular to the longitudinal axis of the nasal bone) (left). Evaluation of the nasal bone should not be attempted with either a 0 or 180-degree angle of insonation (i.e. with the ultrasound beam parallel to the longitudinal axis of the nasal bone) (right).

and the lateral resolution of the ultrasound equipment available today is not sufficient to reliably detect the NB in this view. When the transducer is adjusted to an angle of insonation approaching 45 or 135 degrees, the lateral scatter at the ends of the NB is reduced rendering the NB ends as more sharply delineated (Fig. 80.4). This is helpful in the second trimester to improve the accuracy of NB length measurement. Figs 80.5 to 80.7 show absence, hypoplasia and presence of NB in the mid-second trimester.

Several factors make the ultrasound examination challenging; the foremost of these are maternal habitus and an unfavorable fetal position, such as hyperextension or vertical position. Others factors, such as large uterine fibroids or, early in pregnancy, retroflection of the uterus can also make the examination difficult. Fetal small parts, especially the hand, often lie in a close proximity to the fetal face, especially early in gestation. This can lead to erroneous results in two ways. If the digits are actually resting on the fetal face they can mimic the NB. Small parts in front of the fetal face, but not actually resting on it, can produce an obscuring effect and may create an erroneous impression of NB absence. Finally, fetal face contains other echogenic structures, which are located laterally to the NB,

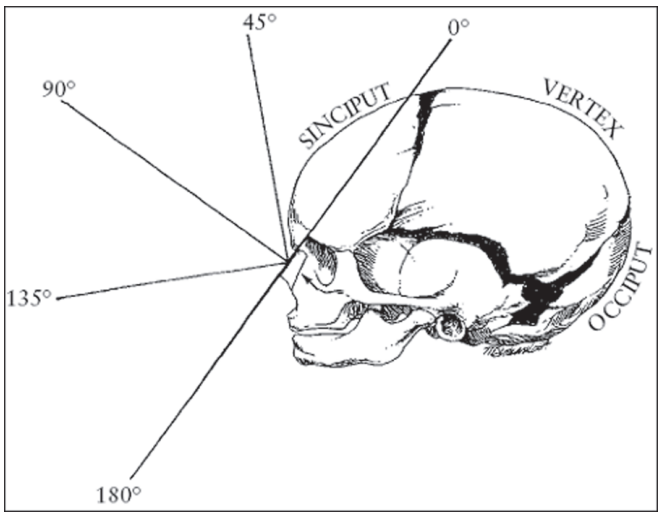


Fig. 80.4: Diagrammatic representation of the bony structures of the fetal face with angles of insonation. (Reprinted and adopted with permission from O'Brien W, Cefalo R. Simpson J, editors. Obstetrics: normal and problem pregnancies. 3rd ed. New York: Churchill Livingstone; 1996. p. 393)

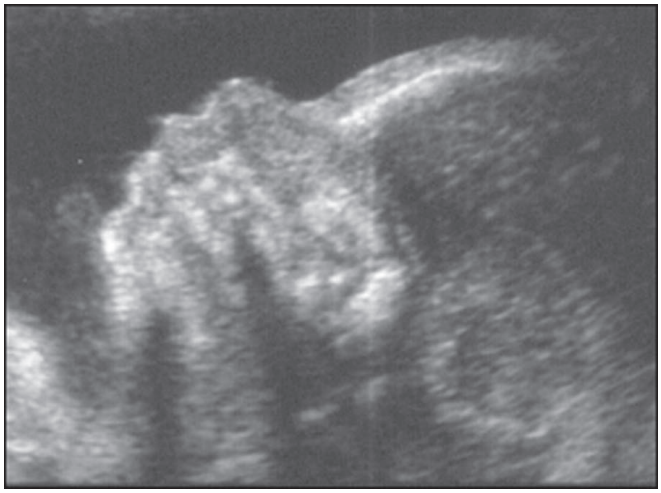


Fig. 80.5: Fetal profile at 20 weeks of gestation in a trisomy 21 fetus showing absence of the nasal bone

which can give the incorrect impression that the NB is present if the correct technique is not followed. These structures include the medial aspect of the orbis oculi and the maxilla. Being able to demonstrate either the presence or the absence of the NB from several different angles will make the correct diagnosis more certain

