Prenatal Diagnosis Over 25 Years – A Different Perspective

Editorial

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Some may not have thought that this year was particularly "interesting" for prenatal diagnosis. But the ramifications of the recent breakthroughs in the commercial and research molecular worlds have put controversy in our clinical footsteps. It is well known that as our ability to isolate abnormalities in ultrasound examinations improves, our false positive rates also rise with the new sonographic technologies. We now see much more than we could decades ago (remember those B-mode scans?). Thus, the storm of testing prenatally is bringing in a new weather front. Many sense that it will get worse before we ride out through the eye and yet another storm comes through - just hopefully not as bad. Our professional societies need to balance the ever advancing technology with often delayed guideline updates while clinicians must balance these advances with an increasingly informed patient population.

Why do I fear that the perfect storm is coming to prenatal diagnosis? Emotion, technology and economics are all heading on a collision course right for us. Fortunately, these are not the three evils, but they do each move against the general momentum of medical culture. The medical world, unlike the business world, does not abide by the mantra, "adapt, innovate, or die." In fact, being clinically conservative is in many ways, embraced as a good thing. To what am I referring? Being clinically conservative is a comfort zone (you know what you know and do what you do) and, in balance, does no harm. In other words, "if it ain't broke, don't fix it." Better mousetraps do come along, so what drives acceptance within the medical community? This depends. Let's look at one example, first trimester screening.

First trimester serum biochemical screening was first introduced into US medical practice in 1999. It took a number of years, even after the FASTER trial, to achieve "bicoastal" acceptance and peak only a decade later. However, clinical acceptance has not been universal. Even today, there are a number of regions in the United States were a "Triple" or "Quad" screen are the primary screening tool for Down syndrome. Excuses for not implementing the "new" first trimester approach (the generally accepted current standard of care) include the lack of ultrasound service, personal (professional and patient) preference, insurer non-coverage, and economics. Does it truly make sense to keep a screening test with a substantially greater false positive rate and lower sensitivity for the same outcome? Regrettably, some believe it is. The data indicate otherwise.

First trimester screening (FTS) with nuchal translucency and biochemical markers is far superior for the detection of Down syndrome than a Triple or Quad screen [1]. The addition of specific ultrasound markers (e.g., a nasal bone presence/absence) makes FTS performance even better, and the argument to adopt technology advances even more compelling. Over the last two decades, a huge and credible amount of literature of what the biochemical markers may mean for pregnancy outcome has been amassed. That information cannot be ignored or cast aside. Some of these markers may actually detect other disorders besides open neural tube defects. For example, Smith Lemli Opitz (SLO) and other inborn errors of fetal cholesterol metabolism can be detected reliably given a low unconjugated estriol (uE3) levels. Those opposed would argue that such findings are so rare that most clinicians will never see one. As it turns out, they are far more common than expected.

It had been generally accepted that the carrier risk for this class of rare disorders was on the order of 1/100; thus, the expected frequency for an affected pregnancy would be close to 1/40,000. Recent population studies prove otherwise. The frequency of affected fetuses is greatly underestimated. Expanded carrier screening documents a carrier frequency, in the Northern European population, of 1/50 and therefore a population frequency for affected fetuses of 1/10,000. Given this information, we should be screening for SLO as we do for other "common" genetic disorders with similar carrier frequencies as suggested by our respective societies. In doing so, how would we address cost, advocacy, education, and complexity? The general obstetrician has much on her or his plate these days. Adding more genetic disorders for them to explain to patients further saturates their busy schedules, limited already by time and suboptimal genetic education. This leads to misinformation or misinterpretation. Who should do this job and how should the information be presented to the patient? These questions are the rain before the storm.

Karyotypes of amniocenteses and chorionic villus samplings are now almost a thing of the past. This is probably a good thing, but not for the reasons that you may be think. The fact is that karyotypes are not as accurate as you may think. The chromosomal material obtained prenatally has far less than 50% of the number of bands that are normally seen in non-prenatal specimens. Chromosomal banding patterns identify and are specific for each chromosome. Condensation of the band patterns reduces

the accuracy of testing and therefore increases the false negative rates for the detection of chromosomal rearrangements, including translocations, deletions and duplications. Even if translocations are identified, the risk of identifying them as balanced or not, is highly improbable.

