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Early diagnosis of fetal abnormalities – the importance of ultrasonographic examination

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ABSTRACT

First trimester screening is a combination of many factors including maternal age, nuchal translucency (NT) thickness, human chorionic beta-gonadotropin (free β-hCG), and pregnancy-associated plasma protein (PAPP-A). An important cause of perinatal morbidity and mortality is represented by fetal abnormalities that have an increasing incidence in both industrialized and developing countries. As the level of education increases, the average age of women who become pregnant has increased and so this may be one of the causes of the increase in the incidence of abnormalities.

Aim. The purpose of this study is to highlight the importance of first trimester screening by analyzing the results obtained over a period of 3 years.

Material and methods. We conducted an observational study that covered several aspects of fetal diagnosis: screening methods for chromosomal abnormalities, early diagnosis of structural abnormalities and their association with chromosomal anomalies.

Results. The study included 6227 patients from two maternal-fetal departments of two private practice clinics from Bucharest and Constanta, the screening being performed by a specialist in maternal fetal medicine. 242 cases had a positive screening for aneuploidies. These and other 118 patients over the age of 35 (a total of 360 patients) opted to perform invasive diagnostic tests to rule out suspicion of aneuploidy. The karyotyping of the 360 cases showed: 18 (5%) cases with trisomy 21 (T21), 2 with trisomy 13 (T13), 1 case with trisomy 18 (T18) and 43 showed polymorphism changes of certain nucleotides (SNPs). A number of 1248 patients followed between 2016 and 2017 chose to perform screening by determining free fetal DNA in maternal blood.

Conclusions. Our study emphasizes the recognized importance of first trimester screening. Even if the first trimester screening is included in the national health insurance, the access and addressability of many pregnant women belonging to disadvantaged social and cultural classes remain limited.

Keywords: first trimester screening, aneuploidies, chromosomal abnormalities, ultrasound

INTRODUCTION

In the last decade, screening for chromosomal abnormalities has developed rapidly and is now one of the key points of the prenatal consultation (1,2), more and more women are taking part in prenatal screening worldwide (3). It was developed as a combination of many risk factors including maternal age, ultrasonographic nuchal translucency thickness (NT), human chorionic beta-gonadotropin (free β-hCG), and pregnancy-associated plasma pro-

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tein (PAPP-A) (2,4). The accuracy of the screening increases considerably by adding a few ultrasound parameters to the previous ones, such as the nasal bone (2,5), the tricuspid flow (6), the blood flow through the venous duct (7) and the heart rhythm (2). Combining the parameters mentioned above, the individual risk for the most frequent aneuploidies is estimated using special certified softwares. The first trimester combined test detects 90%, 97% and 92% of trisomies 21,18 and 13, respectively, and above 95% of cases of monosomy X and triploidies, and more than 50% of other chromosomal abnormalities, at a false positive result of 4% (8).

An important cause of perinatal morbidity and mortality is fetal abnormalities that have an increasing incidence in both industrialized and developing countries. The World Health Organization (WHO) concluded in 2005 that fetal growth impairment is caused by several causes, including genetic factors, maternal factors related to diet, lifestyle, smoking, maternal age, pregnancy complications, and factors that are linked to the social and economic environment (9). As a result of the increase in the education level, the maternal age at birth has increased considerably; in Germany for example, the average maternal age has reached 31 years, increasing in this way the prevalence of fetal abnormalities: in 1990 the prevalence of Down syndrome was 1:722, and in 2000 it reached 1:553 (3).

The latest guide from the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) highlights the importance of fetal ultrasound in early pregnancy. With the help of the ultrasonographic examination, the viability of the pregnancy is confirmed, the gestational age is established with precision and the number of fetuses, chorionicity and amnionicity of multiple pregnancies are certainly and early determined. An important aspect is also the detection of fetal abnormalities, however they can develop later in pregnancy (10). First trimester screening should be performed between 11 and 13 + 6 weeks of gestation.

The purpose of this study is to highlight the importance of first trimester screening by analyzing the results obtained over a period of 3 years.

