

# Estimating the risk of a Down's syndrome term pregnancy using age and serum markers\*

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## Abstract

The risk of an individual woman having a pregnancy associated with Down's syndrome is estimated given her age and her  $\alpha$ -fetoprotein, human chorionic gonadotrophin and gravidity specific  $\beta$ 1-glicoprotein levels. The method of estimation of the risk is based on the likelihood ratio of the joint densities of the three markers for the Down's syndrome pregnancies and for the unaffected pregnancies multiplied by the age dependent prior risk of the Down's syndrome pregnancies. This is the first case when the gravidity specific  $\beta$ 1-glicoprotein level is considered and moreover, this is the first study in this topic in Hungary. The data of 156 affected and more than 15.000 unaffected pregnancies are examined.

**Key Words and Phrases:** Down's syndrome, biochemical markers, screening, risk, likelihood ratio.

## 1. Introduction

Down's syndrome (DS) is the most common cause of mental retardation which is caused by an extra copy of chromosome #21. The IQ of a person having DS usually varies between 20 and 80, mostly below 50.

DS can be diagnosed early in pregnancy by amniocentesis. During this intervention a sample of the amniotic fluid around the baby is taken and the number of chromosomes is examined. However, amniocentesis carries around 1% risk of

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\*Supported by the Bolyai Grant of the Hungarian Academy of Sciences

miscarriage and requires high professional skills, while the chromosome analysis is very expensive.

In the 60's it was observed, that older women more frequently have affected pregnancies than younger ones. By this reason from the 70's amniocentesis became a compulsory examination for pregnant women above the age 35.

Later it was discovered that there is a connection between the levels of some biochemical markers and DS (see e.g. [5]) and that gave the idea of developing different screening tests. The outline of such a test is the following: determine the probability  $p$  of the DS for the given pregnancy from the age and the values of some biochemical markers. Given a cut-off risk  $\pi_0$  the test is positive if  $p \geq \pi_0$ . "Positives" require further examinations, e.g. amniocentesis.

There are also studies where the performance of combined ultrasound and biochemical markers is examined (see e.g. [8]) and this is also a practice at the Department of Obstetrics and Gynecology of the Medical and Health Science Center of the University of Debrecen.

In our study we considered four data variables: age of the pregnant women expressed in years and the levels of three biochemical markers:  $\alpha$ -fetoprotein (AFP), human chorionic gonadotrophin (hCG) and gravidity specific  $\beta$ 1-glicoprotein (SP1). These marker levels are expressed in multiples of the normal median for unaffected pregnancies of the same gestational age. As the marker levels follow a joint log-normal distribution for both the affected and unaffected pregnancies we considered them on common logarithmic (lg) scale. Moreover, the practice says that their values are independent from the age.

AFP was the first marker which was applied for screening starting from the mid 80's (see [2, 3]) and shortly after it the hCG was introduced in this relation (see e.g. [7]).

The idea of applying SP1 for screening came from the Department of Obstetrics and Gynecology of the University of Debrecen. The measurements are started at 1st of January, 1998. The aim of this research is to develop a screening test based on age and levels of the above mentioned markers and to prove that the knowledge of SP1 is a valuable information for screening. Besides this this is the first research of such kind in Hungary

## 2. Estimated probability of Down's syndrome

In this section we describe the method of estimating the probability of DS proposed in [7]. Let  $\pi(t)$  denote the age specific prior risk of DS, i.e. the probability that a woman of age  $t$  has an affected pregnancy and denote by  $X$  the  $r$ -dimensional vector of marker values on lg scale. Denote by  $f(x; m, S)$  the density function of the  $r$ -dimensional normal distribution with mean vector  $m$  and covariance matrix  $S$ , i.e.

$$f(x; m, S) := \frac{(2\pi)^{-d/2}}{\sqrt{\det S}} \exp \left\{ -\frac{1}{2}(x - m)^\top S^{-1}(x - m) \right\}, \quad x \in \mathbb{R}^d.$$

