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## **Clinical determinants of red cell alloimmunization, implications for preventative antigen matching strategies**

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## ABSENCE OF THE SPLEEN AND THE OCCURRENCE OF RED CELL ALLOIMMUNIZATION IN HUMANS

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With its unique anatomy and location amidst the circulatory system, the spleen allows an intimate contact between its resident cells and blood passing the organ. Senescent and damaged red cells are primarily sequestered in the splenic red pulp and consumed by its macrophages.<sup>1</sup> Consequently, this route facilitates presentation of non-self antigens of transfused red cells to splenic immune cells as a first and essential step in red cell alloimmunization. Indeed, the splenic microenvironment has been demonstrated to play a prominent role in red cell alloimmunization in mice.<sup>2,3</sup> Contrasting these animal studies, some observational studies in thalassemic patients suggested splenectomy to be associated to increased red cell alloimmunization,<sup>4,5</sup> while others did not find any association.<sup>6,7</sup>

In the current study, we assessed the association between the anatomic absence of the spleen and (transfusion-related) red cell alloantibody induction in our multicenter case-control R-FACT study cohort. This cohort includes 505 alloimmunized cases and 1,010 non-alloimmunized matched controls among an earlier described, primarily Caucasian, source population of 24,063 patients receiving their first and subsequent red cell transfusions between January 2005 and December 2013 at one of six participating hospitals in the Netherlands.<sup>8</sup> A detailed description of our case-control cohort and our used methodology has been published recently.<sup>9</sup>

Summarizing, cases were identified as all patients who developed a first transfusion-induced alloantibody during the course of their transfusion history against the antigens: c, C, e, E, K, C<sup>w</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, Le<sup>a</sup>, Le<sup>b</sup>, Lu<sup>a</sup>, Lu<sup>b</sup>, M, N, S, or s. Here, we considered the last (documented or assumed) antigen mismatched transfusion preceding the first positive screen (i.e. the *N*th transfusion) to likely have elicited alloimmunization and defined this as the ‘implicated transfusion’. If this last mismatched transfusion could not be identified due to incomplete donor typing, the last non-tested unit preceding the first positive screen was considered as the implicated transfusion. Based on an ‘incidence-density sampling strategy’, for each identified case we randomly sampled two non-alloimmunized control subjects out of the source population, on the precondition that these controls had received at least an equivalent number of (lifetime) red cell transfusion in the same study center as the case. The *N*th transfusion in these sampled controls, corresponding to the implicated transfusion of their matched cases, was then marked. Subsequently, we constructed a so-called ‘alloimmunization risk period’ in both cases and controls, stretching from 30 days before to seven days after this *N*th (implicated) transfusion. Finally, we compared the presence of a history of splenectomy at the time of the alloimmunization risk period in cases and controls.

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

At the alloimmunization risk period, splenectomy had been performed in 20 patients, namely one case (0.2%) versus 19 controls (1.9%) (Table 1). In 12 patients, splenic injury was caused by severe trauma or complicated abdominal surgery, while no patient underwent a splenectomy in the context of an autoimmune disease. Sixteen of the splenectomized

**Table 1** Demographics and splenectomy details of 19 non-alloimmunized and 1 alloimmunized splenectomized patients.

Patient	Age (years) / sex	Allo- immunization	Indication for splenectomy
A	70/M	Yes	orthotopic liver transplantation complicated by splenic damage.
B	30/M	No	total pancreatectomy complicated by retroperitoneal hematoma and splenic infarction.
C	16/F	No	spontaneous splenic rupture shortly following post allogeneic stem cell transplantation.
D	39/M	No	severe trauma with intra-abdominal organ damage.
E	34/F	No	pregnancy complicated by rupture of a splenic artery aneurysm.
F	40/M	No	severe trauma with intra-abdominal organ damage.
G	74/F	No	resection of a large intra-abdominal liposarcoma, including splenectomy.
H	58/F	No	unilateral nephrectomy for renal cell carcinoma complicated by splenic damage.
I	72/F	No	resection of a large intra-abdominal liposarcoma.
J	55/M	No	polycythemia vera associated splenomegaly.
K	82/M	No	distal pancreatectomy with splenectomy.
L	46/M	No	severe trauma with intra-abdominal organ damage.
M	30/M	No	severe trauma with intra-abdominal organ damage.
N	63/M	No	pancreatic necrosis following a history of pancreaticojejunostomy.
O	49/M	No	severe trauma with intra-abdominal organ damage.
P	76/M	No	coronary artery bypass surgery complicated by an incarcerated inguinal hernia with secondary peritonitis and intra-abdominal hemorrhage.
Q	77/F	No	resection of a large intra-abdominal sarcoma.
R	67/F	No	adrenectomy for metastasized adrenal carcinoma complicated by splenic damage.
S	75/M	No	unilateral nephrectomy for renal cell carcinoma complicated by severe intra-abdominal bleeding.
T	73/M	No	infective endocarditis with septic embolism and splenic abscesses.

