PRENATAL SCREENING AND EVALUATION OF FETAL ANOMALIES

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Prenatal Screening

- Uses maternal age and ≥2 markers (biochemical tests) +/- FTUS (NT) to calculate a single result for age-related risk of T21, T18, and ONTD
- Offered to all Canadian women **of all ages**
    - The goal is to identify women with increased risk of having certain chromosomal abnormalities and malformation
- Accurate dating of pregnancy is important
    - US if LMP is unreliable
    - US if abnormal serum screen

Maternal age:

- The probability of trisomy increases with maternal age
- Screening solely based on maternal age at EDD should be abandoned → inferior to use of multiple biochemical markers and US
    - Use of multiple biochemical markers and FTUS decreases false + rate and improves detection rate
- Do not do diagnostic test alone without a screening test
    - Do not offer women ≥40yo amniocentesis without prior screening

Screening and Diagnostic Tests

Screening Tests for trisomies:

Available in Saskatchewan: FTS, IPS, MSS

1. First Trimester Screen (FTS)
2. Quad Screen (MSS)
3. Integrated screening (IPS)
4. 2nd trimester US then cfNDA (cell free DNA) (Harmony)

Diagnostic Tests:

1. Amniocentesis
2. Chorionic villus sampling (CVS)- Calgary
3. RAD (rapid aneuploidy detection)
    - Replaces FISH

Screening Tests for ONTD:

1. Serum AFP
2. 2nd trimester US (18-20w)

- NT is not offered alone without biochemical markers in singleton pregnancies

Screening vs Diagnostic Test

Screening test

- Identifies individuals at increased risk for having certain chromosomal abnormalities and malformation (chance that a condition is present)
  - Done at the population level
  - Uses specific marker(s) and defined screening cut-off levels
  - If screen positive
    - Patient is at increased risk, does not indicate presence of condition
  - Offer counselling and diagnostic testing

Diagnostic test

- Identifies whether the condition is present or absent
  - Done at the individual level
  - Offered only to those who screen +
  - Tend to be invasive
  - Risk of pregnancy loss

SCREENING

Minimum Standard

- **Minimum standard:** any prenatal screen in
  - 1st trimester should have **detection rate of 75% and false positive rate less than 3%**
  - 2nd trimester should have **detection rate of 75% and false positive rate less than 5%**

Risk cut-off value

- **Risk cut-off:** the risk/likelihood of disease being present in the fetus at term or at mid-trimester
  - Ex. The risk of having Down syndrome in mid-trimester is 1:280 vs 1:350 at term. The risk for mid-trimester is higher since 23% of fetuses with Down syndrome are miscarried between mid-trimester and term

Screening Tests

	Trimester	Markers	Term risk cut-off	Detection rate	False + rate	OAPR (odds of being affected if screen +)
First Trimester Screen (FTS)	T1	• **T1 serum** • **NT US** • Maternal age	1 in 325	83%	5%	1:27
Quad screen (MSS)	T2	• **T2 serum** • Maternal age	1 in 385	77%	5.2%	1:50
Integrated prenatal screening (IPS) • Sequential • Contingent	Both T1 and T2	• **T1** (PAPP-A only)**+ T2 serum** (Quad screen) • **NT US** • Maternal age	1 in 200	87%	1.9%	1:10
		IPS without inhibin A: • **T1** (PAPP-A only)**+ T2 serum** (Quad screen without inhibin A; use total HCG) • **NT US** • Maternal age		88%	3.0%	1:20
		Serum IPS (without NT) (Integrated serum screening- ISS): • **T1** (PAPP-A only)**+ T2 serum** (Quad screen or triple screen)		85% (FASTER trial)	4.4% (FASTER trial)	1:26

Components:
- **T1 serum** (11-13:6w): free beta-HCG, PAPP-A
  - Best time for PAPP-A is 9-10w
- **NT US** (11-13+6w) (best time 11-13w)
- **T2 serum** (15+0-20+6w): free beta-HCG, AFP, uE3, inhibin A
- **T1+ T2 serum**: free beta-HCG, PAPP-A, AFP, uE3, inhibin-A

