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Received: 11 July 2024 | Accepted: 20 August 2024

DOI: 10.1111/bjh.19740

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

SHORT REPORT

Transfusion

Major reduction in occurrence of anti-c and anti-E in pregnancy after more than 10 years of preventive matched transfusion with most benefit for c-matching

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Summary

Extension with cE-matching of the transfusion policy for women under 45 years to prevent alloimmunization and hemolytic disease of the foetus and newborn (HDFN) was evaluated. After implementation of cEK-matching, anti-c occurrence decreased from 46.8 to 30.4 per 100 000 pregnancies (RR 0.65, 95% CI 0.54–0.79), while anti-E occurrence decreased from 122.1 to 89.9 per 100 000 pregnancies (RR 0.74, 95% CI 0.66–0.84). The c-negative women showed a higher anti-E occurrence before cEK-matching and a more pronounced decline with the new policy. This indicates that cEK-matched transfusion effectively reduces alloimmunization, and that a cK-matched approach could prevent most transfusion-related alloimmunization and HDFN.

KEYWORDS

hemolytic disease of the fetus and newborn, immunohaematology, pregnancy, red cell antigens, transfusion

INTRODUCTION

Maternal antibodies against foetal red blood cell (RBC) antigens can lead to severe haemolytic disease of the foetus and newborn (HDFN). HDFN can occur early in gestation with signs of anaemia and hydrops, requiring intrauterine transfusions. Perinatal, immune-mediated RBC destruction may lead to hyperbilirubinaemia, requiring intensive phototherapy, exchange transfusions and/or transfusion dependency in the first 3 months of life. To timely detect pregnancies at risk for HDFN, women are screened for RBC alloantibodies early in pregnancy. With respect to HDFN disease severity, after anti-D and anti-K, the Rh-subtype specific anti-c and anti-E are most relevant in Western pregnant populations.[1]

Women can become alloimmunized due to exposure to RBCs via pregnancy, transfusion or in rare cases after transplantation. Additionally, anti-E alloantibodies can occur without previous RBC exposure, as so-called 'naturally occurring'.[2] We previously reported that among pregnant women with anti-c, -E and -K, respectively, 49%, 37% and 83%, had prior ABO-D-matched blood transfusions, which may have induced alloimmunization.[3]

Internationally, next to matching for D, matching for K and other Rh antigens (C, c, E and/or e) is often applied to prevent HDFN[4,5] and 'cEK-matching' was strongly advocated in the Netherlands since the late nineties. Selection of K-negative units for women under 45 years of age was introduced in the Dutch guidelines in 2004 and was extended with preventive c- and E-matching in 2011.[5] K-matching led to 70% reduction in anti-K in pregnancy and 56% decrease in the number of pregnancies at risk for anti-K-mediated HDFN.[6] We anticipated that the effect of matching for c and E would be lower, due to a lower alloimmunization risk and antigen distribution. In the Dutch pregnant population, K-negativity is common (90%), whereas c-negativity is less common—roughly 20%,[7] and almost all these women will be also E negative due to the RHCe haplotype. The rate of E-negativity is high with 73% and anti-E in these women may be 'naturally occurring'.[8]

In this observational cohort study, we assessed the effectiveness of the c- and E-matched transfusion strategy for women under 45 years in preventing alloimmunization in pregnancy.

METHODS

Study design and setting

Laboratory data were used from a national screening programme among pregnant women who has an uptake of 99%. Women are screened for both infectious diseases and RBC alloantibodies before 13 weeks of gestation. Data on RBC alloimmunization were derived from the laboratory information systems of the two reference laboratories, which perform antibody testing when the locally performed screening for alloantibodies yields a positive result.

Implementation of the matched transfusion policy

Selection of cE-matched RBC products for female transfusion recipients under 45 years of age is prescribed by the national transfusion guideline from 2011, but some centres implemented this earlier because the donor's Rh and K phenotype was displayed on all products since 1999. We assessed the proportion of cE-matched transfusions over time using data from an inquiry among all Dutch hospitals and regular hospital RBC usage data.

Data selection

Similar to our previous study,[6] all pregnancies with anti-c and/or anti-E detected in the first trimester of pregnancy between 1 January 2002 and 31 December 2022 were selected. Variables were the year of detection of the antibody, the pregnant women's Rh phenotype as well as the fathers' antigen typing for the implicated antigen. Pregnancies with a c- or E-positive father were considered to be at risk for the development of HDFN. Data on foetal antigen typing and pregnancy outcomes were not available. The occurrence of anti-c and anti-E was compared to that of other RBC alloantibodies to account for effect modifiers, such as a stricter transfusion policy. Pregnancies with antibodies anti-Fy^a, Fy^b, Jk^a, Jk^b, S or s (named 'reference antibodies') were selected from Sanquin Diagnostic Services' laboratory information system. Details of the data selection and technical aspects are described in the Supplemental Materials.

