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PREGNANCY

ORIGINAL RESEARCH

High frequency of critical and rising titers in alloimmunized pregnancies with antigen-negative fetuses

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Abstract

Introduction: In this study we performed a retrospective chart review of alloimmunized pregnancies undergoing monitoring for hemolytic disease of the fetus and newborn (HDFN) in a cohort with known fetal antigen (FA) status. The objective was to compare fetal monitoring with titers and middle cerebral artery peak systolic velocity Doppler (MCA-PSV Doppler) in FA-negative and FA-positive pregnancies and to understand downstream impacts of screening with titers in FA-negative pregnancies.

Methods: Retrospective chart review of alloimmunized pregnant patients who underwent FA cell-free DNA (cfDNA) analysis and participated in a registry in which neonatal genotyping was performed to confirm FA cfDNA results. Patients were divided into FA-positive or FA-negative cohorts based on the FA genotypes. Medical records were reviewed to characterize monitoring for anemia, titer results, MCA-PSV Doppler results, and whether interventions were performed. Fischer's exact or chi-square tests were performed as appropriate to ascertain differences between the cohorts.

Results: Sixty-nine alloimmunized pregnant individuals were included. Forty pregnancies (58%) were FA positive. Of 29 FA-positive pregnancies who had titers more than once, 20 (69%) had titers that rose over two or more time points. There were 29 FA-negative pregnancies (42%). Of 20 FA-negative pregnancies that underwent titers more than once, 10 (50%) had titers that rose over two or more time points. The FA-positive and FA-negative cohorts had similar proportions of pregnancies that reached critical titers—70% (28/40) and 69% (20/29), respectively. In the FA-positive cohort 14 of 34 participants who underwent MCA-PSV Doppler reached greater than 1.5 multiples of the median (MoM), suggestive of fetal anemia. One patient in the FA-negative cohort reached 1.5 MoM.

Conclusions: Titers frequently yield elevations that, while reflective of maternal alloimmunization, are not indicative of positive FA status or risk for HDFN in

antigen-negative pregnancies. Such results lead to unnecessary monitoring, including MCA-PSV Doppler as well as invasive interventions. These outcomes can be avoided using methods including FA cfDNA, which has been shown to be highly accurate. These results suggest that monitoring for fetal anemia should be discontinued if FA cfDNA has determined the FA status is negative, as this will reduce downstream burdens and risks to alloimmunized patients who are not at risk for HDFN.

KEYWORDS
alloimmunization, antibody titers, cell-free DNA (cfDNA), hemolytic disease of the fetus and newborn (HDFN), noninvasive prenatal testing (NIPT)

1 | INTRODUCTION

Hemolytic disease of the fetus and newborn (HDFN) is a potentially life-threatening form of anemia caused by alloimmunization, a sensitization to red blood cell antigens impacting approximately 1.5% of pregnancies in the United States [1]. A fetus is at risk for HDFN only if it is antigen positive for the antigen to which the pregnant patient is alloimmunized. Current guidelines from the American College of Obstetrics and Gynecologists (ACOG) recommend ascertaining fetal risk by determining paternal antigen genotype and/or amniocentesis if paternal testing is not possible or inconclusive [2]. The fetal antigen (FA) status can then be used to guide management, including proceeding with monitoring for fetal anemia only when the fetus is antigen positive [2]. In September 2022, the first assay became clinically available in the United States for determining FA status utilizing cell-free DNA (cfDNA) for the following antigens: Kell, Fy^a [also known as Fy(a+)], big C, little c, big E, and D (RhD) [3]. In August 2024, ACOG issued a clinical practice update noting that when amniocentesis is declined, FA cfDNA may be considered, specifically stating that "it is reasonable to use it [FA NIPT] as an alternative tool for fetal RHD testing among alloimmunized patients with potentially at-risk pregnancies who decline amniocentesis," and "Cell-free DNA for the assessment of selected non–Rh-D red blood cell antigens may be considered for pregnant patients declining amniocentesis, after weighing cost, access, and the encouraging-yet-limited data supporting its use" [4]. This guidance is in contrast with consensus guidelines as well as guidance and practice in many European countries, which rely on the use of cfDNA isolated from maternal plasma to determine the FA genotype [5–7].

