

# Screening for Chromosomal Abnormalities: A review

## Introduction

The last 20 years have seen major changes in the way we understand and screen for chromosomal abnormalities.

South Africa is moving in the right direction towards adopting and implementing these changes. This is in keeping with the rest of the developed world.

Chromosomal abnormalities represent 15% of congenital abnormalities overall.

In the first trimester about 50% of pregnancy losses are due to chromosomal abnormalities. Around 25% of those are due to trisomy 21 (Down's syndrome), and the remaining 25% are due to other chromosomal abnormalities.

The birth incidence of Down's syndrome is approximately 2 /1000 and is responsible for around 25% of severe mental handicap in the developed world.

Most fetuses with chromosomal abnormalities have either external or internal defects which can be recognized by detailed ultrasonographic examination.

The commonest chromosomal defects are

1. Trisomies 21, 18 or 13,
2. Turner syndrome (45X),
3. 47XXX, 47XXY, 47XYY and
4. Triploidy (69XXX or 69 XXY)

With detailed ultrasound surveillance and in experienced hands it is now possible to identify nearly all fetuses with Trisomy 13, Trisomy 18, and as we will see later around 98-99% of fetuses with trisomy 21. Some experts now even question the necessity of performing an amniocentesis when a Trisomy 13 or 18 is obvious sonographically. <sup>1</sup>

It is however very difficult to produce standardized guidelines regarding the investigation and management of such chromosomal abnormalities, because of different levels of expertise in our country and because the ultrasound industry is not yet regulated.

With increasing awareness of the public and the recent surge in litigations, it is only a question of time before the antenatal screening and management of chromosomal abnormalities may only be offered by properly trained and accredited practitioners.

## Features of chromosomal defects

In the first trimester, a common feature of many chromosomal defects is increased Nuchal Translucency (**NT**).

In the 2nd trimester, each chromosomal defect has its own syndromal pattern of abnormalities.

## Historical background

In 1966, 100 years after the original description by Langton Down, it became possible to diagnose trisomy 21 prenatally by karyotyping of amniotic fluid cells. <sup>2</sup> The first basis for screening of trisomy 21 was the observation by Shuttleworth of its association with advanced maternal age. <sup>3</sup>

Until a decade ago, pre-natal karyotyping was restricted to women older than 35 years resulting in detection of only 30% of all trisomy 21 pregnancies.

In the seventies and eighties, in screening by maternal age with a cut-off of 35 years, only 5% of the pregnant population were classified as high risk and this group contained about 30% of trisomy 21 babies. 4

140 amniocenteses would have been required to detect one case of Down's syndrome.

In 2000 with more than 15% of the pregnant population being above 35 years old, the number of invasive procedures would increase 3-fold and still only achieve a detection rate of around 50%.

Clearly there was a need to review such a policy.

### The Nuchal Translucency (NT)

In the 1990s screening by a combination of maternal age and foetal nuchal translucency thickness at 11-14 weeks of gestation was introduced.

In a multicentre study, under the auspices of the Foetal Medicine Foundation, a total of 100311 singleton pregnancies were examined. Follow up was obtained in 96127.

The nuchal translucency was increased in only 4.4% of the normal pregnancies and in almost 72% of those with Trisomy 21. 5

The estimated risk of Trisomy 21 based on maternal age and nuchal translucency was above the cut-off of 1/300 in 8.3% of normal pregnancies and in 82.2% of those with Trisomy 21.

What is the Nuchal Translucency?

It is a collection of fluid under the skin behind the neck of fetuses at 11-14 weeks that can be measured by ultrasound examination. The nuchal translucency normally increases with gestation.

In fetuses with chromosomal abnormalities, cardiac defects and many genetic syndromes, the nuchal translucency measurement appears to be increased.