MATERIAL AND METHODS

We conducted a retrospective observational study that covered several aspects of fetal diagnosis: screening methods for chromosomal anomalies, early diagnosis of structural abnormalities as well as their association with chromosomal abnormalities. The study was conducted over a period of 3 years between January 2014 and July 2017 and included 6,227 patients from two maternal-fetal departments of two private practice clinics from Bucharest and Constanta, the ultrasound screening being performed by a specialist in maternal fetal medicine. The inclusion criteria consisted in pregnant patients who underwent first trimester combined screening and presented structural or biochemical anomalies, signed an informed consent understanding the limitations of this screening and ulteriorly underwent invasive diagnostic procedures. The follow-up of the pregnancy in order to establish the normal course or the early diagnosis of aneuploidies and structural anomalies is realized following the protocols established by the Fetal Medicine Foundation (FMF) as follows: between 11 and 13 gestational weeks and 6 days, patients undergo first trimester ultrasonographic examination simultaneously with determination of biochemical markers (β-hCG and PAPP-A). The results obtained were then introduced in special programs, accredited and FMF certified, the best known being ASTRAIA and VIEW POINT. The analysis of biochemical markers was performed on two types of analyzers: Siemens, with the Prisca program (uncredited FMF), a reduced cost and more accessible database for screening and Kryptor, which is certified, verified and accredited FMF. We followed the risk for aneuploidies after the first trimester combined test screening and the fetal karyotype after amniocentesis.

Patients whose results are not in the low-risk area at the screening test for aneuploidy are offered further investigations depending on their financial disposition, meaning either the determination of cell free fetal DNA in maternal blood, which can be performed from the 10th week of pregnancy in which the fetal fraction of maternal blood is large enough to allow analysis, either a trophoblast biopsy if the pregnancy is no longer than 12 weeks, or amniocentesis in 16th week with karyotype analysis. Positive cases of cell free fetal DNA screening test are also sending to the invasive diagnostic test.

We used Microsoft Excel for the statistical analysis of the results. The data collected retrospectively did not contain personal information and only Ethics Committee approval of the two private practice clinics from Bucharest and Constanta was required and obtained without the need of informed consent of the patient. Procedures we have done respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law.

RESULTS

Our study includes a number of 6,227 patients who underwent biochemical analysis and ultrasound screening between 11 and 13 gestational weeks and 6 days during the period January 2014 - July 2017. This group contained 1415 patients who

were over 35 years of age, representing a percentage of 22.7%. The biochemical results are presented in Table 1 and Figure 1. As we can conclude from the results in Table I, 4.6% of the β-hCG results exceeded 95%; 5.4% of cases presented PAPP-A values below 5%. The fact that decreased values of PAPP-A associated with increased values of β-hCG characterize the trisomy 21, and decreased values of both hormones are found in T13/T18 is well-known.

TABLE 1. The first trimester biochemical results of our patients included in the study

	5%	Median	95%	
Free β-hCG	0.4	0.975	2.564	4.6% >95%
PAPP-A	0.427	1.029	2.335	5.4% <5%
PlGF	0.415	0.919	1.929	11.2% <5%

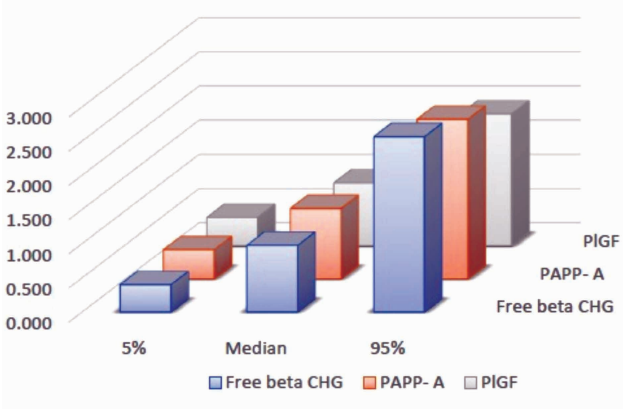


FIGURE 1. The first trimester biochemical results of our patients

The biochemical results correlated with the ultrasound assessment in the first trimester investigation identified a number of 242 patients with positive screening for aneuploidies. Besides the elevated biochemical risk, there were 111 cases, which presented fetal morphology anomalies diagnosed during the ultrasonographic screening. These and 118 other patients over the age of 35, witch personal request, so a total of 360 patients, decided to perform invasive diagnostic tests (amniocentesis followed by fetal karyotyping) to rule out suspicion of aneuploidy.

