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The effect of extended c, E and K matching in females under 45 years of age on the incidence of transfusion-induced red blood cell alloimmunisation

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Summary

Maternal alloantibodies directed against fetal red blood cell (RBC) antigens may cause potentially life-threatening haemolytic disease of the fetus and newborn (HDFN). Dutch transfusion guidelines therefore prescribe preventive cEK matching for all (pre-)fertile females. To quantify the impact of cEK matching, we compared overall and antigen-specific cumulative RBC alloimmunisation incidences in females and males aged <45 years. Among a multicentre cohort comprised of patients who received their first and subsequent RBC unit between 2005 and 2019, first-formed RBC alloantibodies were detected in 47 of 2998 (1·6%) females and 49 of 2507 (2·0%) males. Comparing females and males, overall alloimmunisation incidences were comparable (3·1% [95% confidence interval (CI) 2·1–4·4] *versus* 3·5% (95% CI 2·4–4·9, $P = 0·853$) after 10 units transfused). However, cEK alloimmunisation incidences were significantly lower among females (0·6% (95% CI 0·3–1.5) *versus* 2·2% (95% CI 1·5–3·4, $P = 0·001$) after 10 units transfused). Yet, despite cEK-matching guidelines being in effect, 6·5%, 3·6% and 0·2% of all RBC units remained mismatched for c, E or K antigens respectively. Most of these mismatches were almost always due to emergency settings. Even though cEK alloimmunisation was not prevented completely, implementation of cEK matching resulted in an alloantigen-exposure risk reduction of up to 98%.

Keywords: alloimmunisation, blood transfusion, extended matching, females, HDFN.

Introduction

Haemolytic disease of the fetus and newborn (HDFN) is a rare but severe pregnancy-related condition, caused by maternal red blood cell (RBC) alloantibodies passing the placental barrier and inducing fetal RBC destruction. Depending on the severity of this destruction, anaemia, icterus and its pathophysiological consequences can culminate into severe fetal and neonatal morbidity and even mortality.[1,2] During the first trimester, RBC alloantibodies with

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other specificities than anti-D are detected in ~1·0% of pregnant females,[3] while eventually up to 0·08% of all pregnancies result in severe cases of HDFN (i.e. HDFN that requires intrauterine transfusion or neonatal exchange transfusion or top-up transfusions in combination with phototherapy).[4]

Alloantibodies of the immunoglobulin G (IgG) type that pass the placental barrier may eventually induce HDFN. In this respect, in Caucasian and Black populations alloantibodies directed against the D, K and c antigens are primarily associated with severe HFDN, while to a lesser extent this can be observed for other Rhesus (Rh) blood group system antibodies (mainly anti-E), and rarely for other type of non-Rh antibodies.[5] In alloimmunised females pregnant with a cognate antigen-positive child, most of these alloantibodies originate from primary immunisation following fetomaternal haemorrhage and incompatibility between the RBC antigens of the fetus and mother. In these cases, with a risk of HDFN, the minority of maternal RBC alloantibodies are due to foreign RBC antigen exposure by blood transfusions prior to the pregnancy.[6]

Notwithstanding the latter, given the potentially devastating consequences of HDFN, maximum efforts to prevent RBC alloimmunisation should be aimed for. The latter is dependent on an efficient, large-scale blood supply capable of typing for additional antigens, such as in the Netherlands, where preventive matching for K antigen for all females under the age of 45 years has been mandated since 2004, followed by c and E matching in 2011.[7] Introduction of K matching was recently reported to be associated with a reduction of anti-K prevalences among a general population of pregnant females, although the causal contribution of blood transfusions remains to be established.[8] Notwithstanding, beneficial effects of extended matching on transfusion-induced RBC alloimmunisation seem logical. In this regard, we aimed to quantify the impact of the nationwide implementation of cEK matching on cumulative RBC alloimmunisation incidences in general and per antigen in females aged <45 years.

