BMJ Open

Clinical Utility of Non-Invasive Prenatal Testing in mitigating concerns from Invasive Prenatal Diagnostic Testing

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-057658
Article Type:	Original research
Date Submitted by the Author:	22-Sep-2021
Complete List of Authors:	Craft, Kaitlyn; The University of Utah Kim, Kibum; The University of Utah, Department of Pharmacotherapy; University of Illinois at Chicago, Department of Pharmacy Systems, Outcomes and Policy
Keywords:	OBSTETRICS, Prenatal diagnosis < OBSTETRICS, Fetal medicine < OBSTETRICS, HEALTH SERVICES ADMINISTRATION & MANAGEMENT

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

TITLE

Clinical Utility of Non-Invasive Prenatal Testing in mitigating concerns from Invasive Prenatal Diagnostic Testing

A Short Running Title

Clinical Utility of Non-Invasive Prenatal Testing

Manuscript Size

Word Count: 3,394

Table Count: 3

Figure Count: 1

Authors and Affiliations

Kaitlyn Craft, PharmD.

College of Pharmacy, University of Utah

30 South 2000 East

Salt Lake City, UT 84112

Kaitlyn.Craft@pharm.utah.edu

(408)613-3865

Kibum Kim, BPharm, PhD

Assistant Professor | Department of Pharmacy Systems, Outcomes and Policy, University of Illinois at Chicago

833 South Wood Street, Chicago, IL 60612

Kkim204@uic.edu

(312) 413 - 0152

Adjunct Assistant Professor | Department of Pharmacotherapy, University of Utah

30 South 2000 East

Salt Lake City, UT 84112

kibum.kim@pharm.utah.edu

Correspondence.

Kibum Kim, BPharm, PhD

Assistant Professor | Department of Pharmacy Systems, Outcomes and Policy, University of Illinois at Chicago

833 South Wood Street, Chicago, IL 60612

Kkim204@uic.edu

(312) 413 - 0152

Abstract:

Purpose:

Non-Invasive Prenatal Testing (NIPT) stratifies the risk of congenital disorders from fractions of fetal chromosome without invasive prenatal diagnostic test (IPD), which potentially decrease the risk of IPD-related complications. The objectives of this study were (1) to understand the acceptance of NIPT and (2) to evaluate if Non-Invasive Prenatal Testing (NIPT) is associated with a decrease in the use of Invasive Prenatal Diagnostic Testing (IPD).

Methods:

This was a retrospective observational research consisting of site-level longitudinal analysis and patient-level cross-sectional analysis. The site-level trends of NIPT, IPD, and unique high-risk pregnancies between 2012 and 2018 were descriptively summarized. The patient-level analysis included women with high-risk pregnancy between October 2015-December 2018. Using regression models adjusted for the patient characteristics, the association between the use of NIPT and IPD procedures was tested.

Results:

The rate of increase in the NIPT use exceeded the change in the number of high-risk pregnancies while the annual IPD count has fluctuated without specific trends. Being enrolled in a commercial health plan was significantly associated with both NIPT and IPD. However, there was no significant association between the numbers of NIPT and IPD with the adjusted odds ratios between 0.9 and 1.1 ($p>0.1$). The order of NIPT was not selected as an independent variable predicting the use of IPD.

Conclusion:

Although prenatal care accepted NIPT, the utility of NIPT in mitigating concerns on IPD is unclear and will need further investigation. There was a disparity gap in access to prenatal screening and diagnostics.

Article Summary

Strengths and limitations of this study

- This study includes both cohort-level and patient-level assessments on the utility of non-invasive prenatal testing (NIPT) in the US healthcare setting.
- A rapid increase in the use of NIPT and a gradual expansion of NIPT use outpace the increase in the need for high-risk pregnancy care.
- The use of NIPT is not apparently driven by the clinical utility in mitigating concerns on invasive-prenatal diagnostic testing but directed by the access to the advanced prenatal care.
- Although this study provides variable insight into the use and utility of the advanced prenatal care strategy, general limitations of single-site observational research warrant a multisite health outcomes study.

MAIN TEXT

Introduction

Fetal chromosomal abnormalities (FCA) impose a significant life-long burden, both emotionally and financially.[1-8] People living with congenital disability and their caregivers undergo disarrayed life trajectory and suffer from impaired quality of life.[1 2] Thus, detection of FCA before a child is born helps to mitigate the life-long and societal burden by early termination of pregnancy.

There have been advances in prenatal care that enable expectant parents to learn of congenital disorders earlier, allowing them to make informed medical decisions, including termination of pregnancy. Maternal serum screening (MSS) is a minimally invasive traditional approach to determine the risk of fetal congenital disorders.[9-11] However, MSS has been shown to have low clinical validity, the risk of FCA based on MSS does not well predict the actual rate of chromosome abnormality, as it has a positive predictive value of 70% for pregnancies affected by Down syndrome. [9-12] A better prediction of congenital disorders has been achieved via diagnostic invasive prenatal testing (IPD), which includes amniocentesis, chorionic villus sampling (CVS), and fetal blood sampling (FBS). Though providing patients with clinically valid data with a 99% positive prediction of certain FCAs, IPD is associated with a minor but sizable increase in the rate of miscarriage and infection.[3] Complications after IPD has been a concern to both providers and expectant mothers.[3]

Non-Invasive Prenatal Testing (NIPT), or cell-free DNA (cfDNA) testing, is a screening to help identify potential genetic abnormalities.[10] NIPT relies on the presence of free-floating cfDNA which arise when cells die and release the DNA into the bloodstream from the placenta. If the percentage of cfDNA fragments for a particular chromosome is higher than expected, it indicates that the fetus has an increased likelihood of having a disorder associated with that chromosome, and further testing should be performed. NIPT has been shown to have a sensitivity and specificity above 99% for detecting trisomy 21, as well as a 98% positive predictive value for fetal trisomy 18, and a 99% positive predictive value for fetal trisomy 13 with a combined false-positive rate of 0.13%.[7 10]

NIPT shows promise in reducing unnecessary invasive medical procedures but is associated with a high upfront cost to healthcare plans in the US healthcare setting.[4] When it is covered, NIPT can cost payers upwards of $3,000 and patients with insurance are left with an out-of-pocket cost.[6] In addition, many state Medicaid plans and some health plans are not on board to pay for NIPT.[4 6] While NIPT has upfront costs, implementation of this procedure has the potential to reduce unnecessary medical costs and potential maternal or fetal harm. A previous model-based study demonstrated that NIPT can reduce the number of unnecessary invasive tests by 94.8% and decrease IPD-related miscarriages by 90%.[13] Out of 1,000,000 simulated scenarios, replacing MSS with NIPT would result in an increase in 893 detections of FCA and would be followed by a cost savings of approximately $170 million.[8] Potential cost savings to payers would be achieved when the clinical decision follows the recommended order: IPD performed only for the patients with an increased risk determined by NIPT test results. Nevertheless, NIPT results can be a small addition to a previous standard of care, rather than becomes the most critical component, to determine the needs of IPD. Clinical practice can still be directed by ultrasound assessment, patient preference, and provider's previous training, which may not result in the cost-effectiveness use of the NIPT as simulated. The objectives of this study were to assess the acceptance of NIPT in clinical practice setting, to evaluate if NIPT decreases the utilization of IPD, and to assess the patient-level characteristics that lead to the order of NIPT and IPD. We hypothesized that there would be a negative association between the order of NIPT and frequency of invasive diagnostic procedures performed, which is a strong signal of the clinical utility of NIPT.

Methods

Study Design and Setting:

This is a single-site retrospective observational research consisting of two sections: (1) Site-level longitudinal change analysis and (2) Patient level cross-sectional analysis using data from the University of Utah enterprise data warehouse (EDW) from which comprehensive clinical records and healthcare

resource utilization at the University of Utah Health are available. The University of Utah Institutional Review Board approved this study and deemed it exempt (00115830).

Patient and Public Involvement

Patients were not involved in this study.

NIPT acceptance:

We compared the number of NIPT to the total number of high-risk pregnancies and the number of IPD performed at site-level to visualize the trends of the acceptance of NIPT into the healthcare setting. We looked at the longitudinal variation to see if the numbers of NIPT and IPD testing done over time aligns with the number of new high-risk pregnancies or exceeds the changes in the new high-risk pregnancy cases. Analytic cohort included pregnant women with one or more records of high-risk pregnancy (ICD-10-CM O09.x, V23.x) between January 2012 and December 2018.[14-20] The NIPTs ordered during the study period within the healthcare network were identified using terminology available from institutional treatment records including "NON-INVASIVE PRENATAL", "NIPT FETAL ANEUPLOIDY", "NIPT FETAL MICRODELETION", "CELL-FREE DNA" as well as available brand names of NIPT tests. To be the IPD of interest, the procedure happened at the University of Utah Health and was defined using texts "chorionic villus" and "amniocenteses from pathology, laboratory and procedure records." Descriptive statistics include the number of patients ordering NIPT, receiving IPD procedures, and seeing provider for a new high-risk pregnancy.

Patient Level analysis:

Study cohort identification:

The analytic cohort for the patient-level analysis was a subset of patients from the longitudinal cohort: subjects with a diagnosis of high-risk pregnancy (ICD-10-CM O09.x) at any point between

October 2015 and December 2018.[14-20] NIPT can generally be considered once the gestational age is past 9 weeks, which can be followed by additional CVS before the gestational weeks 11 and 14 of pregnancy.[10] Amniocentesis is usually performed between 15 to 18 weeks of gestational age although more amniocentesis procedures are now being performed at 11-14 weeks' gestation.[21] The first pre-natal visit for a new pregnancy usually happens around the gestational age of 8 weeks.[22] All things considered, eligible subjects had a record of prenatal care from the first-trimester (ICD-10-CM O09.x1, O09.5x1, O09.6x1, O09.8x1) and must be followed by the University of Utah Health for longer than 90 day periods from that first-trimester visit, which allowed for a sufficient window to cover both NIPT and IPD. Sensitivity of NIPT is debatable in patients having more than one fetus. Thus, any expectant mothers with a record of multiple gestation (ICD-10-CM O30.x)[14] were excluded from this study. All the selected subjects were 35 years old or older at the first encounter with the first-trimester prenatal care visit. Clinical characteristics, demographic characteristics and record of NIPT and IPD were collected over the 90-day follow up. Patient characteristics were identified from the review of diagnosis codes, enrollment information, and hospital demographic table. Patient characteristics included maternal age (ICD-9 659.63, ICD-10 O09.51x, O09.52x), insufficient prenatal care (ICD-9 V23.7, ICD-10 O09.3), genitourinary tract infection during pregnancy (ICD-9 646.0x, ICD-10 O23.x), grand multiparity (ICD-9 659.4, ICD-10 O09.40), type 1 or type 2 diabetes (ICD-9 250, ICD-10 O24.01, O24.11), history of hypothyroidism (ICD-9 243, ICD-10 E00,E01,E02), hypertension (ICD-9 642.3x, 642.9x, ICD-10 O13.9), social problems (ICD-10 O09.3, O09.70, O09.71, O09.72, O09.73), drug/alcohol use during pregnancy (ICD-9 649, ICD-10 O99.33), type of health plan and obesity (ICD-10 O99.21).[14-20]

Exposure and Outcomes

The exposure of this study is the order of NIPT. We used the same text-search algorithm used for the Site-level NIPT acceptance to determine the NIPT order. The date of NIPT order was matched with the date of medical encounter for pregnancy to confirm the order was not misplaced and was part of prenatal care. The outcome of this study is the administration of IPD, either CVS or Amniocentesis. The procedure

happened within the institutional healthcare network was defined using texts "chorionic villus" and "amniocentesis, laboratory and procedure records." We also used applicable Current Procedural Terminology (CPT) codes including 59000, 59105, 76945 and 76946 to confirm the IPD performed. To be classified as an exposure or outcome, the procedure or order record had to fall within the 90-day follow-up period.

Statistical Analysis

For the site-level analysis, the number of patients receiving NIPT order, the number of IPD performed, and the number of new high-risk pregnancies within the healthcare system for each calendar year were longitudinally described. The number of patients with NIPT, IPD, and high-risk pregnancy were presented by the calendar year.

Maternal age at the first prenatal visit with a diagnosis of first trimester checkup record was summarized using mean and standard deviation and compared between the NIPT and no-NIPT groups using Student t-test. Categorical variables including type of health plan, grouped age (35 – 39, 40 – 44 and 45 +), and specific risk factors including insufficient prenatal care, social problems, genitourinary infection, gestational diabetes, grand multiparity, hypothyroidism, substance/alcohol abuse, overweight/obese, and hypertension in pregnant women were compared between the NIPT and no-NIPT groups and were summarized using frequency and percentage. Type of health plan was regrouped into two, commercial insurance vs. all the others to address the small number of patients in each non-commercially insured or non-insured subgroups. Age was also categorized into two groups, 35 - 39 vs. 40 or older. To address the influence of the risk factors on IPD, patient characteristics at the date of the first prenatal visit were also compared between IPD and no-IPD groups. Using Chi-square test, or Fisher's Exact test for the small patient counts (<5 count), categorical variables as a clinical characteristic were compared between the NIPT and no-NIPT cohort, and between IPD and no-IPD groups.

We compared the rate of IPD between the patients who received NIPT and those who did not receive NIPT. Proportion of patients receiving IPD during the 90-day assessment period between the

NIPT and no-NIPT groups were statistically compared using Chi-square test. The odds ratio and 95% confidence interval (95CI) estimate from a logistic regression model presented the direction and precision of the association measure. In a multivariable approach, baseline characteristics that were marginally different ($p<0.1$) between the NIPT and no-NIPT were included as regression covariates. Due to the small number of subjects and outcomes in this study relative to the number of covariates that need to be adjusted for (i.e., dimensionality in a regression model), difference in the baseline characteristics may not be simultaneously addressed in the odds ratio estimate.[23] Thus, in addition to running an inclusive multivariable regression model, we calculated the odds ratios of IPD for NIPT in a series of logistic regression models where each regression included a single covariate.

A further assessment tested the significance of NIPT as a predictor out of the clinical factors using multivariable regression model. Variable selection in the logistic regression was performed using a stepwise forward selection approach with significance levels for entering and removing effects of 0.5 and 0.35. The final model including NIPT as a predictor was supposed to indicate that NIPT is a critical factor, to assist providers in determining the need for IPD. All of the statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC).

Results

Site-Level NIPT Acceptance:

A total of 11,562 new high-risk pregnancies were identified between 2012-2018. The number of high-risk pregnancies in 2018 identified from the administrative coding was 2,298 which is 269% of the 2012 (n=1108) and 211% of the 2015 (n=1413) count. The numbers of NIPT and IPD during the longitudinal analysis period were 856 and 110, respectively. There were no specific trends in the number of annual IPD (Figure 1). The annual NIPT order in 2018 was 380 which was 76 times and 5.3 times of the 2012 and 2015 counts (5 and 72), respectively. Overall the rate of increase in NIPT use exceeds the change in the number of high-risk pregnancy. (Figure 1).

Patient-Level Analysis:

The study cohort consisted of 2,057 pregnant women at or older than 35 years with a diagnosis of high-risk pregnancy. We identified a total of 551 NIPT orders for the patients included in the study cohort. The difference in the age distribution between the NIPT and no-NIPT group was not statistically nor clinically significant with the respective proportions of subjects younger than 40 of 84.94% vs. 82.07. The NIPT cohort was more dominated by commercially insured patients (99.27%) compared to the no-NIPT cohort (79.42%). Based on the analysis of clinical characteristics, patients who received NIPT generally carried less risk factors than the no-NIPT patients with the respective proportions of gestational diabetes (11.62% vs. 18.86%, p<0.01), substance or alcohol abuse (1.27% vs. 6.24%, p<0.01), overweight or obese (28.31% vs. 36.06%, p<0.01) and hypertension (13.25% vs. 17.80%, p=0.01). Social problem was the only risk factor more prevalent among the NIPT than the no-NIPT groups (2.9% vs. 1.00%, p<0.01), but the difference in the proportion was nominal from the clinical standpoint. (Table 1*)*.

When the analysis grouped high-risk pregnancy into patients who received IPD (n=56) and patients who did not (n=2,001), the proportion of patients younger than 40 years out of the IPD recipients was significantly less than the proportion among the no-IPD (66.07% vs. 83.31%, p<0.01). The difference in the mean ± SD age was marginally significant between the IPD and no-IPD groups (37.89 ± 2.61 vs. 37.35 ± 2.37). There was a significant difference in the proportion of commercially insured pregnancy (94.64% vs., 84.46, p=0.04, regrouped health plan type) with the larger proportion of commercially insured patients among those who received IPD. The prevalence of clinical risk factors was generally lower among the IPD vs. no-IPD including genitourinary infection (7.14% vs. 11.69%), gestational diabetes (10.71% vs. 17.09%), and hypertension (10.71% vs. 16.74%), but the differences were not statistically significant. The lack of statistical significance might be attributed to the small number of IPD procedures. (Table 2).