The karyotyping of the 360 cases showed:

- 18 (5%) cases with T21
- 2 cases with T13
- 1 case with trisomy 18
- 43 cases showed polymorphism changes of some nucleotides (SNPs)

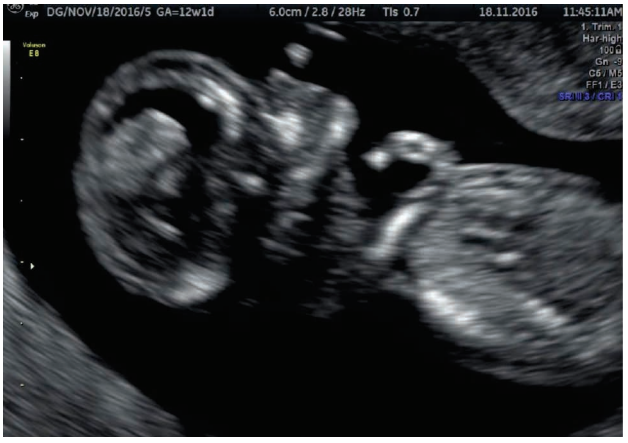
Independently from the β-hCG value, the diminished PAPP-A values correlate with placental disfunction and subsequent intrauterine growth restriction.

A number of 1248 patients followed between 2016 and 2017 chose to perform screening by determining free fetal DNA in maternal blood. Certain patients chose this test known as NIPT (non-invasive prenatal testing) due to:

- Positive result for aneuploidies after the combined screening test;
- Advanced maternal age (knowing the frequent association between advanced maternal age and chromosomal anomalies)

Other patients decided invasive methods for diagnosis, respectively amniocentesis.

Of the 18 cases identified with trisomy 21, 16 had positive first trimester screening for this syndrome; 4 of these cases did not showed structural abnormalities highlighted on ultrasound, 3 cases had echogenic intracardiac focus, 2 cases had absence of the middle phalanx of the V finger, and one case was found with the following: complex neural tube abnormality, cerebellar vermis agenesis, bilateral pyelectasia, ARSA, absent nasal bone (Figure 2), and 2 cases with numerous placental cysts. Below are the ultrasound images (Figure 3) from one of the cases of Down syndrome from our study group who underwent the ultrasound examination at 12 gestational weeks and 5 days and then performed free fetal DNA from maternal blood due to the doctor's recommendation, taking into account the advanced age of the patient – 39 years.