Prevalence of Rh phenotypes

An overview of the risks of antigen exposure by transfusion or pregnancy in the Dutch population is provided in Tables S1 and S2. To estimate the occurrence of naturally occurring anti-E, D-negative women with anti-E were studied. In the absence of an E-matched transfusion strategy, D-negative women will hardly be immunized by transfusion since 97% of D-negative donor blood in the Netherlands is negative for E. We assumed therefore that the anti-E in women with E-negative partners was 'naturally occurring'.

Presentation of data and data analysis

The numbers of antibodies are expressed as occurrences per 100 000 pregnancies. Therefore, yearly numbers of pregnancies were determined from birth rates obtained from Statistics Netherlands, which were adjusted for 3.8% miscarriages after the first-trimester antibody screen.[7] In our analysis of anti-E and the women's Rh phenotypes, the occurrence of anti-E was expressed per 100 000 women with the indicated phenotype. The relative risks (RR) and 95% confidence intervals (CI) were calculated between the situation at baseline before (2002–2004) and after implementation of cE-matching (2020–2022) using data from 3-year periods to reduce variation.

RESULTS

Implementation of matching for c and E antigens

Data on the year of actual implementation of the cE-matched transfusion strategy were obtained from 47 (55%) of 85 hospitals, representing 68% of the national RBC use. According to the data shown in Figure S1, 56% of units were already cE-matched before the prescription in the transfusion guideline in 2011, and 15% in 2002 before the baseline period of 2002–2004.

Occurrence of anti-E, anti-c and reference alloantibodies in pregnancy over time

From 2002 to 2022, approximately four million pregnancies were screened. Anti-c was detected in 1428 pregnancies, anti-E in 3662 pregnancies, and both anti-c and anti-E antibodies in 456 pregnancies. Any (or a combination) of the reference antibodies (anti-Fy^a, Fy^b, Jk^a, Jk^b, S or s) was found in 1938 pregnancies.

Figure 1 shows the declining rates of anti-c, anti-E and reference antibodies and the relative risk (RR) for the presence of these antibodies after full implementation of the cE-matched transfusion strategy (2020–2022) compared to the period before cE-matching (2002–2004). Along with anti-c and anti-E, the occurrence of reference antibodies also decreased over time (from 62.8 to 50.1 per 100 000 pregnancies (RR 0.81, 95% CI 0.69–0.95)), indicating the presence of an effect modifier.

