Predicting anti-Kell mediated hemolytic disease of the fetus and newborn, diagnostic accuracy of laboratory management.

Yolentha M SLOOTWEG (MSc), Leiden, the Netherlands, Department of Obstetrics, Leiden University Medical Center;

Irene T LINDENBURG (MD, PhD), Leiden, The Netherlands, Department of Obstetrics, Leiden University Medical Center;

Joke M KOELEWIJN (PhD), Leiden and Amsterdam, the Netherlands, Department of Immunohematology Diagnostics, Sanquin Diagnostic Services; Department of Clinical Transfusion Research, Sanquin Research;

Inge L. VAN KAMP (MD, PhD) Department of Obstetrics, Leiden, The Netherlands, Leiden University Medical Center;

Dick OEPKES (MD, PhD), Leiden, The Netherlands, Department of Obstetrics, Leiden University Medical Center;

Masja DE HAAS (MD, PhD), Leiden and Amsterdam, the Netherlands, Department of Clinical Transfusion Research, Sanquin Research; Department of Immunohematology and Blood Transfusion, Leiden University Medical Center; Department of Immunohematology Diagnostics, Sanquin Diagnostic Services.

Corresponding author: Yolentha Slootweg, Leiden University Medical Center, Department

Obstetrics, Postbus 10392, postzone K6-P-35, 2300 WB Leiden, The Netherlands. Telephone:

0031 (0) 623604136, Email: y.m.slootweg@lumc.nl

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Condensation

Alloimmunized women with a known K-positive fetus and a titer of 4 or higher need

intensive clinical monitoring.

Short Title

Laboratory tests for predicting anti-Kell mediated HDFN

Implications and contributions

A. To assess the performance of anti-K titer and ADCC measurements in K-alloimmunized

pregnancies with a K-positive fetus, to predict severe hemolytic disease of the fetus and

newborn (HDFN) requiring transfusion therapy.

B. The first titer with a cut-off value of 4 has the best diagnostic accuracy to select

pregnancies at risk for severe HDFN. The ADCC test has no additional value to the titer.

2

1   C. This 16 year unselected cohort actualize that severe K-mediated HDFN can occur with

2   low titers, the importance of fetal K-typing in selection of high risk cases and shows that

3   the K-titer is not changing significantly during pregnancy.

4

5

Abstract

Background: There is controversy on critical cut-off values of laboratory testing to select

pregnancies at increased risk for anti-Kell (K) mediated HDFN (hemolytic disease of the fetus

and newborn). Without early detection and treatment, Anti-K mediated HDFN may result in

progressive fetal anemia, fetal hydrops, asphyxia and perinatal death.

Objective: We aimed to determine the value of repeated anti-K titer determination and

biological activity measurement using the antibody-dependent cellular cytotoxicity (ADCC)

test determination in the management of pregnancies at risk for anti-K mediated HDFN.

Study design: Retrospective cohort study of pregnancies with anti-K and a K-positive fetus,

identified from January 1999 until April 2015. Laboratory test results and clinical outcome

were collected from the Dutch nationwide screening program and the national reference

center for fetal therapy in the Netherlands, the Leiden University Medical Center. Diagnostic

accuracy (ROC-curves, sensitivity, specificity, positive and negative predictive values) of anti-

K titers and ADCC test. The relationship between the titer and ADCC measurements and the

two foregoing measurements were computed with a Pearson product-moment correlation

coefficient.

Results: In a 16 year unselected cohort, representing screening results of 3.2 million

pregnancies resulting in life births in the Netherlands, we identified 1,026 K-immunized

pregnancies. 93 pregnant women had anti-K and a K-positive child, without other red cell

alloantibodies. Forty-nine children (53%) needed intrauterine or postnatal transfusion

therapy. The first anti-K titer showed already a high diagnostic accuracy with an AUC of 91%.

The optimal cut-off point for the titer was 4 (sensitivity 100% (91-100; 95% CI), specificity

4

1   27% (15-43 95% CI) and positive predictive value 60% (49-71%). The ADCC test was not

2   informative to select high-risk pregnancies. Linear regression showed no significant change

3   during pregnancy, when antibody titer and ADCC test results were compared with every two

4   foregoing measurements (p<0.0001).

