

# Nasal bone in screening for T21 at 11–13 + 6 weeks of gestation — a multicenter study

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## ABSTRACT

**Objectives:** Trisomy 21 is one of the most common chromosomal defects diagnosed prenatally. Screening for Down syndrome is based on maternal age, measurement of crown-rump length, nuchal translucency and fetal heart rate, together with free  $\beta$ -hCG and PAPP-A at 11 to 13 + 6 weeks. Introduction of additional ultrasound marker of trisomy 21 (evaluation of the nasal bone) may result in increased DR and decreased invasive diagnostic testing rates (FPR).

**Material and methods:** Ultrasound scan with NB evaluation was performed in 5814 fetuses during routine screening for chromosomal defects at 11 to 13 + 6 weeks of gestation. DR and FPR coefficients were calculated for 4 levels of risk as cut-off points for screening model 1, based on MA, NT, and first trimester biochemistry, as well as for screening model 2, based on MA, NT, first trimester biochemistry and NB.

**Results:** There were 5708 normal cases, 71 cases of trisomy 21 and 35 cases of other chromosomal defects. NB was absent in 46 (64.8%) cases and present in 25 (35.3%) cases of trisomy 21, comparing to present NB in 5463 (95.7%) and absent in 245 (4.3%) of normal cases.

**Conclusions:** First-semester screening with additional NB assessment significantly increases the detection rate for trisomy 21 and decreases the rate of false-positive results. Adding NB evaluation at the risk level of 1:50 causes only a small increase in detection rate. Invasive procedures should be performed in that group regardless NB assessment.

**Key words:** nasal bone, chromosomal defects, screening for trisomy 21

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## INTRODUCTION

Trisomy 21 (Down syndrome) is one of the most common chromosomal defects diagnosed prenatally [1]. Phenotypic characteristics of this disease were described by John Langdon Down in 1866 [2], and are currently used as markers in prenatal ultrasound [3]. Absent nasal bone (NB) is a frequent phenotypic defect in fetuses with trisomy 21 (T21) and affects approximately 70% of fetuses with T21 and 1–3% of fetuses with normal karyotype [1, 4, 5]. Thus, evaluation of

NB presence may constitute an additional marker to increase the efficacy of screening for trisomy 21 [6].

At present, assessment of nuchal translucency (NT), maternal age (MA), and fetal heart rate (FHR), together with first trimester biochemistry (BC) [evaluation of the concentration of free beta subunit of human chorionic gonadotropin (free  $\beta$ -hCG) and pregnancy-associated plasma protein A, (PAPP-A) in maternal blood], constitute a screening standard for Down syndrome at 11 to 13 weeks and 6 days of gesta-

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**Table 1. Description of the study group**

Variable	Mean	SD	Min.	Median	Max.	25% percentile	75% percentile
Maternal age (years)	32.04	5.38	14.00	33.00	46.00	28.00	36.00
CRL [mm]	63.54	8.29	45.00	63.30	84.00	57.77	69.00
NT [mm]	1.86	0.73	0.80	1.80	15.00	1.50	2.00
FHR (BPM)	160.41	16.58	130	160	196	156	164
Free $\beta$ -hCG MoM	1.32	0.90	0.125	1.07	7.68	0.73	1.66
PAPP-A MoM	1.01	0.57	0.115	0.90	6.00	0.61	1.28

tion [6]. This scheme is characterized by a high (approximately 90%) detection rate (DR) of chromosomal defects, mainly T21, with a 5% False Positive Rate (FPR) [5, 7].

Introduction of additional ultrasound markers of trisomy 21 (evaluation of the nasal bone, as well as assessment of tricuspid and ductus venosus blood flow) results in increased DR (up to 95%) and decreased invasive diagnostic testing rates (FPR) [8].

