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## Impact of maternal autoimmune disease on cell-free DNA test characteristics

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### Abstract

**Background**—Maternal biologic factors can affect fetal fraction in cell-free DNA-based prenatal screening assays, thereby limiting effectiveness. Higher rates of indeterminate results from low fetal fraction have been described in cases of autoimmune disease in pregnancy. Existing studies are confounded by the concomitant maternal use of anticoagulants, which may independently influence test characteristics.

**Objective(s)**—To evaluate differences in fetal fraction, indeterminate results, and total cell-free DNA concentration for women with autoimmune disease compared to controls using our in-house developed non-invasive prenatal screening platform in the absence of maternal anticoagulation use.

**Study Design**—A retrospective, single institution cohort study of a previously validated cell-free DNA-based non-invasive prenatal screening assay using a low-pass whole genome sequencing platform between 2017 and 2019. A diagnosis of autoimmune disease included systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and others. Immunomodulator therapies included biologics, corticosteroids, hydroxychloroquine, azathioprine, and intravenous immunoglobulin. Women on anticoagulation were excluded. We evaluated the association between autoimmune disease and fetal fraction, indeterminate results, and total cell-free DNA concentration using univariate and multivariate analyses, stratifying for

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#### DISCLOSURE STATEMENT

The authors report no conflict of interest.

#### PAPER PRESENTATION

Preliminary findings of this study were presented as a poster presentation (#644) at the Society for Maternal Fetal Medicine's 41<sup>st</sup> Annual Pregnancy Virtual Meeting; January 25–30, 2021.

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immunomodulator therapy and adjusting for body mass index, fetal sex, and gestational age at sample collection.

**Results**—1,445 patients met inclusion criteria. Forty-three women had a confirmed autoimmune disease, with 25 not on immunomodulator therapy and 18 on immunomodulator therapy. The mean fetal fraction for women with autoimmune disease was significantly lower compared to controls (9.7% vs. 11.9%,  $p=0.004$ ). The rate of indeterminate results was significantly higher for women with autoimmune disease compared to controls (16.3% vs. 3.5%;  $p<0.001$ ). The total cell-free DNA concentration was not statistically different between groups (94.8 pg/uL for autoimmune disease vs. 83.9 pg/uL for controls,  $p=0.06$ ). In logistic regression, women with autoimmune disease had a significantly higher odds of an indeterminate result compared to controls, (aOR 5.3, 95%CI 2.0, 14.2). Linear regression showed a significant negative association between autoimmune disease and fetal fraction ( $a\beta -2.1$ , 95%CI  $-3.4, -0.6$ ).

Stratifying by treatment status, mean fetal fraction was 9.8%, 9.6%, and 11.9% for women with autoimmune disease not on immunomodulator therapy, autoimmune disease on immunomodulator therapy, and controls, respectively ( $p=0.02$ ). The rate of indeterminate results increased in a stepwise fashion from 3.5% to 11.1% to 20.0% for controls, autoimmune disease on immunomodulator therapy, and autoimmune disease not on immunomodulator therapy, respectively ( $p<0.001$ ).

Logistic regression demonstrated higher odds of an indeterminate result for women with autoimmune disease not on immunomodulator therapy compared to controls, (aOR 7.3, 95%CI 2.3, 22.5). Autoimmune disease not on immunomodulator therapy was negatively associated with fetal fraction compared to controls ( $a\beta -2.2$ , 95%CI  $-4.2, -0.3$ ).

**Conclusion(s)**—Women with autoimmune disease have lower fetal fraction and higher rates of indeterminate results compared to women without autoimmune disease. There was no difference in total cell-free DNA concentration. Treatment of maternal autoimmune disease with immunomodulator therapy may decrease the indeterminate result rate.

## CONDENSATION

Autoimmune disease in pregnancy is associated with higher rates of indeterminate cell-free DNA results due to low fetal fraction, independent of anticoagulation use.

### Keywords

cell-free DNA concentration; fetal fraction; immunomodulator therapy; indeterminate result; non-invasive prenatal screening

## INTRODUCTION:

Before its use for reliable non-invasive prenatal screening (NIPS) for aneuploidy, cell-free DNA (cfDNA) was investigated as a biomarker for several acute and chronic disease states including sepsis, trauma, transplant rejection, cancer, and autoimmune disease (AID).<sup>1-6</sup> Cell-free DNA is present at very low levels in healthy individuals, resulting from physiologic cellular breakdown and active DNA release from living cells.<sup>7</sup> In certain pathologic conditions like AID, cfDNA levels are significantly elevated compared to

healthy controls. Although the underlying mechanisms are not completely understood, proposed etiologies include ineffective clearance of necrotic or apoptotic cellular material and formation of proinflammatory immune complexes.<sup>8</sup> Additionally, studies have shown that cfDNA levels further increase with clinical exacerbations and decrease with immunomodulator (IM) treatments targeted at improving systemic inflammation.<sup>6</sup>

