

Obstetrical Complications Associated With Abnormal Maternal Serum Markers Analytes

This technical update has been reviewed by the Genetics Committee and reviewed and approved by the Executive of the Society of Obstetricians and Gynaecologists of Canada.

PRINCIPAL AUTHORS

Alain Gagnon, MD, Vancouver BC

R. Douglas Wilson (Chair), MD, Philadelphia PA

SOCIETY OF OBSTETRICIANS AND GYNAECOLOGISTS OF CANADA GENETICS COMMITTEE

François Audibert, MD, Montreal QC

Victoria M. Allen, MD, Halifax NS

Claire Blight, RN, Halifax NS

Jo-Ann Brock, MD Halifax NS

Valerie A. Désilets, MD, Montreal QC

Alain Gagnon, MD, Vancouver BC

Jo-Ann Johnson, MD, Calgary AB

Sylvie Langlois, MD, Vancouver BC

Anne Summers, MD, Toronto ON

R. Douglas Wilson (Chair), MD, Philadelphia, PA

Philip Wyatt, MD, Toronto ON

Disclosure statements have been received from all members of the committee.

Evidence: The Cochrane Library and Medline were searched for English-language articles published from 1966 to February 2007, relating to maternal serum markers and perinatal outcomes. Search terms included PAPP-A (pregnancy associated plasma protein A), AFP (alpha-fetoprotein), hCG (human chorionic gonadotropin), estriol, unconjugated estriol, inhibin, inhibin-A, maternal serum screen, triple marker screen, quadruple screen, integrated prenatal screen, first trimester screen, and combined prenatal screen. All study types were reviewed. Randomized controlled trials were considered evidence of the highest quality, followed by cohort studies. Key individual studies on which the recommendations are based are referenced. Supporting data for each recommendation are summarized with evaluative comments and references. The evidence was evaluated using the guidelines developed by the Canadian Task Force on Preventive Health Care.

Values: The evidence collected was reviewed by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada.

Benefits, Harms, and Costs: The benefit expected from this guideline is to facilitate early detection of potential adverse pregnancy outcomes when risks are identified at the time of a maternal serum screen. It will help further stratification of risk and provide options for pregnancy management to minimize the impact of pregnancy complications. The potential harms resulting from such practice are associated with the so called false positive (i.e., uncomplicated pregnancies labelled at increased risk for adverse perinatal outcomes), the potential stress associated with such a label, and the investigations performed for surveillance in this situation. No cost-benefit analysis is available to assess costs and savings associated with this guideline.

Abstract

Objective: To review the obstetrical outcomes associated with abnormally elevated or decreased level of one or more of the most frequently measured maternal serum marker analytes used in screening for aneuploidy. To provide guidance to facilitate the management of pregnancies that have abnormal levels of one or more markers and to assess the usefulness of these markers as a screening test.

Options: Perinatal outcomes associated with abnormal levels of maternal serum markers analytes are compared with the outcomes of pregnancies with normal levels of the same analytes or the general population.

Key Words: Prenatal screening, maternal serum markers, obstetrical complications

Summary Statements

1. An unexplained level of a maternal serum marker analyte is defined as an abnormal level after confirmation of gestational age by ultrasound and exclusion of maternal, fetal, or placental causes for the abnormal level. (III)
2. Abnormally elevated levels of serum markers are associated with adverse pregnancy outcomes in twin pregnancies, after correction for the number of fetuses. Spontaneous or planned multifetal reductions may result in abnormal elevations of serum markers. (II-2)

Recommendations

1. In the first trimester, an unexplained low PAPP-A (< 0.4 MoM) and/or a low hCG (< 0.5 MoM) are associated with an increased frequency of adverse obstetrical outcomes, and, at present, no specific protocol for treatment is available. (II-2A) In the second trimester, an unexplained elevation of maternal serum AFP (> 2.5 MoM), hCG (> 3.0 MoM), and/or inhibin-A (≥ 2.0 MoM) or a

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- decreased level of maternal serum AFP (< 0.25 MoM) and/or unconjugated estriol (< 0.5 MoM) are associated with an increased frequency of adverse obstetrical outcomes, and, at present, no specific protocol for treatment is available. (II-2A)
2. Pregnant woman with an unexplained elevated PAPP-A or hCG in the first trimester and an unexplained low hCG or inhibin-A and an unexplained elevated unconjugated estriol in the second trimester should receive normal antenatal care, as this pattern of analytes is not associated with adverse perinatal outcomes. (II-2A)
 3. The combination of second or third trimester placenta previa and an unexplained elevated maternal serum AFP should increase the index of suspicion for placenta accreta, increta, or percreta. (II-2B) An assessment (ultrasound, MRI) of the placental–uterine interface should be performed. Abnormal invasion should be strongly suspected, and the planning of delivery location and technique should be done accordingly. (III-C)
 4. A prenatal consultation with the medical genetics department is recommended for low unconjugated estriol levels (<0.3 MoM), as this analyte pattern can be associated with genetic conditions. (II-2B)
 5. The clinical management protocol for identification of potential adverse obstetrical outcomes should be guided by one or more abnormal maternal serum marker analyte value rather than the false positive screening results for the trisomy 21 and/or the trisomy 18 screen. (II-2B)
 6. Pregnant woman who are undergoing renal dialysis or who have had a renal transplant should be offered maternal serum screening, but interpretation of the result is difficult as the level of serum hCG is not reliable. (II-2A)
 7. Abnormal maternal uterine artery Doppler in association with elevated maternal serum AFP, hCG, or inhibin-A or decreased PAPP-A identifies a group of women at greater risk of IUGR and gestational hypertension with proteinuria. Uterine artery Doppler measurements may be used in the evaluation of an unexplained abnormal level of either of these markers. (II-2B)
 8. Further research is recommended to identify the best protocol for pregnancy management and surveillance in women identified at increased risk of adverse pregnancy outcomes based on an abnormality of a maternal serum screening analyte. (III-A)
 9. In the absence of evidence supporting any specific surveillance protocol, an obstetrician should be consulted in order to establish a fetal surveillance plan specific to the increased obstetrical risks (maternal and fetal) identified. This plan may include enhanced patient education on signs and symptoms of the most common complications, increased frequency of antenatal visits, increased ultrasound (fetal growth, amniotic fluid levels), and fetal surveillance (biophysical profile, arterial and venous Doppler), and cervical length assessment. (III-A)

