

Research letters

Antenatal screening for Down's syndrome with the quadruple test

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Second trimester screening for Down's syndrome is widely practised throughout the world. We assessed the performance of antenatal serum screening for Down's syndrome with the quadruple test in 46 193 pregnancies from 14 hospitals over 5 years. Women who screened positive (risk ≥ 1 in 300) were offered diagnostic amniocentesis or chorionic villus sampling. Of 88 observed Down's syndrome pregnancies, 71 (81%) had a positive screening result (81% detection rate, 95% CI 72–89), and of 46 105 unaffected pregnancies, 3200 tested positive (7% false-positive rate). These results show that the quadruple test is a better method of screening for Down's syndrome than use of maternal age alone (51% detection rate, 14% false-positive rate) and is more effective than other second trimester screening tests. Therefore, we conclude that the quadruple test should be the test of choice in second trimester screening for Down's syndrome.

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See Commentary page 794

The quadruple test for Down's syndrome^{1,2} calculates the risk of a Down's syndrome term pregnancy from maternal age at term and the concentration of four markers in maternal serum—alphafetoprotein, unconjugated oestriol, human chorionic gonadotropin (total hCG or, more usually, the free β subunit), and inhibin-A—at 14–22 weeks of pregnancy. Existing estimates of the screening performance of this test are based on testing stored serum samples in cases and controls.

We aimed to assess the efficacy of the quadruple test in clinical practice. Between August, 1996, and September, 2001, we prospectively assessed the quadruple test at 14 hospitals, as part of routine screening offered by the UK National Health Service. All four markers were measured and maternal age was recorded for all pregnancies. The screening performance (in terms of the detection rate observed for a 5% false-positive rate) of the quadruple test was then compared with that using maternal age alone, the triple test, and the double test to determine whether the advantage expected from the results of previous observational studies was seen in practice. Such an approach—in which different screening methods are compared in the same women, with every woman acting as her own control—is statistically more powerful than a

randomised trial comparing one method in one group of women with another method in a different group, and it provides far stronger evidence than non-randomised comparisons between women.

The biochemical tests were done at Bart's and the London School of Medicine and Dentistry, London, UK. We defined a positive result as a risk of one in 300 or greater, because research predicted a high detection rate (about 75%)^{1,2} with the same 5% false-positive rate used with other tests. The risk of a Down's syndrome pregnancy was estimated with α (versions 4, 5, and 6), which implements a screening algorithm that uses a multivariate Gaussian model.^{1,2} In 79% of women, gestational age was established by an ultrasound scan. Down's syndrome pregnancies, including those missed by screening, were ascertained from hospital records and cytogenetic laboratories.

The analyses for the double and triple tests were done by omitting the results of markers (inhibin-A for the triple test, inhibin-A and unconjugated oestriol for the double test), re-computing risk for all women, and counting the proportion of affected pregnancies at and above the 95th centile of risk for unaffected pregnancies, which gives the detection rate at a 5% false-positive for each method.

Quadruple screening was undertaken and maternal age recorded in 46 193 pregnancies. There were 149 twin pregnancies, none of which were affected with Down's syndrome. Ascertainment is likely to have been complete because the number of affected pregnancies identified (88, including 20 livebirths) was identical to that expected by applying the age-specific risk of Down's syndrome to the age distribution of screened women, after allowing for the 23% spontaneous fetal loss of affected pregnancies between 16 weeks and term:³ 72 expected at birth, less 20 observed, leaves 52, corresponding to 68 (52/[1–0.23]) at mid-trimester, which, when added to the 20 livebirths, yields 88.³

Table 1 shows the screening results with the quadruple test, and those that would have been achieved if the only screening method used had been maternal age with a cutoff of 35 years. The detection rate with the quadruple test was 81% (95% CI 72–89%) and the false-positive rate was 7%. The odds of being affected in those with a positive screening result were 1:45. With maternal age alone, the detection rate

Screening method	Number of pregnancies screened			Number of screen positives			Detection rate	False-positive rate	Odds of being affected given a positive result
	All	With Down's syndrome	Unaffected	All	With Down's syndrome	Unaffected			
Quadruple test*	46 193	88	46 105	3271	71	3200	71 of 88 (81%)	3200 of 46 105 (7%)	1:45 (71:3200)
Maternal age†	46 193	88	46 105	6659	45	6614	45 of 88 (51%)	6614 of 46 105 (14%)	1:147 (45:6614)

*Risk at term ≥ 1 in 300. † ≥ 35 years.

Table 1: Results of antenatal screening for Down's syndrome with quadruple test (including maternal age) and with maternal age alone

Screening method	Observed		Adjusted*	
	DR (95% CI)	OAPR	DR	OAPR
Maternal age alone	26% (17–35)	1:104	24%	1:135
Double test†	61% (51–72)	1:43	57%	1:56
Triple test‡	66% (56–76)	1:40	62%	1:52
Quadruple test§	75% (66–84)	1:35	70%	1:45

88 Down's syndrome pregnancies observed. *Allowing for spontaneous fetal loss of Down's syndrome pregnancies after about 16 weeks' gestation.³ †Alphafetoprotein and hCG. ‡Alphafetoprotein, hCG, and unconjugated oestriol. §Alphafetoprotein, hCG, unconjugated oestriol, and inhibin A. All tests include maternal age.

