

Rh isoimmunization in Sub-Saharan Africa indicates need for universal access to anti-RhD immunoglobulin and effective management of D-negative pregnancies

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Abstract: Transplacental or fetomaternal hemorrhage (FMH) may occur during pregnancy or at delivery and lead to immunization to the D antigen if the mother is Rh-negative and the baby is Rh-positive. This can result in hemolytic disease of the fetus and newborn (HDFN) in subsequent D-positive pregnancies. The aim of this study is to highlight the challenges associated with the effective management and prevention of Rh alloimmunization among Rh-negative women in Sub-Saharan Africa. In most Sub-Saharan African countries, there is poor and sometimes no alloimmunization prevention following potentially sensitizing events and during medical termination of pregnancy in Rh-negative women. Information about previous pregnancies and termination are often lacking in patients' medical notes due to poor data management. These issues have made the management of Rh-negative pregnancy a huge challenge. Despite the fact that the prevalence of Rh-negative phenotype is significantly lower among Africans than Caucasians, Rh alloimmunization remains a major factor responsible for perinatal morbidity in Sub-Saharan Africa and may result in the compromise of the woman's obstetric care due to the unaffordability of anti-D immunoglobulin. There is the urgent need for the implementation of universal access to anti-D immunoglobulin for the Rh-negative pregnant population in Africa. Anti-D immunoglobulin should be available in cases of potentially sensitizing events such as amniocentesis, cordocentesis, antepartum hemorrhage, vaginal bleeding during pregnancy, external cephalic version, abdominal trauma, intrauterine death and stillbirth, in utero therapeutic interventions, miscarriage, and therapeutic termination of pregnancy. There is also the need for the availability of FMH measurements following potentially sensitizing events. The low-cost acid elution method, a modification of the Kleihauer–Betke (KB) test, can become a readily available, affordable, and minimum alternative to flow cytometric measurement of FMH. Knowledge of anti-D prophylaxis among obstetricians, biomedical scientist, midwives, traditional birth attendants, pharmacists, and nurses in Africa needs to be improved. This will facilitate quality antenatal and postnatal care offered to Rh-negative pregnant population and improve perinatal outcomes.

Keywords: rhesus isoimmunization, Sub-Saharan Africa, universal access, anti-D, management, Rh-negative women

Introduction

The human red blood cell (RBC) membrane is complex and contains a variety of blood group antigens, the most clinically significant being the ABO system and the Rh system. The Rh system consists of two related proteins, RhD and RhCE, which express the D and CE antigens, respectively. People who have the D antigen on their RBCs are said

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to be RhD-positive, whereas those who do not are said to be RhD-negative. If the mother is RhD-negative and the fetus RhD-positive, the mother may react to fetal blood cells in her circulation by developing anti-D antibodies, a process known as RhD sensitization. Sensitization is unlikely to affect the current fetus but may result in hemolytic disease of the fetus and newborn (HDFN) during a second RhD-positive pregnancy. In its mildest form the infant has sensitized RBCs, which are detectable only in laboratory tests; however, HDFN may result in jaundice, anemia, developmental problems, or intrauterine death.¹

The frequency of RhD-negative phenotype in previous studies in Nigeria 4.44%,² 3.9% in Kenya,³ 4.06% in Guinea,⁴ and 2.4% in Cameroon.⁵ These findings are much lower than the $\geq 14\%$ prevalence of Rh-negative phenotype observed in studies among Caucasians.⁶

