Prevalence of red-blood-cell and non-red-blood-cell-targeted autoantibodies in alloimmunized postpartum women

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ORIGINAL PAPER

Prevalence of red-blood-cell and non-red-blood-cell-targeted autoantibodies in alloimmunized postpartum women

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Background and Objectives Alloantibodies against red-blood-cell (RBC) antigens often coincide with alloantibodies against leucocytes and platelets and sometimes with autoantibodies towards various antigens. Chimerism may be one of the factors responsible for the combination of allo- and autoantibodies. Women with alloantibodies against RBC antigens causing haemolytic disease of the fetus and neonate may need to receive intrauterine transfusions. These transfusions increase not only maternal antibody formation but also fetomaternal bleeding and may enhance fetal chimerism. We determined the prevalence of and risk factors for autoantibodies against some common clinical target antigens, in alloimmunized women after IUT.

Materials and Methods We tested for autoantibodies against RBC, anti-thyroid peroxidase, anti-extractable nuclear antigens, anti-cyclic citrullinated proteins and anti-tissue transglutaminase. Women with and without autoantibodies were compared for age; number of RBC alloantibodies, pregnancies and IUTs, and other factors that may play a role in immunization.

Results Non-RBC-targeted autoantibodies were present in 40 of 258 tested women (15·5%, with 90% anti-TPO specificity), comparable to the prevalence reported in healthy Dutch women of these ages. Surprisingly, compared with women who had a single RBC alloantibody, a significantly higher proportion of women with multiple RBC alloantibodies had autoantibodies (5·3% and 18·4%, respectively; odds ratio 4·06, 95% CI: 1·20–13·7). Other characteristics of women with and without autoantibodies were not different.

Conclusion Multiple RBC alloantibodies after extensive allogeneic exposure during pregnancy and presumed increased fetomaternal chimerism are not associated with (selected) autoantibodies. Lack of allo-RBC multi-responsiveness seems associated with decreased auto(-TPO) antibody formation.

Key words: alloantibodies, autoantibodies, red blood cells, TPO antibodies, transfusion.

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Introduction

Autoimmune diseases (AID) include a variety of (organ-specific or systemic) disorders. Its overall prevalence is around 8% in the general European population, with a strong female predominance (female-to-male ratio ≈2·5) [1]. AID are the result of a dysregulated immune response, associated with (the interaction of) environmental and genetic predisposition [2–5]. MHC genes play an important role; for instance, the HLA-A*01/HLA-B*08/HLA-DRB1*03 and HLA-DRB1*15 haplotypes are associated with type I diabetes, systemic lupus erythematosus, multiple sclerosis, aplastic anaemia and myasthenia gravis [6–11].

Patients with AID can possess the disease-related autoantibodies against the target tissue or organ(s) many years before clinical manifestation and also frequently possess antibodies against other autoantigens, which often not result in tissue damage [12,13]. In clinically healthy individuals, increasing with older age, the prevalence of at least one autoantibody can be up to 24%, varying considerably between autoantibody specificities [14].

Alloantibodies against fetal RBC antigens, in particular anti-D, anti-c and anti-K, can cause haemolytic disease of the fetus and newborn (HDFN). Severe HDFN is nowadays successfully treated with intrauterine transfusions (IUT) [15]. Fetomaternal haemorrhage during IUT exposes the mother to foreign antigens of donors providing IUT and may transfer more fetal cells to the mother, possibly increasing FMc and leading to autoantibodies (preceding autoimmunity). The higher incidence of AID in woman compared with men has been attributed to fetomaternal chimerism (FMc) [1,16]. We speculated that the women in our cohort could have produced more autoantibodies, for instance in relation to the number of IUTs.

We previously showed in a follow-up study that 76% of these women possess multiple RBC alloantibodies, and 48%, HLA antibodies. We observed a relationship between high-titre (>8000) anti-D (hyper-sero-responders) and multi-responders, with antibodies against more than one RBC antigen and against different HLAs, respectively [17]. The RBC multi-responsiveness compared with single-antibody responders was associated with the HLA-DRB1*15 phenotype [18]. In addition, several studies also showed high allo- and autoresponses towards HLAs in patients with AID, and numerous studies reported that an immune response to self-antigens may indeed be initiated by alloimmune responses in transfusion and organ transplantation settings [19–27]. Although a relationship between alloantibodies and autoantibodies is well described, a relationship between multiple RBC antibodies and AID in woman has only been described long ago by Ramsey and Smietana [28].

