The ratio of nasal bone length to prenasal thickness: a novel approach for prenatal ultrasound screening of Down syndrome in the second trimester

PhD thesis

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2014.

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List of publications

Publications directly related to the subject of the dissertation

1. Szabó A, Szili K, Szabó J, Isaszegi D, Horváth E, Sikovanyecz J Prenasal thickness, nasal bone length and their ratio: good second trimester sonographic markers for Down syndrome *ULTRASOUND IN OBSTETRICS & GYNECOLOGY* 40:(S1) *pp. 157-158.* (2012). **IF: 3.557**

2. Szabó Andrea, Szili Károly, Szabó János Tamás, Szabó János, Isaszegi Dóra, Horváth Emese Ultrasound screening for Down-syndrome in the second trimester: the prenasal thickness *EUROPEAN JOURNAL OF FETAL MEDICINE AND GENOMICS* 1:(1) *Paper 57.* (2012)

3. Szabó Andrea, Szili Károly, Szabó János Tamás, Isaszegi Dóra, Horváth Emese, Sikovanyecz János, Szabó János A prenazális lágyrész vastagodás a 21-es triszómia ultrahang jele a második trimeszterben *MAGYAR NŐORVOSOK LAPJA* 76: *Paper 751134.* (2013)

4. Szabó Andrea, Szili Károly, Szabó János Tamás, Sikovanyecz János, Isaszegi Dóra, Horváth Emese, Szabó János Nasal bone length: prenasal thickness ratio: a strong 2D ultrasound marker for Down syndrome *PRENATAL DIAGNOSIS* In press 25:(1) (2014) **IF: 2.683**

Publications indirectly related to the subject of the dissertation

1. Khalil A, Arnaoutoglou C, Pacilli M, Szabo A, David AL, Pandya P Outcome of fetal exomphalos diagnosed at 11-14 weeks of gestation ULTRASOUND IN OBSTETRICS & GYNECOLOGY 39:(4) pp. 401-406. (2012) **IF: 3.557**

2. Szabó Andrea, Szili Károly, Szabó János Tamás, Szabó János, Isaszegi Dóra, Horváth Emese Ultrasound screening for Down-syndrome in the second trimester: the prenasal thickness EUROPEAN JOURNAL OF FETAL MEDICINE AND GENOMICS 1:(1) Paper 57. (2012)

3. Szabó J, Szabó A, Szili K, Sikovanyecz J, Horváth E, Orvos H, Pal A Single umbilical artery: is it innocent? ULTRASOUND IN OBSTETRICS & GYNECOLOGY 40:(S1) p. 158. 1 p. (2012)

4. Szili K, Ferencz E, Szabó A, Szabó J, Sikovanyecz J Early embryonic heart rate and pregnancy outcome ULTRASOUND IN OBSTETRICS & GYNECOLOGY 40:(S1) pp. 234-235. (2012)

5. Szabó Andrea, Alasztics Bálint, Bánhidy Ferenc, Valent Sándor A 21-es triszómia szűrése napjainkban *ORVOSI HETILAP* 145:(26) *pp. 1026-030.* (2013)

6. Lipták-Váradi Julianna, Szili Károly, Vanya Melinda, Széll Márta, Szabó János, Szabó Andrea, Kató Lilla Az egészséges élettér—az otthoni mikrokörnyezet vizsgálati modellje ÉPÍTÉS ÉPÍTÉSZETTUDOMÁNY 41:(3) pp. 271-282. (201

1. Introduction

1.1. The frequency of birth defects

In developed or high income countries the estimated rate of abnormal fetal and neonatal conditions at birth is four per cent. However, the rate of birth defects is different in developed, developing and underdeveloped countries ranging from 4% up to 7,9% according to a newly conducted world-wide study carried out by the March of Dimes Birth Defects Foundation*(1)*.

In the developed countries the following numbers have to be considered. Out of the 4 per cent general rate of abnormal fetal conditions 0,8 per cent are chromosomal abnormalities, 1 per cent are monogenic disorders, 2,2 per cent are structural abnormalities (Table 1)*(2)*.

Table 1 Annual Rate of Birth Defects in the Globe (WHO)

Annual Rate of Birth Defects in the Globe	
Total no. of birth in the world	**130 000 000**
Congenital anomalies:	**3 200 000**
Chromosomal anomalies:	**850 000**
Mendelian disorders:	**900 000**
Haemoglobinopathies:	**250 000**
Total:	**5 200 000 (4%)**

Couples deciding to have a child rightly lay claim to have a healthy baby. Therefore, they turn to the obstetrician or to the nearest medical genetic service to have the answer, if their future baby will be healthy. To answer the question correctly the available prenatal counselling, screening and diagnostic methods can be used.

1.2.Primary and secondary prevention

Seventy per cent of neural tube defects can be prevented primarily by periconceptional folate admininistration. The 70% prevention rate of neural tube defects achieved by the only administration of folic acid, can be further increased by adding choline, betaine and inositol to folates, especially in obese women *(3)*. However, the majority of abnormal fetal conditions can not be prevented primarily. This is especially true for chromosomal aneuploidies, which can be prevented by secondary measures such as preimplantation genetic diagnosis *(PGD)*, prenatal screening *(PS)* and prenatal diagnosis *(PD)*.

Before the introduction of intrauterine diagnostics the fetal abnormalities could only be detected after birth or in the neonatal period. However, during the last decades a great development has been experienced in prenatal screening and diagnosis of birth defects. The introduction of high resolution ultrasound, fetal biochemistry and new achievements in maternal and fetal pathophysiology equally contributed to the formation of a new independent field of science, the fetal medicine.

1.2.1. Preimplantation genetic diagnosis.

Pre-implantation genetic diagnosis *(PGD)* is generally defined as the in vitro testing the embryo before embryo transfer and its implantation.

1.2.2. Prenatal screening or non-invasive methods.

Prenatal screening *(PS)* for fetal malformations means to detect embryos or fetuses with normal or abnormal features during their intrauterine life. The methods of PS has been developed during the last decades by recognizing ultrasound soft markers and maternal blood biochemical characteristics of the affected pregnancies. Neither fetal sampling nor transuterine puncture is applied therefore, the *non-invasive* term can also be used. To achieve an efficient screening the whole pregnant population should have been examined with the methods of the possible highest detection rate and at the lowest cost. The recently advocated methods to identify affected pregnancies are the ultrasound anatomy scan and the maternal blood tests. To evaluate the efficacy of a particular screening method one has to calculate sensitivity, specificity, detection rate, positive and negative predictive values and cut off values.

To evaluate the efficacy of a particular screening method one has to calculate sensitivity, specificity, detection rate, positive and negative predictive values, and cut off values.

Sensitivity (detection rate, true positive rate): measures the proportion of actual positives which are correctly identified as true positives. Specificity (true negative rate): measures the proportion of negatives which are correctly identified as true negatives.

Positive predictive value (PPV): proportion of positive results that are true positives. Negative predictive value (NPV): proportion of negative results that are true negatives.

False negative rate and false positive rate (cases) should be as low as possible. If the test is screen negative one should be very careful since this does not mean that the method completely picked up the affected cases, only the estimated risk is below the cut off.

Cut off value is a term at what level of risk is considered to be screen positive where invasive diagnostics is offered. In many countries the threshold of cut off value for high-risk is under 1:250-300. In Hungary, the level of intervention is 1:150, or the presence of two ultrasound soft markers or more. The risk of aneuploidy and risk of postprocedural miscarriage is subjective and relative; Some couples may decide to have amniocentesis at a 1:1000 risk of trisomy 21, while others would not opt for an invasive procedure even if they had a 1:10 risk score.

1.2.2.1. Prenatal diagnosis or invasive methods

Prenatal diagnosis enables a definitive fetal diagnosis usually through fetal sampling and laboratory examination of the sampled material (chorionic villi, amniotic fluid, fetal blood and/or tissue). Because of the needle puncture for sampling the *invasive prenatal method* is the other term used. The disadvantage of the sampling procedure is the 1% procedural risk of abortion, while the advantage is the certainty of the result which is nearly 100 per cent sure. Because of the procedural risk of miscarriage associated with invasive method, couples usually first decide to have a non-invasive prenatal screening test to estimate the risk of a fetal chromosomal abnormality if it is high enough to justify the fetal loss risk. Recent Guideline of the Hungarian College of Clinical Geneticist and of Obstetrics and Gynecology (2010) recommend that all pregnant women of 37 years of age or over be offered invasive testing to obtain a definitive diagnosis of fetal karyotype. However, from ethical

point of view the couples are let to have an autonome decision if they want to have an invasive test or not. At genetic counselling the patient is advised with the possibility that they can skip the expensive screening and can go straight for invasive testing.

1.2.2.2. Prenatal genetic counseling (PGC)

Prenatal screening and diagnosis is not an obligatory medical service like preventing infectious diseases by immunization and vaccination. PS and PD can be offered only through genetic counselling, which is a communication process between the pregnant patients (couple) and the counsellor providing up to date information about the fetal condition and the recent choices of fetal diagnosis *(4)*. The aim of prenatal genetic counselling is to plan timely medical or surgical treatment of abnormal fetal conditions via screening and diagnosis during pregnancy or after birth. An other purpose of prenatal counselling to provide information to the parents to have a decision about their fetus in time. As a result of counselling the couple will be able to prepare psychologically, socially, financially, and medically for a baby with a health problem or disability. For example, Down Syndrome is associated with cardiac defects that may need intervention immediately upon birth (Table 2).

Table 2 Methods of prenatal screening and diagnosis

INVASIVE (DIAGNOSTICS)	NON- INVASIVE (SCREENING)
1. CHORION-BIOPSY **Chorionic Villus Sampling (CVS)**	**1. ULTRASOUND** **I. TRIMESTER** **II. TRIMESTER**
2. AMNIOCENTESIS	**2. MATERNAL SERUM MARKERS** **I. TRIMESTER** **II.TRIMESTER**
3. CORDOCENTESIS	**Fetal DNA in maternal blood and NIPT**
RISK: ~ 1 %	**RISK: 0 %**

2. The Down syndrome

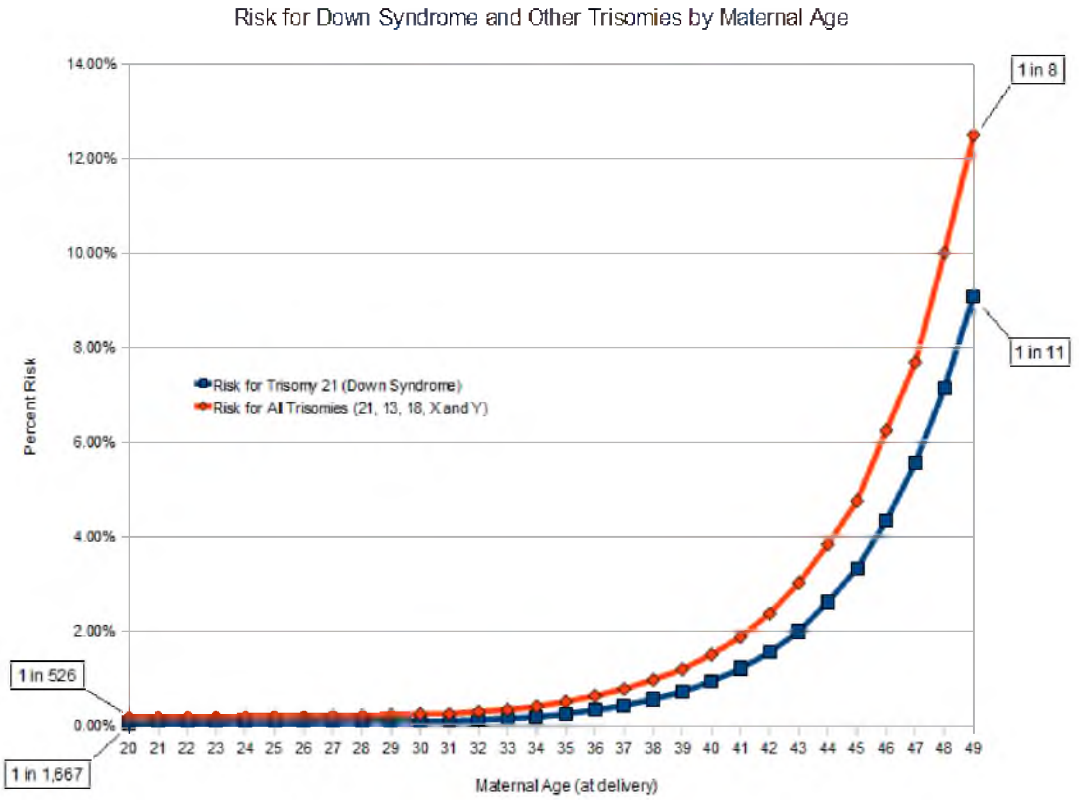
2.1. The prevalence, the discovery, and screening of Down syndrome

The Down syndrome is the most frequent numerical chromosomal abnormality also known as trisomy 21. It results in severe developmental errors, physical and mental handicaps, which can be present already in the fetal life *(5)*. At present, it can not be cured or effectively treated. Therefore, the prevention by early diagnosis and therapeutic abortion is the only choice.

The Down syndrome was first described by Dr. John Langdon Down in 1886 *(6)*. The fact that trisomy 21 is the cause of Down-syndrome was invented by Dr. Jerome Lejuene in 1959. The truth is that Marte Gautier, a woman pediatrician discovered it in 1957. Lejeune only discovered the laboratory where the discovery of 47 chromosome was made by Dr Marthe Gautier using a new tissue culture technique brought back from the United States *(7)*. Anyway, it was an intellectual theft by Jerome Lejuene.