In most Sub-Saharan African countries, there are challenges associated with Rh pregnancies.⁷ A previous report indicated the effectiveness of anti-D prophylaxis in the prevention of HDFN despite poor access.⁸ The utilization rate of anti-Rh antiserum in South African population groups for the years 1983–1985 was investigated. The crude utilization rate of anti-Rh antiserum was 41%–44% for all population groups combined. The rate for Blacks, Whites, Indians, and Coloreds was 14%–20%, 89%–94%, 59%–64%, and 45%–51%, respectively.⁹ The potential risk of rhesus alloimmunization and the ensuing risk of fetal death with increasing parity were investigated in two groups of parturients: primiparous and grand multiparous Mozambican parturients. The difference did not reach statistical significance.¹⁰ A previous report from Zimbabwe indicated that anti-D immunoglobulin remains the most important alloantibody causing HDN, regardless of the availability of anti-D immunoglobulin for prophylaxis and suggests that all patients at booking should have an antibody screen.¹¹ A report from Nigeria has shown that isoimmunization due to Rh incompatibility is poorly studied among Nigerian women and indicates the urgent need for a management protocol for anti-D immunoglobulin for prophylaxis.¹² Care management with anti-D prophylaxis in patients presenting with severe alloimmunization is difficult to access in Sub-Saharan Africa.¹³ Beyond the challenge of access to anti-D prophylaxis, there is lack of alloimmunization prevention during illegal abortions and poor documentation of adequate information in patients' medical notes. These factors are highly responsible for the difficult management of Rh-negative patients.¹⁴ A cross-sectional retrospective study to determine the prevalence of anti-D immunoglobulin among Cameroonian women of

reproductive age has indicated an anti-D prevalence of 4% among Rh-negative African women.¹⁵

To prevent HDFN in most developed countries, RhD-negative women are given anti-D immunoglobulin (IgG) after delivery and often also between 28 and 34 weeks of gestation. At delivery, RhD phenotype of the newborn is determined even if RhD fetal genotype is known. Maternal blood is drawn for quantification of fetomaternal transfusion within 72 hours of delivery of a Rh-positive baby and the optimum amount of anti-D immunoglobulin administered.¹⁶ Anti-D prophylaxis has significantly reduced the incidence of erythroblastosis fetalis caused by sensitization to the D-antigen and perinatal deaths from alloimmunization have fallen 100-fold in the developed world.^{17,18}

The anti-D immunoglobulin is prepared from the plasma of immunized human donors and therefore exists in limited supply. Monoclonal anti-D antibodies have been developed to replace polyclonal anti-D and in vivo assays for these have been predominantly based on their ability to clear erythrocytes from the maternal circulation.¹⁹ Although the implementation of a program of routine antenatal anti-D prophylaxis (RAADP) has led to a significant decline in the residual numbers of women becoming sensitized in most developed countries, a significant number of women are not fortunate enough to have access in Sub-Saharan Africa and thus continue to be affected. This is an ethical issue of utmost public health importance. The aim of this study is thus to highlight the challenges associated with the effective management and prevention of Rh alloimmunization among Rh-negative women in Sub-Saharan Africa.

Anti-D immunoglobulin

Anti-D immunoglobulin is produced by the pooling and fractionation of plasma from large numbers of donors who themselves are RhD-negative and have been exposed to RhD-positive RBCs to stimulate the production of RhD antibodies.^{20,21} The future of anti-D immunoglobulin might involve monoclonal or recombinant products, thus eliminating the risks associated with human blood products. Costs would probably increase if recombinant products were used.²² Anti-D, a polyclonal IgG product, is routinely and effectively used to prevent HDFN. The mechanism of anti-D has not been fully elucidated. However, a correlation has frequently been observed between anti-D-mediated RBC clearance and prevention of the antibody response, suggesting that anti-D may be able to destroy RBCs without triggering the adaptive immune response. Anti-D opsonized RBCs may also elicit inhibitory Fc γ RIIB signaling in B cells and prevent

B cell activation. The ability of antigen-specific IgG to inhibit antibody responses has also been observed in a variety of animal models immunized with a vast array of different antigens, such as sheep RBCs. This effect has been referred to as antibody-mediated immune suppression.²³

Antenatal antibody screening

It is recommended that all women in most developed countries should have a blood group and antibody screening at first antenatal visit. It has been reported that 1.5%–2% of pregnant women show atypical blood group sensitization.²⁴ Opinion is divided as to the clinical importance of a repeat anti-D antibody screen at 28 weeks' gestation. Those in support of 28 weeks' testing argue that there is the potential advantage to identify about 0.18% or fewer women particularly Rh-negative who become alloimmunized after their first antenatal screen possibly as a result of potential sensitizing event occurring after the first antenatal visit.²⁵