If a fetus is identified as being at risk for HDFN or if the risk is uncertain, ACOG advises the use of serial antibody titers to guide management, with the following exceptions: (1) the pregnant patient is alloimmunized to the Kell antigen or (2) the pregnant patient has had a fetus or neonate previously affected with HDFN, stating that "Serial titers are not useful for monitoring fetal status when the mother has had a previously affected fetus or neonate" and "Kell antibodies do not correlate with fetal status" [2]. Of note, the management of alloimmunized pregnant patients—particularly those alloimmunized to Kell—is variable, and many clinicians do employ serial titers for Kell but utilize a lower threshold of 4 for a critical titer (for most antigens, a threshold of 16 is commonly used) [8]. If the patient's titers reach critical levels, then middle cerebral artery peak systolic velocity Doppler (MCA-PSV Doppler) is performed to screen for fetal anemia. MCA-PSV Doppler is also used to screen for fetal anemia for patients alloimmunized to Kell or with prior pregnancies affected with HDFN when their fetus is either antigen positive or the FA status is unknown [2]. Some providers initiate screening for fetal anemia with MCA-PSV Doppler prior to reaching a critical titer, for example, in the setting of rising titers.

Prior to the availability of FA cfDNA testing in the United States, the only option for alloimmunized pregnant patients whose fetus's risk for HDFN could not be clarified by paternal testing was amniocentesis. If they declined invasive testing, they underwent time-consuming and costly monitoring via serial titers and/or MCA-PSV Doppler. For approximately 50% of pregnant patients, this monitoring is unnecessary, as they carry an antigen-negative fetus, which is therefore not at risk for HDFN [9]. However, alloimmunized patients carrying antigen-negative fetuses can reach critical titer levels, despite not being at risk, though the reasons for this phenomenon are not well-described in the medical literature [10]. Additionally, MCA-PSV Doppler has a known false-positive rate of 12% and can lead to unnecessary invasive testing such as percutaneous umbilical cord sampling (PUBS) and intrauterine transfusion (IUT) [11, 12]. False-positive results from monitoring can also lead to iatrogenic late preterm and early term induction of labor

due to concerns for fetal anemia, which increases risk for neonatal complications as well as causing unnecessary stress for prospective parents [13].

For US-based patients alloimmunized to antigens for which FA cfDNA analysis is clinically available, this testing, available as early as 9 weeks' gestational age (GA), presents an opportunity to avoid burdensome and costly monitoring and unnecessary invasive procedures for FA-negative pregnancies. Prior studies have demonstrated 100% accuracy of a next-generation sequencing-based FA cfDNA assay utilizing quantitative counting template technology by determining concordance between clinical FA cfDNA results and postnatal genotyping of the neonate resulting from the alloimmunized pregnancy. These studies included 186 alloimmunized pregnancies for which a total of 558 FA calls were made [3, 9]. This study expands on that previous work utilizing chart reviews of 69 pregnancies from the same registry cohort, all of which had neonatal genotyping that was 100% concordant with FA cfDNA results.

The key objective of this study is to compare fetal monitoring with titers and MCA-PSV Doppler in both the FA-negative and FA-positive pregnancies, and to gain a better understanding of the downstream impact of screening with titers in known FA-negative pregnancies. Our aim is for these data to be used to inform decisions regarding the risks and benefits of continuing or discontinuing screening for fetal anemia in the setting of a fetus predicted to be antigen negative with FA cfDNA testing.

2 | MATERIALS AND METHODS

2.1 | Participants

Participants in this study are part of an IRB-approved patient registry for individuals who underwent clinical FA cfDNA analysis due to an alloimmunized pregnancy. As part of the registry, all participants had their FA cfDNA results confirmed via neonatal genotyping (the patient registry also includes the neonates resulting from the alloimmunized pregnancies). Providers guided patient care and neither the registry nor the observational retrospective chart review entailed any alterations or interventions in clinical care. All participants and/or guardians provided written informed consent to participate in the registry. Details of the inclusion criteria for the registry have been previously published and include that the patient was alloimmunized to at least one of the following antigens for which clinical FA cfDNA analysis is clinically available in the United States: Kell, Fy^a, big C, little c, big E, or D. In the prior study, we demonstrated complete concordance between FA cfDNA results and neonatal antigen genotyping from an independent laboratory for all but one participant who had inconclusive neonatal genotyping results (that participant was excluded from the current study) [9].