Inhibin A
- Increases detection rate for Down Syndrome by 10%
- Risk cut-off 1:230
- Diagnostic rate: 75-80%
- False positive rate: 3-5%

Screening for ONTD

- **2nd trimester US**
  - FTS does not screen for ONTD
  - MSS and IPS screen for ONTD
  - Maternal serum AFP is no longer used if 2nd trimester US is normal

Elevated AFP:
- ONTD
- Gastroschisis
- Omphalocele

	Trimester	Markers
2nd trimester US	2nd (18-20w)	
Maternal serum AFP (MSAFP) • Quad screen (MSS) • Integrated prenatal screening (IPS • *Not in FTS*	2nd	• Serum: AFP • Done if 2nd trimester US is not available

Screening Tests

	Results available	Benefits	Limitations
First Trimester Screen (FTS)	T1		• Needs to be done in 1st trimester • Access to certified NT scan (distance, expertise (need certification)) and 1st trimester diagnostic testing • Reproducibility of NT • Availability of CVS if screen +
Quad screen (MSS)	T2	• Used for late presenters • Highest detection rate, lowest false + rate	
Integrated prenatal screening (IPS)	IPS: T2 (when all testing is completed) Contingent: T1 (small portion T2) Sequential: T1 and T2	• Combines 1st and 2nd trimester screening to improve detection • Also screens for ONTD and trisomy 18 • Lower false + than FTS • Fewer invasive diagnostic procedures compared to FTS	

Contingent and Sequential IPS

	Steps	Benefits	Drawbacks

Contingent Screening	• T1 serum screen (FTS) is performed, results are given to pt
	  • **High risk (risk >1 in 50)**→ offer invasive testing
	  • **Low risk (risk <1 in 1500)**→ no further testing
	  • **Intermediate risk (between 1 in 50 and 1 in 1500) (15.8%)**→ T2 screen, receive combined result
		• Best cost-effectiveness ratio
		• Fewer procedure-related miscarriages and unnecessary terminations
			• Intermediate group: anxiety, wish to have invasive testing immediately, increased false +

Integrated prenatal screening (IPS)	• T1 serum screen (FTS) is performed, results are given to pt
	  • **High risk (risk >1 in 50)**→ offer invasive testing
	  • **Low risk (risk <1 in 1500)**→ offer T2 screen
		• Lower PPV of 2nd trimester screening
		• Performs equally well comparing to full IPS and contingent IPS
			• Increased risk of false + (does not incorporate results of FTS into T2 screen risk analysis)
			• **Should not be offered unless T2 risk incorporates FTS results!**

Prenatal Screening

	Trisomy 13 (Pateau)	Trisomy 18 (Edward's)	Trisomy 21	ONTD	Turner syndrome	Fetal hydrops	Smith-Lemli-Opitz
HCG (free, beta, total)		↓	↑	-----		↑	↓
PAPP-A	↓	↓	↓	-----	↓		
Inhibin-A	↑	↓		-----		↑	
AFP	↓	↓	↓	↑			↓
uE3	↓	↓		-----	↓		↓
NT	↑						

- ONTD: AFP is increased
- Trisomy 21, Trisomy 13
  - Decreased: PAPP-A AFP, Estriol (uE3)
  - Increased: HCG, inhibin A, NT
- Trisomy 18: everything decreased except NT

2nd Trimester US (18-20w)

- All pregnant women should have 2nd trimester US
  - Should be done prior to cfDNA
  - Do not use alone (high false +)→ use in combination with serum screening
- To assess for anatomic abnormalities and soft markers
- Presence/absence of anatomical abnormalities/soft markers is used to modify a priori risk of chromosomal anomalies
  - Absence of soft markers decreases risk of aneuploidy **(risk reduction)** → **Negative likelihood ratio= 0.5!**

Looking for:

- Fetal anatomic abnormalities
  - Most can be detected
- ONTD
- **Soft markers-** features that can increase a priori risk of fetal aneuploidy but may also be normal

