



Maternal Serum Screening: What Do The Results Mean?

MSS can be a valuable screening test for fetal neural tube defects or chromosomal anomalies. As part of this, physicians should counsel their patients about the potential for false-positive or false-negative results.

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Although this article is being written in *The Canadian Journal of Diagnosis*, it must be emphasized that maternal serum screening (MSS) is not a diagnostic test. It is a screening test. The purpose of a screening test is not to determine if a person has a particular condition, but rather to identify individuals who are at high risk for developing that condition.

In the case of MSS, the test is designed to identify women at increased risk for carrying a fetus with a neural tube defect, including spina bifida and anencephaly, or a chromosomal anomaly (*i.e.*, Down syndrome or

Trisomy 18). Although other maternal or fetal conditions may cause abnormal maternal serum screen results, these former conditions are the primary focus of MSS.

Maternal serum alpha-fetoprotein (MSAFP) testing began in the 1970s as a means of screening for fetal neural tube defects. MSAFP is a protein of fetal origin that rises in the maternal serum with advancing gestation from 13 weeks to 32 weeks. MSAFP levels are first determined in biochemical units (*e.g.*, $\mu\text{g/L}$). These are converted to multiples of the median (MOMs) by dividing this value by the

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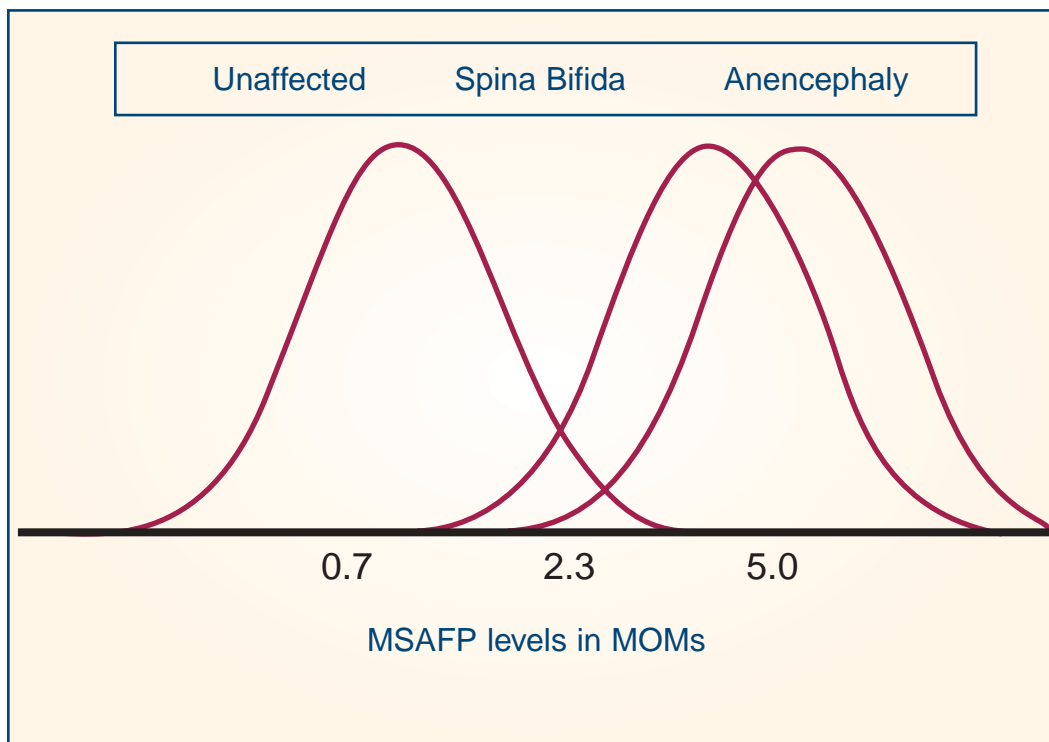


Figure 1. Distribution of MSAFP levels with fetal neural tube defects.

median for the gestational age at which the test was done.