Let  $L_d(x)$  and  $L_n(x)$  denote the joint density functions of the marker levels for affected and unaffected pregnancies, respectively. Then,

$$L_d(x) := f(x; m_d, S_d), \quad L_n(x) := f(x; m_n, S_n), \quad x \in \mathbb{R}^d,$$

where  $m_d$  and  $m_n$  are the mean marker levels while  $S_d$  and  $S_n$  are the covariance matrices of the marker levels. The estimated probability of DS given age  $t$  and marker levels  $x$  can be expressed as a likelihood ratio multiplied the age specific prior risk, that is

$$p(x, t) := \pi(t) \frac{L_d(x)}{L_n(x)}.$$

Now, given a cut-off level  $\pi_0$  (usual values  $1/100$ ,  $1/150$ ,  $\dots$ ,  $1/350$ ), a sample  $(x, t)$  is considered to be "positive" (that is it comes from a woman having an affected pregnancy) if  $p(x, t) \geq \pi_0$ . This means, that a given age  $t$  we decide according to the Bayes discriminating rule with prior probabilities  $\pi(t)$  and  $\pi_0$  (see e.g. [4]). Thus, the detection rate (DR) of the screening test, that is the probability that a sample coming from an affected pregnancy is indicated to be positive, at a given age  $t$  is

$$P(p(x, t) \geq \pi_0 | L_d(x)) = \int_{\{x: p(x, t) \geq \pi_0\}} L_d(x) dx, \quad (1)$$

while the false positive rate (FPR), that is the probability that a sample coming from a normal pregnancy is indicated to be positive, at a given age  $t$  is

$$P(p(x, t) \geq \pi_0 | L_n(x)) = \int_{\{x: p(x, t) \geq \pi_0\}} L_n(x) dx. \quad (2)$$

To determine the overall DR and FPR of the test consider the discrete random variable  $Y$  representing the age of the pregnant women. Let  $p_d(t)$  denote the probability that a pregnant woman is of age  $t$  given the pregnancy is affected and denote by  $p_n(t)$  the same probability given the pregnancy is unaffected. Obviously,

$$p_d(t) = \frac{\pi(t)P(Y = t)}{\sum_t \pi(t)P(Y = t)}, \quad (3)$$

$$p_n(t) = \frac{(1 - \pi(t))P(Y = t)}{\sum_t (1 - \pi(t))P(Y = t)}. \quad (4)$$

Thus, the DR and FPR of the test are

$$\sum_t p_d(t)P(p(x, t) \geq \pi_0 | L_d(x)) \quad \text{and} \quad \sum_t p_n(t)P(p(x, t) \geq \pi_0 | L_n(x)),$$

respectively.

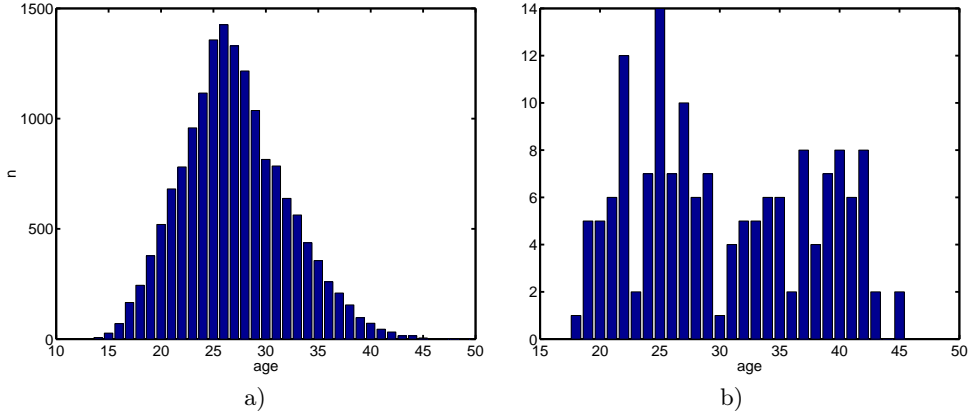


Figure 1: Frequency histogram of maternal age: a) unaffected; b) DS pregnancies.

### 3. Parameter estimation

In practical problems one has to estimate  $\pi(t)$ ,  $m_d$ ,  $m_n$ ,  $S_d$ ,  $S_n$  and the distribution of  $Y$ , and calculate the above quantities using these estimates instead of the theoretical values. An estimate of  $\pi(t)$  can be obtained from the literature, while the distribution of  $Y$  is determined from the given data set. Parameters  $m_d$ ,  $m_n$ ,  $S_d$ , and  $S_n$  are also estimated from the data set, however, they can be adjusted using results given in literature. We remark that the  $r$ -dimensional integrals in (1) and (2) can only be calculated numerically. In this research, where  $r = 3$ , we applied the MATLAB system.