Since 2005, chromosomal microarray (CMA) has been available for prenatal samples and is now the standard of care as a first line diagnostic test in Pediatrics for children requiring cytogenetic evaluation after evolving from array comparative genome hybridization (aCGH). The pediatric literature had documented well over 35 000 cases using a CGH for diagnosis. Since then the number of microdeletions and microduplication syndromes has increased exponentially, all with variable expression. There are now many studies that have demonstrated the accuracy and clinical utility of CMA analysis in the prenatal arena. In 2009, the American Congress of Obstetrics and Gynecology (ACOG) began recommending the use CMA as a second line analysis after a "normal" karyotype result in an anomalous fetus. There is extensive literature to suggest that CMA is definitively important in the interpretation of an anomalous fetus, overall and more so with specific anomalies. Why, then, are many Maternal Fetal Medicine specialists, academic or private, hesitant to use this test, even when it is absolutely better than the "Gold Standard"? Not only is CMA better than the karyotype, but it also detects the deletions at the level a karyotype should with far better accuracy. In other words, karyotyping misses 30% of the deletions and duplications that are larger than 10Mb, which is certainly at the resolution of the microscope. These karyotypes are initially read as normal but the rearrangements were detected and characterized by the CMA serially done thereafter. It is now known that CMA gives an additional 5-10% positive rate when there are anomalies present, after numerical aneuploidy is no longer an issue. It is also found that in populations where the ultrasound showed no findings there is still a 0.5-2% risk of a clinically significant microdeletion or duplication detected in an advanced maternal age, abnormal Down syndrome screen (FTS or Quad), or "worried-well" populations. This clearly exceeds the perceived procedural risk of an amniocentesis or well-trained clinician who does CVS. So the excuse of not doing CMA that I have heard are about unknowns (Variants of Unknown Significance – VOUS). The other excuse is not detecting "balanced" translocations. Since the early days of karyotyping blood in the 1960s, laboratories, laboratory directors and clinicians have been dealing with unknowns. Now that our CMA databases exceed 250 000 samples, prenatally, there is enough experience to say what is thought to be clinically significant and that 1-2% of all samples are classified as VOUS.

Additional anxiety for some clinicians occurs when a dominant gene is involved in a deletion or a recessive gene involved in duplication, because there is a possibility that these may be expressed if there is a mutation present on these alleles. Unknowns are part of the genetics business. There are no absolutes. Definitive lines don't exist and this makes some uneasy in the interpretation and counseling. Should we tell our patients everything that is present, VOUS and all, or should we only give the news that is known to be as true as much as true can be? Some clinicians have a great difficulty dealing with these nuances and there are no good guidelines, or educational tools, in conveying this information to patients.

The commercial and academic laboratories that provide testing for us give as much information as possible. But the art and science of medicine is to interpolate and portray this information to the patient in an understandable way. If the comfort level of a clinician is such that this information makes themselves uneasy, the patient will detect it. Referring patients to someone who knows and is trained in giving this type of information is the proper course of action, and is not a sign of weakness. It provides a clear signal that your patient's well-being is the priority and not one's ego.

Balanced translocations are another excuse that make people shy away from using CMA. Missing a "balanced" translocation is not bothersome to me. Why? 0.5% of the population has balanced translocations, with a good portion of them with a normal variation inversion on chromosome 9. For people who do have balanced translocations, their reproductive history will usually flag them to be detected. Finally, if the fetus has multiple anomalies, then the fetus is not balanced. Current CMA laboratories use single nucleotide polymorphisms (SNP), which help detect microdeletions below 100Kb and microduplications down to 500Kb, or less, in the higher dense builds, then one can be very certain (never 100% though) that the translocation is truly balanced. SNP arrays have other issues, including uniparental disomy (UPD) (segmental or whole chromosome) and can detect degree of relatedness (consanguinity). These may be awkward to the clinician discussing the results and sometimes even embarrassing since fidelity may be an issue. But the clinical significance to loss of heterozygosity among chromosomes that involve mutated autosomal recessive genes may express a disease. Thus, the basic knowledge for the degree of relatedness and the possibility of a recessive disorder is important. In addition, the concept of UPD is important given the number of disorders known to be caused by this phenomenon from specific chromosomes. These disorders usually have corresponding microdeletion syndromes. Again, these patients can be referred to someone who is adept, and comfortable, discussing these issues with patients. But we are not even in the heaviest part of the storm.