A positive screen test for neural tube defects occurs when the MSAFP is greater than a certain cut-off value. Most programs use cut-offs between 2.0 and 2.5

MOMs. This cut-off is chosen as a compromise to get the best detection rate relative to the false-positive rate. As Figure 1 demonstrates, if the only concern was a high detection rate, then a cut-off value of 0.7 MOMs could be used. Although almost all cases of fetal spina bifida and anencephaly would be detected, over 50% of pregnant women would have a positive test. Conversely, if the major concern was the false-positive rate, a cut-off of 5.0 MOMs could be used. There would be very few false-positives, but the detection rate would be very poor. With a cut-off between 2.0 and 2.5 MOMs, a detection rate of 80% for spina bifida and 90% for anencephaly is expected, with a false-positive rate of 2.5% to 4%.¹



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In 1984, one study showed that MSAFP levels in mothers whose babies had Down syndrome were lower than the levels of those in control groups.² This led to the use of MSAFP (combined with maternal age) as a screening test for fetal Down syndrome. Since it was known that advanced maternal age was a major risk factor for having a child with Down syndrome, the original screening test for fetal Down syndrome was to simply ask a woman how old she would be on her due date. If the answer was 35 years or greater, she was considered screen-positive. Using this approach, about 5% of women would be screen-positive (*i.e.*, a 5% false-positive rate) and 20% of babies with Down syndrome would be born to women who were screen-positive (*i.e.*, a 20% detection rate).³ Combining a risk based on the MSAFP level with maternal age improved the screening performance to a 35% detection rate, with a similar false-positive rate.³

The most common form of MSS is triple testing, which is based on MSAFP, human chorionic gonadotropin (HCG) and estriol (E3). HCG is a placental hormone, the levels of which drop between 10 and 20 weeks gestation. E3 is made by the placenta from precursors from the fetal adrenal and liver. The levels of E3 continue to rise after nine weeks gestation.

Since MSAFP levels are lower in mothers carrying fetuses with Down syndrome, a low MSAFP level will statistically increase a woman's risk of having a baby with Down syndrome. The chance of this happening is calculated by comparing the heights of the Down syndrome curve to the unaffected

curve for a given MOM. A MSAFP level of about 0.5 MOMs will double the chance of fetal Down syndrome (likelihood ratio = 2.0) while a level of about 1.4 MOMs will decrease the risk by one-half (Figure 2).

Maternal HCG levels tend to be higher and maternal E3 levels tend to be lower in mothers of Down syndrome fetuses. Likelihood ratios, therefore, are calculated for HCG and E3 in a similar fashion to MSAFP. The risk for fetal Down syndrome then is calculated by multiplying the woman's risk, based on her age, by the three likelihood ratios. A computer program is required for proper interpretation.

With triple testing, the detection rate for fetal Down syndrome is about 60%, with a false-positive rate of 5%.⁴ Other first- and second-trimester markers, including biochemical and ultrasound parameters, have been added by various groups to standard triple testing. This has resulted in even better screening performance.⁵

Fetal trisomy 18 is associated with low levels of all three biochemical parameters, and a similar calculation is made to determine the fetal trisomy 18 risk.

The first step in MSS is to counsel the woman patient. This test should be considered strictly voluntary. She should be informed that the test is associated with significant false-positive and false-negative