The association between maternal age and increasing prevalence of Down-syndrome was first discovered by Suttleworth in 1906 and subsequently confirmed by others *(8)*. The increase in prevalence of Down syndrome after 35 years of age was considered to be worth for indication for karyotyping of the suspected fetus by amniocentesis (AC) or chorionic villus sampling (CVS) (Figure 1).

Figure 1 Age related-risk of Down syndrome



SOURCES:
Hook EB, Cross PK, Schreinemachers DM. Chromosomal abnormality rates at amniocentesis and in live-born infants. JAMA 1983;249(15):2034-38.
Newberger, D., Down Syndrome: Prenatal Risk Assessment and Diagnosis. American Family Physician. 2001.
Down syndrome births in the United States from 1989 to 2001. Egan JF - Am J Obstet Gynaecol - 01-SEP-2004; 191(3): 1044-8.

Table 3 Risk of Down Syndrome and other chromosome abnormalities in Live births by Maternal age

Risk of Down Syndrome and Other Chromosome Abnormalities in Live Births by Maternal Age

MATERNAL AGE (AT TERM)	RISK		MATERNAL AGE (AT TERM)	RISK		MATERNAL AGE (AT TERM)	RISK	
	DOWN SYNDROME	TOTAL CHROMOSOME ABNORMALITY		DOWN SYNDROME	TOTAL CHROMOSOME ABNORMALITY		DOWN SYNDROME	TOTAL CHROMOSOME ABNORMALITY
25	1 in 1,250	1 in 476	32	1 in 637	1 in 323	39	1 in 125	1 in 81
26	1 in 1,190	1 in 476	33	1 in 535	1 in 286	40	1 in 94	1 in 63
27	1 in 1,111	1 in 455	34	1 in 441	1 in 224	41	1 in 70	1 in 49
28	1 in 1,031	1 in 435	35	1 in 356	1 in 179	42	1 in 52	1 in 39
29	1 in 935	1 in 417	36	1 in 281	1 in 149	43	1 in 40	1 in 31
30	1 in 840	1 in 385	37	1 in 217	1 in 123	44	1 in 30	1 in 21
31	1 in 741	1 in 385	38	1 in 166	1 in 105	≥45	≥1 in 24	≥1 in 19

Source: Hecht CA and Hook EB, 1996

The prevalence of trisomy 21 is 1.3/1000 at birth in developed high income countries. In middle- and low-income countries without prenatal screening services the percentage of

women of advanced maternal age (greater than 35 years of age) delivering infants is high (average 12-20 percent) (Table 3). The birth prevalence of Down syndrome can reach 2-3 per 1,000 in these countries: a rate approximately double that currently seen in high-income countries. The demography of motherhood in the western world has shifted strikingly in the past two decades. A new tendency can be observed, that women continue having children up to the end of reproductive life, consequently the birth incidence of chromosomal disorders are increasing by facing new challenges to prenatal diagnosis. *(1)*

Advanced paternal age (greater than 35 years of age), although associated with an increased rate of mutations and a slightly higher birth prevalence of autosomal dominant disorders, is not considered a significant influence on the overall birth prevalence of birth defects. *(1)*

On the base of maternal age approximately 30% of fetal Down syndrome could be detected through invasive procedures, if all women over 35 years (5%) would participate in this diagnostics. However, invasive procedures have one per cent risk of abortion and can not be offered to all women. To avoid fetal loss due to invasive tests stimulated the development of new, not risky screening techniques for defining pregnancies with high and low risk for chromosomal abnormalities.

2.2. Other trisomies: Edwards syndrome: trisomy 18 and Bartholin-Pätau syndrome: trisomy 13

The second and third most frequent chromosomal trisomies after Down syndrome are the Edwards syndrome (the trisomy 18), and Bartholin-Pätau syndrome (the trisomy 13), respectively. They also show an association with advanced maternal age *(Figure 1)*. In 15 per cent of the cases they can be carried to term. In 85 % of the cases the trisomy leads to intrauterine death during the antenatal period *(9-11)*.

2.3. Prenatal (ultrasound) screening of Down syndrome.

With the introduction of the higher resolution vaginal probes ultrasound studies of the embryonic and fetal structures was initiated in the second half of the 80ies. A quite new observation about the association between the first trimester increased nuchal fluid accumulation (also known as *nuchal translucency, NT*) and fetal Down syndrome was reported *(Szabo and Gellén)* in 1990 *(12)*. This observation was approved on a larger pregnant population by *Nicolaides et al, 1992 (9, 13)*. The measurement of NT was standardized and

has become the basis of first trimester screening for Down syndrome. The next step in screening for trisomy 21 is the risk calculation based on maternal age and NT thickness. The sensitivity of this first trimester screening was 80% to 90% in different hands. Subsequently, maternal blood biochemical markers such as free beta-human chorionic gonadotropin (free ß-hCG), and pregnancy associated plasma protein-A (PAPP-A) and other additional ultrasound markers (presence or absence of nasal bone, tricuspidal and ductus venosus flow) was added to the first trimester screening test further increasing the sensitivity up to 93-95% at a 2,5% fals positive rate (Table 4).

Table 4 The efficacy of different screening methods for trisomy 21

The efficacy of different screening methods for trisomy 21		
Screening method	Sensitvity (%)	Fals-positive rate (%)
MA (maternal age)	30	5
First trimester		
MA + NT	75–80	5
MA + NT + free β-hCG + PAPP-A (combined test)	85–95	5
Combined test + NB + tricuspidal or ductus venosus flow	93–96	2,5
Second trimester		
MA + se. AFP + hCG (double test)	55–60	5
MA + se. AFP + free β-hCG (double test)	60–65	5
MA + se. AFP, hCG, uE3 (triple test)	60–65	5
MA + se. AFP, free β-hCG, uE3 (triple test)	65–70	5
MA + se. AFP, hCG, uE3, inhibin A (quadriple test)	65–70	5
MA + se. AFP, free β-hCG, uE3, inhibin A (quadruple test)	70–75	5
MA + NT + PAPP-A (11–13. weeks) + quadriple test (integrated test)	90–94	5
Second trimester ultrasound signs	70	5-15

MA = maternal age; NT = nuchal translucency; NB = nasal bone
β-hCG: β-human choriongonadotropin; PAPP-A = pregnancy-associated plasma protein-A
(Source: Nicolaides, K. H.:Screening for fetal aneuploidies at 11 to 13 weeks. Prenat. Diagn., 2011, 31,7–15.)

Despite the multitude of ultrasound soft markers for Down syndrome fetuses – such as increased nuchal fold thickness, cystic hygroma, cardiac anomalies, echogenic intracardiac foci, nasal bone hypoplasia, ventriculomegaly, widened iliac crest angle, short femur/humerus, duodenal atresia, echogenic bowel, pyelectasis-hydronephrosis, sandal gap sign, choroid plexus cyst, and midphalanx hypoplasia of the fifth finger there are no sensitive ultrasound markers in the second trimester that can be used either alone or in combination.

Furthermore, these markers may not be present in all affected fetuses, and such as all soft markers, they can also be detected in euploid cases *(14)* (Table 5).

Table 5 Ultrasound soft markers for Down syndrome fetuses in the second trimester*(14)*

Nuchal fold thickness (NF)
Cystic hygroma
Cardiac anomalies
Echogenic intracardiac foci/golf ball
Nasal bone hypoplasia (NBL)
Increased prenasal thickness (PT)
Ventriculomegaly
Pyelectasis-hydronephrosis
Duodenal atresia
Echogenic bowel
Sandal gap sign
Choroid plexus cyst
Midphalanx hypoplasia of the fifth
Dilated cavum septi pellucidi
Widened iliac crest angle
Short femur
Short humerus

2.4.Preliminary observations

During the last decades the evaluation of the nasal bone length (NBL) *(15-18)*, its presence, absence or hypoplasia was suggested in second trimester euploid and trisomy 21 fetuses *(15, 17-25)*. Furthermore, a detailed analysis of the facial profile revealed the significance of the prenasal soft tissue thickness (PT) *(26, 27)* as an efficient sonographic marker potentially applicable for ultrasound detection of second trimester trisomy 21 fetuses. Preliminary observation suggested that PT combining with NBL as a ratio would increase the detection rate of second trimester Down-syndrome *(28-32)*.

Our preliminary observations using 2D ultrasound measurements of NBL and PT at our tertiary referral center suggested the potential for the second-trimester identification of euploid and Down syndrome fetuses in a mixed-risk population *(33,34)*.Considering the data from the literature and our good experience with the PT-to-NBL and NBL-to-PT ratio for screening for trisomy 21, a study was started from January 2008 and these markers were incorporated into our second trimester fetal anomaly scan. This prospective study examined the clinical value of 2D ultrasound measurements of NBL, PT, and their ratios for differentiating euploid and Down syndrome fetuses in the second trimester (in an at-risk population).

3. Aims of the study

1) To analyse fetal facial profile for finding new second-trimester markers for Down-syndrome screening.

2) To study the feasibility of the measurements of the fetal nasal bone length (NBL) and prenasal thickness (PT) during the second trimester anatomy scan.

3) To create normograms of fetal nasal bone length (NBL), prenasal thickness (PT) and their ratios for euploid second trimester fetuses.

4) To study the developmental characteristics of PT and NBL in a large second trimester pregnant population to improve the understanding and clinical usage of the normograms.

5) To determine whether the increase in nasal bone length (NBL) and prenasal thickness (PT) between 14-28 weeks of gestation is parallel or divergent and whether the ratio is constant independently from the gestational age.

6) To evaluate the screening performance of nasal bone length (NBL) and prenasal thikness (PT) values and their ratios in the second trimester screening of trisomy 21, and to determine the statistical power of this method with the detection rate, the sensitivity, the false postivie and the false negativ rate, likelihood ratio, posítive and negative predictive values.

7) To determine whether the NBL:PT ratio or its inverse counterpart the PT:NBL ratio have better performance in screening second trimester fetuses with Down syndrome.

8) To incorporate these new markers and their ratios into the second trimester anatomy scan for combined ultrasound screening of Down-syndrome and other fetal defects.

4. Materials and methods

4.1. Materials

Women were referred for genetic counselling and second trimester anomaly scans to our regional prenatal genetics center because of advanced maternal age (≥35 years); positive screening results; intermediate risk of combined, triple, or integrated tests and the presence of one or more aneuploidy soft markers in previous ultrasound examinations. Women were recruited for second-trimester assessment and measurement of the NBL and PT values between January 2008 and April 2013. Chromosomal studies from amniotic fluid were carried out in the Department of Medical Genetics, University of Szeged. Ultrasound anatomy scans were performed at the MEDISONO Fetal and Adult Health Research Center and at the Department of Obstetrics and Gynecology, University of Szeged.

The following criteria determined enrollment into the euploid group: singleton viable pregnancy, 14–28 weeks of gestation, a lack of maternal disease (such as hypertension, toxemia, renal disease, and diabetes mellitus), normal fetal growth, normal amniotic fluid volume, diagnosis of a normal fetal anatomy, and newborns without chromosomal or structural abnormalities between the fifth and 95th percentile birth weight at delivery.

The study included 1330 euploid and 33 Down syndrome fetuses. The protocol was approved by the ethics committee of the University of Szeged. A routine second-trimester anomaly scan in weeks 18–23 and a detailed examination of the fetal anatomy within 14–17 and 23–28 weeks of gestation were performed using a high-resolution 2D transabdominal ultrasound scanner (Voluson E8 Expert, GE Healthcare, Milwaukee, WI, USA).

4.2. Methods

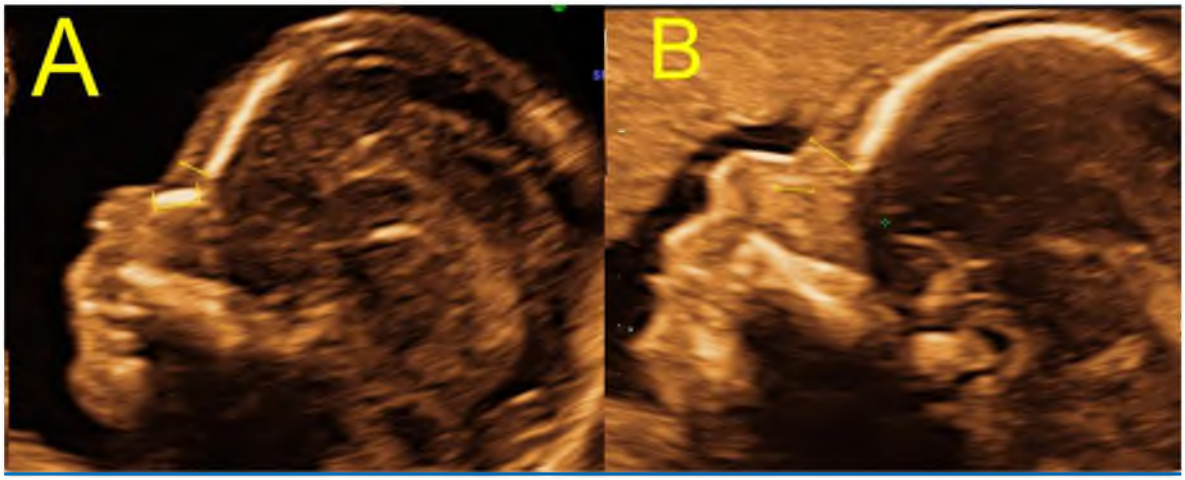
4.2.1. Measurement of prenasal thickness and nasal bone length

The facial profile was assessed at the beginning of the scanning sessions to avoid effects of fetal movements that could alter the fetal position. Three image acquisitions were obtained during one scan session and the best one was used for analysis. If it was not successful, then the patient came back for another scanning session 30–40 min later. The sonographer was blind to the fetal karyotype, and each ultrasound examination was performed before the chromosomal study. Nasal bone length*(15, 32)* and prenasal thickness*(38)* measurements can

be obtained on the same image if the face of the transducer was positioned parallel to the nasal bone. The insonation angle was close to 45°.

