REVIEW ARTICLE

Genetic Screening Tests and Prenatal Diagnosis for Aneuploidies

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Abstract

The genetic screening and prenatal diagnosis panorama have been profoundly modified in the last 30 years. This study analyzes the changes on international level and shares the experience of our maternal-fetal-perinatal medicine center at the Microcitemico Hospital, Cagliari. We observed the evolution of screening tests for fetal aneuploidies. There was an overall reduction of invasive prenatal procedures, probably due to denatality, the innovations in ultrasound imaging technology, and the introduction of noninvasive prenatal testing. Furthermore, we reported the decrease of amniocentesis as compared to chorionic villous sampling (CVS) and the decline of fetal loss rates following both of these procedures. The demand for training fellows in invasive prenatal procedures and especially in CVS is continuously increasing.

Keywords: Amniocentesis, Chorionic villous sampling, Fetal abnormalities, Genetic screening test, Invasive prenatal procedures, Prenatal diagnosis.

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The current scenario of prenatal medicine, ever more aided by the progress of scientific knowledge and the development of innovative technologies, is stirring up new ethical issues; hence, the need to enhance the physician–patient communication and the relational aspects of the clinical approach.[1]

In an attempt to humanize the physician–patient relation further, informed consent is a particular and delicate step of the communication process that can improve the therapeutic alliance and encourage the patient to actively share the screening diagnostic and therapeutic path.[2]

Adequate reproductive risk evaluation and acquirement of major awareness of the risks associated with invasive prenatal diagnosis are essential for patients in order to form an objective choice.[3]

Originally, the evaluation of chromosomopathy risks involved only maternal age, whereas currently many other traits are taken into consideration.

The new approaches depend on new screening methods such as fetal ultrasound evaluation by Doppler velocimetry, 3D-4D imaging, transvaginal approach, ultrasound detection of fetal abnormalities as early as the first trimester, obstetrician and laboratory experience, earliest prenatal diagnosis, low fetal loss risk following prenatal invasive procedures, analysis accuracy, molecular genetic diagnosis, laboratory availability, legal and ethical aspects, public/private healthcare issues and costs discrepancies, and, of course, patient's choices.[4,5]

Any screening test needs several indispensable requirements: it must offer adequate sensitivity in order to limit false-negative results and specificity to minimize false-positives; also, effective treatment for the identified problem must be available and it should be considered acceptable by patients. Moreover, the benefit of its application must justify the financial cost.[2]

The test that currently meets all these requirements for aneuploidy screening is the combined test between 11^{+0} and 13^{+6} weeks of gestation. The risk calculation through the combined test is obtained by a combination of maternal age, fetal nuchal translucency thickness (NT), fetal heart rate, maternal serum-free ß-hCG, and pregnancy-associated plasma protein-A (PAPP-A). The accuracy of the combined screening test is around 90%, 97%, and 92% for trisomies 21, 18, and 13, respectively, with a false-positive rate of 2–4%.[6] The assessment of new markers such as tricuspid regurgitation detected by Doppler velocimetry and increased ductus venosus flow impedance improves the performance of combined screening by increasing the detection rate and reducing the false-positive rate.[7–10]

Less frequently used, the second trimester screening by maternal age and various combinations of total or free ß-hCG, AFP, uE3, and Inhibin A can identify 56–71% of trisomy 21 pregnancies, with a false-positive rate of 5%.[11]

Moreover, biochemical markers can provide indications for selecting a subgroup of pregnant women at increased risk of maternal-fetal obstetric complications.

The following parameters are thus correlated:

Low serum PAPP-A levels (less than 0.5 MoM) and low birth weight; low serum PAPP-A levels (below 0.5 MoM) associated with high or low levels of ß-hCG (below 0.5 MoM or above 2 MoM); and the possibility of gestational complications such as abortion, intrauterine death, pregnancy-induced hypertension, preeclampsia, premature birth, and intrauterine growth retardation as well as many other placental pathologies.