10. Limited information suggests that, in women with elevated hCG in the second trimester and/or abnormal uterine artery Doppler (at 22–24 weeks), low-dose aspirin (60–81 mg daily) is associated with higher birthweight and lower incidence of gestational hypertension with proteinuria. This therapy may be used in women who are at risk. (II-2B)
11. Further studies are recommended in order to assess the benefits of low-dose aspirin, low molecular weight heparin, or other therapeutic options in pregnancies determined to be at increased risk on the basis of an abnormal maternal serum screening analyte. (III-A)
12. Multiple maternal serum markers screening should not be used at present as a population-based screening method for adverse pregnancy outcomes (such as preeclampsia, placental abruption, and stillbirth) outside an established research protocol, as sensitivity is low, false positive rates are high, and no management protocol has been shown to clearly improve outcomes. (II-2D)

When maternal serum screening is performed for the usual clinical indication (fetal aneuploidy and/or neural tube defect), abnormal analyte results can be utilized for the identification of pregnancies at risk and to direct their clinical management. (II-2B) Further studies are recommended to determine the optimal screening method for poor maternal and/or perinatal outcomes. (III-A)

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INTRODUCTION

First and second trimester serum screening has been used for many years in Canada as a method of identifying fetuses at increased risk of open neural tube defects and chromosomal abnormalities, in particular trisomy 21 and trisomy 18. The use of maternal serum screening for aneuploidy is described in the “Prenatal Screening for Fetal Aneuploidy Guidelines” published in this journal in February 2007.¹ Over the years, a variety of other pregnancy outcomes have been associated with abnormal values of the different analytes used in these screening tests. The goal of this guideline is to provide a summary of the obstetrical risks associated with values outside the normal range (see definitions below) for the five common first and second trimester serum screening markers: alphafetoprotein, human chorionic gonadotropin, unconjugated estriol, inhibin-A, and pregnancy associated plasma protein-A. Table 2 shows the typical timing of testing for these analytes. This guideline provides guidance for the evaluation and management of these screening variations. Table 3 provides a summary of levels associated with poor obstetrical outcomes in the second trimester.

MATERNAL SERUM ALPHAFETOPROTEIN

Elevated Maternal Serum AFP

Unexplained elevation of maternal serum AFP has been typically defined as a AFP > 2.5 MoM in the absence of fetal chromosomal abnormalities, fetal structural anomalies (e.g., open neural tube defect, abdominal wall defect), placental anomalies such as chorioangioma, multiple

ABBREVIATIONS

AFP	alphafetoprotein
CI	confidence interval
hCG	human chorionic gonadotropin
IUFD	intrauterine fetal demise
IUGR	intrauterine growth restriction
MoM	multiples of the median
ONTD	open neural tube defects
OR	odds ratio
PAPP-A	pregnancy associated plasma protein A
RR	relative risk
uE ₃	unconjugated estriol

Table 1. Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care

Quality of Evidence Assessment*	Classification of Recommendations†
I: Evidence obtained from at least one properly randomized controlled trial	A. There is good evidence to recommend the clinical preventive action
II-1: Evidence from well-designed controlled trials without randomization	B. There is fair evidence to recommend the clinical preventive action
II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group	C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making
II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category	D. There is fair evidence to recommend against the clinical preventive action
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	E. There is good evidence to recommend against the clinical preventive action
	L. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making

*The quality of evidence reported in these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.¹⁶²

†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the The Canadian Task Force on Preventive Health Care.¹⁶²

pregnancy, or fetal demise or maternal conditions such as ovarian tumour or choriocarcinoma.²

Although the exact cause for an unexplained elevation is not completely understood, placental pathology studies suggest that it is associated with chorionic villitis and placental vascular lesions.³ These lesions allow leakage of the AFP from the high concentration fetal circulation to the low concentration maternal circulation, thereby elevating the maternal serum AFP. It has also been associated with an increased frequency of maternal uterine malformation,⁴ possibly related to abnormal placentation.

