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# Challenging the dogma: Red blood cell-directed autoimmunity as risk factor for red blood cell alloimmunisation after blood transfusion

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## Summary

Red blood cell autoimmunity and alloimmunity are potentially linked. Quantification of this association can tailor extensively matched red blood cell transfusions in patients with autoimmunity. Using an incident new-user cohort comprising 47 285 previously non-transfused, non-alloimmunised patients, we compared transfusion-induced red blood cell alloimmunisation incidences in direct antiglobulin test (DAT)-positive and control patients. Additionally, we performed case–control analyses to handle potential confounding by clinical immunomodulators. Among (IgG and/or C3d) DAT-positive patients ($N = 380$), cumulative red blood cell alloimmunisation incidences after 10 units transfused reached 4.5% (95% confidence interval [CI] 2.5–8.2) versus 4.2% (CI 3.9–4.5, $p = 0.88$) in controls. In case–control analyses, alloimmunisation relative risks among DAT-positive patients increased to 1.7 (CI 1.1–2.8). Additional adjustments for pre-DAT transfusion exposure or the extent of Rh/K mismatching did not impact results. In conclusion, while patients with DAT positivity show an intrinsically increased alloimmune red blood cell response, their absolute risk is comparable to control patients due to counteracting co-existing immunosuppressive conditions. Consequently, isolated DAT positivity in patients lacking overt haemolysis or complicated alloantibody testing does not seem to warrant extended matching strategies.

**KEYWORDS**
autoimmunity, direct antiglobulin test, red blood cell alloimmunisation, red blood cell autoantibodies

Dorothea Evers and Jaap Jan Zwaginga contributed equally to this work.

## INTRODUCTION

Red blood cell autoimmunity and alloimmunity are considered closely linked. Indeed, red blood cell auto- and alloantibodies coincide among various transfused patient populations.[1–4] In agreement, patients with various (chronic) autoimmune diseases[3–6] show higher prevalences of red blood cell alloantibodies. Although the underlying mechanism of such a linkage is not fully understood, a widespread activation of the immune system seems logical. Yet, estimations of true alloimmunisation risks in autoimmune-mediated conditions have been challenging. Moreover, autoimmunisation and alloimmunisation are inherently different: while alloimmunisation is an appropriate default response towards non-self antigens, autoimmunisation represents insufficient tolerance towards heterogenous autoantigens.

In the Netherlands, Rhesus/Kell (Rh/K) matching is selectively applied in specific clinical and diagnostic situations of existing autoantibodies, that is, patients with active immune-mediated haemolytic anaemia (AIHA) and those manifesting strong autoantibodies complicating serology and cross-matching.[7] In this light, evidence for matching in AIHA patients is primarily based on small observational prevalence studies,[8] lacking acknowledgement of important confounders of alloimmunisation such as cumulative transfusion exposure[9] and immunosuppressant usage.[10] Also, the largest multinational study among patients with warm autoantibodies (WAA) reported on transfusion-induced alloimmunisation, but unfortunately omitted to compare findings to the largest majority of patients, that is, controls without WAAs.[4] Hence, the dogma of red blood cell autoimmunity being a risk factor for transfusion-induced alloimmunisation lacks substantiation.

Hypothetically, a positive direct antiglobulin test (DAT), signalling antibody presence on circulating red blood cells, could serve as an early indicator for autoimmunity, potentially qualifying DAT-positive patients for preventative matching. However, due to logistical and financial burdens, implementing such a strategy should be considered only when substantial benefits are validated.

We, therefore, set out to quantify the association between red blood cell-directed autoimmunity as determined by DAT positivity and transfusion-induced red blood cell alloimmunisation, considering important alloimmunisation risk modulators. With this knowledge, we aimed to comprehend how autoimmunity potentially attributes to red blood cell alloimmunisation and contribute to tailoring preventative matching strategies.