a

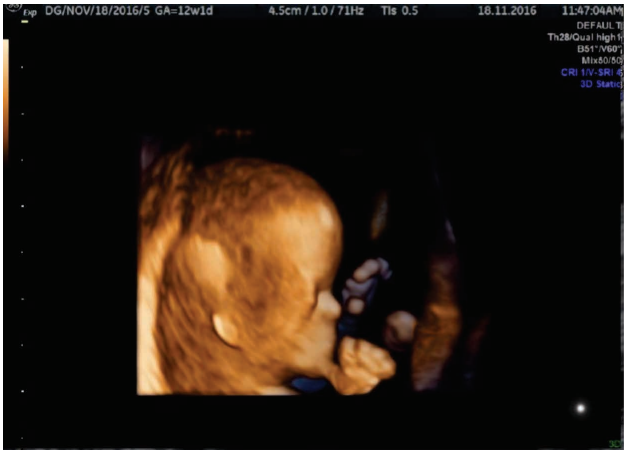


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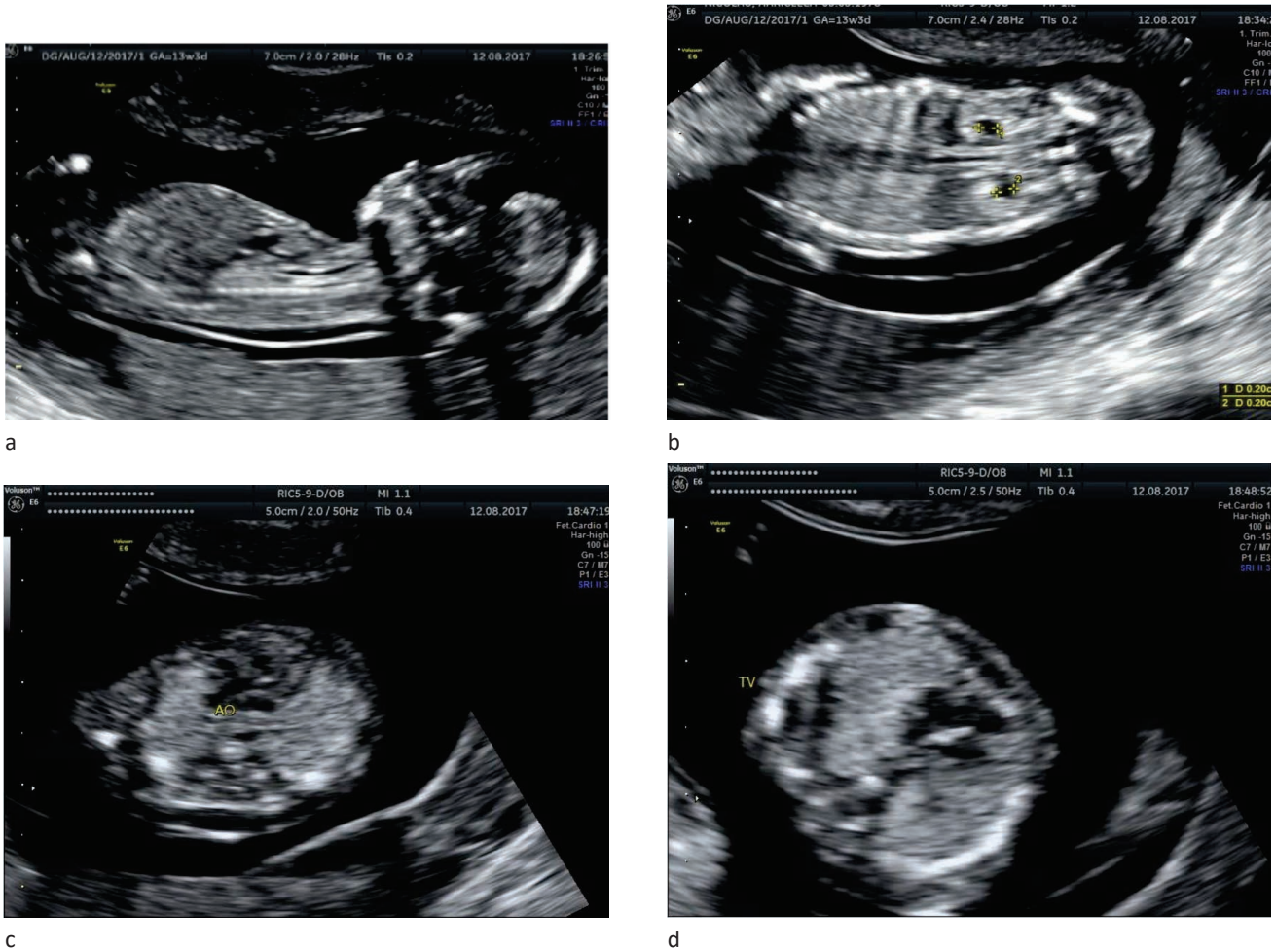
FIGURE 2. Absent nasal bone: (a) 2D ultrasonographic imaging (b) 3D ultrasonographic imaging



a b

c d

FIGURE 3. Ultrasound examination of a suspected T21; (a) sagittal section, absent nasal bone; (b) Coronal section of fetal kidney with 2 mm pyelectasia; (c) Emerging aortic from LV; (d) Apparently enlarged and incompetent tricuspid valve

Fetal karyotype analysis also revealed in one case the existence of trisomy 18. The patient performed amniocentesis due to a combination of risk factors, including advanced maternal age (40 years-old), positive aneuploidy result at the combined screening of the first trimester, as well as certain associated structural abnormalities represented by the absence of stomach visualization, bilateral choroid plexus cysts and polyhydramnios. Due to the amniocentesis positive result and multiple malformations, the patient decided to terminate the pregnancy before the 20th week of gestation.

