# Down's syndrome screening in twins

Maternal serum screening for Down's syndrome in twin pregnancies is fraught with difficulties. Firstly, the standard method of test interpretation, by calculating the risk of an affected pregnancy, requires additional assumptions in twins because information is lacking. Unlike with singletons there is little direct information on the prior maternal age-specific risk and the distribution of marker levels in affected pregnancies. Secondly, the detection rate is relatively poor because in an affected pregnancy feto-placental products from the unaffected cotwin can mask the effect seen in singletons. Lastly, there is the problem of invasive prenatal diagnosis in twins and the selective abortion of the affected fetus.

On theoretical grounds the prior risk of Down's syndrome for each twin pregnancy should be greater than the risk in singleton pregnancies. Because there are two fetuses, if the probability of the second being affected were independent of the first, the risk that at least one twin is affected would be double that of singletons. Actually, the risk will be somewhat less than double because monozygous twins will be concordant for Down's syndrome so reducing the overall risk. Theoretical age-specific risks have been published[1] based on 20% monozygosity, though they incorrectly assume that the monozygosity rate is unrelated to age.

However, the observed prevalence of Down's syndrome in twin pregnancies is much less than the theoretical calculations predict. A meta-analysis of four cohort studies including a total of 64 twins with Down's syndrome yielded a birth prevalence only 18% higher in twin pregnancies than in singletons.[2] When this is extended to include a more recent large study based on notifications to the Office of Populations Censuses and Surveys,[3] taking the combined total of twins with Down's syndrome to 106, the prevalence was only 3% greater than for singletons. None of the five studies was stratified for maternal age and the chance of having a twin increases with age. Therefore the observed small increase in the crude Down's syndrome prevalence rate among twins implies a reduction in the age-specific prevalence rate. Until there is a more precise estimate of these rates it is probably best to assume that the prior term risk for twins does not differ from that of singletons. The prior risk during pregnancy is even more problematic. The discrepancy between the observed crude rate and that expected from theoretical calculations may be accounted for by a particularly high intrauterine lethality for affected twins. If so, the prior risk in a twin during pregnancy may be much higher than for singletons. There are insufficient published data on which to judge this at present.

In unaffected twin pregnancies the median level of the commonly used maternal serum markers is about double that in singletons but the other distribution parameters—standard deviations and correlation coefficients—seem to be the same as in singletons. When a meta-analysis of eight published studies[4–11] is used the unaffected medians for α fetoprotein (AFP), unconjugated oestriol ($uE_3$), human chorionic gonadotrophin (hCG), and free β-hCG are: 2.26 (1314 twins in total[4–6 9–11]), 1.68 (696[4–6 11]), 2.06 (890[4–7 11]), and 2.07 (844[8–10]) multiples of the normal gestation specific median (MoM) respectively. Some centres are beginning to use inhibin A and so far only one series of results from unaffected twins has been published with a median of 1.99 MoM.[12] If each fetus contributed the same amount as a singleton pregnancy the median would be expected to be exactly 2 MoM. The observed deviations from expectation might be due to chance but the effect, particularly for $uE_3$, may be real because it is reasonably consistent between studies.

The marker distribution parameters in twins where one or both of the fetuses has Down's syndrome cannot be estimated directly. There is only one report of multiple marker levels in such pregnancies and this includes just eight cases, all of which were discordant.[9] In the absence of more extensive data it is best to obtain the parameters indirectly. To derive the medians a reasonable approach is to assume that each fetus contributes the expected amount for an affected or an unaffected singleton and that the same deviation from expectation seen in unaffected twins also applies.[11] For example, the median AFP level in affected dizygous twins would be expected to be $(1+0.73)\times(2.26/2)$ or 1.95 MoM and 1.65 MoM for monozygous twins, as the singleton median for Down's syndrome is 0.73 MoM.[13] As with unaffected pregnancies the other distribution parameters can be taken to be the same as the corresponding values in singletons.

Standard multivariate Gaussian modelling techniques can be used to estimate the detection rate for a 5% false positive rate achievable by screening all twins and using the prior risk and medians derived as above. This has been done assuming that the maternal age distribution in unaffected twins is one year more advanced than in singletons, that one third of Down's syndrome pregnancies are monozygous, and that zygosity is unrelated to age.[11] The estimated detection rate for AFP, $uE_3$, and hCG was 51%. However, not only is the assumption of age independence incorrect but if, as the birth prevalence figures suggest, there is high intrauterine lethality of affected twins, monozygosity is likely to be infrequent. In these circumstances it may be better to assume that all are dizygous. For a population with the same maternal age distribution of twins as for England and Wales in 1991–95,[14] the computed risk using a set of twin parameters derived from single parameters obtained by meta-analysis[13] yielded an estimated detection rate for a two marker combination of AFP and free β-hCG of 41%. When $uE_3$ was included as the third marker the detection rate increased to 44%, and with inhibin A as the fourth marker it increased to 47%. None of these rates is as high as for screening in singletons: 62%, 66%, and 72% respectively.

The poor results with maternal serum are due to the interference of the unaffected cotwin, which is not a problem for first trimester ultrasound nuchal translucency screening. In a small series of 392 twin pregnancies nuchal translucency thickness in fetuses with Down's syndrome was similar to that found in affected singletons and much greater than in unaffected twins.[15 16] Using parameters derived from about 60 000 women in the King's College Hospital multicentre prospective intervention trial of nuchal translucency screening,[17 18] it has been estimated that, with maternal age, the detection rate in singletons is 79% for a 5% false positive rate. This may be an overestimate because detected cases were identified in the first trimester and missed ones later, after some might have miscarried.[19] This suggests that if the presence of twins is confirmed early in pregnancy the Down's syndrome screening method of choice is nuchal translucency measurement. A study group of the Royal College of Obstetricians and Gynaecologists has recommended that there are now "sufficient data to consider screening for

Down's syndrome by measurement of nuchal translucency at 10–14 weeks" provided that ultrasound staff are certified and subject to quality control by an external agency.[20] If such screening, either alone or in combination with maternal serum pregnancy associated plasma protein A and free β-hCG, eventually becomes widespread the problem will be resolved.

Antenatal diagnosis in twins is also problematic. In a study of 227 twin pregnancies where amniocentesis had been performed the fetal loss rate was double that in an age and period matched control series of twins.[21] Both fetuses need to be karyotyped and if only one is found to have Down's syndrome selective fetocide may be offered using either air immobilisation or potassium chloride. However, these procedures are associated with a high death rate for the unaffected cotwin. In a large multicentre study, including 169 twins who had been reduced to singletons, the miscarriage rate was 13%, though there was a tendency for the losses to be fewer when the procedure was carried out before 16 weeks' gestation.[22]

Centres offering routine second trimester maternal serum screening for Down's syndrome need to make special provision for twins. Despite the difficulties, testing cannot be avoided even if this is only in those diagnosed as twins after the test has been performed. Laboratories providing risk estimation in twin pregnancies should ensure that their test interpretation software makes reasonable assumptions about prior risk and marker distributions. Also, women with a twin pregnancy should be informed of the low detection rate and the hazardous consequences of a positive result in twins.

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