## METHODS

This study was conducted within our previously published multicenter risk factors for alloimmunisation after red blood cell transfusion (R-FACT) study cohort.[11,12] In short, the cohort constitutes all newly transfused, previously non-alloimmunised patients who received their first and subsequent red blood cell transfusion(s) in six Dutch hospitals between January 2005 and January 2019 (Box S1). Primary transfusion-induced alloimmunisations were defined as directed against the antigens: c, C, e, E, K, Cw, Fya, Fyb, Jka, Jkb, Lua, Lub, M, N, S or s. Herein, we considered the last antigen-mismatched transfusion (based on either a tested or unknown donor phenotype) preceding the first positive screen to have elicited immunisation (Figure S1). Positive antibody screenings without alloantibody identification were included as non-alloimmunised. Patients with previous (pregnancy-induced) alloimmunisation, haemoglobinopathies or an age below 6 months were excluded.

DAT positivity was defined as at least one positive (i.e. 0.5+ till 4+) monospecific DAT result for anti-IgG and/or anti-C3d, preceding at least one red blood cell transfusion and followed by subsequent alloantibody screening. To maintain generalizability between our study population and the general transfusion population, the control group comprised all patients with negative DAT results as well as patients lacking any DAT testing during the study period. If performed, patients with negative eluates were considered DAT negative (Box S2). Positive eluates for anti-A and/or anti-B due to ABO mismatched allogeneic haematopoietic cell transplantation were also considered as DAT negative.

Donor antigen phenotypes of all transfused units were submitted by Sanquin Blood Supply, Amsterdam, while patient phenotypes, if determined, were either electronically or manually obtained in the six hospitals. Notably, in the Netherlands, patient phenotypes are only routinely determined for patients requiring extended matched blood products.[7]

Within this incident new-user cohort and using Kaplan–Meier survival analyses, we then first compared cumulative alloimmunisation incidences between DAT-positive and control group patients. Here, the time-dependent variable was the cumulative number of red blood cell units received up to the first-time alloantibody formation or up to the last negative alloantibody screen. Subsequently, three sensitivity analyses were performed: (1) Considering that the immunological responses of IgG are more closely linked to alloimmune responses as compared to C3d, DAT results were subdivided according to the type of monospecific DAT (i.e. IgG or C3d). Patients with concurrent positive IgG and C3d DATs were alternatively included as IgG DAT positive and as C3d DAT positive in two separate analyses. (2) To determine whether alloimmunisation incidences are impacted by DAT positivity levels, positive DAT results were subdivided into weakly (i.e. 0.5+ till 2+) or strongly (i.e. 3+ till 4+) positive. (3) To determine whether positive DAT results accompanied by a positive eluate (i.e. detecting [non-]specific warm or cold autoantibodies) are more strongly associated with alloimmunisation, positive eluate patients were compared to patients with DAT-positive results without eluate testing, and the control group.

Finally, our control group comprised patients with negative DAT results and those without DAT testing, assuming

general comparability. Considering that DAT testing involves non-routine diagnostics (Box S2), their specific indications might have impacted our results. We therefore performed additional sensitivity analyses, dividing our initial control group into DAT-negative patients and those lacking DAT results and compared them to DAT-positive patients.

Second, an unknown fraction of the DAT-positive group includes AIHA patients, who, in the Netherlands, receive Rh (i.e. RhCcEe)/K extended matched red blood cell transfusions.[7] Thus, reduced alloimmune exposure in these patients likely lowers alloimmunisation risks as compared to unmatched patients. To adjust for this potential reduction in Rh/K alloimmune exposure among DAT-positive patients, we calculated the 'cumulative immunogenic exposure' to Rh/K mismatches for each patient using the following methods and further illustrated in Box S3: (1) Missing values in donor and patient Rh/K phenotypes were handled by multiple imputation (Box S4). (2) For each patient, the cumulative number of Rh and K mismatches was determined (i.e. a mismatch was defined as a *N*-antigen-negative patient receiving a *N*-antigen-positive red blood cell unit). (3) Subsequently and for each Rh/K antigen, the cumulative number of mismatches was interpolated by previously reported cumulative antigen-specific alloimmunisation risks according to cumulative antigen mismatches.[12] (4) Finally, all determined Rh and K antigen-specific alloimmunisation risks were added together and defined as the cumulative immunogenic exposure to Rh/K mismatches per patient. We then compared the cumulative immunogenic exposure received by DAT-positive patients to the general transfusion population, wherein complete preventative Rh/K extended matching would represent zero immunogenic exposure received. Finally, in a Kaplan–Meier analysis, we determined red blood cell alloimmunisation incidences as a function of the cumulative immunogenic exposure to Rh/K mismatches (i.e. if DAT positivity indeed is a risk factor for alloimmunisation, we expect DAT-positive patients to show increased alloimmunisation incidences following the same amount of Rh/K immunogenic exposure compared to the control group).

