First-trimester screening of foetal chromosomal and structural abnormalities

Kornélia Emma Wayda

Ph.D. Thesis

Supervisor: János Szabó, M.D., Ph.D., D.Sc.

Department of Medical Genetics

Faculty of General Medicine

Albert Szent-Györgyi Medical and Pharmaceutical Centre

University of Szeged, Szeged, Hungary

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ABBREVIATIONS

AC	Amniocentesis
AFP	Alpha-foetoprotein
AS	Aortic stenosis
ASD	Atrial septal defect
CC	Chordocentesis
CCC	Continuous cardiac control
CHD	Congenital heart defect
CI	Confidence interval
CRL	Crown-rump length
CVS	Chorionic villus sampling
E_3	Unconjugated oestriol
FISH	Florescence *in situ* hybridisation
hCG	Human choriongonadotropin
HLHS	Hypoplastic left heart syndrome
IUGR	Intrauterine growth retardation
IVF	*In vitro* fertilisation
MS	Mitral stenosis
NICU	Neonatal Intensive Care Unit
NT	Nuchal translucency
OR	Odds ratio
P	Probability value
PAPP-A	Pregnancy-associated plasma protein A
PDA	Patent ductus arteriosus
PS	Pulmonary stenosis
SD	Standard deviation
SLE	Systemic lupus erythematosus
TOP	Termination of pregnancy
VSD	Ventricular septal defect

1. PUBLICATIONS RELATED TO THE THESIS

I. **Wayda K**, Keresztúri A, Orvos H, Horváth E, Pál A, Kovács L, Szabó J. Four years of experience in the first-trimester nuchal translucency screening of fetal aneuploidies with its increasing regional availability. Acta Obstet Gynecol Scand, in press.

II. **Wayda K**, Horváth E, Métneki J, Orvos H, Keresztúri A, Isaszegi D, Szabó J. The pre- and postnatal sex ratio of the main autosomal trisomies. In Hungarian. (A gyakoribb autoszómális triszómiák pre- és posztnatális nemi aránya.) Magy Nőorv L, in press.

III. Orvos H, **Wayda K**, Kozinszky K, Katona M, Pál A, Szabó J. Increased nuchal translucency and congenital heart defects in euploid fetuses. The Szeged experience. Eur J Obstet Gynecol Reprod Biol, in press.

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Scientific abstract:

I. Szabó J, Keresztúri A, Keszthelyi G, Gellén J, Faragó M, Horváth E, **Wayda K**, Szabó-Nagy A, Pál A. First trimester ultrasound screening for fetal chromosomal and other abnormalities. Eur J Obstet Gynecol Reprod Biol 2000;91:S23.

2. PUBLICATIONS NOT RELATED TO THE THESIS

I. Tanaka M, **Wayda K,** Molnár J, Párkányi C, Aaron JJ, Motohashi N. Antimutagenicity of benzo[a]phenothiazines in chemically induced mutagenesis. Anticancer Res 1997;17:839-842.

II. Tanaka M, **Wayda K,** Molnár J, Motohashi N. Antimutagenicity of benzo[a]phenothiazines in chemically induced mutagenesis. Anticancer Res 1996;16:3625-3628.

3. INTRODUCTION

During the past few years there has been a rapid development in prenatal genetic diagnostics. New obstetric and laboratory methods have been developed and introduced into clinical practice. With the introduction of chorionic villus sampling (CVS), transvaginal sonography, early amniocentesis(AC) and first-trimester endoscopy, more and more anomalies can be recognised *in utero* in the first trimester of pregnancy. These new methods can be successfully applied from the 9th week of gestation.

Currently, 4-5% of neonates are born with congenital anomalies. One of the most important groups of these anomalies is the chromosomal aneuploidies, among which the numeric chromosomal aneuploidies have the greatest clinical role (60). The prevalences of Down, Edwards and Patau syndromes at birth are 1/700, 1/3000 and 1/5000, respectively, and they rise with maternal age (60). Prevention plays a great role, as these illnesses cannot be cured or treated efficaciously. The other important group is the structural anomalies, prevention of which should also be emphasised.

The primary prevention is the avoidance of the development of the disease, but this is possible in only a small percentage of congenital anomalies. During the secondary prevention, suspicious cases should be recognised. This secondary prevention should be simple, effective, economically viable, painless, safe and humane.

There is a high-risk group of pregnants:

A. those over 35 years old, as the risk of trisomies gradually rises with the age of the mothers,

B. those with previous chromosomal aneuploidy in their family (parental or neonatal),

C. those whose ultrasound screening shows any type of abnormality.

These pregnants should be screened carefully and invasive testing such as CVS, AC, chordocentesis (CC), or analysis of serum or urine biochemical markers should be offered to them if necessary.

A possible diagnostic method for the detection of trisomy 21, other chromosomal aneuploidies, foetal cardiac defects and certain genetic syndromes in the first trimester is the screening of increased nuchal translucency (NT) thickness. NT is defined as the normal subcutaneous space, observed on first-trimester ultrasound examination, between the skin and

the soft tissue overlying the cervical spine in the foetus (19). (Figure 1,2) This space can be increased in size in the presence of a cystic hygroma or nuchal oedema, both of which can be detected in the first trimester (19). Cystic hygroma are bilateral, septated cystic structures that present a congenital malformation of the foetal lymphatic system, resulting in overdistension of the jugular lymphatic sacs. NT, also known as nuchal oedema, is caused by the subcutaneous accumulation of fluid resulting in a simple, non-septated lesion (19). Although cystic hygroma are often associated with Turner syndrome, a simple NT is more often associated with other aneuploidies, particularly Down syndrome (15,50,55,71,72,76-78). In 1990, Szabó and Gellén first reported the correlation between a nuchal fluid accumulation >3 mm in the first-trimester foetus and trisomy 21 (79). In addition to this association with aneuploidy, multiple studies have now identified an increased NT thickness as a non-specific marker of a range of foetal structural abnormalities and various genetic syndromes (6,7,14,25,30,43,48,74,86).

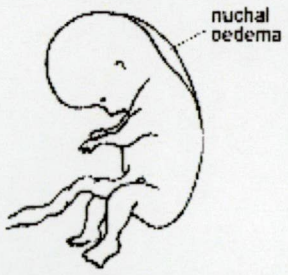


Figure 1. Draft of nuchal translucency

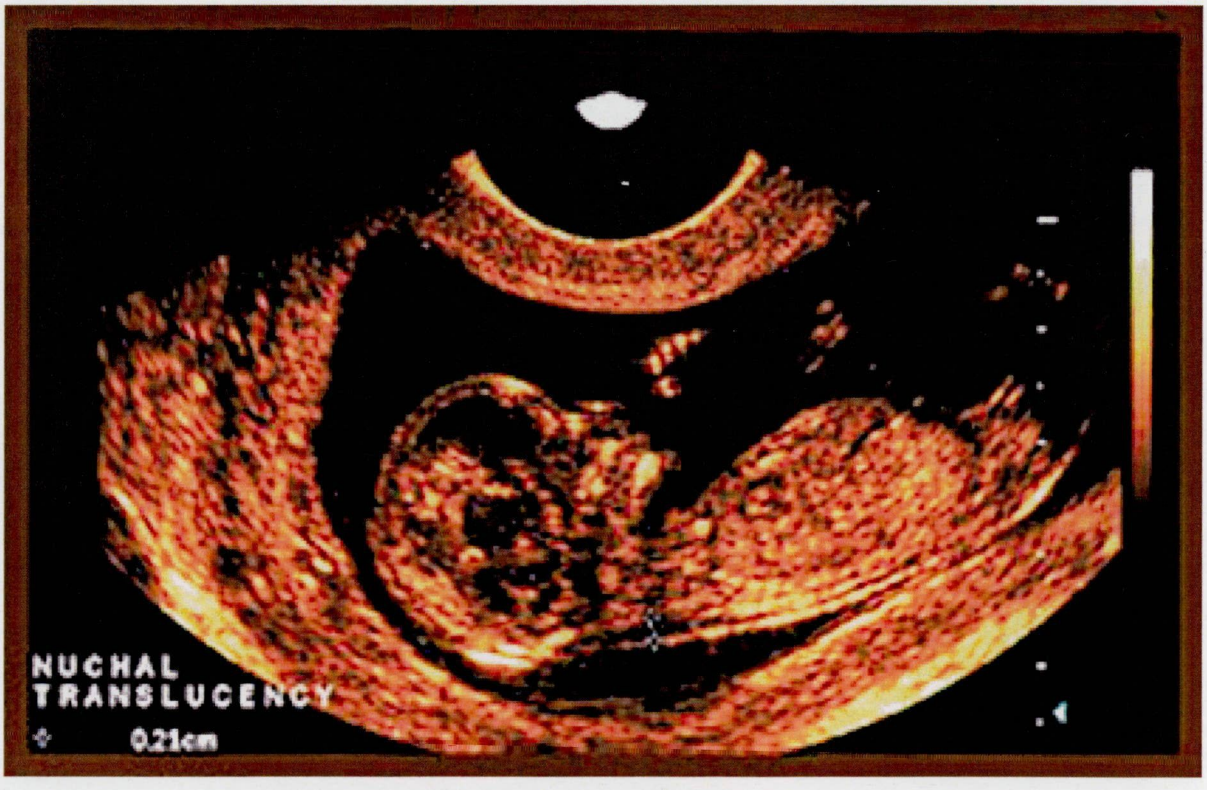


Figure 2. An ultrasound image showing the measurement of first-trimester foetal NT thickness