Fetal cfDNA, originating largely from placental trophoblast cells during pregnancy,<sup>9</sup> is detected in maternal circulation as early as the first trimester, accounting for approximately 10–15% of the total cfDNA concentration.<sup>10–12</sup> Evaluation of cfDNA from maternal plasma has emerged as the most reliable NIPS tool for detecting fetal aneuploidy with a failure rate of 0.8–12.2%.<sup>13</sup> The most common reason for a test failure, or an indeterminate result, is a low fetal fraction (FF).<sup>14,15</sup> Obesity, anticoagulation use, maternal neoplasms, organ transplantation, and fetal aneuploidy are all proposed maternal and fetal factors that may contribute to low FF by either increasing the maternal contribution or decreasing the fetal contribution to total cfDNA.<sup>9,12,16–19</sup>

One small study and two case reports have found an association between maternal AID and higher rates of indeterminate results due to low FF.<sup>20–22</sup> However, the majority of these patients were on anticoagulation, which alone is associated with an increased rate of indeterminate results.<sup>16,17,23</sup> We sought to evaluate differences in FF, indeterminate result rates, and total cfDNA concentration in pregnant women with AID, as well as the potential effect of IM therapies, in the absence of concomitant anticoagulation use. We hypothesized that women with AID have lower FF and a higher rate of indeterminate results compared to controls, owing to an overall increase in maternal cfDNA concentration, and that treatment with IM therapies leads to less indeterminate results from low FF.

## MATERIALS AND METHODS

This is a single institution retrospective cohort study of cfDNA-based NIPS tests performed between May 2017 and December 2019. Since May 2017, the University of Washington has clinically used an internally validated NIPS assay that employs low-pass whole genome sequencing of cfDNA extracted from maternal plasma to provide a high-quality screen for autosomal and sex chromosome aneuploidies. Validation studies prior to clinical use confirmed that our test performance is in line with other commercially available platforms.

### Study Population

All patients with singleton gestations who completed first trimester NIPS through the University of Washington within the study period were eligible for inclusion. We sought to compare samples from women with and without AID. Additionally, we stratified women with AID by use of IM therapy at the time of NIPS, creating four study groups: all AID, AID on IM therapy, AID not on IM therapy, and controls without AID. For all groups we excluded women on anticoagulation, all NIPS results consistent with suspected aneuploidy, those with a missing FF, and those taking IM medications for non-autoimmune conditions (Figure 1). Patients on low-dose aspirin were included in the analysis.

Detailed maternal demographic, medical, and obstetric data was abstracted from the medical record. AIDs included systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and other AID (immune thrombocytopenic purpura, Sjogren's syndrome, Ankylosing spondylitis, Psoriatic arthritis, Guttate psoriasis, Behcet's disease, and AID not otherwise specified). IM therapies included biologics, corticosteroids, hydroxychloroquine, azathioprine, and intravenous immunoglobulin (IVIG). Given the in-house nature of our platform, we have access to detailed test characteristics and medical records, allowing for review of any notable cases. Any such cases, particularly related to abnormal test results, were reconciled by authors, CML and RS. Fetal sex was confirmed via obstetric and neonatal records. Institutional Review Board approval was obtained from the University of Washington (STUDY00005540). Study data was collected and managed using REDCap electronic data capture tools hosted at the University of Washington.<sup>24,25</sup>

### Sample collection and quantification of cfDNA

Whole blood from Streck (BCT1) tubes was centrifuged and plasma was isolated as per the package insert. cfDNA was extracted from plasma using the QIAasymphony Circulating DNA Kit. Total cfDNA concentration was measured by Qubit fluorometry, a highly sensitive method for quantification of intact double-stranded DNA (pg/uL). The Agilent TapeStation workflow, an automated electrophoresis system, was used to assess the size and integrity of the DNA and functions as a critical quality control step throughout next-generation library preparation, hybridization capture, and sample pooling before sequencing.

### Sequencing and quantification of fetal fraction

Following library preparation with KAPA HyperPrep for adapter and index ligation followed by Agencourt AMPureXP purification and amplification, libraries were pooled using an equimolar strategy. Libraries were sequenced using an Illumina NextSeq 500 High Output 75 cycle kit with a 37bp paired-end read configuration. On average, each cfDNA sample was sequenced to a depth of approximately 20 million paired-end reads, corresponding to an average genome depth of 0.5X. Reads were aligned to the human reference genome (hg19) with Bowtie (version 1.1.2), and run metrics were calculated with Picard (version 1.141). The placental-derived fraction for each sample was calculated either by the percent of reads that align to the Y chromosome, or a custom bioinformatic algorithm based on the aggregate length distribution in sequencing reads for samples in which the Y chromosome was not present (i.e. female fetuses).<sup>26</sup> An indeterminate result was defined as fetal fraction <4%. Failures due to technical issues, including samples with insufficient quantities of input DNA, were purposely excluded from the indeterminate group as these were related to collection or processing errors.

### Statistical Analysis

Our primary outcome was to compare cfDNA FF among women with AID compared to controls. Secondary outcomes included differences in the rate of indeterminate results due to low FF and total cfDNA concentration among the groups. Primary and secondary outcomes were additionally analyzed after stratifying for IM use in AID subjects. Demographic and clinical characteristics for the groups were compared with  $\chi^2$  or Fisher's exact test as appropriate. Continuous variables were tested for normality and compared using the t-test

or Kruskal-Wallis test as appropriate. Multivariable logistic regression was performed to determine the odds of an indeterminate result based on the presence of AID and IM treatment status. Linear regression was performed to determine the association between FF and total cfDNA concentrations with AID and IM treatment status. Models were adjusted for fetal sex, gestational age, and BMI at the time of sample collection. All data were analyzed using STATA/IC 16.1 (Statacorp, College Station, TX) and SPSS version 26.0 (IBM Corp).