The American Society of Clinical Pathology recommends that testing for unexpected antibody be carried out before antenatal anti-D is given to Rh-negative pregnant women and that repeat Rh testing be omitted if two documented test results confirming the Rh-negative status of the woman are on her record.²⁶ Prior to 1970, HDFN due to anti-D was a significant cause of morbidity and mortality. By 1990, a reduction in mortality from 1.2 per 1000 births to 0.02 per 1000 births had been achieved in response to the introduction of immunoprophylaxis with anti-D immunoglobulin.²⁷ At that time the sensitization rate dropped to about 1.2%. A further reduction to between 0.17% and 0.28% was achieved by introducing prophylaxis during the third trimester of pregnancy.²⁸ These findings contributed to the National Institute for Clinical Excellence (NICE) recommendation that all D-negative pregnant women who do not have immune anti-D should be offered anti-D immunoglobulin routinely during the third trimester of pregnancy.²⁹ In 2002 the NICE in the United Kingdom assessed the cost effectiveness of routine antenatal anti-RhD prophylaxis with anti-D immunoglobulin.²⁹ Previously anti-D immunoglobulin had been administered antenatally only when events occurred that would be associated with a fetomaternal hemorrhage. NICE recommended that all RhD negative pregnant women should be offered anti-D immunoglobulin at 28 and 34 weeks' gestation. In a predominantly White population, however, about 38% of these women are likely to be carrying an RhD-negative fetus and would receive the treatment unnecessarily. Consequently, NICE also endorsed studies into the feasibility of mass fetal blood group by analysis of fetal DNA in maternal plasma.

The benefits of this testing would be twofold. Firstly, there would be a substantial reduction in the use of anti-RhD immunoglobulin, an expensive blood product in short supply. Secondly, women with an RhD-negative fetus would be spared unnecessary exposure to this pooled human blood product with its associated discomfort and perceived risk from viral or prion contamination.³⁰ Paternal testing of a baby's father may be offered to all Rh-negative pregnant women to eliminate unnecessary blood product administration. Mitchell and colleagues suggest that if the pregnant woman volunteers and confirms in private that her partner is indeed the biological father, and if the said father is documented to be a confirmed Rh-negative, then anti-D may be omitted.³¹ However it is recommended that partners of Rh-negative pregnant women should be routinely tested without this private confirmation. This may avoid creating the potential of a possible conflict for the pregnant woman between privacy in the relationship and the well-being of the fetus. It is being suggested that the most important application of blood group genotyping by molecular genetics is the prediction of fetal RhD phenotype in pregnant women who are Rh-negative and in pregnant women with anti-D, in order to assess the risk of HDFN. This diagnostic test performed on cell-free fetal DNA in the maternal plasma is now available in some laboratories.³² There are, however, no national guidelines, which are required to call it a routine procedure at a national level. High-throughput modifications of this form of fetal D-typing would be valuable for testing fetuses of all D-negative pregnant women to avoid unnecessary antenatal treatment with anti-D immunoglobulin in the 40% of D-negative pregnant women with a D-negative fetus. The results of trials in Bristol and Amsterdam³³ suggest that such routine testing is feasible and accurate. Similarly Finning et al³⁴ recommends that high-throughput RhD genotyping of fetuses in all RhD-negative women is feasible and would substantially reduce unnecessary administration of anti-RhD immunoglobulin to RhD-negative pregnant women with an RhD-negative fetus.

Organized preventive screening programs for antenatal care were first introduced in Western Europe in the twentieth century with the hope that routine antenatal care would contribute to a reduction in maternal and infant mortality rates. Figures on maternal mortality in the developed world show that the risk of death as a result of pregnancy and child birth is approximately 1 in 7000 compared with 1 in 23 for women living in parts of Africa where antenatal care is poor or sometimes nonexistent.³⁵

It is part of modern antenatal care to give all RhD-negative pregnant women an anti-RhD immunoglobulin

