Maternal serum screening for fetal trisomy 18: Benefits of patient-specific risk protocol

W. Allen Hogge, MD,a,c Luanne Fraer, MS,a and Todd Melegari, BSa
Pittsburgh, Pa

OBJECTIVE: Our goal was to evaluate the effectiveness of two approaches to screen pregnancies for trisomy 18.

STUDY DESIGN: We analyzed the outcome of all pregnancies that were screen positive for trisomy 18 by multiple marker screening (α-fetoprotein, unconjugated estriol, and human chorionic gonadotropin) from May 1993 to June 1998. We compared the results of a fixed cutoff protocol to a protocol that incorporates maternal age to generate a patient-specific risk figure.

RESULTS: A total of 45,145 patients were screened. By using the fixed cutoff protocol, 113 patients (0.25%) were screen positive. The risk-based approach was associated with a 0.55% screen-positive rate (250 patients). Eight of 12 cases (67% detection rate) of trisomy 18 were identified by using the risk method, and only 5 cases (42% detection rate) were detected by using the fixed cutoff method. By using the risk-based protocol, 21 pregnancies with chromosomal abnormalities (8, trisomy 18; 7, triploidy; 5, trisomy 21; and 1, mosaic 45X/46XX) were detected. Subsequent fetal death occurred for 42 patients whose fetuses were chromosomally normal and without structural malformations.

CONCLUSION: The patient-specific risk protocol to screen for trisomy 18 is a beneficial adjunct to screening programs already in place for Down syndrome and neural tube defects. Patients found to be screen positive for trisomy 18 are at significant risk for adverse pregnancy outcome. (Am J Obstet Gynecol 2001;185:289-93.)

Key words: Trisomy 18, multiple marker screening, alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin

Trisomy 18 occurs in approximately 1 of 8000 live-born infants. It is associated with severe mental retardation and multiple physical malformations. From an obstetric point of view the most significant issue is that more than 50% of infants affected by trisomy 18 have some form of growth deficiency. Undiagnosed trisomy 18, therefore, is likely to present in the third trimester as unexplained intrauterine growth retardation. Intensive evaluation and monitoring of the pregnancy will be undertaken, and in many cases operative delivery will be performed because of fetal distress, only to result in an infant with a lethal condition.

As is true for Down syndrome, trisomy 18 has been associated with a decrease in second trimester maternal serum α-fetoprotein (AFP)1-4 and unconjugated estriol (uE3).5 Unlike trisomy 21, however, decreased levels of human chorionic gonadotropin have been found in pregnancies with a trisomy 18 fetus.6-8 Based on this information Canick et al suggested a screening protocol using absolute cutoffs at the following levels: serum AFP at or below 0.75 multiples of the median (MoM); serum uE3 at or below 0.60 MoM; and human chorionic gonadotropin (hCG) at or below 0.55 MoM.9

Another approach is to use a risk-based approach to screening for trisomy 18 similar to that in use for trisomy 21.10 Barkai et al retrospectively estimated the detection rate using 15 pregnancies known to have trisomy 18 and 5472 controls.11 By using a risk cutoff of 1:100, 80% of affected pregnancies would have been detected with a false-positive rate of 0.6%.

In order to compare the screening efficiency of the fixed MoM cutoff protocol to the patient-specific risk protocol, herein we report our 5-year experience of serum screening for trisomy 18.

Material and methods

We analyzed the outcome of all pregnancies that were screen positive for trisomy 18 by use of multiple marker screening (AFP, uE3, and hCG) from May 1993 to June 1998 in the pregnancy screening laboratory of Magee-Womens Hospital. Maternal serum AFP and β-hCG were measured by using Abbot (Abbott Park, Ill) or Tosoh (Fos-
ter City, Calif) kits. Unconjugated estriol was measured by using kits from Diagnostic System Laboratories (Webster, Tex). All analyses were recorded as multiples of the unaffected population median on the basis of gestational weeks. Alpha-fetoprotein levels were corrected for race, maternal weight, and diabetes status. Corrections for maternal weight were performed for both uE3 and hCG.

