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CHAPTER B

Risk factors for the presence of non-Rhesus D red blood cell antibodies in pregnancy

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ABSTRACT

Objective: To identify risk factors for the presence of non-RhesusD (RhD) red blood cell (RBC) antibodies in pregnancy. To generate evidence for subgroup RBC antibody screening and for primary prevention by extended matching of transfusions in women <45 years.

Design: Case-control study.

Setting: Nation-wide evaluation of screening programme for non-RhD RBC antibodies.

Population: Cases: consecutive pregnancies (n=900) with non-RhD immunisation identified from Sept 1st 2002 until June 1st 2003 and Oct 1st 2003 until July 1st 2004; controls (n=968): matched for obstetric care worker and gestational age.

Methods: Data collection from the medical records and/or from the respondents by a structured phone interview.

Main Outcome Measure: Significant risk factors for non-RhD immunisation in multivariate analysis.

Results: Significant independent risk factors: history of RBC transfusion (OR 16.7; 95%-Cl:11.4-24.6), parity (para-1 versus para-0 OR 1.3, 95%-Cl:1.0-1.7; para-2 versus para-0 OR 1.4, 95%-Cl:1.0-2.0; para->2 versus para-0 OR 3.2, 95%- Cl:1.8-5.8), haematological disease (OR 2.1; 95%-Cl:1.0-4.2), history of major surgery (OR 1.4; 95%-Cl:1.1-1.8). For the clinically most important antibodies anti-K, anti-c and other Rh-nonD antibodies RBC transfusion was the most important risk factor, especially for anti-K (OR 96.4; 95%-Cl: 56.6-164.1); 83% of the K-sensitized women had a history of RBC transfusion. Pregnancy-related risk factors were a prior male child (OR 1.7; 95%-Cl:1.2-2.3) and caesarean section (OR 1.7; 95%-Cl:1.1-2.7).

Conclusions: RBC transfusion is by far the most important independent risk factor for non-RhD immunisation in pregnancy, followed by parity, major surgery and haematological disease. Pregnancy-related risk factors are a prior male child and caesarean section. Subgroup screening for RBC antibodies, with exclusion of RhD-positive parae-0 without clinical risk factors, is to be considered. This approach will be equally sensitive in detecting severe HDFN compared to the present RBC antibody screening programme without preselection. Primary prevention by extending preventive matching of transfusions in women younger than 45 will prevent more than 50% of pregnancy immunisations.

INTRODUCTION

In pregnancy, the presence of clinically relevant maternal red blood cell (RBC) alloantibodies other than anti-Rhesus D (RhD) is relatively rare; in population studies the prevalence of this so-called non-RhD immunisation is between 0.15% and 1.1%.¹⁻⁶ In this report we use non-RhD immunisation as a basket term, referring to the presence of all maternal RBC antibodies other than RhD antibodies and ABO antibodies that can theoretically cause haemolytic disease of the fetus and newborn (HDFN); this implies all non-RhD RBC antibodies that can cross the placenta and are directed against blood group antigens known to be expressed by the fetal RBCs.

By now, over 250 blood group antigens with their corresponding antibodies have been identified, and grouped into 29 systems: e.g. Kell (with for example the antigens K and k), Rhesus (C, c, C^w, E, e), Duffy (Fy^a and Fy^b), Kidd (Jk^a and Jk^b) etcetera.⁷ The population prevalence of these blood group antigens shows wide variation: e.g. 9% for the K antigen, 99% for the k antigen, 82% for the c antigen, 65% for the C antigen and only 2% for the C^w antigen in Caucasians.⁸ As a consequence, the probability that a woman who lacks a specific blood group antigen, will be exposed to this antigen, varies considerably. The most frequent causes of immunisation are RBC transfusions and fetomaternal haemorrhage (FMH) during pregnancy and delivery, as neither the transfused RBCs nor the fetal RBCs perfectly match those of the recipient. A therapeutic RBC transfusion involves the administration of usually 280 ml (one unit) to 560 ml (in case of two units) of red blood cell concentrate. FMH involves smaller amounts: in 64% of women fetal RBCs are detectable in the maternal circulation after delivery, usually in smaller amounts than in 20 ml of fetal blood. Several conditions increase the risk of FMH such as spontaneous miscarriage, termination of pregnancy, in utero diagnostic procedures, external version, caesarean section, vaginal assisted delivery, and surgical removal of the placenta.9-12