Figure 1



### Pathophysiology of increased NT:

The nuchal translucency may be increased due to a number of reasons:

1. Cardiac failure in association with abnormalities of the heart and great arteries
2. Venous congestion in the head and neck
3. Altered composition of the extracellular matrix
4. Abnormal or delayed development of lymphatic system
5. Failure of lymphatic drainage due to impaired foetal movements
6. Foetal anaemia or hypoproteinaemia
7. Congenital infection, acting through anaemia or cardiac dysfunction. 6

By examining the foetal anatomy and measuring the nuchal translucency at 11-14 weeks gestation around 70% of structural abnormalities and 80% of chromosomal abnormalities can be diagnosed in early pregnancy, provided the guidelines for the measurement of the nuchal translucency are strictly adhered to (figure 2):

1. The gestation should be between 11 w and 13 w 6d
2. The fetal crown-rump length (CRL) should be 45 to 84mm
3. A good sagittal section of the fetus should be obtained, preferably in the horizontal position with the fetal spine at the bottom of the image.
4. The fetus should be in a neutral position with the head in line with the spine, not hyper-extended or flexed.

Figure 2



Increased nuchal translucency at the 11-14 week scan is associated with a wide range of fetal abnormalities.

Souka and associates examined the outcome of 4116 pregnancies with increased nuchal translucency and normal karyotypes. In fetuses with the nuchal translucency measurements marginally increased (<3.4mm) the

prognosis was very good with a 96.3% survival rate. In fetuses however with nuchal translucencies greater than 6.5mm, the overall survival was only 44.4%.<sup>7</sup> Increased nuchal translucency is of particular importance in its association with major abnormalities of the heart and great arteries. In fetuses with nuchal translucency measurements greater than 5.5mm, the prevalence of major cardiac abnormalities appears to be 200 times higher than in the general population.<sup>8</sup>

Increased nuchal translucency above the 99th centile (>3.5mm) therefore constitutes an indication for specialist fetal echocardiography.

Experts agree today that measurement of the nuchal translucency at 11-14 weeks may constitute the most effective method of screening for cardiac defects.

<sup>9</sup>

### The Nasal Bone

In a study by Cicero, around 300 fetuses out of 3788 singleton pregnancies who underwent a Chorionic Villus Sampling (CVS) at 11 to 14 weeks, with a karyotype result available, had a hypoplastic nasal bone. Of those 242 (67%) had trisomy 21. The incidence of absent nasal bone was 2.8% in the 3358 chromosomally normal fetuses.<sup>10</sup>

Nasal bone hypoplasia is associated with a high risk for trisomy 21 and it is a highly sensitive and specific marker for this chromosomal abnormality. It is obvious from the study that absence of the fetal nasal bone therefore indicates an increased the risk of Down's syndrome.

### Maternal first trimester serum screening

In trisomy 21, during the first trimester of pregnancy, the maternal serum concentration of free b-HCG is higher than in chromosomally normal fetuses whereas PAPP-A is lower. The level of free beta-HCG in maternal blood normally *decreases* with gestation and therefore the higher the b-HCG level, the higher the risk of trisomy 21.

The level of PAPP-A in maternal blood normally *increases* with gestation. The lower the PAPP-A, the higher is the risk of trisomy 21.<sup>11</sup>

Measurement of the free beta Human Chorionic Gonadotrophin (beta HCG) and Pregnancy Associated Placenta Protein A (PAPPA-A) between 8-14w gestation has a sensitivity of 63% and a specificity of 94, 5% for the detection of trisomy 21.<sup>12</sup>

### Combined 1<sup>st</sup> Trimester Screening

The breakdown of the various screening modalities discussed so-far, would give the following detection rates individually:

Nuchal Ttranslucency ~ 82%

Biochemistry ~ 63%

Nasal Bone ~ 61.8%

Because there is no correlation between the presence of the nasal bone and nuchal translucency or the serum markers, combination of the three modalities is possible.

Combination of the nuchal translucency measurement with the presence or absence of the nasal bone and the first trimester biochemical markers (free beta HCG and PAPP-A) can achieve a detection rate of around 97% with a false positive rate of 5%. 13

## 2nd Trimester Serum Screening

At 16 weeks of gestation, the maternal serum concentrations of Alpha Feto Protein, beta-HCG and Oestriol E3 are different from normal.

In many centres in South Africa and overseas, 2<sup>nd</sup> trimester serum biochemistry (the so-called triple test: Alpha Feto Protein (AFP), beta-HCG, Oestriol E3) is still considered the gold standard.

