Prenatal screening and diagnostic testing in Ontario: A descriptive analysis of service utilization in the era of cell-free fetal DNA testing

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Abstract:	Abstract Background To assess the impact of publicly funded cell-free fetal DNA (cfDNA) screening on the utilization of multiple marker prenatal screening (MMS), cfDNA screening and prenatal diagnostic testing (PND) for fetal chromosomal aneuploidies in Ontario. Methods A retrospective cohort study based on data collected by the Better Outcomes Registry & Network (BORN) was performed. Descriptive statistics were generated to describe the utilization of MMS, cfDNA screening, and PND. The regional variations in the utilization of MMS and cfDNA screening were assessed by Census divisions and Local Health Integration Networks. Results The study included 534,210 singleton pregnancies. After cfDNA screening was funded for specific indications, the uptake of MMS increased slightly from 66.5% to 68.1%. The uptake of cfDNA screening among MMS positive women increased substantially from 3.2% to 48.8%. In contrast, PND

	among MMS positive women decreased from 54.9% to 30.8%. Follow-up testing (cfDNA/PND) among the MMS positive group increased from under 60% to 75%. Although women ≥40 years are eligible for primary cfDNA screening, there was only a small decrease in the use of MMS in this group (from 75.7% to 72.2%). After cfDNA screening was funded, the greatest use of cfDNA screening and greatest decline in of PND were seen in women with a MMS risk in the range of 1 in 101 to 1 in 200. Interpretation: The changing patterns of uptake of screening and diagnostic tests demonstrate the significant impact of public policy and funding decisions on women's choices regarding prenatal testing.

Prenatal screening and diagnostic testing in Ontario:

A descriptive analysis of service utilization in the era of cell-free fetal DNA testing

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Tianhua Huang contributed to conception and design, performed data analysis, interpreted data and drafted the article, and agreed to act as guarantors of the work presented in this article. Nan Okun, Shelley Dougan and Christine Armour contributed substantially to conception and design, interpretation of data, and revised the manuscript. Mark Walker contributed to the interpretation of data, revised the manuscript critically. All authors gave the final approval of version to be published.

Short title: Utilization of multiple marker screening, cfDNA screening and diagnostic testing

Key words: Prenatal screening, cell free fetal DNA screening, prenatal diagnostic testing, trisomy 21, utilization, uptake rate.

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Abstract

Background

To assess the impact of publicly funded cell-free fetal DNA (cfDNA) screening on the utilization of multiple marker prenatal screening (MMS), cfDNA screening and prenatal diagnostic testing (PND) for fetal chromosomal aneuploidies in Ontario.

Methods

A retrospective cohort study based on data collected by the Better Outcomes Registry & Network (BORN) was performed. Descriptive statistics were generated to describe the utilization of MMS, cfDNA screening, and PND. The regional variations in the utilization of MMS and cfDNA screening were assessed by Census divisions and Local Health Integration Networks.

Results

The study included 534,210 singleton pregnancies. After cfDNA screening was funded for specific indications, the uptake of MMS increased slightly from 66.5% to 68.1%. The uptake of cfDNA screening among MMS positive women increased substantially from 3.2% to 48.8%. In contrast, PND among MMS positive women decreased from 54.9% to 30.8%. Follow-up testing (cfDNA/PND) among the MMS positive group increased from under 60% to 75%. Although women ≥40 years are eligible for primary cfDNA screening, there was only a small decrease in the use of MMS in this group (from 75.7% to 72.2%). After cfDNA screening was funded, the

greatest use of cfDNA screening and greatest decline in of PND were seen in women with a MMS risk in the range of 1 in 101 to 1 in 200.

Interpretation:

The changing patterns of uptake of screening and diagnostic tests demonstrate the significant impact of public policy and funding decisions on women's choices regarding prenatal testing.

Introduction

In Ontario, about 67% of pregnant women had multiple marker screening (MMS) for fetal chromosomal aneuploidies during their pregnancies.[1] MMS incorporates serum biochemical markers, and in most instances, a ultrasound marker nuchal translucency (NT) measured between 11 and 13 weeks gestation. Until recently, the most commonly used screening tests in Ontario have been the Integrated Prenatal Screen (IPS) and the First Trimester Screen (FTS) which has been recently updated to "enhanced" FTS (eFTS). The IPS and FTS provide detection rates (DR) of about 85% with false positive rates (FPR) of 2.5-5%. [2 3] Although MMS is routinely offered to all pregnant women, there has been substantial variation in overall uptake rate across the province, as well as variation in the MMS test undertaken.[4]

