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6.1.3.2 Quality Specifications for S-AFP and S-HCG-β Determinations for Screening of Fetal Abnormalities

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ABSTRACT

High levels of maternal serum α-1-fetoprotein (S-AFP) are associated with fetal structural abnormalities like neutral tube defects and congenital nephrosis. It has a very high detection rate (3,4,5,6) when used for screening of these diseases. On the other hand, the low levels of maternal S-AFP act as a marker of many fetal chromosomal aberrations (2,3,4,8). The detection rate of S-AFP alone for the screening of Down's syndrome is low. Wald et al. (8) have shown that the addition of the determination of maternal serum chorionic gonadotrophin (S-HCG-ß) to S-AFP analysis, and using a computer program for risk estimation increases the detection rate for Down's syndrome to about 57% (2) with a false positive rate of 5%. In this study we present the results of our screening program for structural fetal defects and chromosomal aberrations in Eastern Finland, and describe the standardization and quality control of maternal serum AFP and HCG-ß analysis.

INTRODUCTION

Since 1977 we have analyzed about 12 000 women annually in Eastern Finland for serum AFP concentrations during the 14th-18th weeks of pregnancy. More than 95 percent of all pregnant women living in this area participating in the study. Reference intervals for S-AFP and HCG-ß were determined using serum samples from healthy pregnant women (4) assuming that they gave birth to healthy children. Till 1987 we monitored only elevated S-AFP values, whereas after 1987 even low S-AFP values were taken into account.

In 1987 the RIA assay S-AFP was changed to the new immunoradiometric assay. As a result new medians and cutoff limits were calculated for S-AFP from 1125 healthy

pregnant women during the 14th to 18th weeks of pregnancy (3). During 1987 and 1988 the discrimination limit of 3 MoM (MoM = multiple of medians) for high S-AFP was used to define high S-AFP. In 1989 after reanalysis of the data the discrimination limit was changed to 2.5 MoM. This resulted in a diminished need for amniocentesis without causing any significant loss in the detection rate for structural abnormalities of the fetus. The corresponding cutoff limits for amniotic fluid AFP levels in the detection of fetal structural abnormalities were: mean +5* S.D. or mean +10* S.D. The higher limit indicate a very high probability for fetal abnormality. The gestational age of the pregnancy was verified (and corrected if needed) by a routine ultrasound technique.

Low S-AFP values in association with high S-HCG-ß have been found mothers, who had a fetus with chromosomal aberrations (5,6,8). During 1987 and 1988 we used a 0.4 MoM discrimination limit to define low S-AFP. In 1989 after reanalysis of the data the discrimination limit was changed to 0.5 MoM without any change in the detection efficiency. Following reports of improved results in a new screening program by Wald et al. (8) we added the measurement of serum human chorionic gonadotrophin using a ß-chain specific method to our screening program so as to increase the detection rate for Down's syndrome and other chromosomal abnormalities. Since July 1991 we have gathered one year's experience in the combined use of maternal age, duration of pregnancy, weight corrected S-AFP and S-HCG-ß in the calculation of the risk for either neutral tube defects and other structural defects or for chromosome-linked diseases like Down's syndrome.

METHODS

The prenatal screening for inherited genetic diseases started in the district of Kuopio University Hospital in 1977 (3). During the early years we used a commercial RIA-kit from Behringwerke Ag (Marburg, Germany) to assay S-AFP. Since January 1987 we used the RIA-GNOST AFP® kit from Behringwerke and a gammacounter (Gamma Master®, Wallac Co., Turku, Finland) to measure S-AFP. This immunoradiometric assay has better precision both for high and low AFP values than the earlier methods (4). The coefficients of variation for S-AFP within runs (Vw) and between runs (Vb) are presented in Table 1. The total analytical variation (TV) or the method was calculated as follows: $TV^2 = Vw^2 + Vb^2$.

For the determination of serum HCG-ß the IMX® HCG-ß system (Abbot Laboratories,

Chicago, USA) was selected. The coefficient of variation of the method for S-HCG-ß within runs and between runs as well as the total variation calculated as above for S-AFP, is presented in Table 1.

Table 1. The within, between and total analytical variations of S-AFP and S-HCG-ß measured from commercial control samples C2-M (S-AFP) and Abbot MED (S-HCG-ß)

Variation type	S-AFP			S-HCG-ß		
	No.	mean, kU/L	CV%	No.	mean, U/L	CV%
Within day	54	93.0	2.9	30	141	3.8
Between day	54	92.1	3.5	30	143	5.3
Total	54	-	4.5	30	-	6.5

For the determination of the risk for Down's syndrome, we have used software from two manufacturers. The software from Wallac calculate the risk factor based on the algorithm developed by Wald (8). The aLPHA software from Logical Medical Systems Ltd. (London, U.K.) was also tested in prenatal screening and both software programs gave very similar results in detecting pregnancies with an appreciable risk. The analyzers, gammacounter and the IMX have been connected to a microcomputer (Osborne M386E PC, Microlog Ltd, Espoo, Finland) for automated calculation of the risk factor in neonatal screening using either software from Wallac or from Logical Medical Systems. The installation for the automatic computerization of the results was done by Wallac using a modification of its Multicalc® system.