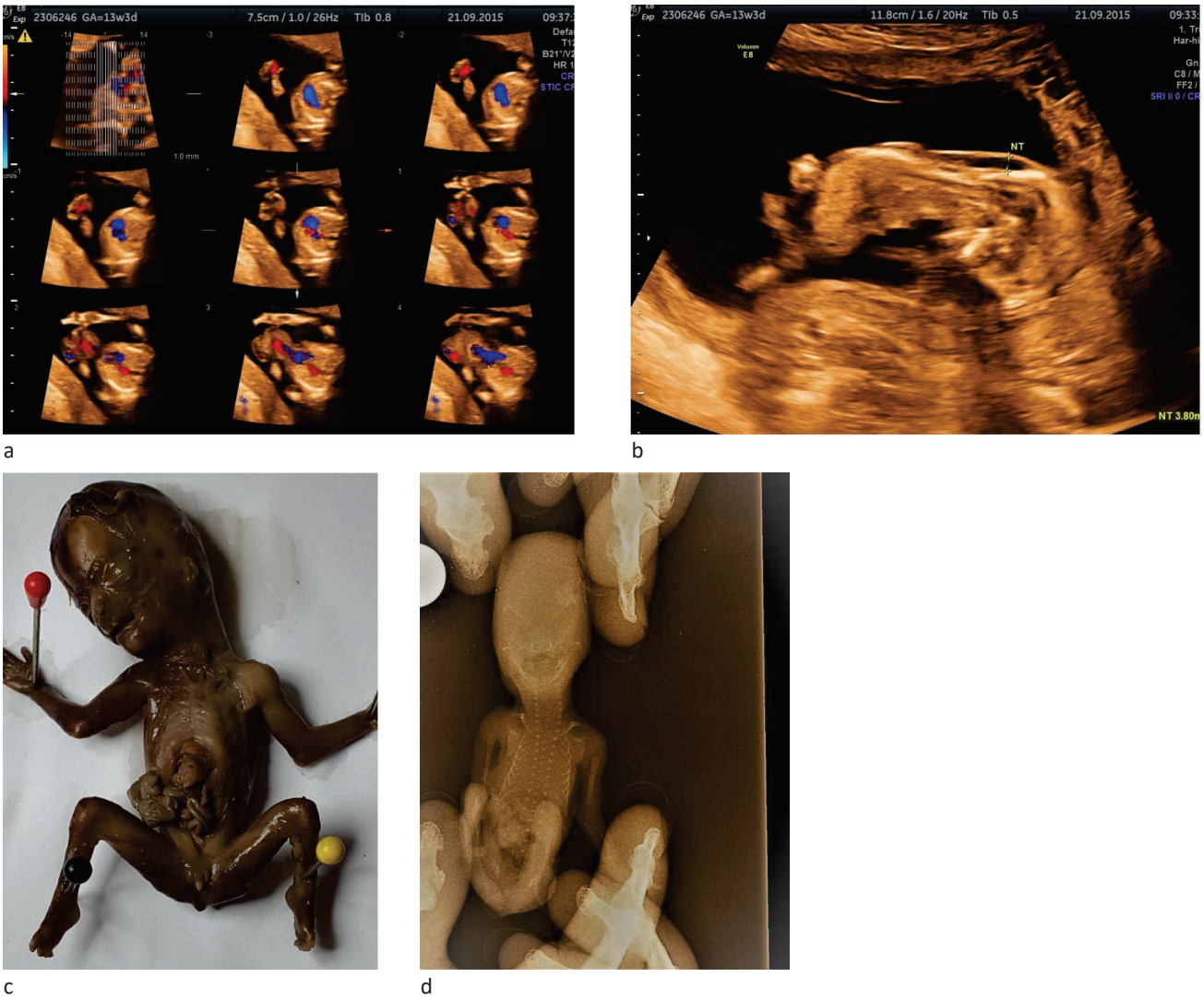
2 cases presented trisomy 13. The first case was referred to our medical unit at 18 weeks of gestation for amniocentesis due to fetal multiple structural abnormalities diagnosis; the amniocentesis confirmed the type of karyotype. Ultrasound examination during the 20th week of gestation describes a fetus with slightly delayed development and numerous structural abnormalities. For religious reasons, the pregnancy was continued. The membranes ruptured spontaneously at 35 weeks and the a 2,700 g fetus was delivered by caesarean section, surviving 5 days postoperatively.

A case of pentalogy of Cantrell was diagnosed at 13 weeks (Figure 4).

The results determined the existence of 43 cases in which amniocentesis revealed polymorphism-like changes of certain nucleotides (SNPs). Of the 43 cases with SNPs, 25 women were over the age of 35; 8 cases presented choroid plexus cysts, 9 presented hyperechoic intestinal loops, one case had bilateral pyelectasia; NT larger than 6 mm was determined in 3 cases, one case in which the stomach could not be seen on repeated examinations and 3 cases presented with heart abnormalities.