Obstetrical Complications

Table 4 provides a summary of the complications that have been described with unexplained elevated maternal serum AFP. This relationship is also present in high-risk groups, such as women with thrombophilias^{5,6} and in cases of fetal echogenic bowel.⁷ The mean maternal serum AFP value is higher in women who develop gestational hypertension with proteinuria and adverse features before 32 weeks' gestation rather than after 32 weeks' gestation.⁸ Higher levels of maternal serum AFP appear to correlate with a higher incidence of poor pregnancy outcome,^{9–12} ranging from 19% at 2.5–2.9 MoM to 70% at ≥ 5.0 MoM.¹¹ An elevation of amniotic fluid AFP in association with an unexplained elevated maternal serum AFP carries a higher risk of preterm delivery (iatrogenic or spontaneous [60%, RR 4.0; 95% CI 2.8–5.7]).¹³ An increased frequency of persistent placenta previa and abnormal placental invasion has been

described in case reports and in case-control and cohort studies in association with maternal serum AFP > 2.0 or 2.5 MoM.^{14–20}

Low Maternal Serum AFP

Low maternal serum AFP, defined as a maternal serum AFP value < 0.25 MoM, has been associated with spontaneous abortion,^{21,22} preterm birth,²² stillbirth,^{22,23} infant death,²² and increased macrosomia.^{24,25}

MATERNAL SERUM HUMAN CHORIONIC GONADOTROPIN

Elevated hCG

Unexplained elevation of second trimester hCG has been defined with cut-offs varying from > 2.0 MoM to > 4.0 MoM in the absence of fetal chromosomal abnormalities, placental anomalies (e.g., molar pregnancy), multiple pregnancy, or fetal demise. Although the exact cause for an unexplained elevation is not completely understood, hypoxic cytotrophoblasts demonstrate increased proliferation and increase hCG production.^{26,27} Case reports have associated elevated hCG with chorioangiomas of the placenta (an abnormal capillary proliferation that has been associated with in utero hypoxia).²⁸ The coexistence of elevated unexplained hCG and confined placental mosaicism has been described.²⁹ Placentas from pregnancies with an elevated hCG were more likely to be large for gestational age and to demonstrate decidual plasma cells and retroplacental hematomas.³⁰

Table 2. Typical timing of testing of different analytes

First trimester (10–14 weeks)	Second trimester (15–21 weeks)
PAPP-A	AFP
hCG	Estriol
	hCG
	Inhibin

Table 3. Summary of level of analytes associated with poor obstetrical outcomes after 24 weeks

	↓	↑
PAPP-A	Yes	No
hCG	Yes in T1	Yes in T2
AFP	Yes	Yes
Estriol	Yes	No
Inhibin	No	Yes

Obstetrical Complications

Table 5 summarizes the complications associated with isolated unexplained elevation of hCG in the second trimester. There appears to be a certain degree of correlation between higher levels of hCG and higher frequency of perinatal complications such as IUGR,^{31–34} gestational hypertension with proteinuria with or without adverse features,^{33,35–37} preterm labour or delivery,^{26,32} and stillbirth.³⁷ The relationship also seems to apply to a population at high risk for gestational hypertension on the basis of previous history.³⁸

In the first trimester, an elevation of hCG has not been associated with a significant increase in pregnancy complications.^{39–42}

Low hCG

Isolated low hCG is usually defined as a value < 0.5 MoM in the context of normal values of the other serum analytes. It is considered “unexplained” when associated with normal fetal anatomy in a viable pregnancy with a normal fetal karyotype. Using these parameters, pregnancy outcomes appear to be similar to those of the general population in the second trimester.^{43–45}

In the first trimester, low levels of hCG (< 0.4 or 0.5 MoM) have been associated with a higher incidence of birthweight below the fifth or tenth centile (OR 1.6–1.7; 95% CI 1.1–2.5)^{46,47} and miscarriage (OR 11.7; 95% CI 6.9–19.8).⁴⁸ Extremely low levels (< 1st centile or < 0.25 MoM) have been associated with an increased risk of spontaneous loss before 24 weeks (adjusted OR range: 3.6–62.0).^{47,48}

MATERNAL SERUM UNCONJUGATED ESTRIOL

Elevated uE₃

High uE₃ has not been associated with adverse perinatal outcomes.

Low uE₃

Low maternal serum uE₃ is defined by most authors as a level ≤ 0.5 MoM.^{49–51} Undetectably low uE₃ is based on the laboratory standard and is typically found in 0.14% of samples.⁵² It has been associated with fetal chromosomal anomalies, fetal structural anomalies (anencephaly), fetal death and a variety of fetal metabolic conditions (e.g., steroid sulfatase deficiency (X-linked ichthyosis), congenital adrenal hypoplasia/hypocortisolism, Smith-Lemli-Opitz syndrome, and placental aromatase deficiency).^{1,53–64} In the majority of these conditions, the level of estriol is reported as undetectable or < 0.2 MoM. Sulfatase deficiency has been associated with prolonged pregnancies and difficulties in inducing labour.⁵⁴ Table 6 provides a summary of complications described with unexplained low level of uE₃. The frequency of these adverse outcomes has an inverse relationship with the uE₃ level.⁶⁵ Two small case series looking at undetectable levels of uE₃ described outcomes similar to those with normal levels, excluding the prolonged pregnancies and increased rates of Caesarean section.^{52,54}

MATERNAL SERUM INHIBIN-A

Inhibin-A is a second trimester marker and is used in the quadruple (quad) screen. Its value is significantly decreased in the presence of primary antiphospholipid antibodies syndrome (median MoM: 0.60 MoM [95% CI 0.4–0.9]).⁶⁶ It has also been described as extremely elevated in pregnancies complicated by triploidy and HELLP syndrome and following the loss of one twin in the first trimester.⁶⁷

Elevated Inhibin-A

Higher levels of inhibin-A are seen in the second trimester in women who subsequently develop gestational hypertension with proteinuria.⁶⁸ No clear cut-off of clinical relevance has been described.⁶⁹ Table 7 summarizes the adverse outcomes associated with unexplained isolated elevated inhibin-A level (≥ 2.0 MoM).

