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REVIEW ARTICLE

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Screening for Down syndrome

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In 1866 J Langdon Down made the observation of a subgroup of patients with particular facial features and mental handicap in the outpatient department of the London Hospital [1]. In the late 1950's, it was shown that an extra acrocentric chromosome was present in persons with Down syndrome, resulting in a diploid chromosome number of 47. It is now known that Down syndrome results when either the whole or a segment of the long arm of chromosome 21 is present in three copies instead of two (Table 1). This can occur as a result of three separate mechanisms: non-dysjunction (94% of cases), Robertsonian translocation (3.6%) and mosaicism (2.4%).

Table 1. Milestones in the history of screening for Down syndrome.

1933	Association between maternal age and Down syndrome noted
1959	Trisomy 21 identified as the cause of Down syndrome
1966	First chromosome analysis from amniotic fluid
1968	Prenatal diagnosis of Down syndrome
1972	Raised amniotic fluid AFP associated with open neural tube defects
1977	Maternal serum AFP screening for open neural tube defects
1988	Triple test
1991	Nuchal translucency

AFP = alpha fetoprotein

The live incidence of Down syndrome is about 1 in 700 births. Approximately 30% of Down syndrome fetuses miscarry between 12 weeks gestation and term, while it is estimated that 24% will miscarry between 16 weeks and term [2]. Affected babies are likely to suffer from severe mental disability and have a high chance of associated physical disabilities, affecting in particular the heart, gastrointestinal tract, eyes, and ears. Individuals with Down syndrome also have a higher incidence of Alzheimer's disease and a 15 to 20 times higher risk of leukemia. Twenty percent die by the age of 5 years, usually from cardiac causes, but over half are expected to survive into their fifties.

Antenatal screening for Down syndrome was first performed in the 1970's using advanced maternal age or a previous history of aneuploidy. In the 1980's, the association of Down syndrome with abnormal levels of certain specific serum markers was discovered, and maternal serum screening was developed which further improved the detection rate [3]. Recent addition of ultrasound as a tool for screening offers several advantages:

1. Fetal nuchal translucency thickness in the first trimester becomes an independent screening tool for Down syndrome [4].
2. Accurate dating of pregnancy facilitates more precise interpretation of serum screening.
3. Second trimester scanning can reveal 'soft markers' for Down syndrome, besides major structural anomalies.

Screening for trisomy 21 should be offered to all women as part of routine antenatal care. This offer should include detailed counseling about the implications and limitations of the test used in the screening program. Women should have the option as to whether they wish to have screening or not. Screening tests will not diagnose whether or not the fetus is affected by Trisomy 21, but simply place the woman in a highrisk or lowrisk category. Being in the lowrisk group does not exclude the possibility of Down syndrome. Women in the highrisk group should be offered diagnostic testing to establish whether or not the fetus is affected.

Invasive prenatal diagnosis is associated with a risk of fetal loss of 0.5-1%. Therefore information gained from the

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various screening technics (biochemical and ultrasound) is combined with maternal age to lower false positive rates and minimise unnecessary invasive testing.

Maternal age

The incidence of trisomy 21 rises with increasing maternal age and falls with advancing gestational age (Figure 1,

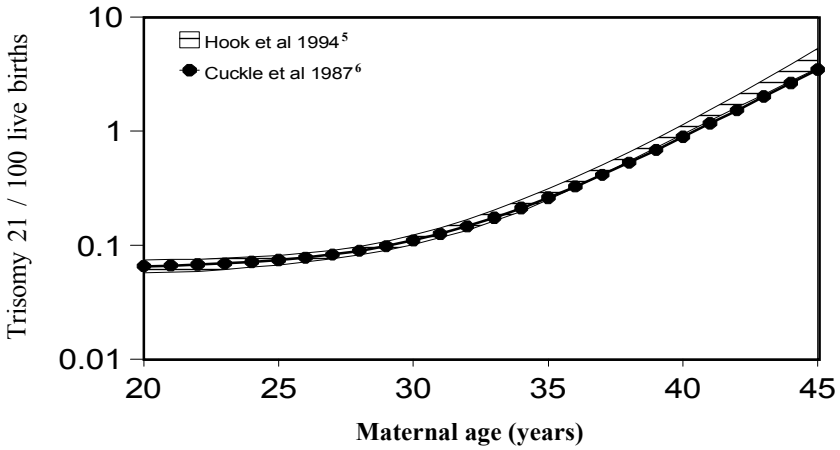

Figure 1. Prevalence of trisomy 21.