The individual blood group antigens differ in their potency to induce an antibody response (immunogenicity), listed in order of decreasing immunogenicity: D, K, E, c, Jk, Fy.¹³⁻¹⁶ Finally, additional variation exists in the ability to cause HDFN. HDFN, severe enough to require treatment by antenatal intra uterine fetal transfusions and/or neonatal (exchange) transfusions occurs in 2-4% of non-RhD immunised pregnancies. Anti-K, anti-c and to a lesser extent other Rh antibodies (C, C^w, E, e) cause the vast majority of cases of severe HDFN.^{6;17;18}

While RhD immunisation has decreased strongly due to the policy of matching RBC transfusions for RhD and to the introduction of anti-D immunoprophylaxis in the late 1960s¹⁹; no primary prevention measures have been taken for other antigens. In most countries RBC transfusions are not matched for other antigens than ABO and RhD. Since 2004 it has been recommended in the Netherlands to only transfuse K-negative blood

Chapter 3

to women aged younger than 45.²⁰ In general only secondary and tertiary prevention measures are taken for HDFN caused by non-RhD RBC antibodies comprising RBC antibody screening of all women during pregnancy, subsequent monitoring of pregnancies at risk of HDFN and - if indicated - antenatal and postnatal treatment of severe HDFN cases. Because of the relatively low prevalence of non-RhD antibodies and of severe HDFN (0.33% and 0.01% respectively in the Netherlands ⁶), this screening policy results in high

Numbers Needed to Screen (15,000 in the Netherlands) and a relatively high number of 'false positives': pregnancies at risk of HDFN that are intensively monitored, where no severe HDFN occurs.⁶

In this study we aimed to identify risk factors for the presence of non-RhD antibodies early in pregnancy. First, to investigate whether it is possible to increase the efficiency and to decrease the burden of the RBC antibody screening programme by restricting this screening to women at risk of having relevant RBC antibodies (subgroup screening). Second, to evaluate the importance of putatively avoidable risk factors and enable primary prevention measurements at reducing the number of non-RhD immunised pregnant women. Since we have previously shown that in 40% of pregnant women with non-RhD RBC antibodies the father did not carry the corresponding antigen ⁶, we were especially interested in the magnitude of the effect of a prior RBC transfusion and the options to prevent alloimmunisation by extended matching of RBC transfusions.

METHODS

National screening programme

Since July 1st 1998 the Dutch screening programme offers free of charge RBC antibody screening as part of the booking visit protocol around the 12th week of pregnancy. This protocol includes typing for ABO and screening for HIV, hepatitis B and syphilis. The obstetric care worker (in the Netherlands: independent midwife [\pm 80%], general practitioner [\pm 5%] or obstetrician [\pm 15%]²¹) is responsible for the administration of the screening test. The coverage of the RBC antibody screening is close to 100%.²² Certified Dutch laboratories (n = \pm 90) process the RBC antibody screening test. A positive screening result is checked by one of two specialized national reference laboratories. After confirmation of the positive RBC antibody screening result, the risk for HDFN is determined by establishing the antibody specificity (occasionally more than one), and by subsequent serological typing of the father for the antigen(s) against which the maternal alloantibodies are directed. If the father has the corresponding antigen, the pregnancy is considered 'at risk of HDFN'.

Study design

A nation-wide case-control study was performed. All pregnant women with clinically

relevant non-RhD RBC antibodies in The Netherlands constituted the cases in two predefined nine-month cohorts (September 1st 2002 until June 1st 2003 and October 1st 2003 until July 1st 2004); for practical reasons no women were included during an interval of four months in summer. We supposed that a seasonal effect on immunisation risk and on risk factors is very unlikely. Women with non-RhD antibodies in combination with RhDantibodies were excluded. Cases were recognized by routine first trimester RBC antibody screening and confirmed in the two national reference laboratories; all screen-positive samples detected in peripheral laboratories were sent to these reference laboratories (n=1,002). During the inclusion phase of the first nine-month cohort, the obstetric care workers in primary care (midwives and general practitioners), asked three controls (with a RBC antibody-negative screen result) to participate in the study; because of time constraints obstetricians (clinical care) recruited only one control. Controls were matched on care practice and on pregnancy duration (\pm one month). No additional matching on e.g. parity or ethnicity was performed to allow these factors to be studied for their possible causal contribution. Information about antibody specificity and antigen typing of the father was collected at the reference laboratories. General and pregnancy-related risk factors were collected by the obstetric care worker with an extensive structured questionnaire which was piloted in advance. If questionnaire completion by the obstetric care worker was not feasible (70%), data were collected in a telephone interview with the pregnant women instead (JK, TV). The study was approved by the relevant professional organisations (obstetricians, midwives, general practitioners, paediatricians, clinical laboratories). Representatives of these organisations monitored the study process. Cases and controls all gave informed consent.

