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Maternal red blood cell alloimmunization prevalence in the United States

Tracking no: ADV-2023-012241R1

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Abstract:
Hemolytic disease of fetus and newborn (HDFN) is a life-threatening disease mediated by maternal alloimmunization to red blood cell (RBC) antigens. Studies of maternal alloimmunization prevalence in the United States (U.S.) lack national data. This study describes prevalence and trends in alloimmunization in pregnancy in the U.S. RBC antibodies (abs) were identified in a large, nationwide, commercial laboratory database from 2010-2021. The cohort comprised pregnancies for which the year of lab collection and patient's state of residence were available. Data were normalized based on U.S. Centers for Disease Control and Prevention estimates of live births and weighted by year and U.S. Census Division. Cochrane-Armitage tests assessed temporal trends of alloimmunization. Of 9,876,196 pregnancies, 1.5% (147,262) screened positive for RBC abs, corresponding to an estimated prevalence of 1,518/100,000 pregnancies. Of identified RBC abs, anti-D comprised 64.1% (586/100,000 pregnancies). Prevalence of other high-risk RBC abs for HDFN included anti-K (68/100,000) and anti-c (29/100,000). Incidence of all three high-risk abs increased from 2010-21 (all $p<0.001$). Among almost 10 million pregnancies in the US, comprising an estimated 14.4% of all pregnancies, 1.5% screened positive for RBC abs. Almost three-quarters (74.3%; 683/100,000) of RBC abs identified were high-risk for HDFN. Though prevalence of anti-D is difficult to interpret without the ability to distinguish alloimmunization from passive immunity, it remains problematic in HDFN, ranking second only to anti-K in critical titers. Given the sequelae of HDFN, new initiatives are required to reduce the incidence of alloimmunization in patients of reproductive potential.

Conflict of interest: COI declared - see note

COI notes: • Louie, Chen, Bare, Alagia, and Kaufman are employees of Quest Diagnostics. • Chen, Bare, Alagia and Kaufman own Quest Diagnostics stock. • Moise is a consultant and investigator for Jannsen Pharmaceuticals, Inc. • Sugrue and Federspiel have no competing financial interests.

Preprint server: No;

Author contributions and disclosures: • The study and statistical methods designs were developed by Sugrue, Moise, Federspiel, Abels, Louie, Chen, Bare, Alagia, and Kaufman. • Louie and Chen performed the primary data analytics. • Sugrue, Moise, Federspiel, Abels, Louie, Chen, Bare, Alagia, and Kaufman participated in data review. • Sugrue authored the initial draft. Moise, Federspiel, Abels, Louie, Chen, Bare, Alagia, and Kaufman edited and reviewed drafts and approved the submission .

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Emails to corresponding author

Clinical trial registration information (if any):

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Funding: Dr. Federspiel was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health under award K12HD103083. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

Data Sharing Statement: For original data or search protocol please contact corresponding author as above.

Text Word Count: 3,289

Abstract Word Count: 245

Key points character count (with spaces): 263

Number of Figures and Tables: 5

Number of References: 63

Key words: alloimmunization, hemolytic disease of the fetus or newborn, prevalence, red blood cells

KEY POINTS

- In this first national sample of red blood cell antibodies (RBC abs) in pregnancies in The United States, 1.5% screened positive for RBC abs
- The prevalence of RBC abs increased from 2010 to 2021. RBC abs high-risk for HDFN were identified in 74.3% of pregnancies

ABSTRACT

Hemolytic disease of fetus and newborn (HDFN) is a life-threatening disease mediated by maternal alloimmunization to red blood cell (RBC) antigens. Studies of maternal alloimmunization prevalence in the United States (U.S.) lack national data. This study describes prevalence and trends in alloimmunization in pregnancy in the U.S. RBC antibodies (abs) were identified in a large, nationwide, commercial laboratory database from 2010-2021. The cohort comprised pregnancies for which the year of lab collection and patient's state of residence were available. Data were normalized based on U.S. Centers for Disease Control and Prevention estimates of live births and weighted by year and U.S. Census Division. Cochrane-Armitage tests assessed temporal trends of alloimmunization. Of 9,876,196 pregnancies, 1.5% (147,262) screened positive for RBC abs, corresponding to an estimated prevalence of 1,518/100,000 pregnancies. Of identified RBC abs, anti-D comprised 64.1% (586/100,000 pregnancies). Prevalence of other high-risk RBC abs for HDFN included anti-K (68/100,000) and anti-c (29/100,000). Incidence of all three high-risk abs increased from 2010-21 (all $p<0.001$). Among almost 10 million pregnancies in the US, comprising an estimated 14.4% of all pregnancies, 1.5% screened positive for RBC abs. Almost three-quarters (74.3%; 683/100,000) of RBC abs identified were high-risk for HDFN. Though prevalence of anti-D is difficult to interpret without the ability to distinguish alloimmunization from passive immunity, it remains problematic in HDFN, ranking second only to anti-K in critical titers. Given the sequelae of HDFN, new initiatives are required to reduce the incidence of alloimmunization in patients of reproductive potential.

