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Prenatal screening options in British Columbia

Counseling is an important requirement before and after a patient undergoes prenatal screening for chromosomal and subchromosomal fetal defects and disorders such as preeclampsia.

ABSTRACT: Prenatal screening in Canada has evolved over the past 40 years along with advances in our understanding of maternal and fetal risk factors, ultrasound technology, and the human genome. Multiple prenatal screening options are now available to patients, including tests that measure biochemical markers in maternal serum, placental DNA fragments, and fetal markers revealed by ultrasound. In BC, tests covered by medical insurance include SIPS (serum integrated prenatal screen), which measures markers in two separate blood tests, IPS (integrated prenatal screen), which measures markers in both serum and on ultrasound, and the Quad screen, which measures four markers in a single second-trimester blood test. Screening tests not usually covered by provincial medical insurance include FTS (first-trimester screening) and NIPT (noninvasive prenatal testing). Knowledge of the human genomic library paired with the commercial availability of sequencing technologies can be expected to produce further advances in prenatal screening. Our greatest challenge in the next 10 years will be to train the genetic counselors needed to provide timely support to patients as they consider the array of screening options available.

This article has been peer reviewed.

The evolution of prenatal screening was slow until relatively recently. In 1866 John Langdon Down published the first reference to a cluster of fetal characteristics that were discovered to be trisomy 21 almost 100 years later,[1,2] and confirmation that each cell normally contains 46 chromosomes was not reported until 1956.[3] Following this discovery, the development of amniocentesis and chorionic villus sampling, along with improved metaphase karyotype resolution, enabled the diagnosis of many more genetic syndromes. Currently, both chromosomal (aneuploidy) and subchromosomal (deletion/duplication/translocation states) can be detected by prenatal screening.[4]

Serum and ultrasound markers

Maternal serum screening for analytes produced by the placenta to detect trisomies 21, 18, and 13 first became available in the 1980s.[4] The

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sensitivity and specificity of screening improved as markers were added. Canadian screening[5,6] tests now employ various combinations of the following biochemical markers:

- Total human chorionic gonadotropin (hCG)[7] and free beta-hCG.[8]
- Alpha fetoprotein (AFP).[9]
- Unconjugated estriol (uE3).[10]
- Pregnancy-associated plasma protein-A (PAPP-A).[11,12]
- Inhibin A.[13]

In 1997, Lo and colleagues published the first paper to describe isolating and amplifying nonmaternal DNA from maternal plasma: at the time, they referred to these fragments as "fetal" although we know now that they are placental DNA fragments.[16] This triggered important clinical applications given that placental DNA largely mirrors fetal DNA (with the exception of mosaicism, which can occur in the placenta). Today, fragments of placental DNA are being referenced against the library created by the Human Genome Project, and new sequencing tools[17] are permitting screening for subchromosomal defects such as microdeletions using commercially available tests.

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In 1992, Nicolaides and colleagues published a seminal article demonstrating that ultrasound measurement of nuchal translucency (the fluid between the fetal skin and skull) could be used to identify the fetus at risk for Down syndrome.[14] Subsequently, new ultrasound markers were identified and these allowed prenatal screening practitioners to flag fetal anomalies beyond just the major aneuploidies of chromosomes 21, 18, and 13.[4] Then, additional ultrasound markers (e.g., uterine artery pulsatility index) expanded screening beyond chromosome errors to disorders of pregnancy such as preeclampsia.[15] This expansion of markers permitted the detection of some more rare anomalies and other syndromes in addition to the major aneuploidies.

Risk factors and tests for prenatal screening

The Society of Obstetricians and Gynaecologists of Canada (SOGC) provides excellent advice on counseling patients before and after prenatal genetic screening.[5] Their committee opinion on the topic states that some patients may have difficulty understanding the difference between a screening test and a diagnostic test, and that counseling will involve explaining that a positive screen means the patient's risk is above a predetermined cutoff set by a regional program, not that her fetus is necessarily affected.[5] The SOGC also provides suggestions for discussing the risk factors to consider and the screening tests available.

Age. Chromosomal aneuploidy increases with maternal age. A study of more than 15 000 embryos from 2701 patients demonstrated that 20.7% of embryos from women age 29 were abnormal, compared with 34.5% of embryos from women age 35, and 58.2% from women age 40. By age 43, over 83.0% of embryos were chromosomally abnormal.[18]

History. Women with a previous pregnancy affected by trisomy 21 have a higher baseline risk of recurrence (approximately 1%) and should therefore be offered prenatal screening regardless of age.