The introduction and rapid diffusion of noninvasive prenatal screening (NIPS) or noninvasive prenatal test (NIPT) into obstetrical

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care, using fetal cell-free DNA (cfDNA) detected in maternal blood, is more recent.[12]

However, the scientific community must carefully consider the economic and ethical issues of NIPS and the impact that it may have on the well-established methods of prenatal screening and diagnosis. It should also reflect on the fact that the modern day society is ever more web-based and that the web, rather than genetic counseling, may be the primary, although misleading, source of information for many prospective parents.[13]

Some cfDNA controversies should also be considered: high costs and commercial pressure by pharmaceutical companies, multiple pregnancies following assisted reproductive techniques, oocyte donorship, 1–2 week delay in receiving results, early gestational age, technical and logistic concerns.[14]

Maternal issues are also relevant, such as consanguinity, bone marrow transplantation, immunological diseases, obesity, malignant tumors, heparin and immunoglobulin therapy, gestational diabetes.[14]

Technique failures and false-positives and negatives must also be taken into account, following NIPS.

In 1–5% of cases, no result is given after first sampling. Most frequent causes of false-positive and negative cfDNA results are low fetal fraction (<4 mg DNA), maternal chromosome abnormality, fetal-placental genetic discordance, fetal mosaicism, or vanishing twin.[15]

Due to all issues mentioned above, the first trimester combined testing remains the cornerstone of prenatal screening, and it still is the starting point which any complex prenatal care algorithm stems from.[9,16–18] With advanced first trimester ultrasound technology, detailed sonographic scans are essential for early evaluation of the fetus, placenta, and maternal anatomy for identifying 15% of clinically relevant gynecologic findings (48% uterine–51% ovarian), 17% of clinically relevant placental findings, and 41% of fetal ultrasound malformations.[19]

Fetal malformations during the first trimester[19] could be distinguished as follows:

- Always detected 30% (acrania, holoprosencephaly, exomphalos, gastroschisis, megacystis, body stalk anomaly)
- Sometimes detected 44% (spina bifida, ventriculomegaly, facial cleft, major cardiac defects, diaphragmatic hernia, lethal skeletal dysplasia, absent hands/feet)
- Undetectable 26% (corpus callosum agenesis, cerebellum/vermis hypoplasia, CCAM/sequestration, esophageal/duodenal atresia, bowel obstruction, hydronephrosis, talipes).

In this context, the fundamental aspect is the epidemiological one. Actually, structural congenital anomalies, many from de novo mutations, are the predominant share of serious congenital malformations. The prevalence is 0.2% for common trisomies 21, 18, 13; 0.4% for other chromosome abnormalities; 1.2% for pathogenic microdeletions and duplications; 0.4% for Mendelian genetic disorders; and 2–3% for structural congenital anomalies.[2]

This is why ultrasound is essential, mostly for detecting the more frequent and severe fetal anomalies such as, for example, congenital heart disease (CHD).

The incidence of severe CHD is about 2.5 to 3/1,000 live births, and the moderately severe forms of CHD account for another 3 per 1,000 live births.[20]

Obviously, abortion after early diagnosis, misdiagnosis due to the intrinsic difficulties of the ultrasound examination, developmental pathologies are factors that could influence the epidemiological data.

These considerations, along with the CHD incidence and its healthcare costs, are the reasons why the advantages of ultrasound must not be underestimated.

Obstetric or genetic counseling is the first step in prenatal invasive procedures; it must be nondirective and should inform patients about the reproductive risks, the diagnostic and therapeutic options, the risks related to the invasive procedures, their diagnostic limitations and the time for receiving the diagnosis, the modes of the procedures, and all the options, including voluntary termination of pregnancy (TOP) and fetal therapy. Patients should also be counseled about delivery time and the choice of maternity unit and possibly given indications on pediatric surgery centers.[2]

The invasive techniques performed in the perinatal centers are opted according to the disease, the physician's experience and hands-on skills, the laboratory availability, and the patient's choice.[3]

Chorionic villus sampling (CVS) should be performed after 11^{+0} gestational weeks, amniocentesis at or after 15^{+0} completed weeks of gestation, and fetal blood sampling (FBS) after 18^{+0} weeks.[21]

Since the evaluation of amniocytes or chorionic villi can often provide similar information as fetal blood, FBS should be limited to clinical situations in which the use of lower risk diagnostic procedures does not provide adequate or sufficiently timely diagnostic information or for mosaicism resulting after CVS or amniocentesis.