INTRODUCTION

Maternal red blood cell (RBC) alloimmunization typically occurs in response to either blood transfusion or exposure to fetal RBC antigens during pregnancy.(1) Hemolytic disease of the fetus and newborn (HDFN) remains a significant public health concern with an estimated incidence of 1.6 per 1000 live births.(2) Rhesus (Rh) alloimmunization has been known to be a primary risk factor for HDFN since the 1940s.(3) Subsequently, maternal abs to more than 50 non-ABO blood group antigens have been implicated with variable incidence and severity of HDFN.(4) Most clinically significant cases result from exposure to antigens in the Rhesus, Kell, Duffy, Kidd, and MNS families.(5, 6) Over the past 60 years, routine administration of antenatal and postnatal anti-D immune-prophylaxis has dramatically reduced anti-D-associated HDFN in the United States (U.S.)(7, 8) Other maternal abs are now emerging as significant contributors to HDFN, and there is no equivalent prophylactic strategy available for non-Rh(D) antigens.(9) National prevalence of RBC abs has been estimated in several countries, ranging from 0.3-3.4% of pregnant individuals.(10, 11, 12, 13, 14, 15, 16, 17, 18) In the U.S., however, prevalence estimates of RBC abs in pregnancy have been confined to single-center reports, with limited applicability to a national population.(19, 20)

In this study, we report prevalence and temporal trends in red cell alloimmunization in pregnancy in a large, national cohort in the U.S..

METHODS

Prenatal RBC abs screening test results during the years 2010-2021 were extracted from the database of a large commercial laboratory (Quest Diagnostics) that serves half of all prescribing physicians and hospitals in the U.S. Data was collected from maternal blood samples sent for an initial obstetric panel; this panel is routinely drawn at prenatal care intake and includes a Type and Screen. Test results where patient's age and state of residence were available were included. Primary payor was reported when available. Data were standardized and maintained at a central repository by data management staff at Quest Diagnostics.

RBC abs screening was performed using patient sera on the Capture-R Ready-Screen® (Immucor; Norcross, GA), an automated solid-phase technology utilizing RBC fragments from three donors pre-coated onto microtiter wells.(21) Per manufacturer's specifications, low-ionic-strength-saline were used as a potentiating agent and indicator RBCs coated with anti-human IgG were utilized for reaction visualization. Screening results were recorded in binary form as positive or negative for RBC antibodies. Antibody identification was performed utilizing the same technology, but with 11-cell panels (Capture-R Ready-ID®, Immucor; Norcross, GA). Antibody titers were determined using traditional hemagglutination techniques, performed in tube without potentiating agents, and recorded in whole numbers as the reciprocal of the lowest dilution at which hemagglutination could be observed macroscopically.(22, 23) All testing took place at laboratory sites owned and managed by Quest Diagnostics. Reagents, testing platforms, and standard operating procedures were consistent across sites. Quality assurance was overseen by the quality control department at Quest Diagnostics. RBC abs were categorized as high, moderate, or low risk for HDFN based primarily on the 2015 Swedish national guideline, as there is no equivalent national guideline in the U.S. (Table 1).(24)