Table 2. Estimated risk for trisomy 21 in relation to maternal age and gestation.

	GESTATION (WEEKS)									
Age (Years)	**10**	**12**	**14**	**16**	**18**	**20**	**25**	**30**	**35**	**Birth**
20	804	898	981	1053	1117	1175	1294	1388	1464	1527
21	793	887	968	1040	1103	1159	1277	1370	1445	1507
22	780	872	952	1022	1084	1140	1256	1347	1421	1482
23	762	852	930	999	1060	1114	1227	1317	1389	1448
24	740	827	903	969	1029	1081	1191	1278	1348	1406
25	712	795	868	933	989	1040	1146	1229	1297	1352
26	677	756	826	887	941	989	1090	1169	1233	1286
27	635	710	775	832	883	928	1022	1097	1157	1206
28	586	655	715	768	815	856	943	1012	1068	1113
29	531	593	648	695	738	776	855	917	967	1008
30	471	526	575	617	655	688	758	813	858	895
31	409	457	499	536	568	597	658	706	745	776
32	347	388	423	455	482	507	559	599	632	659
33	288	322	352	378	401	421	464	498	525	547
34	235	262	286	307	326	343	378	405	427	446
35	187	210	229	246	261	274	302	324	342	356
36	148	165	180	193	205	216	238	255	269	280
37	115	128	140	150	159	168	185	198	209	218
38	88	98	107	115	122	129	142	152	160	167
39	67	75	82	88	93	98	108	116	122	128
40	51	57	62	67	71	74	82	88	93	97
41	38	43	47	50	53	56	62	66	70	73
42	29	32	35	38	40	42	46	50	52	55
43	21	24	26	28	30	31	35	37	39	41
44	16	18	20	21	22	23	26	28	29	30

Estimated risk is one case per number of cases given under corresponding age and gestation.

Table 2) [6,7]. This knowledge could not be utilised until prenatal diagnosis became available in the late 1960's. Unfortunately, prenatal diagnostic methods are associated with a risk of miscarriage in the order of 0.5-1%. This fact, combined with the cost implications, meant that prenatal diagnosis was offered only to women aged 35 and over. This highrisk group constituted 5% of the pregnant population.

Disappointingly, 20 years of screening in the UK using maternal age alone failed to produce a noticeable effect on the birth incidence of Down syndrome. There are several reasons for this failure:

1. The great majority of affected babies are born to women under 35 years of age because a much larger number of babies are born to women in this age group. Women over 35 years of age contribute only 20-30% of all babies born with Down syndrome.
2. The uptake of invasive fetal karyotyping in the highrisk group was less than 50%.
3. The expected fall in the birth prevalence of trisomy 21 may have been counterbalanced by the rise in mean maternal age (from 26.1 years in 1970 to 29 years now) in the UK.

The poor performance of screening for Down syndrome on the basis of advanced maternal age alone is now universally accepted. This has led to the development of newer screening program and the concept of Patient Specific Risk.

Patient specific risk of chromosomal abnormality

Every woman has some risk that her fetus may be affected by a chromosomal defect. In order to calculate this individual risk, it is necessary first to take into account the woman's *a priori* risk based on her age and gestational age (Table 2). This *a priori* risk is then multiplied by a likelihood ratio, calculated from her ultrasound findings and/or serum biochemistry results obtained during the course of the current pregnancy. The product of the *a priori* risk and the likelihood ratio yields the patient specific risk.

Biochemical screening

Second trimester

In 1984 Merkatz et al [3] retrospectively analyzed the maternal serum alpha fetoprotein (AFP) in 44 Down's affected pregnancies and found it to be low. Subsequently, Bogart et al [8] found elevated levels of maternal serum human chorionic gonadotrophin (hCG), and Canick et al [9,10] found low levels of unconjugated estriol (uE3) in Down syndrome pregnancies. The reason for these biochemical changes is not yet fully understood, but may relate to functional immaturity, leading to a delay in the normal gestational rise or fall.

The best combination of maternal serum markers is still debated [11-13]. Screening performance depends on the combination of markers chosen and whether ultrasound has been used to date the pregnancy accurately.

The optimal window for second trimester biochemical screening is between 15 and 22 weeks gestation. Apart from maternal age and gestation, other factors which affect the expected levels of the biochemical markers must be taken into account. These include maternal weight, ethnic origin, the presence of insulin dependent diabetes mellitus, multiple pregnancy, previous Down syndrome pregnancy, smoking, and vaginal bleeding.