Congenital heart defects (CHDs) are the most common congenital abnormalities, with a prevalence of 3-8 per 1000 pregnancies, *i.e.* 30% of the total congenital malformations, but most of them are not identified in the course of routine antenatal scanning (19,22,32). At present, screening for cardiac defects is routinely undertaken as part of the second-trimester scan, at 18 to 22 weeks of gestation, by examining a four-chamber view. Evaluation with foetal echocardiography at 20 weeks of gestation can identify approximately 70% of major cardiac abnormalities (10). However, identification of those pregnancies that require this specialised evaluation remains a challenge. Currently, general population screening for foetal cardiac anomalies is based on maternal and family history, together with examination of the four-chamber view of the heart during routine ultrasound at 18 to 20 weeks of gestation. A recent screening study examining the sensitivity of the four-chamber view at 16 to 20 weeks' revealed an identification rate of only 26% of major cardiac defects (82). Another study reported a sensitivity of 61% on examination of the four-chamber view and the outflow tracts, as well (27). The sensitivity of second-trimester cardiac screening can be increased to approximately 80% by inclusion of the outflow tracts during sonographic evaluation (70).

On the other hand, there is another method, which combines prenatal screening for cardiac defects and the assessment of first-trimester foetal NT thickness. An association between cardiac defects and increased NT thickness has been recognised in the first trimester. Four studies have reported a 81% detection score of foetal cardiac defects by using a first-trimester thickened NT (1,9,26,27). To further assess the relationship between cardiac defects and foetuses with abnormal karyotype and increased NT thickness, Hyett *et al.* (37) reported on a pathological examination of the foetal heart after first-trimester termination of pregnancy (TOP) in 36 foetuses with increased NT thickness and trisomy 21. This evaluation showed atrioventricular or ventricular septal defects (ASD, VSD) in 56% of these foetuses, with the incidence of cardiac septal defects increasing with NT. Studies have also shown that there is an association between cardiac defects and increased NT in chromosomally normal foetuses. Hyett *et al* (39) reported pathological findings observed from the examination of 21 chromosomally normal foetuses with increased NT after TOP. Abnormalities of the heart and great vessels were found in 19 (90%) of the 21 cases. Other studies have reported major cardiac defects in more than 75% of foetuses with trisomy 21 and trisomy 18, in which NT was greater than 4 mm (36-38). Hyett *et al.* (40) also found that the prevalence of major cardiac defects among 1389 chromosomally normal foetuses from a high-risk population with a foetal NT greater than the 95th percentile at 10 to 14 weeks of gestation increased with the NT. In that study, the prevalence of major abnormalities of the heart and great vessels was 17

per 1000. Hyett *et al.* (41) completed a retrospective review evaluating 29,154 singleton pregnancies from an undefined patient population with presumed normal karyotype. Six groups of major cardiac defects were identified in this series. Although increased NT was observed with all types of major abnormalities of the heart and great vessels, there was a stronger association with hypoplastic left heart syndrome (HLHS) and coarctation of the aorta. HLHS is the term used to describe a group of congenital heart lesions characterised by severe underdevelopment of the left heart structures. This association with left-sided defects is supported by a series published by Moselhi *et al.* (48), which identified increased NT as a possible marker for coarctation of the aorta.

Several case reports and small series have also suggested that, in chromosomally normal foetuses, there may be an association between first-trimester increased NT thickness and a wide range of foetal abnormalities and genetic syndromes. The overall incidence of associated structural malformations in these studies ranges from 3% to 75%, with emphasis on cardiac, renal and skeletal anomalies, omphalocele and diaphragmatic hernia (6,25,30,43,48,74,86).

Therefore, it seems that increased NT thickness in the first trimester is associated with an increased risk of major foetal cardiac and structural abnormalities. This increased risk is present in both euploid and aneuploid pregnancies and seems to be proportionate to the degree of NT enlargement (19).

Differences in sex ratios have been noted in cases involving the main trisomies (trisomy 13, 18 and 21). Sex ratio is defined as the number of males divided by the number of females (sometimes multiplied by 100). The primary sex ratio is that observed at conception, and the secondary one is that at birth. Extrapolations suggest that the primary sex ratio for all conceptions of whites may be approximately 1.15 (34); among live births, it is calculated to be 1.06-1.07 (44), the same as in Hungary (18). The major factor affecting the secondary sex ratio appears to be race, with a low of 1.02 in American Indians, and a high of 1.15 reported in Korea (44).

It is frequently suggested that males are more at risk of foetal wastage than females, and that the sex ratio at birth therefore reflects the health of the intrauterine environment, a high sex ratio indicating a good environment, and a low sex ratio an adverse one.

4. AIMS

The main aim of my studies was to gain a better insight into the possibilities provided by first-trimester ultrasound screening for foetal aneuploidies and other structural abnormalities.

1. Examination of the effectiveness of first-trimester ultrasound in the screening of foetal aneuploidies.
2. Standardisation of the conditions of prenatal screening, setting up a cut-off level of NT thickness.
3. The literature contains controversial data on the sex ratio of foetuses and neonates with trisomy 21, 18 and 13. Accordingly, we reinvestigated this issue by analysing these trisomies.
4. Study of the association between first-trimester increased NT thickness and foetal cardiac defects, especially HLHS.
5. Study of the association between first-trimester increased NT thickness and major congenital structural malformations.
6. Establishment of a first-trimester ultrasound scan programme to screen foetal aneuploidies and cardiac defects and major congenital structural abnormalities.
7. Evaluation of the number of pregnants who take part in genetic counselling, and improvement of its efficacy.

5. MATERIALS AND METHODS

All of my examinations were performed at the Department of Obstetrics and Gynaecology, at four regional hospitals and at the Department of Medical Genetics, Faculty of General Medicine, Albert Szent-Györgyi Medical and Pharmaceutical Centre, University of Szeged, Szeged, Hungary.

5.1. NT thickness measurement

The ultrasound examinations were performed by using a 6.5-7.5 MHz vaginal probe (Combison 530, 3D, Kretz Technik). The NT thickness was measured at 10-13 weeks of gestation between the outer skin surface and the soft tissue overlying the foetal spine in the sagittal plane of the foetus in a "quiet or neutral" position. The maximal zoom of the ultrasound screen was used. The foetal NT thickness was evaluated only after embryo movements, in order to distinguish between the foetal skin contour and the amnion. The number of foetuses, the crown-rump length (CRL), the foetal movement and heart activity, the extremities and foetal structural anomalies were also evaluated. NT thicknesses of ≥2.5 mm were defined as positive cases and were referred from the regional hospitals to our tertiary genetic counselling clinic for consideration of an invasive test. Informed consent was obtained before ultrasound examinations and invasive testing, and all the women participated in the study voluntarily.

All the sonographers involved had received a licence for first-trimester scanning after theoretical and practical training at our centre.

5.2. Invasive testing

Invasive tests were offered according to the classical genetic indications, *i.e.* a maternal age ≥35 years, a previous child with chromosomal abnormalities, parental chromosomal abnormalities, and a NT ≥2.5 mm. Women could choose between an earlier rapid test (CVS), or later AC or CC. Invasive tests were offered to patients over 35 years of age, irrespective of the NT, in accordance with the current legal regulations in Hungary. The

invasive procedures were performed transabdominally with ultrasonically guided needles, using a freehand technique. The outer diameters of the CVS, AC and CC needles were 1.1 mm, 0.8 mm and 0.8 mm, respectively.

5.3. Follow-up

For those who declined invasive tests, a thorough ultrasound follow-up was advised in order to seek early second-trimester markers of foetal aneuploidies such as an increased NT thickness, cardiac defects, echogenic bowels, pyelectasis, and other structural defects, combined with the alpha-foetoprotein (AFP) level at 16 weeks of gestation. All other women underwent an anomaly scan at weeks 18 to 20.

Information on the pregnancy follow-up and outcome was obtained through written questionnaires or by telephone from the patients, from the regional hospitals and/or from the nursing midwife network.