Anti-M and anti-E were detected in patient A respectively 23, 23, and 21 days after the first allo-M and allo-E exposure, the splenectomy, and the implicated transfusion.

patients received their implicated (*Nth*) transfusion at or after splenectomy (median 0, range 0-3612 days). In three other patients, splenectomy followed the implicated transfusion by 1-4 days. Consequently, in these patients immunization against the administered blood was considered as being modulated by the splenectomy. Subsequent red cell transfusions beyond splenectomy were received by all, but two (patient L and N) controls (median 19 units; range 0-59, table 2), with one control being further transfused beyond the study period. Red cell alloantibodies were not developed (data available up until april 2017).

Only one splenectomized patient developed alloantibodies (patient A). In this patient, anti-E and anti-M were simultaneously detected 23 days after a combined orthotopic liver transplantation and splenectomy, during and following which he received 6 E-positive and at least 8 M-positive units. Using multivariate logistic regression analysis conditioning on the matched variables plus identified potential confounders (Table S1), we estimated that splenectomized patients had a 20-fold reduced risk of alloimmunization as compared to patients lacking a history of splenectomy (adjusted relative risk (RR) 0.05, 95% confidence interval (CI) 0.01-0.55). Omitting patients L and N who were not further exposed by red cell transfusions following splenectomy did not change the RR (0.05 (95%CI 0.01-0.62)).

Since transfusions were administered both before and after splenectomy, estimation of an alloimmunization risk from the time of splenectomy onwards should be related to both pre- and post-splenectomy red cell exposures. Based on an estimated number of 245 splenectomized patients within the entire source population, we calculated that 13 splenectomized patients instead of only patient A were expected to have developed alloantibodies had splenectomy not influenced alloimmunization (for calculations, see Table 2). We hereby assumed the red cell exposures of the 19 splenectomized controls to represent the red cell exposure pattern of all splenectomized patients within the source population.

To the best of our knowledge, this is the first study in humans reporting red cell alloimmunization to be highly unlikely following splenectomy. Our observation underlines the spleen's function in protective adaptive immunity against non-self antigens present in the circulation and corroborates with earlier studies in splenectomized mice. Even in the setting of poly(I:C) induced inflammation (a condition strongly linked with alloimmunization), murine red cell alloimmune responses were completely abrogated and suggested to be due to a splenectomy induced impairment of CD4<sup>+</sup> T cell priming and expansion.<sup>2,3</sup> Since T cell priming requires efficient antigen-presentation, it seems not surprisingly that splenic conventional CD11c<sup>+</sup> dendritic cells have been strongly implicated in murine red cell alloimmunization.<sup>10</sup> In agreement with these findings, the splenic T cell subsets were shown to be pivotal for antibody production against both autologous and allogeneic platelet membrane antigens.<sup>11</sup>

**Table 2** Illustration of expected versus observed numbers of alloimmunized patients within the splenectomized source population.

**Step 1: Estimation of number of splenectomized patients within the source population**

Among 14,901 patients from the Leiden University Medical Center, University Medical Center Utrecht and Jeroen Bosch Hospital 's Hertogenbosch, 155 patient with a documented history of splenectomy receiving red cell transfusions beyond their splenectomy were identified by searching their clinical files via information technology resources. None of these patients developed red cell antibodies. As these patients represent 62.0 % of the entire source cohort, the total number of splenectomized patients within the source cohort will approximate 245.

**Step 2: Comparison of expected versus observed number of alloimmunized patients within the splenectomized source population**

Based on the cumulative number of red cell units received pre- and post-splenectomy and reported cumulative incidences according to number of red cell units transfused,<sup>8</sup> the expected alloimmunization risk per splenectomized patient encountered from splenectomy onwards ( $\Delta p$ ) can be deduced from the absolute risk at the time of splenectomy ( $pT1$ ) and the risk at the time of last serological follow-up ( $pT2$ ).

