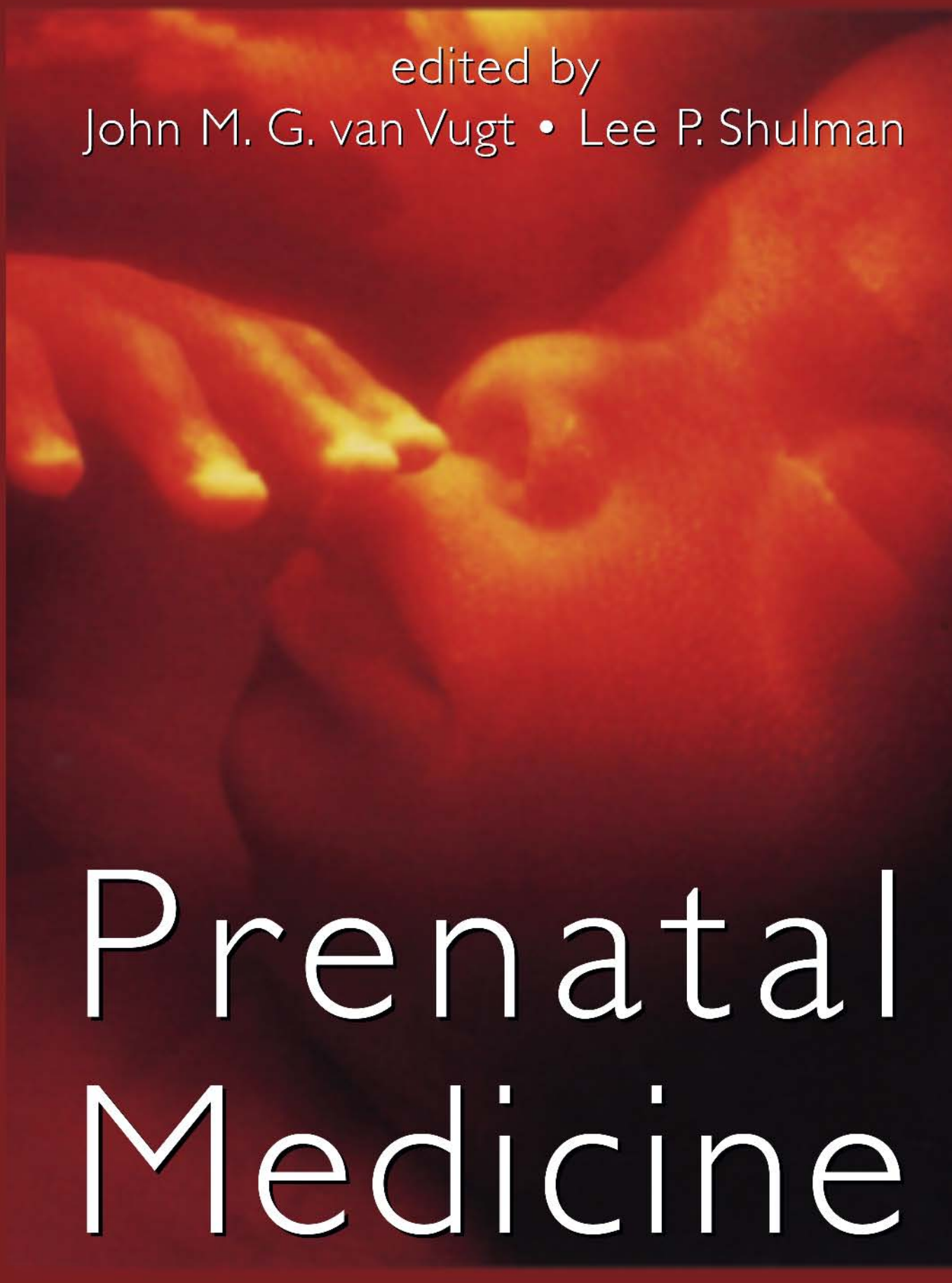

edited by
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Prenatal Medicine

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''L'audace, l'audace, toujours l'audace.''
The greatest honor and privilege bestowed on any person is the responsibility to care for patients. This book is thus dedicated to those teachers, mentors, and colleagues who helped us develop the requisite skills to provide that care and to teach it to others; to our families (Suzanne, Laura, Becky, and Andrew) who provided the foundation for our development as people and physicians and love us for who we are and what we do; and to our patients who honor us everyday with their trust and expectations and challenge us to become better physicians and human beings.

John M. G. van Vugt and Lee P. Shulman
Amsterdam and Chicago
2006

Foreword

In 1970, Henry L. Nadler and Albert B. Gerbie helped usher in the era of prenatal genetic diagnosis in their landmark paper "Role of amniocentesis in the interuterine detection of genetic disorders" (*N Engl J Med* 1970;282:596-9). Since that time, advances in genetics and perinatal medicine have occurred at an amazing pace, allowing physicians to detect and treat genetic disorders in utero with increasing success. There has also been an escalating demand by the public for translational medicine where discoveries in the laboratory are rapidly brought to the bedside. Although scientific breakthroughs in prenatal genetics have been nothing short of dazzling, we must always remember that the benefits they bring to pregnant women and their families is dependent upon the continuation of social attitudes and social support that made these developments possible.

Prenatal Medicine provides a succinct overview of prenatal genetic diagnosis and related topics. This text is particularly valuable because the authoritative contributors of each chapter have put into perspective massive amounts of research data material into clinically relevant information applicable to patient care. The safety and accuracy of state-of-the-art procedures and technologies are covered, including genetic amniocentesis, chorionic villus sampling, maternal serum screening for fetal genetic disorders, and high resolution and three-dimensional ultrasonography. Important topics related to prenatal diagnosis are addressed, including ethical and legal issues, prevention of labor, open fetal surgery, and fetal reduction and selective feticide. Exciting emerging areas of prenatal genetic diagnosis that will undoubtedly change the future of obstetrical care are considered, including fetal cells and DNA in maternal blood, new DNA technologies (e.g., multiplex ligation-dependent probe amplification and fluorescence in situ hybridization), and prenatal pharmaceutical therapy.

The editors of this text, John M. G. van Vugt and Lee P. Shulman, are internationally recognized leaders in prenatal genetic diagnosis, and each has contributed extensively to the literature of this field. Their gravitas and expertise are self-evident as they have carefully orchestrated and edited the contributed chapters into a coherent, cohesive, and highly informative text.

I wish to take this opportunity to add a few personal notes about the editors. I have known John M. G. van Vugt for more than ten years through the International Fetoscopy Work Group and the International Society for Prenatal Diagnosis.

His research in trophoblast cells and chromosome 21-encoded mRNA of placental origin in maternal blood, as well as ultrasonographic markers of fetal genetic disorders, has been pioneering in the quest for non-invasive prenatal diagnosis. He brings a balanced perspective and honesty to our discipline for which we should all be grateful. Finally, I would like to comment about Lee P. Shulman, a reproductive genetics fellow of long ago and an esteemed professional colleague and treasured friend for more than twenty years. His research has bridged prenatal genetic research with family planning and contraception to address the broader needs of women and their families. I know him to be a truly outstanding physician and clinical investigator who always has the best interests of his patients at heart. Lee is my comic relief, and I look forward to working with him each and every day.

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Preface

The goal of obstetrical care—regardless of all the medical, surgical, and technical advances of the past 50 years—still remains the delivery of healthy infants and the maintenance of good maternal health. Because those of us who practice obstetrics usually care for young, healthy women, this goal is one that is usually attained.

However, congenital and acquired maternal and fetal diseases are not uncommon complications during many pregnancies. Although maternal–fetal specialists and geneticists provide the care for those women with maternal conditions that can affect fetal or maternal well being or those women found to be carrying fetuses with congenital and acquired abnormalities, such complications can and do arise during the care of the ostensibly normal pregnancy. Indeed, lifestyle considerations such as nutritional status, maternal age, and an increasing recognition of specific genetic factors in the predisposition of fetal, newborn, and maternal conditions as a result of the Human Genome Project have considerably altered the delineation of low-risk and high-risk pregnancies.

Recognizing that such new and novel advances in our knowledge of maternal and fetal physiology have lead to profound changes in obstetrical management of low- and high-risk pregnancies, we sought to assemble a compilation of the state-of-the-art of prenatal care. We recognized, however, that such advances had led to considerably different approaches to incorporating this information into clinical practice. Therefore, to provide the most accurate presentations of the clinical applications of these novel advances, we utilized our rather disparate clinical and research experiences to recruit an international group of researchers and clinicians who could present an accurate overview of these advances as they apply to a variety of patients, clinical scenarios, and distinct communities of genetically and socially different peoples. We believe that presenting a wide and realistic spectrum of obstetrical practice and research is the best way for our colleagues to determine the best approaches for incorporating this information into their practices. In this way, *Prenatal Medicine* represents a unique and dynamic approach to the challenge of effectively incorporating novel and recent scientific information into the care of pregnant women worldwide.

Although many can agree on the scientific concepts that continue to change and improve our care of pregnant women and fetuses in all stages of pregnancy, one aspect that will assuredly divide clinicians is the legal and political differences

that separate us. Whether the result of geographical boundary or personal conviction, these differences will continue to color and, at times, direct the care of women and their pregnancies. To this end, we also present different legal and political approaches to obstetrical care and seek to engage in dialogue that will hopefully spark lively discussion among colleagues and ultimately improve the care of all pregnant women.

We are indebted to the authors of the chapters, who not only provided outstanding reviews of their particular areas of expertise, but also serve as outstanding clinicians, teachers, mentors, and researchers. Their work improves the care of their patients, and through texts such as *Prenatal Medicine*, may improve the care of women far from their homes, offices, and communities.

The ongoing improvement in medical care relies on all practitioners to commit to lifelong learning. We hope that *Prenatal Medicine* will be a component of that commitment and serve as a resource and motivation for the appropriate incorporation of new and novel scientific advances that will improve the care of women and fetuses no matter where they live.

John M. G. van Vugt
Lee P. Shulman

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1
First Trimester Serum Screening

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INTRODUCTION

The natural frequency of chromosomal abnormalities at birth is around 6 cases per 1000 births among populations without any form of prenatal screening. The aneuploides represent the most frequent of these, with Down syndrome being the most common with a historical birth prevalence of 1 in 800. The other common autosomal trisomies include Edward's syndrome (trisomy 18) and Patau's syndrome (trisomy 13), occurring with historical birth incidences of 1 in 6500 and 1 in 12,500, respectively. The other group of aneuploides include the sex aneuploidies, such as Turner's syndrome (45x), Klinefelter syndrome (47xxy), and those with 47xyy, and the types I and II versions of triploidy.

The incidence of the major trisomies (13, 18, and 21) increases with maternal age, although for the sex aneuploidies and triploidy there is no increased incidence with maternal age (Fig. 1). As a consequence of the changing pattern of childbirth in recent years, with women postponing childbirth until later life, the resulting general prevalence of the age-related trisomies has increased and that for trisomy 21 has changed from 1 in 740 to 1 in 500 in a 23-year period (1).

Although the birth incidence of the major chromosomal abnormalities approaches 6 per 1000, the actual incidence at any one time in pregnancy varies due to the varying intrauterine lethality of the various conditions (2). This means that when screening women in early pregnancy, there is a significantly greater number of fetuses affected than at mid-gestation or at term. (Fig. 2).