## MATERIAL AND METHODS

Ultrasound scan with NB evaluation was performed in 5814 fetuses during routine screening for chromosomal defects at 11–13 + 6 weeks of gestation. The test was carried out using the Voluson Expert VE 730 and Voluson Expert E8 by sonographers with the FMF (Fetal Medicine Foundation) certificate, confirming their skills to evaluate fetuses at 11–13 + 6 wks. The following were investigated: CRL (Crown-rump length), MA, NT, and FHR (Table 1). In addition to the ultrasound examination, free  $\beta$ -hCG and PAPP-A levels were measured in maternal blood. The results of the biochemical test, expressed as multiples of the median (MoMs), were also included in the study. This retrospective, cross-sectional, descriptive, non-interventional study used anonymized data, hence the study protocol did not require ethical approval under local rules.

NB was routinely assessed in each patient with the image of the fetal head in the sagittal plane enlarged to include only the profile and the upper part of the chest (Figure 1). All criteria required by the Fetal Medicine Foundation for imaging were met during the examination [6, 9, 10]. CRL, MA, NT, FHR, free  $\beta$ -hCG and PAPP-A values were entered into the Astraia software to assess the risk for trisomy 21.

In the high-risk group amniocentesis for karyotyping was performed. In remaining cases, neonatologists phenotypically evaluated newborns for follow-up.

DR and FPR coefficients were calculated for 4 levels of risk as cut-off points for screening model 1, based on MA, NT, and first trimester biochemistry (Table 2), as well as screening model 2, based on MA, NT, first trimester biochemistry, and NB (Table 3).



**Figure 1.** Routine assessment of the nasal bone with the image of the fetal head in the sagittal plane enlarged to include only the profile and the upper part of the chest. Present NB

**Table 2. Comparison of DR and FPR in screening model 1 (without NB)**

Risk cut-off for trisomy 21	1:300	1:200	1:100	1:50
DR [%]	84.5	83.1	80.3	78.8
FPR [%]	10.7	8.5	5.7	3.6

**Table 3. Comparison of DR and FPR for screening model 2 (with NB assessment)**

Risk cut-off for trisomy 21	1:300	1:200	1:100	1:50
DR [%]	93.0	91.5	90.1	80.3
FPR [%]	2.0	2.0	1.9	1.5

All generated data were used to verify the usefulness of NB evaluation as the component of the screening test. The results were analyzed with PQStat statistical package, version 1.4.2.324.

CRL, MA, NT, free  $\beta$ -hCG MoM, PAPP-A MoM, and FHR were analyzed in each group with U Mann-Whitney test in relation to karyotype. The presence of NB in the investigated groups was measured with chi-squared test. Maternal age ( $\geq 35$  years), T21 NT risk ( $\geq 1:300$ ), T21 BC

risk ( $\geq 1:300$ ), and T21 BC NT risk ( $\geq 1:300$ ) were compared in both groups using cross tabulation and chi-squared test in relation to karyotype. The sensitivity and FPR were determined. ROC analysis for both models (1 and 2) of T21 risk was performed. The p-value of  $< 0.05$  was considered statistically significant.

### RESULTS

There were 35 cases of other chromosomal defects that were excluded from the study (18 of trisomy 18, 6 of trisomy 13, 5 of Turner syndrome, 3 of chromosomal inversion, 2 of triploid and 1 of trisomy 9). The presence and absence of NB was analyzed only in trisomy 21 and normal cases. There were 71 cases of trisomy 21 (1.22%) and 5708 normal cases. NB was absent in 46 (64.8%) cases (Figure 2), and present in 25 (35.3%) cases of trisomy 21, comparing to present NB in 5463 (95.7%) and absent in 245 (4.3%) of normal cases (Table 4). Study group description is presented in Table 1.

In screening model 1 (without NB) for the cut-off of 1:300, DR was 84.5% and FPR was 10.7%. When the additional marker (NB) was added to screening (model 2), at cut-off of 1:300, DR increased to 93.0% for 2.0% FPR. For the cut-off of 1:100, DR was 80.3% for 5.7% FPR without NB assessment. After adding NB, DR increased to 90.1% at 1.9% FPR. Both screening models were also compared at cut offs of 1:200 and 1:50 (Tables 2 and 3).