The inclusion criteria for this study required that registry participants have obstetrical medical records available from the pregnancy in which FA cfDNA testing was performed via a healthcare interoperability network. The records had to meet the following criteria: (1) include a minimum of four obstetrical records from different time points within the pregnancy and (2) at least one of those records must be from within 5 weeks prior to the estimated date of delivery (or within 1 week of delivery if the patient delivered prior to 35 weeks' GA). The requirement to have at least one record from within 5 weeks prior to the estimated date of delivery was chosen to be consistent with consensus guidance that states IUTs are not recommended past 35 weeks' GA. Therefore, we expect to capture monitoring and/or interventions performed in participants who did not have records available beyond the 35th week of pregnancy, as it is unlikely that any interventions or newly initiated monitoring will take place beyond 35 weeks' GA.

2.2 | Retrospective chart review

Retrospective chart reviews were performed for the pregnancy for which FA cfDNA testing was ordered. Data abstracted during chart review included demographic characteristics, the antigens to which the pregnant patient was alloimmunized, information on monitoring for fetal anemia, and whether any interventions such as PUBS or IUTs were performed. Data regarding patient referral patterns were not included in the analysis; however, observationally, the most common pattern entailed a patient receiving a type and screen from an obstetrician and then being referred to a maternal-fetal medicine specialist following a positive antibody screen. A team of three researchers performed the chart reviews, including two genetic counselors and a trained research associate. All pregnancies underwent two rounds of chart review by two separate reviewers. Reviewers met weekly to discuss and resolve discrepancies.

2.3 | Data analysis

Registry participants were divided into two cohorts: the FA-negative cohort and the FA-positive cohort. Participants alloimmunized to more than one antigen on the FA cfDNA panel were included in the FA-positive cohort if at least one antigen was positive in the fetus.

Data points compared between the two cohorts included the number and percentage of patients who underwent

TABLE 1 Participants' FA status and gestational age.

Fetal antigen (FA) status[a]	Participants (*n*)	Participants (%)	
FA positive	40	58	
FA negative	29	42	
Gestational age (GA) at time of cfDNA	**Antigen-positive cohort**	**Antigen-negative cohort**	**All participants**
Median GA (weeks)	16.4	16.6	16.4
Range of GA (weeks)	10.1–34.7	10.1–33.0	10.1–34.7

Abbreviation: cfDNA, cell–free DNA.

[a]Participants alloimmunized to more than one antigen were classified as FA positive if the fetus was antigen positive for at least one antigen included in the assay. They were classified as FA negative only if the fetus was negative for all alloimmunized antigens. All FA statuses were confirmed with neonatal antigen genotyping in a prior study [9].

titers and MCA-PSV Doppler, the proportion of each cohort who had titers rise over two or more time points, as well as the proportion for whom titers reached the critical threshold (defined as 4 for those alloimmunized to Kell or little c, and 16 for those alloimmunized to the remaining antigens based on recommendations by Moise & Abels published in 2024) [8]. Finally, we determined the number of participants in each cohort who underwent invasive interventions such as amniocentesis, PUBS, or IUT. Summary statistics were calculated. Either a chi-squared test or a Fischer's exact test was performed as appropriate to determine if differences in observations between the two cohorts were statistically significant (https://www.socscistatistics.com, www.medcalc.org).

3 | RESULTS

3.1 | Demographics

The patient registry for alloimmunized individuals who underwent clinical FA cfDNA testing included 72 patients who had sufficient medical records available for review. Of these, 69 were included in the study, and three were excluded due to inconclusive or unavailable neonatal or cfDNA antigen genotyping results or alloimmunization to an antigen for which FA cfDNA was not available (Figure S1).

All pregnancies were singletons. The estimated due dates for the pregnancies included in this study ranged from December 2022 to April 2024. FA cfDNA testing was performed between October 2022 and November 2023. Of the 69 individuals included in the analysis, 40 (58%) were part of the FA-positive cohort. Twenty-nine (42%) were antigen negative and were therefore not at risk of HDFN. FA cfDNA results were confirmed postnatally with neonatal genotyping for all pregnancies and were 100% concordant. The mean GA at the time of FA cfDNA analysis was 16.4 weeks (Table 1).

TABLE 2 Alloimmunized antigens by participant.

