PRENATAL SONOGRAPHIC DIAGNOSIS OF ANEUPLOIDIES IN MULTIPLE

Alexandra Matias, MD PhD, Nuno Montenegro MD PhD
Department of Obstetrics and Gynecology, S. João Hospital, Medicine Faculty of Porto, Porto, Portugal
almatias@mail.telepac.pt

What is Screening All About?

A clear distinction should be established between screening and diagnostic tests. Thanks to **screening,** which comprises a methodical search, in an apparently normal population, for those individuals at a particular high risk of suffering from a defined pathological condition, we can then offer a complimentary **diagnosis** or a direct preventive/curative measure.

The decision to screen low-risk populations requires a series of prerequisites in order to make it useful and effective:

• The **natural history of the disease** should be **well-known**
• The disease should be significantly **prevalent**
• The test should have a low false-positive rate **(high specificity)**
• The test should have a low false-negative rate **(high sensitivity)**
• The test should be **simple, safe, reproducible** and **reliable**
• **Benefits** should outweigh the **risks**
• The test should be **cost-effective**
• The test should have **equal access** to the whole population, irrespective from financial status
• The test should be **acceptable** clinically, socially and ethically

Screening Versus Diagnosis of Aneuploidies

Down´s syndrome is the most prevalent autosomic chromosomal abnormality in human race (birth prevalence of about 1 in 800) and accounts for almost 50% of all aneuploidies. The main impact of Down´s syndrome is its contribution to mental retardation (8-33% of IQ<50%).

The most important factor determining the incidence of trisomy 21 is **maternal age.** Depending on maternal age distribution (5% in the early 1970s and 15% in the beginning of the 21st century of women are aged over 35 years), 50-70% of affected children are born to younger women who would be called "screen negative". Nevertheless, maternal age has been a consistent screening method since it is very cheap, is universally available, has no intra- or inter-observer variation, is non-invasive and is understandable by the women screened.

This background risk for aneuploidies, based on maternal age and gestational age, is redefined in a corrected individual risk depending on the results of further screening. Whenever the final risk is increased, that is, if the screening test is positive, these women may then be offered a suitable diagnostic test. When confronted with the definite diagnosis, the parents must be fully informed to be able to make their decision after careful consideration.

In the late 80s, screening based on the quantification of various fetoplacental products in the maternal circulation **(biochemical screening)** was introduced (Merkatz et al, 1984, Wald et al, 1988). In the 1990s, screening by a combination of maternal age and **fetal nuchal translucency thickness** (NT) at 11-14 weeks was proposed. This method has now been shown to identify about 75% of trisomy 21-affected fetuses for a screen-positive rate of about 5% (Snijders et al, 1998). In a fetus with a given crown-rump length, every NT measurement represents a factor that is multiplied by the background risk to calculate a new risk.

Twenty years have evolved and rules have changed in the screening of trisomy 21 for singleton pregnancies: detection rates were improved and invasive testing rate has decreased. Though preliminary, screening for trisomy 21 at 11-14 weeks of gestation has made its last steps combining the sonographic markers NT and nasal bone and the biochemical markers of free β-hCG and PAPP-A yielding a detection rate of about 95% for a false positive rate of 2% (Cicero et al, 2003, Spencer et al, 1999).

Does Twinning Process Matters?

Twins account for about 1% of all pregnancies and monozygotic (MZ) twinning occurrs in one third of twin pregnancies. Clearly, it is chorionicity rather than zygosity that determines several aspects of antenatal management and perinatal outcome. Zygosity refers to the type of conception, whereas chorionicity denotes the type of placentation, depending on the time of splitting of the fertilized ova.

The first step towards prenatal diagnosis and screening in multiple pregnancies is the establishment of chorionicity (around 100% correct chorionic assignment is possible in the first trimester of pregnancy). Like-sex and monoplacentation strongly suggest but do not prove monozygosity. Zygosity can only be determined by DNA fingerprinting.

Screening in Twin Pregnancies

Twins present unique and problematic issues in prenatal diagnosis. The performance of screening tests designed for singleton pregnancies is lowered. Surprisingly, recent investigation on this topic is limited despite its increasing importance on daily clinical care.

Screening for chromosomal abnormalities in twin pregnancies arise serious clinical, ethical and moral problems that need to be addressed:

- effective methods of screening, such as maternal serum biochemistry, are not applicable and have lower detection rates;
- in the presence of a "screen-positive" result, there is no feature to suggest which fetus may be affected;
- the techniques of invasive testing are more demanding in twins and there is difficulty in ensuring fetal tissue is obtained from each fetus;
- there is increased risk of miscarriage of an invasive test in twins;
- which invasive test to offer;
- the difficulties of clinical management of fetal reduction and the potential increased risk to the unaffected co-twin.