Low Inhibin-A

No adverse obstetrical outcomes have been described with low inhibin-A levels in the second trimester.

MATERNAL SERUM PREGNANCY ASSOCIATED PLASMA PROTEIN-A

PAPP-A is one of the two maternal serum markers currently used for screening between 10 and 14 weeks. It is

Table 4. Complications associated with an unexplained isolated elevation of maternal serum AFP*

Complication	Frequency (range reported, %)	OR or RR (range reported)
Intrauterine growth restriction	10.9–27.1	1.6–4.0
Antepartum hemorrhage (all causes)	12.5	–
Abruption	4–5.6	4.8–11
Preterm delivery	5.8–21.4	1.8–4.8
Fetal death > 24 weeks	3.2–7.2	4.4–9.8
Spontaneous abortion	4.6	3.6–10.1
Gestational hypertension	5.1	1.6–1.9
Gestational hypertension with proteinuria	4.6–13.9	3.8
Infant death	–	1.9
Oligohydramnios	1.2–2.0	3.3
Perinatal morbidity (low Apgar, asphyxia and neonatal intensive care unit admissions)	13.5	–
Spontaneous preterm labour	5.6	3.6

*Only values demonstrating statistical significance were included in this table.

References: 9,11,20,22,23,25,51,73,80,81,116,145,146,149–152. Papers selected if population series > 5000 women or case-control series with > 100 cases.

produced by the placenta and decidua. It increases the bioavailability of insulin-like growth factor, which in turn mediates trophoblast invasion and modulates glucose and amino acids transport in the placenta.

Elevated PAPP-A

No adverse obstetrical outcomes have been described with elevated PAPP-A levels in the first trimester.^{46,47}

Low PAPP-A

Although earlier case-control studies did not report any significant differences in obstetrical outcomes associated with PAPP-A level,⁷⁰ more recent cohort studies describe increased adverse obstetrical outcomes associated with PAPP-A level below the fifth or tenth centile (< 0.4 MoM or 0.5 MoM). Table 8 summarizes the complications associated with unexplained low PAPP-A. The lower PAPP-A levels are associated with higher OR of an adverse outcome.⁴⁷

Summary Statement

1. An unexplained level of a maternal serum marker analyte is defined as an abnormal level after confirmation of gestational age by ultrasound and exclusion of maternal, fetal, or placental causes for the abnormal level. (III)

Recommendations

1. In the first trimester, an unexplained low PAPP-A (< 0.4 MoM) and/or a low hCG (< 0.5 MoM) are associated with an increased frequency of adverse obstetrical

outcomes, and, at present, no specific protocol for treatment is available. (II-2A) In the second trimester, an unexplained elevation of maternal serum AFP (> 2.5 MoM), hCG (> 3.0 MoM), and/or inhibin-A (≥ 2.0 MoM) or a decreased level of maternal serum AFP (< 0.25 MoM) and/or unconjugated estriol (< 0.5 MoM) are associated with an increased frequency of adverse obstetrical outcomes, and, at present, no specific protocol for treatment is available. (II-2A)

2. Pregnant woman with an unexplained elevated PAPP-A or hCG in the first trimester and an unexplained low hCG or inhibin-A and an unexplained elevated unconjugated estriol in the second trimester should receive normal antenatal care, as this pattern of analytes is not associated with adverse perinatal outcomes. (II-2A)
3. The combination of second or third trimester placenta previa and an unexplained elevated maternal serum AFP should increase the index of suspicion for placenta accreta, increta, or percreta. (II-2B) An assessment (ultrasound, MRI) of the placental–uterine interface should be performed. Abnormal invasion should be strongly suspected, and the planning of delivery location and technique should be done accordingly. (III-C)
4. A prenatal consultation with the medical genetics department is recommended for low unconjugated estriol levels (< 0.3 MoM), as this analyte pattern can be associated with genetic conditions. (II-2B)

Table 5. Complications associated with an unexplained isolated elevation of hCG in the second trimester*

Complication	Frequency (range reported, %)	OR or RR (range reported)
Intrauterine growth restriction (< 10th centile or < 3rd centile)	5–15.5	1.5–4.7
Preterm delivery	5.4–11	1.7–2.8
Preterm labour before or at 28 weeks	–	2.74 (1.9–4.8)
Preterm delivery before or at 32 weeks	1.5	–
Spontaneous abortion	1.1–2.5	2.2–4.8
Fetal death > 20 weeks	1.2–5.9	2.7–7.08
Gestational hypertension (overall)	4.1–28	1.4–4.1
Gestational hypertension with proteinuria	1.8–7.4	1.7–6.9
Velamentous cord insertion	3.8	2.6

*Only values demonstrating statistical significance were included in this table.