In 2011, noninvasive prenatal testing (NIPT) became commercially available. The selling of the Holy Grail of Prenatal Diagnosis thus began and a dream of many since the early 1990's with millions in federal and private funding backed the concept. It is now a reality. Today, now five years later, this test is widely recognized for its technologic advances but no longer the pure diagnostic gold we had hoped for in the 1990's. But it has demonstrated itself, over and over, to be by far the best performing screening test for fetal Down syndrome. The source of DNA used in NIPT is the placenta and the placenta is a very active metabolically and genetically. It is the only organ programmed for death, at a very specific time. The placenta is an actively apoptotic organ. How this timing is triggered is not known. The placenta is also the most active endocrinologic organ known. Thus having genetic mosaics, chimeras and other "imbalances" are not so unusual nor should be surprising. NIPT has been studied, in a multitude of techniques, and shown that it is an excellent test detecting trisomy 21, slightly less for trisomy 18 and trisomy 13, for various clinical and technical reasons.

The laboratory developed NIPTs available on the market today

vary in assay technique, ability to provide a result, and detection performance. It is therefore the responsibility of the clinician to understand and know these differences. These tests are not the same. The question is, do you believe what the laboratory data says without additional independent confirmation? In the genetics world, testing is done all the time without independent studies. However, the call for large independent studies for these laboratories is difficult, time consuming, costly and almost impossible due to beta error. Thus, a head-to-head study using all companies/techniques via a National or International study screening of >200 000 people or more to answer the questions of which has better detection, at what level of fetal fraction, no call rates, false negatives, false positives, and general population screen. All with follow up for all pregnancies and done independently with confirmation study groups for surveillance. When this study would be over, the labs would have certainly modified their methodology through typical quality improvement processes. We have seen this countless times such as in the FASTER study not adding nasal bone calculations, or even in the NIPT world where companies have changed methods within the study or after a study has been published, claiming equal or better detection rates. The detection of maternal cancers has been a plus for those who have been discovered serendipitously. The sex chromosomes have been particularly hard in detecting given that aging mothers are themselves known to be mosaic for Turner syndrome physiologically. Mothers who have unknown chromosomal abnormalities, such as 47, XXX, may change the results of the fetal NIPT result. Thus, fetal rescue has been a question, and problem, for most of us who do CVS. This appears to be more common with trisomy 13 and the sex chromosomal abnormalities and less so with Down syndrome and trisomy 18. However, the caveat that is not mentioned or taught is that despite a normal amniocentesis, the fetus still can be mosaic for one of those chromosomes.

I learned a lesson while a fellow in clinical genetics caring for a patient who continued her pregnancy with a child diagnosed as having trisomy 13, in all cells, via blood analysis after birth. I saw him at his yearly appointment of three years old. This child did not have a heart defect, facial clefting nor polydactyly. Skin and blood sampling were redone, over 100 cells counted in each – all trisomy 13. No mosaicism. The child needed a bronchoscopy which we collected cells for interphase FISH. Mosaicism found. Thus the endodermal lineage was mosaic. Was that enough to "protect" the rest of the fetus and child? I cannot tell you what this child's placenta would have been nor NIPT, but I can say there are more things we will need to discover and what the exceptions are and the causes for the false positives and negatives in these tests. To me, as a Maternal Fetal Medicine specialist and Clinical Geneticist, I want to know if the placenta is mosaic for aneuploidy, primarily for growth and abnormal placentation issues. Some MFM specialists don't want to deal with the placental mosaic issues and thus did not learn CVS or shy away from CVS. Guess what? These placental issues, and more, are just beginning to be realized by NIPT and therefore placental genetics is back to haunt you. More to come. This is the storm.