5.4. Foetal aneuploidies

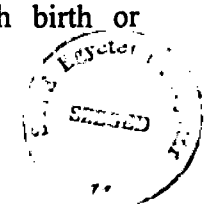
In a prospective screening study between 1995 and 1998 at the Szeged University Prenatal Clinic and at four regional hospitals, the NT thickness was measured at 10 to 12 weeks of gestation by transvaginal ultrasound examination in 7044 women with singleton or multiple pregnancies. Informed consent was obtained before ultrasound examinations and invasive testing, and all the women participated in the study voluntarily.

The study did not cover the whole population in the region, in spite of the increasing quantity of available information on the importance of first-trimester ultrasound screening.

From October 1997, we used in parallel the USS program kindly provided by Professor Nicolaides from the Foetal Medicine Foundation, London, but NT thickness of 2.5 mm remained as an interventional cut-off.

5.5. Cardiac defects

The pre- and postnatal findings, the course and outcome of the pregnancies and the relationship between the first-trimester foetal NT thickness and foetal CHDs in euploid foetuses were retrospectively analysed in 4309 pregnancies that ended with birth or



therapeutic abortion at the Department of Obstetrics and Gynaecology, University of Szeged, between 1 January 1998 and 30 June 2000.

At our Department, routine foetal echocardiography has been performed in high-risk cases since 1 October 1999. The pregnants in the high-risk group have

- A family history of CHD,
- maternal diabetes mellitus,
- maternal systemic lupus erythematosus (SLE),
- teratogen exposure,
- any type of foetal malformation,
- intrauterine growth retardation (IUGR),
- chromosomal anomalies.

However, this examination was also made in suspicious cases during this period.

Newborns were examined on the first and fourth postnatal days by a neonatologist. In the event of any clinical sign of CHD (*i.e.* cyanosis, a murmur, an increased oxygen requirement or fatigue), a paediatric cardiologist was involved in exploring the diagnosis.

5.6. Hypoplastic left heart syndrome

Between 1 January 1998 and 31 August 2001, 6639 births and 78 therapeutic abortions were recorded at our University. During this period, eight euploid cases with HLHS were diagnosed. The relationship between the first-trimester foetal NT thickness and foetal left-sided heart defects and neonatal outcomes were analysed.

Neonates were transmitted to the Neonatal Intensive Care Unit (NICU) because of the suspicion of a congenital vitium, and a paediatric cardiologist established the diagnosis.

5.7. Other major structural abnormalities

This retrospective study was carried out to assess the efficiency of establishing the risk of structural abnormalities by using the foetal NT thickness at 10 to 13 weeks of gestation in chromosomally normal foetuses. Scans were carried out between 1 January 1998 and 31 December 2000. During this period, 5186 births were recorded at our Department. Ninety-seven singleton pregnancies with major congenital malformations were recorded. Of these, 30

were diagnosed *in utero* by sonographers, while the others were diagnosed postnatally by a neonatologist.

The procedure used in cases of CHDs is described above.

A neonatologist postnatally routinely examined all newborns soon after delivery. Neonates who needed intensive care or paediatric surgery were transferred immediately to the NICU for intensive observation or operation.

5.8. Sex ratio

Three sources of data were used for analysis of the sex ratios of the prenatal and live birth cases:

1. the prenatal register of aneuploidies of Szeged between 1990 and 1999,
2. the Down register of Szeged between 1970 and 1999,
3. the Down register of Hungary between 1970 and 1999.

These registers contain data on the mother (name, age and address) and on the foetus or the child (gestational age at karyotyping, karyotype, date of TOP and outcome of pregnancy). During the statistical analysis, chi-square probe was used.

6. RESULTS

6.1. Foetal aneuploidies

Between January 1995 and December 1998, 7044 women were enrolled in the first-trimester ultrasound screening study. Of these, 203 were excluded from further analysis since it was impossible to obtain any written or oral information concerning the pregnancy outcome.

As regards the remaining 6841 pregnancies, 4821 were examined in the regional centres and 2020 (29.5%) were referred to or primarily examined at the Department of Obstetrics and Gynaecology. There were 6750 (98.67%) singleton and 91 (1.33%) multiple pregnancies (85 sets of twins, 3 sets of triplets, 2 sets of quadruplets and 1 set of quintuplets). The 2 sets of quadruplets and the quintuplet pregnancy were each reduced to 2 foetuses at the request of the parents. All these multiplets were euploid. Spontaneous abortion occurred between the NT screening and the planned invasive tests in 8 patients, and up to week 20 in another 15 pregnancies.

The median maternal age was 31 years (range 16-46). The NT was ≥3 mm in 191 (2.8%) and ≥2.5 mm in 308 (4.5%) cases (Table I), to whom invasive tests were offered; however, 54 declined.

Six hundred and seven (8.87%) of the screened women underwent invasive genetic tests: 463 involved CVS, 97 AC and 47 CC. Two hundred and fifty-four of the 607 invasive tests were based on a NT thickness of ≥2.5 mm, 323 were based on maternal age (≥35 years) alone (NT <2.5 mm) and 30 were based on other indications (*e.g.* previous parental or neonatal chromosomal abnormalities). All those women who did not undergo invasive tests gave birth to apparently normal neonates, as checked by paediatricians.

An abnormal karyotype was found in 33 (0.48%) cases, *i.e.* 5.4% of the total number of those who participated in invasive examinations (Table II). A NT ≥2.5 mm was measured in 32 (96.97%) of the foetuses with an abnormal karyotype. For a cut-off of 3 mm, this changed to 84.8%. In the group of invasive tests based on maternal age and previous chromosomal abnormalities (353 cases), only 1 chromosomal aneuploidy was found (Table II, serial number 18).

The mean NT values were 3.76 mm for trisomy 21, 5.75 mm for trisomy 18 and 4.23 mm for trisomy 13. The maternal age was ≥35 years in 18 (54.54%) of the 33 aneuploid pregnancies. A foetal NT thickness <3 mm (2.8 mm) was measured in only 1 of the 15 mothers <35 years of age (33 years old), while 4 of the foetuses of the 18 women aged ≥35 years had a NT thickness <3 mm (1.2, 2.5, 2.8 and 2.9 mm).

In the 191 (2.8%) foetuses where the NT was ≥ 3 mm, the prevalence of chromosomal defects was 14.7% (28 cases). When a cut-off of 2.5 mm was applied (308 pregnants), this figure changed to 10.4% (32 cases).

Tables III and IV present the sensitivity, the specificity, the positive and negative predictive values and the false-positive rate for cut-off values of 2.5 mm and 3 mm in all aneuploid cases and in cases with trisomy 21.

Table I. Distribution of NT thickness among pregnants screened during the first trimester

NT thickness (mm)	<35 years	≥35 years	Total number of cases
0-0.9	773	288	1061
1-1.9	2673	1638	4311
2-2.4	686	475	1161
Total <2.5 mm	4132 (97.43%)	2401 (92.35%)	6533 (95.5%)
2.5-2.9	54	63	117
3-3.9	24	91	115
4-4.9	11	24	35
5-5.9	9	11	20
6-6.9	5	7	12
7-7.9	2	2	4
8-8.9	2	1	3
9+	2	0	2
Total ≥2.5 mm	109 (2.57%)	199 (7.65%)	308 (4.5%)
Total:	4241	2600	6841

Table II. Chromosomal aneuploidies screened by NT measurement at a gestational age of 70 to 91 days

Serial number	Maternal age (years)	Gestational age (days)	NT thickness (mm)	Karyotype
1	42	86	2.5	47,XX+21
2	33	71	2.8	47,XY+21
3	36	91	2.8	47,XY+21
4	42	70	2.9	47,XY+21
5	25	85	3	47,XX+21
6	38	89	3.1	47,XY+21
7	39	74	3.2	47,XX+21
8	36	98	3.2	47,XY+21
9	40	73	3.3	47,XY+21
10	27	73	3.5	47,XY+21
11	30	93	3.6	47,XY+21
12	28	86	3.7	47,XX+21
13	23	91	4	47,XY+21
14	36	92	4.1	47,XX+21
15	38	97	4.3	47,XY+21
16	39	80	6.3	47,XX+21
17	36	96	8.4	47,XY+21
18	41	80	1.2	47,XY+18
19	33	78	4.2	47,XY+18
20	36	78	4.3	47,XX+18
21	33	80	4.6	47,XY+18
22	26	94	5.4	47,XX+18
23	29	87	7.4	47,XY+18
24	40	82	8	47,XX+18
25	24	89	11.1	47,XY+18
26	20	83	3.1	47,XY+13
27	40	73	3.6	47,XX+13
28	42	75	3.9	47,XY+13
29	28	80	6.3	47,XY+13
30	28	88	11	45,XO
31	39	91	3	46XX/46XY/47XXX/47XXY
32	24	89	8.5	69,XXX
33	35	95	3.5	47,XY+14q partial trisomy

