> The objective of our Summer Student Internship Project (SSIP) was to create educational videos that demonstrated the practice of evidence based clinical diagnosis.

Similarly, in our video entitled "Does this patient have appendicitis?" evidence–based clinical diagnostic principles are applied to demonstrate their usefulness. Appendicitis has a baseline prevalence of 25% in an adult population. This is the pre–test probability. With a positive result for tenderness at McBurney's point, which has a likelihood ratio of 3.4, the chance that the patient has appendicitis is now 53%. This is the post–test probability.[2] In this scenario, the clinical exam is used in such a way to guide treatment with more efficiency. This demonstrates the power of using evidence–based physical examination; ultimately these types of manoeuvres may spare patients unnecessary or invasive procedures while promoting more cost-effective clinical practices in the setting of finite healthcare resources.[3]

Exploring these topics in the course of this SSIP project allowed us to accumulate valuable clinical knowledge while developing practical new skills. Evidence–based clinical diagnosis has been helpful for us as students, as we can apply the science we have learned in order to make clinical diagnoses we can feel more confident about. An additional benefit of producing these clinical videos was exposure to the art and science of film-making. We researched, developed and edited a script that was subsequently professionally developed into a collection of short educational videos. We found this to be a creative and enjoyable process that opened our eyes to the many ways multimedia can be used in the undergraduate medical curriculum. This process also emphasized the value of having a diverse skill set in any clinician's practice.

In summary, evidence–based clinical diagnosis is a topic that we believe all physicians–in–training should be exposed to while developing expertise and fluency with the foundational skills of performing physical examinations. We hope that the concepts outlined in this paper as well as the videos produced by this project will spark interest and discussion among our peers and colleagues. The videos will be available to UBC medical students on the Medicine and Dentistry Integrated Curriculum On-Line (MEDICOL) website for viewing. We look forward to expanding our knowledge of this important area in our future training, and we are hopeful that similar content may be considered for incorporation into the upcoming UBC medical curriculum renewal.

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Non-invasive Prenatal Diagnosis – A New Era

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ABSTRACT

Recent advancements in genetics have changed the field of non–invasive prenatal diagnosis (NIPD). Since cell–free fetal DNA (cffDNA) was detected in maternal plasma in the 1990s, researchers have been trying to enhance detection and quantification techniques in order to utilize this DNA in early prenatal diagnosis. As technology advances, there are a number of concerns requiring discussion, including ethical considerations of non–invasive prenatal testing, commercial utilization, and implementation into prenatal screening protocols. This commentary introduces cffDNA, the techniques used for detection, and ethical considerations for the future.

KEYWORDS: *non-invasive prenatal diagnosis, cell-free fetal DNA, chromosomal aneuploidy, genetic screening*

Prenatal diagnostic testing has been available in Canada for years. In British Columbia, it involves non–invasive blood tests during the first and second trimesters that measure hormones in maternal blood indicative of chromosomal abnormalities in the fetus.[1] Further non–invasive screening includes measuring nuchal translucency (fetal nuchal fold thickness) on ultrasound. These results along with ethnicity and maternal age are used to calculate an individual's risk of having a child with Down syndrome, trisomy 18, or open neural tube defects with a cut–off at 1:300 in Canada.[1] Subsequently, definitive prenatal diagnosis depends on fetal karyotyping or DNA analysis through invasive techniques such as chorionic villi

sampling or amniocentesis. The downside to these procedures is a 0.5-1.0 % miscarriage rate.[2]

Consequently, there is active investigation in reducing invasive procedures for definitive prenatal diagnostic testing. In 1997, a breakthrough in the field of non–invasive prenatal diagnosis (NIPD) occurred when cell–free fetal DNA (cffDNA) was detected in maternal blood. The cffDNA is from placental syncytiotrophoblasts that have undergone apoptosis, with a possible secondary source from fetal erythroblasts.[4-6] It consists of short fragments of 143 base pairs on average. Although it can be detected during the fifth week of gestation, it is best quantified after seven weeks.[4-6] Maternal clearance of this DNA is rapid, and provides a real-time prenatal diagnosis early in pregnancy.

