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Review Article

Fetal Medicine the way forward

Zanardini Cristina, Bhide Amar

Fetal Medicine Unit St. George's Hospital, London United Kingdom

Introduction

The past two decades have witnessed significant advances in the field of fetal medicine. This has revolutionized the management of many conditions. This is an attempt to look at advances in the recent past, changes expected to take place in the near future, at the same time identifying future challenges in fetal medicine.

Prenatal screening for chromosomal abnormalities has now reached a high degree of sensitivity and specificity. First trimester screening for trisomy 21 can be provided by a combination of maternal age, nuchal translucency (NT) and maternal serum level of free b-hCG and pregnancy associated plasma protein-A (PAPP-A) at 11^{+0}–13^{+6} weeks of gestation with a detection rate of ~90% for a false positive rate (FPR) of 5%[1]. The performance of the test has been improved recently by the discovery of additional sonographic markers, such as absent nasal bone, wide fronto-maxillary facial angle, abnormal flow in the ductus venosus and tricuspid regurgitation[2,3]. An additional benefit of first trimester screening is the early diagnosis of trisomies 18 and 13, which are the second and the third most common chromosomal abnormalities[4] respectively.

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Correspondence :
Dr. Bhide Amar
Fetal Medicine Unit
Lanesborough Wing, 4th Floor,
St. George's HospitalBlackshaw Road,
London, SWI7 0QT, United Kingdom
Tel. +44 2087250009 Fax : +44 2087250079
Email : abhide@sgul.ac.uk

The incidence of allo-immunization against RhD antigen has been dramatically reduced from 13% to 0.16% with the advent of postnatal Rh immune prophylaxis[5]. This can be reduced further by antenatal Anti-D administration to Rhesus negative mothers. Antenatal Anti-D administration is the national recommendation in the United Kingdom since 2002[5]. The measurement of peak systolic velocity (PSV) of blood flow in the middle cerebral artery (MCA) has been shown to be an accurate test for non-invasive detection of fetal anemia[6]. This technique has also been found to be useful in detecting fetal anemia associated with a variety of other conditions such as Kell allo-immunisation, Parvovirus infection, placental chorioangioma, feto-maternal hemorrhage and intrauterine death of one monochorionic twin[7].

Twin-to-twin transfusion syndrome (TTTS) is a complication occurring in 10 - 15% of monochorionic pregnancies. If left untreated, the mortality of this condition exceeds 90%, with significant neurological morbidity in 30–50% of surviving twins[8]. The treatment options for TTTS include serial amnioreduction and fetoscopic laser ablation of the placental vascular anastomoses. In 2008, a Cochrane review confirmed the significant increase in survival rates and reduction in neurological morbidity with the use of laser ablation compared with amnioreduction in severe TTTS. This review[8] demonstrated that laser coagulation results in less death of both infants per pregnancy (relative risk (RR) 0.49; 95% confidence interval (CI) 0.30 to 0.79), less perinatal death (RR 0.59; 95% CI 0.0.40 to 0.87) and less neonatal death (RR 0.29; 95% CI 0.14 to 0.61) than in pregnancies treated with amnioreduction. However,

laser ablation is a more complex technique and less readily available compared to amnioreduction.

Current developments

(1) *Move from traditional karyotype to PCR/FISH*

When the pregnancy is at an increased risk for chromosome abnormalities prenatal diagnosis involves examination of the karyotype. Karyotyping is performed on fetal cells obtained by amniocentesis or chorionic villus sampling (CVS). Karyotype has traditionally remained the standard method to detect the copy number and structural changes of chromosomes. The disadvantages are that it requires prolonged cell culture and small deletions or rearrangements can be missed. There is also the possibility of flagging up an 'abnormal' karyotype of no clinical significance. The average reporting time is about two weeks.

Rapid methods involving molecular genetics have been investigated and developed for prenatal diagnosis of chromosome disorders. The two most common molecular methods are fluorescent in situ hybridization (FISH) and quantitative fluorescence polymerase chain reaction (qf-PCR). The vast majority of chromosomal abnormalities are limited to abnormal copy numbers of chromosomes 13, 18, 21, X or Y. Both methods are used routinely for rapid diagnosis of aneuploidy (Trisomy 21, Trisomy 18, Trisomy 13) and sex chromosomes abnormalities (e.g. X-Turner Syndrome, XXY-Klinefelter, XYY, XXX). FISH and qf-PCR are performed on uncultured fetal cells using the invasive procedures amniocentesis or CVS. These procedures are capable of detecting more than 95% of the chromosome abnormalities, do not require cell culture and can deliver a diagnosis within 1-2 days[9]. Can molecular genetics tests be used as standalone test for prenatal diagnosis? A recent study reported that in the diagnosis of chromosomal abnormalities after the first trimester screening for trisomy 21, a policy of qf-PCR for all samples and karyotyping, only if the fetal NT thickness is increased would reduce the economic costs, provide rapid delivery of results, and identify 99% of the clinically significant chromosomal abnormalities[9]. Currently many units employing first trimester screening would perform only qf-PCR for chromosomes 13, 18, 21, X and Y unless the NT is increased above 99th percentile or structural abnormalities have been identified on ultrasound scan, in which case a full karyotype is also carried out.