From the tabulate analysis, the proportion of patients who received IPD among the NIPT patients during the 90-day assessment period was 2.90% which was slightly larger than the rate of IPD performed without NIPT record (2.66%, Table 1). The results were not statistically nor clinically significant (p=0.76,

Tables 1 and 2). The logistic regression model, without any adjustment for the baseline characteristics, resulted in the odds ratio and 95% confidence interval [95CI] of 1.10 [0.61 – 1.97]. Patient demographics and clinical risk factors had only a nominal impact on the adjusted odds ratio calculation. When the association was adjusted for all patient characteristics with p <0.1, the odds ratio [95CI] was 0.90 [0.49 – 1.65]. The stepwise model selection process chose age (35-39 vs. 45≤), type of health plan (commercial vs. all non-commercial), social problem, gestational diabetes and hypertension as independent variables in the logistic regression model. Of the selected variables, 40 years or older (OR=2.74 [95CI: 1.54 – 4.81], p <0.01) and commercial insurance (OR=3.19 [95CI: 0.10 – 1.04], p = 0.06) showed a marginally significant association with IPD. NIPT was not considered to be an independent variable that predicts IPD use while the selection process finalized the multivariable regression model.

Discussion

Our assessment confirms that there has been a rapid increase in the use of NIPT and the gradual expansion of NIPT use outpaces the increase in the need for high-risk pregnancy care. Although the acceptance of NIPT was partially explained by the longitudinal changes in the characteristics of pregnancy, such as becoming older and increasing prevalence of pre-existing conditions, it is mainly attributable to coverage expansion, particularly among the patients enrolled in a commercial health plan.[24-31] Our results are comparable to the outcomes of a recent time-series analysis comparing the orders of NIPT and number of IPD in that there has been a significant increase in the order of NIPT with a subtle decrease in the number of IPD, with the adjusted incidence rate ratio of 0.97.[24]

To the best of our knowledge, our study includes the first patient-level assessment to analyze the clinical utility of NIPT in the US healthcare setting. Because IPD is followed by the likelihood of complications, one of the primary aims of NIPT is to diminish the need for diagnostic IPD. To achieve the expected cost saving or cost-effectiveness, NIPT needs to achieve an anticipated decrease in the IPD by 66% to 93%.[25] Not being aligned with the anticipated clinical scenario, our study did not find a strong signal of the negative association between the order of NIPT and the frequency of IPD. We tentatively concluded that the utility NIPT in alleviating IPD-related concerns would be, at best, nominal

in the high-risk pregnancy management based on the odds ratio of 0.9 from our multivariable logistic regression model.

A decision assisted by multiple risk factors, imaging and confirmatory diagnostic procedure partially explains the reason for the subtle influence of NIPT on the following diagnostic tests. A recent chart review showed that the first trimester ultrasonography still provides valuable clinical information about fetal anatomy.[26] Typically, the first trimester ultrasonography determines the presence of trisomy 18 with a sensitivity of 70%, while previous multiple marker test detected 43% of cases.[27 28] In combination with invasive diagnostic testing, the standard screening process without NIPT already achieved 100% sensitivity and negative predictive value.[29] This likely involves providers and patients needed to confirm the presence or absence of a congenital malformation by standard combination screenings, regardless of the results from the first trimester NIPT.[30-33] Thus a substantial proportion of prenatal care would not be altered by the use of NIPT.

Congenital malformation is a subject of environmental and socioeconomic factors. For example, being placed in a lower quartile of social deprivation is associated with a 30% increase in the rate of live-born congenital disease.[34] Therefore, the ultimate goal of prenatal screenings and diagnostics, to minimize invasive procedures and to reduce hereditary malformations, will not be accomplished until underprivileged pregnancies have access to advanced prenatal care strategies. However, Medicaid enrollees still have limited prenatal care as indicated by 20% of the US states that do not cover the cost of NIPT.[35-37] Whereas, the majority of commercial health plans have expanded NIPT coverage to all pregnancies.[35-37] A coverage gap in access to prenatal care was confirmed by our study finding. Not being enrolled in a commercial health plan was also a negative indicator for further IPD to confirm the presence of genetic disorder. Our data obtained from the real-world assessments warrant future research in and revision of the current policy to improve the utility of clinically advanced strategies in prenatal care, particularly in a disadvantaged population.

There are a couple of risk factors that may be associated with the decision to perform IPD based on our administrative data, including having Commercial insurance and being between the ages of 35-39.

This may be due to patients with commercial insurance having greater access to healthcare, which is consistent with results from a previous study[30]. Insufficient prenatal care, social problems, and substance/alcohol abuse may be associated with less likelihood to receive NIPT and/or IPD. These associations may be related to Medicaid and underserved populations that do not have as great of access to healthcare resources.[24 38] It is important that doctors and midwives provide adequate information on the benefits and limitations associated with NIPT, specifically for the minorities and underprivileged population

The interpretation of our data should be considered in light of several limitations. Firstly, the identification of both exposure and outcomes are limited by the procedure and order defined by the administrative records. Although the quality of the study using the institutional EDW was confirmed by multiple researches, the likelihood of misclassification could not be ruled out from a retrospective observational research. The study findings need to confirmed by a detailed medical note review and warrant a confirmatory randomized controlled study. Second, our research was limited to a single healthcare systems data. Future research may include multisite observational databases to establish generalizability of study findings. Lastly, the size of the study cohort was associated with wide confidence intervals, limiting statistical inference. Although the point estimates confirm no-nominal influence of NIPT on IPD, a further assessment using a larger cohort is warranted. Despite the limitations, our study provides valuable insight into the use of NIPT.

In conclusion, our study delineates the acceptance of NIPT in prenatal care. However, the utility of NIPT in mitigating concerns on IPD use has not been established. Future study needs to address disparity in access to advanced prenatal care strategies including NIPT and IPD.

Funding

None.

Acknowledgement

None.

Competing Interests

There are no competing interests to declare.

Ethics approval statement

The University of Utah Institutional Review Board approved this study and deemed it exempt (IRB# 00115830).

Contributorship Statement

LCK and KK equally contributed to this study and the final document.

LCK and KK jointly developed the initial research plan. The initial research protocol was reviewed and modified by both KK and LCK. KK extracted analytic cohorts. LCK and KK performed statistical analyses. LCK compiled this drafted manuscript. KK reviewed and edited this manuscript. The overall research project was supervised and managed by KK, the corresponding author of this manuscript.

Data Availability Statement

No additional data is available.

Accessibility of protocol, raw data, and programming code

Research protocol and programming code will be available from the corresponding author, KK, upon reasonable request. Accessibility of the raw day will be subject to the data use agreement between the publisher and the University of Utah where the raw data were collected and the study was performed.

References

1. Barros ALO, Barros AO, Barros GLM, Santos M. Burden of caregivers of children and adolescents with Down Syndrome. Cien Saude Colet 2017;**22**(11):3625-34 doi: 10.1590/1413-812320172211.31102016[published Online First: Epub Date]|.
2. Geelhoed EA, Bebbington A, Bower C, Deshpande A, Leonard H. Direct health care costs of children and adolescents with Down syndrome. J Pediatr 2011;**159**(4):541-5 doi: 10.1016/j.jpeds.2011.06.007[published Online First: Epub Date]|.
3. Ghi T, Sotiriadis A, Calda P, Da Silva Costa F, Raine-Fenning N, Alfirevic Z, McGillivray G. ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis. Ultrasound Obstet Gynecol 2016;**48**(2):256-68 doi: 10.1002/uog.15945[published Online First: Epub Date]|.
4. Panorama testing BILL $4000, doctor said it would cost $200 - Page 1. . Secondary Panorama testing BILL $4000, doctor said it would cost $200 - Page 1. . https://community.babycenter.com/post/a62696863/panorama-testing-bill-4000-doctor-said-it-would-cost-200.
5. Minear MA, Lewis C, Pradhan S, Chandrasekharan S. Global perspectives on clinical adoption of NIPT. Prenat Diagn 2015;**35**(10):959-67 doi: 10.1002/pd.4637[published Online First: Epub Date]|.
6. Non-Invasive Prenatal Testing (NIPT). . Secondary Non-Invasive Prenatal Testing (NIPT). . http://www.perinatalservicesbc.ca/health-professionals/professional-resources/screening/prenatal-genetic/non-invasive-prenatal-testing-nipt.
7. Petersen AK, Cheung SW, Smith JL, Bi W, Ward PA, Peacock S, Braxton A, Van Den Veyver IB, Breman AM. Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. Am J Obstet Gynecol

2017;**217**(6):691.e1-91.e6 doi: 10.1016/j.ajog.2017.10.005[published Online First: Epub Date]|.

8. Walker BS, Nelson RE, Jackson BR, Grenache DG, Ashwood ER, Schmidt RL. A Cost-Effectiveness Analysis of First Trimester Non-Invasive Prenatal Screening for Fetal Trisomies in the United States. PLoS One 2015;**10**(7):e0131402 doi: 10.1371/journal.pone.0131402[published Online First: Epub Date]|.

9. Maternal Serum Screening, Second Trimester. Secondary Maternal Serum Screening, Second Trimester. https://www.labcorp.com/help/patient-test-info/maternal-serum-screening-second-trimester#:~:text=Maternal%20serum%20screening%20is%20a,or%20a%20condition%20called%20anencephaly.

10. Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226. Obstet Gynecol 2020;**136**(4):e48-e69 doi: 10.1097/aog.0000000000004084[published Online First: Epub Date]|.

11. LeFevre NM, Sundermeyer RL. Fetal Aneuploidy: Screening and Diagnostic Testing. Am Fam Physician 2020;**101**(8):481-88

12. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. Obstet Gynecol 2007;**109**(1):217-27 doi: 10.1097/00006250-200701000-00054[published Online First: Epub Date]|.

13. Kostenko E, Chantraine F, Vandeweyer K, Schmid M, Lefevre A, Hertz D, Zelle L, Bartha JL, Di Renzo GC. Clinical and Economic Impact of Adopting Noninvasive Prenatal Testing as a Primary Screening Method for Fetal Aneuploidies in the General Pregnancy Population. Fetal Diagn Ther 2019;**45**(6):413-23 doi: 10.1159/000491750[published Online First: Epub Date]|.

14. The Web's Free 2021 ICD-10-CM/PCS Medical Coding Reference. Secondary The Web's Free 2021 ICD-10-CM/PCS Medical Coding Reference. https://www.icd10data.com/.

15. The Web's Free ICD-9-CM Medical Coding Reference. Secondary The Web's Free ICD-9-CM Medical Coding Reference. http://www.icd9data.com/2014/Volume1/240-279/240-246/244/244.9.htm.

16. Raskind-Hood C, Saraf A, Riehle-Colarusso T, Glidewell J, Gurvitz M, Dunn JE, Lui GK, Van Zutphen A, McGarry C, Hogue CJ, Hoffman T, Rodriguez Iii FH, Book WM. Assessing Pregnancy, Gestational Complications, and Co-morbidities in Women With Congenital Heart Defects (Data from ICD-9-CM Codes in 3 US Surveillance Sites). Am J Cardiol 2020;**125**(5):812-19 doi: 10.1016/j.amjcard.2019.12.001[published Online First: Epub Date]|.

17. Persson M, Pasupathy D, Hanson U, Westgren M, Norman M. Pre-pregnancy body mass index and the risk of adverse outcome in type 1 diabetic pregnancies: a population-based cohort study. BMJ Open 2012;**2**(1):e000601 doi: 10.1136/bmjopen-2011-000601[published Online First: Epub Date]|.

18. Persson M, Winkvist A, Mogren I. Lifestyle and health status in a sample of Swedish women four years after pregnancy: a comparison of women with a history of normal pregnancy and women with a history of gestational diabetes mellitus. BMC Pregnancy Childbirth 2015;**15**:57 doi: 10.1186/s12884-015-0487-2[published Online First: Epub Date]|.

19. Kuhle S, Muir A, Woolcott CG, Brown MM, McDonald SD, Abdolell M, Dodds L. Maternal pre-pregnancy obesity and health care utilization and costs in the offspring. Int J Obes (Lond) 2019;**43**(4):735-43 doi: 10.1038/s41366-018-0149-3[published Online First: Epub Date]|.

20. Quinley KE, Falck A, Kallan MJ, Datner EM, Carr BG, Schreiber CA. Validation of ICD-9 Codes for Stable Miscarriage in the Emergency Department. West J Emerg Med 2015;**16**(4):551-6 doi: 10.5811/westjem.2015.4.24946[published Online First: Epub Date]|.

21. Olney RS, Moore CA, Khoury MJ, Erickson D, Edmonds LD, Botto LD, Atrash HK. Chorionic Villus Sampling and Amniocentesis: Recommendations for Prenatal Counseling. Secondary Chorionic Villus Sampling and Amniocentesis: Recommendations for Prenatal Counseling. https://www.cdc.gov/mmwr/preview/mmwrhtml/00038393.htm#:~:text=Amniocentesis%20is%20usually%20performed%20at,11%2D14%20weeks'%20gestation.

22. Your First Prenatal Visit. Secondary Your First Prenatal Visit. https://americanpregnancy.org/healthy-pregnancy/planning/first-prenatal-visit-71023/.

23. Cepeda MS, Boston R, Farrar JT, Strom BL. Comparison of logistic regression versus propensity score when the number of events is low and there are multiple confounders. Am J Epidemiol 2003;**158**(3):280-7 doi: 10.1093/aje/kwg115[published Online First: Epub Date]|.

24. Quinlan TAG, Schroeder B, Kwon S, Barlow JF, Sherman MS, Anderson HD, Wright G, McQueen RB. Economic Impact of Coverage Expansion for Non-invasive Prenatal Testing Through a Performance-Based Risk-Sharing Agreement. Pharmacoecon Open 2021:1-10 doi: 10.1007/s41669-021-00261-y[published Online First: Epub Date]|.

25. Garfield SS, Armstrong SO. Clinical and Cost Consequences of Incorporating a Novel Non-Invasive Prenatal Test into the Diagnostic Pathway for Fetal Trisomies. Journal of Managed Care Medicin 2021;**15**(2):34-41

26. Rao R, Han C, Silverman N, Platt L. Utility of first trimester ultrasound in the setting of NIPT incorporation into practice: a retrospective pilot review. American Journal of Obstetrics and Gynecology 2015;**212**(1)

27. Brumfield CG, Wenstrom KD, Owen J, Davis RO. Ultrasound findings and multiple marker screening in trisomy 18. Obstet Gynecol 2000;**95**(1):51-4 doi: 10.1016/s0029-7844(99)00461-5[published Online First: Epub Date]|.

28. Yeo L, Guzman ER, Day-Salvatore D, Walters C, Chavez D, Vintzileos AM. Prenatal detection of fetal trisomy 18 through abnormal sonographic features. J Ultrasound Med 2003;**22**(6):581-90; quiz 91-2 doi: 10.7863/jum.2003.22.6.581[published Online First: Epub Date]|.

29. Park SY, Jang IA, Lee MA, Kim YJ, Chun SH, Park MH. Screening for chromosomal abnormalities using combined test in the first trimester of pregnancy. Obstet Gynecol Sci 2016;**59**(5):357-66 doi: 10.5468/ogs.2016.59.5.357[published Online First: Epub Date]|.

30. Benn P, Borell A, Chiu R, Cuckle H, Dugoff L, Faas B, Gross S, Johnson J, Maymon R, Norton M, Odibo A, Schielen P, Spencer K, Huang T, Wright D, Yaron Y. Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn 2013;**33**(7):622-9 doi: 10.1002/pd.4139[published Online First: Epub Date]|.

31. Raised Maternal Age in Pregnancy. Secondary Raised Maternal Age in Pregnancy. https://www.rdehospital.nhs.uk/media/hrxj1nho/patient-information-leaflet-raised-maternal-age-in-pregnancy-rde-19-089-001.pdf.

32. What are some factors that make a pregnancy high risk? . Secondary What are some factors that make a pregnancy high risk? . https://www.nichd.nih.gov/health/topics/high-risk/conditioninfo/factors.

33. Mackie FL, Allen S, Morris RK, Kilby MD. Cell-free fetal DNA-based noninvasive prenatal testing of aneuploidy. The Obstetrician & Gynaecologist 2017;**19**(3):211-18 doi: https://doi.org/10.1111/tog.12388[published Online First: Epub Date]|.

34. Peyvandi S, Baer RJ, Chambers CD, Norton ME, Rajagopal S, Ryckman KK, Moon-Grady A, Jelliffe-Pawlowski LL, Steurer MA. Environmental and Socioeconomic Factors Influence the Live-Born Incidence of Congenital Heart Disease: A Population-Based Study in California. J Am Heart Assoc 2020;**9**(8):e015255 doi: 10.1161/jaha.119.015255[published Online First: Epub Date]|.

35. Gadsbøll K, Petersen OB, Gatinois V, Strange H, Jacobsson B, Wapner R, Vermeesch JR, Vogel I. Current use of noninvasive prenatal testing in Europe, Australia and the USA: A graphical presentation. Acta Obstet Gynecol Scand 2020;**99**(6):722-30 doi: 10.1111/aogs.13841[published Online First: Epub Date]|.

36. Benoy ME, Iruretagoyena JI, Birkeland LE, Petty EM. The impact of insurance on equitable access to non-invasive prenatal screening (NIPT): private insurance may not pay. J Community Genet 2021;**12**(1):185-97 doi: 10.1007/s12687-020-00498-w[published Online First: Epub Date]|.

37. Löwy I. Non-invasive prenatal testing: A diagnostic innovation shaped by commercial interests and the regulation conundrum. Soc Sci Med 2020:113064 doi: 10.1016/j.socscimed.2020.113064[published Online First: Epub Date]|.