The following image settings were used: low gain, medium dynamic contrast, and maximum magnification so that the fetal head occupied the entire screen. Images were adjusted to ensure correct midsagittal plane *(22, 31)*. Briefly, PT was measured as the shortest distance from the lower margin of the frontal bone to the outer surface of the overlying skin. The margins of the nasal bone are the proximal and the distal ends of the white ossification line. The NBL*(15, 32)* and PT*(31)* were measured using the same view (Figure 2A and B).

Figure 2 Measurements of nasal bone length and prenasal thickness in euploid (A) and in a trisomy 21 (B) fetus



4.2.2. Database

Maternal data and sonographic findings were recorded in a database (Astraia Software GmbH, Münich, Germany). The ultrasound imaging data were stored in the local Digital Imaging and Communications in Medicine (DICOM) format via Astraia. Values of NBL and PT were exported to Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

4.2.3. Statistical analysis

Statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, CA, USA). Scatter plots of NBL and PT with linear polynomial regression lines and percentile curves (third and 97th) were created. Similarly, scatter plots of NBL: PT and PT: NBL ratios with linear polynomial regression lines and percentile curves (fifth and 95th) were produced. Comparisons between euploid and Down syndrome measurements for NBL, PT [in

millimeters (mm) and in multiple of medians (MoMs)], and their ratios (NBL:PT and PT:NBL) were performed using the Mann–Whitney U independent samples test. NBL, PT, and PT:NBL and NBL:PT ratio correlations were analyzed. No analysis of correlation was performed between any other markers.

5. Results

The mean maternal age in euploid and Down syndrome cases was 30.6 years (16.6–47.1 years) and 31.5 years (21.1–42.3 years). The mean gestational age was 19.6weeks (14.0–28.9weeks) for euploid and 20.3 weeks (15.0–25.6 weeks) for Down syndrome cases (Table 6).

Table 6 Distribution of maternal age, gestational age (GA), nasal bone length (NBL) and prenasal thickness (PT) in euploid fetuses

	Min.	Mean	Max.	SD
Age (year)	16.63	33.59	47.06	±4.64
GA (week)	14.00	19.57	28.86	±3.25
NBL (mm)	0.00	5.62	12.00	±1.63
PT (mm)	1.4	3.88	8.5	±1.08

The three consecutive NBL and PT measurements lasted 3 to 6 min and were completed during the first, the second, and the third attempts in 77%, 19%, and 4% of the cases, respectively.

The total number of the screened patients was 1470. Those excluded (107) were the following: fetal structural abnormalities (24), multiple pregnancy (35), maternal conditions listed in the method (41) and chromosomal abnormalities, such as Turner syndrome (n=1), trisomy 18 (n=4) and trisomy 13 (n=2). After exclusion 1330 euploid and 33 Down syndrome fetuses remained.

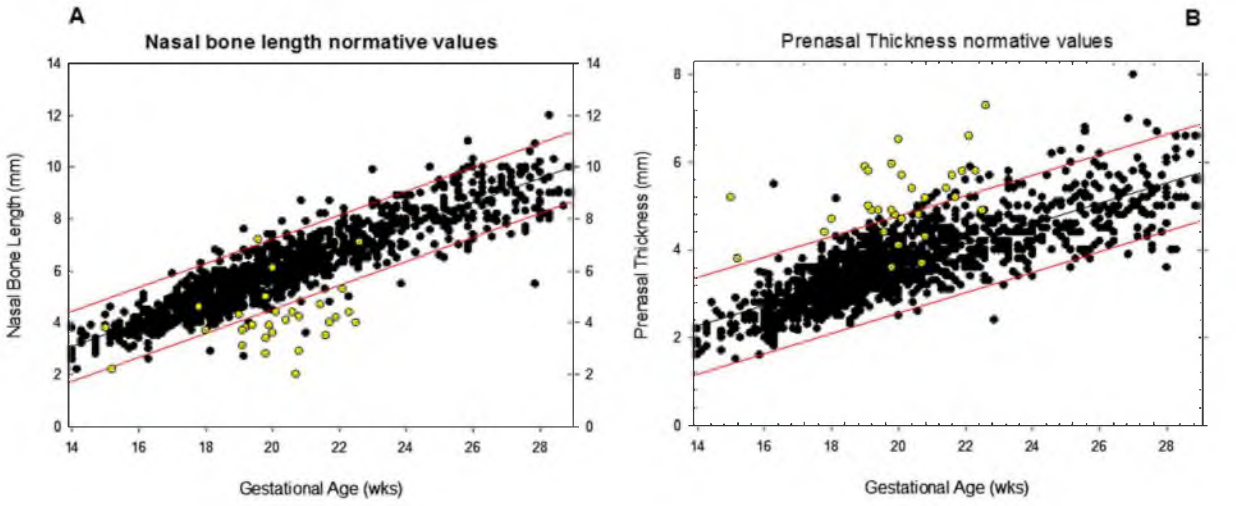
Ultrasound markers found in the Down syndrome group were:increased nuchal fold thickness (n=10), cystic hygroma (n=2), cardiac defects (n=9), echogenic intracardiac focus (n=4),mild ventriculomegaly (n=4),short femur (n=3), duodenal atresia (n=1),hyperechogenic bowel (n=3), pyelectasis-hydronephrosis (n=3), choroid plexus cyst (n=4), sandal gap sign (n=3),and midphalanx hypoplasia of the fifth finger (n=4).

All invasive tests were amniocenteses, either because maternal age (≥35 years) (18 cases), a positive combined test (≥1:250) (12 cases), and second-trimester ultrasound soft markers (three cases).

5.1. Statistics on the screening performance of this method

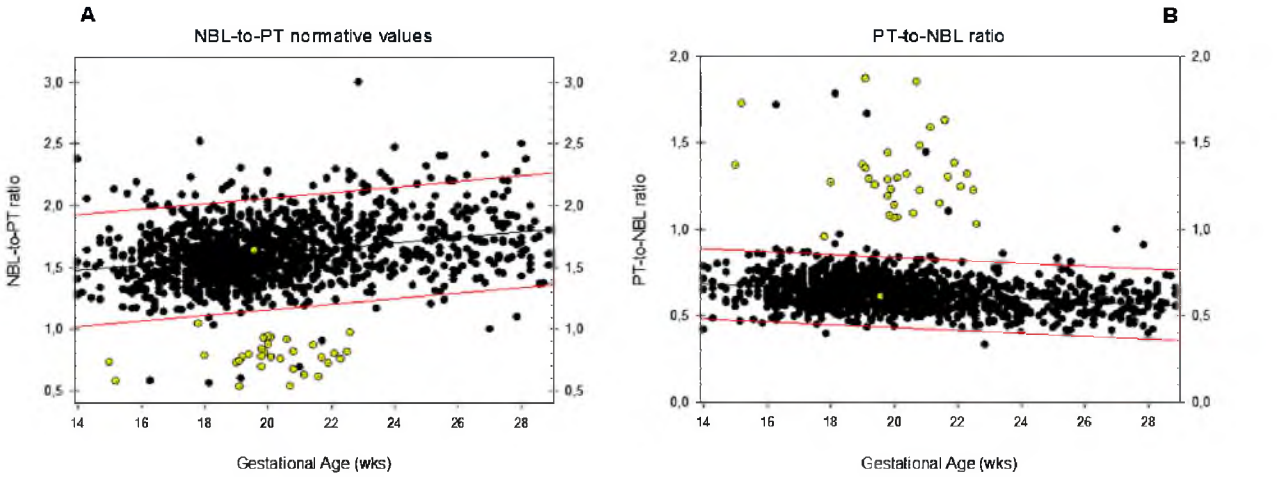
A linear increase was observed in the mean NBL and in the mean PT according to increasing gestational age between the 14th and 28th weeks (Table 8). Both the NBL and PT alone were found to be strong markers (sensitivity of 76% for both markers) for Down syndrome (Figure 3A and B).

Figure 3 (A) Gestational-age-dependent nasal bone length values in 1330 euploid (black filled circles) and 33 Down syndrome (yellow open circles) fetuses. Approximately 76% of cases with Down syndrome fell under the third percentile. (B) Gestational-age-dependent prenasal thickness values in 1330 euploid (black filled circles) and 33 Down syndrome (yellow open circles) fetuses. Approximately 76% of cases with Down syndrome were above the 95th percentile.



The mean NBL:PT ratio showed a gradual increase from 1.48 to 1.79 between the 14th and 28th weeks of gestation (a 21.2% T1 increase) (Table 8). There was a statistically significant difference ($p<0.0001$) in the NBL:PT ratio between the euploid and Down syndrome groups (Figure 4A and B).

Figure 4. (A) Scatterplot of the ratio of nasal bone length to prenasal thickness in 1330 euploid (black filled circles) and 33 Down syndrome (yellow open circles) fetuses. All fetuses, except one, with Down syndrome fell under the fifth percentile. (B) Scatterplot of the ratio of prenasal thickness to nasal bone length in 1330 euploid (black filled circles) and 33 Down syndrome (yellow open circles) fetuses. All fetuses, except one, with Down syndrome were above the 95th percentile.



A total of 14 out of the 1330 euploid pregnancies and 32 out of the 33 Down syndrome cases were under the fifth percentile, with 97% sensitivity, 0.9% false positive rate, and 99% specificity. Evaluating the performance of the ratios, there were 32 true positive and one false negative Down syndrome cases identified. However, using the NBL:PT ratio, the false positive rate was 50% of those using the PT:NBL ratio. Their ratios have different reference ranges because of the inverted counterparts, and the reference range of the NBL:PT ratio is wider than that of the PT:NBL ratio. The positive and negative cases with the calculated sensitivity, specificity, and false positive and negative rate, using NBL, PT, the NBL: PT ratio, and the PT:NBL ratio for screening Down syndrome are summarized in Table 7.

Table 7. Statistical characteristics of the performance of the screening for Down syndrome using NBL, PT, and their ratios.					
	NBL	**PT**	**NBL + PT**	**NBL:PT**	**PT:NBL**
Sensitivity (%)	75.758	75.758	87.879	96.970	96.970
Specificity (%)	98.120	97.651	97.143	99.098	98.421
False Positive Rate (%)	1.880	2.349	2.857	0.902	1.579
False Negative Rate (%)	24.242	24.242	12.121	3.030	3.030
Positive Likelihood Ratio	40.303	32.244	30.758	107.475	61.414
Negative Likelihood Ratio	0.247	0.248	0.125	0.031	0.031

No correlation has been found between PT and NBL with Spearman Rank Order Correlation test (SROC = 0.830 at $p<0.05$) supporting that both markers are independent variables. The PT (PT mean:2.0–5.8mm) has lower values than the NBL (NBL mean: 3.0–10.0mm), and PT (axPT average=1.066) and NBL (axNBL average=1.084) elevation are also different during the second trimester.

Table 8. The mean, the 3rd, and the 97th percentiles of nasal bone length and prenasal thickness and the mean, the 5th, and the 95th percentiles of the ratios of nasal bone length to prenasal thickness and prenasal thickness to nasal bone length of euploid fetuses between 14 and 28 weeks of gestation

Gestational Age (Weeks)	Nasal Bone Length (mm)			Prenasal Thickness (mm)			NBL-to-PT Ratio			PT-to-NBL Ratio		
	3rd Percentile	Mean	97th Percentile	3rd Percentile	Mean	97th Percentile	5th Percentile	Mean	95th Percentile	5th Percentile	Mean	95th Percentile
14	1.867	3.088	4.310	1.265	2.265	3.266	1.023	1.476	1.928	0.486	0.686	0.886
15	2.324	3.545	4.766	1.495	2.496	3.496	1.046	1.498	1.950	0.478	0.678	0.877
16	2.781	4.002	5.223	1.726	2.726	3.726	1.068	1.520	1.972	0.470	0.669	0.869
17	3.239	4.459	5.679	1.957	2.956	3.956	1.090	1.542	1.994	0.461	0.661	0.861
18	3.723	4.943	6.163	2.201	3.200	4.199	1.113	1.565	2.017	0.453	0.652	0.852
19	4.180	5.399	6.619	2.431	3.430	4.429	1.136	1.587	2.039	0.444	0.644	0.844
20	4.636	5.856	7.076	2.661	3.660	4.660	1.158	1.609	2.061	0.436	0.636	0.835
21	5.093	6.313	7.533	2.891	3.891	4.890	1.180	1.632	2.083	0.428	0.628	0.827
22	5.550	6.770	7.990	3.122	4.121	5.120	1.202	1.654	2.105	0.420	0.619	0.819
23	6.033	7.254	8.474	3.365	4.365	5.364	1.225	1.677	2.129	0.411	0.611	0.810
24	6.490	7.711	8.931	3.595	4.595	5.595	1.247	1.699	2.151	0.403	0.602	0.802
25	6.946	8.167	9.388	3.825	4.825	5.825	1.269	1.721	2.173	0.394	0.594	0.794
26	7.403	8.624	9.846	4.055	5.055	6.056	1.291	1.743	2.196	0.386	0.586	0.786
27	7.886	9.108	10.330	4.298	5.299	6.300	1.314	1.767	2.219	0.377	0.577	0.777
28	8.342	9.565	10.788	4.528	5.529	6.531	1.336	1.789	2.242	0.369	0.569	0.769
Correlation Coefficient (r) (95% CI) p = <0.0001	0.916 (−1.000–0.923)			0.815 (−1.000–0.829)			0.285 (−1.000–0.326)			−0.244 (−0.286–1.000)		
Total Increase in Percent	346.87%	209.70%	150.29%	258.00%	144.08%	99.96%	30.54%	21.20%	16.25%	−24.10%	−17.05%	−13.18%

6. Discussion

At present there are no simple and sensitive second trimester ultrasound or biochemical markers for screening trisomy 21 with high performance. The aim of this study was to test the value of ultrasound measurements of NBL and PT for screening Down syndrome.