Counselling based on chorionicity, clinically more feasible than zygosity, results that in monochorionic twins both fetuses can be affected equally. This is, however, an oversimplification since, unlike all monochorionic pregnancies that are always monozygotic, only about 90% of dichorionic pregnancies are dizygotic.

The overall probability that a multiple gestation contains an aneuploid fetus is directly related to its zygosity. In **dizygotic** pregnancies, each fetus has an independent risk of aneuploidy, thus, the maternal age-related risk for chromosomal abnormalities for each twin may be the same as in singleton pregnancies, but the chance that at least one fetus is affected by a chromosomal defect is twice as high as in singleton pregnancies. In dizygotic twin pregnancies, the pregnancy-specific risk is calculated by *summing* the individual risk estimates for each fetus. The risk that both fetuses would be affected is a much rarer event, corresponding to the singleton risk squared. However, with higher risk conditions, such as autosomal recessive disorders, this could be as high as one in 16. For example, in a 40-year-old pregnant woman with a risk for trisomy of about 1 in 100 based on

maternal age, in a dizygotic twin pregnancy the risk that one fetus would be affected would be 1 in 50 (1 in 100 plus 1 in 100), whereas the risk that both fetuses would be affected is 1 in 10,000 (1 in 100x1 in 100). Finally, we should take into consideration that since the rate of dizygotic twinning increases with maternal age, the proportion of twin pregnancies with chromosomal defects is higher than in singleton pregnancies.

In **monozygotic** twins, the risk of an affected fetus approximates the maternal age risk of a singleton pregnancy and the risk for one fetus is, in expectation, the same as the risk for the other. This ignores the small possibility of heterokaryotypic monozygotic twins resulting from a mitotic non-disjunction after the zygote splits. There are occasional reports of monozygotic twins discordant for abnormalities of autosomes or sex chromosomes, most commonly with one fetus presenting a Turner syndrome and other either a normal male or female phenotype, but usually with a mosaic karyotype, or a Klinefelter syndrome.

The relative proportion of spontaneous dizygotic to monozygotic is about 2:1 and, therefore, the prevalence of chromosomal abnormalities affecting at least one fetus in a twin pregnancy would be expected to be about 1.6 times that in singletons. If zygosity is unknown the risk of at least one aneuploid fetus can be approximated as five-thirds that of the singleton risk. This is based on the assumption that a third of all twin pairs are monozygotic (Rodis et al, 1990).

When calculating the risk of higher order multiples, estimates can be made multiplying the singleton risk by the number of fetuses (Jenkins and Wapner, 2000). This method assumes unique chorionicity for each fetus, though monozygosity can occur more frequently than usually thought at higher rates in ART multiple gestations (Blickstein et al, 1999).

Biochemical Screening

Biochemical screening in twins was the first alternative to age derived risk but clearly has a lower detection rate for fetal aneuploidies and higher rates of false positives. Chorionicity does not appear to affect the distribution and level of maternal serum analytes and, therefore, it does not need to be taken into account when interpreting biochemical markers in twin pregnancies.

In twin pregnancies interpretation of serum analytes is clearly more problematic since each serum marker necessarily relates to the pregnancy and is not specific to the fetus, deriving solely a pregnancy and not a fetus-specific risk.

As biochemical screening in twins is still investigational and far less powerful than in singletons, it should not be recommended in general practice without extensive counselling.

Nuchal Tanslucency

The possibility of deriving a risk for trisomy 21 from NT assessment in the first trimester of pregnancy shifted the consideration of a *pregnancy-specific risk* to a fetus-specific risk. This assumption is based on the observation that the distribution of NT measurements in twin fetuses with trisomy 21 is similar to that in singletons (Pandya et al, 1995; Sebire et al, 1996a,b).

In one of the first studies for trisomy 21 in twins involving 448 twin pregnancies, NT was measured in each fetus and the risk was estimated by combining it with maternal age. The NT was above the 95t^{h} centile for gestational age in 65 of the 896 (7.3%) fetuses, including 88% of those with trisomy 21 (Sebire et al, 1996a). Eight out of nine fetuses affected with trisomy 21 were detected for an overall sensitivity of 88% which is comparable to the sensitivity obtained in singletons. When analyzing the false positive results, a higher rate was seen in monochorionic gestations (8.4%) compared with dichorionic gestations (5.4%). In fact, increased NT at 10-14 weeks of gestation was found twice as much as in monochorionic than in singleton pregnancies, but concomitantly the likelihood ratio of developing twin-to-twin transfusion syndrome in those twins with increased NT was higher (3.5x) (Sebire et al, 1997, 2000, Matias et al, 2000). Considering that monochorionic pregnancies do not show a higher prevalence of chromosomal abnormalities, the higher prevalence of increased NT in those twins could be ascribed to cardiac dysfunction.