As NIPT expands into the microdeletion arena and now with the advent of one company making available a full molecular karyotype, with a resolution to 7Mb (at the current karyotype threshold when done via a light microscope), we will learn more about exceptions. The professional societies have had a very difficult time recommending any of these tests. Given the prevalence of aneuploidy in the higher risk population, it is very reasonable, at today's price point, to offer the test to the higher risk population. Now that NIPT is expanding into the general population, will prices fall? When it becomes commonplace, what will become of biochemical testing and first trimester ultrasound? Utilization of first trimester ultrasound should not disappear. Between our newer ultrasound machines and their outstanding resolution along with our better knowledge of fetal anomaly progression, is all due to the FTS nuchal translucencies experience. It is reported that we are now able to detect 60-90% of all major anomalies in the first trimester. But what about biochemical testing? Should we continue to obtain PAPP-A, beta hCG, alpha fetoprotein, uE3, Inhibin? We have learned that abnormalities in these tests not only tell us about possible structural abnormalities and genetic disorders themselves but also about the healthiness of the placenta, fetus and placentation. They have also been used to predict intrauterine growth restriction, placenta accreta, preeclampsia, placental abruption, and fetal demise. This is where the clash occurs. We need to integrate our knowledge to look at maternal and fetal outcomes. The data is there over the last decade. It just needs to be teased out.

There are still a number of "next steps" for NIPT. Deeper and higher density molecular karyotyping, getting to the level seen today by our SNP arrays, and RNA noncoding testing. Recently, microdeletions detected by current NIPT technology, that is publically available, detects to the resolution of 3Mb. This was the resolution of CMA, via array comparative genomic hybridization in the early 2000's [2]. The molecular karyotype available goes to a resolution of 7Mb. So, what is the issue? There are many deletion syndromes where their deletions are below the 7 or 3Mb mark. This is why "detection" rates not only change, but one has to be careful in how to read the literature. For example, the majority (about 85%) of affected individuals with DiGeorge syndrome (22q11 microdeletion) have a 3Mb deletion covering 40 genes. Why is this important? Just like with the light microscope karyotype, the resolution of the molecular testing will miss a number of these patients, depending on the resolution. Even the NIPT tests targeting microdeletions, there is a reasonable proportion of fetuses affected by DiGeorge that will not be detected until the density increases and gets down into the 100's of Kb resolution. Thus, NIPT is not the diagnostic test of choice, CMA is. Moreover, NIPT will not be diagnostic until fetal DNA can be accessed directly, and not just placental DNA. Clearly, DiGeorge syndrome is a significant disorder for all stages of life, including adults. It is said that 60% of all adults affected by DiGeorge will have a psychological diagnosis, of which 25% with Schizophrenia. These are retrospective studies, but suffice it to say, there is a significant dosage affect. The current population frequency is approximately 1/2500 individuals. Most likely, the number is higher since there are many patients who have the deletion but don't demonstrate the classic findings. Again, a general obstetrician does not have the time to discuss all these nuances with patients and once the patient asks specific information about the disorder or life issues, he or she will not have proper armamentarium to relay the proper information. A molecular karyotype, as it stands

now, as with the light microscopic karyotype, will miss the vast majority of DiGeorge syndrome patients, unless there is specific targeting and higher density testing at those sites. As molecular karyotyping becomes better (higher density, detecting small deletions and duplications), and as the clinical world utilizes this testing more so, again we'll go through the pendulum of a learning curve in seeing what chromosomes will be affected by the highly metabolic and genetically active placenta. Also remember there may be a skewness of apoptotic cells with a higher turnover for those cells with aneuploidy. This certainly appears to be the case with Trisomy 13 and 18 (but not necessarily with Trisomy 21). What other chromosomes could be involved with a high, or even low, turnaround time? This is the hurricane.