This 2D ultrasound study demonstrates that NBL and PT measurements can easily be carried out within the routine second trimester anatomy scans. We confirmed in a potentially high risk Caucasian population that both NBL and PT alone are strong markers of Down syndrome, with both having a sensitivity of 76%. The combination of these two markers as a ratio increased the detection rate to 97% with a 0.9% false positive rate. Furthermore, we demonstrated that the NBL:PT ratio performs slightly better than its inverse counterpart. This is new that the NBL:PT ratio is a better marker than the PT:NBL ratio for detecting Down syndrome fetuses, primarily because it produced less false positive cases, and it can be used in cases where the nasal bone is absent. Moreover, the NBL:PT ratio can easily be calculated during the scan. If the NBL:PT ratio is less than the fifth percentile, a search for other aneuploidy soft markers and invasive fetal karyotyping should be considered. In euploid fetuses, the NBL, the PT, and the NBL:PT ratio showed a linear increase with advancing gestational age *(28, 39)*. However, our data do not support previous observations that the ratio is constant throughout the second trimester because the increase is more accelerated in NBL than in PT, and their ratio seems to be dependent on the gestational age *(40)*.

The correlation between nasal bone hypoplasia, absent nasal bone and the correct measurement of NBL in Down syndrome fetuses between 15 and 22 weeks of gestation was published in 2002 *(15, 20, 42)*. The importance of increased PT in second-trimester screening for Down syndrome was first reported by Maymon et al. in 2005 and this technique has a sensitivity of 70% *(28)*. They combined PT and NBL measurements, yielding a 27% higher screening detection rate than NBL alone (43%). Three subsequent studies confirmed the association *(30,39,40)*. De Jong-Pleij et al (2012) in a retrospective study, first reported that the PT:NBL ratio is a strong marker for Down syndrome. In their analysis of 3D volumes of 106 euploid and 30 Down syndrome cases (20 cases on 3D volumes and ten cases on 2D volumes), the detection rate was 100% with 5% false positive rate *(40)*.

Genetic sonography can substantially increase detection rates for combined and quadruple tests, with a more modest increase in sequential protocols *(36,37,43)*. Combining PT and biochemical markers yields an 85% detection rate with 5% false positive rate. When nuchal fold thickness is added to PT, NBL, and serum markers, the sensitivity increases to 93%*(26)*. When PT, NBL, and their ratios, all in MoMs, are combined with the lengths of the second and third digits, a 76% detection rate is achieved with a 6.7% false positive rate using a 1-in-200 risk cutoff *(29)*. The combination of quadruple tests with the measurements of nuchal fold thickness and long bones can yield 90% sensitivity at a 3.1% false positive rate *(35)*.

Two-dimensional measurements of NBL*(41,44)* and PT are feasible in the first trimester*(45)*; therefore, the markers examined in that study could also be beneficial for earlier Down syndrome detection. Using a marker similar to PT (e.g., frontonasal fold thickness), one 2D study showed that the ratio of frontonasal fold thickness to NBL in a Latin American low-risk population (1922 pregnancies with four cases of Down syndrome) can easily be obtained during the second-trimester anatomy scan *(39)*.

This study presents novel evidence that the NBL:PT ratio is a better marker than the PT:NBL ratio for detecting Down syndrome fetuses. Our data indicate that the NBL:PT ratio is superior to currently used investigated ultrasound markers alone or in combination with each other or even in combination with maternal biochemistry. A limitation of our study can be that it was performed on a mixed-risk Caucasian-population. However, a point in favor of this study is that it allowed us to test the performance of these markers on a relatively large group of fetuses with Down syndrome. This study focused on a Caucasian population, and further studies are needed to evaluate the sensitivity of the ratios across different ethnic groups.

6. Summary

Currently, a number of second trimester ultrasound softmarkers for the detection of Down syndrome are in use, but none of them are really efficient and realiable. We know from preliminary 2D and retrospective 3D ultrasound studies that the ratio of prenasal thickness to nasal bone length (PT:NBL) represents a good marker of second-trimester Down syndrome fetuses *(40)*.

In this thesis the results of our prospective study is reported about the importance of the 2D ultrasound measurements of nasal bone length (NBL) and prenasal soft tissue thickness (PT) and their ratios in the second trimester screening for trisomy 21.

We successfully analyzed and characterized these important fetal facial landmarks. In euploid fetuses, the NBL:PT ratio showed gradual increase while the PT:NBL ratio demonstrated a gradual decrease over time and was more prominent in case of NBL:PT ratio than in its inverse counterpart. It was found that both ratios are highly sensitive markers for Down syndrome fetuses. The ratio of nasal bone length to prenasal thickness (NBL:PT) had performed better than its inverse counterpart for the screening of trisomy 21. Furthermore, we examined the feasibility of incorporation of the 2D ultrasound measurements of NBL and PT and the NBL:PT and PT:NBL ratios into the second trimester anomaly scan and evaluated their screening performance for the detection of Down syndrome.

In conclusion, the nasal bone length (NBL) and prenasal thickness (PT) and their ratios was found to be highly sensitive and specific markers for euploid and Down syndrome fetuses and their 2D ultrasound measurements have easily been performed and incorporated into the second trimester anatomy scan.

6.1. New observations in this study

1) In the facial profile of euploid and trisomy 21 fetuses a striking difference was observed. The nasal bone length (NBL) and prenasal thickness (PT) proved to be a sensitive marker for differentiating trisomy 21 and euploid fetuses.

2) We elaborated the method how the fetal nasal bone length (NBL) and prenasal thickness (PT) can be obtained and measured in a single volume acquisition (image) during the second trimester anatomy scan.

3) Validated normograms have been created for NBL:PT and PT:NBL ratios on the base of large number of second trimester euploid pregnancies

4) We first demonstrated and published the gradual increase in nasal bone length (NBL) and prenasal thickness (PT) between 14-28 weeks of gestation in a substantially large euploid pregnant population in contrast to other investigators who concluded their results oppositely from much smaller population.

5) Our data do not support previous observations that the ratio is constant throughout the second trimester because the increase is more accelerated in NBL than in PT, and their ratio seems to be dependent on the gestational age.

6) We confirmed in a potentially high risk Caucasian population that both NBL and PT alone are strong markers of Down syndrome, with both having a sensitivity of 76%. The combination of these two markers as a ratio increased the detection rate to 97% with a 0.9% false positive rate.

7) We first described *in the international literature* that the NBL:PT ratio showed a better sreening performance than its inverse counterpart.

8) We first published *in the international literature* that the ultrasound measurements of these new markers can sucessfully be incorporated into the second trimester fetal anatomy scan

Important note: Highly trained operators, high quality ultrasound machine, and developed data processing are considered to be the most important personal and technical conditions for performing this level of ultrasound anatomy scan extended with the measurements of NBL and PT.

7. Acknowledgements

This to acknowledge with thanks to my supervisor, Professor János Szabó,MD, DSc, allocating me this fantastic research topic and supporting me to realize it. I am grateful for his devoted supervisor activity and for helping me to perform the practical and theoretical part of my study. Thanks for his kind assistance in prepararion of the manuscripts and my PhD dissertation. I have learnt a lot from him especially the patient care and scientific mentality in ultrasound studies and modern patient-centred medical genetic activity especially the fully informed patients counselling.

I would like to thank Professor Márta Széll, DSc, the head of the Department of Medical Genetics for her kind support in the last period of my fellowship.

Special thanks to my PhD-fellow, Károly Szili, MD for his outstanding knowledge in medical IT and statistics and his dedicated work in preparation of the manuscripts.Thanks for the colleagues in the Cytogenetic Laboratory: Dr. Emese Horváth, MD, PhD, Zsuzsanna Horváth Dr. Raskóné, Dóra Isaszegi and for all members of the Department of Medical Genetics.

Thanks to my colleagues in the Department of Obstetrics and Gynecology: János Sikovanyecz, MD, PhD and János Tamás Szabó, MD for the scientific collaboration.

I am extremely grateful to all members of my family who guaranteed me the steady and quiet background.

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9. Abbreviations

2D: two-dimensional

3D: three-dimensional

AC: Amniocentesis

CVS: Chorionic Villus Sampling

DICOM: Digital Imaging and Communications in Medicine

DNA: deoxyribonucleic acid

DR: detection rate

FNR: false negative rate

FPR: false positive rate

GA: gestational age

GE: General Electric

LR: likelihood ratio

MA: maternal age

mm: millimeter

MoM: multiple of median

NBL: nasal bone length

NIPT: non-invasive prenatal test

NPV: negative predictive value

NT: nuchal translucency

PAPP-A: pregnancy associated plasma protein-A

PD: prenatal diagnosis

PGC: prenatal genetic counselling

PGD: pre-implantation genetic diagnosis

PPV: positive predictive value

PS: prenatal screening

PT: prenasal thickness

SROC: Spearman Rank Order Correlation Test

ß-hCG:beta-human chorionic gonadotropin

TNR: true negative rate

TPR: true positive rate

uE3: unconjugated Estriol

Appendix

I.

ORIGINAL ARTICLE

Nasal bone length: prenasal thickness ratio: a strong 2D ultrasound marker for Down syndrome

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ABSTRACT

Objectives To evaluate the feasibility of incorporating two-dimensional ultrasound measurements of nasal bone length (NBL) and prenasal thickness (PT) into the second-trimester anomaly scan and to determine whether the NBL : PT ratio could help in differentiating euploid and Down syndrome fetuses.

Method Two-dimensional measurements of NBL and PT were obtained from the midsagittal plane of the fetal head at 14–28 weeks of gestation in a Caucasian population at risk for aneuploidy. The screening performances of NBL, PT, and the ratios NBL : PT and PT : NBL were analyzed in euploid ($n = 1330$) and Down syndrome ($n = 33$) fetuses.

Results Nasal bone length and PT alone showed strong correlations with Down syndrome (sensitivity: 76% at 1.88% and 2.35% false positive rate, respectively). However, the NBL : PT ratio showed an even stronger correlation with Down syndrome (false positive rate: 0.9%, sensitivity: 97%). The mean NBL : PT ratio showed a gradual increase from 1.48 to 1.79 (a 21.2% increase) between 14 and 28 weeks of gestation.

Conclusion Two-dimensional ultrasound measurements of NBL and PT, particularly the NBL : PT ratio, are highly sensitive markers for Down syndrome fetuses.

Funding sources: None
Conflicts of interest: None declared

INTRODUCTION

Differences in nasal bone length (NBL), determined by ultrasound, have been suggested to differentiate second-trimester euploid and Down syndrome fetuses.[1–6] From analyses of the facial profile, a thickening of the prenasal soft tissue [prenasal thickness (PT)] is also apparent in the vast majority of second-trimester fetuses with Down syndrome. There is evidence that the combination of NBL and PT measurements as a ratio improves the detection of fetal Down syndrome by ultrasound.[6–10]

Despite the multitude of ultrasound soft markers for Down syndrome fetuses – such as increased nuchal fold thickness, cystic hygroma, cardiac anomalies, echogenic intracardiac foci, nasal bone hypoplasia, ventriculomegaly, widened iliac crest angle, short femur/humerus PT, duodenal atresia, echogenic bowel, pyelectasis-hydronephrosis, sandal gap sign, choroid plexus cyst, and midphalanx hypoplasia of the fifth finger (Appendix 1) – there are no very sensitive ultrasound markers in the second trimester that can be used either alone or in combination.[11–13] Furthermore, these markers may not be present in all affected fetuses, and such as all soft markers, they can also be detected in euploid cases.[11]

Preliminary observations using 2D ultrasound measurements of NBL and PT at our tertiary referral center suggested the potential for the second-trimester identification of euploid and Down syndrome fetuses in a mixed-risk population.[14] Therefore, we proposed that both markers and their ratios should be incorporated into the second-trimester fetal anomaly scan for a reliable, cheap, and efficient screening of Down syndrome. The current prospective study examined the clinical value of 2D ultrasound measurements of NBL, PT, and their ratios for differentiating euploid and Down syndrome fetuses in the second trimester (in an at-risk population).

METHODS

Women were referred for genetic counseling and second-trimester anomaly scans to our regional prenatal genetics center because of advanced maternal age (≥35 years); positive screening results; intermediate risk of combined, triple, or integrated tests; and the presence of one or more aneuploidy soft markers in previous ultrasound examinations. Women were recruited for second-trimester assessment and measurement of the NBL and PT values between January 2008 and April 2013. Informed consent was obtained from the mothers before examination at the MEDISONO Fetal and Adult Health Research Center or at the Department of Obstetrics and Gynecology in Szeged, Hungary.

The following criteria determined enrollment into the euploid group: singleton viable pregnancy, 14–28 weeks of gestation, a lack of maternal disease (such as hypertension, toxemia, renal disease, and diabetes mellitus), normal fetal growth, normal amniotic fluid volume, diagnosis of a normal fetal anatomy, and newborns without chromosomal or structural abnormalities between the fifth and 95th percentile birth weight at delivery.

The study included 1330 euploid and 33 Down syndrome fetuses. The protocol was approved by the ethics committee of the University of Szeged. A routine second-trimester anomaly scan in weeks 18–23 and a detailed examination of the fetal anatomy within 14–17 and 23–28 weeks of gestation were performed using a high-resolution 2D transabdominal ultrasound scanner (Voluson E8 Expert, GE Healthcare, Milwaukee, WI, USA). The facial profile was assessed at the beginning of the scanning sessions to avoid effects of fetal movements that could alter the fetal position. Three image acquisitions were obtained during one scan session, and the best one was used for analysis. If it was not successful, then the patient came back for another scanning session 30–40 min later. The sonographer was blind to the fetal karyotype, and each ultrasound examination was performed before the chromosomal study.

