Chapter 7 Integrated testing for trisomy 18

7.1 introduction

In the 1990s there was a real division in prenatal care providers between those offering serum-based second trimester testing for Down syndrome, and others who were convinced that first trimester NT measurements w/wo biochemistry was at least as efficient, and provided an opportunity for earlier diagnostic testing. In 1999, Wald and colleagues (Wald *et al.*, 1999) redirected the discussion from first or second trimester testing, to a combination of first and second trimester testing. He theorized that if both first and second trimester screening were each effective tests, then the combination of the two must be even better. Interpreting biochemical and/or ultrasound marker results from both trimesters into a single interpretation for Down syndrome was called 'integrated' testing and is covered by patent (U.S. Pat No. 6,573,103, issued 6/3/03 to Nicholas J. Wald).

Integrated testing comes in two flavors, serum integrated, where NT measurements are not included and full integrated, when NT measurements are included in the interpretation. Although not in this chapter, additional twists in implementation include sequential and contingent integrated testing (Chapter 10). In the original description of integrated screening, no interpretation is provided until all data is available for the interpretation in the second trimester. Waiting until the second trimester has been criticized for two reasons. First, it does not allow for any first trimester diagnostic testing, a leading reason for combined testing in the first trimester. Second, it can appear to some that the laboratory is 'withholding' information from the woman that could be useful in decision-making. Sequential testing avoids much of the controversy by allowing for a very high risk group (usually about 0.5%) to receive their results after first trimester testing is completed (Palomaki *et al.*, 2006). In order to be efficient, an NT measurement is required for sequential testing. In this group, nearly 50% of the Down syndrome cases can be detected. The remainder of the population (99.5%) received a full integrated test, as planned. Contingent testing goes a step further, but not only having a high risk cut-off, but also a low risk cut-off (usually about 1:2000 to 1:5000). Women with first trimester risks below this level are told that testing is completed and there is no need to return for the second trimester portion of the test. Sequential testing, when implemented correctly, is nearly as efficient as full integrated testing and is becoming more commonly offer as part of routine prenatal care in the US. Contingent screening is not as widely used.

7.2 Modeling serum integrated test performance

Serum integrated screen for Down syndrome has been reported from Maine (Knight *et al.*, 2005), and a serum integrated algorithm for trisomy 18 has been published (Palomaki *et al.*, 2003). That modeling relied on first trimester PAPP-A measurements along with second trimester AFP, uE3 and hCG measurements from a previous publication (Palomaki *et al.*, 1995). Performance was estimated to be 90% at a 0.1% false positive rate. This is similar to the performance estimated for the quadruple test for trisomy 18 in the second trimester (AFP, uE3, hCG and PAPP-A) of 90% at 0.2% (Table 3.9-2). However, it is better than the performance of the combined test in the first trimester 0.5% to 0.8%, depending on the hCG subunit tested (Table 6.2.-1). The advantage of a serum integrated test over the second trimester quadruple test for trisomy 18 is that an additional assay (PAPP-A in the second trimester) is not necessary.

The one missing parameter is between trimester correlation coefficients for trisomy 18. In the previous modeling (Palomaki *et al.*, 2003) it was assumed that the correlation coefficients between trimesters for these markers was 0. For unaffected pregnancies, data now exist (Wald *et al.*, 2003) that show the correlations are low (first trimester PAPP-A correlations with second trimester AFP, uE3, free β, and hCG were all positive and less than 0.12). For trisomy 18 pregnancies, unpublished data are available from the FaSTER Study (Malone *et al.*, 2005) to determine the correlation coefficients between PAPP-A in the first trimester and AFP, uE3 and hCG measurements in the second trimester (personal communication, Dr Geralyn Messerlian-Lambert). Figure 7.2-1 shows this data collected from 12 trisomy 18 pregnancies having both first and second trimester serum samples obtained and tested as part of that study. For comparison, the 93 available matched controls for these 12 trisomy 18 pregnancies had correlations of -0.0625, 0.01777 and 0.1251, respectively. The modeling uses correlations in unaffected pregnancies as derived in the SURUSS report (Wald *et al.*, 2003), and the values are 0.0114, 0.1003 and 0.2198, respectively. This high level of agreement in the unaffected pregnancies provides some additional confidence that the correlations found for the 12 trisomy 18 pregnancies are accurate, albeit, imprecise.

