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Study protocol for a descriptive analysis of non-invasive prenatal testing uptake and performance in singleton and twin pregnancies using Ontario birth registry data

Erin Collins ,[1] Bounhome Soukkhaphone,[2] Kara Bellai-Dussault,[3,4] Lynn Meng,[3] Mahin Ahmadi Pishkuhi,[1] Michele Rubini,[5] Shelley Dougan,[3,6] Julian Little [7]

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For numbered affiliations see end of article.

Correspondence to
Dr Erin Collins;
ecoll098@uottawa.ca

ABSTRACT

Introduction Despite the availability of funded first-tier non-invasive prenatal testing (NIPT) for twin pregnancies in Ontario, Canada, research gaps persist regarding the feasibility and effectiveness of NIPT in this demographic. This protocol documents our planned comprehensive overview of twin data from the large Ontario provincial registry and evaluates the performance of NIPT among singleton and twin pregnancies.

Methods and analysis We will conduct a descriptive study using routinely collected data housed in the Better Outcomes Registry & Network Ontario. The study population will include all singleton and twin pregnancies with an estimated date of delivery between 1 September 2016 and 31 March 2023. We will compare patient characteristics, NIPT uptake and test performance metrics (including sensitivity, specificity, positive predictive value and negative predictive value) between singleton and twin pregnancies. Subgroup analyses will be conducted, including assessment by the mode of conception, trimester of initial screening, age of the pregnant individual and eligibility for publicly funded first-tier NIPT.

Ethics and dissemination This study has received approval from the Research Ethics Boards of the Children's Hospital of Eastern Ontario (24/01PE) and the University of Ottawa (H-04-24-10309). Results will be disseminated through scientific conferences and publication in a peer-reviewed journal. By making our protocol and findings publicly available, we aim to establish a foundational reference for future investigations in Ontario. Additionally, we seek to support the design and implementation of further studies on NIPT in twin pregnancies in Canada and elsewhere.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study will use data from a large prescribed perinatal registry, which provides comprehensive coverage in Ontario, Canada, enabling robust analyses of all eligible singleton and twin pregnancies.
⇒ Our team of investigators possesses extensive multidisciplinary expertise, encompassing genetics, maternal health, epidemiology and statistics, and has a longstanding history of collaboration.
⇒ A limitation of the study is its retrospective design, relying on the interpretation of medical records, clinical forms and self-reported data from patients.
⇒ Furthermore, we anticipate the need to rely on existing literature or expert opinion for certain estimates due to small value suppression, where data cells with counts fewer than six will be withheld.

INTRODUCTION

Non-invasive prenatal testing (NIPT), also referred to as prenatal cell-free DNA (cfDNA) screening, is a method used to detect certain chromosomal abnormalities in the developing foetus.[1–3] Since its clinical introduction in 2011, NIPT has been widely adopted across healthcare systems in Canada and globally, offering high sensitivity and specificity for common aneuploidies. In a systematic review, Badeau et al[4] reported pooled sensitivities (95% CI) among high-risk populations using massively parallel shotgun sequencing (MPSS) of 99.7% (98.0% to 100%) for trisomy 21 (T21), 97.8% (92.5% to 99.4%) for trisomy 18 (T18) and 95.8% (86.1% to 98.9%) for trisomy 13 (T13). Corresponding specificities (95% CI) were 99.9% (99.8% to 100%) for T21 and T18 and 99.8% (99.8% to 99.9%) for T13. Similar performance metrics were observed with targeted MPS (TMPS). Other systematic reviews[5 6] have reported comparable findings. Through a meta-analysis, Gil et al[5] found high pooled detection rates for singleton pregnancies: 99.7% (99.1% to 99.9%) for T21, 97.9% (94.9% to 99.1%) for T18 and 99.0% (65.8% to 100%) for T13, with a combined false-positive rate of 0.13%. More recent primary studies

Table 1 Comparison of prenatal screening strategies in Ontario, Canada[1 3 13 43 44]

Enhanced first trimester screening (eFTS)	Non-invasive prenatal testing (NIPT)
Can screen for T21 (Down syndrome) and T18 (Edwards syndrome)	Can screen for T21 and T18, as well as T13, SCAs (including 45,X/Turner syndrome, 47,XXY/Klinefelter syndrome, 47,XYY/Jacobs syndrome and 47,XXX/triple X syndrome) and microdeletion syndromes
Requires a blood test and nuchal translucency ultrasound	Requires a blood test
Less accurate than NIPT*	More accurate than eFTS†
Less expensive than NIPT	More expensive than eFTS
Both are safe and pose no risk to the foetus	
Test failure is less of an issue than for NIPT	Risk of test failure, especially among true cases of chromosomal anomalies
OHIP-funded if specific criteria are not met‡	NIPT for common trisomies and SCAs (for singletons) is OHIP-funded if specific criteria are met, while publicly funded for all twin pregnancies since December 2021‡