Table III. Efficiency of NT screening with a cut-off level of ≥2.5 mm for all aneuploidies and trisomy 21

	All aneuploidies (%)	Trisomy 21 (%)
Sensitivity	96.97	100
Specificity	95.94	95.74
Positive predictive value	10.39	5.52
Negative predictive value	99.98	100
False-positive rate	4.05	4.26

Table IV. Efficiency of NT screening with a cut-off level of ≥3 mm for all aneuploidies and trisomy 21

	All aneuploidies (%)	Trisomy 21 (%)
Sensitivity	84.85	76.47
Specificity	97.61	97.39
Positive predictive value	14.66	6.81
Negative predictive value	99.92	99.94
False-positive rate	2.39	2.61

6.2. Cardiac defects

Between January 1998 and June 2000, 4251 births and 58 therapeutic abortions (altogether 4309 pregnancies) were recorded at our Department of Obstetrics and Gynaecology. At birth, 151 congenital malformations were diagnosed; 34 of them were known prenatally. In the 58 pregnancies that ended with therapeutic abortion, there were 22 foetal chromosomal abnormalities, 19 neural tube defects and 17 severe multiple foetal malformations. Altogether, 209 (4.9%) congenital malformations were detected, 39 (18.7%) of which were CHDs. Eight of the 39 CHDs were diagnosed by foetal echocardiography, while the others were suspected on neonatal examination and were confirmed by ultrasonography. The prevalence of major CHDs was 9 per 1000 pregnancies (39/4309).

First-trimester foetal NT was measured in 35 of the 39 foetuses with CHDs: it was ≥3 mm in 18 (51.4%) and <3 mm in 17 (48.6%) (Tables V and VI). Table V shows the CRL at which the NT thickness was measured in 36 pregnancies. The types of CHDs, the gestational age in weeks at term and the outcomes are also listed. The NT was not measured in 4 foetuses with CHDs. The NT thickness was ≥3 mm in 15 and ≥2 mm in 20 of 32 foetuses with CHDs. Table VI presents data on 3 additional foetuses with prenatally detected severe CHDs combined with other structural abnormalities. The NT thickness was >3 mm in all 3 cases. With regard to the virtual certainty of extremely unfavourable outcomes of these 3 pregnancies, therapeutic abortion was offered (after careful and extensive non-directive counselling) by members of the University Foetal Board (obstetrician-sonographer, paediatric cardiologist, neonatologist, heart surgeon and geneticist). In all 3 cases, the couples opted for therapeutic abortion, which was performed in weeks 17, 22 and 23, respectively. Autopsy confirmed the prenatal findings.

The CHDs were classified into 5 main groups: HLHS; VSD and ASD; tetralogy of Fallot; transposition of the great vessels; and others (Table VII). An increased first-trimester NT was found predominantly in foetuses with defects in the left-heart tract (75%) or with ASD or VSD (58.8%). All foetuses with transposition of the great vessels had a NT <3 mm. The numbers indicate only the tendency, and are insufficient for a statistical evaluation.

A sensitivity of 51.4%, a specificity of 97.6%, a positive predictive value of 17.8%, a negative predictive value of 99.5% and a false-positive rate of 4.1% were found at a cut-off of 3 mm (Table VIII). The measurement of increased NT for the detection of foetal cardiac abnormalities had sensitivities of 51.4% and 71.9 % at a cut-off of 3 and 2 mm, respectively.

The outcomes of pregnancies involving foetal CHDs are detailed in Table IX. The mean neonatal birth weight was 3141.7±549.7 grams and the mean neonatal gestational age was 38.83±1.3 weeks. There were 24 (66.7%) spontaneous deliveries, one (2.8%) forceps delivery and 11 (30.6%) Caesarean sections. The rates of premature delivery and IUGR were 11.1% and 16.7%, respectively. The 5-minute Apgar score was <7 in only 1 (2.8%) case. The umbilical cord blood pH was less than 7.2 in 10 (27.8%) cases. Fifteen newborns (41.7%) were transferred to the NICU. Two neonates underwent heart surgery in the first week and another 2 during the first year. Three babies (8.3%) died in the perinatal period, and 4 (11.1%) during the first year of life, *i.e.* perinatal and neonatal mortalities of 8.3% (3/36) and 11.1% (4/36), respectively.

Table V. First-trimester NT thickness and CRL in neonates with major defects of heart and great vessels

No.	NT thickness (mm)	CRL	CHDs	Gestational age (weeks)	Outcome
1	5	63	ASD,PS	39	NICU
2	4.8	43	ASD	38	CCC
3	4.3	42	Tetralogy of Fallot	32	Intrauterine death
4	4.2	39	VSD	40	CCC
5	4.1	55	VSD	40	CCC
6	4	62	HLHS	40	NICU, death
7	4	40	HLHS	37	NICU, death
8	3.5	37	ASD, club-foot	39	CCC
9	3.4	37	ASD	36	CCC
10	3.4	40	ASD, PDA	39	CCC
11	3.2	43	ASD	36	CCC
12	3.2	42	Tetralogy of Fallot	38	NICU
13	3.1	41	ASD	40	CCC
14	3.1	48	Tetralogy of Fallot	40	NICU
15	3	45	AS	39	NICU
16	2.4	39	ASD, PDA	40	CCC

17	2.3	45	ASD	36	CCC
18	2.1	55	VSD	37	CCC
19	2.1	43	PDA	40	CCC
20	2	50	Transposition of pulmonary vein	40	NICU
21	1.9	51	Tetralogy of Fallot	41	NICU
22	1.7	39	Transposition of pulmonary vein	38	NICU, death
23	1.6	42	VSD	39	CCC
24	1.6	34	ASD, VSD	39	CCC
25	1.5	49	Tetralogy of Fallot	39	NICU
26	1.5	38	VSD	40	CCC
27	1.4	43	Tetralogy of Fallot	40	CCC
28	1.3	34	Transposition of great arteries	39	NICU
29	1.3	39	HLHS	37	NICU, death
30	1.2	45	AS, MS	39	NICU, death
31	1.2	45	ASD	39	CCC
32	0.9	37	PS	39	NICU
33	*	50	Tetralogy of Fallot, hypospadiasis	39	NICU, death
34	*	63	ASD	38	CCC
35	*	38	VSD	41	CCC
36	*	43	ASD	39	CCC

*: not measured

Table VI. First-trimester NT thickness and CRL in foetuses who underwent therapeutic abortion because of severe cardiac and other structural abnormalities

No.	NT thickness (mm)	CRL	Foetal abnormalities	Gestational age (weeks)	Outcome
1	4.8	42	VSD, hernia diaphragmatica, hypoplastic left lung	23	TOP
2	4.2	38	HLHS, dextrocardia	22	TOP
3	3.2	39	Cong. cardiomyopathy, bilateral renal agenesis, pulmonary hypoplasia, oesophageal atresia, micropenis	17	TOP

Table VII. Detection of specific cardiac defects using a NT thickness cut-off of 3 mm

Cardiac defect	No. of foetuses	NT thickness		Detection rate (%)
		<3 mm	≥3 mm	
HLHS	4	1	3	75
ASD and VSD	20*	7	10	58.8
Tetralogy of Fallot	7**	3	3	50
Transposition of great vessels	3	3	0	0
Other defects	5	3	2	40
Total	39	17	18	51.4

*: In 3 cases the NT thickness was not measured
**: In 1 case the NT thickness was not measured

Table VIII. Sensitivity, specificity, positive and negative predictive values, and false-positive rate of screening for major defects of heart and great vessels using NT cut-off of 3 mm

	At a 3 mm cut-off level (%)
Sensitivity	51.4
Specificity	97.7
Positive predictive value	17.8
Negative predictive value	99.5
False-positive rate	4.05

Table IX. Outcomes of pregnancies with foetal cardiac defects

Birth weight (g) (mean ± SD)	3141.71 ± 549.72	
Gestational age at birth (weeks) (mean ± SD)	38.83 ± 1.34	
Mode of delivery:	No.	%
Spontaneous vaginal	24	66.6
Operative vaginal	1	2.8
Caesarean section	11	30.6
Premature labour	4	11.1
IUGR	6	16.7
Apgar score < 7 at 5 min	1	2.8
Umbilical cord pH < 7.20	10	27.8
NICU	15	41.7
Cardiac defects operated on in the first week	2	5.6
Cardiac defects operated on in the first year	2	5.6
Perinatal mortality	3	8.3
Neonatal mortality	4	11.1

6.3. Hypoplastic left heart syndrome

Between January 1998 and August 2001, a total of 287 congenital malformations were diagnosed, 79 of which were CHDs; 8 of them were HLHS. The prevalence of HLHS was 1.2 per 1000 pregnancies (8/6717).