38. Goudie A, Martin B, Li C, Lewis K, Han X, Kathe N, Wilson JC, Thompson J. Higher Rates of Preventive Health Care With Commercial Insurance Compared With Medicaid: Findings From the Arkansas Health Care Independence "Private Option" Program. Med Care 2020;**58**(2):120-27 doi: 10.1097/mlr.0000000000001248[published Online First: Epub Date]|.

Table 1. Clinical characteristics and demographics of NIPT vs. no NIPT groups.

	NIPT (n=551)	No-NIPT (n=1506)	p-value*
Demographic Information			
Age, mean(SD)	37.23 (2.25)	37.41 (2.42)	0.11**
Grouped Age (3 groups)			0.17
35 – 39	468 (84.94)	1236 (82.07)	
40 – 44	80 (14.52)	251 (16.67)	
45 ≤	3 (1.26)	19 (0.54)	
Grouped Age (2 groups)			0.13
35 – 39	468 (84.94)	1236 (82.07)	
40 ≤	83 (15.06)	270 (17.93)	
Health plan			<0.01
Commercial Insurance	547 (99.27)	1196 (79.42)	
Government	0 (0)	3 (0.20)	
Medicaid	2 (0.36)	270 (17.93)	
Medicare	2 (0.36)	20 (1.33)	
Other Insurance/Unknown	0 (0)	17 (1.13)	
Health plan – two grouped			<0.01
Commercial	547 (99.27)	1196 (79.42)	
All non-Commercial	4 (0.73)	310 (20.58)	
Clinical Characteristics and Risk factors			
Insufficient Prenatal Care	4 (0.73)	22 (1.46)	0.18
Social Problem	16 (2.9)	15 (1.00)	<0.01
Genitourinary Infection	58 (10.53)	180 (11.95)	0.37
Gestational Diabetes	64 (11.62)	284 (18.86)	<0.01
Grand Multiparity	0	0	n/a
Hypothyroidism	90 (16.33)	216 (14.34)	0.26
Substance Abuse/Alcohol Abuse	7 (1.27)	94 (6.24)	<0.01
Overweight/Obese	156 (28.31)	543 (36.06)	<0.01
Hypertension	73 (13.25)	268 (17.80)	0.01
IPD during the 90-day follow up	16 (2.90)	40 (2.66)	0.76

Abbreviations: NIPT, Non-Invasive Prenatal Testing; IPD – Invasive Prenatal Diagnostic Testing including amniocentesis and chorionic villus sampling

*p-value from chi-square test or Fisher's exact test if an expected count of patient is less than 5 from a tabulate analysis.

** p-value from student t-test

Table 2. Clinical characteristics and demographics of IPD vs. no IPD groups.

	IPD (n=56)	No-IPD (n=2,001)	p-value*
Demographic Information			
Age, mean(SD)	37.89 (2.61)	37.35 (2.37)	0.09**
Grouped Age (3 groups)			<0.01
35 – 39	37 (66.07)	1667 (83.31)	
40 – 44	19 (33.93)	312 (15.59)	
45 ≤	0 (0)	22 (1.10)	
Grouped Age (2 groups)			<0.01
35 – 39	37 (66.07)	1667 (83.31)	
40 ≤	19 (33.93)	334 (16.69)	
Health plan			0.34
Commercial Insurance	53 (94.64)	1690 (84.46)	
Government	0 (0)	3 (0.15)	
Medicaid	3 (5.36)	269 (13.44)	
Medicare	0 (0)	22 (1.10)	
Other Insurance/Unknown	0 (0)	17 (1.0.85)	
Health plan - regrouped			0.04
Commercial	53 (94.64)	1690 (84.46)	
All non-Commercial	3 (5.36)	311 (15.54)	
Clinical Characteristics and Risk factors			
Insufficient Prenatal Care	0 (0)	26 (1.30)	0.39
Social Problem	2 (3.57)	29 (1.45)	0.19
Genitourinary Infection	4 (7.14)	234 (11.69)	0.29
Gestational Diabetes	6 (10.71)	342 (17.09)	0.21
Grand Multiparity	0	0	n/a
Hypothyroidism	8 (14.29)	298 (14.89)	0.90
Substance Abuse/Alcohol Abuse	3 (5.36)	98 (4.90)	0.88
Overweight/Obese	21 (37.50)	678 (33.88)	0.57
Hypertension	6 (10.71)	335 (16.74)	0.23
NIPT during the 90-day follow-up	16 (28.57)	535 (26.74)	0.76

Abbreviations: NIPT, Non-Invasive Prenatal Testing; IPD – Invasive Prenatal Diagnostic Testing including amniocentesis and chorionic villus sampling

*p-value from chi-square test or Fisher's exact test if an expected count of patient is less than 5 from a tabulate analysis.

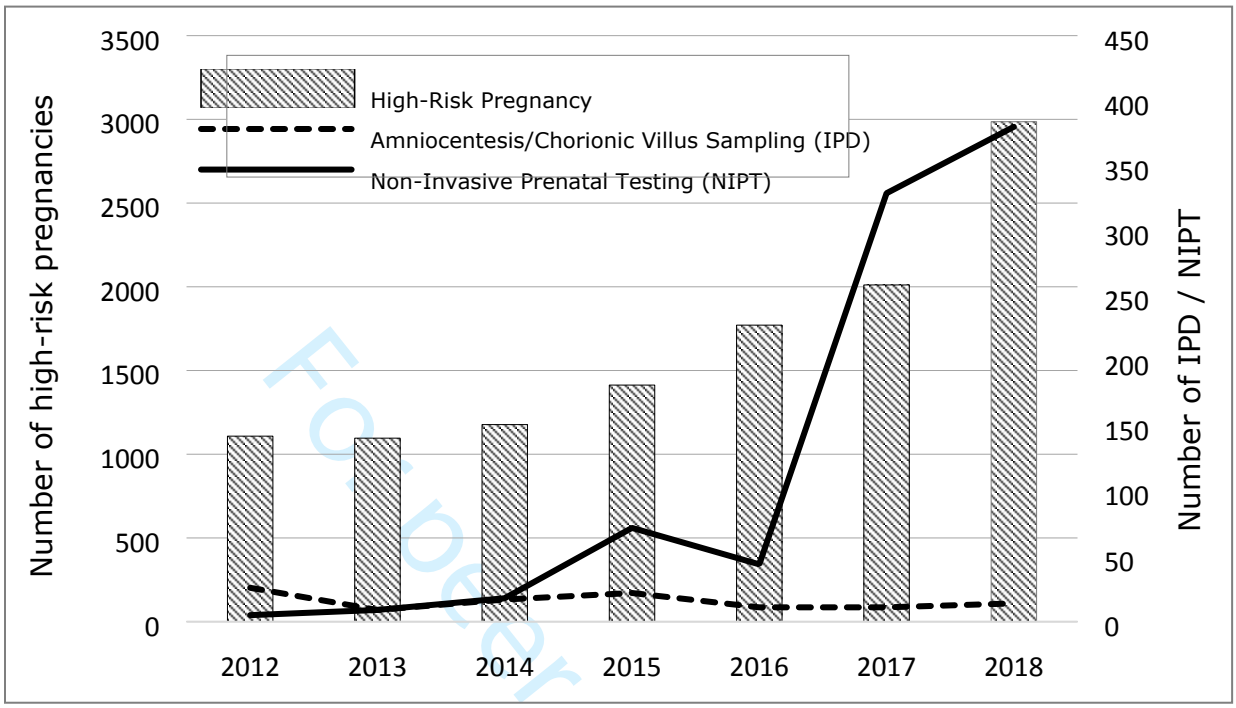
** p-value from student t-test

Table 3. Odds ratio of IPD for NIPD from logistic regression with single and multiple covariate adjustments

Covariates	OR [95CI]
No covariate adjustment	1.10 [0.61 - 1.97]
Grouped Age (35-39 vs. 40≤)	1.14 [0.63 - 2.05]
Insufficient Prenatal Care	1.09 [0.60 - 1.96]
Social problem	1.07 [0.59 - 1.93]
Genitourinary Infection	1.09 [0.60 - 1.96]
Gestational Diabetes	1.06 [0.59 - 1.91]
Hypothyroidism	1.10 [0.61 - 1.98]
Substance or Alcohol abuse	1.10 [0.61 - 1.99]
Overweight or Obese	1.11 [0.62 - 2.00]
Hypertension	1.07 [0.60 - 1.94]
Health plan (commercial vs. all non-commercial)	0.94 [0.52 - 1.71]
All variables with p<0.1*	0.90 [0.49 - 1.65]

*Regression model includes type of health plan (commercial vs. all non-commercial), Social Problem, Gestational Diabetes, Hypothyroidism, Substance/Alcohol abuse, and Overweight/Obese as covariates for the NIPT-IPD association

Figure 1: Longitudinal trends in the number of high-risk pregnancies, IPD, and NIPT



The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract Page and Page 6	RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 5		
Objectives	3	State specific objectives, including any prespecified hypotheses	Page6		
Methods					
Study Design	4	Present key elements of study design early in the paper	Page 7		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Pages 5 and 6		

Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case	Pages 7 and 8	RECORD 6.1: The methods of study population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	Pages 10 and 11	RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Pages 10 and 11
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	Pages 8 and 9		

Bias	9	Describe any efforts to address potential sources of bias	Page 10		
Study size	10	Explain how the study size was arrived at	Not applicable		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Page 9		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses	Page 9		
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population.	12.1: Pages 6 and 7 12.2: Pages 7 and 8

				RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	Page 7
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram	Pages 10 and 11	RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)	Page 11		
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure	Page 11		

		category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures			
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 11 and 12		
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Not applicable		
Discussion					
Key results	18	Summarise key results with reference to study objectives	Page 12		
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	Page 14	RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	
Interpretation	20	Give a cautious overall interpretation of results considering objectives,	Page 14		

		limitations, multiplicity of analyses, results from similar studies, and other relevant evidence			
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 14		
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	Not applicable		
Accessibility of protocol, raw data, and programming code		..	Page 15	RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	Page 15

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.

BMJ Open

Clinical Utility of Non-Invasive Prenatal Testing in mitigating concerns from Invasive Prenatal Diagnostic Testing in an Academic Healthcare System in the US

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-057658.R1
Article Type:	Original research
Date Submitted by the Author:	07-Mar-2022
Complete List of Authors:	Kim, Kibum; University of Illinois at Chicago, Department of Pharmacy Systems, Outcomes and Policy; The University of Utah, Department of Pharmacotherapy Craft, Kaitlyn; The University of Utah
Primary Subject Heading:	Health services research
Secondary Subject Heading:	Evidence based practice, Health informatics, Obstetrics and gynaecology, Diagnostics
Keywords:	OBSTETRICS, Prenatal diagnosis < OBSTETRICS, Fetal medicine < OBSTETRICS, HEALTH SERVICES ADMINISTRATION & MANAGEMENT

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

TITLE

Clinical Utility of Non-Invasive Prenatal Testing in mitigating concerns from Invasive Prenatal Diagnostic Testing in an Academic Healthcare System in the US

A Short Running Title

Clinical Utility of Non-Invasive Prenatal Testing

Manuscript Size

Word Count:

Abstract: 296

Main Text: 3,696

Table Count: 3

Figure Count: 1

Reference Count: 50

Authors and Affiliations

Kibum Kim, BPharm, PhD

Assistant Professor | Department of Pharmacy Systems, Outcomes and Policy, University of Illinois at Chicago

833 South Wood Street, Chicago, IL 60612

Kkim204@uic.edu

(312) 413 - 0152

Adjunct Assistant Professor | Department of Pharmacotherapy, University of Utah

30 South 2000 East

Salt Lake City, UT 84112

kibum.kim@pharm.utah.edu

Kaitlyn Craft, PharmD.

College of Pharmacy, University of Utah

30 South 2000 East

Salt Lake City, UT 84112

Kaitlyn.Craft@pharm.utah.edu

(408) 613-3865

Correspondence.

Kibum Kim, BPharm, PhD

Assistant Professor | Department of Pharmacy Systems, Outcomes and Policy, University of Illinois at Chicago

833 South Wood Street, Chicago, IL 60612

Kkim204@uic.edu

(312) 413 - 0152

Abstract:

Purpose:

Non-Invasive Prenatal Testing (NIPT) is a front-line screening for fatal chromosomal aneuploidy. In pregnant women with a risk of having fetal congenital disorders, NIPT is anticipated to reduce the needs of invasive prenatal diagnostic test (IPD) that increases the risk of rare but serious complications. The objectives of this study were to understand the acceptance of NIPT and the utility of NIPT to mitigate concerns about IPD in the US high-risk pregnancy management.

Methods:

This was a retrospective observational research using the data obtained from an academic healthcare system. The study consisted of site-level longitudinal analysis and patient-level cross-sectional analysis. The site-level trends of NIPT order, IPD procedure, and the number of patients diagnosed with high-risk pregnancy with age ≥ 35 years between 2012 and 2018 were descriptively summarized. The patient-level analysis included women with high-risk pregnancy between October 2015 – December 2018. We tested the association between the use of NIPT and IPD using multivariable regression analysis and odds ratios.

Results:

The rate of increase in the NIPT use exceeded the changes in the number of high-risk pregnancies with age ≥ 35 years, while the number of annual IPD procedures has fluctuated without specific trends. There was no significant association between the numbers of NIPT and IPD with the adjusted odds ratios between 0.9 and 1.1 ($p > 0.1$). The order of NIPT was not selected as an independent variable predicting the use of IPD. Clinical characteristics indicating low socioeconomic status and limited healthcare coverage are associated with less use of NIPT and lower clinical utility.

Conclusion:

Although prenatal care accepted NIPT over the last decade, the utility of NIPT in mitigating concerns on IPD is unclear and needs further investigation. Limited clinical utility should be addressed in the context of disparity in prenatal care.

Article Summary

Strengths and limitations of this study

- This study includes both healthcare system level and patient-level assessments on the utility of non-invasive prenatal testing (NIPT) in the US healthcare setting.
- A rapid increase in the use of NIPT outpaced the increase in the number of patients diagnosed with high-risk pregnancy with age ≥ 35 years .
- The use of NIPT is not apparently driven by the clinical utility in mitigating concerns on invasive-prenatal diagnostic testing but directed by the capacity to advance prenatal care access indicated by the type of healthplan.
- Although this study provides viable insight into the use and utility of the advanced prenatal care strategy, general limitations of single-site observational research warrant a multisite health outcomes study.

MAIN TEXT

Introduction

Fetal chromosomal anomalies (FCA) has a significant influence on the personal and familier life trajectory, both emotionally and financially.[1-8] People living with congenital disability and their caregivers suffer from impaired quality of life.[1, 2] Despite major improvements in medical management and social support, long-term morbidities, particularly neurodevelopmental and mental health issues, remain a cause for concern.[9, 10] In the era of patient-centric medical care, the early detection of FCA enhances reproductive autonomy and helps expectant parents to contemplate before making an irrevocable conclusion.[11, 12]

There have been advances in prenatal care that enable expectant parents to learn of congenital disorders, allowing them to have the power to control pregnancy and childbearing earlier and make informed medical decisions. Maternal serum screening (MSS) was a minimally invasive traditional approach to determine the risk of fetal congenital disorders.[13-15] However, the risk of FCA based on MSS does not well predict the actual chromosomal anomalies, as it has a positive predictive value inferior to the predictive accuracy of a combination of other non-invasive measures, including maternal age, fetal nuchal translucency and fetal heart rate.[13-17] A better prediction of congenital disorders has been achieved via diagnostic invasive prenatal testing (IPD), which includes amniocentesis, chorionic villus sampling (CVS), and fetal blood sampling. Although providing patients with clinically valid data with a 99% positive prediction of certain FCAs, IPD is associated with a minor but sizable increase in the rate of miscarriage and infection.[3] Complications after IPD has been a concern to both providers and expectant mothers.[3]

Non-Invasive Prenatal Testing (NIPT), or cell-free DNA (cfDNA) testing, is a screening to help identify potential genetic concerns.[14] NIPT relies on the presence of free-floating cfDNAs which arise when cells die and release the DNA into the bloodstream from the placenta. If the percentage of cfDNA fragments for a particular chromosome is higher than expected, it indicates that the fetus has an increased

likelihood of having a disorder associated with that chromosome, and is generally followed by further testing. NIPT has been shown to have a sensitivity and specificity above 99% for detecting trisomy 21, as well as a 98% positive predictive value for fetal trisomy 18, and a 99% positive predictive value for fetal trisomy 13 with a combined false-positive rate of 0.13%.[7, 14]

NIPT showed promise in reducing unnecessary invasive medical procedures but is associated with a high upfront cost to healthcare plans in the US healthcare setting.[4] When it is covered, NIPT can cost payers upwards of $3,000 and patients with insurance are left with an out-of-pocket cost.[6] In addition, many state Medicaid plans and some health plans are not on board to pay for NIPT.[4, 6] While NIPT has upfront costs, implementation of this procedure has the potential to reduce unnecessary medical costs and potential maternal or fetal harm. A previous model-based study demonstrated that NIPT can reduce the number of unnecessary invasive tests by 94.8% and decrease IPD-related miscarriages by 90%.[18] Out of 1,000,000 simulated scenarios, replacing MSS with NIPT would result in an increase in 893 detections of FCA and would be followed by a cost savings of approximately $170 million.[8]

A screening test has clinical utility, beyond analytical validity and clinical validity, in a practice when it potentially influences and improves clinical decisions.[19-21] Thus, potential cost savings to payers would be achieved when analytically valid test is translated into a clinical utility: NIPT significantly influences clinical decision and outcomes as hypothesized. Nevertheless, NIPT results may be a small addition to a previous standard of care, rather than become the most critical component, to determine the needs of further actions. Clinical practice can still be directed by ultrasound assessment, patient preference, and provider's previous training, which may not result in the cost-effective use of the NIPT as simulated. A study performed in early 2010's showed a decline in the number of amniocenteses coincided with the use of NIPT.[22] Similarly, a recent time-series assessment on the use of invasive diagnostic test in Austrailian healthcare system demonstrated that the decrease in IPD since 2000 continued after NIPT started being covered by the public sector since 2013.[23] Nevertheless, the lack of assessment on the patient-level association on the NIPT and IPD left the downstream effect of NIPT from the clinical utility standpoint unanswered.

