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TITLE: Maternal Serum Screening for Chromosomal Aneuploidies

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Slide 1: Title Slide

Slide 2: Introduction

Maternal Screening is a grouping of prenatal tests incorporating ultrasound measurements and laboratory data obtained from maternal serum. The goal of screening is to identify women at high risk for carrying a fetus affected by either an aneuploidy such as trisomy 21, commonly known as Down syndrome, trisomy 18, also known as Edwards syndrome, and trisomy 13, also known as Patau syndrome as well as neural tube defects such as spina bifida.

The purpose of screening for these conditions is to target invasive diagnostic testing only to those pregnancies that are calculated to be at high risk.

While maternal screening strategies such as the second semester Quad screen are also used for screening for open neural tube defects, the focus of this presentation will be screening for the chromosomal aneuploidies and to begin I will briefly discuss trisomies 21, 18 and 13.

Slide 3: Chromosome Aneuploidies

Individuals with Down syndrome carry an extra copy of chromosome 21 and often display characteristic physical traits such as upward slanting eyes, a broad flat nose and macroglossia, or a large tongue. Down syndrome individuals also display mild to moderate developmental delay, as well as an increased incidence of thyroid disease, cardiovascular disease, and megakaryoblastic leukemia and acute lymphoblastic leukemia.

It is commonly appreciated that there is a maternal age-related increase in the incidence of Down syndrome. The incidence of Down syndrome live births is approximately 1:2000 at a maternal age of 20; this risk increases to 1:350 live births at a maternal age of 35, and continues to increase beyond that age. The incidence of Down syndrome at mid-gestation is slightly higher at 1:270; however, some pregnancies will of course end prematurely, resulting in the slightly lower incidence at birth.

Some individuals are at greater risk of having an affected pregnancy, such as those with a family history of Down syndrome or if they have had a previously affected pregnancy. It is important to recall that all women are at risk of carrying a child with Down syndrome and the overall incidence is approximately 1:730 live births.

The second most common autosomal aneuploidy for which screening is also performed is trisomy 18, or Edwards syndrome. The effect of this trisomy is more severe for the fetus than that of trisomy 21. The affected fetus and neonate will have multiple defects including atrioventricular septal defects, horseshoe kidney, omphalocele, esophageal atresia, and severe developmental delay. Trisomy 18 is the second most common autosomal trisomy with an incidence of 1:5,500 live births. As with trisomy 21, the mid-gestation incidence is much higher but many affected fetuses die in utero. Similar to trisomy 21, there is also an age-related increase in the incidence of trisomy 18 with advancing maternal age.

Of those affected fetuses that do survive to delivery, approximately half will not survive the first week of life and 90% will die within their first year.

The last autosomal trisomy that we will discuss is trisomy 13, or Patau syndrome. As with trisomy 21 and trisomy 18, there is an age associated risk of having an affected pregnancy. The prevalence of trisomy 13 is less than both trisomy 21 and 18, with approximately 1 in 10,000 live births.

Trisomy 13 affected individuals display a number of defects including but not limited to holoprosencephaly, a sloping forehead, severe eye malformations, deafness, cleft lip and/or palate, polydactyly, and congenital heart defects. Approximately 80% of affected neonates do not survive past one month of age and only 5% survive to six months. For those that do survive beyond one year of age, they display severe intellectual disability, seizures, and failure to thrive.

Slide 4: Diagnostic Testing for Aneuploides

Prior to beginning our discussion of the different maternal screening options available we will first discuss how a definitive diagnosis is established. The only conclusive diagnosis of aneuploidies comes from obtaining an adequate amount of fetal material to perform Fluorescent In-Situ Hybridization (FISH) analysis and/or a karyotype spread to determine if an extra copy of one of the chromosomes is present.

While either a karyotype or FISH analysis will establish a definitive diagnosis, obtaining sufficient fetal material for analysis is not without risk. Chorionic villius sampling (CVS) and amniocentesis have a small but quantifiable risk of inducing pregnancy loss. For CVS, the risk is on the order of 1 loss per 100 procedures, while amniocentesis carries a lower risk of approximately 3 pregnancy losses per 1000 procedures.

As there is an unavoidable risk of pregnancy loss associated with the diagnostic procedures and to adequately target diagnostic testing to those pregnancies at high risk of carrying an aneuploid fetus, screening tests have been developed.

Currently patients are screened via a combination of 1st trimester ultrasound and maternal serum markers alone or in combination with 2nd trimester maternal serum markers to assess risk. The choice of which screening strategy to choose will depend on the time of patient presentation. For example, if the patient presents early in gestation, the first trimester screen will be carried out, but if the patient does not present until the second trimester, the screening test used would be the Quad screen.