Nasal bone length[1,10] and PT[9] measurements can be obtained on the same image if the face of the transducer was positioned parallel to the nasal bone. The insonation angle should be close to 45°. The following image settings were used: low gain, medium dynamic contrast, and maximum magnification so that the fetal head occupied the entire screen. Images were adjusted to ensure correct midsagittal plane and sharp margins of the skin and the nasal bone. The diencephalon, nasal bone, lips, maxilla, and mandible were used as reference points for the correct measurements of NBL and PT in the midsagittal plane.[3,9] Briefly, PT was measured as the shortest distance from the lower margin of the frontal bone to the outer surface of the overlying skin. The margins of the nasal bone are the proximal and the distal ends of the white ossification line. The NBL and PT were measured using the same view (Figure 1A and B).

Maternal data and sonographic findings were recorded in a database (Astraia Software GmbH, Munich, Germany). The ultrasound imaging data were stored in the local Digital Imaging and Communications in Medicine (DICOM) format via Astraia. Values of NBL and PT were exported to Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, CA, USA). Scatter plots of NBL and PT with linear polynomial regression lines and percentile curves (third and 97th) were created. Similarly, scatter plots of NBL:PT and PT:NBL ratios with linear polynomial regression lines and percentile curves (fifth and 95th) were produced. Comparisons between euploid and Down syndrome measurements for NBL, PT [in millimeters (mm) and in multiple of medians (MoMs)], and their ratios (NBL:PT and PT:NBL) were performed using the Mann–Whitney U independent samples test. NBL, PT, and PT:NB and NB:PT ratio correlations were analyzed. No analysis of correlation was performed between any other markers.

RESULTS

The total number of the screened patients was 1470. The mean maternal age in euploid and Down syndrome cases was 30.6 years (16.6–47.1 years) and 31.5 years (21.1–42.3 years). The mean gestational age was 19.6 weeks (14.0–28.9 weeks) for euploid and 20.3 weeks (15.0–25.6 weeks) for Down syndrome cases.

Those excluded were the following: fetal structural abnormalities (24), multiple pregnancy (35), maternal conditions listed in the method (41), and chromosomal abnormalities, such as Turner syndrome ($n=1$), trisomy 18 ($n=4$), and trisomy 13 ($n=2$) (Appendix 2).

Ultrasound markers found in the Down syndrome group were increased nuchal fold thickness ($n=10$), cystic hygroma ($n=2$), cardiac defects ($n=9$), echogenic intracardiac focus ($n=4$), mild ventriculomegaly ($n=4$), short femur ($n=3$), duodenal atresia ($n=1$), hyperechogenic bowel ($n=3$), pyelectasis-hydronephrosis ($n=3$), choroid plexus cyst ($n=4$), sandal gap sign ($n=3$), and midphalanx hypoplasia of the fifth finger ($n=4$) (Appendix 3).

All invasive tests were amniocenteses, either because maternal age (≥35 years) (18 cases), a positive combined test (≥1:250) (12 cases), and second-trimester ultrasound soft markers (three cases).

The three consecutive NBL and PT measurements lasted 3 to 6 min and were completed during the first, the second, and the third attempts in 77%, 19%, and 4% of the cases, respectively.

There was a statistically significant difference ($p<0.0001$) in the NBL:PT ratio between the euploid and Down syndrome groups. Both the NBL and PT alone were found to be strong markers (sensitivity of 76% for both markers) for Down syndrome (Figure 2A and B).

A linear increase was observed in the mean NBL, the mean PT, and the mean NBL:PT ratio according to increasing gestational age between the 14th and 28th weeks. The mean NBL:PT ratio showed a gradual increase from 1.48 to 1.79 between the 14th and 28th weeks of gestation (a 21.2% increase) (Table 1). A total of 14 out of the 1330 euploid pregnancies and 32 out of the 33 Down syndrome cases were under the fifth percentile, with 97% sensitivity, 0.9% false positive rate, and 99% specificity (Table 2.)

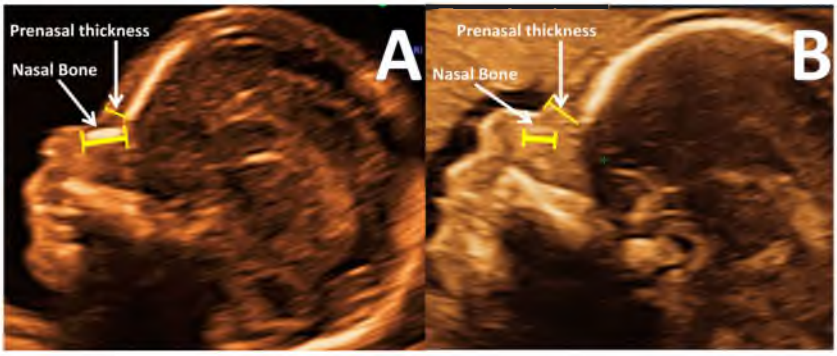

Figure 1 Examples of nasal bone length and prenasal thickness measurements obtained in euploid (A) and Down syndrome (B) fetuses

Evaluating the performance of the ratios, there were 32 true positive and one false negative Down syndrome cases identified. However, using the NBL : PT ratio, the false positive rate was 50% of those using the PT : NBL ratio (Figure 3A and B). The positive and negative cases with the calculated sensitivity, specificity, and false negative rate, using NBL, PT, the NBL : PT ratio, and the PT : NBL ratio for screening Down syndrome are summarized in Table 2.

No correlation has been found between PT and NBL with Spearman Rank Order Correlation test (SROC = 0.830 at $p < 0.05$) supporting that both markers are independent variables. The PT (PT mean: 2.0–5.8 mm) has lower values than the NBL (NBL mean: 3.0–10.0 mm), and PT (axPT average = 1.066) and NBL (axNBL average = 1.084) elevation are also different during the second trimester. Their ratios have different reference ranges because of the inverted counterparts, and the reference range of the NBL : PT ratio is wider than that of the PT : NBL ratio.

DISCUSSION

This 2D ultrasound study demonstrates that NBL and PT measurements can easily be incorporated into routine second-trimester anatomy scans. We confirmed in a potentially high risk Caucasian population that both NBL and PT alone are strong markers of Down syndrome, with both having a sensitivity of 76%. The combination of these two markers as a ratio increased the detection rate to 97% with a 0.9% false positive rate.

Furthermore, the NBL : PT ratio performs slightly better than its inverse counterpart. This is new that the NBL : PT ratio is a better marker than the PT : NBL ratio for detecting Down syndrome fetuses, primarily because it produced less false positive cases, and it can be used in cases where the nasal bone is absent. Moreover, the NBL : PT ratio can easily be calculated during the scan. If the NBL : PT ratio is less than the fifth percentile, a search for other aneuploidy soft markers and invasive fetal karyotyping should be considered.

In euploid fetuses, NBL, PT, and the NBL : PT ratio showed a linear increase with advancing gestational age. However, our data do not support previous observations[7,15] that the ratio is constant throughout the second trimester because the increase is more accelerated in NBL than in PT, and their ratio seems to be dependent on the gestational age (Table 1).[15]

The correlation between nasal bone hypoplasia, absent nasal bone, and the correct measurement of NBL in Down syndrome fetuses between 15 and 22 weeks of gestation was published in 2002.[1–3] The importance of increased PT in second-trimester screening for Down syndrome was first reported by Maymon *et al.* in 2005, and this technique has a sensitivity of 70%.[7] They combined PT and NBL measurements, yielding a 27% higher screening detection rate than NBL alone (43%). Three subsequent studies confirmed the association.[8,15,16]

De Jong-Pleij *et al.*, in a retrospective study, first reported that the PT : NBL ratio is a strong marker for Down syndrome. In their analysis of 3D volumes of 106 euploid and 30 Down syndrome cases (20 cases on 3D volumes and ten cases on 2D volumes), the detection rate was 100% with a 5% false positive rate.[16]

Genetic sonography can substantially increase detection rates for combined and quadruple tests, with a more modest increase in sequential protocols.[17] Combining PT and biochemical markers yields an 85% detection rate with a 5% false positive rate. When nuchal fold thickness is added to PT, NBL, and serum markers, the sensitivity increases to 93%.[18] When PT, NBL, and their ratios, all in MoMs, are combined with the lengths of the second and third digits, a 76% detection rate is achieved with a 6.7% false positive rate using a 1-in-200 risk cutoff.[19] The combination of quadruple tests with the measurements of nuchal fold thickness and long bones can yield 90% sensitivity at a 3.1% false positive rate.[20]

Two-dimensional measurements of NBL[21] and PT are feasible in the first trimester[22]; therefore, the markers examined in that study could also be beneficial for earlier Down syndrome detection.

Using a marker similar to PT (e.g., frontonasal fold thickness), one 2D study showed that the ratio of frontonasal fold thickness to NBL in a Latin American low-risk population (1922 pregnancies with four cases of Down syndrome) can easily be obtained during the second-trimester anatomy scan.[15]

This study presents novel evidence that the NBL : PT ratio is a better marker than the PT : NBL ratio for detecting Down syndrome fetuses. Our data indicate that the NBL : PT ratio is

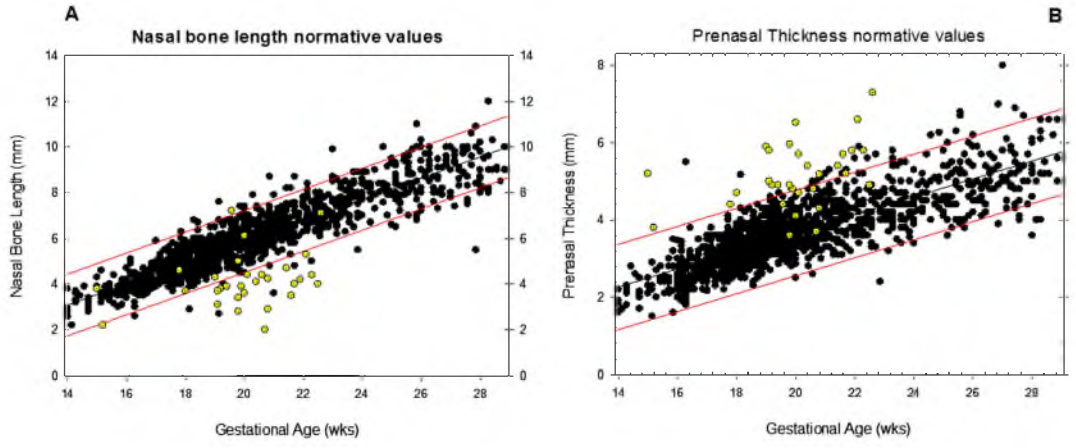

Figure 2 (A) Gestational-age-dependent nasal bone length values in 1330 euploid (black filled circles) and 33 Down syndrome (black open circles) fetuses. Approximately 76% of cases with Down syndrome fell under the third percentile. (B) Gestational-age-dependent prenasal thickness values in 1330 euploid (black filled circles) and 33 Down syndrome (black open circles) fetuses. Approximately 76% of cases with Down syndrome were above the 95th percentile

Table 1 The mean, the third, and the 97th percentiles of nasal bone length and prenasal thickness (PT) and the mean, the fifth, and the 95th percentiles of the ratios of nasal bone length to PT and PT to nasal bone length of euploid fetuses between 14 and 28 weeks of gestation

	Nasal bone length (mm)			Prenasal thickness (mm)			NBL-to-PT ratio			PT-to-NBL ratio		
Gestational age (weeks)	3rd percentile	Mean	97th percentile	3rd percentile	Mean	97th percentile	5th percentile	Mean	95th percentile	5th percentile	Mean	95th percentile
14	1.867	3.088	4.310	1.265	2.265	3.266	1.023	1.476	1.928	0.486	0.686	0.886
15	2.324	3.545	4.766	1.495	2.496	3.496	1.046	1.498	1.950	0.478	0.678	0.877
16	2.781	4.002	5.223	1.726	2.726	3.726	1.068	1.520	1.972	0.470	0.669	0.869
17	3.239	4.459	5.679	1.957	2.956	3.956	1.090	1.542	1.994	0.461	0.661	0.861
18	3.723	4.943	6.163	2.201	3.200	4.199	1.113	1.565	2.017	0.453	0.652	0.852
19	4.180	5.399	6.619	2.431	3.430	4.429	1.136	1.587	2.039	0.444	0.644	0.844
20	4.636	5.856	7.076	2.661	3.660	4.660	1.158	1.609	2.061	0.436	0.636	0.835
21	5.093	6.313	7.533	2.891	3.891	4.890	1.180	1.632	2.083	0.428	0.628	0.827
22	5.550	6.770	7.990	3.122	4.121	5.120	1.202	1.654	2.105	0.420	0.619	0.819
23	6.033	7.254	8.474	3.365	4.365	5.364	1.225	1.677	2.129	0.411	0.611	0.810
24	6.490	7.711	8.931	3.595	4.595	5.595	1.247	1.699	2.151	0.403	0.602	0.802
25	6.946	8.167	9.388	3.825	4.825	5.825	1.269	1.721	2.173	0.394	0.594	0.794
26	7.403	8.624	9.846	4.055	5.055	6.056	1.291	1.743	2.196	0.386	0.586	0.786
27	7.886	9.108	10.330	4.298	5.299	6.300	1.314	1.767	2.219	0.377	0.577	0.777
28	8.342	9.565	10.788	4.528	5.529	6.531	1.336	1.789	2.242	0.369	0.569	0.769
Correlation coefficient (r) (95% CI) $p=<0.0001$	0.916 (−1.000–0.923)			0.815 (−1.000–0.829)			0.285 (−1.000–0.326)			−0.244 (−0.286–1.000)		
Total increase in percent	346.87%	209.70%	150.29%	258.00%	144.08%	99.96%	30.54%	21.20%	16.25%	−24.10%	−17.05%	−13.18%

Table 2 **Statistical characteristics of the performance of the screening for Down syndrome using NBL, PT, their multiple of medians and their ratios**

	NBL	PT	NBL + PT	NBL MoMs	PT MoMs	NBL : PT	PT : NBL
Sensitivity (%)	75.75	75.75	87.88	69.70	69.70	96.97	96.97
Specificity (%)	98.12	97.65	97.14	98.34	96.84	99.10	98.42
False positive rate (%)	1.88	2.35	2.86	1.65	3.16	0.90	1.58
False negative rate (%)	24.24	24.24	12.12	30.30	30.30	3.03	3.03

NBL, nasal bone length; PT, prenasal thickness; MoMs, multiple of medians.