The aim of prenatal screening programs is to identify a subgroup of women who may be at a higher risk of carrying a fetus with a chromosomal anomaly. This group could then be offered an invasive diagnostic test such as amniocentesis or chorionic villus sampling (CVS) followed by karyotyping of the fetal cells. Such invasive procedures themselves carry a potential fetal loss rate of 0.5–1% above the background fetal loss rate. At the same time, prenatal screening programs aim to provide information with which couples can make appropriate informed choices about reproductive decisions, rather than focusing on disabilities and their eradication (3).

Screening for Down syndrome (trisomy 21) over the past two decades has become an established part of obstetric practice in many developed countries,

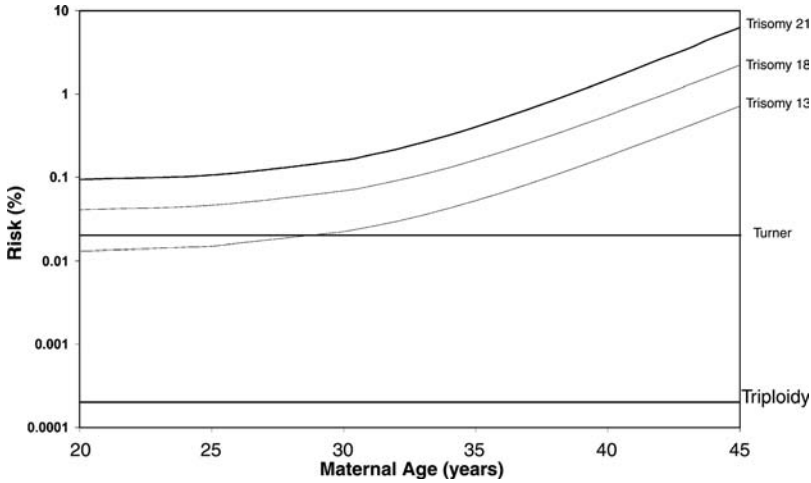

Figure 1 Variation of risk for various chromosomal anomalies with maternal age.

primarily through the use of maternal serum biochemical screening in the second trimester of pregnancy. In the second trimester, a range of maternal serum biochemical markers have been investigated, but routine screening has come to rely on the use of a combination of 2, 3, or 4 markers. The concentration of many of the biochemical markers varies with the duration of pregnancy. By expressing the observed concentration as a ratio of the median value observed in a normal pregnancy of the same gestation to obtain a multiple of the median (MoM), these gestational fluctuations are removed. The distributions of the MoM values in normal and Down pregnancies usually follow a gaussian distribution when the MoM is log transformed; however, with all markers there is a significant overlap of the two populations, but it is possible to establish from the gaussian distributions, the likelihood of any one result coming from the population of results associated with fetal Down syndrome. An individual patient-specific risk is then calculated by multiplying the a priori risk (usually based on maternal age) with the likelihood ratio. Unfortunately, no one individual marker alone has sufficient discriminatory power and a more efficient screening program can be achieved by combining information from

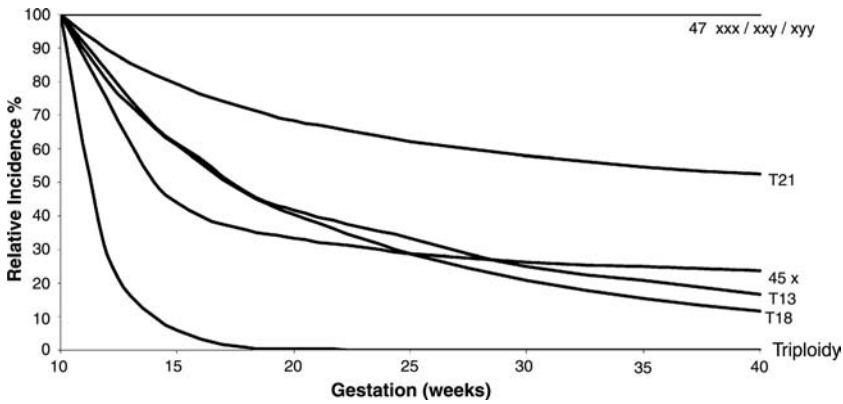

Figure 2 Gestational age-related risk for various chromosomal anomalies relative to the risk at 10 weeks. *Source*: From Ref. 2.

Table 1 Modeled Expected Detection Rates at a 5% False-Positive Rate Using a Variety of Combinations of Second-Trimester Biochemical Markers

Marker combination	Detection rate (%)
AFP, free β-hCG	63.2
AFP, free β-hCG, unconjugated estriol	66.8
AFP, free β-hCG, unconjugated estriol, inhibin A	72.1

Abbreviations: AFP, alpha-fetoprotein; β-hCG, β human chorionic gonadotropin.
Source: From Ref. 5.

more than one marker. The detailed mathematics of this multimarker approach is beyond the scope of this review but can be found in other publications (4). A summary of the modeled expected second trimester screening performance using various marker combinations (5) is shown in Table 1. Apart from Down syndrome, the only other major aneuploidy that is routinely screened for in some second trimester screening programs is Edward's syndrome (trisomy 18). In all other regards the biochemical patterns observed with the other aneuploidies are unremarkable—perhaps with the exception of triploidy types I and II (Table 2).

The past decade has seen a considerable focus on moving screening earlier into the first trimester. Earlier screening is anticipated to provide women with an earlier reassurance and, if termination of pregnancy is required, this can often be completed before fetal movements are evident. Also termination of pregnancy in the first trimester is safer than later in pregnancy (6). The fact that some Down syndrome pregnancies detected in the first trimester will be spontaneously lost before term is not a valid argument against early screening. For these women, it is important to have this information with regard to future reproductive decisions, so that a late miscarriage can be prevented.

A range of maternal serum biochemical markers has been investigated in both the first and second trimesters of pregnancy in normal and chromosomally abnormal pregnancies. Table 3 summarizes a meta-analysis of published cases with trisomies 13, 18, and 21 in the first trimester. For trisomy 21, of the markers of value in the second trimester, only the elevated free β human chorionic gonadotropin (β-hCG) is of any value in the first and second trimesters, and is reduced in both trimesters when trisomy 18 is present. The only other biochemical marker of value is the lowered levels of pregnancy-associated plasma protein A (PAPP-A) seen in cases with trisomies 13, 18, and 21. A guide to the scale of clinical effectiveness in discriminating normal

Table 2 Second Trimester Marker Patterns in Common Aneuploidies

Anomaly	HCG	AFP	UE3	Inhibin A
T21	High	Low	Low	High
T18	Low	Low	Low	Small decrease
T13	Normal	Small increase	Normal	Normal
Turner's	High/low +/− hydrops	Small decrease	Small decrease	High/low +/− hydrops
Other sex	Normal or high	Normal or high	Normal	
Triploidy I	High	High		
Triploidy II	Low	Normal		

Abbreviations: AFP, alpha-fetoprotein; HCG, human chorionic gonadotropin; UE3, unconjugated estriol.

Table 3 Meta-Analysis of Published Maternal Serum Biochemical Markers in Cases with Trisomies 21, 18, and 13 in the First Trimester

	Trisomy 21		Trisomy 18		Trisomy 13	
Serum marker	Median MoM	*N*	Median MoM	*N*	Median MoM	*N*
AFP	0.80	611	0.91	53	0.92	42
Total hCG	1.33	625	0.39	53	0.74	42
Unconjugated estriol	0.71	210				
Free β-hCG	1.98	846	0.27	126	0.51	45
Inhibin A	1.59	112	1.41	235	0.74	45
Free α-hCG	1.00	163				
CA125	1.14	34				
PAPP-A	0.45	777	0.20	119	0.25	42
SP1	0.86	246				
Activin	1.36	45	1.23	45		

Abbreviations: AFP, alpha-fetoprotein; hCG, human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein A.

pregnancies and those affected by trisomy 21 can be obtained using the Mahalanobis distance (7), calculated from:

$$\text{mean(unaffected)} - \text{mean(affected)}/\text{SD(unaffected)}$$

where the mean and standard deviation (SD) are in the log domain (2). Table 4 summarizes this clinical effectiveness scale and includes, for comparison, the ultrasound marker nuchal translucency (NT) thickness, which is the single most prominent marker for fetal aneuploidy at the 11- to 14-week period.

FREE β-HCG

General Biology

Intact hCG is a 39.5 kDa dimeric glycoprotein of two different subunits. The α subunit is a 15 kDa protein identical to the common α subunits of the other pituitary

Table 4 Relative Clinical Effectiveness (Mahalanobis Distance) of Markers in Discriminating for Trisomy 21 in the First Trimester

Marker	Mahalanobis distance
NT	6.46
PAPP-A	2.08
Free β-hCG	1.45
Unconjugated estriol	0.68
Total/intact hCG	0.38
Dimeric inhibin A	0.35
AFP	0.23

Abbreviations: NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein A; hCG, human chorionic gonadotropin; AFP, alpha-fetoprotein.

glycoprotein hormone family. The 23 kDa β subunit of this family confers biological activity and there is an 80% sequence homology between the β subunits of luteinizing hormone and hCG. hCG synthesis occurs in the synctiotrophoblast of the placenta and involves an independent translation of the respective mRNAs for the α and β subunits. The β subunit is coded for by at least six genes on chromosome 19 while only one gene on chromosome 6 codes for the α subunit. Post-translational glycosylation of the subunits occurs before the subunits are released in the form of free α-hCG or free β-hCG along with the intact dimeric molecule. Control of secretion of the β subunit is thought to be the rate-limiting step in the production of the intact molecule and this is influenced in a positive way by cyclic adenosine monophosphate, insulin, calcium, interleukin-1, fibroblast growth factor, and placental gonadotropin-releasing hormone. Inhibitory influences include prolactin, progesterone, and inhibin.

In the placenta, maternal pregnancy serum, and urine, hCG is present in multiple related forms including degraded hCG molecules, hyper- and hypoglycosylated hCG, free subunits, and fragments (8). Urine is the major route for clearance of hCG from the circulation, with the major breakdown product β-core being produced within the kidney. The clearance half-life of the intact dimer is of the order of 24–36 hours, while the free subunits are more rapidly cleared in 2–5 hours. It is thought that the degradation process of intact hCG may involve peptide nicking of the β subunit at peptide linkages 47–48 (less frequently at 43–44 and 44–45) and that the nicked intact hCG is less stable than non-nicked hCG and rapidly dissociates to the free subunit, which is then cleared. In pregnancies affected by trisomy 21, there is some limited evidence that both the nicked forms of β-hCG and the hyperglycosylated forms may be increased in both maternal serum and urine.

Since free β-hCG is present in serum in a milieu of other hCG-related molecules in the first trimester, it is important to ensure that the assay that is used in clinical practice measures the component of interest and not any of the other potential species (8). In the first trimester it appears that only the free β-hCG subunit is of proven clinical value, and while assays should also measure the nicked and non-nicked form, cross-reactivity with the intact molecule may reduce the clinical discrimination.