Methods

Study design and study population

This study was performed among the incident new-user Risk Factors for Alloimmunisation after red blood Cell Transfusion (R-FACT) study cohort.[9] For this study, data from five Dutch hospitals were available from January 2005 until January 2019 (Table SI). Methods on the establishment of this study cohort have been published previously.[10] To summarise, the incident new-user cohort consisted of newly transfused, previously non-alloimmunised patients who received their first and subsequent RBC unit during our study period, provided the availability of at least one pre- and post-transfusion antibody screen. Second, immunisations to clinically relevant RBC alloantigens present on standard three-cell screening panels were defined as RBC alloimmunisation (i.e. C, c, E, e, K, C^w, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, Lu^a, Lu^b, M, N, S and s). Other antibodies were not included because these are not routinely screened for, and their inclusion would thus have led to selection bias. Over the course of our study period, antibody screening and subsequent alloantibody detection were performed using column agglutination systems in all participating centres. Patients in whom an alloimmunisation event could not be linked to a preceding RBC transfusion expressing the cognate antigen were excluded. Third, we excluded patients with a positive screen within 7 days after a first mismatched transfusion, as we considered these reactions likely to be caused by secondary boosting rather than a primary alloimmune response. Fourth, we then nominated the last RBC unit prior to the first positive antibody screen and containing the cognate antigen mismatch as the transfusion that most likely had elicited RBC alloimmunisation, i.e. the implicated transfusion. Here, we assumed units with missing cognate antigen phenotypes to be positive. Although it is likely that in addition to the final mismatched unit preceding alloimmunisation detection, earlier mismatched units also contributed to immunisation, the here chosen implicated transfusion strategy at least prevented underestimation of exposure and thus overestimation of cumulative alloimmunisation incidences. Fifth, we excluded infants aged <6 months, as young children have been reported to have poor antibody responses during the first months of life. If data were available, we excluded patients with haemoglobinopathies, as these patients had received preventive extended matching strategies beyond indications evaluated in the present analysis.

As almost all study centres performed cEK matching long before implementation of national matching guidelines (Table SI), we were unable to determine the impact of cEK matching in females by directly comparing alloimmunisation incidences before and after implementation. We therefore compared RBC alloimmunisation incidences in cEK matched females to non-matched males. For this reason, we only included males and females who received RBC transfusions under the age of 45 years. One study centre did not perform cEK matching before the national implementation, thus in this study centre only females who received their first and subsequent RBC unit(s) according to cEK-matching guidelines were included in our analyses.

The study protocol was approved by the Ethical Review Board in Leiden and by the board of each participating centre.

Data collection

Pseudonymised general and transfusion data were collected by the participating centres and contained: sex, date of birth, RBC products' unique identification numbers [Eenheid Identificatie Nummer (EIN)], dates and results of alloantibody screening, and alloantibody specificity. In addition, antigen phenotypes of all transfused RBC products were supplied by

Sanquin Blood Supply, Amsterdam. Data on race and/or ethnicity in the patient and donor population were not routinely registered.

Data analysis

Cumulative numbers of transfused RBC units received up to the implicated transfusion for alloimmunised patients and up to the last negative screen for non-alloimmunised patients were determined.

To quantify the impact of cEK matching, we compared cumulative overall and antigen-specific (here cEK) alloimmunisation incidences for females and males aged <45 years using Kaplan–Meier analyses. Age per individual corresponded to the date at which the first RBC unit had been received. As the number of patients at risk decreases with the number of transfusions received, we present cumulative incidences up to a total of 20 units received. In addition, to assess whether matching for specific RBC group antigens also protects against immunisation against other (non-matched, non-linked) antigens, we compared alloimmunisation incidences against cEK and non-cEK antigens in females and males. Finally, we assessed general adherence to the nationwide guideline by determining the ratio of cEK-positive RBC units transfused to cEK-negative females (i.e. mismatched transfusions) and performed clinical chart review to evaluate underlying reasons for these protocol violations.

Results

Among 85 510 newly transfused patients, 38 810 patients fulfilled the study criteria, of whom 2998 females and 2507 males below the age of 45 (Figure S1). In addition, 373 females were excluded because these females received their first RBC unit before local implementation of cEK-matching policies. General characteristics of this study cohort and the types of alloantibodies are presented in Table I. Patients received a median [interquartile range (IQR)] of 3 [2–8] RBC units during a median (IQR) follow-up of 122 (18–584) days. Transfusion-induced alloimmunisation occurred in 47 (1·6%) females and 49 (2·0%) males, including eight and 35 cEK alloantibodies formed in these females and males respectively. A complete overview on numbers and specificities of alloantibodies formed during our study period is presented in Table SII.