We determined the prevalence of and risk factors for autoantibodies against some common clinical target antigens, in RBC-alloimmunized women after fetal treatment with IUT. Besides autoantibodies to red cells, we selected to test for autoantibodies associated with (later) diseases and with a clear (>2 to >10 times) female predominance, for example thyroid disease (anti-TPO, anti-thyroid peroxidase), Sjögren's syndrome (anti-ENA, anti-extractable nucleated antigen), rheumatoid arthritis (anti-CCP, anti-cyclic citrullinated proteins) and coeliac disease (anti-tTG, anti-tissue transglutaminase). Of these, anti-TPO antibodies are the most frequent autoantibodies in healthy individuals and in patients with AID. However, only a small proportion of individuals with anti-TPO will develop autoimmune thyroid disease and anti-TPO is also found in up to 40% of patients with other autoimmune diseases [12]. Therefore, the presence of anti-TPO may be considered as a sensitive marker for decreased regulation of immunity towards self-antigens, but has limited clinical relevance.

Our cohort of women with multiple RBC antibodies and increased risk for FMc after IUT would allow to investigate both factors on a possible association with autoimmunity.

Materials and methods

All women and their children who received one or more IUTs for HDFN from 1987 to 2008 were asked to participate in a long-term follow-up cohort study (LOTUS, LOng-Term follow-Up after intrauterine transfusionS). After informed consent was obtained, blood samples were taken from participating mothers. Participants were included from February 2008 to September 2010. The full protocol of the study has been published [29]. The following data were collected: age, number and specificity of RBC alloantibodies, number of pregnancies, number of IUTs, HLA-DRB1*15 genotype, HLA antibodies and for how long the mothers were breastfed. The study was approved by the ethics committee of the LUMC (P08·080). The present analysis, based on data from the LOTUS study, is cross-sectional in design.

At this follow-up, women were tested for the presence of allo- and autoantibodies against RBC antigens and for non-RBC-targeted autoantibodies. RBC alloantibodies were tested with an indirect antiglobulin test using the LISS/Coombs gel microcolumn technique (LISS DiaMed ID gel system, Murten, Switzerland). The presence of RBC autoantibodies was tested using the direct antiglobulin test with anti-IgG and anti-complement.

IgG anti-TPO (Thyroid PerOxidase for thyroid disorders) has been measured with a chemiluminescent test from Siemens (Cat Nr L2KT012) on a Siemens Immulite 2000 XPi analyser. IgG anti-ENA (Extractable Nucleic Antigen for mixed connective tissue disease, Sjögren's syndrome and SLE) was measured with an Enzyme-linked immunoAssay (EliA Symphony, Cat Nr 14-5508-01) for screening and detection of the most prevalent antibodies against extractable nuclear antigens. IgG anti-CCP (Cyclic Citrullinated Peptide for rheumatoid arthritis) was measured with a second-generation Enzyme-linked immunoAssay (EliA CCP, Cat Nr 14-5515-01). IgA anti-tTG (tissue TransGlutaminase for coeliac disease) was measured with an Enzyme-linked immunoAssay (EliA Celikey Cat Nr 14-5517-01). Anti-ENA, anti-CCP and ant-tTG were all tested on an ImmunoCap 250 analyser from Phadia (Phadia, Uppsala, Sweden).

Statistical analyses

Categorical variables are presented as numbers and percentages with 95% confidence intervals (CIs), and continuous variables are presented as median and range for non-normally distributed data. Univariable logistic regression was used to estimate odds ratio (OR) and 95% CIs. The presence of autoantibodies was used as the dependent variable. The following variables were considered potential risk factors for autoantibodies: maternal age at time of autoantibody measurement (categorized as 25–34, 35–39, 40–44, 45–49 and >50 years); number of RBC alloantibodies (categorized as 1, 2 and ≥3); number of pregnancies (categorized as ≤2, 3 and >3); number of IUTs (categorized as 1, 2, 3, 4 and >4); maternal HLA-DRB1*15 genotype; the co-presence of HLA antibodies; and for how long the mothers were breastfed (categorized as 0, 1, 2, 3, 4–6 and >6 months).

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA).

Results

A total of 258 women of children with HDFN treated with IUTs (median age 43 years; range 25–60) were screened for the presence of autoantibodies (six women were not screened for anti-TPO) at a median follow-up of 9·0 years (range 0·7–22·5; 44% >10 years) after the last complicated pregnancy. The women had been pregnant a median of three times (range 1–14) and received a median of three IUTs (range 1–18).

In 258 women, 545 RBC alloantibodies (Table S1) were found: one in 57 women, two in 134 women and three to six different RBC alloantibodies in 67 women. HLA antibodies were present in 118 (46%) women [20]. No women had a positive direct antiglobulin test, excluding RBC autoantibodies. Non-RBC-targeted autoantibodies were present in 40 women (15·5%), of which anti-TPO test was positive in 36 of the 252 women who were tested for anti-TPO. One woman had anti-ENA and anti-CCP antibodies (Table 1).

