Second Trimester Maternal Quadruple Screening for Fetal Defects
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Maternal serum screening is a valuable tool in prenatal management and is used to identify pregnancies at increased risk for certain birth defects and chromosomal abnormalities. Alpha-fetoprotein (AFP) was the earliest serum marker used to detect open neural tube defects and abdominal wall defects (ONTD and AWD, respectively) and with time it was extended to the screening for Down syndrome.\(^1\)

Continued advancements in scientific research resulted in the introduction of a multiple marker screening panel, or the "triple test", in 1991.\(^1\) In addition to AFP, the panel included unconjugated estriol (uE3) and total-human chorionic gonadotropin (t-hCG).\(^1\) The triple screen is now widely employed in obstetrical practice to detect neural tube defects, trisomy 18 and Down syndrome (trisomy 21). The quadruple test was introduced in 2000, when inhibin A was added to the triple test panel.\(^1,2\)

Inhibins are glycoproteins produced mainly by testicular and ovarian cells. They consist of disulfide linked subunits, either \(\alpha\) and \(\beta A\) (dimeric inhibin A or DIA) or \(\alpha\) and \(\beta B\) (dimeric inhibin B). Both forms suppress follicular stimulating hormone produced by the anterior pituitary gland. During pregnancy, the placenta secretes significant amounts of inhibin, particularly after the first trimester. Maternal serum inhibin is usually increased in pregnancies with Down syndrome.

The recommended screening interval for the triple or quadruple test is between 15 and 22 weeks of pregnancy. Timing of the prenatal assay and dating of pregnancy should be accurate to obtain optimum value of each analyte.\(^3\) Gestational dating may be obtained by the last menstrual period, although early ultrasound examination is more reliable in decreasing errors caused by estimation of gestational age by the former method.\(^2,4\)

Serum analytes vary widely with patients’ demographic status such as gestational age, number of fetuses, maternal weight, diabetic status, race and individual history of in vitro fertilization.\(^2,3,4\) Along with serum analyte values, these variables are incorporated into a mathematical model that calculates a risk value for an individual pregnancy. For Down syndrome and trisomy 18, maternal
and gestational ages, race, weight and diabetic status are taken into consideration. Gestational age, race, weight, diabetic status and family history are important variables for neural tube defects. The calculated risk value depends on the accuracy of these demographic factors.\textsuperscript{2,3,4}

Levels of the analytes change with gestational age; the serum value of each analyte is therefore expressed as MoM (multiples of the median), obtained by dividing the serum analyte concentration at a particular gestational age by the population median concentration at the same gestational age.\textsuperscript{2,3,4} Therefore, MoM is a unitless function and serves as a convenient, common basis for transforming all values across different gestational ages, racial groups, maternal weights and various laboratories.\textsuperscript{3}

Laboratories must establish reference values that consist of a set of median values calculated for each week and each day of gestation for the relevant population. The values above the established cutoff are considered positive and indicate the need for additional evaluation.\textsuperscript{3,4} Confirmatory testing is recommended, since a positive screening result could indicate normal variation, erroneous gestational age, multiple fetuses, structural or placental defects or other fetal abnormalities.\textsuperscript{3} The serum variations of the quadruple screening panel with specific clinical entities are shown in the table below.

<p>| Patterns of second trimester marker concentrations that may indicate specific clinical entities |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Trisomy 21</th>
<th>Trisomy 18</th>
<th>Neural Tube Defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Estriol</td>
<td>Low</td>
<td>Very Low</td>
<td>-</td>
</tr>
<tr>
<td>hCG</td>
<td>High</td>
<td>Very Low</td>
<td>-</td>
</tr>
<tr>
<td>DIA</td>
<td>High</td>
<td>High</td>
<td>-</td>
</tr>
</tbody>
</table>

Maternal AFP continues to be a single marker in screening for increased risk of ONTD and AWD. It has a detection rate of 75-80\% for open spina bifida and 95\% for anencephaly, with a false-positive rate of less than 3\%.\textsuperscript{2} Using the triple screening panel, the detection rate for Down syndrome varies from 30\% to approximately 69\%, with a false-positive rate of 5\%.\textsuperscript{1,5} The introduction of the quadruple test has significantly increased the detection in cases of Down syndrome to 76\%; the false-positive rate has remained at 5\%.\textsuperscript{5} In cases of trisomy 18 the detection rate is approximately 80\% with this panel.\textsuperscript{2}

Some experts believe that due to the increased sensitivity of the quadruple test, the triple test should no longer be used as a screening tool. However, a recent CAP survey (FP-A 2005: Participant Summary Report) demonstrates that 209 laboratories continue to perform the triple test, while only about 98 laboratories offer quadruple testing.

Newer integrated screening profiles include first trimester markers PAPP-A (pregnancy associated placental protein A) and nuchal translucency (NT).\textsuperscript{1,3,5} Optimally, this testing is recommended between 10 and 13 weeks of pregnancy. This serum integrated screen that includes PAPP-A requires two-stage blood
testing. If NT testing is available, it can be incorporated into the serum integrated screen. The first trimester results are then incorporated with the quadruple test (performed in the second trimester, 15-22 weeks), producing a single calculated risk value.\textsuperscript{1,3,5}

Studies have demonstrated that the newer integrated screen has a markedly increased sensitivity rate for Down syndrome and a significantly decreased false-positive rate, which would result in a decrease of invasive procedures such as amniocentesis.\textsuperscript{5} The serum integrated assay has a detection rate of 85\% with a false-positive rate of 3\%, while the full integrated assay that includes nuchal testing has a similar detection rate but a significantly lower false-positive rate of 1.7\%.\textsuperscript{1,5,6} Hence, the newer integrated tests that incorporate the quadruple test appear to be much more effective and safer than traditional triple testing.

With the use of NT, focus has been shifted toward first trimester screening, with combined and contingent screening, as an alternative to integrated screening.\textsuperscript{6} The combined test includes free or total-hCG, PAPP-A and NT at 10 weeks. However, for a detection rate of 85\%, the false-positive rate is 6.1\%. If the first trimester screen is positive, a second trimester test would also be performed; so called “contingent” screening. Contingent testing, used with the combined test, may eliminate the necessity for testing in the second trimester, depending on the results of the first trimester combined test, and provide earlier diagnosis and reassurance.\textsuperscript{6} However, large scale prospective studies are needed to evaluate the psychological impact, statistical validity and practicality of this screening method before it is employed widely in obstetric practice.

In summary, maternal serum screening has evolved from a single marker analysis to a multiple marker panel over the years. The quadruple test has increased in sensitivity, especially in cases of Down syndrome. Experts believe that quadruple screen should be used as a standard screening tool instead of the triple test. Furthermore, the newer integrated screening profiles, which incorporate both the first and second trimester markers have several advantages, to include higher detection rates with very low false positive results, as well as early and safer diagnoses.

References

1. Prenatal screening program, Physician information. \textit{MSU Human Genetics}.