Occasionally, the addition of transvaginal sonography can be a helpful adjunct. However, the directions and angles with which the fetus can be

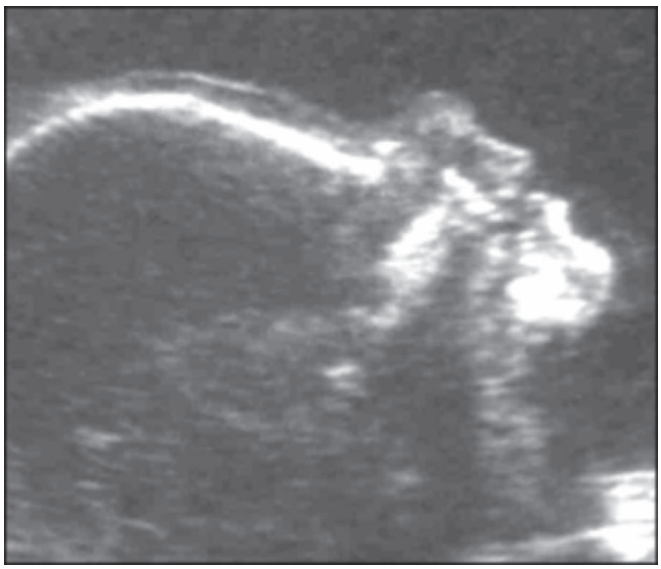


Fig. 80.6: Fetal profile at 20 weeks of gestation in a trisomy 21 fetus showing nasal bone hypoplasia

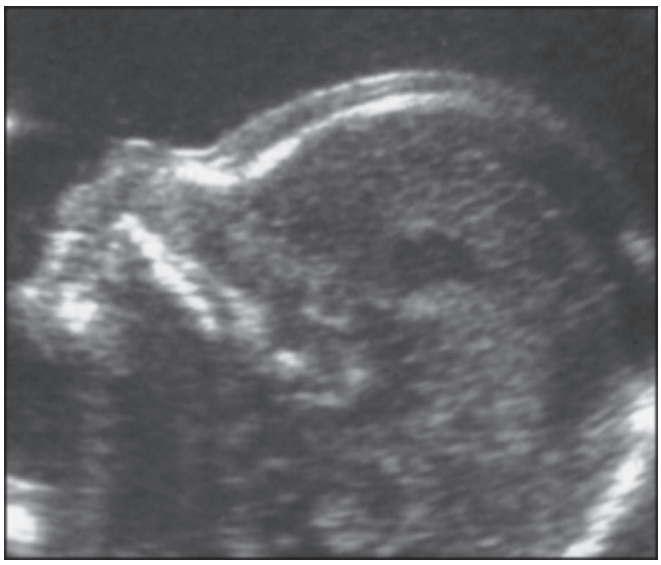


Fig. 80.7: Fetal profile at 20 weeks of gestation in a normal fetus showing presence of the nasal bone

viewed using this approach are limited. Rarely, the patient may need to be asked to return for a follow-up if the initial examination is not satisfactory.

THREE-DIMENSIONAL ASSESSMENT OF THE NASAL BONES

The role of three-dimensional (3D) sonography and its potential benefit in the assessment of the fetal NB, has been investigated in the last two years in the first, second and third trimesters of pregnancy. The published studies have focused on trying to overcome the technical difficulties encountered during routine two-dimensional (2D) sonography, and on further investigating aspects of the NB development, by using different techniques such as multiplanar imaging and 3D rendering of the facial bones.