Levels in Cases with T21, T18, T13, and Other Aneuploidies

In 1992, Spencer et al. (9) first reported that levels of free β-hCG were elevated in cases with trisomy 21 and subsequently many other studies have confirmed that levels approach 2 MoM, being slightly lower than the average value of 2.20 seen in the second trimester. The largest series to date (10) with 210 cases of trisomy 21 showed a median MoM of 2.15. In studies which have investigated total hCG, the median in the first trimester is considerably lower than that in the second trimester with a median of 1.33 compared to 2.06 in the second trimester (11).

In cases with trisomy 18, early studies (9) indicated that free β-hCG levels were reduced. Since then a number of studies have confirmed these findings with levels approaching 0.27 MoM (12), which is very close to the 0.33 average seen in the second trimester (13). Total hCG levels in cases with trisomy 18 are also reduced by a similar amount (14).

In cases with trisomy 13, the median free β-hCG MoM is decreased (15) to around 0.51 MoM, unlike in the second trimester, when levels are normal to slightly elevated (K. Spencer, unpublished). Total hCG is also reduced at the same time, albeit to a lesser extent than free β-hCG (14).

In cases with Turner's syndrome, free β-hCG levels are not particularly different from normal (1.11 MoM), as was the case in other cases with sex aneuploidy (16).

In cases with triploidy, free β-hCG levels are supra-elevated (8.04 MoM) in cases with triploidy type I (17) and to a lesser extent for total hCG (4.91). In triploidy type II, levels are dramatically reduced (0.18 MoM), as is total hCG (0.16).

PREGNANCY-ASSOCIATED PLASMA PROTEIN A

General Biology

PAPP-A is a large (800 kDa) dimeric zinc containing metalloglycoprotein synthesized by the syncytiotrophoblast tissue of the placenta in an initial pro-form approximately 80 amino acids longer than the mature subunit. Each mature subunit consists of 1547 amino acid residues, and in pregnancy serum, PAPP-A exists as a 2:2 complex with the pro-form of eosinophil major basic protein (ProMBP) (18). ProMBP also circulates bound to angiotensinogen, and this new complex can also bind complement 3dg. In pregnancy, ProMBP concentrations exceed PAPP-A by 10-fold. PAPP-A is encoded by a gene on the long arm of chromosome 9. Although the biological function of PAPP-A is not clearly defined, it has recently been shown to be an insulin-like growth factor 4 (IGF4) protease (19) and it is speculated that PAPP-A, therefore, may have some form of regulatory role in the growth of the fetus by controlling the amount of bioavailable IGF1 and IGF2.

Levels in Cases with T21, T18, T13, and Other Aneuploides

Brambati et al. (20) first observed that PAPP-A levels were reduced in cases with aneuploidy (including trisomy 21) during the early first trimester. Subsequently, many studies have been published which, although showing quite wide variation of median MoM, have confirmed that levels are on average reduced to around 0.45 MoM. The variation in median MoM from study to study can now be attributed to the temporal variation of marker levels, since in early first trimester studies, median levels of PAPP-A were very low (0.3), and in studies performed around 12–13 weeks, levels were higher (0.65) (10). This temporal variation also results in a loss of clinical discrimination for PAPP-A by the time one reaches the 17th week of gestation (21,22).

In cases with trisomy 18 in the first trimester, levels of PAPP-A are reduced (12) significantly with a median MoM from accumulated world series of 0.20. Unlike with trisomy 21, levels slightly reduce in the second trimester making PAPP-A a useful marker of trisomy 18 at this time (23,24).

In cases with trisomy 13 in the first trimester, levels of PAPP-A are also reduced (15) to around 0.25, making it difficult to discriminate between trisomies 13 and 18 because of the similar biochemical pattern (25).

In Turner's syndrome, PAPP-A levels are lower (0.49 MoM) in the first trimester, but for other sex aneuploides, levels are not significantly different (16).

In triploidy type I, PAPP-A levels are slightly decreased (0.75 MoM), while in type II, they are dramatically reduced to almost immeasurable levels (0.06 MoM) (17).

COMBINED MODELED DETECTION RATES

When used as a single marker in combination with maternal age, at a fixed 5% false-positive rate, the best estimates for detection of cases with trisomy 21 range from

42% to 46% for free β-hCG (10,26) and 48% to 52% for PAPP-A (10,26) for specimens collected between the 10- and 14-week period. When the two markers are combined together with maternal age, the detection rates increase to from 65% to 67%.

For trisomies 13 and 18, it is not possible to produce individual specific risks since the biochemical patterns and the NT patterns are quite similar; however, it is possible to provide a combined trisomy13/18 risk estimate (25). Modeling has shown that for a 0.3% false-positive rate, 95% of cases could be identified (25).

Temporal Variation

It has become evident over time that many markers show a different pattern of variation in cases with aneuploidy across the first and second trimester. Berry et al. (27) collected samples from 45 cases with trisomy 21 in the first and second trimesters. They showed that in these same patients the first trimester free β-hCG median was 1.99 compared with 2.79 in the second trimester. Similarly, for PAPP-A the corresponding values were 0.50 and 0.94 MoM. In the second trimester, Spencer and Macri (28–33) have demonstrated that median free β-hCG levels and detection rates for trisomy 21 are higher at around 14–16 weeks than at 17–19 weeks. A similar pattern was shown for free β-hCG in the first trimester when levels increased from 1.75 at 11 weeks to 2.25 at 13 weeks, and for PAPP-A, the levels increased from 0.44 at 11 weeks to 0.69 at 13 weeks (10). In a comprehensive analysis of data from between 700 and 1000 cases with trisomy 21 and over 100,000 unaffected pregnancies, Spencer et al. (21,22) have described in detail the temporal variation across the first and second trimesters for the markers alpha-fetoprotein (AFP), PAPP-A, free β-hCG, and total hCG. The result of this temporal variation is that the separation between normal pregnancies and those with trisomy 21 is changing all the time, and unless this is taken into account in the screening algorithm by using a variable separation model (21,22) rather than the constant median separation model assumed by Wald et al. (34–36), then significant errors in individual patient-specific risks can be created. The other feature of such temporal variation is that for individual markers, there are key measuring periods. For example PAPP-A is a better marker before 10 weeks, but free β-hCG is a better marker at around 12–14 weeks. The consequence of this opposing changing pattern is to some extent to balance detection rates so that across the 8- to 13-week window the variation is from 72.5% at 8 weeks to 62.6% at 13 weeks (22).

Temporal variation also exists for other aneuploidies. For example, in cases with trisomy 18, levels of PAPP-A are low in the first trimester and get progressively lower throughout the second trimester. Indeed for trisomy 18, PAPP-A is probably the best second trimester clinical discriminator, and a two-stage screening program has been proposed (23,24). In cases with trisomy 13, the low first trimester levels of free β-hCG increase such that by the 18th week, levels are elevated (K. Spencer, unpublished).

In order to compare detection rates between the first and second trimesters, it is necessary to allow for the intrauterine lethality of fetal aneuploidy between the two gestational periods. In the first trimester, there are more cases of trisomy 21, for example, than in the second trimester (Fig. 2). A statistical methodology for this has been developed by Dunstan and Nix (37), but this relies on having a suitable measure of the fetal loss rate in cases with trisomy 21 between the 12- and 16-week period. Typical fetal loss rates have been constructed from studies looking at the incidence of trisomy 21 at the time of CVS and the incidence at the time of amniocentesis (38). Unfortunately these data may well be biased because they are

observations in a group which are largely of advanced maternal age (39), and it is known that advancing maternal age per se is a factor that increases fetal loss rates (40). Probably one of the more secure estimates of fetal loss is from the UK National Down Syndrome register, which collects data from all pre- and postnatally detected cases (41). Assuming a 75% second trimester detection rate, a detection rate would need to be 3.5% higher in the first trimester in order to be considered better. Clearly, first trimester maternal serum biochemistry and age together cannot achieve the detection rates of second trimester maternal serum biochemistry. Indeed it is questionable whether NT in conjunction with maternal age can achieve any better detection than first trimester maternal serum biochemistry. Fortunately, NT and maternal serum biochemical markers in the first trimester are not correlated (10), and a much more effective screening program can be obtained by combining both screening modalities together either in a one-stop clinic (OSCAR) (42–47) or delivered in a sequential screening program (48). The benefits of screening at the point of care (OSCAR) have been outlined (43,44).

MODELED AND ACHIEVABLE PERFORMANCE IN STUDIES OF MATERNAL SERUM BIOCHEMISTRY WITH NT

A retrospective study of 210 cases of trisomy 21 and approximately 1000 controls showed that the combined approach could achieve a detection rate of 89% for a 5% false-positive rate (10). Other studies using modeling or observed data have also shown that the combined approach can achieve detection rates of the order of 85% (26,48–53). In addition to cases with trisomy 21, combined screening can identify pregnancies complicated by trisomy 13 (15), trisomy 18 (12), Turner's syndrome and other sex aneuploidies (16), and triploidy types I and II (17). It is expected that 90% of these other chromosomal anomalies can be identified for an additional 1% false-positive rate.

In prospective screening in two OSCAR clinics over a 5-year period (46,47) that screened over 45,000 women, a detection rate of 92% (145/158) was achieved for trisomy 21 with a further 107 other aneuploidies identified for a total screen positive rate of 5.3% (44). Similarly, in a sequential prospective screening program (54), 93% of cases with trisomy 21 were identified with a false-positive rate of 5.9%, and 96% (25/26) of other anomalies identified for an overall false-positive rate of 6.3%.

SAMPLE COLLECTION CONDITIONS

Consideration should be given to preanalytical variables that can affect the maternal serum biochemical markers. Free β-hCG stability at room temperature was originally queried based on anecdotal evidence of the thermal degradation of intact hCG leading to elevated levels of the free β subunit. Accelerated thermal degradation studies have shown that serum intact hCG is stable for up to 70 hours at room temperature, although the free β-hCG levels can increase in whole blood after 36 hours at room temperature (55). Others have also shown that such limitations have no impact on screening performance (56–58). Provided samples are transported as serum, then stability is not an issue. We recommend to all our outlying hospitals and clients that serum samples should not take longer than 72 hours to reach the laboratory (unless sent refrigerated), and that whole blood should be with us within 36 hours. The transport of samples as whole blood filter paper spots has also been shown to be a reliable alternative (51,55).

Blood collection tube type is also known to influence measured PAPP-A levels (59). Samples collected as heparinized plasma and citrated plasma all had significantly lower values than samples collected as native serum, while those collected as ethylenediaminetetraacetic acid plasma had dramatically reduced levels. Levels of free β-hCG were not affected. Serum is thus the medium of choice for first trimester biochemical screening.

COVARIABLES

A range of factors is known to affect the biochemical marker levels and some of these factors may have a sufficiently large effect to warrant some form of correction. Correction for such variables aims to reduce the spread of the normal and affected populations, thus leading to a smaller overlap of the gaussian distributions and hence improvement in detection rate or reduction of false-positive rate. In many instances, although correcting for these covariables by themselves will have little impact on population detection rates, they can be quite significant for the individual and result in significantly different individual risks, which in turn may lead to different clinical interventions.