To provide better advice for women about the risks and benefits of prenatal invasive procedures, and to ensure that women are given sufficient information/counseling to make a decision about screening, it is useful to describe not only to the emerging data in literature but also to share the experience of the referral center of the patient.

In 1986, pregnancy outcome after amniocentesis was reported in a randomized controlled trial of 4,606 women, age range 25–34 years, without any known risk of genetic diseases. Spontaneous abortion rate was 1.7% in the study group after amniocentesis and 0.7% in the control group after ultrasound (relative risk 2.3).[22]

In 2008, Odibo et al. reported a retrospective cohort study including all women undergoing CVS and a control group that had no invasive procedure in a single center over a 16-year period. 5,243 women who had CVS were compared with 4,917 women seen before 14 weeks who had no invasive procedure; there were 138 (2.7%) fetal losses before 24 weeks of gestation in the CVS group compared with 161 (3.3%) in the control group (relative risk 0.80, 95% confidence interval, 0.64–1.0). The difference in the loss rate of -0.7% (95% confidence interval, -0.02 to 1.3) between the CVS group and those who had no procedure was not statistically significant at $p < 0.05$. The authors concluded that the estimated fetal loss rate after CVS was not significantly different from the group that had no procedure. Significant predictors of fetal loss after CVS were identified but the accuracy of the final model for predicting fetal loss was only modest.[23]

The systematic literature review and meta-analysis by Akolekar et al. in 2015 estimated a controlled study of 324 losses in 42,716 women who underwent amniocentesis and 207 losses in 8899 women who underwent CVS. The risk of miscarriage prior to 24 weeks in women who had amniocentesis and CVS was 0.81% (95% CI, 0.58–1.08%) and 2.18% (95% CI, 1.61–2.82%), respectively. The background rates of miscarriage in women from the control

group that did not undergo any procedures were 0.67% (95% CI, 0.46–0.91%) for amniocentesis and 1.79% (95% CI, 0.61–3.58%) for CVS. The weight-pooled procedure-related risks of miscarriage for amniocentesis and CVS were 0.11% (95% CI, −0.04 to 0.26%) and 0.22% (95% CI, −0.71 to 1.16%), respectively.[24]

Finally, a more recent systematic review by Salomon et al. in 2019 showed that when studies including only women with similar risk profiles for chromosomal abnormality in the intervention and control groups were considered, the procedure-related risk for amniocentesis was 0.12% (95% CI, −0.05 to 0.30%; I2 = 44.1%), and for CVS, it was −0.11% (95% CI, −0.29 to 0.08%; I2 = 0%). These findings indicated that the procedure-related risks of miscarriage following amniocentesis and CVS were much lower than those currently quoted to women. The risk appeared to be negligible when these interventions were compared to control groups of the same risk profile. Currently, there is no evidence that CVS is less safe than amniocentesis[25] (Table 1).

The benefits of current and modern approaches in the invasive prenatal procedures and analysis such as preimplantation genetic diagnosis (PGD), quantitative fluorescence-polymerase chain reaction (QF-PCR), and microarray techniques should also be considered.[4]

PGD or preimplantation genetic testing (PGT) is a very early form of prenatal diagnosis performed for the first time by Handyside in 1989 for cystic fibrosis.[26]

This technique aims to avoid the transmission of specific genetic or chromosomal birth defects[27] and to bypass the obvious issue of TOP. Thus, only the healthy embryos obtained *in vitro* by *in vitro* fertilization and intracytoplasmic sperm injection get to to be transferred.[28]

PGD is employed for the genetic diagnosis of autosomic recessive disorders[29] for woman at risk for chromosomal disorders, recurrent pregnancy loss and repeat IVF failure, severe sperm factors, carrier of chromosomal rearrangement, for human leukocyte antigen (HLA) compatibility by HLA matching in case of bone marrow transplantation after birth,[30] and in the last indications, it is named "preimplantation genetic screening" (PGS).[31]