References: 20,26,31,33,51,80,81,145,152–156. Papers selected if population series > 5000 women or case-control series with > 100 cases.

Table 6. Complications associated with an unexplained isolated low uE₃*

Complication	Frequency (range reported, %)	OR or RR range (95% CI)
Intrauterine growth restriction (birthweight < 10th centile)	4.6–16	1.79–2.89
Fetal death > 24 weeks	2.1	3.3 (2.2–4.9)
Pregnancy loss	1.8–8.4	3.3–7.0
Oligohydramnios	14	3.85 (1.53–9.68)

*Only values demonstrating statistical significance were included in this table. References: 49,51,54

COMBINED ASSESSMENT OF MULTIPLE MARKERS

The combination of multiple abnormal markers and their association with adverse perinatal outcomes has been studied in a variety of ways since the introduction of multiple marker screening. While some authors have described the obstetrical data looking at the screen positive status for trisomy 21, trisomy 18, and ONTD, others have looked at combinations based on the cut-offs described above for isolated unexplained elevations or reductions of the different markers.

Maternal Screen Positive for Trisomy 21

The serum marker pattern leading to a positive screen result represents a variation of one or more of the markers. For example, in the second trimester, a positive screen for trisomy 21 may be associated with an abnormally low maternal serum AFP or uE₃ and/or an abnormally high maternal serum hCG. Overall, in case-control studies, a false positive screen for trisomy 21 has been associated with an increased risk of gestational hypertension with

proteinuria (3.6–6.9%, OR 1.3), small-for-gestational age (5.2%, OR 1.4), and spontaneous fetal loss (1.5–1.7%, OR 1.8).^{71–73} This relationship may not be true for some subpopulations, such as Hispanic women where a small case-control study did not demonstrate any significant differences in obstetrical outcomes.⁷⁴

Maternal Screen Positive for Trisomy 18

Two studies have been published regarding the obstetrical outcome of women who screened positive for trisomy 18 only. A non-statistically significant tendency toward a higher frequency of fetal growth restriction (4.9% vs. 3.3%) and gestational hypertension with proteinuria (2.9% vs. 1.2%) was identified.⁷⁵ In a review of over 250 000 triple markers screening tests performed in Ontario, a false positive risk of trisomy 18 of 1 in 1110 or higher was associated with an overall risk of fetal loss of 14% (2.0% if risk between 1 in 491 and 1 in 1110, 35.7% if risk between 1 in 46 and 1 in 70 and up to 70% if the risk was 1 in 8 or greater).⁷³

Table 7. Complications associated with an unexplained isolated elevation of inhibin A (≥ 2.0 MoM)*

Complication	Frequency (%)	OR or RR (95% CI)
Intrauterine growth restriction	16.9	1.53 (1.27–1.85)
Preterm delivery before or at 32 weeks	3.1	2.38 (1.44–3.95)
Fetal death > 24 weeks	0.9	2.41 (1.04–5.56)
Gestational hypertension with proteinuria	6.63	2.39 (1.75–3.26)

*Only values demonstrating statistical significance were included in this table.

Reference: 51

Table 8. Complications associated with an unexplained isolated low PAPP-A (below the 5th centile [0.4 MoM])*

Complication	Frequency (range reported, %)	OR or RR range (95% CI)
Intrauterine growth restriction (birthweight < 5th centile)	9.3–13.3	2.8 -3.1
Preterm delivery	6.9–10.5	2.2–2.4
Fetal death > 24 weeks	0.9–2	3.9 -9.2
Gestational hypertension (overall)	–	1.47 (1.20–1.82)
Gestational hypertension with proteinuria	6.7–14.1	2.1 (1.6–3.2)
Spontaneous abortion	11.26	2.5 -13.3

*Only values demonstrating statistical significance were included in this table

References: 40,46–48,128, 146,157

Dual Positivity (Maternal Screen Positive for Trisomy 21 and ONTD)

This situation is typically associated with an increase of maternal serum AFP and hCG in the second trimester, leading to the dual positivity. It is seen in about 1 in every 1000 second trimester screens performed.^{76,77} The largest case-control study revealed an association with an increased risk of at least one obstetrical complication (43%, OR 6.0; 95% CI 4.3–8.5). The risk of specific complications were as follows: IUFD: 4.8% (OR 11.8; 95% CI 3.2–52.7), preterm delivery: 23% (OR 5.9; 95% CI 3.8–9.3), gestational hypertension with proteinuria: 15.9% (OR 6.7; 95% CI 3.8–11.6), IUGR: 12.7% (OR 9.7; 95% CI 4.9–19.1) and preterm premature rupture of membranes: 10% (OR 1.8; 95% CI 1.01–3.2).⁷⁶ An association with confined placental mosaicism for trisomy 16 has also been described in up to 30% of these cases.⁷⁸

Dual Positivity (Maternal Screen Positive for Trisomy 21 and Trisomy 18)

In a population of 32 women with a positive triple marker screen for trisomy 21 and trisomy 18, 14 had a fetus with a chromosomal or structural anomaly, eight were lost spontaneously and five had pregnancy complications such as preterm labour and abruption.⁷⁹