Cell-free fetal DNA (cfDNA) screening is based on sequencing of cell-free fetal DNA in maternal plasma. The test provides a DR of approximately 99% and a FPR of < 0.1% for trisomy 21.[5] CfNDA for specific aneuploidies became available in Ontario in late 2012 on a private-pay basis. In early 2014, the Ontario Ministry of Health and Long Term Care (MOHLTC) began to fund cfDNA screening for women whose pregnancies were identified to be at increased risk for one of the common aneuploidies including women with a positive MMS result, certain abnormal ultrasound findings or advanced maternal age (≥40 years), among other eligibility criteria (Appendix 1). Other jurisdictions have also incorporated cfDNA screening into traditional prenatal screening paradigms,[6] though there is limited literature describing the effect of the systematic incorporation of cfDNA screening on prenatal screening and diagnostic test utilization at a population level.

Before cfDNA screening was publicly funded, women whose MMS result indicated an increased risk for trisomy 21 or 18 were offered genetic counselling and the option of confirmatory invasive prenatal diagnostic testing (PND), via amniocentesis or chorionic villus sampling (CVS). The introduction of cfDNA screening, with its low FPR and false negative rates, has been shown to reduce the usage of diagnostic testing.[7 8]

The current study aims to assess the utilization pattern of MMS and PND prior to, and after cfDNA screening was introduced into a general population setting in Ontario, Canada using data collected by the Better Outcomes Registry & Network (BORN) Registry.

Methods

Datasets

This is a retrospective cohort study based on a secondary analysis of data collected by BORN. BORN is a prescribed registry under the Personal Health Information Protection Act (PHIPA). The BORN information system (BIS) routinely collects information on pregnancy (including MMS), labour, birth, and early newborn care from all hospitals, midwifery practices, and prenatal screening centres in Ontario, as well as from Newborn Screening Ontario. The real-time data collection into the BIS began on January 1 2012. In addition, BORN has retrospectively collected data on all cfDNA screening provided to Ontario residents, as well as data on diagnostic testing from all but one cytogenetic laboratory in Ontario. The study population includes all pregnant women who had a singleton pregnancy and an expected date of delivery (EDD) between July 1 2012 and March 31, 2016.

To link legacy cfDNA screening and cytogenetic testing data with the BIS records, each cfDNA screening and cytogenetic record was assigned to a series of BIS identifiers including maternal person ID, pregnancy ID, birth ID and infant person ID through record matching. The record matching was performed by BORN data analysts and achieved through deterministic and probabilistic matching using maternal and newborns' health care number, date of birth, name, sex and postal code. Overall, 91.1 % (12,755/13,999) of cfDNA screening and 81.3% (12,279/15,107) of cytogenetic records could be matched to a pregnancy record in the BIS. In over 95% of occasions, cfDNA screening and cytogenetic records were matched to a correct

pregnancy. Unmatched records were mostly likely miscarriages or terminations before 20 weeks of gestation, as these data points were not systematically captured by BORN during this time period. As well, they may also be records from non-Ontario patients. Records of MMS, amniotic fluid alpha-fetoprotein (AFP) testing, cfDNA screening and cytogenetic testing were subsequently linked with pregnancy records by pregnancy ID. The linked data set was used in the study.

In Ontario, amniocentesis is the main form of prenatal diagnostic testing. Chorionic villus sampling is performed in a small number of high risk centres. To maximize ascertainment, the number of amniocenteses in this study was ascertained through a combination of amniotic fluid AFP records (as AFP was measured on almost all amniotic fluid samples) and cytogenetic records indicating amniocentesis as the tissue type. As entry of CVS testing as a data point is not systematically collected in the BIS, the use of CVS was based on information collected from cytogenetic records.

Data analysis

In this study, we used uptake rates to describe the utilization of MMS screening, cfDNA screening and PND. The numerator of uptake rate was number of singleton pregnancies in Ontario that had a MMS, cfDNA screening or PND. The denominator was singleton pregnancies in Ontario with a defined character (e.g. all pregnancies, MMS positive pregnancies etc). Expected date of delivery was used to describe date ranges. The regional variations in the

utilization of MMS and cfDNA screening were described by Census division (CD) and Local Health Integration Networks (LHIN). Census division describes provincially legislated geographic areas that are intermediate between the province/territory level and the municipality; they have been established in provincial law to facilitate regional planning and service provision.[9] LHINs were established under the Local Health System Integration Act, 2006 for health care services planning, funding and management. There are 49 CDs and 14 LHINs in Ontario.[10] To assess how women may be using MSS to make decisions, the utilization of cfDNA screening and PND by MSS risk cut-off were also examined.