Serum markers

AFP was the first serum marker used in screening program for trisomy 21 [3,14]. It is a fetal-specific protein produced by the yolk sac and fetal liver. Traditionally, it was performed between 15 and 21 weeks of gestation to screen for open neural tube defects. AFP levels are reduced in pregnancies affected by Down syndrome. Adding maternal serum AFP to maternal age increased the detection rate of screening to approximately 30%.

Other serum markers used in the second trimester include human chorionic gonadotrophin (hCG) or its free beta subunit (free β-hCG), both of which are increased in Down pregnancies; unconjugated estriol (uE3) which is decreased; and inhibin A which is increased.

Multiple of Median (MoM)

In screening program, marker levels are described in terms of Multiple of the Median (MoM). This is to allow for the fact that marker levels vary with gestational age. MoM values are calculated by dividing an individual's marker level by the median level of that marker for the entire population at that gestational age in that laboratory. Using MoM values, rather than absolute levels, also allows results from different laboratories to be interpreted in a consistent way.

Second trimester biochemical screening tests

The performance of different screening tests can be compared by evaluating their detection rates (DR) and false positive rates (FPR). The DR is a measure of the proportion of affected pregnancies which will be picked up by the test. The FPR is a measure of the number of pregnancies

incorrectly identified as highrisk. The higher the FPR the greater the number of unnecessary invasive tests and therefore the greater the number of unnecessary miscarriages of normal fetuses.

The available second trimester screening tests are the Double, Triple and Quadruple Tests. They are compared in Table 3.

Table 3. Second trimester biochemical tests.

Test	Markers	DR	FPR
Double	Age + AFP + hCG	59%	5%
Triple	Age + AFP + hCG + uE3	63%	5%
Quadruple	Age + AFP + hCG + uE3 + inhibin A	72%	5%

AFP = alpha fetoprotein hCG = human chorionic gonadotrophin
DR = detection rate uE3 = unconjugated gestriol
FPR = false positive rate

First trimester biochemical screening

Pregnancy-associated plasma protein A (PAPP-A) and free β-hCG are two serum markers used in screening for Down syndrome in the first trimester [15,16]. PAPP-A levels are reduced in affected pregnancies while free β-hCG levels are raised. Adding maternal age to PAPP-A and free β-hCG gives a DR of 60% and a FPR 5%, using a risk cut-off level of 1 in 250 (i.e. any woman with a risk greater than 1 in 250 is defined as highrisk, and offered invasive testing).

Ultrasound screening

Second trimester

Thirty percent of fetuses with trisomy 21 have a major structural malformation. Congenital cardiac anomalies are the commonest (up to 40%) and of these atrioventricular canal defects and ventricular septal defects are the most frequent.

Trisomy 21 in the second trimester is also associated with nasal bone hypoplasia, increased nuchal fold thickness, duodenal atresia, echogenic bowel, mild hydronephrosis, shortening of the femur or humerus, sandal gap, and clinodactyly or midphalanx hypoplasia of the fifth finger.

If the second trimester scan demonstrates major defects, it is advisable to offer fetal karyotyping, even if these defects are apparently isolated. The prevalence of such defects is low and therefore the cost implications are small. If the defects are either lethal or associated with severe handicap, such as hydrops or duodenal atresia, fetal karyotyping constitutes one of a series of investigations to determine the possible cause and thus the risk of recurrence. If the defect is potentially correctable by surgery (either intrauterine or postnatal) such as diaphragmatic hernia, it may be logical to exclude an underlying chromosomal abnormality. This is especially important as in many of these conditions, the associated chromosomal abnormality is trisomy 18 or 13.

Minor fetal defects or soft markers are common and not usually associated with any handicap unless there is an underlying chromosome abnormality [17-20]. Routine karyotyping of all pregnancies with these markers, therefore, would have major implications, both in terms of miscarriage and financial cost. In this situation, it is best to base counseling on an individual estimated risk for chromosomal abnormality. The overall risk for chromosomal abnormalities increases with the total number of defects identified. It is therefore recommended that when a defect/marker is detected, a thorough ultrasound examination is done for other features of the chromosomal abnormality known to be associated with that defect, because the presence of additional defects increases the risk substantially.