## RESULTS

A total of 1,520 women with singleton pregnancies completed cfDNA-based NIPS during the study period. Of the 1,445 women who met inclusion criteria, 3% (n=43) had a confirmed AID with 58% (n=25) not on IM therapy and 42% (n=18) on IM therapy (Figure 1). Of those on IM therapy, 13 were on single agent therapy and 5 were on more than one agent. There were higher rates of pregestational hypertension and low-dose aspirin use in women with AID on IM therapy. Other characteristics including maternal age at delivery, gravidity, body mass index (BMI), gestational age at sample collection, fetal sex, use of assisted reproductive technologies, and indication for testing were similar across groups (Table 1). The most common indication for screening was advanced maternal age (> 35 years) while other less common indications included first-line screening, ultrasound abnormalities, and abnormal serum screening results.

The mean FF for women with AID was significantly lower compared to controls (9.7% vs. 11.9%,  $p=0.004$ ) and, correspondingly, the indeterminate rate was significantly higher compared to controls (16.3% vs. 3.5%;  $p<0.001$ ) (Table 2). Although the median total cfDNA concentration was higher for women with AID compared to controls, this did not reach statistical significance (94.8 pg/uL [IQR 68.7–138.0] vs. 83.9 pg/uL [IQR 59.0–117.0];  $p=0.06$ ).

Logistic regression analyses demonstrated that for women with AID the odds of an indeterminate result was approximately 5-fold higher compared to controls (OR 5.4, 95% CI 2.3,12.7). This remained significant after controlling for the gestational age at sample collection, BMI, and fetal sex (aOR 5.3, 95% CI 2.0, 14.2). In linear regression analysis, FF was negatively associated with maternal AID, controlling for previously listed confounders ( $\beta -2.1$ , 95% CI  $-3.4$ ,  $-0.6$ ). We found no significant association between total cfDNA concentration and AID (Table 3).

After stratifying women with AID based on IM treatment status, we found that the mean FF remained significantly lower for women with AID compared to controls (9.8% AID not on IM, 9.6% AID on IM, and 11.9% controls,  $p=0.02$ ) (Table 4, Figure 2).

Correspondingly, there was a stepwise increase in the rate of indeterminate results from 3.5% to 11.1% to 20.0% for controls, women with AID on IM therapy and women with AID not on IM therapy, respectively ( $p=0.001$ ) (Figure 3). Of those with indeterminate results, 36 in the control group and 4 in the AID group not on IM therapy underwent repeat testing with 31% and 75% having a second indeterminate result, respectively. The 2 women in the AID group on IM with indeterminate results did not have repeat testing. Although there

was no significant difference in the total cfDNA concentration between groups, there was a trend towards increasing total cfDNA concentration from a median of 83.9 pg/uL (IQR 59.0–117.0) to 94.8 pg/uL (IQR 63.1–163.0) to 96.4 pg/uL (IQR 73.5–134.0) for controls, women with AID on IM therapy, and women with AID not on IM therapy, respectively ( $p=0.08$ ) (Figure 4).

Logistic regression analyses demonstrated that women with AID not on IM therapy had an approximately 7-fold higher odds of an indeterminate result compared to controls (OR 6.90, 95% CI 2.5–19.1). This remained significant in the adjusted analysis (aOR 7.3, 95% CI 2.3–22.5;  $p=0.001$ ). For women with AID on IM therapy, there was no significant difference in the odds of an indeterminate result compared to controls in both unadjusted and adjusted analyses (OR 3.5, 95% CI 0.8, 15.4 and aOR 2.4, 95% CI 0.3, 19.4). In linear regression analysis, FF was negatively associated with AID not on IM therapy, controlling for the aforementioned confounders ( $a\beta -2.2$ , 95% CI  $-4.2, -0.3$ ). For women with AID on IM therapy, there was no significant association with FF compared to controls ( $a\beta -1.8$ , 95% CI  $-4.1, 0.5$ ). Additionally, we found no significant association between total cfDNA concentration and AID among the stratified groups (Table 5).

## COMMENT

### Principal Findings

We found that women with AID had a significantly lower mean FF on cfDNA-based NIPS compared to controls, with correspondingly higher indeterminate result rates, without differences in total cfDNA concentration. These findings were obtained in the absence of concomitant anticoagulation. The indeterminate result rate progressively increased from the lowest rate in controls, to women with AID on IM therapy, followed by women with AID not on IM therapy. Stratified regression analysis demonstrated that maternal AID not on IM therapy negatively correlated with FF and that these women had a 7-fold increased odds of an indeterminate result compared to controls. Treatment of AID with IM therapy tempered these effects, as this group was statistically similar to controls. We found no statistical difference in total cfDNA concentration between groups, however, there may be a trend towards higher concentrations in women with AID with or without IM therapies.

### Results in the context of what is known

Our findings add to a growing body of literature suggesting the complex role maternal medical conditions, specifically AID, may play in cfDNA-based NIPS testing. To our knowledge, no prior studies have specifically assessed the effect of maternal AID on such tests in the absence of anticoagulation. This is important as anticoagulation is independently associated with low FF and higher test failure.<sup>16,17,23</sup> We elected to include patients on aspirin, as this has not been demonstrated to influence cfDNA prenatal screening test characteristics.<sup>27</sup> Additionally, we utilized our robust medical records to clinically confirm AID, followed by stratification based on confirmation of IM use at the time of sample collection. As such, our data demonstrate that not only does the presence of maternal AID influence test characteristics, but so does IM treatment of these diseases.