Noncoding RNA (ncRNA) and methylation studies are on the forefront of disease and gene regulation. It is known that maternal RNA does cross the placenta as well as RNA from plant and other food stuffs. Is it possible that truly we are what our mother's ate and exposed to in utero? And our maternal grandmother's exposures had influenced our mother's ovum to adapt to their environment whilst we were in utero. Epigenetics drives diversity in our genome. It also shows the consequence of exposures (teratogens, inflammatory agents, nutrients, etc.). The lack of a particular nutrient, the way it's metabolized, or being available may cause a particular disorder as well. For example, the folate and open neural tube defects story. There are a number of studies utilizing whole transcriptomes sequencing from maternal blood to help detect congenital anomalies via gene expression profiling. Since there are multiple drivers for gene expression at multiple salient points in expression and production, it will be a long time coming before a blood test will be able to tell you if your child has a multifactorial cardiac malformation or the like. Separation from maternal, environmental and fetal noncoding RNA will be difficult, but eventually will be done. Noncoding RNA is not just for prenatal diagnosis. There is some evidence that ncRNA may be involved in preeclampsia and other maternal disorders that will affect the pregnancy, fetus and offspring. It is this epigenetic phenomenon that will help prove the Barker hypothesis and hopefully someday help offspring and pregnancies proactively.

Currently, if I have a patient who has already had NIPT and they elect to have a CVS or amniocentesis since the fetus has multiple anomalies and the NIPT is negative, I do not spend time getting all sorts of tests. I utilize the NIPT as a "FISH", since their sensitivities are similar, and go directly to the CMA and bypass a regular karyotype. This way I get an answer in 7-10 days. There are some labs that would do an abbreviated karyotype and run it in parallel to the CMA preparation. If the abbreviated karyotype is negative, then the CMA will be done. If positive for a trisomy, then the karyotype will be then completed and the CMA discarded. If I am doing a diagnostic test for confirmation of a positive NIPT, then I order the karyotype without a FISH. Expanding carrier screening, as eluded earlier, has been available for over five years. Acceptance for this test has been varied throughout the US [3]. Again the bicoastal areas are quicker to adopt this technology then the Midwest or South. The difference here is that the American Societies have not adopted this technology for reasons given above. Knowledge of one's carrier status does not preclude anyone from reproduction, nor should they be discriminated against. Guilt plays the mother's role, but the reality is that we all carrier multiple mutated genes that are potentially detrimental to our progenity, and to ourselves. There is no one person who has a "perfect" gene pool. It is this admixture 'roll of the dice' that brings two half cells together to create a zygote. The system is not very efficient in utero where fetal wastage is high, but once born, the system is rather efficient at 93%. Meaning, about 7% of the world's population has a genetic-based disorder. The general public, and clinicians, need to understand there are very few absolutes in genetics. Nothing is perfect and I no longer accept the word "normal" anymore. If one is uncomfortable with this concept, then allow someone who is. Yes, counseling patients can be challenging. And one method that works with some will never work with others. Directive counseling should always be the last resort and never used unless necessary. And when it is done, then the patient should know of your bias. Some physicians feel that they are doing the patient a service by directing them into a decision. It is not. Nonjudgmental, nondirective counseling should always be the goal. Patients do not want to hear that their fetus has something wrong with it or worse yet, possibly wrong and we don't know. And they don't want to hear that they passed along a gene that caused their child harm.

Expanded carrier screening has many unresolved issues, such as which genes should we look at, at what price, what detection rates are there for each of the disorders looked at, how many genes (100, 200, 1000, 6000)? As the technology becomes quicker and more informative, it will become easier and less costly to look at these genes for carrier status. Whole exome sequencing (WES) for parental carrier status, and for fetuses, are here but used sparely so. Next Generation Sequencing (NGS) has opened this world in both the Pediatric and Prenatal arenas. We have learned a great deal from doing expanded carrier screening thus far, such as what was previously cited for SLO. Time for universal expanded carrier screening is near. But as with everything, one needs to understand the test and its flaws and strengths. Just like with CMA, VOUS may play even a more important role. Will this push the obstetrician over the proverbial edge? The other question in the back of everyone's mind is if you don't offer it, and it's available, is one "at fault" for a wrongful birth or wrongful life lawsuit? If we as a society perform carrier screening for everyone and the detection rate for the disorders covered is excellent, should we just do away with newborn screening of metabolic disorders? Luckily, we are a long way from this. Hopefully, we will learn from our past on how to better utilize and present this data to patients.