Alloimmunized antigens	Antigen-positive cohort (*n*)	Antigen-negative cohort (*n*)	All participants (*n*)
big E	9	11	20
Kell	1	14	15
little c	6	1	7
big C, D	6	1	7
D	7	0	7
Fy^a	3	0	3
big C	2	0	2
big E, D	0	1	1
little c, big E	2	0	2
Fy^a, Kell	1	0	1
Fy^a, Kell, big C	1	0	1
big C, D, G	1	0	1
big C, D, Jka, G	1	0	1
Fy^a, big E	0	1	1
Total	40	29	69

The most common antigen to which participants were alloimmunized was big E ($n = 24$, 36.2%), followed by Kell and D ($n = 17$ for each, 24.6%), big C ($n = 12$, 17.4%), little c ($n = 9$, 13.0%), and Fy^a ($n = 6$, 8.7%). Fifteen participants were alloimmunized to more than one antigen (Tables 2 and 3). Two participants were alloimmunized to antigens not included on the FA cfDNA assay, however, they were also alloimmunized to antigens that are included in the assay and tested positive, and so they were included in the study in the FA-positive cohort.

3.2 | Fetal monitoring

All participants underwent titers at least once, usually as a reflex to their initial antibody screen. Of the

TABLE 3 Proportion of participants alloimmunized to each antigen.

Alloimmunized antigens	*N*	%
big E	24	34.8
Kell	17	24.6
D	17	24.6
big C	12	17.4
little c	9	13.0
Fy[a]	6	8.7
G	2	2.8
Jka	1	1.4

Note: A total of 15 participants were alloimmunized to multiple antigens, and as such the percentages alloimmunized to each antigen will add up to more than 100%.

29 participants with FA-negative pregnancies, 20 (69%) had serial titers (more than one titer result), compared with 29 (73%) in the FA-positive cohort. In the FA-negative cohort, 18 participants (62%) underwent MCA-PSV Doppler, compared with 34 (85%) in the FA-positive cohort.

Twenty of 29 (69%) participants in the FA-negative cohort reached a critical titer, and importantly, 18 of those reached critical titer prior to receiving their FA-negative cfDNA results. Of the 40 participants in the FA-positive cohort, 28 (70%) reached a critical titer, 26 prior to receiving FA-positive cfDNA results. There was not a statistically significant association between FA status and reaching critical titers ($X^2[1, N = 69] = 0.009$, $p = 0.927$). One participant from the FA-negative cohort who reached critical titer underwent amniocentesis despite receiving negative FA cfDNA results in order to confirm fetus's antigen status (it was confirmed negative). In the FA-negative cohort, there were 20 individuals who underwent titers at least twice during their pregnancy, and of those, 10 (50%) had titers rise over two or more time points. In the FA-positive cohort, there were 29 individuals who underwent titers at least twice during their pregnancy, and of those, 20 (69%) had titers rise over two or more time points during their pregnancy (Figure 1, Table S1). There was not a statistically significant association between FA status and rising titers ($X^2[1, N = 49] = 1.79$, $p = 0.181$).

In the FA-positive cohort, 14 of the 34 participants (41%) who underwent MCA-PSV Doppler reached greater than or equal to 1.5 multiples of the median (MoM), suggestive of fetal anemia. Ten of those underwent PUBS and/or IUTs, and the remaining four did not, either because the patient was close to delivery or the clinician decided to repeat the MCA-PSV Doppler prior to intervening and the subsequent result was below the threshold. Of the 18 participants in the FA-negative cohort who underwent monitoring with MCA-PSV Doppler, one (5.6%) received a false-positive result (1.68 MoM), suggestive of fetal anemia, at 29 weeks 2 days' GA. The participant was alloimmunized to Fy[a] and big E and the fetus was antigen negative for both. That participant had a subsequent MCA-PSV result below 1.5 MoM and no interventions were performed. There was a statistically significant positive association between positive FA status and reaching the 1.5 MoM threshold concerning for fetal anemia (Fisher's exact test, $p = 0.009$).

4 | DISCUSSION

In this retrospective chart review, we analyzed the results of monitoring done for fetal anemia in alloimmunized pregnant patients in the United States who underwent FA cfDNA testing between 2022 (when FA cfDNA first became available in the United States) and 2023. All participants underwent titers at least once, and the same proportion of participants with FA-negative pregnancies (which were therefore not at risk of HDFN) reached critical titer levels as the proportion of the FA-positive cohort (69% and 70%, respectively). Of the pregnancies in which serial titers were performed, a similar proportion of FA-negative (50%) and FA-positive (69%) pregnancies rose over two or more time points. These findings indicate that titer increases or critical thresholds may occur frequently, even when the fetus is antigen negative and not at risk for HDFN. This raises questions about the cost–benefit balance of continuing monitoring after an FA-negative cfDNA result.