In univariate analysis, the frequency of autoantibodies increased with higher age (OR, 1·21; 95% CI, 0·90–1·63) and with higher number of RBC alloantibodies (OR, 1·60; 95% CI, 0·97-2·65). All other variables showed no association with autoantibodies (see Table 2 and Table S2 for more detailed information). For a sensitivity analysis, the number of RBC alloantibodies was dichotomized in one and more than one antibody. The analysis revealed that, compared with women who had a single RBC alloantibody, a higher proportion of women with multiple RBC alloantibodies had autoantibodies (5·3% and 18·4%, resp.; OR 4·06; 95% CI 1·20–13·7). In multivariate analysis, including age and dichotomized number of RBC alloantibodies only the presence of multiple alloantibodies was significantly associated with autoantibodies (aOR, 4·06; 95% CI, 1·20–13·7) (Table 2).

Discussion

We determined the prevalence of autoantibodies against some clinical target antigens in 258 women, who were alloimmunized by pregnancy and/or (intrauterine) transfusion. These women, who possessed 78% multiple RBC alloantibodies and had an increased risk for FMc, showed a frequency of non-RBC-targeted autoantibodies of 15·5% (14% anti-TPO and 1·5% other autoantibodies). We found no association of autoantibodies with the number of

Table 1 Frequency and prevalence of autoantibodies in 258 women with red-blood-cell alloantibodies

	Reference value of the test	N (%; 95% CI)[a]
DAT	Absent	0 (0·0; 0·0–1·8)
IgG anti-TPO	<35 kU/L	36 (14·3; 10·1–18·9)[b]
IgG anti-ENA	Negative	2 (0·8; 0·1–3·1)
IgG anti-CCP	<7 U/ml	2 (0·8; 0·1–3·1)
IgA anti-tTG	<7 U/ml	1 (0·4; 0.0–2·5)
All	n.a.	40 (15·5; 11·4–20·6)

CCP, cyclic citrullinated protein; DAT, direct antiglobulin test; ENA, extractable nuclear antigen; n.a., not applicable; TPO, thyroid peroxidase; tTG, tissue transglutaminase.

[a]Number (%; 95% confidence interval) of women with results above the reference value.

[b]252 of 258 women were tested for anti-TPO; one woman had anti-ENA and anti-CCP.

Table 2 Uni- and multivariable analyses of risk factors associated with non-RBC-targeted autoantibodies in 258 women with RBC alloantibodies

Covariate		Number of women and autoantibodies		OR (95% CI)	*P*-value
		Absent (%)	Present (%)		
Age categorized[a]		n.a.	n.a.	1·21 (0·90–1·63)	0·22
RBC antibodies (1, 2, ≥3)		n.a.	n.a.	1·60 (0·97-2·65)	0·066
Dichotomized	1	54 (94·7)	3 (5·3)	Reference	
	>1	164 (81·6)	37 (18·4)	4·06 (1·20–13·7)	0·02
Pregnancies (≤2, 3 and >3)		n.a.	n.a.	1·12 (0·74–1·69)	0·61
IUTs (1, 2, 3, 4 and >4)		n.a.	n.a.	1·11 (0·87–1·41)	0·39
HLA-DRB1*15[b]	Absent	135 (82·3)	29 (17·7)	Reference	
	Present	70 (86·4)	11 (13·6)	0·73 (0·35–1·55)	0·42
HLA antibodies	Absent	119 (85·0)	21 (15·0)	Reference	
	Present	99 (83·9)	19 (16·1)	1·09 (0·55–2·14)	0·81
Breastfeeding	Absent	37 (82·3)	8 (17·8)	Reference	
	Present	65 (83·3)	13 (16·7)	0·93 (0·35–2·44)	0·88
Breastfeeding period[c]		n.a.	n.a.	1·12 (0·87–1·45)	0·39

Multivariate analysis, including age (categorized) and the number of RBC antibodies (dichotomized), revealed that having multiple RBC alloantibodies significantly increased the risk for autoantibodies (OR: 4·07; 95% CI: 1·20–13·8), while age was suggestive of an association (OR, 1 21; 95% CI, 0·89–1·65).

HDFN, haemolytic disease of the fetus and newborn; IUT, intrauterine transfusion; n.a., not applicable; RBC, red blood cell.

[a]Age was categorized as <35; 35–39; 40–44; 45–49 and ≥50 years.

[b]245 (95%) women were HLA-DRB1*15-typed.

[c]Data available for 123 (48%) women, for how long the mothers were breastfed was categorized as 0, 1, 2, 3, 4–6 and >6 months.

pregnancies and the presence of concomitant HLA antibodies. In accordance with a population-based survey in randomly selected healthy Dutch women of comparable ages, (TPO) autoantibodies were not significantly more frequent with increasing age [30]. Relevant to our study population, no relationship was present between autoantibodies and the number of IUTs, the presence of HLA-DRB1*15 and the period of the mother being breastfed.