IgG injection at about 28 weeks' gestation with or without a booster at 34 weeks' gestation. This reduces the effect of the vast majority of sensitizing events which mostly occur after 28 weeks' gestation. Anti-RhD immunoglobulin is also given to non-sensitized Rh-negative women immediately within 72 hours after potentially sensitizing events that occur during pregnancy. All these advances in antenatal management of Rh-negative pregnant women in developed countries are beyond the reach of a vast majority of women in Sub-Saharan Africa. In most Sub-Saharan African countries, the recommendation is that women should have an ABO and Rh blood group test done at the time of antenatal booking. Women found to be Rh-negative and who are married to Rh-positive men and run the risk of carrying an Rh-positive fetus and who can afford treatment are offered prophylactic D immunoglobulin of 500 IU at 28 weeks' gestation. Tests to determine the presence of clinically significant alloantibodies in antenatal care patients are seldom carried out in most settings. At delivery the blood group of the baby is determined. If the baby is found to be Rh-positive, 500 IU of D immunoglobulin is administered. Facilities for determination of FMH are seldom available in most settings. Provision of prophylactic D immunoglobulin following potential sensitizing events during pregnancy is only available in an insignificant number of Rh-negative women due to unaffordability. The net result is that anti-D remains the most important alloantibody causing HDFN in Sub-Saharan Africa despite the availability of anti-D immunoglobulin for prophylaxis. Only in an insignificant number of centers do Rh D-negative women have an antibody screen at booking and repeat antibody screens during the rest of their pregnancy.

Rh-negative women should be given an injection of human anti-D immunoglobulin after the termination of pregnancy procedure to prevent blood incompatibility complications in future pregnancies. However, unsafe abortion, defined by the World Health Organization as a procedure for terminating an unwanted pregnancy either by persons lacking the necessary skills or in an environment lacking the minimal medical standards, or both, is prevalent and continues to put Rh-negative women who cannot afford anti-D immunoglobulin at risk of Rh isoimmunization.³⁶ A broad array of personnel perform unsafe termination of pregnancy in Africa. Aside from the woman herself, others include physicians working at clandestine sites or in hospital operating theaters after normal working hours. Others with medical experience include midwives, traditional birth attendants, pharmacists, and nurses. Most worrisome are 'untrained quacks' whose motives may be financial and their skills negligible. Women

who are Rh-negative should be given an injection of human anti-D immunoglobulin after the termination of pregnancy procedure unless the father of the fetus is also Rh-negative. This prevents blood incompatibility complications in future pregnancies.³⁷

The costs associated with providing routine antenatal anti-D prophylaxis are the cost of the anti-D immunoglobulin IgG and the cost of treatment administration. The price of anti-D Immunoglobulin differs according to its manufacturers: Bio Products Laboratory (BPL; Elstree, UK) offers anti-D IgG at a unit price of £27 (US\$41) for 500 IU vial³⁸ while Baxter Healthcare (Deerfield, IL) anti-D IgG is offered at a unit price of £23.90 (US\$36) for a 1250 IU vial.³⁹ Offering antenatal anti-D prophylaxis will cost an Rh-negative woman £47.80 (US\$72) to £54 (US\$82) per pregnancy depending on whether she is administered the BPL or Baxter product at 28 and 34 weeks. Cost-effective analysis indicates that offering routine antenatal anti-D prophylaxis to RhD-negative women is economical and results in a marked impact upon the death rate associated with hemolytic disease of the newborn.⁴⁰ Drug manufacturers need to be more humane by reducing the cost of providing anti-D prophylaxis particularly in low-income countries in Sub-Saharan Africa. Cost constraints have remained a limiting factor preventing people from access to best possible treatment and care in Sub-Saharan African countries like their counterparts in most developed countries. There is also the urgent need for African leaders to take up the bold challenge to provide universal access to anti-D prophylaxis for Rh-negative women. Per capita income in most settings in Sub-Saharan Africa is low and continues to affect affordability to prophylactic anti-D treatment.