5   **Conclusion(s):** Early determination of the anti-K titer is sufficient to select pregnancies at

6   increased risk for HDFN with need for transfusion therapy. If the K status of the fetus is

7   known to be positive, a titer of 4 or higher can be used to target intensive clinical

8   monitoring.

9   **Keywords:** alloimmunization, anti-K, diagnostic accuracy, hemolytic disease of the fetus and

0   newborn (HDFN), intra-uterine blood transfusion, laboratory tests, red blood cell

1   antibodies, screening program.

2

1 Introduction

2 Hemolytic Disease of the Fetus and Newborn (HDFN) is caused by red blood cell (RBC)

3 antibodies developed by the mother and transferred to the foetus.[1, 2] Kell (K) alloantibodies

4 are second to RhD alloantibodies in importance as the cause of severe HDFN.[1, 2] K

5 alloantibodies cause hemolysis of fetal erythrocytes and also inhibit the fetal

6 erythropoiesis.[3-5] Without treatment, HDFN may result in progressive fetal anemia, fetal

7 hydrops, asphyxia and perinatal death.[6] After birth, neonatal hyperbilirubinemia may lead to

8 'kernicterus', cause of neurodevelopmental impairment with athetoid cerebral palsy,

9 hearing problems and psychomotor handicaps.[7-13] Even though the incidence of fetal

0 alloimmune hydrops has declined in the last decades,[14] this condition is still a well-known

1 risk factor for adverse perinatal and long-term outcomes.[15] Severe anti-K-mediated HDFN

2 may develop early in pregnancy, and often presents with hydrops before 20 weeks

3 gestation.[5, 15, 16] Postnatally, anti-K-mediated HDFN is characterized more frequently by

4 anemia than by hyperbilirubinemia, compared with HDFN caused by anti-D or other type of

5 Rh alloantibodies.[6]

6

7 Red blood cell (RBC) alloimmunization should ideally be detected early in pregnancy upon

8 routine RBC antibody screening. In most centers, to identify pregnancies at risk for severe

9 HDFN, the titer of clinically relevant RBC alloantibodies is determined.[1, 8, 17, 18] If the titer is

0 above a certain threshold, patients are referred to a maternal-fetal medicine center for

1 close surveillance and, if needed, for fetal or neonatal treatment. [17, 18] High-risk pregnancies

2 are monitored with ultrasound and Doppler middle cerebral artery peak systolic velocity

(MCA-PSV) measurements, to predict the presence of fetal anaemia.[19-21] Severe fetal

anemia can be successfully treated using intrauterine transfusions (IUT). Neonates may

require phototherapy or neonatal (exchange) transfusions.[22]

In the Netherlands, fetal K (Kell) genotyping is performed with cell-free fetal DNA isolated

from maternal plasma.[23] K-alloimmunized pregnancies with a K-positive fetus are monitored

by serial antibody titer measurements and by the Antibody Dependent Cellular Cytotoxicity

(ADCC) bioassay, a monocyte based assessment of the destructive capacity of the

antibodies.[24-26] However, there is still controversy on which critical titers and ADCC cut-off

levels indicate a high risk for anti- K-mediated HDFN.[17, 18, 27-31]

The aim of this study was to assess the performance of anti-K titer and ADCC measurements

in K-alloimmunized pregnancies with a K-positive fetus, to predict severe HDFN requiring

transfusion therapy.