Quadruple marker (Quad) screen. The use of maternal serum markers for prenatal screening has evolved over time to produce the current Quad screen, which requires that blood be drawn between 15 and 22 weeks of gestation to measure levels of uE3, AFP, free beta-hCG, and inhibin A. These levels are analyzed along with maternal age. Described first in 1996 by Wald and colleagues, the Quad screen provides improved detection rates for trisomies 21, 18, and 13 and lower false-positive rates than double marker and triple marker screening.[19]

Nuchal translucency (NT) ultrasound. The measurement of fluid between the fetal skin and skull seen on an ultrasound performed between 11 and 14 weeks of pregnancy can reveal anomalies. As a single measurement, NT has a detection rate of 75% and a screen positive rate of 5%.[20]

Open neural tube defects (ONTDs) screen. Most open neural tube defects will be picked up either at the first-trimester nuchal translucency ultrasound scan (if the patient receives this) or the second-trimester anatomy scan at 20 weeks. However, AFP determination between 15 and 22 weeks (alone or as part of the Quad screen) is an excellent method for detecting ONTDs.

Serum integrated prenatal screen (SIPS). The addition of a first-trimester PAPP-A measurement to the Quad panel in SIPS enhances screening. Blood must be drawn in the first and second trimesters for PAPP-A measurement, and in the second trimester for uE3, AFP, free beta-hCG/total hCG, and inhibin A measurement.[5]

Integrated prenatal screening (IPS). The addition of nuchal translucency ultrasound to the serum integrated prenatal screen blood tests in IPS has been shown to increase the detection rate by 2% and reduce the false-positive rate by 2.5%.[5]

First-trimester screening (FTS). FTS combines more detailed ultrasound assessment with serum sampling for levels of free beta-hCG and PAPP-A. The Fetal Medicine Foundation recommends using the following first-trimester ultrasound markers in addition to nuchal translucency: fetal nasal bone, facial angle, and ductus venosus flow (DV). By clearly visualizing the limbs, cranial structures, heart, stomach, and bladder, ultrasound allows assessment of early fetal anatomy and major organ systems. The addition of DV assessment to FTS in 2009 led to detection rates of 96% with a screen positive rate of 3%.[21] At that time, FTS became the most sensitive and specific screening tool available, able to screen for monosomy X, trisomies 21, 18, and 13, and cardiac defects.[21,22] FTS also offers the advantage of providing results much earlier in the pregnancy (before 14 weeks) compared with the 18 to 20 weeks required for the Quad screen or IPS.

Noninvasive prenatal testing (NIPT). Commercial use of NIPT in British Columbia started in 2012 following the availability of massively parallel DNA sequencing. However, the testing was time-consuming and expensive, and therefore limited in uptake.[23,24] In 2013, targeted sequencing using specific regions of chromosomes 21, 18, and 13 was utilized to provide a faster and less-expensive alternative.[23] Also in 2013, the identification of single nucleotide polymorphism (SNP) using microarray was described. SNP array allowed for the detection of subchromosomal errors, including deletion and duplication of sequences. Most recently, whole genome sequencing has been employed to give much higher resolution of the placental genome.[25] Some limitations of NIPT are that the commercial products narrow the focus to trisomy 21 and, to a lesser extent, trisomies 18 and 13. The other limitation is that these products are being marketed directly to the patient as the "best" type of prenatal screening available, while all current clinical guidelines on prenatal screening state that NIPT should be considered an adjunct to currently available screening.

Preeclampsia screening. In 2011, Karagiannis and colleagues published a seminal paper on the use of first-trimester placental growth factor levels, uterine artery Doppler velocimetry, and mean arterial blood pressure as a screening tool for preeclampsia.[26] Despite detection rates of 95% and screen positive rates of only 10%,

Prenatal screening considerations

- Maternal serum sampling and ultrasound can be used to identify fetuses at increased risk of open neural tube defects and trisomies 21, 18, and 13.
- Serum sampling alone has problems of lower detection rates, and higher false-positive rates.
- NT combined with first- and second-trimester serum testing provides improved detection rates and lower screen positive rates, but has the drawback of second-trimester reporting.
- NT combined with nasal bone, ductus venosus flow, serum PAPP-A, and free beta-hCG provides the higher detection rates, with results available in the first trimester.
- Screening options offered will depend on maternal age and pregnancy history as well as on gestational age and other risk factors.
- Perinatal Services BC provides information on provincially insured screening practices: www.perinatalservicesbc.ca/health-professionals/professional-resources/screening/prenatal-genetic.
- Perinatal services BC does not discuss in detail the availability of first-trimester screening or NIPT.
- NIPT has excellent screening detection rates, but should not be used in isolation—NIPT is an adjunct to currently available screening.

widespread adoption of this screening tool has not occurred.

Detection rates

The detection and screen positive rates for screening tests currently used in BC are presented in **Table 1**.[27] Clinicians should be aware that the way these rates are reported for SIPS and IPS results can be confusing for patients and health care providers.

First, what is the difference between a screen positive and a false-positive? Of all women undergoing a screening test who have a positive result (i.e., they are screen positive), some will have the disorder (i.e., they are truly positive) and most will not (i.e., they are falsely positive). If the screening test is positive, it means that the patient requires further testing. For example, a patient with a screen positive SIPS would be offered NIPT, and a patient with a screen positive NIPT would be offered a diagnostic test such as amniocentesis.[27]

Second, why are screen positive and false-positive numbers so variable? This is the result of using data obtained from large prospective prenatal screening trials such as SURUSS,[28] which aimed at the standardization of *detection rates* with *variable screen positive rates*. Generally, screening tests will aim to have high detection rates with low screen positive rates. False-positive rates in serum screening, for example, increase substantially in women over 40. This is because the prevalence of aneuploidy in this population is high at baseline, which increases the pretest probability of a positive result.