First Trimester of Pregnancy

The two major technical problems in the assessment of the fetal NB during the 11-13^{+6} weeks scan are the need to obtain a mid-sagittal view of the fetal profile and the need for the angle between the ultrasound beam and the fetal profile to be about 45° (i.e. ultrasound beam perpendicular to the NB). In the acquired 3D volume, multiplanar imaging permits to obtain both a perfect midsagittal section and a perfect angle. However, in the first study published on 3D assessment of the NB in the first trimester of pregnancy, Rembouskos et al[41], found that the visualisation of the fetal NB in a perfect reconstructed view of the nasal bridge by 3D multiplanar imaging, is entirely dependent on the initial 2D section. In this study, the authors demonstrated that in order to obtain a good quality volume, the initial section should be taken with a sagittal view of the fetal profile facing upwards, and that the angle between the fetal profile and the ultrasound beam should be in a range of 30° to 60°, with maximum quality at 45°. In volumes obtained in any other initial 2D view, the visualisation of the fetal NB was poor at this gestation, and could lead to an erroneous diagnosis of absent NB. Consequently, the inability to examine the NB by 2D scanning because of the fetal position cannot be overcome by 3D ultrasound.[41]

In correctly acquired volumes, 3D multiplanar mode permits further assessment of both NB. Peralta et al[42], used this technique to examine the gap between the two NB at 11-13^{+6} weeks of gestation, and compared the 3D findings to those obtained following 2D examination. The authors found that a gap between the NB is present in about 20% of the fetuses examined by 3D. When the gap measured 0.6 mm or more (40% of cases), it was possible to obtain a perfect mid-sagittal plane where the NB could erroneously be considered absent. By contrast, in all cases in which the gap was less than 0.6 mm, the NB was visualized in the perfect mid-sagittal plane. These findings can be explained by the limit of the lateral resolution of the ultrasound equipment. However, none of the cases with a gap was associated with a diagnosis of absent NB when the examination was performed with the 2D scan, and therefore the false positive rate would not increase. Furthermore, the authors observed that, when using the multiplanar mode, unilateral or bilateral absence of the NB could be demonstrated in about 1% of the chromosomally normal fetuses, and in 61% of the fetuses with trisomy 21. In about 10% of trisomy 21 fetuses only one of the two NB was absent.

However, all the cases with unilateral absence demonstrated with the 3D scan, were classified as 'absent' NB during the 2D ultrasound examination, and therefore the sensitivity of the test, when performed by 2D sonography, would not decrease.[42]

In the original description of the technique for examination of the fetal NB by 2D ultrasound[16], it was suggested that, once obtained the mid-sagittal section of the fetus, the transducer should be gently tilted from one side of the fetal profile to the other, in order to adequately examine the NB. Therefore, by using this technique, it is extremely unlikely that the presence of a gap can lead to a false positive diagnosis of absent NB when 2D sonographic assessment of the NB is undertaken.[16]

Second and Third Trimesters of Pregnancy

The role of multiplanar imaging and 3D rendering of the NB have also been evaluated in the second and third trimester of pregnancy. It has been suggested that 3D ultrasonography allows a better description of normal, absent, hypoplastic and unilaterally absent NB.[43-45]

Lee et al[43], used the multiplanar mode to evaluate the NB of 20 fetuses with Down syndrome and 20 fetuses with normal karyotype, between 16 and 30 weeks' gestation. Two examiners independently evaluated the same images. The incidence of absent NB in the fetuses with Down's syndrome was 40% (8 of 12) and 45% (9 of11) by examiner #1 and examiner #2 respectively. These results were similar to those observed by 2D sonography. However, the prevalence of absent NB in the normal population was 20% (4 of 16) and 10% (2 of 18) by examiner #1 and examiner #2 respectively. These prevalences are much higher than those reported using 2D sonography during this gestational time period (<1.3%). These data suggest that routine application of 3D assessment of the NB in screening for trisomy 21 could increase the false positive rate.[43]