*NIPT performance metrics (OHIP-funded only) estimated using cytogenetic testing data from the Born Information System (estimated delivery dates: 1 September 2016–31 March 2023): trisomy 21 - sensitivity 99.13% (95% CI, 98.48 to 99.55), specificity 99.91% (95% CI, 99.89 to 99.94), false-positive rate 0.09% (95% CI, 0.06 to 0.11); trisomy 18 – sensitivity 95.76% (95% CI, 92.98 to 97.66), specificity 99.97% (95% CI, 99.96 to 99.99), false-positive rate 0.03% (95% CI, 0.01 to 0.04); trisomy 13 – sensitivity 92.52% (95% CI, 85.80 to 96.72), specificity 99.95% (95% CI, 99.93 to 99.97), false-positive rate 0.05% (95% CI, 0.03 to 0.07).
†eFTS performance metrics estimated using cytogenetic testing data from the Born Information System (estimated delivery dates: 1 September 2016–31 March 2023): trisomy 21 – sensitivity 88.23% (95% CI, 86.38 to 89.91), specificity 93.94% (95% CI, 93.87 to 94.01), false-positive rate 6.06% (95% CI, 5.99 to 6.13); trisomy 18 – sensitivity 87.31% (95% CI, 83.61 to 90.43), specificity 99.71% (95% CI, 99.70 to 99.73), false-positive rate 0.29% (95% CI, 0.27 to 0.30).
‡Criteria include: a positive prenatal screening result from multiple marker screening; maternal age ≥40 years at the expected date of delivery; nuchal translucency measurement ≥3.5 mm; history of a previous pregnancy or child with T21, T18 or T13; ongoing twin pregnancy.
OHIP, Ontario Health Insurance Plan; SCA, sex chromosome aneuploidies; T13, trisomy 13 ; T18, trisomy 18; T21, trisomy 21.

(2018–2024), including large-scale analyses from South Korea, China and Italy,[7–10] continue to support the high accuracy of NIPT in diverse populations.

In Ontario, Canada, the Ontario Health Insurance Plan (OHIP)-funded NIPT has been available for common trisomies (T13, T18 and T21) in singleton pregnancies meeting specific criteria (eg, positive multiple marker screen, maternal age ≥40 years at the expected date of delivery, history of a previous pregnancy or child with aneuploidy) since 2016,[3 11 12] and in December 2021, it was extended to include all twin pregnancies.[13] For pregnancies not meeting the specific criteria, OHIP-funded Enhanced First Trimester Screening is available, or pregnant individuals may choose to self-pay for the screen.[14] Table 1 compares these prenatal screening strategies.

In Canada, twin pregnancies have been steadily rising, due to factors such as increasing maternal age and use of fertility treatments.[15–17] Consequently, the demand for NIPT in twin pregnancies is expected to rise. However, published data on NIPT in twin pregnancies remains more limited than in singletons.[3 17–21] Canadian studies on this topic are sparse, and small sample sizes do not allow for robust exploration of NIPT uptake and performance in this population. Notably, data related to anomalies other than T21 are often lacking or exhibit wide uncertainty.[18 21–25] Additionally, the integration of twins in NIPT studies adds complexity to the analysis due to factors such as the following:

- Necessity to identify vanishing twins, as NIPT is not recommended for these cases.[1 20 26 27]
- Higher NIPT failure rates associated with twin pregnancies than with singleton pregnancies.[17]
- Foetal fraction, which may be lower and less stable in twin pregnancies, influences NIPT performance.[2 20 28]
- Additional variables within the twin population, such as zygosity, can introduce fluctuations in NIPT performance.[20 27 28]

Currently, the limited understanding of how to identify and integrate eligible twin pregnancies in analyses has led to the exclusion of this population in many NIPT-related studies. Nevertheless, there remains a need for further research on the performance of NIPT in twins, both in Canada and globally. To address this, we will conduct a comprehensive descriptive analysis comparing the use and performance of NIPT among eligible twin pregnancies with those among singleton pregnancies, using data from the BORN Ontario registry. This study aims to present a descriptive analysis of patient characteristics and screening uptake, comparing singleton with twin populations, which may serve as a foundational reference for future studies and facilitate the inclusion of twins in NIPT-related research.