Gestational Age

Nearly all maternal serum marker levels vary with gestational age. As noted previously, comparison of levels at different gestations is achieved by conversion of the marker concentration to a MoM. However, the precision of this estimate depends upon the accuracy of the gestational estimate—whether this be derived from the last menstrual period or more preferably from an ultrasound assessment of fetal maturity. With the first trimester markers, free β-hCG levels rise to a peak at around 9 weeks and then fall, while PAPP-A levels increase in a fairly linear fashion (Fig. 3).

Maternal Weight

Maternal serum biochemical markers have a tendency to be lower than normal in women with maternal weights larger than normal and conversely tend to be higher

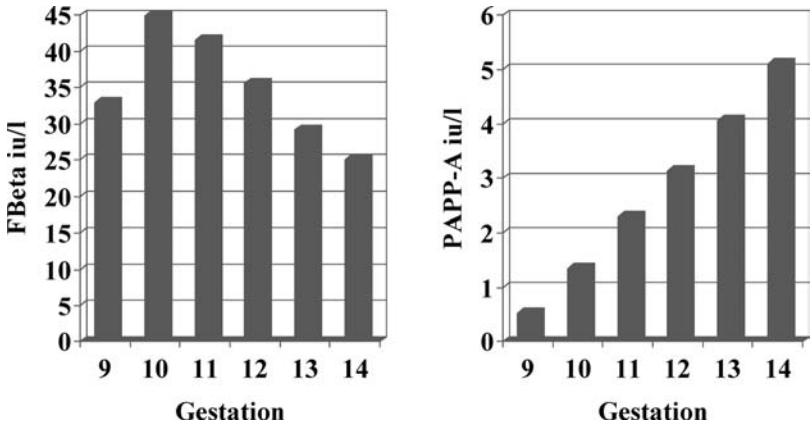

Figure 3 Gestational age variation of free β-hCG and PAPP-A in the first trimester.

than normal in women with lower than average maternal weight. This phenomenon is due to the fact that in women of larger weight the circulating blood volume is greater than normal, hence the placenta producing markers at the normal rate will result in the marker being diluted in a larger blood volume. In smaller women, with a lower blood volume, the effect is to concentrate the marker level. Correction for maternal weight can be made by dividing the result in MoM by the expected value for the weight based on a regression curve. Regression curves can be of two types, either a linear regression method (60) or a reciprocal regression procedure. In the first trimester both methods are seen to fit the data reasonably well, with perhaps the linear regression procedure having a slightly better fit to the data.

The importance to an individual of taking maternal weight into account is shown in Table 5, which also shows the importance of accurate dating in calculating risk. In most risk algorithms if no weight is given, the algorithm uses the average pregnancy population weight, which in Europe is around 69 kg. Thus, if the mother is 55 kg rather than 69 kg, the risk she will be given in this example is close to 1:280 when it should be 1:224, but if she were a 120-kg woman, then her risk should have been 1:419, changing her from high risk to low risk.

Multiple Pregnancy

Several complex issues are associated with screening for chromosomal anomalies in twin pregnancies, namely: how to interpret the marker values, the paucity of data in abnormal affected pregnancies when the fetuses are either concordant or discordant for an anomaly, the dilemmas regarding which invasive test to offer, the perceived increased risk of such procedures in twins, the technical difficulties of ensuring fetal tissue is obtained from each fetus, the need to ensure each fetus can be clearly differentiated at a later date, and finally, the difficulties of clinical management of fetal reduction and potential risk to the unaffected co-twin. These concerns form the basis of arguments that screening in twins poses such a serious clinical, ethical, and moral dilemma that it should be discouraged. Despite such reservations, screening programs for twin pregnancies have been successfully implemented in both the second and first trimesters, in units that have strong links with specialized fetal medicine centers (61).

The biochemical markers in twin pregnancies are on average twice that in normal singleton pregnancies. In a summary of the world literature, the median MoM PAPP-A in 707 cases was 1.826 and for free β-hCG was 2.035 from 825 cases (61). Wald et al. (62) proposed a pseudo-risk approach for risk assessment in twins, whereby the measured result (in MoM) is divided by the corresponding median

Table 5 Influence of MWt and GA on Risk for a 25-Year-Old with NT of 1.9 mm, FBeta 100 IU/L, and PAPP-A 1.0 IU/L

MWt (kg)	11 wk 1 day	11 wk 4 day
55	224	173
65	263	209
75	302	245
85	339	280
95	372	314
120	419	374

Abbreviations: MWt, maternal weight; GA, gestational age; PAPP-A, pregnancy-associated plasma protein A.

MoM value found in twin pregnancies and treating the risk calculation as for a singleton pregnancy. Although such an approach leads to lower detection rates in twins (compared to singleton pregnancies), it is thought to be a valuable procedure in the second trimester (63–65). In the first trimester it is predicted that adding in twin biochemistry correction will improve the detection rate by NT alone from 75% to 80%—some 10% less than achieved in singleton pregnancies (66). In prospective practice, this does seem to also be achievable (67,68). However, the median MoM twin-corrected free β-hCG was only 1.39 in 19 cases discordant for trisomy 21 while that for PAPP-A was 0.56 (61). When chorionicity or zygosity is considered, there does appear to be measurable differences in the marker levels, particularly for PAPP-A, which appear 10% lower in monochorionic twins (69). Further studies are needed to confirm these differences. It remains to be seen whether screening in twins in the first trimester is more widely accepted using ultrasound alone or ultrasound in combination with maternal serum biochemistry. Little data are available in higher order multiple pregnancies.

Insulin-Dependent Diabetes Mellitus

In the second trimester, women with insulin-dependent diabetes mellitus (IDDM) were shown to have reduced levels of AFP, and correction for this was considered appropriate by dividing the measured MoM by the median MoM observed in IDDM pregnancies. The validity of such correction is now questioned, since more recent data show that in women with IDDM, the difference is much smaller, and it has been suggested that this is because of improved diabetic control in patients over the past 25 years. For first trimester markers, there is very little evidence to support the need for correction. Levels of PAPP-A were shown to be reduced in IDDM mothers in one study (70) and in those with pre-existing or gestational diabetes free β-hCG was reduced by 20% and PAPP-A by 25% (71). In another smaller study (72), both free β-hCG and PAPP-A levels were 14% lower in women with gestational diabetes. If such reductions are confirmed in other studies, then it may be necessary to make correction in women with IDDM.

Fetal Sex

In the second trimester of pregnancy, free β-hCG levels are around 7% higher in women carrying a female fetus (73). In the first trimester, levels in normal pregnancies are 15% higher in the presence of a female fetus and 11% higher in the presence of female fetuses with trisomy 21, while PAPP-A levels were 10% and 13% higher (74). The potential impact of such changes would be a reduction in the detection rate in female fetuses of the order of 1–2% (74). To make correction for such sex differences would require an accurate method of sex determination at the 11- to 14-week scan, ultrasound at best can only provide a 75% level of accuracy at this time.

Assisted Conception

An important point to remember when estimating risks in in vitro fertilization (IVF) pregnancies is that in cases where a donor egg is used, the prior risk should be based on the maternal age of the donor at the time of egg collection rather than the recipient's age.

First trimester marker levels have been investigated in a few studies. The general consensus is that in IVF pregnancies, free β-hCG was increased by about 14% (75),

while PAPP-A was reduced by some 8%, although other smaller studies have not confirmed this. In a small number of cases having intracytoplasmic sperm injection, PAPP-A levels were 20% lower. The overall impact of such changes has been estimated to increase the false-positive rate in the IVF group by around 1%. Further studies are needed to make secure correction factors by which to reduce the false-positive rate and potential invasive testing rate in this important group of individuals.

Ethnicity

When maternal weight is taken into account, in the second trimester it has been reported that free β-hCG levels are 12% higher in Afro-Caribbean women than in Caucasians, and in Asian and Oriental women, levels are also known to be higher. In the first trimester in one study (76), weight-corrected free β-hCG levels were 21% higher and PAPP-A 57% higher in Afro-Caribbean women than in Caucasians. In Asian women the levels were 4% higher for free β-hCG and 17% higher for PAPP-A. Such large differences, if confirmed in other studies and other ethnic groups, would seriously warrant correction. Watt et al. (77) have proposed a method of correcting for ethnicity in the second trimester which could be applied in the first trimester (76).

Smoking

In the second trimester of pregnancy, maternal cigarette smoking has been shown to influence the levels of maternal serum biochemical markers. In unaffected pregnancies, smoking is associated with a mean increase in serum AFP (4%) and inhibin A (45–62%) and a decrease in unconjugated estriol (3%), total hCG (24%), and free β-hCG. On the whole, the limited data in pregnancies with Down syndrome suggest a similar level of change. On the whole, correcting for smoking status by dividing the measured MoM by that found in a group of smokers results in a reduction in the false-positive rate of less than 1%. In the first trimester, preliminary data suggested that PAPP-A levels in smokers were reduced by 15% but were unaltered for free β-hCG in normal pregnancies (78) and were perhaps reduced by 13% in pregnancies with Down syndrome with PAPP-A being 6% higher (79). A much extended study of nearly 30,000 nonsmokers and 4000 smokers has shown that free β-hCG levels in unaffected pregnancies are reduced by 3% while PAPP-A is reduced by 18% (80). Furthermore, it has been demonstrated that this effect does not seem to be related to the number of cigarettes smoked (80). The false-positive rate when screening using first-trimester biochemistry and age alone or in combination with NT was shown to be 0.7–1.5% higher than in the nonsmoking group, and after correction the rates in the two groups were the same (80).

Gravidity/Parity

In the second trimester, free β-hCG levels are decreased by a small amount with an increasing number of pregnancies (gravidity) or an increasing number of births (parity), but the effect is so small as to not warrant correction (81). In the first trimester, it also appears that gravidity or parity is associated with a small but progressive increase in both free β-hCG and PAPP-A. None of these small changes are significant or warrant correction (82).

Vaginal Bleeding

The presence of vaginal bleeding in early pregnancy may complicate the interpretation of screening results, partly because vaginal bleeding is often related to unfavorable pregnancy outcome, and low levels of PAPP-A and increased levels of free β-hCG are known to be associated with such adverse events. In a study of 253 cases, which reached term and who had early vaginal bleeding, the median free β-hCG was significantly higher (9%) than in 2077 cases with no vaginal bleeding, but levels of PAPP-A were not significantly different (3% higher) (83). In a similar but unpublished analysis of 89 cases with early vaginal bleeding and 1047 without, no statistically significant difference was observed, but levels of free β-hCG were increased by 8% and PAPP-A was decreased by 5% (72, unpublished data therein).

Previous Pregnancy Results

In women who have an increased second trimester Down syndrome risk in a first pregnancy, there is a fivefold greater chance of them also having an increased risk in a second or subsequent pregnancy (84). Between pregnancies, there is a significant correlation between the same marker in a subsequent pregnancy, and such association suggests that there are additional maternal or genetic factors influencing the levels of the serum markers. In the first trimester, a significant between-pregnancy correlation for free β-hCG ($r = 0.3976$) and PAPP-A ($r = 0.4371$) has been shown (85,86). The end result is that women who have an increased first-trimester risk of Down syndrome are two to three times more likely to repeat this event in their next pregnancy (85,86). Although the impact of correcting for previous results is unlikely to have more than a 1% improvement in population detection rates, some have argued that correction would be worthwhile (87), and others proposed methods for taking previous results into account (88).