Red blood cell alloimmunisation and the impact of cEK matching in females aged <45 years

Among females, the cumulative incidence of RBC alloimmunisation after 10 units transfused was 3·1% [95% confidence interval (CI) 2·1–4.4] as compared to 3·5% (95% CI 2·4–4·9, $P = 0·853$) in males (Fig 1A). In addition, incidence of cEK alloimmunisation among females after receiving 10 units was 0·6% (95% CI 0·3–1.5) as compared to 2·2% (95% CI 1·5–3·4, $P = 0·001$) among males (Fig 1B). Separately, cumulative anti-K incidences were only 0·1% (95% CI 0·0–0.6) *versus* 0·7% (95% CI 0·3–1·4, $P = 0·003$) amounting to anti-c, or anti-E incidences of 0·5% (95% CI 0·2–1.4) *versus* 1·6% (95% CI 1·0–2·7, $P = 0·017$) after 10 units (Figure S2). Among females, the incidence of non-cEK antibodies was 2·5% (95% CI 1·7–3.7) after 10 units transfused, whereas among males this was 1·5% (95% CI 0·9–2·6, $P = 0·003$) (Figure S3). An overview of cumulative alloimmunisation incidences after different number of RBC units transfused is presented in Table SIII.

Adherence to cEK matching in females aged <45 years

For 448 of 2998 females (14·9%) and 880 of 18 403 RBC units (4·8%), c, E and/or K antigen phenotypes were untraceable. All these females and the RBC units they received were thus omitted from the analysis on matching adherence. Overall, only 278 (1·6%) of 17 523 RBC units were c, E and/or K incompatible and were transfused to 126 (4·2%) females, of whom eight (0·3%) subsequently formed cEK alloantibodies. More than half of the mismatched units were transfused during emergency settings (Table II). In total, 6·5% (220/3404), 3·6% (61/12 613) and 0·2% (29/16 289) of all RBC units transfused to females aged <45 years were mismatched for the c, E, or K antigen respectively. Figure 2 shows that only for a minor fraction of these mismatched transfusions no valid reason was available (i.e. unexplained transfusions).

Discussion

Following the nationwide implementation of cEK matching in females aged <45 years, the present study reports a fourfold lower incidence of transfusion-induced alloimmunisation to c, E, or K antigens in females as compared to males in the Netherlands. Consequent to a strict adherence to this matching policy, only 1·7% of all RBC products were incompatible for c, E and/or K antigens, which were predominantly transfused in emergency settings. Ultimately, transfusion-induced cEK alloantibody formation occurred only in a few (0·3%) females as compared to 1·4% of males.

The present study is the first of its kind quantifying the impact of cEK matching on transfusion-induced RBC alloimmunisation incidences in newly transfused female patients. In addition, detailed evaluation of cEK-matching guideline adherence and further analysis to why mismatched RBC units were transfused in these females have, to the best of our knowledge, not been published before.

In agreement with our expectations, antigen-specific matching (here cEK) results in a decreased alloantibody formation to that specific antigen. Notably, matching against specific high-immunogenic RBC antigens did not seem to protect against alloimmunisation to other (non-matched,

Table I. General characteristic of study cohort.

	Females <45 years (n = 2998)	Males <45 years (n = 2507)
Age at first transfusion, years, median, IQR	30·1 (17·2–36·8)	23·7 (10·4–37·2)
Academic hospital, n (%)	1951 (65·1)	1849 (73·8)
Number of cumulative units transfused	18 403	21 885
Number of units per patient, median (IQR)	3 (2–6)	4 (2–9)
Follow-up, days, median (IQR)	156 (18–732)	96 (18–399)
Red blood cell alloimmunisation, n (%)		
Alloimmunised patients	47 (1·6)	49 (2·0)
Patients with >1 alloantibody*	2 (4·3)	7 (14·3)
Alloantibodies formed	49	56
K alloantibodies	1 (2·0)	12 (21·4)
c alloantibodies	3 (6·1)	4 (7·1)
E alloantibodies	4 (8·2)	19 (33·9)

*As patients were censored at the time of first positive antibody screen, these included patients had more than one alloantibody simultaneously detected.