METHODS AND ANALYSIS

Study design and data source

This study will use data from BORN Ontario,[29] including data collected directly in the BORN Information System and linked data from the Canadian Institute for Health Information (CIHI). BORN Ontario is a prescribed registry established under the Personal Health Information Protection Act 2004 (PHIPA) to facilitate and enhance the provision of healthcare in the province. As a registry, BORN has the authority to collect personal health information without consent for these purposes, subject to PHIPA, its regulation (O. Reg. 329/04) and procedures approved of by the Information and Privacy Commissioner of Ontario.

Study population

The study will include all singleton and twin pregnancies during years of OHIP-funded NIPT for common aneuploidies in Ontario (ie, with an estimated date of delivery between 1 September 2016 and 31 March 2023).

- ***Twin cohort:*** this study will capture all pregnancies with ≥1 encounter identifying a twin pregnancy in the twin cohort.
- ***Singleton cohort:*** remaining pregnancies with all encounters identifying a singleton pregnancy will be captured in the singleton cohort.

Follow-up data on singleton and twin cohorts will be obtained. We will perform sensitivity analyses limited to a) 3 months and b) 1 year of follow-up data available after birth. The number of women with multiple eligible pregnancies and/or multiple NIPT tests within a single pregnancy, along with test failure rates over time for each cohort, will be reported. In cases that multiple pregnancies are eligible, a sensitivity analysis will be performed using the most recent pregnancy per individual. For pregnancies with more than one NIPT test, the most recent test with an available result will be used to calculate performance metrics. All NIPT tests will be considered when deriving test failure rates. Any pregnancies with >2 foetuses identified at any encounter will be excluded, as NIPT is not recommended in these cases.[1]

Study characteristics and outcomes

The primary exposure is twin pregnancy. Maternal characteristics, screening/prenatal characteristics and pregnancy complications will be compared between the twin and singleton cohorts. Results will be reported both before and after the removal of vanishing twins to assess the effect of including these records. Subgroup analyses will be conducted among predefined groups, including the mode of conception (eg, use of in vitro fertilisation), trimester of initial screening, maternal age and eligibility for OHIP-funded screening.[1 2 19 30–38] The variables to be assessed are outlined in table 2. Further details can be found in online supplemental file 1.

Table 2 Characteristics and outcomes to be extracted for the twin cohort and compared with the singleton cohort, where appropriate

Category	Characteristics and outcomes (counts and %, unless otherwise stated)*
Maternal characteristics	► Age (<25, 25–29, 30–34, 35–39 and ≥40 years at the expected date of delivery)[19 23 25 27–30 33–38 40] ► Self-reported smoking[2 31 40] ► Pre-pregnancy BMI (median, IQR)[19 21 25 28 31 33 34] ► Parity (number of previous live and stillbirths; median, IQR)[21 22 28] ► Neighbourhood income quintile[32 35–37] ► Neighbourhood education quintile[32 35–37] ► Rural/urban residence[35 37]
Foetal–placental and screening factors	► Gestational age at the time of the first screening (<14 vs 14–21 weeks)[18 19 26–28] ► Eligibility for publicly funded NIPT[19 29–33 35 36] ► Vanishing twins[19 20 22 26 27 31] ► Foetal growth restriction[19 33 34 38] ► IVF status[21–25 28 30 31 33 40] ► Screening modality[25 28 33] ► Numbers a) eligible for and b) to undertake the first screening, second screening and confirmatory testing[30 33–37] ► Number to have negative, positive or inconclusive results for the first screening, second screening and confirmatory testing (median, IQR)[31–33 38]
Pregnancy complications/ outcomes	► Stillbirths[19 38 40] ► Spontaneous loss (related vs not related to a procedure)[19 20 27 30–33 38 40] ► Selective termination of pregnancy[22 26 38 40] ► Preterm labour[19 20 33 34 38] ► Unaffected vs affected foetuses[26 37 38]

*The references supporting the rationale for inclusion are cited.
BMI, body mass index; IQR, interquartile range; IVF, in vitro fertilisation; NIPT, non-invasive prenatal testing.

Analysis plan

The analysis will be conducted in the BORN secure environment. Data processing and statistical analyses will be performed by BORN Analysts in SAS (SAS V.9.4, SAS Institute). The analysis will include a description of cohort characteristics and derivation of performance metrics.

Description of cohort characteristics

Continuous variables will be reported as medians (interquartile range) and categorical variables as counts (%). Characteristics will be compared using χ^2 for categorical variables (or Fisher's exact test if ≥1 cell has ≤5 observations) and the Mann–Whitney U test for continuous variables. A p-value <0.05 will be considered to be statistically significant. Characteristics will be derived for 1) all singleton vs all twin pregnancies, 2) all singleton vs NIPT-eligible twin pregnancies (ie, after excluding vanishing twins) and 3) subgroups of interest (including the mode of conception, trimester of the initial screening, maternal age and eligibility for OHIP-funded screening) as data permit.