Previous Trisomy

The risk for trisomies in women who have had a previous pregnancy with a trisomy is higher than that expected on the basis of age alone. One estimate for women with a previous Down syndrome pregnancy is the addition of 0.75% to the maternal and gestational age-related risk for Down syndrome. Similar corrections also apply for trisomies 18 and 13 (89). However, this assumes that the marker distributions are the same in women with and without a family history of aneuploidy. A recently published study provides evidence that this may not be the case (90). In this study of 375 women with a previous aneuploidy (303 with Down syndrome, 63 with Edwards' syndrome, and 9 with Patau's syndrome), in a subsequent pregnancy free β-hCG was significantly increased (10%) as was PAPP-A (15%). In the series with Edwards' syndrome if anything the increase was greater (25%) than with those with Down syndrome. Such difference may require correction.

DETECTION RATE AND FALSE-POSITIVE RATE BY MATERNAL AGE

One factor that is often overlooked in screening programs is the fact that detection rates and false-positive rates vary considerably with maternal age. In younger women the detection rate using second-trimester screening falls quite dramatically,

Table 6 Detection Rate and False-Positive Rates for Trisomy 21 in the First Trimester Combined Program and the Second Trimester Triple Test Program

	First trimester		Second trimester	
Maternal age (yr)	False-positive rate (%)	Detection rate (%)	False-positive rate (%)	Detection rate (%)
20	78.8	2.3	44.7	3.2
25	80.6	2.9	47.5	3.8
30	83.8	4.0	56.0	6.1
35	89.7	8.7	73.8	15.8
38	93.9	15.9	85.5	28.6
40	96.1	24.4	91.6	40.9
44	98.8	47.1	98.1	70.0

as does the false-positive rate. Screening programs that quote only global detection and false-positive rates could be misleading to patients. When counseling women on the test and its results, these issues need to be considered (91). Two studies have used modeling to calculate expected detection rates and false-positive rates at various maternal ages using either the second-trimester triple-marker approach (91) or the combined ultrasound and biochemical approaches in the first trimester (92). Table 6 summarizes these data. The odds of an increased risk result being Down is 1 in 55 in the second trimester compared with 1 in 29 in the first trimester in general population terms.

ADVERSE OUTCOME

In second-trimester screening, there are conflicting views on the relationship between biochemical marker levels and the incidence of adverse outcomes such as preeclampsia, intrauterine growth restriction (IUGR), low birth weight, preterm delivery, and stillbirth (93). An increased free β-hCG may be associated with an increased incidence of preeclampsia, but when examined in the first trimester, levels were not elevated in cases developing preeclampsia (71). Low levels of PAPP-A, however, were found to be associated with subsequent miscarriage, the development of pregnancy-induced hypertension, and growth restriction, although the authors concluded that the sensitivity and specificity of these were low and were not useful predictors of adverse outcome. Other studies have also shown this association between low levels of PAPP-A and IUGR or low birth weight (94–97) and one has found no evidence of this (98).

POTENTIAL FUTURE DEVELOPMENTS

One new and exciting ultrasound marker of aneuploidy is the observation of an absent nasal bone at the 11- to 14-week scan. The initial studies found an absent nasal bone in about 70% of fetuses with trisomy 21 and in 0.5% of normal fetuses (99). The findings in this preliminary study were confirmed in other smaller studies (100). An extension of the original study in 430 cases with an abnormal karyotype confirmed

that the nasal bone was absent in 67% of cases with trisomy 21 and in 2.8% of cases with a normal karyotype. However, there were some differences in incidence between different ethnic groups and a relationship between crown–rump length and NT (101). One way in which this marker may be used in the future is to incorporate it into the existing first-trimester scan as part of the combined ultrasound and biochemistry screening at 11–14 weeks. Preliminary studies have shown (102) that absent nasal bone is not significantly correlated with the biochemical markers. Modeling would suggest that a detection rate of 97% could be achieved at a 5% false-positive rate. Alternatively, if one wanted to focus on reducing the invasive testing rate, then at a 0.5% false-positive rate, the detection rate would still be 90%.

Other biochemical markers may have a role to play in the future. ADAM 12, a metalloprotease which cleaves IGF–binding proteins 3 and 5, has been recently shown to be such a potential new marker (103). In this one study, levels of ADAM 12 were 0.14 MoM in early first-trimester cases with Down syndrome, and a project detection rate using this marker alone with maternal age gave an 81.5% detection at a 3.2% false-positive rate, and combined with NT, PAPP-A, and free β-hCG, a detection rate of 94.1% at a 1.5% false-positive rate.

Another area of potential for the future is the developments associated with the isolation and quantitation of fetal DNA (104–106) or placental mRNA (107–110) in maternal serum/plasma. Whether such techniques become viable as alternatives to invasive diagnostic procedures or whether they may be used as adjuncts to existing ultrasound and biochemical screening techniques, remains to be established.

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Maternal Serum Screening for Down Syndrome

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INTRODUCTION

Over recent years, an ever increasing number of pregnant women have undergone Down syndrome screening based either on maternal serum markers (MSM) or on ultrasound, and a considerable amount of scientific literature has been published on the subject. However, one should bear in mind that screening for Down syndrome is by no means mandatory. Many women do not wish to undergo prenatal screening for trisomy 21, and would not consider terminating the pregnancy of an affected baby. Although it is accepted that women should be made aware of the existence of screening, their ethical positions should be respected while offering MSM screening. Besides, it would be unfair to implement large-scale prenatal screening policies without also implementing voluntary policies facilitating the integration of trisomy 21–affected persons into society.

Down syndrome screening targets patients at increased risk of chromosomal abnormality. Unequivocal diagnosis is made by fetal karyotyping using samples of chorionic villi, amniotic cells or occasionally fetal blood. Till the mid-1980s, the only screening strategy was to offer amniocentesis to older women (35 or $\geq$38 years), since maternal age was the longest established chromosomal abnormality risk factor. Development of ultrasonography subsequently enabled detection of fetal malformations, thus opening the way to screening for chromosomal abnormalities in younger women. The identification of biochemical markers in maternal serum in the second trimester then extended Down syndrome screening to all pregnant women, regardless of their age and whether or not fetal malformations are visible by ultrasound.

PRINCIPLE

In maternal serum screening for Down syndrome, individual risk associated with age is corrected using a factor related to serum marker concentrations. The relation between MSM and Down syndrome was discovered fortuitously. In 1984, levels of maternal serum alpha-fetoprotein (AFP) were found to be lower in Down syndrome–affected pregnancies (1). In 1987 and 1988, hCG, its free β fraction, and unconjugated estriol (uE3) were also found to be valuable markers for Down syndrome (2–5). Various combinations of maternal age with one or more of these markers were suggested to improve their predictive value. In 1988, Wald proposed a risk calculation, which is now used in all software programs (6). Many other markers have been investigated since, but in practice the four original markers are widely used: total hCG, or free β-hCG, AFP, and/or uE3 (7) with on average, a fetal Down syndrome detection rate of 60%, for a 5% false-positive rate among women under 35 (7,8).

MATHEMATICAL BASIS OF THE CALCULATION OF DOWN SYNDROME RISK

Calculation of a mother's risk of a Down syndrome-affected pregnancy incorporates the age-related risk and the risk determined from serum marker levels. Several markers can be combined. It is also possible to take into account sonographic data such as nuchal translucency measurement, provided these factors are proven to be independent of one another

In practice, this risk is calculated using computer programs. The mathematical model is based on the comparison of two populations, one of women with a Down syndrome-affected pregnancy and another with a non-Down syndrome-affected child.

Establishing the Age-Related Risk

The age-related risk of delivering a baby affected by Down syndrome has been established by a number of observational studies (9). Using such age-related risk reference values in the computerized calculation of the trisomy 21–derived risk has the advantage of predicting the odds of delivering an affected liveborn. It has the disadvantage of underestimating the actual risk of bearing an affected child at the time of screening, due to the relatively high spontaneous fetal loss rate in trisomy 21–affected pregnancies. It is usually estimated that the risk of bearing an affected infant at midtrimester is 1.2-fold greater than the risk of delivering an affected liveborn (10). This has a practical consequence when choosing a cutoff. It is expected that a 1/250 cutoff taking into account the midtrimester risk is equivalent to a 1/300 cutoff when computing the risk at birth.

Multiple of Median

Levels of all serum markers vary during gestation. However, if expressed in multiple of median (MoM), the marker value no longer depends on gestational age. To express a serum marker value in MoM, a median value (or 50th percentile) must first be determined in a control population for various gestational ages. The median is more accurately defined when the number of controls is high. It is generally accepted

that to establish MSM normal values, at least 300 patients are needed per week of gestation. The raw values for each marker are converted into MoM by dividing them by the median value at the same gestational age. Because serum markers expressed in MoMs follow a log normal distribution, their value is deemed low when under 0.5 MoM, high when above 2.5 MoM, and otherwise normal.

Likelihood Ratio

The likelihood ratio (Fig. 1) determined for each marker (expressed in MoM) is calculated based on the comparison of the distribution of a given marker in a control population and in a Down syndrome population. Maternal age-related risk is adjusted by multiplying it by the likelihood ratio determined for the marker, giving a new risk. If the markers are independent, the corresponding likelihood ratios can be multiplied.

A 1/300 to 1/250 risk cutoff is used in many countries to determine which patients should be offered amniocentesis.

Sensitivity, Specificity, and Positive Predictive Value

Down syndrome screening efficiency is assessed using two main criteria. Detection rate or sensitivity indicates the percentage of Down syndrome cases detected. Screen-positive rate (expressed in practice by 100-specificity) indicates the amniocentesis rate that would be produced by screening using a given risk cutoff. The false-positive rate is often used instead of the screen-positive rate, an approximation that is possible because of the low prevalence of Down syndrome. Screening efficiency is influenced by the choice of the cutoff used to decide whether to offer amniocentesis and maternal age distribution in the population.

In a given screening method, the detection rate and amniocentesis rate vary inversely and depend on the chosen cutoff. In patients under 35 years of age, a 1/250 cutoff allows a detection rate of 60% to 65%, at the cost of a 5% amniocentesis rate. If a 1/370 cutoff was used, the Down syndrome detection rate would be higher, but the number of resulting amniocenteses would also rise.

Because of the exponential increase in Down syndrome risk with maternal age, sensitivity and screen-positive rate are higher in older women (11).

Other criteria derived from the previous ones are used to define screening efficiency. Positive predictive value (PPV) is the percentage of infants with Down syndrome observed in women whose risk is above the cutoff. In practice, a balance is sought between the probability that amniocentesis will detect a chromosomal abnormality (PPV, approximately 1/100) and the risk of amniocentesis-related complications (iatrogenic risk, approximately 1/100 to 1/200) (12).

Theoretically, screening efficiency increases as more markers are tested. If the screen-positive rate is set at 5%, the detection rate ranges from 36% to 49% using a single marker combined with maternal age, but is 63% to 68% when four markers are used. However, variations in analytical efficiency for each marker may considerably attenuate this effect. For example, the coefficients of variation are around 5% for AFP, 3% for hCG or its free β-hCG fraction, but reach 10% to 20% for estriol. It may be preferable to use two well-controlled markers rather than four technically dubious ones.

