In case you missed it: the *Prenatal Diagnosis* editors bring you the most significant advances of 2015

Jan Deprest[1,2], Alessandro Ghidini[3], Tim Van Mieghem[1,2], Diana W. Bianchi[4*],

Brigitte Faas[5], Lyn S. Chitty[6]

[1]*Department of Obstetrics and Gynecology, University Hospitals Leuven, Leuven, Belgium*

[2]*Academic Department Development and Regeneration, Biomedical Sciences, KU Leuven, Leuven, Belgium*

[3]*Department of Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC, USA*

[4]*Mother Infant Research Institute, Tufts Medical Center, Boston, MA, and Floating Hospital for Children, Boston, MA, USA*

[5]*Department of Human Genetics, Radboud University Medical Centre, Nijmegen, the Netherlands*

[6]*UCL Institute of Child Health, Great Ormond Street Hospital for Children and NHS Foundation Trust, London, UK*

*Correspondence to: Diana W. Bianchi. E-mail: dbianchi@tuftsmedicalcenter.org

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At our annual meeting, it has become a tradition for the editors of *Prenatal Diagnosis* to reflect upon the advances in prenatal diagnosis that have occurred over the previous year, assess all of the relevant journals and summarize what we think were the key events in a brief year-end review. Although this represents the opinions of the editors, we believe that our readers appreciate this summary, as our prior reflection on the advances in 2014 was one of our most frequently downloaded articles. This year the editors of *Prenatal Diagnosis* met in October in a mild and sunny Boston to make plans for the Journal's next year and to celebrate the significant increase in our impact factor to 3.27. We thank our authors and our reviewers for submitting excellent work, and through their constructive criticism, improving the submissions during the review process.

Here we present our highlights of 2015. We begin with non-invasive prenatal testing (NIPT) based on cell free DNA, including the unexpected surprises and dilemmas it poses for patients, their physicians and commissioners of health care. We then move to summarize the recent literature on placental function evaluation and assessment of fetal well-being before ending where we started last year with *capita selecta* from the fetal surgical literature.

Advances in Cell-Free DNA Testing

As reported previously, NIPT for aneuploidy is now available across the globe, largely through the private healthcare sector, but there are several countries contemplating adoption into their public sector maternity care pathways [1]. Until recently the majority of studies reported only on the performance of NIPT in high risk populations, but two publications in 2015 confirmed strong performance in pregnant women with general risk. The first was a study designed to compare NIPT with standard first trimester Down syndrome screening. It showed that the positive predictive value (PPV) for the women with a positive NIPT test for Down syndrome was 80.9% (66.7-100), and 50% (24.7 – 75.3) for low risk (<1/270) compared with a 3.4% (2.3 – 4.8) PPV for standard first trimester combined testing[2]. The second study was a report on the clinical performance of NIPT for the detection of the major autosomal trisomies in both high and low risk pregnancies in a cohort in which pregnancy outcomes or karyotyping results were available in 76% of the 147,314 pregnancies tested[3]. These authors showed that there were no significant differences in sensitivity and specificity in the high risk (maternal age >35 years, Down syndrome screening risk >1/270 or 1/300, sonographic abnormalities etc.) versus low risk. The results of these studies open the way for more widespread clinical application of NIPT. In fact, the American College of Obstetrics and Gynecology's most recent recommendation no longer precludes the use of NIPT in low risk women[4]. The clinical availability of NIPT has largely been driven by

commercial organizations that promote their individual tests. The excellent performance of NIPT and in particular the very high negative predictive values have resulted in a dramatic reduction in the need for invasive diagnostic procedures[5]. These successes inevitably attract media attention and commercial organizations compete for business with widespread advertising in a variety of media. In addition to the counseling received from their health professionals, these sources may thus influence couples considering non-invasive testing. Unfortunately, evaluation of the quality of patient information leaflets from five commercial providers of NIPT found that none of the pamphlets included all of the content recommended by professional bodies[6]. Furthermore, surveys of newspaper articles describing non-invasive testing in the UK[7] and the USA[8], as well as a review of 40 different websites[9] found that whilst some articles contained balanced information, the majority focused on the benefits of NIPT. These included avoiding the risk of miscarriage but did not always address concerns or limitations of the technology, such as the need for an invasive test to confirm a positive NIPT result or the possibility of inconclusive or failed test results. These findings highlight the need for high quality education so that health professionals are in a position to accurately inform expectant couples of both the benefits and risks when considering testing.