Descriptive statistics were generated to describe the utilization of MMS, cfDNA screening, and PND. The Chi-square test was used to compare the uptake rates of these tests prior and after the introduction of funded cfDNA screening for higher risk pregnancies. The Cochran-Armitage test was used examine the temporal changes in the utilization of these tests.

The study was approved by the Research Ethics Board of Children's Hospital of Eastern Ontario.

Results:

There were 534,210 singleton pregnancies during the study period. 359,066 had a MMS yielding an overall MMS uptake rate of 67.2%. Figure 1 shows the uptake of MSS by census division and in the LHINs with lowest and highest MMS uptake rates. There were substantial variations in different geographic areas in Ontario; from under 30% in North West LHIN to over 83% for Toronto Central LHIN.

Over the study period, 11,102 (2.1%) singleton pregnancies underwent cfDNA screening, with a dramatic increase after funding from 854 in 2013, to 6,298 in 2015. Figure 2 shows the uptake rate of cfDNA screening by census division among pregnancies with a positive MMS result following MOHLTC funding for high risk pregnancies in 2014. Also shown are the LHINs with the lowest and highest utilization of cfDNA screening following a positive MMS. Again there are substantial regional variations noted, with a 2.7- fold higher cfDNA screening uptake rate in Toronto Central LHIN (64.5%) compared to that for North West LHIN (24.2%).

During the study period, 11,261 (2.1%) singleton pregnancies had a PND. There were with 10,312 amniocenteses and 949 CVS. Figure 3 shows key pathways of prenatal screening before and after cfDNA screening was funded. After cfDNA screening was funded, the update rate of MMS increased slightly ($p<0.001$). PND among MMS positive women decreased substantially from 54.9% to 30.8% ($p<0.001$).

After women age≥40 years became eligible for primary cfDNA screening in 2014. The uptake of cfDNA screening in this age group increased from 2.4% to 33%, yet only 7.2% had primary

cfDNA screening (data not shown in the figure). This was accompanied by only a small decrease (from 75.7% to 72.2%, p<0.001) in the uptake of MMS in this group.

Figure 4 demonstrates graphically the uptake rates of MMS, cfDNA screening and/or PND among all pregnancies over the study period. CfDNA screening uptake increased from under 1.0% in 2012 to over 4.0% in 2016 (p<0.001). The uptake of PND decreased by over 50%, and the proportion of women who had a follow up testing (either NIPT screening or PND) more than doubled (p<0.001).

Figure 5 shows the changes in the uptake rates of cfDNA screening and PND in pregnancies with a positive MMS over the study period. There was a steady increase in the uptake of cfDNA screening with a consistent decrease in the uptake of diagnostic testing after cfDNA screening was funded (p<0.01). The proportion of women who had a follow up testing increased from about 60% to 75% (p<0.001).

Table 1 shows the highest uptake of PND was seen in women with a MMS risk ≥ 1 in 10 both before and after cfDNA screening was funded. There was a relatively smaller decrease in the rate of PND as the follow-up test in this group compared to those with lower risk MMS results, The greatest decline in the uptake of PND after funding was seen in women with a MMS risk in the range of 1 in 101 to 1 in 200 (from 51% to 23.5%). This group also had the highest uptake of cfDNA screening (55.1%) and biggest increase in the uptake of a follow-up testing (from 56.3% to 76%).

Interpretation:

Our study examined utilization patterns of MMS, cfDNA screening and PND testing in Ontario and specifically evaluated the impact of publicly funded cfDNA screening on the utilization of prenatal screening and diagnostic testing.

Over the study period, 67.6% of pregnant women had a prenatal screening test (whether MMS, cfDNA screening or both). Substantial variations were observed in overall uptake rates of prenatal screening across the province. There was a small increase in the overall uptake of MMS, which became more marked after publically funded cfDNA screening was introduced for higher risk pregnancies. Accompanying this trend was a decrease in PND following a positive MMS, with a more marked decline after funding and an increase in the uptake cfDNA screening secondary to a positive MMS. Possible explanations for increased MMS uptake include heightened awareness of screening following advertising of cfDNA screening. Another possible reason is that women were interested in cfDNA screening and underwent MSS to determine if they would be eligible via a positive MMS. Furthermore, a positive test for MSS, followed by a second blood test, cfDNA, may be more appealing than having to have an invasive procedure such as amniocentesis.