Risk factors

Risk factors underlying RBC antigen immunisation were divided into general and pregnancy-related risk factors.

Risk factors were only taken into account in cases if these were present prior to the first discovery of the RBC antibodies. General risk factors were listed as: age, history of RBC transfusion, history of thrombocyte transfusion, parity, gravidity, ethnicity (based on the racial classification which is routinely used in national obstetric registration) and variables related to increased risk of blood transfusion: presence of haematological diseases specified as sickle cell disease, thalassaemia, autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpurae, or history of surgery or more specifically major surgery which was defined as: orthopaedic surgery, cardiac surgery, transplantation, extirpation of the spleen, surgery after trauma. Finally, variables which may influence the immune response after exposition to allogeneic RBCs, such as the use of drugs/medication and the presence of immunological diseases (and of haematological diseases, such as listed

above) were investigated.

Pregnancy-related risk factors were all factors possibly related to increased FMH, such as miscarriage, termination of pregnancy, blood loss, trauma, invasive diagnostics in utero, external version, twin pregnancy, postmaturity, caesarean section, instrumental delivery, surgical removal of the placenta. Active work and active sport during pregnancy were also recorded. Moreover factors possibly related to the clearance of fetal cells after FMH were included: blood group (grouped into A or AB/B or O), and administration of anti-D immunoglobulin after birth of an RhD-positive child.

Analysis

First, univariate analysis of risk factors was performed (Pearson's chi square test, Fisher's exact test (n<5) or Student's t-test, depending on the measurement level of the variable). All cases and controls were used in the analyses for the general risk factors. For analysis of the pregnancy-related risk factors, only data of multigravid women (for the risk factor abortion) or parous women (for the other risk factors) without a history of nonpregnancy-related RBC transfusion and with an antigen-positive partner (implying the possibility of an antigen-positive prior child) were used. This subset allowed analysis of isolated FMH-related factors. Next, multivariate logistic regression analysis was performed. Univariate factors shown to be important (p < 0.10) were offered stepwise to the model. As the proportion of non-Dutch women in controls (8.7%) was not representative for the pregnant Dutch population (18.7% in 2003²¹), ethnicity was offered to the multivariate models as a fixed factor to adjust for the difference between cases (15% non-Dutch) and controls, if present. To determine the contribution of RBC transfusion and pregnancy, the analyses were performed in two different groups of cases and controls. Group I: all cases and controls to determine the contribution of the risk factors in the general population. Group II: all cases with an antigen-positive partner and all controls to determine the independent contribution of RBC transfusion and a pregnancy from an antigen-positive partner. As an outcome we used both the presence of any non-RhD immunisation (900 cases) and of the following specific immunisations with a substantial risk of severe HDFN: K-immunisation (221 cases) c-immunisation (154 cases) and Rh immunisation, other than RhD or Rhc (457 cases).

By design, controls under primary care in early pregnancy (with a lower prevalence of potential risk factors such as a prior caesarean section or a pregnancy-related RBC transfusion) were over-represented, which could contribute to over-estimation of the effect of potential risk factors. We therefore restored the proportion of primary care pregnancies in the control group (843/968=87%) to the population proportion of 80% ²² by weighting the primary care controls with 0.59 ((0.8*125)/(0.2*843). These weighted data were used in all analyses. Missing values (<1%) were not substituted. Goodness of fit

of the logistic regression models was assessed by the standard Hosmer and Lemeshow test. All statistical analysis were performed in SPSS 11.0.

We modelled the risk for non-RhD immunisation for combinations of significant risk factors, based on a 0.33% population prevalence of non-RhD antibodies ⁶ (calculations of the model available from the authors).

RESULTS

Description of the study population

In this study 1,002 pregnant women with non-RhD RBC antibodies were consecutively included. Data on risk factors were available from 900 of these cases (response rate of 90%), i.e. 527 cases at risk of HDFN (paternal antigen positive or unknown) and 373 cases not at risk (paternal antigen negative), and from 968 controls (primary care: n=843, secondary care n=125).⁶

The presence of RBC antibodies was already known before screening in the ongoing pregnancy in 36% of the cases (322/900, of which 185 at risk and 137 not at risk for HDFN); the paternal antigen was already known before pregnancy in 209 of these cases (126 at risk, 83 not at risk).

In the case group 21 cases (4%) of severe HDFN occurred: five cases received an intra uterine fetal transfusion, ten other cases received a neonatal exchange transfusion and the remaining six cases only received an RBC transfusion during the first week of life. Moderate HDFN, treated with phototherapy only, was seen in 71 cases (14%).