## Clinical Implications

Given its prolific use, understanding biologic factors influencing cfDNA-based NIPS is important for providers counseling patients and triaging failed tests for women with underlying conditions like AID. The initial clinical concern in the setting of an indeterminate result is the risk of fetal aneuploidy.<sup>28</sup> However as mounting evidence reveals additional factors that increase the risk of a failed test from low FF, providers should be thoughtful when considering next steps of management given the additional cost, anxiety and potential diagnostic delay that could result from repeated indeterminate results. More information is needed to develop appropriate clinical algorithms for approaching failed tests.

We found differences in test results for all women with AID regardless of IM treatment status. Although heterogeneity of AID diagnoses in our cohort precludes precise assessment of individual disease severity, it is reasonable to posit that IM therapy was indicated in cases with more severe disease. Interestingly, our data suggest that differences in cfDNA test characteristics are more pronounced in women with AID not on IM therapy, who presumably have mild or quiescent disease. Thus, it seems that the presence of AID alone, regardless of its apparent clinical severity, may impact cfDNA test characteristics. The precise interaction between underlying AID, IM therapy and disease severity is unknown at this time. Despite the assumption that IM treatment may indicate increased underlying AID severity, this group was statistically similar to controls in stratified analysis. Although the regression analyses were non-significant for AID on IM compared to controls, the absolute value of indeterminate result rates fell between that of controls and AID not on IM therapy. Future studies with a larger sample sizes may provide additional power to further evaluate the potential modulating effect of IM therapy on cfDNA FF and indeterminate results for women with AID.

## Research Implications

Limited data is currently available regarding the potential mechanisms contributing to altered cfDNA test characteristics in AID. Dabi *et al.* recently investigated the relationship between heparin treatment and cfDNA-based NIPS results and found that the presence of maternal AID was associated with an increased rate of an indeterminate result independent of anticoagulation use.<sup>20</sup> Although this is consistent with our findings, all patients in this smaller cohort had concurrent heparin use, as such, this association was determined based on inclusion of AID as a confounder in regression analyses. Two additional case reports have described patients with AID and multiple failed cfDNA assays, although results in one case are confounded by anticoagulation use.<sup>21</sup> In the second, a result was obtained on cfDNA-based NIPS on the third attempt, coinciding with the initiation of IM therapies.<sup>22</sup> Although this may suggest a treatment-related effect, the known serial increase in FF with advancing gestation may have also played an important role.

Studies in both non-pregnant and pregnant women suggest an increased amount of circulating total cfDNA in the setting of various AIDs.<sup>29–35</sup> This has led others to posit that this observed low FF results from excess maternal contribution to the total cfDNA concentration in the setting of systemic inflammation or impaired clearance mechanisms. Interestingly, we found no significant difference in total cfDNA concentration among

women with AID compared to controls; however, a trend towards increasing cfDNA concentration in AID suggests possible limitations of our small sample size. Although the FF was not different in stratified analysis between women with AID on IM and women with AID on no IM in our cohort, the indeterminate rate was significantly lower for women with AID on IM therapy. This suggests that IM therapies may improve assay performance via currently unknown mechanisms. Comparisons across currently available studies are limited by differences in clinical cohorts, test methodology, and confounders, such as concomitant anticoagulation use.

### Strengths and Limitations

Our study has multiple strengths. To specifically delineate relationships between AID and NIPS test characteristics, we carefully selected our clinical cohort to exclude all subjects on anticoagulation at the time of sample collection. Additionally, we utilized a single validated platform in current clinical use with a uniform FF cutoff for interpretation (<4%), removing any issues related to test performance of differing platforms. Due to the in-house nature of our testing platform, we have access to detailed laboratory and clinical data, allowing us to reconcile all cases with an indeterminate result to identify the underlying reason, such that all indeterminate results are truly due to a low FF and not other potential errors along the testing pipeline. Access to our robust and detailed medical records allowed us to clinically confirm every AID case and the presence of IM use at the time of sample collection, in turn allowing us to investigate these two clinical scenarios (AID on IM therapy and those not on IM therapy) separately. Importantly we did not exclude pregnancies based on fetal sex and included fetal sex as a biologic variable in all regression analyses, in line with contemporary research guidelines.<sup>36,37</sup>

Our study is limited by its retrospective design and small sample size of women with AID. Although we present novel findings, our study does not provide mechanisms to explain how AID or IM therapy affect cfDNA-based NIPS test characteristics. Finally, although we adjusted for known confounders, our results may be explained by additional unmeasured factors not gleaned from the medical records.

### Conclusions

Maternal AID was associated with lower FF, higher indeterminate result rates and no difference in total cfDNA concentration compared to controls. Women with AID not on IM therapy had an increased odds of an indeterminate result due to low FF, while those on IM treatment did not, suggesting a mitigating role of IM therapy. We did not find significant differences in total cfDNA concentration between the groups, suggesting contribution of additional factors aside from dilution from maternal sources. Future studies directed at identifying biologic mechanisms that influence indeterminate cfDNA results in the setting of maternal AID and the effects of various IM therapies on NIPS test characteristics are needed.