Methods

Setting and Prevention program in the Netherlands

In the Netherlands, all pregnant women are typed for ABO, RhD and Rhc blood group

antigens and screened for RBC antibodies at the first trimester booking visit. All screen-

positive samples are sent to one of two national reference laboratories for confirmation and

determination of the antibody specificity. These laboratories are Sanquin Diagnostics,

Amsterdam (90% of the pregnant population) and the Special Institute for Blood Group

Investigations (BIBO), Groningen (10% of the pregnant population). When clinically relevant

RBC antibodies are detected, i.e. antibodies with the potency to destroy fetal RBC's, the

father of the fetus is typed for the cognate antigen(s). In case the father is antigen-positive,

7

or his type is not known, non-invasive fetal typing with cell-free fetal DNA isolated from

maternal plasma is offered (for *RHD*, *RHC*, *RHc*, *RHE* and *K*), since 2004. [23] If the fetus is

antigen-positive, serial testing (starting with every four weeks, from 24 weeks every three

weeks, from 36 weeks every two weeks) of maternal antibody titers and the ADCC test is

performed. Following current Dutch guidelines, a K-antibody titer ≥ 2 and/or an ADCC-test

result ≥30% indicate a substantial risk for K-mediated HDFN, and the fetus will be weekly or

every two weeks monitored with MCA Doppler measurements.[17] Laboratory follow-up is

stopped if these thresholds are reached. Severe fetal anemia is treated with intrauterine

transfusion(s) (IUT's) at the Leiden University Medical Center (LUMC), which is the national

Dutch reference center for fetal therapy. The threshold for suspected severe fetal anemia

requiring IUT was 1) a MCA-PSV of 1.5 multiples of the median for gestational age (MoM),

detected by Doppler measurement, and/or 2) the presence of other signs of anemia at

ultrasound examination (cardiomegaly, ascites, hydrops), or 3) amniotic fluid delta optical

density measurements reaching the upper part of Liley's zone II or zone III (only in the early

years of this study). [19, 32]

Laboratory testing

Both reference laboratories assess antibody titers, in phosphate-buffered saline by doubling

dilutions, with the indirect antiglobulin test (IAGT), using an anti-IgG reagent and

heterozygous K-positive RBCs.[33]

The ADCC test, as described by Engelfriet and Ouwehand, is only performed at Sanquin

Diagnostics in Amsterdam.[24] Fetal K typing is also only performed at Sanquin Diagnostics.[23]

1   *Study design*

2   We performed a retrospective cohort study, including all pregnancies diagnosed with anti-K

3   in the Netherlands, between January 1st 1999 and April 1st 2015. All K-immunization cases

4   were identified at the two national reference laboratories. Women with K alloimmunization

5   and antibody titers ≥2 and/or ADCC test results > 30% were usually referred to the LUMC

6   for monitoring or treatment. All these cases could therefore also be identified in the LUMC

7   database. We only included pregnancies with a K-positive fetus.

8   *Outcomes*

9   The primary outcome was the diagnostic accuracy (sensitivity, specificity and predictive

0   values) of antibody titers and ADCC tests to predict severe K-mediated HDFN, which was

1   defined as the need for intrauterine or postnatal transfusion.

2   *Data collection*

3   We collected the results of laboratory monitoring during pregnancy from Sanquin

4   Diagnostics and data concerning clinical monitoring and IUT treatment during pregnancy,

5   from the LUMC databases. Neonatal outcome data on treatment with blood transfusion(s)

6   or phototherapy during the first three months of life were extracted from their medical files,

7   by contacting the obstetric care provider, the pediatrician or the local hospital laboratories.

8   *Analysis*

9   Categorical variables were described as number and percentage and continuous variables by

0   median and interquartile range P25-75%. To establish the optimal cut-off for antibody titer

1   and ADCC test results, Receiver Operating Characteristics (ROC) curves were constructed for

2   both the first and the highest measurement. Subsequently, the sensitivity, specificity and

positive and negative predictive values for the prediction of fetal and neonatal hemolytic

disease were calculated with 2x2 tables for different cut-off levels. To determine the best

interval between consecutive titer and ADCC measurements, in order to adequately predict

severe HDFN, a linear regression analysis (Pearson product-moment correlation coefficient)

was performed. All analyses were performed with SPSS Statistics (version 23).

Ethical considerations

Clinical data were provided by the health care professionals as part of the quality evaluation

of the routine laboratory monitoring of RBC alloantibody-complicated pregnancies The data

were stored according to the Dutch established codes of conduct for responsible use of

patient material and data, as approved by the Leiden University Medical Center. Ethical

approval was not necessary according to the Dutch law on medical scientific research

involving human subjects and according to the rules published by the Central Committee on

Research involving Human Subjects (http://www.ccmo.nl/nl/niet-wmo-onderzoek).