Outcome data about RBC transfusion around the current delivery were available of 897/900 cases and of 412/968 controls. An RBC transfusion around delivery was administered to 6.1% (55/897) of the cases and to 1.7% of the controls (7/412).⁶

Univariate analysis

Significant general risk factors for the presence of non-RhD red cell antibodies in pregnancy were prior gravidity, parity (delivery \geq 16 weeks), a history of RBC transfusion, of thrombocyte transfusion, of surgery, and the presence of an haematological or immunological disease (Table 3.1). To determine the influence of pregnancy-related risk factors we selected women with antigen-positive partners and excluded women who had a history of non-pregnancy-related RBC transfusion. Significant pregnancy-related risk factors were a pregnancy-related RBC transfusion, the birth of a prior male child, a caesarean section, surgical removal of the placenta and postmaturity (Table 3.1). It might have been expected that the enhanced clearance of fetal red cells by the administration of anti-D prophylaxis or ABO-antagonism would have a preventive effect on non-RhD antibody formation. However, no difference was observed in anti-D immunoglobulin

	cases	weighted	p-value
		controls*	
General risk factors			
Total N	900	625	
Age (mean) [†]	30.91	30.85	0.82
RBC transfusion (% yes)	50.1	5.1	< 0.001
not pregnancy-related (% yes)	16.3	1.9	< 0.001
pregnancy-related (% yes)	34.8	3.4	< 0.001
Thrombocyte transfusion (% yes)	4.9	0.3	< 0.001
Surgery (% yes)	80.8	64.3	< 0.001
major surgery [‡] (% yes)	35.3	24.3	< 0.001
Medication/drugs use (% yes)	18.2	16.5	0.37
Immunological disease (% yes)	5.7	3.4	0.04
Haematological disease ⁹ (% yes)	5.5	1.9	0.001
Gravidity (%)			
1	23.2	34.6	
2	33.3	37.0	
3	23.6	18.4	
>3	19.9	10.1	< 0.001
Parity (%)			
Ō	29.7	43.5	
1	42.1	39.0	
2	19.2	14.6	
>2	9.0	2.9	< 0.001
Pregnancy-related risk factors			

TABLE 3.1 GENERAL AND PREGNANCY-RELATED UNIVARIATE RISK FACTORS FOR NON-RHD ANTIBODIES IN PREGNANCY

(cases and controls without history of non-pregnancy-related RBC transfusion/ cases with antigen-positive

partner)			
N of multigravid women	419	402	
Abortion (< 16 weeks), non invasive (% yes)	19.6	21.1	0.58
Abortion (< 16 weeks), invasive (% yes)	19.3	19.2	0.95
N of parous women (≥16 weeks)	395	348	
ABO-blood group (% A or AB)	45.6	44.5	0.78
Blood loss in pregnancy (% yes)	24.1	19.6	0.14
Trauma (% yes)	6.8	7.8	0.63
Chorionvillusbiopsy (% yes)	1.5	0.6	0.21
Amniocentesis (% yes)	2.5	0.9	0.08
Version (% yes)	2.5	2.6	0.94
Twins (% yes)	2.3	0.6	0.06
Postmaturity (≥42 weeks; % yes)	12.9	8.0	0.03
Male child (% yes)	62.0	47.6	< 0.001
Female child (% yes)	55.2	50.1	0.17
Breech delivery (% yes)	3.3	2.9	0.75
Instrumental delivery (% yes)	17.2	16.4	0.76
Surgical removal placenta (% yes)	13.7	5.7	< 0.001
Caesarean section (% yes)	21.8	11.0	< 0.001
RBC transfusion (pregnancy-related)	38.0	5.2	< 0.001
Active work	11.1	12.6	0.53
Active sport **	1.5	2.0	0.61

* Primary care controls (n=843) were weighted by 0.593 = 500, secondary care controls: 125

[†] Age cases= age at the moment of first detection of antibodies; age controls = age at antibody screening in last pregnancy

Major surgery: orthopaedic surgery, cardiac surgery, transplantation, spleen extirpation, surgery after trauma, abdominal surgery not pregnancy-related

§ Haematological disease: e.g. sickle cell disease, thalassaemia, autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpurae

Non-invasive abortion: miscarriage without curettage, extra-uterine pregnancy non-surgical or non complicated surgery

Invasive abortion: spontaneous miscarriage with curettage, termination of pregnancy, mola pregnancy, extrauterine pregnancy with complicated surgery

¶ Work requiring walking/cycling, \geq 5 days/week until week 30 or longer

** High impact sport, ≥ 2 times/week until week 20 or longer

administration after birth between RhD-negative cases and controls (65.0% versus 64.9%). The ABO blood group of the mother did not have an effect on her immunisation risk either.

