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Justifiability of amniocentesis on the basis of positive findings of triple test, ultrasound scan and advanced maternal age

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Objective. To assess the effectiveness of antenatal screening for chromosomal abnormalities based on maternal age (≥35 years), positive ultrasound findings or a positive triple test. **Materials and methods.** Retrospective six-year study. The pregnant women routinely underwent established clinical and laboratory practice at the Department of Medical Genetics between 1997 and 2003. The women's case notes were examined to identify indications for karyotyping, gestation period and the outcome of karyotyping and pregnancy. **Results.** Invasive antenatal tests were performed on 1440 cases, 1168 (81.11%) age 35(a), 72 (5.00%) positive triple test (b), 24 (1.67%) positive ultrasound scanning (c) and 176 (12.2%) other (psychological, personal reasons, etc) (d). The overall positive predictive value was 1.67% (1.6%(a), 1.4% (b), 12.5% (c), 0.0% (d). The constructed model of logistic regression gave an odds-ratio of 8.647 for the "positive ultrasound result vs. maternal age ≥35" indication, while the odds-ratio for the triple test vs. maternal age ≥35 was 0.854. **Conclusions.** Amniocentesis and cytogenetic analysis of foetal karyotype should be presented as a diagnostic possibility to all women over 35 years. The application of biochemical markers was far from the expected results. If we compare results for indication positive ultrasound scanning vs. maternal age, an odds-ratio of ~9 was obtained. These results demonstrate that the likelihood of obtaining positive results (i.e. the presence of chromosome alterations) from an amniocentesis having this indication is almost 9 times higher than from having an amniocentesis performed solely for advanced maternal age.

Key words: Chromosomopathy, Triple test, Ultrasound, Maternal age, Prenatal screening.

Introduction

Detection of abnormalities in an unborn child is the subject of interest of prenatal diagnosis, which includes all instrumental and laboratory procedures or techniques used for pregnancy monitoring from the moment of

conception up until the period immediately before delivery. So, this is a matter of preventing and identifying hereditary diseases and congenital anomalies of an unborn foetus/child, which is simultaneously altering the attitudes of medical practice, changing established physician-patient relations in modern perinatology.

Potentially detectable abnormalities can be divided into chromosome, gene and genomic mutation, and those changes (mutations) could cause different consequences i.e. mental retardation and/or the appearance of different somatic malformations. Chromosome abnormalities are present in approximately 50% of all spontaneous miscarriages (1). The most commonly used invasive procedures to obtain material for subsequent genetic diagnostic are villocentesis (Chorionic Villus Sampling-CVS) and amniocentesis (Amniotic Fluid Sampling-AFS) with the rate of spontaneous foetal loss related to amniocentesis, on average, about one in every 200 procedures (2).

There is a direct relationship between foetal trisomies and maternal age, so, as a consequence this can be viewed as the first "screening test" for foetal chromosome abnormalities (3). However, the use of maternal age alone does not appear to be an effective screening method and the traditional estimate is that 30% of Down's syndrome cases can be detected using maternal age alone (3). Furthermore, observations that women younger than 35 years old give birth to about 70 percent of infants with Down's syndrome (4)indicates that it could be necessary to provide younger (<35) pregnant women with non-invasive screening tests. In the last decade there has been a strong development of non-invasive techniques, biochemical serum markers, such as Free Estriol, βHCG, AFP etc., applicable in the first and second trimesters of pregnancy, which has become an established part of non-invasive first-step obstetric practice in many countries.

Four advantages were suggested compared with solely using maternal age (5): It can detect twice as many affected pregnancies for the same rate of amniocentesis; It can identify affected pregnancies in women below the age cut-off; it can reassure older women whose risk was lower than that predicted by age alone, so that they might avoid the need for amniocentesis. Detection rates of these tests are approximately 60%, but in combination with ultrasound examination, the detection rate rises to 85-90% (6).

In the 1990s, Nicolaides et al. realized that the excess skin of individuals with Down's syndrome can be visualized by ultrasonography as increased nuchal translucency in the first 3 months of intrauterine life (6). Foetal nuchal translucency thickness at the 11–14-week scan has been combined with maternal age to provide an effective method of screening for trisomy 21; for an invasive testing rate of 5%, about 75% of trisomic pregnancies can be identified. Other benefits of the 11–14-week scan include confirmation that the foetus is alive, accurate dating of the pregnancy, early diagnosis of major foetal defects, and the detection of multiple pregnancies (6).