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**AJOG AT A GLANCE****A: Why was this study conducted?**

- Interpreting cell-free DNA (cfDNA) results in the context of biologic factors is important when counseling and managing indeterminate results.
- The impact of maternal autoimmune disease, independent of anticoagulation use, on cfDNA screening is poorly understood.

**B: What are the key findings?**

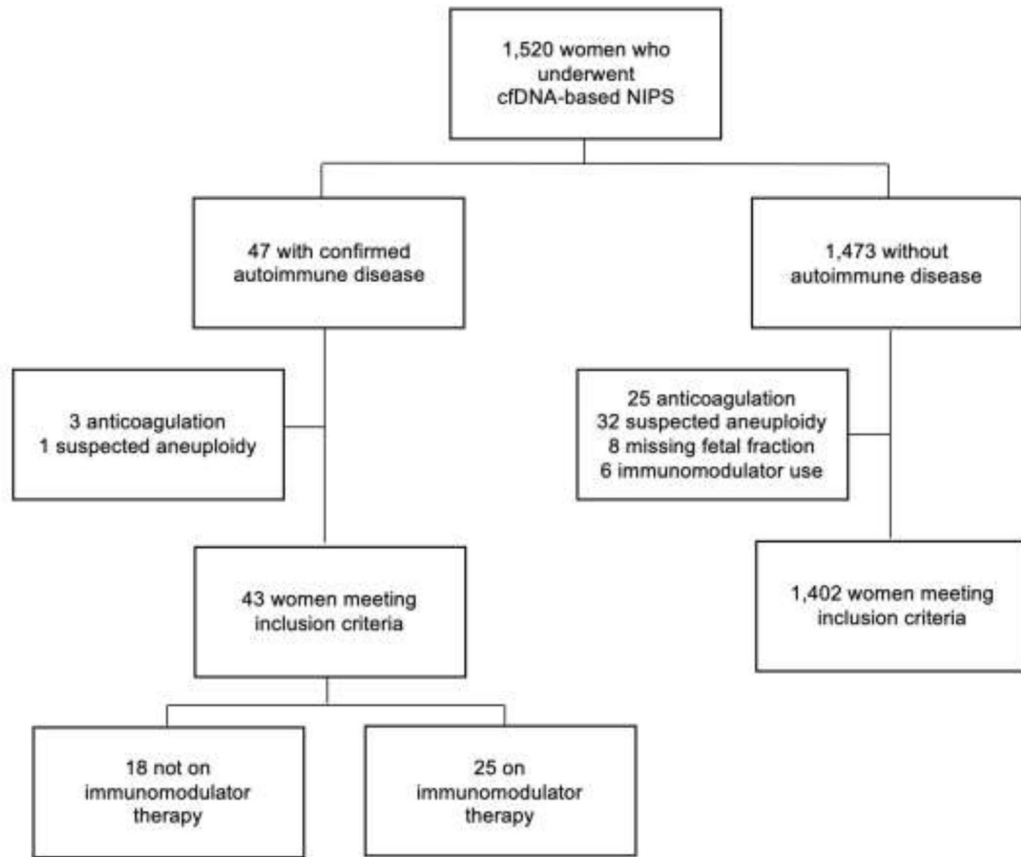
- Fetal fraction was lower in autoimmune disease versus controls.
- Indeterminate result rates were higher in autoimmune disease, regardless of immunomodulator use, versus controls.
- Total cfDNA concentration was not different in autoimmune disease versus controls.

**C: What does this study add to what is already known?**

- In the absence of anticoagulation, maternal autoimmune disease alone contributes to lower fetal fraction and higher indeterminate result rates.
- Low fetal fraction in the presence of autoimmune disease may not be solely explained by an excess of maternally-derived cell-free DNA.
- Use of immunomodulatory therapy in maternal autoimmune disease may lower indeterminate result rates.