Third, to take into account that interpatient differences in red blood cell exposure prior to the first positive DAT likely influences estimated alloimmunisation risks,[9] a first nested matched cohort was established (i.e. DAT-landmark cohort). For this, all DAT-positive patients were randomly matched to four control group patients, based on their cumulative number of red blood cell units received and the hospital. For each DAT-positive patient, the cumulative red blood cell exposure prior to the first positive DAT (i.e. landmark) was determined. Subsequently, for each matched cluster, all red blood cell units received up to this landmark were censored. Finally, we compared cumulative alloimmunisation incidences between DAT-positive patients and their matched controls, using Kaplan–Meier landmark survival analyses. Here, the cumulative red blood cell exposure excluding the censored units prior to the landmark served as a time-dependent variable (Figure S2).

Fourth and finally, to take into account confounding by clinical immunomodulating conditions, we compared alloimmunisation risks between DAT-positive patients and their controls using multivariate logistic regression models in a second matched cohort (i.e. case–control alloimmunisation cohort). Descriptions of the establishment and used methodology of this case–control have been previously published[13] and are summarised in Figure S1 and Box S5. Here, DAT positivity was defined as a positive result during and/or before the alloimmunisation risk period. Missing data were handled by multiple imputations (Box S6). Crude RRs were conditioned on the cumulative number of red blood cell units received and the hospital. Adjusted RRs were additionally conditioned on identified (relevant) clinical immunomodulating confounders by using propensity scores.

An overview of all used study designs is provided in Table S1.

## RESULTS

Among 103 124 newly transfused patients, 47 825 patients fulfilled all inclusion criteria (Figure S3). Table 1 presents the general characteristics of this study cohort and their DAT results. Red blood cell alloimmunisation occurred in 1127 (2.4%) patients, who formed a total of 1280 alloantibodies, including 876 (68.4%) directed against Rh/K. DATs were performed in 4456 (9.3%) patients. Of those, 380 (0.8%) had positive results for IgG and/or C3d. All others, including most of the patients in whom no DAT was performed (i.e. 90.7%), were included in the control group (Figure S3).

### Alloimmunisation incidences according to DAT result (incident new-user cohort)

In general, cumulative red blood cell alloimmunisation incidences were comparable between patients with and without positive DAT results, that is, 4.5% (95% confidence interval [CI] 2.5–8.2) as compared to 4.2% (CI 3.9–4.5, $p = 0.88$) after 10 units received (Figure 1A). In detail, as compared to the control group, patients with different types of DAT reached similar alloimmunisation incidences after 10 units received, that is, 4.2% (CI 2.0–8.4, $p = 0.88$) for IgG positive DATs and 5.9% (CI 2.2–15.2, $p = 0.53$) for C3d positive DATs (Figure S4A,B). Furthermore, the degree of DAT positivity did not significantly impact results as alloimmunisation incidences after 10 units received increased to 3.2% (CI 1.3–7.8, $p = 0.30$) and 6.8% (CI 2.9–15.4, $p = 0.25$) in weak and strong positive results, compared to the control group (Figure S4C). Cumulative incidences among control patients were comparable to DAT-positive patients with or without eluate testing, that is, 7.1% (CI 1.0–40.9, $p = 0.94$) and 4.2% (CI