Benoit and Chaoui[45] assessed the unilateral absence of NB by using 3D rendering of the facial bones. In their study, similarly to what Peralta et al[41] found in the first trimester, in all Down's syndrome fetuses with unilateral absent NB, the two dimensional assessment of the nasal bridge had diagnosed 'absent' NB[45]

In conclusion, 3D ultrasound evaluation in those cases with suspicious findings of hypoplastic/absent NB may improve the accuracy of the test. However the extend to which 3D ultrasound could be demonstrated essential in effective screening using the NB at 11 to 13^{+6} weeks needs to be further investigated.

INTEGRATED FIRST-TRIMESTER SONOGRAPHIC AND BIOCHEMICAL SCREENING

A retrospective case-control study[46] comprising of 100 trisomy 21 and 400 chromosomally normal singleton pregnancies at 11-13^{+6} weeks of gestation, and a subsequent study which extended the previous series of data[47], examined the potential performance of screening for trisomy 21 by a combination of sonography for measurement of fetal NT and assessment of the presence or absence of the fetal NB, and measurement of maternal serum free β-hCG and PAPP-A. It was concluded that, as no relationship between an absent fetal NB and the levels of maternal serum PAPP-A or free b-hCG in trisomy 21 fetuses was demonstrated, for a false positive rate of 5%, the detection rate of trisomy 21 would be 96%.[46,47]

NASAL BONE REFERENCE RANGES BY ULTRASOUND

First Trimester

Cicero et al[40], reported that in the chromosomally normal group the fetal NB length increases significantly with crown-rump length (CRL) from a mean of 1.3 mm at a CRL of 45 mm to 2.1 mm at CRL of 84 mm. In the fetuses with Down's syndrome in which the NB was present, even though these were found to be shorter than in chromosomally normal fetuses, the difference in the NB lengths was not sufficiently great to be of clinical utility.[40]

Second Trimester

Guis et al[48] published reference ranges for NB lengths, based on 376 cases, between 14-34 weeks' gestation. Sonek et al[49], provided reference range based on a larger number of patients (3547) and for a wider gestational range (11-40 weeks) using the ultrasound technique described above was published in 2003.

IMPACT OF THE NASAL BONE IN SCREENING FOR TRISOMY 21 IN THE FIRST TRIMESTER OF PREGNANCY

In this chapter, we have already reported on the prospective study of 5,918 fetuses[18], in which assessment of the fetal profile for absence or presence of the NB was performed during the routine ultrasound examination at 11-13^{+6} weeks, carried out before chorionic villus sampling for fetal karyotyping. In all cases there was prior screening for chromosomal defects by a combination of maternal age and fetal NT[49] and after counselling the parents elected to have invasive testing. The NB was absent in 129 of 5,223 (2.5%) chromosomally normal fetuses and in 229 of 333 (68.8%) fetuses with trisomy 21. Logistic regression analysis was used to examine the effect of maternal ethnic origin, fetal CRL and NT on the incidence of absent NB in the chromosomally normal and trisomy 21 fetuses. This study demonstrated that the incidence of absent NB is higher in fetuses of Afro-Caribbean origin than in Caucasians, it decreases with fetal CRL and increases with fetal NT. Therefore, when calculating an individual patient-specific risk for trisomy 21, it is necessary to take into account these demographic and ultrasound findings. The likelihood ratio for trisomy 21 with absent NB is considerably higher in Caucasians than in those of Afro-Caribbean origin, it is lower at 11 than at 13 weeks and it is higher for low than high NT. The relationship between absent NB and ethnic group, fetal CRL and fetal NT are shown in Tables 80.3 to 80.5.