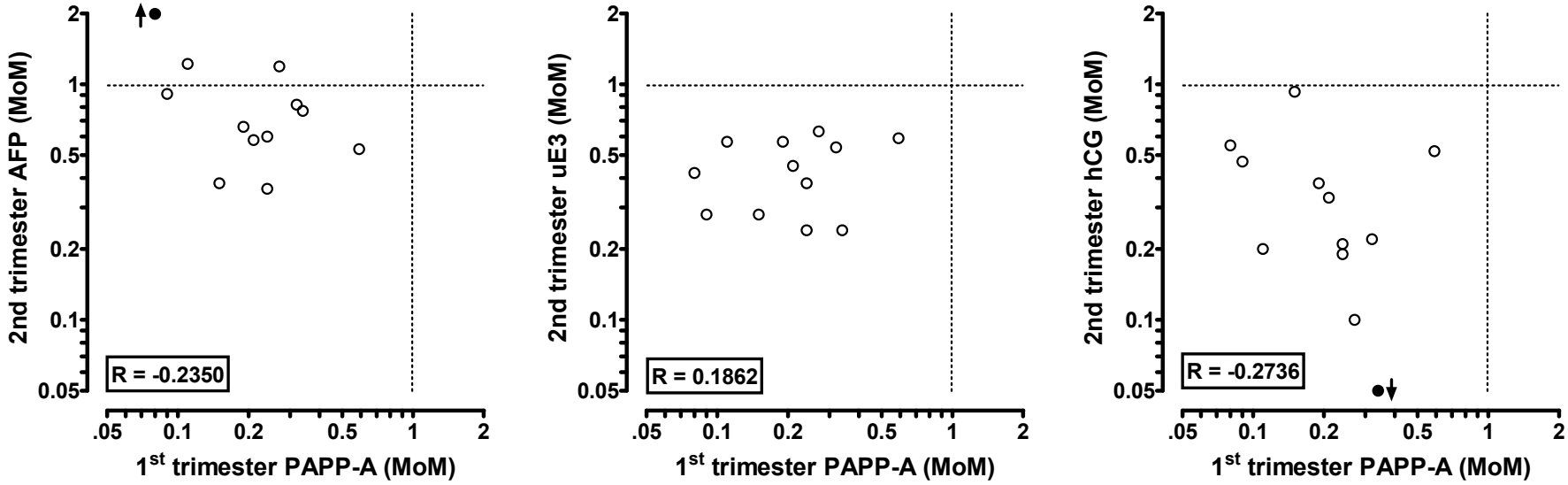

Figure 7.2-1 Correlation between first trimester PAPP-A and second trimester AFP, uE3 and hCG measurements from 12 trisomy 18 pregnancies. The three figures show the pair-wise correlation coefficients (after a logarithmic transformation) and the exclusion of the paired observations. All the correlations are relatively small. The vertical and horizontal dotted lines are shown at the median level for unaffected pregnancies. All of the markers examined are significantly lower than 1.00 MoM.

Table 7.2-1 shows the detection and false positive rates for serum integrated testing for trisomy 18 using updated age related risks (Chapter 2) and serum parameters (Chapters 3 and 6) and the between-trimester correlations in unaffected pregnancies from SURUSS (Wald *et al.*, 2003) and in affected pregnancies as shown in Figure 7.2-1. At a second trimester risk cut-off level between 1:70 and 1:140, the detection rates are in the mid 80% range, with about a 0.2% false positive rate. The OAPR is about 1:2 or 1:3. As described earlier, it is likely that the observed positive rate in practice will be higher that the modeling suggests, due to the identification of other abnormalities, such as existing fetal deaths.

There is insufficient information to create the corresponding model using free β measurements instead of total or intact hCG measurements in the second trimester. However, were the correlations assumed to be the same for PAPP-A and free β as for PAPP-A and hCG, the performance would only be slightly improved (e.g., at a given false positive rate, the detection would be, at most, 1% or 2% higher (modeling results not shown).