One promising marker for Down syndrome, which was recently described, is nasal bone hypoplasia. This is defined as a nasal bone that is not visible or has a length of less than the 3rd centile for gestational age in the second trimester. One study examined 1046 pregnancies undergoing amniocentesis for fetal karyotyping at 15-22 weeks gestation [21]. The nasal bone was hypoplastic in 62% of fetuses with trisomy 21, but in only 1% of chromosomally normal fetuses. Nasal bone hypoplasia was commoner in normal Afro-Caribbean fetuses (8.8%) than in normal Caucasian fetuses (0.5%). Although much more evidence needs to be gathered, it seems likely that this marker will have a major impact on Down syndrome screening, and should be incorporated into the detailed second trimester anomaly scan.

The estimated risk can be derived by multiplying the *a priori* maternal age related risk by the likelihood ratio of the specific defect. The best estimates of both the positive and negative likelihood ratios for each of the common markers of trisomy 21 are given in Table 4. On the basis of these data the likelihood ratio for trisomy 21 if there is no detectable defect or marker is 0.30. In each case the likelihood ratio is derived by dividing the incidence of a given marker in trisomy 21 pregnancies by its incidence in chromosomally normal pregnancies. For example, an intracardiac echogenic focus is found in 28.2% of trisomy 21 fetuses and in 4.4% chromosomally normal fetuses, resulting in a positive likelihood ratio of 6.41 (28.2 / 4.4) and a negative likelihood ratio of 0.75 (71.8 / 95.6). Consequently, the finding of an echogenic focus increases the background risk by a factor

of 6.41, but at the same time absence of this marker reduces the risk by 25%. It is important to bear in mind that the same logic applies to each one of the six markers in Table 4. Thus, in a 25 year old woman undergoing an ultrasound scan at 20 weeks of gestation, the *a priori* risk is around 1 in 1000. If the scan demonstrates an intracardiac echogenic focus, but the nuchal fold is not increased, the humerus and femur are not short and there is no hydronephrosis, hyperechogenic bowel or major defect, the combined likelihood ratio should be 1.1 (6.41 x 0.67 x 0.68 x 0.62 x 0.85 x 0.87 x 0.79) and consequently her risk remains at around 1 in 1,000.

Table 4. Incidence of major and minor defects or markers in the second trimester scan in trisomy 21 and chromosomally normal fetuses in the combined data of two major series.

Sonographic marker	Trisomy 21	Normal fetus	Positive LR	Negative LR	LR for isolated marker
Nuchal fold	107/319 (33.5%)	59/9331 (0.6%)	53.05 (39.37-71.26)	0.67 (0.61-0.72)	9.8
Short humerus	102/305 (33.4%)	136/9254 (1.5%)	22.76 (18.04-28.56)	0.68 (0.62-0.73)	4.1
Short femur	132/319 (41.4%)	486/9331 (5.2%)	7.94 (6.77-9.25)	0.62 (0.56-0.67)	1.6
Hydronephrosis	56/319 (17.6%)	242/9331 (2.6%)	6.77 (5.16-8.80)	0.85 (5.16-8.80)	1.0
Echogenic focus	75/266 (28.2%)	401/9119 (4.4%)	6.41 (5.15-7.90)	0.75 (0.69-0.80)	1.1
Echogenic bowel	39/293 (13.3%)	58/9227 (0.6%)	21.17 (14.34-31.06)	0.87 (0.83-0.91)	3.0
Major defect	75/350 (21.4%)	61/9384 (0.65%)	32.96 (23.90-43.28)	0.79 (0.74-0.83)	5.2

LR = likelihood ratio

First trimester

Nuchal translucency (NT) refers to the fluid-filled space between the fetal skin and the soft tissue overlying the cervical spine. It is measured between 11 and 13 weeks gestation, and the criteria for measurement include:

- crown-rump length between 45 and 84 mm
- midsagittal view
- neutral position
- measure of the maximal lucency
- the ultrasound machine with 0.1mm callipers
- 'on-to-on' measure
- fetal neck away from the amnion (it is important to distinguish between the fetal skin and the amnion).

A number of studies have demonstrated that an increased nuchal translucency is associated with abnormal karyotype [22,23]. One large multicenter study concluded that maternal age in combination with nuchal translucency measurement achieved a 77% DR for a 5% FPR [4].

Concerns have been expressed that the measurement of nuchal translucency may be difficult or time-consuming if the fetal position is incorrect. It is clear that adequate training is essential to ensure that the measurement is reproducible in different centres [24].

Combined test

Recent advances include using a combination of NT and biochemical markers. The combination of first trimester free β-hCG, PAPP-A, NT and maternal age is known as the Combined Test, and is measured between 11 and 13 weeks. This has been reported in some studies to have a DR of 80-89% with a FPR of 5% [25, 26] .