From May 1993 to June 1996, a fixed cutoff protocol according to the approach of Canick et al.9 was used. All patients with results at or below these fixed cutoff levels (AFP, ≤0.75 MoM; uE3, ≤0.60 MoM; and hCG, ≤0.55 MoM) were classified as “increased risk for trisomy 18” and offered genetic counseling and amniocentesis. From July 1996 to June 1998 the risk-based protocol as described by Palomaki et al.12 was used, and all patients having a risk of 1:100 or greater were classified as “increased risk for trisomy 18” and were offered genetic counseling and amniocentesis. Repeat samples were not requested. Follow-up was obtained by review of delivery records at Magee-Womens Hospital or by follow-up letters to referring physicians. Cytogenetic records of all trisomy 18 diagnoses, either from prenatal diagnosis or newborn samples were cross-referenced with the pregnancy screening laboratory database.

For the purposes of this study, the entire population was analyzed by using both the fixed cutoff and the risk-based protocols in order to assess the screening efficiency of both protocols on the same population.

To compare detection rates, statistical analysis was performed by using 95% CIs, with application of the formula for proportion (detection rate – 1.96 x standard error to detection rate + 1.96 x standard error). Significance was determined by assessing overlap of the 95% CIs.

Results

A total of 45,145 patients were screened, 90% of whom were less than 35 years old. According to the fixed cutoff protocol, 113 patients (0.25%) were screen positive. The risk-based approach had an initial 0.55% screen-positive rate (250 patients). There were 12 known cases of trisomy 18, which is the expected number of cases in a population of this size. Eight of the 12 cases (67% detection rate) of trisomy 18 were identified by using the risk method, and only 5 cases (42% detection rate) were detected by using the fixed cutoff method (Table I). Complete follow-up was obtained for 93.2% of the patients who were screen positive according to the risk-based method. (These outcomes are depicted in Table II.) There were 21 pregnancies associated with chromosomal abnormalities (8, trisomy 18; 7, triploidy; 5, trisomy 21; and 1, mosaic 45X/46XX). Of the five patients with trisomy 21, four had extremely low risk for Down syndrome on the basis of their serum results (1:49,000 to 1:99,999). All four pregnancies ended in fetal demise. One patient, age 44, was at increased risk for both Down syndrome (1:10) and trisomy 18 (1:100) and had a liveborn child with trisomy 21.

An additional 12 patients had other nonchromosomal fetal malformations. The abnormalities included anencephaly, renal malformations, and several unidentified multiple malformation syndromes. For 42 patients, subsequent fetal death occurred in fetuses that were chromosomally normal and without structural malformations. Twenty patients developed a pregnancy complication (preeclampsia, preterm delivery, or intrauterine growth retardation). Therefore, the true false-positive rate according to the risk-based method was only 0.29%. Finally, 32 of the 235 patients who were screen positive for trisomy 18 also had an AFP of ≥2.0 MoM. None of the pregnancies had normal outcomes.

Follow-up was available on 90.3% of the patients who were screen-positive according to the fixed cutoff methods (Table I). Only six chromosome abnormalities were detected (5 trisomy 18 and 1 triploidy). Subsequent fetal death occurred for 10 patients, and 7 patients had pregnancy complications.

Comment

An effective screening method for trisomy 18 has significant benefits for the prenatal detection and the obstetric management of trisomy 18 fetuses. Our study confirms the exceptionally low false-positive rate (0.25%), as predicted by Canick et al.9 However, with use of these fixed cutoff values the detection rate in our population (42%) was quite low. These results, however, are consistent with those reported by Benn et al, who found a 23% detection rate at a 0.19% false-positive rate.13 Likewise Yankowitz et al were able to detect only 12.5% of their cases when using the fixed cutoff approach.14 Although Palomaki et al reported an estimated 85% detection rate in a prospective study of 19,491 women, the 95% CI was 40% to 95% because of the small number of cases.15 Our study confirms that the fixed cutoff approach has a very low detection rate.