Assessment Based on Individual Values of the Markers

Table 9 summarizes the adverse obstetrical outcomes described in association with multiple abnormal maternal serum markers. In general, the tendency for their frequency or odds ratio is increased when more than one abnormal marker is identified.^{20,51,80,81}

Recommendation

5. The clinical management protocol for identification of potential adverse obstetrical outcomes should be guided by one or more abnormal maternal serum marker analyte value rather than the false positive screening results for the trisomy 21 and/or the trisomy 18 screen. (II-2B)

MULTIPLE PREGNANCIES

Although screening for trisomy 21 based on maternal serum is not recommended in multiple pregnancies,⁸² for a variety of reasons (unknown multiple pregnancy at the time of screening, screening for ONTD, maternal choice), many women with multiple pregnancies will have results of maternal serum screening. In twin pregnancies, the value of each analyte appears to be about 1.7–2.1 times the value in a singleton pregnancy.^{83–85} Consequently, abnormal elevation of maternal serum AFP and hCG has been studied at cut-offs of ≥ 3.5 MoM for AFP⁸⁶ and ≥ 5.0 MoM for hCG³³

Table 9. Multiple serum screening anomalies and associated adverse pregnancy outcomes (list of stronger associations than for each marker alone)

↑MSAFP, ↑MShCG	Gestational hypertension, Intrauterine growth restriction (< 10th centile and < 3rd centile), fetal death, medically indicated and spontaneous preterm deliveries, abdominal pregnancy, non-immune hydrops at the time of screening, spontaneous abortion, abruption, absent or reversed end diastolic flow (AREDF) on UA Doppler ^{20,51,80,81, 91,152,158,159}
↑MSAFP, ↑MShCG, ↓MSuE ₃	Low birth weight, ¹⁶⁰ small for gestational age, ¹⁵² spontaneous abortion, ¹⁵² fetal demise after 24 weeks ¹⁵²
↑MSAFP, ↑Inhibin A	Preterm birth ≤ 32 weeks, Intrauterine growth restriction, gestational hypertension with proteinuria, spontaneous abortion, fetal demise ⁵¹
↑MShCG, ↑Inhibin A	Preterm birth ≤ 32 weeks, Intrauterine growth restriction, gestational hypertension with proteinuria, fetal demise ⁵¹
↑MSAFP, ↑MShCG, ↑Inhibin A	Preterm birth ≤ 32 weeks, Intrauterine growth restriction, gestational hypertension with proteinuria, spontaneous abortion, fetal demise ⁵¹
↑MSAFP, ↓MSuE ₃	Small for gestational age, ^{152,161} spontaneous abortion, ¹⁵² fetal demise after 24 weeks ¹⁵²
↑MSAFP, ↓PAPP-A	Small for gestational age, preterm labour and delivery, fetal demise after 24 weeks ¹⁴⁶
↑MShCG, ↓MSuE ₃	Small for gestational age, spontaneous abortion, fetal demise after 24 weeks ¹⁵²

in the second trimester. Unexplained elevation of AFP has been associated with increased preterm deliveries (38% vs. 17%, OR 3.0; 95% CI 1.5–6.2) and increased spontaneous preterm deliveries (31% vs. 16%, OR 2.4; 95% CI 1.1–5.0).⁸⁶ Isolated elevation of hCG in the second trimester has been associated with an increased frequency of preterm deliveries (55% vs. 43%, OR 1.6; 95% CI 1.1–2.6), miscarriages (3% vs. 0.5%, OR 7.1; 95% CI 1.2–31.6), and overall adverse perinatal outcomes (71% vs. 55%, OR 2.0; 95% CI 1.2–3.3).³³

Multifetal Reduction

Transabdominal multifetal reduction performed between 10 and 13 weeks has been associated with significant increases in maternal serum AFP, with a mean AFP varying between 4.6 MoM and 9.3 MoM,^{87–89} although other authors failed to demonstrate such increase.⁹⁰ A spontaneous reduction (“vanishing twin”) within four weeks of testing has been associated with higher levels of PAPP-A and hCG in the first trimester.⁹¹

Summary Statement

2. Abnormally elevated levels of serum markers are associated with adverse pregnancy outcomes in twin pregnancies, after correction for the number of fetuses. Spontaneous or planned multifetal reductions may result in abnormal elevations of serum markers. (II-2)

FACTORS AFFECTING THE LEVELS OF VARIOUS MATERNAL SERUM MARKERS

Maternal factors such as ethnicity, smoking, consanguinity, geographical altitude, and hemoglobin level, as well as fetal gender, have been described as influencing the level of the maternal serum markers. Women who are undergoing renal

dialysis or who have had a renal transplant have significantly higher levels of maternal serum hCG than control groups.^{92,93} Assisted reproductive technologies have been associated with a slight increase in hCG levels.^{94–97} In women infected with HIV, variations in hCG and AFP have been demonstrated and have depended on the viral load, CD4 count, and protease inhibitors intake.^{98,99} Although these differences were statistically significant, they cannot be considered clinically significant in the context of assessment for obstetrical complications of pregnancy.^{100–107} Ethnicity and pre-existing diabetes influence these values^{108–114} and are already taken into account in the standard risk calculations. Diet may be influential, since women on a vegetarian diet who have low levels of vitamin B₁₂ demonstrate higher hCG levels.¹¹⁵ However, at this point, levels of hCG should not be corrected for a vegetarian diet.