The primary study outcome was an estimated nationwide prevalence of RBC alloimmunization. A secondary study outcome was estimated prevalence of critical RBC abs titers for all high- and moderate-risk RBC abs, defined as $\geq$4 for K antibody and $\geq$16 for all other RBC abs.(25, 26, 27) To improve the national representativeness of our estimates, we obtained birth data from the Centers for Disease Control and Prevention Wide-Ranging Online Data for Epidemiologic Research (CDC WONDER) U.S. Natality Index – a comprehensive, anonymized dataset of all certified live births in the U.S.(28, 29) The total number of pregnancies including live births, stillbirths, miscarriages and terminations were estimated using U.S. National Vital Statistics Data.(30) Quest Diagnostics data were stratified by year and U.S. Census Divisions (9 geographic regions) and calendar year (Table 2).(21) To calculate weights, we adjusted the distribution of patients across divisions to match the national birth cohort (first fraction), and for the fact that the Quest Diagnostics cohort represents a subset of the national birthing population (second fraction). This is noted in the equation below:

$$\text{Weight} = \frac{\text{Proportion of national births in division}}{\text{Proportion of Quest observations in division}} \text{x} \frac{\text{Total number of national births}}{\text{Total number of Quest observations}}$$

Estimates of prevalence were reported per 100,000 pregnancies. To estimate the prevalence of critical RBC abs titer for high- and moderate-risk RBC abs, we used a random sampling method to impute missing critical RBC ab titers for each antibody in a calendar year.

Noting the potential for weighting to be skewed by data limitations, we developed two sensitivity analyses of our weighting method. The first (Supplementary Figure 2) was undertaken to account for the possibility that twin and higher order multiples in the livebirth data may only have one record in the Quest Diagnostics dataset. Using birth certificates from the U.S. Natality index from the years 2016-2021, we excluded second and subsequent births from the same delivery (U.S. natality data from 2010-2015 did not include a variable allowing for removal of multiple observations for the same delivery). We then recalculated the weights using the same approach as outlined above. The second sensitivity analysis (Supplementary Figure 3) was performed in recognition that U.S Natality Index data only includes pregnancies which results in live birth, in contrast to Quest Diagnostics data which also includes pregnancies ending in loss or termination after completion of the prenatal panel. To assess whether this affected our weights, we used the most recently available at time of analysis (2012-2017) state-specific data regarding estimated number of total pregnancies from the Guttmacher Institute, a research and policy non-governmental organization.(31) We then compared weights for 2012-2017 observations by our live-birth-based methodology to those that would be generated using the total pregnancy methodology.

All estimates were calculated using methods accounting for weighting and stratification of the resulting dataset and reported with 95% confidence intervals. Cochrane-Armitage tests based on linear regression were used to assess temporal trends in alloimmunization; $P < 0.05$ was considered statistically significant. All statistical analyses were performed using Stata Statistical Software, Version 17 (StataCorp, College Station, TX) and R Software, Version 4.2.1 (https://www.R-project.org/). The study

protocol was reviewed by Western Copernicus Group institutional review board and deemed exempt. Requests for access to the data sets used in this study should be addressed to the senior author.

RESULTS

Of the 10.2 million pregnancies in our dataset from 2010-2021, 9.9 million met inclusion criteria. Age was recorded for almost all patients (99.99%), state of residence for 9.9 million (96.3%) and primary payor for 7.0 million (71.1%). Mean (SD) patient age was 29.5 (7.2) years with 6.8% <20 years, 74.5% 20-34 years, and 18.7% >35 years. During this period, birth certificate data confirmed 46.4 million births in the U.S., corresponding to approximately 71.4 million total pregnancies. Data describing number of unique pregnancies from 2010-2021 stratified by age, primary payor, calendar year, and all nine U.S. Census divisions (Table 2).

From January 2010 through December 2021, 147,262 of 9,876,196 (1.5%) pregnancies in our cohort had a positive antibody screen, corresponding to a national estimate of 1,518/100,000. Antibody specificity was conclusively identified in 89,249 (60.6%) of these, or 0.9% of total pregnancies while the remainder had abs screens of uncertain specificity (AUS), *e.g.*, weak abs. Of identified RBC abs in the cohort, high-risk abs accounted for 74.3%, moderate-risk abs for 13.5%, and low-risk abs in 12.3% of cases. Anti-D was the most common antibody, accounting for 64.1% of all RBC abs, corresponding to an estimated national rate of 586/100,000 pregnancies. The prevalence of other high-risk RBC abs for HDFN was 68/100,000 for anti K), and 29/100,000 for anti-c. Critical titers were observed in 10.2% of high-risk and 5.5% of moderate-risk abs; titers were missing in 5.2% of high-risk and 13.8% of moderate-risk abs. Most pregnancies (67.6%) with anti-K abs had critical titers; a small proportion of pregnancies with other high-risk RBC abs had critical antibody titers (Anti-D: 3.6%; Anti-c: 6.6%). The overall prevalence of critical titers for anti-D was estimated at 21/100,000 pregnancies (Table 3).