The aim of this study is to establish, based on our experience, the most justified diagnostic approach with a special interest in contributing to drawing up guide lines and directions for the most effective clinical practice, especially important in societies with limited economic resources.

Materials and methods

Patients

The pregnant women were selected and enrolled in the study group at the "DIMIMP -Medical Genetic Section, University of Bari, between 1997 and 2003. All pregnant women who had been undergoing routinely established clinical and laboratory practices, consisting of genetic counselling (pre-test

counselling-before AFS), amniocentesis, cytogenetics and if necessary (positive result on cytogenetic analysis) genetic counselling once again (post-test counselling). Where necessary, medical or psychological support/consultation was given.

Pregnant women with indications for amniocentesis such as positive triple test, positive ultrasound scan and advanced maternal age were included in this study (95% of all examined cases). Pregnant women with other indications (molecular prenatal diagnosis) or those with motivations such as personal decision (not psychological nature) were excluded from this study. For ultrasound and biochemical tests, the maternal age was taken into consideration, and in addition, for the triple test a cut-off level (1:250) was used as recommended (7, 8). Furthermore, results of cytogenetic analysis were evaluated in relationwith above mentioned indications.

Statistical analysis

A descriptive statistical analysis was undertaken, which took into consideration the age of the pregnant women as well as indications for amniocentesis. The indications for amniocentesis were divided into: maternal age ≥35, a positive triple test, a positive ultrasound test, others (a child with previous genetic disorder, families with positive anamnesis and reasons of a psychological nature). The evaluation of specificity and sensitivity of biochemical analysis could not be carried out because of the unavailability of information regarding the total number of patients who undertook the examination (i.e. those that took the test but had a negative result). On the other hand, it was possible to carry out the evaluation of the positive predictive values for every single indication, with a particular emphasis on the result of the positive triple tests. Furthermore, a model of logistic regression was constructed to ascertain the probability of getting a positive (pathological) result with the foetal karyotype as a function of the different indications. Statistical analysis was performed using the statistical package SPSS 12.0 for Windows and SAS.

Results

During a period of 6 years (1997-2002) a total of 1440 amniocentesis tests were performed; 82.9% of the examined pregnant women (n=1194) belong to the group of those over 35 years old, while the group of less than 35 years accounts for only 17.1% (n=246) of the patients. 30% of patients were actively working (n=432), while 5% of patients (n=72) smoked during the pregnancy (despite their doctor's recommendation). Figure 1 presents the distribution of pregnant women according to each indication for amniocentesis, in terms of the age of the patient.

Table 1 shows the data regarding the distribution of pregnant women in relation to the indications and obtained results of cytogenetic analyses with the predictive positive value of each indication particularly.

Of the total number (n=1440) of amniocentesis, 81.1% were those done for the maternal age as the only indication (≥35 years old), 5% (n=72) were with the positive triple test as an indication, 1.7% (n=24) for a positive ultrasound result and 12.2% (n=176) other indications. Cytogenetic analysis of the pregnant women with the indication "maternal age ≥35" gave positive results in 19 cases (1.6% of 1.168 amniocentesis tests or 1.3% of the total performed amniocentesis); 15 (78.9%) cases were Down's syndrome, 2 cases (10.5%) were Klinefelter's Syndrome (47,XXY); 1 case was Edwards' Syndrome (5.3%); 1 case was 47,XXX (5.3%) as represented in Table 2.

The positive triple test was the indication in 72 cases (5%) and the largest number of tests were done on pregnant women in the age group from 31 to 34 years (37 cases or 51.4%). Only one case (1.4% of 72 amnio-

