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Selecting a Sensitive Immunoradiometric Method of Maternal Serum Alphafetoprotein for Prenatal Screening of Abnormalities in the Fetus

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It is well known that the value of alphafetoprotein in maternal serum (S-AFP) can be used as a marker of the fetal abnormalities. In many diseases like in anencephalus, spina bifida and congenital nephrosis the value is increased (3,4), while low values indicate rearrangement or other chromosomal abnormalities like Down's syndrome (5).

In the district of Kuopio university central hospital the prenatal screening for inherited genetic diseases was started in 1978. During the early years in late 70'ies we pointed out our interest for high alphafetoprotein (S-AFP) values in maternal serum and used an ordinary commercial RIA-kit of Behrinwerke Ag. In 1984 this RIA-method was changed to a double-antibody technique with glass-bead coated first antibody. Since January 1987 we have utilized the RIA-GNOST AFP of Behringwerke. This immunoradiometric assay has better precision for both high and low values than the earlier methods. The screening system was also enlarged to the whole Eastern Finland.

From the year 1988 annually about 13 000 women from the Eastern Finland have been examined for S-AFP concentration during 14th-18th weeks of pregnancy. This means that at least 95 % of pregnant women has been studied for S-AFP. Reference values for sera were analyzed using samples of 1125 healthy pregnant women presupposing and confirming that the children born had no abnormalities. With this reference population the mean and median values were calculated as well as the discrimination limits for both low and high values. The gestational age of the pregnancy was verified (and corrected if needed) by a routine ultrasound technique.

For the measurement of S-AFP we used the Ria-Gnost AFP immunoradiometric method of Behringwerke Ag. (Marburg, F.R.G.) with the sensitivity of 2 kU/l. The measurements were performed by using an automated gamma counter (Gamma master, Wallac Co., Turku, Finland). The statistical calculations were made by a microcomputer using standard statistical methods.

In the Table 1 the medians for S-AFP during the weeks of 14th to 18th of pregnancy in the maternal serum with the discrimination limits are presented both for high and low values by using the immunoradiometric method (2). At first from 1987 to 1988 with this method the discrimination limits of 0.4 * median and 3 * median were used, while in 1989 the limits were changed to 0.5 * median and 2.5 * median for security reasons (diminished need to amniocentesis) without causing any noticeably loss in the detection of fetal abnormalities. The corresponding discrimination limits for high values in amniotic fluid were: means + 5 * S.D. and means 0+ 10 * S.D., the highest one indicating a very probable abnormality in the fetus.

The precision of the method used was followed by analyzing pooled serum samples, low, normal and high levels, in every series of analyses. The results are presented in Table 2. In addition, our results in Welcome Immunoassay Quality Assessment Programme 1 (Wellcome Diagnostics, Dartford, England) have shown that the method has been well adapted for routine clinical laboratory practice being well comparable to the results of other laboratories in Europe.

During the last 12 years we have found many kinds of fetal abnormalities like neural tube defects, congenital nephrosis and anencephalus when using the earlier RIA-methods. With the new immunoradiometric method also low values have been measured more precisely. When a variation coefficient of about 5.0 % has been achieved it has been possible to find the pregnancies with inherited chromosomal abnormalities like Down's syndrome. From the women with the abnormal S-AFP values samples from amniotic fluid were taken (only on voluntary basis) to make the final decision of the abnormality by using Am-AFP values and chromosome analyses from cells in amniotic fluid or from villus biopsies. Every fetus, if found, has been carefully studied for

final diagnosis.

When only S-AFP and the age of the pregnant women were used for screening, about 38 % of pregnancies with chromosome abnormalities like Down's syndrome were found. In 1991 we will add to our screening programme the measurements of chorionic gonadotrophin (S-HCG-b) and in selected cases also free estriol (S-Estriol). This combination has been reported by Noergaard-Pedersen & al. (1) to increase the detection rate of abnormalities to about 55-60 %. For the general improvement of the diagnostic value of prenatal screening by S-AFP, S-HCG-b and perhaps by free S-Estriol it would be necessary to start a common Nordic project to evaluate the methods available and the best combination of tests, age included.

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Table 1. The median values and the discrimination limits of S-AFP in maternal serum during 15th to 18th week of pregnancy of healthy women.

Week of pregnancy	No. of women	Serum AFP, kU/l median	0.5 * median	2.5 * median
14	38	19.0	9.5	47.5
15	789	21.0	10.5	52.5
16	217	23.0	11.5	57.5
17	54	27.0	13.5	67.5
18	27	29.6	14.8	74.0

Table 2. The precision of the immunoradiometric methodfor S-AFP. In every series of analyses three levels of pooled frozen sera have been analyzed. As an example the results from three different months in 1989 are expressed.

	S-AFP, kU/l			Coefficient of variation, %		
Month	Low	Medium	High	Low	Medium	High
II/1989	8.4	78	155	5.9	4.0	5.0
VI/1989	8.2	79	158	6.1	6.0	4.5
X/1989	9.1	80	153	5.5	4.5	5.0

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