The first-trimester foetal NT thickness was measured in 7 of the 8 foetuses with HLHS and was ≥3 mm in 6 cases (Table X). Table X shows the CRL at which the NT thickness was measured in the pregnancies, the gestational age in weeks at term and the outcomes. One case ended with therapeutic abortion at the request of the parents, which was performed in the 22nd week. The autopsy justified the prenatal finding.

Invasive testing was offered in the first trimester for all cases with an increased NT thickness, but was accepted by only one couple. *In utero* 2 were diagnosed; one couple decided to continue the pregnancy, while in the other case the parents refused both the invasive genetic testing and the foetal echocardiography. In the other 6 cases, the screens were reported to be normal and their heart defects were picked up at a routine postnatal check. The women who gave their informed consent participated in the ultrasound study voluntarily.

The mean neonatal birth weight was 2711.43±759.022 grams and the mean neonatal gestational age was 38.1±1.95 weeks. There were 2 spontaneous deliveries and 4 Caesarean sections. One of them was a premature delivery; 3 cases involved IUGR. The 5-minute Apgar score was <7 in only one case. All newborns were transferred to the NICU; 5 were died in the perinatal period, one in the neonatal period and one newborn is treated at the NICU.

Table X. First-trimester NT thickness and CRL in foetuses with HLHS

No.	NT thickness (mm)	CRL	Foetal abnormalities	Gestational age (weeks)	Outcome
1	4.2	38	HLHS, dextrocardia	22	TOP
2	4	62	HLHS	40	NICU, death
3	4	40	HLHS	37	NICU, death
4	1.3	39	HLHS	37	NICU, death
5	4.1	72	HLHS	40	NICU, death
6	*	59	HLHS	38	NICU, death
7	6.4	52	HLHS	40	NICU, death
8	3.5	45	HLHS	35	NICU, alive

*: not measured

6.4. Other major structural abnormalities

Between 1 January 1998 and 31 December 2000, 5186 newborns were delivered at our Department; 201 of them had congenital malformations, 97 of which were major abnormalities. The prevalence of foetal malformations was 18.7 per 1000 (97/5186) live births and that of major cardiac defects was 9.1 per 1000 (47/5186) live births.

Foetal malformations were divided into 8 essential groups. Table XI shows the foetal NT thickness and the types of the major defects of the neonates. Table XII presents the detailed list of congenital malformations with NT thicknesses. The NT thickness was measured in 75 cases (77.32%); it was ≥2.5 mm in 41 cases (54.67%).

A sensitivity of 54.67%, a specificity of 96.24%, a positive predictive value of 17.6%, a negative predictive value of 99.31% and a false-positive rate of 3.75% were found at a cut-off value of 2.5 mm in 75 cases where the NT thickness was measured (Table XIII).

The neonatal outcomes of newborns with congenital structural defects are demonstrated in Table XIV. The mean birth weight was 3038.247±632.895 grams and the mean gestational age 38.18±1.926 weeks. Vaginal delivery occurred in 67 cases (69.07%), vacuum extraction in one case (1.05%) and Caesarean section in 29 cases (29.9%). Premature delivery resulted in 16 cases (16.49%); 5 of them had IUGR. Ten mature neonates had IUGR.

The total rate of IUGR was 15.46%. The frequency of an Apgar score <7 at 5 minutes was 5.5%, and the umbilical cord blood pH was less than 7.2 in 25.77% of the neonates with congenital structural malformations. Forty-five newborns (46.39%) were transferred to the NICU. Five newborns died during the first week, *i.e.* a perinatal mortality of 5.15%, and 4 neonates (4.12%) died before the first year of age.

Table XI.: The groups of major structural malformations in chromosomally normal foetuses according to NT thickness

Groups	Number of cases	NT <2.5 mm	NT ≥2.5 mm
Major cardiac defects	47	22	16
Abdominal wall and gastrointestinal system	12	2	8
Face, eyes, lip	10	3	6
Skeleton	9	4	2
Urinary system and genitalia	7	0	5
Diaphragmatic hernia	4	0	2
Central nervous system	4	1	2
Spina bifida	4	2	0
Total	97	34	41

Table XII. Foetal abnormalities and genetic syndromes in chromosomally normal foetuses according to NT thickness

Groups	Number of cases	Recognised *in utero*	NT <2.5 mm	NT ≥2.5 mm
Brain cyst	1	1	0	1
Choroid plexus cyst	3	3	1	1
Major cardiac defects	47	10	22	16
Diaphragmatic hernia	4	1	0	2[1]
Gastroschisis	3	3	1	1
Choledochus cyst	1	1	0	1
Exomphalos	4	2	1	3[2,3]
Duodenal atresia	4	2	0	3
Spina bifida	4[4]	1	2[5]	0
Ovary cyst	1	1	0	1
Multicystic kidneys	4	2	0	2
Urethra and pyelon duplex	1	0	0	1
Hydronephrosis	1	0	0	1
Microcephalia	1	0	0	0
Microphthalmia	1	0	0	1
Palato- and/or gnathoschisis and cheiloschisis	8	1	3	4[6]
Sublingual cyst	1	1	0	1
Ectrodactyly	2	0	0	1
Rudimentary limbs	6	1	4	1

Symbols 1-6 refer to one case in each group that had additional abnormalities: [1] lung cyst, [2] gastroschisis, [3] Beckwith-Wiedemann syndrome, [4] extrophy of urinary bladder, [5] club-foot, [6] syndactyly and club-foot.

Table XIII. Sensitivity, specificity, positive and negative predictive values, and false-positive rate of screening for major structural defects using a NT thickness cut-off of 2.5 mm

	At a 2.5 mm cut-off level (%)
Sensitivity	54.67
Specificity	96.24
Positive predictive value	17.6
Negative predictive value	99.31
False-positive rate	3.75

Table XIV. Neonatal outcomes with major structural abnormalities

	n	%
Birth weight (g) (mean±SD)	3038.247±632.895	
Gestational age (weeks) (mean±SD)	38.187±1.926	
Mode of delivery		
vaginal	67	69.07
vacuum extraction	1	1.05
Caesarean section	29	29.9
Premature	16	16.49
IUGR	15	15.46
Apgar score <7at 5 minutes	5	5.5
Umbilical cord blood pH <7.20	25	25.77
NICU	45	46.39
Mortality		
perinatal mortality	5	5.15
between the 7th day and the first year of age	4	4.12

6.5. Sex ratio

In the cases of prenatally diagnosed chromosomal abnormalities between 1990 and 1999, the sex ratios were 1.48 (43/29) for trisomy 21, 0.375 (6/16) for trisomy 18 and 2.0 (6/3) for trisomy 13. In cases of postnatal karyotyping, the sex ratios were 1.44 (118/82), 0.5 (2/4) and 3.0 (3/1), respectively (Table XV).

The Down register of Szeged contained Down's cases between 1970 and 1999; in the early years, it contained only the data on the postnatally diagnosed cases, but later on both the pre- and postnatally diagnosed ones. The total number of cases was 441 and their sex ratio was 1.41. Taking into consideration only the prenatally diagnosed ones (72 cases), the sex ratio was 1.48; for the postnatal cases (369 cases) it was 1.4 (Table XVI).

The Down register of Hungary contained 5531 cases. The number of prenatally diagnosed cases in this register was 505 (the sex was unknown in 20 cases), with a sex ratio of 1.16 (260/225), while in the 5026 postnatally diagnosed cases (the sex was unknown in 21 cases) it was 1.18 (2709/2296). These data suggested that there is no significant difference in intrauterine lethality between males and females (Table XVI).

When the data in the registers of Szeged and Hungary were compared, no significant difference was found either prenatally (P=0.886, OR=0.922, 95% CI=0.526-1.613) or postnatally (P=0.669, OR=0.869, 95% CI=0.496-1.522).