(2) *Non-invasive prenatal diagnosis*

Current prenatal diagnosis of aneuploidy and single-gene requires a fetal sample collected through amniocentesis, chorionic villus sampling or cordocentesis. As these invasive procedures carry a risk of miscarriage noninvasive prenatal diagnosis is an actively researched area in prenatal medicine.

One potential noninvasive approach utilizes fetal cells within the maternal circulation as a source of fetal DNA. Another approach is analysis of cell free fetal DNA (ffDNA) in maternal plasma or serum. The third approach is using placental RNA. The main advantage of using fetal cell within maternal blood is that they offer a pure source of the entire fetal genome. Therefore inclusion of any maternal genetic material can be avoided[10]. However, the major problems are the limited number of circulating fetal cells and the absence of a reliable detection system that could avoid the confusion with similar maternal cells. Since only a small number of fetal cells circulate within maternal blood, procedures to enrich the cells and enable single cell analysis with high sensitivity are required. Recently, separation methods, including a lectin based method and automatic image analyzing, have been developed, which have improved the sensitivity of genetic analysis[10].

The reported presence of free fetal DNA in maternal serum has opened up exciting opportunities. Cell free fetal DNA represents only 3-5% of the total circulatory DNA in maternal plasma[10]. This is not a limiting factor for very sensitive molecular genetics techniques. Fetal gender in sex linked disease[11] and fetal RhD status determination in pregnancies involving RhD negative pregnant women[12] can now be reliably determined through analysis of maternal plasma using PCR strategies on cell free fetal DNA. This approach has also been used for the detection of mutations that the fetus may have inherited from the father, but which are not present in its mother (e.g. beta thalassemia when mother and father carry different mutations).

The detection of maternally inherited genetic disease, fetal single gene disorder and aneuploidy is still limited and difficult using this approach because these analyses involve fetal genetic changes that differ only slightly from the maternal genome[13]. However, in an early report all nine cases of Trisomy 21, two of Trisomy 18, one case of Trisomy 13 and six normal singleton

pregnancies were correctly identified using a 'shotgun DNA sequencing' technique[14].

A promising alternative to free DNA is provided by the presence of placentally derived cell free fetal RNA. The main advantage of free RNA over free DNA is that it is possible to select for placental-specific RNA species not expressed by any maternal tissue[10].

This area holds great potential, and it is estimated that noninvasive prenatal diagnosis will be used to diagnose a full range of chromosomal and genetic disorders.

(3) *Interventions to reduce preterm birth*

Preterm birth (PTB) is a major cause of perinatal morbidity and mortality, infant death and long term neurological morbidity. The risk for PTB is inversely related to the length of the cervix. Cervical length (CL) measured by transvaginal ultrasound (TVU) is known to be an effective screening test for the prediction of PTB[15]. The cervix starts to shorten after 28 weeks of gestation in women destined to deliver at term. Several studies have shown that 18-22 weeks is the mean gestational age when shortening occurs. Therefore, one TVU CL screening at the routine second trimester scan may be sufficient[16]. TVU CL is also predictive of PTB in women with symptoms.

It has been demonstrated that TVU CL screening has a higher detection rate and positive predictive value in women with a prior PTB and a singleton pregnancy[17]. In this group, another advantage of ultrasound screening is that it can easily detect high risk women who do not need intervention. In fact, more than 60% of the high risk women, such as those with a prior PTB, maintain a normal CL up to 24 weeks and need no intervention. Interventions that may be effective in specific populations include cerclage and progesterone.

