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

Summary of PhD thesis

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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 *(WHO 1985, 1989).* 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 *(Global Report On Birth Defects,2006).*

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 *(Simpson et al, 2011).* 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).* 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 is generally defined as the in vitro testing the embryo before embryo transfer.

1.2.2. Prenatal screening or non-invasive methods means to detect embryos or fetuses with normal or abnormal features during their intrauterine life. The recently advocated methods to identify affected pregnancies are the ultrasound anatomy scan and the maternal blood tests.

1.2.3. Prenatal diagnosis or invasive methods 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).

1.2.4. Prenatal genetic counseling (PGC) 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 *(Tóth A,et al., 2008)*.

2. The 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 *(Bianchi, 2010 Fetology)*.

The prevalence of trisomy 21 is 1,3/1000 at birth in developed, high income countries, but it can reach 2-3 per 1,000 in middle- and low-income countries: a rate approximately double that currently seen in high-income countries *(Global Report On Birth Defects, 2006).*

Avoiding the 1% risk abortion stimulated the development of new, not risky screening techniques for defining pregnancies with high and low risk for chromosomal abnormalities.

2.1.2. Other trisomies: The second and third most frequent chromosomal trisomies are the Edwards syndrome (the trisomy 18), and Bartholin-Pätau syndrome (the trisomy 13), respectively. They also show an association with advanced maternal age.

2.2. Prenatal screening of Down syndrome: the beginning

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 *(Szabó and Gellén)* in 1990. This observation was approved on a larger pregnant population by *Nicolaides et al, 1992.* The measurement of NT was standardized and has become the basis of first trimester screening for Down syndrome. The free beta choriogonadotropin (free B-hCG), and pregnancy associated plasma protein-A (PAPP-A) in maternal blood and other ultrasound markers (presence or absence of nasal bone, tricuspidal and ductus venosus flow) was added to the test further increasing the sensitivity first trimester screening up to 93-95% at a 2,5% fals positive rate.

Despite the multitude of ultrasound soft markers for Down syndrome fetuses such as increased nuchal fold thickness, cardiac anomalies, nasal bone hypoplasia, ventriculomegaly, widened iliac crest angle et cet. there are no sensitive ultrasound markers in the second trimester that can be used either alone or in combination *(Benacerraf et al,2010,Cuckle H et al, 2013,Agathokleous et al,2013).*

2.3. Preliminary observations

During the last decades the nasal bone length (NBL), its presence, absence and the hypoplasia and the prenasal thickness (PT) and their ratio was suggested increase the detection rate of second trimester Down-syndrome *(Sonek et al,2001,2003Maymon et al,2005).*

Considering the data from the literature and our good experience with the PT:NBL and NBL:PT ratio for trisomy 21 screening, a study was started from January, 2008 and these markers were incorporated into our second trimester fetal anomaly scan.

2.4. 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) within 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 paralell 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 trimster 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.

3. Materials and methods

3.1. Materials

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.

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 and anatomy, and newborns without chromosomal or structural abnormalities between the fifth and 95th percentile birth weight.

3.2. Methods

3.2.1. Measurement of prenasal thickness and nasal bone length

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. Nasal bone length and prenasal thickness 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°. Images were adjusted to ensure correct midsagittal plane. 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 NBL and PT were measured using the same view.

3.2.2. 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.

3.2.3. 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:NB and NB:PT ratio correlations were analyzed. No analysis of correlation was performed between any other markers.

4. 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. 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.

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).

4.1. Statistics on the screening performance of this method

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. 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% T1 increase).

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.

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. 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. 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.

5. Discussion

This 2D ultrasound study demonstrates that NBL and PT measurements can easily be carried out wthin 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.

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.

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 *(Sonek J et al 2002, Bromley B,2002).* 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% *(Maymon R,et al 2005).* They combined PT and NBL measurements, yielding a 27% higher screening detection rate than NBL alone (43%). Three subsequent studies confirmed the association. *(Maymon R, et al 2008, Gonzalez R,et al 2013, De Jong-Pleij et al 2012). 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 a 5% false positive rate. *(De Jong-Pleij et al, 2012).*

Two-dimensional measurements of NBL *(Casasbuenas A et al 2009)* and PT are feasible in the first trimester *(Miron JP et al 2012)* ; 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 *(Gonzalez R,2013).*

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

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.

6. 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 screening 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.

7. 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, Alasztics Bálint, Bánhidy Ferenc, Valent Sándor
A 21-es triszómia szűrése napjainkban ***ORVOSI HETILAP*** **145:**(26) *pp. 1026-030.* (2013)
4. 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)
5. 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.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. (2013)
2. 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**
3. 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)
4. 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)
5. 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)

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