Multivariate analysis

A history of RBC transfusion was the strongest independent general risk factor, in the population (OR 16.7) as well in the group with an antigen-positive partner (OR 11.6). Other independent risk factors were parity, major surgery and haematological disease (Table 3.2). The importance of parity as a risk factor increased if only pregnancies from an antigen-positive father were included (OR 6.2 parae>2 versus parae-0), but an RBC transfusion was also the strongest risk factor in this group (OR 11.5). Haematological disease and major surgery were significant risk factors in the population as well in the group with an antigen-positive father.

Multivariate analysis of pregnancy-related risk factors established a pregnancy-related RBC transfusion as the strongest risk factor (OR 10.5). Other independent risk factors were the birth of at least one prior male child and a history of caesarean section (Table 3.2).

	Prevalence risk	All cases	Cases with
	factor in control		antigen-positive partner
	group	N = 900	N = 527
		N controls = 625	N controls = 625
	%	OR (95%-CI)	OR (95%-CI)
General risk factors*			
Parity			
0	43.5	1	1
1	39.0	1.3 (1.0-1.7)	2.6 (1.9-3.6)
2	14.6	1.4 (1.0-2.0)	3.1 (2.1-4.5)
> 2	2.9	3.2 (1.8-5.8)	6.2 (3.3-11.6)
RBC transfusion	5.1	16.7 (11.4-24.6)	11.6 (7.7-17.4)
Major Surgery	12.6	1.4 (1.1-1.8)	1.4 (1.0-1.9)
Haematological disease	1.9	2.1 (1.0-4.2)	2.5 (1.1-5.5)
Pregnancy-related risk	Cases with antigen-po	sitive partner, no history	of non-pregnancy-related
factors [†]	RBC transfusion		
	N = 395		
	N controls = 348		
RBC transfusion	5.2	10.5 (6.2-17.7)	
Caesarean section	11.0	1.7 (1.1-2.7)	
Male child	47.6	1.7 (1.2-2.3)	

 TABLE 3.2 MULTIVATIATE GENERAL AND PREGNANCY-RELATED RISK FACTORS FOR NON-RHD ANTIBODIES IN THE POPULATION AND

 IN WOMEN AT RISK FOR HDFN

Goodness of fit tests showed no evidence of lack of fit (P>0.50 for the two logistic models)

* Adjusted for ethnicity [†] Adjusted for parity

			No RBC tra	ansfusion			RBC tran	sfusion	
		No Haem	disease	Haem. d	lisease	No Haem	. disease	Haem.	disease
		no MS	MS	no MS	MS	no MS	MS	no MS	MS
Parity	0	0.14%	0.27%	0.37%	0.57%	2.50%	2.70%	2.79%	2.99%
	1	0.27%	0.47%	0.56%	0.76%	2.69%	2.89%	2.99%	3.19%
	2	0.28%	0.48%	0.58%	0.78%	2.71%	2.90%	3.00%	3.20%
	>2	0.54%	0.74%	0.84%	1.04%	2.96%	3.16%	3.26%	3.46%

 TABLE 3.3 MODEL BASED RISK FOR NON-RHD IMMUNISATION IN THE POPULATION, ACCORDING TO THE PRESENCE OF RISK

 FACTORS

MS: Major Surgery

The four identified general risk factors RBC transfusion, presence of haematological disease or major surgery in medical history and parity were used to calculate their contribution for the presence of non-RhD immunisation in the population. Table 3.3 shows the model-based risk of non-RhD immunisation, varying from 0.14% if no risk factors are present, to 3.46% in presence of all significant risk factors.

Analyses for the different antibody specificities

Table 3.4 presents the results of the specific multivariate analyses for the antibody specificities anti-K, anti-c and Rh antibodies, other than D or c. These specificities were separately investigated, because we have previously shown that the majority of severe cases of HDFN due to non-RhD antibodies is caused by anti-K (23%) and anti-c (59%), and by other Rh-antibodies (18%).⁶ For each specificity a history of RBC transfusion is the most important risk factor both in the population and in women with an antigenpositive partner. The relative contribution of RBC transfusion is most pronounced for anti-K (OR 96.4). 83% of all women with anti-K had a history of RBC transfusion. Because the population prevalence of K positivity is low (only 9%⁸), parity is only a significant risk factor in pregnancies with a K-positive partner (OR 2.63), and not in the population (OR 1.43 95%-Cl: 0.84-2.46). For the anti-c antibody, which is the other clinically most relevant antibody, almost 50% of the women had received a RBC transfusion. As the population prevalence of a c-positive partner is higher (81.5%), parity was also a significant risk factor in the population (OR 6.18). RBC transfusion and parity are also significant risk factors for the other anti-Rh (non-cD) antibodies in both the population and in pregnant women with an antigen-positive partner, although the ORs in the last group are somewhat lower compared to the clinically more relevant c- and K-antibodies. This might be related to the fact that the largest group of these antibodies consisted of anti-E (71%) which antibody is frequently found as a naturally occurring antibody, not preceded by any contact with the E-antigen.