The uptake patterns of cfDNA screening demonstrate the significant impact of public policy and funding decisions on patterns of prenatal testing. The utilization of cfDNA testing increased markedly following funding amongst women age ≥ 40 years and those with a positive MMS result when it became a funded alternative to PND. Interestingly, only 33% of women >40 years

of age had cfDNA screening and only 7.2% had it as a primary screening test. In this study, we were not able to assess any details on counselling provided to pregnant women choosing prenatal testing options. However, these results suggest that previous Canadian policy guidelines to exclude maternal age alone as an indicator for invasive PND [11] continues to be operative, with most women aged 40 or older still undergoing MMS first, vs primary cfDNA screening. MMS can provide additional information on pregnancy health that cfDNA alone cannot provide, and thus may have been chosen by providers for that reason. As well, primary providers are challenged by rapidly changing information in this genomic era of prenatal testing and some of the patterns of utilization demonstrated over the study period may reflect delayed incorporation of these newer technologies. Lastly, there have been anecdotal reports of choosing two screens given that women age ≥ 40 years were eligible for both tests.

Provincial funding of cfDNA screening has been accompanied by a decreased utilization of PND, with the largest decline among those with a risk for Down syndrome lower than 1 in 50 (e.g. 1 in 100) , The uptake of any form of follow-up testing following a positive MMS increased substantially across all MMS risk groups, but especially among women with a MMS risk lower than 1 in 50 where follow-up PND was less likely prior to funded cfDNA This suggests that cfDNA screening as a non-invasive and highly accurate alternative to amniocentesis or CVS is positively regarded by pregnant women, or more likely to be suggested by providers, particularly for women at 'intermediate' or 'lower' risk groups.

There is a wide range in reported uptake rates of MMS in studies from different countries and screening programs; from 35.2% in the Netherlands, to about 76.0% in UK and 91.6% in

Denmark.[6 12 13] In Ontario, the overall uptake rate increased from 63% to about 68% over the past 5-7 year period, [14 15] but regional variations in the uptake of MMS remain. Because MMS is funded for all Ontario residents by the MOHLTC, it is unlikely the variable regional uptake rates were cost-driven as reported by some studies .[15] While we were not able to account for this variation, lower screening rates have been associated with living in a rural area, receiving first-trimester care from a family physician or midwife and being in a lower income quintile in Ontario suggesting there are areas to target for increased awareness and knowledge translation to support patient choice.[14]

Multiple recent studies have assessed the impact of cfDNA screening on prenatal screening and diagnostic services.[6 7 8 16 17] Because of the variations in prenatal screening tests and policies for cfDNA screening in these studies, it is difficult to directly compare the magnitude of the impacts. However, we have observed some similar patterns in the utilization of these tests in our population compared to others. In a study by Chitty et al. (2016), the proportion of women having a PND following a positive FTS decreased from 60% to 17.8% after the implementation of cfDNA screening. Of women in the same FTS risk group, 74.4% had cfDNA screening. Chan et al. (2015) reported a 45% decline in the rate of refusal of further testing and a one third reduction in PND after a positive MMS following introduction of cfDNA screening. [16] This marked decline in the utilization of PND following the implementation of cfDNA screening has also been reported by a number of other studies. [7 8 17 18 19]

Gil et al. (2015) examined the impact of FTS result on the uptake of cfDNA screening.[20] In women with FTS risk ≥1:100, 40% opted for CVS, 57% for cfDNA screening and 3% did not want any further testing. In women with risk between 1:101 to 1:1000, the uptake of cfDNA screening was 91.7%. Similar results were reported by Manegold-Brauer et al. (2015) [21] In our population, we observed a reduction of PND in all risk groups, with the biggest reduction seen in women with risk between 1:101 and 1:200.

Our study only included women with an EDD up to March 31 2016, therefore we cannot comment on more recent trends to determine a more long term view of the impact of funded cfDNA screening on prenatal screening and diagnostic testing. As well, we could not assess factors accounting for variations in uptake; such as lack of informed choice or sub-optimal counseling/access to MMS, cfDNA screening and PND. We were not able to assess how many women were offered prenatal screening tests and then declined, or the indications for cfDNA screening. Further studies are needed to evaluate the referral and utilization patterns as well as costs and effectiveness of MMS, cfNDA and PND.

In conclusion, our study described the utilization patterns of MMS, cfDNA screening and PND testing in Ontario prior to and after cfDNA screening was funded by MOHLTC. The results are useful for screening program planning and further analysis on the costs and benefits of different screening strategies. Further studies are warranted to investigate the factors that affect the utilization of MMS, cfDNA screening and PND in order to improve the efficacy and performance of prenatal screening.