The estimated detection rate of trisomy 21 by combining maternal age and serum biochemistry increased from 30% based on maternal age alone to around 65% with 5, 2% false positive rate. 11

However, with a detection rate of around 65%, 60 amniocenteses would be required to detect one case of Down's syndrome.

The triple test has some advantages:

It doubles detection rate for the same screen positive rate, it identifies affected pregnancies in young women, it allows some older women to avoid invasive tests and it is more cost effective than age-based screening alone as explained above.

In addition it provides an effective screening for Open Neural Tube Defects.

There are however, some strong disadvantages with the triple test as well: It may provide false reassurance to 35-40% of women with a Down syndrome fetus who are screen negative, it may cause anxiety to women who are screen positive with a normal karyotype, it may lead to increased anxiety in around 25% of screen positive women who do not opt for invasive testing and additional resources are needed for counselling, both pre- and post serum screening.

Certain factors may affect the second trimester biochemical screening, and one may need to make adjustments.

| <b>Variable marker</b>        | <b>Serum</b>            |
|-------------------------------|-------------------------|
| Gestational age with GA       | increases               |
| Weight proportional to weight | inversely               |
| IDDM reduced                  | uE3 and inhibin A       |
| unchanged                     | Total/free $\beta$ -hCG |
| effect                        | AFP? significant        |

|                               |                           |
|-------------------------------|---------------------------|
| Recent bleeding in AFP        | variable increase         |
| Afro-Caribbean race increased | AFP and hCG               |
| Smoking reduced               | total & free $\beta$ -hCG |
| Multiple pregnancy increased  |                           |

## 2<sup>nd</sup> trimester ultrasound and soft markers

Phenotypic expression of chromosomal defects

### Trisomy 21

Is associated with a tendency towards brachycephaly, mild ventriculomegaly, flattening of the face with a hypoplastic or absent nasal bone, nuchal oedema, cardiac abnormalities such as atrioventricular septal defects, duodenal atresia, echogenic bowel, mild hydronephrosis, shortening of the limbs, sandal gap clinodactyly and/or mid-phalanx hypoplasia of the fifth finger.

### Trisomy 18

Common abnormalities include: strawberry-shaped head, choroid plexus cysts, absent corpus callosum, Dandy–Walker complex, facial cleft, micrognathia, nuchal oedema, cardiac defects, diaphragmatic hernia, oesophageal atresia, exomphalos, renal defects, myelomeningocele, growth retardation and shortening of the limbs, radial aplasia, overlapping fingers and talipes or rocker bottom feet.

### Trisomy 13

Common defects include: holoprosencephaly and associated facial abnormalities, microcephaly, cardiac and renal abnormalities (often enlarged and echogenic kidneys), exomphalos and postaxial polydactyly.

### Triploidy

When an extra set of chromosomes is *paternally derived*, it is associated with a molar placenta and the pregnancy rarely persists beyond 20 weeks. When there is a double *maternal chromosome* contribution, the pregnancy may persist into the third trimester. The placenta may be of normal consistency and the foetus demonstrates severe asymmetrical growth retardation.

Common findings are: early severe intrauterine growth restriction, mild ventriculomegaly, micrognathia, cardiac abnormalities, myelomeningocele, syndactyly, ‘hitch-hiker’ toe deformity.

### Turner’s syndrome

There are two types of this syndrome: the lethal and non-lethal types. The rate of intrauterine death between 12 and 40 weeks is about 75%.

The *lethal* type of Turner syndrome presents with

1. large nuchal cystic hygromas,
2. generalized oedema,
3. pleural effusions
4. ascites,
5. cardiac abnormalities

The *non-lethal* type usually does not demonstrate any ultrasonographic abnormalities and may therefore remain undiagnosed.