Beyond aneuploidy testing there have been further developments in non-invasive prenatal diagnosis (NIPD) for monogenic disorders. The use of sequencing panels to screen for multiple alleles for the exclusion of the paternal mutation has facilitated the clinical implementation of NIPD for some dominantly inherited skeletal dysplasias[10], for cystic fibrosis where parents carry different mutant alleles[11] and for beta-thalassaemia[12]. In addition, the feasibility of reliable NIPD for Huntington disease by detection of the paternally inherited expanded CAG repeat in maternal plasma has been demonstrated[13]. It is good to see increased efforts directed towards the development of NIPD for monogenic disorders as families are often at extremely high risk and very keen to access NIPD. However, as there is likely to be increased demand for these safer tests with parents requesting NIPD to prepare themselves for the birth of an affected child rather than interrupt the pregnancy, there is potential to add significantly to the cost of prenatal diagnostic services[11].

The Biological Basis for Discordant NIPT Results

During the past year, knowledge has increased significantly regarding the underlying biological bases for discordance between NIPT results and the diagnostic fetal karyotype. Confined placental mosaicism (CPM) is thought to occur in up to 1% of pregnancies. Because the cell-free DNA in maternal plasma derives from the trophoblast, it would be expected that CPM is the underlying basis for many discordant NIPT cases. Whenever aneuploidy is

detected by NIPT, the international standard of care is to perform a diagnostic procedure to confirm the fetal karyotype. However, until this year, it was not known whether the type of follow-up diagnostic procedure (amniocentesis or CVS) was important. In 2015 Grati and colleagues used an existing database of 52,673 fetal karyotypes in which cytotrophoblast, mesenchyme, and amniocyte karyotypes were available to estimate the frequency with which CPM was present[14]. The surprising results showed that there was a difference in the likelihood of finding cytotrophoblast mosaicism according to the fetal karyotype. Mosaicism was relatively low for trisomies 21 (2%) and 18 (4%), but significantly higher for trisomy 13 (22%) and monosomy X (59%). These results have an immediate practical application in that they provide evidence upon which to base recommendations for the type of follow-up test for abnormal NIPT results: for trisomies 21 and 18 a CVS can be performed (with a 2-4% chance of detection of mosaicism), but for trisomy 13 and monosomy X it is better to wait to perform an amniocentesis to determine the true fetal karyotype.

During the past year maternal DNA copy number variations (CNVs) were also shown to be an important reason for discordant NIPT results[15, 16]. In one study, a single nucleotide polymorphism (SNP)-based NIPT assay had a "no-call" result for chromosome 18 because of an "atypical finding outside the current scope of the test"[16]. On amniocentesis, a very large (16.1 Mb) pathogenic duplication of 18q12.1-18q21.1 was present in the fetus. Detailed work-up showed an identical duplication in 20% of maternal peripheral blood lymphocytes. The mother was clinically asymptomatic, likely because she was mosaic for the duplication. In another study, peripheral blood DNA samples from four women with discordant results for trisomies 18 (n=3) and 13 (n=1) were sequenced and compared with infant cord blood DNA for the presence of copy-number variants (CNVs) of greater than 250 kb. In two of the cases, smaller maternal CNVs (1.15 Mb and 487 kb) were identified on the short arm of chromosome 18. These appeared to have no clinical consequences for the mother. In the setting of a maternal CNV, the following additional factors might influence whether or not a sample is called as "aneuploidy detected': the total number of reads per sample, the coefficient of variation for the chromosome being analyzed, the fetal DNA fraction, and whether or not the fetus inherited the maternal CNV. Importantly, CNVs are unaffected by maternal age, so their clinical significance as a cause for false positive results may increase as DNA testing expands to the general risk population. Accordingly, individual laboratories are adjusting and refining the bioinformatics algorithms used to analyze their data[17].

A third explanation for discordant NIPT results, maternal malignancy, was first described in *Prenatal Diagnosis* in 2013[18]. This finding received heightened attention during the past year, with the retrospective analysis of genome-wide sequencing data in eight women who had false positive NIPT results and a clinical diagnosis of cancer[19]. Detailed bioinformatics analyses showed unique patterns of copy-number gains and losses across

multiple chromosomes. In three of the eight women, the unusual NIPT results (that included autosomal monosomies and multiple aneuploidies) prompted additional testing that resulted in the diagnosis of malignancy. In Belgium, three women whose NIPT genome-wide results were suspicious for malignancy were referred for whole-body diffusion magnetic resonance imaging[20]. These studies revealed an ovarian carcinoma, and follicular and Hodgkin lymphomas. CNVs in the tumor biopsies were concordant with the genome-wide abnormalities detected in maternal plasma. To further confound the issues, discordant results have also been found in the presence of benign maternal uterine leiomyoma[21].