Comparison of screening models 1 and 2 and was also performed with the use of the ROC analysis (Figures 3 and 4).

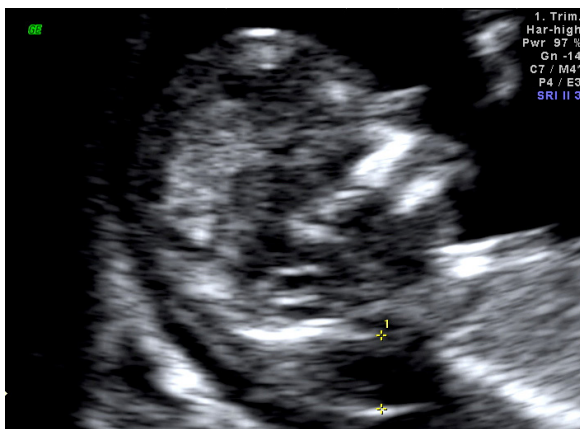


Figure 2. Nasal bone is less echogenic than overlying skin, hence classified as “absent”

	Total number	Present NB (%)	Absent NB (%)
Normal neonates	5708	5463 (95.7%)	245 (4.3%)
Trisomy 21 cases	71	25 (35.2%)	46 (64.8%)

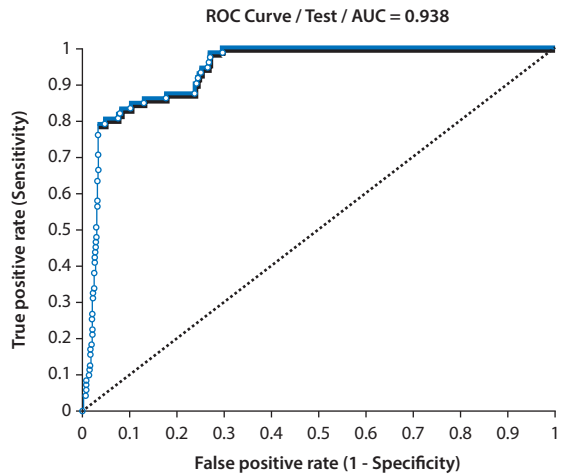


Figure 3. ROC analysis for model 1

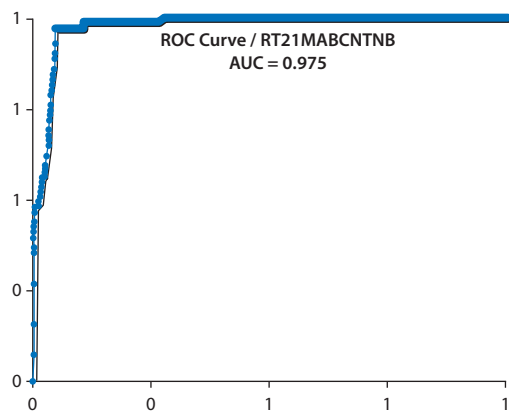


Figure 4. ROC analysis for model 2

### DISCUSSION

Absent or hypoplastic NB is one of the characteristic features of trisomy 21. NB may be evaluated on ultrasound at 11–13 + 6 weeks. It is visualized as a thin line, more echogenic than the overlying skin. NB assessment has been applied to optimize screening for trisomy 21 [8, 9]. In 2001, Cicero et al., proved an important correlation between absent NB and the presence of trisomy 21 in their study on over 3800 fetuses [4]. The results of a study by Orlandi et al., who also investigated the effect of NB evaluation during routine first-trimester screening, are comparable with our findings. These authors assessed over 2300 fetuses and found 15 cases with Down syndrome, including 8 with absent NB. During statistical analysis, a comparison of the results with the risk cut-off level of 1:250 for standard screening was performed and revealed DR and FPR of 87% and 4.3%, respectively, and of 90% and 2.5%, respectively with additional NB evaluation [10]. Zoppi et al., investigated NT and NB in 5532 fetuses [1], and found absent NB in 70%, 80% and 66% of the fetuses with Down's,