The prevalence of all three high-risk abs increased incrementally over the 12-year period for positive antibody detection (all P <.001; Figure 1). This was most notable for anti-D antibodies, where prevalence rose 43.0% from 481/100,000 pregnancies in 2011 to 688/100,000 in 2021. Similar rises

occurred for the other high-risk abs over the same period (anti-c 63.6%, anti-K 36.4%). The prevalence of critical titers of both anti-K and anti-c increased while that of anti-D decreased over the 12-year period (*P* <.001 for both anti-K and anti-D, *P* =.03 for anti-c; Figure 2). The most significant change was for the critical titer of anti-K with its prevalence increased 25.6% from 39/100,000 in 2011 to 49/100,000 in 2021. It is also worth noting that the prevalence of the critical titer of anti-D decreased 21.7% from 23/100,000 in 2010 to 18/100,000 in 2021.

Our first sensitivity analysis accounting for multiple births at the same delivery found that the weights estimated by our original approach were extremely well-correlated with weights based on exclusion of multiple births from 2016-2021 U.S. natality data. This suggested that our original weighting was unlikely to significantly alter our estimates of alloimmunization rates. These findings are presented in Supplementary Figure 2. In our second sensitivity analysis, weights were higher when using Guttmacher pregnancy data, since there are more pregnancies than births and thus the Quest data are being asked to represent a larger number of pregnancies. However, our weights remained strongly correlated ($R^2 = 0.97$), suggesting that using natality data was unlikely to significantly bias our estimates of the prevalence of alloimmunization. The findings are presented in Supplementary Figure 3.

DISCUSSION

In this large, nationwide study in the U.S., estimated prevalence of positive RBC antibody screen was 1518/100,000 pregnancies at the initiation of prenatal care. Of RBC abs identified, anti-D was the most common, comprising more than half (64.1%). Most RBC abs identified (74.3%) are high-risk for development of HDFN. The majority of anti-K abs (67.6%) were at critical titer at the time of early prenatal care (time of initial obstetric panel testing).

The estimated prevalence of RBC abs was consistent with that of a previously reported rate from a single center in the U.S.(19) This is higher than antibody prevalence reported in another single center U.S. study (0.74%) or other high-income countries (HIC), including Spain (0.63%), Australia (0.73%), Sweden (0.38%), Norway (0.73%), Iceland (1.04%) and Canada (0.36%).(13, 14, 15, 17, 32) Our data

suggests that prevalence of maternal alloimmunization to all antigens at high-risk for development of HDFN is rising in the U.S.. Passively acquired anti-D following Rhesus immunoglobulin (RhIg) administration may contribute, but does not explain consistent increases in the prevalence of anti-Kell and anti-c. Possible explanations may include increasing ethnographic and genetic diversity in the United States, in prenatal antibody screening, in survival from congenital hemoglobinopathies requiring blood transfusion, and in maternal age giving rise to a longer period for potential antigen exposure prior to conception.(38, 39) In addition, though the use of blood transfusion in the U.S. has been declining in recent years, use of allogenic blood transfusion has been significantly higher in women than men, even when controlling for age and comorbidities.(33, 34, 35, 40)

An unexpected result from the current study was the particularly high rate of anti-D detected, inconsistent with reports from the other U.S. based or international reports from high-income countries.(41) Given the inability to distinguish between passively acquired anti-D due to RhIg (anti-DRG) and patient produced anti-D (aka anti-D alloimmunization) with testing data alone, it is difficult to interpret the increasing prevalence of anti-D antibody observed in this study's cohort. Indeed, one possible explanation is increased use of RhIg and therefore detection of anti-DRG abs.(42) In addition, the decrease in alloimmunization rates cited by other countries could be due to variations in screening or RhIg delivery practices over time. For example, in several European countries, only Rh(D) negative patients pregnant with cell-free fetal DNA (cffDNA) proven Rh(D) positive fetuses are given RhIg, a possible cause of lower RhIg delivery resulting in less anti-DRG abs interfering with testing.(43, 44) Further, some high-income countries have national patient databases allowing for more comprehensive tracking of RhIg delivery and other patient testing data.(17, 44) This level of granularity would allow for researchers to more accurately predict and report on active versus passive anti-D rates. Possible reasons for a true increase in anti-D alloimmunization rates in the U.S. would include: A more ethnically diverse patient population with varying rates in antigen expression allowing for increased incidence of antigen exposure in antigen negative individuals, a diverse socioeconomic patient population and lack of universal access to healthcare causing variable adoption of RhIg use at the ideal times in pregnancy, or an