Recommendation

6. Pregnant woman who are undergoing renal dialysis or who have had a renal transplant should be offered maternal serum screening, but interpretation of the result is difficult as the level of serum hCG is not reliable. (II-2A)

EVALUATION AND MANAGEMENT OF WOMEN WITH ONE OR MORE ABNORMAL SERUM MARKERS

Evaluation and subsequent patient management need to be based on the potential complications associated with the serum marker pattern and the overall risk associated with this pattern. The more the pattern of abnormalities implies a specific adverse outcome, the more targeted the management will need to be.

Second Trimester Evaluation

The first step in the evaluation process is to confirm that the findings are truly unexplained via history, physical examination, and prenatal ultrasound, as well as invasive prenatal testing when indicated, in order to rule out a cause for the abnormal serum findings. An ultrasound should be performed to confirm the gestational age and exclude common causes of abnormal levels of analytes, such as wrong dating, fetal anomalies, fetal demise, and multiple pregnancy. If the abnormal level remains unexplained, a referral for a detailed anatomical ultrasound should be considered.

1. Detailed ultrasound

In a detailed ultrasound, particular attention should be paid to the placental appearance and location. Three authors have associated the presence of placental pathology on ultrasound (periplacental hemorrhage, intraplacental maternal venous lakes ≥ 1 cm in diameter, thickened placenta, and echogenic cystic lesions) with worse prognosis for these pregnancies.^{116–118} In contrast, Kuo et al. suggested a lower pregnancy complications rate for pregnancies with increased maternal serum AFP when sonolucencies (anechoic focus measuring at least 3 mm without blood flow detected) were noted on ultrasound (44.3% vs. 22.2%, $P = 0.06$).¹¹⁹

2. Doppler assessment

Uterine artery Doppler at 22–24 weeks has been particularly studied in women with abnormal maternal serum marker levels. In the presence of an unexplained elevation of maternal serum AFP, uterine artery notching and/or high resistance index (> 95 th centile or 0.6) were associated with higher rates of gestational hypertension with proteinuria (56–58%) and preterm births (44–45%)^{120,121} or overall adverse outcomes (composite score including gestational hypertension with proteinuria, IUGR, IUFD, and preterm delivery).^{122,123}

In women with elevated hCG (≥ 2.0 – 4.0 MoM) in the second trimester, the finding of unilateral or bilateral uterine artery notching is associated with a 25% to 67% risk of IUGR and/or gestational hypertension with proteinuria.^{124–126}

Alkazaleh et al. described improved positive predictive value when uterine artery Doppler with or without placental ultrasound was added to a combination of elevated serum AFP (> 2.0 MoM) and elevated serum hCG (> 2.5 MoM) in the second trimester.¹¹⁸

When inhibin-A is elevated (> 2.8 MoM) and uterine artery notching is noted in at least one artery, Emine et al. described a 100% positive predictive value and overall

71% sensitivity for preeclampsia in an unselected population of 178 women.¹²⁷

Spencer et al. and Pilalis et al. have published extensively on PAPP-A and uterine artery Doppler as a screening test for adverse pregnancy outcomes, mainly preeclampsia and IUGR. In their populations, the combination of low PAPP-A (< 5 th centile) and abnormal uterine artery Doppler (either increased PI or notching) provided a higher sensitivity (up to 62%) than PAPP-A alone (14–23%) or uterine artery Doppler alone (23–55%).^{40,128,129} In this population of women with abnormal levels of maternal serum marker analytes, umbilical artery Doppler at 18–20 weeks (usual initial assessment) does not help in identifying a higher risk group.¹²²

3. Presence of placenta previa

When placenta previa is present in the context of increased maternal serum AFP (see above), a thorough assessment of the placental bed by ultrasound and/or MRI should be done to identify areas suspicious for abnormal invasion.¹³⁰ This situation should lead to planning for the likelihood of significant blood loss at the time of delivery and discussion about hysterectomy. In addition, planned conservative methods to address this potential obstetrical complication should also be discussed.

Recommendations

3. The combination of second or third trimester placenta previa and an unexplained elevated maternal serum AFP should increase the index of suspicion for placenta accreta, increta, or percreta. (II-2B) An assessment (ultrasound, MRI) of the placental–uterine interface should be performed. Abnormal invasion should be strongly suspected, and the planning of delivery location and technique should be done accordingly. (III-C)
7. Abnormal maternal uterine artery Doppler in association with elevated maternal serum AFP, hCG, or inhibin-A or decreased PAPP-A identifies a group of women at greater risk of IUGR and gestational hypertension with proteinuria. Uterine artery Doppler measurements may be used in the evaluation of an unexplained abnormal level of either of these markers. (II-2B)

Maternal and Fetal Surveillance

There is a paucity of studies assessing the effect of altered pregnancy management on adverse outcomes associated with unexplained abnormal maternal serum markers. In a retrospective study, Huerta-Enochian et al. did not find any benefits in terms of earlier or improved detection of perinatal complications from a heightened surveillance program of 25 women (including twice weekly non-stress test and amniotic fluid index determination) compared with

routine care provided to 88 women (without non-stress test and amniotic fluid index determination unless otherwise indicated).¹³¹ A group at higher risk was identified by repetitively abnormal serial umbilical artery Doppler.^{132–134}