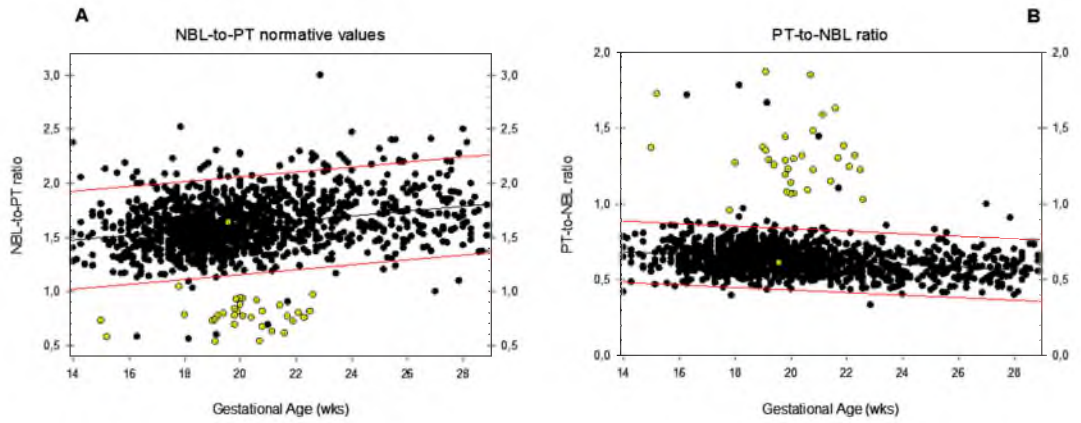

Figure 3 (A) Scatterplot of the ratio of nasal bone length to prenasal thickness in 1330 euploid (black filled circles) and 33 Down syndrome (black open circles) fetuses. All fetuses, except one, with Down syndrome fell under the fifth percentile. (B) Scatterplot of the ratio of prenasal thickness to nasal bone length in 1330 euploid (black filled circles) and 33 Down syndrome (black open circles) fetuses. All fetuses, except one, with Down syndrome were above the 95th percentile

superior to currently used investigated ultrasound markers alone or in combination with each other or even in combination with maternal biochemistry. A limitation of our study is that it was performed on a mixed-risk Caucasian population. However, a point in favor of this study is that it allowed us to test the performance of these markers on a relatively large group of fetuses with Down syndrome. This study focused on a Caucasian population, and further studies are needed to evaluate the sensitivity of the ratios across different ethnic populations.

CONCLUSION

In conclusion, 2D ultrasound measurements of NBL and PT can easily be performed within the second-trimester anomaly scan, and their ratios appear to be highly sensitive and specific markers for euploid and Down syndrome fetuses. The 2D measurements of these markers and their ratios can be incorporated into the second trimester anatomy scan.

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Preliminary 2D and retrospective 3D studies show that the ratio of prenasal thickness to nasal bone length (PT : NBL) is a strong marker of second-trimester Down syndrome fetuses.

WHAT DOES THIS STUDY ADD?

- The ratio of nasal bone length to prenasal thickness (NBL : PT) had performed better than its inverse counterpart for the screening of trisomy 21.
- In euploid fetuses, the PT : NBL and the NBL: PT ratios showed gradual increases over time.
- Two-dimensional ultrasound measurements of NBL and PT were successfully incorporated into the routine second-trimester anomaly scan.

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APPENDIX 1. Ultrasound soft markers for Down syndrome fetuses in the second trimester

Nuchal fold thickness (NF)
Cystic hygroma
Cardiac anomalies
Echogenic intracardiac foci/golf ball sign
Nasal bone hypoplasia (NBL)
Increased prenasal thickness (PT)
Widened iliac crest angle
Short femur
Short humerus
Ventriculomegaly
Duodenal atresia
Pyelectasis-hydronephrosis
Echogenic bowel
Sandal gap sign
Choroid plexus cyst
Midphalanx hypoplasia of the fifth finger

APPENDIX 2. Excluded euploid pregnancies

	Number of cases (*N*)
Multiple viable pregnancy	35
MATERNAL disease	18
Abnormal amniotic fluid volume	10
Fetal structural abnormalities	24
Chromosomal or structural abnormalities	7
Abnormal birth weight at delivery(<5th and >95th)	41
Fetal loss/death in second and third trimester	3

APPENDIX 3. Trisomy 21 cases scan results

	Number of cases (*N*)
Increased nuchal fold thickness	10
Cardiac defects	9
Echogenic intracardiac focus	4
Mild ventriculomegaly	4
Choroid plexus cyst	4
Midphalanx hypoplasia of the fifth finger	4
Hyperechogenic bowel	3
Pyelectasis-hydronephrosis	3
Short femur	3
Sandal gap sign	3
Cystic hygroma	2
Duodenal atresia	1

APPENDIX 4. Interobserver and intraobserver variability

Table A. Interobserver and intraobserver variability of nasal bone length (NBL) and prenasal thickness (PT) in absolute (mm) and relative (%) values at 95% limits of agreement (LoA) and their confidence intervals (CI)

Intraobserver ($n = 1.330$)						
Measurement	Mean relative difference	95% CI	95% Lower LoA	95% CI	95% Upper LoA	95% CI
NBL	0.59%	0.398% to 0.786%	−6.48%	0.398% to 0.786%	7.66%	7.330% to 7.993%
PT	0.97%	0.787% to 1.163%	−5.86%	−6.185% to −5.543%	7.81%	7.493% to 8.134%
Measurement	Mean difference	95% CI	95% Lower LoA	95% CI	95% Lower LoA	95% CI
NBL (mm)	0.028	0.0164 to 0.0403	−0.406	−0.4268 to −0.3860	0.463	0.4427 to 0.4835
PT (mm)	0.039	0.0320 to 0.0464	−0.223	−0.2351 to −0.2105	0.301	0.2889 to 0.3135
Interobserver ($n = 102$)						
Measurement	Mean relative difference	95% CI	95% Lower LoA	95% CI	95% Upper LoA	95% CI
NBL	−0.14%	−0.769% to 0.494%	−6.47%	−7.551% to −5.386%	6.19%	5.112% to 7.276%
PT	−0.11%	−0.649% to 0.436%	−5.55%	−6.477% to −4.617%	5.33%	4.404% to 6.264%
Measurement	Mean difference	95% CI	95% Lower LoA	95% CI	95% Upper LoA	95% CI
NBL (mm)	−0.010	−0.067 to 0.047	−0.442	−0.540 to −0.344	0.421	0.324 to 0.519
PT (mm)	−0.004	−0.030 to 0.023	−0.204	−0.249 to −0.159	0.197	0.151 to 0.242

Intraobserver and interobserver variability

These are preliminary data for intraobserver and interobserver variability; a Bland–Altman analysis[23] was used to describe intraobserver and interobserver variability.

Methodology: Two images were saved: one with calipers and one without calipers. To assess interobserver variability, the measurements of these two markers were repeated after the scanning by another operator, who remeasured the markers as previously described.The intraobserver variability analysis was performed on the three images, which were taken during the scan session.

Results: The limits of agreement (LOA: 95% CI) were −6.48% to 7.66% and −5.86% to 7.81% for NBL and PT, respectively. The respective interobserver variability 95% limits of agreement were −6.47% to 6.19% and −5.55% to 5.44% (Appendix Table A). There is a very low and not significant difference that has been confirmed.

Conclusion: There is a need to have further studies on the measurement education and on the intraobserver and interobserver variability of PT and NBL, and have it published as a separate article.

II.

A prenazális lágyrész-vastagodás a 21-es triszómia ultrahangjele a második trimeszterben

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Összefoglaló

Célkitűzés: A 21-es triszómia szűrése az első trimeszterben a tarkóredő mérésével és a biokémiai markerek, nevezetesen a szabad b-hCG és a PAPP-A, segítségével nagy hatékonyságú módszer, míg a második trimeszterben - megbízható ultrahangjelek hiányában - igen nagy kihívást jelent a szakemberek számára. Az utóbbi években a prenazális lágyrész vastagságának mérésére terelődött a figyelem a második trimeszterben. Tanulmányunk célja az említett, ultrahanggal mérhető jel normogramjának és szűrővizsgálati hatékonyságának meghatározása euploid és 21-es triszómiában szenvedő magzatok esetén.

Betegek és módszerek: A méréseket prospektív vizsgálatok keretében, euploid és 21-es triszómiában szenvedő magzatokon végeztük el a várandósság 16-24 hetében. A prenazális lágyrész-vastagság mérését a magzati arcprofil közép-szagittális síkjában, hasi ultrahangvizsgálattal (Voluson E8 és Voluson 730 Expert, GE Medical Systems, Zipf, Austria) 2D-ben végeztük. Prenazális lágyrész-vastagságnak az *os frontale* alsó csontos pólusától a felette lévő bőr külső felszíne közötti legrövidebb távolságot tekintettük. Az adatokat *Astraia* programban és excel file-ban rögzítettük. A következő statisztikai módszereket alkalmaztuk: regressziós analízis, Shapiro-Wilk teszt, Kolmogorov-Smirnov-teszt és a Mann-Whitney U-teszt, SigmaStat statisztikai programban.

Eredmények: Ötszáz euploid és tizenkilenc 21-triszómiás magzat adatainak elemzése során megállapítottuk, hogy a különbség a két csoport értékei között nem véletlenszerű. A Mann-Whitney U-teszt alapján a különbség a két csoport között statisztikailag szignifikánsnak bizonyult. A 21-es triszómiában szenvedő magzatok túlnyomó többségében a prenazális lágyrész-vastagság az euploid magzatok 95 percentilise fölé esik.

Következtetés: A prenazális lágyrész-vastagság értéke a várandósság második trimeszterében önmagában is igen hatékony ultrahangjele a 21-es triszómiának. Alkalmazásával még hatékonyabbá válhat a Down-szindróma szűrése.

Kulcsszavak: Down szindróma, 21-es triszómia, ultrahangszűrés, második trimeszter, prenazális lágyrészvastagság, orrcsonthosszúság

Szabó A, Szili K, Szabó JT, Isaszegi D, Horváth E, Sikovanyecz J, Szabó J
The prenasal thickness a good second trimester marker of trisomy 21.

Summary

Objective: Down-syndrome screening in the first trimester based on increased nuchal translucency (NT) and biochemical markers of free b-hCG and PAPP-A has been established to be a very efficient method. However, in the absence of efficient ultrasound marker, the screening for trisomy 21 is a great challenge in the second trimester. Therefore in the last several years research has been focused on the measurement of

the prenasal soft tissue thickness and the nasal bone length. In this period the purpose of our study was the evaluation of the prenasal thickness in the second trimester screening for trisomy 21.

Patients and Methods: In a prospective study prenasal thickness (PT) was measured in euploid and in trisomy 21 fetuses with transabdominal 2D ultrasound (GE Voluson E8 and GE- 730). Measurement of the prenasal thickness was performed in mid-sagittal plane of the fetal head identifying the appropriate landmarks. Prenasal thickness was defined as the shortest distance from the lower edge of the *os frontale* to the outer surface of the overlying skin. Data were registered in Astraia programme and in excel files. For statistical methods regression analysis, Shapiro-Wilk test, Kolmogorov-Smirnov-test and Mann-Whitney U-test were used in Sigmastat, statistical software.

Results: We analyzed the results of 500 euploid and 19 fetuses with trisomy 21. The difference in the median PT values between the two groups was greater than it would be expected by chance. There was a statistically significant difference (P = <0,001) according to Mann-Whitney Rank Sum Test. The majority of the PT values of 19 fetuses with trisomy 21 fell above the 95th percentile of the euploid group.

Conclusion: The ultrasound measurement of prenasal soft tissue thickness alone was found to be highly efficient marker for trisomy 21. Using this sonographic marker in the second trimester the trisomy 21 screening may become more effective.

Keywords: Down syndrome, trisomy 21, second trimester, ultrasound screening, prenasal thickness, nasal bone length

Bevezetés

A 21-es triszómia az első trimeszterben a tarkóredő vastagsága (*nuchal translucency, NT*), az orrcsont *(nasal bone)* vizsgálata és a biokémiai markerek alapján nagy hatékonysággal szűrhető, míg a második trimeszteri ultrahangszűrés - megbízható jelek hiányában - igen nagy kihívást jelent a szakemberek számára [1-4].

Bár a 21-es triszómiának a második trimeszterben is vannak jól meghatározott ultrahangjelei, amelyek felhívják a figyelmet a szindróma lehetőségére, az érintett magzatok egy része az euploidoktól alig mutat eltérést, így emiatt az esetek jelenős részét a második trimeszterben sem ismerik fel [4]. Ismert tény az is, hogy a szűrés eredményessége jelentősen függ a vizsgáló jártasságától [5].

Az utóbbi években, főként a magzati arcprofil elemzése vezetett oda, hogy finomabb jelek, mint például az orrcsont hypoplasia, aplasia, az orrgyök-homlokcsont szöglet és az úgynevezett prenazális lágyrész-vastagodás (PLV) - angol nevén *prenasal thickness (PT)* - mérése hasznos adatokat szolgáltathat a kórkép második trimeszteri szűrésében [6, 7].