Results

Study population

In 16 years, 1,026 K-immunized pregnancies were identified, including three pair of twins

(Figure 1). After exclusion of pregnancies with K-negative fathers (n=743) and/or K-negative

fetuses/children (n=83), miscarriages (<16 weeks, n=2) and loss-to follow up (n=6), 192

pregnancies with 195 K-positive fetuses remained for analysis. In another 70 pregnancies,

the K type of the father and that of the child were unknown (after 2008 all fetal K status

were known), but the absence of any sign of HDFN could be confirmed in all cases. After

exclusion of these pregnancies, 124 pregnancies with 125 K-positive fetuses remained.

Another seven of these 125 fetuses were excluded from our analysis, because of perinatal

death, clearly not related to K-immunization (n=2) or unknown neonatal outcome (n=5).

One case with severe HDFN (hydrops) detected late in pregnancy was excluded, because of

lack of laboratory data. Furthermore, we excluded 24 cases with additional red cell

alloantibodies, which may have contributed to the severity of HDFN. The remaining 93 (92

pregnancies) K-positive fetuses were used in our diagnostic accuracy analysis.

Fifty-six percent (49/93) of children received either intrauterine (48/93; 52%) or neonatal

(1/93; 1%) transfusion, whereas the remaining 47% (44/93) had no signs of HDFN or was

only treated with phototherapy . There were three cases with two pregnancies, all women

had in all pregnancies titers above 4.

There were three cases of perinatal death related to the K-immunization (titers variating

from 128-1024). One case was closely monitored every two weeks with ultrasound. At 23

weeks the fetus was unexpectedly found to be hydropic, with an MCA Doppler result

indicating fetal anemia. Fetal demise occurred just before the first IUT could be given. One

case was a very early onset of fetal anemia with hydrops detected at 15+3 weeks of

gestation. The fetus died at 16 weeks; intrauterine transfusion was not performed because

of the poor condition and prognosis at this early stage of the pregnancy. In the last case, the

fetus was initially predicted to be K-negative with non-invasive fetal K antigen typing, at 12

weeks of gestation. Awaiting the repeated and definitive K-typing result, clinical monitoring

was not performed and the fetus died at 18 weeks of gestation. At 19 weeks 'gestation, non-

invasive fetal K typing showed the fetus to be K-positive.

The median gestational age at the first laboratory testing was 14 weeks (P25-P75: 13-18

weeks). The median gestational age at the last measurement of titer and ADCC test was 26

weeks (P25-75: 18-35 weeks). The median first K-titer was 64 (P25-75: 8-256) and the

highest median titer was 128 (P25-75: 16-256). The median first ADCC was 35% (P25-75: 10-

57.5%) and the highest median ADCC was also 35% (P25-75: 15-65%). The median number

of days from the last test to the first IUT or to delivery was 26 days (P25-P75: 9-77 days).

The first IUT was performed in week 24 (P25-75: 22-28 weeks). The median number of

laboratory tests performed per pregnancy was 5 (P25-75: 3-8).

Diagnostic accuracy of the K-antibody titer and ADCC test

The ROC curves for respectively the first and the highest anti-K titer, correlating with severe

HDFN, with need for transfusion therapy (n=93) The Area Under the Curve (AUC) for the first

measured K antibody titer to predict the need for transfusion therapy was 0.917, for the

highest titer during pregnancy the AUC was 0.906. We defined the optimal cut-off point at a

sensitivity of 100% (91-100 95% CI) and combined with the highest specificity of 36% (23-52,

95% CI). Thus, an optimal cut-off for the first and highest titer was assessed at 4 (table 1).

The AUC for the highest ADCC test result was 0.843. If a sensitivity of 100% was taken, the

optimal cut off value appeared to be below the first test outcome of ADCC <10% (data not

shown). Therefore, additional ADCC testing seems to be not informative for the prediction

of severe HDFN (data not shown).