Acknowledgements

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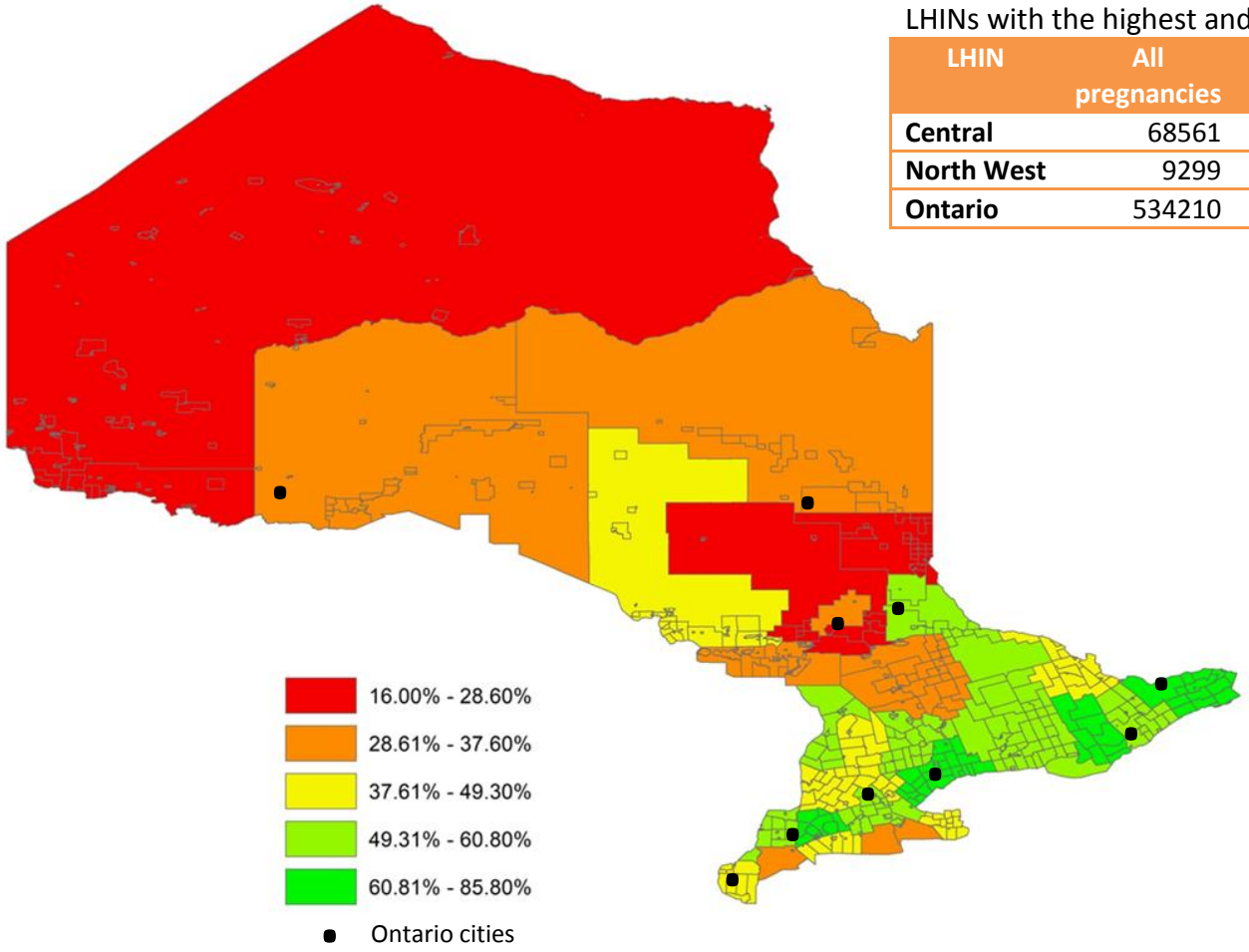

LHINs with the highest and lowest utilization of MMS

LHIN	All pregnancies	Pregnancies with MMS	Uptake of MMS (%)
Central	68561	57289	83.6 (Highest)
North West	9299	2576	27.7 (Lowest)
Ontario	534210	359066	67.2 (All)

Figure 1. Utilization of MMS Among all Singleton Pregnancies with an EDD Between July 1 2012 and March 31 2016 by Census Division

LHINs with the highest and lowest utilization of cfDNA screening Following positive MMS

LHIN	All pregnancies	Pregnancies with cfDNA	Uptake of cfDNA (%)
Toronto Central	775	500	64.5 (Highest)
North West	33	8	24.4 (Lowest)
Ontario	6036	2947	48.8 (All)

LHIN (Local Health Integration Networks)