QF-PCR (or, more rarely, fluorescence in situ hybridization) may be carried out on villi or amniotic fluid to test for specific chromosomes (21, 13, 18, X,Y). These tests provide results in 1–2 days and are commonly employed after a positive screening result or in fetuses with ultrasound findings or markers of common aneuploidies. In some settings, the use of QF-PCR has replaced the full karyotype. However, inaccuracies of the rapid testing results (false-positives or negatives) are reported occasionally. On this basis, abnormal rapid testing should be confirmed by metaphase culture or should be associated with ultrasound anomalies before opting for TOP.[21,32]

Microarray techniques, such as microarray-based comparative genomic hybridization (aCGH), can discern submicroscopic chromosomal deletions and duplications or smaller pathogenic chromosomal variants that are undetectable with standard cytogenetic analyzes.[33]

Different platforms are available, including genome-wide (10–400 kb resolution), targeted, and mixed arrays. Incremental diagnostic yields of 7.0% and 5.0% were reported with the use of aCGH in fetuses with CHD or NT >3.5 mm. The choice of women in the field of prenatal diagnosis has been largely modified in the last decade.

Two studies, from Denmark and England, analyze the impact of the introduction of a national Down syndrome screening policy on invasive prenatal diagnosis.[34,35] Both studies confirm these advancements by carefully documenting a considerable reduction of the total number of invasive prenatal diagnoses performed for

Table 1: Antenatal screening and diagnosis of aneuploidies

Screening test	*Gestation weeks*	*Days to receiving results*	*Sensibility (%)*	*False positive (%)*	*False negative (%)*	*Sampling success (%)*	*Analysis failure (%)*	*Fetal loss*
Fetal nuchal translucency+ free beta and PAPP-A+ other US markers	11–14	The same day	90–95	2–3	–	100	0	No
NIPS/NIPT, for trisomies	>11	7–10 days			–	100	About 5	No
21			Tr. 21 99*	0.09				
18			Tr. 18 95*	0.13				
13			Tr. 13 92*	0.13				
Triple test	>15	The same day	70	5	–	100	0	No
Quadruple test	>15	The same day	80	5	–	100	0	No

Diagnostic test	*Gestation weeks*	*Days to receiving results*	*Sensibility (%)*	*False positive (%)*	*False negative (%)*	*Sampling success (%)*	*Analysis failure (%)*	*Fetal risk*
Preimplantation genetic diagnosis and screening	Before pregnancy	3–5	98	1–2	–	–	2	–
Chorionic villous sampling	After 11 weeks	In 4 days if abnormal in 10 days if normal	100	1	–	100	0	1:1000
Amniocentesis	After 16 weeks	In 10–15 days	100	0.5	–	100	0	1:1000
Fetal blood sampling	After 18 weeks	1 week	100	–	–	99	1	1–2:100

*99-95-92% sensibility only in the cases with results for trisomies 21-18-13 (no result in 5% of cases)

karyotype analysis and a redistribution of the relationship between the number of procedures performed by amniocentesis and CVS. In the Danish study, CVS was performed in 66% of cases in 2006, while in England, the amniocentesis/CVS ratio of 3:1 in 2003 improved to 1:1 in 2011.

Women's choice varies and changes over time. It can depend on several maternal parameters such as sociocultural and religious background, maternal and gestational age, occupation, health welfare, medical history, and screening test outcome.[3,36]

Currently, women generally prefer early prenatal diagnosis to reduce the anxiety associated with waiting for the results, and this is why they tend to opt for the first trimester CVS rather than second trimester amniocentesis or FBS.[37]

Women's preference of CVS is due to the vast experience of the operators in our center who have been performing CVS since 1983 in early gestation in high-risk women.[38]

Furthermore, innovations in ultrasound technology allow a more detailed fetal anatomy screening at an earlier pregnancy stage and detect about 50% of fetal abnormalities that require karyotype analysis. All these factors contribute to the women's preference of CVS to amniocentesis.