**TABLE 1** Basic characteristics of the incident new-user cohort and DAT results.

| | Study population (*N* = 47 825) |
|---|---|
| Male | 25 123 (52.5) |
| Age at first red blood cell unit, years, median (IQR) | 66.8 (53.5–76.3) |
| Academic centre | 24 094 (50.4) |
| Number of transfused cumulative red blood cell units | 320 204 |
| Number of red blood cell units per patient, median (IQR) | 4 (2–8) |
| Follow-up days, median (IQR) | 92 (13–527) |
| Red blood cell alloimmunisation | 1127 (2.4) |
| Number of red blood cell alloantibodies formed | 1280 |
| Rh/K alloantibodies | 876 (68.4) |
| Non-Rh/K alloantibodies | 404 (31.6) |
| DAT performance and results | |
| DAT not performed | 43.369 (90.7) |
| DAT negative, based on | 4.076 (8.5) |
| Negative DAT result | 3929 (8.2) |
| Negative eluate | 120 (0.3) |
| Positive DAT due to anti-A/B post-allogeneic stem cell transplantation | 27 (0.1) |
| DAT positive | 380 (0.8) |
| Type of monospecific DAT | |
| IgG | 272 (0.6) |
| C3d | 76 (0.2) |
| IgG and C3d | 32 (0.1) |
| Level of positivity[a] | |
| Weak positive | 232 (0.5) |
| Strong positive | 148 (0.3) |
| Eluate results | |
| Non-specific warm autoantibodies | 21 (0.0) |
| Non-specific cold autoantibodies | 1 (0.0) |
| Eluate not performed | 358 (0.8) |

*Note*: Values are *n* (%), unless otherwise stated.

Abbreviations: DAT, direct antiglobulin test; Ig, immunoglobulin; IQR, interquartile range; K, Kell; Rh, Rhesus.

[a]Positive DAT results were classified as weakly positive (i.e. 0.5+ till 2+) or strongly positive (i.e. 3+ till 4+).

2.2–7.9, $p = 0.89$) (Figure S4D). Finally, upon dividing our control group into DAT-negative patients (≈8% of initial controls) and those without DAT results (≈90% of initial controls), we observed that DAT-negative patients had the lowest alloimmunisation incidences (1.8% [CI 1.3–2.3] after 10 units received), distinct from both DAT-positive patients and patients lacking DAT testing (incidences 4.5% [CI 2.5–8.2] and 4.7% [CI 4.3–5.0] after 10 units received, $p = 0.00$, Figure 1B).

## Alloimmunisation incidences as a function of the immunogenic exposure (incident new-user cohort)

Of all units transfused during the study period, about 3% of Rh/K phenotypes were untraceable, whereas approximately 90% of patient Rh/K phenotypes were not determined (Table S2). Table S3 presents an overview of the estimated cumulative immunogenic exposure to Rh/K mismatches in the incident new-user cohort. The general transfusion population received a median of 0.7 (IQR 0.0–1.9) immunogenic Rh/K exposure, whereas DAT-positive patients received slightly more Rh/K incompatibility (0.9, IQR 0.0–3.2), contradicting the hypothesis that Rh/K extended matching was more heavily applied across the DAT-positive cohort. As illustrated in Figure 2, according to cumulative Rh/K immunogenic exposure, DAT-positive patients do not significantly form more Rh/K alloantibodies as compared to control group patients (i.e. cumulative incidences of Rh/K alloimmunisation 9.0% [CI 7.0–11.4] among DAT-positive patients as compared to 5.4% [CI 5.2–5.6, $p = 0.41$] among matched controls following a cumulative immunogenic exposure of 3.0).

## Alloimmunisation incidences after censoring of pre-positive DAT exposure (DAT-landmark cohort)

Table S4 presents the basic characteristics of the 380 DAT-positive patients and their 1520 matched controls. Patients received a median of 3 (0–13) units prior to their first positive DAT or matched landmark. Red blood cell alloimmunisation occurred in 15 (4.0%) of DAT-positive patients compared to 35 (2.3%) control patients, resulting in alloimmunisation incidences of 5.5% [CI 3.2–9.2] after 10 units received among DAT-positive patients as compared to 3.2% [CI 2.2–4.8, $p = 0.07$] in matched controls (Figure 3).