We will also capture and compare rates of obstetric complications (eg, preterm delivery and stillbirth) among singleton and twin cohorts, as evidence suggests that twin pregnancies have a higher risk of these outcomes than singleton pregnancies.[39] Various factors contribute to this disparity, including uterine overdistension and placental insufficiency.[39 40] However, comprehensive data, particularly from population-based registries in Canada, remain insufficient. This gap impedes our understanding of the specific risks faced by women with twin pregnancies. Moreover, this analysis can lay the groundwork for future research and educational endeavours aimed at advancing our understanding, prevention and management of these conditions across different pregnancy groups.

Derivation of diagnostic performance metrics

The sensitivity, specificity, positive predictive value and negative predictive value for common trisomies, SCAs and 22q11.2 deletion syndrome, along with test failure rates, will be derived for 1) singleton pregnancies, as compared with eligible twin pregnancies (after excluding vanishing twins), and 2) subgroups of interest. The test failure rates will also be analysed according to the gestational age within each cohort. Results will be presented with 95% CIs. To derive performance measures, NIPT results will be compared with findings from prenatal diagnostic testing or postnatal clinical assessment (ie, unaffected or affected). Hence, to derive measures of NIPT performance, pregnancies must have 1) NIPT results available at ≥1 encounter and 2) ≥1 of the following available: a) aneuploidy diagnoses through cytogenetic testing, b) documentation of clinical examination where no features of aneuploidy were noted or c) follow-up data available ≥3 months after birth. We will assume that infants and linked pregnancies are unaffected if birth records show no identification of aneuploidy by a minimum of 3 months after birth.

Sample size

Given the descriptive nature of this study, we are not planning a formal sample size calculation. As described above, we will include all eligible singleton and twin pregnancies. The planned subgroup analyses will include all eligible pregnancies, appraise the size of subgroups and summarise potential limitations of available data (eg, wide uncertainty).

Missing data

The extent and patterns of missing data for all study variables will be reported. Based on prior analyses using BORN data,[37 41] we anticipate that most missingness will be random and will address it through multiple imputation. For variables with substantial missingness (ie, >50%), we will consult with co-authors to assess the appropriateness of using expert-informed estimates. Where applicable, sensitivity analyses will be conducted to compare findings based on expert-informed vs data-derived estimates.

Patient and public involvement

The study involves secondary data routinely collected in Ontario's prescribed perinatal registry BORN and linked databases (CIHI). There will be no direct patient involvement in the design, conduct, reporting or dissemination of our research.

ETHICS AND DISSEMINATION

The protocol has been approved by the Research Ethics Boards of the Children's Hospital of Eastern Ontario (24/01PE) and the University of Ottawa (H-04-24-10309). Data preparation is underway.

Anticipated risks and benefits

In developing this protocol, we thoroughly evaluated the expected risks and benefits associated with the proposed study and determined that the anticipated risks are minimal. The personal health information to be used is routinely collected by BORN Ontario, and its use in this study neither poses risk to the participants nor will affect or alter the current standard of care. All BORN Ontario policies and procedures governing the protection of individual privacy and the safeguarding of personal health information will be rigorously adhered to throughout the study. Notably, BORN Ontario recommends the suppression of any data cells with counts fewer than six, although zero counts may be reported, to minimise the risk of identifying individuals. Should we encounter cell counts below six, we will suppress these results and instead rely on relevant literature and expert opinion to provide estimates.

In addition to addressing privacy concerns, we acknowledge the potential risks of drawing unsubstantiated conclusions about specific participant populations. To mitigate these risks, the interpretation and reporting of study results will be thoroughly scrutinised. A multidisciplinary team of experts from the University of Ottawa and BORN Ontario will conduct a comprehensive review of

results, ensuring rigour in the study's conceptualisation, terminology and analysis prior to dissemination.

Regarding the anticipated benefits, we expect our study to significantly contribute to future research by facilitating the identification and inclusion of twin pregnancies eligible for NIPT, addressing the current lack of insight into these data. By improving our understanding of twin pregnancies in NIPT-related research, we can enhance the overall accuracy and applicability of study findings, ensuring that they reflect the diverse realities of pregnant individuals and their families.

This protocol provides detailed guidance on how to identify eligible twin pregnancies in a provincial birth registry. By transparently and thoroughly reporting our methods, we endeavour to facilitate the design and implementation of future studies evaluating prenatal screening methods among different populations. Finally, we aim to raise awareness about the complexities of including this population in NIPT research efforts.