Expression of genes is not always straight forward. It has been discovered, just because you have a particular mutation(s) to cause a disease, one may not even express that disease. We have to be careful on how disorders, mutations and VOUS are portrayed and reported (and their follow-up). There are other problems with carrier screening, such as phenotype-genotype correlation and population risks. For example, mutations found in Tay Sachs from different populations are not the same (Ashkenazi, French Canadians, Cajun, Irish) and thus biochemical testing may be better than molecular (or combination thereof). And even in the general population, the carrier risk for Tay Sachs is still present, albeit much less. Therefore, detection rates may differ in different populations and therefore coverage, if possible, should be

panethnic and not just for one group. We don't have enough data yet for this to happen. Expanded screening and its implications can be the second storm coming. It will be up to us how to utilize this information and portray it to patients. The bright side of expanding screening is cost. What used to cost over $5000 for one particular panel of 11 disorders now costs less than $100 with an additional 100 other conditions. To me, this is a bargain but I know the test's limitations.

We are moving to a Pregnancy Risk Profile for our patients. Something that has been done in the past by history taking and checking a box. Now it is being refined and rebranded. A computer-generated computation would be best, gathering all the information and highlighting individualized risks for that particular pregnancy, broken down by genetic and obstetric risk. We should be doing this now. We can gather this information and act on it proactively. Nothing is ideal, but until the next generation of technology, it will be the best we know. Remember that when it comes to living organisms, nothing is 100% either way. Should general obstetricians send their patients to see a genetic counselor for everyone? Probably not, but I am sure there can be an easy computer program to help further identify patients who need to be seen. As we go deeper into this world of prenatal genetics, patients need to realize what is out there and what can be offered. This does not mean that every patient will utilize the technology available to them but it should be explained, as an informed consent, and offered what is available to them. Perhaps just an office visit dedicated to prenatal genetics and genetic screening would be appropriate for every patient. Incorporate it into a second visit in the first trimester.

In my 25 years of practice, I have never seen more personal emotion and directive counseling from clinicians about this subject. It's more defensive than logic. I feel that not offering these technologies to patients is directive counseling in a covert way since it shows one's own personal bias. We all have to be honest with oneself. Who are we to say that a patient does not want this type of testing? Some physicians cite time, complexity, and feeling out of control by companies, patients and other external influences. Politics and religion do not belong in a private discussion between a patient and her physician but they must be respected. When a physician tells me that he or she knows their patient population and they are not going to want this XYZ test, I think they are just kidding themselves. I have never experienced my patient's life and don't pretend to say to them that I know best for them personally. I give them information, as much as I can, and allow them to make their decisions. Yes, this takes a tremendous amount to time. At times, it raises the patient's anxiety bar considerably. Acknowledge this with them. I encourage patients to go home and discuss it, come back with more questions, and think it over again. These decisions should not be taken lightly and the fetus and patient both need advocates. The patient knows their preferences, not the clinician. If I feel my bias is coming through, I let the patient know. I help direct them through their conversation, but do not interject my bias. If asked, depending on the situation, I generally decline my personal opinion. If I feel that pregnancy management would change with a particular diagnosis, such as mode of delivery, or the need for neonatal comfort care, then I mention this to them since they may not realize the availability or need for these options. I also offer them to get a second opinion, or more. And reassure them their decision is the best for them at this time of their life. They need to hear this. Listen to and respect your patients and allow them to make decisions. Given the current ethical environment of patient autonomy, it is up to the clinician to give patients the best information available to them and allow them to make the choice. It is not your life, it's the patient's.

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