By using the patient-specific risk protocol as described by Palomaki et al,12 we detected 8 of 12 trisomy 18 cases for a detection rate of 67%. There were 250 patients who were screen positive (an initial 0.55% false-positive rate). Although the confidence interval is wide (40%-94%) and overlaps the fixed cutoff rate, these results are almost identical to those of Benn et al,13 who screened 41,565 pregnancies and detected 69% of the trisomy 18 fetuses with an initial false-positive rate of 0.47%. The results of both studies compare favorably with those predicted by Palomaki et al (60% detection with a 0.2% false-positive rate), and the detection rate (65%) these investigators found in a retrospective evaluation of 89 trisomy 18 pregnancies.12

In addition to the cases of trisomy 18 detected by screening, pregnancies complicated by triploidy and
Down syndrome, as well as numerous nonchromosomal malformations were identified by this protocol. This pattern of low levels of all markers also appears to reflect pregnancies with significant fetal or placental compromise, with a fetal loss rate of 19.3% in apparently normal pregnancies. Overall, of patients who have an initial positive screen for trisomy 18, less than 60% will have a normal pregnancy outcome.

Our results support previous studies that conclude that the use of a patient-specific risk protocol for trisomy 18 is a beneficial adjunct to screening programs already in place for Down syndrome and neural tube defects. Unlike the fixed cutoff protocols, this protocol has a high detection rate (67%) and a very low false-positive rate (0.3%). Although three separate studies have shown remarkably similar detection rates (65%-69%), a large multicenter study is needed to attain the statistical significance necessary to confirm the benefits of risk-based screening. However, there are benefits in addition to the detection and management of trisomy 18. The patient-specific risk protocol improves detection of Down syndrome and provides a method for detecting triploidy, another lethal chromosome abnormality. Early detection of both trisomy 18 and triploidy has significant benefits for both the obstetrician-gynecologist and the patient. Both disorders are associated with significant intrauterine growth retardation that would lead to intensive fetal monitoring and a high likelihood of cesarean delivery. Knowledge of the fetal chromosomal abnormality in the second trimester provides the family with information to consider options: either pregnancy termination or a noninterventional approach to the remainder of the pregnancy and delivery, should they choose not to terminate. Because of the extremely low false-positive rate and the significant benefits of early detection of trisomy 18, we believe all serum screening programs should incorporate a patient-specific risk protocol for trisomy 18 with their present Down syndrome and neural tube defect screening protocols.

REFERENCES


Table I. Comparison of screening protocols for trisomy 18

<table>
<thead>
<tr>
<th>Detection (CIs)</th>
<th>False positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 (14-70)</td>
<td>0.25</td>
</tr>
<tr>
<td>67 (40.3-93.7)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Data are percentages unless indicated otherwise.

Table II. Outcomes of screen-positive patients according to protocol

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Fixed cutoff</th>
<th>Risk-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome abnormality</td>
<td>6 (5.3)</td>
<td>21 (8.4)</td>
</tr>
<tr>
<td>Fetal death</td>
<td>10 (8.8)</td>
<td>42 (16.8)</td>
</tr>
<tr>
<td>Other fetal abnormality</td>
<td>0</td>
<td>12 (4.8)</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>7 (6.2)</td>
<td>20 (8.0)</td>
</tr>
<tr>
<td>Normal</td>
<td>79 (69.9)</td>
<td>138 (55.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>11 (9.7)</td>
<td>17 (6.8)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>113</td>
<td>250</td>
</tr>
</tbody>
</table>

Data are presented as n (%).


Discussion

Dr Steven R. Wells, Durham, North Carolina. I would like to commend Dr Hogge on a well thought out and well-written study of a comparison of fixed cutoffs and patient-specific protocol in serum screening for trisomy 18. Reports such as this add to the growing body of work in the literature that allows us to increase the detection of this abnormality and provide for better counseling and management options in pregnancy. Because many patients will make decisions about invasive testing on the basis of information provided by serum screening, it is
vital that we have accurate information concerning detection rates and false-positive rates.