But are prenatal centers that offer invasive diagnosis prepared for this significant shift toward CVS determined by the widespread of first trimester screening?[39]

Our Department of Maternal-Fetal-Perinatal Medicine is a referral center in Sardinia with a population of 1.6 million inhabitants. It has been offering a highly efficient program for prenatal screening and diagnosis of ß-thalassemia since 1977.[29,40] The first trimester NT screening was introduced in 1996, and more than 60,000 prenatal invasive procedures have been performed since 1977.[38]

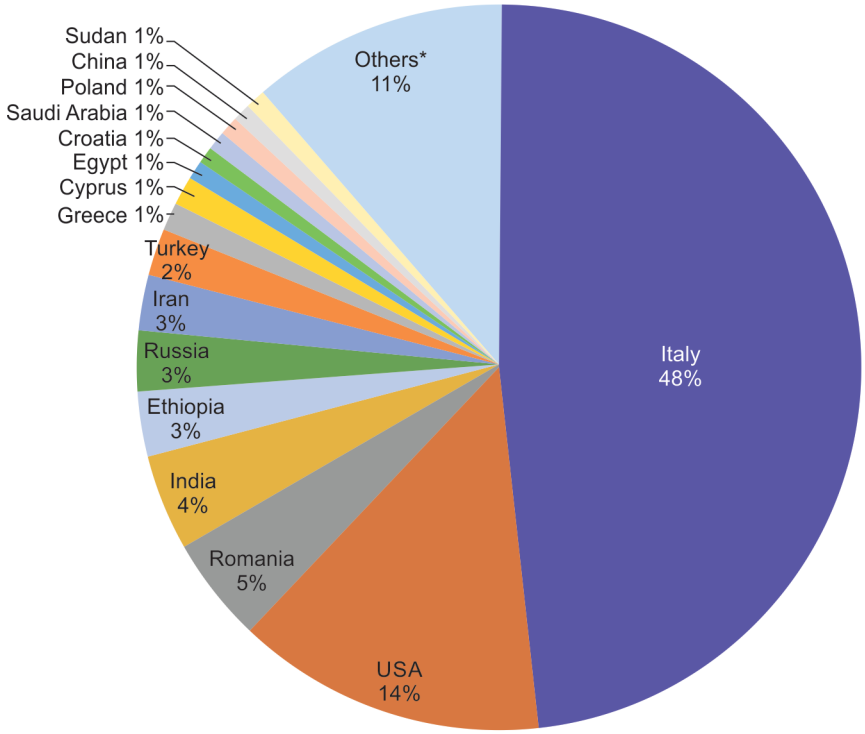
We recently conducted a retrospective cohort study of all deliveries from 2010 to 2018 in Sardinia to determine the rate of change by the type of invasive diagnostic procedure, transabdominal (TA) CVS vs TA amniocentesis, all conducted in our referral center at the Microcitemico Hospital.

During the study period, the number of deliveries (13,413 to 9,143, $p < 0.0001$) and total invasive diagnostic procedures (1,506 to 858 per year, $p = 0.019$) declined significantly, and this is consistent with the international data. However, the percentage of total deliveries undergoing invasive diagnostic procedures has not been changed (mean: 12.2%, R2 = 0.347, $p = 0.095$).

There has been a significant increase in the rate of early diagnostic testing with TA-CVS compared to amniocentesis and in the ratio of women receiving earlier definitive diagnosis.[41]

These results are possibly related to increasing maternal age, expansion of NIPS, and the first trimester detailed ultrasound screening for anomalies in our practice, among others, but also to the cultural frameset of Sardinian women who are familiar with it always due to the early diagnosis for ß-thalassemia.

Pregnant women should be informed and offered all screening and diagnostic risk assessment programs available in our respective countries and healthcare systems, thus providing them with high-standard tailored paths. We must conduct constant studies and research, focusing on communicating the correct information to the patient. Continuing to tutor fellow physicians skilled in CVS is also essential. Currently, the demand for training in the invasive prenatal procedures and especially in CVS is continuously increasing. Our Department of Maternal-Fetal-Perinatal Medicine at Microcitemic Hospital has continuously offered maximum availability for tutoring to all requests received from any part of the world[42,43] (Fig. 1).



*Others: n = 1 from Argentina, Azerbaijan, Bosnia, Czech Republic, Canada, Georgia, Japan, France, Germany, Kosovo, Lebanon, Mongolio, Morocco, Nigeria, Netherlands, Pakistan, Portugal, Qatar, Slovenia, Spain, Sudan, Emirates, Venezuela

Fig. 1: Percentage of country origin of 236 fellows rotating in international TA-CVS (1985–2018)

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