In the absence of anti-D prophylaxis to prevent incidence of HDFN, options such as exchange blood transfusion and intrauterine transfusion (IUT) can significantly reduce mortality and prevent stillbirths. However, safety of blood and blood products remains a great concern. One of the biggest challenges to blood safety particularly in Sub-Saharan Africa is accessing safe and adequate quantities of blood and blood products. Societies in Africa face several enduring challenges: chronic blood shortages, high prevalence of transfusion-transmissible infection, absence of national blood transfusion service, recruitment and retention of voluntary non-remunerated donors, lack of appropriate infrastructure, trained personnel, and financial resources to support the running of a safe blood transfusion service.⁴¹ Although not available in most settings in Sub-Saharan Africa, the introduction of ultrasonographically guided IUT has improved the ability to treat severely anemic fetuses earlier in gestation

and has increased the chances of survival of more severely affected fetuses with the potential for poor neurodevelopmental outcomes.⁴² Around 10%–12% of fetuses affected by HDN will require IUT and a relatively high proportion of IUT survivors may suffer neurodevelopmental problems such as cerebral palsy, deafness, and motor and speech delay that will require specialist input and, in some cases, special education; others will suffer some degree of developmental delay requiring physiotherapy or speech therapy.⁴³

Antepartum and postpartum prophylaxis

Current guidelines in the United Kingdom recommend that a minimum of 500 IU anti-D IgG be offered to all non-sensitized RhD-negative women at 28 and 34 weeks gestation in order to prevent the risk of RhD sensitization in pregnancy.⁴⁴ It is recommended that a minimum anti-D immunoglobulin of 250 IU be administered after miscarriage or threatened abortion or induced abortion, ectopic pregnancy, following chorionic villous sampling, amniocentesis, cordocentesis, placental abruption, blunt trauma to the abdomen, placenta previa with bleeding, external cephalic version, and any other potentially sensitizing events at less than 20 weeks gestation in non-sensitized D-negative women. However in the event of any sensitizing event after 20 weeks' gestation, a minimum of 500 IU of anti D is administered and blood is tested for FMH and if the estimated fetal bleed is greater than 4 mL, additional anti-D is administered (125 IU per 1 mL bleed). Before termination of pregnancy, blood type and antibody screen is done and if lady or mother is a confirmed RhD-negative 250 IU of anti D is given. A previous report⁴⁵ in England had investigated the clinical effectiveness and cost-effectiveness of RAADP for RhD-negative women. Results showed that RAADP reduces the incidence of sensitization and hence of hemolytic disease of the newborn. The economic model suggests that RAADP given to all RhD-negative pregnant women is likely to be cost-effective at a threshold of around £30,000 per quality of life years (QALY) gained. The total cost of providing RAADP to RhD-negative primigravidae in England and Wales is estimated to be around £1.8–3.1 million per year, depending upon regimen, and to all RhD-negative pregnant women in England and Wales around £2–3.5 million.

In France, targeted prophylaxis is applied regardless of the gestational age and a dose of 100 g anti-D immunoglobulin is usually enough (200 g is the lowest dosage currently available). However it is recommended to quantify the volume of FMH to avoid administration of a dose of anti-D IgG less

than 20 g/mL of fetal RBCs. Efficacy of prophylaxis relies also on the delay of less than 72 hours between the sensitizing event and the injection of anti-D. Intravenous administration (IV) of anti-D allows for the immediate neutralization of D-positive fetal RBCs and should be, if possible, preferred to intramuscular administration (IM).⁴⁶