The objectives of this study were to assess the acceptance of NIPT in clinical practice setting, to evaluate the role of NIPT in alleviating the need for IPD, and to explore the patient-level characteristics that lead to the order of NIPT and IPD. We hypothesized that there would be a negative association between the order of NIPT and the frequency of IPD performed, which is a strong signal of the clinical utility of NIPT in high-risk pregnancy management.

Methods

Study Design and Setting:

This is a retrospective observational research consisting of two sections: (1) A healthcare system-level longitudinal change analysis and (2) Patient-level cross-sectional analysis using data from the University of Utah enterprise data warehouse from which comprehensive clinical records and healthcare resource utilization at the University of Utah Health are available. The University of Utah Institutional Review Board approved this study and deemed it exempt (00115830).

Patient and Public Involvement:

Patients were not involved in this study.

NIPT acceptance:

We compared the number of NIPT to the total number of high-risk pregnancies with age ≥ 35 years (advanced maternal age) and the number of IPD performed at a site-level to visualize the acceptance of NIPT into the healthcare setting. We looked at the longitudinal variation to see if the numbers of NIPT and IPD over time align with or exceeds the changes in the number of new high-risk pregnancies with advanced maternal age. Analytic cohort included pregnant women with one or more records of high-risk pregnancy (ICD-10-CM O09.x or ICD-9-CMV23.x) between January 2012 and December 2018.[24-30] Eligible subjects were 35 years old or older at the first date of the high-risk pregnancy diagnosis. The NIPTs ordered during the study period within the healthcare network were identified using terminology

available from institutional treatment records including “NON-INVASIVE PRENATAL”, “NIPT FETAL ANEUPLOIDY”, “NIPT FETAL MICRODELETION”, “CELL-FREE DNA” as well as available brand names of NIPT tests. To be labled as the IPD of interest, the procedure happened at the University of Utah Health and was defined using texts “chorionic villus” and “amniocenteses” from pathology, laboratory and procedure records. Descriptive statistics include the number of patients ordering NIPT, receiving IPD procedures, and seeing providers for a new high-risk pregnancy.

Patient-level analysis:

Study cohort identification:

The analytic cohort for the patient-level analysis was a subset of patients from the longitudinal cohort: subjects with a diagnosis of high-risk pregnancy (ICD-10-CM O09.x) at any point between October 2015 and December 2018 with the patient aged 35 years or older.[24-30] NIPT can generally be considered once the gestational age is past 9 weeks, which can be followed by additional CVS before the gestational weeks 11 and 14 of pregnancy.[14] Amniocentesis is usually performed between 15 to 18 weeks of gestational age although more amniocentesis procedures are now being performed at 11-14 weeks' gestation.[31] The first prenatal visit for a new pregnancy usually happens around the gestational age of 8 weeks.[32] All things considered, eligible subjects had a record of prenatal care from the first-trimester (ICD-10-CM O09.x1, O09.5x1, O09.6x1, O09.8x1) and must be followed by the University of Utah Health for longer than 90-day period from that first-trimester visit, which allowed for a sufficient window to cover both NIPT and IPD. The accuracy of NIPT results is debatable in patients having more than one fetus. Thus, any expectant mothers with one or more records of multiple gestation (ICD-10-CM O30.x) were excluded from this study[24]. All the selected subjects were 35 years old or older at the first encounter with the first-trimester prenatal care visit. Clinical characteristics, demographic characteristics and records of NIPT and IPD were collected over the 90-day follow-up period. Patient characteristics were identified from the review of diagnosis codes, enrollment information, and demographic tables.

Patient characteristics as underlying conditions for the high-risk pregnancy included maternal age (ICD-9 659.63, ICD-10 O09.51x, O09.52x), insufficient prenatal care (ICD-9 V23.7, ICD-10 O09.3), genitourinary tract infection during pregnancy (ICD-9 646.0x, ICD-10 O23.x), grand multiparity (ICD-9 659.4, ICD-10 O09.40), type 1 or type 2 diabetes (ICD-9 250, ICD-10 O24.01, O24.11), history of hypothyroidism (ICD-9 243, ICD-10 E00,E01,E02), hypertension (ICD-9 642.3x, 642.9x, ICD-10 O13.9), social problems (ICD-10 O09.3, O09.70, O09.71, O09.72, O09.73), drug/alcohol use during pregnancy (ICD-9 649, ICD-10 O99.33), type of health plan and obesity (ICD-10 O99.21).[24-30]

Exposure and Outcomes

The exposure of the patient-level analysis is the order of NIPT. We used the same text-search algorithm used for the Site-level NIPT acceptance to determine the NIPT order. The date of NIPT order was matched with the date of medical encounter for pregnancy to confirm the order was not misplaced and was part of prenatal care. The outcome of this study is the administration of IPD, either CVS or Amniocentesis. The procedure performed within the institutional healthcare network was defined using texts "chorionic villus" and "amniocentesis, laboratory and procedure records." We also used applicable Current Procedural Terminology (CPT) codes including 59000, 59105, 76945 and 76946 to confirm that the IPD was performed. To be classified as an exposure or outcome, the procedure or order record had to fall within the 90-day follow-up period.

Statistical Analysis

For the site-level analysis, the number of patients receiving NIPT order, the number of IPD performed, and the number of new high-risk pregnancies within the healthcare system for each calendar year were longitudinally described. The number of patients with NIPT, IPD, and high-risk pregnancy with advanced maternal age were presented by the calendar year.

Maternal age at the first prenatal visit with a diagnosis of first trimester checkup record was summarized using mean and standard deviation and compared between the NIPT and no-NIPT groups using Student t-test. Categorical variables including type of health plan, grouped age (35 – 39, 40 – 44 and 45 +), and specific risk factors including insufficient prenatal care, social problems, genitourinary infection, gestational diabetes, grand multiparity, hypothyroidism, substance/alcohol abuse, overweight/obese, and hypertension in pregnant women were compared between the NIPT and no-NIPT groups and were summarized using frequency and percentage. Type of health plan was regrouped into two, commercial insurance vs. all the others to address the small number of patients in each non-commercially insured or uninsured subgroups. Age was also categorized into two groups, 35 - 39 vs. 40 or older. To address the influence of the clinical factors on the decision to perform IPD, patient characteristics at the date of the first prenatal visit were also compared between IPD and no-IPD groups. Using Chi-square test, or Fisher's Exact test for the small patient counts (<5 count), categorical variables as a clinical characteristic were compared between the NIPT and no-NIPT groups, and between IPD and no-IPD groups.

We compared the rate of IPD between the patients who received NIPT and those who did not receive NIPT. Proportion of patients receiving IPD during the 90-day assessment period between the NIPT and no-NIPT groups were statistically compared using Chi-square test. The odds ratio and 95%CI estimate from a logistic regression model presented the direction and precision of the association measure. In a multivariable approach, baseline characteristics that were marginally different ($p<0.1$) between the NIPT and no-NIPT were included as regression covariates. Due to the small number of subjects and outcomes relative to the number of covariates that need to be adjusted for (i.e., dimensionality in a regression model), the multivariable approach may not address all the differences in the baseline characteristics simultaneously.[33] Thus, in addition to running an inclusive multivariable regression model, we calculated the odds ratios of IPD for NIPT in a series of logistic regression models where each regression included each single covariate.

A further assessment tested the significance of NIPT as a predictor out of the clinical factors using a multivariable regression model selection process. Variable selection in the logistic regression was performed using a stepwise forward selection approach with significance levels for entering and removing effects of 0.5 and 0.35. The final model including NIPT as a predictor was supposed to indicate that NIPT is a critical factor, to assist providers in determining the need for IPD. Statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC).

Results

Site-Level NIPT Acceptance:

A total of 5,660 new high-risk pregnancies with advanced maternal age were identified between 2012-2018. The number of high-risk pregnancies with advanced maternal age in 2018 was 977 which is 158% of the 2012 (n=616) and 116% of the 2015 (n=841) count. The numbers of NIPT and IPD performed within the selected pregnant women were 436 and 126, respectively. There were no specific trends in the number of annual IPD (Figure 1). The annual NIPT order in 2018 was 203 which was 7 times 29 cases in 2015. Overall the rate of increase in NIPT use exceeded the change in the number of high-risk pregnancy with advanced maternal age. (Figure 1).

Patient-Level Analysis:

The study cohort consists of 2,057 pregnant women at or older than 35 years with a diagnosis of high-risk pregnancy. We identified a total of 551 NIPT orders for the patients included in the study cohort. The difference in the age distribution between the NIPT and no-NIPT group was not statistically nor clinically significant with the respective proportions of subjects younger than 40 of 84.94% vs. 82.07. The NIPT cohort was more dominated by commercially insured patients (99.27%) compared to the no-NIPT cohort (79.42%). Based on the analysis of clinical characteristics, patients who received NIPT generally carried less risk factors than the no-NIPT patients with the respective proportions of gestational diabetes (11.62% vs. 18.86%, $p<0.01$), substance or alcohol abuse (1.27% vs. 6.24%, $p<0.01$), overweight

or obese (28.31% vs. 36.06%, p<0.01) and hypertension (13.25% vs. 17.80%, p=0.01). Social problem was the only risk factor more prevalent among the NIPT than the no-NIPT groups (2.9% vs. 1.00%, p<0.01), but the difference in the proportion was nominal from the clinical standpoint. (Table 1).

When the analysis grouped high-risk pregnancy into patients who received IPD (n=56) and patients who did not (n=2,001), the proportion of patients younger than 40 years out of the IPD recipients was significantly less than the proportion among the no-IPD (66.07% vs. 83.31%, p<0.01). The difference in the mean ± SD age was marginally significant (p=0.09) between the IPD and no-IPD groups (37.89 ± 2.61 vs. 37.35 ± 2.37). There was a significant difference in the proportion of commercially insured pregnancy (94.64% vs., 84.46, p=0.04, regrouped health plan type) with the larger proportion of commercially insured patients among those who received IPD. The prevalence of clinical risk factors was generally lower among the IPD vs. no-IPD, including genitourinary infection (7.14% vs. 11.69%), gestational diabetes (10.71% vs. 17.09%), and hypertension (10.71% vs. 16.74%), but the differences were not statistically significant. The lack of statistical significance was likely attributed to the small number of IPD procedures. (Table 2).

From the tabulate analysis, the proportion of patients who received IPD among the NIPT patients during the 90-day assessment period was 2.90% which was slightly larger than the rate of IPD performed without NIPT record (2.66%, Table 1). The results were not statistically nor clinically significant (p=0.76, Tables 1 and 2). The logistic regression model, without any adjustment for the baseline characteristics, resulted in the odds ratio [95%CI] of 1.10 [0.61 – 1.97]. Patient demographics and clinical risk factors had only a nominal impact on the adjusted odds ratio calculation. When the association was adjusted for all patient characteristics with p <0.1, the odds ratio [95%CI] was 0.90 [0.49 – 1.65]. The stepwise model selection process chose age (35-39 vs. 45≤), type of health plan (commercial vs. all non-commercial), social problem, gestational diabetes and hypertension as independent variables in the logistic regression model. Of the selected variables, 40 years or older (OR=2.74 [95%CI: 1.54 – 4.81], p <0.01) and commercial insurance (OR=3.19 [95%CI: 0.10 – 1.04], p = 0.06) showed a significant or marginally

significant association with IPD. (Table 3) NIPT was not considered to be an independent variable that predicts IPD use while the selection process finalized the multivariable regression model.

Discussion

Our assessment confirms that a rapid and gradual increase in the use of NIPT outpaced the increase in the need for a maternity care for the high-risk pregnancy with advanced age. Although the acceptance of NIPT was partially explained by the longitudinal changes in the characteristics of pregnancy, such as becoming older and increasing prevalence of pre-existing conditions, it is mainly attributable to coverage expansion, particularly among the patients enrolled in a commercial health plan.[24-31] Our results are comparable to the outcomes of a recent time-series analysis comparing the orders of NIPT and number of IPD in that there has been a significant increase in the order of NIPT with a subtle decrease in the number of IPD, with the adjusted incidence rate ratio of 0.97.[34]

To the best of our knowledge, our study includes the first patient-level assessment to analyze the clinical utility of NIPT in the US healthcare setting. Because IPD is followed by the likelihood of complications, one of the expected benefits of NIPT is to diminish the need for diagnostic IPD. To achieve the expected cost saving or cost-effectiveness, NIPT needs to achieve an anticipated decrease in the IPD by 66% to 93%.[35] Not being aligned with the anticipated clinical scenario, our study did not find a strong signal of the negative association between the order of NIPT and the frequency of IPD. We tentatively concluded that the utility NIPT in alleviating IPD-related concerns would be, at best, nominal in managing high-risk pregnancy with advanced maternal age based on the odds ratio of 0.9 from our multivariable logistic regression model.

A decision assisted by multiple risk factors, imaging and confirmatory diagnostic procedure partially explains the reason for the subtle influence of NIPT on the following diagnostic tests. A recent chart review showed that the first-trimester ultrasonography still provides valuable clinical information about fetal anatomy.[36] Typically, the first-trimester ultrasonography determines the presence of trisomy 18 with a sensitivity of 70%, while a previous multiple marker test detected 43% of cases.[37, 38] In combination with invasive diagnostic testing, the standard screening process without NIPT already

achieved 100% sensitivity and negative predictive value.[39] This likely involves clinical scenarios that providers and patients confirm the presence or absence of a congenital malformation by standard combination screenings witnout NIPT in many cases. [40-43] Thus, a substantial proportion of prenatal care would not be altered by the use of NIPT.

Congenital malformation is a subject of environmental and socioeconomic factors. For example, being placed in a lower quartile of social deprivation is associated with a 30% increase in the rate of live-born congenital disease.[44] Therefore, the ultimate goal of prenatal screening, to achieve the reproductive autonomy mediated by reducing complications and herediatary malformation with a properly informed decision, will not be accomplished until underprivileged pregnancies have access to advanced prenatal care strategies. However, Medicaid enrollees still have limited prenatal care as indicated by 20% of the US states that do not cover the cost of NIPT.[45-47] Whereas, the majority of commercial health plans have expanded NIPT coverage to all pregnancies.[45-47] Not being enrolled in a commercial health plan was also a negative indicator for further IPD to confirm the presence of genetic disorder. Considring the significant changes in the pranal care strategy coincided with the beginning of a nationwide coverage for advanced prenatal screenings,[48] any coverage gap in access to prenatal care and the potential influence of the disparity has to be addressed to achieve the equity in reproductive autonomy, specifically in the US healthcare setting. Our data obtained from the real-world assessments warrant future research in and revision of the current policy to improve the utility of clinically advanced strategies in prenatal care, particularly in a disadvantaged population.

There are a couple of factors that may be associated with the decision to perform IPD based on our administrative data, such as having commercial insurance and being aged between 35 and 39 years. This may be due to patients with commercial insurance having greater access to healthcare, which is consistent with results from a previous studies.[42, 44] Insufficient prenatal care, social problems, and substance/alcohol abuse may be associated with less likelihood to receive NIPT and/or IPD. These associations may be related to Medicaid and underserved populations that do not have as great of access to healthcare resources, as well as types of providers that patient will see.[34, 49, 50] It is important that

doctors and midwives provide adequate information on the benefits and limitations associated with NIPT, specifically for the minorities and underprivileged population.

The interpretation of our data should be considered in light of several limitations. Firstly, the identification of both exposure and outcomes are limited by the procedures and orders defined by the administrative records. Although the quality of the study using the institutional data was confirmed by multiple observational studies, the likelihood of misclassification could not be ruled out. The study findings need to be confirmed by a detailed medical note review and warrant a confirmatory randomized controlled study. Second, our research was limited to a single healthcare system in the US healthcare setting. Future research may include multisite observational databases to establish the generalizability of study findings. Also, the use of both NIPT and IPD in the US healthcare setting would be significantly influenced by the patient socioeconomic status that were not fully controlled in this study. Any future attempts have to further investigate the disparity in achieving informed decisions and its influence on the overall utility of the advanced prenatal care technologies. Lastly, the size of the study cohort was associated with wide confidence intervals, limiting statistical inference. Although the point estimates confirm the no-to-nominal influence of NIPT on IPD, a further assessment using a larger cohort is warranted. Despite the limitations, our study provides valuable insight into the use of NIPT.

In conclusion, our study delineates the acceptance of NIPT in prenatal care. However, the utility of NIPT in mitigating concerns on IPD use has not been established. Future study needs to address inequal access to advanced prenatal care strategies, including NIPT and IPD.

Funding

None.

Acknowledgement

None.

Competing Interests

There are no competing interests to declare.

Ethics approval statement

The University of Utah Institutional Review Board approved this study and deemed it exempt (IRB# 00115830).