Slide 5: Maternal Serum Screening Options

As you can see, there is more than one screening strategy, and the decision on which tests are available will depend on the time of maternal presentation for prenatal care. The first trimester screen is a combination of ultrasound measurement and maternal serum markers. The second trimester screen is solely an assessment of risk based on the concentration of four maternal serum markers. This screen is popularly called the Quad screen; however, it is also known by many other common names.

There are also a few options that combine the risk calculation of the 1st and 2nd trimester screens, including an integrated screen, serum integrated screen, and sequential screening.

Slide 6: Multiple of Median

The data obtained by any of these screening choices which we will be discussing shortly, are reported as a Multiple of Median (MoM). The MoM is used for these screening programs as the ultrasound and serum marker values vary significantly throughout gestation. By converting the marker concentrations into a MoM, the reporting of values is standardized for gestational age, race, and for differences in measurement platforms. Establishing a database for MoM requires a large number of well characterized singleton pregnancies that are known to be unaffected by chromosomal aneuploidy. The use of the MoM does not obviate the need for the establishment of a reference range by race and by platform for each week of gestation but it does simplify the reporting of values.

Converting a serum marker value into a MoM is simply done by dividing the obtained value by the gestational age-specific median value of the analyte to be reported. The calculated MoM value can then be used to determine the likelihood ratio for carrying an affected fetus. In this example, the hCG MoM was determined to be 2.0. This value is plotted on a distribution of hCG values in normal and Down syndrome-affected pregnancies. As you can see in this simplified example, an hCG value of 2.0 MoM is equal to a likelihood ratio of 2 of carrying a Down syndrome fetus vs. an unaffected fetus. This likelihood ratio is then multiplied by the patient's age-related risk to determine the risk of carrying an affected fetus. In this highly simplified example, we are only considering one marker; however, calculating the overall risk of carrying a Down syndrome or trisomy 18 fetus involves sophisticated formulas incorporating the nuchal translucency (if measured), the other biochemical markers as well as whether the patient has type I diabetes mellitus, their body mass, and smoking status to generate an overall relative risk.

Slide 7: 1st Trimester Screen

Now that we have discussed how the measurements are normalized to MoM, we can discuss the various screening options that are available. The 1st trimester screen is ideally performed between the 11th and 13th weeks of gestation, although it may be performed as early as the 10th week and as late as the 14th week. This screen is a combination of an ultrasound measurement of nuchal translucency (NT), which is a fluid filled space at the back of the fetus's neck (as can be observed in the photograph at right), pregnancy associated plasma protein A (PAPP-A), and the free beta chain of hCG.

Slide 8: 1st Trimester Screen Performance

In the 1st trimester screen, trisomy 21fetuses will typically display an elevation in both the NT measurement and the hCG beta chain concentration in respect to unaffected pregnancies, while the PAPP-A concentration will be decreased. Trisomy 18-affected pregnancies will also display an increase in NT and all serum marker concentrations are typically lower than in unaffected pregnancies with PAPP-A and hCG significantly lower than unaffected. Trisomy 13-affected pregnancies will also display an increased NT and moderately to severely decreased PAPP-A MoM and decreased hCG. A screen positive result for the 1st trimester screen is a calculated risk of 1:270 for Down syndrome or 1:100 for trisomy 18. At these cutoffs, the 1st trimester screen reportedly will detect between 79% and 92% of Down syndrome affected pregnancies at a false detection rate of 5% depending on the publication cited. At a false positive rate of 1%, the 1st trimester screen will detect 89% of trisomy 18-affected pregnancies. The 1st trimester screen will also detect 90% of trisomy 13-affected pregnancies at a false positive rate of 0.5%. While these values are from published peer-reviewed studies, individual laboratories may report varying detection and false positive rates.

The 1st trimester screen also offers the patient the advantage of increased privacy as the screening takes place early in pregnancy and it also offer a wider window in which diagnostic testing can be done to either rule in or rule out the diagnosis of a chromosomal aneuploidy. The 1st trimester screen does however have the disadvantage that it cannot screen for neural tube defects, and a return visit and blood draw would be required to screen for open neural tube defects.

Slide 9: 2nd Trimester Screen

Another option, the second trimester screen, commonly known as the Quad screen, is a combination of four maternal serum markers: Alpha-fetoprotein, total hCG, unesterified Estriol, and Inhibin A. This screen is performed between 15 and 22 weeks of gestation, and is designed to screen for both chromosomal aneuploidies and neural tube defects.