	A FUNCTION OF ANTIBODY SI	PECIFICITY, A	VDJUSTED FO	JR ETHNICITY						
			Parity	· (dichotomous)	RB(C transfusion	Ma	ijor surgery	Haema	tological disease
			prevalen	ICE	prevalen	ce	prevalend	Ge	prevalence	a
			RF in cas	ies	RF in cas	ies	RF in case	SS	RF in case	S
		z	%	OR (95%-CI)	%	OR (95%-CI)	%	OR (95%-CI)	%	OR (95%-CI)
An	ti-K									
	Population	221	70	1.43 (0.84-2.46)	83	96.4 (56.6-164.1)	43	1.10 (0.62-1.93)	9	1.92 (0.47-7.93)
	Group at risk	37	78	2.63 (1.13-6.13)	46	13.5 (6.23-29.2)	43	1.57 (0.73-3.35)	e	1.39 (0.13-15.2)
	(K-positive partner)									
An	ti-c									
	Population	154	06	6.18 (3.35-11.4)	49	16.0 (9.60-26.8)	28	1.09 (0.67-1.79)	7	4.28 (1.48-12.4)
	Group at risk	148	06	5.98 (3.24-11.0)	47	15.5 (9.22-26.1)	28	1.06 (0.64-1.74)	9	4.22 (1.44-12.3)
	(c-positive partner)									
Rh	esus antibodies, non-c/D									
Ŋ	E, e, C ^w , f)									
	Population	457	66	1.16 (0.88-1.53)	37	10.3 (6.80-15.5)	32	1.27 (0.94-1.72)	9	1.99 (0.91-4.35
	Group at risk	249	80	2.67 (1.84-3.86)	33	7.72 (4.90-12.2)	29	1.19 (0.83-1.72)	S	2.26 (0.91-5.62)
	(Rh-positive partner)									

Table 3.4 The independent contribution of parity, blood transfusion, hematological disease and main surgery to non-RHD antibodies in the population and in groups at risk as

RF Risk factor

Goodness of fit tests showed no evidence of lack of fit (P>0.35 for the three logistic models)

DISCUSSION

This is the first report of a study on risk factors for the presence of RBC alloantibodies other than anti-RhD in pregnancy, in an unselected population. A history of RBC transfusion (of which 70% was pregnancy-related) was the most important independent risk factor, even in women with an antigen-positive partner, followed by parity, haematological disease, and major surgery. A prior male child and caesarean section were the only pregnancy-related risk factors. The effect of an RBC transfusion was very strong, especially in the case of K-antibodies, while parity merely had only a relatively small effect in the group with an antigen-positive partner.

RBC transfusion and parity both are known risk factors for RBC immunisation.^{10;13;17;23;24} Major surgery may be an indicator of non-reported RBC transfusion, since only 32% of the women with a history of previous major surgery reported having received a transfusion. This might also be the case for haematological diseases (only 39% reported RBC transfusion), but it is conceivable that these conditions are related to increased susceptibility to red cell antibody formation, as is shown in sickle cell patients.²⁵ RBC transfusion and parity were shown to be independent risk factors without significant interaction. However, because 70% of transfusions in our study was given around delivery, it is difficult to completely disentangle the RBC transfusion effect from that of delivery. However, as (1) the amount of allogeneic RBCs in case of a RBC transfusion is much higher than in case of FMH, and (2) non-RhD antigens show much lower immunogenicity ¹³⁻¹⁵ compared to RhD, it is likely that RBC transfusions rather than FMH are the main contributor to RBC immunisation after a pregnancy-related RBC transfusion to a woman with an antigen-positive partner. An extra argument for the major role of blood transfusion comes from our observation that of the 103 women with anti-K antibodies and a prior pregnancy-related blood transfusion, only nine (9%) had a K-positive partner, which is completely identical to the frequency of K-positivity in the total population. For women with c- and E-antibodies and a pregnancyrelated blood transfusion the proportion of antigen-positive partners was higher than the population prevalence, but lower than the prevalence in parous women without a RBC transfusion (Supplemental Table). This points to an effect of both RBC transfusion and FMH in the development of these antibodies. Nevertheless, some indirect effect of blood transfusion on alloimmunisation against fetal RBC antigens cannot be completely excluded, since we have previously shown that pregnancy-related blood transfusion is also a weak risk factor for RhD immunisation, whereas these RBC transfusions are always matched for RhD (Koelewijn, unpublished observation).