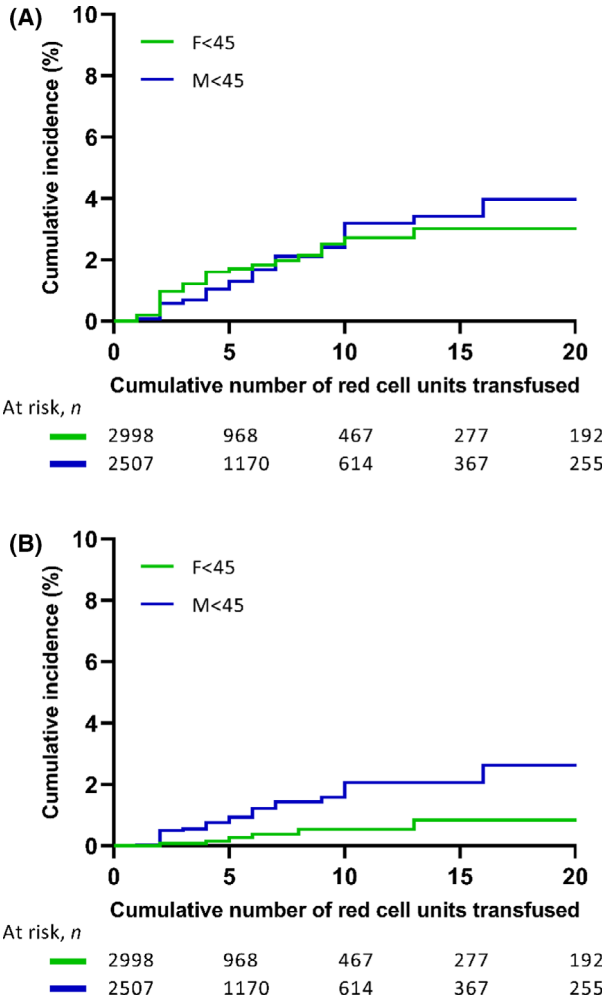

Fig 1. (A) Cumulative alloimmunisation incidence as a function of cumulative number of red blood cell units exposed. (B) Cumulative alloimmunisation incidence against cEK-antigens as a function of cumulative number of red blood cell units exposed. [Colour figure can be viewed at wileyonlinelibrary.com]

non-linked) 'weaker' RBC antigens by a mechanism of epitope spreading as suggested by others.[11] Comparing the standard distribution of RBC antigen phenotypes among the Caucasian population[12] to the one in our extended matched study population, the probability for a c-negative female to randomly receive a c-positive RBC unit in non-matching conditions was 80%, whereas in matching conditions it was 6·5%. Thus, the overall risk reduction of receiving a mismatched RBC unit was 91·9% for c, and similarly calculated 87·6% for E, and 97·8% for K. Despite that the active cEK-matching guidelines were applied throughout our entire study cohort, a fraction of females at risk were still exposed to cEK-incompatible RBC units. Naturally, c-mismatches exceeded E and K mismatches, likely due to a higher distribution of E- and K-negative phenotypes amongst the mostly Caucasian donor population. In addition, over 50% of mismatched units were transfused under critical (massive haemorrhagic) emergency circumstances, where mismatches were forced due to pending antigen phenotyping results or exhaustion of inventories.

Supporting our present findings, the effect of K matching on K alloimmunisation incidences in pregnant females and its ultimate effect on the development and severity of HFDN has been recently described.[8] That study demonstrated a 70% reduction of K alloimmunisation in the first trimester of pregnancy since the implementation of K-matching guidelines in the Netherlands in 2004, although other explanatory conditions associated with a reduction in allo-K exposure over time should be considered as well. In addition, the benefit of preventive extended matching on the reduction of alloantibody formation has been extensively demonstrated in other high-risk patients, e.g. in patients with haemoglobinopathies[13,14] and myelodysplastic syndrome.[15] Importantly, most studies did not directly compare extended with non-extended matched patients, and reported prevalence rather than incidence.[16,17] Finally, preventive extended matching for

Table II. Characteristics of cEK mismatched red blood cell units.