In Canada, it is recommended that anti-D immunoglobulin 300 µg IM or IV should be given within 72 hours of delivery to a postpartum non-sensitized Rh-negative woman delivering an Rh-positive infant. Additional anti-D immunoglobulin may be required for FMH greater than 15 mL of fetal RBCs (about 30 mL of fetal blood). If anti-D immunoglobulin is not given within 72 hours of delivery or other potentially sensitizing event, anti-D immunoglobulin should be given as soon as the need is recognized, for up to 28 days after delivery or other potentially sensitizing events. Anti-D immunoglobulin 300 µg should be given routinely to all Rh-negative non-sensitized women at 28 weeks' gestation when fetal blood type is unknown or known to be Rh-positive. Alternatively, 2 doses of 100–120 µg may be given (120 µg being the lowest currently available dose in Canada): one at 28 weeks and one at 34 weeks. All pregnant women (D-negative or D-positive) should be typed and screened for alloantibodies with an indirect antiglobulin test at the first prenatal visit and again at 28 weeks. When paternity is certain, Rh testing of the baby's father may be offered to all Rh-negative pregnant women to eliminate unnecessary blood product administration. Non-sensitized D-negative women are given a minimum anti-D of 120 µg after miscarriage or threatened abortion or induced abortion during the first 12 weeks of gestation, ectopic pregnancy at less than 12 weeks' gestation, molar pregnancy, and following chorionic villous sampling. After 12 weeks' gestation, they should be given 300 µg. At therapeutic termination of pregnancy, blood type and antibody screen is done unless results of blood type and antibody screen during the pregnancy are available, in which case antibody screening need not be repeated. Anti-D of 300 µg is given to all non-sensitized D-negative women, following amniocentesis, placental abruption, blunt trauma to the abdomen, cordocentesis, placenta previa with bleeding, external cephalic version, and placenta previa with bleeding. There is a substantial risk of FMH over 30 mL with such events, especially with blunt trauma to the abdomen. If FMH is in excess of the amount covered by the dose given (6 mL or 15 mL fetal RBC), 10 µg additional anti-D should be given for every additional 0.5 mL fetal RBCs.¹⁸

A report on Dutch women that evaluated the acceptance by pregnant women in a perinatal screening program showed that women highly accept the program for prenatal

screening for RBC antibodies.⁴⁷ Similarly a nationwide Dutch antenatal study evaluated the risk factors for RhD immunization in pregnancy, despite adequate antenatal and postnatal anti-D prophylaxis in the previous pregnancy in a bid to generate evidence for improved primary prevention by extra administration of anti-D immunoglobulin in the presence of a risk factor. The report indicated that in at least half of the failures of anti-D immunoglobulin prophylaxis, a condition related to increased FMH and/or insufficient anti-D immunoglobulin administration was observed. The authors suggested that RhD immunization may be further reduced by strict compliance to guidelines for determination of FMH and anti-D immunoglobulin prophylaxis adjusted accordingly, or by routine administration of extra anti-D immunoglobulin after a non-spontaneous delivery and/or a complicated or prolonged third stage of labor.⁴⁸

Facilities for the determination of FMH to allow for optimum dosing of anti-D immunoglobulin are often lacking in most settings in Africa. Countries in Sub-Saharan Africa could learn from good practices in developed countries to help reduce the incidence of Rh isoimmunization and hemolytic disease of the newborn. The proposal to use human Anti-D immunoglobulin prophylactically in pregnancy should not detract from the most expedient approach to further the reduction of Rh disease; that is, to ensure that every eligible woman is given Anti-D immunoglobulin after delivery, abortion, and other potentially sensitizing events like their counterparts in the developed world. Family planning by Rh-negative women at risk has the potential to limit the number of pregnancies in women already immunized. This is likely to be an effective way to reduce the current incidence of hemolytic disease in Sub-Saharan Africa. Present evidence shows that blanket antepartum Anti-D immunoglobulin prophylactic treatment may be very costly but beneficial to a significant number of women who may not be fortunate enough to have access as a result of unaffordability. There is need for sensitive and practical laboratory testing for FMH to be clinically available to provide new data on FMH. It is suggested that the KB testing should become the minimal cost-effective alternative to flow cytometric testing of FMH in low-income countries in Sub-Saharan Africa because of the cost implication of procuring flow cytometric equipment and lack of trained personnel. There is the urgent need for pregnant women truly at risk for Rh isoimmunization to be identified by analysis of their blood during first antenatal visit and that this should become the rational basis for antepartum Anti-D immunoglobulin treatment. There are compelling advantages in cost, risks, and benefits for an approach of

selective antepartum Anti-D immunoglobulin therapy as opposed to routine prophylaxis for all Rh-negative gravid women.⁴⁹ The knowledge of anti-D prophylaxis among obstetricians can be improved. A continual system of education to raise awareness of evidence-based practices as well as clinical audit can be implemented to address this.⁵⁰ Rh-negative women in Sub-Saharan Africa will benefit immensely from programs such as the RAADP, but costs remains a major hindrance.