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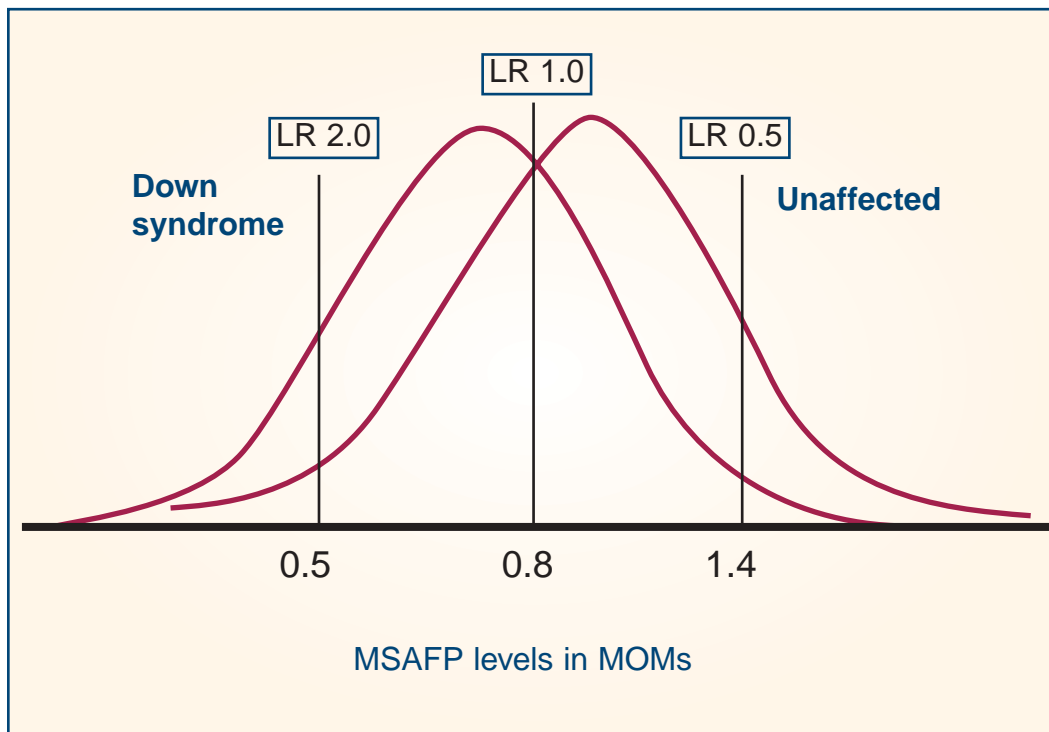


Figure 2. Distribution of MSAFP levels with fetal Down syndrome.

Small errors in assignment of gestational age can cause major errors in risk determination.

rates. She should be made aware that an abnormal test result does not mean that the fetus is affected, while a normal test result does not guarantee an unaffected fetus.

Currently, the policy in Canada is that all women who will be over 35 years of age on their due date should be offered an invasive test for fetal chromosomal abnormalities (*i.e.*, an amniocentesis or chorionic villus sampling). This policy may change in the future. If a woman chooses to have MSS, care must be taken to send the sample at the appropriate gesta-

tion point. Some programs will require ultrasound dating before a sample is tested. Most Canadian centers prefer ultrasound-derived dates, but will use gestation calculations based on the patient's last period, provided these dates are reliable. The ideal time to send the blood sample is between 15 and 18 weeks. The author would not recommend sending a sample at exactly 15 weeks, as the computer used by the MSS program may determine the gestation to be 14.9 weeks, and recommend repeat samples. Samples could be drawn later—at up to 22 weeks—but this can cause unnecessary delays.

It is important that requisitions for MSS be filled in as completely and accu-

rately as possible. Small errors in assignment of gestational age can cause major errors in risk determination. The interpretation also should take into consideration the maternal age, maternal weight, diabetic status and the patient's ethnic origin, all of which can influence the interpretation.

A series of six case presentations are provided here to illustrate the possible outcomes after MSS. The reader is invited to try and predict the outcome before reading the actual outcome. All risks quoted are term risks, *i.e.*, the chance of having a live child born with a specific condition.

Case 1

A 26-year-old gravida 2 (G2) para 1 (P1) woman has an MSS drawn at 16.2 weeks gestation. The MSAFP is 0.5 MOMs, the HCG is 2.0 MOMs and the E3 is 0.4 MOMs. The gestational age is based on her last menstrual period (LMP). The risk for fetal Down syndrome went from an age-related risk of 1/1,300 to 1/80.

Outcome

Further review determined that she has had only one period since her last pregnancy due to breast feeding. An ultrasound examination showed that she was only 12.5 weeks pregnant when the blood was drawn. The original blood test was, therefore, drawn too early. A repeat blood sample was drawn at 15.5 weeks by ultrasound dates, and was screen-negative. No amniocentesis was done and the mother gave birth to a healthy baby.

Comment

This case illustrates the importance of an accurate determination of gestation. Since AFP and E3 levels rise and HCG levels fall with advancing gestation, overestimation of the gestational age will show an MSS pattern identical to that expected with fetal Down syndrome. Conversely, the underestimation of dates will result in elevated MSAFP levels. Inaccurate estimation of the gestation is one of the most common explanations for false-positive test results.