Other

- **Do not meet the minimum standard:**

	Trimester	Markers	Term risk cut-off	Detection rate	False + rate	OAPR (odds of being affected if screen +)
Maternal age	1st, 2nd	• Maternal age alone	1 in 385	44%	16%	1:218
Triple screening	2nd	• Serum: AFP, uE3, total HCG (no inhibin A) • Maternal age		71%	7.2%	1:59
Nasal bone	1st or 2nd	• Presence/absence of nasal bone at 11-14w US		68.8%	9% Afro-Carribeans 5% Asians 2.2% Caucasians ↑ with ↑NT ↑ with ↓ CRL Inter-operator variability	

- Nasal bone should not be incorporated as a screen unless it is performed by trained and accredited sonographers with ongoing quality assurance
- Triple screening also screens for ONTD, open fetal defects (gastroschisis, omphalocele), placental dysfunction, Smith-Lemli-Opitz syndrome

Factors that Affect Screening Performance

Accuracy of GA	• Need accurate dating • For all serum markers, false positive is decreased by 2% when GA is estimated by US
Maternal weight	• Need to adjust for maternal weight • ↑ weight results in physiological increase in blood volume dilutes both 1st and 2nd trimester serum markers • Not clinically significant for NT (do not need to adjust weight for NT)
Ethnicity	• Use ethnic origin chart • Differences in levels of serum markers between ethnic groups • Adjusting for ethnicity increases detection rate and equalizes false + rate • African American: ↑ AFP (by 15%), ↑ HCG (by 18%), ↑ PAPP-A (by 35%), ↓ inhibin A (by 8%) • South Asian: ↓ AFP (by 6%), ↑ uE3 (by 7%), ↑ HCG (by 6%), ↑ PAPP-A (by 17%) • Asian:↑ PAPP-A, ↑ HCG • Aboriginal: ↑ uE3 • There are statistically significant differences in NT between ethnic groups, but they are not enough to be corrected

Factors that Affect Screening Performance

IDDM	• ↓ AFP (10%), ↓ uE3 (5%) • No difference in free beta-HCG, PAPP-A, NT • Pseudo-risk of Down syndrome is calculated by dividing observed MoM for a pt with diabetes by median MoM in diabetic pt without Down syndrome
Accuracy of NT and serum marker measurements	

ART (IVF)	• Age at the time when egg was harvested is used as maternal age to determine risk
	• ↓ uE3, ↑ free beta-HCG and total HCG (results are conflicting)
	  • Have almost double the false + rate in 2nd trimester screening
	  • Due to continuing high progesterone concentrations after hormonal treatment
	• Whether need adjustment needs further investigation

Counselling

- Disclose results to pt
- Possibility of false +/false-
- Discuss options
  1. Conservative management
  2. Further screening: 2nd trimester US to assess for structural anomalies then cfDNA
  3. Diagnostic testing
- Refer to MFM

Meaning of positive screening

- A screen is positive when the risk of ≥1 screened disorders is above the level of risk cut-off
- Does not mean the baby has the disease → it means that the baby is at increased risk of having the disease
- The result may also be false positive

FURTHER SCREENING

2nd trimester US then cfDNA

DIAGNOSTIC TESTING

Indications for Diagnostic Testing

- Invasive prenatal diagnosis for cytogenetic analysis is performed under these circumstances:

- Positive screening test (risk above cut-off)

Offer diagnostic testing without prior screening due to increased risk of aneuploidy due to:

- Ultrasound findings
- IVF pregnancy with intracytoplasmic sperm injection
- Pt or partner has a Hx of previous child/fetus with a chromosomal abnormality or a carrier of chromosome rearrangement that increases risk of having a fetus with a chromosomal abnormality

RAD (replaces FISH)

- Used to get results rapidly (within 4 days) for pts with very high risk of aneuploidy for patients to decide whether or not to terminate pregnancy
- Criteria
  - >20w
  - Risk of chromosomal abnormality >1 in 50

Counselling

- Disclose results to pt
- Discuss options
  1. Genetic counselling
  2. Terminate pregnancy
  3. Continue pregnancy → consult NICU

Recurrence Risk in Future Pregnancies

- Depends on cause
- Nondisjunction: 1% unless age-related risk exceeds this
- Robertsonian translocation
    - 10-15% if mother is the balanced translocation carrier
    - 2-5% if father is the translocation carrier

References

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