A tanulmányunk célja volt, hogy prospektiv vizsgálat során összehasonlítsuk az euploid és a 21-es triszómiában szenvedő magzatok esetén a prenazális lágyrész- vastagságot és megállapítsuk ennek jelentőségét a Down szindróma szűrésében.

Betegek és módszerek

Ötszáz euploid és tizenkilenc, a későbbiekben citogenetikai módszerrel igazolt, 21-es triszómiában szenedő magzat PT mérését végeztük el prospektív tanulmány keretében, a várandósság 16-24 hete között.

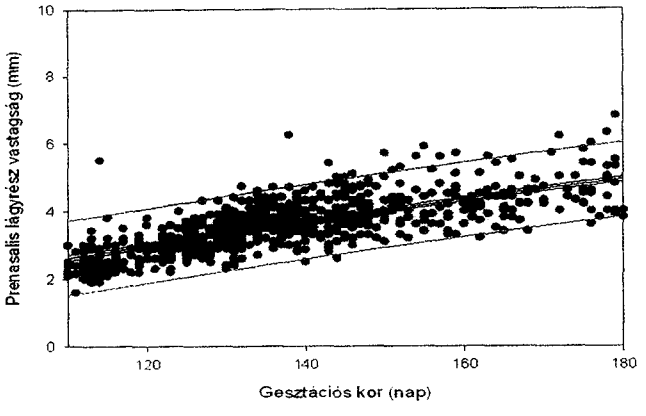
A prenazális lágyrész-vastagság mérését a magzati arcprofil közép-szagittális síkjában 2 dimenziós hasi ultrahangvizsgálattal (Voluson E8 és Voluson 730 Expert, GE Medical Systems, Zipf, Austria) végeztük. Az orrhegy, az orrcsont, az ajkak, a maxilla, a mandibula és a diencephalon egy síkba állítása után a beeső ultrahangnyalábbal az orrcsontot merőlegesen pásztáztuk. Beállítási nehézség esetén legfeljebb 30 fokos homlokirányú eltérést tartottunk megengedhetőnek. A prenazális lágyszövet vastagságnak az *os frontale* alsó csontos pólusától a felette lévő bőr külső felszíne közötti legrövidebb távolságot tekintettük. A nagyító (zoom) funkcióval a képet akkorára növeltük, hogy a magzat arcprofilja a képernyőnek csaknem az egészét elfoglalja (*1. ábra*). Ugyanebből a beállításból az orrcsont hosszúsága is mérhető, így ezzel a mérési módszerrel a hiányzó orrcsont esetén is meg lehet mérni a prenazális lágyrész- vastagságot.

Statisztikai analízis

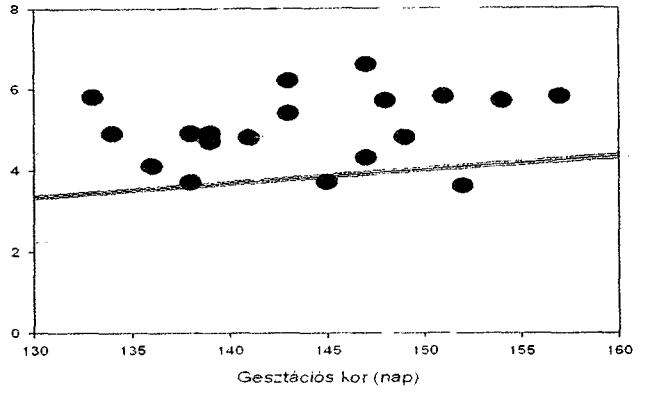
A PT értékeket az Astraia szülészeti és nőgyógyászati software adatbázisából (astraia GMBH, Germany, Münich) kérdeztük le és ellenőrzés céljából MicroSoft Excel programba importáltuk, majd statisztikai elemző programmal vizsgáltuk annak érdekében, hogy regressziós analízist végezzünk és hogy meghatározzuk az eloszlást és annak normalitási fokát, valamint a gesztációs kor (GA) és a prenazális lágyrész-vastagság (PT) közötti kapcsolatot. A Shapiro-Wilk normálitási vizsgálat bizonyítot-

1. ábra 19 hetes euploid magzat prenazális lágyrész-vastagsága. A fehér vonal jelzi a mérendő lágyrész-vastagságot és a helyes mérést.

2. ábra 19 hetes 21-es triszómiás magzat prenasalis lágyrész-vastagsága. A fehér vonal jelzi a mérendő lágyrész-vastagságot és a helyes mérést.



3. ábra Euploid magzatok prenazális lágyrész-vastagság nomogramja a terhességi napok függvényében



4. ábra A 21-es triszómiás magzatok prenazális lágyrész-vastagságának (PLV) értékei az euploid nomogramon

ta a normál eloszlást az euploid és a 21-es triszómiában szenvedő magzatok között. A delta értékre ellenőrzés céljából a Kolmogorov-Smirnov-tesztet alkalmaztuk, amely megerősítette feltevésünket azáltal, hogy a delta értékek eloszlása mind az euploid, mind a 21-es triszómiás magzatokon szignifikáns korrelációt mutatott.

Mann-Whitney-U-teszttel hasonlítottuk össze az euploid és a 21-es triszómiában szenvedő magzatok PT-értékeit. Az adatok statisztikai elemzéséhez SigmaStat (SigmaStat és SigmaPlot; San Jose, California, USA) programot használtunk, amely szignifikáns értékeket mutatott ($p < 0,005$) és polynomális súlyozott eloszlás szerint ábrázoltuk. Ezután a grafikonokat és a diagramokat szerkesztés és publikálás céljából Excel 2007 programba (Microsoft Corp., Redmond, WA, USA) importáltuk.

Eredmények

Ötszáz euploid és tizenkilenc, 21-es triszómiában szenvedő magzatnál végeztük el a méréseket a várandósság 16-24. hete között a 2009-2011-es években. A vizsgálatban euploid egyes terhességeket tanulmányoztunk, ahol nem volt sem kromoszóma-, sem strukturális rendellenesség. Az *1. ábra* egy euploid magzat, a *2. ábra* pedig egy 21-es triszómiában szenvedő magzat prenazális lágyrész-vastagsága mérésének módját, a mérőkeresztek (caliper) helyes elhelyezését mutatja.

A terhességi kort a prenazális lágyrész-vastagság függvényében ábrázolva egy normogramot kaptunk (*3. ábra*). A normogramról leolvasható, hogy a prenazális lágyrész-vastagság terhességi korral arányosan növekszik. Hasonló normogramon ábrázoltuk a 21-es triszómiában szenvedő magzatok PT-értékeit. A 21-es triszómiás magzatok PT-értéke a 95 percentilis fölé esett, jelentősen meghaladva az euploid magzatok prenazális lágyrész-vastagságát (*4. ábra*).

Megbeszélés

A prenazális lágyrész-vastagodás (PT) jelentőségére triszómiás magzatoknál először *Maymon és mtsai.* (2005) hívták fel a figyelmet [6]. A 14 -27. hét között húsz, 21-

es triszómiában szenvedő és 500 euploid magzat esetében mérték a prenazális lágyrész- vastagságot. Megállapították, hogy a 21-es triszómiás magzatoknál a PT lényegesen nagyobb, mint az ép kariotípussal rendelkező magzatokban. Tanulmányuk szerint a prenazális lágyrész-vastagság és az orrcsont hosszúságának együttes mérésével 70 %-os volt a felismerési arány, 5 %-os álpozitív arány mellett, míg az orrcsont hosszának mérésével önmagában csak 43 %-os szűrési hatékonyságot értek el. A két marker együttes alkalmazását hatékonyabbnak találták a Down-szindróma szűrésében.

Persico és mtsai. (2008) 135 euploid és 26, cytogenetikailag bizonyított 21-es triszómiás magzat arcprofiljának elemzése kapcsán, a 16-24. hét közötti prenazális lágyrész-vastagság méréseinek eredményeit közölték [7]. Megállapították, hogy a 21-es triszómiás magzatok 73,1%-ánál a PT 95 percentilis feletti tartományba esett. Arra a következtetésre jutottak, hogy a PT vizsgálata érzékeny módszer a Down-szindróma második trimeszteri szűrésében. A 21-es triszómia egyéb ultrahangjelei, mint a ventriculomegalia, nuchalis oedema, hiányzó orrcsont, vagy szívdefektus nem befolyásolták ezt a hatékonyságot.

Maymon és mtsai. korábbi eredményüket azzal egészítették ki, hogy az anyai életkoron és a PT-értéken alapuló táblázatot készítettek a triszómia kockázatának „bedside" értékelése céljából [8]. Vizsgálatuk szerint jól elkülönülnek a 21-es triszómiás és az euploid magzatok multiples-of-median (MoM)-ban kifejezett medián PT-értékei: a 21-es triszómiásoknál 1,31 MoM volt, míg 1,01 MoM az euploid magzatok esetén adódott.

Miguelez és mtsai. a prenazális lágyrész-vastagság mérését biokémia markerekkel kombinálva a 21-es triszómia magas detekciós arányát találták [9].

De Jong-Pleij és mtsai., 106 euploid és harminc, 21-es triszómiában szenvedő magzatban mérték a prenazális lágyrész-vastagságot, az orrcsont hosszúságát 3D-ben a második és harmadik trimeszterben [10]. Az elmentett képek utólagos elemzése során azt találták, hogy a PT és az orrcsont hosszúsága (*nasal bone legth, NBL*) a terhességi korral együtt nő és a kettő aránya 21-es triszómiás magzatoknál 95 percentilis fölé esett.

Prospektív tanulmányunk megerősíti az irodalmi adatokat, miszerint a prenazális lágyrész-vastagság (PLV) a terhességi korral arányosan növekszik euploid magzatok esetében. A prenazális lágyrész-vastagság a 21-es triszómiában szenvedő magzatok jelentős részében 95 percentilis felett van. Ez a viszonylag könnyen mérhető ultrahangjel rutinszerűen alkalmazható a várandósság második trimeszterében és jelentősen növeli a 21-es triszómia méhen belüli szűrésének hatékonyságát.

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Levelezés

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III.

OP33.11
A new scoring system for the diagnosis of placenta accreta by ultrasound

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Objectives: Our aim was to determine the accuracy of a novel simple scoring system based on sonographic markers in differentiating between low and high risk forplacenta accreta (PA).
Methods: All women who were referred to the Sheba Medical Center due to suspected PA were included, underwent a detailed ultrasound examination. A score was given based on the common sonographic findings of PA: loss of the hypoechoic retroplacental zone and placental lacunae. A score of 0–2 was defined as low risk and 3 was defined as high risk. Patients assigned to the high risk category underwent prophylactic pelvic artery catheterization before cesarean section and embolization if needed, whereas patients in the low risk category underwent simple cesarean section.
Results: 71 women were included. PA was diagnosed clinically during surgery in 28 women, of whom 31 had a score of 3, and ruled out in 43 women, of whom only one had a score of 3. The sensitivity, specificity, positive predictive value and negative predictive value of our ultrasound-based scoring system in predicting PA were 90%, 97.5%93 and 95% respectively.
Conclusions: A simple scoring system based on ultrasound alone can identify accurately a high risk population for PA who can benefit from prophylactic pelvic artery catheterization and embolization.

OP34: SCREENING FOR ANEUPLOIDY AND CONSULTING IN THE SECOND TRIMESTER

OP34.01
Extracellular chromosome 21: derived microRNAs in maternal circulation: evaluation of their diagnostic potential for screening of Down syndrome

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Objectives: In this pilot study we focused on the detection of extracellular microRNAs in maternal circulation, whose genes are located on human chromosome 21 (miR-99a, let-7c, miR-125b-2, miR-155 and miR-802). Subsequently, we studied if plasmatic concentrations and/or expression profile of extracellular chromosome 21-derived microRNAs would distinguish between pregnancies bearing euploid foetuses and those affected with Down syndrome.
Methods: 12 women with normal course of gestation (mean 16.4 weeks, median 16.0 weeks), 12 pregnancies bearing Down syndrome foetus (mean 18.2 weeks, median 18.5 weeks) and 6 non-pregnant individuals were involved in the retrospective study. RNA enriched for small RNAs (including microRNAs) was isolated from 1 ml of plasma sample. Consequently relevant microRNA was transcribed into cDNA using specific stem-loop primer and detected by specific real-time PCR assay.
Results: Commercial systems enabled reliable detection of 4 out of 5 extracellular chromosome 21-derived microRNAs (miR-99a, let-7c, miR-125b-2 and miR-155). Expression profile of extracellular miR-99a, miR-125b-2 and miR-155 was significantly higher in the cohort of pregnant women than in non-pregnant individuals. Also plasmatic levels of miR-99a and miR-125b-2 were significantly increased in pregnant women. Unfortunately, the concentrations and gene expression of extracellular chromosome 21-derived microRNAs (miR-99a, let-7c, miR-125b-2 and miR-155) did not differ between the cohorts of pregnancies bearing euploid foetuses and those affected with Down syndrome.
Conclusions: Analysis of extracellular chromosome 21-derived microRNAs does not distinguish between pregnancies with euploid and aneuploid foetuses and has no benefit for screening programmes.
Acknowledgement: The work was supported by GAUK no. 434011.