Cell-free DNA collected during pregnancy is mainly comprised of maternal DNA, with a 9% fetal component in early gestation that increases to 20% later in pregnancy.[4] Therefore, technologies capable of isolating fetal DNA from maternal DNA have been sought out. An example is the discovery of cffDNA using polymerase chain reaction (PCR) technology to search for chromosome Y–specific genes of fetal origin in maternal plasma.[3] Other techniques include using PCR to identify paternally–inherited genes, or to detect varying amounts of methylation between fetal and maternal DNA.[3-6] To detect single–gene disorders with a component of maternal inheritance (e.g. β–thalassemia), more specific techniques are required to determine allelic differences in cffDNA from maternal cell–free DNA such as digital PCR, massively parallel sequencing, and relative mutation dosages.[4-6] These new technologies that quantify maternal versus fetal allelic DNA changes are essential for detection of autosomal dominant disorders or autosomal recessive disorders.[4]

The applications of cffDNA are numerous. First and foremost, it can be used to detect fetal sex as early as seven weeks gestational age.[3,4] Digital PCR technology enhances this detection and completes many PCRs in parallel. By the tenth week, PCR technology can detect the Y chromosome with 100% sensitivity and specificity.[3,4] Thus, this technology can be applied in the detection of sex–linked diseases such as hemophilia and congenital adrenal hyperplasia. It may also be used to Rh type the fetus, and prevent unnecessary immunoglobin vaccinations as well as unnecessary monitoring.[4]

Secondly, cffDNA can be used for the detection of single–gene disorders.[4,6] Detection of paternally-inherited genes is the easiest to accomplish with PCR technology, and has high specificity and sensitivity.[4,6] Maternally–inherited disorders or autosomal recessive disorders require new technology to quantify the allelic differences between cell–free fetal DNA and maternal DNA. The difference can be found by quantifying relative mutation dosages.[4,6] However, this technology is of lower accuracy, and further research is required to improve the sensitivity and specificity.[4-6]

Thirdly, cffDNA may be used to detect chromosomal aneuploidy. NIPD using placental hormone detection in maternal serum exists for the detection of trisomy 18 and 21; it has a 70-90% detection rate, but a high false positive rate (approximately

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90% of fetuses that test positive are unaffected).[1,2] The hope is that cffDNA NIPD will replace this detection method and approach sensitivity and specificity levels similar to those of invasive procedures (90-100%).[1,2] Quantifying the extra genetic material from trisomic diseases is difficult because of the normal pair of chromosomes present in the sample. Currently, massively parallel sequencing detects the short fragments of cffDNA and calculates the fetal versus maternal DNA distribution. This technique has been shown to be 99-100% sensitive and specific for diagnosis of trisomy 21 and has the best detection rate so far, but other technologies may be easier to use and less resource intensive.[4,6]

The development and implementation of cffDNA NIPD into Canadian prenatal screening protocols would be difficult. The aforementioned technologies such as digital PCR would need to be available. Additionally, cffDNA NIPD results would have to be confirmed with invasive testing because its accuracy is currently only established for a small number of diagnoses (i.e., RhD status and some X–linked genetic disorders).[7] Eventually, with increasing accuracy and effectiveness, NIPD may actually help reduce costs by replacing invasive prenatal diagnosis and decreasing complications from these procedures.

Finally, the lucrative market of prenatal diagnosis has commercial companies already looking to capitalize on NIPD via direct–to–consumer marketing, thus raising ethical issues regarding cost, informed consent, and gender selection.[8] In 2011, a California–based company Sequenom, Inc., who claims to hold the intellectual property rights to using cffDNA, introduced its prenatal test for trisomy 21, available for order on the internet.[9] Its cost is $1900 USD; similar products from other companies are available and estimated to range from $795 to $1100 USD.[9] This price is beyond the reach of most women without extended health insurance, creating a cost barrier to universally available NIPD.[9]

With the nature of NIPD being "just a blood test" many women will not have sufficiently considered the implications of an abnormal result before undergoing this test, thereby undermining informed consent.[7] Currently, the consequences of further testing are only discussed with women who have a positive screen result.[7] Direct–to–consumer marketing bypasses this process and makes adequate pre–test counseling more difficult.[7] With the expanding scope of conditions, including early and late onset diseases, covered by NIPD, obstetricians will find it increasingly difficult to help patients answer, "Do you want this testing?"[8]

Direct–to–consumer marketing over the internet has given NIPD the potential to be used for non–clinical applications such as sex selection.[9] In societies where females are devalued, standard regulation practices may not be in place to protect expectant women from being coerced into testing and terminating

pregnancy based on gender.[8] The issue of gender selection raises concerns particularly in countries with skewed sex ratios, namely India and China.[9]

Overall, improved safety, earlier detection, and ease of testing will make NIPD an exciting option for prenatal diagnosis in the future. Commercialization has raised various ethical concerns, and there is an urgent need for standardized regulations and guidelines that can harness the potential benefits and minimize the risks of NIPD.

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