Consequently, would splenectomy not have influenced alloimmunization, one would have expected 1.059 alloimmunizations per 20 splenectomized patients. This number corresponds to an estimated total of 13 alloimmunizations among the estimated 245 splenectomized patients (5.3%). As only one splenectomized patient within the source population developed alloantibodies, it seems conceivable that approximately 12 patients were protected from alloimmunization due to splenectomy, corresponding to a crude relative risk of 0.08.

Patient	T1: number of red cell units received before splenectomy	T2: cumulative number of red cell units received up till last screen	pT1	pT2	$\Delta p$
A	0	19	0.000	0.063	0.063
B	0	2	0.000	0.016	0.016
C	15	30	0.061	0.084	0.023
D	0	11	0.000	0.051	0.051
E	0	16	0.000	0.063	0.063
F	0	8	0.000	0.037	0.037
G	0	31	0.000	0.084	0.084
H	0	19	0.000	0.063	0.063
I	4	31	0.027	0.084	0.057



**Table 2** Continued.

Patient	T1: number of red cell units received before splenectomy	T2: cumulative number of red cell units received up till last screen	pT1	pT2	Δ p
J	0	34	0.000	0.089	0.089
K	0	19	0.000	0.063	0.063
L	2	2	0.016	0.016	0.000
M	0	10	0.000	0.047	0.047
N	21	21	0.067	0.067	0.000
O	0	59	0.000	0.104	0.104
P	30	53	0.084	0.104	0.019
Q	4	13	0.027	0.058	0.031
R	0	21	0.000	0.067	0.067
S	1	38	0.010	0.089	0.079
T	0	53	0.000	0.104	0.104
SUM					1.059

*pT1 = the chance to have developed red cell alloantibodies following the number of red cell exposures at T1. pT2 = the chance to have developed red cell alloantibodies following the number of red cell exposures at T2. Δ p = the chance to have developed red cell alloantibodies between T1 and T2 (i.e. following splenectomy). P-values were deduced from reported cumulative incidences according to number of red cell units transfused.<sup>8</sup>*

Contrary to our results, observational studies in patients with major thalassemia and sickle cell disease (a population not included in the current study) so far did not find any abrogation of red cell antibody development with splenectomy. Some even concluded these patients to be more prone to red cell alloimmunization.<sup>4,5</sup> Yet, hemoglobinopathy patients in need of splenectomy are often highly transfusion dependent, causing a beforehand high exposure related cumulative alloimmunization risk.<sup>8</sup> As such, exposure related confounding cannot be excluded as most of these studies did not correct for the cumulative exposure at the time of primary alloimmunization. Second, none reported the timing of alloimmunization to splenectomy nor the transfusion burden at the time of splenectomy, leaving the question whether alloimmunization, or even only CD4<sup>+</sup> T cell sensitization,<sup>12</sup> had not already occurred prior to splenectomy. With regard to the latter, alloimmunization following splenectomy could as such represent a T cell dependent process and may explain why some hemoglobinopathy patients still develop alloantibodies despite absence of the spleen. In addition, it is unknown how a functional deficiency of the spleen, as is known to be frequent in sickle cell disease patients, modulates red cell alloimmunization. As such, we argue it of importance to re-evaluate primary alloimmunization potentials in hemoglobinopathy patients with either anatomic or functional asplenia

by carefully taking into account the above mentioned methodological issues, in order to elucidate the spleen's role in immunization against allogeneic blood cells in this specific patient population.

Concerning the anti-E and anti-M formed by the splenectomized patient A, we should first recognize that they might have developed independent of red cell exposure, i.e. as so-called "naturally occurring antibodies". Second, the induction of anti-M (if from the IgM class) might implicate a T cell-independent humoral immune response, for which the spleen is known to be essential.<sup>13</sup> Although an accessory spleen, present in over 10% of humans, was not identified via post-splenectomy CT scanning of the abdomen, some functional splenic tissue might have remained after splenectomy mediating alloimmunization. Third, the specific combination of a donor liver transplant with splenectomy could have caused red cell alloimmunization via pre-primed lymphocytes derived from the donor's liver transplant (i.e. passenger lymphocyte syndrome). A similar mechanism has been reported in a patient developing non-hemolytic anti-M after multiorgan transplant.<sup>14</sup> Unfortunately, we could not retrieve the red cell antigenic phenotype of the liver donor to corroborate this hypothesis. Finally, we do not imply an absolute abolishment of red cell alloimmunization after splenectomy. Indeed, substantial evidence shows that at least a few asplenic patients are still capable to mount a protective immune response following non-conjugated polysaccharide vaccination.<sup>15</sup> In addition, the absence of a functional spleen can, at least partly, be compensated by vaccines targeting a germinal center B cell response.<sup>16</sup> Yet, the non-intravenous route of vaccines and the common use of conjugates differ considerably from the administration of donor red cells, facilitating epitope presentation and efficient induction of T cell dependent alloimmune responses in non-splenic lymphoid organs.