Trisomy 18 is an essentially lethal karyotypic abnormality characterized by a host of possible fetal malformations including central nervous system, craniofacial, cardiac, diaphragmatic, gastrointestinal, renal, and skeletal anomalies. On occasion, fetuses with trisomy 18 will have no obvious anomalies or have simply growth restriction in the latter part of pregnancy. There is a very high attrition rate in the case of trisomy 18, with up to 70 percent of affected fetuses lost in the third trimester. Schneider et al reported a 50% cesarean section rate in pregnancies that involve fetuses lost in the third trimester. Schneider et al reported their experience in 1992. In this study, 85% of cases of trisomy 18 were detected by using fetuses with trisomy 18 not diagnosed before delivery. The indications for operative delivery relate to growth restriction, fetal distress, and other problems associated with this condition. For these reasons, it is easy to understand why screening for trisomy 18 would be appealing to many patients and their health care providers.

Serum screening for neural tube defects and aneuploidy is a fairly recent phenomenon; Brock et al introduced maternal serum AFP screening for spina bifida and anencephaly in 1972. This screening protocol was originally implemented in Great Britain because of the high incidence of open neural tube defects in that population. In fact, in the 1970s, because of the low incidence of the condition in the United States there was considerable controversy as to whether this screening should be offered. The first reports of abnormalities in serum screening being associated with aneuploidy began to appear in the 1980s. The seminal article on this subject was published by Merkatz et al in 1984. This was a classic example of serendipity, with a case report of a woman with an undetectable level of AFP on a serum screen. Since there were essentially no reports of fetal abnormalities being associated with this finding, the woman declined any invasive testing and subsequently delivered a baby with trisomy 18. This prompted a retrospective review that revealed markedly lower maternal serum AFP levels in pregnancies associated with autosomal trisomies, including trisomy 18. This article ushered in the era of serum screening for aneuploidy. From this grew the expansive literature showing the association of abnormal serum markers with chromosomal abnormalities. The addition of hCG, uE3, and in some cases a fourth marker has increased the sensitivity for detecting aneuploidy.

Most of the early screening protocols for trisomy 18 were based on fixed cutoffs for the serum markers routinely used (maternal serum AFP, hCG, and uE3). Palomaki et al reported their experience in 1992. In this study, 85% of cases of trisomy 18 were detected by using the fixed cutoff method, although the number affected was very small. In a second prospective study, Palomaki et al compared a patient-specific risk protocol with the fixed cutoff method. This study revealed that in terms of the efficiency of ascertaining cases of trisomy 18, the patient-specific method was superior to the fixed cutoff method.

Dr Hogge’s study agrees with the findings reported by Palomaki et al. Dr Hogge reports a 67% detection rate when using the patient-specific risk method and a 42% rate using the fixed cutoff protocol. Admittedly the numbers of affected fetuses in all of these studies are small. Trisomy 18 is a much less common chromosomal abnormality than trisomy 21, with a second trimester incidence of 1 in 2400 and a live-born rate of 1 in 8000. Of over 45,000 patients screened in Dr Hogge’s study, there were only 12 cases of trisomy 18. This is in keeping with the known prevalence rates. Thus with the low numbers it is impossible to show statistical significance when comparing methods, but every study published so far has revealed a higher detection rate by using a patient-specific protocol when compared to a fixed cutoff method. Therefore, I would agree with Dr Hogge’s conclusion that all serum screening programs should incorporate this type of protocol in screening for trisomy 18, just as it is done for trisomy 21.

I would like to ask Dr Hogge two questions. Because all the studies report low numbers of affected fetuses as a result of the low prevalence of trisomy 18, is there any move afoot to perform larger multicenter prospective studies to test the accuracy of the reported detection rates? (Perhaps a meta-analysis of existing studies may also prove to be beneficial.) Secondly, it is well known that ultrasound is a valuable tool in detecting anomalies associated with trisomy 18, with 60% to 80% of fetuses exhibiting some type of abnormality. How would you suggest combining serum screening and ultrasound to help patients make decisions about invasive testing? Obviously, this is important because amniocentesis is associated with some risk and the majority of patients who are screen positive for trisomy 18 do not have an affected fetus.