## Alloimmunisation risks with adjustment for clinical confounders (case–control alloimmunisation cohort)

Basic characteristics of the nested case–control study cohort, consisting of 505 alloimmunised cases and 1010 matched control patients, as well as the number of missing values, and identified confounders used for propensity score calculations are presented in Table S5. Noticeably and as previously reported,[10] the use of immunosuppressants was higher among alloimmunised as compared to non-alloimmunised patients (42.4% vs. 30.9%). After adjusting for clinical confounders, DAT-positive patients demonstrated a 1.7-fold increased risk for red blood cell alloimmunisation (RR 1.7 [95% CI 1.1–2.8]; Table 2).

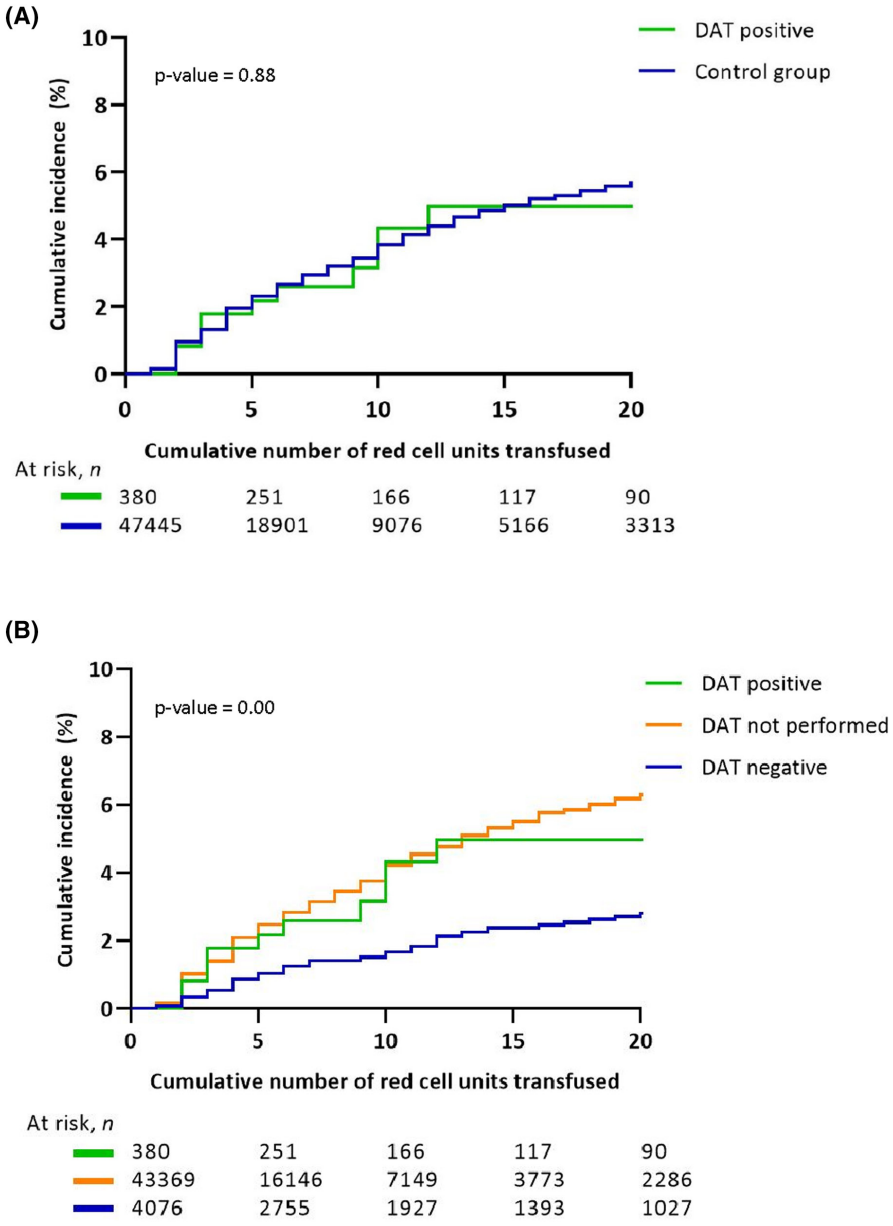


**FIGURE 1** Cumulative red blood cell alloimmunisation incidences in the incident new-user cohort. (A) Cumulative alloimmunisation incidences among newly transfused patients. (B) Sensitivity analysis on DAT-negative control group with additional subdivision of this control group into patients in whom no DAT was performed during the study period, and patients with negative DAT results. DAT, direct antiglobulin test.

