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Invasive or non-invasive prenatal genetic diagnosis?

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About 40 years ago, invasive prenatal diagnosis techniques were introduced in obstetrics. Initially, amniocentesis was performed followed by placentalcentesis, fetoscopy, fetal blood sampling (FBS), and chorionic villus sampling (CVS). These procedures, while invading the uterine environment, have made it possible to proceed with the retrieval of biological tissue of fetal origin for analysis and definitive diagnosis [6, 20, 21, 27]. The development of such techniques was facilitated by improvements in instrumentation and technology, and was further propelled by the advancement of cytogenetics and molecular genetic techniques [3, 13, 14, 22].

However, invasive prenatal diagnosis carries the inherent risk of fetal loss, which is low, but not negligible (amniocentesis and CVS approximately 0.3%–0.5% and FBS 1%–2%). In addition, there is a significant economic burden from the costs associated with laboratory techniques. Public health programs of nations interested in the utilization of invasive procedures have generally limited their use to high-risk cases, where the risk of procedure related loss is comparable to the risk of an affected fetus for a given condition [25, 26]. As a result, in the 1980s amniocentesis was offered to women at higher risk of trisomy 21 based on maternal age alone, if there was an increased risk due to prior complications or pre-existing conditions (i.e., chromosomal alterations in the parents or in prior offspring), or because of prenatal detection of fetal malformations or other abnormal ultrasound findings. The initial policies led to an offering of invasive prenatal diagnosis to 5% of all pregnant women (positive screen rate), with a 40% detection rate for trisomy 21.

With the advent of biochemical screening tests using maternal serum in the second trimester, the “triple” and “quadruple screen”, the detection rate of trisomy 21 increased to 60% [28]. Meanwhile, with advancements in ultrasound, numerous reports were published that identified sonographic “markers” for trisomy 21, leading to additional screening with a “genetic ultrasound” in the second trimester [1]. However, second trimester ultrasound for the purposes of screening an unselected population never gained universal acceptance, and was primarily used in higher-risk populations (i.e., a woman with advanced maternal age that wished to avoid invasive testing). At this time, second trimester amniocentesis was the primary invasive diagnostic test practiced, while fetal blood sampling by cordocentesis was utilized when a diagnostic test was desired later in the second trimester, often in the setting of identification of a fetal anomaly in the second trimester ultrasound. As mean maternal age at childbirth continued to increase, especially in Western countries, alongside increasing scientific advancements, the number of indications for prenatal diagnosis rose. This trend led to an increased rate of invasive prenatal diagnosis, based on maternal age alone up to 15–20% in some nations, and spurred a search for new solutions.

In the mid-1990s, an important turning point was the use of ultrasound to measure fetal nuchal translucency at 11–14 weeks [17], along with maternal serum biochemical screening, pregnancy associated plasma protein-A (PAPP-A), and free beta subunits of human chorionic gonadotropin (free beta-hCG), for the screening of trisomy 21 [24]. With a similar invasive testing rate as maternal age alone of 5%, the combined test led to a detection rate of approximately 90% for trisomy 21 (in the setting of a positive test, the odds of an affected fetus were 1:24). The introduction of the nuchal translucency measurement raised the issue of certification, reproducibility, and reliability of
measurements. Additionally, it helped launch the positive and ambitious process of ultrasound accreditation, which served as a reference for many other systems of accreditation in obstetric ultrasound (however, it was criticized for the level of cost and difficulty) [15].

First trimester screening and the measurement of nuchal translucency, in particular, changed the course of prenatal care. From the latter half of the 1990s, obstetric guidelines of many nations placed the 11–14 weeks ultrasound as a routine part of prenatal care. The importance of this evaluation was further reinforced after multiple reports demonstrated an association between an abnormal nuchal translucency and major congenital malformations, especially cardiac defects [23]. Additional benefits from the first trimester ultrasound that were explored included early recognition of major fetal malformations, or their early exclusion. After the passage of time, however, it must be admitted that ultrasound in the first trimester never gained widespread recognition for anatomical assessment [8].