The primary reason to consider continuing monitoring a pregnancy with negative FA cfDNA testing is concern that a false-negative result could lead to undetected fetal anemia. However, extensive evidence from both Europe and the United States has demonstrated the accuracy of FA cfDNA, suggesting minimal benefits and potential harms to such ongoing monitoring [3, 5, 6, 9]. These harms include the financial and emotional toll on the patient, downstream risks if titers are rising or at critical levels, false-positive MCA-PSV Doppler results leading to unnecessary interventions, and the cost to the healthcare system. Indeed, we see evidence in this study of such unnecessary and burdensome monitoring and interventions in the 29 patients who had FA-negative pregnancies, including 20 who underwent titers more than once (20/29, 69%), one who underwent amniocentesis due to rising titers (1/29, 3.4%), and 18 who underwent MCA-PSV Doppler (18/29, 62%), including one with a false-positive MCA-PSV Doppler (1/18, 5.6%).

Current recommendations state that titers should be performed monthly through 24 weeks' GA in at-risk

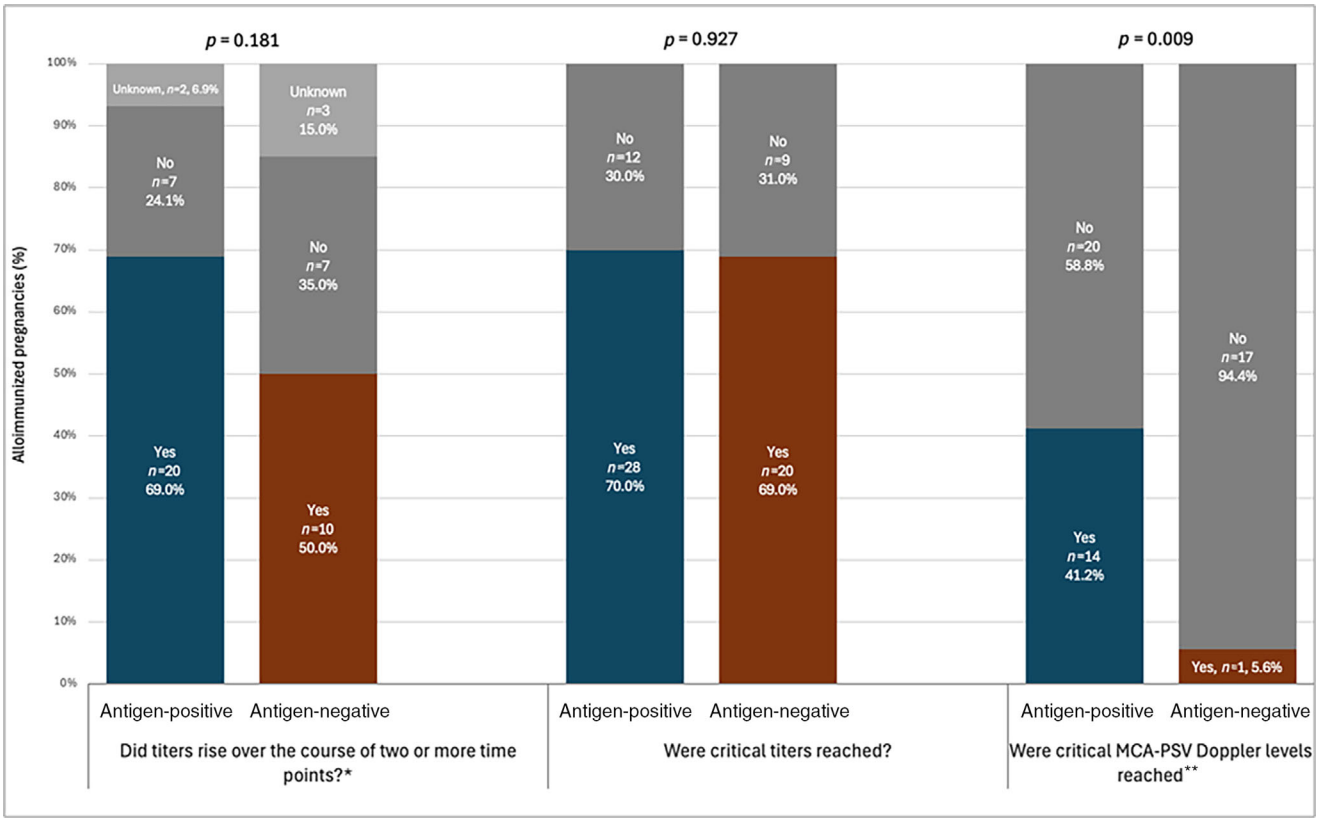

FIGURE 1 Rising and critical titers and MCA-PSV Doppler by cohort. *Includes only patients who underwent serial titers. **Includes only patients who underwent MCA-PSV Doppler. MCA-PSV, middle cerebral artery peak systolic velocity.