OP34.02
Ultrasound screening of Down-syndrome in the second trimester: the prenasal thickness alone

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Objectives: Down-syndrome screening in first trimester based on increased nuchal translucency (NT) and biochemical markers is very effective. However, in second trimester it is a great challenge in the absence of efficient ultrasound marker. During the last years several reports suggested that prenasal soft tissue thickness and nasal bone hypoplasia could be sonographic markers for Down-syndrome screening. We measured and compared the prenasal thickness (PT) in euploid and in fetuses with Down-syndrome prospectively.
Methods: Transabdominal 2D ultrasound (Voluson E8) measurement of the prenasal thickness was performed in mid-sagittal plane of the fetal head identifying diencephalon, tip of the nose, lips, maxilla, nasal bone. The insonation angle was 90° to the nasal bone or maximum 30 degree of lifting to the frontal bone was allowed. The prenasal thickness was defined as the shortest distance from the lower edge of the os frontale to the outer surface of the overlying skin. The nasal bone can also be determined from this view. The magnification of the view (zoom) was zoomed such that the fetal profile occupied the whole screen.
Results: We analyzed the results of 810 euploid and 19 fetuses with trisomy 21 measured between the 16–23 gestational weeks. In euploid fetuses the mean PT (and NBL) increased steadily between 16 and 33 weeks' gestation. The difference in the median PT values between the two groups was greater than it would be expected by chance. There was a statistically significant difference ($P < 0.001$) according to Mann-Whitney Rank Sum Test. All of the 19 fetuses with trisomy 21 the PT values were lower than 5th percentile curve of the euploid group.
Conclusions: The ultrasound measurement of prenasal soft tissue thickness was found to be highly efficient marker alone for trisomy 21. The Down-syndrome screening with this marker can become more effective.

OP34.03
Prenasal thickness, nasal bone length and their ratio: good second trimester sonographic markers for Down syndrome

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Objectives: Down syndrome screening in first trimester based on nuchal translucency (NT) and biochemical markers is very efficient, while in second trimester it is a great challenge. Measurement of nasal bone length and prenasal soft tissue thickness was found to be promising facial landmarks in second trimester screening

for Down-syndrome. We prospectively measured and compared prenasal soft tissue thickness (PT) and nasal bone length (NBL) in second trimester euploid and trisomy-21 fetuses.
Methods: Using 2D abdominal ultrasound the measurement of PT and NBL was taken in mid-sagittal plane of the fetal head identifying diencephalon, tip of the nose, lips, maxilla, mandible, nasal bone in weeks 16–23 in the second trimester. The PT is the shortest distance from the bottom edge of the os frontale to the outer surface of the overlying skin. The nasal bone was measured from this view. The insonation angle was 90° (perpendicular) to the nasal bone. NBL/PT ratios of euploid fetuses between 16–23 weeks were analyzed and validated by Shapiro-Wilke test. We used software (SigmaStat 12 & SigmaPlot 12) to create graphs, the regression line and the percentiles curves (5^{th}–25^{th}–75^{th}–95^{th}) have been calculated from the normal values. We used the normal graphs to visualize and compare trisomy 21 cases to euploids ($P < 0.0001$).
Results: Analyzes of 810 euploid and 19 fetuses with trisomy 21 measured between the 16–23 gestational weeks were done. In euploid fetuses the mean PT and NBL increased steadily between 16 and 33 weeks' gestation. The difference in the median PT values between the two groups was greater than would be expected by chance. There was a statistically significant difference ($P = <0.001$) Mann-Whitney Rank Sum Test. All of the 19 fetuses with trisomy 21 the NBL/PT values were lower than 5^{th} percentile curve of the euploid group.
Conclusions: CIn fetuses with trisomy 21 the NBL/PT ratio was significantly lower compared to euploid ones. The NBL/PT ratio was found to be very sensitive and specific marker for trisomy 2.

OP34.04
Fetuses with single umbilical artery: a seven year study

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Objectives: Study of incidence, fetal development, anatomy and birth of single umbilical artery (SUA) fetuses during seven years.
Methods: A retrospective study of 6 148 low risk singleton pregnancies. Number of umbilical arteries was determined using color flow imaging of the fetal pelvis between 11+0 and 13+6 gestation weeks. Medical, obstetric ultrasound records and postnatal maternal questionnaires were reviewed.
Results: SUA was antenataly diagnosed in 15 pregnancies (incidence 0.24%), and isolated SUA in 73% of the cases ($n = 11$). 93.3% of the pregnancies resolved in live birth. In one case pregnancy was terminated due to multiple anomalies (omphalocela, anasarca, bilateral choroid plexus cyst, regurgitation of atrioventricular valves). Amniocentesis was performed in 5 cases of the US verified isolated SUA. In all the results were normal. Screening for neural abnormalities showed choroid plexus cyst in 13% of the cases, dilated posterior ventricle in 6.7%, same as absent corpus callosum. Fetal echocardiography revealed no increase in incidence of heart defects while hyperechogenic focus of the left ventricle prompted genetic exploration and yielding normal results in one case. Antenatal pyelon abnormalities were detected in 6.7%. Estimated fetal weight was distributed as follows: below 10^{th} percentile 15.4%, 10–49^{th} percentile 49.1%, 50–90^{th} percentile 30.8% and above 90^{th} percentile 7.7%. At birth 4 fetuses had pelvic presentation, and Cesarean section performed in all (26.7%). Postnatal questionnaires revealed presence of birth defects in two babies (absence of an ear lobe in the first, atresia of the biliary duct in the second) which were not diagnosed prenatally.
Conclusions: Vigilant and frequent antenatal monitoring of SUA fetuses focusing on fetal anatomy is warranted, knowing chromosopathies are linked to anatomical abnormalities. Incidence may be statistically small, but the consequences of prenatally undiagnosed abnormalities on the quality of life are great.

OP34.05
Single umbilical artery: is it innocent?

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Objectives: Single umbilical artery (SUA) is found in 1% of pregnancies. It can be diagnosed in the first and second trimester. The two vessel cord is associated with chromosomal trisomies and a number of structural abnormalities such as spina bifida, renal and heart defects, intrauterine growth retardation, intrauterine demise and impaired school achievements. Some severe defects can only be diagnosed after birth.
Methods: Referrals of couples with single umbilical artery were made from the Department of Obstetrics and Gynecology of University and from the regional obstetric hospitals and private clinics. A database was established and analyzed with statistical methods. Associated anomalies were classified according to severity and organ system occurrence.
Results: Eighty eight couples with diagnosis of SUA attended our Prenatal Clinic between 2005 and November, 2011. Sixteen of them were first trimester diagnosis, and 4 out of them proved to be three vessels (3-VC) at the control examination. In 59 cases the SUA was recognized in weeks 19–23, two of them proved to be false diagnosis. Mean age was 29,86 years, mean body weight was 69 kg. Male/female ratio was 46/36. Genetic advice was accepted by most of the pregnant, except three, and one of them gave birth to a newborn with trisomy 21. Chromosomal aberration was revealed in two cases: a trisomy 18 and a trisomy 21. Kidney and heart defects were found in four cases. A lethal tracheal stenosis was revealed month after birth.
Conclusions: Majority of SUAs are recognized in weeks 18–23. Genetic counseling is suggested and chromosomal study is indicated except in very low risk cases for chromosomal defects calculated according to ultrasound and biochemical tests. Our data shows that the risk of trisomies is high in cases of SUA and the clinician should consider for sevear adverse fetal outcome, too.

OP34.06
Etiology and perinatal outcome of pregnancies with polyhydramnios

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Objectives: Polyhydramnios occur in 1–2% of pregnancies. While the majority is idiopathic, conditions like gestational diabetes (GDM), congenital malformations and viral infections may be associated. This work presents etiology of polyhydramnios and the respective perinatal outcome.
Methods: Etiology and perinatal outcome of pregnancies diagnosed with polyhydramnios at the Department of Obstetrics and Gynecology, Medical University Graz, Austria, between 2003 and 2011 were retrospectively analyzed.
Results: 976 affected pregnancies were identified, from which 166 (17.0%) were excluded due to incomplete data. 152 (18.8%) of the remaining 810 cases were associated with GDM, 73 (9.0%) with congenital malformations and 24 (3.0%) with viral infections, while 560 (69.1%) were idiopathic. The latter had the best outcome, while those with malformations had higher rates of preterm delivery, lower Apgar scores and low birth weight. The group with viral infections had nearly the same outcome as the idiopathic group. Elective Caesarean sections were equally

IV.

Identified fetal heart malformations in the first trimester. Experiences after 1000 examination

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Objectives:

According to the literature, 80-90% of congenital heart defects (CHD) are a product of low-risk pregnancies. The international datas also demonstrate that in a high-risk population succesful transabdominal ultrasound assessment of the fetal heart can be carried out at 11-13 weeks by well trained obstetricians in >95% of cases and that >90% of major cardiac defects can be identified at this gestation. The aim of this study was to evaluate the feasibility and accuracy of fetal echocardiography at the first trimester extended screening performed by well trained obstetricans in a group of unselected, consecutive pregnants.

Patients and Method:

In this retrospective study, fetal echocardiography was performed between January 2010 and March 2012. Within this period we examined 1007 unselected, consecutive patients, singletons, twins and triplets, (1033 fetuses) presented in our private fetal medicine centre for first trimester extended screening. The vast majority of examinations were carried out transabdominally using a 4-8 MHz convex transducer (Accuvix V20, Samsung-Medison). The examinations were performed by two obstetricians with extensive experience in first and second trimester screen. Digital videoclips and photo documentations of the cardiac scan were stored. All abnormal findings during the routine assessment were reviewed by a fetal cardiologist, and a genetic counsellor was also involved in the consultation with the patients. Follow-up US evaluations during the second trimester were offered to all patients, the high risk group was also referred to fetal cardiologist.

Results:

In the group of 1007 unselected pregnancies 983 were singeletons, 22 were twins and 2 were triplets. The median maternal age was 33,4 (range 20-44) years. Among the 1033 fetuses included in this study the obstetricans identified 6 cases with severe heart malformations: two AV septal deffect, one VSD, one pulmonary stenose, one pulmonary vein transposition and one aortic stenose(?). In the group classified as normal only 353 patients chose our centre for subsequent second trimester scan. Among them, we identified cardiac defect in two cases: one aortic stenose at 17 weeks, and one dislocated heart, related to a right sided diaphragmatic hernia at 20 weeks.

Conclusion:

First trimester assesment of the fetal heart is not only feasible, but also very profitable in an unselected population, when performed by experienced obstetricians. To determine our centre's degree of accuracy in the first trimester fetal heart screening, further data analysis is necessary. However, altough most types of severe CHDs can be diagnosed early in pregnancy, some may become apparent later in gestation. Our experiences also suggest that all cases classified as abnormal should be referred to cardiologist; either for better evaluation of the abnormality or to confirm the diagnosis, in order to give more appropiate counselling to the parents.

Ultrasound screening for Down-syndrome in the second trimester: the prenasal thickness

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Objective:

Down-syndrome screening in first trimester based on nuchal translucency (NT) and biochemical markers is very effective, while in second trimester the screening is a great challenge. Despite of the fact that Down-syndrome has ultrasound soft markers, which are remarkable, a small proportion of of fetuses with Down-syndrome do not show marked alterations from euploid ones. That's why some

cases of Down-syndrome is not detected even in the second trimester. In the last years, especially analyzing the fetal face called attention to nasal bone hypoplasia, aplasia, and thickening of the prenasal soft tissue indicating them, useful tools in sonographic screening for trisomy 21. Our aim was to elaborate a better screening protocol. We measured prenasal soft tissue thickness and nasal bone length in euploid and aneuploid fetuses.

Methods:
The measurement of the prenasal thickness was taken in a mid-sagittal plane of the fetal head indentifying lips, maxilla, mandible, diencephalon, nasal bone and tip of the nose with abdominal ultrasound. The insonation was perpendicular (90°) to the nasal bone or maximum a 30 degree inclination towards the *os frontale* was acceptable. The prenasal thickness is the shortest distance from the bottom part of the *os frontale* to the outer surface of the overlying skin. The nasal bone can also be measured from this view. The magnification of the view (zoom) should be such that the fetal profile occupy the two-third of the image.
Statistical analysis:
The data were analyzed according to polynomial distribution.
Results: We analyzed the results of 500 euploid and 19 fetuses with 21 trisomy measured between the 16-24 gestational weeks. In fetuses with trisomy 21 the prenasal thickness was found to be significantly larger, i e it was above 95 percentile in trisomy 21 fetuses.
Conclusion:
The prenasal soft tissue thickness measured in the nasal bridge is a strong marker for trisomy 21. Using these marker the Down-syndrome screening can become more effective.

Septostomy vs. amniocentesis in case of twin-to-twin transfusion syndrome
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Twin to twin transfusion syndrome (TTTS) is a disease of the placenta that effect only identical twin pregnancies. The placenta contains abnormal blood vessels which connects the circulations of the twins. Depending on the number, type and direction of the connecting vessels, blood can be transfused disproportionately from one twin (the donor) to the other twin (recipient). The recipient twin becomes overloaded with blood with the consequence of heart failure and polyhydramnion. The transfusion causes the donor twin to have decreased blood volume, which leads to growth retardation and poor urinary output as a consequence of oligohydramnion. The polyhydramnion causes prematurity very often. The fetal and neonatal loss rate is very high, without treatment it's mortality rate is 90-100%. The neonatal morbidity rate is 10-30% resulted from fetal hypoxia. The frequency of TTTS is 15% among monochorionic twins.
Treatments:
1. Reduction amniocentesis: Removal of the excessive amniotic fluid from the sac of the recipient twin.
2. Septostomy: Creation of a hole in the membrane between the babies' sacs using a needle. This procedure allows the amniotic fluid to be equalized between the two amniotic sacs. Septostomy can slow down the development of polyhydramnion hereby avoiding prematurity. Amnionreduction and septostomy are not causal treatment of TTTS.
3. Laser ablation of the placental anastomotic vessels: Laser ablation of the communicating vessels on the placenta between the twin fetuses. This is a causal treatment of TTTS, but fetal and neonatal loss rate is similarly high to the previous procedure. Other disadvantage of the procedure is the need of a special and expensive instrument.
The author would like to share his experience about amnionreduction and septostomy.