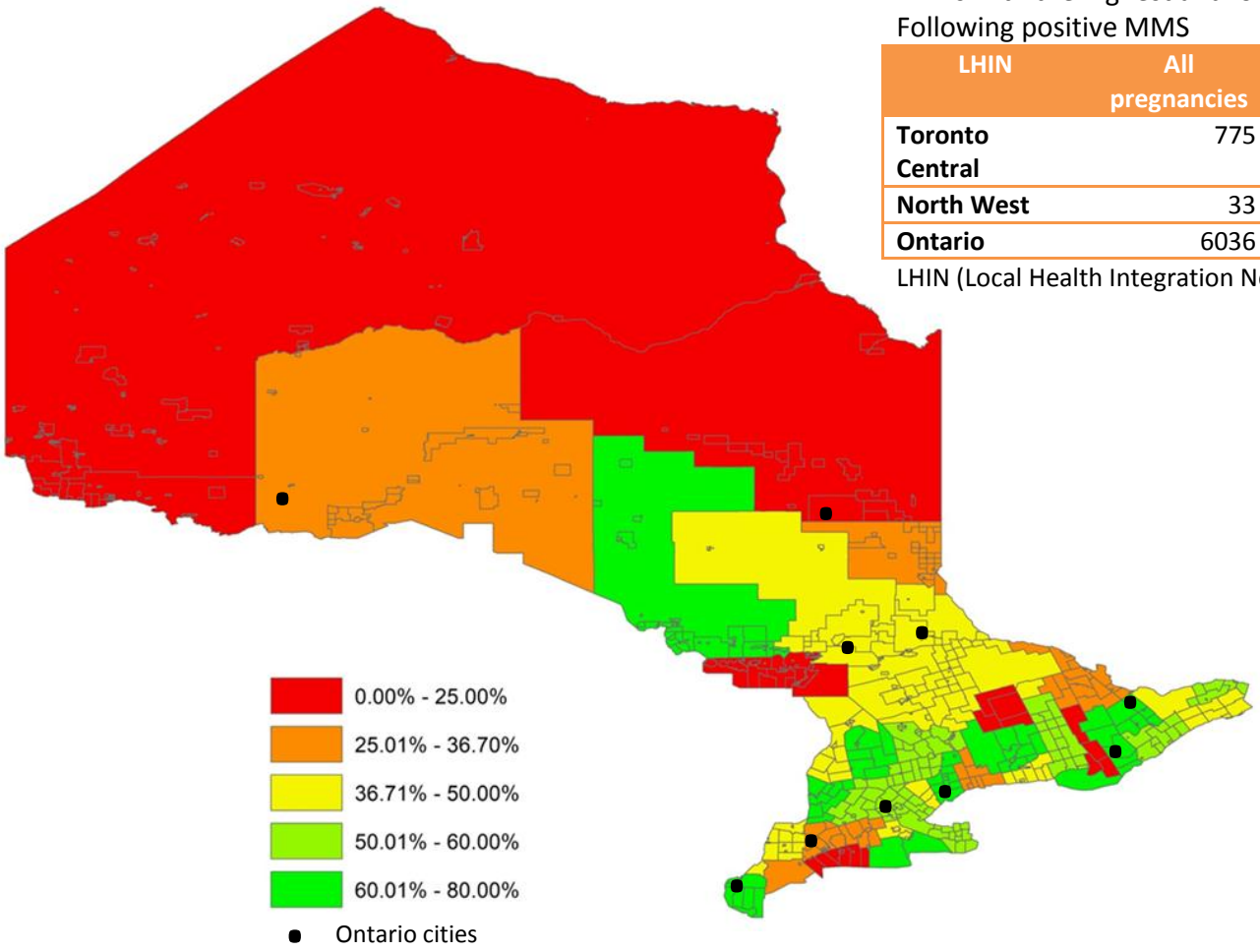

Figure 2 .Utilization of cfDNA screening Among MMS Positive Singleton Pregnancies with an EDD Between July 1 2014 and March 31 2016 by Census Division

Figure 3. Prenatal screening pathways before and after cfDNA screening was funded. Women who did not have a follow-up testing following a positive MMS were not shown