Testing for FMH

The KB test is a blood test used to measure the amount of fetal hemoglobin transferred from a fetus to a mother's bloodstream.⁵¹ It is usually performed on Rh-negative mothers to identify women with a large fetomaternal hemorrhage (>4 mL of packed fetal RBCs) who may need additional anti-D immunoglobulin to ensure complete clearance of all fetal RBCs from maternal circulation and thus prevent them from being sensitized to produce immune antibodies against D- antigen on the surface of the fetal RBCs. A standard dose of 125 IU is the required dose of Anti-D immunoglobulin required to inhibit 1 mL bleed of fetal RBCs and thus prevent the formation of Rh- antibodies in the mother and prevent Rh- disease in future Rh-positive children. The KB test is the standard method of detecting FMH. It takes advantage of the differential resistance of fetal hemoglobin to acid elution. A standard blood smear is prepared from the mother's blood, and exposed to an acid bath. This removes adult hemoglobin, but not fetal hemoglobin, from the RBCs. Subsequent staining with eosin makes fetal cells (containing fetal hemoglobin) appear rose-pink in color, while adult RBCs are only seen as 'ghosts'. A large number of cells (>5000) are counted under the microscope and a ratio of fetal to maternal cells generated. In those with positive tests, follow-up testing as a postpartum check should be done to rule out the possibility of a false positive. This could be caused by a process in the mother which causes persistent elevation of fetal hemoglobin, for example; sickle cell trait and hereditary persistence of fetal hemoglobin (HPFH). Comparison with other more expensive or technologically advanced methods such as flow cytometry has shown that the KB test, like the more advanced methods, is sensitive for the detection of FMH.⁵² Background counting errors can result in estimates of as much as 5 mL fetal blood loss when there actually is no such blood loss, but standard methods available in most laboratories admit an extremely low probability of the return of a false positive when more severe FMH has taken place. Performance indicators for the KB test during antepartum period in most developed

countries include: unexpected/unexplained still birth, significant maternal abdominal trauma, post 20 weeks' gestation vaginal bleed, post 20 weeks' therapeutic termination of pregnancy, miscarriage, in utero therapeutic interventions, external cephalic version, and antepartum hemorrhage.⁵³ Testing at the time of birth and postpartum is indicated if baby is Rh-positive. A cord sample is collected from all babies born of Rh-negative mothers. Where the cord sample is Rh(D)-positive, a KB or flow cytometric determination of FMH is carried out and anti-D immunoglobulin optimal to clear the volume of FMH is administered preferably within 72 hours of delivery.⁵⁴ If recurrent uterine bleeding occurs in a D-negative woman after 20 weeks' gestation, anti-D immunoglobulin will be required at a minimum of 6-weekly intervals. An FMH test should be performed every 2 weeks and if FMH is detected, additional anti-D will be required.⁵⁵

Since fetal and maternal blood cells have the same life expectancy in the maternal bloodstream, it is possible to obtain informative results from a KB stain for a fair period of time after a stillbirth. However, if the mother and fetus are ABO incompatible, it is more crucial to quickly perform the KB stain following a stillbirth, as the fetal RBCs will be eliminated from the maternal bloodstream very quickly, causing the KB stain to underestimate the degree of FMH, if any. The KB technique, based on acid elution of maternal RBCs, is the most widely used technique in the developed world for estimating the volume of FMH and for determining the need for additional doses of anti-D immunoglobulin to prevent maternal alloimmunization.⁵⁶ Finally, anything that causes persistence of fetal hemoglobin in maternal blood cells will make interpretation much trickier. Certain hemoglobinopathies, the most common of which is sickle cell trait, and HPFH do this. The KB test has been used worldwide since the 1950s to quantify the FMH and to ensure that an appropriate dose of anti-D immunoglobulin is administered both antenatally and postnatally to RhD-negative women to prevent Rh alloimmunization.¹⁶ Although apparently a simple test to perform, recent reports have suggested that unless meticulous attention is paid to both technique and interpretation, the accuracy of the test cannot be guaranteed and that it should be replaced with a flow cytometric test which would give more relevant and accurate results.⁵⁷ Flow cytometers are not, however, available to all laboratories performing estimations of FMH. The comparability of results was assessed using a standardized KB technique with flow cytometry with a total of 957 samples were analyzed. Results suggest that if careful attention is paid to performing a standardized KB