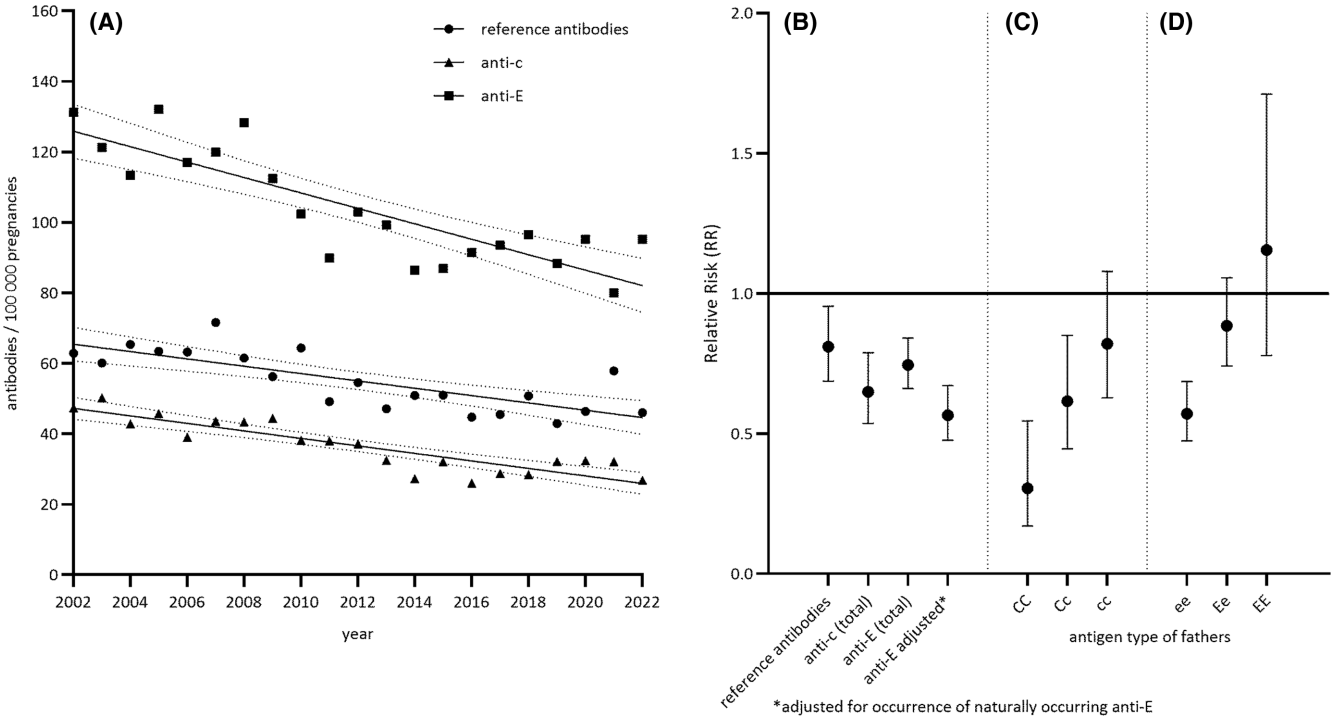

FIGURE 1 Occurrence (A) and Relative Risk (RR) (B–D) on c-, E- and reference antibodies in 2020–2022 compared to 2002–2004.

Occurrence of anti-c and risk for HDFN

The occurrence of anti-c was 46.8 per 100 000 pregnancies in 2002–2004 and decreased to 30.4 per 100 000 pregnancies in 2020–2022 (RR 0.65, 95% CI 0.54–0.79; Figure 1A,B).

The c phenotype of the fathers ($n = 1267$) was homozygous c positive: $n = 678$, heterozygous c positive: $n = 524$ and c negative: $n = 22\,665$ registered and 161 assumed to be c negative (see also Supplemental Materials). The occurrence of pregnancies with a c-positive biological father and thus a risk to develop HDFN decreased from 38.1 per 100 000 pregnancies in 2002–2004 to 27.8 per 100 000 pregnancies in 2020–2022 (RR 0.73, 95% CI 0.59–0.89; Figure 1C). Figure S2 shows the yearly occurrence of anti-c over time per antigen type of fathers. The decrease in anti-c was highest in pregnancies with a c-negative father (RR 0.31, 95% CI 0.17–0.54), indicating a decrease attributable to c-matching.

Occurrence of anti-E and risk for HDFN

The occurrence of anti-E decreased from 122.1 per 100 000 pregnancies in 2002–2004 to 89.9 per 100 000 pregnancies in 2020–2022 (RR 0.74, 95% CI 0.66–0.84; Figure 1A,B). The E phenotype of the fathers ($n = 3662$) was homozygous E positive: $n = 261$, heterozygous E positive: $n = 1623$ and E negative: $n = 1778$ (1353 registered and 425 assumed to be E negative). The pregnancies with a risk for HDFN decreased from 60.1 per 100 000 pregnancies in 2002–2004 to 55.2 per 100 000 pregnancies in 2020–2022 (RR 0.92, 95% CI 0.79–1.09; Figure 1D). The yearly occurrence of anti-E over time per antigen type of fathers is depicted in Figure S3. The decrease of anti-E was highest for the pregnancies with an E-negative father (RR 0.57, 95% CI 0.47–0.69).

'Naturally occurring' anti-E

We calculated that 'naturally occurring anti-E' was present in on average 45.9 per 100 000 D-negative pregnancies (dashed area in Figure 2A, calculation in Supplemental Materials). When a stable number of 45.9 naturally occurring anti-E/100 000 pregnancies is presumed in any given E-negative population, and the occurrence of anti-E is adjusted by this number, this would reflect a slightly higher reduction of 76.2 per 100 000 pregnancies in 2002–2004 to 44.0 per 100 000 pregnancies in 2020–2022 of anti-E caused by previous exposure to the E antigen (RR 0.56, 95% CI 0.48–0.67).