It has been estimated that if examination of the fetal profile for the absence / presence of the NB is incorporated in first trimester screening for trisomy 21 by fetal NT thickness or NT and maternal serum free β-hCG and PAPP-A the detection rates for trisomy 21 would increase substantially and the false positive rate would decrease.[16-18,46,47]

Very recently, the potential role of the NB in screening fro trisomy 21 in the first trimester of pregnancy, has been further investigated in a large series by Nicolaides et al.[51] The authors proposed a new policy for first-trimester screening, based on two-stage individual risk. After having evaluated the performance of first-trimester screening for trisomy 21 by a combination of maternal age, fetal NT and maternal serum free â-hCG and PAPP-A (Combined test) in prospective study of 75,821 singleton pregnancies, they examined the potential impact of a new individual risk orientated two-stage approach to first-trimester screening (Fig. 80.8), based on the additional examination of the fetal NB in the group of women who fell in an intermediate risk following

Table 80.3: Incidence of absent nasal bone (NB) in chromosomally normal and trisomy 21 fetuses and likelihood ratio (LR) according to ethnic group (from Cicero et al[18])

Ethnic group	*Trisomy 21 (n (%))*	*Normal karyotype (n (%))*	*LR (95% CI) for Trisomy 21*	
			NB absent	*NB present*
Total (n = 5851)	229/333 (68.8)	129/5223 (2.5)	27.8 (23.1-33.5)	0.32 (0.27-0.37)
Caucasian (n = 5384)	207/303 (68.3)	105/4811 (2.2)	31.3 (25.5-38.4)	0.32 (0.27-0.38)
Afro-Caribbean (n = 170)	11/14 (78.6)	13/145 (9.0)	8.8 (4.7-15.5)	0.24 (0.08-0.52)
Asian* (n = 201)	10/14 (71.4)	9/179 (5.0)	14.2 (6.8-28.4)	0.30 (0.12-0.58)
Chinese/Japanese (n = 69)	1/2 (50.0)	2/61 (3.3)	15.3 (2.1-73.4)	0.52 (0.10-0.94)
Mixed (n = 27)	–	0/27 (–)	–	–

*People originating from India, Pakistan, Bangladesh, Sri Lanka and Philippines

Table 80.4: Incidence of absent nasal bone (NB) in chromosomally normal and trisomy 21 fetuses and likelihood ratio (LR) according to crown-rump length (CRL) (from Cicero et al[18])

CRL (mm)	*Trisomy 21 (n (%))*	*Normal karyotype (n (%))*	*LR (95% CI) for Trisomy 21*	
			NB absent	*NB present*
Total (n=5851)	229/333 (68.8)	129/5223 (2.5)	27.8 (23.1-33.5)	0.32 (0.27-0.37)
45-54	41/49 (83.7)	32/675 (4.7)	17.6 (12.3-25.2)	0.17 (0.09-0.30)
55-64	78/118 (66.1)	63/1850 (3.4)	19.4 (14.7-25.5)	0.35 (0.27-0.44)
65-74	85/118 (72.0)	25/1805 (1.4)	52.0 (34.8-77.8)	0.28 (0.21-0.37)
75-84	25/48 (52.1)	9/893 (1.0)	51.8 (25.8-102.8)	0.48 (0.35-0.62)

the initial screening test. The detection and false-positive rates were calculated for different risk cut-offs and the screened population was then classified in three groups: a high risk group, which included patients with a risk estimate of 1 in 100 or more; a low risk group, which included those with a risk estimate of less than 1 in 1000; and the intermediate-risk category, with a risk estimate of between 1 in 101 and 1 in 1000. The authors proposed that patients in the high-risk category are offered karyotyping by chorionic villus sampling (CVS), and those in the low risk category are reassured that their fetus is unlikely to be chromosomally abnormal. Those in the intermediate-risk category have further assessment of risk by first-trimester ultrasound examination to determine absence / presence of the NB, and CVS is offered if their adjusted risk becomes 1 in 100 or more.[51]

Following the combined screening test, for a false positive rate of 2% the detection rate was 80%. When the nasal bone examination is performed into the two-stage screening, for a risk cut-off of 1 in 100 the total false-positive rate would be 2.1%, and the detection rate would be 92.0%. The authors confirmed that first-trimester combined screening for trisomy 21 is associated with a detection rate of about 90% for a false-positive rate of 5%[52-54] and concluded that individual risk-orientated two-stage screening for trisomy 21 can potentially identify, in the first trimester of pregnancy, more than 90% of affected fetuses for a false-positive rate of about 2%.