Table XV. The pre- and postnatal sex ratios of trisomies 21, 18 and 13 on the basis of the register of aneuploidies of Szeged between 1990 and 1999

	Prenatal				Postnatal			
	Trisomies			Control	Trisomies			Control
	21	18	13		21	18	13	
Total	72	22	9		200	6	4	
Sex ratio	43/29	6/16	6/3		118/82	2/4	3/1	
(male/female)	1.48	0.375	2	1.08	1.44	0.5	3	1.06-1.07
Chorionbiopsy	34/24	6/12	6/2					
(weeks 11 to 13)	1.42	0.5	3					
Amniocentesis	9/5	0/4	0/1					
(weeks 16 to 20)	1.8	0	0					

Table XVI. The pre- and postnatal sex ratios of trisomy 21 on the basis of the Down registers of Szeged and Hungary between 1970 and 1999

		All cases	Males (XY)	Females (XX)	Sex ratio
Register of Szeged:	Prenatal (1990-1999)	72	43	29	1.48
	Postnatal (1970-1999)	369	215	154	1.4
	Total	441	258 (58.5%)	183 (41.5%)	1.41
Register of Hungary (1970-1999)	Prenatal	485	260	225	1.16
	Postnatal	5005	2709	2296	1.18
	Total	5490	2969 (54.1%)	2521 (45.9%)	1.2

7. DISCUSSION

Efforts are currently being made worldwide to achieve the recognition of foetal chromosomal aneuploidies at the earliest possible gestational age. Increased NT have a prognostic significance, especially when found in association with other foetal anomalies (19). Although the exact pathophysiology of increased NT is uncertain, the cause of this transient event may be connected with alterations in foetal haemodynamics and/or alterations in lymphatic drainage (19).

The heterogeneity in conditions associated with increased NT thickness suggested that there may not be a single underlying mechanism for the subcutaneous oedema in the foetal neck. Possible mechanisms include:

- Cardiac failure in association with abnormalities of the heart and great arteries;
- Venous congestion in the head and neck in association with the constriction of the foetal body in amnion rupture sequence, or superior mediastinal compression found in diaphragmatic hernia, or the narrow chest in skeletal dysplasia;
- Failure of lymphatic drainage due to impaired foetal movements in various neuromuscular disorders;
- Abnormal or delayed development of the lymphatic system;
- Altered composition of the subcutaneous connective tissue (74).

7.1. Foetal aneuploidies

Though a number of second-trimester ultrasonic markers of foetal aneuploidies have been described, such as a thickened nuchal pad, heart defects, a shorter humerus and femur, echogenic bowels, pyelectasis, *etc.* (3,20,56), none of them is specific and of value for efficient screening. Moreover, the search for these sonographic markers is time-consuming, and demands exceptional training, qualifications, experience and competence in ultrasound examinations.

The report by Szabó and Gellén (79) that the accumulation of nuchal fluid ≥3 mm thick as measured by high-resolution ultrasonography in the first-trimester embryo is

associated with trisomy 21 is gaining increasing acceptance worldwide (50-53,75,76,91). First-trimester NT screening for chromosomal aneuploidies is ever more widely utilised, but the efficiency is extremely variable: between 30 and 90% (8,16,30,43,50,57-59,71,80,84,90). In Szeged, from the start we have placed special emphasis on the analysis of factors influencing the efficiency, and in the learning years we have maintained a high rate of efficiency (88%) (77).

The Department of Medical Genetics is the only centre in South Hungary where all prenatal counselling and cytogenetic studies are performed and to which all neonates with any indications of abnormality are referred. The ultrasound clinic is a third-level screening centre.

Cut-off levels: The cut-off levels of first-trimester NT thickness used by different authors range from 2 to 5 mm (8,24,30,43,50,57,65,71,80,90). In practice, we use a 2.5 mm cut-off. Since the frequency of a NT thickness >3 mm in the general population is 2-3%, invasive testing of these selected pregnancies means an appreciable load for genetic centres. If we use a lower cut-off, we can detect more foetal aneuploidies, but the work-load increases significantly. A practically acceptable cut-off value is therefore necessary to distinguish affected cases from normal ones. It is known that the NT thickness normally increases with the CRL (65,67), and the use of a single cut-off may bias the sensitivity. If the NT screening concentrates on weeks 11 to 12, a cut-off of 2.5 mm, which yields more than 90% sensitivity, is acceptable in practice.

With a cut-off of 3 mm or higher (15,65,80), the reported sensitivity for all aneuploidies ranges between 46% and 69%, and that for trisomy 21 between 54% and 77%. The sensitivity we obtained by using a 2.5 mm cut-off (96.97% for all aneuploidies and 100% for trisomy 21) is highly comparable with the literature data.

Recent studies have used cut-off levels calculated via percentile curves or the risk calculation program of the Foetal Medicine Foundation, UK. This program calculates the risk of aneuploidies from a combination of the maternal age and the gestational age-related prevalence, multiplied by a likelihood ratio depending on the deviation from the normal in NT thickness for the CRL. Use of a risk calculation involving a combination of the maternal age, gestational age, NT thickness and former aneuploidy allowed the setting-up of a risk cut-off level ≥1:300 (71). The method of risk calculation reported by Snijders *et al.* (71) is a more comprehensive approach, which collects the known factors responsible for the actual risk of aneuploidy. Their risk calculation program led to a detection rate of 82% for Down syndrome and of 78% for other chromosomal aneuploidies.

In our opinion, it is useful to set up a standard of first-trimester ultrasound screening with a well-defined cut-off level, as there are centres in the developing areas which do not have the facilities to establish a computer calculation network. In addition, our study was conducted before the Foetal Medicine Foundation risk calculation was available. A well-defined cut-off level is of practical value for sonographers trained in NT measurement who screen in underdeveloped regions far from the centres and who need a practical solution. Our results suggest that 2.5 mm at 10 to 12 weeks of gestation is a reasonable cut-off level for those who do not have the programme, as this yields an acceptable rate of first-trimester detection of foetal aneuploidies.

Efficiency of NT screening with a cut-off of 2.5 mm: Both in our regional perinatal centre and in Hungary overall, the proportion of pregnants aged over 35 years has been around 7% during the last decade. The regulations require that they should be offered invasive tests, which result in only a 12-15% detection rate of trisomy 21 at 50% uptake. With a NT thickness of ≥2.5 mm, in the present study 4.5% of all pregnancies screened positive and they included 100% of the trisomy 21 cases and 96% of all aneuploidies. The sensitivity obtained in the literature studies lay in the range 50-92% for all aneuploidies and in the range 43-88% for trisomy 21 (15,21,30,50,71,77,80).

In experienced hands, ultrasonographic evaluation of the foetus at 10 to 12 gestational weeks is one of the most efficient methods of screening chromosomal abnormalities. In the group of 353 women with a NT thickness of <2.5 mm who underwent invasive tests, only one aneuploidy (trisomy 18) was found; this suggests that the measurement of NT thickness alone is a good selection criterion for invasive tests. A NT cut-off of ≥2.5 mm is a sensitive and effective criterion of selection of women for invasive tests.

The recognition of foetal aneuploidies at this gestational age and the early termination of the affected pregnancy may be less traumatic for couples who choose this option and they may have a healthy offspring sooner. A potential disadvantage of this early diagnosis is the identification of chromosomally abnormal pregnancies that are destined to miscarry. About 40% of trisomy 21 foetuses die between 12 weeks of gestation and term (72). Other benefits of the first-trimester scan include the confirmation that the foetus is alive, accurate dating of the pregnancy, the early diagnosis of multiple pregnancies and the detection of major structural abnormalities and missed abortion (19,24,63,73,74). General evaluation of the foetal structure simultaneously with NT measurement can reveal other structural abnormalities and allow efficient genetic counselling to be extended to more and more women, irrespective of their age.

7.2. Cardiac defects

Screening for CHDs at the earliest possible stage of gestation is an important task, but a great challenge in prenatal diagnostics. Foetal echocardiography in the second or (more recently) the first trimester of pregnancy has been found to be a promising screening method for CHDs. Evaluation of the four-chamber view of the heart and the outflow tracts in the second trimester of pregnancy yielded a sensitivity as high as 61% (64,82).

An earlier and a higher rate of detection of foetal CHDs was found when ultrasound measurement of increased foetal NT thickness (≥3 mm) at weeks 10 to 13 of gestation was demonstrated to be associated with foetal cardiac malformations (1,5,13,40,41,54,92). The findings in most of the publications are in accord from this respect, but Schwärzler *et al.* were not able to confirm such an association in an unselected population (66).

Cut-off levels: The prevalence of major CHDs increases in parallel with the NT thickness (40,41) and Zosmer *et al.* (92) suggest the 95th centile of NT thickness as a cut-off at which the follow-up of such pregnancies by sonographers and specialists in foetal echocardiography is recommended. Our data indicate that screening on the basis of an increased NT thickness, with a cut-off of 3 mm, identifies 51.4% of the major abnormalities of the heart and great arteries at weeks 10 to 13 of gestation. The results compare favourably with the data reported by Hyett *et al.* (40,41). An even higher detection rate of 71.9% (23/32) can be obtained at a cut-off value of ≥2 mm. Since the rate of a NT thickness >3 mm in the general population is between 2 and 3%, ultrasound follow-up of these selected pregnancies means an extra load for specialists in foetal echocardiography. If we use a lower cut-off, we can detect more foetal CHDs, but the work-load will increase significantly. A practically acceptable cut-off value is therefore necessary to distinguish cases marked for follow-up. It is known that the NT thickness normally increases with CRL and the 95th centile is 2.2 mm for a CRL of 38 mm, and 2.8 mm for a CRL of 84 mm (41) and the use of a single cut-off may bias the sensitivity. We consider that a 3 mm cut-off yielding a 51.4% sensitivity is practically acceptable, especially if the NT screening concentrates on the period between weeks 11 and 12.