The effect of a prior male child is intriguing and has never been described before for non-RhD antibodies. It has previously been observed that male fetuses are more severely affected by anti-D antibodies.²⁶ Furthermore the neonatal death rate from SUPPLEMENTAL TABLE CHAPTER 3: ANTIGEN GENOTYPES OF PARTNERS OF WOMEN WITH K, C OR E ANTIBODIES, COMPARED WITH THE POPULATION DISTRIBUTION, ACCORDING TO RBC TRANSFUSION HISTORY * ÷.

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	Genotypes in the		C	ases with non-RhD) antibodies		
	population	history of RB(C transfusion	parous women, r	no RBC transfusion	0-parae, no RBC	transfusion
		pregnancy-related [‡]	pregnancy-unrelated	HD or MS †	no HD or MS	HD or MS †	no HD or MS
	%	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
K-antibodies		n=103	n=61	n=8	n=17	n=3	n=5
K-negative	91	91 (86-97)	90 (83-98)	87(65-100)	41 (18-65)	100	80 (45-100)
heterozygous	80	8 (3-13)	10 (2-17)	13 (0-35)	47 (23-71)	0	20 (0-55)
homozygous K-positive	-	1 (0-3)	0	0	12 (0-27)	0	0
c-antibodies		n=47	n=15	n=17	n=46	n=3	n=2
c-negative	19	8 (1-16)	0	0	0	0	0
heterozygous	49	45 (30-59)	40 (15-65)	59 (35-82)	33 (19-46)	67 (13-100)	50 (0-100)
homozygous c-positive	32	47 (33-61)	60 (35-85)	41 (18-65)	67 (54-81)	33 (0-87)	50 (0-100)
E-antibodies		n=80	n=33	n=30	n=82	n=20	n=41
E-negative	72	50 (39-61)	58 (41-74)	7 (0-16)	21 (12-30)	70 (50-90)	58 (43-74)
heterozygous	26	47 (37-58)	33 (17-49)	80 (66-94)	65 (54-75)	30 (10-50)	37 (22-51)
homozygous E-positive	2	3 (0-6)	9 (0-19)	13 (1-25)	14 (7-22)	0	5 (0-11)
	-		<i>a</i> :r				

* Excluded are women if the paternal antigen is missing or with pregnancies from different partners

[†] MS Major surgery HD Hematological Disease [‡] Including women with pregnancy-related + -unrelated transfusion Bold: significant difference compared to the population

i.

Chapter 3

kernicterus was twice as high among boys as among girls before the introduction of anti-D immunoprophylaxis.²⁹ Although the exact mechanism is unknown, it is tempting to speculate that the immune response is stimulated by male-specific antigens, thereby increasing the response to RBC antigens. This fits with the observation that gender mismatch in allogeneic stem cell transplantation influences the outcome of the transplantation. Especially in patients with less severe immune suppressive therapy, male transplant to female recipient was shown to be the strongest risk factor for Graft versus Host Disease and failure of engraftment.^{27 28}

The only specific FMH-related risk factor within the group of parous women was a prior caesarean section. Other supposed risk factors such as instrumental delivery, shown to be a risk factor for RhD immunisation (Koelewijn, unpublished results) did not emerge here. Perhaps the lower immunogeniticy of non-RhD antigens compared to RhD is responsible; only a minority of cases with an increased FMH gave rise to non-RhD immunisation. As parity in itself is a clear risk factor, risk factors within the group of parous women, have no consequences for the RBC antibody screening policy.

Some possible caveats in our study were: a) The presence of recall bias, caused by the knowledge about the presence of non-RhD. We judge this to be unlikely, as the data were retrieved by the obstetric care worker from the medical records or collected directly from the pregnant women by telephone, while most women do not have any knowledge about immunisation and its risk factors. b) Lack of representativeness of the control group. The controls were comparable with the population on parity and gravidity; however, despite the instruction to select controls at random, non-Dutch women were under-represented in the control group. We have no indication that the control group was not representative on other variables that could be related to risk factors after adjustment for this difference in ethnicity.