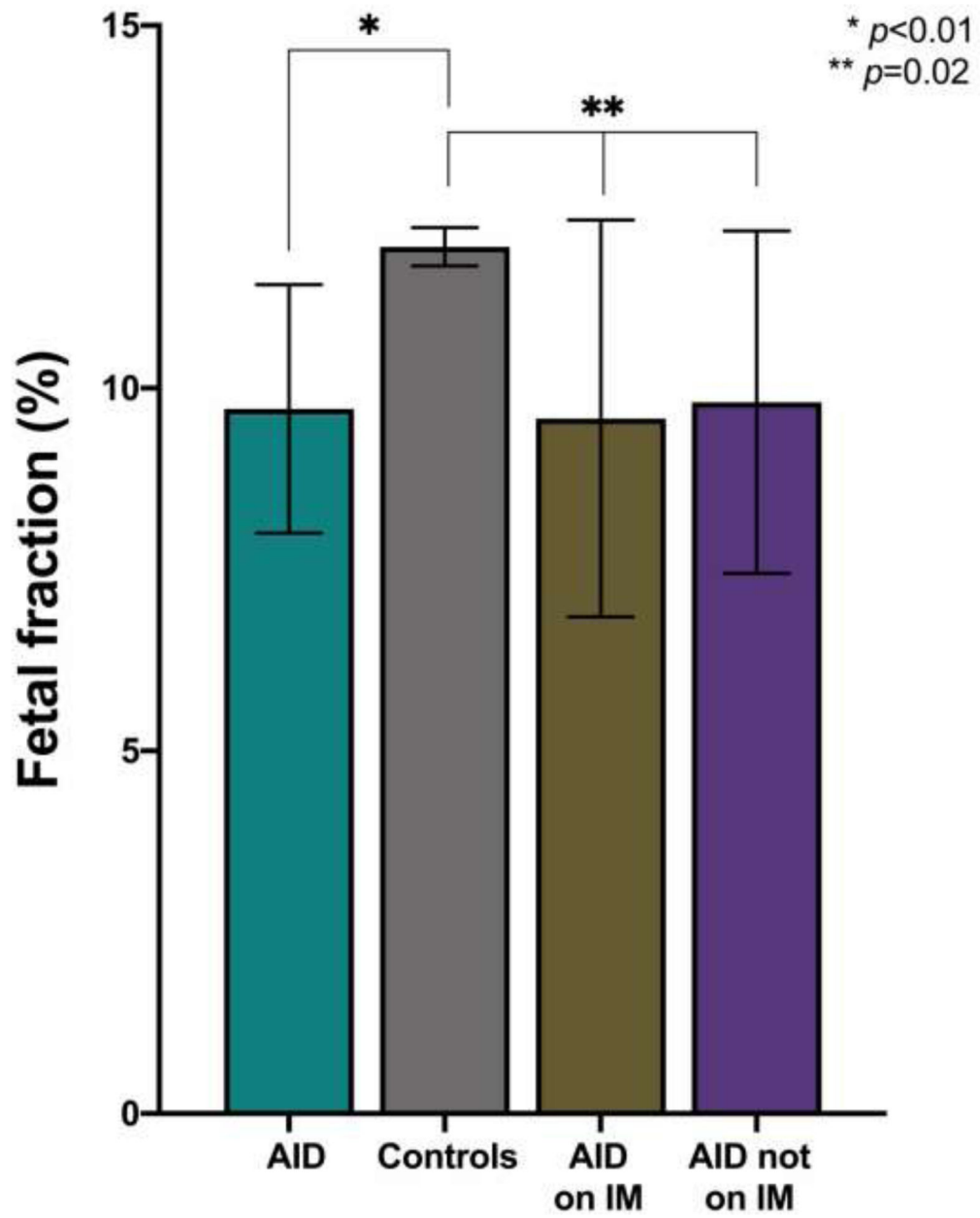
**HIGHLIGHTS**

- Maternal autoimmune disease contributes to low cell-free DNA fetal fraction and higher rates of indeterminate results in non-invasive prenatal screening.
- Immunomodulator therapy in maternal autoimmune disease may mitigate the increased odds of indeterminate cell-free DNA prenatal screening results.
- Total cell-free DNA concentration is not significantly different in pregnant women with and without autoimmune disease.

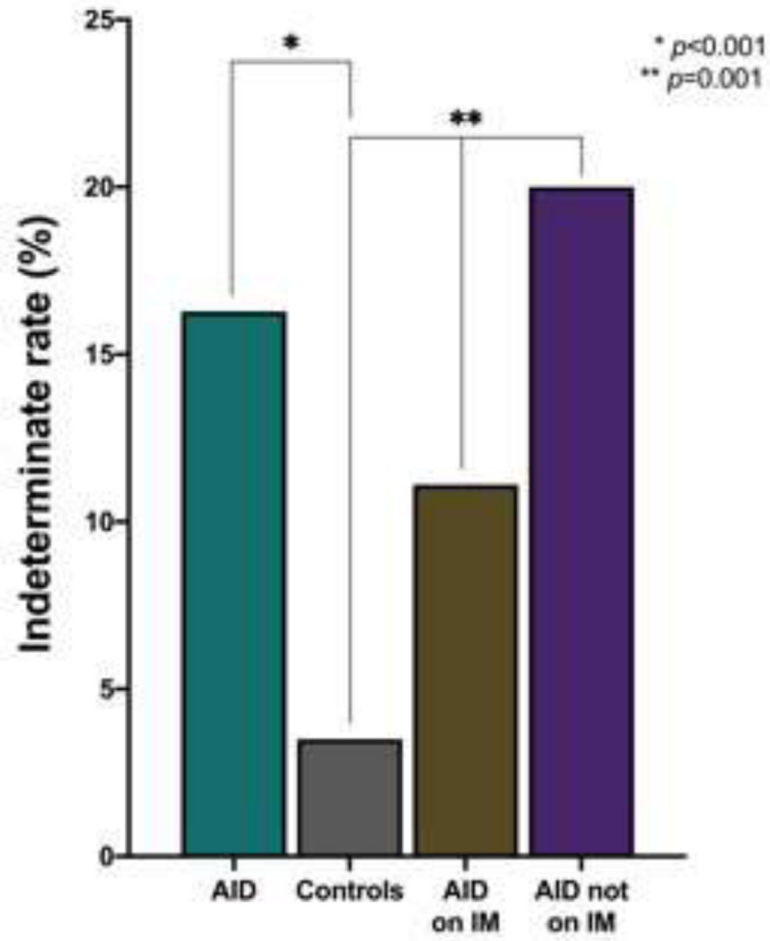


**Figure 1: Flowchart of study population**

Flow chart of the study population. cfDNA, cell-free DNA; NIPS, non-invasive prenatal screening.