This prevalence of 14% anti-TPO is within the range of 14·7–18·8 found in 1845 randomly selected Dutch women of comparable ages without thyroid disease [30]. The higher frequency of anti-TPO versus other autoantibodies was also in line with the results from a European study in 503 healthy women screened for various autoantibodies (i.e. 10·7% and 3·4%, resp.) [14]. In both these studies, the prevalence of RBC alloantibodies was not studied, but presumed very low.

In the general population, anti-TPO ranges from 5 to 24%, highest in the elderly and women [14,30]. Only a small proportion of anti-TPO-positive individuals, in particular with higher antibody concentration, develop autoimmune thyroid disease. Although not always equivocal, studies reported that increased parity is associated with FMc, which may be a cause of thyroid and other autoimmunity [31,32]. Even a relationship between the quantity of fetal cells in the maternal thyroid and thyroid disease was reported [33]. We speculated that the women in our cohort could have produced more autoantibodies in relation to the number of IUTs, which was not the case. This similar prevalence of anti-TPO may indicate that our study cohort has the same genetic risks to develop anti-TPO compared with the random Dutch age-matched women. Genetic studies indeed showed that single nucleotide polymorphisms in TPO and some other genes are associated with the presence of low- or high-level anti-TPO [34–36].

Another aspect we intended to evaluate in our alloimmunized cohort was the relationship between alloantibody and autoantibody formation. So far, only three studies investigated a relationship between the formation of (multiple) RBC alloantibodies and the occurrence of AID. More than 20 years ago, Ramsey and Smietana, in a retrospective 15-year hospital-based case–control study, reported that women (mean age 55 years) with three or more RBC alloantibodies had a higher prevalence of AID (12 of 43; 28%) compared with women with one (6 of 43; 14%) or no (3 of 43; 7%) RBC antibodies. The prevalence of AID differed statistically significant only between women with multiple compared with women without RBC antibodies. Rheumatoid arthritis and SLE were in particular increased in their cohort [28]. Ryder and colleagues, in an over 50-year retrospective hospital-based case–control study, found a variety of chronic autoimmune disorders significantly more frequent in a cohort of

alloimmunized (predominantly) male military veterans, compared with non-immunized transfusion recipients (15·9% and 8·4%, resp.) [37]. In a study by Karafın *et al.* [38], after excluding women with anti-D as sole specificity, systemic lupus erythematosus and rheumatoid arthritis (without discriminating between women and men and with a median age between 61 and 70 years) were significantly more frequent in RBC alloantibody responders (23% multiple antibodies) compared with non-responders (8·0% and 6·1%, resp.). These three studies differed from our study in patients and study design. First, the presence of AID was determined, while we tested for common autoantibodies. Ramsey and Smietana studied female, Ryder male and Karafın mixed-sex hospital populations, while we tested for the presence of autoantibodies in a selected group of healthy women exposed to multiple alloantigens by pregnancy and transfusion. As the women are not under routine control in our medical centre, it is unknown whether AID might have developed later on. Second, the women in our study were on average more than ten years younger, while advancing age is a risk factor for occurrence of autoantibodies, which may precede the onset of AID by many years [39]. Last, Ryder and Karafın only considered the absence or presence of RBC alloantibodies and did not discriminate between patients with single and multiple alloantibodies. Altogether, these three studies showed an association between (multi-)RBC-alloimmunized hospital patients and AID, while our immunized, but otherwise healthy, women did not have an increased risk for common non-RBC-targeted autoantibodies. We agree with Ramsey and Smietana presuming that RBC transfusions do not elicit AID. Rather, the transfusion reveals a group of high-responder patients and this responder capacity may also affect self-reactivity.

Although our immunized women did not have an increased risk for common non-RBC-targeted autoantibodies, an unexpected finding was that autoantibodies were four times more frequent in women with multiple RBC alloantibodies compared with women with a single RBC alloantibody. We cannot exclude coincidence, but encourage studies evaluating autoantibodies in single and multiple antibody responders to evaluate this confusing observation.

In conclusion, the overall prevalence of non-RBC-targeted autoantibodies in women after IUT, promoting FMc, did not differ from the prevalence reported in healthy Dutch women of comparable ages. However, compared with women with a single RBC alloantibody, the proportion of women who produced multiple RBC alloantibodies also showed an increased risk for (anti-TPO) autoantibodies, suggestive that a shared pathway leading to antibodies to allogeneic and self-antigens in individuals with a high immune response potential cannot be excluded. The findings pose a challenge to investigate and further elucidate a possible allo- and autoimmune relationship.

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Conflict of interests

The authors declare no conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Alloantibody specificities in 258 women tested for RBC and non-RBC targeted autoantibodies.
Table S2. Uni- and multivariable analysis of risk factors associated with non-RBC targeted autoantibodies in 258 women with RBC alloantibodies.