pregnancies that have not yet reached critical titer levels, and then every 2 weeks after 24 weeks, requiring frequent blood draws for patients [8]. In addition, recommendations state that titers should be performed at the same laboratory, as variability in laboratory practices can confound results—this too puts additional burden on patients who have less flexibility in where their blood is drawn [8]. However, even this approach does not ensure consistency—a recent study evaluating the accuracy and reliability of titers found a striking lack of consistency in results in both interlaboratory *and* intralaboratory evaluations [14]. These inconsistencies can lead to false and concerning results suggestive of risk for anemia even in a fetus that is antigen negative.

For those patients who proceed to MCA-PSV Doppler, monitoring is even more burdensome, as consensus guidelines state that MCA-PSV Doppler should be performed approximately weekly starting as early as 16 weeks and continuing through delivery [15]. Patients undergoing this monitoring may need to travel long distances to a maternal-fetal medicine subspecialist, and/or bear the cost of childcare and/or time off work [16]. A recent study addressing the nonmedical burdens of receiving care at a fetal care center at a major medical center found that more than half of participants traveled over 100 miles for care, and 38% reported a moderate-to-severe financial burden associated with receiving that care [17]. These costs extend beyond patients and also impact the healthcare system broadly. An economic analysis by Gajic-Veljanoski et al. suggests improved clinical outcomes for alloimmunized patients who undergo FA cfDNA testing as opposed to usual care, and also estimated a cost savings of $7903 per alloimmunized pregnancy when FA cfDNA is used to guide management [18].

In addition to logistical and financial burdens, unnecessary monitoring in FA-negative alloimmunized pregnancies can pose additional risks by leading to unnecessary invasive testing and/or interventions such as amniocentesis, PUBS, or IUTs. In this study, one participant in the FA-negative cohort reached critical titers and proceeded with an amniocentesis, which confirmed her negative FA cfDNA results. This invasive testing poses a risk for pregnancy loss as well as sensitization to additional antibodies [19, 20]. Another participant in the FA-negative cohort had a false-positive MCA-PSV Doppler result, which reflects the known 12% false-positive rate of MCA-PSV Doppler, and which can lead to unnecessary invasive interventions such as PUBS and IUT [11, 12].

While the rates of both serial titers and MCA-PSV Doppler usage were decreased in the FA-negative cohort

compared to the FA-positive cohort, titers were still utilized extensively across both cohorts. Of note, in the majority of FA-negative cases where critical titers were reached, the first critical titer occurred prior to the FA-negative cfDNA result, suggesting that providers may have been utilizing FA cfDNA to guide next steps after reaching critical titers. This hypothesis is bolstered by the lower utilization of MCA-PSV Doppler in the FA-negative group, despite this group having similar percentages of critical titers as the FA-positive cohort. This suggests that FA cfDNA results were already being used for the clinical decision-making in alloimmunized pregnancies, despite this analysis taking place on pregnancies that delivered prior to the recent changes in ACOG's guidance, which included cfDNA as an option for determining FA status [4]. Given these recent changes, the results shown in this publication may drive FA cfDNA results to be incorporated even more fully into clinical decision-making. In such a model, FA cfDNA testing could be performed immediately following a positive maternal antibody screen, as early as 9 weeks' GA, removing the need for serial titers for alloimmunized pregnancies that are FA negative and therefore not at risk of HDFN.

This study has limitations. The registry only included patients alloimmunized to the clinically significant antigens for which FA cfDNA testing was available. In cases where FA cfDNA is not available, the traditional approach of paternal testing and/or amniocentesis or proceeding straight to monitoring remains the best option for identifying fetuses at risk for HDFN. In addition, the pregnancies included in this analysis took place prior to the recent change in ACOG guidance, which now includes FA cfDNA as an option for determining FA status when paternal testing is unavailable or inconclusive and amniocentesis is declined, and therefore may not reflect the latest approach to management for all providers [4]. The landscape of FA cfDNA testing is evolving rapidly, and as it becomes more integrated into clinical practice, unnecessary monitoring for fetal anemia in FA-negative pregnancies should decrease over time.