## DISCUSSION

This current study is the first large incidence cohort study on the association between red blood cell-directed autoimmunity as determined by DAT and transfusion-induced red blood cell alloimmunisation. As the potential impact of autoimmunisation on alloimmunisation is largely determined by cumulative (mismatched) exposure and clinical conditions, we employed various methodological approaches. After adjusting for immunomodulating confounders, patients with DAT positivity demonstrated a 1.7-fold increased alloimmunisation risk. This intrinsically increased humoral functioning toward allogeneic red blood cells, however, seems nullified by a co-existence of immune-depressing conditions, including a widespread use of immunosuppressants. This is illustrated by comparable (non-adjusted) alloimmunisation incidences among DAT-positive and non-tested patients in both our incidence and nested matched cohorts. Translating to a clinical advice, in our opinion, an isolated positive DAT without specific clinical context does not warrant preventative matching. Yet, we do not debate the current practice of extensive matching in cases of active AIHA, as the immense complexity of alloantibody screening and cross-matching in these patients justifies this approach.

Our findings certainly require methodological discussion.

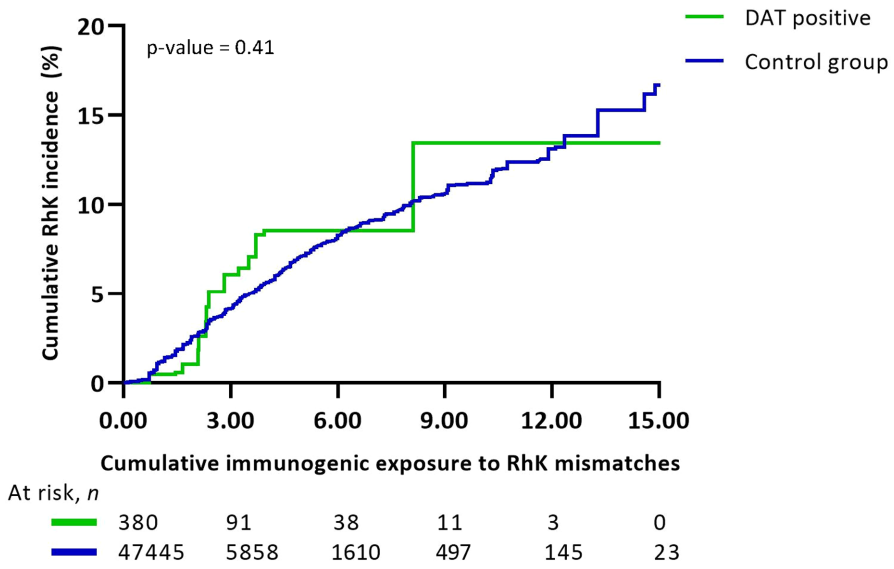


**FIGURE 2** Cumulative red blood cell alloimmunisation incidences in the incident new-user cohort as a function of the cumulative immunogenic exposure to Rh/K mismatches. The cumulative immunogenic exposure to Rh/K mismatches was calculated by the sum of all determined Rh/K antigen-specific alloimmunisation risks, that is, for each patient, the cumulative number of Rh/K antigen mismatches was interpolated by previously reported antigen-specific alloimmunisation risks (Box S3).