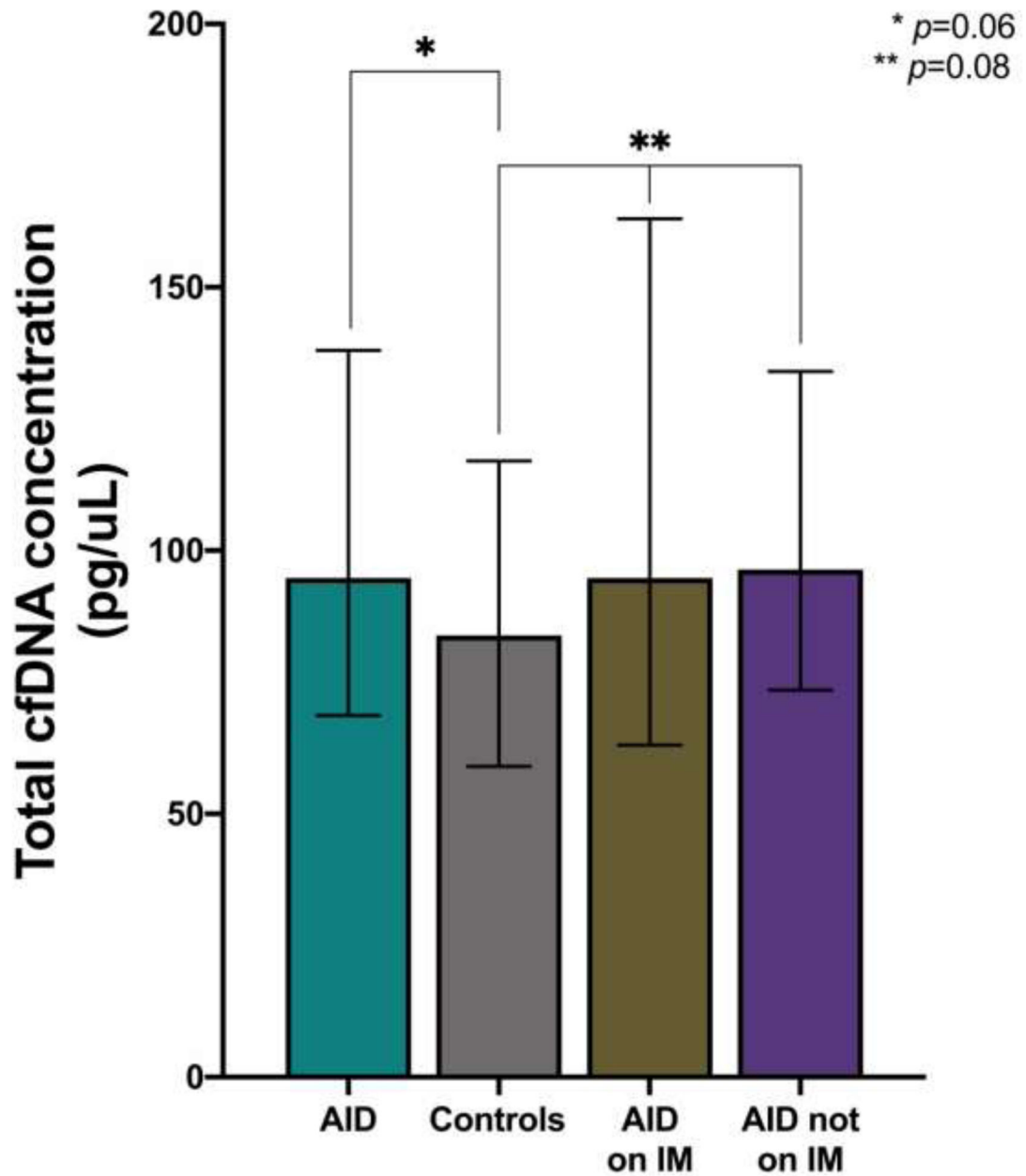
**Figure 2: Fetal fraction in maternal autoimmune disease**  
 Mean fetal fraction, demonstrating comparisons between controls and study groups. AID, autoimmune disease; IM, immunomodulator.



**Figure 3: Indeterminate result rate in maternal autoimmune disease**

Indeterminate result rates, demonstrating comparisons between controls and study groups. AID, autoimmune disease; IM, immunomodulator.





**Figure 4: Total cfDNA concentration in maternal autoimmune disease**  
Total cfDNA, demonstrating comparisons between controls and study groups. AID, autoimmune disease; IM, immunomodulator.