Contributorship Statement

LCK and KK jointly developed the initial research plan. The initial research protocol was reviewed and modified by both KK and LCK. KK extracted analytic cohorts. LCK and KK performed statistical analyses. LCK and KK compiled the drafted manuscript together. KK reviewed and edited this manuscript. KK revised this manuscript in response to the reviewers' comments. The overall research project was supervised and managed by KK, the corresponding author of this manuscript.

Data Availability Statement

No additional data is available.

Accessibility of protocol, raw data, and programming code

Research protocol and programming code will be available from the corresponding author, KK, upon reasonable request. Accessibility of the raw data will be subject to the data use agreement between the publisher and the University of Utah where the raw data were collected and the study was performed.

References

1. Barros ALO, Barros AO, Barros GLM, Santos M. Burden of caregivers of children and adolescents with Down Syndrome. Cien Saude Colet. 2017;22(11):3625-34.

2. Geelhoed EA, Bebbington A, Bower C, Deshpande A, Leonard H. Direct health care costs of children and adolescents with Down syndrome. J Pediatr. 2011;159(4):541-5.

3. Ghi T, Sotiriadis A, Calda P, Da Silva Costa F, Raine-Fenning N, Alfirevic Z, et al. ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis. Ultrasound Obstet Gynecol. 2016;48(2):256-68.

4. Panorama testing BILL $4000, doctor said it would cost $200 - Page 1. San Francisco, CA [cited 2021 March 18]. Available from: https://community.babycenter.com/post/a62696863/panorama-testing-bill-4000-doctor-said-it-would-cost-200.

5. Minear MA, Lewis C, Pradhan S, Chandrasekharan S. Global perspectives on clinical adoption of NIPT. Prenat Diagn. 2015;35(10):959-67.

6. Non-Invasive Prenatal Testing (NIPT). Vancouver, BC [cited 2021 March 18]. Available from: http://www.perinatalservicesbc.ca/health-professionals/professional-resources/screening/prenatal-genetic/non-invasive-prenatal-testing-nipt.

7. Petersen AK, Cheung SW, Smith JL, Bi W, Ward PA, Peacock S, et al. Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. Am J Obstet Gynecol. 2017;217(6):691.e1-.e6.

8. Walker BS, Nelson RE, Jackson BR, Grenache DG, Ashwood ER, Schmidt RL. A Cost-Effectiveness Analysis of First Trimester Non-Invasive Prenatal Screening for Fetal Trisomies in the United States. PLoS One. 2015;10(7):e0131402.

9. Derridj N, Bonnet D, Calderon J, Amedro P, Bertille N, Lelong N, et al. Quality of life of children born with a congenital heart defect. J Pediatr. 2022.

10. Mebius MJ, Kooi EMW, Bilardo CM, Bos AF. Brain Injury and Neurodevelopmental Outcome in Congenital Heart Disease: A Systematic Review. Pediatrics. 2017;140(1).

11. Robertson JA. Children of Choice: Freedom and the New Reproductive Technologies. Princeton, UK: Princeton University Press; 1996.

12. Mackenzie C, Stoljar N. Relational Autonomy: Feminist Perspectives on Autonomy, Agency, and the Social Self Oxford, UK: Oxford University Press; 2000.

13. Maternal Serum Screening, Second Trimester Burlington, NC [cited 2021 March 16]. Available from: https://www.labcorp.com/help/patient-test-info/maternal-serum-screening-second-trimester#:~:text=Maternal%20serum%20screening%20is%20a,or%20a%20condition%20called%20anencephaly.

14. Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226. Obstet Gynecol. 2020;136(4):e48-e69.

15. LeFevre NM, Sundermeyer RL. Fetal Aneuploidy: Screening and Diagnostic Testing. Am Fam Physician. 2020;101(8):481-8.

16. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. Obstet Gynecol. 2007;109(1):217-27.

17. Santorum M, Wright D, Syngelaki A, Karagioti N, Nicolaides KH. Accuracy of first-trimester combined test in screening for trisomies 21, 18 and 13. Ultrasound Obstet Gynecol. 2017;49(6):714-20.

18. Kostenko E, Chantraine F, Vandeweyer K, Schmid M, Lefevre A, Hertz D, et al. Clinical and Economic Impact of Adopting Noninvasive Prenatal Testing as a Primary Screening Method for Fetal Aneuploidies in the General Pregnancy Population. Fetal Diagn Ther. 2019;45(6):413-23.

19. Kim K, Magness JW, Nelson R, Baron V, Brixner DI. Clinical Utility of Pharmacogenetic Testing and a Clinical Decision Support Tool to Enhance the Identification of Drug Therapy Problems Through Medication Therapy Management in Polypharmacy Patients. J Manag Care Spec Pharm. 2018;24(12):1250-9.

20. Lesko LJ, Zineh I, Huang SM. What is clinical utility and why should we care? Clin Pharmacol Ther. 2010;88(6):729-33.

21. Quinn B. Payers and the assessment of clinical utility for companion diagnostics. Clin Pharmacol Ther. 2010;88(6):751-4.

22. Beamon CJ, Hardisty EE, Harris SC, Vora NL. A single center's experience with noninvasive prenatal testing. Genet Med. 2014;16(9):681-7.

23. Hui L, Hutchinson B, Poulton A, Halliday J. Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy. Genet Med. 2017;19(12):1338-45.

24. The Web's Free 2021 ICD-10-CM/PCS Medical Coding Reference [cited 2021 March 18]. Available from: https://www.icd10data.com/.

25. The Web's Free ICD-9-CM Medical Coding Reference [cited 2021 March, 18]. Available from: http://www.icd9data.com/2014/Volume1/240-279/240-246/244/244.9.htm.

26. Raskind-Hood C, Saraf A, Riehle-Colarusso T, Glidewell J, Gurvitz M, Dunn JE, et al. Assessing Pregnancy, Gestational Complications, and Co-morbidities in Women With

Congenital Heart Defects (Data from ICD-9-CM Codes in 3 US Surveillance Sites). Am J Cardiol. 2020;125(5):812-9.

27. Persson M, Pasupathy D, Hanson U, Westgren M, Norman M. Pre-pregnancy body mass index and the risk of adverse outcome in type 1 diabetic pregnancies: a population-based cohort study. BMJ Open. 2012;2(1):e000601.

28. Persson M, Winkvist A, Mogren I. Lifestyle and health status in a sample of Swedish women four years after pregnancy: a comparison of women with a history of normal pregnancy and women with a history of gestational diabetes mellitus. BMC Pregnancy Childbirth. 2015;15:57.

29. Kuhle S, Muir A, Woolcott CG, Brown MM, McDonald SD, Abdolell M, et al. Maternal pre-pregnancy obesity and health care utilization and costs in the offspring. Int J Obes (Lond). 2019;43(4):735-43.

30. Quinley KE, Falck A, Kallan MJ, Datner EM, Carr BG, Schreiber CA. Validation of ICD-9 Codes for Stable Miscarriage in the Emergency Department. West J Emerg Med. 2015;16(4):551-6.

31. Olney RS, Moore CA, Khoury MJ, Erickson D, Edmonds LD, Botto LD, et al. Chorionic Villus Sampling and Amniocentesis: Recommendations for Prenatal Counseling Atlanta, GA: Centers for Disease Control and Prevention; [cited 2021 March, 15]. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/00038393.htm#:~:text=Amniocentesis%20is%20usually%20performed%20at,11%2D14%20weeks'%20gestation.

32. Your First Prenatal Visit [cited 2021 March 18]. Available from: https://americanpregnancy.org/healthy-pregnancy/planning/first-prenatal-visit-71023/.

33. Cepeda MS, Boston R, Farrar JT, Strom BL. Comparison of logistic regression versus propensity score when the number of events is low and there are multiple confounders. Am J Epidemiol. 2003;158(3):280-7.

34. Quinlan TAG, Schroeder B, Kwon S, Barlow JF, Sherman MS, Anderson HD, et al. Economic Impact of Coverage Expansion for Non-invasive Prenatal Testing Through a Performance-Based Risk-Sharing Agreement. Pharmacoecon Open. 2021:1-10.

35. Garfield SS, Armstrong SO. Clinical and Cost Consequences of Incorporating a Novel Non-Invasive Prenatal Test into the Diagnostic Pathway for Fetal Trisomies. Journal of Managed Care Medicin. 2021;15(2):34-41.

36. Rao R, Han C, Silverman N, Platt L. Utility of first trimester ultrasound in the setting of NIPT incorporation into practice: a retrospective pilot review. American Journal of Obstetrics and Gynecology. 2015;212(1).

37. Brumfield CG, Wenstrom KD, Owen J, Davis RO. Ultrasound findings and multiple marker screening in trisomy 18. Obstet Gynecol. 2000;95(1):51-4.

38. Yeo L, Guzman ER, Day-Salvatore D, Walters C, Chavez D, Vintzileos AM. Prenatal detection of fetal trisomy 18 through abnormal sonographic features. J Ultrasound Med. 2003;22(6):581-90; quiz 91-2.

39. Park SY, Jang IA, Lee MA, Kim YJ, Chun SH, Park MH. Screening for chromosomal abnormalities using combined test in the first trimester of pregnancy. Obstet Gynecol Sci. 2016;59(5):357-66.

40. Benn P, Borell A, Chiu R, Cuckle H, Dugoff L, Faas B, et al. Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn. 2013;33(7):622-9.

41. Raised Maternal Age in Pregnancy [cited 2021 March 18]. Available from: https://www.rdehospital.nhs.uk/media/hrxj1nho/patient-information-leaflet-raised-maternal-age-in-pregnancy-rde-19-089-001.pdf.

42. What are some factors that make a pregnancy high risk? [cited 2021 March 18]. Available from: https://www.nichd.nih.gov/health/topics/high-risk/conditioninfo/factors.

43. Mackie FL, Allen S, Morris RK, Kilby MD. Cell-free fetal DNA-based noninvasive prenatal testing of aneuploidy. The Obstetrician & Gynaecologist. 2017;19(3):211-8.

44. Peyvandi S, Baer RJ, Chambers CD, Norton ME, Rajagopal S, Ryckman KK, et al. Environmental and Socioeconomic Factors Influence the Live-Born Incidence of Congenital Heart Disease: A Population-Based Study in California. J Am Heart Assoc. 2020;9(8):e015255.

45. Gadsbøll K, Petersen OB, Gatinois V, Strange H, Jacobsson B, Wapner R, et al. Current use of noninvasive prenatal testing in Europe, Australia and the USA: A graphical presentation. Acta Obstet Gynecol Scand. 2020;99(6):722-30.

46. Benoy ME, Iruretagoyena JI, Birkeland LE, Petty EM. The impact of insurance on equitable access to non-invasive prenatal screening (NIPT): private insurance may not pay. J Community Genet. 2021;12(1):185-97.

47. Löwy I. Non-invasive prenatal testing: A diagnostic innovation shaped by commercial interests and the regulation conundrum. Soc Sci Med. 2020:113064.

48. Vinante V, Keller B, Huhn EA, Huang D, Lapaire O, Manegold-Brauer G. Impact of nationwide health insurance coverage for non-invasive prenatal testing. Int J Gynaecol Obstet. 2018;141(2):189-93.

49. Goudie A, Martin B, Li C, Lewis K, Han X, Kathe N, et al. Higher Rates of Preventive Health Care With Commercial Insurance Compared With Medicaid: Findings From the Arkansas Health Care Independence "Private Option" Program. Med Care. 2020;58(2):120-7.

50. van der Steen SL, Houtman D, Bakkeren IM, Galjaard RH, Polak MG, Busschbach JJ, et al. Offering a choice between NIPT and invasive PND in prenatal genetic counseling: the impact of clinician characteristics on patients' test uptake. Eur J Hum Genet. 2019;27(2):235-43.

Table 1. Clinical characteristics and demographics of NIPT vs. no NIPT groups.

	NIPT (n=551)	No-NIPT (n=1506)	p-value*
Demographic Information			
Age, mean(SD)	37.23 (2.25)	37.41 (2.42)	0.11**
Grouped Age (3 groups)			0.17
35 – 39	468 (84.94)	1236 (82.07)	
40 – 44	80 (14.52)	251 (16.67)	
45 ≤	3 (1.26)	19 (0.54)	
Grouped Age (2 groups)			0.13
35 – 39	468 (84.94)	1236 (82.07)	
40 ≤	83 (15.06)	270 (17.93)	
Health plan			<0.01
Commercial Insurance	547 (99.27)	1196 (79.42)	
Government	0 (0)	3 (0.20)	
Medicaid	2 (0.36)	270 (17.93)	
Medicare	2 (0.36)	20 (1.33)	
Other Insurance/Unknown	0 (0)	17 (1.13)	
Health plan – two grouped			<0.01
Commercial	547 (99.27)	1196 (79.42)	
All non-Commercial	4 (0.73)	310 (20.58)	
Clinical Characteristics and Risk factors			
Insufficient Prenatal Care	4 (0.73)	22 (1.46)	0.18
Social Problem	16 (2.9)	15 (1.00)	<0.01
Genitourinary Infection	58 (10.53)	180 (11.95)	0.37
Gestational Diabetes	64 (11.62)	284 (18.86)	<0.01
Grand Multiparity	0	0	n/a
Hypothyroidism	90 (16.33)	216 (14.34)	0.26
Substance Abuse/Alcohol Abuse	7 (1.27)	94 (6.24)	<0.01
Overweight/Obese	156 (28.31)	543 (36.06)	<0.01
Hypertension	73 (13.25)	268 (17.80)	0.01
IPD during the 90-day follow up	16 (2.90)	40 (2.66)	0.76

Abbreviations: NIPT, Non-Invasive Prenatal Testing; IPD – Invasive Prenatal Diagnostic Testing including amniocentesis and chorionic villus sampling

*p-value from chi-square test or Fisher's exact test if an expected count of patient is less than 5 from a tabulate analysis.

** p-value from student t-test

Table 2. Clinical characteristics and demographics of IPD vs. no IPD groups.

	IPD (n=56)	No-IPD (n=2,001)	p-value*
Demographic Information			
Age, mean(SD)	37.89 (2.61)	37.35 (2.37)	0.09**
Grouped Age (3 groups)			<0.01
35 – 39	37 (66.07)	1667 (83.31)	
40 – 44	19 (33.93)	312 (15.59)	
45 ≤	0 (0)	22 (1.10)	
Grouped Age (2 groups)			<0.01
35 – 39	37 (66.07)	1667 (83.31)	
40 ≤	19 (33.93)	334 (16.69)	
Health plan			0.34
Commercial Insurance	53 (94.64)	1690 (84.46)	
Government	0 (0)	3 (0.15)	
Medicaid	3 (5.36)	269 (13.44)	
Medicare	0 (0)	22 (1.10)	
Other Insurance/Unknown	0 (0)	17 (1.0.85)	
Health plan - regrouped			0.04
Commercial	53 (94.64)	1690 (84.46)	
All non-Commercial	3 (5.36)	311 (15.54)	
Clinical Characteristics and Risk factors			
Insufficient Prenatal Care	0 (0)	26 (1.30)	0.39
Social Problem	2 (3.57)	29 (1.45)	0.19
Genitourinary Infection	4 (7.14)	234 (11.69)	0.29
Gestational Diabetes	6 (10.71)	342 (17.09)	0.21
Grand Multiparity	0	0	n/a
Hypothyroidism	8 (14.29)	298 (14.89)	0.90
Substance Abuse/Alcohol Abuse	3 (5.36)	98 (4.90)	0.88
Overweight/Obese	21 (37.50)	678 (33.88)	0.57
Hypertension	6 (10.71)	335 (16.74)	0.23
NIPT during the 90-day follow-up	16 (28.57)	535 (26.74)	0.76

Abbreviations: NIPT, Non-Invasive Prenatal Testing; IPD – Invasive Prenatal Diagnostic Testing including amniocentesis and chorionic villus sampling

*p-value from chi-square test or Fisher's exact test if an expected count of patient is less than 5 from a tabulate analysis.