Table 7.2-1. Serum integrated testing for trisomy 18: Modeled detection rates (DR) and false positive rates (FPR)

	DR (%)	FPR (%)	OAPR[2]
DR (%)			
50	-	<0.1	>1: 3
60	-	<0.1	>1: 2
70	-	<0.1	>1: 2
80	-	0.1	1: 2
90	-	0.6	1: 9
FPR (%)			
0.3	87	-	1: 4
0.5	89	-	1: 7
0.7	92	-	1:10
1.0	93	-	1:14
1.5	94	-	1:21
Risk cut-off level at term (in second[1])			
1:100 (1: 35)	76	<0.10	>1: 2
1:150 (1: 52)	80	<0.10	>1: 2
1:200 (1: 70)	82	0.10	1: 2
1:250 (1: 88)	83	0.13	1: 2
1:300 (1:105)	84	0.16	1: 3
1:350 (1:123)	85	0.19	1: 3
1:400 (1:140)	86	0.21	1: 3

Serum integrated testing is defined as a first trimester PAPP-A measurement combined with second trimester AFP, uE3 and hCG measurements

[1] assuming a 65% fetal loss from 16-18 weeks to term (Table 2.4-1)

[2] OAPR = second trimester odds of being affected given a positive result (assumes 1:1300, derived from the birth prevalence of 2.69/10,000, with adjustment for 65% fetal loss from 16-18 weeks to term).

7.3 Modeling full integrated test performance

The same parameters using in modeling serum integrated performance (Chapter 7.2) can be used to model full integrated performance, with the addition of information about NT distributions in trisomy 18 and unaffected pregnancies (Chapter 5.2). Studies have consistently found that NT measurements have little or no correlation with serum markers in either unaffected or unaffected pregnancies, and the model will assume these correlations are zero. Given that the ultrasound measurement of NT is the strongest predictor of trisomy 18, the full integrated test should have the highest possible performance.

Table 7.3-1 shows the results of modeling full integrated test performance for trisomy 18. The detection rates are 90% or higher, with corresponding false positive rates of 0.2% or lower. Expected OAPRs are on the order of 1:2, but in practice, lower odds might be expected due to other outcomes with this pattern such as fetal death.

Comparing this performance with first trimester combined testing

Full integrated screening can detect 90% of trisomy 18 at a 0.1% false positive rate, but requires waiting until the second trimester for the interpretation and subsequent offering of diagnostic testing. To achieve a 90% detection rate in the first trimester, the false positive rate is higher at 0.5%. Although there are 5 times as many false positives, the advantage is that diagnostic testing can be offered several weeks earlier, sometimes in the first trimester. Fortunately, there is a way to keep the higher performance of full integrated screening while allowing for most of the diagnoses to be made in the first trimester. Conceptually, the first step of integrated testing identifies pregnancies at very high risk and offers first trimester diagnostic testing immediately. That cut-off is so high that subsequent testing in the second trimester would be unlikely to re-classify them as being test negative. Such a protocol has been labeled 'sequential' and has been well described for Down syndrome screening (Palomaki *et al.*, 2006). That paper demonstrates that it is always possible to create a sequential strategy with only slightly lower performance than full integrated, and that such a protocol usually results in most cases identified in the first trimester. In Section 10, modeling sequential testing for trisomy 18 is performed and compared with full integrated as well as first trimester testing.

Table 7.3-1. Full integrated testing for trisomy 18: Modeled detection rates (DR) and false positive rates (FPR)

	DR (%)	FPR (%)	OAPR[2]
DR (%)			
50	-	<0.1	>1: 3
60	-	<0.1	>1: 2
70	-	<0.1	>1: 2
80	-	<0.1	>1: 2
90	-	0.1	1: 1
FPR (%)			
0.3	94	-	1: 4
0.5	95	-	1: 7
0.7	96	-	1: 9
1.0	97	-	1:13
1.5	98	-	1:20
Risk cut-off level at term (in second[1])			
1:100 (1: 35)	84	<0.1	>1: 2
1:150 (1: 52)	86	<0.1	>1: 2
1:200 (1: 70)	88	<0.1	>1: 2
1:250 (1: 88)	89	<0.1	>1: 2
1:300 (1:105)	89	<0.1	>1: 2
1:350 (1:123)	90	<0.1	>1: 2
1:400 (1:140)	91	0.11	1: 2

Full integrated testing is defined as first trimester PAPP-A and NT measurements combined with second trimester AFP, uE3 and hCG measurements.

[1] assuming a 65% fetal loss from 16-18 weeks to term (Table 2.4-1)

[2] OAPR = second trimester odds of being affected given a positive result (assumes 1:1300, derived from the birth prevalence of 2.69/10,000, with adjustment for 65% fetal loss from 16-18 weeks to term).