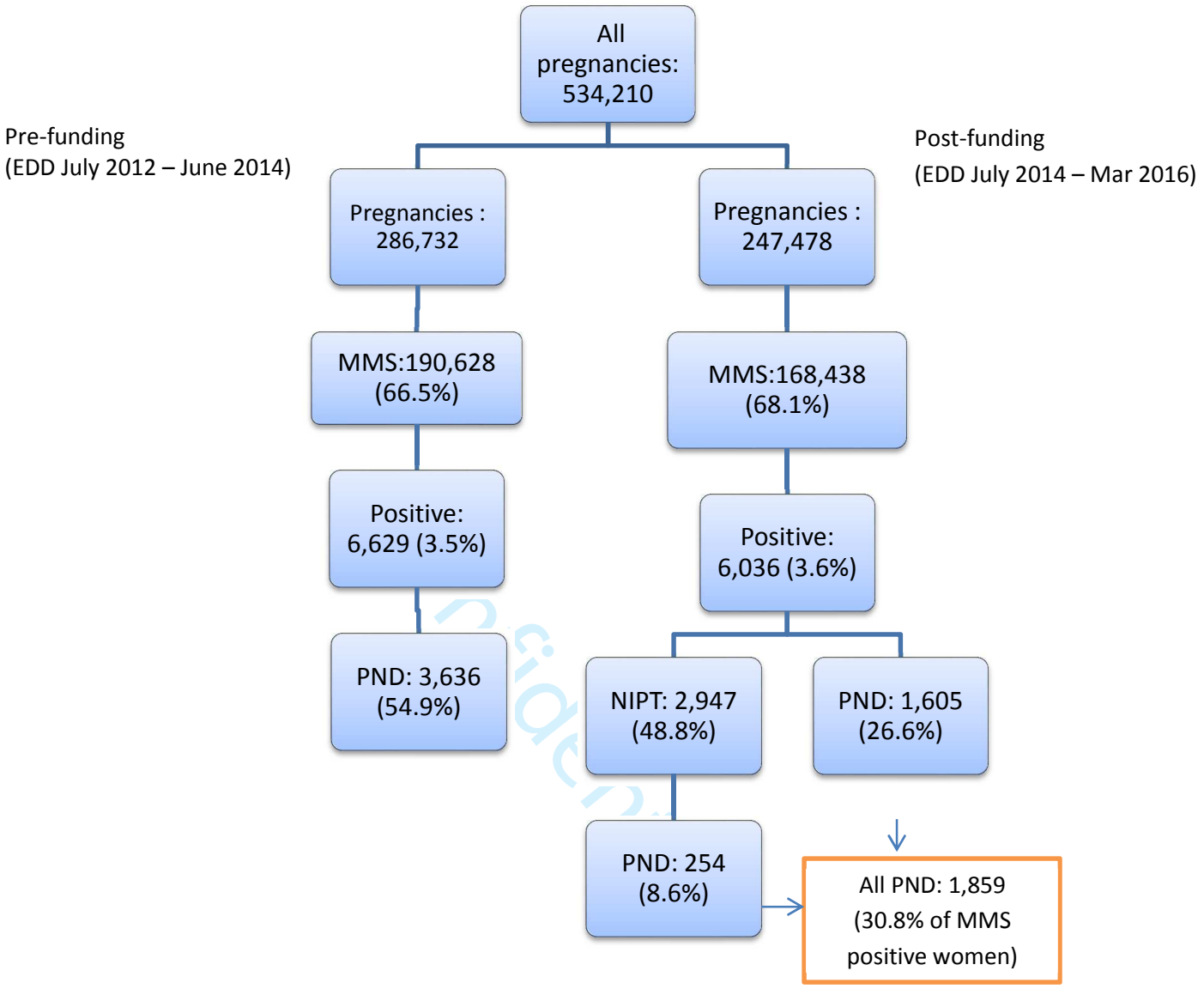

Figure 4. Uptake rates of MMS, cfDNA screening, and PND among all pregnancies (Ontario, Singleton, EDD between July 2012 and March 2016)