Although discordant NIPT results are uncommon, they highlight the need for thorough pre-test counseling[22] and in particular, a description of the possible incidental findings that can be detected in the maternal DNA that is sequenced at the same time as the fetal (placental) DNA[23].

Prenatal Evaluation of Placental Function

2015 has been an important year for prenatal assessment of the placenta. Despite its crucial role in the health of both the fetus and the mother during pregnancy, as well as lifelong effects on their health, the placenta is the least understood human organ. To overcome this lack of knowledge, the National Institute of Child Health and Human Development (NICHD) has launched the Human Placenta Project, with the ultimate goal of understanding human placental structure, development, and function in real time[24] (http://www.nichd.nih.gov/hpp). The initial awards have been funded through this project, which will ultimately involve an investment of 46 million US dollars.

Abnormal placental implantation is thought to be responsible for a proportion of clinical manifestations of placental dysfunction, particularly those occurring during the late second and early third trimester. Demographic, clinical, maternal serum, ultrasonographic and Doppler factors can be combined to identify pregnancies with abnormal ongoing placental implantation, and are thus at high risk for complications. Subsequent imbalance of angiogenic factors can be monitored via promising biophysical and biochemical markers [25,26]. However, most cases of placenta-related obstetric complications that occur during the 3rd trimester, including fetal death, are not due to primary placenta pathologies[27], but rather to acquired or secondary placental disorders (e.g. thrombi in the basal plate, extensive fibrin deposition, chronic villitis, and villous fibrosis, among others). Another emerging concept is that of the capacity of the uteroplacental circulation. Appropriately implanted placentas may have an upper limit of such capacity, which contributes to the increased rates of adverse perinatal outcomes noted at or near term in the presence of appropriately grown fetuses[27].

New approaches and technologies will be required to achieve the goal of understanding how the placenta changes over time and what normal development and

function look like so as enable the timely diagnosis of pathology. For example, in animal models shear wave elastography has been shown to provide quantitative elasticity measurements of the placenta[28], as increased placenta stiffness may be indicative of pathology and indeed seems to be associated with fetal growth restriction. Magnetic resonance spectroscopy can provide information on placental metabolism non-invasively[29]. Transcriptome analysis of placental gene expression can also be used to identify useful biomarkers of placental pathologies[30]. However, such techniques of direct functional assessment of the placenta are too cumbersome or expensive for mass screening purposes. Meanwhile, available technologies may provide some indirect functional assessment of the placenta in real time. For example, Doppler assessment showing an abnormal cerebroplacental ratio is associated with increased risk of adverse pregnancy outcome and indicates the need for urgent delivery and NICU admission independent of birth weight[31].

The completion of the Pregnancy Outcome Prediction (POP)-study and the Truffle-trial were two more steps forward to overcome the challenge of managing intrauterine growth restriction (IUGR). The POP-study showed that, in nulliparous women, routine 3rd trimester ultrasound studies detect growth restricted fetuses better (57% sensitivity) than selective ultrasound examinations limited to women with a clinically small fetus (20% sensitivity), thereby making a strong case for routine 3rd trimester imaging[32]. On the other hand, the Truffle study was a randomized controlled trial comparing computerized cardiotocographic (cCTG) assessment of short term heart rate variability with ultrasound assessment of ductus venosus flow to time delivery of the IUGR fetus[33]. The primary outcome of this trial (survival at 2 years without neurodevelopmental impairment) was not significantly different between the two management options (77 versus 85%). Moreover, no matter what method of surveillance was used, the clinical outcomes were exceptionally good, with much lower than expected rates of perinatal death and neurologic impairment, suggesting that cCTG and ultrasound are both valid monitoring options. This trial certainly provides strong data with which to counsel expectant couples. However, its limitations should also be noted: first, over 50% of women in the trial were delivered for reasons other than their indicated monitoring method (maternal pre-eclampsia, 'safety-net' criteria, out of protocol). Second, the lack of a clear difference between study groups was partly due to a divergence in the two components of the composite primary outcome: a small increase in deaths in the group monitored by ultrasound examination was offset by a reduction in neurodevelopmental impairment in that group. Some parents may therefore prefer one monitoring method over the other. Further reports on this cohort are eagerly awaited.