CONCLUSIONS

Trisomy 21 is the most common chromosomal abnormality found at birth, with an incidence of about 1:600. However, the sonographic appearance of individuals with Down's syndrome, is often quite similar to those with normal karyotype, making screening for Down syndrome more difficult compared to that of other chromosomal abnormalities. Hence the importance of a continued search for markers, that would accurately discriminate between affected and unaffected fetuses.

The sonographic appearance of increased nuchal translucency and absent/hypoplastic nasal bone could be due to connective tissue abnormalities.[55-57] The increased thickness of subcutaneous tissues in association with Down syndrome has lead to the

Table 80.5: Incidence of absent nasal bone (NB) in chromosomally normal and trisomy 21 fetuses and likelihood ratio (LR) according to nuchal translucency thickness (NT) (from Cicero et al[18])

NT (mm)	*Trisomy 21 (n (%))*	*Normal karyotype (n (%))*	*LR (95% CI) for Trisomy 21*	
			NB absent	*NB present*
Total (n=5851)	229/333 (68.8)	129/5223 (2.5)	27.8 (23.1-33.5)	0.32 (0.27-0.37)
<95th	23/38 (60.5)	53/3245 (1.6)	37.1 (25.0-52.5)	0.40 (0.26-0.56)
>95th-3.4	48/83 (57.8)	40/1500 (2.7)	25.1 (16.7-37.4)	0.45 (0.34-0.56)
3.5-4.4	49/67 (73.1)	16/294 (5.4)	13.4 (8.2-22.1)	0.28 (0.19-0.41)
4.5-5.4	26/41 (63.4)	5/84 (6.0)	10.7 (4.6-25.3)	0.39 (0.25-0.55)
=5.5	83/104 (79.8)	15/100 (15.0)	5.3 (3.4-8.7)	0.24 (0.16-0.34)