In 36% of cases the RBC antibodies were known before screening in the ongoing pregnancy, in most cases from screening in a prior pregnancy.⁶ Thus, in the majority of the pregnant women with non-RhD antibodies, screening in pregnancy reveals the presence of these antibodies. Our study shows that it is at least theoretically possible to increase the efficiency of the RBC antibody screening programme by introduction of subgroup screening (see also Figure 3.1). If only parous women and women with a history of RBC transfusion, major surgery or haematological disease (69% of all pregnants), and RhD-negative women are screened, a 25-30% reduction of the number of screened women is achieved, leading to a cost reduction of the RBC antibody screening programme (inclusive monitoring and treatment of cases at risk) of about 20% (calculations available from the first author). This policy accepts a maximum risk of 0.17% (138/95,000=0.14%; 95%-CI 0.12%-0.17%) of missed early detection of a case of non-RhD immunisation in women without clinical risk factors (Table 3.3). With this policy 5.2% of all non-RhD immunisations



FIGURE 3.1 FLOW DIAGRAM OF RBC ANTIBODY SCREENING PROGRAMME, BASED ON PRE-SELECTION OF WOMEN WITH CLINICAL RISK FACTORS

would have been missed: 47 out of 900 cases in our study, with 52 antibodies: anti-E (n=19), anti-C^w (n=19), anti-S (n=4), anti-K (n=3), anti-Wr^a (n=2), anti-C (n=1), anti-Kp^a (n=1), anti-M (n=1), anti-P (n=1) and anti-P^k (n=1). Severe HDFN did not occur in any of these cases. Remarkably, the majority of these antibody specificities (like anti-E, anti-C^w and anti-Wr^a) can occur naturally, implying development of these antibodies without a prior immunizing event. Only three anti-K, but with K-negative fathers, and no anti-c would have escaped from detection. Another purpose of the RBC antibody screening programme is to increase transfusion safety and to save time, needed for the identification of antibody specificities if an emergency RBC transfusion is necessary around delivery. Evidently, women with risk factors for non-RhD immunisation like a history of pregnancy-related RBC transfusion, are especially at risk of a transfusion around delivery. In our study only one of the cases potentially missed by subgroup screening, received a (non-emergency) RBC transfusion around delivery. In emergency situations RBCs with blood group O, RhD-negative and K-negative are selected for transfusion. In general, these will also be C- and E- negative. Assuming that it were possible to select appropriate antigen-negative blood in an emergency situation (but this depends on the availability of a fully typed RBC inventory), subgroup screening would place < 1% (1/897) of pregnant women at risk of a haemolytic transfusion reaction.

In our view subgroup RBC antibody-screening based on clinical risk factors is justified, which is in contrast to current guidelines in most western countries.²⁹ Although apparently efficient, this policy requires careful documentation of risk factors. It should be considered that the introduction of subgroup screening always harbours the risk that the algorithm is not correctly followed.

Apart from the primary prevention option a secondary option also emerged from our study, which is in our view the major conclusion of this study. In pregnant women who could have been immunised by either a former antigen-positive child or by RBC transfusion a transfusion was by far the strongest risk factor, especially for anti-K and anti-c, which are the antibody specificities that can cause severe HDFN. Extended matching of transfusions can therefore prevent a substantial proportion of non-RhD immunisations and of severe HDFN. The use of K-negative RBCs in transfusions to women younger than 45 is prescribed by the current Dutch guidelines since 2004. As 91% of them is K-negative and at risk for K-immunisation²⁰, it is obvious that this policy has decreased the K-immunisation risk considerably. Additional typing of girls and women of child bearing age for RhCcEe antigens and transfusion of c- and possibly E-matched RBCs should be considered in view of our results as well. In the Netherlands more than 80% of anti-K immunisations and almost 50% of anti-c immunisations might be prevented by such a policy. In about 37% of these avoidable cases the fetus is at risk of HDFN because of an antigen-positive father. Since many hospitals in the Netherlands had already the policy to transfuse K-negative blood to women <45 yrs, which was facilitated by all K-negative RBC units being labelled as such, we expect that in most other countries the impact of matched transfusion will be even higher.

In conclusion, we are, to our knowledge, the first to determine the contribution of different risk factors for alloimmunisation against red blood cell antigens in pregnant women. Moreover, our risk factor analysis shows that it is theoretically possible to increase the efficiency of the RBC antibody screening in pregnant women by the introduction of subgroup screening, without the risk of missing severe cases of HDFN and without a significant increase of the risk of a haemolytic transfusion reaction in case of emergency RBC transfusions around delivery. Moreover, since more than 50% of women with clinically relevant RBC antibodies had a prior history of blood transfusion, the introduction of Rhcmatched K-negative blood transfusion to women under the age of 45 years will over time contribute to a major reduction of immunisations and cases of severe HDFN.

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