Linear correlation between consecutive measurements of titer and ADCC test

Since the AUCs for the highest titer and the first titer hardly differed, we investigated

whether the titer and ADCC test results changed significantly during pregnancy. Linear

regression showed no significant change, when antibody titer and ADCC test results were

compared with every two foregoing measurements. A Pearson product-moment correlation

coefficient was computed to assess the relationship between the titer and ADCC

measurements and the two foregoing measurements (for scatterplots see appendix 1

a,b,c,d). Overall, there was a strong correlation between titer and ADCC measurements with

the two foregoing measurements. The small, non-clinical relevant difference between the

measurements is explained for 94% and 91% (titer) and 87% and 84% (ADCC) by the two

foregoing measurements ($p<0.0001$).

Comments

Main Findings

In a 16 year unselected cohort, representing screening results of 3.2 million pregnancies

resulting in life births in the Netherlands, we identified 93 pregnancies complicated by the

presence of anti-K in the presence of a K-positive fetus. We determined that, if the K status

of the fetus is positive, an anti-K titer of 4 identifies all cases with a high risk for severe

HDFN, defined as the need for intra-uterine or postnatal transfusions. Test results of both

titer and ADCC-test did not change significantly during pregnancy. The first titer appeared to

have the highest power to predict the necessity of transfusion therapy in K-alloimmunized

pregnancies.

Strengths and weaknesses

To our knowledge this is the first large registry-based cohort study, including an unselected

complete population of K-alloimmunized pregnant women with a K-positive child. Most

other studies included a selected group of women with an increased risk for severe HDFN,

for example women referred to a regional or national referral centre.[27-29]

In our study, 93 out of 1,026 (9%) of the K-immunized pregnancies were considered as at

risk for HDFN (fetus K-positive) and included in the analysis. A weakness of our study is that

the K status was not known for all fetuses; yet we think no severe cases of HDFN with need

for intra-uterine transfusion were missed as they would have been referred to the LUMC.

Clinical implications

Overall, we observed in this unselected population, that over 50% of K-positive fetuses of K-

alloimmunized mothers need either intrauterine or postnatal transfusion therapy. At a cut-

off of 4 for the first titer the specificity is 27% and the positive predictive value for

transfusion therapy is 60%. This relatively high positive predictive value implicates that

transfusion therapy is needed in two out of three K-positive children of mothers with a K

titer of 4 or above, and may be an argument to accept this relatively low cut-off titer. In

order to fine tune the selection of high-risk cases, it would making it worthwhile to add fetal

K typing to the diagnostic algorithm. With higher cut-off titers, for example 16, still 96% of

severe cases will be followed, raising test specificity to 66% and the positive predictive value

to 76%. With this cut-off titers of 16 the number of missed cases of severe HDFN is three.

Our proposed optimal cut-off point of the anti-K antibody titer of 4 is only one dilution step

lower than suggested by Moise et al., who proposed a threshold of 8. It is also lower than

McKenna et al.[27] who proposed a threshold of 32, based on a smaller cohort with eight

cases of severe HDFN, all with titers of >=32. In contrast, Leggat et al., including 16 K-

positive fetuses, and our previous study, as reported by Van Wamelen et al. including 41 K-

positive fetuses, reported one case each needing intrauterine transfusion therapy at anti-K

titers of 2. [28, 29] These studies thus support our finding that also a low anti-K titer can be

responsible for a severe course of anti-K-mediated HDFN. However, in our study only 16

pregnancies of 93 cases had titers below 4, we didn't find severe cases of HDFN in this

group. Therefore K-mediated HDFN with need for transfusion therapy in cases with titers

below 4 is very rare.

Although titer measurements can vary between laboratories, also with established

techniques, in general a comparison be made with one-fold dilution difference between

observers and between laboratories. It is advised to use an indirect antiglobulin test without

additives.[33] Non-invasive fetal K-typing with cell-free DNA isolated from maternal plasma is

not available in all countries. Fetal K-typing can be performed with DNA obtained via

amniocentesis. Amniocentesis is an invasive procedure, with risks for the pregnancy and a

possible rise in anti-K titers. Therefore, it should be considered, if non-invasive fetal K typing

is not possible via a reference laboratory, if close monitoring of anti-K complicated

pregnancies with MCA-Doppler can be used for timely detection of the occurrence of fetal

anemia.