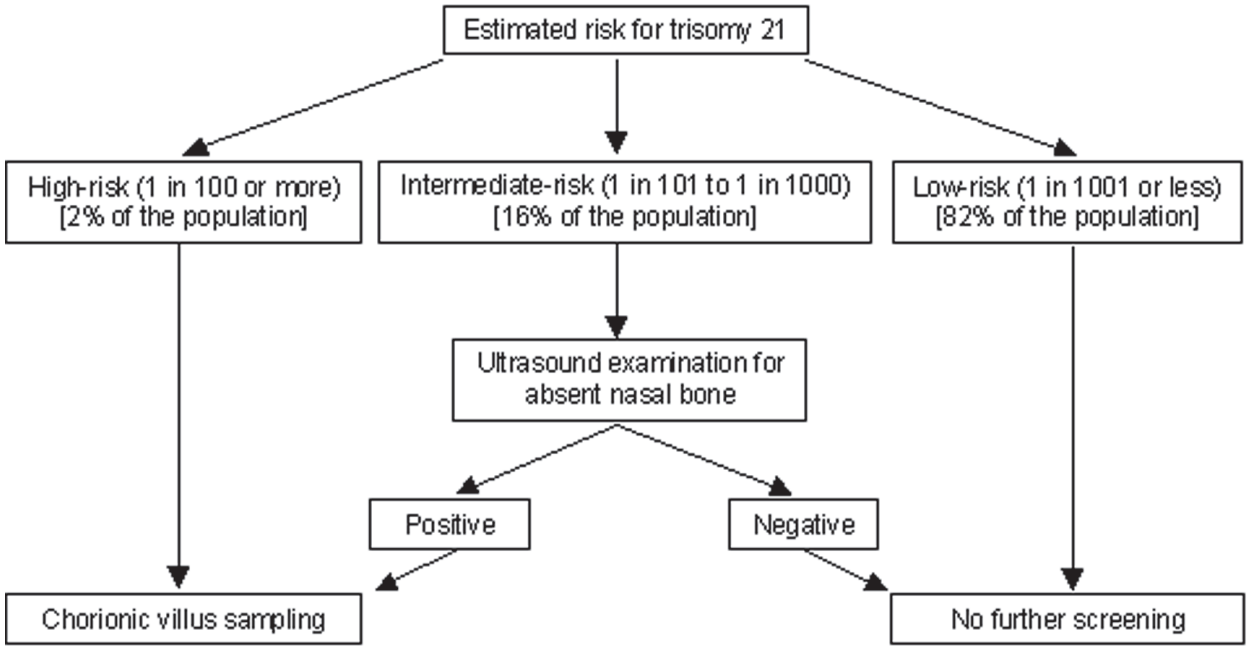


Fig. 80.8: New individual risk orientated two-stage approach to first-trimester screening, (Nicolaides et al[50]). Screening by maternal age, fetal nuchal translucency and maternal serum free â-human chorionic gonadotropin and pregnancy-associated plasma protein-A

development of the NT measurement between 11 and 13^{+6} weeks of gestation, which is the most sensitive and specific ultrasound marker for this condition.[58]

Several studies have demonstrated that the best screening test for trisomy 21 is given by the combination, in the first trimester of pregnancy, of maternal age, fetal NT thickness and maternal serum free â-hCG and PAPP-A. This test allows a detection rate of 90% for a false positive rate of 5%.[51-54] By adding the ultrasonographic evaluation of the NB, a sensitivity of over 90% could be achieved for a false positive rate of about 2%.[46,47,51]

Although extensive studies have demonstrated that absence of the NB is highly sensitive and specific marker of trisomy 21, its accurate examination requires highly skilled operators and at present it is unlikely that this assessment will be incorporated into the routine first-trimester scan. Nevertheless, this sonographic marker could be used in specialist centres to re-evaluate the risk, in patients with intermediate risk after screening by fetal NT and maternal serum biochemistry.[51]

If the individual risk-orientated two-stage screening for trisomy 21[51], which includes examination of the fatal NB in those women with a individual risk between 1:101 to 1:1000, is to be introduced in the routine clinical practice, the detection rate could be potentially be increased to more that 90%, and the false positive rate could be decreased to about 2%. This aspect is extremely important for three main reasons: firstly, such a screening test would reduce the economic costs due to unnecessary invasive testing; secondly, it would reduce the number of miscarriage of chromosomally normal fetuses due to the invasive testing[59,60]; thirdly would provide an early reassurance to pregnant women, and at the same time, would allow them to have an early termination of pregnancy should they wish so in case their baby is found to be affected.

However, in order to reproduce the same results, it is imperative that sonographers receive appropriate training and adhere to a standard technique for the measurement of NT and the assessment of the NB. Furthermore, the success of a screening program necessitates the presence of a system for regular audit

of results and continuous assessment of the quality of images. The Fetal Medicine Foundation, which is a UK registered charity, has established a process of training and quality assurance for the appropriate introduction of NT screening into clinical practice.[61]

In conclusion, improvements in ultrasound resolution have allowed us to evaluate and measure very minute fetal structures with a concomitant decrease in the room for error. If they are to be included in prenatal screening protocols, their evaluation must be done with a high degree of precision and accuracy. This can be accomplished only through strict standardization of the fetal image and with appropriate training and an ongoing quality assurance. Just like the NT measurements, it is only by strictly adhering to these principles that the evaluation of the NB can be incorporated into any screening protocol using prenatal sonography.

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