Integrated test

The Integrated Test is the most recent screening test for Down syndrome [27]. This combines maternal age with the following:

1. 11-14 weeks: NT + PAPP-A
2. 15-22 weeks: AFP + hCG + uE3 + Inhibin A

The performance of this test is reported to be better than that of all others. The model of screening described by Wald and Hackshaw [25] has the major theoretical advantage of a high DR of 94% for a FPR of 5% or alternatively 85% DR with a 1% FPR. The SURUSS trial [27] found that for a fixed DR of 85% the FPR for the integrated test was 1.2%. However the data available at present is from a single retrospective study and large prospective trials are currently being conducted.

The integrated test requires two stage screening and a proportion of women may fail to attend for the second stage test. In addition, for women who complete the test, the result

is not obtained until after 16 weeks. Termination of pregnancy is more traumatic at this stage since it usually requires a medical abortion rather than surgical, and the mother may have already felt the fetal movements. The same argument could of course be applied to the second trimester anomaly scan at 18 to 23 weeks. Although the combined test may have a lower DR than the integrated test, it does yield a result in the first trimester, thus allowing an early surgical (or medical) termination, which may be less traumatic for the mother.

Recent developments

First trimester ultrasound markers other than NT

More recent studies have examined the role of first trimester ultrasound markers other than NT. They suggest that absence of the nasal bone, increased impedance to flow in the ductus venosus (DV) and tricuspid regurgitation (TR) are highly sensitive and specific first-trimester markers for trisomy 21. [28-30].

Two stage screening process

In 2005, Nicolaides et al [31] proposed a two-stage screening process in the first trimester.They suggested using the combined test to triage women into high risk (1 in 100 or greater), intermediate risk (between 1 in 101 and 1 in 1000) and low risk (less than 1 in 1000). Intermediate risk women were offered further assessment of risk by first trimester ultrasound examination to determine the presence or absence of the nasal bone, presence or absence of TR and normal/ abnormal doppler velocity waveform in the DV. They concluded that using this approach, more than 90% of trisomy 21 fetuses can potentially be identified in the first trimester, for a FPR rate of 2-3%.

The concept of sequential screening

As discussed earlier, a woman will start with an *a priori* risk of Down syndrome, based on her age and gestation period.This can then be modified to a more patient specific risk by serum screening – the combined or integrated test. The risk obtained can then be further modified by a second trimester scan looking for major fetal malformations (such as cardiac defects) or soft markers for Down syndrome (such as short femur or humerus). The information from this second trimester scan, including major and minor defects (particularly nasal bone hypoplasia and nuchal edema), will contribute to identification of further cases.

It is of utmost importance to consider the results of any previous screening during the 18-23 weeks scan, as this will help to accurately calculate the adjusted patient specific risk. There are some exceptions to this process of sequential screening results. It seems obvious that the findings of increased nuchal edema or a cardiac defect at the second trimester scan cannot be considered as risk factors independent from an increased NT in the first trimester. Similarly, hyperechogenic bowel (which may be due to intra-amniotic bleeding) and relative shortening of the femur (which may be due to placental insufficiency) may well be related to abnormal serum biochemistry (high hCG and inhibin A, and low uE3 may be markers of placental damage). They cannot, therefore, be considered as independent risk factors while screening for the risk of trisomy 21.

Conclusion

The continuing debate as to whether screening should be performed solely in the first trimester or should incorporate second trimester markers remains mostly unanswered with no prospective randomized trials to compare first versus second trimester screening. One of the main focus for screening is to achieve a high DR with a low FPR rate. It would appear that the integrated test may be the most effective test available at present. However, new first trimester markers, such as fetal nasal bone hypoplasia and TR, are being evaluated. These may prove even more effective.

The resources available and limited opportunity for antenatal care may limit usefulness of some of these tests in certain settings. When resources are limited, the best first trimester test is combining maternal age with the measurement of nuchal translucency while in the second trimester the quadruple test outperforms the other available tests.

The role of the obstetrician is to help the woman and her partner decide what they wish to do, in accordance with their personal values. Some will prefer an earlier test in order to allow an early termination in case of a positive result. Others will accept the late result provided by the integrated test, in order to minimise the chances of an unnecessary amniocentesis. And some will not wish to have any screening tests, often because they would not consider termination of pregnancy even if the baby was diagnosed to have trisomy 21. Our role is to provide accurate advice and counseling and then to support the woman in whatever choice she makes.

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