increase in immigrant populations from low-middle income countries (LMIC) with high rates of alloimmunization and limited access to RhIg prior to entry into the U.S.(31, 33, 39, 40, 45) Varied dose regimens of RhIg are unlikely to be a contributor, as the typical routine antenatal dosage given in Europe is the same or less than that in North America (300 μg [1500 IU] at 28-30 weeks).(46) Though the overall trend in anti-D alloimmunization is unable to be adequately characterized in this cohort, the decreasing rates of anti-D critical titer cases - which almost certainly represent real alloimmunization cases - while cases of critical titer alloimmunization in anti-c and anti-K increase would suggest that rates of anti-D alloimmunization are declining.

Anti-K is the antibody most often associated with critical titers in our study. This is an expected finding, given widespread use of K-incompatible blood in both child and adult transfusion, and the lower critical titer threshold used for anti-K abs.(47, 48) More surprisingly, despite ongoing efforts to reduce alloimmunization, the overall prevalence of high-risk RBC abs and critical titers in the U.S. continues to rise. The true burden of RBC alloimmunization in the U.S. is poorly understood and understudied. It is established, however, that newborns with HDFN are more likely to require transfusion, cesarean delivery, neonatal intensive care admission and longer length of stay than healthy and other sick newborns; neonates who are black, female and from the American South are disproportionately affected.(49) In this context, several initiatives to reduce alloimmunization in the U.S. are worthy of consideration. In the Netherlands, routine national use of K-compatible blood has been shown to reduce the incidence of K alloimmunization from 67.9 to 20.2 per 100,000 pregnancies, and the total number of pregnancies at high risk of HDFN from 9.7 to 4.2 per 100,000.(50) This finding has been replicated for anti-c and anti-E in several studies.(51, 52, 53)

Strengths of our study include our large, nationwide cohort, representing nearly 10 million of all pregnancies in the U.S. from a wide spectrum of health insurers (primary payors) from 2010-21. Given the absence of nationalized data, we believe this is by far the largest prenatal antibody cohort reported in the U.S. to date, representing a wide geographic and socioeconomic sample of the U.S. population. Our statistical weighting methods were also a strength, adjusting for potential regional and temporal variation

in the proportion of pregnant patients using Quest Diagnostics services. Our sensitivity analyses using U.S. birth certificate and state-specific pregnancy data demonstrated that, while multiple gestations and pregnancies that do not result in live birth are not distinguishable in our dataset, the accuracy of our geographical and temporal weighting is unaffected.

This study has several significant limitations. Firstly, our sample, though large, may not be representative of the U.S. population as a whole. Quest Diagnostics serves a full spectrum of both privately and publicly insured patients nationwide. The proportion of patients served by Quest Diagnostics, however, varies by geography and calendar year. Secondly, individual patient-level data on race, ethnicity, gestational age at time of initial presentation, gravidity, parity, medical comorbidities, and clinical course of pregnancy are unavailable, as these data are not made available to Quest Diagnostics at the time of client request. As a result, demographic data are limited only to patient's age, address, primary payor and calendar year in which the blood specimens were obtained. We acknowledge that lack of data on race and ethnicity in particular limits our ability to study important demographic differences in alloimmunization prevalence in the U.S., given recipient race is known to independently predictive of RBC alloimmunization.(49, 54) In this regard, we noted that U.S. Census data from 2020, demonstrates significant variation in race and ethnicity between the nine U.S. census divisions (see Supplementary Table 5).(55) For example in 2020, 78.5% of people in West North Central Division (North Dakota, South Dakota, Minnesota, Iowa, Missouri, Nebraska, Kansas) were white, versus 47% in Pacific (Washington, Oregon, California); 21.1% of people in South Atlantic (West Virginia, Delaware, Maryland, Virginia, North Carolina, South Carolina, Georgia, Florida) were Black versus 4.0% in Mountain (Montana, Idaho, Wyoming, Nevada, Utah, Colorado, Arizona, New Mexico); 14% of Pacific were Asian versus 1.6% in East South Central (Alabama, Mississippi, Tennessee, Kentucky). Thus, we believe our geographically and temporally weighted estimates by calendar year and U.S. Census Division represent the best available partial control both for varying proportions of patients served by year and geography, and for regional variation in race and ethnicity.