	Mismatched units (n = 278)
Age at time of mismatched transfusion, years, median (IQR)	30·7 (20·7–38·4)
Academic hospital, n (%)	178 (64·0)
Transfused prior to national cE matching guideline implementation, n (%)	107 (38·5)
Reasons for non-matched unit selection, n (%)	
Emergency transfusions	145 (52·2)
Gynaecology/obstetrics	41 (14·8)
Surgery	7 (2·5)
Trauma	52 (18·7)
Other	45 (16·2)
Phenotype unknown at time of transfusion	41 (14·8)
Compatible red blood cell units unavailable in storage	27 (9·7)
Other	16 (5·8)
Unknown reasons	49 (17·6)

selected antigens as compared to standard ABO/RhD matching reduced the primary alloimmunisation rate by 64% in a cohort of patients undergoing elective (cardiac) surgery.[18]

Coinciding of transfusion-induced RBC alloimmunisation and severe HDFN is a very rare event (i.e. RBC alloimmunisation occurred in 1·6% of our young females and only 3% of severe HDFN is caused by transfusion-induced alloantibodies[6]). Ultimately, implementation of cEK-matching guidelines resulted in a significant reduction of cEK-mismatched transfusions, but importantly did not reduce c, E, or K alloantibody formation after blood transfusion to zero. An ultimate cost–benefit analysis of these alloimmunisation preventions should include the effect on HDFN incidences and their associated health effects. Naturally, the effect on HDFN prevention will not transfer to all (pre-)fertile females receiving matched transfusions as some of them for various reasons will not become pregnant after transfusion. Unfortunately, quantification of the impact of extended matching on HDFN is challenging as, at least in the Netherlands, data on pregnancy history is for most patients not registered. Lastly, each prevented case of transfusion-induced HDFN related with serious morbidity and even mortality is vital. Thus, we hope our study motivates others to follow our extended matching guidelines in these young females. However, we acknowledge that the required donor and patient typing and an adequate sufficiently typed blood supply, as are implemented in the Netherlands, can be cumbersomely restricted elsewhere by logistic (blood supply) capacities and financial challenges.

Our present study is important in several aspects. We performed our analyses in a large incident new-user cohort comprising all first-time transfused patients from five large Dutch hospitals with up to 14 years of follow-up. Furthermore, exposure-related confounding was handled by using the cumulative number of RBC units transfused as the time-dependent variable in our survival model. Primary transfusion-induced RBC alloimmunisations were only included after careful selection (i.e. identification of the implicated transfusion and usage of a lag period to exclude boosting events). Finally, the Netherlands is a small country with well-regulated and extensively documented blood supply ensuring broad, reliable data of donor phenotypes, RBC unit identification numbers and transfusion dates.

Our present study design, findings and interpretations require additional remarks:

First, because matching implementation varies, our data set did not allow a direct comparison between alloimmunisation incidences before and after implementation of cEK matching in females, we compared young females to young males. Naturally, such a comparison assumes a similar potency of the male and female immune systems to react to foreign RBC antigens. However, at least for older females, studies have reported increased alloimmunisation incidences compared to older males, possibly related to intrinsic patient-related factors associated with the female gender (i.e. oestrogen levels, persisting fetomaternal chimerism).[19,20] Such an intrinsic difference between female *versus* male immunisation might be reflected by a slightly higher female immunisation incidence observed for the non-cEK antigens. If so, our reported impact of cEK matching might even underestimate the real impact of cEK matching for young females. On the other hand, our data did not include indications for RBC transfusion and thus, differences in the underlying disorders necessitating transfusions may have influenced patients' immune responses differently (e.g. females receiving RBC units after postpartum haemorrhage compared to males receiving RBC units after trauma). However, gender-specific conditions are to date not known to be associated with RBC alloimmunisation risks. Furthermore, demographic and ethnic conditions are not gender-specific and thus will not have influenced our conclusions. Hence, the slightly higher non-cEK alloimmunisation incidences among females should be interpreted considering known gender-related intrinsic biological differences (e.g. oestrogen