5 | CONCLUSION

In conclusion, this study provides evidence that titers incorrectly suggest pregnancies are at risk of HDFN, leading to unnecessary downstream monitoring of alloimmunized pregnancies with additional titers and/or MCA-PSV Doppler. Consequences of this unnecessary monitoring may include the financial and emotional burden to the patient, the cost to the healthcare system, and the potential risks of the patient undergoing unnecessary invasive procedures such as amniocentesis, PUBS, or IUT. This evidence validates the utility of the recent movement in guidance toward the use of cfDNA for determining FA status, particularly for RhD, but also for non-D antigens, for which ACOG noted cfDNA "may be considered" when amniocentesis is declined [4]. It also suggests value in additional changes to guidance to recommend the discontinuation of fetal monitoring for anemia after the FA status has been determined to be negative in order to reduce the burden to patients and the healthcare system and to prevent negative downstream implications.

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CONFLICT OF INTEREST STATEMENT

Shannon Rego, Julia Wynn, and Rachel Levan are employees of BillionToOne, Inc. and have options/equity in BillionToOne, Inc. Olaide Ashimi Balogun, Kirsten Emanuel, and Rachael Overcash received research funding from BillionToOne, Inc. Shannon Rego received National Society of Genetic Counselor National Meeting Speaker compensation. The other authors did not report any potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Anonymized individual participant data will be available. The data will include structured data from the medical records such as alloimmunized antigens, fetal/neonatal antigen genotype, and relevant details of monitoring from the alloimmunized pregnancy, including titer values and MCA-PSV Doppler results. Data will be available at the time of publication and for 5 years after. Access can be requested by contacting the author, and sharing will be determined by the author and the clinical laboratory where the study was conducted. Data will be shared in a secure electronic format for replication purposes.

ETHICS STATEMENT

This research study was approved by WCG-IRB (WCG IRB protocol No. 20225380).