Advances in the Treatment of Maternal Conditions

Reassuring data regarding maternal cancer was published in 2015. The International Network on Cancer, Infertility and Pregnancy (INCIP) reported on the outcomes of 129 fetuses of mothers diagnosed with cancer during pregnancy[34]. Over 80% of them underwent chemo- and/or radiotherapy while pregnant. Developmental and cardiac outcomes in fetuses exposed to treatment were similar to controls matched for gestational age at delivery. The main determinant of adverse neurodevelopment in this study was prematurity, which should be prevented whenever compatible with maternal care.

2015 started also with an absolute "premiere", a true new perspective in fertility. The Swedish team around Brännström reported on the first baby born after uterine transplantation in a woman with congenital absence of the uterus (Rokitansky syndrome)[35] . Although this birth was a huge scientific success, concluding years of well-planned and well executed basic and human research, the ethical challenges for the medical community will now need to address the complications and potential harms caused by this procedure[36, 37] . From the perspective of the offspring, this includes finding ways to prevent, diagnose and treat preterm birth, pre-eclampsia, and IUGR.

Fetal Therapy

Spina bifida repair

Despite the highest level of evidence provided by the *Management of Myelomeningocele Study* (MOMS), fetal surgery for spina bifida has not been equally enthusiastically embraced in all parts of the world for several possible reasons. First, fetal surgery may improve outcomes yet it is not a cure. Further, there are the associated risks of preterm delivery (13% deliver <30 weeks and 33% <34 weeks) and cardiac failure during surgery[38, 39] . Then there is the maternal morbidity associated with open repairs, which can now be (partly) avoided if the surgery is done fetoscopically. A substantial literature on this modality has become available, the largest (n=71) by Graf *et al,* using a three-port technique[40]. In the Americas, Pedreira *et al* also translated her experimental work into a clinical program[41], and just a few months ago, the fetoscopic approach was also revived in North-America[42]. At present it is difficult to judge the exact place of this investigational approach to fetal spina bifida repair. The debate remains intense[43, 44] with the published literature reporting overlapping series, none of which are controlled. In addition, there is variation in operator experience, technical approach and outcome reporting, all of which make inter-study comparisons very difficult.

In a systematic review comparing results of cases operated on by either open or by fetoscopy "beyond the learning curve" techniques, fetoscopic repair had a comparable perinatal mortality (7.8% vs. 2.6%, p=0.212) and shunt rate at 12 months (45% vs. 40%,

p=0.619), yet took longer (223 vs. 105 min, p<0.001) and had a higher preterm pre-labor membrane rupture rate (84% vs. 46%, p<0.001), earlier gestational age at birth (32.9 vs. 34.1 weeks, p=0.03), and higher postnatal reoperation rate (28% vs. 2.56%, p<0.001)[45] . Though fetoscopic repair may offer neuroprotection, it has not yet solved the problems with prematurity and membrane rupture, so currently the advantages are only to the mother. To address these issues, many groups are working hard on innovation of instruments, coverage techniques and cell therapeutic and tissue engineering approaches in preclinical settings[46-51]. It is to be hoped that these newer methods will not be implemented without proper comparisons to the standard technique.

This year, secondary analyses and longer term follow-up reports on layered neurosurgical repair through hysterotomy became also available. These clearly demonstrated that the need for postnatal shunting is predicted by ventricular size at the time of surgery with the strongest effect when ventricles are < 10 mm (shunt rate 45% as compared to 79% for postnatal repair). When the ventricles measured >15 mm there is no difference in shunt rates. The level of the lesion as well as hindbrain herniation does not have an impact on shunt rate[52]. Prenatal surgery did not reduce the need for intermittent catheterization at 30 months but did reduced bladder trabeculation and the presence of an open bladder neck[53]. However, the clinical relevance of these findings is an open question.

Congenital Diaphragmatic Hernia (CDH)

The outcome of CDH is usually predicted by imaging to measure liver herniation and lung size. The predictive value of stomach position was first described when using Magnetic Resonance (MR)[54] , later revived for ultrasound (US)[55] , and has now been confirmed[56-58]. Whether this is an independent predictor remains uncertain. Others suggest using serial measurements for prognostication, but this may not be practical[59,60]. Far less is known about the uncommon right sided CDH, however lung size, determined by US or MR, seems to be predictive but the values for severity of hypoplasia used for left sided lesions cannot be interchanged with those used for right sided lesions[61]. Studies dedicated to prenatal prediction of short-term morbidity remain scarce but it seems that the above predictors, as well as vascular reactivity, can predict pulmonary hypertension and short term pulmonary morbidity[62,63]. One very large French study came to the conclusion that gestational age at diagnosis can predict both mortality and morbidity[64].