For higher order multiple´s more credible data using NT alone to assess risk in triplets or more are described by Maymon and co-workers (1999) who attempted to perform trisomy 21 screening in higher order twin pregnancies (≥3) compared to consecutively matched singleton controls. Not only was it feasible and reproducible, but also mean NT was similar for both groups (1.41±0.41 mm and 1.35±0.39 mm, respectively, and 0.87±0.23 MoM and 0.83±0.25 mm, respectively) (Maymon et al, 1999).

In a monochorionic twin pregnancy, both will be affected or both will be unaffected. It is therefore appropriate to take the average of the two NT measurements, so that a single risk estimate can be calculated *(averaging method).*

In a dichorionic twin pregnancy, the twins are dizygotic in about 90% of the cases, which means that one of the fetuses, or, much more rarely, both fetuses could be affected. Both fetuses have an independent risk, so that it is reasonable to sum the risks on the basis of the NT measurements **(summing method).** The 10% of dichorionic twin pregnancies that are monozygotic will incorrectly have their risks calculated by the summing rather than the averaging method. However, the ultimate effect on screening performance will be a negligible one.

Ductus Venosus Flowmetry

In recent studies of vascular haemodynamics in fetuses with increased NT at 10-14 weeks, abnormal flow in the ductus venosus (DV) was more frequently recorded in fetuses with chromosomopathies, with or without cardiac defects, probably related to heart dysfunction (Montenegro et al, 1997; Matias et al, 1998). These findings are in good agreement with the overt haemodynamic alterations found in TTTS later in pregnancy. Therefore, accumulated evidence suggests that increased NT along with abnormal flow in the DV, even in the presence of a normal karyotype, may be early signs of cardiac impairment or defect (Montenegro et al, 1997; Matias et al, 1998, 2000).

During a four-year period 55 monochorionic diamniotic pregnancies were identified in our Ultrasound Unit during routine ultrasonographic assessment at 11-14 weeks of gestation. Nuchal translucency and Doppler blood flow waveforms in the DV were recorded in both twins between 11-14 weeks of gestation. TTTS was recorded in those fetuses which combined increased NT and abnormal flow in the DV. Until now, in all cases with both discrepant NT and abnormal blood flow in the DV, TTTS eventually developed. In contrast, whenever NTs were discrepant but with normal flow in the DV, no cases of TTTS were found (Matias et al, 2005). In dizygotic twin pairs, increased NT had a different meaning and detected some cases of Down´s syndrome.

Combination of NT and biochemical markers for screening of trissomy 21 at 11-14 weeks: the gold standard?

Considering that biochemical screening alone cannot specifically identify the fetus at risk in the presence of twins discrepant for Down´s syndrome, it seems reasonable to combine NT and maternal biochemical markers, as suggested by Spencer (2000). In this modelled study it was demonstrated that biochemical screening would add a further 5% to the detection rate obtained by using NT alone and thus offering a detection rate for Down´s syndrome of about 80% compared to the 90% in singleton pregnancies.

In prospective screening in the first trimester using combined ultrasound and biochemical screening over a three year period, Spencer and Nicolaides (2003) offered screening to 230 women with twins. The risk for trisomy 21 was calculated for each fetus based on the individual NT and the maternal serum biochemistry corrected for twins. Four cases were observed with twins discordant for trisomy 21 and in 3 cases combined screening identified the affected pregnancy. Of the twin fetuses screened, 6.8% had risks greater than the cut-off and 9.2% of pregnancies had at least one fetus with an increased risk.

Interestingly, the combined test is more discriminative in monochorionic than in dichorionic twin pregnancies, since in DC pregnancies the median serum marker level in affected pregnancies will be artificially lowered by the unaffected twin, whereas in MC pregnancies no dilution of the serum markers is expected from an unaffected pregnancy.

Conclusions

First or second trimester screening in twin pregnancies is feasible and still efficacious, either by using the combination of ultrasound and maternal serum biochemistry in the first trimester (80% detection rate), or maternal serum biochemistry in the second trimester (50-55% detection rate) (Wald et al, 2003). However, these "pseudo risks" have been challenged for their scientific and clinical validity.

Until more data are available from larger studies on the distribution of markers in concordant or discordant twins, NT estimated for each fetuses should be the predominant factor by which women presenting with increased risk should be counselled regarding invasive testing. In dizygotic pregnancies pregnancy specific risk should be calculated by summing the individual risk estimates for each fetus. In monozygotic twins, the risk should be calculated based on the geometric mean of both NT measurements, not forgetting that the false positive rate of NT screening is expectantly higher than in singletons. Nevertheless the calculated detection rates modelled using this method are still 10% lower than in singleton pregnancies.

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