Serum marker levels vary during pregnancy, particularly between 14 and 15 weeks of gestation: β-hCG falls from 25 ng/mL at 14 weeks to 18 ng/mL at 15 weeks; AFP rises from 22 to 27 IU/mL. For this reason, gestational age must be accurately determined by ultrasound (7).

FACTORS AFFECTING MSM LEVELS

Screening efficiency can be improved by taking into account factors that influence serum marker levels.

Maternal Weight

Concentrations of AFP, hCG, and uE3 vary with maternal weight. An increase of 20 kg reduces the level of AFP by approximately 17%, uE3 by 7%, and hCG

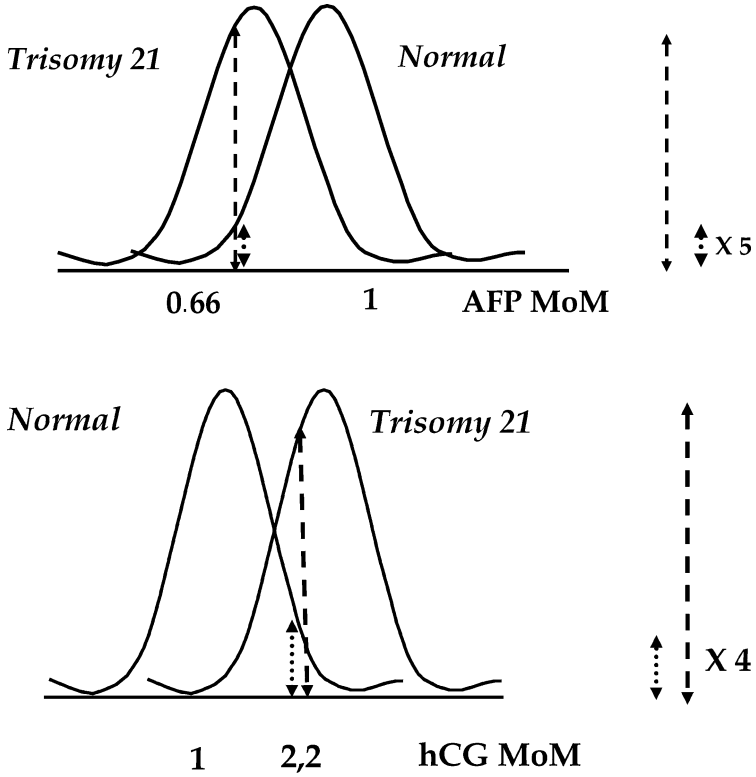

Figure 1 Distribution of maternal serum AFP and hCG (expressed in logarithm of MoM) in two groups of women, one with a Down syndrome–affected fetus, the other with an unaffected fetus. In the normal population, the median value is by definition equal to 1 MoM. In Down syndrome, AFP distribution is shifted toward low values and hCG distribution toward high values. In the example shown, the maternal serum concentrations were 0.66 MoM for AFP and 2.2 MoM for hCG. The software risk calculation is done by multiplying the age-related Down syndrome prevalence by the likelihood ratios generated by each marker. For a given value of a marker, the likelihood ratio is the ratio of the probability of having this concentration of marker while belonging to the trisomy 21–affected group to the probability of having the same concentration while belonging to the unaffected group. In the example, the likelihood ratio is 5 for AFP and 4 for hCG. If the patient is 20 years of age, the age-related risk is 1/1500. Taking AFP alone into account, the patient's risk becomes $1/1500 \times 5 = 1/300$. Taking hCG alone into account, the risk becomes $1/1500 \times 4 = 1/375$. Taking into account AFP and hCG yields a risk of $1/1500 \times 4 \times 5 = 1/75$. Likelihood ratios derived from nonbiochemical markers, such as nuchal translucency measurement, can be incorporated into a similar calculation. *Abbreviations*: AFP, alpha-fetoprotein; MoM, multiple of median.

by 16% (13). All risk calculation software packages incorporate maternal weight in the risk calculation.

Diabetes

After adjustment for maternal weight, diabetes has no significant effect on serum markers (14).

Ethnic Background

Adjustment for maternal weight considerably reduces the impact of ethnic factors on serum markers (15,16). In some countries, such as France, ethnic background is not currently taken into account by calculation software.

Smoking

Smoking has a large impact on levels of hCG and free β-hCG (−18%) and a smaller effect (3–4%) on concentrations of AFP and estriol (17). The effect of smoking is independent of the number of cigarettes smoked, and is apparent from one cigarette a day. However, smoking is not yet taken into account by most software programs. The effect of passive smoking, on the other hand, is unknown.

Pregnancies Following Ovarian Stimulation or In Vitro Fertilization

A study of a large number of cases has shown that serum markers are unaffected in such pregnancies, in contrast to data reported in small study populations (18).

FACTORS INFLUENCING RISK CALCULATION

History of Down Syndrome

If the patient has had a previous Down syndrome pregnancy, performing an amniocentesis during subsequent pregnancies is a widely accepted policy, as the overall risk of recurrence is around 1% (19). However, the patient may wish to have a more precise evaluation of her risk prior to undergoing an invasive procedure. If the patient opts for MSM screening, this additional risk must be incorporated into the risk calculation, by altering the maternal age-related risk. An additional risk of 0.54% in the second trimester and 0.77% in the first is generally used (20).

This strategy is, however, not suitable when the history of Down syndrome is due to familial translocation, since the risk of transmission due to linkage disequilibrium is far greater than 1%.

Twin Pregnancies

Down syndrome screening cannot simply be applied to twin pregnancies since serum marker concentrations are physiologically higher. Furthermore, serum assays reflect the placental and fetal metabolism of both twins and this may limit screening sensitivity when only one fetus is affected by Down syndrome. Serum marker data are scarce for twin pregnancies with one or both fetuses affected. Marker concentrations in twins are "normalized" by dividing them by the median value defined for a control population of twin pregnancies. The normalized values are then entered in software programs calibrated for singleton pregnancies. The distribution of hCG varies with chorionicity, which should be taken into account to improve screening specificity

(21,22). In addition, Down syndrome screening in twin pregnancies is complicated by estimation of maternal age-related risk. Although in monozygotic twin pregnancies this risk is identical to that of singleton pregnancies, in dizygotic twin pregnancies (information unavailable prenatally: the chorionicity is known but not the zygosity) the risk of having at least one affected child is almost doubled compared with a singleton pregnancy (23). This theoretical risk is, however, not observed in study populations and is, therefore, not use in practice (24).

Despite all these limitations, if one considers predicting the "at least one affected twin event," maternal serum screening in twins may achieve a 54% detection rate with a screened positive rate of 8% (22). This is better than using maternal age alone, but not as good as what can be achieved based on first-trimester nuchal translucency measurement (25).

SERUM MARKERS AND SCREENING FOR OTHER ANOMALIES

Screening for Spina Bifida

Abnormally high maternal serum AFP is suggestive of open neural tube defects (NTD) including spina bifida and anencephaly, whose frequency varies with ethnic and geographical background. With a cutoff of 2.5 MoM, 1% of patients will have a serum AFP above this cutoff and the NTD detection rate will be 80%. Depending on the prevalence of NTD, a 1/25 to 1/50 PPV can be reached. Most software provides an NTD risk calculation. This risk may be underestimated when the patient's medical history and geographical background are not taken into account.

Once the patient is considered at risk for open NTD, the diagnostic strategy tends to be based on ultrasound. However, when fetal imaging remains inconclusive, amniocentesis can provide amniotic fluid for electrophoresis of cholinesterases.

Screening for Trisomy 18

AFP and hCG (or β-hCG) are simultaneously reduced to below 0.5 MoM in approximately 75% of trisomy 18–affected pregnancies. In such cases, ultrasonography should be used to search for morphological signs associated with trisomy 18 (26).

Other Risks

Other maternal or fetal diseases are associated with changes in serum markers, including pre-eclampsia (high hCG), fetal death (high AFP and/or low hCG), triploidy (very high hCG), Smith–Lemli–Opitz syndrome (low estriol). However, the markers for these diseases are of low specificity and cannot be used in practice (27–29).

QUALITY CONTROL AND SCREENING POLICIES

As for most laboratory assays, quality insurance procedures have been implemented for maternal serum screening. This includes internal quality control of assays generally provided by the manufacturers. In addition, an external quality control procedure is widely used as the U.K. NEQAS (30). The principle of this quality control designed for MSM screening consists of providing laboratories with selected sera and studying the interlaboratory agreement of the calculated risks and the distribution of the raw and MoM marker concentrations.

Screening policies vary greatly among countries and are usually based on hospitals or regions. In some countries such as France, stringent national screening policies are implemented.

THE EXAMPLE OF A NATIONAL SCREENING POLICY

In France, strict regulatory measures were put in place in January 1997 to govern MSM (31). The law stipulates that the gestational age at screening should range between 14 and 18 weeks, the pregnancy should be dated as accurately as possible, and a written informed consent is mandatory. Since doctors have been sued and condemned for not having informed pregnant women of the availability of MSM, it is widely accepted that this screening should be universally offered. MSM and subsequent fetal karyotyping are available free of charge. The MSM assay and risk calculation can only be performed by laboratories accredited by the ministry of health. Assay kits and software must be approved by a national health products safety agency. In addition to the quality controls mentioned above, each accredited laboratory must submit a yearly activity report to the ministry of health.

Using such a stringent national policy, the rate of patients electing to undergo MSM screening rose from 54% in 1997 to over 80% in 2004.

The major advantage of a standardized national policy is that it allows for large-scale evaluation of screening. For example, the national average Down syndrome detection rate has been shown to be of 70%, with a 6.5% amniocentesis rate based on a study of 854,902 patients (31). These good year-on-year results mean that great caution is exercised before making any methodological changes.

The drawback of such a standardized national policy is that any innovation, even minor or evidence-based, must be authorized by the national health agency following a long and complex procedure.

SECOND-TRIMESTER MSM RESULTS

Table 1 presents French data on MSM for Down syndrome (1997–2001). As in other countries, around 75% of laboratories assay two serum markers (AFP and hCG or β-hCG), whereas the remaining 25% use triple test screening, which includes the assay of estriol.

Patients Under 38 Years of Age

Between 1997 and 2001, 2,450,449 patients below 38 years of age chose to undergo maternal serum screening. Overall, 2332 cases of Down syndrome were diagnosed, either prenatally or at birth. Of these, 1676 were detected by MSM with an action threshold of 1/250, giving a detection rate of 72% and 159,334 patients had a calculated risk above 1/250, corresponding to a screened positive rate of 6.5%. The detection rate and the screened positive rate did not change between 1997 and 2001.

These results confirm that serum markers are more effective than maternal age alone, since their PPV of 1/95 is greater than that for maternal age 38, 39, or even 40 years (1/200). In other words, 95 amniocenteses are needed to detect one Down syndrome case by maternal serum screening, compared with 200 using maternal age alone.