A recently reported novel approach for predicting severity is to use biomarkers. When analyzing the microRNA profile of severely hypoplastic lungs from fetuses undergoing tracheal occlusion (TO), there seems to be a specific miR-200/miR-10a signature[65]. Whilst not predictive at baseline, survival after occlusion was associated with increased miR-200

expression, a factor linked to TGF-beta signaling. Modern genetic techniques were also used for the further elucidation of the pathogenesis of CDH as well as exploring the mechanisms underlying TO. Whole transcriptome analysis to compare the effects of TO on surgically induced hypoplasia demonstrated induction of a gene expression pattern that was largely comparable of that of normal lungs[66]. Transcriptome analysis will also help to identify novel targets for future therapies.

Fetal endoscopic tracheal occlusion (FETO) is currently being investigated in a global randomized trial (www.totaltrial.eu). The first interim analysis in moderate cases (n=80) will be used to determine the ultimate sample size. This year, centers in Brisbane (Australia) and Toronto (Canada) obtained ethical approval, and the FDA approved the use of the study instruments and devices. As most centers are participating in this trial, they cannot report on their data. Thus, as yet, there are relatively few clinical studies on FETO. One study reassessed the relationship of gestational age at FETO and the subsequent increase in lung volume, demonstrating a lower response as pregnancy proceeds[67]. This supports the strategy of the TOTAL trial to perform FETO earlier in severe than in moderate cases. A side-effect of FETO remains preterm delivery, and an attempt to prolong gestation using a pessary postoperatively failed[68]. Further work is needed to identify medical solutions. Transplacental sildenafil is probably the closest to clinical application given that its efficacy, previously demonstrated in rodents, is now confirmed in a larger animal model, justifying a clinical trial[69,70].

Twin-Twin Transfusion Syndrome (TTTS)

Laser coagulation of placental anastomoses for TTTS has been available for 25 years, yet only became the standard of care just over 10 years ago[71]. This year, a number of studies were published on medium-term outcomes. Gschliesser *et al.* found that TTTS predisposes to retinopathy to a greater extent than can be explained by prematurity[72]. Chmait *et al.* showed that the cerebro-placental ratio (CPR), a semi-quantitative marker for fetal brain-sparing, predicted a poor neurological outcome at the age of two years when performed postoperatively, but neither the CPR pre-operatively or Quintero stage were predictive[73] . Though improvement of surgical technique by lining the vascular equator (Solomon technique) reduces the risk for feto-fetal transfusion syndromes, it apparently does not further improve neurologic morbidity[74,75]. TTTS also has an impact on fetal hemodynamics. Recipients are prone to pressure and volume overload, while donors may develop hypovolemia and are more often exposed to increased placental resistance. Some of these changes may persist or incompletely reverse after laser treatment, hence they have postnatal consequences. Chmait's group demonstrated higher blood pressures in both donor

and recipient survivors at the age of two years[76]. Another consequence is right ventricular outlet tract obstruction (RVOT). In a very large study by Ville's group (n=1052), RVOT occurred in 2.1% of cases, which was less than anticipated[77,78]. On the technical side, several groups are working on training models as well as new imaging methods, with the purpose of training new teams and/or improving results even further[49,79,80]. Indeed, the first hands-training course was held this year at the annual International Society for Prenatal Diagnosis meeting that was held in Washington, DC.

Preimplantation Genetic Testing

In the preimplantation setting, microarrays have already been introduced as a replacement for FISH in many laboratories. Recently, some groups compared the sensitivity and specificity of next generation sequencing (NGS) as a replacement for arrays and concluded that indeed both sensitivitities and specificities are comparable[81-83]. Moreover, Fan *et al.*[84] reported on the validation and clinical application of a NGS technique that not only allows for a comprehensive aneuploidy screening but also for the detection of pathogenic subchromosomal copy number variations.

The use of NGS requires more DNA and is more time-consuming than FISH or arrays. Therefore, for the time being, it can only be carried out on 5-day old blastocysts in combination with cryopreservation. Furthermore, NGS equipment is not available in all IVF laboratories. Broad implementation will therefore probably require more time, but will eventually find its way into clinical application in the preimplantation setting. Whether or not NGS (or another technique) should be used as a preimplantation genetic screening tool, to select the most viable embryo, is still a matter of debate[85].

Conclusions

Our selection of research papers was certainly biased, highly influenced by our own research and clinical interests. If we have forgotten other fields, please forgive us, but feel free to point out our omissions. Better still, submit your work for publication in *Prenatal Diagnosis* and become a feature in our reflections on 2016, or join our community on Facebook at https://www.facebook.com/pages/Prenatal-Diagnosis-Journal/131137730416347). Happy 2016!

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