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REFERENCES

1. Sugrue, R. P., K. J. Moise, J. J. Federspiel, E. Abels, J. Z. Louie, Z. Chen, L. Bare, D. P. Alagia, and H. W. Kaufman. 2024. "Maternal Red Blood Cell Alloimmunization Prevalence in the United States." *Blood Advances* 8(16): 4311–9. https://doi.org/10.1182/bloodadvances.2023012241.
2. Holmgren, C. and T. Flint Porter. 2018. "ACOG Practice Bulletin No. 192: Management of Alloimmunization During Pregnancy." *Obstetrics and Gynecology* 131(3): e82–e90. https://doi.org/10.1097/AOG.0000000000002528.
3. Alford, B., B. P. Landry, S. Hou, X. Bower, A. M. Bueno, D. Chen, B. Husic, et al. 2023. "Validation of a Non-Invasive Prenatal Test for Fetal RhD, C, c, E, K and Fy^a Antigens." *Scientific Reports* 13(1): 12786. https://doi.org/10.1038/s41598-023-39283-3.
4. The American College of Obstetricians and Gynecologists. 2024. "Paternal and Fetal Genotyping in the Management of Alloimmunization in Pregnancy." *Obstetrics & Gynecology* 144(2): e47–e9. https://doi.org/10.1097/AOG.0000000000005630.
5. Scheffer, P. G., C. E. van der Schoot, G. Page-Christiaens, and M. de Haas. 2011. "Noninvasive Fetal Blood Group Genotyping of Rhesus D, c, E and of K in Alloimmunised Pregnant Women: Evaluation of a 7-Year Clinical Experience." *BJOG* 118(11): 1340–8. https://doi.org/10.1111/j.1471-0528.2011.03028.x.
6. Royal College of Obstetricians and Gynaecologists. 2014. "Green-Top Guideline No. 65: The Management of Women With Red Cell Antibodies During Pregnancy." *Obstetrics & Gynecology* 16(3): 224. https://doi.org/10.1111/tog.12125.
7. Gutensohn, K., S. Müller, K. Thomann, W. Stein, A. Suren, S. Körtge-Jung, G. Schlüter, and T. Legler. 2010. "Diagnostic Accuracy of Noninvasive Polymerase Chain Reaction Testing for the Determination of Fetal Rhesus C, c and E Status in Early Pregnancy." *BJOG* 117(6): 722–9. https://doi.org/10.1111/j.1471-0528.2010.02518.x.
8. Moise, K. J. Jr, and E. A Abels. 2024. "Management of Red Cell Alloimmunization in Pregnancy." *Obstetrics and Gynecology* 144(4): 465–80. https://doi.org/10.1097/AOG.0000000000005709.
9. Rego, S., O. Ashimi Balogun, K. Emanuel, R. Overcash, J. M. Gonzalez, G. A. Denomme, J. Hoskovec, et al. 2024. "Cell-Free DNA Analysis for the Determination of Fetal Red Blood Cell Antigen Genotype in Individuals With Alloimmunized Pregnancies." *Obstetrics and Gynecology* 144(4): 436–43. https://doi.org/10.1097/AOG.0000000000005692.
10. Moise, K. J. Jr. 2024. "D Alloimmunization in Pregnancy: Management." *UpToDate*. Last updated March 25, 2025. https://www.uptodate.com/contents/d-alloimmunization-in-pregnancy-management?source=mostViewed_widget.
11. Mari, G., R. L. Deter, R. L. Carpenter, F. Rahman, R. Zimmerman, K. J. Moise, K. F. Dorman, et al. 2000. "Noninvasive Diagnosis by Doppler Ultrasonography of Fetal Anemia due to Maternal Red-Cell Alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses." *New England Journal of Medicine* 342(1): 9–14. https://doi.org/10.1056/NEJM200001063420102.
12. Abels, E., B. D. Adkins, K. Cedeno, G. S. Booth, E. S. Allen, L. D. Stephens, J. S. Woo, C. A. Tormey, and J. W. Jacobs. 2024. "Assessing Recommendations for Determining Fetal Risk in Alloimmunized Pregnancies in the United States: Is It Time to Update a Decades-Old Practice?." *Transfusion Medicine Reviews*. 38(2): 150810. https://doi.org/10.1016/j.tmrv.2023.150810.
13. Spong, C. Y., B. M. Mercer, M. D'Alton, S. Kilpatrick, S. Blackwell, and G. Saade. 2011. "Timing of Indicated Late-Preterm and Early-Term Birth." *Obstetrics and Gynecology* 118(2 Pt 1): 323–33. https://doi.org/10.1097/AOG.0b013e3182255999.
14. Karafin, M. S., R. A. DeSimone, J. Dvorak, R. A. Metcalf, M. B. Pagano, Y. A. Park, J. Schwartz, et al. 2023. "Antibody Titers in Transfusion Medicine: A Critical Reevaluation of Testing Accuracy, Reliability, and Clinical Use." *Archives of Pathology & Laboratory Medicine*. 147(12): 1351–9. https://doi.org/10.5858/arpa.2022-0248-CP.
15. Mustafa, H. J., E. V. Sambatur, A. A. Shamshirsaz, S. Johnson, K. J. Moise, A. A. Baschat, E. J. T. Joanne Verweij, et al. 2024. "Monitoring and Management of Hemolytic Disease of the Fetus and Newborn Based on an International Expert Delphi Consensus." *American Journal of Obstetrics and Gynecology* 232(3): 280–300. https://doi.org/10.1016/j.ajog.2024.11.003.
16. van der Schoot C., D. Winkelhorst, and F. B. Clausen. 2018. *Noninvasive Fetal Blood Group Typing*. Edited by H. G. K. Lieve Page-Christiaens. Academic Press. Accessed December 14, 2024. https://www.sciencedirect.com/science/article/abs/pii/B9780128141892000086?via=ihub.
17. Gregory, E. J., A. H. Abbasi, and K. B. Markham. 2024. "Patient Survey Regarding Non-Medical Burdens of Care at a Parental Fetal Care Center." *Journal of Patient Experience* 11: 23743735241231693. https://doi.org/10.1177/23743735241231693.
18. Gajic-Veljanoski, O., C. Li, A. K. Schaink, J. Guo, N. Shehata, G. S. Charames, B. de Vrijer, et al. 2022. "Cost-Effectiveness of Noninvasive Fetal RhD Blood Group Genotyping in Nonalloimmunized and Alloimmunized Pregnancies." *Transfusion* 62(5): 1089–102. https://doi.org/10.1111/trf.16826.
19. Murray, J. C., L. E. Karp, R. A. Williamson, E. Y. Cheng, and D. A. Luthy. 1983. "Rh Isoimmunization Related to Amniocentesis." *American Journal of Medical Genetics* 16(4): 527–34. https://doi.org/10.1002/ajmg.1320160411.
20. Bowman, J. M. and J. M. Pollock. 1985. "Transplacental Fetal Hemorrhage After Amniocentesis." *Obstetrics and Gynecology* 66(6): 749–54. https://www.ncbi.nlm.nih.gov/pubmed/3934608.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.