Finally, we note that our dataset includes all pregnancies whether culminating in live birth, miscarriage, termination of pregnancy or fetal demise. By contrast, U.S. Natality index only includes pregnancies resulting in a live birth. There is also no way to definitively determine if a second RBC Abs screening panel on the same patient in the same year reflects repeat screening later in pregnancy or initial screening in a subsequent pregnancy, though our sensitivity analyses suggest the effect on our weighting is minimal. Thirdly, despite standardization, inherent limitations of RBC abs testing and reporting error remain. For example, in our cohort, only 60.6% of positive antibody screens resulted in conclusive antibody identification. Antibody titers were missing in 5.2% of high-risk abs and 13.8%of moderate-risk abs, the majority due to the test not being performed or an insufficient quantity of patient specimen volume for titration. To that end, we employed an imputation method for missing titers based on the distribution of observed titers for each antibody by calendar year.

RBC alloimmunization increases burden of disease, maternal and fetal mortality and healthcare costs.(56) We believe the results of our study should refocus national attention towards improved prenatal antibody testing techniques and measures to prevent of RBC alloimmunization in the U.S. Several initiatives to reduce alloimmunization merit further consideration, including development of: A national blood donor database for better allocation of antigen matched blood to chronically transfused patients, routine extended prophylactic antigen matching for RhD, Rhc and Kell in females under age 50, RhD antigen genotyping for patients with serologically weak D antigens to identify possible weak D variants which would not require administration of RhIg, prenatal fetal RhD screening in RhD women to target prenatal RhIg prophylaxis, restrictions in use of low-titer group O+ whole blood in females under 50, routine transfusion of Kell negative blood in females under 50, and routine optimization of preoperative hemoglobin levels to reduce transfusion exposure (43, 52, 57, 58, 59, 60, 61) Lastly, prevention of trauma such as motor vehicle accidents and gun violence can play an important part in reducing need for transfusion.(45, 62, 63)

CONCLUSIONS

Our findings suggest that the prevalence of alloimmunization as detected by screening in pregnancy in the U.S. may be higher than in other high-income countries and has continued to rise since 2010. RBC abs conferring high-risk of development of HDFN constitute the majority of RBC abs identified. Alloimmunization to RBC abs is an acquired and largely preventable condition, that represents an ongoing challenge to transfusion, preconception, prenatal and neonatal care. Given the significant sequelae for patients requiring transfusion of blood products and the risk of developing HDFN during pregnancy, new initiatives are required to reduce alloimmunization in the U.S.

ACKNOWLEDGEMENTS

Dr. Moise serves as the overall principal investigator for the phase 2 trial of nipocalimab (UNITY); has received funding from Momenta Pharmaceuticals, Inc. paid on his behalf to the McGovern Medical School – UT Health and from Janssen Pharmaceuticals, Inc paid on his behalf to Dell Medical School at The University of Texas at Austin for a clinical trial on a monoclonal antibody for the treatment of HDFN; has served on the steering committees and advisory boards for clinical studies for Momenta Pharmaceuticals, Inc., and Janssen Pharmaceuticals but has not received funding for these activities; receives royalty funding from UpToDate, Inc. for authorship on various chapters, consulting fees from Health Management Associates, Inc. for consultation on the formation of fetal centers, consulting fees from BillionToOne, Inc. paid on his behalf to Dell Medical School at The University of Texas at Austin, honoraria from GLC Healthcare, Inc. for podcast content on HDFN; and serves as a nonpaid consultant for Immunology for Janssen Pharmaceuticals, Inc.

Dr. Federspiel was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health under award K12HD103083. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

AUTHORSHIP CONTRIBUTIONS AND CONFLICTS OF INTEREST

- The study and statistical methods designs were developed by Sugrue, Moise, Federspiel, Abels, Louie, Chen, Bare, Alagia, and Kaufman.
- Louie and Chen performed the primary data analytics.
- Sugrue, Moise, Federspiel, Abels, Louie, Chen, Bare, Alagia, and Kaufman participated in data review.
- Sugrue authored the initial draft. Moise, Federspiel, Abels, Louie, Chen, Bare, Alagia, and Kaufman edited and reviewed drafts and approved the submission.
- Louie, Chen, Bare, Alagia, and Kaufman are employees of Quest Diagnostics.