Edwards and Turner's syndromes, respectively, as well as 0.2% of fetuses with normal karyotype [1]. According to Otano et al. [11], agenesis of the nasal bone is diagnosed in 60% of T21 and 0.6% of healthy fetuses, what is consistent with our findings. In our study, 71 cases with Down syndrome were confirmed, including 46 (64.7%) with absent NB.

In 2006, Cicero et al. proved in another study that combination of NB evaluation with other basic parameters of the first-trimester screening decreases the FPR by half, from 5% to 2.5% for cut-off level of 1:100 [5], which is similar to our findings (FPR decreased from 5.7% to 1.9% for cut-off level of 1:100).

Also in 2006, Kozłowski et al. studied approximately 300 fetuses and found no significant advantages of including NB evaluation into the first-semester routine screening. In fact, sensitivity was higher when that marker was excluded from the calculations. According to these authors, DR with and without NB evaluation was 77.8% and 94.4%, respectively for cut-off level of 1:300 [12]. Our data showed higher efficacy (DR of 84.5 and 93.0% respectively), with low rates of false-positive results (FPR of 10.7 and 2.0% respectively). Possibly, divergent findings were the result of a small sample in the paper of Kozłowski et al.

Our prospective study demonstrates that NB is absent in approximately 4% of the fetuses with normal karyotype and 65% of the fetuses with Down syndrome at 11–13 + 6 weeks. Effective first-semester screening is based on MA, NT, FHR, CRL as well as maternal levels of  $\beta$ hCG and PAPP-A, resulting in FPR of 5.7% with risk cut-off of 1:100. Inclusion of NB into the screening test reduced FPR to 1.9% without the necessity to change the cut-off level. That allows to lower the number of invasive tests, and consequently of iatrogenic miscarriages and the related costs. Kagan et al., assessed the usefulness of NB evaluation as an additional marker of chromosomal defects (trisomy 21, 18, and 13) [13].

The suggested risk division into the cut-offs of 1:50, 1:100, 1:200 and 1:300 demonstrated the usefulness of NB as an additional marker of trisomy 21 in each group. Similar observations were made in our study. Owing to a high rate of trisomy 21 in the 1:50 risk group, invasive diagnostic testing is immediately advised in these patients. In case of risk lower than 1:50 NB marker is applicable due to significantly decreased FPR and higher DR [13]. Our study showed increased DR (from 78.7% to 80.3%) and lower FPR in each risk group (from 3.6% to 1.5%), depending on the model (1 or 2). Comparisons of models 1 and 2 at the risk level of 1:50 revealed a slightly higher DR and significantly lower number of indications for invasive testing.

The FMF management protocol recommends invasive diagnosis for trisomy 21 risk  $\geq$  1:50, regardless of the presence of the NB, similarly to other additional ultrasound markers (for example fronto-maxillary facial angle evaluation) [14]. The

sonographer assessing the fetal nasal bone has to have an appropriate experience. Lack of appropriate training, certification and auditing has significant negative impact of the FPR [15]. Proper ultrasound evaluation of ultrasound markers of chromosomal defects, including the nasal bone, should lead to more selective referral to invasive testing, and, as a consequence, fewer procedure-related complications and pregnancy losses.

## CONCLUSIONS

First-semester screening with additional NB assessment significantly increases the detection rate for trisomy 21 and decreases the rate of false-positive results.

Adding NB evaluation at the risk level of  $\geq$  1:50 (based on ultrasound and first trimester biochemistry) causes only a small increase in detection rate. Invasive procedures should be performed in that group regardless NB assessment.

## Conflict of interest

Authors don't have any sources of financial support to disclose.

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