Slide 10: 2nd Trimester Screen Performance

As you can see in this table, the detection rate for Down syndrome in the 2nd trimester Quad screen is similar to the first trimester combined screen at the same false positive detection rate. As for detection of trisomy 18-affected pregnancies, the two cannot be directly compared as their false positive rates differ, and the Quad screen does not detect trisomy 13-affected pregnancies. Since this screen incorporates the measurement of alpha-fetoprotein, it may also be used to screen for open neural tube defects. A MoM greater than 2.0 or 2.5, depending on the laboratory and prevalence of disease in the population being tested, is a screen positive result. This screen will detect ~85% of open neural tube defects at a false positive rate between 2% and 5%, depending on the testing laboratory and literature source.

Slide 11: Sequential and Integrated Screens

In addition to the two separate 1st and 2nd trimester screening options, there is also the option of one of three cross-trimester screens to assess risk. The first we will discuss is the Integrated screen, which is a combined risk assessment of the 1st trimester NT measurement and PAPP-A concentration and the 2nd trimester quad screen components. In the Integrated screen, all of these values are used to calculate an

overall risk of carrying a trisomy 21 or trisomy 18 fetus. In the Integrated screen, there is no disclosure of the patient's risk after the first trimester screen.

Slide 12: Sequential and Integrated Screens

The serum integrated screen is identical to the full integrated screen with the exception of the NT measurement. The NT measurement must be performed by a certified ultrasonographer. In some communities, access to a certified ultrasonographer may not be possible, and at times the NT measurement may not be obtainable due to fetal position, maternal habitus and possible lack of insurance coverage. Despite the lack of the NT measurement, the 1st trimester PAPP-A concentration can still be incorporated into the calculation with the second trimester serum markers.

The third cross-trimester screening option is the Sequential screen. As with the Integrated screen, the Sequential screen incorporates both the NT measurement as well as serum markers from the first and second trimester to calculate risk. The Sequential screen does however differ from the Integrated screen by disclosing the calculated risk result to the patient if the calculated risk is greater than or equal to a predetermined cut-off in the range of 1:50 to 1:40. If the risk of carrying a Down syndrome fetus is less than the cut-off, the results typically are not revealed to the patient and the values are combined with the risk assessment from the 2nd trimester.

Slide 13: Sequential and Integrated Screen Performance

If you compare the detection rate of either the full Integrated and Sequential screens, you'll note that either of these two options will result in the highest detection rate and lowest false positive rate for Down syndrome of any of the screening protocols. In literature sources, the serum sequential screen, which does not include the NT measurement, does not perform to the same level as the full integrated screen. In regards to the detection of trisomy 18, the data for this condition is not as well characterized in literature but the detection rate in the serum integrated screen is 92%. As the NT measurement is also increased in trisomy 18 it is likely that the use of the NT measurement would increase the detection rate but those publications are lacking.

Slide 14: When to Proceed to Diagnostic Testing

Following a screen positive result from any of the screening programs, the first step should be to verify gestational age to avoid an inaccurate determination of relative risk. Should the gestational age be accurate, genetic counseling and diagnostic testing should be offered to the patient, and this follow-up testing may include the use of a high resolution ultrasound. For the 1st, 2nd, and integrated screens a screen positive result for trisomy 21 is a calculated risk of greater than or equal to 1:270, the same as the age-based risk of a 35-year-old woman. For trisomy 18, the threshold for offering diagnostic testing is a calculated risk of 1:100. As mentioned, the sequential screen is slightly different due to the 2 stage testing.

Slide 15: Summary

According to the American College of Obstetricians and Gynecologists, all women, regardless of age, should be educated on screening options and be offered both screening and diagnostic testing. Which testing is pursued will depend on the time of patient presentation for prenatal care.

It is important to recall that these screening programs are not diagnostic tests. As the overall prevalence of trisomy 21 and trisomy 18 is fairly low, these screening tests will falsely identify more unaffected pregnancies than affected ones. For instance, if 1000 individual women are screened at a false positive rate of 5%, 50 patients will receive a high risk result. That value of 50:1000 is the same as 1:20, and the overall Down syndrome prevalence, not taking into consideration maternal age, is 1:730. It is also important to recall that screening will not detect all cases of fetal chromosomal aneuploidy.

Regardless of the screening result the risk estimate should always be communicated to the patient as the patient's interpretation of the risk and the choice of whether to proceed to diagnostic testing is important.

Slide 16: References