Case 2

A 24-year-old woman has an MSS drawn at 15.5 weeks, according to her LMP. Her periods have been regular, occurring every 28 days, and the last period was normal. Her MSAFP level was 2.5 MOMs, the HCG was 1.2 MOMs and the E3 level was 0.9 MOMs. The Down syndrome risk was 1/14,000. The MSS was done as part of her routine pregnancy blood work without any explanation or consent. She is extremely anxious, as she believes her baby has spina bifida.

Outcome

The woman was told that, although an elevated MSAFP level is a risk factor for various problems, including fetal spina bifida, this MSAFP level is associated most

Elevated MSAFP levels can be a sign of placental compromise, and may indicate later problems, such as maternal hypertension.



all these conditions, most women with elevated MSAFP levels—especially those with minimal elevations—go on to have normal pregnancies and healthy babies.

Case 3

An 18-year-old woman from Northern Manitoba had an MSAFP drawn at 16 weeks by sure dates. The MSAFP levels was 7.8 MOM, the HCG was 1.1 MOM and the E3 was 0.1 MOM.

often with a normal outcome. A fetal assessment showed no abnormality and confirmed the gestational age. She was seen again at 31 weeks gestation and the assessment was again normal. Her baby was born healthy.

Comment

This case illustrates the worst aspects of MSS. The patient was not properly counseled in advance of testing or about the meaning of a positive result. Only about one in 50 women with an elevated MSAFP level will have a baby with spina bifida.⁶ Elevated MSAFP can be seen with other fetal anomalies, including ventral wall defects. Elevated MSAFP levels also can be a sign of placental compromise, and can be indicators of later problems, such as maternal hypertension, intrauterine growth retardation, oligohydramnios and subsequent fetal demise. Although an elevated MSAFP level is a risk factor for

Outcome

Extremely high MSAFP levels in association with very low E3 levels strongly suggest fetal anencephaly. The E3 levels are low because the fetal adrenals are underdeveloped. The primary-care physician was notified about this and an ultrasound examination was arranged in Northern Manitoba, rather than in Winnipeg. The ultrasound confirmed fetal anencephaly. The woman chose to have a pregnancy termination in her hometown. She was subsequently seen for genetic counseling in Winnipeg.

Comment

Extremely high MSAFP levels (*i.e.*, > 5.0 MOM) are usually associated with poor outcomes. In this case, the low E3 level suggested anencephaly. One cause of an extremely elevated MSAFP level that has a good prognosis is gastroschisis, which can be corrected post-natally.

Case 4

A 36-year-old woman had an MSS at 16.5 weeks gestation, as determined by an early ultrasound examination. The MSAFP is 0.8 MOM, the HCG is 1.3 MOM and the E3 is 0.9 MOM. The risks for fetal Down syndrome went from 1/340 to 1/370. The fetal assessment confirms the gestation, and no abnormalities suggestive of Down syndrome are seen.

Outcome

After appropriate counseling, the woman decided to have an amniocentesis. The fetal karyotype was 47,XY, +21, confirming Down syndrome. After much deliberation, the woman decided to terminate the pregnancy.

Comment

This is an example of a true-positive screen. Although the risks of fetal Down syndrome were lowered by the MSS, the results were considered screen-positive since the risks were greater than the cut-off used by the MSS program. The Manitoba MSS program uses a risk of 1/384 as a cut-off. This is the risk of an average 35-year-old having a live-born child with Down syndrome. Some programs base their risk cut-off on the risk of diagnosing fetal Down syndrome at amniocentesis. A mid-trimester risk of 1/270 is equivalent to a term risk of 1/384, since about one-third of fetuses with Down syndrome will die before term. This case also illustrates that applying a second screening test, such as ultrasound, to a positive test is inappropriate. Since less than 70% of fetuses with

Down syndrome have any detectable signs on a second-trimester ultrasound,⁷ relying on the ultrasound as a second layer screen would decrease the overall detection rate to 60% times 0.7, or 42%.