We observed that the monocyte-based ADCC test was not suitable to accurately select high-

risk K-alloimmunized pregnancies. This might be due to the pathogenesis of anti-K-mediated

HDFN, in which both the suppression of erythropoiesis and hemolysis of fetal RBC may be of

importance.[3, 4, 34] Recently, it was also shown that the glycoprofile of alloantibodies may

influence antibody pathogenicity and therefore a putative diagnostic marker.[34] Therefore, it

may be that other type of bioassays, testing such antibody characteristics, may improve test specificity.[26, 35, 36 37]

A first step in the diagnostic algorithm, that we currently use, is non-invasive fetal K typing with cell-free DNA isolated from maternal plasma.[38] Prediction of K-negativity warrants a high sensitivity of this PCR-based testing and the confirmation on sufficient levels of fetal DNA; also in our series early prediction of K-negativity was incorrect once in week 12 of pregnancy.[39] Since cell-free fetal DNA levels raise in the first trimester of pregnancy, for fetal K typing a conclusive result can in general be provided around week 18 of pregnancy.[23]

Conclusion

To select pregnancies with an increased risk for anti-K-mediated HDFN requiring frequent monitoring to detect fetal anemia, determination of the anti-K titer once early in pregnancy is sufficient. The optimal cut-off value is a titer of 4. Following the detection of anti-K, fetal K-typing, preferably using a non-invasive method, is an important step in efficient management. In pregnancies with an anti-K titer of 4 or higher and a positive fetus, 60% of fetuses or neonates requires transfusion therapy. Since the ADCC test is not useful in the prediction of fetal hemolysis in the presence of an anti-K we recommend discontinuing its use for these pregnancies.

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Reference List

1. KOELEWIJN JM, VRIJKOTTE TGM, VAN DER SCHOOT CE, BONSEL GJ, DE HAAS M. Effect of screening for red cell antibodies, other than anti-D, to detect hemolytic disease of the fetus and newborn: a population study in the Netherlands. Transfusion 2008;48:941-52.

2. DE HAAS M, THURIK FF, KOELEWIJN JM, VAN DER SCHOOT CE. Haemolytic disease of the fetus and newborn. Vox Sang 2015;109:99-113.

3. VAUGHAN JI, WARWICK R, LETSKY E, NICOLINI U, RODECK CH, FISK NM. Erythropoietic suppression in fetal anemia because of Kell alloimmunization. American Journal of Obstetrics and Gynecology 1994;171:247-52.

4. WEINER CP, WIDNESS JA. Decreased fetal erythropoiesis and hemolysis in Kell hemolytic anemia. American Journal of Obstetrics and Gynecology 1996;174:547-51.

5. DANIELS G, HADLEY A, GREEN CA. Causes of fetal anemia in hemolytic disease due to anti-K. Transfusion 2003;43:115-6.

6. KAMPHUIS MM, LINDENBURG I, VAN KAMP IL, MEERMAN RH, KANHAI HH, OEPKES D. Implementation of routine screening for Kell antibodies: does it improve perinatal survival? Transfusion 2008;48:953-7.

7. DANIELS GL. Blood group antibodies in haemolytic disease of the fetus and newborn; in: Hadley A, Soothill P (eds): Alloimmune disorders of pregnancy. Anaemia, thrombocytopenia and neutropenia in the fetus and newborn. Cambridge: Cambridge university press, 2002.

8. MOISE KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Seminars in fetal & neonatal medicine 2008;13:207-14.

9. MOISE KJ, JR. Management of rhesus alloimmunization in pregnancy. Obstetrics and gynecology 2008;112:164-76.

10. FRANKLIN I. Prevention of rhesus haemolytic disease of the fetus and newborn. Lancet 2009;373.

11. KLEIN HG AD. Haemolytic disease of the fetus and the newborn. In: Klein HG, Anstee DJ, editors. Mollison's blood transfusion in clinical Medicine. 2012:499-548.