A marker is a structural anomaly visible on ultrasound scan, rarely of postnatal significance **but** associated with a chromosomal abnormality. Every marker has a likelihood ratio attached to it and the calculation of the new (adjusted) risk for chromosomal abnormalities is as follows: <sup>13</sup>

**Background risk x Factor(s) derived from screening test(s) = Adjusted risk**

Some of the common soft markers are listed below with their likelihood ratios (LR):

|   |           |
|---|-----------|
| No soft marker present                                  | LR 0.3x   |
| Mild hydronephrosis                                     | LR 1.0 x  |
| Short femur   | LR 1.6 x  |
| Short humerus   | LR 4.1 x  |
| Echogenic bowel   | LR 3.0 x  |
| Echogenic focus   | LR 1.1 x  |
| Major Defect  | LR 5.0 x  |
| Nuchal fold >6 mm                                       | LR 10.0 x |
| Hypoplastic nasal bone with a length of less than 2.5mm | LR 50.0 x |

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So, if for example a patient with a background risk (age related risk) of 1 in 1000 has a fetus with a nuchal fold greater than 6mm, her new adjusted risk will be calculated as follows:  $1/1000 \times 10 = 1/100$ . The adjusted risk is now 1 in 100. The same patient, in the absence of any soft markers would have an adjusted risk of  $1/1000 \times 3 = 1/3000$ . This means that in the absence of any soft markers the background risk may be reduced 3 fold.

If a patient had a 1<sup>st</sup> trimester screening by means of a nuchal translucency measurement, the adjusted risk after the 2<sup>nd</sup> trimester soft marker scan may only be reduced by 2 fold. So, if for example a patient had a background risk of 1 in 100, and her adjusted risk became 1 in 1000 after the nuchal translucency measurement, the new adjusted risk after a negative soft marker scan will become 1 in 2000. The detection rate after the 1<sup>st</sup> trimester scan combining the nuchal translucency, the nasal bone, and biochemistry (NT + NB + Biochemistry) is 97%. Out of the remaining 3% of undetected Trisomy 21 cases, a further 50% (1.5%) will be detected during the soft marker scan. The overall detection rate becomes 98.5%.

Time for a change

In view of the current evidence as shown above, it is good clinical practice to offer all pregnant women 1<sup>st</sup> trimester screening. There is no need to perform 2<sup>nd</sup> trimester serum screening (integrated/sequential screening) but every patient should be offered a detailed soft marker scan between 18week and 23 weeks. If the patient did not have 1<sup>st</sup> trimester screening, she should be offered second trimester biochemistry screening, together with a detailed soft marker scan. In the presence of a major defect, even if isolated, an amniocentesis should be offered.

In women between 35 and 39 years, with a background risk of anything between 1 in 100 to 1 in 300 the adjusted risk, in the absence of any markers becomes 1 in 300 to 1 in 900 (reduction by a factor of 3) and therefore still screen negative. In women who are older than 40 years old, with no 1<sup>st</sup> trimester screening and a background risk of 1 in 70, the adjusted risk after the second trimester scan becomes **1 in 210** and therefore still remains screen positive for trisomy 21. An invasive test here could be considered.

## Conclusion

Until a decade ago, pre-natal karyotyping was usually restricted to women of 35 years or older. Screening based on this criterion identified only 30% of all trisomy 21 fetuses. Screening protocols combining maternal age, alpha-fetoprotein, free  $\beta$ -chorionic gonadotrophin ( $\beta$ -hCG) and (Estriol) E3 have improved detection rates up to 65% with a 5.2% false positive rate in the second trimester.

Before the introduction of serum screening, 140 amniocenteses would have been required to identify one case of Down's syndrome based on a maternal age of more than 35 years. This figure has been reduced to one in 60 amniocenteses after the introduction of serum screening.

Today, with the introduction of the nuchal translucency and the nasal bone in the first trimester the detection rate for Trisomy 21 is around 92% and by adding the first trimester biochemistry (PAPP-A and free  $\beta$ HCG) the detection rate for Trisomy 21 is around 97%.

In the second trimester a detailed "soft marker" scan can increase the detection rate for chromosomal abnormalities to 98.5%.

The use of such screening has enabled prenatal karyotyping by amniocentesis, chorionic villus sampling (CVS) or cordocentesis with a risk of miscarriage of about 1 % to be focused on pregnancies at highest risk for chromosomal abnormalities regardless of age.

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Professor Ermos Nicolaou  
 Maternal Fetal Medicine Specialist  
 Fetal Medicine Centre  
 Chris Hani Baragwanath Hospital  
 University of the Witwatersrand