Data access, storage and retention

All linked database files will be de-identified, stored, accessed and analysed exclusively within the secured network environment at BORN. Access will be password-protected and limited to the BORN team. The BORN servers are located at the Children's Hospital of Eastern Ontario in Ottawa, Canada, and study data in an identifiable form (linked data set/analytic data set with pregnancy identifier) will be retained for 10 years after study completion and then destroyed.

Future uses of data/data sharing

Data collected for this research may be used in future related research projects that are either an extension of the original project or in the same general area of research (secondary use of data). Researchers outside of this specific study may request access to the coded data for new research purposes.

Dissemination of findings

The results of this study will be disseminated through conferences and meetings, with a minimum of two manuscripts submitted for peer review. Results will be published according to the Reporting of studies Conducted using Observational Routinely-collected health Data statement.[42] By making our protocol and study results publicly available, we aim to provide a foundational reference for future investigations in Ontario and assist other Canadian provinces and territories in adapting our methods for similar studies. Our protocol may also benefit research teams in other countries grappling with the need for further investigation into the feasibility and effectiveness of NIPT among diverse populations. Indeed, we have shared our protocol with a research team in Italy, where, similar to Canada, increasing twinning rates coincide with the scarcity of data on NIPT in twin pregnancies.[2] We are currently collaborating with this team to launch a similar initiative.

DISCUSSION

We anticipate several challenges in conducting our descriptive analysis. The retrospective nature of the study imposes inherent limitations, as it relies on the interpretation of medical records, clinical documentation and patient self-reported data. Consequently, we are restricted to information that has already been collected. One key limitation is the current unavailability of data on zygosity, which may influence NIPT performance. We expect this information may become available in the future, at which point we plan to repeat the analysis, stratifying results by monozygotic and dizygotic status. Another consideration is the use of multiple NIPT providers in Ontario (Harmony and Panorama), which is reflective of real-world conditions. While we expect the test performance to be comparable, we are unable to analyse results by test provider due to data-sharing agreements that restrict access to commercially sensitive information. Additionally, while maternal health conditions, such as hypertension, diabetes and cancer, may have offered further insights into cohort characteristics and pregnancy outcomes, these data were not accessible as they were considered to be beyond the scope of this study's primary objectives. Finally, due to small cell suppression policies, whereby data with cell counts fewer than six are withheld for privacy reasons, we anticipate the need to rely on published literature or expert opinion for certain estimates. For instance, case counts are expected to be low when evaluating the screening performance for rarer genetic anomalies.

Research gaps persist regarding the feasibility and effectiveness of NIPT for twin pregnancies, compared with singleton pregnancies. By conducting a comprehensive descriptive analysis of a large provincial data registry, we aim to facilitate the inclusion of eligible twin pregnancies in future NIPT-related studies in Ontario, Canada, addressing the current issue of exclusion due to data limitations. By publishing our protocol, we seek to assist jurisdictions in Canada and internationally in better understanding data pertaining to twin pregnancies, integrating this population into prenatal screening analyses and facilitating the design and implementation of future studies.

Author affiliations
[1]School of Epidemiology and Public Health, University of Ottawa Faculty of Medicine, Ottawa, Ontario, Canada
[2]Lao Tropical and Public Health Institute, Vientiane, Lao People's Democratic Republic
[3]Prenatal Screening Ontario for Better Outcomes Registry & Network, Ottawa, Ontario, Canada
[4]Children's Hospital of Eastern Ontario Research Institute, Ottawa, Ontario, Canada
[5]Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Emilia-Romagna, Italy
[6]Children's Hospital of Eastern Ontario (CHEO) Research Institute, Ottawa, Ontario, Canada
[7]School of Epidemiology and Public Health Ottawa, University of Ottawa Faculty of Medicine, Ottawa, Ontario, Canada

Contributors EC, BS, KB-D, SD and JL contributed to the study conception and design. JL supervised all stages of this work and is the guarantor. JL was the PI at the University of Ottawa, and KB-D served as the PI at CHEO. SD, KB-D and LM continue to lead data preparation efforts at BORN Ontario. EC and BS drafted the manuscript. All of the authors revised it critically for important intellectual content, gave final approval of the version to be published and agreed to be accountable for all aspects of the work. The content and views expressed in this article are those of the authors and do not necessarily reflect those of BORN Ontario.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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ORCID iDs
Erin Collins http://orcid.org/0000-0002-4209-1786
Julian Little http://orcid.org/0000-0001-5026-5531

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