12. WATCHKO J, TIRIBELLI C. Bilirubin-induced neurologic damage--mechanisms and management approaches. N Engl J Med 2013;369:2021-30.

13. BHUTANI VK, ZIPURSKY A, BLENCOWE H, et al. Neonatal hyperbilirubinemia and Rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels. Pediatric research 2013;74 Suppl 1:86-100.

14. VAN KAMP IL, KLUMPER FJCM, MEERMAN RH, OEPKES D, SCHERJON SA, KANHAI HHH. Treatment of fetal anemia due to red-cell alloimmunization with intrauterine transfusions in the Netherlands, 1988 - 1999. Acta Obstetricia et Gynecologica Scandinavica 2004;83:731-37.

15. LINDENBURG IT SWV, VAN KLINK JM, VERDUIN E, VAN KAMP IL, WALTHER FJ, ET AL. Long-term neurodevelopmental outcome after intrauterine transfusion for hemolytic disease of the fetus/newborn: the LOTUS study. Am J Obstet Gynecol 2012;206:141-48.

16. RATH MEA, SMITS-WINTJENS VEHJ, LINDENBURG ITM, et al. Exchange transfusions and top-up transfusions in neonates with Kell haemolytic disease compared to Rh D haemolytic disease. Vox Sanguinis 2011;100:312-16.

17. NVOG DUTCH ASSOCIATION OBSTETRICS AND GYNAECOLOGY. *Red blood cell immunisation and pregnancy.* Number of pages.

18. ACOG Practice Bulletin No. 75: Management of alloimmunization during pregnancy. Obstetrics and gynecology 2006;108:457-64.

19. OEPKES D, SEAWARD PG, VANDENBUSSCHE FP, WINDRIM R, KINGDOM J, BEYENE J. Doppler ultrasonography versus amniocentesis to predict fetal anemia. N Engl J Med 2006;355:156-64.

20. ILLANES S, SOOTHILL P. Management of red cell alloimmunisation in pregnancy: the non-invasive monitoring of the disease. Prenatal Diagnosis 2010;30:668-73.

21. MARI G, DETER RL, CARPENTER RL, et al. 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. The New England journal of medicine 2000;342:9-14.

22. SMITS-WINTJENS VEHJ. Rhesus haemolytic disease of the newborn: Postnatal management, associated morbidity and long-term outcome. Seminars in fetal and neonatal medicine 2008;13:265-71.

23. SCHEFFER PG VDSC, PAGE-CHRISTIAENS GC, DE HAAS M. 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 2011;118:1340-48.

24. ENGELFRIET CO, WH. . ADCC and other cellular bioassays for predicting the clinical significance of red cell alloantibodies. Balliere's Clin Haematol 1990;3:321-37.

25. URBANIAK SJ, GREISS MA. ADCC (K-cell) lysis of human erythrocytes sensitized with rhesus alloantibodies. III. Comparison of IgG anti-D agglutinating and lytic (ADCC) activity and the role of IgG subclasses. British journal of haematology 1980;46:447-53.

26. HADLEY AG. Laboratory assays for predicting the severity of haemolytic disease of the fetus and newborn. Transplant immunology 2002;10:191-8.

27. MCKENNA DS, NAGARAJA HN, O'SHAUGHNESSY R. Management of pregnancies complicated by anti-Kell isoimmunization. Obstetrics & Gynecology 1999;93:667-73.

28. LEGGAT HM, GIBSON JM, BARRON SL, REID MM. Anti-Kell in pregnancy. BJOG: An International Journal of Obstetrics & Gynaecology 1991;98:162-65.

29. VAN WAMELEN D, KLUMPER FJ, DE HAAS M, MEERMAN RH, VAN KAMP IL, OEPKES D. Obstetric History and Antibody Titer in Estimating Severity of Kell Alloimmunization in Pregnancy. Obstetrics & Gynecology 2007;Volume 109, :1093-98.

30. MOISE KJ. Non-anti-D antibodies in red-cell alloimmunization. European journal of obstetrics, gynecology, and reproductive biology 2000;92:75-81.