Table 2: Detection rates (DR) and odds of being affected given a positive result (OAPR) for a fixed 5% false-positive rate by method of screening

would have been much lower (51%, 95% CI 41–62%), the false-positive rate more than twice as high (14%), and the odds of being affected in those with a positive result less favourable (1:147) than those obtained with the quadruple test.

Table 2 shows detection rates for a 5% false-positive rate for all methods, before and after adjustment for the spontaneous fetal loss of affected pregnancies after about 16 weeks of gestation.³ The adjusted results from the screening programme were similar to the estimates from statistical modelling of data from the original study with stored serum samples⁴ (30%, 58%, 68%, and 75% detection rates for a 5% false-positive rate for age alone, double, triple, and quadruple tests, respectively). The adjusted detection rates in table 2 show that the quadruple test was substantially better than the double test (70% *vs* 57%) and moderately better than the triple test (70% *vs* 62%). Statistical significance testing is inappropriate in table 2 because, in expectation, each method is better than the one above it, so the null hypothesis (that there is no difference between the methods) does not apply. The table shows that the more markers that are used, the better the predictive value of the test. The additional advantage of the quadruple test over the triple test is that, at the same 5% false-positive rate, it detected 21% of the cases missed by the triple test at a cost that involved no more than the measurement of another marker in a blood sample already collected.

Uptake of amniocentesis (or sometimes chorionic villus sampling) increased with increasing risk (trend test $p < 0.0001$). Only 216 of 501 (43%) women with risks of 1 in 250–300 had an amniocentesis, whereas 105 of 141 (74%) did so if they had risks higher than 1 in 50. Among women who tested positive and had affected pregnancies (who tended to be at very high risk) 87% had an amniocentesis (62 of 71), and of these 95% (59 of 62) had a termination of pregnancy. The influence of an anomaly scan in women with screen-positive results might have led to a lower overall uptake of amniocentesis than anticipated (60%). The uptake of serum screening could not be established because the number of women who were offered such testing was not recorded.

Our results, from a routine screening service, confirm the value of early second trimester serum screening over screening using maternal age alone, in contradiction to recent opinion,⁵ and lend support to the decision of the UK government to offer serum screening to all pregnant women. We have also confirmed that in the second trimester the quadruple test is sufficiently more effective than the double or triple tests that it should be regarded as the test of choice at this time of pregnancy.

Contributors

N Wald is in charge of the screening programme, and was the main author of this report. W Huttly manages the screening programme and assembled the database. A Hackshaw did the statistical analysis.

Conflict of interest statement

N Wald is a director of Logical Medical Systems, which produces the software alpha, for antenatal screening for Down's syndrome and neural tube defects, and of Intema, which holds rights to the Integrated test. He has interests in patents (granted and pending) in antenatal screening.

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- 1 Wald NJ, Densen JW, George L, Muttukrishna S, Knight PG. Prenatal screening for Down's syndrome using inhibin-A as a serum marker. *Prenat Diagn* 1996; **16**: 143–53.
- 2 Wald NJ, Densen JW, George L, et al. Inhibin-A in Down's syndrome pregnancies: revised estimate of standard deviation. *Prenat Diagn* 1997; **17**: 285–90.
- 3 Morris JK, Wald NJ, Watt HC. Fetal loss in Down syndrome pregnancies. *Prenat Diagn* 1999; **19**: 142–45.
- 4 Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. *J Med Screen* 1997; **4**: 181–246.
- 5 Wellesley D, Boyle T, Barber J, Howe DT. Retrospective audit of different antenatal screening policies for Down's syndrome in eight district general hospitals in one health region. *BMJ* 2002; **325**: 15–17.

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Alternative lengthening of telomeres and survival in patients with glioblastoma multiforme

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Despite advances in the molecular pathogenesis of glioblastoma multiforme, no reliable prognostic markers have been identified. We analysed telomerase activity and telomere lengths in glioblastoma multiformes from 77 patients. 19 patients (25%) had tumours with the alternative-lengthening-of-telomere (ALT) phenotype. Median survival for patients with this phenotype was 542 days (95% CI 114–970) compared with 247 days (224–270) for glioblastoma multiformes with normal telomeres ($p=0.0003$). Cox's regression analysis showed that this association is independent of age. In patients with non-ALT tumours, telomerase activity did not affect survival (median 287 [199–375] *vs* 236 [230–242] days, $p=0.275$). We conclude that ALT is a prognostic indicator for patients with glioblastoma multiforme.

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Patients with glioblastoma multiforme have a poor prognosis, with median survival being less than 9 months from diagnosis.¹ However, length of survival varies greatly, suggesting that intrinsic factors could differentially affect outcome. At present, the molecular basis for this variation is