Types of foetal CHDs screenable via an increased NT: We found an association between the type of foetal CHDs and an increased NT. A HLHS and septal defects displayed stronger associations with an increased NT. This observation is comparable with previous reports of the highest detection rate in cases involving a HLHS (54,92).

Follow-up and clinical management of pregnancies with an increased NT and a normal karyotype: An increased foetal NT thickness at weeks 10 to 13 of gestation is associated with foetal aneuploidies (50,79), cardiac (40,41,50,92) and other structural abnormalities (54) and certain genetic syndromes (54,74). Since an increased NT accompanies such a wide range of foetal abnormalities, careful clinical management should be observed. Our policy is first to determine the foetal karyotype; in chromosomally normal foetuses, a thorough ultrasound follow-up is recommended, including targeted foetal echocardiography and scanning for structural abnormalities and genetic syndromes.

Spontaneous resolution of nuchal oedema occurs in 90% of pregnancies with an increased NT and a normal karyotype. These pregnancies will result in normal neonates (85). Only the remaining 10% of foetuses with an increased NT need further evaluation (with targeted foetal echocardiography), which does not mean too great an overload for the medical personnel.

Though the issue of whether the antenatal diagnosis of CHDs affects the postnatal outcome is still controversial, most authors favour an early prenatal diagnosis to avoid long-term cardiac and neurological consequences. Newborns with cardiac abnormalities should be born in hospitals where all appropriate cardiological facilities are available. Adequate care can then be provided in due time for the neonatal cardiac patient.

In conclusion, an increased foetal NT at weeks 10 to 13 is an important predictor and efficient method of screening, not only for foetal aneuploidies (54,77), but also for foetal cardiac abnormalities, and particularly left-sided defects (54,92).

7.3. Hypoplastic left heart syndrome

The birth incidence of HLHS has been estimated to be 0.1-0.25/1000 live births (22). Foetal echocardiography is usually performed during the second trimester of pregnancy. Most of the major abnormalities of the heart and great vessels can be identified at 18 to 22 weeks of gestation by specialist foetal echocardiography. However, it is possible nowadays to visualise the complete four-chamber view of the foetal heart during the 11^{th} gestational week, and several reports have been published about the early detection of cardiac anomalies (10, 45). HLHS may be accurately diagnosed during foetal life. Prenatal diagnosis provides an opportunity for parents to make an informed choice about their options, including surgery, non-intervention postnatally or TOP.

At the regional perinatal centre in Szeged, the mean birth prevalence of CHDs during the past 5 years has been 8.2 per 1000 live births. Since first-trimester screening for foetal abnormalities is rapidly spreading in this region in consequence of the regular practical training of the obstetric health-care personnel, we were interested in looking at the current first-trimester prediction rate of foetal left-sided heart defects by means of an increased NT in chromosomally normal foetuses.

Our results suggest that an increased NT in karyotypically normal foetuses in the first trimester is closely associated with HLHS. Our observation is comparable with previous reports of the highest detection rate in cases involving HLHS (54,92).

7.4. Other major structural abnormalities

We can confirm the findings of studies reporting that an increased foetal NT at 10 to 13 gestational weeks is associated with a wide range of foetal malformations, especially major cardiac defects, diaphragmatic hernia, exomphalos, body stalk anomaly and foetal akinesia deformation sequence (1,9,11,17,19,26,35,68,69,74,87).

Diaphragmatic hernia is a sporadic defect with a birth prevalence of about 1 in 4000. In this study, its prevalence (4 in 5186) was higher than expected in the general population as our Department is a tertiary screening centre. In accordance with a published study (68), our 2 cases of diaphragmatic hernia had a NT over the 2.5 mm cut-off level (2.9 mm; 3.3 mm). One of them was associated with a lung cyst (68).

Defects of the gastrointestinal system, abdominal wall, urinary system, genitalia, face, lip and eyes also exhibited a strong association with an increased NT thickness of ≥2.5 mm. Exomphalos is a sporadic defect with a birth prevalence of about 1 in 3000. In this study, its prevalence (4 in 5186) was also higher. The 12^{th} week of gestation is a cut-off point for the visualisation of physiologic herniation of the gut, and persistent herniation of the abdominal contents after that time usually reflects exomphalos. Three of the four cases had a NT thickness of over 2.5 mm (1.7 mm; 3.0 mm; 3.1 mm; 3.6 mm). The case with a NT of 3.6 mm involved Beckwith-Wiedemann syndrome. This result correlates with the findings in the international literature (87).

Four cases with palato- and/or gnathoschisis and cheiloschisis had an increased NT thickness (4.5 mm; 3.8 mm; 4.5 mm; 2.8 mm). In one of these cases (a NT of 3.8 mm at 10 gestational weeks), there were also associated syndactyly and club-foot, which could have

been ectrodactyly-ectodermal dysplasia-clefting syndrome. In the literature only one case of this syndrome has been reported, with a NT of 3.4 mm at 12 gestational weeks (6).

The recognition of foetal structural malformations in the first trimester is useful for the counselling of parents with affected pregnancies, and it can be an indication for further screening and investigations. In the event of incompatibility with life, an early diagnosis makes it possible for the couples to choose an early termination of the affected pregnancy, which is less traumatic than later. If they wish to continue the pregnancy, the information on structural defects can also be important in the later course of pregnancy and also during perinatal care. Although, early ultrasound techniques have developed considerably, this does not reduce the importance of the second-trimester scan at 18 to 22 weeks of gestation.

The prevalence data are higher than in the normal population because our Department is a tertiary centre. Almost half of the neonates with major congenital malformations were transferred to the NICU and the perinatal mortality was 3 times higher than the average for the country; early diagnosis of the malformations is therefore necessary. The affected newborns should born in well-equipped hospitals (with a NICU), where surgery is also available. Our data indicated that more than 15% of the major congenital malformations were associated with IUGR, which corresponds to the literature finding (46).

With the recent advances in ultrasound technology, first-trimester screening and the diagnosis of foetal malformations will play a greater role in obstetrics management. First-trimester ultrasound provides a sensitive method of screening for structural abnormalities. Although, the number of affected cases in this study was too small for definite conclusions to be drawn, a NT thickness of 2.5 mm can be a sign of structural foetal anomalies.

An increased NT thickness is an important early sonographic marker for foetal aneuploidies and structural malformations, especially defects of the heart, gastrointestinal system, abdominal wall, urinary system, genitalia, diaphragmatic hernia and face (74). In conclusion, the greater the NT thickness, the greater the likelihood of abnormalities, both chromosomal and structural foetal malformations. In chromosomally normal foetuses, an increased NT can be used to identify about 55% of the cases with structural abnormalities.

7.5. Sex ratio

The excess of males in Down syndrome has long been recognised (4). An earlier study reported a value of 1.23 for the sex ratio of Down syndrome in England and Wales (49). In Edwards syndrome, a low sex rate was noted as early as 1962 (23) and has since been

confirmed in other studies (28,81). In the study by Goldstein and Nielsen (28) in 1988, the sex ratio for trisomy 18 was 0.64. Combined data from these two studies suggest that the sex ratio in prenatally diagnosed cases (0.82) may be greater than in postnatally diagnosed ones (0.52), with the implication that the *in utero* survival in male trisomy 18 is poorer than in females.

Huether *et al.* (33) suggested that the foetal sex ratio estimate for trisomy 21 was 1.16, while in the prenatal controls it was 1.07. The live birth sex ratio estimate for Down syndrome was 1.15, while in the postnatal controls it was 1.05. Both the pre- and postnatal data were significantly different from the pre- and postnatal controls.

We can confirm earlier reports (12,49) of the male excess in Down syndrome, using different Down registers. Both our pre- (1.48) and our postnatal (1.44) ratios were higher. On the basis of the Hungarian Down register, the prenatal sex ratio was 1.16 and the postnatal one was 1.18. The cause of the male excess in trisomies 21 and 13 has not been established, but there are some possible explanations.

Sex ratios in trisomy 21:

Hassold *et al.* (31) proposed that the increased sex ratio for Down syndrome is associated with a paternal non-disjunction, where the extra 21st chromosome preferentially segregates with the Y chromosome.

Petersen *et al.* (62) provided data based upon DNA polymorphisms and gave strongly supportive evidence. They found a sex ratio of 3.5 in those cases where the extra 21st chromosome was paternally derived through a meiotic non-disjunction.