test, then it is of value in estimating the size of FMH, and that flow cytometry may be of additional value for cases in which the Kleihauer result is equivocal or indicates that a large FMH has occurred which requires the administration of additional anti-D immunoglobulin.⁵⁷ Similarly Johnson and colleagues⁵⁸ evaluated an indirect immunofluorescence flow cytometry technique in a series of patients with large FMH. Patient samples identified by KB testing as having FMH > 4 mL were sent for flow cytometric analysis. The report indicated that flow cytometry is helpful for the accurate quantification and management of patients with large FMH, and in cases where the presence of maternal hemoglobin F-containing cells renders the KB technique inaccurate, worthwhile reductions in the use of anti-D immunoglobulin can be achieved.⁵⁹

Discussion

Despite the fact that the prevalence of Rh-negative phenotype is significantly lower among Africans than in Caucasians, alloimmunization to RhD remains a major factor in perinatal morbidity and continues to compromise women's obstetric care in Sub-Saharan Africa due to the unaffordability of anti-D immunoglobulin. A preliminary study of 67 RhD-negative women over a 2-year period in Nigeria has shown that isoimmunization due to Rh incompatibility is poorly studied among Nigerian women, with many questions unanswered, and that there is an urgent need for a management protocol for this condition, which will include both the clinicians and the laboratory biomedical scientist.¹² Similarly a previous report in Cote d'Ivoire has indicated that the lack of alloimmunization prevention during illegal abortions and the lack of information about patients' medical files are highly responsible for the difficult management of Rh-negative patients.¹³ There are several possible reasons for continuing cases of Rh isoimmunization among the Rh-negative pregnant population in Sub-Saharan African: cost of procuring anti-D immunoglobulin; absence of a universal access program for all Rh-negative women; failure to recognize potential sensitizing events in pregnancy as such and to treat them appropriately; failure and absence of facilities to assess the extent of FMH; poor and sometimes absence of alloimmunization prevention during illegal termination of pregnancy in Rh-negative women; a dearth of information about previous pregnancies and termination in patients' medical files due to poor data management; failure to comply with postpartum prophylaxis guidelines to offer further anti-D immunoglobulin to all Rh-negative women delivered of Rh-positive babies with 72 hours of

delivery depending on the extent of FMH; failure to offer Rh-negative pregnant women anti-D immunoglobulin following any potentially sensitizing event during pregnancy; and failure of obstetrician to offer these Rh-negative women the maximum standard of antenatal and postnatal care. Antenatal management of Rh-negative pregnant women in Sub-Saharan Africa is suboptimal. There are several health system challenges: socioeconomic realities, lack of adequate qualified staff, inadequate referral services, shortage of supplies, and shortages of midwives, counselors, laboratory, and obstetrics and gynecology personnel. In the midst of these challenges, anti-D remains the most important alloantibody causing HDN in Sub-Saharan Africa.¹¹ Evidence has shown that prophylaxis of the alloimmunization to the antigen D is effective among Rh-negative African women fortunate enough to have access.⁶⁰ Investment in health infrastructure, personnel, and research both for innovation and to improve implementation as well as universal access to anti-D immunoglobulin is what countries in Sub-Saharan Africa desperately need to facilitate the reduction in the incidence of Rh isoimmunization.⁶¹ Innovative low-cost devices and diagnostic methods such as the use of the KB test for determination of FMH could improve the quality of care offered these women.⁶² Improving the uptake of quality antenatal, intrapartum, and postpartum care as well as innovative community-based strategies, combined with health systems strengthening and the development of an evidenced-based protocol for the management of Rh isoimmunization, are critical for evidence-based interventions required to deliver interventions to improve screening and treatment for risk factors and reduce the risks of Rh isoimmunization.

Disclosure

The authors declare no conflicts of interest in this work.

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