The quality control of the methodology was checked by analyzing pooled serum samples - as low, normal and high level controls - in every series of assays. In addition, our results from the Wellcome Immunoassay Quality Assessment Programme 1 (Wellcome Diagnostics, Dartford, England) have showed that our methodology is suitable for routine clinical laboratory practice and that the quality of results is comparable to that from other laboratories in Europe. It is very important that the accuracy and precision of the methods is very high because the analytical variations of S-AFP and S-HCG-ß markedly affect the variation of the risk factors (Table 2). Ideally the coefficients of variation for S-AFP and S-HCG-ß should be below 4.0% to avoid increasing the variation for the risk factors markedly.

Table 2. The effect of the variation of maternal age and serum AFP and HCG-ß levels on Down risk factor. The weight and pregnancy week of the mother are unchanged (no weight corrections on the 14th week of pregnancy).

Maternal age	Down risk, HCG-ß = 1.0 MoM	S-AFP variation 1.0 MoM ±1.0%	S-AFP variation 1.0 MoM ±5.0%	S-AFP variation 1.0 MoM ±10.0%
20 yrs	Down risk	-1.8%, +1.8%	-9.1%, +9.5%	-17.8%, +19.4%
30 yrs	Down risk	-1.8%, +1.8%	-9.1%, +9.5%	-17.8%, +19.4%
40 yrs	Down risk	-1.9%, +1.6%	-9.1%, +9.3%	-17.9%, +19.2%
50 yrs	Down risk	-0.0%, +0.0%	-8.7%, +8.7%	-17.4%, +17.4%
Maternal age	**Down risk, S-AFP = 1.0 MoM**	**HCG-ß variation 1.0 MoM ±1.0%**	**HCG-ß variation 1.0 MoM ±5.0%**	**HCG-ß variation 1.0 MoM ±10.0%**
20 yrs	Down risk	+2.2%, -2.1%	+11.6%, -10.1%	+25.1%, -18.8%
30 yrs	Down risk	+2.2%, -2.1%	+11.6%, -10.1%	+25.1%, -18.8%
40 yrs	Down risk	+2.1%, -2.1%	+11.5%, -10.1%	+25.1%, -18.9%
50 yrs	Down risk	+0.0%, -4.4%	+13.0%, -8.7%	+26.1%, -17.4%

RESULTS AND DISCUSSION

During the last 13 years we have detected several fetal abnormalities such as congenital nephrosis, neutral tube defects and exomphalos by using only S-AFP cutoff limits. During this period a total of 109 structural and chromosomal abnormalities were detected. Only seven children (6.4%) with known abnormality were missed by the screening system. By using the present immunoradiometric AFP method, the elevated S-AFP seems to detect almost all of the pregnancies with structural fetal defects. In addition, a low S-AFP alone detects about 25% of pregnancies positive for Down's syndrome. A low S-AFP combined with the age of the pregnant women increased the percentage of detectable Down's syndrome pregnancies to about 37% (3).

During the one year's period from July 1991 to June 1992 only about 30% of the health centres participated in the extended screening program, while practically all women in the whole region of Eastern Finland were screened by S-AFP alone. Using the extended screening program we have studied 2751 pregnant women from July 1991 to June 1992. The software of Wald (8) has revealed seven cases with Down's syndrome and two with Turner's syndrome, one with 45X and one with trisomy 13. During this period only one

child was born with Down's syndrome, that was not detected by the screening procedure. Thus, our results agree well with the Down's syndrome screening results published by others (2,8).

Women with abnormal S-AFP values or with a high risk for Down's syndrome were studied further to reveal the nature of any possible fetal abnormality. Amniotic fluid samples were taken on a voluntary basis for AFP determination and karyotyping of cells from amniotic fluid or from villus biopsies was done in all cases. All serum samples with abnormal results were stored at -20°C for the measurement of possible new risk parameters in the future.

Utilizing the aLPHA program and reference values based on a larger reference population we have succeeded in decreasing the number of required amniocentesis without loss of screening efficiency. In the future the determination of serum free ß-subunit of HCG (1) may probably replace the S-HCG-ß analyses. This may increase the detection of true positive cases. On the other hand, this may also lead to analytical problems, at least due to the difficulties in standardization when different methods for free ß-subunit of HCG are used (7). Our results from one year's study show that a large part of fetal chromosomal abnormalities can be detected by the present screening procedures. Thus we will continue with the existing methodology and screening procedures until the advantages of newer methods is firmly established.

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