Insured and uninsured tests

Currently, Perinatal Services BC focuses on the detection of open neural tube defects and trisomies 21 and 18 using the tests already described: SIPS, NT, IPS, and NIPT.[27] For patients meeting the eligibility criteria based on age and other risk factors, the tests are insured under MSP, as shown in **Table 2**.

In BC there are also some private pay options for prenatal screening. These include FTS, NIPT, and DuO (FTS plus NIPT). The detection rates, screen positive rates, and costs for these are shown in **Table 3**.

Counseling requirements

The last 40 years have seen tremendous growth in the array of screening options. Unfortunately, we have not seen a parallel growth in the training of genetic counselors in North America to serve the growing population needing to understand specific tests such as NIPT, which can involve many complexities that must be explained for fully informed consent. Our greatest challenge in the next 10 years will be to ensure we can provide education and knowledge translation for prenatal screening that supports patients as they consider the options available.

Summary

The evolution of prenatal screening has advanced along with our understanding of the human genome, ultra-

Table 1. Detection and false-positive rates for prenatal screening in British Columbia, 2012 to 2015.

		Serum integrated prenatal screen (SIPS)	Integrated prenatal screen (IPS)	Quadruple marker screen (Quad screen)	First-trimester screening	Noninvasive prenatal testing (NIPT)
Trisomy 21	**Screen cutoff**	**1:300**	**1:200**	**1:385**	**1:100**	
	Detection rate (by age in years)	73% (< 35) 83% (35–39) 100% (≥ 40)	86% (< 35) 96% (35–39) 100% (≥ 40)	78% (< 35) 80% (35–39) 100% (≥ 40)	96% (all ages)	> 99.0% (all ages)
	False-positive rate (by age in years)	3% (< 35) 8% (35–39) 19% (≥ 40)	4% (< 35) 7% (35–39) 18% (≥ 40)	4% (< 35) 14% (35–39) 27% (≥ 40)	3% screen positives (all ages)	< 0.1% (all ages)
Trisomy 18	**Screen cutoff**	**1:300**	**1:300**	**1:300**	**1:100**	
	Detection rate	90.0%	90.0%	90.0%	92% (all ages)	97.0%
	False-positive rate	0.4%	1.7%	0.4%	3% screen positives (all ages)	< 0.1%
Trisomy 13					> 96% (all ages)	

Adapted from Perinatal Services BC. Obstetric guideline: Prenatal screening for Down syndrome, trisomy 18 and open neural tube defects. June 2016.[27]

sound technology, and maternal and fetal risk factors. Screening methods now available can detect the likelihood of chromosomal and subchromosomal defects and disorders such as preeclampsia. Perinatal Services BC focuses on detecting open neural tube defects and trisomies 21, 18, and 13 with tests such as SIPS, NT, and IPS, which are provided under MSP for patients meeting eligibility criteria. Additional private pay options for testing include FTS and NIPT. The current clinical challenge is to ensure patients are informed about the wide array of screening options, that they receive timely genetic counseling to sort out the options, that they understand the difference between screening and diagnostic testing, and that they end up with the screening test that gives them the information they seek. BCMJ

Table 2. Eligibility criteria for MSP-insured prenatal tests in British Columbia.

Test	Eligibility criteria
Serum integrated prenatal screening (SIPS)	• Any woman
Integrated prenatal screening (IPS)*	• Age over 35 • Twin pregnancy • Previous pregnancy affected by trisomy 21, 18, or 13 • HIV-positive • IVF or ICSI conception
Noninvasive prenatal testing (NIPT)	• Screen positive with IPS, SIPS, or quadruple marker (Quad) screen • Previous pregnancy affected by trisomy 21, 18, or 13 • Risk higher than 1:300 after Quad, SIPS, or IPS

*IPS = SIPS combined with nuchal translucency ultrasound

Table 3. Uninsured prenatal tests available in British Columbia.

	Tests	Detection rate	Screen positive rate	Cost	Provider
First-trimester screening (FTS)	Ultrasound + maternal serum sampling between 11 and 14 weeks	96.0%	3.0%	$550	PCRM[a]
Noninvasive prenatal testing (NIPT)	Maternal serum sampling after 9 to 10 weeks	99.9%	0.1%	$400–$1000	LifeLabs[b] PCRM Olive[c]
DuO (FTS plus NIPT)	Ultrasound + maternal serum sampling after 9 to 10 weeks + maternal serum sampling between 11 and 14 weeks	> 99.9%	0.1%	$1000	PCRM

[a] Pacific Centre for Reproductive Medicine, www.pacificfertility.ca
[b] LifeLabs, www.lifelabsgenetics.com
[c] Olive Fertility Centre, www.olivefertility.ca

Competing interests
None declared.

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