#### 3.1. Age specific probability of Down's syndrome

As an estimate of  $\pi(t)$  we applied the regression function  $\hat{\pi}(t)$  given in [3]. It is calculated on the basis of medical records from various parts of the world containing the data of 4528 affected and over 5 million unaffected pregnancies from the period between 1958–1976. At the time when these data were recorded there was no screening for DS so the relative frequencies of the affected pregnancies at different ages calculated from these datasets show the "natural" situation. The estimated probability of DS at age  $t$  is

$$\hat{\pi}(t) = q(t)/(1 - q(t)), \quad \text{where} \quad q(t) := 0.000627 + \exp(-16.2395 + 0.286t).$$

#### 3.2. Unaffected pregnancies

For our study we have the data of 15817 unaffected pregnancies. The ages vary between 14 and 49 years with mean age 27 years and standard deviation 5 years (see Figure 1a).

The estimates of the mean  $m_n$  and variance  $S_n$  for AFP, hCG and SP1 (on lg scale) calculated from our data are

$$\hat{m}_n = (-0.005504 \ 0.006754 \ -0.031093)^\top, \hat{S}_n = \begin{pmatrix} 0.026517 & 0.003915 & 0.004974 \\ 0.003915 & 0.067406 & 0.020022 \\ 0.004974 & 0.020022 & 0.044492 \end{pmatrix}.$$

### 3.3. Affected pregnancies

The dataset of affected pregnancies is much smaller than the dataset of the unaffected ones. We have 113 cases with full data and 43 cases with age and AFP and hCG levels only. Here the ages vary between 18 and 45 years with mean age 30.5 years and standard deviation 7.4 years (see Figure 1b).

The estimates of the mean  $m_n$  and variance  $S_n$  for AFP, hCG and SP1 (on lg scale) calculated from our data (for AFP and hCG 156 observations, for SP1 113 cases) are

$$\tilde{m}_d = (-0.136735 \ 0.283611 \ 0.188923)^\top, \tilde{S}_d = \begin{pmatrix} 0.041993 & 0.002675 & 0.002080 \\ 0.002675 & 0.050991 & 0.005769 \\ 0.002080 & 0.005769 & 0.049300 \end{pmatrix}.$$

The lognormality of the individual marker values were tested using the Kolmogorov-Smirnov test. The corresponding significance levels are the following:

$$\text{AFP: } 0.5272; \quad \text{hCG: } 0.8724; \quad \text{SP1: } 0.9243.$$

To test the joint lognormality we applied Mardia's multivariate tests of skewness and kurtosis (see e.g. [6]) where we considered  $\tilde{m}_d$  and  $\tilde{S}_d$  as estimates of the mean and covariance matrix. The significance level for the skewness test is 0.587376 while for the kurtosis test it is equal to 0.080353.

However,  $\tilde{m}_d$  and  $\tilde{S}_d$  are just raw estimates and we would like to adjust them using the results of Cuckle [1]. Using 1140 observations for AFP and 850 observations for hCG he obtained that the the mean marker level for AFP is  $-0.136677$  while for hCG it is equal to  $0.305351$ . By taking the weighted average of the above values and the ones obtained from our dataset with weights  $(156/1296, 1140/1296)$  (AFP) and  $(156/1006, 850/1006)$  (hCG) we obtain the following estimate of the mean

$$\hat{m}_d = (-0.136684 \ 0.301980 \ 0.188923)^\top.$$

Cuckle also suggests to apply the estimated covariance matrix of marker levels for normal pregnancies in order to adjust the estimate for the affected ones. He observed that the difference in covariance matrix of AFP and hCG levels between Down's syndrome and unaffected pregnancies (lg scale) is:

$$\begin{pmatrix} 0.005497 & -0.005387 \\ -0.005387 & 0.018549 \end{pmatrix}.$$

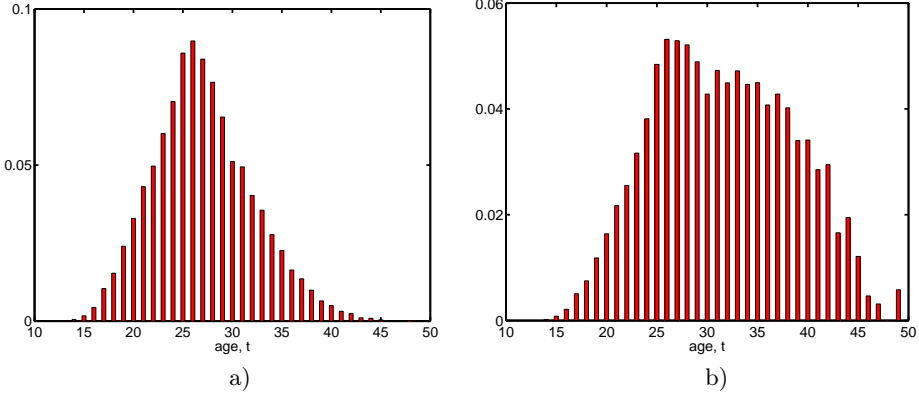


Figure 2: Estimated posterior distributions of ages: a)  $\hat{p}_n(t)$  ; b)  $\hat{p}_d(t)$  .

To estimate the variances of AFP and hCG levels he considered 542 and 383 observations, respectively, while the estimate of the covariance between AFP and hCG levels was obtained from the data of 269 pregnancies. The variances and covariances in DS pregnancies are calculated by adding the appropriate difference to the corresponding parameter observed in unaffected pregnancies. By taking the weighted average of the obtained matrix and the the appropriate part of  $\tilde{S}_d$ , in a similar way as we did for the mean marker levels, we obtain the following estimate of the covariance matrix:

$$\hat{S}_d = \begin{pmatrix} 0.034244 & 0.000050 & 0.002080 \\ 0.000050 & 0.075835 & 0.005769 \\ 0.002080 & 0.005769 & 0.049300 \end{pmatrix}.$$

By applying  $\hat{m}_d$  and  $\hat{S}_d$  as estimates of the mean and covariance matrix in Mardia's multivariate skewness and kurtosis tests the obtained significance levels of the hypothesis that the markers follow jointly lognormal distribution are 0.578029 and 0.917592, respectively.

### 3.4. Age distributions

The estimates  $\hat{P}_Y(t)$  of the probabilities  $P(Y = t)$  where  $Y$  is the random variable representing the age of pregnant women are estimated from the data of affected and unaffected pregnancies (15973 observations,  $14 \leq t \leq 49$ ). Using  $\hat{P}_Y(t)$  and  $\hat{\pi}(t)$  from (3) and (4) we can obtain the estimates  $\hat{p}_n(t)$  and  $\hat{p}_d(t)$  of  $p_n(t)$  and  $p_d(t)$ , respectively. Figures 2a and 2b show these estimated distributions.

	AFP & hCG & SP1		AFP & hCG		AFP & SP1		hCG & SP1	
Cut-off	DR	FPR	DR	FPR	DR	FPR	DR	FPR
1/100	45.14	1.59	37.33	1.54	36.18	1.48	33.44	1.63
1/150	52.59	2.79	44.68	2.73	43.52	2.63	42.06	3.10
1/200	57.96	4.06	50.18	4.05	49.05	3.91	48.66	4.67
1/250	62.11	5.36	54.55	5.41	53.46	5.26	53.92	6.21
1/300	65.44	6.62	58.16	6.72	57.12	6.64	58.24	7.77
1/350	68.21	7.82	61.20	8.02	60.21	8.02	61.86	9.31

	AFP		hCG		SP1	
Cut-off	DR	FPR	DR	FPR	DR	FPR
1/100	19.83	1.18	26.94	1.30	24.26	1.38
1/150	26.37	2.24	34.53	2.71	31.64	2.58
1/200	31.77	3.47	40.58	4.09	37.64	3.95
1/250	36.38	4.83	45.61	5.57	42.70	5.44
1/300	40.46	6.30	49.88	7.11	47.07	7.01
1/350	44.10	7.84	53.58	8.69	50.91	8.64

Table 1: DR (%) and FPR (%) for maternal age and combinations of AFP, hCG and SP1 according to risk cut-off level.

## 4. Results

Using the estimates given in Section 3 one can calculate the DR (%) and FPR (%) of the screening test described in Section 2. Table 1 shows these values for maternal age and combinations of AFP, hCG and SP1 according to risk cut-off level. We remark that the results for age & AFP & hCG are very close to the ones given in [7]. Our results clearly indicate that it is possible to screen pregnant women for DS using maternal age and biochemical markers AFP, hCG and SP1 with a test that identifies approximately 60% of all affected pregnancies with a FPR of 5%. The performance of this test is nearly the same as that of the test given in [7] where besides the maternal age, AFP and hCG the unconjugated oestriol concentration is considered.