REFERENCES

DR ED HORGER, Columbia, South Carolina. Dr Hogge, I certainly enjoyed that. I think we should all pay attention to the risk-based screening, which we currently are not doing in South Carolina right now. However, I want to ask why you chose the 1:100 or greater risk. Is the fixed
screening protocol that you compared, the one that picked up 42%, is that also based on 1:100? Because, frankly, maybe that is why I have increased my amniocentesis numbers, we use a higher number. Obviously, we are going to come up with more false positives. You have done a wonderful job with picking up 80% with all of your data, but I can’t help but wonder if you would pick up 99% if you would move that risk up to 1:150.

**Dr Wade Neiman**, Lynchburg, Virginia. It was alluded to, but could you comment on the utility of a fourth marker and could you comment on whether there is any utility in doing first trimester markers combined with things like nuchal translucency?

**Dr Hogge** (Closing). Dr Wells, thank you for a wonderful summary. I would comment that what we should all remember about Dr Merkatz’s patient: she is the individual who got us going because she came back and said “Is there any relationship between my trisomy 18 and my very low AFP?” Most of us would have simply said in 1983, when Dr Merkatz saw her, “No there isn’t m’am. You’re just unlucky.” He took the risk of looking at all of his patients and found this information. Dr Wells, to this point, no one has tried to put all the data together and I think it is a very important thing. The only thing we can say right now is that everything is consistent with the models. That is not good science, and I think at some point we need to combine studies of 45,000 and 41,000 and a few others to get some absolute numbers that can be useful from that perspective. Having said that, as I get to the end of the comments, it may be something that is not going to be useful, on the basis of what lies in the future for us. Yes, ultrasound can detect between 60% and 80% in good centers, there is no question about that, and one of the benefits of this screening is that it allows the option to look at the fetuses, to look for anomalies. I would remind you that in that 60% to 80% is growth retardation, and one of the big problems we get into is deciding if babies are undergrown because they have trisomy 18 or because they are misdiagnosed, it often becomes difficult to make that judgement in the circumstance. But having good detailed ultrasound following the screening results certainly can lower the false-positive rate.

Dr Hogge, the answer to the 1:100 is that it has to do with trying to keep the false positive at the same level that we did with the fixed cutoff protocols. The false-positive rate is quite low for trisomy 18 simply because the condition is quite uncommon. If we raise the risk level, even though we pick up substantially more of the trisomy 18s, we bring more patients in for evaluation with the risk that goes with amniocentesis in that particular group and the anxiety produced. The fixed cutoff risk, in general, is quoted at 1:15 to 1:16 depending on what you read so that for those individuals who are in that category have a very high risk of trisomy 18. Taking it to the 1:100, we get the risk value or the false positive going up to about .5% or .6%. So, overall, we think it detects about 80%, which is probably the best we can do and keep the false-positive at a realistic rate.

Dr Neiman, the comments you made are well taken. We would love to have the fourth marker be helpful. We are doing inhibin-A screening as part of our screening at Magee-Womens Hospital, and what we have found as well as what has been reported by Lambert-Messerlian and colleagues is that there is no association between trisomy 18 and inhibin-A. We looked at all these patients and hoped we could use the screening to lower false positives. It does not do anything for us, so the fourth marker is not helpful in that respect.

First trimester screening is clearly the wave of the future. At Magee-Womens Hospital we have just started offering it as a clinical procedure, no longer a research procedure, because it is likely to have a 90% detection rate for trisomy 18 as opposed to the 67% to 80% we have talked about today. The difficulty with first trimester screening is that it requires that all of the technicians be certified by the Fetal Medicine Program in London and, again, without that certification, the detection rates are markedly lower. I think in the future we will be looking at first trimester screening as our best method for trisomy 18. Again, thank you very much.

**REFERENCE**