Many critical issues in first trimester ultrasound remain unresolved, most importantly, that fetal structures are often too small or undeveloped for proper evaluation (little of which is ameliorated by the transvaginal approach). Furthermore, assigning a pathologic diagnosis to a global abnormality is complicated because the etiology may not be discovered until later in gestation (e.g., disproportion of the cardiac chambers). A definitive diagnosis is difficult to come by and pathological anatomy analyses cannot be performed until later in pregnancy. Indeed, the utility of performing early ultrasound anatomical screening has been questioned because such screening rarely offers a comprehensive diagnosis, and abnormal findings only serve to raise anxiety of both practitioners and expecting parents.

In cases of abnormal nuchal translucency measurement, complex algorithms have been devised for prenatal care. Included in most algorithms are assessment of fetal karyotype, early echocardiography and early second trimester sonographic assessment of fetal anatomy, repeat sonographic assessment later in the second trimester, and neonatal follow-up. The increasing use of nuchal translucency screening, and of the combined test, has reduced the importance of genetic ultrasound in the second trimester and replaced the use of the triple or quadruple screen unless risk screening was not performed in the first trimester. Concurrently, elaborate programs for second trimester testing were suggested, such as the integrated test, which involves the questionable policy of non-disclosure until the second trimester [5, 29]. However, integrated testing did provide the unequivocal benefit of reducing the screen-positive rate to 1%, while maintaining a sensitivity around 90%.

In regards to invasive prenatal diagnosis, the identification of cases at greater risk of aneuploidy by first trimester screening led to a general rate of decline in invasive procedures, thereby reducing both the economic burden and the number of fetal losses. Another effect of first trimester screening was the shift in prenatal diagnosis from the second to the first trimester. As a result of the greater predictive value of first trimester screening, compared to the maternal age alone, the request of prenatal diagnosis by CVS increased [18, 31]. Hence, an important consequence was the greater demand for CVS and those trained to perform the procedure. The number of practitioners, once performing predominantly amniocentesis, who have had to improve in CVS, has risen consistently over the last number of years. As a result, educational organizations have faced new challenges in providing such training [11].

The effects of introducing the combined test went beyond the single issue of screening for trisomy 21 and created the recently expressed concept of reversing the traditional prenatal care pyramid [16]. Some additional information can be obtained by collecting other fetal-placental biochemical markers (i.e., PI GF Placental Growth Factor), placental growth factor). However, it remains to be shown that this can translate into more effective monitoring and intervention for certain complications of pregnancy, such as spontaneous abortion, preterm delivery, preclampsia, intrauterine growth restriction, and gestational diabetes.

In addition to the developments discussed above in the 1990s, pre-implantation genetic diagnosis (PGD) technology became available and allowed couples that are carriers for, or affected by, genetic diseases to select unaffected embryos for transfer before implantation, after in vitro fertilization (IVF) [10]. The option of PGD offered the opportunity to circumvent wrestling with the option of pregnancy termination in affected fetuses [12]. The practice of PGD has evolved rapidly, in terms of both diagnostic techniques and biopsy methods. Indeed, testing for a specific gene mutation can be performed in concert with 24-chromosome aneuploidy screening [7]. However, this technique has also stirred much ethical debate about the interests of the prospective parents, the embryo, the child, and the people affected by these diseases.