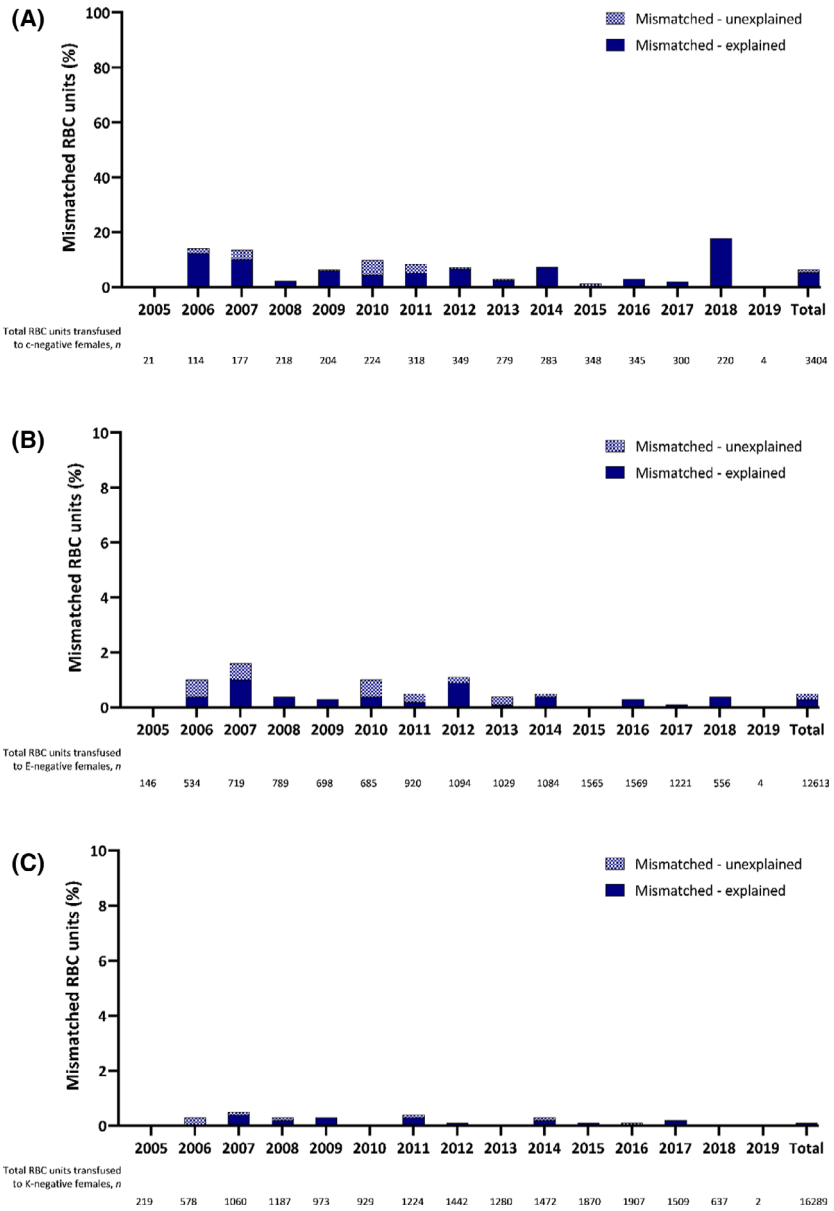

Fig 2. Proportions of cEK-mismatched red blood cell (RBC) units transfused to cEK-negative females aged <45 years during the study period. (A) Proportions of c-mismatched RBC units received by c-negative females. (B) Proportions of E-mismatched RBC units received by E-negative females. (C) Proportions of K-mismatched RBC units received by K-negative females. Explained mismatches were defined as: emergency transfusions, unknown phenotype at the time of transfusion, unavailability of compatible units and other. Mismatched transfusions received for unclear reasons were defined as 'unexplained'. [Colour figure can be viewed at wileyonlinelibrary.com]

related), and at most minutely influenced by an uneven distribution of clinical conditions among males and females. Finally, differences in alloimmunisation incidences caused by misclassification of boosting of pregnancy-induced alloantibodies *versus* primary transfusion-induced alloimmunisation were handled by an uniform lag period of 7 days strategy. Also, as data on pregnancies prior or during the transfusion episode are not routinely registered in Dutch patients' electronic files, alloimmunisation incidences might partly be caused by boosting of pregnancy-induced alloantibodies. Because a primary immune response and a subsequent detectable antibody titre need a certain time, we excluded very early post-transfusion (within 7 days) formation of alloantibodies, as possibly reflecting a boosting response. However, true primary alloimmunisation on the other hand might take <7 days while boosting sometimes takes longer, depending on the type of RBC antigen and the timing of previous immunisation. Hence, to investigate possible modulation of our results by this arbitrarily chosen lag period, we performed an additional sensitivity analyses extending our lag period to 14 days. This excluded 12 patients but did not alter our overall results on alloimmunisation incidences (data

not shown). We therefore regard the influence of misclassification of primary *versus* secondary boosting responses to be minimal.