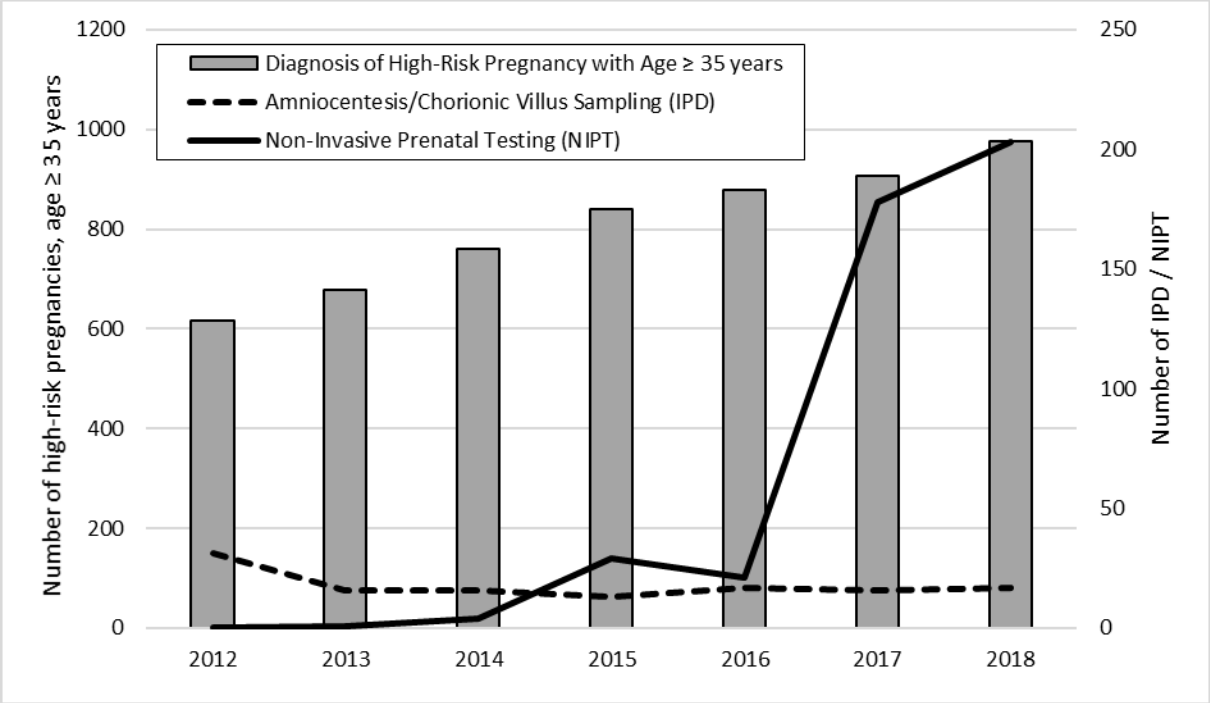
** p-value from student t-test

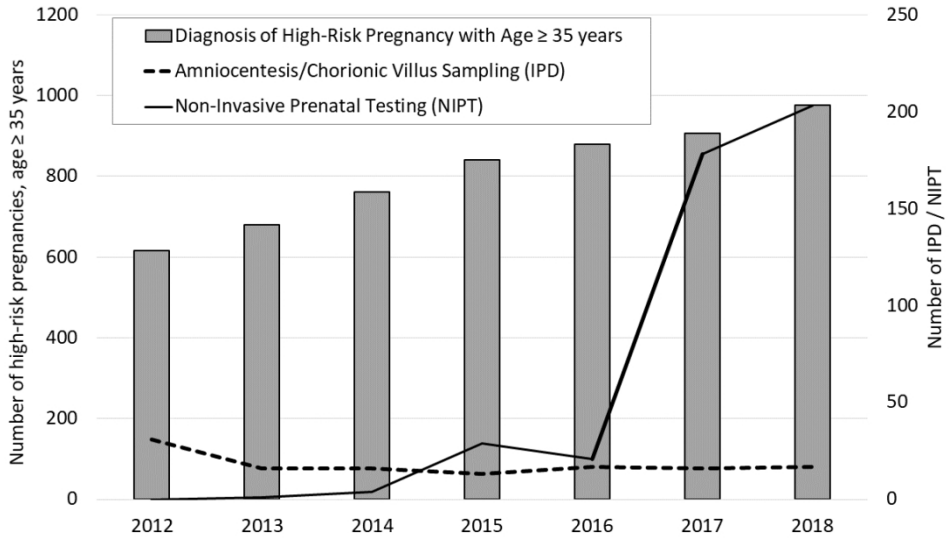
Table 3. Odds ratio of IPD for NIPD from logistic regression with single and multiple covariate adjustments

Covariates	OR [95%CI]
No covariate adjustment	1.10 [0.61 - 1.97]
Grouped Age (35-39 vs. 40≤)	1.14 [0.63 - 2.05]
Insufficient Prenatal Care	1.09 [0.60 - 1.96]
Social problem	1.07 [0.59 - 1.93]
Genitourinary Infection	1.09 [0.60 - 1.96]
Gestational Diabetes	1.06 [0.59 - 1.91]
Hypothyroidism	1.10 [0.61 - 1.98]
Substance or Alcohol abuse	1.10 [0.61 - 1.99]
Overweight or Obese	1.11 [0.62 - 2.00]
Hypertension	1.07 [0.60 - 1.94]
Health plan (commercial vs. all non-commercial)	0.94 [0.52 - 1.71]
All variables with p<0.1*	0.90 [0.49 - 1.65]

*Regression model includes type of health plan (commercial vs. all non-commercial), Social Problem, Gestational Diabetes, Hypothyroidism, Substance/Alcohol abuse, and Overweight/Obese as covariates for the NIPT-IPD association

Figure 1: Longitudinal trends in the number of high-risk pregnancies with advanced maternal age (age ≥ 35 years), IPD, and NIPT





Longitudinal trends in the number of high-risk pregnancies with advanced maternal age (age ≥ 35 years), IPD, and NIPT

451x254mm (72 x 72 DPI)

The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract Page and Page 6	RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 5		
Objectives	3	State specific objectives, including any prespecified hypotheses	Page6		
Methods					
Study Design	4	Present key elements of study design early in the paper	Page 7		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Pages 5 and 6		

Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case	Pages 7 and 8	RECORD 6.1: The methods of study population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	Pages 10 and 11	RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Pages 10 and 11
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	Pages 8 and 9		

Bias	9	Describe any efforts to address potential sources of bias	Page 10		
Study size	10	Explain how the study size was arrived at	Not applicable		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Page 9		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses	Page 9		
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population.	12.1: Pages 6 and 7 12.2: Pages 7 and 8

				RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	Page 7
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram	Pages 10 and 11	RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)	Page 11		
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure	Page 11		

		category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures			
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 11 and 12		
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Not applicable		
Discussion					
Key results	18	Summarise key results with reference to study objectives	Page 12		
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	Page 14	RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	
Interpretation	20	Give a cautious overall interpretation of results considering objectives,	Page 14		

		limitations, multiplicity of analyses, results from similar studies, and other relevant evidence			
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 14		
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	Not applicable		
Accessibility of protocol, raw data, and programming code		..	Page 15	RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	Page 15

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.

BMJ Open

Non-Invasive Prenatal Testing in Mitigating Concerns from Invasive Prenatal Diagnostic Testing: Retrospective Assessment of Utility in an Academic Healthcare System in the US

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-057658.R2
Article Type:	Original research
Date Submitted by the Author:	27-May-2022
Complete List of Authors:	Kim, Kibum; University of Illinois at Chicago, Department of Pharmacy Systems, Outcomes and Policy; The University of Utah, Department of Pharmacotherapy Craft, Kaitlyn; University of Utah
Primary Subject Heading:	Health services research
Secondary Subject Heading:	Evidence based practice, Health informatics, Obstetrics and gynaecology, Diagnostics
Keywords:	OBSTETRICS, Prenatal diagnosis < OBSTETRICS, Fetal medicine < OBSTETRICS, HEALTH SERVICES ADMINISTRATION & MANAGEMENT

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

TITLE

Non-Invasive Prenatal Testing in Mitigating Concerns from Invasive Prenatal Diagnostic Testing: Retrospective Assessment of Utility in an Academic Healthcare System in the US

A Short Running Title

Clinical Utility of Non-Invasive Prenatal Testing

Manuscript Size

Word Count:

Abstract: 300

Main Text: 3,696

Table Count: 3

Figure Count: 1

Reference Count: 50

Authors and Affiliations

Kibum Kim, BPharm, PhD

Assistant Professor | Department of Pharmacy Systems, Outcomes and Policy, University of Illinois at Chicago

833 South Wood Street, Chicago, IL 60612

Kkim204@uic.edu

(312) 413 - 0152

Adjunct Assistant Professor | Department of Pharmacotherapy, University of Utah

30 South 2000 East

Salt Lake City, UT 84112

kibum.kim@pharm.utah.edu

Kaitlyn Craft, PharmD.

College of Pharmacy, University of Utah

30 South 2000 East

Salt Lake City, UT 84112

Kaitlyn.Craft@pharm.utah.edu; kaitlyn.craft27@gmail.com

(408) 613-3865

Correspondence.

Kibum Kim, BPharm, PhD

Assistant Professor | Department of Pharmacy Systems, Outcomes and Policy, University of Illinois at Chicago

833 South Wood Street, Chicago, IL 60612

Kkim204@uic.edu

(312) 413 - 0152

Abstract:

Objective:

Non-Invasive Prenatal Testing (NIPT) is a front-line screening for fatal chromosomal aneuploidy. In pregnant women with a risk of having fetal congenital disorders, NIPT is anticipated to reduce the needs of invasive prenatal diagnostic test (IPD). The objectives of this study were to understand the acceptance of NIPT and the utility of NIPT to mitigate concerns about IPD in the US high-risk pregnancy management.

Design and Setting:

This was a retrospective observational research using healthcare records obtained from an academic healthcare system in the US. The study consisted of site-level longitudinal analysis and patient-level cross-sectional analysis.

Participant:

A total of 5,660 new high-risk pregnancies with age ≥ 35 years were identified for the longitudinal trend analysis. Cross-sectional utility assessment included 2,057 pregnant women.

Exposure and outcome measures:

Longitudinal trends of NIPT order, IPD procedure, and the number of patients diagnosed with high-risk pregnancy were descriptively summarized. In the cross-sectional assessment, we tested the association between the use of NIPT and IPD using multivariable regression..

Results:

The rate of increase in the NIPT use exceeded the changes in the number of high-risk pregnancies with age ≥ 35 years, while the number of annual IPD procedures has fluctuated without specific trends. There was no significant association between the numbers of NIPT and IPD with the adjusted odds ratios between 0.9 and 1.1 ($p > 0.1$). The order of NIPT was not selected as an independent variable predicting the use of IPD. Clinical characteristics indicating low socioeconomic status and limited healthcare coverage are associated with less use of NIPT and lower clinical utility.

Conclusion:

Although prenatal care accepted NIPT over the last decade, the utility of NIPT in mitigating concerns on IPD is unclear and needs further investigation. Limited clinical utility should be addressed in the context of disparity in prenatal care.

Article Summary

Strengths and limitations of this study

- This study includes both healthcare system level and patient-level assessments on the utility of non-invasive prenatal testing (NIPT) in the US healthcare setting.
- Summary statistics segmented by calendar year specifically demonstrated the acceptance of NIPT in a US academic medical center driven by the expansion of insurance coverage.
- Factors other than the clinical motivations are descriptively and inferentially tested in the assessment of NIPT utility and access.
- Although this study provides viable insight into the use and utility of the advanced prenatal care strategy, general limitations of single-site observational research warrant a multi-site health outcomes study.

MAIN TEXT

Introduction

Fetal chromosomal anomalies (FCA) has a significant influence on the personal and familier life trajectory, both emotionally and financially.[1-8] People living with congenital disability and their caregivers suffer from impaired quality of life.[1, 2] Despite major improvements in medical management and social support, long-term morbidities, particularly neurodevelopmental and mental health issues, remain a cause for concern.[9, 10] In the era of patient-centric medical care, the early detection of FCA enhances reproductive autonomy and helps expectant parents to contemplate before making an irrevocable conclusion.[11, 12]

There have been advances in prenatal care that enable expectant parents to learn of congenital disorders, allowing them to have the power to control pregnancy and childbearing earlier and make informed medical decisions. Maternal serum screening (MSS) was a minimally invasive traditional approach to determine the risk of fetal congenital disorders.[13-15] However, the risk of FCA based on MSS does not well predict the actual chromosomal anomalies, as it has a positive predictive value inferior to the predictive accuracy of a combination of other non-invasive measures, including maternal age, fetal nuchal translucency and fetal heart rate.[13-17] A better prediction of congenital disorders has been achieved via diagnostic invasive prenatal testing (IPD), which includes amniocentesis, chorionic villus sampling (CVS), and fetal blood sampling. Although providing patients with clinically valid data with a 99% positive prediction of certain FCAs, IPD is associated with a minor but sizable increase in the rate of miscarriage and infection.[3] Complications after IPD has been a concern to both providers and expectant mothers.[3]

Non-Invasive Prenatal Testing (NIPT), or cell-free DNA (cfDNA) testing, is a screening to help identify potential genetic concerns.[14] NIPT relies on the presence of free-floating cfDNAs which arise when cells die and release the DNA into the bloodstream from the placenta. If the percentage of cfDNA fragments for a particular chromosome is higher than expected, it indicates that the fetus has an increased

likelihood of having a disorder associated with that chromosome, and is generally followed by further testing. NIPT has been shown to have a sensitivity and specificity above 99% for detecting trisomy 21, as well as a 98% positive predictive value for fetal trisomy 18, and a 99% positive predictive value for fetal trisomy 13 with a combined false-positive rate of 0.13%.[7, 14]

NIPT showed promise in reducing unnecessary invasive medical procedures but is associated with a high upfront cost to healthcare plans in the US healthcare setting.[4] When it is covered, NIPT can cost payers upwards of $3,000 and patients with insurance are left with an out-of-pocket cost.[6] In addition, many state Medicaid plans and some health plans are not on board to pay for NIPT.[4, 6] While NIPT has upfront costs, implementation of this procedure has the potential to reduce unnecessary medical costs and potential maternal or fetal harm. A previous model-based study demonstrated that NIPT can reduce the number of unnecessary invasive tests by 94.8% and decrease IPD-related miscarriages by 90%.[18] Out of 1,000,000 simulated scenarios, replacing MSS with NIPT would result in an increase in 893 detections of FCA and would be followed by a cost savings of approximately $170 million.[8]

A screening test has clinical utility, beyond analytical validity and clinical validity, in a practice when it potentially influences and improves clinical decisions.[19-21] Thus, potential cost savings to payers would be achieved when analytically valid test is translated into a clinical utility: NIPT significantly influences clinical decision and outcomes as hypothesized. Nevertheless, NIPT results may be a small addition to a previous standard of care, rather than become the most critical component, to determine the needs of further actions. Clinical practice can still be directed by ultrasound assessment, patient preference, and provider's previous training, which may not result in the cost-effective use of the NIPT as simulated. A study performed in early 2010's showed a decline in the number of amniocenteses coincided with the use of NIPT.[22] Similarly, a recent time-series assessment on the use of invasive diagnostic test in Austrailian healthcare system demonstrated that the decrease in IPD since 2000 continued after NIPT started being covered by the public sector since 2013.[23] Nevertheless, the lack of assessment on the patient-level association on the NIPT and IPD left the downstream effect of NIPT from the clinical utility standpoint unanswered.

The objectives of this study were to assess the acceptance of NIPT in clinical practice setting, to evaluate the role of NIPT in alleviating the need for IPD, and to explore the patient-level characteristics that lead to the order of NIPT and IPD. We hypothesized that there would be a negative association between the order of NIPT and the frequency of IPD performed, which is a strong signal of the clinical utility of NIPT in high-risk pregnancy management.

Methods

Study Design and Setting:

This is a retrospective observational research consisting of two sections: (1) A healthcare system-level longitudinal change analysis and (2) Patient-level cross-sectional analysis using data from the University of Utah enterprise data warehouse from which comprehensive clinical records and healthcare resource utilization at the University of Utah Health are available. The University of Utah Institutional Review Board approved this study and deemed it exempt (00115830).

Patient and Public Involvement:

Patients were not involved in this study.

NIPT acceptance:

We compared the number of NIPT to the total number of high-risk pregnancies with age ≥ 35 years (advanced maternal age) and the number of IPD performed at a site-level to visualize the acceptance of NIPT into the healthcare setting. We looked at the longitudinal variation to see if the numbers of NIPT and IPD over time align with or exceeds the changes in the number of new high-risk pregnancies with advanced maternal age. Analytic cohort included pregnant women with one or more records of high-risk pregnancy (ICD-10-CM O09.x or ICD-9-CMV23.x) between January 2012 and December 2018.[24-30] Eligible subjects were 35 years old or older at the first date of the high-risk pregnancy diagnosis. The NIPTs ordered during the study period within the healthcare network were identified using terminology

available from institutional treatment records including “NON-INVASIVE PRENATAL”, “NIPT FETAL ANEUPLOIDY”, “NIPT FETAL MICRODELETION”, “CELL-FREE DNA” as well as available brand names of NIPT tests. To be labled as the IPD of interest, the procedure happened at the University of Utah Health and was defined using texts “chorionic villus” and “amniocenteses” from pathology, laboratory and procedure records. Descriptive statistics include the number of patients ordering NIPT, receiving IPD procedures, and seeing providers for a new high-risk pregnancy.

Patient-level analysis:

Study cohort identification:

The analytic cohort for the patient-level analysis was a subset of patients from the longitudinal cohort: subjects with a diagnosis of high-risk pregnancy (ICD-10-CM O09.x) at any point between October 2015 and December 2018 with the patient aged 35 years or older.[24-30] NIPT can generally be considered once the gestational age is past 9 weeks, which can be followed by additional CVS before the gestational weeks 11 and 14 of pregnancy.[14] Amniocentesis is usually performed between 15 to 18 weeks of gestational age although more amniocentesis procedures are now being performed at 11-14 weeks' gestation.[31] The first prenatal visit for a new pregnancy usually happens around the gestational age of 8 weeks.[32] All things considered, eligible subjects had a record of prenatal care from the first-trimester (ICD-10-CM O09.x1, O09.5x1, O09.6x1, O09.8x1) and must be followed by the University of Utah Health for longer than 90-day period from that first-trimester visit, which allowed for a sufficient window to cover both NIPT and IPD. The accuracy of NIPT results is debatable in patients having more than one fetus. Thus, any expectant mothers with one or more records of multiple gestation (ICD-10-CM O30.x) were excluded from this study[24]. All the selected subjects were 35 years old or older at the first encounter with the first-trimester prenatal care visit. Clinical characteristics, demographic characteristics and records of NIPT and IPD were collected over the 90-day follow-up period. Patient characteristics were identified from the review of diagnosis codes, enrollment information, and demographic tables.