· Chen, Bare, Alagia and Kaufman own Quest Diagnostics stock.

· Moise is a consultant and investigator for Jannsen Pharmaceuticals, Inc.

· Sugrue, Federspiel, and Abels have no competing financial interests.

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Figure 1: Temporal trends of prevalence of and critical titer of high-risk alloantibodies at initial prenatal visit 2010-2021

- Figure 1A depicts the temporal increase in positive detection of the three alloantibodies (Anti-D, anti-c and anti-Kell) considered high-risk for alloantibodies over a 12-year period. This temporal trend was significant for all three alloantibodies (all P <.001), and most notable for Anti-D, where prevalence rose 43.0% from 481/100,000 pregnancies in 2011 to 688/100,000 in 2021.
- Figure 1B depicts the frequency of critical titers for all three high-risk antibodies. These frequencies changed over the 12-year period (P < .001 for both anti-K and anti-D and P = .03 for anti-c). The prevalence of critical titer of anti-K increased 25.6% from 39/100,000 in 2011 to 49/100,000 in 2021 while that of anti-D decreased 21.7% from 23/100,000 in 2010 to 18/100,000 in 2021.

TABLES

Table 1 - Red blood cell (RBC) antibodies and critical titers according to Swedish national guidelines, 2015

Risk for HDFN	Types of RBC antibodies
High risk	Anti-D
	Anti-K
	Anti-c
Moderate risk	Anti-C
	Anti-e
	Anti-E
	Anti-k
	Anti-Fya
	Anti-U
Low risk	Anti-Cw
	Anti-f
	Anti-Jka
	Anti-Jkb
	Anti-M
	Anti-Kpa
	Anti-Kpb
	Anti-Yta
	Anti-Coa
	Anti-Cob
	Anti-Ge2,3
Titer	**Management Implication**
1–8	Not critical for the fetus, but an indication for continued surveillance with antibody titers. The exception is anti-K where monitoring with Doppler interrogation of MCA PSV should start at titer ≥8
16–32	Low risk of severe HDFN, but neonatal hyperbilirubinemia requiring phototherapy is possible
≥64	Close monitoring, including Doppler interrogation of MCA PSV. Risk of need for neonatal exchange transfusion

≥128	Close monitoring, including Doppler interrogation of MCA PSV. Intrauterine blood transfusion/neonatal exchange transfusion may be required

Abbreviations: HDFN, hemolytic disease of the fetus and newborn; MCA, middle cerebral artery; PSV, peak systolic velocity. Reproduced from: Liu S, Ajne G, Wikman A, Lindqvist C, Reilly M, Tiblad E. Management and clinical consequences of red blood cell abs in pregnancy: A population-based cohort study. *Acta Obstet Gynecol Scand*. 2021;100(12):2216-25.

Table 2 – Cohort Demographic Characteristics

	Pregnancies (Number = 10,252,880)		United States Birth Certificate Data (Number = 46,423,127)	
Age (years)				
Younger than 20	697,063	6.8%	2,847,328	6.1%
20-34	7,637,635	74.5%	35,782,150	77.1%
35 or older	1,917,065	18.7%	7,793,659	16.8%
Overall mean in years	29.5		29	
Primary Payor				
Commercial Insurer	4,264,026	41.6%	11,252,650	49.7%
Medicaid	1,111,368	10.8%	9,478,566	41.9%
Self-pay	86,160	0.8%	913,660	4.0%
Other	1,823,790	17.8%	973,690	4.3%
Census Division				
New England	415,265	4.1%	1,772,902	3.8%
Mid Atlantic	1,412,147	13.8%	5,665,326	12.2%
South Atlantic	2,260,046	22.0%	8,832,494	19.0%
East South Central	304,503	3.0%	2,781,122	6.0%
West South Central	1,372,830	13.4%	6,419,230	13.8%
East North Central	809,709	7.9%	6,558,943	14.1%
West North Central	471,243	4.6%	3,190,253	6.9%
Mountain	838,531	8.2%	3,585,200	7.7%
Pacific	1,991,922	19.4%	7,617,667	16.4%
Calendar Year				
2010	900,589	8.8%	3,999,386	8.6%
2011	868,422	8.5%	3,953,590	8.5%
2012	848,041	8.3%	3,952,841	8.5%
2013	880,091	8.6%	3,932,181	8.5%
2014	879,959	8.6%	3,988,076	8.6%
2015	890,489	8.7%	3,978,497	8.6%
2016	852,857	8.3%	3,945,875	8.5%
2017	840,425	8.2%	3,855,500	8.3%
2018	844,965	8.2%	3,791,712	8.2%
2019	841,918	8.2%	3,747,540	8.1%
2020	802,106	7.8%	3,613,647	7.8%
2021	803,018	7.8%	3,664,292	7.9%