With regard to the recent molecular evidence (2) which indicates that only ~5% of Down syndrome non-disjunctions are paternally derived, and assuming a sex ratio of 1.06 for all maternally derived cases, a sex ratio of 3.5 is exactly that needed in paternally derived cases to reach an overall sex ratio of 1.18. Huether *et al.* (33) confirm that the lack of any maternal age effect for either foetuses or live births is also evidence in support of the higher sex ratio being paternally derived in Down syndrome.

James (42) considers that the cause of this male excess in Down syndrome is not the sex-selective spontaneous abortion, but rather the timing of insemination in relation to ovulation is not optimal. It is widely believed (at least among non-geneticists) that the timing of fruitful coitus within the human menstrual cycle is associated with the offspring sex ratio, male zygotes being preferentially formed when the fruitful insemination is either early or late. In a meta-analysis on this point, Gray (29) estimated that fruitful inseminations around

ovulation have a relative risk of only 90% of yielding males as contrasted with early or late inseminations.

Sex ratios in trisomy 18:

In the study by Goldstein and Nielsen (28), the sex ratio for trisomy 18 was 0.64, which means a female excess. Combined data from the studies by Taylor (81) and Goldstein and Nielsen (28) suggest that the sex ratio in prenatally diagnosed cases (0.82) may be greater than that in postnatally diagnosed ones (0.52), with the implication that the *in utero* survival in male trisomy 18 is poorer than in females; however, the difference was only marginally significant.

Huether *et al.* (33) demonstrated that in trisomy 18 the foetal sex ratio estimate was 0.9, while in the prenatal controls it was 1.07. The live birth sex ratio estimate was 0.63, while in the postnatal controls it was 1.05. Both the pre- and the postnatal data were significantly different from the pre- and postnatal controls. Our data were considerably lower than in the former studies: the prenatal sex ratio for Edwards syndrome was 0.375, and the postnatal one was 0.5.

Sex ratios in trisomy 13:

In the study by Huether *et al.* (33), the foetal sex ratio estimate was 0.88, while in the prenatal controls it was 1.07. The corresponding data in the live birth cases were 0.9 and 1.05, respectively. Our estimated sex ratios for Patau syndrome were the opposite of the literature data, though the number of cases was quite low. The prenatal sex ratio was 2, and the postnatal one was 3.

Study of the differences between the sex ratios of the autosomal trisomies, and molecular genetic study of the non-disjunction of the chromosomes of trisomies 13, 18 and 21, can furnish further data about the genesis of these trisomies.

8. CONCLUSIONS

Recent advances in medical technology have allowed more emphasis to be placed on first-trimester screening and the diagnosis of foetal congenital malformations and chromosomal abnormalities. As these screening programs are implemented, sonographic evaluation of foetal anatomy will potentially also occur at an earlier gestational age. Improved ultrasound resolution has allowed us to evaluate foetal anatomy in the late first trimester, including the detection of ultrasound markers for foetal aneuploidy and structural malformations. The NT thickness has been identified as a sonographic marker for foetal aneuploidy and structural malformations, especially cardiac abnormalities. NT screening has a function for the identification of patients at high risk of cardiac anomalies who may benefit from foetal echocardiography or follow-up evaluation. In addition, an increased NT can be useful for counselling patients concerning the prognosis for pregnancy.

We hope that NT thickness measurement will be a useful screening tool to identify patients from the low-risk population for further examination, such as targeted foetal ultrasound and possibly foetal echocardiography. In the interim, it is reasonable to recommend that if the NT thickness is ≥2.5 mm at 10 to 13 gestational weeks, referral should be made to a tertiary care centre for targeted sonographic examination to assess for foetal anomalies.

First-trimester screening is currently offered in more than 200 centres in 41 countries throughout the world. A total of more than 100,000 women have been examined and 80% of foetuses with chromosomal abnormalities have been identified. This compares favourably with the detection rates achieved with screening based on maternal age (a detection rate of about 30%) or maternal age and serum biochemistry (a detection rate of about 60%).

Besides the detection of chromosome defects, the scan provides important information in twin pregnancies and early scans help to identify pregnancies with an increased risk of cardiac defects or genetic syndromes.

Conclusions of the thesis:

1. NT thickness measured by ultrasound in the first trimester is a reliable marker of foetal chromosomal abnormalities.

2. First-trimester ultrasound screening is one of the most effective methods of screening foetal anomalies, especially major cardiac defects, which are detectable by ultrasound examination.
3. The method is safe and has no adverse effects on the foetus or the pregnancy.
4. A NT thickness of 2.5 mm or higher is a warning sign of a possible chromosomal abnormality or of some other foetal structural abnormality, and careful follow-up of each case is therefore mandatory.
5. I suggest a cut-off level of 2.5 mm for the first-trimester NT thickness at 10 to 13 weeks of gestation.
6. If the NT thickness is increased, genetic counselling can be essential for the parents concerning the prognosis of pregnancy.
7. The number of pregnants taking part first-trimester screening should be increased.
8. In order to furnish further data on the genesis of trisomies, study of the differences between the sex ratios of the autosomal trisomies and molecular genetic study of the non-disjunction of the chromosomes of trisomies 13, 18 and 21 is essential.

On the basis of our results, I recommend the overall introduction of the method suggested above during routine first-trimester ultrasound screening.

9. PERSPECTIVES

In prenatal services, cytogenetics risk estimates will no longer be based on advanced maternal age alone, but rather on a combination of sonographic screening, family history, maternal age and biochemical parameters in the maternal serum as early as in the first trimester of pregnancy. This more complex approach will generate a considerable additional demand for counselling. Fluorescence *in situ* hybridisation (FISH) has dramatically improved the diagnostic performance with specific indications (83). The new FISH-based techniques for differential chromosome identification, however, have not replaced conventional chromosome analysis as a routine or primary diagnostic tool. The growing demand for rapid karyotyping leads to the complementary use of interphase FISH or molecular genetic approaches to aneuploidy testing. With the progress in automatisation, the latter might replace conventional chromosome analysis for the selective exclusion of aneuploidy in routine diagnosis (47).

Some studies have made use of the combination of maternal age, ultrasonography and biochemical parameters for risk estimation (61,88). In the second trimester of pregnancy, the tripletest of AFP, human choriongonadotropin (hCG), and unconjugated oestriol (E_3) in the maternal serum are generally nowadays used for risk estimation. Two new parameters have been introduced: the free β-hCG and the pregnancy-associated plasma protein A level(PAPP-A). These two parameters make it possible to recognise more cases of Down syndrome in the first trimester of pregnancy than previously (89). Recent results suggest that the best method for deciding on whether to make an invasive test or not is the combination of ultrasonography and maternal serum screening in early pregnancy; with these methods, the interest in chorionbiopsy will increase. In the second and third trimesters of pregnancy, AC using FISH on the interphase cells or the direct chromosome preparation with placentapuncture can be a beneficial alternative in the high-risk cases for chromosomal anomalies. In the third trimester, prenatal chromosome diagnostics can furnish data for the perinatal care (47).

During the prenatal care, the pregnant should receive genetic counselling about the possibility of estimating the risk to the foetus as early as possible, and this counselling should be independent of maternal age. This will cause an increase in the number of genetic counselling, but this seems essential if we wish to reduce the uncertainty of the mothers. For this, a trustful cooperation between the obstetrician and the human geneticist is essential.

Some experimentally used methods are applied in prenatal chromosome diagnostics, such as coelocentesis or cervical lavage, but it is doubtful whether these can complement or supply each other. The modern methods of molecular cytogenetics (*e.g.* FISH on the interphase cells) permit the diagnosis of some chromosomal anomalies in the early embryonic stage of single cells. Preimplantational diagnostics is forbidden, so this procedure with the essential *in vitro* fertilisation (IVF) is out of the question as a routine procedure, but it could be introduced in cases of IVF if there is a high risk of diagnosable chromosomal anomaly (47).

As the number of theoretically diagnosable diseases is continuously, even in prenatal cytogenetics, indication-oriented procedures of monogenic disorders will be essential in molecular genetic diagnostics. The risk of the remaining "exotic" chromosomal abnormalities after interphase-FISH or molecular genetic diagnostics of aneuploidies is marginal in the low-risk cases. The targeted exclusion of numeric chromosomal abnormalities will be better in the future and will be cheaper (e.g. Microarray-techniques). The introduction of microsatelite diagnostics will perhaps have a great role in risk cases, involving factors such as a small amount of bloody amniotic fluid, poorly growing cultures, a low number of colonies, etc. (47).

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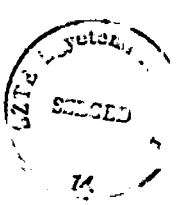

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12. ANNEX

Full papers accepted in international journals.