Besides the theoretical calculations we classified the cases of the dataset we considered for estimation and counted the correctly classified DS cases and the errors performed during the classification of the unaffected ones. In this way we obtained the empirical DRs and FPRs corresponding to different cut-off levels. Three different combinations of markers were examined. Besides the maternal age in the first case we classified with the help of AFP and hCG, in the second, hCG and SP1, while in the third case we considered all three markers. The results are given in Table 2 where both the theoretical and the empirical DRs and FPRs are indicated. While the corresponding empirical and theoretical FPRs are very close to each other, the empirical DRs are much worse than the theoretical ones. One reason of this difference might be that we have a rather small dataset of affected pregnancies in comparison with the unaffected ones. The other reason is that the distribution of maternal ages in our sample containing the data of DS cases

Cut-off	AFP & hCG					hCG & SP1				
	theoretical		empirical			theoretical		empirical		
	DR	FPR	DR	SDR	FPR	DR	FPR	DR	SDR	FPR
1/100	37.33	1.54	28.21	33.83	1.71	33.44	1.63	25.66	31.24	1.25
1/150	44.68	2.73	37.18	41.72	2.69	42.06	3.10	36.28	38.83	2.57
1/200	50.18	4.05	46.15	47.38	3.79	48.66	4.67	43.36	44.35	3.86
1/250	54.55	5.41	48.72	52.27	4.88	53.92	6.21	47.79	49.24	5.29
1/300	58.16	6.72	53.85	56.17	6.06	58.24	7.77	53.10	53.79	6.85
1/350	61.20	8.02	58.33	59.92	7.06	61.86	9.31	57.52	58.10	8.27

Cut-off	AFP & hCG & SP1				
	theoretical		empirical		
	DR	FPR	DR	SDR	FPR
1/100	45.14	1.59	31.86	38.15	1.61
1/150	52.59	2.79	38.05	45.47	2.64
1/200	57.96	4.06	44.25	51.65	3.69
1/250	62.11	5.36	53.10	56.89	4.79
1/300	65.44	6.62	59.29	60.61	6.06
1/350	68.21	7.82	60.18	63.39	7.05

Table 2: Theoretical and empirical DRs, FPRs and empirical SDRs according to risk cut-off levels.

is completely differs from the theoretical one. This can be obviously seen if we compare Figures 1b and 2b. A reason of this difference is might be that the data of normal pregnancies came from Hajdú-Bihar county only, while the data of DS pregnancies from a larger population, from several counties in East-Hungary.

Thus, we simulated 500 samples of length 156 from the distribution indicated on Figure 2b. These samples were used as ages in the dataset of DS pregnancies (age and marker levels are independent), and for these simulated data the DRs were calculated. For a given cut-off risk the simulated detection rate (SDR) indicated on Table 2 is the mean DR of the 500 simulated datasets. Table 2 shows that these SDRs are much closer to the theoretical DRs than the ones calculated from the original dataset.

## 5. Conclusions

The introduction of serum markers brought a real break-through in the estimation of the risk of DS. Earlier this risk was determined using only maternal age which means that in practice, embryonal chromosome analysis was performed merely for women aged 35 years or more. Using this method 25 – 30% of DS pregnancies of the whole population can be diagnosed, theoretically (in practice, less). The remaining 70 – 75% of DS newborns are born by women under age 35, as in their case – due to the relatively low age-specific risk – embrional karyotyping is not performed.

However, using serum markers an effective risk estimation can be performed



even in this age-group and in this way women under 35 have similar chances to give birth to chromosomally sound babies as their older fellows.

In this paper – besides AFP and hCG that are widely used in international practice – we have examined the risk estimating power of SP1 concentration on a large number of patients for the first time in the literature..

By performing calculations markerwise and using various combinations of markers we have noted that the introduction of SP1 in the risk estimation yields a 7–8% increase in DR together with a small decrease of FPR.

Thus DS risk estimation based on maternal age, AFP, hCG and SP1 levels is more than twice as effective as using merely maternal age. Moreover, it can be applied for the whole population of pregnant women and reduces the number of false positive cases. Therefore, its application in practice seems to be reasonable.

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