*Primary payor was only available in US Birth Certificate data from 2016-21

Table 3: Estimates of national prevalence of alloimmunization in early pregnancy

Risk of HDFN	Rate per 100,000 pregnancies (95% CI)	
	Pregnancies (Number = 9,876,196)	
	RBC Antibody Detected	Critical Titer*
High-risk		
anti-D	586 (553, 618)	21 (20, 22)
anti-K	68 (64, 71)	46 (44, 48)
anti-c	29 (26, 31)	1.9 (1.5, 2.2)
Any[#]	679 (645, 714)	69 (66, 71)
Moderate-risk		
anti-E	110 (105, 116)	8 (7, 9)
anti-C	30 (26, 33)	2.6 (2.2, 3.0)
anti-e	4 (3, 5)	0.06 (0.01, 0.12)
anti-k	0.01 (0, 0.03)	0.01 (0, 0.03)
anti-Fya	13 (12, 14)	3.1 (2.7, 3.6)
anti-U	0.03 (0, 0.07)	0
Any[##]	123 (116, 130)	6.8 (5.8, 7.7)
Low-risk		
Any	112 (103, 121)	Not applicable

* >4 for anti-K and >16 for all other high-risk and moderate-risk antibodies

** Includes: anti-Cw, anti-f, anti-Jka, anti-Jkb, anti-M, anti-S, anti-s, anti-Fyb, anti-Lua, anti-Lub, anti-Kpa, anti-Kpb, anti-Yta, anti-Coa, anti-Cob, anti-Ge2,3

[#] Accounted for any high-risk antibody combination detected in a test (0.3%)

[##] Accounted for any moderate-risk antibody combination detected in a test (0.5%) and excluded those combinations with high-risk antibodies (3.1%)

Figure 1A: Temporal trends of prevalence of alloantibodies high-risk for HDFN

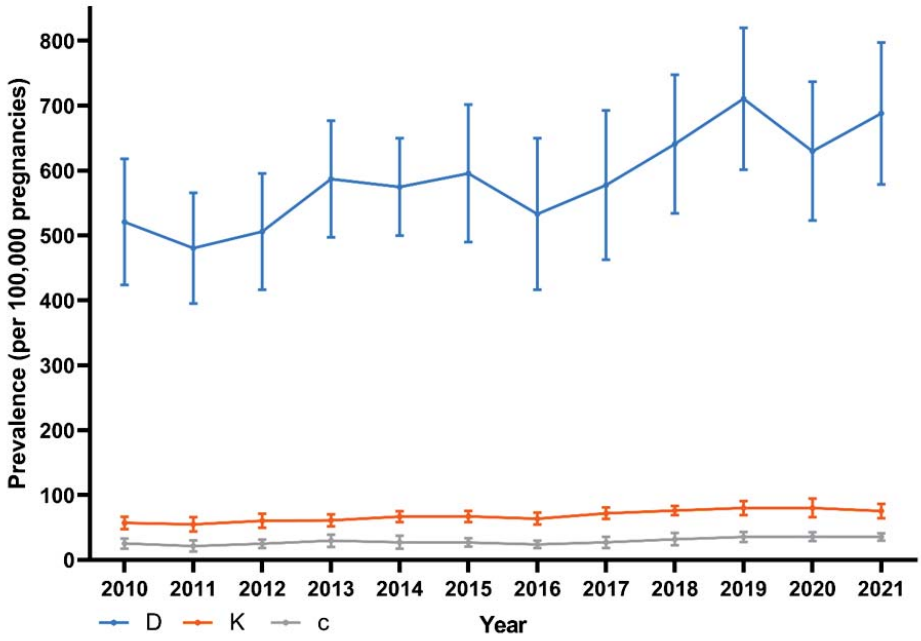

Figure 1B: Temporal trends of frequency of critical titers of alloantibodies high-risk for HDFN (1:16 for Anti-D, Anti-c; >1:4 for Anti-Kell)