Patient characteristics as underlying conditions for the high-risk pregnancy included maternal age (ICD-9 659.63, ICD-10 O09.51x, O09.52x), insufficient prenatal care (ICD-9 V23.7, ICD-10 O09.3), genitourinary tract infection during pregnancy (ICD-9 646.0x, ICD-10 O23.x), grand multiparity (ICD-9 659.4, ICD-10 O09.40), type 1 or type 2 diabetes (ICD-9 250, ICD-10 O24.01, O24.11), history of hypothyroidism (ICD-9 243, ICD-10 E00,E01,E02), hypertension (ICD-9 642.3x, 642.9x, ICD-10 O13.9), social problems (ICD-10 O09.3, O09.70, O09.71, O09.72, O09.73), drug/alcohol use during pregnancy (ICD-9 649, ICD-10 O99.33), type of health plan and obesity (ICD-10 O99.21).[24-30]

Exposure and Outcomes

The exposure of the patient-level analysis is the order of NIPT. We used the same text-search algorithm used for the Site-level NIPT acceptance to determine the NIPT order. The date of NIPT order was matched with the date of medical encounter for pregnancy to confirm the order was not misplaced and was part of prenatal care. The outcome of this study is the administration of IPD, either CVS or Amniocentesis. The procedure performed within the institutional healthcare network was defined using texts "chorionic villus" and "amniocentesis, laboratory and procedure records." We also used applicable Current Procedural Terminology (CPT) codes including 59000, 59105, 76945 and 76946 to confirm that the IPD was performed. To be classified as an exposure or outcome, the procedure or order record had to fall within the 90-day follow-up period.

Statistical Analysis

For the site-level analysis, the number of patients receiving NIPT order, the number of IPD performed, and the number of new high-risk pregnancies within the healthcare system for each calendar year were longitudinally described. The number of patients with NIPT, IPD, and high-risk pregnancy with advanced maternal age were presented by the calendar year.

Maternal age at the first prenatal visit with a diagnosis of first trimester checkup record was summarized using mean and standard deviation and compared between the NIPT and no-NIPT groups using Student t-test. Categorical variables including type of health plan, grouped age (35 – 39, 40 – 44 and 45 +), and specific risk factors including insufficient prenatal care, social problems, genitourinary infection, gestational diabetes, grand multiparity, hypothyroidism, substance/alcohol abuse, overweight/obese, and hypertension in pregnant women were compared between the NIPT and no-NIPT groups and were summarized using frequency and percentage. Type of health plan was regrouped into two, commercial insurance vs. all the others to address the small number of patients in each non-commercially insured or uninsured subgroups. Age was also categorized into two groups, 35 - 39 vs. 40 or older. To address the influence of the clinical factors on the decision to perform IPD, patient characteristics at the date of the first prenatal visit were also compared between IPD and no-IPD groups. Using Chi-square test, or Fisher's Exact test for the small patient counts (<5 count), categorical variables as a clinical characteristic were compared between the NIPT and no-NIPT groups, and between IPD and no-IPD groups.

We compared the rate of IPD between the patients who received NIPT and those who did not receive NIPT. Proportion of patients receiving IPD during the 90-day assessment period between the NIPT and no-NIPT groups were statistically compared using Chi-square test. The odds ratio and 95%CI estimate from a logistic regression model presented the direction and precision of the association measure. In a multivariable approach, baseline characteristics that were marginally different ($p<0.1$) between the NIPT and no-NIPT were included as regression covariates. Due to the small number of subjects and outcomes relative to the number of covariates that need to be adjusted for (i.e., dimensionality in a regression model), the multivariable approach may not address all the differences in the baseline characteristics simultaneously.[33] Thus, in addition to running an inclusive multivariable regression model, we calculated the odds ratios of IPD for NIPT in a series of logistic regression models where each regression included each single covariate.

A further assessment tested the significance of NIPT as a predictor out of the clinical factors using a multivariable regression model selection process. Variable selection in the logistic regression was performed using a stepwise forward selection approach with significance levels for entering and removing effects of 0.5 and 0.35. The final model including NIPT as a predictor was supposed to indicate that NIPT is a critical factor, to assist providers in determining the need for IPD. Statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC).

Results

Site-Level NIPT Acceptance:

A total of 5,660 new high-risk pregnancies with advanced maternal age were identified between 2012-2018. The number of high-risk pregnancies with advanced maternal age in 2018 was 977 which is 158% of the 2012 (n=616) and 116% of the 2015 (n=841) count. The numbers of NIPT and IPD performed within the selected pregnant women were 436 and 126, respectively. There were no specific trends in the number of annual IPD (Figure 1). The annual NIPT order in 2018 was 203 which was 7 times 29 cases in 2015. Overall the rate of increase in NIPT use exceeded the change in the number of high-risk pregnancy with advanced maternal age. (Figure 1).

Patient-Level Analysis:

The study cohort consists of 2,057 pregnant women at or older than 35 years with a diagnosis of high-risk pregnancy. We identified a total of 551 NIPT orders for the patients included in the study cohort. The difference in the age distribution between the NIPT and no-NIPT group was not statistically nor clinically significant with the respective proportions of subjects younger than 40 of 84.94% vs. 82.07. The NIPT cohort was more dominated by commercially insured patients (99.27%) compared to the no-NIPT cohort (79.42%). Based on the analysis of clinical characteristics, patients who received NIPT generally carried less risk factors than the no-NIPT patients with the respective proportions of gestational diabetes (11.62% vs. 18.86%, $p<0.01$), substance or alcohol abuse (1.27% vs. 6.24%, $p<0.01$), overweight

or obese (28.31% vs. 36.06%, p<0.01) and hypertension (13.25% vs. 17.80%, p=0.01). Social problem was the only risk factor more prevalent among the NIPT than the no-NIPT groups (2.9% vs. 1.00%, p<0.01), but the difference in the proportion was nominal from the clinical standpoint. (Table 1).

When the analysis grouped high-risk pregnancy into patients who received IPD (n=56) and patients who did not (n=2,001), the proportion of patients younger than 40 years out of the IPD recipients was significantly less than the proportion among the no-IPD (66.07% vs. 83.31%, p<0.01). The difference in the mean ± SD age was marginally significant (p=0.09) between the IPD and no-IPD groups (37.89 ± 2.61 vs. 37.35 ± 2.37). There was a significant difference in the proportion of commercially insured pregnancy (94.64% vs., 84.46, p=0.04, regrouped health plan type) with the larger proportion of commercially insured patients among those who received IPD. The prevalence of clinical risk factors was generally lower among the IPD vs. no-IPD, including genitourinary infection (7.14% vs. 11.69%), gestational diabetes (10.71% vs. 17.09%), and hypertension (10.71% vs. 16.74%), but the differences were not statistically significant. The lack of statistical significance was likely attributed to the small number of IPD procedures. (Table 2).

From the tabulate analysis, the proportion of patients who received IPD among the NIPT patients during the 90-day assessment period was 2.90% which was slightly larger than the rate of IPD performed without NIPT record (2.66%, Table 1). The results were not statistically nor clinically significant (p=0.76, Tables 1 and 2). The logistic regression model, without any adjustment for the baseline characteristics, resulted in the odds ratio [95%CI] of 1.10 [0.61 – 1.97]. Patient demographics and clinical risk factors had only a nominal impact on the adjusted odds ratio calculation. When the association was adjusted for all patient characteristics with p <0.1, the odds ratio [95%CI] was 0.90 [0.49 – 1.65]. The stepwise model selection process chose age (35-39 vs. 45≤), type of health plan (commercial vs. all non-commercial), social problem, gestational diabetes and hypertension as independent variables in the logistic regression model. Of the selected variables, 40 years or older (OR=2.74 [95%CI: 1.54 – 4.81], p <0.01) and commercial insurance (OR=3.19 [95%CI: 0.10 – 1.04], p = 0.06) showed a significant or marginally

significant association with IPD. (Table 3) NIPT was not considered to be an independent variable that predicts IPD use while the selection process finalized the multivariable regression model.

Discussion

Our assessment confirms that a rapid and gradual increase in the use of NIPT outpaced the increase in the need for a maternity care for the high-risk pregnancy with advanced age. Although the acceptance of NIPT was partially explained by the longitudinal changes in the characteristics of pregnancy, such as becoming older and increasing prevalence of pre-existing conditions, it is mainly attributable to coverage expansion, particularly among the patients enrolled in a commercial health plan.[24-31] Our results are comparable to the outcomes of a recent time-series analysis comparing the orders of NIPT and number of IPD in that there has been a significant increase in the order of NIPT with a subtle decrease in the number of IPD, with the adjusted incidence rate ratio of 0.97.[34]

To the best of our knowledge, our study includes the first patient-level assessment to analyze the clinical utility of NIPT in the US healthcare setting. Because IPD is followed by the likelihood of complications, one of the expected benefits of NIPT is to diminish the need for diagnostic IPD. To achieve the expected cost saving or cost-effectiveness, NIPT needs to achieve an anticipated decrease in the IPD by 66% to 93%.[35] Not being aligned with the anticipated clinical scenario, our study did not find a strong signal of the negative association between the order of NIPT and the frequency of IPD. We tentatively concluded that the utility NIPT in alleviating IPD-related concerns would be, at best, nominal in managing high-risk pregnancy with advanced maternal age based on the odds ratio of 0.9 from our multivariable logistic regression model.

A decision assisted by multiple risk factors, imaging and confirmatory diagnostic procedure partially explains the reason for the subtle influence of NIPT on the following diagnostic tests. A recent chart review showed that the first-trimester ultrasonography still provides valuable clinical information about fetal anatomy.[36] Typically, the first-trimester ultrasonography determines the presence of trisomy 18 with a sensitivity of 70%, while a previous multiple marker test detected 43% of cases.[37, 38] In combination with invasive diagnostic testing, the standard screening process without NIPT already

achieved 100% sensitivity and negative predictive value.[39] This likely involves clinical scenarios that providers and patients confirm the presence or absence of a congenital malformation by standard combination screenings witnout NIPT in many cases. [40-43] Thus, a substantial proportion of prenatal care would not be altered by the use of NIPT.

Congenital malformation is a subject of environmental and socioeconomic factors. For example, being placed in a lower quartile of social deprivation is associated with a 30% increase in the rate of live-born congenital disease.[44] Therefore, the ultimate goal of prenatal screening, to achieve the reproductive autonomy mediated by reducing complications and herediatary malformation with a properly informed decision, will not be accomplished until underprivileged pregnancies have access to advanced prenatal care strategies. However, Medicaid enrollees still have limited prenatal care as indicated by 20% of the US states that do not cover the cost of NIPT.[45-47] Whereas, the majority of commercial health plans have expanded NIPT coverage to all pregnancies.[45-47] Not being enrolled in a commercial health plan was also a negative indicator for further IPD to confirm the presence of genetic disorder. Considring the significant changes in the pranal care strategy coincided with the beginning of a nationwide coverage for advanced prenatal screenings,[48] any coverage gap in access to prenatal care and the potential influence of the disparity has to be addressed to achieve the equity in reproductive autonomy, specifically in the US healthcare setting. Our data obtained from the real-world assessments warrant future research in and revision of the current policy to improve the utility of clinically advanced strategies in prenatal care, particularly in a disadvantaged population.

There are a couple of factors that may be associated with the decision to perform IPD based on our administrative data, such as having commercial insurance and being aged between 35 and 39 years. This may be due to patients with commercial insurance having greater access to healthcare, which is consistent with results from a previous studies.[42, 44] Insufficient prenatal care, social problems, and substance/alcohol abuse may be associated with less likelihood to receive NIPT and/or IPD. These associations may be related to Medicaid and underserved populations that do not have as great of access to healthcare resources, as well as types of providers that patient will see.[34, 49, 50] It is important that

doctors and midwives provide adequate information on the benefits and limitations associated with NIPT, specifically for the minorities and underprivileged population.

The interpretation of our data should be considered in light of several limitations. Firstly, the identification of both exposure and outcomes are limited by the procedures and orders defined by the administrative records. Although the quality of the study using the institutional data was confirmed by multiple observational studies, the likelihood of misclassification could not be ruled out. The study findings need to be confirmed by a detailed medical note review and warrant a confirmatory randomized controlled study. Second, our research was limited to a single healthcare system in the US healthcare setting. Future research may include multisite observational databases to establish the generalizability of study findings. Also, the use of both NIPT and IPD in the US healthcare setting would be significantly influenced by the patient socioeconomic status that were not fully controlled in this study. Any future attempts have to further investigate the disparity in achieving informed decisions and its influence on the overall utility of the advanced prenatal care technologies. Lastly, the size of the study cohort was associated with wide confidence intervals, limiting statistical inference. Although the point estimates confirm the no-to-nominal influence of NIPT on IPD, a further assessment using a larger cohort is warranted. Despite the limitations, our study provides valuable insight into the use of NIPT.

In conclusion, our study delineates the acceptance of NIPT in prenatal care. However, the utility of NIPT in mitigating concerns on IPD use has not been established. Future study needs to address inequal access to advanced prenatal care strategies, including NIPT and IPD.

Funding

None.

Acknowledgement

None.

Competing Interests

There are no competing interests to declare.

Ethics approval statement

The University of Utah Institutional Review Board approved this study and deemed it exempt (IRB# 00115830).

Contributorship Statement

LCK and KK jointly developed the initial research plan. The initial research protocol was reviewed and modified by both KK and LCK. KK extracted analytic cohorts. LCK and KK performed statistical analyses. LCK and KK compiled the drafted manuscript together. KK reviewed and edited this manuscript. KK revised this manuscript in response to the reviewers' comments. The overall research project was supervised and managed by KK, the corresponding author of this manuscript.

Data Availability Statement

No additional data is available.

Accessibility of protocol, raw data, and programming code

Research protocol and programming code will be available from the corresponding author, KK, upon reasonable request. Accessibility of the raw data will be subject to the data use agreement between the publisher and the University of Utah where the raw data were collected and the study was performed.

References

1. Barros ALO, Barros AO, Barros GLM, Santos M. Burden of caregivers of children and adolescents with Down Syndrome. Cien Saude Colet. 2017;22(11):3625-34.

2. Geelhoed EA, Bebbington A, Bower C, Deshpande A, Leonard H. Direct health care costs of children and adolescents with Down syndrome. J Pediatr. 2011;159(4):541-5.

3. Ghi T, Sotiriadis A, Calda P, Da Silva Costa F, Raine-Fenning N, Alfirevic Z, et al. ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis. Ultrasound Obstet Gynecol. 2016;48(2):256-68.

4. Panorama testing BILL $4000, doctor said it would cost $200 - Page 1. San Francisco, CA [cited 2021 March 18]. Available from: https://community.babycenter.com/post/a62696863/panorama-testing-bill-4000-doctor-said-it-would-cost-200.

5. Minear MA, Lewis C, Pradhan S, Chandrasekharan S. Global perspectives on clinical adoption of NIPT. Prenat Diagn. 2015;35(10):959-67.

6. Non-Invasive Prenatal Testing (NIPT). Vancouver, BC [cited 2021 March 18]. Available from: http://www.perinatalservicesbc.ca/health-professionals/professional-resources/screening/prenatal-genetic/non-invasive-prenatal-testing-nipt.

7. Petersen AK, Cheung SW, Smith JL, Bi W, Ward PA, Peacock S, et al. Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. Am J Obstet Gynecol. 2017;217(6):691.e1-.e6.

8. Walker BS, Nelson RE, Jackson BR, Grenache DG, Ashwood ER, Schmidt RL. A Cost-Effectiveness Analysis of First Trimester Non-Invasive Prenatal Screening for Fetal Trisomies in the United States. PLoS One. 2015;10(7):e0131402.

9. Derridj N, Bonnet D, Calderon J, Amedro P, Bertille N, Lelong N, et al. Quality of life of children born with a congenital heart defect. J Pediatr. 2022.

10. Mebius MJ, Kooi EMW, Bilardo CM, Bos AF. Brain Injury and Neurodevelopmental Outcome in Congenital Heart Disease: A Systematic Review. Pediatrics. 2017;140(1).

11. Robertson JA. Children of Choice: Freedom and the New Reproductive Technologies. Princeton, UK: Princeton University Press; 1996.

12. Mackenzie C, Stoljar N. Relational Autonomy: Feminist Perspectives on Autonomy, Agency, and the Social Self Oxford, UK: Oxford University Press; 2000.

13. Maternal Serum Screening, Second Trimester Burlington, NC [cited 2021 March 16]. Available from: https://www.labcorp.com/help/patient-test-info/maternal-serum-screening-second-trimester#:~:text=Maternal%20serum%20screening%20is%20a,or%20a%20condition%20called%20anencephaly.

14. Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin, Number 226. Obstet Gynecol. 2020;136(4):e48-e69.

15. LeFevre NM, Sundermeyer RL. Fetal Aneuploidy: Screening and Diagnostic Testing. Am Fam Physician. 2020;101(8):481-8.

16. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. Obstet Gynecol. 2007;109(1):217-27.

17. Santorum M, Wright D, Syngelaki A, Karagioti N, Nicolaides KH. Accuracy of first-trimester combined test in screening for trisomies 21, 18 and 13. Ultrasound Obstet Gynecol. 2017;49(6):714-20.