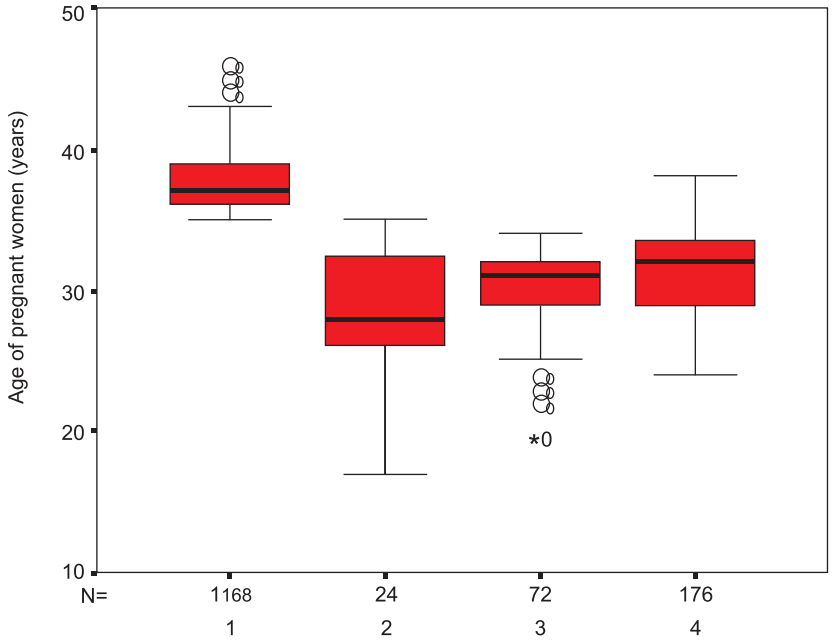

Figure 1 Distribution of the pregnant women according to the age and indications for amniocentesis (N = number of pregnant women; 1 = indication for age group of pregnant women ≥35; 2 = indication for positive ultrasound scanning 3 = positive triple test; 4 = other indications.)

Table 1 Distribution of the pregnant women frequency in relation to the indication and result of the cytogenetic analysis (positive/negative) with the positive predictive value (italic) regarding each indication

Indications	Cytogenetic analysis (n=1440)		Total
	Negative (n=1417; 98.4%)	Positive (n=23;1.6%)	
	n (%)	n (%)	n (%)
Age ≥35	1149 (98.4)	19 (*1.6*)	1168 (81.0)
Ultrasound	21 (87.5)	3 (*12.5*)	24 (1.7)
Triple test	71 (98.6)	1 (*1.4*)	72 (5.0)
Other	176 (100.0)	-	176 (12.2)

Table 2 Distribution of "positive" cytogenetic analysis for indication "maternal age ≥ 35"

Kariotype (n)	Age (Years)								Σ	Positive results (%)
	35	36	37	38	39	40	41	42		
+21*	2	2	1	2	3	1	2	2	15	78.9
+18*	-	-	-	-	-	-	1	-	1	5.3
XXY	-	1	-	1	-	-	-	-	2	10.5
XXX	-	-	-	-	-	1	-	-	1	5.3
Total	2	3	1	3	3	2	3	2	19	100
Positive results (%)	10.5	15.8	5.3	15.8	15.8	10.5	15.8	10.5		100

*Trisomy for the chromosome 18 and 21

centesis tests or 0.07% of the total performed amniocentesis) resulted in the abnormal foetal karyotype (Down's syndrome).

A positive ultrasound marker was found in 24 cases (1.7%) of the total number of amniocenteses performed and for all patients subsequent amniocentesis and cytogenetic analysis of foetal karyotype was performed. A positive result of karyotype analysis was confirmed in 3 cases (12.5% of 24 amniocentesis tests) with the above-mentioned indication (0.3% of total performed amniocenteses). One case of Patau Syndrome (Ultrasound result of multiple malformations) was found; 2 cases of Down's syndrome (the positive NT result); 1 case of Robert's Syndrome (tetraphocomelia).

The constructed model of logistic regression gives an odds-ratio of 8.647 for the positive ultrasound result indication with respect to maternal age (≥35). It actually means that the risk of getting a pathological foetal karyotype result after amniocentesis is almost 9 times higher than the risk in a pregnant woman with only the indication of advanced maternal age ("maternal age ≥35"). On the other hand, the odds-ratio for the triple test vs. maternal age (≥35) is only 0.854.

Discussion

The aim of this retrospective study is to justify the performing of amniocentesis as an invasive diagnostic technique by analyzing a group of 1440 patients with the following indications: maternal age, positive triple test and positive ultrasound result. The total positive predictive values of all amniocenteses performed in this study was 1.67%, which is completely in accordance with the observations of Howe et al. who reported positive predictive values of 1.8% (3).