Table 1 Cumulated Results for Maternal Serum Screening in France from 1997 to 2001

Patients <38 years of age (singleton pregnancies)	
Total patients	2,450,449
Patients at risk (≥1/250)	159,334 (6.5%)
Total number of Down syndrome cases	2,332
Down syndrome cases in the at-risk group (1 ≥ 250)	1,676
Detection rate	71.9%
PPV	1/95
Patients ≥38 years of age (singleton pregnancies)	
Total patients	50,707
Patients at risk (≥1/250)	17,897 (35%)
Total number of Down syndrome cases	319
Down syndrome cases in the at-risk group (≥1/250)	304
Detection rate	95.3%
PPV	1/59

Abbreviation: PPV, positive predictive value.
Source: Data from medical laboratory technologists accredited for Down syndrome screening.

Patients Aged ≥38 Years

Serum markers can also be used in patients aged ≥38 years, who wish to avoid amniocentesis if they can be shown to be at low risk. Between 1997 and 2001, 20% ($n = 50{,}707$) of French pregnant women aged ≥38 years elected to undergo MSM. Only one-third of these patients had a risk >1/250. Amniocentesis was avoided in two-thirds of cases; however, a Down syndrome detection rate of 95% would still have been achieved (11,32).

CURRENT PROBLEMS IN DOWN SYNDROME SCREENING

Two aspects of screening need to be addressed. First, second-trimester MSM is performed relatively late in pregnancy (14–17 weeks of amenorrhea) and many women wish to have an earlier screening (33). Second, the risk calculated from second-trimester maternal serum screening is likely to be substantially overestimated in women who underwent first-trimester nuchal translucency screening.

First-Trimester Maternal Serum Screening

First-trimester screening for Down syndrome does not use the same markers as second-trimester screening, since total hCG, AFP, and estriol are less effective or ineffective before 14 weeks of gestation. Only free β-hCG is a good marker in both the first and second trimesters (34). Pregnancy-associated plasma protein A (PAPP-A), whose level is reduced in Down syndrome, can only be used before 14 weeks (35,36). Retrospective studies show that the most effective combination is PAPP-A plus free β-hCG, with a 60% detection rate and 5% amniocentesis rate (8). Quality control of these markers can be easily implemented (37).

Early screening for Down syndrome would enable diagnosis of chromosomal abnormality from 12 to 13 weeks, which has clear medical and psychological advantages. These advantages should nonetheless be balanced against technical considerations in fetal karyotyping. Before 15 weeks, amniocentesis carries an increased risk of fetal loss or rupture of the membranes and chorionic villi sampling (CVS) is preferable (38).

A meta-analysis including all available trials (39) shows that transabdominal CVS, when performed by very experienced operators, carries a risk of fetal loss similar (1.3-fold) to that of second-trimester amniocentesis.

This drawback of early diagnosis is counterbalanced by the fact that Down syndrome screening combining nuchal translucency and serum markers is highly specific and, therefore, assigns fewer patients to the at-risk group. However, generalization of first-trimester screening and prenatal diagnosis might necessitate additional medical training of doctors in performing CVS and of cytogeneticists for first-trimester methods. Another potential drawback of first-trimester Down syndrome screening is that it could not be used jointly with screening for spina bifida, since high maternal serum AFP is a good marker of neural tube closure defects, but is unusable before 14 weeks.

Sequential Screening

Three methods of screening for Down syndrome are available to most pregnant women in most industrialized countries: nuchal translucency measurement, second-trimester MSM, and second-trimester ultrasound. Considered separately, each method yields an amniocentesis rate of the order of magnitude of 5%, which seems reasonable. However, when these methods, which are deemed independent, are used sequentially on the same patients, almost 15% of women undergoing this multiple step screening will be offered amniocentesis (40).

Therefore, the results of first-trimester nuchal translucency and first- or second-trimester maternal markers should be analyzed together to produce a single integrated risk in order to maintain an acceptable screened positive rate (41–44). However, before considering the risk derived from nuchal translucency measurement, a specific quality insurance procedure is mandatory to ensure the quality of the measurement.

It is also possible to take into account second-trimester ultrasound markers to assess the risk of aneuploidy. For each "soft second-trimester ultrasound marker," estimations of a specific likelihood ratio have been published, and commercially available software is designed to incorporate MSM, first-trimester ultrasound, and second-trimester ultrasound to provide a single integrated risk assessment. However, there is no consensus regarding practical strategies of quality control of second-trimester ultrasound.

The best strategy in the short-term is probably to combine likelihood ratios derived from nuchal translucency measurement with MSM in the first and or second trimester. This can only be done if there is specific medical training in nuchal translucency measurement together with standardized quality control.

CONCLUSION

Prospective studies have shown that MSM detects at least 60% of Down syndrome-affected pregnancies, for a 5% amniocentesis rate. This screening strategy enables Down syndrome screening in younger women. Serum markers can also be used in patients aged 38 to 40 years, who wish to avoid unnecessary amniocentesis.

Maternal serum screening has been available free of charge in France since January 1997. Its use has spread rapidly in the general population and it has been applied to close to 80% of pregnancies since 1998. The aim now is to reduce the number of amniocentesis generated by currently available screening methods by

performing a single–risk calculation combining nuchal translucency measurement with first- or second-trimester maternal serum screening.

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3
Nuchal Translucency Screening

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INTRODUCTION

Down syndrome (DS) is the most frequent severe chromosomal anomaly in live born infants, with a frequency of 1 in about 600 births. Its name originates from the British physician Langdon Down who first described the syndrome in 1866 (1). The association of DS with maternal age was known from the beginning of the 20th century. Since the 1970s, maternal age-based screening for DS has been introduced in most developed countries. Invasive diagnostic procedures (amniocentesis and, chorion biopsies) were offered to all pregnant women above a certain age threshold. Every country has variably set, from 35 years onward, the age limit for offering DS screening, the choice in cutoff depending on public health, economic, and/or social argumentations. Disadvantages of a maternal age-based selection are (i) the high screen-positive rate—due to the increasing number of women postponing reproduction to a later phase in life, (ii) the low positive predictive value, and (iii) the unfavorable ratio between detected DS cases and iatrogenic abortions caused by the invasive diagnostic procedure. In fact, for each detected DS case one healthy baby is lost as a consequence of the invasive procedures (2). Moreover, as the majority of babies are still born from younger mothers, a screening strategy based on maternal age only leads to the detection of about one-third of all DS cases. Over the last decade, the need for a safer and more efficient screening strategy has been a major challenge for researchers and health policy makers. In his *Observation of an Ethnic Classification of Idiots*, Down described the typical features of affected individuals as "... their skin appears to be too large for their bodies, the nose is small and the face is flat..." One hundred and thirty years later, these features have been proposed as, at the moment, the best available strategies for an early ultrasound-based screening for DS. In this chapter, the various aspects and implications of first-trimester screening for chromosomal anomalies by nuchal translucency (NT) measurement will be discussed.

METHODS OF SCREENING

Every woman has a risk that her fetus/baby is affected by a chromosomal defect. With the knowledge of the natural history of chromosomal anomalies, it is possible to calculate for every maternal age and gestational age the background risk of carrying a chromosomally abnormal fetus (3). When additional measurements are taken, measurement specific likelihood ratios are derived and these are applied to adjust the background risk. Based on these concepts new screening strategies have been developed. The first has been second-trimester maternal serum screening (4). Since the late 1980s, the idea of an early and ultrasound-based screening strategy has become attractive, considering that many women undergo ultrasound examination from the early stages of pregnancy.

Nuchal Translucency

In 1990, Szabo first described the association between increased nuchal fluid in early gestation and trisomy 21 (5). A few years later, Nicolaides proposed NT screening as an early ultrasound screening strategy for chromosomal anomalies (6). Nuchal translucency, named in view of its ultrasound aspect, is a common feature observed in all fetuses between 10 and 14 weeks' gestation (7). Normal ranges for the measurement have been constructed (8). The measurement can be carried out transabdominally or transvaginally (9). Another relevant aspect is that in addition to being associated with chromosomal abnormality, increased NT is associated with perinatal death, major cardiac defects, and other structural defects, and has been reported to be associated with a number of genetic syndromes (10,11). The pathophysiology of a normal and increased NT is not yet fully understood. The anatomical substrate seems to be the presence of two rhomboidal cavities, symmetrically situated with respect to the sagittal plane and appearing at ultrasound investigation as a single black space, due to the lateral resolution of the ultrasound beam. The two spaces may be the superficial recesses of the jugular lymphatic sacs at a stage when they are not yet connected to the system. The connection occurs physiologically from 9 to 10 weeks onward and may be completed by 12 to 13 weeks (12). The pathophysiological background of an increased NT will be the subject of another chapter.

STUDIES ON NT SCREENING

Since the early 1990s, numerous studies on the association between fetal NT thickness and chromosomal anomalies have been published (13–16).

When it became clear that NT could be used as an early screening method for DS, studies have focused especially on aspects such as success rate in obtaining the measurement, and sensitivity of the screening method. Almost all studies have shown an association between trisomy 21 and increased NT in the late first trimester and early second trimester; however, the variation in reported performance was considerable due to variation in study design, population, gestational age, time assigned for the measurement, and cutoffs used to define an abnormal measurement. Table 1 presents studies with a great variation in the methodological approach in chronological order (17–26). Gestational age ranged from as early as 8 weeks up to 16 weeks. Success in obtaining the measurement ranged from 66% to 100%, false-positive rate (FPR) from 0.4% to 6.3%, and detection rate (DR) from 30% to 100%. Table 2 presents studies where the

Table 1 Studies Investigating the Value of NT Measurement as Screening Method for Chromosomal Anomalies and Showing a Great Variation in Methodology (Cutoff and Gestational Age Window)

Author	GA (wk)	*N*	Success rate measurement (%)	Used cutoff (mm)	FPR (%)	DR of trisomy 21 (%)
Bewley et al. (17)	$8–13^{+6}$	1704	66.1	3	6.2	33.3
Szabo et al. (18)	$9–12^{+6}$	3380	100	3	1.6	90
Kornman et al. (19)	$8–13^{+6}$	923	58.2	3	6.3	50
Haddow (26)	$9–15^{+6}$	4049	83	95th percentile	5	31
Economides (20)	$11–14^{+6}$	2256	100	95th percentile	0.4	75
Schuchter (25)	$10–12^{+6}$	9342	100	2.5	2.1	57.9
Wayda (23)	10–13	6841	100	2.5	4.1	100
Crossley (21)	$10–14^{+6}$	17,229	72.9	95th percentile	5	48.6
Rozenberg (24)	$12–14^{+6}$	6234	98.6	3	2.8	61.9
Wald (22)	$6–16^{+6}$	47,053	76.6	95th percentile	5	38.7

Abbreviations: GA, gestational age; FPR, false-positive rate; DR, detection rate; NT, nuchal translucency.

measurement has been performed in the same gestational age window (8,27–33). In spite of using different cutoffs for defining an abnormal measurement, the success rate has increased remarkably and the DR is about 75%.