**Table 1:**

## Patient Demographics

	AID (-) Immunomodulator Therapy (n=25)	AID (+) Immunomodulator Therapy (n=18)	No AID (n=1402)	p-value
Age (years)	36.9 ± 4.5	34.7 ± 3.7	34.7 ± 5.0	0.1
BMI (kg/m <sup>2</sup> ) at sample collection	27.9 ± 8.0	26.8 ± 4.8	27.3 ± 6.6	0.8
Gravidity	3 (1–5)	3 (1–4)	2 (1–4)	0.2
Pregestational hypertension	2 (8.0)	3 (16.7)	86 (6.1)	0.03
ART pregnancy	3 (12.0)	1 (5.6)	92 (6.6)	0.41
Gestational age at sample collection	13.6 ± 3.9	14.0 ± 3.3	14.2 ± 4.4	0.8
Female fetal sex	13 (52.0)	8 (44.4)	537 (38.3)	0.9
Aspirin use	4 (16.0)	4 (22.2)	53 (3.8)	<0.001
<b>Indication for testing</b>				0.8
Advanced maternal age	19	12	833	
First-line screen	3	4	303	
Ultrasound abnormality	1	1	163	
Abnormal serum screen	2	1	78	
Other	0	0	16	
<b>Type of AID</b>				
Systemic lupus erythematosus	3 (12.0)	5 (27.8)	0 (0)	
Rheumatoid arthritis	5 (20.0)	1 (5.6)	0 (0)	
Multiple sclerosis	3 (12.0)	0 (0)	0 (0)	
Inflammatory bowel disease	4 (16.0)	7 (38.9)	0 (0)	
Other *	11 (44.0)	5 (27.8)	0 (0)	

Data presented as mean ± SD, N (%), or median (interquartile range) AID, autoimmune disease; BMI, body mass index; ART, assisted reproductive technologies

\* Includes immune thrombocytopenic purpura, Sjogren's syndrome, Ankylosing spondylitis, Psoriatic arthritis, Guttate psoriasis, Behcet's disease, autoimmune disease not otherwise specified

**Table 2:**

Cell-free DNA test characteristics by maternal disease status

	AID (n=43)	No AID (n=1402)	p-value
<b>Fetal fraction (%)</b>	9.7 ± 5.6	11.9 ± 5.0	0.004
<b>Indeterminate rate</b>	7 (16.3)	49 (3.5)	<0.001
<b>Total cfDNA (pg/uL)</b>	94.8 (68.7–138.0)	83.9 (59.0–117.0)	0.06

Data are mean ± SD, N (%), or median (interquartile range)

AID, autoimmune disease; cfDNA, cell-free DNA

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**Table 3:**

Association of maternal disease status with fetal fraction, rate of indeterminate result and total cell-free DNA concentration

	AID (n=43)	No AID (n=1402)
<b>Fetal fraction % β (95% CI)</b>		
Unadjusted	-2.2 (-3.7, -0.7)	0 (Reference)
Adjusted *	-2.1 (-3.4, -0.6)	0 (Reference)
<b>Indeterminate rate OR (95% CI)</b>		
Unadjusted	5.4 (2.3, 12.7)	1.0 (Reference)
Adjusted *	5.3 (2.0–14.2)	1.0 (Reference)
<b>Total cfDNA β (95% CI)</b>		
Unadjusted	27.8 (-15.1, 70.6)	0 (Reference)
Adjusted *	24.7 (-19.2, 68.7)	0 (Reference)

AID, autoimmune disease; CI, confidence interval; cfDNA, cell-free DNA

\* Adjusted for BMI, fetal sex, and gestational age at sample collection

**Table 4:**

Cell-free DNA test characteristics by maternal autoimmune disease and immunomodulator therapy status

	<b>AID (-) Immunomodulator Therapy (n=25)</b>	<b>AID (+) Immunomodulator Therapy (n=18)</b>	<b>No AID (n=1402)</b>	<b>p-value</b>
<b>Fetal fraction (%)</b>	9.8 ± 5.7	9.6 ± 5.5	11.9 ± 5.0	0.02
<b>Indeterminate rate</b>	5 (20.0)	2 (11.1)	49 (3.5)	0.001
<b>Total cfDNA (pg/uL)</b>	96.4 (73.5–134.0)	94.8 (63.1–163.0)	83.9 (59.0–117.0)	0.08

Data are mean ±SD, N (%), median (interquartile range)

AID, autoimmune disease; cfDNA, cell-free DNA

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**Table 5:**

Association of immune modulator therapy with fetal fraction, indeterminate result rate and total cfDNA concentration

	<b>AID (-) Immunomodulator Therapy (n=25)</b>	<b>AID (+) Immunomodulator Therapy (n=18)</b>	<b>No AID (n=1402)</b>
<b>Fetal fraction % <math>\beta</math> (95% CI)</b>			
<b>Unadjusted</b>	-2.1 (-4.1, 0.2)	<b>-2.4 (-4.7, -0.02)</b>	0 (Reference)
<b>Adjusted *</b>	<b>-2.2 (-4.2, -0.3)</b>	-1.8 (-4.1, 0.5)	0 (Reference)
<b>Indeterminate rate OR (95% CI)</b>			
<b>Unadjusted</b>	<b>6.9 (2.5, 19.1)</b>	3.5 (0.8, 15.4)	1.0 (Reference)
<b>Adjusted *</b>	<b>7.3 (2.3, 22.5)</b>	2.4 (0.3, 19.4)	1.0 (Reference)
<b>Total cfDNA <math>\beta</math> (95% CI)</b>			
<b>Unadjusted</b>	38.5 (-19.2, 92.2)	15.5 (-50.5, 81.5)	0 (Reference)
<b>Adjusted *</b>	-44.0 (-12.6, 100.7)	-3.5 (-71.8, 64.8)	0 (Reference)

AID, autoimmune disease; CI, confidence interval; cfDNA, cell-free DNA

\* Adjusted for BMI, fetal sex, and gestational age at sample collection