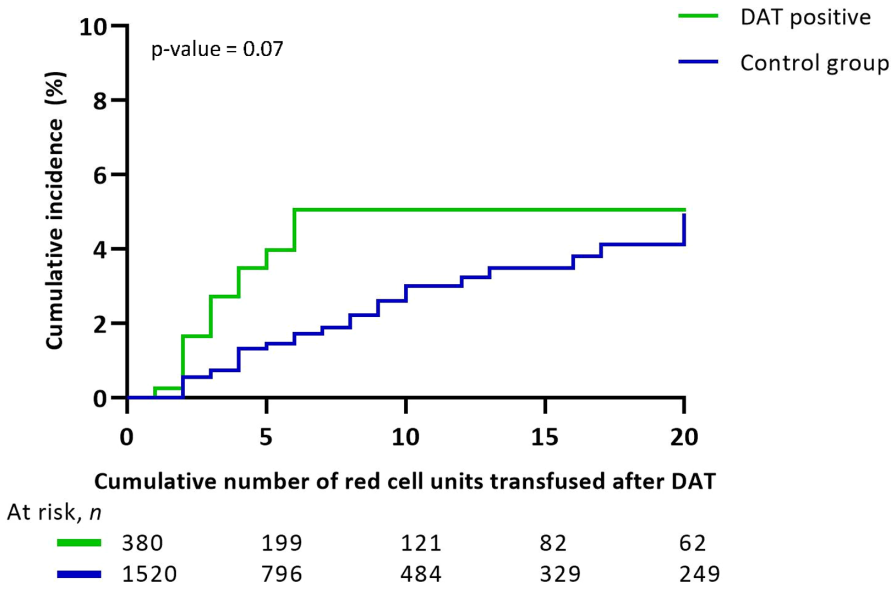


**FIGURE 3** Cumulative red blood cell alloimmunisation incidences in the DAT-landmark cohort, with censoring of red blood cell units received prior to the first positive DAT. Each DAT-positive patient was matched to four randomly selected control patients based on the matching variables: cumulative number of red blood cell units transfused and hospital. For each cluster a landmark was installed, that is, the cumulative number of red blood cell units prior to the first positive DAT in the DAT-positive patient. Units transfused prior to this landmark were censored from this analysis (Figure S2). DAT, direct antiglobulin test.

First, our additional sensitivity analyses, which divided our initial control group into DAT-negative patients and those lacking DAT results, unexpectedly revealed that DAT-negative patients exhibited the lowest alloimmunisation incidences compared to both DAT-positive patients and the general transfusion population. Such specific comparisons have not been reported earlier. Among specific patient populations, mainly thalassaemia, an association between red blood cell auto- and alloimmunisation has been suggested,[1,14,15] but conclusions remain challenging as cumulative exposure, an important confounder, was not considered. Furthermore, the large study of Delaney et al. confined analyses to patients with WAAs, thereby excluding important comparisons to the non- or negatively tested general transfusion population.[4] Importantly, the observed risk difference, which stems from the significantly lower alloimmunisation risk among DAT-negative patients rather than an elevated risk linked to DAT positivity, is specific to this

**TABLE 2** Association between positive DAT results and red blood cell alloimmunisation in the case–control alloimmunisation cohort.

| | Cases | Controls | Crude RR | Adjusted RR |
|---|---|---|---|---|
| | *N* (%) | *N* (%) | 95% CI[a] | 95% CI[b] |
| DAT[c] | | | | |
| Negative/not performed | 500 (99.0) | 1000 (99.0) | Ref. | Ref. |
| Positive | 5 (1.0) | 10 (1.0) | 1.1 (0.7–1.6) | 1.7 (1.1–2.8) |

Abbreviations: CI, confidence interval; DAT, direct antiglobulin test; RR, relative risk.

[a]Crude RR adjusted for cumulative number of red blood cell units transfused and hospital.

[b]Additionally adjusted for identified potential confounders (for details, see Table S5).

[c]First positive DAT for IgG and/or C3d during or before the alloimmunisation risk period.

patient group and not applicable to the general transfusion population.