Anti-E and Rh phenotype of pregnant women

Since previous reports indicated an overrepresentation of women with the CCDee phenotype among women with anti-E,[8,9] we studied the occurrence of anti-E for women with different Rh phenotypes, expressed per 100 000 pregnancies of women with that specific Rh phenotype (Figure 2A,B). As presumed, the occurrence of anti-E in D-negative women is lowest and did not significantly change (RR 0.96, 95% CI 0.68–1.37). When comparing D-positive phenotypes, the occurrence of anti-E was remarkably higher in the women with

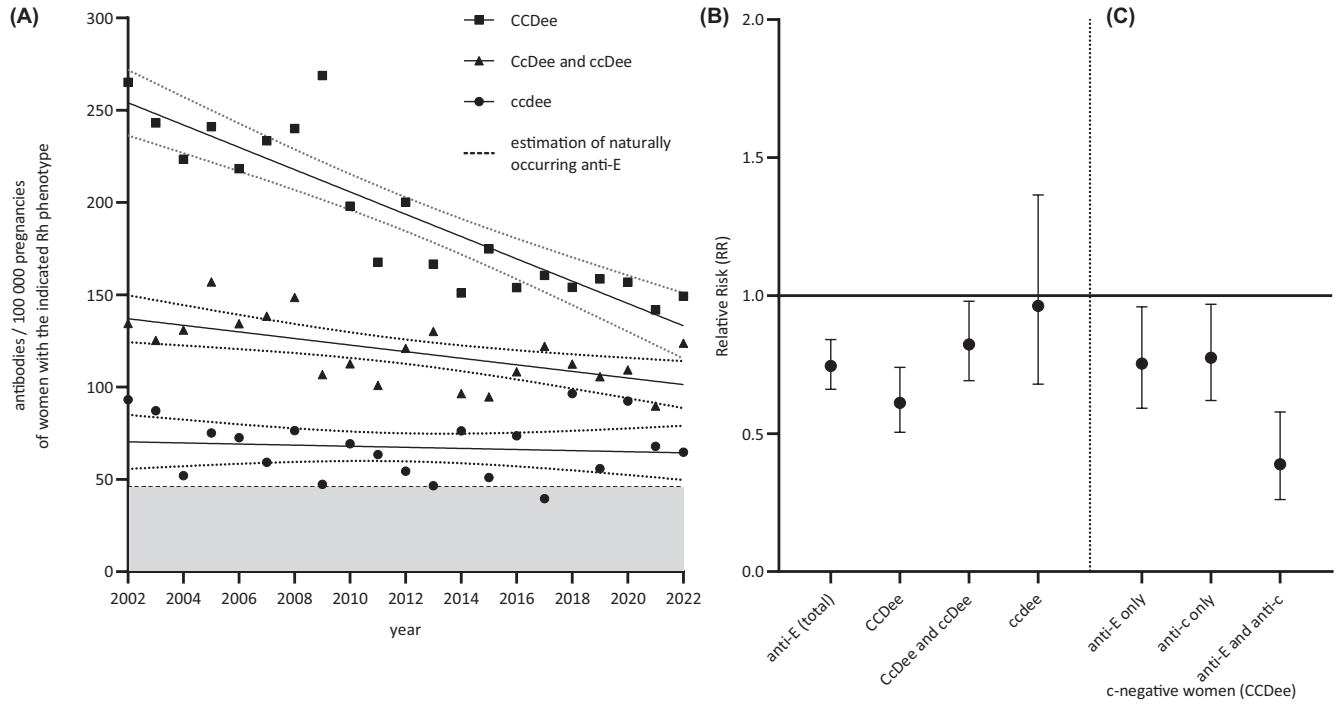

FIGURE 2 Occurrence (A) and relative risk (B) on anti-E per indicated women's Rh phenotype and antibody combinations (C).

Rh phenotype CCDee and the decrease in anti-E was more pronounced in this population. The occurrence of anti-E decreased from 244.2 in 2002–2004 to 149.2 in 2020–2022 (RR 0.61, 95% CI 0.50–0.74) per 100 000 pregnancies of women with Rh phenotype CCDee and from 130.3 to 107.3 (RR 0.82, 95% CI 0.69–0.98) per 100 000 pregnancies of women with other D-positive phenotypes. We did not observe this correlation of Rh phenotypes and alloimmunization rate for anti-K (Figure S4). In line with this, for women with the CCDee phenotype the decrease of the combination of anti-E and anti-c was more than that of anti-E or anti-c alone (Figure 2C). The yearly occurrence over time of anti-c, anti-E and combinations of both antibodies is shown in Figure S5. The occurrence of pregnancies with anti-E and a risk to develop HDFN decreased from 125.1 to 98.0 per 100 000 pregnancies of women with the CCDee phenotype (RR of 0.78, 95% CI 0.61–1.00).

DISCUSSION

This observational cohort study reveals a decrease in c- and E-alloimmunization during pregnancy following the introduction of a c- and E-matched transfusion protocol for female recipients under 45 years of age. After 10 years of preventive cE-matching, anti-c showed a low occurrence of 30.4 per 100 000 pregnant women and an RR of 0.65 (95% CI 0.54–0.79). The occurrence of anti-E remained higher at 89.9 per 100 000 pregnancies, but by subtracting an estimated amount of 'naturally occurring anti-E' the frequency was assessed as 44.0 per 100 000 pregnancies and an RR of 0.56 (95% CI 0.48–0.67).