Cervical cerclage has been available for a while, but remains controversial as an intervention to prevent preterm birth. Current evidence shows that cerclage does not lead to statistically significant reduction in preterm birth in women identified by TVU CL in population screening programs[15]. There is a statistically significant reduction in the preterm birth rate in asymptomatic women with short cervix and prior PTB (= 3 prior PTBs or second trimester losses) with cerclage. The incidence of PTB <35 weeks was 23% in the cerclage group versus 39% in no cerclage group [relative risk (RR) 0.61, 95% CI 0.40–0.92]. It should not be used in twin pregnancies for short TVU CL as cerclage is associated with higher incidence of PTD in this population[15].

Different forms of progesterone have been used to prevent PTB in asymptomatic women with short cervix. A recent trial has shown that vaginal progesterone (200mg daily from 24 to 34 weeks) effectively prevents PTB in women with CL = 15mm, regardless of their history[16].

There have been recent reports of an increasing preterm birth rate from epidemiological studies[18]. One of the causes is the higher multiple birth rate due to infertility treatment. Even if the reason for the increased risk of PTB following reproductive techniques is unclear, single embryo transfer is increasingly considered a workable clinical option, particularly in young women[19].

Future challenges

Inspite of several advances, there are challenges ahead. Some of these are as below.

(1) A large proportion of stillbirths remain 'unexplained'

Stillbirth remains a relatively common complication in pregnancy. Different classifications have been designed to better understand the possible causes. Fetal congenital anomalies, multiple gestation, alteration in fetal growth, infections, prematurity/immaturity, maternal diseases, placental abnormalities and obstetric factors have been recognized as important causes of stillbirth. However, up to two thirds of these deaths are reported as unexplained even after complete pathologic examination[20]. It has also been noticed that the proportion of fetal deaths without a known cause increases as gestational age advances[21].

Some authors have described a number of possible risk factors for unexplained stillbirth, including advanced maternal age, low educational attainment, nulliparity, parity =3, body mass index =25 and smoking[21]. Infertility and fertility treatment are also associated with an increase in unexplained fetal death[22].

The sequence of events leading to stillbirth is unknown. Consequently, neither antenatal monitoring nor preventive strategies are available at present. As the prevalence of unexplained stillbirth is very low, a test

with very high accuracy is required in order to do more good than harm.

(2) *Preterm birth rates have remained unchanged or may even have increased*

Preterm delivery can be due to spontaneous labour with intact membranes, preterm prelabor rupture of membranes (PPROM) or iatrogenic. About 30-35% of PTB are indicated (labour induction or Cesarean section for maternal or fetal indication), 40-45% follow spontaneous preterm labor and 25-30% follow PPROM. In Europe and in the USA, the PTB rate is 5-9% and 12-13%, respectively, but has risen in many locations. This may be due to an increasing indicated PTB or a consequence of multiple pregnancy conceived resulting from assisted reproductive techniques (ART). PTB accounts for 75% of perinatal mortality and =50% long term morbidity[23]. Despite the availability of many drugs to inhibit uterine activity there is little evidence that they can stop preterm birth.

Progesterone treatment has been shown to reduce the risk of preterm birth in women with a history of preterm delivery. However, majority of women who deliver preterm do not have a previous history of preterm birth. A randomised controlled trial published in 2007[16] used ultrasound cervical length measurement as a screening tool in the general pregnant population. The trial reported that in women with a short cervix, treatment with progesterone reduces the rate of spontaneous early preterm delivery. Preterm delivery rate in the placebo group was 34.4% versus 19.2% in the progesterone group (Relative risk 0.56, 95% CI: 0.36 to 0.86). While this finding is extremely encouraging, there are limitations to this strategy. Only a third of all the women who delivered preterm had a short cervix. This intervention will have no effect on the remaining two-third, who delivered preterm despite not having a short cervix on ultrasound. The overall impact of a screening program on the rates of preterm birth is likely to be modest. Given that care of premature infants is very expensive, even a modest reduction in the rate of preterm delivery is likely to lead to major savings. A model combining obstetric history and cervical length can improve the prediction of spontaneous preterm birth rather than either factor alone[24].

(3) *Prevention of preeclampsia*

Preeclampsia, along with preterm birth, remains a major cause of perinatal and maternal morbidity. Consequently, research is directed towards developing effective screening test with a view to improving the pregnancy outcome with earlier diagnosis.

Preeclampsia (PET) affects 2-4% of pregnancies. Its underlying pathological mechanism is thought to be inadequate trophoblastic invasion of the maternal spiral arteries, leading to an impaired placentation. This process of abnormal placental remodelling has been documented by both histological and Doppler ultrasound studies of the uterine arteries[25]. It is also known that risk factors for PET are maternal ethnic origin, parity, body mass index, family history of PET and smoking. In addition the maternal serum concentration of some molecules, such as pregnancy associated plasma protein A (PAPP-A) and pro angiogenic protein placental growth factor (PIGF), has been reported to be different in women who develop PET compared to women with normal pregnancy[26].