In conclusion, our findings suggest that splenectomy is strongly associated to protection from primary red cell alloimmunization in the general transfused patient population.

## References

1. Mebius RE, Kraal G. Structure and function of the spleen. *Nat Rev Immunol.* 2005;5(8):606-16.
2. Hendrickson JE, Saakadze N, Cadwell CM, et al. The spleen plays a central role in primary humoral alloimmunization to transfused mHEL red blood cells. *Transfusion.* 2009;49(8):1678-84
3. Hendrickson JE, Chadwick TE, Roback JD, Hillyer CD, Zimring JC. Inflammation enhances consumption and presentation of transfused RBC antigens by dendritic cells. *Blood.* 2007;110(7):2736-43.
4. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. *Blood.* 2000; 96(10): 3369-73.
5. Thompson AA, Cunningham MJ, Singer ST, et al. Red cell alloimmunization in a diverse population of transfused patients with thalassaemia. *Br J Haematol.* 2011;153(1):121-8.
6. Al-Mousawi MM, Al-Allawi NA, Alnaqshabandi R. Predictors of Red Cell Alloimmunization in Kurdish Multi Transfused Patients with Hemoglobinopathies in Iraq. *Hemoglobin.* 2015;39(6):423-6.
7. Nickel RS, Horan JT, Fasano RM, et al. Immunophenotypic parameters and RBC alloimmunization in children with sickle cell disease on chronic transfusion. *Am J Hematol.* 2015;90(12):1135-41
8. Evers D, Middelburg RA, de Haas M, et al. Red-blood-cell alloimmunisation in relation to antigens' exposure and their immunogenicity: a cohort study. *The Lancet Haemat.* 2016;3(6):e284-292
9. Evers D, Zwaginga JJ, Tijmensen J, et al. Treatments for hematological malignancies in contrast to those for solid cancers are associated with reduced red cell alloimmunization. *Haematologica* 2017; 102(1):52-9.
10. Calabro S, Gallman A, Gowthaman U, et al. Bridging channel dendritic cells induce immunity to transfused red blood cells. *J Exp Med.* 2016;213(6):887-96.
11. Gilson CR, Zimring JC. Alloimmunization to transfused platelets requires priming of CD4+ T cells in the splenic microenvironment in a murine model. *Transfusion.* 2012;52(4):849-59.
12. Martin F, Oliver AM, Kearney JF. Marginal zone and B1 B cells unite in the early response against T-independent blood-borne particulate antigens. *Immunity.* 2001;14(5):617-29.
13. Hudson KE, Lin E, Hendrickson JE, Lukacher AE, Zimring JC. Regulation of primary alloantibody response through antecedent exposure to a microbial T cell epitope. *Blood.* 2010;115(19): 989-96.
14. Makuria AT, Langeberg A, Fishbein TM, Sandler SG. Nonhemolytic passenger lymphocyte syndrome: donor-derived anti-M in an M+ recipient of a multiorgan transplant. *Immunohematology.* 2009;25(1):20-3.
15. Cherif H, Landgren O, Konradsen HB, Kalin M, Bjorkholm M. Poor antibody response to pneumococcal polysaccharide vaccination suggests increased susceptibility to pneumococcal infection in splenectomized patients with hematological diseases. *Vaccine.* 2006;24(1):75-81
16. Meerveld-Eggink A, de Weerd O, van Velzen-Blad H, Biesma DH, Rijkers GT. Response to conjugate pneumococcal and Haemophilus influenzae type b vaccines in asplenic patients. *Vaccine.* 2011;29(4):675-80.

## Supplementary Material

**Table S1** Characteristics of 1,010 non-alloimmunized sampled controls during the alloimmunization risk period according to a history of splenectomy.