We observed a more pronounced decline in anti-c and anti-E in women with pregnancies with antigen-negative fathers, underscoring that the decrease attributable to cE-matching. But while RBC alloantibody frequencies reduce by preventive matching,[6,10] a beneficial effect on the prevention of HDFN is often questioned.[11,12] However, we also observed a decrease in alloimmunization rate in c-negative women with c- and/or E-positive partners, and thus a risk on HDFN: for anti-c an RR of 0.73 (95% CI 0.59–0.89) and for anti-E an RR of 0.78 (95% CI 0.61–1.00) was observed, without taking naturally occurring anti-E into account.

In line with older literature,[2,9] we observed a higher occurrence of anti-E before preventive matching in women with a c-negative phenotype (CCDee), and a remarkably more pronounced decline of anti-E after matching. This group showed, without adjustment for naturally occurring anti-E, an RR of 0.61 (95% CI 0.50–0.74). Apparently, c-negative women have a higher risk for activation of their immune system to produce anti-E. We postulate that immune-induced formation of anti-E by women with the CCDee phenotype will mostly result from exposure to RBCs expressing cE-type polypeptides, since the CE type is rare.[8] Hence, c-matching results in simultaneous matching for E. In mice, several studies have shown that CD4+ Th cells providing help to B cells for RBC alloantibody formation do not have to respond to a peptide derived from the same antigen as the B-cell receptor is recognizing.[13,14] In women-typed CCDee, if a D-positive blood

transfusion is not matched for c, or if a D-positive pregnancy is c-incompatible, there will be exposure to both a c antigen and an E antigen on the same Rh polypeptide. It is conceivable that this leads to Rhc-peptide responsive Th cells also providing help to B cells recognizing the RhcE polypeptide and producing anti-E.

In this study, reference antibodies were used to account for other changes over time. Data in older literature, as well as work in mouse models, highlight that alloimmunization against certain RBC targets may provide help in the immune response to other RBC targets.[13–15] Hence, K-matching, cE-matching and a more restrictive transfusion policy possibly all contributed to the observed reduction in alloimmunization in pregnant women. This may even affect the production of alloantibodies for which no matching strategy is in place. Therefore, the comparison with reference antibodies may lead to underestimating the effect of preventive cE-matching.

A strength of this study is the long study period of 21 years, covering over 99% of Dutch pregnancies, including four million pregnancies within a situation of a centralized transfusion policy. In this period, the number of first pregnancies and average number of pregnancies per woman remained relatively constant.[6] A limitation is that by 2011, 56% of blood usage was already cE-matched, potentially resulting in an underestimation of the effect of cE-matching. Additionally, we made predictions to estimate the frequency of Rh phenotypes among Dutch pregnant women, which may have introduced a minor degree of inaccuracy.

In conclusion, we demonstrate that a reduction in the occurrence of anti-c and anti-E is achieved by a cE-matched transfusion policy. Notably, the decrease in anti-E was more prominent among women with a c- and E-negative phenotype, whereas we could not clearly demonstrate the effectiveness of E-matched transfusion for c-positive women. Therefore, a change of the current cEK-matched transfusion strategy to a cK-matched approach could be considered to balance efforts for matched transfusion with optimal prevention of severe HDFN.

AUTHOR CONTRIBUTIONS

MdH, JSL and CCF designed the study; JSL, MVL and JHM collected the data; JSL performed the analysis; MdH, CCF and CEvdS contributed to the analysis and review of the data; JSL, CCF and MdH wrote the manuscript. All authors participated in revising and finalizing the manuscript and approved it for submission.

FUNDING INFORMATION

No direct funding has been received for this research.

CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ETHICS APPROVAL AND PATIENT CONSENT STATEMENT

Pregnant women are informed about data registration and testing in the screening programme by their obstetric caregiver. Pseudonymized data were used which are allowed without informed consent according to the Dutch Code of Conduct for Health Research and Medical Treatment Contracts Act (WGBO).

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SUPPORTING INFORMATION

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

How to cite this article: Luken JS, Folman CC, Meekers JH, Lukens MV, van der Schoot CE, de Haas M. Major reduction in occurrence of anti-c and anti-E in pregnancy after more than 10 years of preventive matched transfusion with most benefit for c-matching. Br J Haematol. 2024;00:1–6. https://doi.org/10.1111/bjh.19740