31. DE HAAS M, KOELEWIJN JM, BILGIN K, VRIJKOTTE TGM, VAN DER SCHOOT CE, BONSEL GJ. Diagnostic performance of laboratory monitoring to predict severe haemolytic disease of the fetus and newborn in non-RhD-alloimmunised pregnancies. Detection and prevention of pregnancy immunisation, The OPZI-study, Academic Thesis 2009;Chapter 4:73-85.

32. LILEY AW. Intrauterine transfusion. Annali di ostetricia, ginecologia, medicina perinatale 1971;92:539-42.

33. DOWNES KAS, I.A. Pretransfusion Testing. In: Fung MG, B.J.; Hillyer, C.D.; Westhoff, C.M.;, ed. *Technical manual*. Bethesda: AABB, 2014.

34. SONNEVELD ME, KOELEWIJN J, DE HAAS M, et al. Antigen specificity determines anti-red blood cell IgG-Fc alloantibody glycosylation and thereby severity of haemolytic disease of the fetus and newborn. British journal of haematology 2017;176:651-60.

35. HADLEY AG, KUMPEL BM, LEADER KA, POOLE GD, FRASER ID. Correlation of serological, quantitative and cell-mediated functional assays of maternal alloantibodies with the severity of haemolytic disease of the newborn. British journal of haematology 1991;77:221-8.

36. NANCE SJ, NELSON JM, HORENSTEIN J, ARNDT PA, PLATT LD, GARRATTY G. Monocyte Monolayer Assay: An Efficient Noninvasive Technique for Predicting the Severity of Hemolytic Disease of the Newborn. American Journal of Clinical Pathology 1989;92:89-92.

37. CONTRERAS M, ENGELFRIET CP, OUWEHAND WH. Blood Transfusion: The Impact of New Technologies6 ADCC and other cellular bioassays for predicting the clinical significance of red cell alloantibodies. Baillière's Clinical Haematology 1990;3:321-37.

38. ELLEN VAN DER SCHOOT C, MARTINE TAX GH, RIJNDERS RJP, DE HAAS M, CHRISTIAENS GCML. Prenatal typing of Rh and kell blood group system antigens: The edge of a watershed. Transfusion Medicine Reviews 2003;17:31-44.

39. FINNING K, MARTIN P, SUMMERS J, DANIELS G. Fetal genotyping for the K (Kell) and Rh C, c, and E blood groups on cell-free fetal DNA in maternal plasma. Transfusion 2007;47:2126-33.

1   Table 1. Number of positive tests, sensitivity, specificity and predictive values

2   of K-mediated pregnancies without additional antibodies to predict the need for

3   transfusion by cut-off first titre (N=93).

First titre Cut-off	Positive tests	Need for transfusion					
		True positive	*Missed HDFN cases	Sens % (95%-CI)	Spec % (95%-CI)	PPV % (95%-CI)	NPV % (95%-CI)
≥2	81	49	0	100 (91-100)	27 (15-43)	60 (49-71)	100 (70-100)
≥4	77	49	0	100 (91-100)	36 (23-52)	64 (52-74)	100 (76-100)
≥8	70	47	2	96 (85-99)	48 (33-63)	67 (55-78)	91 (70-98)
≥16	62	47	2	96 (85-99)	66 (50-79)	76 (63-85)	94 (77-99)
≥32	57	45	4	92 (80-97)	73 (57-85)	79 (66-88)	89 (73-96)
≥64	50	43	6	88 (75-95)	84 (70-93)	86 (73-93)	86 (71-94)
≥128	43	38	11	78 (63-88)	89 (75-96)	88 (74-96)	78 (64-88)

4   *Cases with necessity for transfusion therapy that would be missed when cut-off used.

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1   FIGURE 1: FLOWCHARTS OF INCLUSION K-IMMUNIZED PREGNANCIES WITH A K-ANTIGEN POSITIVE FETUS.

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3   APPENDIX 1: SCATTERPLOTS OF RELATION BETWEEN TITER AND ADCC MEASUREMENTS WITH THE TWO

4   FOREGOING MEASUREMENTS.