Screening on the basis of maternal characteristics alone can identify only 30% of the pregnancies destined to develop PET. At present, Doppler screening studies at 12 and 22 weeks' gestation have reported that for a 10% false positive rate the sensitivity for preeclampsia were about 40% and 50%, respectively. It is increasingly getting clear that no single test has a high accuracy. A higher detection rate can be achieved using a combination of tests. A combination of maternal history, uterine artery Doppler, maternal cardiac function, mean arterial pressure and maternal serum markers can improve the detection rate up to 95% at a screen positive rate of 5%[26].

The only proven strategy for prevention of PET appears to be the use of low dose aspirin. A large trial and a subsequent meta analysis have shown that low dose aspirin reduces the incidence of PET compared to placebo, but the reduction is modest (15%)[27]. The advantage of this treatment is that there are no major harmful effects for the mother or the fetus.

(4) *Ultrasound detection of structural abnormalities: Dilemmas in counselling*

Identification of fetal abnormalities with ultrasound can lead to difficulties with counselling. Many prospective parents are very concerned with the finding, and choose pregnancy termination as an option. However, the natural history of many of these 'malformations' is incompletely understood. Counselling should be based

on the natural history of the disorder. Sometimes it is not fully understood because it is modified by intervention, and it is impossible to predict the course of postnatal events. This is particularly true of malformations of the fetal central nervous system (CNS). The current medical uncertainty regarding long term outcome of some disorders (e.g. agenesis of corpus callosum, posterior fossa malformation) is reflected in high rates of pregnancy termination.

In women with an antenatal diagnosis of agenesis of fetal corpus callosum (ACC), 79% opted for termination of pregnancy[28]. The termination rate was slightly lower in pregnancies with isolated ACC compared to complex ACC. Therefore, only a small proportion of the population is available to study the neuro developmental outcome. The same is true for long term neurological development related to posterior fossa malformation. This is also mainly due to high rate of pregnancy termination[29].

(5) *Loss rate in monochorionic twins is still high*

Monochorionic diamniotic (MC DA) twin pregnancy is associated with a higher risk of perinatal complications, compared with dichorionic and singleton pregnancy. This excess risk is partly due to a higher rate of congenital malformations. The main reason for the risk is thought to be related to the presence of placental vascular anastomoses connecting the two fetal circulations. These connections can cause twin to twin transfusion syndrome (TTTS). MC twins always share a single placenta but not always equally, leading to placental insufficiency in one twin (selective IUGR and birth weight discordance).

An accurate diagnosis of chorionicity can be made sonographically in the first trimester. Laser coagulation of vascular anastomosis in TTTS has improved survival rate and neurological outcome. In spite of these advances the mortality of MC twins remains high. The survival rate reported after laser treatment for TTTS is 60-70%[8]. Most losses in MC twins are during the second trimester and are mainly due to TTTS.

(6) *Fetal interventions*

Intrauterine fetal transfusion for Rhesus disease is one of the greatest success stories of invasive fetal intervention. The aim of fetal surgery is to interrupt the in-utero progression of a fetal disease. In many situations neither in-utero nor neonatal intervention are available to treat the anomaly, and termination of pregnancy may be the only possible intervention.

Fetal obstructive uropathy was a condition for prenatal intervention using a vesico amniotic shunt. This intervention reduced the risk of lung hypoplasia, and made survival a real possibility. Unfortunately, medium to long term outcome of such fetuses is not encouraging[30]. There is a high risk of end stage renal disease requiring renal transplantation and voiding difficulties. A randomised trial assessing the benefit of prenatal intervention in congenital diaphragmatic hernia was halted early due to the unexpectedly high rate of survival in the control arm[31]. Over the past two decades successful fetal treatment has also been performed for spina bifida, congenital diaphragmatic hernia, cystic adenomatoid malformations, and stenotic cardiac valves[32-34]. However, it is not clear if the invasive treatment can cause additional disabilities. Therefore, more studies are required in order to establish if in utero treatment is better than optimal postnatal care.

Conclusion -

Major advances have taken place in the field of fetal medicine, and there are exciting times in the future. Newer advances have created their own set of problems. Despite the improvement in our understanding many challenges remain unconquered. Improvement in our understanding reveals the complexity of the problems, and the realisation that no single strategy is likely to work for complex problems such as preeclampsia and preterm birth.

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