Recommendations

8. Further research is recommended to identify the best protocol for pregnancy management and surveillance in women identified at increased risk of adverse pregnancy outcomes based on an abnormality of a maternal serum screening analyte. (III-A)
9. In the absence of evidence supporting any specific surveillance protocol, an obstetrician should be consulted in order to establish a fetal surveillance plan specific to the increased obstetrical risks (maternal and fetal) identified. This plan may include enhanced patient education on signs and symptoms of the most common complications, increased frequency of antenatal visits, increased ultrasound (fetal growth, amniotic fluid levels), and fetal surveillance (biophysical profile, arterial and venous Doppler), and cervical length assessment. (III-A)

Therapeutic Approaches and Interventions

Low-dose aspirin has been studied in a secondary analysis of a study looking at its influence on birth weight. Newborns of women with a maternal serum hCG ≥ 2.0 MoM in the second trimester who had taken aspirin 60 mg daily starting before 22 weeks' gestation had an average birthweight of $3275\text{g} \pm 412\text{g}$ compared with $2859\text{g} \pm 770\text{g}$ for those born of women with a hCG ≥ 2.0 MoM in the second trimester who had taken a placebo during the same period ($P = 0.02$).¹³⁵ This appeared to be related to higher gestational age at delivery but also to higher birthweight for a specific gestational age. There was no statistically significant difference in the incidence of gestational hypertension with proteinuria between these two groups (0/20 in the aspirin group vs. 3/28 in the placebo group, $P = 0.26$) but the study was not powered to test for this difference.

When uterine artery Doppler has been performed, an anomaly of the Doppler (defined either as an increased resistance index or unilateral or bilateral nearly diastolic notching, depending on the study) likely identifies a group of women who may benefit from low dose aspirin therapy (81 mg daily) if initiated between 17 and 24 weeks. In a meta-analysis including five studies, Coomarasamy et al. described a reduction in the rate of gestational hypertension with proteinuria with an OR of 0.55 (95% CI 0.32–0.95).¹³⁶ The number of women needing to be treated to prevent one case of gestational hypertension with proteinuria was calculated at 16 (95% CI 8–316). No other improvements in outcomes were noted as statistically significant. No maternal adverse outcomes were identified in these studies.

Low molecular weight heparin has been used in a small cohort of six women presenting with abnormal maternal serum hCG and/or AFP in the second trimester, abnormal uterine artery Doppler and placental echogenic cystic placental lesions on ultrasound at 18–26 weeks. All had liveborn infants at 33 to 37 weeks' gestation with birthweights varying from 1000 g to 3200 g. A cohort of 14 women with a similar condition seen at the same centre during the study period was used for comparison. Nine of the 14 women experienced a perinatal death.¹¹⁷

Recommendations

10. Limited information suggests that, in women with elevated hCG in the second trimester and/or abnormal uterine artery Doppler (at 22–24 weeks), low-dose aspirin (60–81 mg daily) is associated with higher birthweight and lower incidence of gestational hypertension with proteinuria. This therapy may be used in women who are at risk. (II-2B)
11. Further studies are recommended in order to assess the benefits of low-dose aspirin, low molecular weight heparin, or other therapeutic options in pregnancies determined to be at increased risk on the basis of an abnormal maternal serum screening analyte. (III-A)

MULTIPLE MARKERS SCREEN AS A SCREENING TEST FOR OBSTETRICAL COMPLICATIONS

Despite the clear associations (summarized above) between abnormal serum marker values and adverse pregnancy outcomes, no study has currently shown that such screening methods are sensitive and specific enough to be used in isolation as a screening test for hypertensive disorders of pregnancy, intrauterine growth restriction, preterm labour, perinatal mortality, and placenta accreta.^{14,22,25,39,40,47,51,125,126,128,129,137–146} Studies have described sensitivities varying from 5% to 43% with false positive rates from 3% to 30%, the highest sensitivities being associated with the highest false positive rates. These values were not significantly modified by the addition of uterine artery Doppler.¹²⁴ The opposite approach may not be true. The addition of inhibin-A to uterine artery Doppler screening for gestational hypertension with proteinuria improved the screening value in a small unselected cohort. Aquilina et al. described a sensitivity of 60% with a false positive rate of 3% and a positive likelihood ratio of 20.8 when uterine artery Doppler and inhibin-A were combined in a screening test for pre-term gestational hypertension with proteinuria.⁶⁹ Spencer reported similar improvements in sensitivity when adding PAPP-A to uterine artery Doppler in a screening program (sensitivity improved from 55% to 62%).⁴⁰ Wald et al. published the only two case-control studies describing a theoretical detection rate of

preeclampsia of 34% to 55% with a 5% screen positive rate.^{147,148}

Recommendation

12. Multiple maternal serum markers screening should not be used at present as a population-based screening method for adverse pregnancy outcomes (such as preeclampsia, placental abruption, and stillbirth) outside an established research protocol, as sensitivity is low, false positive rates are high, and no management protocol has been shown to clearly improve outcomes. (II-2D)

When maternal serum screening is performed for the usual clinical indication (fetal aneuploidy and/or neural tube defect), abnormal analyte results can be utilized for the identification of pregnancies at risk and to direct their clinical management. (II-2B) Further studies are recommended to determine the optimal screening method for poor maternal and/or perinatal outcomes. (III-A)

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