The detection rate for Down syndrome increases with advancing maternal age, but so does the false-positive rate. The detection rate for 20-year-olds is about

40%, with a false-positive rate of 2.4%, as compared to the detection rate of 75% and a false-positive of 16% for 36-year-olds.⁸

MSAFP and HCG levels tend to be about twice as high in twin pregnancies.

Case 5

A 22-year-old has an MSS at 16.0 weeks by sure dates. The MSAFP is 2.5 MOM, the HCG is 2.1 MOM and the E3 is 1.7 MOM. The Down syndrome risk is 1/26,400.

Outcome

A fetal assessment shows that this is a twin pregnancy. The results are considered normal for twins.

Comment

MSAFP and HCG levels tend to be about twice as high in twin pregnancies. E3 levels are about 1.67 times higher. This MSS pattern should strongly suggest a twin pregnancy. Physicians should be careful not to rely on MSS as a screening test for twins, as only about 50% to 60% of cases

of twins are associated with elevated MSAFP levels.⁹ Occasionally, the elevated HCG levels in twin pregnancies will cause the test to be screen-positive for Down syndrome. The MSS should not be considered reliable in predicting the chances of fetal Down syndrome when twins are present. Some programs will calculate a “pseudo-risk” estimate in this situation.

Case 6

A 31-year-old has an MSS at 16 weeks by certain dates. The MSAFP is 0.3 MOM, the HCG is 0.2 MOM and the E3 is 0.3 MOM. The Down syndrome risk is 1/736 by age and 1/1,011 by MSS. The risk for fetal trisomy 18 is quoted as 1/11.

Outcome

The woman is counseled about the possibility of trisomy 18, and consents to an amniocentesis. The ultrasound, however, shows a fetal demise. The fetus appears to be about 13 weeks size.

Comments

The pattern of low levels of all three parameters is suggestive of trisomy 18, but also can be seen with a fetal demise as levels return to the non-pregnant state. Since fetal demise is much more common, it may be wise to suggest an ultrasound examination be done locally before the woman travels long distances to the tertiary center for an amniocentesis. Low levels of E3 can be seen in a variety of other conditions, including a relatively

benign skin disorder known as X-linked ichthyosis. A recent fetal demise will often cause elevated MSAFP levels before the levels begin to drop.

Conclusion

MSS can be a valuable screening test for fetal neural tube defects or chromosomal anomalies. Physicians should counsel their patients about the potential for false-positive or false-negative results. There are many causes for positive MSS results. The pattern of the MSS levels may suggest an underlying cause and may be useful in providing post-test counseling. **Dx**

References

1. Rose NC, Mennuti MT: Maternal serum screening for neural tube defects and fetal chromosome abnormalities. *West J Med* 1993; 159:312-7.
2. Merkatz IR, Nitowsky HM, Macri JN, et al: An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. *Am J Obstet Gynecol* 1984; 148:886-94.
3. Knight GJ, Palomaki GE, Haddow JE: Use of maternal serum alpha-fetoprotein measurements to screen for Down's syndrome. *Clin Obstet Gynecol* 1988; 31:306-27.
4. Canick J, Knight GJ: Multiple marker screening for fetal Down syndrome. *Contemporary Ob/Gyn* 1992; 36:25-42.
5. Wald NJ, Watt HC, Hackshaw AK: Integrated screening for Down's syndrome based on tests performed during the first and second trimester. *N Engl J Med* 1999; 341:461-7.
6. Haddow JE, Palomaki G, Knight GJ, et al: The Foundation for Blood Research Handbook. Volume 1. The Foundation for Blood Research, Maine, 1990, pp. 5-8.
7. Smith-Bindman R, Homer W, Feldstein VA, et al: Second-trimester ultrasound to detect fetuses with Down syndrome. *JAMA* 2001; 285:1044-55.
8. Haddow JE, Palomaki GE: Prenatal screening for Down syndrome. In: Simpson JL, Elias S (eds.): *Essentials of Prenatal Diagnosis*. Churchill Livingstone, New York, 1993, p. 209.
9. Johnson JM, Harman CR, Evans JA, et al: Maternal serum alpha-fetoprotein in twin pregnancy. *Am J Obstet Gynecol* 1990; 162:1020-5.