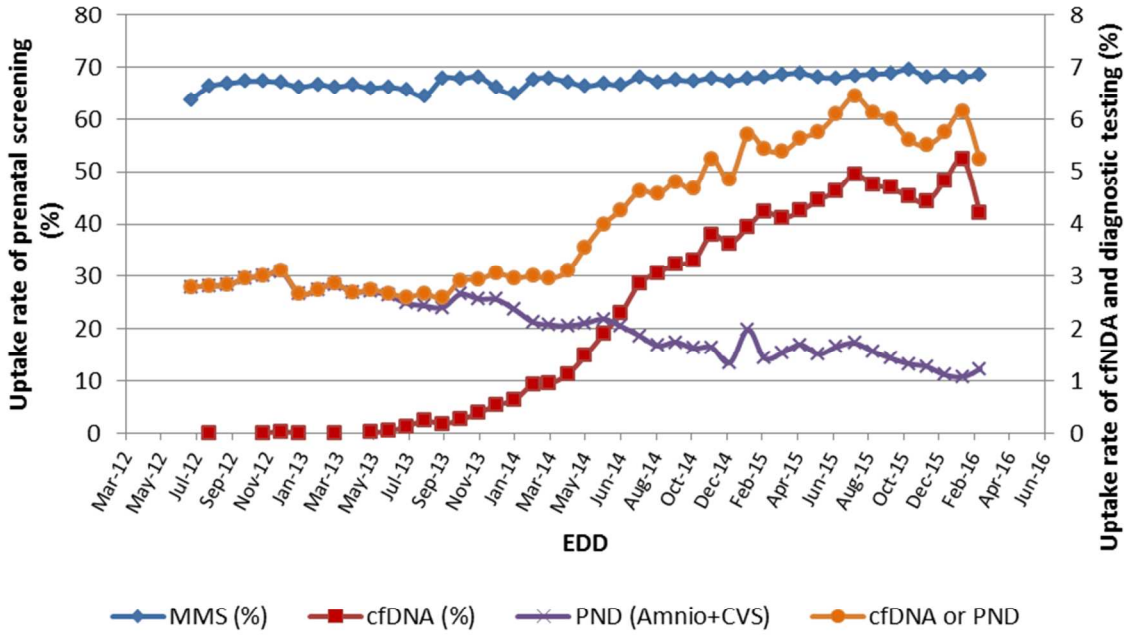

Figure 5. Utilization of cfDNA screening and PND among MMS positive pregnancies (Ontario, Singleton, EDD between July 2012-March 2016)

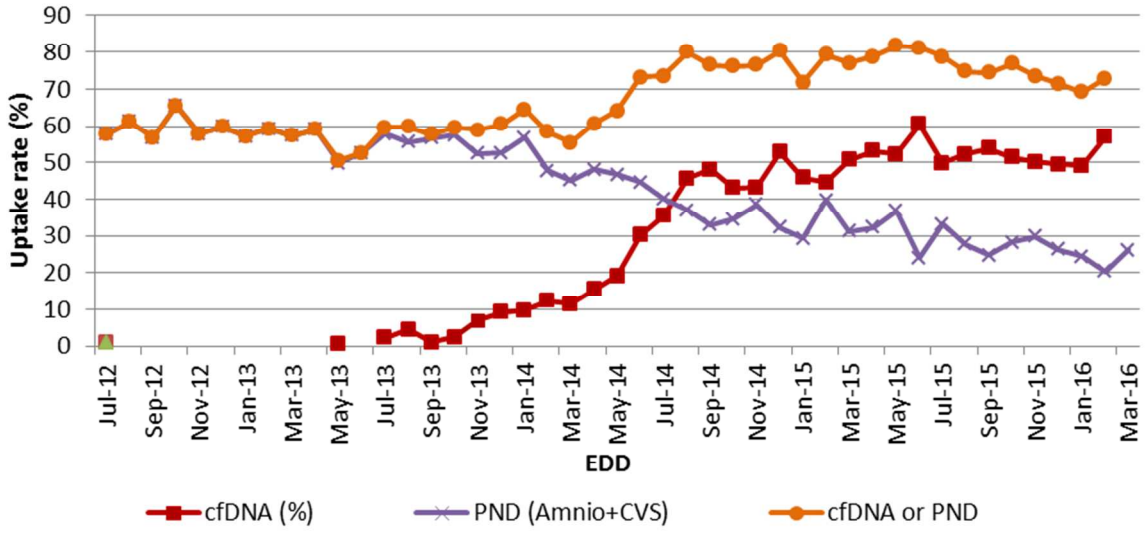

Table 1. Utilization of cfDNA screening and PND by the risk group of MMS (Ontario, singleton)

	Before cfDNA screening was funded #			After cfDNA screening was funded ##		
MMS risk for Trisomy 21	Uptake of cfDNA screening (%)	Uptake of PND#	Uptake of cfDNA screening or PND testing (%)	Uptake of cfDNA screening (%)	Uptake of PND (%)	Uptake of cfDNA screening or PND testing (%)
≥ 1 in 10	4	67.53	70.19	30.5	54.08	76.07
1 in 11- 1 in 50	6.08	63.07	67.8	44.28	40.47	78.77
1 in 51- 1 in 100	7.98	55.89	62.62	51.13	29.22	77.28
1 in 101- 1 in 200	6.39	50.88	56.26	55.1	23.51	76.28
1 in 201- 1 in 350	3.1	13.07	15.94	25.2	6.02	29.94
1 in 351- 1 in 500	1.35	6.43	7.47	12.5	2.31	14.62
1 in 501- 1 in 1000	1.11	4.1	5.15	9.29	1.64	10.64
1 in 1000- 1 in 5000	0.63	1.97	2.56	5.34	1.01	6.19
< 1 in 5000	0.23	0.65	0.88	2.03	0.45	2.44
All risk group*	0.58	3.25	3.77	4.96	1.89	6.61

* all risk groups plus no MMS result

EDD before July 2014

EDD between July 2014 and March 2016

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page Number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7-9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7-8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	8-9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	Figure 3
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	Report numbers of outcome events or summary measures over time	10-11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear	N/A

		which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	11 and Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.