The Role of the Fetal Medicine Foundation in Standardizing NT Screening

Since 1994, the Fetal Medicine Foundation (FMF) has played a crucial role in promoting a uniform measurement technique by holding courses aimed at teaching the principles of first-trimester ultrasound screening. The FMF provides certified ultrasonographers with a free software program that allows risk calculation based on the background risk (maternal age), NT measurement, and gestational age [crown–rump length (CRL)]. By combining these three parameters, it is possible to calculate for each fetus its individual risk of being affected by trisomy 21 (32). The license for the risk assessment is subject to renewal on a yearly basis. Condition for renewal is that the affiliated centers participate in an audit of their screening activities. This enables pooling of data and continuous evaluation of the technical skills. The

Table 2 Studies Investigating the Value of NT Measurement as Screening Method for Chromosomal Anomalies at the Same Gestational Age Window

Author	GA (wk)	*N*	Success rate measurement (%)	Used cutoff (mm)	FPR (%)	DR of trisomy 21 (%)
Pandya et al. (8)	$10–13^{+6}$	1763	100	2.5	3.4	75
Taipale et al. (27)	$10–13^{+6}$	6939	98.6	3	0.7	66.7
Pajkrt et al. (28,29)	$10–13^{+6}$	3614	100	3	4.2	69.6
Theodoropoulos et al. (31)	$10–13^{+6}$	3550	100	95th percentile	2.3	90.9
Schwarzler et al. (30)	$10–13^{+6}$	4523	100	2.5	2.7	66.7
Panburana et al. (32)	$10–13^{+6}$	2067	100	2.5	2.9	100

Abbreviations: GA, gestational age; FPR, false-positive rate; DR, detection rate; NT, nuchal translucency.

Table 3 Guidelines of the Fetal Medicine Foundation

- Ultrasound equipment of good quality with zoom, cineloop facility, and possibility of measuring in decimals of millimeters
- The measurement should be performed (preferably) transabdominally or vaginally
- The fetus has to be in a midsagittal plane with head in neutral position (not extended or flexed) (Fig. 1)
- The picture has to be magnified so that the fetus occupies 75% of the picture (every movement apart of the callipers should be equivalent to 0.1 mm) (Fig. 2)
- Distinction between amniotic membrane and fetal skin must be possible
- The callipers have to be placed on the maximal black thickness "on-to-on," which means on the white line at the limit of the black space (Fig. 2)

A few practical tips on how to obtain a good NT measurement:

a. As a general rule, the ultrasonographer should be patient and only be satisfied when the measurement is as good as possible
b. Fetal immobility: the woman can be asked to cough and, in case of no success, the scan should be repeated after a while (send her for a short walk)
c. Unfavorable fetal position (standing fetus): In this case the vaginal route should be tried
d. Impossibility to clearly visualize the nuchal area: the umbilical cord may be around the fetal neck preventing visualization of the optimal place to measure the NT (to verify this, turn color Doppler on). Avoid measuring too low (neck or upper back) as the measurement will be underestimated. Wait for a change in position or reschedule the scan for another day
e. In case there is an impression that the NT may be increased but a good measurement cannot be obtained, the nuchal area should be looked at in the transversal plane. This may confirm the presence of small septations. Wait then until a good sagittal measurement is obtained

Abbreviation: NT, nuchal translucency.

guidelines of the FMF are provided in Table 3. Examples of normal and enlarged NT and correct measurement techniques are provided in Figures 1–3. In 1998, a large multicenter study including 100,000 pregnancies based on the FMF criteria was published (34). When measurements are obtained according to the guidelines of the FMF, about 70% of fetuses with trisomy 21 have a measurement above the 95th percentile for gestational age. When risk calculation is used, it is expected that, in an unselected population, a risk cutoff of 1 in 300 will identify 75% of trisomy 21 fetuses for a 5% FPR. While the risk assessment focuses on trisomy 21, the group with a high risk of trisomy 21 is also known to contain the majority of other chromosomal defects (34).

Thanks to uniformity in the methodological approach, comparison of results of studies from different centers has become possible (Table 4) (34–40). DR is about 85% for an FPR of about 6%. A meta-analysis of studies reporting on NT as a screening method for chromosomal anomalies without description of used methodology reports a DR of 76% for an FPR of 6% (41).

Fetal Loss in Chromosomally Abnormal Pregnancies

It is known that chromosomally abnormal fetuses have a high spontaneous intrauterine lethality (3). About 40% to 50% of conceived DS fetuses will not end in live births. It is well known that chromosomally abnormal pregnancies relatively often result in spontaneous intrauterine loss. Therefore, if screening is applied in early pregnancy, it cannot be excluded that some degree of verification bias inflates the

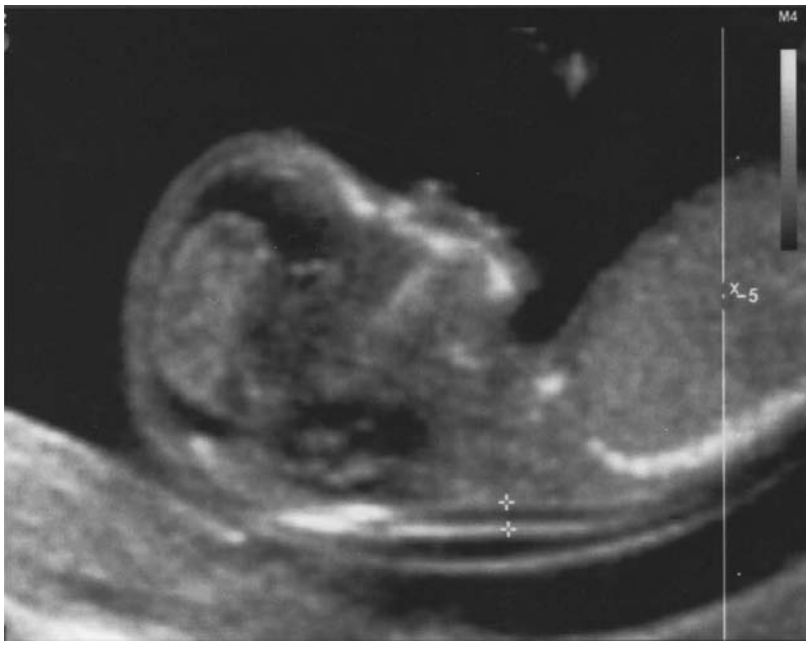

Figure 1 Correct fetal position to perform the NT measurement. *Abbreviation*: NT, nuchal translucency.

performance (3). The degree by which results are biased depends on the association between marker levels and spontaneous loss rate (41,42). In chromosomally normal fetuses increased NT is associated with an increased spontaneous fetal loss rate (43,44). However, in a study from Brasil the association between increased NT and intrauterine death in DS pregnancies seemed limited (45). Further studies are needed to assess to what extend NT thickness affects the chances of spontaneous loss. In the mean time, screening performances may be compared using the observed number of affected pregnancies and the expected number based on the maternal age and gestational age distribution of the population (34). Using this approach it is

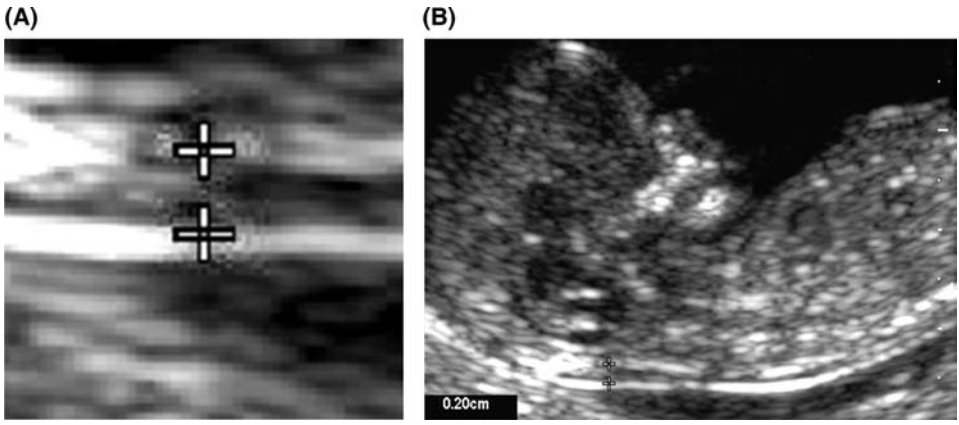

Figure 2 Example of a good NT measurement. Detail on callipers placement on the white lines. *Abbreviation*: NT, nuchal translucency.

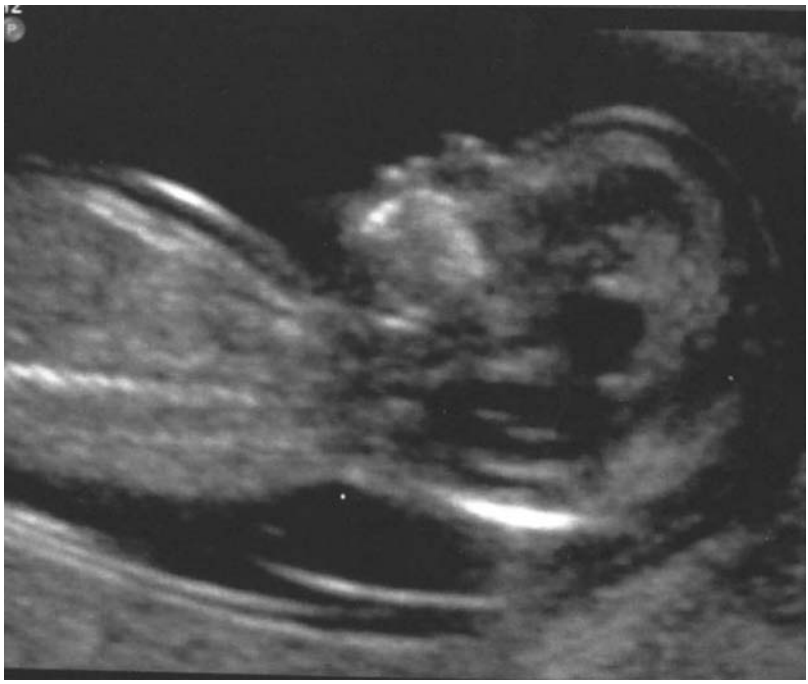

Figure 3 Example of a trisomy-21 fetus showing an extremely increased NT, generalized edema, and absent nasal bone. *Abbreviation*: NT, nuchal translucency.

estimated that screening based on fetal NT reduces the prevalance of DS among live born children by about 80% (34).

Quality Control of NT Measurements

The original FMF audit scheme entailed regular quantitative assessment of the distribution of NT measurements complemented by global qualitative examination of five randomly selected images (23). In a recently modified approach, the qualitative

Table 4 Studies Where the Methodological Approach Has Been Standardized According to the Guidelines of the FMF

Author	Mean maternal age	Population (*N*)	Cutoff	FPR (%)	DR of trisomy 21 (%)	DR of other chromosomal anomalies (%)
Snijders et al. (34)	31	96,127	1:300	8.3	82.2	77.8
Thilaganathan et al. (35)	29	9753	1:300	7.8	81	89.3
Gasiorek-Wiens et al. (38)	33	21,475	1:300	13	87.6	88.2
Zoppi et al. (36)	33	10,001	1:300	8.9	90.6	84.8
Brizot et al. (37)	28	2470	1:300	7.4	90	75
Chasen et al. (40)	33	2216	1:300	7.5	83.3	75
Prefumo et al. (39)	31	11,820	1:300	4.8	81.5	—
Total	31	153,862	1:300	8.3	85.2	82.2

Abbreviations: FPR, false-positive rate; DR, detection rate; FMF, Fetal Medicine Foundation.