As the present study reports absolute alloimmunisation incidences for the overall transfused population, the net benefit of extended matching for the individual female patient will also depend on immunomodulating conditions present, of which some diminish and others increase alloimmunisation risks.[21–25] Thus, the presented absolute risk reductions should be only extrapolated when taking the specific clinical context of the patient into account.

Second, we cannot fully rule out the possibility that included patients received their first RBC unit in another, non-participating centre prior to our present study period due to the lack of such data in transfusion records. Taken into account that in the Netherlands all detected RBC alloantibodies are centrally registered in a national database,[26,27] our study centres can be assumed to have had knowledge of the presence of previous detected alloantibodies. Finally, to optimise the selection of only primary alloimmunisations, we excluded all patients with alloantibody formations before the first positive antibody screen date in the study centre.

Third, a large fraction of the newly transfused patients had to be excluded because these patients never underwent a follow-up antibody screen. Furthermore, post-transfusion screens are not routinely performed in the Netherlands resulting in a wide variety of intervals between initial transfusion and subsequent antibody screen testing. Reports have estimated that one-quarter of alloantibodies can become serological undetectable within 1 month after initial detection, and even halved after 6 months.[28] Due to the absence of standardised mandated post-transfusion screening and antibody evanescence, only 31·6% of alloantibodies are regularly detected by antibody screening.[29] Thus, the present studies presented alloimmunisation incidences might be an underestimation of true incidences.

Fourth, most of the Dutch patients as well as the donor population were of Caucasian origin. Thus, our present results should be interpreted with caution for other populations, for which due to differences in antigen prevalence and (constitutionally determined) clinical phenotypes, the immune response to cEK antigens may differ with related impacted on matching-induced prevention.

In conclusion, RBC alloimmunisation incidences to cEK antigens were importantly reduced by preventive matching of female patients when compared with males under 45 years of age. Despite a strict adherence to matching guidelines, alloimmunisation against cEK antigens was not completely prevented due to inevitable transfusions of mismatched RBC units in emergency settings. Considering our comparison between females and males, we must finally acknowledge that our present results may be further influenced by possible sex-related differences in immune response. With indeed females having a higher incidence in forming alloantibodies against non-cEK antigens, we hypothesise that the observed effect of cEK matching might be even underestimated. In-depth evaluation of the cost-effectiveness of cEK alloimmunisation reduction at present among only females, and with this the prevention of the most severe forms HFDN, is warranted.

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Author contributions

Jaap Jan Zwaginga and Johanna G. van der Bom designed the study. Josine A. Oud, Karen M. K. de Vooght, Daan van de Kerkhof, Nel Som, Nathalie C. V. Péquériaux and Francisca Hudig collected the data. Josine A. Oud, Dorothea Evers, Masja de Haas, Jaap Jan Zwaginga and Johanna G. van der Bom analysed and interpreted the data. Josine A. Oud, Dorothea Evers, Masja de Haas, Jaap Jan Zwaginga and Johanna G. van der Bom wrote the manuscript. All the authors revised and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest relevant to the work presented in this manuscript.

Fig S1. Study flow diagram.
Fig S2. Cumulative alloimmunisation incidence against cE-antigens as a function of cumulative number of red cell units exposed.
Fig S3. Cumulative alloimmunisation incidence against non-cEK-antigens as a function of cumulative number of red cell units exposed.
Table SI. Implementation of K and cE matching per participating center.
Table SII. Overview of all alloantibodies formed in our study population.
Table SIII. Overview of cumulative alloimmunisation incidences (i.e. general, cEK and non-cEK) after different cumulative number of red blood cell units transfused.

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