18. Kostenko E, Chantraine F, Vandeweyer K, Schmid M, Lefevre A, Hertz D, et al. Clinical and Economic Impact of Adopting Noninvasive Prenatal Testing as a Primary Screening Method for Fetal Aneuploidies in the General Pregnancy Population. Fetal Diagn Ther. 2019;45(6):413-23.

19. Kim K, Magness JW, Nelson R, Baron V, Brixner DI. Clinical Utility of Pharmacogenetic Testing and a Clinical Decision Support Tool to Enhance the Identification of Drug Therapy Problems Through Medication Therapy Management in Polypharmacy Patients. J Manag Care Spec Pharm. 2018;24(12):1250-9.

20. Lesko LJ, Zineh I, Huang SM. What is clinical utility and why should we care? Clin Pharmacol Ther. 2010;88(6):729-33.

21. Quinn B. Payers and the assessment of clinical utility for companion diagnostics. Clin Pharmacol Ther. 2010;88(6):751-4.

22. Beamon CJ, Hardisty EE, Harris SC, Vora NL. A single center's experience with noninvasive prenatal testing. Genet Med. 2014;16(9):681-7.

23. Hui L, Hutchinson B, Poulton A, Halliday J. Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy. Genet Med. 2017;19(12):1338-45.

24. The Web's Free 2021 ICD-10-CM/PCS Medical Coding Reference [cited 2021 March 18]. Available from: https://www.icd10data.com/.

25. The Web's Free ICD-9-CM Medical Coding Reference [cited 2021 March, 18]. Available from: http://www.icd9data.com/2014/Volume1/240-279/240-246/244/244.9.htm.

26. Raskind-Hood C, Saraf A, Riehle-Colarusso T, Glidewell J, Gurvitz M, Dunn JE, et al. Assessing Pregnancy, Gestational Complications, and Co-morbidities in Women With

Congenital Heart Defects (Data from ICD-9-CM Codes in 3 US Surveillance Sites). Am J Cardiol. 2020;125(5):812-9.

27. Persson M, Pasupathy D, Hanson U, Westgren M, Norman M. Pre-pregnancy body mass index and the risk of adverse outcome in type 1 diabetic pregnancies: a population-based cohort study. BMJ Open. 2012;2(1):e000601.

28. Persson M, Winkvist A, Mogren I. Lifestyle and health status in a sample of Swedish women four years after pregnancy: a comparison of women with a history of normal pregnancy and women with a history of gestational diabetes mellitus. BMC Pregnancy Childbirth. 2015;15:57.

29. Kuhle S, Muir A, Woolcott CG, Brown MM, McDonald SD, Abdolell M, et al. Maternal pre-pregnancy obesity and health care utilization and costs in the offspring. Int J Obes (Lond). 2019;43(4):735-43.

30. Quinley KE, Falck A, Kallan MJ, Datner EM, Carr BG, Schreiber CA. Validation of ICD-9 Codes for Stable Miscarriage in the Emergency Department. West J Emerg Med. 2015;16(4):551-6.

31. Olney RS, Moore CA, Khoury MJ, Erickson D, Edmonds LD, Botto LD, et al. Chorionic Villus Sampling and Amniocentesis: Recommendations for Prenatal Counseling Atlanta, GA: Centers for Disease Control and Prevention; [cited 2021 March, 15]. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/00038393.htm#:~:text=Amniocentesis%20is%20usually%20performed%20at,11%2D14%20weeks'%20gestation.

32. Your First Prenatal Visit [cited 2021 March 18]. Available from: https://americanpregnancy.org/healthy-pregnancy/planning/first-prenatal-visit-71023/.

33. Cepeda MS, Boston R, Farrar JT, Strom BL. Comparison of logistic regression versus propensity score when the number of events is low and there are multiple confounders. Am J Epidemiol. 2003;158(3):280-7.

34. Quinlan TAG, Schroeder B, Kwon S, Barlow JF, Sherman MS, Anderson HD, et al. Economic Impact of Coverage Expansion for Non-invasive Prenatal Testing Through a Performance-Based Risk-Sharing Agreement. Pharmacoecon Open. 2021:1-10.

35. Garfield SS, Armstrong SO. Clinical and Cost Consequences of Incorporating a Novel Non-Invasive Prenatal Test into the Diagnostic Pathway for Fetal Trisomies. Journal of Managed Care Medicin. 2021;15(2):34-41.

36. Rao R, Han C, Silverman N, Platt L. Utility of first trimester ultrasound in the setting of NIPT incorporation into practice: a retrospective pilot review. American Journal of Obstetrics and Gynecology. 2015;212(1).

37. Brumfield CG, Wenstrom KD, Owen J, Davis RO. Ultrasound findings and multiple marker screening in trisomy 18. Obstet Gynecol. 2000;95(1):51-4.

38. Yeo L, Guzman ER, Day-Salvatore D, Walters C, Chavez D, Vintzileos AM. Prenatal detection of fetal trisomy 18 through abnormal sonographic features. J Ultrasound Med. 2003;22(6):581-90; quiz 91-2.

39. Park SY, Jang IA, Lee MA, Kim YJ, Chun SH, Park MH. Screening for chromosomal abnormalities using combined test in the first trimester of pregnancy. Obstet Gynecol Sci. 2016;59(5):357-66.

40. Benn P, Borell A, Chiu R, Cuckle H, Dugoff L, Faas B, et al. Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn. 2013;33(7):622-9.

41. Raised Maternal Age in Pregnancy [cited 2021 March 18]. Available from: https://www.rdehospital.nhs.uk/media/hrxj1nho/patient-information-leaflet-raised-maternal-age-in-pregnancy-rde-19-089-001.pdf.

42. What are some factors that make a pregnancy high risk? [cited 2021 March 18]. Available from: https://www.nichd.nih.gov/health/topics/high-risk/conditioninfo/factors.

43. Mackie FL, Allen S, Morris RK, Kilby MD. Cell-free fetal DNA-based noninvasive prenatal testing of aneuploidy. The Obstetrician & Gynaecologist. 2017;19(3):211-8.

44. Peyvandi S, Baer RJ, Chambers CD, Norton ME, Rajagopal S, Ryckman KK, et al. Environmental and Socioeconomic Factors Influence the Live-Born Incidence of Congenital Heart Disease: A Population-Based Study in California. J Am Heart Assoc. 2020;9(8):e015255.

45. Gadsbøll K, Petersen OB, Gatinois V, Strange H, Jacobsson B, Wapner R, et al. Current use of noninvasive prenatal testing in Europe, Australia and the USA: A graphical presentation. Acta Obstet Gynecol Scand. 2020;99(6):722-30.

46. Benoy ME, Iruretagoyena JI, Birkeland LE, Petty EM. The impact of insurance on equitable access to non-invasive prenatal screening (NIPT): private insurance may not pay. J Community Genet. 2021;12(1):185-97.

47. Löwy I. Non-invasive prenatal testing: A diagnostic innovation shaped by commercial interests and the regulation conundrum. Soc Sci Med. 2020:113064.

48. Vinante V, Keller B, Huhn EA, Huang D, Lapaire O, Manegold-Brauer G. Impact of nationwide health insurance coverage for non-invasive prenatal testing. Int J Gynaecol Obstet. 2018;141(2):189-93.

49. Goudie A, Martin B, Li C, Lewis K, Han X, Kathe N, et al. Higher Rates of Preventive Health Care With Commercial Insurance Compared With Medicaid: Findings From the Arkansas Health Care Independence "Private Option" Program. Med Care. 2020;58(2):120-7.

50. van der Steen SL, Houtman D, Bakkeren IM, Galjaard RH, Polak MG, Busschbach JJ, et al. Offering a choice between NIPT and invasive PND in prenatal genetic counseling: the impact of clinician characteristics on patients' test uptake. Eur J Hum Genet. 2019;27(2):235-43.

Table 1. Clinical characteristics and demographics of NIPT vs. no NIPT groups.

	NIPT (n=551)	No-NIPT (n=1506)	p-value*
Demographic Information			
Age, mean(SD)	37.23 (2.25)	37.41 (2.42)	0.11**
Grouped Age (3 groups)			0.17
35 – 39	468 (84.94)	1236 (82.07)	
40 – 44	80 (14.52)	251 (16.67)	
45 ≤	3 (1.26)	19 (0.54)	
Grouped Age (2 groups)			0.13
35 – 39	468 (84.94)	1236 (82.07)	
40 ≤	83 (15.06)	270 (17.93)	
Health plan			<0.01
Commercial Insurance	547 (99.27)	1196 (79.42)	
Government	0 (0)	3 (0.20)	
Medicaid	2 (0.36)	270 (17.93)	
Medicare	2 (0.36)	20 (1.33)	
Other Insurance/Unknown	0 (0)	17 (1.13)	
Health plan – two grouped			<0.01
Commercial	547 (99.27)	1196 (79.42)	
All non-Commercial	4 (0.73)	310 (20.58)	
Clinical Characteristics and Risk factors			
Insufficient Prenatal Care	4 (0.73)	22 (1.46)	0.18
Social Problem	16 (2.9)	15 (1.00)	<0.01
Genitourinary Infection	58 (10.53)	180 (11.95)	0.37
Gestational Diabetes	64 (11.62)	284 (18.86)	<0.01
Grand Multiparity	0	0	n/a
Hypothyroidism	90 (16.33)	216 (14.34)	0.26
Substance Abuse/Alcohol Abuse	7 (1.27)	94 (6.24)	<0.01
Overweight/Obese	156 (28.31)	543 (36.06)	<0.01
Hypertension	73 (13.25)	268 (17.80)	0.01
IPD during the 90-day follow up	16 (2.90)	40 (2.66)	0.76

Abbreviations: NIPT, Non-Invasive Prenatal Testing; IPD – Invasive Prenatal Diagnostic Testing including amniocentesis and chorionic villus sampling

*p-value from chi-square test or Fisher's exact test if an expected count of patient is less than 5 from a tabulate analysis.

** p-value from student t-test

Table 2. Clinical characteristics and demographics of IPD vs. no IPD groups.

	IPD (n=56)	No-IPD (n=2,001)	p-value*
Demographic Information			
Age, mean(SD)	37.89 (2.61)	37.35 (2.37)	0.09**
Grouped Age (3 groups)			<0.01
35 – 39	37 (66.07)	1667 (83.31)	
40 – 44	19 (33.93)	312 (15.59)	
45 ≤	0 (0)	22 (1.10)	
Grouped Age (2 groups)			<0.01
35 – 39	37 (66.07)	1667 (83.31)	
40 ≤	19 (33.93)	334 (16.69)	
Health plan			0.34
Commercial Insurance	53 (94.64)	1690 (84.46)	
Government	0 (0)	3 (0.15)	
Medicaid	3 (5.36)	269 (13.44)	
Medicare	0 (0)	22 (1.10)	
Other Insurance/Unknown	0 (0)	17 (1.0.85)	
Health plan - regrouped			0.04
Commercial	53 (94.64)	1690 (84.46)	
All non-Commercial	3 (5.36)	311 (15.54)	
Clinical Characteristics and Risk factors			
Insufficient Prenatal Care	0 (0)	26 (1.30)	0.39
Social Problem	2 (3.57)	29 (1.45)	0.19
Genitourinary Infection	4 (7.14)	234 (11.69)	0.29
Gestational Diabetes	6 (10.71)	342 (17.09)	0.21
Grand Multiparity	0	0	n/a
Hypothyroidism	8 (14.29)	298 (14.89)	0.90
Substance Abuse/Alcohol Abuse	3 (5.36)	98 (4.90)	0.88
Overweight/Obese	21 (37.50)	678 (33.88)	0.57
Hypertension	6 (10.71)	335 (16.74)	0.23
NIPT during the 90-day follow-up	16 (28.57)	535 (26.74)	0.76

Abbreviations: NIPT, Non-Invasive Prenatal Testing; IPD – Invasive Prenatal Diagnostic Testing including amniocentesis and chorionic villus sampling

*p-value from chi-square test or Fisher's exact test if an expected count of patient is less than 5 from a tabulate analysis.

** p-value from student t-test

Table 3. Odds ratio of IPD for NIPD from logistic regression with single and multiple covariate adjustments

Covariates	OR [95%CI]
No covariate adjustment	1.10 [0.61 - 1.97]
Grouped Age (35-39 vs. 40≤)	1.14 [0.63 - 2.05]
Insufficient Prenatal Care	1.09 [0.60 - 1.96]
Social problem	1.07 [0.59 - 1.93]
Genitourinary Infection	1.09 [0.60 - 1.96]
Gestational Diabetes	1.06 [0.59 - 1.91]
Hypothyroidism	1.10 [0.61 - 1.98]
Substance or Alcohol abuse	1.10 [0.61 - 1.99]
Overweight or Obese	1.11 [0.62 - 2.00]
Hypertension	1.07 [0.60 - 1.94]
Health plan (commercial vs. all non-commercial)	0.94 [0.52 - 1.71]
All variables with p<0.1*	0.90 [0.49 - 1.65]

*Regression model includes type of health plan (commercial vs. all non-commercial), Social Problem, Gestational Diabetes, Hypothyroidism, Substance/Alcohol abuse, and Overweight/Obese as covariates for the NIPT-IPD association

Figure 1: Longitudinal trends in the number of high-risk pregnancies with advanced maternal age (age ≥ 35 years), IPD, and NIPT

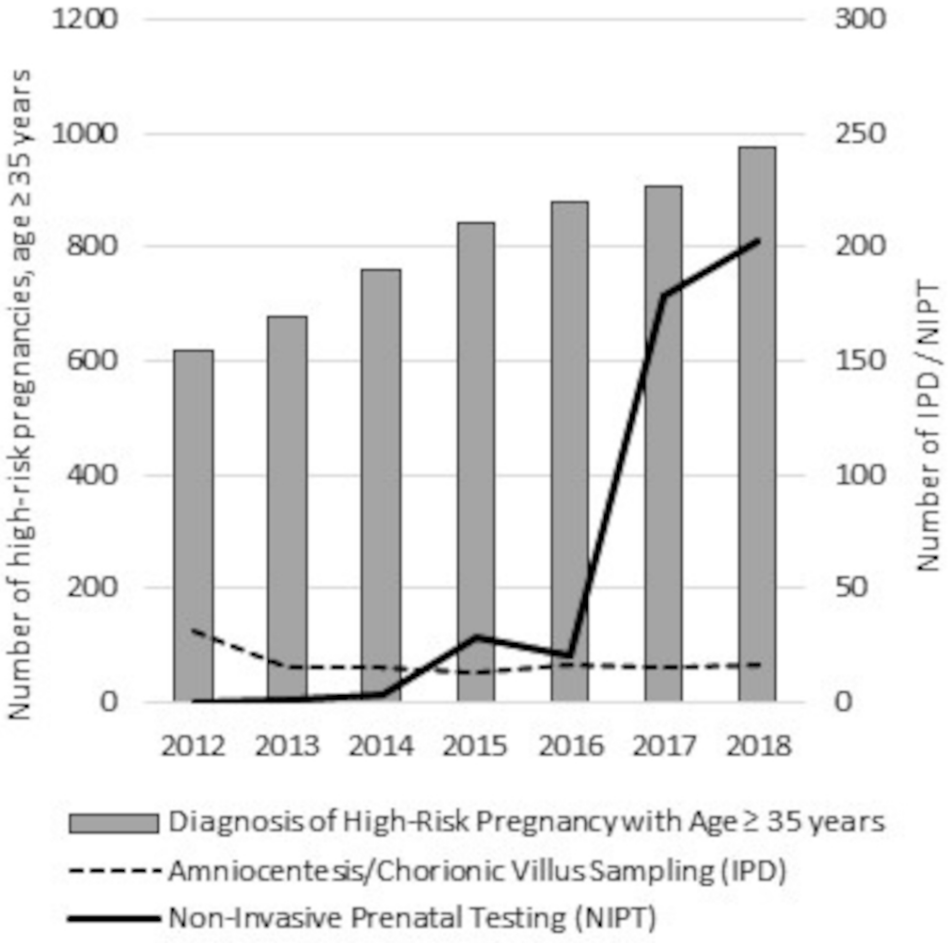


Figure 1: Longitudinal trends in the number of high-risk pregnancies with advanced maternal age (age ≥ 35 years), IPD, and NIPT

90x90mm (300 x 300 DPI)

The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract Page and Page 6	RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 5		
Objectives	3	State specific objectives, including any prespecified hypotheses	Page6		
Methods					
Study Design	4	Present key elements of study design early in the paper	Page 7		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Pages 5 and 6		

Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case	Pages 7 and 8	RECORD 6.1: The methods of study population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	Pages 10 and 11	RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Pages 10 and 11
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	Pages 8 and 9		

Bias	9	Describe any efforts to address potential sources of bias	Page 10		
Study size	10	Explain how the study size was arrived at	Not applicable		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Page 9		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses	Page 9		
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population.	12.1: Pages 6 and 7 12.2: Pages 7 and 8

				RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	Page 7
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram	Pages 10 and 11	RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)	Page 11		
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure	Page 11		

		category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures			
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 11 and 12		
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Not applicable		
Discussion					
Key results	18	Summarise key results with reference to study objectives	Page 12		
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	Page 14	RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	
Interpretation	20	Give a cautious overall interpretation of results considering objectives,	Page 14		

		limitations, multiplicity of analyses, results from similar studies, and other relevant evidence			
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 14		
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	Not applicable		
Accessibility of protocol, raw data, and programming code		..	Page 15	RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	Page 15

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.