The percentage of analyzed patients with the "maternal age ≥35" indication is 81.1% (n=1168), which is very similar to the observation of Chaabouni et al., quoting that precisely maternal age appeared in 63% cases as the main indication for foetal karyogram analysis. Ferguson–Smith and Yater reported similar observations (60%) (9, 10). In contrast, Howe et al. presented data with only 10% of cases with the mentioned indication for amniocentesis (3).

Within this group, 19 abnormal foetal karyotypes were observed which represents 1.6% of all analyses performed in our study, while Chaabouni et al. reported 3.9% and Dupont and Carles 3.2% (7, 9). The most frequent pathological result was Down's syndrome with 15 cases (78.9% of all abnormal karyograms). Our data is in accordance with those given by Howe et al., reporting 66% of Down's syndrome diagnosed (3).

The triple test was an indication in 72 cases or 5% of all performed analyses which completely concurs with the observations of Spenser et al. (6.1%), Wald et al. (5%) while Webley and Halliday reported higher results (9.8%), and Ayme et al. (9.1%) and finally Chaabouni et al. quoted in their study only 1.97% (8, 9, 11, 12, 13). One case of Down's syndrome (or 1.4%) was confirmed by foetal karyotype analysis. This result is in total accordance with those publicized by Dupont and Carles of 2.1%, Spenser of 2.8%, Chaabouni et al. of 3.3%, Ben et al. of 5% and Nyberg et al. of 5.3% (7, 9, 14, 15, 16). A higher percentage was observed by Baenna et al. of 22.8% and Howe et al. of 16.6% of studied cases (3, 17). During the period of six years, a positive ultrasound result as an indication was a means of foetal karyotype analysis in 24 cases, which is 1.7% of all analyses; in contrast, Webley and Halliday observed 20% and Chaabouni et al. gave data of 8.2% of foetal karyotype analysis (9, 12). After cytogenetic analysis, there were 3 pathological cases of foetal karyotype, which was 12.5% of the discovered cases. Baenna et al. quoted data of 45.6%, while Howe et al. presented data of 20.4% of positive cases (17, 3). Nicolaides et al. in their

work reported 64% of discovered trisomy 21 after doing NT, followed by 4.1% false positive results (18). It is necessary to underline the fact that in this study there was total of 4 pregnant women with a positive NT ultrasound result, of which 2 cases (50%) were with the positive abnormal foetal karyotype (Down's syndrome), which is very close to the observations of Nicolaides et al. (18). In contrast, Chaabouni et al. quoted only 9.0% of cases with abnormal karyotypes and Dupont and Carles had similar reports (9.5%) and Benacerraf et al. presented only 1.4% of cases detected (7 , 9, 19).

A limitation of the study was certainly the absence of an evaluation of the specificity and sensitivity of the biochemical analysis, but, as explained in the statistical methods, the information was missing regarding the patients that underwent the examination but had a negative result. In fact these patients generally do not go through genetic counselling and do not have an amniocentesis

Conclusions

The constructed model of logistic regression gave an odds-ratio of 8.647 for the positive ultrasound result vs. maternal age (≥35) as the only indication. When we add the low price of US, and practically no risk to the foetus and mother, the application of this technique imposes itself as the "gold standard" in prenatal diagnosis. Amniocentesis and cytogenetic analysis of foetal karyotype should be presented as a diagnostic possibility to all women over 35 years of age. Our results completely confirm the indicated observation, taking into consideration the fact that all 19 cases of chromosomopathy discovered belong to that age group. The issue of biochemical markers and application of this non-invasive diagnostic method, at least for the period we studied (1997-2002), is, in our opinion (74/75 cases were found to be false-positive), far from the expected results, as was also confirmed by Howe et al. (3). De Vore and Romero, furthermore, considered that it would be desirable to offer serum marker tests to all younger women (≥ 35 years old) as a routine analysis in pregnancy (20). We could express our agreement especially considering its low price, but with the remark that those tests require setting the laboratory criteria for their conduct, as well as establishing so-called corrected factors (algorithms) which would ensure the adequate increase in quality and sensitivity of these tests (only accredited institutions should perform it).

It is evident that the important goal for future parents, and society in general, is giving birth to healthy offspring, or if the diagnoses is positive and the parents decide to continue with the pregnancy, this permits preparation for the delivery in the best way, with the best hospital/personal organization to obtain optimal results.

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