The explanation of our observed mitigated risk of DAT-negative patients remains unclear and intriguing. Possibly, the patients' clinical conditions warranting DAT testing represent an overall immune depression, impacting both auto- and alloimmunisation in these DAT-negative patients. However, if we extrapolate our obligatory study design to a hypothetical prospective study where DATs are performed in all patients undergoing transfusion, the DAT-negative group would be diluted by a large majority of patients lacking such immune depression (i.e. the non-tested population in our study). Thus, under these circumstances, DAT negativity would likely no longer demonstrate a protective effect against alloimmunisation. Similarly, we cannot dismiss the possibility that our eluate comparison analyses may have been biased by specific clinical indications warranting eluate testing, potentially affecting the generalizability of our results to the overall DAT-positive cohort.

Second, as cumulative red blood cell exposure is known to impact alloimmunisation risks,[9] it might similarly impact autoimmunisation. Autoantibody prevalence among multi-transfused patients, mainly studied in small Asian cohorts with haemoglobinopathy and myelodysplastic syndrome,[1,2,14–21] have an overall tendency to exceed prevalences among the general population.[3,4] This association between transfusion exposure and subsequent immunisation might reflect a common immune activating mechanism. In this respect, in various animal and human experimental models, the introduction of incompatible red blood cells induced both red blood cell auto- and alloantibody formation.[22] Moreover, reports have shown a temporal association between the development of autoantibodies (most often accompanied by haemolytic anaemia), exposure to red blood cell transfusions and alloimmunisation.[23,24] Although unclear, transfusion-associated alloimmunisation might, for example, be induced by the donor-recipient incompatibility of non-exofacial polymorphisms, which contribute to the immunogenicity of blood group antigens via enhancement of CD4+ helper T-cell responses. The latter has been implicated to enhance both alloimmunisation and autoimmunisation after blood transfusion.[25] Importantly, our study excluded multi-transfused patients with haemoglobinopathy and adjusted for cumulative red blood cell exposure before and after DAT. This eliminates possible confounding related to transfusion burden and its timing, as well as to the frequency of antibody screenings. Regarding the latter, multi-transfusions inevitably result in multiple indirect antiglobulin tests, thereby increasing the likelihood of detecting auto- and alloantibodies compared to less intensively transfused patients.

On the contrary, DAT-positive patients, particularly those with AIHA, are more likely to restrictively receive transfusions. Together with an often accompanying use of immunosuppressants by such patients, this will result in the mitigation of their intrinsically enhanced alloimmune response. Such factors likely explain our intuitively discrepant findings of comparable alloimmunisation incidences (i.e. absolute risk) between DAT-positive patients and control patients. Indeed, after adjustment for clinical conditions including immunosuppressants use, these patients do demonstrate an increased relative risk. Notably, DAT-positive patients, unlike the small subset of AIHA patients, were shown not to receive extensively matched transfusions at large scale. The immunogenic exposure to Rh/K mismatches did not differ between the general population and DAT-positive patients, thus excluding an impact of Rh/K matching on our results.

In conclusion, DAT positivity is associated with an intrinsically increased red blood cell alloimmune response. Despite this, but explained by counteracting co-existing immune conditions, overall alloimmunisation incidences among DAT-positive patients are comparable to patients lacking DAT testing, while patients with a negative DAT result exhibit particularly low incidences of alloimmunisation. Taken together, in our opinion, DAT positivity does not warrant extensive red blood cell matching. Notwithstanding, the broadly applied strategy of preventative matching in AIHA remains prudent given its complicated red blood cell diagnostics and crossmatching induced by interfering autoantibodies. Whether overt AIHA is accompanied by a general alloimmune activation and even further enhances alloimmunisation risk remains unclear and hard to determine. Indeed, we could not deduce the calculated risk among AIHA patients as only seven were present in our case–control alloimmunisation cohort. This is likely because, except for a few patients with fulminant haemolysis, AIHA patients are not or very restrictively transfused, and if they do, it is typically with extensively matched units.

## AUTHOR CONTRIBUTIONS

JJZ designed the study. JAO and DE collected the data. JAO analysed the data. JAO, DE and JJZ interpreted the data. JAO, DE and JJZ wrote the manuscript. All other authors revised and approved the final manuscript.

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## CONFLICT OF INTEREST STATEMENT

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

## ETHICS STATEMENT

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

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## SUPPORTING INFORMATION

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

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