In the field of prenatal invasive diagnostic techniques, there was no major news for many years. Recently, however, two developments have appeared, and they will likely cause a great stir. These techniques are the array comparative genomic hybridization (aCGH) or chromosomal microarray (CMA) in the field of the invasive prenatal diagnosis, and in the field of the screening, non-invasive prenatal tests (NIPT) [4, 30].
Comparative genomic hybridization has emerged in recent years as a primary diagnostic tool for the evaluation of developmental anomalies and structural malformations in children. Chromosomal copy number variation (CNV), detectable by aCGH, is a variation from the expected number of DNA segment copies when compared to a reference genome.

aCGH allows detection of smaller pathogenic chromosomal variants that are undetectable using standard cytogentic analyses. An important aspect is the significant frequency of aCGH findings in fetuses with particular structural abnormalities, and a lower but still notable frequency of aCGH abnormalities in cases with other findings, such as fetal hydrops, cystic hygroma, and abnormal nuchal translucency. Although currently quite expensive, this technology has the advantage of being automated, requiring less personnel and providing faster turnaround time. Of note, the traditional G-banding technique used in aCGH does not detect balanced chromosomal rearrangements, triploidy, and some instances of mosaicism. The biggest clinical challenge presented by aCGH is the detection of chromosomal variants of unknown clinical significance (VOUS) [19]. Currently, there are two policies about the implementation of the aCGH analysis in prenatal diagnosis: the first, more traditional approach, suggests aCGH only in cases with specific indications such as fetal malformations, and the second approach proposes aCGH as an alternative, and not necessarily as a replacement, to traditional karyotype screening even in low-risk cases [30]. There is a general consensus on the need for proper genetic counselling prior to performing this test.

The second recent advancement is the use of cell free fetal DNA (cff-DNA) in maternal blood for non-invasive prenatal testing [4]. Non-invasive prenatal diagnosis for fetal sex determination, RhD antigen, paternal inherited genes or some de novo autosomal dominant diseases have been validated but there are many concerns about non-invasive prenatal diagnosis for monogenic diseases (autosomal recessive diseases, X linked diseases, and autosomal dominant diseases of maternal origin) [2].

Regarding autosomal trisomies, such as trisomy 21, 18, 13, sex chromosome aneuploidies, and triploidy, NIPT is employed mostly using shotgun massively parallel sequencing (s-MPS) [2]. For trisomy 21, individual studies showed the detection rate (DR) ranges between 94.4% and 100%, and false positive rates (FPR) ranges between 0 and 2.1% [9]. Fairly good results have been demonstrated for detection of trisomy 18, while screening for trisomy 13 and monosomy X has a poorer performance due to the highly variable amplification of these chromosomes with lower guanosine and cytosine content. The main limitations of NIPT for chromosomal abnormalities are the frequency of failure to provide results (1–5% of cases), the delay in obtaining the results (1–2 weeks), and the high cost of the technique.

The expectations regarding cff-DNA for fetal genetic anomalies are very high, as it may have the potential to change the landscape of prenatal diagnosis. However, to the disappointment of many, cff-DNA does not have the ability to function as a diagnostic test but is considered a “super” screening test. Use of NIPT has been proposed for use as either screening for the general obstetric population or as a contingent screening test in high-risk groups. The first option would be quite expensive given the sheer volume of tests performed and the subsequent invasive testing required for positive cases (number of false positives expected is about 1%) [9]. However, this option would maximize the prenatal detection rate (99%). The second approach implies a lower number of invasive procedures (0.4–0.8%) with a very high detection rate (86–97%), depending on how the “high-risk” group is classified. Karyotype in the high-risk group can alternatively be obtained by the QF-PCR method, which has the advantage of offering very rapid results (useful in cases where the likelihood of abnormal karyotype is very high). It can also be studied by aCGH methods, whose rationale is intuitive, in cases of malformations or with an enlarged nuchal translucency and normal karyotype. At present, a few groups are studying implementation of NIPT after the use of first trimester screening. Under these circumstances, a position of watchful waiting appears reasonable.

The scientific community must carefully consider the economic and ethical issues of NIPT and the impact that it may have on well-established methods of prenatal screening and diagnosis [2]. However, for those who have the duty of this consideration, should reflect on the fact that modern society is ever more web-based and that the web, rather than the counselling that geneticists can offer, may be the primary, although misleading, source of information for many prospective parents.

References


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