DISCUSSIONS

Searching the literature, in a study including 452,901 women, published in 2015 (11), 3.8% tested positive for Down syndrome, 433 (0.1%) for trisomy 18, 1689 (0.4%) for both and 0.7% for neural tube defects, Smith-Lemli-Opitz, or for other multiple conditions. The detection rates were 92.9% for Down syndrome, 93.2% for trisomy 18 and for trisomy 13 the detection rate was 80.4% (95% CI 73.9–86.9); for 45X the detection rate was 80.1% and for



a b c d

FIGURE 4. Fetus with pentalogy of Cantrell. (a) ultrasound image of ectopia cordis; (b) enlarged NT; (c) anatomopathological examination - macroscopic piece; (d) X-ray examination of the fetus showing the absence of the sternum in the midline malformation

triploidy 91.0%. Thus, it was concluded that the first trimester screening is a sensitive and specific test for a wide range of anomalies, their detection rate being 81.6%, with an overall false-positive rate of 4.5%.

In our study the incidence for trisomy 21 was 2.89 per 1,000 patients compared to 2 per 1000 live births in the absence of screening, diagnosis and termination reported in a study published in 2021 (12). The slightly higher incidence in our group is an argument in favor of first trimester screening and its advantages, thus informing the mother about the implications and possible neonatal complications and also giving her the choice of pregnancy termination.

Kevin et al. (13) analyzed data from 1,339 patients who underwent first trimester screening. The mean maternal age was 30 years, based on the age distribution. The prevalence of Down syndrome cases was 26. Among the women surveyed, 577 had a higher risk than 1 in 300 cutoff. A number of 51 aneuploidies were diagnosed: 23 cases of Down syndrome, 15 cases of trisomy 13 or 18, 4 cases of 45X, 2 cases of 47XXY/ XXX and 5 cases of triploidy. Invasive diagnostic tests were performed in 77% of cases. The rate of false positive screening results was only 5%.

One of the key points of the first trimester screening is the measurement of nuchal translucency, studies have shown that only this marker can detect 65% of chromosomal abnormalities such as Down syndrome, with a false positive rate of only 5%. If biochemical markers are added to this measurement, the detection rate increases to 88% (14); by using the additional ultrasound parameters, the detection rate reach 90% (8). If the screening suspects an abnormality, the clinician must recommend further investigations and the most frequently used is represented by cell-free DNA (CF-DNA) analysis from the mother's blood. A recent meta-analysis re-

ported that in the case of CF-DNA the detection rate for Down syndrome was 99.7% and the false positive rate was only 0.04% (15,16). It is currently under discussion if CF-DNA testing should be extensively used as a first-line screening method because this could lead to a much higher detection rate and a much lower invasive testing rate, thus reducing the associated costs of diagnostic methods (16-18). In some countries such as Netherlands and Belgium, CF-DNA has already been implemented in screening programs for all women (14), and we believe that this screening attitude is beneficial and should be applied in more and more countries in order to increase the detection rate. If positive, the next step consists in performing an invasive diagnosis test, respectively classic or molecular karyotyping by amniocentesis. Perception has changed in this regard as well, because controlled studies have shown that ultrasound-guided amniocentesis is associated with a lower rate of pregnancy loss (0.6%), and with a decrease in the bleeding incidence than previous reported (19). Therefore, first trimester ultrasound screening is essential in order to triage the high-risk pregnancies for chromosomal anomalies and fetal structural anomalies to the invasive karyotype testing.

CONCLUSIONS

Our study emphasizes the recognized importance of first trimester screening. The first trimester screening, especially the ultrasonographic evaluation is fundamental for detection of structural anomalies that are not associated with chromosomal anomalies, either isolated or included in a syndrome such as pentalogy of Cantrell which is incompatible with survival.

Even if the first trimester screening is included in the national health insurance system, the access and addressability of many pregnant women belonging to disadvantaged socio-cultural classes remain limited. Every pregnant woman should undergo first trimester screening for the early detection of chromosomal anomalies, structural malformations or even complex syndromes with a diminished chance of survival; only prenatal counseled women, based on the results of screening, are able to take an informed decision regarding the continuation or early affected pregnancy termination.

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