Characteristics	Splenectomized (N=19)	Non- splenectomized (N=991)	Missing	Possible confounder Y/N
<b>General</b>				
Men	12 (63.2)	556 (56.1)	0 (0)	Y
Age in years (median, IQR)	58.0 (38.5-74.3)	65.2 (51.9-75.1)	0 (0)	Y
Cumulative (lifetime) number of red cell units up till implicated transfusion (median, IQR)	4 (2-14)	4 (2-8)	0 (0)	NA
<b>Diagnoses</b>				
Atherosclerosis *	4 (21.1)	310 (31.3)	13 (1.3)	Y
Diabetes mellitus type 1	0 (0)	7 (0.7)	0 (0)	N
Diabetes mellitus type 2	4 (21.1)	172 (17.4)	1 (0.1)	Y
GFR $\leq$ 30 ml/min †	2 (10.5)	147 (14.8)	0 (0)	Y
Dialysis (chronic or acute) ‡	2 (10.5)	96 (9.7)	0 (0)	N
Chronic obstructive airway disease §	2 (10.5)	87 (8.8)	19 (1.9)	N
Organ transplant (in past or during risk period)	0 (0)	33 (3.3)	0 (0)	Y
Liver cirrhosis	0 (0)	24 (2.4)	2 (0.2)	N
Hematological malignancy	1 (5.3)	209 (21.1)	5 (0.5)	Y
Carcinoma	3 (15.8)	180 (18.2)	5 (0.5)	N
Sarcoma	3 (15.8)	15 (1.5)	1 (0.1)	Y
<b>Interventions</b>				
ICU admission	15 (78.9)	354 (35.7)	0 (0)	Y
days at ICU (median, IQR)	3 (1-8)			
Surgery				
thoracic, including CABG	3 (15.8)	141 (14.2)	0 (0)	N
abdominal	17 (89.5)	164 (16.5)	0 (0)	Y
back or spinal cord	1 (5.3)	10 (1.0)	0 (0)	Y

ABSENCE OF THE SPLEEN PROTECTS AGAINST RED CELL ALLOIMMUNIZATION

Immunosuppressant medication	9 (47.4)	414 (41.8)	13 (1.3)	Y
Chemotherapy	0 (0)	215 (21.7)	4 (0.4)	Y
Radiotherapy	0 (0)	37 (3.7)	0 (0)	Y
Hematopoietic stem cell transplantation (in past or during risk period) ¶	1 (5.3)	63 (6.4)	0 (0)	N
<b>Treatment-related complications</b>				
Leukopenia **	5 (26.3)	303 (30.6)	24 (2.4)	Y
Infections				
(tissue-invasive) severe bacterial ††	8 (42.1)	161 (16.2)	32 (3.2)	Y
Gram-positive bacteremia	1 (5.3)	82 (8.3)	7 (0.7)	Y
Gram-negative bacteremia	1 (5.3)	43 (4.3)	0 (0)	N
disseminated viral ††	1 (5.3)	19 (1.9)	0 (0)	Y

Values are n (%), unless otherwise stated. Numbers of patients for whom data on certain conditions were not documented are presented as missing. Covariates were identified as a possible confounder of splenectomy when considered to be a potential risk factor for alloimmunization and being associated with splenectomy among the source population (i.e., represented by the sampled controls). An association with splenectomy was considered present in case of a  $\geq 3\%$  difference in covariate presence between controls exposed and controls.

IQR = interquartile range. NA = not applicable, as this represents a matched variable.

\*systemic or coronary atherosclerosis. † glomerular filtration rate (GFR) below 30 ml/min during at least one week of the risk period (with GFR calculated using the Modification of Diet in Renal Diseases (MDRD) equation). ‡ hemodialysis, peritoneal dialysis, or continuous veno-venous hemofiltration needed for at least one day during the risk period. § chronic asthma bronchiale or chronic obstructive pulmonary disease. || medication under subcategory H02 (corticosteroids) or L04 (other immunosuppressants) within the World's Health Organization Anatomical Therapeutic Chemical (ATC) classification index. ¶ autologous or allogeneic. \*\* at least once measured leukocyte counts below lower limit of normal. †† defined as: abscesses, cardiac infections, infected foreign material, intra-abdominal infections, lower respiratory tract infections, meningitis, osteomyelitis, soft tissue infections, spondylodiscitis, upper urinary tract infections. ‡‡ defined as: viremia and varicella zoster infections.

